Designation: ~~E2871 – 12~~ E2871 – 13

Standard Test Method for Evaluating Disinfectant Efficacy ~~against~~Against *Pseudomonas aeruginosa* Biofilm Grown in CDC Biofilm Reactor ~~using~~Using Single Tube Method[1]

This standard is issued under the fixed designation E2871; 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 perform a quantitative liquid disinfectant efficacy test against biofilm bacteria.

1.2 The test method was developed using a *Pseudomonas aeruginosa* biofilm grown in the CDC Biofilm Reactor (Test Method E2562), modified to include borosilicate glass coupons as a hard nonporous surface and *P. aeruginosa* ATCC 15442.

1.3 Disinfectant preparation and contact time are used in the assessment according to the manufacturer's instructions for use.

1.4 The test method uses a closed system to treat biofilm. A coupon is placed in a single tube for the treatment, neutralization, and sampling steps to prevent the loss of cells.

1.5 Verification of disinfectant neutralization is determined prior to conducting the test method.

1.6 This test method describes how to sample and analyze treated and untreated control biofilms for viable cells. Biofilm population density is recorded as $\log_{10}$ colony-forming units per coupon. Efficacy is reported as a $\log_{10}$ reduction of viable cells.

1.7 Basic microbiology training is required to perform this assay.

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

1.9 *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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2562 Test Method for Quantification of ***Pseudomonas aeruginosa*** Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor

2.2 *Other Standards:*

Method 9050 C.1.a Buffered Dilution Water Preparation according to Eaton et al **(1)**[3]

3. Terminology

3.1 *Definitions:*

3.1.1 *biofilm, n*—microorganisms living in a self-organized community attached to surfaces, interfaces, or each other, embedded in a matrix of extracellular polymeric substances of microbial origin, while exhibiting altered phenotypes with respect to growth rate and gene transcription.

[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.

Current edition approved ~~April 1, 2012~~Oct. 1, 2013. Published ~~June 2012~~November 2013. Originally approved in 2012. Last previous edition approved in 2012 as E2871–12. DOI: ~~10.1520/E2871–12.~~10.1520/E2871–13.

[2] 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.

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

3.1.1.1 *Discussion*—

Biofilm may be comprised of bacteria, fungi, algae, protozoa, viruses, or infinite combinations of these microorganisms. The qualitative characteristics of a biofilm including, but not limited to, population density, taxonomic diversity, thickness, chemical gradients, chemical composition, consistency, and other materials in the matrix that are not produced by the biofilm microorganisms, are controlled by the physicochemical environment in which it exists.

3.1.2 *contact time, n*—predetermined time that the biofilm is exposed to the activity of a disinfectant.

3.1.3 *coupon, n*—biofilm growth surface.

3.1.4 *disinfectant, n*—a chemical that destroys vegetative forms of microorganisms, but does not ordinarily kill bacterial spores.

3.2 *Acronyms:*

3.2.1 *ATCC*—American Type Culture Collection.

3.2.2 *CDC*—Centers for Disease Control and Prevention.

3.2.3 *CFU*—colony-forming unit.

4. Summary of Test Method

4.1 This test method describes the use of the single tube method to evaluate the efficacy of a liquid disinfectant against a *Pseudomonas aeruginosa* biofilm on a hard nonporous surface grown in the CDC Biofilm Reactor. The test method consists of adding a disinfectant (treated) or a control buffer (untreated) to individual coupons held in 50-mL conical tubes. Three coupons are treated with disinfectant and three coupons receive buffered dilution water. Neutralizer is added to the tubes after the appropriate contact time. A combination of vortexing and sonication are used to remove the biofilm from the coupon and disaggregate the clumps. The cell suspension is serially diluted and plated on agar medium. Viable plate counts from treated and untreated control coupons are used to calculate the $\log_{10}$ reduction of viable cells.

5. Significance and Use

5.1 Vegetative biofilm bacteria are phenotypically different from suspended planktonic cells of the same genotype. Biofilm growth reactors are engineered to produce biofilms with specific characteristics (**2**). Altering either the engineered system or operating conditions will modify those characteristics as well as the physicochemical environment. The goal in biofilm research and efficacy testing is to choose the growth reactor and operating conditions that generate the most relevant biofilm for the particular study.

5.2 The test method was developed using *Pseudomonas aeruginosa* ATCC 15442 biofilm grown on borosilicate glass coupons in the CDC Biofilm Reactor and liquid disinfectants. Efficacy data developed using other bacteria, different shear, different coupons, or other standardized biofilm reactor systems, and/or other forms of disinfectants may result in different $\log_{10}$ reduction (LR) values and repeatability and reproducibility standard deviations.

5.3 The efficacy test was designed to determine the $\log_{10}$ reduction in bacteria after exposure to a disinfectant in a closed system.

5.4 The test method was developed using 50-mL conical tubes. The conical geometry allows for disinfectant exposure to biofilm on all surfaces of the coupon.

5.5 Each efficacy test includes a single contact time and temperature for three untreated control coupons (exposed to buffered dilution water) and three treated coupons (per disinfectant/concentration combination).

6. Apparatus

6.1 *Conical centrifuge tubes,* sterile, any with 50-mL volume capacity and secure leakproof lids.

6.2 *Ultrasonic water bath,* any capable of maintaining a homogeneous sound distribution at 45 kHz with a variable power setting and a volume large enough to accommodate 50-mL conical tubes in a wet environment.

