

Aspiration techniques for bronchoalveolar lavage in translational respiratory research: Paving the way to develop novel therapeutic moieties

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Abbreviations used: BAL, bronchoalveolar lavage; BALf, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity of the lungs for carbon monoxide; ELISA, enzyme-linked immunosorbent assay; IPF, idiopathic pulmonary fibrosis; MA, manual aspiration; NMR, nuclear magnetic resonance; qPCR, quantitative polymerase chain reaction; SPA, suction pump aspiration

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ABSTRACT

Bronchoalveolar lavage (BAL) is a simple, yet informative tool in understanding the immunopathology of various lung diseases *via* quantifying various inflammatory cells, cytokines and growth factors. At present, this traditional method is often blended with several robust and sophisticated molecular and biological techniques sustaining the significance and longevity of this technique. Crucially, the existence of slightly distinct approaches and variables employed at different laboratories around the globe in performing BAL aspiration indeed demands an utmost need to optimize and develop an effective, cost-effective and a reproducible technique. This mini review will be of importance to the biological translational scientist, particularly respiratory researchers in understanding the fundamentals and approaches to apply and consider with BAL aspiration techniques. This will ensure generating a meaningful and clinically relevant data which in turn accelerate the development of new and effective therapeutic moieties for major respiratory conditions.

Keywords: bronchoalveolar lavage, aspiration, respiratory

INTRODUCTION

Globally, chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis are among the leading causes of mortality and morbidity. Currently available treatments for these diseases only focus on alleviating various symptoms instead of completely preventing/treating the diseases, which warrants ongoing investigations for novel targeted treatments [1]. To establish an effective therapeutic moiety for various lung diseases, it is crucial to have a thorough knowledge on various cellular and immunological processes and parameters in lungs [2,3]. One such approach includes sampling bronchoalveolar lavage fluid (BALf), which aids in the assessment of immune cells, as well as various cytokines and mediators produced by these immune cells and the respiratory epithelial cells.

With bronchoalveolar lavage (BAL) method, various cellular and non-cellular components from the respiratory tract (epithelial lining fluid)

can be recovered [4]. Despite being a traditional approach, it avoids lung biopsies in various human diseases like pulmonary proteinosis, alveolar haemorrhage and eosinophilic pneumonia. Apart from these, the application of BAL includes diagnosis of various infectious diseases such as ventilator-associated pneumonia, mycobacterial infection, aspergillus fumigatus infection, pneumocystis pneumonia, chlamydia pneumoniae pneumonia, cryptococcal infection and non-infectious diseases like idiopathic lung fibrosis, connective tissue disorders, alveolar haemorrhage, alveolar proteinosis, *etc.*

The historical concept of BAL started in early 1920s, when it was found particularly useful in the treatment of phosgene gas poisoning [5]. At present, evaluation of BAL remains one of the most commonly employed techniques in both diagnosis and research in respiratory disorders to gain meaningful cytological information [6,7]. The major advantage of collecting BALf is minimal invasion of patient, which is the most important basis of human and veterinary medicine [8].

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Despite the immense clinical utility of BAL in the evaluation of respiratory diseases, our understanding is limited to establishing and optimizing a simple, effective, affordable and reliable BAL aspiration method at laboratory scale. Thus, it is of utmost importance to review the currently employed common practices of BAL acquisition in laboratory animals and how this useful technique enables the in-depth understanding of respiratory diseases and research. This mini review primarily focuses on the importance and approaches employed in various published studies for BAL collection and will benefit various biological translational and medical researchers, particularly in the field of respiratory diseases.

CLINICAL UTILITY OF BALf COLLECTION

The BALf collection in human patients involve inserting a flexible tube connected to a mini-camera (commonly known as fiberoptic bronchoscope) into the trachea to enable real-time visualization and operating the instrument at the appropriate site, post administration of local anesthesia [9]. Rosell *et al.* also recommended using plastic tubing between the 50 ml syringe and the working channel of the flexible bronchoscope when carrying out manual instillation and suction [10]. In most cases, three to five aliquots of 50 ml of sterile, phosphate-buffered saline solution at 37°C is instilled and after each instillation, the fluid is gently aspirated with a negative pressure of -40 to -50 mm Hg [11]. Recently, a systemic review of published literature upto 2016 concluded that the process of collecting BALf *via* flexible bronchoscope in humans is generally safe, however, it also asserted the need of more research to document complications of procedure in greater details [12].

