FINAL SCRIPT: APPROVED FOR FILMING

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Project page Link: https://review.jove.com/account/file-uploader?src=20885228

Title: Modification of a 3D Printed Flexipeg Device to Model Catheter-Associated Urinary Tract Infection

Q: Filming location: Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 19
Number of Shots: 41

Ethics Disclaimer

Pooled human urine used in this study was collected under ethical approval from the University of Liverpool Central University Research Ethics Committee (Reference: 1854)

Protocol

Protocol

1.1. To begin, obtain pooled human urine sample **[1].** Aliquot 135 microliters of pooled human urine into each on-test well of a 96-well plate following the designated layout **[2-TXT]**.

1.1.1. WIDE: Talent holding a tube with pooled human urine sample. **Videographer's Note:** Unfortunately I was unable to frame out the windows for this shot. Part of the window is overexposed. I did a second take of this shot with a higher aperture in case the windows was way too overexposed in the first take.

1.1.2. Talent pipetting pooled human urine into the wells of a 96-well plate as per the layout. **TXT: Maintain 3 technical replicates per biological replicate** **Videographer's Note:** The second take of this shot is a close up.

1.2. Inoculate each on-test well with 15 microliters of normalized overnight bacterial culture to achieve a final volume of 150 microliters per well **[1]**. Reserve at least 100 microliters of this culture for subsequent serial dilution validation steps **[2]**.

1.2.1. Talent adding overnight culture to each well using a micropipette. **Videographer's Note:** Take 1 of this shot is a close up. Not an ECU (error on the clapperboard). Take 1 can be cut with take 3 of this shot if the editor(s) deems it fit.

1.2.2. Talent transferring 100 microliters of overnight culture for later use. **Videographer's Note:** Take 3 is a close up

1.3. Prepare negative control wells by adding 150 microliters of sterile pooled human urine into designated wells **[1]**.

1.3.1. Talent filling control wells with sterile urine.

1.4. Now, insert the pegs into their corresponding wells **[1]**. Press down the peg lid until it sits flush on the plate **[2]** and secure it with the microplate lid, taping it down if necessary **[3]**.

1.4.1. Talent inserting the peg lid into the wells of the microplate.

1.4.2. Talent pressing down on the peg lid. **Videographer's Note:** Take 3 is a close up

1.4.3. Talent covering the peg lid with the microplate lid and securing it with tape.

1.5. Then place the peg models in a sealed plastic tub or bag containing a damp blue roll to prevent evaporation **[1].** Incubate statically for 24 hours at 37 degrees Celsius **[2]**.

1.5.1. Talent placing the sealed plate setup into a humidified container.

1.5.2. Talent placing the setup in an incubator.

1.6. For inoculum validation, aliquot 180 microliters of PBS into rows 2 through 6 of the first column of a 96-well plate before adding the reserved culture **[1]**. ~~Add 100 microliters of reserved overnight culture to the first well in the column **[2].**~~

1.6.1. Talent pipetting phosphate-buffered saline into designated wells of a new microplate.

1.6.2. Talent adding reserved overnight culture to the first well. **Videographer's Note:** Shot not filmed at author's request

1.7. Now, transfer 20 microliters from the first well into the second well, mixing gently by pipetting up and down **[1]**. Continue serial dilutions until the initial inoculum has been diluted to the final well **[2]**.

1.7.1. Talent performing serial dilution by transferring and mixing the culture in successive wells

1.7.2. Talent completing the dilution series up to the final well.

1.8. Then plate 10 microliter spots from each dilution onto appropriate solid media in triplicate **[1]**. After overnight incubation at 37 degrees Celsius, count the colonies from each spot **[2-TXT].**

1.8.1. Talent transfer 10 µL spots from each dilution onto appropriate solid media.

1.8.2. Talent counting colonies on the agar plate. **TXT: Calculate CFU/mL inoculum**

1.9. For biofilm quantification, remove the peg model from the microplate **[1]** and invert it under a flame or inside a microbiological safety cabinet, with pegs facing upwards **[2]**. Discard the used microplates **[3]**.

1.9.1. Talent removing the peg lid from the microplate.

1.9.2. Talent inverting it under a flame.

1.9.3. Talent disposing of the used microplate in a designated biohazard bin.

1.10. Transfer the peg lid to a fresh microplate containing 180 microliter aliquots of sterile PBS to rinse off unadhered cells **[1-TXT]**.

