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## **Title: Murine Nasal Lavage Fluid Collection Without Blood Contamination**

### **Authors and Affiliations:**

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## **Author Questionnaire**

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
  
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
  
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

### **Current Protocol Length**

Number of Steps: 07

Number of Shots: 20

## Introduction

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*Videographer: Obtain headshots for all authors available at the filming location.*

- 1.1. **Fumitaka Sato**: Our research focuses on developing a method to collect nasal lavage fluid from mice in large volumes, addressing two main challenges: achieving high yield and preventing blood contamination [1].

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.2.* Videographer's Note: Use Take 1

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

- 1.2. **Ijaz Ahmad**: There are two approaches to collecting nasal lavage fluid: the pharyngeal route, which yields larger volumes, and the tracheal route, which reduces blood contamination [1].

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer's Note: Use Take 2

What advantage does your protocol offer compared to other techniques?

- 1.3. **Alfredo Hinay**: Our method enables the collection of a large volume of nasal lavage fluid from the pharynx, while placing cotton balls in the rodent's mouth helps minimize blood contamination. The approach is both simple and cost-effective [1].

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1.* Videographer's Note: Use Take 3

What are the most recent developments in your field of research?

- 1.4. **Ah-Mee Park**: To detect blood contamination in the sample, we employed a forensic method, known as the luminol reaction. The luminol reaction is a simple and more sensitive method than other methods [1].

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 2.* Videographer's Note: Use Take 2

How will your findings advance research in your field?

- 1.5. **Ikuo Tsunoda:** The concentration of IgG in blood is much higher than that in nasal lavage fluid; our method, which avoids blood contamination, is crucial in immunological research. It is also suitable for analyzing other molecules, such as the microbiome, where blood contamination can compromise accuracy [1].

- 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 3.*  
**Videographer's Note: Use Take 2**

***Videographer: Obtain headshots for all authors available at the filming location.***

**Testimonial Questions:**

*Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.*

How do you think publishing with JoVE will enhance the visibility and impact of your research?

- 1.6. **Ikuo Tsunoda, Professor and Chair, Department of Microbiology, Kindai University Faculty of Medicine:** (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer's Note: Use Take 2

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE?

- 1.7. **Fumitaka Sato, Associate Professor, Department of Microbiology, Kindai University Faculty of Medicine:** (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer's Note: Use Take 2

**Ethics Title Card**

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) of Kindai University Faculty of Medicine

# Protocol

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## 2. Nasal Lavage Fluid Collection

**Demonstrator:** Ijaz Ahmad

- 2.1. To begin, place the euthanized mouse on its back on a surgical plate [1-TXT] and fix all four limbs using pins [2]. Using a 1-milliliter syringe, collect blood from the heart [3].
  - 2.1.1. WIDE: Talent positioning the mouse on its back on a surgical plate. **TXT: Euthanasia: 5% isoflurane. Videographer's Note: 2.1.1-2.1.2 shot together. Use Take 1**
  - 2.1.2. Talent pinning the limbs.
  - 2.1.3. Talent collecting cardiac blood with a 1 milliliter syringe. **Videographer's Note: Use Take 1**
- 2.2. Using scissors, open the abdominal cavity to expose the visceral organs and diaphragm [1]. Incise the diaphragm and ribs [2] to reveal the right atrium [3]. Then, puncture the right atrium with scissors to drain remaining blood [4].
  - 2.2.1. Talent using scissors to open the abdominal cavity, exposing the visceral organs and diaphragm. **Videographer's Note: Use Take 1**
  - 2.2.2. Talent incising the diaphragm and ribs. **Videographer's Note: Use Take 1**
  - 2.2.3. A shot of the exposed right atrium. **Videographer's Note: Use Take 1**
  - 2.2.4. Talent puncturing the right atrium with scissors. **Videographer's Note: Use Take 1**
- 2.3. Place three cotton balls into the mouth of the animal while pulling the tongue forward [1]. Then, raise the head into a nose-up position and use scissors to separate the lower jaw to prevent blood contamination in the nasal lavage fluid [2]. Replace the cotton balls with fresh ones to avoid blood contamination [3].
  - 2.3.1. Talent inserting cotton balls into the mouth while pulling the tongue. **Videographer's Note: 2.3.1-2.3.3 shot together. Use Take 1**
  - 2.3.2. Talent raising the head and separating the lower jaw with scissors.
  - 2.3.3. Talent replacing used cotton balls with new ones.
- 2.4. After bleeding has stopped, inject 200 microliters of sterile PBS into the choana [1] and collect the nasal lavage fluid ejected from the nostrils into a 1.5-milliliter tube [2-TXT].

