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Title: Modeling Ascending Vaginal Infection, Preterm Birth, and Neonatal Morbidity 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? **Yes**

We are not able to use the screen capture software as the equipment cannot be connected to the internet. We can take static screen shots or the videographer can try to capture this instead.

Videographer: Please film the screen for all SCREEN shots.

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   How far apart are the locations? **5 minute walk**

Current Protocol Length
Number of Steps: 07
Number of Shots: 24

Introduction

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

Videographer's NOTE: The authors made a slight change to the words spoken in the interviews

1.1. **Ashley Boyle:** We are investigating the mechanisms that lead to preterm labour and developing treatments that could prevent the early delivery of babies. To do this, we need clinically relevant animal models **[1].**

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

What are the current experimental challenges?

1.2. **Ashley Boyle:** Modelling the adverse neurodevelopmental outcomes experienced by premature babies has proved challenging, as the methods used to induce labour in animals often lead to fetal death **[1]**.

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

What significant findings have you established in your field?

1.3. **Natalie Suff:** We have developed a mouse model of preterm birth in which bacteria ascend from the vagina into the uterus, which is believed to be a common route of infection in humans. Importantly, this model produces live pups that exhibit neuroinflammation **[1]**.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 6.*

What advantage does your protocol offer compared to other techniques?

1.4. **Natalie Suff:** As we can model brain damage in premature pups, we can study the mechanisms leading to this outcome and develop interventions to prevent or treat this, which hasn't been possible with the traditional lipopolysaccharide models of preterm birth **[1]**.

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

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

Ethics Title Card

This research was conducted under a UK Home Office License in accordance with the Animal Scientific Procedures Act (1986) and the ARRIVE guidelines

Protocol

2. Preparation and Administration of Midlog-Phase *Escherichia coli* for Vaginal Colonization and Bioluminescence Imaging

Demonstrators: Ashley Boyle and Natalie Suff

2.1. To begin, add 10 milliliters of antibiotic-treated Luria-Bertani or LB *(L-B)* broth to a sterile 30-milliliter universal container **[1]**. Retrieve a frozen glycerol stock of *Escherichia coli* from minus 80 degrees Celsius storage **[2]**. Using a sterile pipette tip, scrape the surface of the frozen stock to lightly coat the tip with bacteria **[3]**, then eject the tip directly into the universal container containing the LB broth **[4]**.

2.1.1. WIDE: Talent adding Luria-Bertani broth to the 30-milliliter universal container using a pipette.

2.1.2. Talent opening the freezer and retrieving the frozen glycerol stock.

2.1.3. Talent scraping the glycerol stock using a sterile pipette tip. Videographer's NOTE: 2.1.3 and 2.1.4 are combined.

2.1.4. Talent dropping the used tip into the universal container.

2.2. Seal the container with parafilm **[1]**, label it as a biohazard **[2]**, and place it in an orbital shaker set at 200 rpm and 37 degrees Celsius overnight **[3]**.

2.2.1. Talent sealing the container with parafilm. Videographer's NOTE: 2.2.1 and 2.2.2 are combined.

2.2.2. Talent labeling the container as biohazard.

2.2.3. Talent placing the container into the orbital shaker. Videographer's NOTE: Shot 2.2.3 Take 2 is a medium shot, and Take 3 is a close-up shot

2.3. The next morning, dilute the overnight bacterial culture 1 to 100 by adding 0.1 milliliters of the culture to 9.9 milliliters of fresh LB broth **[1]**. Return the diluted culture to the orbital shaker at 200 rpm and 37 degrees Celsius for 1.5 to 3 hours **[2]**.

2.3.1. Talent pipetting 0.1 milliliters of culture into fresh LB broth. Videographer's NOTE: 2.3.1 Take 3 is a close-up to show the different colour & transparency of the liquid. **Don't use Take 2**

2.3.2. Talent placing the diluted culture back into the orbital shaker. Videographer's NOTE: 2.3.2 Take 1 is a close-up shot, and take 2 is a medium shot

2.4. Now, pipette 100 microliters of the culture in duplicate into a clear, flat-bottomed 96-well plate **[1]**, and measure the absorbance using a spectrophotometer with pathlength correction activated **[2-TXT]**.