1.10.1. Talent placing peg lid into a plate with 180 µL PBS. **TXT: Repeat wash in fresh PBS 2x**

1.11. Next, working aseptically, place a sterile glass test tube rack onto the bench **[1]** and attach the fitted rack top that holds the peg lid **[2].** **Videographer's Note:** Shots 1.11.1 and 1.11.2 were filmed together

1.11.1. Talent placing sterile glass test tube rack onto the bench.

1.11.2. Talent attaching the rack top under aseptic conditions.

1.12. With a micropipette, aliquot 600 microliters of sterile PBS into each test tube **[1]**. Now, insert the peg lid into the rack, ensuring the pegs are aligned with the test tubes **[2]**. Remove any autoclave tape from the lid **[3]**.

1.12.1. Talent pipetting sterile phosphate-buffered saline into the glass tubes. **Videographer's Note:** Take 2 is a close up

1.12.2. Talent placing the peg lid over the rack and aligning the pegs with tubes.

1.12.3. Talent peeling off autoclave tape from the peg lid.

1.13. Use sterile forceps to push each peg through the lid into the test tubes directly below **[1]**. Then replace the test tube caps **[2]** and vortex each tube for 60 seconds to dislodge biofilm-associated cells **[3]**.

1.13.1. Talent using forceps to insert pegs into tubes

1.13.2. Talent capping the tubes.

1.13.3. Talent vortexing each tube.

1.14. Remove 100 microliters from each vortexed tube **[1]** and perform serial dilutions in sterile PBS as done previously **[2]**.

1.14.1. Talent pipetting out 100 µL from a vortexed tube.

1.14.2. Talent performing serial dilution of the vortexed biofilm samples.

1.15. ~~Now use a serological pipette to spot 10 microliters of each dilution on solid media in triplicate and incubate **[1 TXT].** The next day, count the colonies on each plate and calculate the colony-forming units per milliliter for biofilm-associated cells **[2].**~~ **Videographer's Note:** Step 1.15 not filmed at author's request

~~1.15.1. Talent plating dilution spots. **TXT: Incubation: 37 °C, overnight**~~

~~1.15.2. Talent counting colonies on biofilm dilution plates.~~

1.16. To stain the cells with crystal violet, pipette 180 microliters of 0.1 percent weight per

volume crystal violet in sterile distilled water into the 24 on-test wells of a 96-well plate **[1].** Insert the rinsed peg model into the corresponding wells and incubate at room temperature for 20 minutes **[2]**.

1.16.1. Talent pipetting crystal violet solution into designated wells of the microplate.
Videographer's Note: Take 1 is a close up

1.16.2. Talent placing the rinsed peg lid into the wells.

1.17. After staining, remove the peg model from the plate and discard the used plate **[1]**. In a clean tub, rinse the entire peg model in running tap water with gentle agitation **[2]**.
Videographer's Note: Shots 1.17.1-1.17.2 were filmed together

1.17.1. Talent lifting peg model and discarding the stained plate.

1.17.2. Talent gently swirling the peg lid in a container of tap water.

1.18. After 2 more washes, invert the peg model and let it dry completely at room temperature **[1]**. Then add 180 microliters of 30% acetic acid to a new 96-well plate **[2.** Insert the dried peg model into the corresponding wells **[3-TXT]**.

1.18.1. Talent placing the peg lid inverted on a clean surface to dry.

1.18.2. Talent pipetting acetic acid solution into a fresh plate.

1.18.3. Talent placing the dry peg lid into the wells and setting a timer. **TXT: Incubation: RT, 30 min**

Added shot 1.18.4 : ECU shot of timer

Videographer's Note: This shot was split into two shots because it was difficult to get all of the action in one take. So there is an extra shot filmed. Which is 1.18.4, an ECU shot of the timer

1.19. When incubation is complete, remove the peg model from the microplate **[1].** Measure the optical density of the stained wells at 540 nanometers using a microplate reader or spectrophotometer **[2-TXT]**.

1.19.1. Talent removing the peg model from the microplate.

1.19.2. Talent placing the plate in a microplate reader. **TXT: Subtract OD_{540nm} of stained negative control pegs from average on-test pegs for biofilm formation**

Pronunciation Guide:

1. FlexiPeg

- **Pronunciation link: No confirmed link found**
- **IPA: /ˈflɛk.si.pɛg/**
- **Phonetic Spelling: flek-see-peg**

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2. Catheter

- **Pronunciation link: https://www.merriam-webster.com/dictionary/catheter**
- **IPA: /ˈkæθ.ə.tɚ/**
- **Phonetic Spelling: kath-uh-ter**

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3. Urinary

- **Pronunciation link: https://www.merriam-webster.com/dictionary/urinary**
- **IPA: /ˈjʊr.əˌnɛr.i/**
- **Phonetic Spelling: yoor-uh-nair-ee**

