Designation: E1874 – 14

Standard Test Method for Recovery of Microorganisms From Skin using the Cup Scrub Technique[1]

This standard is issued under the fixed designation E1874; 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 is designed to recover microorganisms from the skin of human subjects or human subject surrogates (animal skin, isolated porcine skin, human skin equivalents, and other such surfaces).

1.2 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) is required.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *contralateral, adj*—on or relating to the opposite side (of the body).

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

3.1.3 *scrub cups, n*—sterile cylinders of suitable composition (that is, glass, ceramic, stainless steel, plastic, etc.) used to isolate a sample area of skin (or skin equivalent) and confine a aliquot of liquid which is used to facilitate the scrubbing of the skin and removal of microorganisms from the skin surface by pipetting.

3.1.4 *transient organisms, n*—organisms from the environment that contaminate but do not normally colonize skin.

4. Summary of Test Method

4.1 This test method describes a technique suitable for the recovery of resident and transient microorganisms from human or animal skin; the technique may be used in situ within clinical protocols or *in vitro* for studies using isolated skin or skin equivalents.

4.1.1 Resident microorganisms or transient microorganisms (previously applied to a test site), are recovered from the site by pressing a rigid cylinder against the skin with sufficient pressure to form a seal and instilling recovery liquid into the cylinder. The surface of the skin is then mechanically 'scrubbed' with a glass rod, rubber policeman, or some other suitable device for a prescribed period of time. The fluid is pipetted from the cylinder into a test tube, or other suitable receptacle, for further analysis.

5. Significance and Use

5.1 The procedure can be incorporated into protocols used to evaluate test materials containing antibacterial ingredients that are intended to reduce significantly the number of organisms on intact skin. It also may be used to provide an indication of residual antibacterial activity. Examples of test materials, for which this method is applicable, include hand-washes, surgical scrubs, acne reduction products, and others. For each type of test material, types of resident flora or transient organisms, or a combination thereof, may differ and should be considered (this is, aerobic bacteria, anaerobic bacteria, yeast, or mold).

5.2 The procedure may be used in protocols intended to evaluate and identify resident flora from the skin.

5.3 Performance of this technique may require the knowledge of regulations pertaining to the protection of human subjects if the protocol involves application of the technique to the skin of human subjects.

[1] This tests method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents 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.

6. Apparatus

6.1 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization.

7. Reagents and Materials

7.1 *Scrub Cups*—Sterile cylinders of suitable composition, preferably with rod handles to facilitate stabilization, height approximately 2.5 cm, inside diameter of convenient size. Useful sizes range from approximately 1.5 to 4.0 cm.

7.2 *Polished Glass Rod or Rubber Policeman*—Can be fashioned in the laboratory or purchased.

7.3 *Pipettor*—With disposable tips to deliver appropriate volume(s).

7.4 *Sterile Beakers, Test Tubes or other container,* to receive the cup scrub fluid.

7.5 *Appropriate Bacterial Cultures*—If this test method will be used within a protocol targeting transient organisms.

7.6 *Sampling and Dilution Fluid*—Sterile Butterfield's phosphate buffered water or other recovery fluid of suitable composition; this should contain an antimicrobial inactivator specific for any antimicrobial that might be on the test site; inactivator efficacy should be determined by Test Method E1054.

8. Test Control and Baseline Skin Sites

8.1 Select skin sites appropriate for target flora and the protocol objectives; where possible, contralateral sample sites are recommended for use as controls.

9. Sample Site

9.1 *Subjects*—The number of subjects (human or animal) required (if the protocol is *in vivo*) depends on the statistical confidence needed for the expected test results, the variability encountered in the study, and the relative efficacy of any antibacterial agent that may be evaluated. There may be multiple sites available on subjects; randomization is required to suppress sample bias.

9.2 *Isolated Skin or Equivalents*—The number of replicates required to discriminate effects will depend in part on the appropriateness and design of controls within the protocol.

