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Antibacterial Activity Assessment of Textile Materials: Agar Plate Method

Developed in 1958 by AATCC Committee RA31; revised 1962, 1965, 2010; reaffirmed 1970, 1974, 1977, 1982, 2016; editorially revised 1971, 1972, 1974, 1982, 1985, 1986; removed 1989; reactivated/revised 2011.

Foreword

The Agar Plate Method is a qualitative method to determine antibacterial activity of diffusible antimicrobial agents on treated textile materials. This method is an adaptation of a test method described by Ruehle and Brewer (see 12.1) and was originally adopted as an AATCC tentative test method in 1958. RA31 revised this method in 1962 in order to clearly define the objectives and limitations of this method. In 1989, the method was discontinued and removed from the TECHNICAL MANUAL. The reason cited for removal was that pertinent aspects of the agar plate method were incorporated in the re-write of AATCC Test Method 147, Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method. In 2011, RA31 updated and reactivated the method in part because ASTM cites this method in their standard ASTM E1115, Standard Test Method for Evaluation of Surgical Hand Scrub Formulations, and because the agar plate method was found to be beneficial when testing odd shape test samples, batting, fiber fill, etc. This method is used to demonstrate bacteriostatic activity by the diffusion of the antibacterial agent through agar. Molten agar is seeded with the test organisms and allowed to become semi-solid or solid depending on the test specimen to be tested. The test specimen is then placed in intimate contact with the agar surface. An advantage of the Agar Plate Method is that a non-embedded test specimen can be removed to determine inhibition in the contact area without fear of removing the test organism with the test sample. Both Gram positive and Gram negative bacteria are tested.

1. Purpose and Scope

1.1 The objective is to qualitatively detect bacteriostatic activity on products that are treated with antimicrobials and are capable of producing a zone of inhibition. Some test samples do not have a flat surface, therefore, this method provides

the advantage of testing odd shaped and non-flat surfaces by partially embedding them into molten agar prior to solidification. This provides the intimate contact required between the test sample and the seeded agar.

2. Principle

2.1 Specimens of the test material, including corresponding untreated controls of the same material if available, are placed in intimate contact with agar which has been previously seeded with an inoculum of a test bacterium. After incubation, a clear area of interrupted growth underneath and/or adjacent to the test material indicates antibacterial activity of the specimen. A standard strain of bacteria is used that is specific to the requirements of the material under test. If no other bacterial species is specified, *Staphylococcus aureus* and *Klebsiella pneumoniae* may be used as representative Gram positive and Gram negative organisms. Other recommended strains can be used.

3. Terminology

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

3.2 **antibacterial agent**, n.—in textiles, 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.

tions. 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 12.2).

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 12.3).

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, and DSM 799, NBRC 13276, NCIMB 9518 or equivalent strain (see 12.4)

6.1.2 *Klebsiella pneumoniae*, ATCC