



Standard Test Method for Evaluating Disinfectant Efficacy Against *Pseudomonas aeruginosa* Biofilm Grown in CDC Biofilm Reactor Using Single Tube Method¹

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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 log₁₀ colony-forming units per coupon. Efficacy is reported as a log₁₀ 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.

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*:

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 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.