Standard Operating Procedures Clinical Protocol III Bronchoalveolar Lavage isolation and processing

NOTE: The following procedure is to be performed wearing laboratory coat, gloves, eye protection, and mask.

The bronchoalveolar lavage (BAL) must be processed immediately after collection.

1.Label all the tubes and slides needed for this procedure BEFORE the start of the protocol.

2.ICU Doc's will collect BALF in sterile traps (tri-trap assembly). Tubes should be documented for volume of normal saline infused, anatomical area lavaged (i.e. right middle lobe, and volume aspirated back into trap) for the bronchopist.

3.Give one aliquot to the ICU nurse to be sent to Microbio/Virology/Cyto. (They will need approximately 20ml of fluid.)

4.Place the two remaining traps on ice.

5.After the procedure, wait for the bronchopist to fill out the paperwork, obtain a copy immediately and transport the specimens back to the lab for processing.

6.Pool two traps of BALF and record the volume obtained.

7.Filter the lavage sample through a 40µm nylon filter into a sterile 50mL falcon tube.

8.Record the volume obtained after filtering (some fluid loss is expected).

9.Transfer 2mL of filtered lavage sample to a clean and identified 15mL Falcon tube.

10.Centrifuge the remaining fluid at 1200rpm (235 g) for 15 minutes with brakes at 4°C.

11.In the mean time, determine the number of cells present in the sample:

a.Mix 10µl of lavage fluid (from the 0.5 ml saved above) with 80l PBS + 10l Turks.

b.Load 10µl of this mix into each chamber of a hemocytometer. Count four squares in each chamber. Maintain two counts, one viable, one non-viable.

c.Calculate and record cell concentration for every chamber, as follows:

i.(Total cells counted/4) x 10 x 4000 = number of cells per mL

d.Do this for each chamber and average the numbers.

e.Calculate and record total cell number by multiplying “cells per ml” by the volume of BAL after filtering (less 2mL fraction removed).

f.Cell viability will be recorded as % of viable cells: (viable cells/total cells) x 100

12.Pour the centrifuged lavage supernatant into a 50mL Falcon tube and place on ice.

13.Put 1.5mL of supernatant into each of the ten cryovials and store the remaining supernatant in 10mL aliquots in the 15mL identified conical Falcon tubes.

14.Place all aliquoted supernatant into a cryopreservation box into a -70C freezer.

15.Set up the slides with the cytofunnels in the cytospin.

16.Compute the volume needed for 50,000 cells per slide.

a.(5x104cells/slide) / (Y x 104cells/mL) x 1000µL/mL = X µL/slide

b.X = µL to give 50 000 cells/slide.

c.Y = cell concentration from Step 6.

17.Multiply X by 12.

18.Remove this volume from the 2mL BAL aliquot.

19.Bring total volume to 6mL with PBS in a separate tube. Mix well.

20.Load 500µL into each cytofunnel.

21.Centrifuge the slides in the cytospin at 600 rpm (Cytospin2 by Shandon) for 10 minutes

22.Discard funnels and allow the slides to air dry at least two hours.

23.Stain two slides with Hemacolor stains as follows:

a.30 seconds in fixative #1

b.15 seconds in eosin Y (red) #2

c.15 seconds in thiazine (blue) #3

d.Rinse in distilled water

24.All remaining slides are fixed. At least two slides should be fixed in each of the following ways: Methanol, acetone or formalin. Indicate on the slide with #2 pencil how slide was fixed.
