FINAL SCRIPT: APPROVED FOR FILMING

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Title: Conjunctival Commensal Isolation and Identification 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 something similar? **No**

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

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 videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, 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? **No.**

Current Protocol Length

Number of Steps: 18
Number of Shots: 47

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Kirsten Smith-Page:** This method identifies viable ocular conjunctival microorganisms, which is challenging due to the hostile microbial environment. It can help answer whether an ocular microbiome exists and how bacterial presence changes in eye disease.

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

1.2. **Kirsten Smith-Page:** In contrast to DNA sequencing, which identifies both viable and nonviable microorganisms, this method identifies only viable microorganisms, resulting in a clearer understanding of the ocular commensal community.

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

OPTIONAL:

1.3. **Kirsten Smith-Page**: Studies suggest that difficult to diagnose eye diseases, such as autoimmune and non-autoimmune Dry Eye, have different ocular bacterial presence. This method could be used to diagnose eye diseases with distinctive microbial signatures.

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

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Harvard Medical School.

Protocol

2. Preparation of Sterile Eye Swabs

2.1. Begin by autoclaving the appropriate amount of cotton batting and toothpicks for the number of mice to be swabbed **[1]**. Then, pinch off a half a centimeter-long piece of cotton batting **[2]** and tease it out by pulling on the edges to form a flat single porous layer, stopping just before the batting falls apart **[3]**. *Videographer: This step is important!*

2.1.1. WIDE: Establishing shot of talent taking materials out of the autoclave.

2.1.2. Talent pinching off cotton.

2.1.3. Talent pulling the edges of the cotton.

2.2. Swirl the batting around one of the sharp ends of the toothpick by lightly holding the stretched-out piece on the toothpick tip as it is twisted **[1]**. The finished eye swab will have a very thin layer of cotton stretched over the tip, extending approximately one half to one centimeter away from the tip **[2]**. *Videographer: This step is important!*

2.2.1. Talent swirling the batting around the toothpick.

2.2.2. Finished eye swab.

2.3. Insert the swabs into a small beaker, swab side down **[1]**, then cover and autoclave them **[2]**.

2.3.1. Talent inserting the swab into the beaker.

2.3.2. Talent putting the covered beaker in the autoclave.

3. Workspace Setup

3.1. Clean the work area with disinfectant to minimize contamination **[1]** and aliquot 0.5 milliliters of sterile Brain Heart Infusion, or BHI, media into labeled 1.5-milliliter sterile microcentrifuge tubes **[2-TXT]**. Cap the tubes in a rack **[3]** and set the rack on ice **[4]**.

3.1.1. Talent disinfecting the workspace.

3.1.2. Talent aliquoting media. **TEXT: 1 tube per mouse eye swab, 1 tube per control**

3.1.3. Talent capping the tubes in a rack.

3.1.4. Talent putting the rack on ice.

3.2. Set up workflow from left to right, starting with the mouse anesthetizing station, which contains the cage with the experimental mice, empty sterile cage, room temperature anesthesia, 25-gauge needle, and a 1-milliliter syringe **[1]**.

3.2.1. Anesthetizing station. *Videographer: Shots 3.2.1, 3.3.1, and 3.4.1 need to be filmed in a hood.*

3.3. Next, prepare an eye swabbing station that contains the aliquoted BHI on ice, sterilized eye swabs, clean paper towels, and 70% isopropanol spray **[1]**.

3.3.1. Eye swabbing station.

3.4. Finally, set up the plating station with room temperature blood agar plates, a 10-microliter pipette, 10-microliter sterile disposable tips, and a biohazard waste container **[1]**.

3.4.1. Plating station.

4. Eye Swabbing

4.1. Make sure that the mouse is properly anesthetized by squeezing a hindfoot pad. Only proceed if there is no movement **[1]**.

