**Submission ID #: 67966**
**Scriptwriter Name: Poornima G**
**Project Page Link: https://review.jove.com/account/file-uploader?src=20729678**

# Title: An In Vitro Bladder Model of Catheter-Associated Urinary Tract Infection

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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: 25
Number of Shots: 56

# Introduction

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

**Videographer's NOTE:** The authors didn't say the lines on the script word for word. They altered it slightly.
The slating for Brian's introduction is incorrect. The same could be for Ocean's introduction.
I think there is an extra introduction piece from Ocean due to accidently following the draft script initially.

1.1. **Ocean Clarke:** Our research is focuses on catheter-associated urinary tract infections. We use *in vitro* models, such as the bladder model, to learn more about the pathogens that cause CAUTIs.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1*

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

1.2. **Vicky Bennett:** The *in vitro* bladder model provides a controlled environment to investigate the practical applications of diagnostic tools and therapeutic interventions for CAUTI treatment

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What are the current experimental challenges?

1.3. **Ocean Clarke:** The catheterized urinary tract is a complex environmental niche, with important parameters like the catheter surface and the nutritional environment of urine which make it hard to replicate *in vitro*.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

What research gap are you addressing with your protocol?

1.4. **Brian Jones:** A key aim of our research is to find ways to control biofilm formation on medical devices, like urinary catheters, in order to improve outcomes for individuals who can benefit from these devices.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.1*

What research questions will your laboratory focus on in the future?

1.5. **Brian Jones:** We are also adapting our models to study the development of antimicrobial resistance in bacterial communities, and answer questions about how this arises and what we can do to prevent it.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 6.4.1*

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

# Protocol

**Videographer's NOTE:** Many apologies here. I forgot to switch my camera settings to 1080p when we started the Protocol filming. Shots 2.1.1 to 2.1.4 and 6.1.1 to 6.1.4 were shot in 4K. I realised my mistake after this and switched my camera settings to 1080p. All other protocol shots were filmed in 1080p.

## 2. In Vitro Bladder Model Setup

**Demonstrator:** Ocean E. Clarke

2.1. To begin, insert a Foley catheter into the central glass chamber of the in vitro bladder model **[1]** and secure the catheter in place after inflating the balloon using the syringe of sterile water provided **[2]**. Then, attach the waste bag to the catheter port **[3]** before connecting the in vitro bladder model device to the AUM reservoir **[4]**.

2.1.1. WIDE: Talent inserting the Foley catheter into the central glass chamber of the in vitro bladder model.

2.1.2. Talent inflating the balloon with a syringe filled with sterile water to secure the catheter.

2.1.3. Talent attaching the waste bag to the catheter port.

2.1.4. Talent connecting the in vitro bladder model device to the AUM reservoir. **Videographer's NOTE**: Shot 2.1.4 was split into two takes

2.2. Now, attach the in vitro bladder models to clamp stands **[1].** Connect the media circuit tubing to the peristaltic pump **[2]** and secure the waste bags in the stands positioned below the models **[3]**.

2.2.1. Talent securing the in vitro bladder models onto clamp stands.

2.2.2. Talent connecting the media circuit tubing to the peristaltic pump. **TXT: Follow the manufacturer's instructions**

2.2.3. Talent positioning and securing the waste bags beneath the models.

2.3. Connect the outer chamber of the bladder model to a circulating water bath using silicone tubing **[1]**. Set the water bath to heat to 37 degrees Celsius **[2]** and turn on the tank circulators **[3]**.

2.3.1. Talent connecting the outer chamber of the bladder model to the circulating water bath with silicone tubing.

2.3.2. Talent setting the water bath to 37 degrees Celsius. **Videographer's NOTE**: Take 2 was boarded on the end due to the time constraints of the machine changing temperature sporadically

2.3.3. Talent activating the tank circulators.

**3. Inoculation of In Vitro Bladder Model**

**Demonstrator:** Vicky Bennett

3.1. Sterilize the in vitro bladder model using 70 percent ethanol **[1]**. Remove the stopper at the media inlet to make the inner chamber accessible **[2]**. Using a serological pipette, remove 10 milliliters of AUM from the bladder model volume **[3]**.

