Designation: E1882 – 05

Standard Test Method for Evaluation of Antimicrobial Formulations by the Agar Patch Technique[1]

This standard is issued under the fixed designation E1882; 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 determines the antibacterial activity and persistence of test formulations, as measured by the inhibition of a test organism on an agar surface exposed to test sites on human skin treated with the formulations.

1.2 A knowledge of microbiological techniques is required for these procedures.

1.3 It is the responsibility of the investigator to determine if Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) are required and to adhere to these practices, as appropriate.

1.4 In this test method, metric units are used for all applications except linear measure. In that case, inches are used and metric units follow in parentheses.

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 and health practices and determine the applicability of regulatory limitations prior to use.* Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects (see 21 CFR, Ch. I, Parts 50 and 56).

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1874 Test Method for Evaluation of Antibacterial Washes by Cup Scrub Technique

[3]

2.2 *Federal Standard* [4]

21 CFR, Ch. I, Parts 50 and 56 Protection of Human Subjects

3. Terminology

3.1 *active test formulation*—a substance containing active ingredient(s).

3.2 *active ingredient*—a substance performing a function defined in the test method; in this test method, a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.3 *active plate*—inoculated agar plate that has been attached to a skin site treated with an active formulation.

3.4 *antibacterial activity*—killing of bacteria or supression of their growth or reproduction.

3.5 *control formulation*—a formulation that does not contain an active ingredient.

3.6 *control plate*—inoculated agar plate that has been attached to an untreated skin site, or one treated with a control formulation.

3.7 *inhibition*—prevention of bacterial population growth, either through lethality or through prevention of bacterial reproduction.

3.8 *inoculum determination plate*—an inoculated plate that has not been exposed to any skin test site.

3.9 *persistence*—effectiveness of a test formulation in inhibiting bacteria, defined in terms of time elapsed between application of test formulation and application of test plates.

3.10 *resident microorganisms*—microorganisms that live and multiply on skin, forming a permanent population.

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides 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.

[3] Withdrawn. The last approved version of this historical standard is referenced on www.astm.org.

[4] Available from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

3.11 *transient microorganisms*—microorganisms from the environment that contaminate, but do not normally permanently colonize skin.

3.12 *volar aspect of the forearms*—the inside of the forearm on the same plane as the palm of the hand.

4. Summary of Test Method

4.1 This test method is conducted on subjects selected from a group of volunteers who have refrained from using topical antimicrobials for at least one week and have minimal hair on the test site. The test site should normally have a low number of resident microorganisms (approximately 10^4 CFU/cm^2 or fewer) and be easily sampled.

4.2 The surfaces of agar contact plates are inoculated with the selected organism and placed in contact with skin sites that have been treated with active or control formulations, or left untreated. After contact with the treated skin sites, these plates are incubated and the colonies enumerated. Inhibitory activity of the active test formulation is measured by comparing differences in microbial colony counts between plates that were in contact with sites treated with an active formulation and plates that were in contact with untreated sites, or sites treated with a control formulation. Results are expressed as percent inhibition.[5]

5. Significance and Use

5.1 This procedure can be used to evaluate formulations containing ingredients intended to inhibit growth of bacteria on intact skin and measures the difference, post-product-exposure, between numbers of bacterial colonies on active test formulation plates and numbers on control plates, expressed as percent inhibition.

5.2 This procedure may also be used to test for persistence of activity, as a function of time elapsed between application of active test formulation and application of active test plates.

5.3 Because no procedure for neutralization of the antimicrobial action of active ingredients can be included in the test, the agar patch method is limited to the extent that results expressed as percent inhibition do not differentiate between bacteriostatic and bacteriocidal effects and, hence, must not be portrayed as "reductions."

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used. A magnifying device, such as a dissecting microscope, may be used for manual enumeration of colonies.

6.2 *Incubator*—Any incubator capable of maintaining a suitable temperature ± 2°C may be used.

6.3 *Sterilizer*—Any steam sterilizer capable of producing the conditions of sterility.

6.4 *Timer (Stop Watch)*—One that can be read for hours, minutes, and seconds.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*, 10.0 and 2.2 or 1.1 mL capacity.

NOTE 1—Presterilized/disposable bacteriological pipettes are available from most laboratory supply houses.

7.2 *Pipetter*, with disposable tips capable of delivering 10 µL.

7.3 *Plating Medium*, soybean-casein digest agar, or equivalent.[6]

7.4 *Dilution Fluid*, Butterfield's phosphate buffer[7], or equivalent.

7.5 *Isopropanol or Ethanol, 60 to 75% (v/v)*

7.6 *Sterile Disposable Culture Dishes*, 1.4 × 0.4 in (35 mm by 10 mm) and 4.0 × 0.8 in (100 mm by 20 mm).

7.7 *Sterile Test Tubes*.

7.8 *Surgical Adhesive Tape*, or equivalent.

7.9 *Disposable Examining Gloves*.

7.10 *Inoculating Loop or Glass Spreader*.

7.11 *Appropriate Bacterial Cultures*.

7.12 *Test Formulations*—Directions for application of active and control formulations should be followed, if available. If directions are not available, the directions provided in this test method may be applied.

8. Test and Control Skin Sites

8.1 The volar aspect of the forearm is commonly used as the location of the skin sites, but other areas such as the back or forehead may be used for test sites. Application of active test and control formulations (or no treatment) will be assigned by a predetermined randomization so that either forearm (or either side, right or left, of other anatomical areas) may receive active or control formulations (or none).**Warning—**Application of agar patch plates and alcohol to the forehead risks contaminating the eyes, and extra precautions must be exercised.

9. Subjects

9.1 *Number of Subjects*—The number of subjects used in the test depends on the statistical significance required for the expected results, the sampling variability encountered in the study, and the relative efficacy of the active formulation being evaluated.

9.1.1 Recruit a sufficient number of healthy adult subjects who have no clinical evidence of dermatoses, open wounds, or other skin disorders that may affect the integrity of the test.

9.2 Instruct the subjects to avoid contact with antimicrobials for at least the week prior to testing and, other than the active formulation, for the duration of the test. This restriction includes spray antiperspirants and deodorants, shampoos, lotions, dishwashing detergents, and soaps containing antimicrobial compounds, and materials such as acids, bases, and solvents. Bathing in biocide-treated pools, hot tubs, or spas should be avoided. Subjects may be provided with a kit of

[5] Yackovich, F., C.A. Wagner, and J.E. Heinze. 1989. Validity of the agar patch test with an antibacterial liquid soap and comparison with the finger imprint method. *J. Soc. Cos. Chem.* 40:263-271.

[6] *U.S. Pharmacopeia* XXIV, NF 19. 2000. United States Pharmacopeial Convention Inc., Rockville, MD. Chapter 61, entitled " Microbial Limits Test."

[7] Horowitz, W. (Ed.). 1980. *Official Methods of Analysis of the AOAC*, 13th Ed. Sec. 46.013(m), p. 825. Assoc. of Official Analytical Chemists, Washington, D.C. 1018 pp.