2.4.1. Talent dispensing 100 microliters of culture in duplicate into a 96-well plate.

2.4.2. Talent inserting the plate in a spectrophotometer. **TXT: An OD_{600} between 0.5 and 0.7 signifies the mid-logarithmic phase of growth**

2.5. When the optical density at 600 nanometers reaches between 0.5 and 0.7, remove 100 microliters of the bacterial culture **[1]**. Dilute this in 900 microliters of sterile PBS **[2]**. Centrifuge the sample at 14,000 *g* for 1 minute to wash the bacterial pellet and remove the broth **[3]**. Discard the supernatant **[4]**, resuspend the pellet in sterile PBS **[5]**, and then dilute the suspension 1 to 10,000 in sterile PBS to achieve 100 to 1,000 colony-forming units **[6]**.

2.5.1. Talent removing 100 microliters from the culture using a pipette. Videographer's NOTE: 2.5.1, 2.5.2, 2.5.3 are combined.

2.5.2. Talent mixing the culture with 900 microliters of PBS.

2.5.3. Talent placing the tube into the centrifuge.

2.5.4. Talent discarding supernatant. Videographer's NOTE: 2.5.4, 2.5.5, 2.5.6 are combined. I felt like I wasn't seeing clearly what was going on in Takes 1 and 2. I changed the camera position in Takes 3 and 4. I felt these takes showed what was happening better. Take 4 is the best to use in my opinion.

2.5.5. Talent resuspending the pellet in PBS.

2.5.6. Talent performing a 1:10,000 dilution in PBS.

2.6. After anesthetizing the mouse, administer 20 microliters of midlogarithmic-phase *E. coli* suspension into the vagina using a sterile 200 microliter pipette tip **[1-TXT]**. Then, using a fresh sterile 200 microliter pipette tip, deliver 20 microliters of 20 percent Pluronic F127 *(F-one-twenty-seven)* gel into the vagina to prevent bacterial leakage **[2]**. Dab quinine powder onto the introitus using sterile gauze to prevent the mouse from cleaning the area **[3-TXT]**.

2.6.1. Talent administering bacterial suspension into the vaginal tract of the anesthetized mouse. **TXT: Anesthesia:; Induction: 5% Isoflurane in O_2; Maintenance: 1.5% Isoflurane in O_2; Administer 20 µL of sterile PBS for vehicle control** Videographer's NOTE: 2.6.1, 2.6.2, 2.6.3 are combined.

2.6.2. Talent administering Pluronic gel into the vagina with a fresh tip.

2.6.3. Talent dabbing quinine powder onto the introitus. **TXT: Record the date and time of treatment**

2.7. After launching the imaging software, click on **Initialize** and wait for the camera to reach the operating temperature indicated by a green and locked temperature bar **[1].** After 48 hours, place the animal in the supine position on the heated stage of a bioluminescence imaging machine while maintaining anesthesia **[2]**. Check the **Luminescent imaging** box, ensure the **Excitation Filter** is set to **Block**, and the **Emission Filter** is set to **Open [3]**. Then, select **Auto exposure time [4]**. NOTE: VO is swapped for the reordered shots.

2.7.2. SCREEN: **Initialize** being clicked on the software interface, and the camera reaches the operating temperature indicated by a green and locked temperature bar. Author's NOTE: 2.7.2 is placed before 2.7.1 according to the correct order of the steps.

Videographer's NOTE: Shots 2.7.2, 2.7.3, and 2.7.4 did not have a clapperboard and are combined. An extra shot is also available (computer monitor fully in frame with an image of the mouse and the bacteria glowing in the mouse's stomach. These are found in the last 3 video files within the Step 2 folder)

2.7.1. Talent positioning the anesthetized animal on the imaging stage.

2.7.3. SCREEN: Check the Luminescent imaging box, verify **Excitation Filter** is **Block**, and **Emission Filter** is Open.

2.7.4. SCREEN: Click on **Auto exposure time**.

Videographer: Please film the screen for all the SCREEN shots.

Results

3. Results

3.1. This figure illustrates the time-dependent progression of bioluminescent *E. coli* infection from the vaginal tract to uterine and fetal tissues in pregnant mice **[1]**.

