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Title: Longitudinal Follow-up of Urinary Tract Infections and Their Treatment in Mice using Bioluminescence Imaging

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? **YES, done**

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, all locations are within same building.**

Current Protocol Length

Number of Steps: 21
Number of Shots: 54 (12 SC)

Introduction

1. Introductory Interview Statements

1.1. **Noémie Luyts:** The enumeration of CFU was limiting the reproducibility of research in the UTI field. BLI will advance in vivo UTI research on bladder physiology, UTI pathogenesis and susceptibility **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Figure 1B and Figure 2.*

1.2. **Greetje Vande Velde:** BLI enables longitudinal follow-up with real-time insight into the evolution of the infection. Moreover, it drastically reduces the number of animals needed **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.2.*

OPTIONAL:

1.3. **Helene De Bruyn:** BLI facilitates research on recurrent or chronic infections, which are frequent in humans. Additionally, researchers can identify ascending infections or dissemination to the blood with BLI **[1]**.

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

Ethics Title Card

1.4. All animal experiments were conducted in accordance with the European Union Community Council guidelines and were approved by the Animal Ethics Committee of KU Leuven.

Protocol

2. Culturing Bacteria

2.1. Obtain single colonies by streaking out the glycerol stock of bacteria with an inoculation loop on LB *(spell out)* plates supplemented with kanamycin sulfate **[1-TXT]** and culturing overnight at 37 degree Celsius **[2].**

2.1.1. Talent streaking bacteria on LB plates. **TEXT: LB with 50 µg/mL Km**

2.1.2. Talent putting the plates in the incubator.

2.2. Fill a sterile 14-milliliter polystyrene round-bottom tube with dual-position snap caps with 5 milliliters of LB broth supplemented with kanamycin sulfate **[1]**. Pick a single bacterial colony with an inoculation loop **[2]** and add this to the LB broth **[3]**. Vortex for 10 seconds to ensure proper mixing **[4].**

2.2.1. Talent filling LB broth in sterile polystyrene RB tube and adding 5µL of Km sulfate.

2.2.2. Talent picking a single bacterial colony.

2.2.3. Talent adding the colony to the LB broth in the tube.

2.2.4. Talent vortexing the tube.

2.3. Culture statically with the snap cap in the open position at 37 degree Celsius overnight **[1].**

2.3.1. Talent incubating the tube overnight.

2.4. After the incubation, vortex the tube for 10 seconds to ensure proper homogenization of the bacterial culture **[1]**. Make a sub-culture in the Erlenmeyer by adding 25 microliters of the bacterial suspension to 25 milliliters of fresh LB medium without antibiotics **[2].** Close the Erlenmeyer **[3]** and culture statically overnight **[4].**

2.4.1. Talent vortexing the culture tube.

2.4.2. Talent adding 25 µL of the bacterial suspension to fresh LB medium without antibiotics.

2.4.3. Talent closing the Erlenmeyer tube.

NOTE: Shot 2.4.3. was an extra shot that was not necessary but was filmed in case the video editor wanted to keep it was a separate shot.

2.4.4. Talent incubating the tube.

2.5. On the day of instillation, pour the culture from the Erlenmeyer into a 50-milliliter culture tube **[1]** and centrifuge **[2-TXT]**. Decant the supernatant **[3]** and resuspend the bacterial pellet in 10 milliliters of sterile PBS **[4].**

2.5.1. Talent pouring the culture from the Erlenmeyer to culture tube.

NOTE: For shot number 2.5.1., the flash card used has '2.5' written on it and not '2.5.1.'

2.5.2. Talent centrifuging the tube. **TEXT: at 3,000 x g and 4 °C for 5 min,**

NOTE: The centrifuge settings in the shot are different than the settings mentioned in the text overlay.

2.5.3. Talent discarding the supernatant.

2.5.4. Talent adding PBS to the pellet.

NOTE: Author – "Please cut this fragment before throwing away the pipet, it was placed back into the paper, but this is not ideal".

2.6. Vortex the tube again for 10 seconds **[1].** Choose the experimental concentration of the inoculum and determine the corresponding OD *(spell out)* at 600 nanometers using the standard curve **[2].**

2.6.1. Talent vortexing the tube.

2.6.2. Talent determining the OD of the culture.

2.7. Add 1 milliliter of the resuspended bacterial culture into 9 milliliters of sterile PBS **[1]** and adjust until the desired OD 600 nanometer is reached **[2].**

2.7.1. Talent adding culture into PBS.

2.7.2. Talent adjusting the desired OD of the culture. *Videographer: This step is important!*

NOTE: Shot number 2.7.2. was filmed as shot number 2.6.2. Take 2.