9.2.1 The use of this technique on isolated skin or equivalents is dependent on securing the test site in order to effectively perform the procedure.

10. Sampling Live Subjects (Human or Animal)

10.1 *Method:*

10.1.1 Quantitative microbial counts are obtained by the cup scrub technique.[3] This procedure is used at test and control sites.

10.1.2 Subjects are positioned for site sampling.

10.1.3 The area to be sampled is delineated by a sterile sampling cylinder. The cylinder is pressed firmly against the skin surface during sampling to ensure that the sampling fluid does not leak from the sampling site.

10.1.4 A minimum 1.5-mL aliquot of sterile sampling fluid, with or without product neutralizers, is pipetted into the cylinder. The entire area is then scrubbed with moderate pressure for 60 ± 6 s using a sterile polished glass rod or policeman. After scrubbing, the sampling fluid is transferred by pipette into a sterile sample tube. This procedure is repeated once more with a fresh aliquot of sampling fluid. The sampling fluids are pooled. This procedure is repeated for each sampling site.

10.1.5 The same pipettes, cylinders, glass rods, and policeman are used for both washes of a site, but new sterile equipment is used for each site. After samples are collected, paper toweling is used to blot the site dry.

10.1.6 Care must be taken during this process to prevent the sampling fluid from spilling into an adjacent site that has not been sampled.

10.1.7 Following all sampling, when using marker organisms, the sampling site should be decontaminated using 70 to 90 % isopropanol (or equivalent), followed by a 4 % chlorhexidine scrub (or equivalent).

11. Sampling Isolated Skin or Skin Equivalents

11.1 *Method:*

11.1.1 Quantitative microbial counts are obtained by the cup scrub technique.[3] This procedure is used for test and control samples.

11.1.2 Samples are positioned and secured as necessary to enable placement and effective use of the sampling cylinder.

11.1.3 The area to be sampled is delineated by a sterile sampling cylinder. The cylinder is pressed firmly against the sample surface during sampling to ensure that the sampling fluid does not leak from the sampling site.

11.1.4 A minimum 1.5-mL aliquot of sterile sampling fluid, with or without product neutralizers, is pipetted into the cylinder. The entire area is then scrubbed with moderate pressure for 60 ± 6 s using a sterile polished glass rod or policeman. After scrubbing, the sampling fluid is transferred by pipet into a sterile sample tube. This procedure is repeated once more with a fresh aliquot of sampling fluid. The sampling fluids are pooled. This procedure is repeated for each sampling site.

11.1.5 The same pipettes, cylinders, glass rods, and policeman are used for both washes of a site, but new sterile equipment is used for each site.

11.1.6 If there are multiple sample sites on the same piece of isolated tissue, care must be taken during this process to prevent the sampling fluid from spilling into an adjacent site that has not been sampled.

12. Microbial Counts

12.1 Each sample is mixed thoroughly. Tenfold serial dilutions of each sample are prepared in dilution fluid. Duplicate quantitative pour or spread plates are prepared. Selection of agar is dependent upon purpose of method execution. For

[3] Williamson, P., and Kligman, A. M., "A New Method for the Quantitative Investigation of Cutaneous Bacteria," *Journal of Investigative Dermatology*, Vol 46, 1965, pp. 498–503.

determination of antibacterial effectiveness or residual antimicrobial activity, or both, agar shall contain suitable neutralizer. Type(s) of resident flora of interest in the study shall also be considered in selecting media (that is, aerobic bacteria, anaerobic bacteria, yeast or mold). Incubate plated samples at suitable growth temperature and atmosphere conditions for organism(s) of interest, or until colonies are visible on the plates.

12.2 If the intended evaluation is for identification of resident flora, the collected samples may be frozen and utilized for sequencing or other methods for direct (that is, without first growing samples on agar) organism identification.

13. Precision and Bias

13.1 A precision and bias statement cannot be made for this test method at this time.

14. Keywords

14.1 cup scrub; resident flora; skin; skin equivalent; transient organism