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4. Inoculate

- **Pronunciation link: https://www.merriam-webster.com/dictionary/inoculate**
- **IPA: /ɪˈnɑː.kjəˌleɪt/**
- **Phonetic Spelling: ih-nok-yuh-layt**

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5. Micropipette

- **Pronunciation link: No confirmed link found**
- **IPA: /ˌmaɪ.kroʊ.paɪˈpɛt/**
- **Phonetic Spelling: my-kroh-pie-pet**

6. Phosphate-buffered saline

- **Pronunciation link: No confirmed link found**
- **IPA: /ˈfɑs.feɪt ˈbʌf.ɚd ˈseɪ.lin/**
- **Phonetic Spelling: foss-fate buff-erd say-leen**

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7. Microplate

- **Pronunciation link: No confirmed link found**
- **IPA: /ˈmaɪ.kroʊ.pleɪt/**
- **Phonetic Spelling: my-kroh-plate**

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8. Vortex

- **Pronunciation link: https://www.merriam-webster.com/dictionary/vortex**
- **IPA: /ˈvɔːr.tɛks/**
- **Phonetic Spelling: vor-teks**

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9. Serological

- **Pronunciation link: https://www.merriam-webster.com/dictionary/serological**
- **IPA: /ˌsɪr.əˈlɑː.dʒɪ.kəl/**
- **Phonetic Spelling: seer-uh-loj-ih-kul**

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10. Acetic acid

- **Pronunciation link: https://www.merriam-webster.com/dictionary/acetic%20acid**
- **IPA: /əˈsiː.tɪk ˈæs.ɪd/**
- **Phonetic Spelling: uh-see-tik ass-id**

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11. Spectrophotometer

- **Pronunciation link: https://www.merriam-webster.com/dictionary/spectrophotometer**
- **IPA: /ˌspɛk.trə.foʊˈtɑː.mə.tɚ/**
- **Phonetic Spelling: spek-troh-fuh-tom-uh-ter**

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12. Colony-forming units

- **Pronunciation link: No confirmed link found**
- **IPA: /ˈkɑː.lə.ni ˈfɔːr.mɪŋ ˈjuː.nɪts/**
- **Phonetic Spelling: kol-uh-nee for-ming yoo-nits**

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13. Crystal violet

- **Pronunciation link: No confirmed link found**
- **IPA: /ˈkrɪs.təl ˈvaɪ.lət/**
- **Phonetic Spelling: kris-tuhl vie-let**

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14. Biofilm

- **Pronunciation link: https://www.merriam-webster.com/dictionary/biofilm**
- **IPA: /ˈbaɪ.oʊ.fɪlm/**
- **Phonetic Spelling: bye-oh-film**

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15. Pseudomonas aeruginosa

- **Pronunciation link: https://www.merriam-webster.com/dictionary/Pseudomonas%20aeruginosa**
- **IPA: /ˌsuː.dəˈmoʊ.nəs ˌɛə.ruː.dʒəˈnoʊ.sə/**
- **Phonetic Spelling: soo-duh-moh-nuhs air-oo-juh-noh-suh**

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16. Klebsiella pneumoniae

- **Pronunciation link: https://www.merriam-webster.com/dictionary/Klebsiella%20pneumoniae**
- **IPA: /ˌklɛb.siˈɛl.ə nuːˈmoʊ.ni.iː/**
- **Phonetic Spelling: kleb-see-ell-uh new-moh-nee-ee**

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17. Escherichia coli

- **Pronunciation link: https://www.merriam-webster.com/dictionary/Escherichia%20coli**
- **IPA: /ˌɛʃ.əˈrɪk.i.ə ˈkoʊ.laɪ/**
- **Phonetic Spelling: esh-uh-rik-ee-uh koh-lie**

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18. Polydimethylsiloxane

- **Pronunciation link: https://www.merriam-webster.com/dictionary/polydimethylsiloxane**
- **IPA: /ˌpɒl.i.daɪˌmɛθ.əlˈsɪ.lɒk.seɪn/**
- **Phonetic Spelling: pol-ee-dye-meth-uhl-sih-lox-ane**

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19. Autoclave

- **Pronunciation link: https://www.merriam-webster.com/dictionary/autoclave**
- **IPA: /ˈɔː.təˌkleɪv/**
- **Phonetic Spelling: aw-tuh-klayv**

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20. Aliquot

- **Pronunciation link: https://www.merriam-webster.com/dictionary/aliquot**
- **IPA: /ˈæl.ɪ.kwɒt/**
- **Phonetic Spelling: al-ih-kwot**