3. Inoculation of the Animals

3.1. Fill the syringe with the prepared bacterial solution **[1].** Mount a sterile 24-gauge angiocatheter tip on a 100-microliter syringe **[2].**

3.1.1. Talent mounting the angiocatheter tip on a syringe. *Videographer: This step is important!*

3.1.2. Talent filling the syringe with bacterial solution.

NOTE: Shot number 3.1.2. should be placed before shot number 3.1.1.

3.2. Place one animal on a working surface in the supine position **[1-TXT]** and maintain a stable isoflurane anesthesia using a nose cone during the instillation **[2]**. Apply the eye-ointment **[3].**

3.2.1. Talent placing the animal on the working surface. **TEXT: Anesthesia: Isoflurane with pure oxygen as a carrier gas (induction at 3% and maintenance at 1.5%)**

3.2.2. Talent placing the nose cone with isoflurane anesthesia.

NOTE: Shot number 3.2.2. was not filmed according to its description. Instead of that footage of the talent pinching the animal's toe has been added.

3.3. Expel the residual urine by applying gentle compression and making circular movements on the suprapubic region **[1]**, then clean the lower abdomen with 70% ethanol **[2].**

3.3.1. Talent applying gentle compression on the suprapubic region of the animal.

NOTE: It is important to see that some urine is coming out of the urethra.

3.3.2. Talent cleaning the lower abdomen with ethanol and Iso-betadine.

3.4. Lubricate the catheter tip with normal saline **[1]**. Put the index finger of the non-dominant hand on the abdomen and push it gently upwards **[2]**. Start the catheterization of the urethra vertically in a 90-degree angle **[3]**. Once resistance is encountered, tilt it horizontally before inserting it further **[4-TXT]**.

3.4.1. Talent lubricating the catheter tip with saline.

3.4.2. Talent gently pushing the abdomen of the animal upwards with the index finger.

3.4.3. Starting of the catheterization of the urethra. *Videographer: This step is important!*

NOTE: Shots 3.4.3. to 3.5.3 were filmed continuously as a single shot. There are 2 takes for this long shot. Please use parts of each take that look the best.

3.4.4. Talent tilting the catheter horizontally before inserting. **TEXT: 0.5 cm**

3.5. Perform a slow instillation of 50 microliters of the bacterial inoculum **[1-TXT].** After the instillation, keep the syringe and catheter in place for a few more seconds **[2]** and then slowly retract to prevent leakage **[3].**

3.5.1. Talent performing slow instillation of bacterial inoculum. **TEXT: 5 µL/s** *Videographer: This step is important!*

3.5.2. Talent keeping the syringe and catheter undisturbed.

3.5.3. Talent slowly retracting the syringe and catheter.

3.6. Position the animal in a supine position at the nose cone of the imaging chamber **[1]** and prepare it for imaging using one catheter per experimental group **[2].** If necessary, administer antibiotics or experimental drugs as mentioned in the text manuscript **[3].**

3.6.1. Talent positioning the animal in the CCD camera.

3.6.2. Talent preparing the animal for imaging. *Videographer: This step is important!*

NOTE: Shot number 3.6.2. was not filmed separately but was combined with shot 3.6.1.

3.6.3. Talent administering antibiotics to the animal.

NOTE: Shot number 3.6.3. and its corresponding VO text should be moved and placed after shot number 3.5.3.

4. Bioluminescence Imaging

4.1. Open the BLI acquisition software **[1-TXT]** and click on **Initialize** in the imaging device to test the camera and stage controller system and to cool the charge-coupled device camera to minus 90 degrees Celsius **[2-TXT].** Click on acquisition **Auto-save to [3].**

4.1.1. SCREEN: 62614_screenshot_1.mp4. 00:03-00:20. **TEXT: BLI-Bioluminescence Imaging**

4.1.2. SCREEN: 62614_screenshot_1.mp4. 00:20-00:25. **TEXT: CCD-Charge-coupled device.**

4.1.3. SCREEN: 62614_screenshot_1.mp4. 00:28-00:40.

4.2. Select **Luminescence** and **Photograph**. Check the default luminescence settings by setting the excitation filter to **Block** and emission filter to **Open [1].**

4.2.1. SCREEN: 62614_screenshot_1.mp4. 00:45-00:48.

4.3. Set the exposure time to **Auto** when taking the first image. For *in vivo* measurements and bright signals, set the **Exposure Time** to around 30 seconds. Reduce the exposure time if a warming appears due to a saturated image **[1].**

4.3.1. SCREEN: 62614_screenshot_1.mp4. 00:40-00:45 and 00:52-00:59. *Videographer: Take multiple shots of the talent sitting on the computer and clicking the mouse to be used as b-roll throughout the shot.*

4.4. Select the medium **Binning, F/stop** 1 **[1]** and choose the correct **FOV [2-TXT].** Set the **subject height** to 1 centimeter when imaging mice **[3]**.

