



Standard Test Method for Applying Aerosolized *Bacillus* Spores as Dry Inocula to Inanimate Surfaces¹

This standard is issued under the fixed designation E2894; 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.

INTRODUCTION

Bacillus anthracis, the causative agent of anthrax, poses a formidable threat as an infectious bioagent. A combination of significant environmental stability, ease of production,² and high mortality through the inhalational route³ makes *B. anthracis* an ideal bioagent. Aerosolized powders are expected to be the primary mechanism for disseminating *B. anthracis* spores, likely delivered as an aerosol cloud from either a line-source, for example, low-flying aircraft, or point-source, for example, spray device. The characteristics of the aerosol (particle size and composition) will influence its behavior, thereby affecting its potency as a threat agent. This test method is designed to perform surface deposition of aerosolized spores with the primary objective of evaluating the utility of self-sanitizing antimicrobial materials (SSAMs). However, it could be used for other types of research, for example, decontamination, reaerosolization, fate and transport, contamination avoidance, collective protection, and individual protection, as well. We are aware of other consensus standards for applying spores to surfaces (Test Method E2149, AATCC Test Method 100, Test Method E2180), but these methods are based on liquid inocula. SSAMs are more active in the presence of moisture,⁴ and thus standard test methods using water-based inocula may overestimate their effectiveness. Protocols for the preparation, aerosolization, extraction, viable enumeration, and calculation of spores for testing and determining the effectiveness of SSAMs are described.

1. Scope

1.1 This test method is designed to uniformly apply fluidized spores to surfaces as an aerosol under defined conditions.

1.2 This test method is specific to *B. anthracis* Delta (Δ)Sterne (BA Δ S), but could be adapted for work with other types of *Bacillus* species.

1.3 This test method is suitable for working with any type of environmental surface.

1.4 This test method should be performed only by those trained in aerobiology, microbiology, or a combination thereof.

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:⁵

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2149 Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions

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 Oct. 1, 2013. Published March 2014. Originally approved in 2012. Last previous edition approved in 2012 as E2894–12. DOI: 10.1520/E2894-13.

² Woods, J. B., Darling, R. G., Dembek, Z. F., Carr, B. K., Cieslak, T. J., and Lawler, J. V., et al., *USAMRIID's Medical Management of Biological Casualties Handbook*, 6th edition, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD, 2005.

³ LeClaire, R. D., and Pitt, M. L. M., "Biological Weapons Defense: Effect Levels," *Biological Weapons Defense: Infectious Diseases and Counterterrorism*, Humana Press Inc., Totowa, NJ, 2005, pp.41–61.

⁴ Prugh, A., and Calomiris, J. J., "Inactivation of *Bacillus anthracis* Spores Delivered as Liquid Suspension or Aerosol to Self-Decontaminating Fabric," Defense Technical Information Center (DTIC), AFRL-HE-WP-TP-2006-0060, 2006.

⁵ 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.