

Murine Bronchoalveolar Lavage

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[Abstract] A basic Bronchoalveolar lavage (BAL) procedure in mouse is described here. Cells and fluids obtained from BAL can be analyzed by Hema3-staining, immunostaining, Fluorescence-activated cell sorting (FACS), PCR, bicinchoninic acid protein assay, enzyme-linked immunosorbent assay (ELISA), luminex assays, *etc.*, to examine the immune cells, pathogens, proteins such as cytokines/chemokines, and the expression levels of inflammation-related and other genes in the cells. This will help to understand the underlying mechanisms of these lung diseases and develop specific and effective drugs.

Keywords: Bronchoalveolar lavage, Lung cancer, Hema3-staining

[Background] Bronchoalveolar lavage (BAL) is a simple and typical method commonly performed to diagnose pulmonary diseases including lung cancer (Daubeuf and Frossard, 2012). It is used to sample pulmonary components to determine the protein composition, immune cells and pathogens in the lung. Pulmonary chronic inflammation plays a critical role in lung cancer initiation and progression. To clarify the underlying mechanism of inflammation in lung tumorigenesis, a basic BAL protocol in mice is used in our laboratory to determine the pulmonary immune response (Qu *et al.*, 2015; Zhou *et al.*, 2015; Sun *et al.*, 2016; Zhou *et al.*, 2017).

Materials and Reagents

1. Needles (BD, catalog number: 305167) or tapes
2. Nylon string (Dynarex, catalog number: 3243)
3. 22 G x 1" Exel Safelet Catheter (Exel International, catalog number: 26746)
4. 1 ml syringe (BD, catalog number: 309659)
5. 1.5 ml Eppendorf tubes (VWR, catalog number: 87003-294)
6. 0.22 μ m filter (EMD Millipore, catalog number: SLGP033RS)
7. Mice (THE JACKSON LABORATORY)
8. 70% ethanol (Decon Labs, catalog number: 2701)
9. Protease inhibitor cocktail (Roche Diagnostics, catalog number: 11697498001)
10. Phenylmethylsulfonyl fluoride (PMSF) (Thermo Fisher Scientific, Thermo Scientific™, catalog number: 36978)
11. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S9625)

12. Potassium chloride (KCl) (Sigma-Aldrich, catalog number: P9541)
13. Disodium hydrogen phosphate heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) (Fisher Scientific, catalog number: BP331-500)
14. Potassium phosphate monobasic (KH_2PO_4) (Acros Organics, catalog number: 205925000)
15. Ammonium chloride (NH_4Cl) (Sigma-Aldrich, catalog number: A9434-1KG)
16. Potassium bicarbonate (KHCO_3) (Sigma-Aldrich, catalog number: P9144-1KG)
Note: This product has been discontinued.
17. Ethylenediaminetetraacetic acid disodium salt dihydrate ($\text{Na}_2\text{-EDTA} \cdot 2\text{H}_2\text{O}$) (Sigma-Aldrich, catalog number: E5134-100G)
18. Phosphate-buffered saline (PBS) (see Recipes)
19. ACK lysis buffer (see Recipes)

Equipment

1. CO_2 chamber
2. Biosafety cabinet
3. Styrofoam board
4. Forceps (Roboz Surgical Instrument, catalog number: RS-5135)
5. Scissors (Roboz Surgical Instrument, catalog number: RS-6802)
6. Centrifuge (Eppendorf, model: 5417 R)
7. Hemocytometer (Hausser Scientific, catalog number: 3110)
8. Microscope (Olympus, model: CK30)

Procedure

1. Sacrifice a mouse by CO_2 inhalation in a CO_2 chamber.
2. Bring the mouse to a biosafety cabinet.
3. Dampen the mouse with 70% ethanol.
4. Place the mouse front side up on styrofoam panel and fix the arms and legs with needles or tapes (Figure 1A).
5. Use scissors to make incision in the skin from abdomen to neck, and tear skin with forceps to expose thoracic cage and neck (Figure 1A).
6. Gently remove the muscle around the neck to expose trachea (Figure 1B).
7. Use forceps to put a ~10 cm-long nylon string under the trachea (Figure 1C).
8. Carefully cut the ribs to expose the heart and the lungs (Figure 1D).
Note: Do not cut the trachea and lungs.
9. Insert a 22 G x 1" Exel Safelet Catheter into the trachea, remove the stylet hub, and tie the catheter and the trachea together firmly with a nylon string (Figures 1E-1G).
10. Load a 1 ml syringe with 0.8 ml of PBS and place it in the end of the catheter (Figure 1H).

