Designation: E3321 – 21

Standard Test Method for Intraluminal Catheter Model used to Evaluate Antimicrobial Urinary Catheters for Prevention of *Escherichia coli* Biofilm Growth[1]

This standard is issued under the fixed designation E3321; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method specifies the operational parameters required to assess the ability of antimicrobial urinary catheters to prevent or control biofilm growth. Efficacy is reported as the log reduction in viable bacteria when compared to a repeatable (1)[2] *Escherichia coli* biofilm grown in the intra-lumen of a urinary catheter under a constant flow of artificial urine.

1.2 The test method is versatile and may also be used for growing and/or characterizing biofilms and suspended bacteria of different species, although this will require changing the operational parameters to optimize the method based upon the growth requirements of the new organism.

1.3 This test method may be used to evaluate surface modified urinary catheters that contain no antimicrobial agent.

1.4 This test method describes how to sample and analyze catheter segments and effluent for viable cells. Biofilm population density is recorded as log colony forming units per surface area. Suspended bacterial population density is reported as log colony forming units per volume.

1.5 Basic microbiology training is required to perform this test method.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D5465 Practices for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 *Definitions:* For definitions of terms used in the test method see Terminology E2756.

4. Summary of Test Method

4.1 This test method is used for evaluating the growth of *Escherichia coli* ATCC 53498 biofilm in an antimicrobial Foley catheter. Antimicrobial catheters are tested in parallel to control catheters. Prior to inoculation with an overnight culture, sterile artificial urine media (AUM) is flowed through the catheter for 2 h. During the entire test, the biofilm is exposed to a continuous, slow flow of AUM through the Foley catheter. At the end of each 24 h time period, bacteria are quantified by collecting 10 mL of the effluent and a 2 cm segment from the distal end of the catheter. The biofilm is harvested by scraping the lumen of the catheter segment into 10 mL neutralization broth then placing the scraped segment into the neutralization broth to be processed. Both effluent flow and biofilm samples are vortexed and sonicated to disaggregate the clumps and diluted and plated for viable cell enumeration.

5. Significance and Use

5.1 In the battle to reduce medical device and implant-related infections, prevention of bacterial colonization of

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] The boldface numbers in parentheses refer to a list of references at the end of this standard.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

surfaces is a logical strategy. Bacterial colonization of a surface is a precursor to biofilm formation. Biofilm is the etiological agent of many implant and device-related infections and once established, microorganisms in biofilm can be up to 1000 times more tolerant to antibiotic therapy. Often the best treatment strategy is removal of the implant or device at a high socioeconomic cost. Catheter associated urinary tract infections (CAUTI) are the most prevalent of the device-related healthcare associated infections. Catheter associated infections account for 37 % of all hospital acquired infections (HAI) and 70 % of all nosocomial urinary tract infections (UTI) in the U.S. (**2**, **3**). The Intraluminal Catheter Model (ICM) was developed to evaluate the ability of antimicrobial catheters to inhibit biofilm growth on the catheter lumen.

5.2 The purpose of this test method is to direct a user in how to grow, sample, and analyze an *E. coli* biofilm in a urinary catheter under a constant flow of artificial urine. The test method incorporates operational parameters utilized in similar published methods (**4**). The *E. coli* biofilm that grows has a patchy appearance that varies across the catheter. Microscopically, the biofilm is heterogenous, with large clusters in some areas, and flat sheets of cells or even single cells in others. By 24 h, the biofilm is developed in the control catheters. If the goal is to monitor early stage biofilm development, then tubing and effluent samples need to be collected prior to the 24 h sample collection. Monitoring biofilm development requires sampling. The biofilm generated in the Intraluminal Catheter Model is suitable for comparison testing between antimicrobial and control catheters.

6. Apparatus

6.1 *Aluminum foil*.

6.2 *Analytical Balance*—Sensitive to 0.01 g.

6.3 *Applicator Sticks, wooden*—sterile.

6.4 *Bacterial Air Vent (Filter)*—autoclavable, 0.2 µm pore size.

6.5 *Barbed Bulkhead Fittings*—autoclavable, plastic, inner diameter (ID) 0.25 in.

6.6 *Biosafety Cabinet*.

