Designation: E 1173 – 01

Standard Test Method for Evaluation of a Preoperative, Precatheterization, or Preinjection Skin Preparations[1]

This standard is issued under the fixed designation E 1173; 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 The test method is designed to measure the reduction of the resident microbial flora of the skin.

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 except for linear measure, in which case inches are used, and metric units follow in parentheses.

1.4 *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.*

1.5 Performance of this procedure requires a knowledge of regulations pertaining to the protection of human subjects **(1)**.[2]

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 1874 Test Method for Evaluation of Antibacterial Washes by Cup Scrub Technique[3]

3. Terminology

3.1 *active ingredient*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.2 *test formulation*—a formulation containing an active ingredient(s).

3.3 *internal reference formulation*—a formulation with demonstrated performance characteristics within a specific laboratory.

3.4 *sampling fluid*—a recovery fluid that may or may not contain a neutralizer to inactivate the active ingredient(s) in test and internal reference formulations.

3.5 *persistence*—prolonged or extended antimicrobial activity after treatment that prevents or inhibits the proliferation and/or survival of microorganisms.

3.6 *neutralization*—a process that results in quenching the antimicrobial activity of a formulation. This may be achieved through dilution of the formulation to reduce the antimicrobial activity, or through use of chemical agents, called neutralizers, to curtail antimicrobial activity.

4. Summary of Test Method

4.1 These test methods are conducted on human subjects selected randomly from a group of volunteers who, after refraining voluntarily from using topical and oral antimicrobials for at least two weeks (14 days), exhibit acceptably high normal flora counts on the skin sites to be used in testing (see Section 8).

4.2 The antimicrobial activity of the preoperative, precatheterization, or preinjection skin preparations is measured by comparing microbial counts, obtained at various time intervals after application of a test formulation to skin sites, to counts obtained from those same sites prior to application of the test formulation. Skin sites recommended for use in testing are: 1) the inguinal region and the abdomen for preoperative skin preparations; 2) the inguinal region, the subclavian (clavicular) region, and/or the median cubital region of the arm for precatheterization preparations; and 3) the median cubital region of the arm for preinjection skin preparations.

4.2.1 *Preoperative Skin Preparation*—Microbial samples are collected from the test sites a minimum of 3 times after treatment application on both moist and dry skin sites. The sample times are 10 min, 30 min, and 6 h (or other appropriate times) post-treatment.

4.2.2 *Precatheterization Preparation*—Microbial samples are collected from the test sites a minimum of 3 times after treatment application on both moist and dry skin sites. The sample times are "immediate," 12 h, and 24 h post-treatment. The immediate sample may be 30 sec to 10 min, depending on the test material being evaluated.

4.2.3 *Preinjection Preparation*—A microbial sample is collected from the test site 30 sec post-treatment.

4.3 The fluid used for sampling the test sites must effectively quench (neutralize) the antimicrobial action of all formulations tested. The effectiveness of the inactivator must be demonstrated prior to initiation of product-testing, as

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard..

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

described in Practices E 1054, and using in vivo techniques consistent with the cup-scrub technique (see Section 10).

4.4 To ensure the internal validity of the test, an internal reference formulation having performance characteristics known to the laboratory should be tested in parallel with the test formulation.

5. Significance and Use

5.1 These procedures should be used to test topical antimicrobial-containing preparations that are intended to be fast-acting and to reduce significantly the number of organisms on intact skin immediately and, for preoperative and precatheterization preparations, to maintain reductions for an extended time.

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used; for example, Quebec colony counters and similar devices, or automated, computerized plater/counter systems.

6.2 *Incubator*—Any incubator that can maintain a temperature of 30° ± 2°C may be used.

6.3 *Sterilizer*—Any steam sterilizer that can produce the conditions of sterilization is acceptable.

6.4 *Timer (stopwatch)*—One that displays hours, minutes, and seconds.

6.5 *Examining Table*—Any elevated surface, such as a 3-by-6-ft (0.9-by-1.8-meter) table with mattress or similar padding to allow the subject to recline.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*—10.0 and 2.2-mL or 1.1-mL capacity, available from most laboratory supply houses.

7.2 *Petri Dishes*—100 mm by 15 mm, for performing standard plate counts, available from most laboratory supply houses.

7.3 *Scrubbing Cups*—Autoclavable cylinders, height approximately 1 in (2.5 cm), inside diameter of convenient size to place on anatomical area to be sampled. Useful diameters range from approximately 0.5 to 1.5 in (1.3 to 3.8 cm), depending on sites to be sampled.

7.4 *Rubber Policeman or Teflon® Scrubbers*—Can be fashioned in the laboratory or purchased from most laboratory supply houses (whichever type is selected, it should be used throughout the course of testing).

7.5 *Testing Formulation, including directions for use.*

7.6 *Sterile Gauge Pads*—Used to cover treated skin sites.