The main rationale and objective for collecting the BALf from widely used mice models of respiratory diseases is to evaluate differential cell counts, which is clinically significant in understanding and underpinning various process and mechanisms involved in inflammation, primarily at cellular level, but also in elucidating disease pathology at large [13-17]. This is an approach which also helps in obtaining a correlation between the BAL differential cell counts and various airway responsiveness parameters such as airway resistance, diffusing capacity of the lungs for carbon monoxide (DLCO) *etc.*, which ultimately predicts the pathophysiological consequences occurring during various stages of the chronic inflammatory airway diseases.

Several attempts have been made to critically evaluate various aspiration techniques to optimize and have a reliable approach for the collection of BALf in various animal models of airway diseases. One such study by Woods *et al.* [8] involved collection the BALf using manual aspiration (MA) and suction pump aspiration (SPA) in dogs with pulmonary disease, and then comparing the sample quality of the collected BALf. They have shown that SPA aids in better BALf retrieval than MA, however, it does not affect the diagnostic parameters. Similarly, Hoffman have investigated various sampling techniques and developed guidelines for cytologic preparation and analysis in equine airways [18].

Many researchers have postulated various methods to carry out intubation of the mouse lung in order to assess both pulmonary function and BAL in the individual mice with longitudinal studies [19-23]. Such techniques appear to be largely successful but they are quite expensive and require intense training for the operator. Notably, Das *et al.* reported a simplified approach for intubating mice in longitudinal studies where the study involved limited number of expensive research animals [24]. They

demonstrated usage of fiber-optic light source to visualize trachea which makes the technique convenient to the researcher and aids in measurement of lung mechanisms for relatively longer duration (approximately several weeks) [24]. Crucially, this method could facilitate repeated BALf collection in rats that could reveal real time cellular/cytokine profile changes in the models of chronic respiratory diseases [25].

Maxeiner and co-workers standardized a method in murine model of lung carcinoma and melanoma which assists in understanding and analyzing various cells and released mediators in natural state [26]. Analyzing BALf is one of the prime parameters to understand the underlying disease pathology in prominent mice models of major respiratory diseases, including asthma [27-29], COPD [30,31], and pulmonary fibrosis [32]. Moreover, the application of BALf is not only restricted to quantifying the inflammatory cells, it is also employed to determine the viral titre in various viral infections [3,30].

VARIABLES AFFECTING BALf COLLECTION

The most common problem encountered by researchers during the BALf collection is the variability in the sampling volume [33], which, in turn, significantly affects the amount of cells and fluid returned. It has also been reported that various sections of the lungs have dissimilar BALf findings [34,35]. There are various factors which may explain this variability (Table 1) such as: the underlying disease [36], amount and the nature of instillation used [37], size of syringes [38,39], amount of “dwell” time of instillation before aspiration [38-41], extent of pressure used while carrying out the BALf collection [42], number of aliquots and handling of the collected BALf. Importantly, there are set of recommendations been laid down by The European Respiratory Society (ERS) which clearly explains the major causes of the variability and the strategies to overcome these variabilities [43], as well as various methods to perform BAL [44,45].

Table 1. Various factors causing variability with bronchoalveolar lavage sampling.

Serial No.	Factor causing variability with BAL sampling
1	The underlying disease
2	Extent of pressure applied while collecting BALf
3	Amount and nature of instillation used
4	Number of aliquots
5	Size of syringes used
6	Amount of “dwell” time of instillation before aspiration
7	Handling and storage of collected BALf

The various solution and media generally employed for collecting BALf include normal sterile saline (0.9% NaCl) [46]/phosphate buffer saline (PBS) [47,48] and Hanks buffered salt solution (HBSS) [49]. Also, the volume and number of aliquots employed for BALf varies from one study to another, ranging from 500 µl [47] to 1000 µl [49]. All these parameters need to be thoroughly considered based on the previously reported studies to ensure the reproducibility and accuracy of the BALf technique.

Song *et al.* standardized BAL method based on the suction frequency

number and lavage fraction number in rats [50]. Similarly, Singletary *et al.* [41] compared two different aspiration techniques: conventional method which involves the use of syringe by manually installing and aspirating the fluid and modified method where insertion of a sterile intravenous extension tubing was done between the syringe tip and bronchoscope biopsy channel. Among both the methods, the modified method was found to be statistically significant in the amount of instillation collected and cells recovered (8.3% increase) as compared to the conventional method, which proved to be highly reproducible and of clinical importance in understanding the disease pathology [41].

RECENT DEVELOPMENTS IN BALf ANALYSIS

The recent advances in the BALf analysis include the proteomic analysis of various proteins which have provided information on the mechanism involved with ultrafine carbon black-induced lung injury in mice [51]. Remarkably, considerable progress has been made in the development of a validated computer program based on polychotomous logistic regression model, which is a validated model and have better diagnostic power of BALf analysis and reliable prediction of the current diagnosis in patients with interstitial lung diseases [52,53]. Such models can also be efficiently employed in various translational studies where the set of reliable and reproducible BALf data can be obtained.

