Designation: E3371 – 22

# Standard Test Method for Measuring the Ability of a Synthetic Polymeric Material to Resist Bacterial Adherence[1]

This standard is issued under the fixed designation E3371; 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

Synthetic polymeric materials, used in packaging for food containers, personal care products and other items, may have inherent antimicrobial properties. Others may contain antimicrobial additives. Many methods, such as Test Method E2180 are used to determine quantitative bacterial reductions caused by these additives. Test Method E3151 specifically examines the ability of tubular, yarn and fiber specimens to resist bacterial colonization/adherence. However, these methods do not quantitatively measure the number of culturable bacteria adhering to the flat surfaces of these synthetic polymeric materials, as measured in this test method.

## 1. Scope

1.1 This method is designed to evaluate (quantitatively) the number of bacteria attached to the flat, two-dimensional surfaces of synthetic polymeric materials and polymeric coatings on various substrates that may or may not contain bound or incorporated anti-adherent agents. The method focuses on assessing the ability of the surface to reduce bacterial attachment. Other microorganisms such as yeast and fungal conidia may be tested using this method.

1.2 This test method quantitatively determines the differences in bacterial adherence seen between synthetic polymeric surfaces that allow bacterial adherence and those that do not, comparing the number of organisms recovered from the control surface to the number recovered from the test specimen surface after the contact time. Knowledge of microbiological techniques is required for these procedures.

1.3 This test method specifies proper methods for measuring the ability of a synthetic polymeric material to resist adherence against specified organism. Due to individual sensitivities, the result of one test organism might not be applicable for other organisms.

1.4 This test method is designed to measure the potential ability to resist bacterial adherence of a non-porous surface compared directly to a polyester control panel known to support bacterial adherence under specific testing conditions.

1.5 Antimicrobial treated non-porous surfaces may demonstrate ability to resist bacterial adherence in this method. This method does not purport to differentiate between anti-adherence and antimicrobial activity nor is it designed to reflect specific end-use or environmental conditions. Any product that demonstrates ability to resist bacterial adherence in this method should be measured for antimicrobial activity using a separate test technique such as Test Method E2180 or ISO 22196.

1.6 The method focuses on assessing the ability of synthetic polymeric materials and polymeric coatings on various substrates to reduce bacterial attachment. The specimen with absorbing or adhesive surfaces may be unable to be disinfected properly before testing, or may trap inoculated organism during recovery process and thus lead to a false result. This method does not apply to specimens with absorbent or adhesive surfaces.

1.7 The values stated in SI units are to be regarded as standard. The values given in parentheses after SI units are provided for information only and are not considered standard.

1.8 *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.*

1.9 *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]

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E2180 Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E3151 Test Method for Determining Antimicrobial Activity and Biofilm Resistance Properties of Tube, Yarn, or Fiber Specimens

2.2 *ISO Standard:*[3]

ISO 22196 Measurement of Antibacterial Activity on Plastic and Non-Porous Surfaces

2.3 *FDA Manuals:*[4]

Bacteriological Analytical Manual (BAM)

## 3. Terminology

3.1 *Defintions:*

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 *anti-adherence, n*—term describing a state where the attachment of bacteria to the surface of products is suppressed or describing the effect of an agent which reduces the ability of bacteria to attach to product surfaces.

3.2.2 *anti-adherent, adj*—term describing a state where the attachment of bacteria to the surface of products is suppressed or describing the effect of an agent which reduces the ability of bacteria to attach to product surfaces.

3.2.3 *ability to resist bacterial adherence, n*—percent reduction, calculated from the number of bacteria recovered from the surface of the test specimen and the reference control after inoculation with bacteria, incubation, saline wash, and quantification of attached organisms.

3.2.4 *antimicrobial, n*—difference in the logarithm of the culturable cell count found on supernatant of an antibacterial-treated product and an untreated product after inoculation with and incubation of bacteria.

3.2.5 *reference control material, n*—synthetic polymeric material, that does not inhibit the attachment of the test bacteria.

3.2.5.1 *Discussion*—This material is commonly referred to as the untreated control and is used for test validation calculations.

## 4. Summary of Test Method

4.1 A fixed inoculum volume (0.4 mL ± 0.02 mL) at a known concentration is pipetted onto the surface of the test specimens and controls and then incubated under appropriate conditions for the test organism.

4.2 After the specified contact time (24 h), the testing surface is gently washed with sterile saline with the aid of rocker to remove the unattached bacteria.

4.3 The remaining attached bacteria are collected by sonication and then counted.

4.4 Ability of a material to resist bacterial adherence is calculated from the numbers of bacteria recovered from the control and specimen surfaces.

## 5. Significance and Use

5.1 This method can be used to evaluate the effectiveness of incorporated or bound anti-adherent agents in synthetic polymeric materials and polymeric coatings intended to reduce the attachment of bacteria to the substrate surface.

5.2 The synthetic polymeric substrate surface may be tested repeatedly over time for assessment of persistent ability of a material to resist bacterial adherence.

5.3 This method is to quantify the degree of bacteria colonization of a surface to assess a materials ability to resist bacterial adherence because biofilm formation can contribute to material degradation and malfunction.

## 6. Apparatus

6.1 Autoclave, capable of producing 103 kPa (15 psi) of steam pressure at 121 °C and maintaining it for a minimum of 15 minutes.

6.2 Balance, capable of weighing to ± 0.01 g.

6.3 Binder clip, 15 mm to 25 mm width, or equivalent.

6.4 Cell counting chamber (optional).

6.5 Clear polyester panels (Leneta – Order #P300-7G or equivalent)

6.6 Colony counter (optional).

6.7 Cotton swabs, sterile.

6.8 Culture tubes, screw top, sterile, or equivalent.

6.9 Cuvettes.

6.10 Forceps, sterile.

6.11 Incubator, set at required temperature (35 °C ± 1 °C).

6.12 Inoculating loops, sterile, 4 mm ring diameter.

6.13 Petri dishes, (15 mm× 100 mm), sterile.

6.14 pH meter, any capable of measuring to 0.2 units.

6.15 Pipettes, (100 µL and 1000 µL) positive displacement.

6.16 Pipette tips, sterile.

6.17 Rocker, with rocking angle of 8° - 10°, and shaking speed of 30 - 50 rpm.

6.18 Round stick, 4 mm in diameter and min 30 mm, or equivalent.

[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 International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, https://www.iso.org.

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