BONCHOALVEOLAR LAVAGE FLUID SAMPLE PREPARATION FOR DIVERSE PROTEOMIC APPROACHES IN PULMONARY DISEASES

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The proteomic analysis of bronchoalveolar lavage fluid (BALF) protein content may reveal changes in lung protein expression and secretion during the course of pulmonary diseases. Sample preparation is a critical step in proteomic analysis of BALF. Procedures such as iTRAQ™, 2-D Electrophoresis and Mass Spectrometry require a sample with specific characteristics and BALF samples need to be carefully prepared in order to obtain consistent and reproducible results. Our intention is to generate a workflow that would allow for a small sample of BALF to be used in different proteomic analysis methods such as iTRAQ™ and 2-D electrophoresis.

In order to obtain a more concentrated BALF sample, acetone precipitation and a vacuum concentrator were tested. High abundance plasma proteins are present in BALF samples therefore the depletion of plasma proteins is necessary. Several affinity resin/bead columns were examined: ProteoMiner™ (Bio-Rad), Proteoprep Immunoaffinity Albumin and IgG Depletion Kit (SIGMA) and Albumin and IgG Depletion SpinTrap (GE Healthcare). The removal of salts and other contaminants is essential to prepare a sample for 2-D electrophoresis and iTRAQ™ experiments followed by mass spectrometry. To clean the samples, a 2-D Clean-Up kit (GE Healthcare) was used.

The comparison of different procedures suggests a specific technical workflow to improve the quantity and quality of protein samples required for the experimental setting. For example, concentrating samples by speed vac resulted in a relatively smaller protein loss than acetone precipitation. Although effective in reducing the amount of the major plasmatic proteins, ProteoMiner™ columns (Bio-Rad) equalize the concentration of all the proteins present in a sample, making them disadvantageous for our studies. In the present work we present the final workflow obtained from our experiments that allows for limited BALF samples to be used in different proteomic analysis methods such as iTRAQ™ and 2-D electrophoresis.