Submission ID #: 61929
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Title: In Vivo Evaluation of Mucociliary Clearance in Mice

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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 similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **28**

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Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Kyle Feldman**: Our protocol allows the accurate measurement of mucociliary clearance in an animal model using radionuclides that are tracked via dual modality SPECT and CT imaging to accurately localize and measure airway clearance **[1]**.

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

REQUIRED:

1.2. **Kyle Feldman**: This technique is a reproducible method for measuring MCC that can be applied to different animal and disease models due to the prevalence of dual SPECT/CT **[1]**.

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

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pittsburgh.

Protocol

2. Mouse Intubation and Instillation

2.1. After confirming a lack of response to pedal reflex **[1-TXT]**, equip the stand with a nose cone to maintain anesthesia **[3]**, and suspend the anesthetized mouse by the front incisors on an intubation stand at a 45-degree angle **[2]**.

2.1.1. WIDE: Talent pinching toe *Videographer: More Talent than mouse in shot*
TEXT: Anesthesia: 1.5% isoflurane

2.1.2. Teeth being hooked onto wire

2.1.3. Cone being placed NOTE: Show 2.1.3 before 2.1.2.

2.2. Connect one end of a 50-micron fiber optic wire to a light source **[1]** and thread a 20-gauge cannula over the wire **[2]**.

2.2.1. Wire being connected
2.2.2. Cannula being threaded

2.3. Use blunt forceps to pull out the tongue **[1]** and illuminate the guide wire to visualize the vocal cords **[2]**.

2.3.1. Tongue being pulled out NOTE: This and next shot together
2.3.2. Guide wire/vocal cords being illuminated *Videographer: Important/difficult step*

2.4. Pass the guide wire through the vocal cords so that the wire is just beyond the vocal cords resting in the upper trachea **[1]** and slide the 1-inch cannula forward along the wire until it is deep enough that the hub is against the animal's incisors **[2]**.

2.4.1. Guide wire being inserted *Videographer: Important/difficult step*
2.4.2. Cannula being inserted

2.5. Remove the wire while leaving the cannula in place **[1]** and use a finger to briefly plug the cannula to check for changes in breathing **[2-TXT]**.

2.5.1. Wire being removed

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2.5.2. Cannula being briefly plugged **TEXT: If no breathing pattern change, re-position cannula**

2.6. Then add 10 microliters of freshly prepared 0.2-millicurie-99m (ninety-nine-M) technetium sulfur colloid to the cannula **[1-TXT]** and allow the mouse to spontaneously inhale the radionuclide into the lungs over a period of 1-2 minutes **[2]**.

2.6.1. Colloid being added to cannula *Videographer: Important step* **TEXT: See text for all solution preparation details** NOTE: This and next shot together

2.6.2. Colloid being inhaled into cannula OR Talent setting timer *Videographer: More Talent than mouse in shot*

3. Single Photon Emission Computed Tomography and Computed Tomography (SPECT/CT) Imaging

3.1. For SPECT-CT (spect [like expect]-C-T) imaging, after removing the cannula, use tape to secure the mouse to a 25-millimeter pallet with a nose cone **[1-TXT]** and tape a 200-microliter PCR tube containing 0.05-millicure radioactive phantom under the lower abdomen of the mouse below the lungs **[2]**.

3.1.1. WIDE: Talent taping mouse to pallet *Videographer: More Talent than mouse in shot* **TEXT: Caution: Do not tape chest and abdomen too tightly** Added shots: 3.1.1 A – 3.1.1 C NOTE: No accompanying narrative text provided for screenshots_1 to _3. Need text from authors and review from science editor for inclusion

3.1.2. Tube being taped

3.2. Place the mouse into the SPECT-CT system **[1]**, select the imaging workflow **[2]**, and click **Setup [3]**.

3.2.1. Talent placing mouse into system *Videographer: More Talent than mouse in shot; Important step*

3.2.2. Talent selecting workflow, with monitor visible in frame

3.2.3. SCREEN: screenshot_4: 00:16-00:45

3.3. Set up the positioning of the detectors on the mouse **[1]** and run the imaging workflow **[2- 1A]**.

3.3.1. SCREEN: screenshot_4: 01:32-01:40 *Video Editor: please speed up*

3.3.2. SCREEN: screenshot_4: 02:16-02:24

3.3.1A Added shot: click run

3.4. Upon completion of the workflow, transfer the mouse into a new cage with monitoring and unrestricted access to food and water and a radiation safety sticker for 6 hours between scans **[1]**.