6.7 *Bacterial Air Vent (Filter)*—Autoclavable 0.2 µm pore size, to be attached into tubing on waste and nutrient carboy (recommended diameter is 37 mm) and each reactor channel top (recommended diameter is 15 mm).

6.8 *Bacterial Air Vent (Filter)*—autoclavable, 0.2 µm pore size.

6.9 *Barbed Bulkhead Fittings*—autoclavable, plastic, ID 0.25 in.

6.10 *Bunsen Burner*—Used to flame sterilize inoculating loop and other instruments.

6.11 *Carboys*—One 5 L glass and one 20 L autoclavable carboy for nutrients and waste, respectively.

6.11.1 *Carboy Lids*—One 5 L glass carboy lid with at least two barbed fittings to accommodate tubing ID 3.1 mm (one for nutrient line and one for bacterial air vent). One carboy lid with at least two 1 cm holes bored in the same fashion (one for effluent waste and one for bacterial air vent).

NOTE 1—Carboy tops can be purchased with fittings.

6.12 *Chemical Spatulas.*

6.13 *Clamp Stand*—Height no less than 76.2 cm, used with clamp to suspend glass flow break vertically and stabilize tubing.

NOTE 2—If a clamp stand does not fit in the incubator, tape may be used.

6.14 *Colony Counter*—Any one of several types may be used. A hand tally for the recording of the bacterial count is recommended if manual counting is done.

6.15 *Conical Centrifuge Tubes*—Sterile, any with 15 mL and 50 mL volume capacity.

6.16 *Culture Tubes and Culture Tube Closures*—Any with a volume capacity of 10 mL and a minimum diameter of 16 mm. Recommended size is 16 mm by 125 mm borosilicate glass with threaded opening.

6.17 *Disposable Bottle Top Filtration Units and 1 L Receiver Flasks*—sterile, nylon filter with 0.2 µm pore size.

6.18 *Ethanol, 95 %*—Used to flame sterilize hemostats or forceps.

6.19 *Glass Flow Breaks*—Any that will connect with tubing of ID 3.1 mm and withstands sterilization.

6.19.1 *Clamp*—Used to hold flow breaks, extension clamp with 0.5 cm minimum grip size.

6.20 *Incubator*—that can maintain a temperature of 36 °C - 38 °C.

6.21 *Inoculating Loop*—10 µL.

6.22 *Magnetic Stir Plate, Heated*—Used to dissolve chemicals in stock solutions.

6.23 *Norprene*[4] Tubing—size 16 with ID 3.1 mm and outer diameter (OD) 3.2 mm. Must withstand sterilization.

6.24 *Peristaltic Pump*—One pump head capable of holding tubing with ID 3.1 mm and OD 3.2 mm and operating at a flow rate of 0.75 mL per minute.

NOTE 3—A digital pump, or pump with high sensitivity will be beneficial for maintaining a low flow rate. Pump must be calibrated following the manufacturer's recommendations using the same tubing configuration that will be utilized in the experiment.

6.25 *Petri Dish*—100 mm by 15 mm, plastic, sterile, and empty for transporting samples from the incubator to the workstation.

6.26 *pH Meter and Electrode*—Used for adjusting pH of AUM.

6.27 *Pipettes*—Serological sterile single-use pipettes with volume capacity of 5 mL, 10 mL, 25 mL, and 50 mL.

6.28 *Pipettor*—Continuously adjustable pipette with volume capability of 1 mL.

[4] Trademarked by the Saint-Gobain Performance Plastics Corporation.

6.29 *Ruler*—Used to measure the correct length of catheter tubing.

6.30 *Silicone Tubing*—Three sizes of silicone tubing: size 16 with ID 3.1 mm and OD 3.2 mm, size 18 with ID 7.9 mm and OD 9.5 mm, size 25 with ID 4.8 mm and OD 4.9 mm. All sizes must withstand sterilization.

6.31 *Size 16 French Foley catheter*—Sterile, silicone. Antimicrobial and control catheters that are 30 cm in length with the same inner diameter should be used.

6.32 *Stainless Steel Scissors*—For aseptic cutting of catheter tubing.

6.33 *Stainless Steel Hemostat Clamp or Forceps*—For aseptic handling of catheter tubing.

6.34 *Sterilizers*—Any steam sterilizer capable of producing the conditions of sterilization.

