



Designation: E3092 – 18

Standard Practice for Evaluating Efficacy of Vaporous Decontaminants on Materials Contaminated with *Bacillus* Spores and Contained Within 0.2µm Filter-Capped Tubes¹

This standard is issued under the fixed designation E3092; 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 practice is used to quantify the efficacy of vaporous decontaminants on *Bacillus* spores dried on the surface of coupons made from porous and non-porous materials and contained within 0.2µm filter-capped tubes.

1.2 This practice should be performed only by those trained in microbiological techniques, are familiar with antimicrobial (sporicidal) agents and with the end use of such products.

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

1.4 *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.*

1.5 *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

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2111 Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporocidal Potencies of Liquid Chemicals

E2197 Quantitative Disk Carrier Test Method for Determin-

ing Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporocidal Activities of Chemicals

E2414 Test Method for Quantitative Sporocidal Three-Step Method (TSM) to Determine Sporocidal Efficacy of Liquids, Liquid Sprays, and Vapor or Gases on Contaminated Carrier Surface (Withdrawn 2014)³

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 For definitions of terms used in this guide, see Terminology **E2756**.

3.2 For inactivators and neutralizers of decontaminants see Test Methods **E1054**.

3.3 *Definitions of Terms Specific to This Standard:*

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

3.3.2 *endospore, n*—a dormant, robust and non-metabolically active structure produced by certain bacteria from the Firmicutes phylum.

3.3.3 *exosporium, n*—the outermost layer of spores of *Bacillus anthracis* and its close relatives *Bacillus thuringiensis* and *Bacillus cereus*.

3.3.4 *macrobacillus, n*—a *Bacillus* endospore that possess an exosporium.

3.3.5 *microbacillus, n*—a *Bacillus* endospore that does not possess an exosporium.

3.3.6 *vapor, n*—a substance in the gas phase at a temperature lower than its critical temperature, such that it can be condensed back into a liquid by increasing the pressure on it without reducing the temperature.

3.3.7 *vaporous decontaminant, n*—for the purpose of this practice, a vaporous decontaminant can be interpreted to include gases, vapors, fogs, mists and thermal decontaminants.

¹ This practice 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 last approved version of this historical standard is referenced on www.astm.org.

4. Summary of Practice

4.1 This practice quantitatively evaluates the efficacy of vaporous decontaminants on coupons contaminated with *Bacillus* spores (pathogenic and non-pathogenic strains). Spores are dried on coupon surfaces, and the coupons are then transferred to 0.2 µm filter-capped 50-ml conical tubes (1-3).⁴

4.2 Coupon material is selected according to the claims or intended use of the decontaminant. Coupons may be made of any material – hard, flexible, porous, non-porous, metallic, or non-metallic. Flat (2 × 2 cm) coupons are preferred; however non-flat coupons and smaller coupons have been tested using this practice.

4.3 Fifty-ml conical tubes are used for extraction vessels. This allows for greater flexibility in coupon material selection, accommodating materials that are difficult to manufacture in extremely small sizes, for example, concrete, asphalt, carpet and wallboard.

4.4 Contaminated test coupons are subjected to decontamination procedures. Control coupons are subjected to identical procedures without the decontaminant.

4.5 Solution controls (spores suspended in aqueous solution) will represent the 100 % recovery reference for calculating spore survival after decontamination treatment and analysis.

4.6 Spore extraction percentage will be calculated by dividing the number of spores recovered from each spore-inoculated control coupon by the number of spores recovered from the solution controls.

4.7 The number of surviving spores from decontamination tests will be divided by the extraction percentage to determine the number of surviving spores in CFU ml⁻¹. This spore concentration is then multiplied by 10 ml to give a total number of spores surviving (CFU) from each test sample. A log₁₀ transformation of the total surviving spores will then be performed (log₁₀ (total CFU + 1)).

5. Significance and Use

5.1 The practice can be used to evaluate coupon materials of any composition, insofar as the coupon can be prepared small enough to fit inside a 50-ml conical tube.

5.2 This practice defines procedures that are quantitative, scalable, rapid, sensitive, safe, reduces consumables, minimizes labor and addresses statistical confidence (1, 2, 4).

5.2.1 *Quantitative*—The total number of spores per coupon is determined by dilution-plating, and all spores remaining on the coupon are assayed for activity in the extraction tube to provide confidence that 100 % of spores were assayed.

5.2.2 *Statistical Confidence*—The use of five independent preparations of spore inoculum for a statistical N of 5.

5.2.3 *Sensitivity*—Allows for complete detection of all viable spores inoculated on a coupon, including the spores that remain attached to the coupon.

5.2.4 *Safety*—Inoculated coupons are contained within 0.2 µm filter-capped 50-ml conical tubes. The 0.2 µm filter allows vaporous decontaminants to pass through while preventing escape of spores, thereby providing an important level of containment when working with pathogenic strains.

5.2.5 *Simplicity of Testing*—Tests and extractions are performed in the same 50-ml conical tube to minimize handling steps.

5.2.6 *Scalable and Rapid*—A maximum of 36 samples can be processed in 1 h by two technicians; a total of 300 samples have been processed by six technicians in 5 h (1-3).

5.2.7 Wide application for numerous *Bacillus* species and strains.

NOTE 1—This practice differs from similar quantitative methods (E2111, E2197 and E2414) in the size and variety of coupon materials available for testing, in the practical confidence of the statistics, the application of the decontaminant, scalability and sensitivity.

6. Apparatus

6.1 Autoclave.

6.2 Shaking Incubator.

6.3 Incubator.

6.4 Phase-Contrast Microscope.

6.5 Centrifuge.

6.6 Water Bath.

6.7 Single-tube Vortex Mixer.

6.8 Multi-tube Vortex Mixer.

6.9 Analytical Balance.

6.10 -80 °C Freezer.

6.11 Stopwatch or Electronic Timer.

6.12 Manual or Electronic Pipettes.

6.13 Bio-Safety Cabinet (BSC).

6.14 *Environmental Chamber*, capable of maintaining temperature ±2 °C and relative humidity ±5% of target parameters; must be capable of maintaining vapor concentration.

6.15 *Appropriate PPE*, for example, gloves, safety glasses, lab coats, etc. (5).

7. Reagents and Materials

7.1 *Reagents*⁵:

7.1.1 *Bacillus anthracis*—Ames, Sterne, ΔSterne.

7.1.2 *Bacillus thuringiensis*—Al Hakam, cry⁺ HD-1.

7.1.3 *Tryptic Soy Broth (TSB)*.

7.1.4 *Tryptic Soy Agar (TSA)*.

7.1.5 *Nutrient Broth (NB)*.

7.1.6 *Tween 80*.

7.1.7 *L-Alanine*.

⁴ The boldface numbers in parentheses refer to the list of references at the end of this standard.

⁵ *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see *Annual Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.