4.1.1. Talent squeezing the hindfoot and mouse not moving.

4.2. Assign one hand to handle anesthetized mice and the other hand to handle the eye swab and the culture. Remove the mouse from the cage **[1]** and place it on top of the work surface positioned on its side with the left eye exposed **[2]**. Spray gloved hands with isopropanol and dry them with a paper towel **[3]**.

4.2.1. Talent picking up a mouse from the cage.

4.2.2. Talent positioning the mouse on work surface

4.2.3. Talent spraying hands with isopropanol and drying them.

4.3. Uncap a labeled BHI microcentrifuge tube with the dedicated media handling hand **[1]** and place the tube back in the rack **[2]**. Dip the cotton coated tip of the eye swab in the BHI, then withdraw the swab from the tube while swirling the tip twice against the inner tube to remove excess liquid and remove it **[3 and added]**. *Videographer: This step is important!*

4.3.1. Talent uncapping the tube.

4.3.2. Talent placing the tube back in the rack.

4.3.3. Talent dipping the swab in the BHI.

Added shot: removing swab

4.4. With the mouse handling hand, gently hold the mouse by the scruff of the neck **[1]**. With the other hand, place the tip of eye swab against the medial conjunctival region of the left eye. Lightly depress the eyeball and move the swab in a window washing motion between the lower eyelid and eye 10 times, maintaining constant pressure **[2-TXT]**. *Videographer: This step is difficult and important! Please capture placement,* *depression and swabbing.* Author NOTE: This step was a difficult shoot. We had to raise the mouse off of the work surface and film in mid air. Please use the last shot in this series.

4.4.1. Talent holding the mouse.

4.4.2. Talent swabbing the eye. NOTE: 4.4.2 and 4.5.1 shot together. **TXT: Swab back and forth 10 X**

4.5. Without touching the fur, gently remove the tip of the swab, perpendicular to where it was inserted **[1]**. Place the swab cotton side down directly into a labeled micro-centrifuge tube with BHI media **[2]**. Apply eye to swabbed eye **[2b]**. If desired, acquire skin or fur swabs for control samples **[4.7.1]**, sterilizing gloves appropriately between each swab **[4.7.2]**. When finished, return the mouse to the cage **[3]**. *Videographer: This step is important!*

4.5.1. Talent removing the swab.

4.5.2. Talent placing the swab in the BHI.

4.5.2 b. Added: Talent applying eye drop to swabbed mouse eye.

NOTE: Please insert the shots from 4.7 here.

4.5.3. Talent placing the mouse in the cage.

4.6. Let the swab stand for 10 to 15 minutes on ice **[1]**, then sterilize gloved hands **[2]** and remove the swab while mixing the tip in the media for 10 rotations. Withdraw the swab by swirling the tip against the inner wall of the tube for 5 rotations **[3]** and dispose of it in a biohazard container **[4]**. Repeat the process for each mouse **[5]**. *Videographer: This step is important!*

4.6.1. Swab in the BHI on ice.

4.6.2. Talent sterilizing gloved hands with isopropanol

4.6.3. Talent mixing the swab in the media, then withdrawing it.

4.6.4. Talent placing the swab in a biohazard container.

4.6.5. Talent capping microcentrifuge tube

4.7. **[1]**, **[2]**. NOTE: Please move entire step 4.7 to below 4.5.2 b. (control swabs should be done prior to returning the mouse to the cage).

4.7.1. Talent making a control swab.

4.7.2. Talent sterilizing hands.

5. Enrichment

5.1. Enrich the sample by incubating the tube statically for 1 hour at 37 degrees Celsius **[1]**. During incubation, label one room temperature TSA plate per mouse **[2-TXT]** and divide it in half **[3]**.

5.1.1. Talent putting the samples in an incubator and closing the door.

5.1.2. Talent labeling a plate. **TEXT: TSA: Trypticase Soy with 5% sheep's blood agar** NOTE: difficult to show labelling- labeled in film as wild type female or control female

5.1.3. Talent dividing the plate in half. Author NOTE: Please note that shots, 5.1.2 and shots 5.1.3 may have been switched.