6.35 *Stir Bars, Magnetic*—Used to dissolve chemicals in stock solutions.

6.36 *Straight Tubing Connectors*—ID 3.1 mm and ID 7.9 mm. Must withstand sterilization.

6.37 *System Components*—A schematic of the tubing from the AUM carboy to where the tubing connects to the catheter is shown in Fig. 1. The Intraluminal Catheter Model is shown in Fig. 2.

6.38 *Three way Stop-Cock Valve*—4 mm and autoclavable.

6.39 *Timer*—Used to time the effluent collection.

6.40 *Tubing, Silicone*—ID 3.1 mm, size 16, OD 3.2 mm.

6.41 *Vacuum source*—in-house line or suitable vacuum pump for filtering.

6.42 *Vortex*—Any vortex that will ensure proper agitation and mixing of culture tubes.

6.43 *Water Bath, Ultrasonic*—any capable of maintaining a homogeneous sound distribution at 45 kHz ± 5 kHz with a volume large enough to accommodate 50 mL conical tubes in a wet environment.

NOTE 4—Verify that the sonicating bath does not kill viable cells by placing the standardized broth culture into sonicator for 60 s, serially dilute, and plate. The sonicated and non-sonicated counts should be within 0.5 logs.

6.44 *Weigh Boats or Papers*.

7. Reagents and Materials

7.1 *Purity of Water*—All reference to water as diluent or reagent shall mean distilled water or water of equal purity.

7.2 *Culture Media:*

7.2.1 *Bacterial Liquid Growth Broth*—Artificial Urine Medium (**5**) is required.

7.2.1.1 *Solution 1*—1.85 g $CaCl_2 \times 2H_2O$, 26.0 g NaCl, 2.45 g $MgSO_4 \times 7H_2O$, 8.45 g Na_2SO_4, 6.5 g NH_4Cl, 5 g peptone in 4580 mL deionized water in 5 L glass vessel, mixed with a magnetic stir bar and stir plate, autoclaved and cooled to room temperature.

7.2.1.2 *Solution 2*—0.5 g/L yeast extract, 5 g/L lactic acid, 40 g/L citric acid in distilled water, mixed and filter sterilized.

7.2.1.3 *Solution 3*—25 g/L creatinine, 0.06 g/L $FeSO_4 \times 7H_2O$ in distilled water, mixed and filter sterilized.

7.2.1.4 *Solution 4*—95 g/L KH_2PO_4 in distilled water, mixed and filter sterilized.

7.2.1.5 *Solution 5*—120 g/L K_2HPO_4 in distilled water, mixed and filter sterilized.

7.2.1.6 *Solution 6*—400 g/L urea in distilled water, mixed and filter sterilized.

7.2.1.7 *Solution 7*—105 g/L $NaHCO_3$ in distilled water, mixed and filter sterilized.

