



Standard Test Method for Evaluation of Antimicrobial Formulations by the Agar Patch Technique¹

This standard is issued under the fixed designation E 1882; 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, Parts 50 and 56).

2. Referenced Documents

2.1 *Federal Standard: Protection of Human Subjects*, 21 CFR Ch. I, Parts 50 and 56².

2.2 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.

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

2.4 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.

¹ This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

Current edition approved May 10, 2000. Published August 2000. Originally published as E 1882 – 97. Last previous edition E 1882 – 97.

² Available from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

3. Terminology

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

3.2 *active ingredient*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

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

3.4 *antibacterial activity*—bacteriostatic and/or bactericidal activity.

3.5 *control formulation*—a formulation that does not contain an active ingredient.³

3.6 *control plate*—inoculated 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 attached 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 normally live and multiply on skin, forming a permanent population.

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

3.12 *volar aspect of the forearms*—the inside of the forearm on the same side 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 10⁴ CFU/cm² 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

³ Yackovich, F., Wagner C.A., Heinze J.E. 1989.