Designation: E3285 – 22

Standard Test Method for Evaluation of Contact-Mediated Microbial Transference[1]

This standard is issued under the fixed designation E3285; 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 designed to evaluate the contact-mediated microbial transference on two-dimensional material surfaces. Material surfaces intended to reduce microorganism adherence or contamination may be evaluated using this test method. Additionally, this test method can be utilized on a wide variety of material surfaces for other downstream processes.

1.2 This test method is designed to quantify the fomite transfer of bacteria on material surfaces through touch-transfer, be inexpensive, require limited training in practice, and be adaptable to many surfaces or microorganisms. Non-porous surfaces are contaminated with inoculated filter paper as a carrier and surface contamination is quantified by sampling with Replicate Organism Detection and Counting (RODAC) plates.

1.3 Basic microbiology training is required to perform this test method.

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

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 *Definitions*—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 *microbial transference, n*—relocation of a microorganism from a previously contaminated surface to a different surface by contact.

4. Significance and Use

4.1 This test method utilizes filter paper as a medium for evaluating touch-transfer of bacteria to material surfaces. Sample surfaces are exposed to filter papers saturated with a *Staphylococcus aureus* suspension, followed by recovery of the bacteria from the surface with Replicate Organism Detection and Counting (RODAC) plates. This test method reports the $\log_{10}$ reduction of bacterial transfer on an intended test surface compared to a control surface. The test and control surfaces can differ by texture, coating, treatment, or any other desired variables, as long as they are the same material.

5. Apparatus

5.1 *Calibrated timer*—readable in minutes and seconds,

5.2 *Cell spreader*—sterile,

5.3 *Centrifuge*—capable of 2500 × g,

5.4 *Forceps*—sterile,

5.5 *Incubator*—capable of maintaining a temperature of 35 °C ±2 °C,

5.6 *Thermometer or thermocouple*,

5.7 *Variable volume pipettors and sterile tips,* capable of dispensing volumes from 10 µL-1000 µL, with current calibration,

5.8 *Vortex mixer*—any vortex mixer that will ensure proper agitation and mixing of the contents of test tubes.

6. Reagents and Materials

6.1 *Reagents:*

6.1.1 *Ethanol*—commercially available 95 % ethanol.

[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] 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.

6.1.2 *Phosphate Buffered Saline (PBS)*—Prepare according to manufacturer's instructions to produce 1× PBS.

6.1.3 *Trypticase Soy Agar with Lecithin and Polysorbate 80*—purchased pre-made plates or prepared according to manufacturer's instructions for a concentration of 45.1 g/L and pipette 16 mL into each sterile RODAC plate.

NOTE 1—It might be necessary to perform a neutralizer effectiveness test if there is a possibility of the material leaching antimicrobials. If deemed necessary, perform this test in compliance with Practices E1054.

6.1.4 *Tryptic Soy Agar (TSA)*—purchased pre-made plates or prepared according to manufacturer's instructions for a concentration of 40 g/L and dispensed into sterile 100 mm × 15 mm Petri plates.

6.1.5 *Tryptic soy broth (TSB)*—purchased pre-made or prepared according to manufacturer's instructions for a concentration of 30 g/L.

6.2 *Materials:*

6.2.1 *Conical tubes*—Sterile, 15 mL and 50 mL volume capacity with threaded cap,

6.2.2 *Double-sided adhesive tape*,

6.2.3 *Filter paper*—sterilized cellulose, Example Whatman grade 2, 8 µm particle retention, 190 µm nominal thickness, 97 g/m^2 nominal basis weight. Cut filter paper to two sizes, 45 mm × 45 mm (+2 mm) and 60 mm × 60 mm (+2 mm),

6.2.4 *Inoculating loops*—Sterile 10 µL,

6.2.5 *Petri plates*—sterile, 100 mm diameter,

6.2.6 *RODAC plates*—sterile, 65 mm × 15 mm,

6.2.7 *Serological pipettes*—Sterile, 10 mL and 25 mL volume,

6.2.8 *Test tubes*—Sterile, any with a volume capacity of 10 mL, a minimum diameter of 16 mm, and a cap that can withstand 100 °C. Recommended size is 18 mm × 150 mm borosilicate glass.