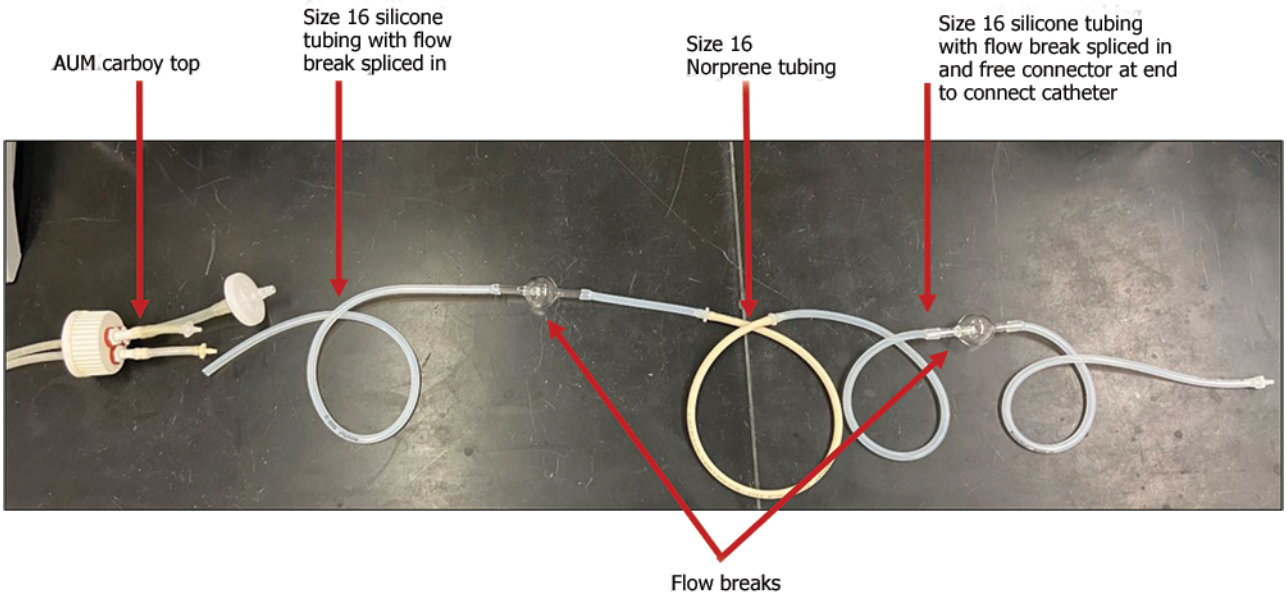

FIG. 1 Tubing Set-up from the AUM Carboy to Where the Tubing Connects to the Catheter

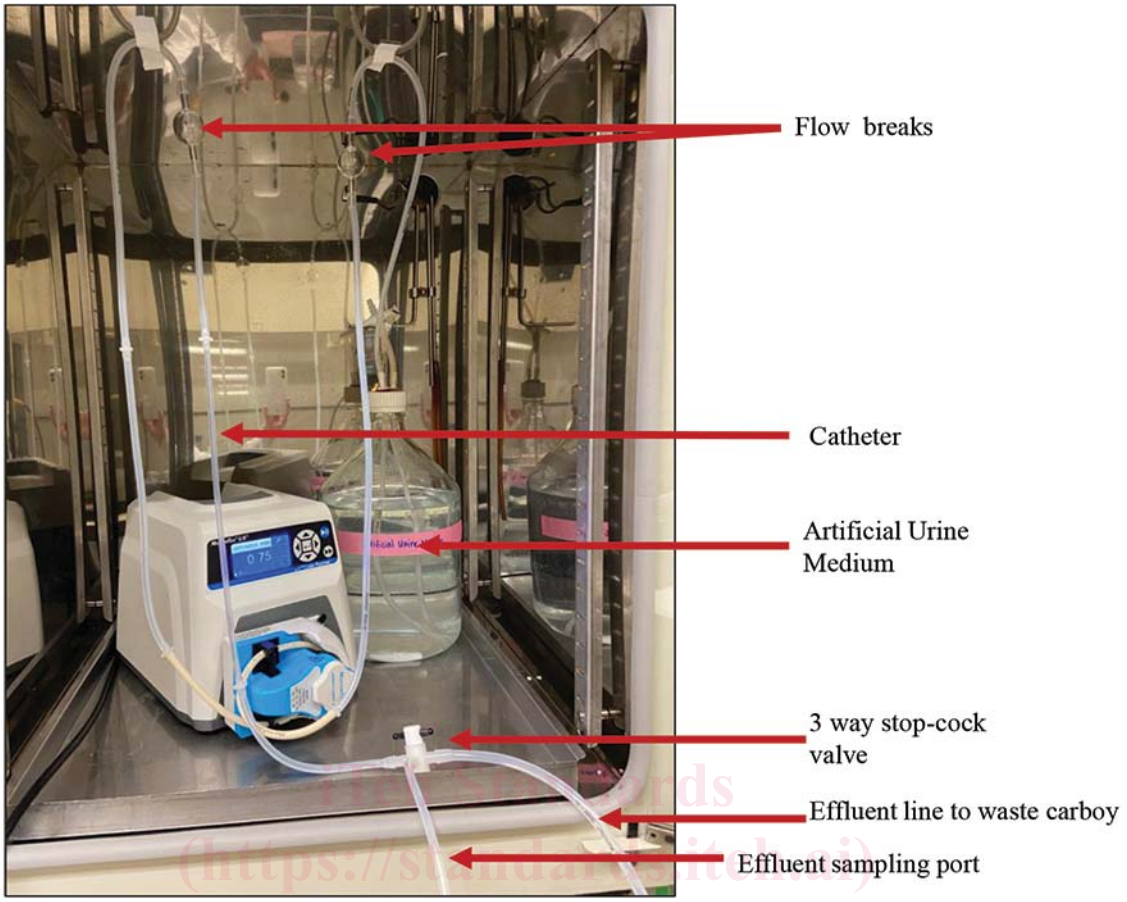

FIG. 2 Intraluminal Catheter Model

7.2.1.8 *Solution 8*—83 mL/L HCl in distilled water, mixed and filter sterilized.

