



Standard Test Method for Evaluation of a Pre-Operative Skin Preparation¹

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1. Scope

1.1 The test method is designed to determine the ability of a pre-operative skin preparation to reduce the resident microbial flora on the skin when used in a skin pre-operative preparation procedure.

1.2 In this test method, metric units are used for all applications except for distance, in which case inches are used and metric units follow in parentheses.

NOTE 1—A knowledge of microbiological techniques is required for these procedures.

1.3 *This standard does not purport to address all of the safety problems, 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:

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products²

3. Summary of Test Method

3.1 This test method is conducted on panelists selected from a group of volunteers who, after refraining from using topical and oral antimicrobials for at least two weeks, exhibit high skin flora counts on the abdomen and groin.

3.2 Activity of the pre-operative skin preparation is measured by comparing microbial counts obtained at various time intervals after application of the pre-operative treatment to skin sites located on the abdomen and in the groin to counts obtained from the same sites prior to treatment application.

NOTE 2—Microbial samples are collected a minimum of three times after treatment application. The first two collections are made 10 min and 30 min post treatment, and the third collection is made no less than 4 h post treatment but may be made later.

4. Significance and Use

4.1 The procedure should be used to test antimicrobial-

containing preparations that are intended to be fast-acting and to significantly reduce the number of organisms on intact skin.

4.2 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.³

5. Apparatus

5.1 *Colony Counter*—Any of several types may be used, for example, Quebec colony counter.

5.2 *Incubator*—Any incubator capable of maintaining a temperature of $30 \pm 2^\circ\text{C}$ may be used.

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

5.4 *Timer (stop-clock)*—One that can be read for hours and minutes.

5.5 *Examining Table*—Any elevated surface such as a 3 by 6-ft table with mattress or similar padding to allow the subject to recline.

6. Reagents and Materials

6.1 *Bacteriological Pipette*—10.0 and 2.2-mL or 1.1-mL capacity.⁴

6.2 *Water Dilution Bottles*—Any sterilizable glass container having a 150 to 200-mL capacity and tight closure may be used.⁵

6.3 *Scrubbing Cups*—Sterile glass cylinders, height approximately 2.5 cm, inside diameter of convenient size to place on anatomical area to be sampled. Useful sizes range from approximately 1.5 to 4.0 cm.

6.4 *Rubber Policeman*—Can be fashioned in the laboratory or purchased from most laboratory supply houses.

6.5 *Test Formulation*—With directions for use.

6.6 *Sterile Drape or Dressing*⁶—Used to cover treated skin sites.

6.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4 , 10.1 g Na_2HPO_4 and 1.0 g isocetylphenoxypolyethoxyethanol⁷ in 1 L of distilled water. Include in this formulation an inactivator specific for the antimicrobial in the test formulation. Adjust to

³ See *Federal Register*, Vol 46, No. 17, Jan. 27, 1981.

⁴ Presterilized/disposable bacteriological pipettes are available from most laboratory supply houses.

⁵ Milk dilution bottles of 160-mL capacity having a screw-cap closure are available from Corning Glass Co., Kimble Glass Co. or most local laboratory supply houses.

⁶ A suitable covering is TELFA Non-Adherent Dressing, No. 3279, from the Kendall Co.; Hospital Products; Boston, MA 02101.

⁷ Triton X-100, is available from Rohm and Haas Co., Philadelphia, PA.

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.

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² *Annual Book of ASTM Standards*, Vol 11.05.

pH 7.8. Dispense in 100-mL volumes and sterilize for 20 min at 121°C.

6.8 *Dilution Fluid*—Butterfield's⁸ phosphate buffered water adjusted to pH 7.2 and containing an antimicrobial inactivator specific for the test formulation. (See Practices E 1054.)

6.9 *Plating Medium*—Soybean-casein digest agar.⁹

7. Test and Control Skin Sites

7.1 The skin sites selected for use in evaluating the effectiveness of the pre-operative skin preparation should represent body areas that are common surgical sites and should include both dry and moist skin area. The sites should possess bacterial populations large enough to allow demonstrations of bacterial reduction of up to 2.0 log₁₀ cm² on dry skin sites and up to 3.0 log₁₀ cm² on moist sites. Baseline populations of at least 4.5 log₁₀/cm² on wet skin sites and of at least 3.5 log₁₀/cm² on dry skin sites are recommended. A suitable dry skin area is the abdomen, and a suitable moist area is the axilla or groin.