6.3 *Test organism:*

6.3.1 *Staphylococcus aureus*, ATCC 6538

7. Hazards

7.1 Handling and manipulation of microorganisms that are potentially hazardous requires a high degree of technical competence and may be subject to legislation and regulations. Only personnel trained in microbiological techniques should carry out such tests. Appropriate practices for disinfection, sterilization, and personal hygiene must be strictly observed.

8. Culture Preparation

8.1 *Staphylococcus aureus* ATCC6538 is the organism used in this test.

8.1.1 A frozen culture shall be used as the source for establishing the suspension used in testing.

8.1.2 Streak a TSA plate for isolation with the source culture and incubate at 35 °C + 2 °C for 18 h-24 h.

8.1.3 Aseptically transfer one loopful of bacteria from the surface of a TSA plate and place into 5 mL of TSB in a sterile culture tube. Incubate at 35 °C + 2 °C for 18 h-24 h with agitation to ensure adequate aeration of the culture by shaking at a minimum of 200 revolutions per minute on a standard laboratory shaker.

NOTE 2—Additional challenge species may be substituted to evaluate the breadth of a material's antimicrobial activity versus species to which it may be exposed. If an alternative challenge species is used in testing, it must be identified in the final report, along with any accommodative modifications made to the method (that is, changes to culture media, buffers, etc.). The test report also must indicate that ASTM Test Method E3285, modified, was used. The precision statistics reported in this method will not apply.

9. Preparation of Apparatus

9.1 Prepare flat test samples of the control and test surfaces by cutting into (50 ± 2) mm × (50 ± 2) mm squares. The test and control surfaces must be assayed at least in triplicate. Sample size can be determined from preliminary data by performing a power calculation.

9.2 Adhere test and control surfaces to the inside bottom of a 100 mm Petri plate (with double-sided tape, if necessary), ensuring that the test surface is facing up.

9.3 Wipe surfaces with 95 % ethanol 3 times, if compatible with surface.

9.4 If surface is not compatible with ethanol, an alternative method of cleaning and disinfection may be used. Additionally, sample cleaning and disinfection may be omitted, however any modification to the cleaning and disinfection step shall be noted in the test report.

10. Procedure

10.1 *Inoculum Preparation:*

10.1.1 Centrifuge culture from step 8.1.3 at 2500 × g for 5 minutes at room temperature. Discard supernatant.

10.1.2 Resuspend cell pellet in PBS.

10.1.3 Vortex thoroughly to generate a single-cell suspension.

10.1.4 Dilute in PBS to reach a culturable cell titer of 6.0×10^3 CFU/mL (range 1.0×10^3 to 1.2×10^4). Each sample requires 25 mL of inoculum, so this dilution step may be scaled according to the number of samples tested. Cell density shall be confirmed by serial dilution and plating onto TSA. This serves as a purity control, as colony morphology can be evaluated in the diluted samples.

10.1.5 Check PBS for sterility by plating 100 µL of PBS on a TSA plate and incubate with the inoculation plates from 10.2.3.

NOTE 3—If necessary, the inoculum titer may be adjusted to produce approximately 100 CFU/RODAC-250 CFU/RODAC on the control specimen samples. Modification of the inoculation titer shall be noted in the test report.

10.2 *Inoculation of Test Specimens:*

10.2.1 Pour 25 mL of the challenge inoculum into a sterile Petri plate.

10.2.2 Transfer a 45 mm × 45 mm sterile filter paper (carrier) into the inoculum with sterile forceps and immerse for 5 s.

10.2.3 Remove carrier from inoculum with sterile forceps and hold vertically for 25 s to drain excess suspension. Tap corner of the carrier against the side of the Petri plate to remove droplets.

10.2.4 Place one inoculated carrier on the apical surface of each of the test and control specimens carefully to avoid bubbles.

10.2.5 Place a 60 mm × 60 mm sterile, dry filter paper on top of the carrier within 5 s of placing the inoculated carrier onto the test or control surface.

10.2.6 Use a sterile spreader to press gently across the sample once. Rotate the sample and press once, perpendicular to the first pass. The entire 60 mm × 60 mm filter paper should be pressed twice.