7.2.2 *Bacterial Plating Medium*—Tryptic Soy Agar (TSA[5]) is required.

7.3 *Buffered Water*—0.0425 g/L KH2PO4 distilled water, filter sterilized and 0.405 g/L $MgCl{\cdot}6H_2O$ distilled water, filter sterilized (prepared according to Method 9050 C.1a).

7.4 *Neutralizer*—Dey/Engley Neutralizing Broth or one specific to the antimicrobial being evaluated as determined for effectiveness and toxicity according to Practices E1054 in accordance with Section 8 or Section 10. Conduct neutralization testing to confirm and document the neutralizer's effectiveness for the antimicrobial.

8. Preparation of Artificial Urine Medium (AUM) (5)

NOTE 5—Preparation of one container of Artificial Urine Medium will yield approximately 5000 mL, enough volume for two urinary catheters running continuously for two days or up to four urinary catheters for one day.

8.1 *Artificial Urine Medium Vessel Preparation:*

8.1.1 Plumb 5 L vessel lid with three barbed bulkhead fittings (two for nutrient lines and one for bacterial air vent), see Fig. 3.

8.1.2 Attach a length of silicone tubing to the underside of two of the bulkhead fittings to reach the bottom of the vessel.

8.1.3 Attach short pieces of tubing to the top of all three bulkhead fittings. Fit a tubing connector to the two with tubing interior to the vessel. Fit a bacterial air vent to the remaining piece of tubing on the top of the vessel lid.

8.1.4 Cover the two connectors and interior tubing with aluminum foil. Leave the bacterial air vent uncovered.

8.1.5 Place the lids in an autoclavable container and sterilize.

8.2 *Preparation of Artificial Urine Medium:*

8.2.1 Working in the biosafety cabinet, add 50 mL each Stock Solution 2, 3, 4, and 5 to Solution 1.

8.2.2 Add 100 mL each Stock Solution 6 and 7 to Solution 1.

8.2.3 Place vessel on stir plate to mix.

8.2.4 Aseptically adjust Solution 1 to pH 6.5 using Stock Solution 8 and mix.

NOTE 6—Ruggedness testing demonstrated the pH of the AUM is a critical parameter that must be tightly controlled to achieve repeatable results.

8.2.5 Aseptically remove 1.0 mL of AUM for sterility confirmation on TSA. Plate must show no growth.

8.3 *Procedure:*

[5] Atlas, R. M., Parks, L. C., Eds., *Handbook of Microbiological Media*, 2nd ed., CRC Press, Boca Raton, FL, 1997.

