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Title: Processing optimization of exhaled breath condensate previous to the analysis by mass spectrometry

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Body: The Exhaled Breath Condensate (EBC) is a representative and non-invasive lung sample so the determination of its proteome might be useful to find disease-specific biomarkers. Most of the works published up to the date about this issue describe several problems to identify proteins by mass spectrometry. Aims: To evaluate three methods of EBC concentration as a pre-processing step for the use of mass spectrometry protein identification. To optimize the storage conditions for the EBC. Methods: EBC samples were collected with the EcoScreen Device (Viasys GmbH, Germany) and stored at -80°C. Protein quantification was performed by BCA methodology. Sample concentration was performed by liofilization, centrifugation with Amicon Ultra-2 filters (Millipore) or Reverse phase chromatography with POROS R2 resin. Proteins were identified by mass spectrometry. Results: After processing, there were no differences between liofilization and filtration which yielded an insufficient concentration for mass spectrometry (64.38±25.97; average μg/mL±standard deviation). Protein purification with POROS R2 followed by tryptic digestion gave place to the identification of 13 proteins. Long term storage of EBC affected dramatically the protein stability. Discussion: Protein concentration by reverse phase chromatography is necessary to determinate the EBC proteome by mass spectrometry. EBC samples should be analyzed within one year period to avoid protein degradation. This optimization is crucial to determinate the protein profile in EBC samples from different respiratory pathologies.