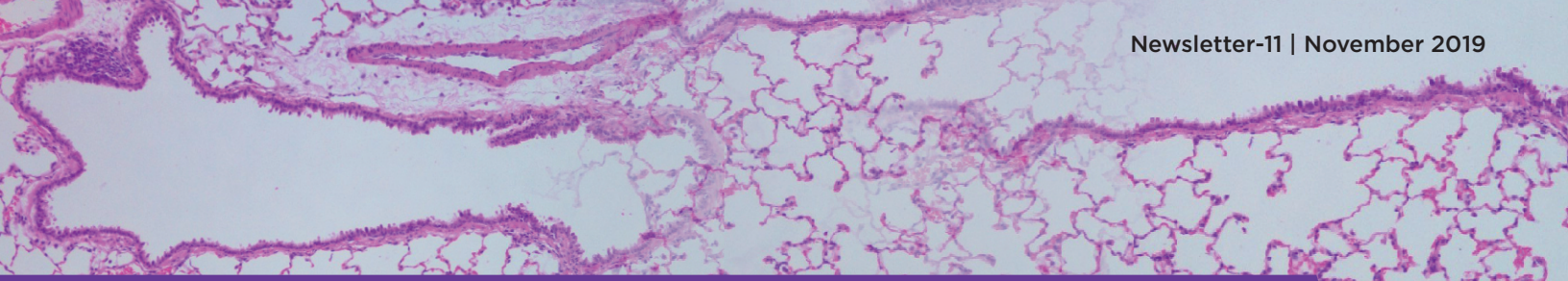

Broncho-alveolar Lavage (BAL) in Inhalation Studies

Air pollution nowadays is becoming a major challenge around the globe which may contain aerosols or nanoparticles. The lung is the first major vital organ exposed to these aerosols and nanoparticles. When there is evidence that, lower respiratory tract (alveoli/tracheobronchial region) is the site of deposition and retention of a test article, then broncho-alveolar lavage (BAL) fluid analysis can give an idea about pathological conditions of lungs such as alveolitis, etc.

BAL fluid analysis is useful when dose-response is occurring particularly in the lower respiratory tract. This analysis has limited relevance where lavage fluid cannot reach in areas such as focal responses, interstitial spaces, severe inflammatory reactions, neoplastic lesions, etc. Therefore, it is believed that BAL fluid analysis can go in parallel with histopathology of the lung but cannot replace it. When there is evidence of the limitation of BAL fluid analysis for lung toxicity, then histopathology becomes more relevant needing a strong approach towards lung toxicity assessment.

BAL fluid analysis gives two types of information, one is from the acellular component including levels of immunoglobulins, enzymes, inflammatory mediators and surfactant whereas, the other is the cellular component consisting of levels of various types of cells.

A procedure to collect the BAL fluid is as follows: After euthanasia of the animal by intraperitoneal administration of Thiopentone sodium, it should be positioned on its back and skin incision should be made to open the abdominal cavity. Exsanguinate the animal by cutting abdominal aorta/posterior vena cava or taking out the blood with the help of a syringe. Open the thoracic cavity, ligate/clamp the left lung lobe posterior to the bifurcation of the trachea (carina) to prevent the flow of fluid in the left lung which means broncho-alveolar lavage should be performed on the right lung after the lung weight has been recorded and before the lungs are inflated with a fixative. Both sides of lungs can be used for lavage fluid collection if the study is having another group assigned for histopathology of lungs.

For BAL fluid collection phosphate buffer saline (PBS) with a pH of approximately 7.4, should be used. A syringe with the needle should be placed in trachea and fluid is gently flushed into the lungs at the rate of approximately 25-30 mL/kg body weight. Over-inflation of lungs is to be avoided. This flush should be consisting of three up and down movements. Care should be taken to avoid any blood contamination of fluid and accidental damage before or during flushing, which may affect the data significantly by giving faulty cell count results. Only BALF data with a recovery >60% total flush volume should be considered. Recovered bronchoalveolar lavage fluid (BALF) should be stored on ice until processed. After completion of the BAL, the clamp/ ligation should be removed from the left mainstem bronchus and the lungs should be fixed with fixative for possible future histopathological examinations. The collected fluid should then be centrifuged at 400 x g at 4°C for 10 minutes as soon as possible. After refrigerated centrifugation, the supernatant from lavage should be carefully removed and placed in a tube which can be used for the analysis of lactate dehydrogenase (LDH), albumin, and total protein. The sediment should be resuspended in 1 mL of phosphate buffer saline for total and differential cell count. The differential count might be done by identifying a minimum of 100 cells using a low power light microscopy. The number of alveolar macrophages, lymphocytes, and neutrophils is quantified by multiplying the % per cell type by the total number of BAL cells.

According to OECD guideline 412 and 413, it is mandatory to analyse LDH activity, albumin or total protein in acellular BALF and cell counts and differentials for alveolar macrophages, lymphocytes and neutrophils from the cellular component of BALF.

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