Designation: E2871 – 21

Standard Test Method for Determining Disinfectant Efficacy Against Biofilm Grown in the CDC Biofilm Reactor Using the 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 bacterial biofilm.

1.2 The test method was optimized and validated for a *Pseudomonas aeruginosa* or *Staphylococcus aureus* biofilm grown in the CDC Biofilm Reactor (E3161). The method is suitable for evaluating additional bacteria grown using the procedures outlined in methods with comparable coupon dimensions such as Practice E3161, Test Method E2562, or Test Method E2196.

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 harvesting steps to prevent the loss of cells.

1.5 This test method describes a harvesting and analysis procedure which includes vortexing and sonicating treated and untreated control biofilm, and recovery of culturable cells using filtration to lower the limit of detection. Biofilm population density is recorded as $\log_{10}$ colony-forming units per coupon. Efficacy is reported as a $\log_{10}$ reduction of culturable cells.

1.6 Basic microbiology training is required to perform this assay.

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

1.8 *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.*

1.9 *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:*[2]

E2196 Test Method for Quantification of ***Pseudomonas aeruginosa*** Biofilm Grown with Medium Shear and Continuous Flow Using Rotating Disk Reactor

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

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E3161 Practice for Preparing a ***Pseudomonas aeruginosa*** or ***Staphylococcus aureus*** Biofilm using the 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 For definitions of terms used in this test method refer to Terminology E2756.

3.2 *Definitions:*

3.2.1 *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.

3.2.1.1 *Discussion*—Biofilm may be comprised of bacteria, fungi, algae, protozoa, viruses, or infinite combinations of these microorganisms. The qualitative characteristics of a

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[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.

***A Summary of Changes section appears at the end of this standard**

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.

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

3.2.3 *coupon, n*—biofilm growth surface.

3.2.4 *disinfectant, n*—a physical or chemical agent or process that destroys pathogenic or potentially pathogenic microorganisms in/on surfaces or objects.

3.3 *Acronyms:*

3.3.1 *ATCC*—American Type Culture Collection.

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

3.3.3 *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* or *Staphylococcus aureus* biofilm on a hard, non-porous surface grown in the CDC Biofilm Reactor. The test method consists of adding disinfectant (treated) or buffered dilution water (untreated) to individual coupons held in conical tubes. Five 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 is used to remove the biofilm from the coupon and disaggregate the clumps. The cell suspension is serially diluted and recovered on an agar medium. The difference in viable plate counts from treated coupons and untreated control coupons is used to calculate the $\log_{10}$ reduction of viable cells.

5. Significance and Use

5.1 The Single Tube Method is designed to evaluate the efficacy of disinfectants against biofilm grown in the CDC biofilm reactor following the procedures outlined in Practice E3161. Biofilm grown in the CDC reactor is representative of biofilm that forms under high fluid shear on surfaces conducive to biofilm formation.

5.1.1 Vegetative biofilm bacteria are phenotypically different from suspended planktonic cells of the same genotype. Biofilm growth reactors are engineered to produce biofilm 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 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 designed to determine the $\log_{10}$ reduction in bacteria after exposure to a disinfectant in a closed system.

5.3 The test method uses 50 mL conical tubes. The conical geometry allows for disinfectant exposure to biofilm on all surfaces of the coupon. For foaming disinfectants or for disinfectants requiring a larger volume of neutralizer, 250 mL conical tubes are used which preserve the required geometry and allow for greater neutralization capacity.

5.4 Each test includes three untreated control coupons (exposed to buffered dilution water) and five treated coupons (per disinfectant/concentration/contact time combination).

6. Apparatus

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

NOTE 1—There are slight differences in the dimensions of the conical tubes between manufacturers. The tubes selected for testing must properly accommodate the splashguard inserts (that is, appropriate interior diameter and length) (see Fig. 1).

6.2 *Ultrasonic water bath,* any capable of maintaining a homogeneous sound distribution at 45 kHz ± 5 kHz with a volume large enough to accommodate 50 mL or 250 mL conical tubes in a wet environment.

NOTE 2—Prior to using the sonicating bath for the first time, verify that

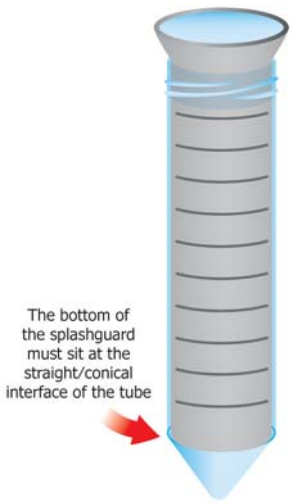

FIG. 1 Conical tube with splashguard insert