Radhakrishnan *et al.* briefed a translational research on BALf which have provided a detailed information on the identification of clinically relevant microbiologic pathogen and cellular analysis in pediatric respiratory diseases [54]. Also, a candidate molecular biomarkers in idiopathic pulmonary fibrosis (IPF) namely, S100A9 protein is been identified in the BALf proteins [55]. Additionally, Correlating Outcomes With Biochemical Markers to Estimate Time to Progression in Idiopathic Pulmonary Fibrosis (COMET) study [56-58] analysis with BALf have shown an association in the development of IPF and presence of *Staphylococcus* and *Streptococcus* genera [57] in the United States. Furthermore, Molyneaux and co-workers have shown increased pathogenic load of *Haemophilus*, *Streptococcus*, *Neisseria* and *Veillonella spp.* in IPF patients as compared to control groups (healthy smokers, non-smokers, and patients with moderate COPD) [59].

Ortea *et al.* had used SWATH MS data-independent acquisition and targeted data extraction where they have discovered a protein biomarker in BALf which can be used in the diagnosis, prognosis and subtyping of lung cancer including the treatment response monitoring [60]. All such listed BALf analysis studies have provided a great platform and foundation for understanding various other respiratory diseases so as to identify the mechanisms involved and new therapeutic interventions.

CONCLUSION AND FUTURE PROSPECTS

BAL is an extremely important respiratory clinical sample and is useful in extracting a great level of information on the disease markers by blending with various modern and sophisticated molecular and biological techniques like -omics approach such as proteomic and transcriptomics analysis (aids in establishing the detailed mapping of the cellular output and gene displays in the BAL fluid and cells in both naïve and patient group) [61,62], enzyme-linked immunosorbent assay (ELISA) [63], flow cytometric analysis [64], immunohistochemistry [65],

quantitative polymerase chain reaction, (qPCR) [66] mass spectrophotometry [67,68] and nuclear magnetic resonance (NMR) [68] (Table 2). Optimizing BALf collection and combining it with modern advanced techniques would ensure higher sensitivity in diagnosing and evaluating a variety of inflammatory processes in lungs by quantifying the different inflammatory cells, assessing various inflammatory cytokines and growth factor expression to underpin the mechanism involved, disease features and pathology.

Table 2. Advances in analysis of bronchoalveolar lavage.

BAL aspiration	
Conventional approaches	Modern approaches
Cytology	Proteomic analysis
	Enzyme-linked immunosorbent assay
	Flow cytometric analysis
	Immunohistochemistry
	Quantitative polymerase chain reaction
	Mass spectrophotometry
	Nuclear magnetic resonance

Remarkably, various special cell types such as dendritic cells could also be specifically isolated from BALf which would further our understanding on specific innate immune pathways [69-71].

In order to have a clinically relevant and meaningful data, it is essential to validate and standardize the lavage process and subsequent handling/processing to warrant greater reproducibility and minimum variability for future applications in respiratory research. The recent advances in technology, various computer assisted programs also have enhanced reproducibility and accuracy in the BALf aspiration techniques.

References

1. World Health Organisation (2014) The top 10 causes of death. Geneva: World Health Organisation. updated 2017 Jan. Available from: <http://www.who.int/mediacentre/factsheets/fs310/en/>.
2. Dua K, Hansbro NG, Foster PS, Hansbro PM (2017) MicroRNAs as therapeutics for future drug delivery systems in treatment of lung diseases. *Drug Deliv Transl Res* 2016: 1-11. doi: [10.1007/s13346-016-0343-6](https://doi.org/10.1007/s13346-016-0343-6). PMID: 27848224
3. Starkey M, Hanish I, Dua K, Nair P, Haw T, et al. (2014) 175: Interleukin-13 predisposes mice to more severe influenza infection by suppressing interferon responses and activating microRNA-21/PI3K. *Cytokine* 70: 70. doi: [10.1016/j.cyto.2014.07.182](https://doi.org/10.1016/j.cyto.2014.07.182).
4. Daubeuf F, Frossard N (2012) Performing bronchoalveolar lavage in the mouse. *Current protocols in mouse biology. Curr Protoc Mouse Biol* 2: 167-175. doi: [10.1002/9780470942390.mo110201](https://doi.org/10.1002/9780470942390.mo110201). PMID: 26069010
5. Gee JB, Fick Jr RB (1980) Bronchoalveolar lavage. *Thorax* 35: 1-8. doi: [10.1136/thx.35.1.1](https://doi.org/10.1136/thx.35.1.1). PMID: 7361279
6. Hsu AC, Starkey MR, Hanish I, Parsons K, Haw TJ, et al. (2015) Targeting PI3K-p110 α Suppresses Influenza Virus Infection in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 191: 1012-1023. doi: [10.1164/rccm.201501-0188OC](https://doi.org/10.1164/rccm.201501-0188OC). PMID: 25751541
7. Eftekhari P, Hajizadeh S, Raoufy MR, Masjedi MR, Yang M, et al. (2013) Preventive effect of N-acetylcysteine in a mouse model of steroid resistant acute exacerbation of asthma. *EXCLI J* 12: 184-192. PMID: 26417226
8. Woods KS, Defarges AMN, Abrams-Ogg ACG, Viel L, Brisson BA, et al. (2014) Comparison of manual and suction pump aspiration techniques for performing bronchoalveolar lavage in 18 dogs with respiratory tract disease.