3.1.1. Talent decontaminating the external surfaces of the bladder model with 70 percent ethanol. **Videographer's NOTE**: Shots 3.1.1 & 3.1.2 were joined together in one take

3.1.2. Talent removing the stopper from the media inlet.

3.1.3. Talent extracting 10 milliliters of AUM using a serological pipette.

3.2. Inoculate the in vitro bladder models through the central chamber using the entire normalized 10 milliliter suspension and mix thoroughly **[1]**. Using the pipette, remove 10 milliliters of the inoculated sample from the central chamber **[2]** and aliquot them into a 15-milliliter universal tube for later assays **[3-TXT]**.

3.2.1. Talent inoculating the central chamber with 10 milliliters of normalized suspension and mixing with a pipette. **Videographer's NOTE**: Shots 3.2.1, 3.2.2 & 3.2.3 were joined together in one take

3.2.2. Talent removing 10 milliliters of the mixed suspension.

3.2.3. Talent aliquoting suspension into a 15 milliliter universal tube. **TXT: Perform CFU/mL calculations and pH measurement**

3.3. After decontaminating the external surfaces, replace the stopper and briefly resume media flow to ensure that the AUM level in the central chamber reaches the eyehole **[1]**, then stop the flow **[2-TXT]**. Following a 1-hour incubation, switch on the media flow using the peristaltic pump to begin running the in vitro bladder models **[3-TXT]**.

~~3.3.1. Talent wiping the bladder model and bung with 70 percent ethanol.~~ **Videographer's NOTE**: Not filmed, VO deleted and merged to the next shor

3.3.2. ~~Talent replacing the stopper securely~~.

3.3.3. Talent resuming media flow and the AUM reaching the eyehole.

3.3.4. Talent stopping the flow. **TXT: Note the time**

3.3.5. Talent switching on the peristaltic pump. **TXT: Note the time**

## 4. Sampling Planktonic Cells

**Demonstrator:** Ocean E. Clarke

4.1. After decontaminating the bladder model stopper, remove it to access the central chamber **[1]**.

4.1.1. Shot of removing stopper to expose the central chamber.

4.2. Using a serological pipette, gently mix the contents of the central chamber, avoiding the catheter tip and balloon **[1-TXT]**.

4.2.1. Talent carefully mixing the contents of the central chamber with a serological pipette. **TXT: Transfer 10 mL of sample from the chamber into a fresh tube**

4.3. ~~Next, transfer 10 milliliters of sample from the chamber to a sterile tube **[1]**. Immediately decontaminate the stopper with 70 percent ethanol **[2]** and replace it to complete the sterile, closed drainage circuit **[3]**~~. **Videographer's NOTE**: Not filmed, VO moved as on screen text to the previous shot

~~4.3.1. Talent withdrawing 10 milliliters of sample and putting it to a sterile tube.~~

~~4.3.2. Talent wiping the stopper.~~

4.3.3. ~~Talent replacing the stopper after sampling~~.

4.4. Then, remove 1 milliliter from the sample using a pipette and aliquot it into a 1.5 milliliter microcentrifuge tube for serial dilutions **[1]**. Measure the pH of the remaining sample using a pH meter **[2]**.

4.4.1. Talent pipetting 1 milliliter from the sample into a 1.5 milliliter microcentrifuge tube.

4.4.2. Talent operating the pH meter to measure the pH of the sample.

## 5. Sampling Biofilm-Associated Cells

**Demonstrator:** Vicky Bennett

5.1. Disconnect the waste bag from the catheter **[1]** and drain any residual urine from the

model by deflating the balloon **[2]**. Remove the catheter from the bottom of the model using sterile forceps **[3]**.

5.1.1. Talent disconnecting the waste bag.

5.1.2. Shot of deflating the balloon to drain residual urine. **Videographer's NOTE**: Shots 5.1.2 & 5.1.3 were done in one take

5.1.3. Talent removing the catheter from the model using sterile forceps.

5.2. Now, place the removed catheter onto a sterile board **[1]** and, using a sterile scalpel, cut the catheter at the required points for sectioning **[2]**.

5.2.1. Talent placing the catheter onto a sterile board. **Videographer's NOTE**: 5.2.1 & 5.2.2 were done in one take. I couldn't show this on the clapperboard due to technical issues I was experiencing with the app at that specific moment.

