



Designation: ~~E 1874–97~~ Designation: E1874 – 09

# Standard Test Method for ~~Evaluation of Antibacterial Washes by Cup Scrub~~ ~~Technique~~ Recovery of Microorganisms From Skin using the Cup Scrub Technique<sup>1</sup>

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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

~~1.1 This test method is designed to demonstrate the effectiveness of an antibacterial wash product in reducing the resident microbial flora or a marker organism (representing transients) when used as recommended. Microbial activity can be compared with either a bland soap control or to a baseline organism count. Microbial samples can be collected either manually or by the mechanical Thran spray gun sampler.~~

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

~~1.3 In this test method, metric units are used for all applications.~~

~~1.4~~

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.

~~1.5 It is the responsibility of the investigator to determine if Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) is required.~~

## 2. Referenced Documents

2.1 *ASTM Standards*:<sup>2</sup>

E1054 ~~Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products~~

~~E1173 Test Method for Evaluation of a Pre-Operative Skin Preparation~~ Test Methods for Evaluation of Inactivators of Antimicrobial Agents

## 3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 ~~bland control soap~~ contralateral, *n*—a formulation that does not exhibit antimicrobial activity under the test parameters. adj—on or relating to the opposite side (of the body).

3.1.2 ~~marker organism~~ resident flora, *n*—an applied inoculum of an organism that has characteristics that allow it to be readily identified or differentiated. Marker organisms are used to simulate transient microorganisms. It also is referred to as a simulant or bacterial contaminant.—microorganisms that live and multiply on skin, forming a permanent population.

3.1.3 ~~resident flora~~ scrub cups, *n*—microorganisms that live and multiply on skin, forming a permanent population.—sterile

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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<sup>2</sup> Thran spray gun bacterial sampler may be obtained through Dr. Volker Thran, Kommission der Europäischen Gemeinschaften, Rue de la Loi 200, B-1049 Brussel, Belgium.

<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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 permanently colonize skin.

#### 4. Summary of Test Method

##### 4.1 *Resident Flora*:

4.1.1 This test method is conducted on a group of volunteer subjects who have refrained from using oral and topical antimicrobials for at least one week and exhibit skin flora counts of at least  $1 \times 10^3/\text{cm}^2$  on the target sites.

4.1.2 Activity of the antibacterial wash is measured by comparing bacterial counts obtained at a specified time interval after application of the test material to one test site with the activity of a bland soap control or with a baseline value. The sites used for comparison should be contralateral to the test site whenever possible.

##### 4.2 *Transient Organisms (Marker)*:

4.2.1 This test method is conducted on a group of volunteer subjects who have refrained from using oral and topical antimicrobials for at least one week.

4.2.2 Activity of the antibacterial wash is measured by comparing microbial counts of a marker organism applied to test sites after application of the test material with counts obtained after application of a bland soap control to a contralateral site.

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 should 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.

5.2 Performance of this procedure requires technique may require the knowledge of regulations pertaining to the protection of human subjects (see Practices E 1054E-1054 and Test Method E 1173E-1173). if the protocol involves application of the technique to the skin of human subjects.

#### 6. Apparatus

6.1 *Colony Counter*—Any of several types may be used.

6.2 *Incubator*—Any incubator capable of maintaining a suitable temperature  $\pm 2^\circ\text{C}$  may be used.

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

6.4 *Timer (Stop Clock)*—One that displays hours and minutes.

6.5 *Examining Table*—Any elevated surface, (such as a 3 by 6-ft (1 by 2-m) table with mattress or similar padding to allow the subject to recline, when applicable).

6.6 *Thran Spray Gun Bacterial Sampler*:

6.6.1 *Filtered Air Source*, capable of maintaining 30 psi.

6.6.2 *Sterile Thran Reservoir Jar*, with 3-hole stopper (one/sample):

6.6.3 *Sterile Thran Collector Jar*, with 5-hole stopper (one/sample):

6.6.4 *1-L Vacuum Flask*, with trap.

#### 7. Reagents and Materials

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

7.2 *Water Dilution Bottles*—Any sterilizable glass container having a 100 to 200-mL capacity and tight closure may be used.

7.3 *Scrub Cups*—Sterile glass cylinders with glass 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.

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

7.5

7.3 *Pipettor*—With disposable tips capable of delivering 25  $\mu\text{L}$ .

7.6 *Sterile 100-mL Graduated Cylinders*.

7.7 *Sterile 400-mL Beakers*:

7.8 —*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*:

7.9 *Test Formulations*, with directions for use.