- J Vet Intern Med 28: 1398-1404. doi: [10.1111/jvim.12403](https://doi.org/10.1111/jvim.12403). PMID: 25056240
9. Du Rand IA, Blaikley J, Booton R, Chaudhuri N, Gupta V, et al. (2013) British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: accredited by NICE. *Thorax* 68 Suppl 1: doi: [10.1136/thoraxjnl-2013-203618](https://doi.org/10.1136/thoraxjnl-2013-203618). PMID: 23860341
 10. Rosell A, Xaubet A, Agustí C, Castella J, Puzo C, et al. (2005) A new BAL fluid instillation and aspiration technique: a multicenter randomized study. *Respir Med* 100: 529-535. doi: [10.1016/j.rmed.2005.06.002](https://doi.org/10.1016/j.rmed.2005.06.002). PMID: 16023336
 11. Balfour-Lynn IM, Spencer H (2002) Bronchoscopy--how and when?. *Paediatr Respir Rev* 3: 255-264. doi: [10.1016/S1526-0542\(02\)00195-1](https://doi.org/10.1016/S1526-0542(02)00195-1). PMID: 12376063
 12. Leiten EO, Martinsen EMH, Bakke PS, Eagan TML, Grønseth R (2016) Complications and discomfort of bronchoscopy: a systematic review. *Eur Clin Respir J* 3: 33324. doi: [10.3402/ecrj.v3.33324](https://doi.org/10.3402/ecrj.v3.33324). PMID: 27839531
 13. Girkin JL, Hatchwell LM, Collison AM, Starkey MR, Hansbro PM, et al. (2016) TRAIL signaling is proinflammatory and viral in a murine model of rhinovirus IB infection. *Am J Physiol Lung Cell Mol Physiol* 312: 10-1152. doi: [10.1152/ajplung.00200.2016](https://doi.org/10.1152/ajplung.00200.2016). PMID: 27836899
 14. Jarnicki AG, Schilter H, Liu G, Wheeldon K, Essilfie A, et al. (2016) The inhibitor of semicarbazide-sensitive amine oxidase, PXS-4728A, ameliorates key features of chronic obstructive pulmonary disease in a mouse model. *Br J Pharmacol* 173: 3161-3175. doi: [10.1111/bph.13573](https://doi.org/10.1111/bph.13573). PMID: 27495192
 15. Han H, Ziegler SF (2013) Bronchoalveolar Lavage and Lung Tissue Digestion. *Bio Protoc* 3: PMID: 27390755
 16. Takaishi M, Awaya Y, Ishioka S, Hozawa S, Oyama T, et al. (1991) Analyses of bronchoalveolar lavage fluid (BALF) in MRL-lpr/lpr mice. *Autoimmunity* 8: 183-186. doi: [10.3109/08916939108997104](https://doi.org/10.3109/08916939108997104). PMID: 1932506
 17. Okada S, Hasegawa S, Hasegawa H, Aina A, Atsuta R, et al. (2013) Analysis of bronchoalveolar lavage fluid in a mouse model of bronchial asthma and H1N1 2009 infection. *Cytokine* 63: 194-200. doi: [10.1016/j.cyto.2013.04.035](https://doi.org/10.1016/j.cyto.2013.04.035). PMID: 23706975
 18. Hoffman AM (2008) Bronchoalveolar lavage: sampling technique and guidelines for cytologic preparation and interpretation. *Vet Clin North Am Equine Pract* 24: 423-435. doi: [10.1016/j.cveq.2008.04.003](https://doi.org/10.1016/j.cveq.2008.04.003). PMID: 18652963
 19. Walters DM, Wills-karp M, Mitzner W (2000) Assessment of cellular profile and lung function with repeated bronchoalveolar lavage in individual mice. *Physiol Genomics* 2: 29-36. PMID: 11015579
 20. Rivera B, Miller SR, Brown EM, Price RE (2005) A novel method for endotracheal intubation of mice and rats used in imaging studies. *Contemp Top Lab Anim Sci* 44: 52-55. PMID: 15773778
 21. Zhao X, Wu N, Zhou J, Yang Y, Fang Y, et al. (2006) A technique for retrograde intubation in mice. *Lab Anim (NY)* 35: 39-42. doi: [10.1038/labani0306-39](https://doi.org/10.1038/labani0306-39). PMID: 16505825
 22. Vergari A, Polito A, Musumeci M, Palazzesi S, Marano G (2003) Video-assisted orotracheal intubation in mice. *Lab Anim* 37: 204-206. doi: [10.1258/002367703766453047](https://doi.org/10.1258/002367703766453047). PMID: 12869282