10.2.7 Incubate for 2 min at room temperature.

10.2.8 Remove filter paper and carrier from test and control surfaces with sterile forceps and discard.

10.2.9 Allow the surfaces to stand for 5 min.

10.3 *Sampling of Contamination:*

10.3.1 Press a RODAC plate onto test and control specimen with 500 g of downward pressure for 5 s, ensuring even contact between the media and test specimen.

10.3.2 Invert the inoculum plates from 10.2.3 and incubate at 35 °C + 2 °C for 18 h-24 h. Incubate RODAC plates upright at 35 °C + 2 °C for 18 h-24 h to prevent condensation from dripping onto media.

10.4 *Enumeration:*

10.4.1 Count the appropriate number of colony-forming units (CFU) according to the plating method used. For RODAC plates, count all colonies contained within the 40 mm × 40 mm square outlined on the plate.

10.4.2 Record colony counts from the inoculum plates containing 25 CFU-250 CFU. Plates with more than 250 colonies are recorded as too numerous to count (TNTC).

10.4.3 Record colony counts from the RODAC plates. Plates with over 250 colonies are recorded as too numerous to count (TNTC).

10.5 *Calculations:*

10.5.1 Include counts from 25 CFU-250 CFU for inoculum plates and 1 CFU-250 CFU for RODAC plates in calculations. RODAC plates with 0 colonies should be entered as 0.5 CFU for calculation purposes.

10.5.2 Calculate the $\log_{10}$ transformation of each CFU/specimen replicate.

10.5.3 Average the $\log_{10}$ transformed CFU/specimen ($\log_{10}$ density, LD) data for the control specimens ($AvgCFU_{con}$) and for the test specimens ($AvgCFU_{test}$) from three independent experiments:

$$Avg_{CFUx} = \frac{LOG_{CFUx1} + LOG_{CFUx2} + LOG_{CFUx3}}{3} \quad (1)$$

where:

LOG_{CFUx} = control or test log transformed CFU/specimen counts

1, 2, 3 = Independent experiment from which CFUs were counted

10.5.4 Determine the microbial transference reduction on test specimens compared to control specimens using the equation:

$$LR = Avg_{CFUcon} - Avg_{CFUtest} \quad (2)$$

where:

Avg_{CFUcon} = the average of the LOG(CFU/sample) replicate values from the control specimens

$Avg_{CFUtest}$ = the average of the LOG(CFU/sample) replicate values from the test specimens

10.5.5 Determine the percent reduction of microbial transference on test specimen compared to control specimen using the equation:

$$\%\ \text{Reduction} = \left(1 - 10^{(-LR)}\right) * 100 \quad (3)$$

11. Report

11.1 The test report shall include the following information, at a minimum:

11.1.1 A reference to this method.

11.1.2 Detailed information on material surface specifications.

11.1.3 Detailed information on filter paper specifications.

11.1.4 The ATCC numbers of other ID of the species and strains of test microbes.

11.1.5 The titer of the inoculum suspension.

11.1.6 The control and test specimen LD and test specimen LR values.

11.1.7 Percent transference reduction on the test specimen compared to control specimen.

11.1.8 Details of any deviation from this test method.

11.1.9 Identification of the test laboratory and experimental operator.

11.1.10 Date of the experiment.

12. Precision and Bias

12.1 *Repeatability Limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the "*r*" value for that material; "*r*" is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory. Additionally, $r = 2.8\ s_r$, where sr is the repeatability standard deviation.

12.1.1 Preliminary repeatability was determined based on six replicate determinations performed in a single laboratory (Sharklet Technologies, Inc.) by three operators on a single day (Appendix X1). This replicate number was determined from preliminary data indicating an Effective Sample Size of at least 5, as described by Killip, et al.[3] Operators are trained to this procedure prior to performing the experiment, and no deviations from this procedure are recorded. For Log_{10} CFU/specimen, $s_r = 0.15\ X$ and $r = 0.42$ X, where X is Log_{10} CFU/sample.

12.2 *Reproducibility*—An interlaboratory study of this test method is being conducted and a complete precision statement is expected to be available within five years after approval of this test method.

[3] Killip, S., Mahfoud, Z. & Pearce, K. What is an intracluster correlation coefficient? Crucial concepts for primary care researchers.*Ann Fam Med* 2, 204–208 (2004).