4.4.1. SCREEN: 62614_screenshot_1.mp4. 00:48-00:52.

4.4.2. SCREEN: 62614_screenshot_1.mp4. 00:59-01:01. **TEXT: FOV-Field of view**

4.4.3. SCREEN: 62614_screenshot_1.mp4. 01:01-01:09.

4.5. Image up to 5 mice simultaneously **[1]** and separate the animals using the light baffle to prevent reflection **[2].** Close the door **[3]** and click on **Acquire** to start the imaging sequence. Then, fill in detailed information about the experiment **[4].**

4.5.1. Talent imaging 5 mice.

NOTE: Use footage from 3.6.1. for shot 4.5.1.

4.5.2. Talent separating the animals using light baffle.

4.5.3. Talent closing the door.

4.5.4. Starting of imaging sequence. *Videographer: Take a shot of the talent sitting on the computer and clicking the mouse.*

4.6. Remove mice from the imaging chamber and return them to their cage **[1]**. Check for full recovery after anesthesia **[2-TXT]**, then return the cages to the ventilated racks until the next imaging cycle **[3].**

4.6.1. Talent returning the mice to their cage from imaging chamber.

4.6.2. Talent checking for full recovery of the mice and placing them back into the group housed IVC cage **TEXT: Do not provide analgesia**

4.6.3. Talent returning the cages to the ventilated racks.

4.7. Start the imaging software **[1]** and load the experimental file by clicking on **Browse [2].**

4.7.1. SCREEN: 62614_screenshot_2.mp4. 00:05-00:20.

4.7.2. SCREEN: 62614_screenshot_2.mp4. 00:24-00:38.

4.8. Use the **Tool Palette** to adjust the color scale of the image **[1].** Use the **ROI tools** to draw a region of interest on the image, ensuring that it is large enough to cover the complete area and using the same dimensions for all the images **[2]**. Then, click on **ROI measurement** to quantify the light intensity **[3].**

4.8.1. SCREEN: 62614_screenshot_2.mp4. 00:40-00:56.

4.8.2. SCREEN: 62614_screenshot_2.mp4. 01:00-01:15.

4.8.3. SCREEN: 62614_screenshot_2.mp4. 01:15-01:25.

Results

5. The Analysis of BLI Correlation with CFU at the Time of Instillation and Longitudinal Follow-up During Treatment

5.1. Subsequent images of mice obtained immediately post-instillation showed that bioluminescence was robustly detectable above 20,000 CFU *(Colony forming units)* **[1-TXT]** and a linear correlation between the CFU of the inoculum and the bioluminescence *in vivo* was established **[2].**

5.1.1. LAB MEDIA: Figure 1A. **TEXT: CFU-Colony forming unit.**

5.1.2. LAB MEDIA: Figure 1B.

5.2. The natural evolution of a urinary tract infection was studied in a control group that did not receive any treatment. The effect of antibiotic treatment on the infection kinetics was visualized in detail in a group of antibiotic-treated animals. **[1].**

5.2.1. LAB MEDIA: Figure 2.

5.3. An immediate decrease in bacterial load, as measured by total photon flux, was seen after the first dose of enrofloxacin. None of the treated animals had a subsequent rise in bacterial load **[1]**.

5.3.1. LAB MEDIA: Figure 2A. *Video editor focus on the blue line graph.*

5.4. The overall bacterial load for each animal was calculated using the area under the curve of the log-transformed total photon flux, showing a significant difference between animals treated with enrofloxacin and untreated animals over the time course of 10 days **[1]**.

5.4.1. LAB MEDIA: Figure 2B.

5.5. These results provide proof-of-concept that BLI can be used to evaluate differences in UTI infection kinetics **[1]**.

5.5.1. LAB MEDIA: Figure 2B.

Conclusion

6. Conclusion Interview Statements

6.1. **Matthias Vanneste:** BLI is a non-invasive technique that can be combined with other methods such as regular collection of urinary samples for bacterial or biochemical analysis or voiding experiments **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Figure 2.*

6.2. **Wouter Everaerts:** Here we have demonstrated that BLI is a powerful tool to evaluate new therapeutic strategies on the disease course of UTIs **[1]**.

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

NOTE: Shot number 6.2.1A is to be used for this interview statement. This shot has Prof. Everaerts delivering the statement in a green OR outfit with white UZ Leuven coat.