



Designation: E3383 – 24

Standard Test Method for Determining the Microbial Barrier Properties of Wound Dressing – in vitro Wound Model¹

This standard is issued under the fixed designation E3383; 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 is used to determine the microbial barrier properties of wound dressing. The test is designed to measure the ability of microorganisms to penetrate through a material (wound dressing) under specific conditions. This qualitative in vitro test will demonstrate results such as “Growth” or “No Growth” of the test organism on the agar surface after a specified contact time.

1.2 The test method includes an initial inoculum of $3.0 \times 10^6 \pm 2.0 \times 10^6$ CFU/mL and a contact time of 24 h to 72 h or per the wound dressing label claim. To show barrier properties, wound dressing should not allow the challenged organism to penetrate and reach the media surface.

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 Testing is to be performed by individuals trained in microbiological techniques under appropriately controlled conditions to ensure the integrity of results and personnel safety.

1.5 *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.*

1.6 *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.*

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. Referenced Documents

2.1 *ASTM Standards*:²

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *FDA Documents*:³

FDA Executive Summary Classification of Wound Dressings Combined with Drugs - Prepared for the Meeting of the General and Plastic Surgery Devices Advisory Panel September 20-21, 2016

3. Terminology

3.1 *Definitions*:

3.1.1 For definitions of terms used in this test method, refer to Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard*:

3.2.1 *contact time, n*—length of time, during which a wound dressing is applied to the skin in a clinical setting.

3.2.2 *microbial barrier, n*—a chemical or physical material used to prevent microbes from reaching or colonizing wounds.

3.2.3 *wound dressing, n*—a sterile covering used to protect wounds.

4. Summary of Test Method

4.1 The microbial barrier effectiveness procedure starts by placing the wound dressing on the surface of an appropriate agar medium plate.

4.2 The dressing surface is then inoculated with a population $3.0 \times 10^6 \pm 2.0 \times 10^6$ CFU/mL of an appropriate challenge organism.

4.3 Positive and negative controls are also included to demonstrate the viability of the challenge organism (positive control) and the aseptic processing (negative control).

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 Available from U.S. Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993, <http://www.fda.gov>.

4.4 Test and control plates are incubated upright at $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 24 h to 72 h or any other time point listed on the label claim. After incubation, the wound dressing is carefully removed and the agar media plate is re-incubated for 48 h or other suitable time for the growth of the target microorganism.

NOTE 1—If the media contains the growth indicator, re-incubation is not needed.

4.5 After the additional incubation period, the agar plate is examined for growth of the challenge organism, and the result is documented as “Growth” or “No Growth” to measure the effectiveness of the dressing’s barrier properties.

5. Significance and Use

5.1 An ideal wound dressing absorbs wound exudates, reduces the bioburden within/on the dressing, and protects wounds from microbial penetration through the dressing. Microbial barrier effectiveness testing is performed to measure the ability of microorganisms to penetrate through a material under specific conditions.

5.2 The test may be used for solid wound dressings with or without the addition of antimicrobial agents where the manufacturer is claiming microbial barrier properties in their performance claim. Barriers can be physical (polyurethane) and/or chemical (antimicrobial barrier).

5.2.1 Five (5) commonly used solid wound dressings are (1) hydrogel, (2) hydrocolloid, (3) film, (4) foam, and (5) alginate. Liquid adhesives also protect the wound from being infected by microorganisms. Liquid adhesive dressings contain cyanoacrylate or polyacrylate, which polymerizes quickly to create a solid barrier and protect the wound from contamination.

5.3 This qualitative test method can be used to determine the microbial barrier properties of solid wound dressings including liquid adhesive.

5.3.1 The test represents an in vitro wound model by using a nutrient-rich medium to mimic the wound, the contact time will represent the maximum period of use for a single dressing application on the wound, and an incubation temperature of $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ represents the human body temperature.

5.4 Results are documented as “Growth” or “No Growth.”

5.4.1 No growth underneath the dressing indicates the barrier properties of the wound dressing are effective at preventing microbial penetration through the dressing.

5.4.2 Growth underneath the dressing demonstrates that the challenge organism was able to penetrate the dressing and reach the media surface, indicating that microorganisms can reach the wound by penetrating through the dressing from the outside environment.

6. Apparatus

6.1 *Centrifuge*, non-refrigerated benchtop capable of running $2000 \times g$.

6.2 *Cover film*, that does not affect the growth of the test organism or absorb water, made of polyethylene, polypropylene, or polyester (poly(ethylene terephthalate)). A film that is 0.05 mm to 0.1 mm thick.

NOTE 2—Films cut from sterile lab blender bags are also suitable.

6.3 *Cuvette*, sterile, appropriate size that can fit into spectrophotometer cuvette chamber.

6.4 *Forceps*, sterile.

6.5 *Glass Wool*.

6.6 *Gloves*, sterile.

6.7 *Hypodermic Needle*, 16G.

6.8 *Incubators*, capable of maintaining temperatures of $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, $22.5\text{ }^{\circ}\text{C} \pm 2.5\text{ }^{\circ}\text{C}$.

6.9 *Inoculating Loops*, (10 μL).

6.10 *ISO Class 5 Environment: Laminar Airflow Hood (LAF) / Biosafety Cabinet (BSC)*.

6.11 *Micropipetor and Sterile Pipette Tips*, (1.0 mL, 0.1 mL, and 0.01 mL).

6.12 *McFarland Standard*, (1.0).

6.13 *Paraffin Film*.

6.14 *Petri Plates*, 15 mm by 60 mm, 15 mm by 100 mm, 15 mm by 150 mm.

6.15 *Polypropylene Tubes*, 50 mL.

6.16 *Refrigerator*, capable of maintaining the temperature of $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$.

6.17 *Scissors*, sterile.

6.18 *Spectrophotometer*, capable of measuring an absorbance of 475 nm.

6.19 *Tubes*, sterile, ~14 mL (screw cap or snap cap).

6.20 *Timer*.

6.21 *Vortex Mixer*.

7. Reagents and Materials

7.1 *Growth Media (1)*:⁴

7.1.1 *Blood Agar*.

7.1.2 *Cetrimide Agar Plates*.

7.1.3 *Dey-Engley Agar (D/E Agar)*.

7.1.4 *Sabouraud Dextrose Agar (SDA) Plates*.

7.1.5 *Tryptic Soy Agar (TSA)*.

7.1.6 *Tryptic Soy Broth (TSB)*, 10 mL.

7.2 *Sterile Physiological Saline*, (0.9 % sodium chloride, pH 5.5).

7.3 *Sterile Saline*, containing 0.05 % polysorbate 80.

8. Test Organisms

8.1 The study should include at least three gram-positive, three gram-negative (motile and non-motile), one yeast, and one mold. Cultures shall not be more than five passages removed from the original ATCC culture. Organisms listed below are used as examples as other organisms can also be included in the test.

8.2 *Gram-Positive Bacteria*:

⁴ Atlas, R.M., *Handbook of Microbiological Media*, fourth edition, CRC Press, Boca Raton, FL, 2010, pp. 343, 589, 1530, 1821, and 1831.