3.1.1. LAB MEDIA: Figure 6.

3.2. Bioluminescent *E. coli* reached the uterine horns within 48 hours post-infection, with strong luminescent signals detected at embryonic day 18.5 **[1]**, in contrast to the absence of signal at embryonic day 16.5 **[2]**.

3.2.1. LAB MEDIA: Figure 6A. *Video Editor: Highlight the image of the mouse on the right (colored one).*

3.2.2. LAB MEDIA: Figure 6A. *Video Editor: Highlight the image of the mouse on the left.*

3.3. Dissected uterine horns revealed clear bioluminescent signals as early as 18 hours after infection, indicating rapid intrauterine colonization **[1]**.

3.3.1. LAB MEDIA: Figure 6B. *Video Editor: Highlight the colored areas throughout the coiled structure.*

3.4. Infection had also reached fetal compartments within 18 hours, as shown by luminescent signals **[1]** in the placenta **[2]**, fetal membranes **[3]**, and amniotic fluid **[4]**.

3.4.1. LAB MEDIA: Figure 6C.

3.4.2. LAB MEDIA: Figure 6C. *Video Editor: Highlight the label "Placenta" and the corresponding white arrow.*

3.4.3. LAB MEDIA: Figure 6C. *Video Editor: Highlight the label "Fetal membranes" and the corresponding white arrow.*

3.4.4. LAB MEDIA: Figure 6C. *Video Editor: Highlight the label "Amniotic fluid" and the corresponding white arrow.*

Pronunciation Guide:

1. *Luria-Bertani* (as in Luria-Bertani broth)

Pronunciation link: https://www.pronouncekiwi.com/Luria-Bertani%20broth How To Pronounce+7How To Pronounce+7How To Pronounce+7Biology Stack Exchange+5Pronounce Kiwi+5Microbiology Class+5
IPA: /ˌlʊri.ə bərˈtɑni/
Phonetic: loo-REE-ə ber-TAH-nee

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2. *Escherichia coli*

Pronunciation link: https://www.merriam-webster.com/dictionary/escherichia — note: E. coli is defined there How To Pronounce+2How To Pronounce+2How To Pronounce+2YouGlish+11Merriam-Webster+11Dictionary.com+11
IPA: /ˌɛʃəˈrɪkiəˈkoʊlaɪ/
Phonetic: esh-uh-RICK-ee-ə KO-lye

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3. *Midlogarithmic*

Pronunciation link: Wiktionary entry used as trusted fallback (no Merriam-Webster) Wiktionary
IPA: /ˌmɪdˌlɑgəˈrɪθmɪk/
Phonetic: mid-lah-gah-RITH-mik

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4. *Orbital shaker*

Pronunciation link: YouGlish example page for “orbital shaker”
IPA: /ˈɔrbɪtəl ˈʃeɪkər/
Phonetic: OR-bi-təl SHAY-kər

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5. *Parafilm*

Pronunciation link: HowToPronounce.com listing for “parafilm”
IPA: /ˈpærəˌfɪlm/
Phonetic: PAR-ə-film

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6. *Introitus*

Pronunciation link: Merriam-Webster Medical entry for introitus
IPA: /ɪnˈtroʊətəs/
Phonetic: in-TROH-uh-təs

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7. *Bioluminescent*

Pronunciation link: Cambridge Dictionary pronunciation for “bioluminescent”
IPA: /ˌbaɪoʊˌluːməˈnɛsənt/
Phonetic: BY-oh-loo-muh-NES-uhnt

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8. *Chemiluminescence*

Pronunciation link: Merriam-Webster “chemiluminescence” entry
IPA: /ˌkɛmɪˌluːməˈnɛsəns/
Phonetic: kem-ih-loo-muh-NES-ens

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9. *Supine*

Pronunciation link: Merriam-Webster dictionary for "supine"
IPA: /suːˈpaɪn/
Phonetic: soo-PINE

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10. *Pluronic*

Pronunciation link: YouTube clip “How to Pronounce Pluronic”
IPA: /plʊˈrɒnɪk/ (American variant often /plʊˈroʊnɪk/)
Phonetic: plu-RON-ik