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Test Method for Antibacterial Activity of Textile Materials: Agar Plate

1. Foreword

1.1 The Agar Plate Method is a qualitative method to determine antibacterial activity of diffusible antimicrobial agents incorporated into treated articles. This method is an adaptation of a test method described by Ruehle and Brewer (see 16.1) 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 *Manual of International Test Methods and Procedures*. The reason cited for removal was that pertinent aspects of the agar plate method were incorporated in the rewrite of AATCC TM147, Test Method for 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-shaped or non-flat test samples, such as batting, fiber fill, and yarn, as well as non-textile materials such as foam or plastics. 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.

2. Purpose and Scope

2.1 The objective is to detect bacteriostatic activity on samples that are treated with antimicrobials and are capable of producing a zone of inhibition.

2.2 Some test samples do not have a flat surface and are not easily tested using AATCC TM147. This method provides the advantage of testing odd-shaped and non-flat samples such as batting, fiber fill, foam, plastic, yarn, etc. by partially embedding them into molten agar prior to

solidification. This provides the intimate contact required between the test sample and the seeded agar.

3. Principle

3.1 Test samples, 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 sample indicates antibacterial activity of the specimen. A standard strain of bacteria is used that is specific to the requirements of the sample 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.

4. Referenced Documents

NOTE: Use current versions of all publications unless otherwise specified.

4.1 AATCC TM147, Test Method for Antibacterial Activity of Textile Materials: Parallel Streak (see 16.2)

4.2 ASTM E1115, Standard Test Method for Evaluation of Surgical Hand Scrub Formulations (see 16.3)

4.3 ASTM E1402, Standard Guide for Sampling Design (see 16.3)

4.4 Centers for Disease Control. Biosafety in Microbiological and Biomedical Laboratories (see 16.4).

5. Terminology

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

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

5.3 **zone of inhibition**, n.—clear area of no visible 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.

6. Safety Precautions

6.1 The safety precautions specified in the method/procedure are ancillary to the testing procedures and are not intended to be all inclusive.

6.2 It is the user's responsibility to reference applicable safety data sheets, use safe and proper techniques, and wear appropriate personal protective equipment in handling materials in this standard.

6.3 Users MUST consult manufacturers for specific details such as equipment operating instructions and other recommendations. Consult and follow all applicable health and safety regulations (e.g. OSHA standards and rules).

6.4 See 4.4, 9.1, 12.1 for additional safety precautions and personnel training related to handling bacteria.

7. Uses and Limitations

7.1 The method is not suitable for materials which tend to encapsulate and prevent the diffusion of the antibacterial agent. Not suitable for materials that contain substances that may neutralize the antibacterial agent. Not suitable for antimicrobial finishes that will react with the agar.

7.2 Being a qualitative method, the efficacy of the antimicrobial is not determined by zone size.

7.3 Some standard textile finishing chemicals, especially crease resistant and permanent-press reagents, will give antibacterial activity. 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.

8. Apparatus (see 16.5)

8.1 Incubator maintained at $37 \pm 2^\circ\text{C}$ ($99 \pm 4^\circ\text{F}$).

8.2 Water bath maintained at $45\text{--}50^\circ\text{C}$ ($113\text{--}122^\circ\text{F}$).

8.3 Bunsen Burner or equivalent.

8.4 Stereomicroscope, minimum 40x magnification.

8.5 Autoclave.

8.6 Vortex or equivalent.

9. Materials (see 16.5)

9.1 Bacteria species suitable for the intended end-use of the test sample. Some