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Title: Non-Invasive Endotracheal Administration of Lipopolysaccharide to Induce Acute Lung Injury in Rodents

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **10/28/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare

Current Protocol Length

Number of Steps: 09
Number of Shots: 19

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Sheikh Rayees:** The scope of our research is to develop a clinically relevant, non-invasive rat model of acute lung injury to improve preclinical testing.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B-roll: 2.4.3.*~~

~~What technologies are currently used to advance research in your field?~~

1.2. **Sheikh Rayees:** Modern lung biology research integrates advanced lung models, nanocarriers, and aerosol technologies to optimize and study targeted drug delivery within physiologically relevant systems.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What significant findings have you established in your field?~~

1.3. **Sheikh Rayees:** Using noninvasive endotracheal pulmonary delivery, we created a self-limiting acute lung injury model ideal for testing targeted therapeutics and studying lung repair.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B-roll: Figure 1*~~

~~What advantage does your protocol offer compared to other techniques?~~

1.4. **Sheikh Rayees:** The key benefit of this method is its precise non-traumatic delivery, which facilitates repeated dosing in the same animal and customization for peculiar research objectives.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. ~~*Suggested B roll: 2.6.2*~~

~~What questions will future research focus on?~~

1.5. **Sheikh Rayees:** Future acute lung injury research will focus on developing targeted therapies and understanding lung injury and repair mechanisms.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Ethics Committee at CSIR-Indian Institute of Integrative Medicine

Protocol

2. Preparation and Endotracheal Instillation of Lipopolysaccharides

Demonstrators: Sheikh Rayees and Yassir Arfath

2.1. To begin, weigh each animal using a calibrated balance **[1]**.

2.1.1. WIDE: Talent placing a mouse on a digital balance and recording the weight.
NOTE: Use C0051, C0052

2.2. Prepare the lipopolysaccharide solution by diluting the lyophilized *Escherichia coli* lipopolysaccharide in sterile PBS to achieve the desired concentration **[1]**.

2.2.1. Talent adding PBS into a vial containing lyophilized *Escherichia coli* lipopolysaccharide.
NOTE: Use C0141, C0142

2.3. After anesthetizing the mouse, check the depth of anesthesia **[1-TXT]** and position the mouse in a semi-recumbent position with its incisors suspended on a solid supporting platform **[2]**.

2.3.1. Talent pinching the mouse's toe gently with forceps to check for reflex. **TXT: Anaesthesia: Ketamine-xylazine cocktail**
NOTE: Use C0046, C0047, C0048

2.3.2. Talent placing the anaesthetized mouse with its incisors hooked over a bar in a semi-recumbent posture.
NOTE: Use C0049, C0050, C0053, C0054

2.4. Then, open the mouth of the anaesthetized animal **[1]**, gently grasp the tongue using forceps **[2]**, and carefully insert the laryngoscope **[3]**, aligning it precisely for optimal visualization of the tracheal opening **[4]**.

2.4.1. Talent opening the mouth of the anaesthetized mouse.
NOTE: Use C0057, C0058, C0061, C0062, C0063, C0064

2.4.2. Talent holding the tongue with forceps, pulling it forward gently.
NOTE: Use C0065, C0066, C0067, C0068, C0069, C0070, C0071, C0072 for 2.4.2 and 2.5.2, since same technical step is shown in both shots

2.4.3. Talent inserting the laryngoscope carefully into the oral cavity.
NOTE: Use C0073, C0075, C0076, C0077, C0078, C0079, C0080, C0081, C0082

2.4.4. Talent adjusting the angle of the laryngoscope for clear visualization of the tracheal opening.
NOTE: Use C0088, C0089, C0090, C0091, C0092, C0093, C0094, C0095,

2.5. Using curved, blunt-ended forceps, delicately grasp the tongue and gently retract it in

an upward and lateral direction **[1]**.

2.5.1. Talent holding the tongue with curved, blunt-ended forceps and carefully pulling it upward and to the side.
NOTE: Same as 2.4.2

2.6. Now, draw 100 microliters of lipopolysaccharide solution into a syringe and attach it to a 16-gauge rat endotracheal tube **[1]**. Administer the lipopolysaccharide solution near the tracheal opening **[2]**. Immediately after instillation, gently occlude the nostrils using blunt-ended forceps for 2 to 4 seconds to promote aspiration of the solution into the lungs through the trachea **[3]**.

2.6.1. Talent drawing 100 microliters of lipopolysaccharide solution into the syringe and attaching it to a 16-gauge endotracheal tube. **NOTE: Use** C0098, C0099, C0100, C0101, C0102

2.6.2. Talent placing the tube near the tracheal opening and delivering the solution. **NOTE: Use** C0105, C0106 for 2.6.2 and 2.6.3

2.6.3. Talent closing the animal's nostrils with blunt-ended forceps for a few seconds.

2.7. Then, carefully reposition the tongue to its normal anatomical orientation following lipopolysaccharide delivery **[1]**. Transfer the animal back to its cage and closely monitor for any signs of respiratory compromise or choking for at least 1 minute **[2]**.

2.7.1. Talent repositioning the tongue inside the mouth to its natural resting position. **NOTE: Use** C0119

2.7.2. Talent placing the animal into its cage. **NOTE: Use** C0121

2.8. After performing bronchoalveolar lavage and tracheotomy **[1]**, gently infuse 2 milliliters of cold PBS into the lungs and retrieve the fluid carefully **[2]**.