6.3 *Test tube rack,* any capable of holding 50-mL conical centrifuge tubes.

6.4 *Micropipettes,* continuously adjustable pipettes with volume capacity of 100 µL and 1000 µL.

6.5 *Sterile pipette tips,* 100-µL and 1000-µL volumes.

6.6 *Bunsen burner,* used to flame-sterilize Allen wrench and plate spreader.

6.7 *95 % Ethanol,* used to flame-sterilize Allen wrench and plate spreader.

6.8 *Small Allen wrench,* for loosening set screws and pushing coupons out of reactor rods.

6.9 *Timer,* any that can display time in seconds.

6.10 *Vortex mixer,* any vortex that will ensure proper agitation and mixing of centrifuge tubes.

6.11 *Serological pipettes,* sterile single-use pipettes with volume capacity of 1, 5, 10, 25, and 50 mL.

6.12 *Plate spreader,* for spreading serial dilutions on agar plates.

6.13 *Water bath,* any capable of maintaining a constant temperature of 20 ± 1°C.

6.14 *Sterilizer,* any steam sterilizer capable of producing the conditions of sterilization.

6.15 *Colony counter,* any one of several types may be used. A hand tally for recording of the bacterial count is recommended if manual counting is done.

6.16 *Environmental incubator,* any capable of maintaining a temperature of 36 ± 2°C.

6.17 *Appropriate glassware/plasticware,* as required to make media and agar plates.

6.18 *Volumetric flasks,* used for preparing disinfectants.

6.19 *Magnetic stir bars,* sterile, for mixing prepared disinfectant.

6.20 *Magnetic stir plate,* any capable of mixing.

7. Reagents and Materials

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

7.2 *Bacterial Plating Medium*—R2A agar is recommended.

7.3 *Buffered Water*—0.0425 g KH_2PO_4/L distilled water, filter-sterilized and 0.405 g $MgCl{\cdot}6H_2O$/L distilled water; filter-sterilized (prepared according to Method 9050 C.1.a Buffered Dilution Water Preparation (**1**)).

7.4 *Disinfectant*—product to be tested.

7.5 *Neutralizer*—Dey/Engley Neutralization Broth or one specific to the disinfectant being evaluated as determined for effectiveness and toxicity according to Test Method E1054.

8. Culture/Inoculum Preparation

8.1 Borosilicate glass coupons with mature *Pseudomonas aeruginosa* ATCC 15442 biofilm grown according to Test Method E2562 through step 10.2.4.

9. Procedure

9.1 The test is conducted with three treated and three untreated control coupons.

9.2 An overview of the procedure is shown in Fig. 1.

9.3 *Prepare Disinfectant:*

9.3.1 Prepare disinfectant according to manufacturer's specifications in sterile volumetric glassware. Ensure that the disinfectant is adequately mixed. Use within 3 h of preparation or as specified in the manufacturer's instructions.

9.3.2 Place prepared disinfectant in water bath equilibrated to 20 ± 1°C for 10 to 15 min.

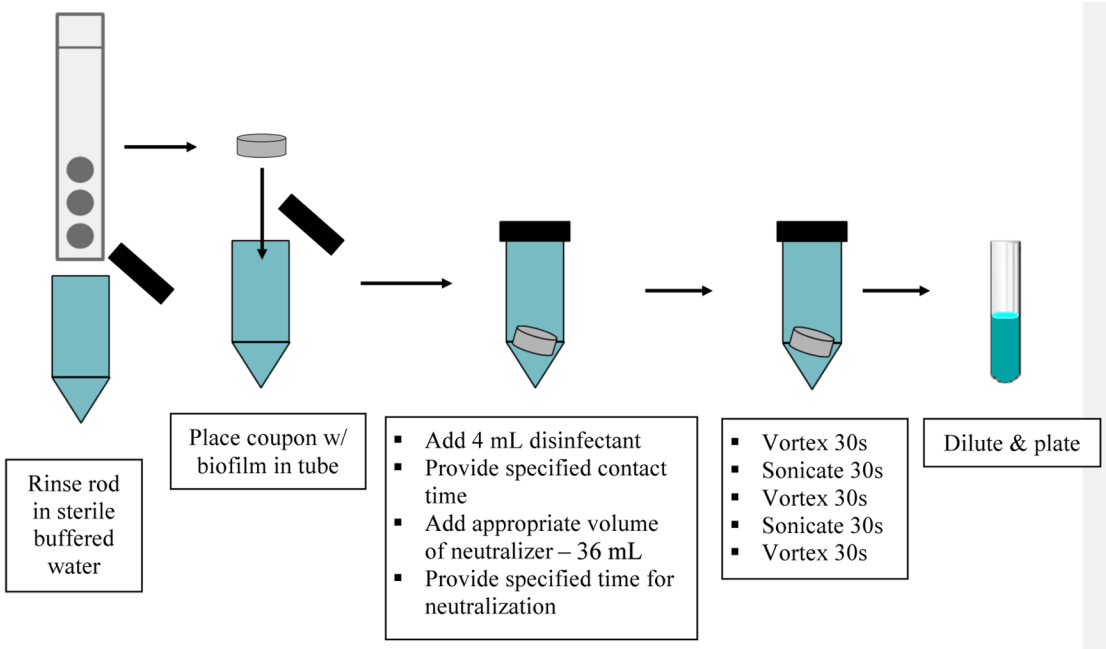

FIG. 1 Single Tube Method Overview