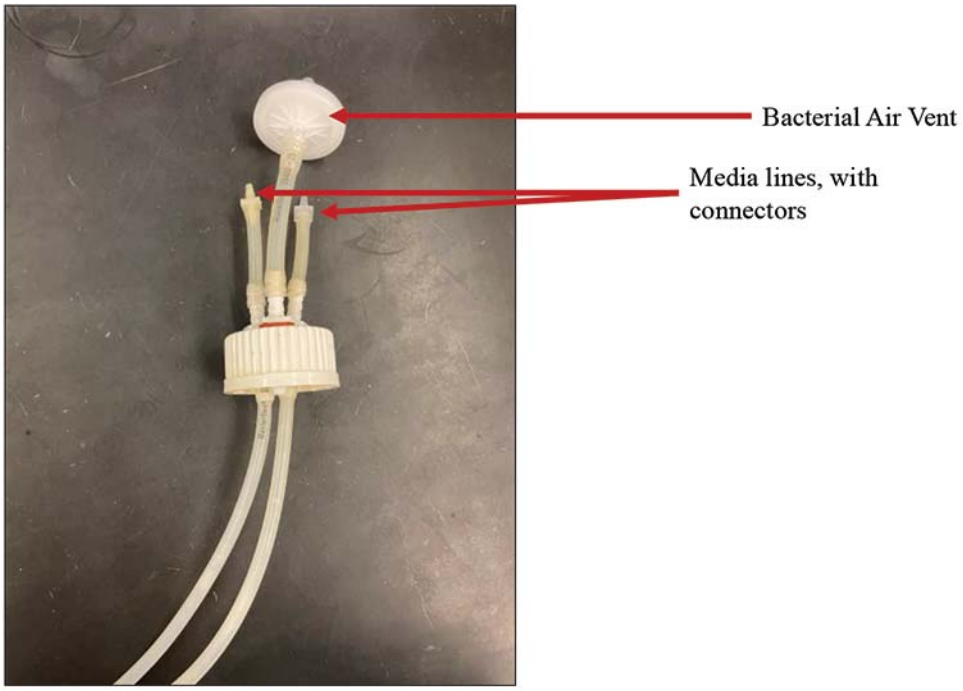

FIG. 3 Carboy Top for AUM Carboy

8.3.1 Aseptically pipet 10 mL AUM into sterile 15 mL vial for growth of culture in ICM.

8.3.2 Aseptically pipet AUM into 5 sterile dilution tubes for Culture Preparation in ICM.

8.3.3 Aseptically place sterile plumbed lid into vessel.

8.3.4 Place vessel in incubator set at 36 °C - 38 °C.

9. Culture/Inoculum Preparation

9.1 *Escherichia coli* (ATCC 53498) is the organism used in this test. Aseptically remove 2-3 isolated colonies from a TSA plate and inoculate into 10 mL of sterile Artificial Urine Medium. Incubate bacterial suspension in an incubator set at 36 °C - 38 °C for 20 h to 24 h. Viable bacterial density should equal 108 CFU/mL and may be checked by serial dilution and plating.

10. Preparation of Apparatus

10.1 *Preparation of Influent Line:*

10.1.1 From media side to catheter: Connect size 16 (ID 3.1 mm, OD 3.2 mm) silicone tubing with flow break spliced in, to segment of Norprene tubing. On the opposite side of the Norprene tubing, connect another segment of silicone tubing with flow break spliced in, and a free connector at the end. See Fig. 1.

NOTE 7—The use of two flow breaks helps to prevent the *E. coli* from contaminating the AUM.

10.2 *Preparation of Effluent Line:*

10.2.1 Connect size 16 tubing, with free connector at one end, to a 3 way stop-cock valve that has another length of size 16 tubing on the perpendicular fitting and is connected to size 25 silicone tubing and the third fitting, ensuring that tubing is long enough to reach waste carboy.

10.3 *Sterilizing the Model System:*

10.3.1 Wrap all exposed tubing ends with aluminum foil and place assembled reactor into an autoclave tray. Cover entire tray with aluminum foil.

10.3.2 Sterilize the reactor system for 20 min on dry cycle.

11. Procedure

11.1 *Model Setup:*

11.1.1 Connect influent tubing to nutrient carboy so flow breaks are oriented correctly with the direction of flow.

11.1.2 Feed Neoprene tubing through pump and clamp in place.

11.1.3 Clamp, or tape, second flow break in upright position, so free connector is pointed downward.

11.1.4 Open sterile catheter, with flame sterilized scissors, cut the proximal tip directly below the drainage hole and distal end of the catheter.

11.1.5 Connect the proximal end of the catheter to the free connector on the end of the influent tubing, and the distal end of the catheter to the 3 way stop-cock valve to drain to effluent waste carboy.

11.2 *Sterile Flow Phase:*

11.2.1 Prime the tubing with AUM and pump a continuous flow of AUM into the catheter at a flow rate equal to 0.75mL/min for two hours.

11.3 *Inoculation of ICM:*

11.3.1 Pause pump.

11.3.2 Aseptically remove the proximal end of the catheter from connector and pipette 2 mL of bacteria from the 5th dilution (10^3 CFU/mL) from the overnight culture through the catheter. Confirm bacterial count in the 5th dilution of the inoculum.