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Test Method for Antibacterial Activity of Textile Materials: Parallel Streak

Developed in 1976 by AATCC Committee RA31; reaffirmed 1977, 1982, 1998, 2016; editorially revised 1980, 1982, 1983, 1986, 2010, 2019 (with title change); revised 1987, 1988 (with title change), 1993, 2011; editorially revised and reaffirmed 2004.

Foreword

The Parallel Streak Method has filled a need for a relatively quick and easily executed qualitative method to determine antibacterial activity of diffusible antimicrobial agents on treated textile materials.

AATCC TM100, Test Method for Antibacterial Finishes on Textile Materials, Assessment of, is a quantitative procedure which is adequately sensitive but is cumbersome and time consuming for routine quality control and screening tests. Therefore, when the intent is to demonstrate bacteriostatic activity by the diffusion of the antibacterial agent through agar, AATCC TM147 fulfills this need. In the Parallel Streak Method, the agar surface is inoculated making it easier to distinguish between the test organism and contaminant organisms which may be present on the unsterilized specimen. The Parallel Streak Method has proven effective over a number of years of use in providing evidence of antibacterial activity against both Gram positive and Gram negative bacteria.

1. Purpose and Scope

1.1 The objective is to detect bacteriostatic activity on textile materials. The results of using this procedure have been demonstrated by Committee RA31 to be reproducible by various laboratories working with materials containing residual amounts of antibacterial agents (as determined by chemical assay) after multiple standard washings. The method is useful for obtaining a rough estimate of activity in that the growth of the inoculum organism decreases from one end of each streak to the other and from one streak to the next resulting in increasing degrees of sensitivity. The size of the zone of inhibition and the narrowing of the streaks caused by the presence of the antibacterial agent permit an estimate of the residual antibacterial activity after multiple washings.

2. Principle

2.1 Specimens of the test material, including corresponding untreated controls of the same material, are placed in intimate contact with the agar surface which has been previously streaked with an inoculum of a test bacterium. After incubation, a clear area of interrupted growth underneath and along the sides of the test material indicates antibacterial activity of the specimen. A standard strain of bacteria is used which is specific to the requirements of the material under test. If no other bacterial species is specified, *Staphylococcus aureus* may be used as a representative Gram positive organism. Other recommended strains are listed below in Section 6.

3. Terminology

3.1 **activity,** n.—*of an antibacterial agent*, a measure of effectiveness of the agent.

3.2 **antibacterial agent,** n.—any chemical which kills bacteria (bactericide) or interferes with the multiplication, growth or activity of bacteria (bacteriostat).

3.3 **zone of inhibition,** n.—clear area of no growth of a microorganism, cultured onto the surface of an agar growth medium, in proximity to the borders of a specimen placed in direct contact with this agar surface.

NOTE: A zone of inhibition occurs as a result of the diffusion of an antimicrobial agent from the specimen.

4. Safety Precautions

NOTE: These safety precautions are for information purposes only. The precautions are ancillary to the testing procedures and are not intended to be all inclusive. It is the user's responsibility to use safe and proper techniques in handling materials in this test method. Manufacturers MUST be consulted for specific details such as material safety data sheets and other manufacturer's recommendations. All OSHA standards and rules must also be consulted and followed.

4.1 This test should be performed only by trained personnel. The U.S. Department of Health and Human services publication *Biosafety in Microbiological and Biomedical Laboratories* should be consulted (see 12.1).

4.2 CAUTION: Some of the bacteria used in this test are pathogenic; i.e., capable of infecting humans and producing disease. Therefore, every necessary and reasonable precaution must be taken to eliminate this risk to the laboratory personnel and to personnel in the associated environment. Wear protective clothing and respiratory protection that prevents penetration by the bacteria.

4.3 Good laboratory practices should be followed. Wear safety glasses in all laboratory areas.

4.4 All chemicals should be handled with care.

4.5 An eyewash/safety shower should be located nearby for emergency use.

4.6 Sterilize all contaminated samples and test materials prior to disposal.

4.7 Exposure to chemicals used in this procedure must be controlled at or below levels set by government authorities (e.g., Occupational Safety and Health Administrations [OSHA] permissible exposure limits [PEL] as found in 29 CFR 1910.1000; see web site: www.osha.gov for latest version). In addition, the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) comprised of time weighted averages (TLV-TWA), short term exposure limits (TLV-STEL) and ceiling limits (TLV-C) are recommended as a general guide for air contaminant exposure which should be met (see 12.2).

5. Uses and Limitations

5.1 The method is not suitable for materials which tend to encapsulate and prevent the diffusion of the antibacterial agent or contain antibacterial-neutralizing substances.

6. Test Organisms

6.1 Test bacteria:

6.1.1 *Staphylococcus aureus*, ATCC 6538, CIP 4.83, DSM 799, NBRC 13276, NCIMB 9518 or equivalent strain (see 12.3).

6.1.2 *Klebsiella pneumoniae*, ATCC 4352, CIP 104216, DSM 789, NBRC 13277, NCIMB 10341 or equivalent strain (see 12.3).

6.1.3 Other suitable species can also be used depending on the intended end-use of the test sample.

6.2 Maintain test organisms according to good laboratory practice standard (see 12.4).

6.3 Whenever possible, test the activity

of the culture to be used against a standard control specimen (a positive control) with known antibacterial activity.