5.2.2. Talent cutting the catheter at specific points with a sterile scalpel.

5.3. Using sterile forceps, dip the catheter section three times into sterile PBS to remove non-adherent cells **[1]** and incubate the prepared catheter section in 0.25 percent trypsin at 37 degrees Celsius for 30 minutes **[2]**.

5.3.1. Talent dipping the catheter section into sterile PBS using sterile forceps.

5.3.2. Talent placing the catheter section in 0.25 percent trypsin in an incubator set at 37 degrees Celsius.

5.4. After incubation, sonicate the solution containing the catheter for 10 minutes **[1]**.

5.4.1. Talent sonicating the catheter-containing solution for 10 minutes.

## 6. Antimicrobial Susceptibility Testing in the Model

**Demonstrators:** Vicky Bennett; Ocean E. Clarke

6.1. Using the aseptic technique, insert the end of the syringe containing the lubricating agent into the neck of the bladder model and inject 5 milliliters of gel **[1-TXT]**. Rotate the model to ensure the base is evenly coated with gel **[2]**. To insert a Foley catheter into the model, push the catheter tip through the gel **[3]** and secure it after filling the balloon as normal **[4]**.

6.1.1. Talent inserting the syringe into the neck of the bladder model and injecting lubricating gel. **TXT: Let the gel fill the neck and enter the base of the chamber**
**Videographer's NOTE**: Shots 6.1.1 & 6.1.2 were joined together in one take

6.1.2. Talent rotating the model to coat the base evenly with gel.

6.1.3. Talent inserting the Foley catheter through the gel.

6.1.4. Shot of inflating the balloon to secure it.

6.2. Connect the model to the rest of the setup, then fill the model lumen with urine until it starts to drain through the catheter eyehole **[1]**. As soon as urine begins to drain, stop the pump to prevent loss of gel before inoculation **[2]**.

6.2.1. Talent connecting the bladder model to the experimental setup and filling it with urine. **Videographer's NOTE**: Shot 6.2.1 also covered 6.2.2 but this wasn't on the slate

6.2.2. Talent stopping the pump once urine drains through the catheter eyehole.

6.3. ~~After a 30-minute incubation, inoculate the models **[1]** and leave them to incubate for 1 hour at 37 degrees Celsius prior to switching on the peristaltic pumps **[2]**.~~ At the point of switching on the pump after incoulations, plate out dilutions for colony forming unit counts **[1-TXT]**.

~~6.3.1. Talent inoculating the model by adding the sample. **Videographer's NOTE**: Not filmed~~

6.3.2. ~~Talent placing the model in incubator~~.

6.3.3. Talent collecting 1 milliliter planktonic sample into a separate tube. **TXT: Inoculate the models and incubate (1 h); Take a 1 mL planktonic sample**

6.4. If using an individual irrigation pouch, disconnect the waste bag from the catheter and use the cap provided with the pouch to seal the waste bag **[1]**. Immediately attach the irrigation solution pouch to the catheter **[2]**, and using light pressure, squeeze the product into the central lumen following the manufacturer's instructions for 15-minutes **[3]**.

6.4.1. Talent disconnecting the waste bag and sealing it with the provided cap.

6.4.2. Talent attaching the irrigation pouch to the catheter. **Videographer's NOTE**: Shots 6.4.2 & 6.4.3 were done in one take. There is an additional shot of 6.4.3 which is a close up

6.4.3. Shot of squeezing the solution into the bladder model.

6.5. Once the recommended treatment time is completed, allow the residual solution to drain from the inner chamber via the catheter eyehole into the irrigation pouch **[1]**, then disconnect the pouch **[2]**.

6.5.1. Shot of solution draining into the pouch.

6.5.2. Talent disconnecting the pouch.

6.6. Sterilize the catheter drainage port and the waste bag connection port with 70 percent ethanol before reconnecting them **[1]**.

6.6.1. Talent wiping both ports with 70 percent ethanol and reconnecting the waste bag to the catheter.

6.7. To terminate the experiment, turn off the water bath flow and disconnect the media circuit and water bath circuit tubing from the in vitro bladder models **[1]**.

6.7.1. Talent turning off the water bath and disconnecting the tubing from the bladder models.

6.8. After removing the stoppers, collect the entire volume of residual urine **[1-TXT]**.

6.8.1. Talent removing the stoppers and extracting residual urine with a serological pipette. **TXT: Discard any contaminated media appropriately**

6.9. Drain the waste bags and discard any residual media **[1]**. Then, disconnect the catheter waste bags from the catheter port and discard them **[2]**.

