SOP 3.13 Processing of Bronchoalveolar Lavage

SOP Number: 3.13
Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose

This SOP describes the procedure for the processing of a bronchoalveolar lavage sample.

Responsibility

It is the responsibility of all medical and research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

Procedure:

1. During processing and transport keep BAL sample in a container on ice at 4°C.

2. Measure the volume of BAL fluid on receipt at the processing laboratory. Record the volume of BAL fluid in the study specific documentation and/or data management system.

3. Filter BAL fluid through sterile gauze to remove aggregated debris

4. Centrifuge the fluid for 10 min at 300g at RT as soon as possible after collection.

5. Pour off and store the supernatant on ice at 4°C. If enzymatic activity, is to be measured in the supernatant it should be done immediately. Supernatant protein or lipid concentration can also be measured.

5. Re-suspend the cell pellet in 1mL of 1X PBS.

6. Perform a total cell count on an aliquot of the sample as outlined below.

Cell count

- A haemocytometer slide is used to perform cell counts using trypan blue exclusion dye stain to count viable cells.
- Add 20µL trypan blue to 100µL cell suspension, mix by gently vortexing or by aspirating the full volume of the suspension at least twice and incubate for 2 min at RT.
- A 20µL sample of the mixture is applied to the haemocytometer counting chamber and the cells are visualised by light microscopy.
- Viable cells exclude the dye and remain clear while dead cells stain blue. Cells in the four outer quadrants are counted and an average obtained.
- The number of cells is determined as follows: total count /4 (average number of viable cells) x 1.2 (dilution factor) x 1 x 10^4 (area under coverslip) = viable cells/mL.

7. Similarly, perform a differential cell count using for example Wright's stain. Express each cell type as a % of total cells. At least 200 cells should be counted when doing a differential, preferably from different areas on the slide.

8. Aliquot fluid into labeled storage tubes and store at -80°C. To avoid excess freeze/thaw cycles it is recommended that 50% of collected fluid be stored in a single cryostorage tube and the remainder in smaller aliquots.

9 The cell pellet may be used immediately in cell culture experiments or can be stored at -80°C for future RNA analysis. The cell pellet should be re-suspended in 1mL of TRIZOL or equivalent solution for RNA analysis and follow the procedure outlined in SOP 3.5.

10. Consideration should be given to the analysis to be performed on the BAL sample. If protease sensitive proteins are to be measured, the sample should be incubated with protease inhibitor prior to freezing. Samples to be used for nucleic acid extraction should be incubated with a specific agent to facilitate extraction (such as RNAlater) prior to freezing.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.