7.7 *Sterile Drape or Dressing*[4]—Used to cover treated skin sites.

7.8 *Sampling Fluid*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, and 1.0 g isooctylphenoxypolyethoxyethanol[5] in 1 L of distilled water. Inactivator(s) specific for the antimicrobial active(s) in the test and reference formulations may be included, if appropriate. Adjust to pH 7.8. Dispense in appropriate volumes and sterilize.

7.9 *Dilution Fluid*—Butterfield's **(2)** phosphate-buffered water adjusted to pH 7.2, or other suitable diluent, which may or may not contain antimicrobial inactivators specific for the test and reference formulations (see Practices E 1054).

7.10 *Plating Medium*—Soybean-casein digest agar **(3)**, with or without antimicrobial inactivators.

7.11 *Sterile Template Material*—Used to demarcate the skin sites; made from paper, plastic, or cloth, for example.

7.12 *Surgical Skin Marker*—Used to mark the skin sites.

NOTE 1—Some markers contain crystal violet, which is inhibitory to many bacteria.

8. Skin Sites to be Used in Testing

8.1 *Preoperative Skin Preparations*

8.1.1 The skin sites selected for evaluation of the effectiveness of preoperative skin preparations should include body areas that may be surgical sites and should include both moist and dry skin areas. Bacterial baseline populations should be at least 3.0 $\log_{10}/cm^2$ greater on moist skin sites than the detection limit of the sampling procedure and at least 2.0 $\log_{10}/cm^2$ greater than the detection limit on dry skin sites. High baseline counts are desired, because variability in the bacterial counts may be reduced. The preferred moist-skin areas are the inguina, and the preferred dry-skin area is the lower abdomen below the umbilicus.

8.1.2 Using a 1.5-by-5-in (3.8-by-12.7-cm) sterile template (for example, paper, plastic, cloth), treatment sites are delineated on the uppermost inner aspects of both thighs (the inguina), centering the long axis of the template along the inguinal crease immediately below the groin and marking the corners, using a surgical skin marker. If, due to a subject's anatomy, the treatment site cannot be centered along the inguinal crease, the site should be positioned on the upper, inner thigh as close to the crease as possible. In no instance should sampling be performed on areas not having skin-to-skin contact. The site is then divided on the long axis into 1-by-1.5-in (2.5-by-3.8-cm) sampling areas, allowing for spaces of about 0.25 in (about 0.6 cm) between each of the four areas.

8.1.2.1 Sampling areas are numbered from anterior to posterior, beginning with 1 and proceeding perineally to 4. To test a "worst-case scenario" for efficacy of preoperative skin preparations (see Note 2, below), the areas are not randomized for baseline and exposure times. Area 1 is always used for baseline, and areas 2 through 4 for product exposure times of 10 min, 30 min, and 6 ≥ h, respectively.

NOTE 2—Bacterial populations in the inguina are known to be heterogeneous, with counts tending to increase proceeding from the upper reaches of the inguinal crease perineally toward convergence of the inguina at the gluteal fold, and to decrease proceeding from the inguinal crease laterally onto the (dry) surface of the upper thigh. Hence, to provide a fair test of a formulation's antimicrobial efficacy, a "worst-case" sampling scheme is suggested. Baseline sampling is performed in sampling area 1 (lowest count area), with post-treatment sampling progressively deeper, and sampling areas must be confined to the inguinal crease where skin-to-skin contact provides the moist environment conducive to bacterial growth. If preferred, however, the sampling areas may be randomized to baseline and post-treatment sampling times. Because of the increased variance of the count data, it is likely that such a design will require testing of substantially more subjects in order to demonstrate

[4] A suitable covering is TELFA® non-adherent dressing, No. 3279, from the Kendall Co.; Hospital Products; Boston, MA 02101.

[5] Triton X-100, is available from Rohm and Haas Co., Philadelphia, PA.

statistical significance of post-treatment reductions.

8.1.2.2 Because of constraints imposed by the anatomical area, sampling cylinders used for the inguinal sites must be ≤ 1 in (≤ 2.54 cm) in diameter.

8.1.2.3 The test formulation and reference material are randomized one to each side.

8.1.3 Abdominal treatment sites are to be located within 5-by-5-in (12.7-by-12.7-cm) sites below and to the right or left of the umbilicus, approximately midway between the umbilicus and the pubis. Using a 5-by-5-in (12.7-by-12.7-cm) sterile template (for example., paper, plastic, cloth), the corners of each site are numbered 1, 2, 3, and 4 directly on the skin, using a 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. Three quadrants of each site are used for the different treatment exposure times of 10 min, 30 min, or ≥ 6 h, and the remaining quadrant is used for a baseline count. The test formulation or reference material should be randomized to the four quadrants of each site.

