Designation: E 1874 – 97

Standard Test Method for Evaluation of Antibacterial Washes by Cup Scrub Technique[1]

This standard is issued under the fixed designation E 1874; 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 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[2] 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 *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:*

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[3]

E 1173 Test Method for Evaluation of a Pre-Operative Skin Preparation[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *bland control soap*, *n*—a formulation that does not exhibit antimicrobial activity under the test parameters.

3.1.2 *marker organism*, *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.

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

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 $1 \times 10^3/cm^2$ 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.

5. Significance and Use

5.1 The procedure should be 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 the knowledge of regulations pertaining to the protection of human subjects (see Practices E 1054 and Test Method E 1173).

6. Apparatus

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

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[1] This tests 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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[2] Thran spray gun bacterial sampler may be obtained through Dr. Volker Thran, Kommission der Europaischen Gemeinschaften, Rue de la Loi 200, B-1049 Brussel, Belgium.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

6.2 *Incubator*—Any incubator capable of maintaining a suitable temperature ±2°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.*[4]

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.[5]

7.2 *Water Dilution Bottles*—Any sterilizable glass container having a 100 to 200-mL capacity and tight closure may be used.[6]

7.3 *Scrub Cups*—Sterile glass cylinders with glass rod handles, 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 *Pipettor*—With disposable tips capable of delivering 25 µL.

7.6 *Sterile 100-mL Graduated Cylinders*.

7.7 *Sterile 400-mL Beakers*.

7.8 *Appropriate Bacterial Cultures*.

7.9 *Test Formulations*, with directions for use.

7.10 *Sampling and Dilution Fluid*—Sterile Butterfield's phosphate buffered water,[5] containing an antimicrobial inactivator specific for the test formulation as determined by Test Method E 1054.

7.11 *Plating Medium*—Soybean-casein digest agar with 0.5 % polysorbate 80 and 0.07 % lecithin, as determined by Test Method E 1054.

7.12 *Sterile Culture Tubes*, or equivalent.

8. Test Control and Baseline Skin Sites

8.1 Select skin sites appropriate for target flora and the test material's intended use. Where possible, a contralateral site is recommended for application of bland soap (control).

9. Subjects

9.1 *Number of Subjects*—The number of subjects required depends on the statistical confidence for the expected test results, the variability encountered in the study, and the relative efficacy of the antibacterial agent being evaluated.

9.1.1 Recruit a sufficient number of healthy adult volunteers 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, other than the test formulation, for the duration of the test and for at least the week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, and soaps, also such material as acids bases and solvents. Bathing in biocide-treated pools, hot tubs, spas, and so forth, should be avoided. Volunteers may be provided with a kit of nonantimicrobial personal care products for exclusive use during the test, and rubber gloves should be worn when contact with antimicrobial agents cannot be avoided.

10. Sampling

10.1 *Manual Method*:

10.1.1 Quantitative microbial counts are obtained by the detergent cup scrub technique.[7] This procedure is used at test, control, or baseline sites.

10.1.2 Subjects are positioned for site sampling.

10.1.3 The area to be sampled is delineated by a sterile glass 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 of 1.5-mL aliquot of sterile sampling fluid 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 removed and pipetted 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 4 % chlorhexidine scrub.

10.2 *Thran Spray Gun Method*:

10.2.1 Quantitative microbial counts are obtained by the Thran spray gun sampler technique.[4,8]

10.2.2 Subjects are positioned for site sampling.

10.2.3 The head of the Thran Gun is placed firmly against the test site covering an area of approximately 2.4 cm^2.

10.2.4 The gun is turned on, spraying 100 ± 2 mL of sterile sampling solution in a fine mist against the site and collecting

[4] Theiler, R. F., Schmit, C. L., and Rogeim, J. R., "Application of a New Microbiological Technique to the Study of Antiperspirant and Deodorant Soap Efficacy," *Journal of the Society of Cosmetic Chemistry*, 34, pp. 351–359 (1983).

[5] Presterilized/disposable bacteriological pipettes are available from most laboratory supply houses.

[6] Milk dilution bottles of 160-mL capacity have a screw-capped closure and are available from most local laboratory supply houses.

[7] 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.

[8] Fearnley, C., and Cox, A. R., A New Microbiological Approach to the Assessment of Underarm Deodorants, *Internation Journal of Cosmetic Science*, Vol 5, 1983, pp. 97–109.

it in the collector bottle.