- 2.4.1. Talent injecting sterile PBS into the choana. **Videographer's Note: 2.4.1-2.4.2 shot together. Use Take 1**
- 2.4.2. Talent collecting expelled nasal lavage fluid into a 1.5 milliliter tube. **TXT: Repeat this step**

### **3. Detection of Blood Contamination by Luminol Chemiluminescence**

- 3.1. To prepare the stock solution for chemiluminescence detection, dissolve 1 milligram of luminol and 5 milligrams of sodium peroxide in 1 milliliter of sterile water inside a 1.5-milliliter tube [1]. Cover the tube with aluminum foil [2] and store it at 4 degrees Celsius until use [3].
  - 3.1.1. Talent dissolving luminol and sodium peroxide in sterile water inside a 1.5 milliliter tube. **Videographer's Note: 3.1.1-3.1.2 shot together. Use Take 1**
  - 3.1.2. Talent covering the tube with foil.
  - 3.1.3. Talent placing the tube in a refrigerator. **Videographer's Note: Use Take 1**
- 3.2. Next, prepare a working solution by diluting the stock solution ten-fold with deionized water [1]. Pipette 2 microliters of the nasal lavage fluid sample into wells of a 96-well plate [2].
  - 3.2.1. Talent diluting the stock solution with deionized water. **Videographer's Note: Use Take 1**
  - 3.2.2. Talent pipetting nasal lavage fluid into the wells of a 96-well plate. **Videographer's Note: 3.2.2-3.3.2 shot together. Mis-slated as 3.2.2 at the beginning, but did tail slate to show 3.2.2 - 3.3.2**
- 3.3. Add 100 microliters of the diluted luminol solution to each well [1] and briefly shake the 96-well plate to mix the contents [2]. Directly observe the chemiluminescence reaction in the dark [3-TXT].
  - 3.3.1. Talent adding diluted luminol solution to the well.
  - 3.3.2. Talent gently shaking the plate to mix contents.
  - 3.3.3. LAB MEDIA: Luminol-Reaction.mp4. **TXT: Semi-quantify the Hb concentration using a luminometer, if necessary**



# Results

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## 4. Results

- 4.1. This figure presents a comparison of immunoglobulin A concentrations across different sample types [1]. In nasal lavage fluid, the concentration of immunoglobulin A is lower in samples collected using the novel method compared to the conventional method [2], whereas serum and bronchoalveolar lavage fluid samples show no substantial differences between the two methods [3]. This data suggests that the novel method reduces contamination in nasal samples, leading to more accurate immunoglobulin A measurements [4].

4.1.1. LAB MEDIA: Figure 3A.

4.1.2. LAB MEDIA: Figure 3A. *Video Editor: Highlight the NLF bars and emphasize the black bar when the VO says “is lower in samples collected using the novel method” and emphasize the white bar when the VO says “the conventional method”.*

4.1.3. LAB MEDIA: Figure 3A. *Video Editor: Highlight the Serum and BALF bars (both black and white).*

4.1.4. LAB MEDIA: Figure 3A.

- 4.2. A similar pattern is observed for immunoglobulin G [1], with significantly lower concentrations in nasal lavage fluid collected using the novel method [2] compared to the conventional method [3]. In contrast, serum and bronchoalveolar lavage fluid samples again show no meaningful differences between the two methods [4], suggesting that the novel approach consistently reduces contamination in nasal samples and improves the reliability of immunoglobulin G measurements [5].