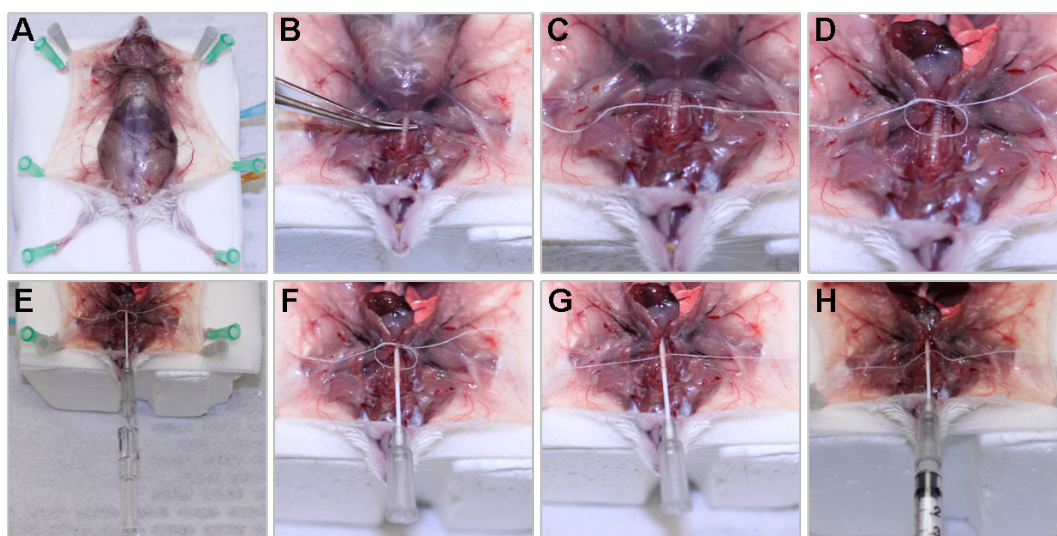


Figure 1. Mouse bronchoalveolar lavage steps

11. Slowly inject and aspirate the PBS 4 times, remove the syringe from catheter, and save the recovered lavage fluid in 1.5 ml Eppendorf (EP) tubes on ice.
12. Repeat steps 10-11 for additional three times.
13. Record BAL volume according to the scales on the 1.5 ml EP tubes.
14. Centrifuge at $800 \times g$ for 10 min at 4°C .
15. Transfer the supernatant to a new 5 ml tube, add protease inhibitor cocktail to a final concentration of 1x and PMSF to a final concentration of 1 mM, and mix well. Store at -80°C if not used immediately. Pathogens, proteins such as cytokines/chemokines, and other components in the supernatants of BAL fluid (BALF) can be analyzed by bicinchoninic acid protein assay, ELISA, luminex assays, and/or other assays.
16. Resuspend BAL cell pellets in $200\ \mu\text{l}$ of PBS and combine the cell suspensions.
17. Centrifuge at $800 \times g$ for 10 min at 4°C .
18. Resuspend BAL cell pellets in $100\text{--}200\ \mu\text{l}$ ACK lysis buffer, lyse red blood cells on ice for 10 min, and then add 1 ml of PBS to stop lysis.
19. Centrifuge at $800 \times g$ for 10 min at 4°C .
20. Resuspend BAL cell pellets in $400\ \mu\text{l}$ of PBS, count the cells by taking about $20\ \mu\text{l}$ of the cell sample to a hemocytometer and counting the cells under a microscope. Record the cell number. These BAL cells can then be used for FACS analysis, spun onto slides for staining (Figure 2), and/or lysed for RNA or protein extraction.

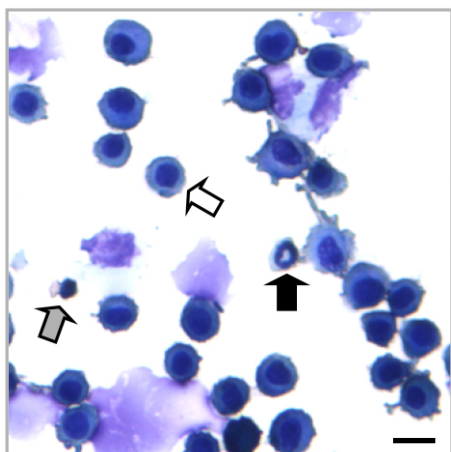


Figure 2. Hema3-staining of immune cells in murine BAL. White arrow: macrophage; grey arrow: lymphocyte; black arrow: neutrophil. Scale bar = 10 μ m.

Data analysis

Murine BAL cells are retrieved and cytopspun onto slides, which are Hema3-stained to determine the immune cells.

Notes

Although pelleted BAL cells from all four washes are combined to analyze the cells, you can combine the supernatant from only the first two washes (Procedure step 11) to analyze the BAL fluid components, as the first two washes contain a high concentration of these components.

Recipes

1. Phosphate-buffered saline (PBS) (1 L)
 - 8.0 g NaCl
 - 0.2 g KCl
 - 1.15 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$
 - 0.2 g KH_2PO_4
 - Adjust to pH 7.4
2. ACK lysis buffer (1 L)
 - 1 L deionized water
 - 8.29 g NH_4Cl
 - 1 g KHCO_3
 - 37.2 mg $\text{Na}_2\text{-EDTA}$
 - Adjust pH to 7.2-7.4, sterilize using 0.22 μ m filter, store at 4 $^\circ\text{C}$

Acknowledgments

The authors thank the members in Xiao-Qu lab for the helpful discussion. This study was supported in part by the National Institute of Health (NIH)/National Cancer Institute (NCI) grants R01 CA172090, R21 CA175252, P50 CA090440, P30 CA047904, as well as the American Lung Association (ALA) Lung Cancer Discovery Award LCD 259111 and American Cancer Society (ACS) Fellowship Award PF-12081-01-TBG.

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