2.8.1. Shot of the animal after performing tracheotomy. **NOTE: Use** C0122, C0123

2.8.2. Talent slowly injecting 2 milliliters of cold PBS into the lungs and collecting the lavage fluid. **NOTE: Use** CO126

2.9. Centrifuge the collected lavage fluid at 5000 *g* for 5 minutes at 4 degrees Celsius **[1]**. Use the supernatant to detect inflammatory markers **[2]**. Collect the lungs for histopathological examination or homogenize them for myeloperoxidase activity **[3]**.

2.9.1. Talent placing the lavage sample into the centrifuge. **NOTE: Use** C0143, C0145

2.9.2. Talent collecting the supernatant after centrifugation. **NOTE: Use** C0146, C0147

2.9.3. Talent placing the lungs into labeled containers for histopathology or

homogenization. **NOTE: Use** C0138, C0139

Results

3. Results

3.1. Compared to the control group, the group euthanized 24 hours after lipopolysaccharide instillation showed a significant increase in interleukin-1 beta levels **[1],** elevated myeloperoxidase activity **[2],** and higher total protein content **[3].** At 120 hours post-lipopolysaccharide instillation, interleukin-1 beta, myeloperoxidase activity, and total protein levels had returned to near-baseline values **[4]**.

3.1.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the bar for "LPS 24 h," which is visibly higher than the "Control" bar.*

3.1.2. LAB MEDIA: Figure 1B. *Video editor: Highlight the bar for "LPS 24 h," which is visibly higher than the "Control" bar.*

3.1.3. LAB MEDIA: Figure 1C. *Video editor: Highlight the bar for "LPS 24 h," which is visibly higher than the "Control" bar*.

3.1.4. LAB MEDIA: Figure 1A–C. *Video editor: Highlight the bars for "LPS 120 h," which are close to the "Control" bars*

3.2. Lung histopathology at 24 hours demonstrated disintegrated alveolar structure**,** swelling of the alveolar wall**,** hemolysis, and severe neutrophil infiltration **[1].**

3.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the "LPS 24 h" panel*.

3.3. Lung architecture was restored at 120 hours post-lipopolysaccharide instillation **[1].**

3.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the "LPS 120 h"*

Pronunciation Guide:

 Endotracheal
Pronunciation link: https://www.merriam-webster.com/dictionary/endotracheal
IPA: /ˌɛndoʊˈtreɪkiəl/
Phonetic Spelling: en·doh·tray·kee·uhl
 Lipopolysaccharide
Pronunciation link: https://www.merriam-webster.com/dictionary/lipopolysaccharide
IPA: /ˌlɪpoʊˌpɑːlɪˈsækəˌraɪd/
Phonetic Spelling: lip·oh·pol·ee·sak·uh·ride
 Noninvasive
Pronunciation link: https://www.merriam-webster.com/dictionary/noninvasive
IPA: /ˌnɑːnɪnˈveɪsɪv/
Phonetic Spelling: non·in·vay·siv
 Acute
Pronunciation link: https://www.merriam-webster.com/dictionary/acute
IPA: /əˈkjuːt/
Phonetic Spelling: uh·kyoot
 Rodents
Pronunciation link: https://www.merriam-webster.com/dictionary/rodent
IPA: /ˈroʊdənt/
Phonetic Spelling: roh·dent
 Pharmacology
Pronunciation link: https://www.merriam-webster.com/dictionary/pharmacology
IPA: /ˌfɑːrməˈkɑːlədʒi/
Phonetic Spelling: far·muh·kol·uh·jee
 Lyophilized
Pronunciation link: https://www.merriam-webster.com/dictionary/lyophilized
IPA: /laɪˈɑːfɪˌlaɪzd/
Phonetic Spelling: lye·ah·fuh·lized
 Escherichia
Pronunciation link: https://www.merriam-webster.com/dictionary/Escherichia
IPA: /ˌɛʃəˈrɪkiə/
Phonetic Spelling: esh·uh·rik·ee·uh
 Anesthetizing
Pronunciation link: https://www.merriam-webster.com/dictionary/anesthetize
IPA: /əˈnɛsθəˌtaɪz/
Phonetic Spelling: uh·nes·thuh·tize
 Semi-recumbent
Pronunciation link: https://www.merriam-webster.com/dictionary/recumbent
IPA: /rɪˈkʌmbənt/
Phonetic Spelling: ruh·kum·bent

 Laryngoscope
Pronunciation link: https://www.merriam-webster.com/dictionary/laryngoscope
IPA: /ləˈrɪŋgəˌskoʊp/
Phonetic Spelling: luh·ring·guh·skohp
 Tracheal
Pronunciation link: https://www.merriam-webster.com/dictionary/tracheal
IPA: /ˈtreɪkiəl/
Phonetic Spelling: tray·kee·uhl
 Microliters
Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪkroʊˌliːtər/
Phonetic Spelling: my·kroh·lee·ter
 Endotracheal tube
Pronunciation link: https://www.merriam-webster.com/dictionary/endotracheal
IPA: /ˌɛndoʊˈtreɪkiəl tuːb/
Phonetic Spelling: en·doh·tray·kee·uhl toob
 Aspiration
Pronunciation link: https://www.merriam-webster.com/dictionary/aspiration
IPA: /ˌæspəˈreɪʃən/
Phonetic Spelling: as·puh·ray·shun
 Bronchoalveolar
Pronunciation link: https://www.merriam-webster.com/dictionary/bronchoalveolar
IPA: /ˌbrɑːŋkoʊˌælˈviːələr/
Phonetic Spelling: bron·koh·al·vee·uh·ler