



Designation: E3321 – 21

Standard Test Method for Intraluminal Catheter Model used to Evaluate Antimicrobial Urinary Catheters for Prevention of *Escherichia coli* Biofilm Growth¹

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

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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.