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Standard Practice 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.

ε[1] NOTE—The standard type was editorially corrected in April 2023.

1. Scope

1.1 This practice 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

E2752 Guide for Evaluation of Residual Effectiveness of Antibacterial Personal Cleansing Products

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Federal Document:*[3]

CFR Parts 50 and 56 Code of Federal Regulations: Protection of Human Subjects; Institutional Review Boards

3. Terminology

3.1 *Defintions*—For definitions of terms used in this document, see Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

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

3.2.2 *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.

4. Summary of Practice

4.1 This practice 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.

4.2 Resident and transient microorganisms and/or surrogate 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 polished 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.

[1] This practice 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.

[3] Available from DLA Document Services, Building 4/D, 700 Robbins Ave., Philadelphia, PA 19111-5094, http://quicksearch.dla.mil.

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 (as in Guide E2752). Examples of test materials, for which this practice is applicable, include pre-operative skin preparations, hand-washes, surgical scrubs, acne reduction products, and others. For each type of test material, types of resident flora or surrogate 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.

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 practice 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 Practice E1054.

7.7 *Agar*—Selection of agar is dependent upon purpose of the method execution and should be appropriate for growth of the microorganisms. For determination of antibacterial effectiveness or residual antimicrobial activity, or both, agar shall contain suitable antimicrobial inactivator; inactivator efficacy should be determined by Practice 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.

8.2 When using this practice to evaluate a test material in reducing microorganisms, a baseline sample must be collected. A baseline site shall be identified adjacent to identified sample site.

8.3 While designing a study following this practice, proper controls must be taken into consideration. Some examples are:

8.3.1 *Test article control*—A formula similar to that under test with antimicrobial ingredients removed.

8.3.2 *Inert test sample*—Sampling fluid used to measure the amount of bacteria removed following sampling process.

8.3.3 *Surrogate microorganism*—Similar to a baseline sample including application of the study organism prior to recovery.

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

10.1 *Method:*

10.1.1 Quantitative microbial counts are obtained by the cup scrub technique.[4] 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 The volume of sterile sampling fluid must be defined based on study purpose, neutralization, and plan for sample processing. It is critical that the fluid be sufficient to fully cover the delineated sample area. A larger volume may be needed for sufficient neutralization of test sample and/or needed for robust organism recovery onto multiple media types.

10.1.5 A defined aliquot of sterile sampling fluid, with or without product neutralizers, is pipetted into the cylinder. The skin area inside the cylinder is then massaged/scrubbed, with moderate pressure using a sterile polished glass rod or policeman. (It is important that uniform pressure and repetitive scrub pattern be used for every sample site.) 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.

10.1.6 The same pipettes, cylinders, glass rods, and policeman are used for both scrubs of a site, but new sterile

[4] 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.