



Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown Using Drip Flow Biofilm Reactor with Low Shear and Continuous Flow¹

This standard is issued under the fixed designation E2647; 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 grow a repeatable² *Pseudomonas aeruginosa* biofilm close to the air/liquid interface in a reactor with a continuous flow of nutrients under low fluid shear conditions. The resulting biofilm is representative of generalized situations where biofilm exists at the air/liquid interface under low fluid shear rather than representative of one particular environment.

1.2 This test method uses the drip flow reactor. The drip flow reactor (DFR) is a plug flow reactor with laminar flow resulting in low fluid shear. The reactor is versatile and may also be used for growing and/or characterizing biofilms 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 describes how to sample and analyze biofilm for viable cells. Biofilm population density is recorded as log colony forming units per surface area.

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

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

1.6 *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:³

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

2.2 Other Standard:

Method 9050 C.1.a Buffered Dilution Water Preparation, according to Eaton et al⁴

3. Terminology

3.1 Definitions:

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

3.1.1.1 *Discussion*—Biofilms 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 *coupon, n*—biofilm sample surface.

3.1.3 *chamber, n*—reactor base containing four rectangular wells or channels.

3.1.4 *channel, n*—one of four rectangular wells in reactor chamber (base) where coupon is placed.

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, 2013. Published May 2013. Originally approved in 2008. Last previous edition approved in 2008 as E2647 – 08. DOI: 10.1520/E2647-13.

² Ellison, S. L. R., Rosslein, M., and Williams, A., Eds., *Quantifying Uncertainty in Analytical Measurement*, 2nd Edition, Eurachem, 2000.

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.

⁴ Eaton, A. D., Clesceri, L. S., and Greenberg, A. E., Eds., *Standard Methods for the Examination of Water and Waste Water*, 19th Edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, 1995.