10.2.5 The gun is decontaminated between each sampling by dipping the nozzle into sterile deionized water and pumping sterile water through for approximately 10 s. This is repeated using 70 to 90 % isopropanol or equivalent followed by a second sterile water rinse.

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

11. Procedure

11.1 *Resident Flora*:

11.1.1 To ensure a valid baseline count of resident flora for each subject, sample one baseline site adjacent or contralateral to the target site prior to the initial application of test materials.

11.1.2 Application of test material will be assigned by a predetermined randomized application schedule. Each subject will have a test and control site.

11.1.3 Application of test and control test material will consist of an equal number of supervised washes (1 to 5). Washes should occur a minimum of 1 h apart.

11.1.3.1 More than one application may be required to demonstrate residual activity.

11.1.4 Under supervision, subjects wash the test site for 30 ± 3 s with either test or control test material and follow with a warm (38 ± 2°C) water rinse for 30 ± 3 s. Freshly laundered undergarments will be distributed and worn following the treatment application, when applicable.

11.1.5 Microbial recovery samples can be collected either manually or by the mechanical Thran Spray Gun sampler.

11.2 *Transient Organism(s) (Marker)*:

11.2.1 *Preparation of Test Organism(s)*:

11.2.1.1 Marker organism(s) representative of the bacterial flora encountered under the conditions of use should be selected.

11.2.1.2 Transfer culture(s) 2 times (once every 18 to 24 h) into appropriate liquid growth media. The second transfer must be into a volume of media large enough to provide enough organism(s) for the test.

11.2.1.3 Alternatively, the second transfer may be to an agar plate or slant.

11.2.1.4 If preparing inoculum from broth, wash culture 2 times by means of centrifugation and resuspend in Butterfield's phosphate buffered water.[9]

11.2.1.5 If preparing inoculum from agar plate or slant, resuspend organisms in Butterfield's phosphate buffered water.

11.2.1.6 Final concentration of test organism should be adjusted to 1.0×10^5 to 1.0×10^7 cfu/mL. Inoculum should be well mixed to break clumps.

11.2.2 Application of test material will be assigned by a predetermined randomized application schedule. Each subject will have an active and control (bland soap) test site.

11.2.3 Application of test and control test material will consist of an equal number of supervised washes (1 to 5). Washes should occur a minimum of 1 h apart.

11.2.3.1 For demonstration of residual activity, more than one test material application may be required.

11.2.4 Under supervision, subjects wash the test site for 30 ±3 s with either test test material or bland soap and follow with a warm (38 ± 2°C) water rinse for 30 ±3 s. Freshly laundered undergarments will be distributed and worn following the treatment application, when applicable.

11.2.5 Delineate one area adjacent or contralateral to the wash sites, which has not received an application of either active test material or bland soap. Determine marker organism baseline count at this site.

11.2.6 Inoculate test and baseline sites with the appropriate bacterial suspension using a micropipettor. A suspension of 25 µL is deposited on the test site.

11.2.7 Using a sterile polished end glass rod, spread the bacterial suspension on the skin in an area approximately 1.2 cm in diameter.

11.2.8 Determine by a standard plate count method, or equivalent, bacterial titer of the inoculating suspension.

11.2.9 Allow the area to air dry. Sampling should be done within 30 min of inoculum application.

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 using soybean-casein digest agar with suitable neutralizer are prepared. Incubate plated samples at suitable growth temperature, ±2°C for 24 to 72 h, or until colonies are visible.

13. Determination of Microbial Reduction Compared to Control Soap

13.1 Colony forming units (cfu) are calculated for the baseline site and for all other sites before and after treatment.

$$\text{Percent reduction test} = \text{Geometric mean of baseline count} - \frac{\text{Geometric mean test count}}{\text{Geometric mean of baseline count}} \times 100$$

$$\text{Percent reduction control soap} = \text{Geometric mean of baseline count} - \frac{\text{Geometric mean control count}}{\text{Geometric mean of baseline count}} \times 100$$

Reduction also may be reported as $\log_{10}$ of cfu.

14. Determination of Reduction Versus Baseline

$$\text{Percent reduction active versus baseline} = \text{Geometric mean of baseline count} - \frac{\text{Geometric mean of active application}}{\text{Geometric mean of baseline count}} \times 100$$

Reduction may also be reported as $\log_{10}$ of cfu.

15. Precision and Bias

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

16. Keywords

16.1 antibacterial wash; cup scrub; resident flora; Thran spray gun; transient organism

[9] Butterfield's Phosphate Buffer, *Journal of the Association of Official Analytical Chemists*, Vol 22, No. 625, 1939.