 23. Hastings RH, Summers-Torres D (1999) Direct Laryngoscopy in Mice. *Contemp Top Lab Anim Sci* 38: 33-35. PMID: 12086445
 24. Das S, MacDonald K, Chang HS, Mitzner W (2013) A simple method of mouse lung intubation. *J Vis Exp* : doi: [10.3791/50318](https://doi.org/10.3791/50318). PMID: 23542122
 25. Novák Z, Peták F, Bánfi A, Tóth-Szuki V, Baráti L, et al. (2006) An improved technique for repeated bronchoalveolar lavage and lung mechanics measurements in individual rats. *Respir Physiol Neurobiol* 154: 467-477. doi: [10.1016/j.resp.2005.12.004](https://doi.org/10.1016/j.resp.2005.12.004). PMID: 16413833
 26. Maxeiner JH, Karwot R, Hausding M, Sauer KA, Scholtes P, et al. (2007) A method to enable the investigation of murine bronchial immune cells, their cytokines and mediators. *Nat Protoc* 2: 105-112. doi: [10.1038/nprot.2007.8](https://doi.org/10.1038/nprot.2007.8). PMID: 17401344
 27. Essilfie A, Horvat JC, Kim RY, Mayall JR, Pinkerton JW, et al. (2015) Macrolide therapy suppresses key features of experimental steroid-sensitive and steroid-insensitive asthma. *Thorax* 70: 458-467. doi: [10.1136/thoraxjnl-2014-206067](https://doi.org/10.1136/thoraxjnl-2014-206067). PMID: 25746630
 28. Burgess JK, Boustany S, Moir LM, Weckmann M, Lau JY, et al. (2009) Reduction of tumstatin in asthmatic airways contributes to angiogenesis, inflammation, and hyperresponsiveness. *Am J Respir Crit Care Med* 181: 106-115. doi: [10.1164/rccm.200904-0631OC](https://doi.org/10.1164/rccm.200904-0631OC). PMID: 19875687
 29. Yang M, Kumar RK, Hansbro PM, Foster PS (2012) Emerging roles of pulmonary macrophages in driving the development of severe asthma. *J Leukoc Biol* 91: 557-569. doi: [10.1189/jlb.0711357](https://doi.org/10.1189/jlb.0711357). PMID: 22293472
 30. Beckett EL, Stevens RL, Jarnicki AG, Kim RY, Hanish I, et al. (2013) A new short-term mouse model of chronic obstructive pulmonary disease identifies a role for mast cell tryptase in pathogenesis. *J Allergy Clin Immunol* 131: 752-762. doi: [10.1016/j.jaci.2012.11.053](https://doi.org/10.1016/j.jaci.2012.11.053). PMID: 23380220
 31. Haw TJ, Starkey MR, Nair PM, Pavlidis S, Liu G, et al. (2015) A pathogenic role for tumor necrosis factor-related apoptosis-inducing ligand in chronic obstructive pulmonary disease. *Mucosal Immunol* 9: 859-872. doi: [10.1038/mi.2015.111](https://doi.org/10.1038/mi.2015.111). PMID: 26555706
 32. Moeller A, Ask K, Warburton D, Gauldie J, Kolb M (2007) The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis?. *Int J Biochem Cell Biol* 40: 362-382. doi: [10.1016/j.biocel.2007.08.011](https://doi.org/10.1016/j.biocel.2007.08.011). PMID: 17936056
 33. Baughman RP (1997) The uncertainties of bronchoalveolar lavage. *Eur Respir J* 10: 1940-1942. doi: [10.1183/09031936.97.10091940](https://doi.org/10.1183/09031936.97.10091940). PMID: 9311482
 34. Garcia JG, Wolven RG, Garcia PL, Keogh BA (1986) Assessment of interlobar variation of bronchoalveolar lavage cellular differentials in interstitial lung diseases. *Am Rev Respir Dis* 133: 444-449. doi: [10.1164/arrd.1986.133.3.444](https://doi.org/10.1164/arrd.1986.133.3.444). PMID: 3954253
