Designation: E3031 – 15

Standard Test Method for Determination of Antibacterial Activity on Ceramic Surfaces[1]

This standard is issued under the fixed designation E3031; 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 method is designed to quantitatively evaluate the antibacterial activity of glazed ceramic surfaces that have been specifically designed to contain an antibacterial treatment as part of the glaze. This test method is meant to compare the efficacy of one ceramic surface to another ceramic surface using the stated conditions and is not meant to be extrapolated to other conditions.

1.2 Knowledge of microbiological techniques is required for this test.

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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

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

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

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

2.2 *ISO Standard:*[3]

ISO 22196 Measurement of Antibacterial Activity on Plastics and Other Non-porous Surfaces

3. Terminology

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

4. Summary of Test Method

4.1 This test method is used for evaluating the antibacterial effect of antimicrobials incorporated into a ceramic glaze. This standard does not seek to imitate all possible real world scenarios but to provide a standardized method to compare multiple antimicrobial technologies that can be incorporated or coated on a ceramic surface. The inherent nature of the ceramic tile allows for desiccation, therefore each ceramic specimen is equilibrated to the testing environment for 18- 24 h. Once the tiles are equilibrated, bacteria are inoculated onto the surface followed by a 24-h exposure time. Bacteria are recovered in a neutralizer broth and enumerated according to a validated method. Log reductions are calculated for a treated versus an untreated sample.

5. Significance and Use

5.1 Current solid surface test methodologies, such as the Test Method E2180 and ISO 22196, do not take into account the complexities associated with a ceramic surface. This includes, but is not limited to, differing chemistries incorporated into the glaze and desiccation due to water absorption through the bisque body. Each point will be elaborated below:

5.1.1 The glaze composition of ceramic tiles can vary between manufacturers, lots, and product lines. Some glaze chemistries such as tin, silver and copper can negatively impact the testing conditions. Therefore, an untreated tile from the same lot is not always suitable for comparison. The control tile proposed herein is capable of supporting growth over the indicated time frame and nutrient level (see Section 9).

5.1.2 Desiccation is a common problem when testing tile surfaces. This can be overcome by pre-hydrating the tile by placing the specimen on a moistened wipe and allowing

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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] Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

incubation for 18 to 24 h before beginning the test. This reduces the number of false positive results and more accurately measures the ability of the antimicrobial to inhibit growth.

5.2 This test method utilizes a low inoculum load and requires growth on the control substrate to demonstrate a valid testing environment. In addition, while some antimicrobials demonstrate activity against static cultures, others require growth of the bacteria to maintain activity. A low inoculum level will allow for both types of antimicrobials to be examined with the same testing conditions.

6. Apparatus

6.1 *Incubator*—capable of maintaining a temperature of 35 ± 2ºC and >75% RH.

6.2 *Pipetter*—continuously adjustable between 100 µL and 1000 µL.

6.3 *Sterilizer*—any suitable steam sterilizer with conditions that produce sterility of samples.

6.4 *Petri dish*—sterile 150 mm by 15 mm for holding the samples

6.5 *Culture tubes and closures*—any with a volume capacity of 10 mL and a minimum diameter of 16 mm. Recommended size is 16 mm by 125 mm borosilicate glass with a threaded opening.

6.6 *Cover film*—25 mm by 25 mm sterile polyethylene or other suitable material that does not impact bacterial growth.

6.7 *Large Water Absorbent Laboratory wipe*—to facilitate pre-hydration of samples similar to a Kimwipes Kimtech[4]delicate task wiper 30 cm by 30 cm.

6.8 *Vortex mixer*—to provide a homogenous bacterial suspension prior to inoculation of samples and prior to the enumeration technique that will be used.

6.9 *Plastic screw top jar*—150 ml capacity that has an opening large enough to insert the sample as a vessel for recovery.

6.10 *Wrist action shaker*—to recover bacteria from samples.

6.11 *Petri dish*—100 mm by 15 mm for enumeration.

6.12 *Shaking incubator*—capable of maintaining 35 ± 2ºC.

7. Reagents and Materials[5]

7.1 *Dilution fluid or diluent*—sterile Butterfield's buffered phosphate.

7.2 *Growth medium.*

7.2.1 *Overnight culture*—brain heart infusion broth prepared according to the manufacturer's instruction.

7.2.1.1 Alternative media may be used for overnight culture of the organism, such as tryptic soy broth, but details shall be included in the final report.

7.2.2 *Inoculation broth*—1:500 dilution of nutrient broth as defined below:

7.2.2.1 Prepare nutrient broth by dissolving 3.0 g of meat (beef) extract, 10.0 g peptone, and 5.0 g of sodium chloride in 1000 mL of distilled or deionized water.

7.2.2.2 Dilute the nutrient broth with distilled or deionized water to a 500-fold volume and adjust the pH to a value between 6.8 and 7.2 with sodium hydroxide or hydrochloric acid.

7.2.2.3 Sterilize by autoclaving at 120°C for 30 min.

7.3 *Solid growth media*—tryptic soy agar plates.

7.4 *Sterile deionized water*—or equivalent.

7.5 *Neutralizer*—A neutralizer should be selected that has been shown to effectively neutralize the active according to Test Methods E1054.

8. Culture Preparation

8.1 *Escherichia coli* American Type Culture Collection, ATCC No. 8739 is the organism to be utilized for this test. Grow a fresh 18 ± 1 h culture in sterile brain heart infusion broth at 35 ± 2ºC and shaking at 110 r/min prior to beginning the test. Dilute this suspension appropriately in the inoculation broth described in 7.2.2 to obtain 1-5 × 10^4 CFU/mL. This will be the working bacterial stock solution.

9. Untreated Control Specimen

9.1 Control tiles suitable for testing purposes may be prepared from glaze ingredients that are free of elements that contribute to antimicrobial activity. One example of a product that meets this criterion is F-524.[6] However, glazed tiles are generally acceptable as controls if they can be shown to meet the following criterion:

9.1.1 Can support > 1.5 log growth under the test conditions given herein as calculated in 12.4.

9.1.1.1 If a control tile, as described above, is not available then the use of borosilicate glass squares, cut to the same dimensions as described in 10.1, can be substituted as control specimens. Glass squares shall meet performance specifications indicated in 9.1.1.

10. Sample Preparation

10.1 Prepare five (5) replicates of each specimen, measuring 50 mm by 50 mm ± 1 mm^2 (see Section 9). Wipe test specimens to remove any debris from processing, place in a sterilization pouch/container and autoclave for at 120°C for 1 h.

NOTE 1—If the active ingredient is affected by autoclaving, then other

[4] Kimwipe is a registered trademark of Kimberly-Clark Dallas TX, USA

[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.

[6] The sole source of supply of the of the control tiles (F-524) suitable for testing purposes prepared from glaze ingredients that are free of elements that contribute to antimicrobial activity and known to the committee at this time is Fusion Ceramics, Inc. (Carrollton, Ohio USA). If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.