7.2 Abdominal treatment areas are to be located within a 5 by 5-in. (12.7 by 12.7-cm) site located in the vicinity below the umbilicus, one either to the right or left of the median. Using a 5 by 5-in. (12.7 by 12.7-cm) sterile paper template, the corners of each site are marked as 1, 2, 3, and 4 directly on the skin, using a sterile surgical skin marker. Numbering is to be the same for all abdominal sites; number 1 is placed at the top corner to the subject's right, and numbers 2, 3, and 4 are assigned in order clockwise from 1. Each quadrant of each site represents a different treatment exposure of either 10 min, 30 min, or ≥4 h. The remaining fourth quadrant is used as a baseline count site.

7.3 Similarly, using a 2 by 5-in. (5.1 by 12.7-cm) sterile template, 2 by 5-in. (5.1 by 12.7-cm) sites are delineated on the inner aspect of both upper thighs within 2 in. and parallel to the leg crease below the groin. The top corners of the sites are numbered beginning on the subject's right lateral with number 1 and in order through 4.

7.3.1 Three 2 by 2-in. (5.1 by 5.1-cm) areas represent different exposures of 10 min, 30 min, or ≥4 h, and the fourth area is used as a baseline site.

7.3.2 The abdominal and groin sites are illustrated in Fig. 1. A panelist may have two treatments applied to his abdomen, provided that a 5 by 5-in. (12.7 by 12.7-cm) site is defined both on the right and left side of the median. Only one treatment, however, may be applied to the groin, as the right and left groin sites are to receive the same treatment.

8. Procedure

8.1 Recruit healthy adult human volunteers who have no clinical evidence of dermatosis, open wounds, or other skin disorders which may affect the integrity of the study and in sufficient numbers such that 12 qualified abdominal sites and 12 qualified groin sites are available for treatment. This may require entering more than six volunteers into the study, as in all probability not everyone entered into the study will exhibit

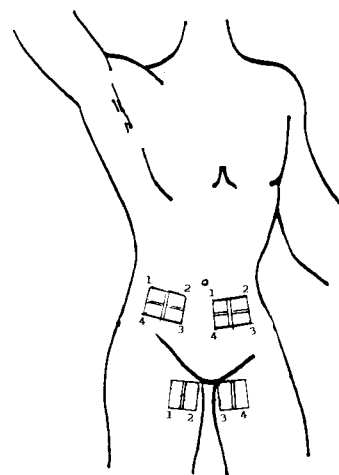


FIG. 1 Illustration of Approximate Location of Test Areas

in the designated skin sites the bacterial population required to demonstrate the log reduction specified in 7.1.

8.2 Instruct the volunteers to avoid contact with antimicrobials (other than the test formulation) for the duration of the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, bathing soaps and body powders. Volunteers also are to refrain from wearing clothes that have been treated with a fabric softener. Bathing in biocide treated pools, hot tubs, spas, and so forth, should be avoided.

8.2.1 Provide volunteers with a kit of non-antimicrobial personal care products for exclusive use during the test. Volunteers are not to shower or tub bathe in the 24-h period prior to the application of test material or microbial sampling. Sponge baths may be taken, but the skin sites to be used in the study are to be excluded.

8.2.2 If the skin sites to be used include areas that would require shaving prior to surgery, for example, the axilla, these sites should be shaved no later than 48 h prior to the application of test formulation or microbial sampling.

8.3 After volunteers have refrained from using antimicrobials for at least 2 weeks, obtain an estimate of baseline bacterial population from a 2 by 2-in. (5.1 by 5.1-cm) area of each 5 by 5-in. (12.7 by 12.7-cm) abdominal site and from a 2 by 2-in. (5.1 by 5.1-cm) area of the left and right groin site. Collect these samples at least 72 h prior to entering subjects into the study. Sampling and enumeration techniques described in Sections 9 and 109 and 10 should be used.

8.4 Based on the initial estimate of baseline bacterial population, select subjects with high abdominal site counts and high groin counts. A total of 12 groin sites and 12 abdominal sites are required.

NOTE 3—A given treatment is to be applied to only one abdominal site on a given subject.

8.5 A second baseline sample is to be collected the day treatment is applied. A single sample is obtained from one 2 by 2-in. (5.1 by 5.1-cm) site in one groin for this baseline estimate and from a 2 by 2-in. (5.1 by 5.1-cm) site within each abdominal area.

8.6 *Treatment Application Procedure*—Immediately after taking the second baseline sample, the treatment is applied

⁸ Butterfield's Phosphate Buffer, *Journal of the Association of Official Analytical Chemists*, Vol 22, No. 625, 1939.

⁹ U.S. Pharmacopeia, XXI: United States Pharmacopeial Convention, Inc., Rockville, MD, see Chapter entitled "Microbial Limits Test," 1985.