 35. Levine SJ, Kennedy D, Shelhamer JH, Kovacs A, Feuerstein IM, et al. (1992) Diagnosis of *Pneumocystis carinii* pneumonia by multiple lobe, site-directed bronchoalveolar lavage with immunofluorescent monoclonal antibody staining in human immunodeficiency virus-infected patients receiving aerosolized pentamidine chemoprophylaxis. *Am Rev Respir Dis* 146: 838-843. doi: [10.1164/ajrccm/146.4.838](https://doi.org/10.1164/ajrccm/146.4.838). PMID: 1416407
 36. Thorpe JE, Baughman RP, Frame PT, Wessler TA, Staneck JL (1987) Bronchoalveolar lavage for diagnosing acute bacterial pneumonia. *J Infect Dis* 155: 855-861. doi: [10.1093/infdis/155.5.855](https://doi.org/10.1093/infdis/155.5.855). PMID: 3559289
 37. Dohn MN, Baughman RP (1985) Effect of changing instilled volume for bronchoalveolar lavage in patients with interstitial lung disease. *Am Rev Respir Dis* 132: 390-392. doi: [10.1164/arrd.1985.132.2.390](https://doi.org/10.1164/arrd.1985.132.2.390). PMID: 4026063
 38. King TE (1992) The handling and analysis of bronchoalveolar lavage specimens. *BAL*. 1992: 3-29.
 39. Crystal RG, Reynolds HY, Kalica AR (1986) Bronchoalveolar lavage. The report of an international conference. *Chest* 90: 122-131. doi: [10.1378/chest.90.1.122](https://doi.org/10.1378/chest.90.1.122). PMID: 3720374
 40. Rosell A, Xaubet A, Agustí C, Castella J, Puzo C, et al. (2005) A new BAL fluid instillation and aspiration technique: a multicenter randomized study. *Respir Med* 100: 529-535. doi: [10.1016/j.rmed.2005.06.002](https://doi.org/10.1016/j.rmed.2005.06.002). PMID: 16023336
 41. Singletary ML, Phillippi-Falkenstein KM, Scanlon E, Bohm RP Jr, Veazey RS, et al. (2008) Modification of a common BAL technique to enhance sample diagnostic value. *J Am Assoc Lab Anim Sci* 47: 47-51. PMID: 18947171
 42. Thompson AB, Robbins RA, Ghafouri MA, Linder J, Rennard SI (1988) Bronchoalveolar lavage fluid processing. Effect of membrane filtration preparation on neutrophil recovery. *Acta Cytol* 33: 544-9. PMID: 2750443
 43. Haslam PL, Baughman RP (1999) Report of ERS Task Force: guidelines for measurement of acellular components and standardization of BAL. *Eur Respir J* 14: 245-248. doi: [10.1034/j.1399-3003.1999.14b01.x](https://doi.org/10.1034/j.1399-3003.1999.14b01.x). PMID: 10515395
 44. Higgenbottam T (1989) Technical recommendations and guidelines for bronchoalveolar lavage (BAL). Report of the European Society of Pneumology Task Group. *Eur Respir J* 2: 561-585. PMID: 2292291
 45. Fabbri LM, De Rose V, Godard P, Rossi GA (1990) Clinical guidelines and indications for bronchoalveolar lavage (BAL): bronchial asthma. *Eur Respir J* 3: 958-959. PMID: 2292306
 46. Mei SHJ, McCarter SD, Deng Y, Parker CH, Liles WC, et al. (2007) Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. *PLoS Med* 4. doi: [10.1371/journal.pmed.0040269](https://doi.org/10.1371/journal.pmed.0040269). PMID: 1780352
 47. Liu G, Cooley MA, Jarnicki AG, Hsu AC, Nair PM, et al. (2016) Fibulin-1 regulates the pathogenesis of tissue remodeling in respiratory diseases. *JCI Insight* 1: e86380. doi: [10.1172/jci.insight.86380](https://doi.org/10.1172/jci.insight.86380). PMID: 27398409
 48. Polikepahad S, Barranco WT, Porter P, Anderson B, Kheradmand F, et al. (2010) A reversible, non-invasive method for airway resistance measurements and bronchoalveolar lavage fluid sampling in mice. *J Vis Exp*: E1720. doi: [10.3791/1720](https://doi.org/10.3791/1720). PMID: 20389278
 49. Asquith KL, Horvat JC, Kaiko GE, Carey AJ, Beagley KW, et al. (2011) Interleukin-13 promotes susceptibility to chlamydial infection of the respiratory