6.9.1. Talent draining and discarding the waste bag contents.

6.9.2. Talent disconnecting and disposing of the catheter waste bags.

6.10. Finally, using a syringe, slowly deflate catheter balloons before removing catheters from the models **[1]**.

6.10.1. Talent deflating the catheter balloon with a syringe and removing the catheter from the model. **Videographer's NOTE**:6.10.1 Take 3 is a close up

# Results

**7. Representative Results**

7.1. After inoculating the bladder model, the blockage time was significantly extended in the mini-Tn5 *(T-N-5)* mutant strain of *Proteus mirabilis* **[1]** compared to the wild-type strain, indicating reduced biofilm formation **[2]**.
7.1.1. LAB MEDIA: Figure 4A. *Video editor: Mark the bar graph for "Mutant"*.
7.1.2. LAB MEDIA: Figure 4A. *Video editor: Mark the bar graph for "wild type"*

7.2. Planktonic colony-forming units per milliliter at the time of blockage were significantly reduced in the mini-Tn5 mutant strain **[1]** compared to the wild-type **[2]**.
7.2.1. LAB MEDIA: Figure 4C. *Video editor: Mark the bar graph for "Mutant"*.
7.2.2. LAB MEDIA: Figure 4C. *Video editor: Mark the bar graph for "wild type"*

7.3. Crystal violet staining demonstrated that *Proteus mirabilis* isolates passaged in chlorhexidine exhibited a significant reduction in biofilm biomass **[1]** compared with those passaged without chlorhexidine **[2]**.
7.3.1. LAB MEDIA: Figure 5. *Video editor: Mark the bar graphs for "2, 4 , 6"*. "passaged"
7.3.2. LAB MEDIA: Figure5. *Video editor: Mark the bar graphs for " 1, 3, 5""controls"*

7.4. Treatment of in vitro bladder models with 0.02% chlorhexidine irrigation significantly extended the time to blockage **[1]** and reduced the planktonic colony-forming units per milliliter compared with untreated and saline-treated controls **[2]**.
7.4.1. LAB MEDIA: Figure 6A *Video editor: Mark the bar graph for "Treated"*.
7.4.2. LAB MEDIA: Figure 6B *Video editor: Mark the bar graph for "Treated"*.

## 1. Foley catheter

**Pronunciation link:**
https://www.howtopronounce.com/foley-catheter
**IPA:** /ˈfoʊli ˈkæθətər/
**Phonetic Spelling:** foh-lee kath-uh-ter

## 2. Peristaltic

**Pronunciation link:**
https://www.howtopronounce.com/peristaltic
**IPA:** /ˌperəˈstɔltɪk/
**Phonetic Spelling:** peh-ruh-stawl-tik

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## 3. Serological

**Pronunciation link:**
https://www.howtopronounce.com/serological
**IPA:** /ˌsɪrəˈlɑːdʒɪkəl/
**Phonetic Spelling:** sih-ruh-laa-juh-kuhl

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## 4. Aliquot

**Pronunciation link:**
https://www.howtopronounce.com/aliquot
**IPA:** /ˈælәˌkwɑt/
**Phonetic Spelling:** al-uh-kwaht

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## 5. Proteus mirabilis

**Pronunciation link:**
https://www.howtopronounce.com/proteus-mirabilis
**IPA:** /ˈproʊtiəs mɪˈræbəlɪs/
**Phonetic Spelling:** proh-tee-uhs mih-ra-buh-lis

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## 6. Trypsin

**Pronunciation link:**
https://www.howtopronounce.com/trypsin
**IPA:** /ˈtrɪpsɪn/
**Phonetic Spelling:** trip-sin

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## 7. Crystal violet

**Pronunciation link:**
https://www.howtopronounce.com/crystal-violet
**IPA:** /ˈkrɪstəl ˈvaɪələt/
**Phonetic Spelling:** kris-tuhl vai-uh-luht

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## 8. Chlorhexidine

**Pronunciation link:**
https://www.howtopronounce.com/chlorhexidine
**IPA:** /klɔːrˈhɛksəˌdiːn/
**Phonetic Spelling:** klor-hek-suh-deen