3.4.1. Talent placing mouse into cage *Videographer: More Talent than mouse in shot*

4. Analysis

4.1. After imaging, histogram the SPECT images using the factory standard settings for 99m technetium sulfur colloid **[1]** and use a MAP3D (map 3-D) algorithm and point spread function reconstruction to reconstruct 3D stack images **[2]**.

4.1.1. WIDE: Talent histogramming images, with monitor visible in frame
4.1.2. SCREEN: screenshot_5: 00:04-00:10

4.2. Reconstruct the CT images using the Feldkamp algorithm and a Shepp-Logan filter **[1]** and open the CT and SPECT images in FIJI **[2]**.

4.2.1. SCREEN: screenshot_5: 00:16-22:35 *Video Editor: almost no action, please speed up*
4.2.2. SCREEN: screenshot_6: 00:00-00:23 *Video Editor: please speed up*

4.3. Use the re-slice tool to generate coronal view images from the default axial images and perform a z-stack sum projection on the SPECT image to add the count data from each slice **[1]**.

4.3.1. SCREEN: screenshot_6: 00:23-01:06 *Video Editor: please speed up*

4.4. Generate a single image for ease of analysis and **[1]**, using the phantom Eppendorf tube as a reference, resize and co-register the CT and SPECT images **[2-TXT]**.

4.4.1. SCREEN: screenshot_7: 00:00-00:19 *Video Editor: please speed up*
4.4.2. SCREEN: screenshot_7: 00:45-01:57 *Video Editor: please speed up* **TEXT: Track and use consistent resize measurements across all samples**

4.5. Use auto thresholding to binarize the CT image, invert the stack, and perform a z-stack sum projection to generate an outline of the lungs for analysis **[1]**.

4.5.1. SCREEN: screenshot_8: 00:00-01:15 *Video Editor: please speed up*

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4.6. Rotate the CT and SPECT images and use the channel tools to merge them into one image **[1]**. Then draw a region of interest around the right lung to determine the mucociliary clearance **[2]**.

4.6.1. SCREEN: screenshot_9: 00:03-00:28 *Video Editor: please speed up*
4.6.2. SCREEN: screenshot_11: 00:11-01:08 *Video Editor: please speed up*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.3.2., 2.4.1., 2.6.1., 3.2.1.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
2.3.2., 2.4.1.

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Results

5. Results: Representative Mouse Mucociliary Clearance (MCC) Evaluation

5.1. After image acquisition and processing **[1]**, the CT and SPECT images can be colocalized **[2]** using the phantom tube as a landmark **[3]**.

5.1.1. LAB MEDIA: Figures 2A and 2B
5.1.2. LAB MEDIA: Figures 2A and 2B *Video Editor: please emphasize Figure 2A*
5.1.3. LAB MEDIA: Figures 2A and 2B *Video Editor: please emphasize tube in Figure 2B*

5.2. Masks of the lungs **[1]** can be generated from the CT image **[2]** and used to draw regions of interest around the lung for their analysis at different time points **[3]**.

5.2.1. LAB MEDIA: Figures 2C-2F
5.2.2. LAB MEDIA: Figures 2C-2F *Video Editor: please emphasize Figures 2C and 2E*
5.2.3. LAB MEDIA: Figures 2C-2F *Video Editor: please emphasize ROIs in Figures 2D and 2F*

5.3. In this analysis, a total of 8 mice were scanned two times on different days under identical experimental conditions to test the reproducibility of the protocol **[1]**.

5.3.1. LAB MEDIA: Figure 3A

5.4. Paired t-test analysis showed no significant difference between the repeat scans **[1]**.

5.4.1. LAB MEDIA: Figures 3A *Video Editor: please add/emphasize p=0.9904 text*

5.5. An additional 2 mice were also scanned three times on different days under identical experimental conditions **[1]**. One-way ANOVA analysis showed significant matching between the repeat scans **[2]**.

5.5.1. LAB MEDIA: Figure 3B
5.5.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize p=0.0041 text*

5.6. Here representative images from two of the scanned mice can be observed **[1]**.

5.6.1. LAB MEDIA: Figure 4

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Conclusion

6. Conclusion Interview Statements

6.1. **Kyle Feldman**: It is essential to be able to visualize the airway and to verify that intubation is successful. If necessary, remove the cannula and re-intubate to avoid erroneous esophageal intubation **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.4.1.)

6.2. **Kyle Feldman**: This protocol can be applied to disease models, to assess the effects of different drugs on airway clearances, and to aid in the development of novel drug therapies **[1]**.

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

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