- and genital tracts. *PLoS Pathog* 7:e1001339. doi: [10.1371/journal.ppat.1001339](https://doi.org/10.1371/journal.ppat.1001339). PMID: [21573182](https://pubmed.ncbi.nlm.nih.gov/21573182/)
50. Song J, Yang H, Lee J, Kwon S, Jung KJ, et al. (2010) Standardization of bronchoalveolar lavage method based on suction frequency number and lavage fraction number using rats. *Toxicol Res* 26: 203-208. doi: [10.5487/TR.2010.26.3.203](https://doi.org/10.5487/TR.2010.26.3.203). PMID: [24278525](https://pubmed.ncbi.nlm.nih.gov/24278525/)
 51. Chang C, Chen S, Ho S, Yang C, Wang H, et al. (2007) Proteomic analysis of proteins from bronchoalveolar lavage fluid reveals the action mechanism of ultrafine carbon black-induced lung injury in mice. *Proteomics* 7: 4388-4397. doi: [10.1002/pmic.200700164](https://doi.org/10.1002/pmic.200700164). PMID: [17963277](https://pubmed.ncbi.nlm.nih.gov/17963277/)
 52. Drent M, van Nierop MA, Gerritsen FA, Wouters EF, Mulder PG (1996) A computer program using BALF-analysis results as a diagnostic tool in interstitial lung diseases. *Am J Respir Crit Care Med* 153: 736-741. doi: [10.1164/ajrccm.153.2.8564126](https://doi.org/10.1164/ajrccm.153.2.8564126). PMID: [8564126](https://pubmed.ncbi.nlm.nih.gov/8564126/)
 53. Drent M, Jacobs JA, Cobben NA, Costabel U, Wouters EF, et al. (2001) Computer program supporting the diagnostic accuracy of cellular BALF analysis: a new release. *Respir Med* 95: 781-786. doi: [10.1053/rmed.2001.1153](https://doi.org/10.1053/rmed.2001.1153). PMID: [11601741](https://pubmed.ncbi.nlm.nih.gov/11601741/)
 54. Radhakrishnan D, Yamashita C, Gillio-Meina C, Fraser DD (2014) Translational research in pediatrics III: bronchoalveolar lavage. *Pediatrics* 134: 135-154. doi: [10.1542/peds.2013-1911](https://doi.org/10.1542/peds.2013-1911). PMID: [24982109](https://pubmed.ncbi.nlm.nih.gov/24982109/)
 55. Hara A, Sakamoto N, Ishimatsu Y, Kakugawa T, Nakashima S, et al. (2011) S100A9 in BALF is a candidate biomarker of idiopathic pulmonary fibrosis. *Respir Med* 106: 571-580. doi: [10.1016/j.rmed.2011.12.010](https://doi.org/10.1016/j.rmed.2011.12.010). PMID: [22209187](https://pubmed.ncbi.nlm.nih.gov/22209187/)
 56. Huie TJ, Moss M, Frankel SK (2010) What can biomarkers tell us about the pathogenesis of acute exacerbations of idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 299:1-2.
 57. Han MK, Zhou Y, Murray S, Tayob N, Noth I, et al. (2014) Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. *Lancet Respir Med* 2: 548-556. doi: [10.1016/S2213-2600\(14\)70069-4](https://doi.org/10.1016/S2213-2600(14)70069-4). PMID: [24767767](https://pubmed.ncbi.nlm.nih.gov/24767767/)
 58. Naik PK, Bozyk PD, Bentley JK, Popova AP, Birch CM, et al. (2012) Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 303: 1046-1056. doi: [10.1152/ajplung.00139.2012](https://doi.org/10.1152/ajplung.00139.2012). PMID: [23043074](https://pubmed.ncbi.nlm.nih.gov/23043074/)
 59. Molyneux PL, Cox MJ, Willis-Owen SAG, Mallia P, Russell KE, et al. (2014) The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 190: 906-913. doi: [10.1164/rccm.201403-0541OC](https://doi.org/10.1164/rccm.201403-0541OC). PMID: [25184687](https://pubmed.ncbi.nlm.nih.gov/25184687/)
 60. Ortea I, Rodríguez-Ariza A, Chicano-Gálvez E, Arenas Vacas, MS, Jurado Gámez, B. (2016) Discovery of potential protein biomarkers of lung adenocarcinoma in bronchoalveolar lavage fluid by SWATH MS data-independent acquisition and targeted data extraction. *J Proteomics* 138: 106-114. doi: [10.1016/j.jprot.2016.02.010](https://doi.org/10.1016/j.jprot.2016.02.010). PMID: [26917472](https://pubmed.ncbi.nlm.nih.gov/26917472/)