8.2 *Precatheterization Skin Preparations*

8.2.1 The skin sites selected for evaluation of the effectiveness of precatheterization skin preparations should include body areas that may be catheterization sites and should include both moist and dry skin areas. Bacterial baseline populations should be at least 3.0 $\log_{10}/cm^2$ greater on moist skin sites than the detection limit of the sampling procedure and at least 1.0 $\log_{10}/cm^2$ greater than the detection limit on dry skin sites. High baseline counts are desired, because variability in the bacterial counts may be reduced. The preferred moist-skin areas are the inguina, and the preferred dry-skin areas are the clavicular region and the median cubital region of the arm.

8.2.2 Test sites in the inguina are to be located and evaluated as specified for testing of preoperative skin preparations (see 8.1.2, Note 1, and Fig. 1).

8.2.3 The dry skin sites and sampling configurations used in testing precatheterization preparations are illustrated in Fig. 1 Detail A. Sterile templates (for example, paper, plastic, cloth) are fashioned for the sampling configuration such that they accommodate the diameter of the sampling cylinder, plus at least 0.5 in (1.25 cm) between the 4 sampling areas. The template is applied to the treatment site, and a surgical skin marker is used to demarcate the sampling areas. These are numbered 1 through four at outside corners, beginning at the subject's upper right and proceeding clockwise in the clavicular region, and beginning proximally and proceeding distally on the arm. Three sampling areas of the site are used for different treatment exposure times of "immediate" (30 sec to 10 min, depending on test product), 12 h, or 24 h, and the remaining sampling area is used for a baseline count. The test formulation and reference material should be randomized to the treatment sites, right or left, and exposure times and baseline should be randomized to the four quadrants of each site.

8.3 *Preinjection Skin Preparations*

8.3.1 The skin site selected for use in evaluating the effectiveness of preinjection skin preparations should represent a body area that is commonly used for transepidermal injection

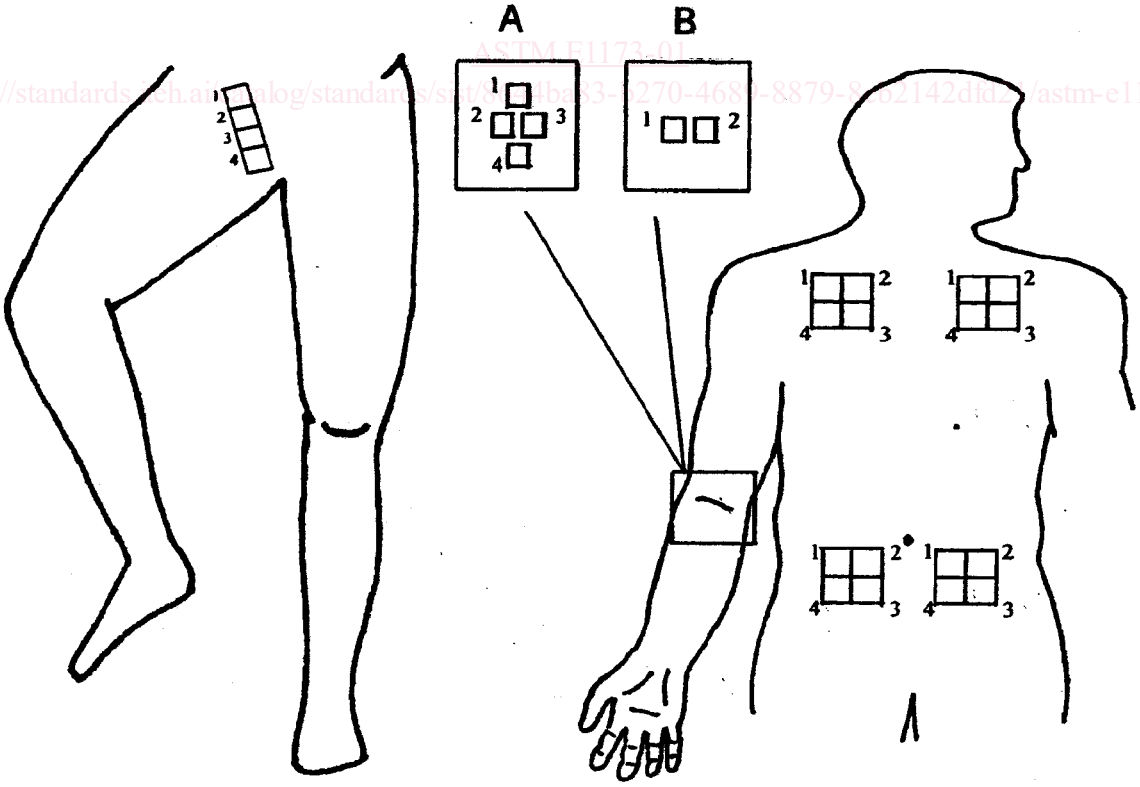

FIG. 1 Illustration of Approximate Sampling Locations on Treatment Sites: Inguinal Crease, Abdomen, Clavicular Region, and Median Cubital Region of Arm