6.4 To determine whether the antibacterial activity is due to the antibacterial agent, test a specimen of the same material treated in exactly the same way with whatever other finishing agents were used, but without the antibacterial agent. Many standard textile finishing chemicals, especially crease resistant and permanent press reagents, will often give strong antibacterial activity even after many washes.

7. Materials, Media and Reagents

7.1 Media and Reagents. Suitable broth/agar media are:

7.1.1 Nutrient broth/agar.

7.1.2 Trypticase Soy broth/agar.

7.1.3 Brain-Heart Infusion broth/agar.

7.1.4 Müller-Hinton broth/agar.

7.1.5 Other appropriate broths/agars can be used depending on test organisms selected.

7.2 Materials.

7.2.1 Incubator maintained at 37 ± 2°C (99 ± 4°F).

7.2.2 Inoculating loop.

7.2.3 Bunsen Burner or equivalent.

7.2.4 Water bath maintained at 45-50°C (113-122°F).

7.2.5 Pipets, 1 mL, sterile.

7.2.6 Culture Tubes with caps; minimum 10 mL capacity.

7.2.7 Petri dishes, 100 mm diam. × 15 mm deep, sterile.

7.2.8 Forceps, sterile.

7.2.9 Stereomicroscope, minimum 40× magnification.

7.2.10 Ruler.

8. Test Specimens

8.1 Test specimens (non-sterile) are cut by hand or with a die. They may be any convenient size. Rectangular specimens cut 25 × 50 mm are recommended. A 50 mm length permits the specimens to lie across five parallel inoculum streaks each of diminishing width from about 8 to 4 mm wide.

9. Procedure

9.1 Dispense appropriate sterilized agar [cooled to 47 ± 2°C (117 ± 4°F)] by pouring 15 ± 2 mL into each standard (15 × 100 mm) flat bottomed petri dish. Allow agar to gel firmly before inoculating.

9.2 Prepare inoculum by transferring 1.0 ± 0.1 mL of a 24 h broth culture into 9.0 ± 0.1 mL of sterile distilled water contained in a test tube or small flask. Mix well using appropriate agitation.

9.3 Using a 4 mm inoculating loop, load one loopful of the diluted inoculum and transfer to the surface of the sterile agar plate by making five streaks approximately 60 mm in length, spaced 10 mm apart covering the central area of a standard petri dish (see 10.1) without refilling the loop. Take care not to break the surface of the agar while making the streaks.

9.4 Gently press the test specimen transversely across the five inoculum streaks to ensure intimate contact with the agar surface. This may be accomplished more easily by pressing the specimen to the agar surface with a biological section lifter or with a spatula which has been sterilized by flaming and then air cooled immediately before use.

9.5 If the specimen curls, preventing intimate contact with the inoculated surface, place sterile glass slides on the ends of the specimen to hold it in place.

9.6 Incubate at 37 ± 2°C (99 ± 4°F) for 18-24 h.

10. Evaluation

10.1 Examine the incubated plates for interruption of growth along the streaks of inoculum beneath the specimen and for a clear zone of inhibition beyond its edge. The average width of a zone of inhibition along a streak on either side of the test specimen may be calculated using the following equation:

$$W = (T - D)/2$$

where:

W = width of clear zone of inhibition in mm

T = total diameter of test specimen and clear zone in mm

D = diameter of the test specimen in mm

10.2 The size of the zone cannot be construed as a quantitative evaluation of antibacterial activity. Treated materials should be compared to an untreated corresponding material and a material specimen with known bacteriostatic activity. Report of results will include an observation of zones of inhibition and growth under the specimen if present. The criterion for passing the test must be agreed upon by the interested parties. To constitute acceptable antibacterial activity, there must be no bacterial colonies directly under the sample in the contact area.

11. Precision and Bias

11.1 Precision for this test method has not been established. Until a precision statement is generated for this test method, use standard statistical techniques in making any comparisons of test results for either *within-laboratory* or *between-laboratory* averages.

12 Notes and References

12.1 Publication available from U.S. Department of Health and Human Services, CDC/NIH-HHS Publication No. (CDC) 84-8395; web site: www.hhs.gov.

12.2 Available from Publications Office, ACGIH, Kemper Woods Center, 1330 Kemper Meadow Dr., Cincinnati OH 45240; tel: +1.513.742.2020; web site: www.acgih.org.

12.3 ATCC is the American Type Culture Collection (USA), P.O. Box 1549, Manassas VA 20108; tel: +1.703.365.2700; fax: +1.703.365.2701; CIP is the Pasteur Institute Collection (France); DSM is the German Collection of Microorganisms and Cell Cultures (Germany); NBRC is the NITE Biological Resource Center (Japan); and NCIMB is the National Collection of Industrial Bacteria (UK). Equivalent bacteria strains obtained from agencies of the World Federation of Culture Collection (WFCC) may be used by agreement between the interested parties. The strains used in the test shall be documented with their supply source.

12.4 Consistent and accurate testing requires maintenance of a pure, uncontaminated, non-mutant test culture. Avoid contamination by using good sterile technique in plating and transferring. Avoid mutation by strict adherence to monthly stock transfers. Check culture purity by making streak plates periodically and observing for a single species-characteristic type of colonies.