 61. Gharib SA, Nguyen E, Altmeier WA, Shaffer SA, Doneanu CE, et al. (2009) Of mice and men: comparative proteomics of bronchoalveolar fluid. *Eur Respir J* 35: 1388-1395. doi: [10.1183/09031936.00089409](https://doi.org/10.1183/09031936.00089409). PMID: [20032019](https://pubmed.ncbi.nlm.nih.gov/20032019/)
 62. Kahn N, Granzow M, Meister M, Eberhardt R, Muley T, et al. (2015) Transcriptome analysis in endobronchial epithelial lining fluid compared to bronchoalveolar lavage in idiopathic pulmonary fibrosis. *Eur Respir J* 2015: 59-3053.
 63. Chen Z, Xu Z, Sun S, Yu Y, Lv D, et al. (2014) TGF- β 1, IL-6, and TNF- α in bronchoalveolar lavage fluid: useful markers for lung cancer?. *Sci Rep* 4: 5595. doi: [10.1038/srep05595](https://doi.org/10.1038/srep05595). PMID: [24999009](https://pubmed.ncbi.nlm.nih.gov/24999009/)
 64. van Rijjt LS, Kuipers H, Vos N, Hijdra D, Hoogsteden HC, et al. (2004) A rapid flow cytometric method for determining the cellular composition of bronchoalveolar lavage fluid cells in mouse models of asthma. *J Immunol Methods* 288: 111-121. doi: [10.1016/j.jim.2004.03.004](https://doi.org/10.1016/j.jim.2004.03.004). PMID: [15183090](https://pubmed.ncbi.nlm.nih.gov/15183090/)
 65. Rong B, Cai X, Liu H, Fu T, Gao W, et al. (2016) Increased level of Hsp90-beta in bronchoalveolar lavage fluid correlates with lymphatic invasion and advanced stage of lung cancer patients. *Am J Transl Res* 8: 4147-4159. PMID: [27829999](https://pubmed.ncbi.nlm.nih.gov/27829999/)
 66. Canonne AM, Billen F, Tual C, Ramery E, Roels E, et al. (2016) Quantitative PCR and Cytology of Bronchoalveolar Lavage Fluid in Dogs with *Bordetella bronchiseptica* Infection. *J Vet Intern Med* 30: 1204-1209. doi: [10.1111/jvim.14366](https://doi.org/10.1111/jvim.14366). PMID: [27461723](https://pubmed.ncbi.nlm.nih.gov/27461723/)
 67. Pounds JG, Flora JW, Adkins JN, Lee KM, Rana GSJB, et al. (2008) Characterization of the mouse bronchoalveolar lavage proteome by micro-capillary LC-FTICR mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 864: 95-101. doi: [10.1016/j.jchromb.2008.01.044](https://doi.org/10.1016/j.jchromb.2008.01.044). PMID: [18313996](https://pubmed.ncbi.nlm.nih.gov/18313996/)
 68. Bessonneau V, Bojko B, Azad A, Keshavjee S, Azad S, et al. (2014) Determination of bronchoalveolar lavage bile acids by solid phase microextraction liquid chromatography-tandem mass spectrometry in combination with metabolite profiling: comparison with enzymatic assay. *J Chromatogr A* 1367: 33-38. doi: [10.1016/j.chroma.2014.09.061](https://doi.org/10.1016/j.chroma.2014.09.061). PMID: [25305784](https://pubmed.ncbi.nlm.nih.gov/25305784/)
 69. Bratke K, Lommatzsch M, Julius P, Kuepper M, Kleine H, et al. (2006) Dendritic cell subsets in human bronchoalveolar lavage fluid after segmental allergen challenge. *Thorax* 62: 168-175. doi: [10.1136/thx.2006.067793](https://doi.org/10.1136/thx.2006.067793). PMID: [16928719](https://pubmed.ncbi.nlm.nih.gov/16928719/)
 70. van Rijjt LS, Prins J, Leenen PJM, Thielemans K, de Vries VC, et al. (2002) Allergen-induced accumulation of airway dendritic cells is supported by an increase in CD31(hi)Ly-6C(neg) bone marrow precursors in a mouse model of asthma. *Blood* 100: 3663-3671. doi: [10.1182/blood-2002-03-0673](https://doi.org/10.1182/blood-2002-03-0673). PMID: [12393720](https://pubmed.ncbi.nlm.nih.gov/12393720/)
 71. van Rijjt LS, Jung S, KleinJan A, Vos N, Willart M, et al. (2005) et al. In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J Exp Med*: 2005-201. doi: [10.1084/jem.20042311](https://doi.org/10.1084/jem.20042311). PMID: [15781587](https://pubmed.ncbi.nlm.nih.gov/15781587/)