5.2. Remove the enriched samples from the incubator and place them on ice **[1]**. Briefly vortex the samples to mix **[2]**, then aliquot 10 microliters of the sample onto the TSA plate **[3]** and tilt the plate to form a strip. Repeat this twice **[4]**.

5.2.1. Talent transferring samples on ice.

5.2.2. Talent vortexing a sample.

5.2.3. Talent aliquoting 10 microliters onto a plate. Author NOTE: Please note that this shot may have been combined with 5.2.4.

5.2.4. Talent tilting the plate.

5.3. On the other side of the plate's dividing line, create 10 dots with 10 microliters of sample each **[1]**.

5.3.1. Talent dotting the sample onto the other side of the plate.

5.4. Incubate the plates at 37 degrees Celsius for 18 hours, 2 days, and 4 days in a clean chamber that prevents agar plates from drying **[1]**. Count the colonies in the strips **[2]**. Note morphology and calculate colony forming units per swab for morphologically similar isolates **[3]**. Look at the dots for unique organisms not captured in the strips **[4]**.

5.4.1. Talent putting the plates in the incubator and closing the door.

5.4.2. Colonies in the strips.

5.4.3. Talent writing down morphologies and CFU numbers. Author NOTE: Changed the filming of this step- the goal was to point out morphologically distinct colonies which is difficult to show in the video. There are 3 representative plates in this shot and 5.4.2 and 5.4.4. From left to right, the plates are **wild type female, wild type male** and **control**. It may be best to cut the video in this section and 5.4.2. and 5.5.4 to show the first plate only.

5.4.4. Colonies in the dots.

Results

6. Results: Ocular Microbiome of C57BL/6 Mice

6.1. A representative eye swab plate with morphologically diverse isolates from a C57BL/6 *(pronounce 'C fifty-seven black six')* mouse is shown here. For each distinct isolate, the colonies were counted in the strip and the relative abundance was calculated and plotted **[1]**.

6.1.1. LAB MEDIA: Figure 3 A.

6.2. For microbiological characterization, bacteria were picked from individual mouse eye swab plates to produce a master TSA plate. When growth appeared, additional tests were run to characterize or identify the microbes. The master plate was used to provide enough inoculum to expand the respective isolates **[1]**.

6.2.1. LAB MEDIA: Figure 3 B.

6.3. To identify isolates, a TSA plate was streaked and incubated overnight and MALDI-TOF MS was performed **[1]**. The isolates were identified as *Streptococcus acidominimus* and *Aerococcus viridans* with a confidence level of 99.9 with no unidentifiable species **[2]**.

6.3.1. LAB MEDIA: Figure 4.

6.3.2. LAB MEDIA: Figure 4. *Video Editor: Label A "**Streptococcus acidominimus**" and B "**Aerococcus viridans**".*

6.4. Significantly different levels of commensal organisms were recovered from male and female C57BL/6 mice **[1]**. *Streptococcus acidominimus*, *Aerococcus viridans,* coagulase negative staphylococcus isolate number 1, *and E. coli* were found in all mice **[2]**. Males showed higher relative abundance and greater diversity **[3]**.

6.4.1. LAB MEDIA: Figure 5.

6.4.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize Streptococcus acidominimus, Aerococcus viridans, CNS isolate 1, and E. coli in the legend.*

6.4.3. LAB MEDIA: Figure 5. *Video Editor: Emphasize the M bar.*

Conclusion

7. Conclusion Interview Statements

7.1. **Kirsten Smith-Page:** When attempting this protocol, keep in mind that the best outcome will be achieved by coating the swab thinly and taking time during the eye swab step.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1 and 4.4.2.*

7.2. **Kirsten Smith-Page:** If access to Maldi-Tof MS is limited, then the isolates may be identified by microbiological and biochemical testing.

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