4.2.1. LAB MEDIA: Figure 3B.

4.2.2. LAB MEDIA: Figure 3B. *Video Editor: Highlight the NLF bars and emphasize the black NLF bar.*

4.2.3. LAB MEDIA: Figure 3B. *Video Editor: Highlight the NLF bars and emphasize the white NLF bar.*

4.2.4. LAB MEDIA: Figure 3B. *Video Editor: Highlight the Serum and BALF bars (both black and white).*

4.2.5. LAB MEDIA: Figure 3B.

### Pronunciation Guide:

#### 1. euthanized

- Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanize>  
[youtube.com+1youtube.com+1merriam-webster.com](https://www.youtube.com/watch?v=1merriam-webster.com)
  - IPA: /'ju:θəˌnaɪzd/
  - Phonetic spelling: **yoo-thuh-nyzd**
- 

#### 2. visceral

- Pronunciation link: <https://www.merriam-webster.com/dictionary/visceral>  
[collinsdictionary.com+1youtube.com+1dictionary.cambridge.org+10merriam-webster.com+10merriam-webster.com+10](https://www.collinsdictionary.com+1youtube.com+1dictionary.cambridge.org+10merriam-webster.com+10merriam-webster.com+10)
  - IPA: /'vɪsərəl/
  - Phonetic spelling: **vis-er-uhl**
- 

#### 3. diaphragm

- Pronunciation link: <https://www.merriam-webster.com/dictionary/diaphragm> [merriam-webster.com+2merriam-webster.com+2merriam-webster.com+2](https://www.merriam-webster.com+2merriam-webster.com+2merriam-webster.com+2)
  - IPA: /'daɪəˌfræm/
  - Phonetic spelling: **dy-uh-fram**
- 

#### 4. atrium

- Pronunciation link: <https://www.merriam-webster.com/dictionary/atrium> [merriam-webster.com+3youtube.com+3merriam-webster.com+3collinsdictionary.com+3merriam-webster.com+3dictionary.cambridge.org+3](https://www.merriam-webster.com+3youtube.com+3merriam-webster.com+3collinsdictionary.com+3merriam-webster.com+3dictionary.cambridge.org+3)
  - IPA: /'ɛtriəm/
  - Phonetic spelling: **ay-tree-uhm**
- 

#### 5. syringe

- Pronunciation link: <https://www.merriam-webster.com/dictionary/syringe> [merriam-webster.com+11merriam-webster.com+11merriam-webster.com+11](https://www.merriam-webster.com+11merriam-webster.com+11merriam-webster.com+11)
  - IPA: /səˈrɪndʒ/
  - Phonetic spelling: **suh-rinj**
- 

#### 6. pipette

- Pronunciation link: <https://www.merriam-webster.com/dictionary/pipette>  
[youtube.com+9merriam-webster.com+9oed.com+9](https://www.youtube.com+9merriam-webster.com+9oed.com+9)
  - IPA: /ˌpɪˈpɛt/
  - Phonetic spelling: **pi-pet**
- 

#### 7. microliter

- Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>  
[dictionary.cambridge.org+9dictionary.cambridge.org+9youtube.com+9merriam-](https://www.merriam-webster.com/dictionary/microliter)  
[webster.com+15merriam-webster.com+15youtube.com+15](https://www.merriam-webster.com/dictionary/microliter)
  - IPA: /ˌmaɪkrəˈliːtər/
  - Phonetic spelling: **my-kroh-lee-ter**
- 

#### **8. isoflurane**

- Pronunciation link: <https://www.collinsdictionary.com/dictionary/english/isoflurane>  
[merriam-webster.com+6merriam-webster.com+6merriam-](https://www.collinsdictionary.com/dictionary/english/isoflurane)  
[webster.com+6collinsdictionary.com+10collinsdictionary.com+10openmd.com+10](https://www.collinsdictionary.com/dictionary/english/isoflurane)
  - IPA: /ˌaɪsoʊˈflʊərɪn/
  - Phonetic spelling: **ahy-soh-floo-rayn**
- 

#### **9. luminol**

- Pronunciation link: <https://www.merriam-webster.com/medical/luminol> [merriam-](https://www.merriam-webster.com/medical/luminol)  
[webster.com+8wordpanda.net+8merriam-webster.com+8en.wiktionary.org+7merriam-](https://www.merriam-webster.com/medical/luminol)  
[webster.com+7youtube.com+7](https://www.merriam-webster.com/medical/luminol)
  - IPA: /ˈluːmɪnəl/
  - Phonetic spelling: **loo-min-ol**
- 

#### **10. chemiluminescence**

- Pronunciation link: <https://www.merriam-webster.com/dictionary/chemiluminescence>  
[merriam-webster.com+4en.wiktionary.org+4merriam-webster.com+4](https://www.merriam-webster.com/dictionary/chemiluminescence)
- IPA: /ˌkɛmɪˈluːməˈnɛsəns/
- Phonetic spelling: **kem-ih-loo-muh-nes-uhns**