Designation: E1173 – 23

Standard Practice for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations[1]

This standard is issued under the fixed designation E1173; 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 practice is designed to measure the reduction of the microflora of the skin.

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

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3.1 *Exception*—In this practice, 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 Performance of this procedure requires a knowledge of regulations pertaining to the protection of human subjects (**1**).[2]

NOTE 1—Importantly, it must be noted that the FDA currently does not accept data resulting from procedures in this Method for testing products for approval as Vascular Precatheterization Skin Preparations.

1.5 *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, 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:*[3]

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

E1874 Practice for Recovery of Microorganisms From Skin using the Cup Scrub Technique

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 Terms used in this standard are defined in E2756, Standard Terminology Relating to Antimicrobial and Antiviral Agents. Others defined below are specific to their use in this document.

3.2 *Definitions of Terms Specific to This Standard:*

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

3.2.2 *inguen, n*—groin: the junctional region between the abdomen and thigh; pl. *inguina*.

3.2.3 *inguinal crease, n*—the discrete region of flexure between the abdomen and the thigh.

3.2.4 *sampling fluid, n*—a recovery fluid that contains a neutralizer demonstrated to inactivate or quench the active ingredient(s) in the test formulation and the positive and negative control formulations. See Practices E1054.

3.2.5 *test formulation, n*—a formulation containing an active ingredient(s).

4. Summary of Test Method

4.1 This practice is 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 preoperative, vascular precatheterization, or preinjection skin preparations and of controls is measured by comparing microbial counts, obtained at various time intervals after application of a formulation to skin sites to counts obtained from those same sites prior to application of a 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

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

[3] 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.

subclavian (clavicular) region, and/or the median cubital region of the arm for vascular 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 three (3) times after treatment application on both moist and dry skin sites. The recommended sample times are 30 s, 10 min, and 6 h post-treatment, but other relevant times may be selected.

4.2.2 *Vascular Precatheterization Preparation*—Microbial samples are collected from the test sites a minimum of three (3) times after treatment application on skin sites. The recommended sample times are "immediate," 12 h, and 24 h post-treatment, but other relevant times may be selected.

4.2.3 *Preinjection Preparation*—Microbial samples are collected from the skin sites 30 s 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 described in Practices E1054, and using in-vivo techniques consistent with the cup-scrub technique (see Section 10).

4.4 A positive control formulation having performance characteristics known to the laboratory (internal validity of test) and a negative control (test formulation without active ingredient or a physiological saline solution) must 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 in reducing significantly the number of microorganisms on intact skin immediately and, for preoperative and vascular precatheterization preparations, maintenance of some degree of reduction 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 comfortably.

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) and inside diameter of a size convenient to placement on the skin of the 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, TFE-fluorocarbon Scrubbers, or other appropriate device*—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 Dressings*[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 in 1 L of distilled water. Inactivator(s) specific for the antimicrobial active(s) in the test and control formulations must be included (See Practices E1054). 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 must contain antimicrobial inactivators specific for the test, positive and negative control formulations (see Practices E1054).

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 delineate mark the skin sites to be used in testing.

NOTE 2—Because some markers contain crystal violet or other fluids that are inhibitory to many skin microflora, a marker should be proven non-antimicrobial prior to use in testing.

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 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. The preferred moist-skin areas are the inguina, in which skin-to-skin contact results in a moist environment conducive to higher populations of microflora. The preferred dry-skin area is the lower abdomen adjacent to the umbilicus. These areas are illustrated in Fig. 1.

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 in the

[4] The sole source of supply of the apparatus (TELFA non-adherent dressing, No. 3279) known to the committee at this time is Kendall Co.; Hospital Products; Boston, MA 02101. This product is not sterile, but can be steam-sterilized prior to use. If you are aware of alternative suppliers of appropriate dressings, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

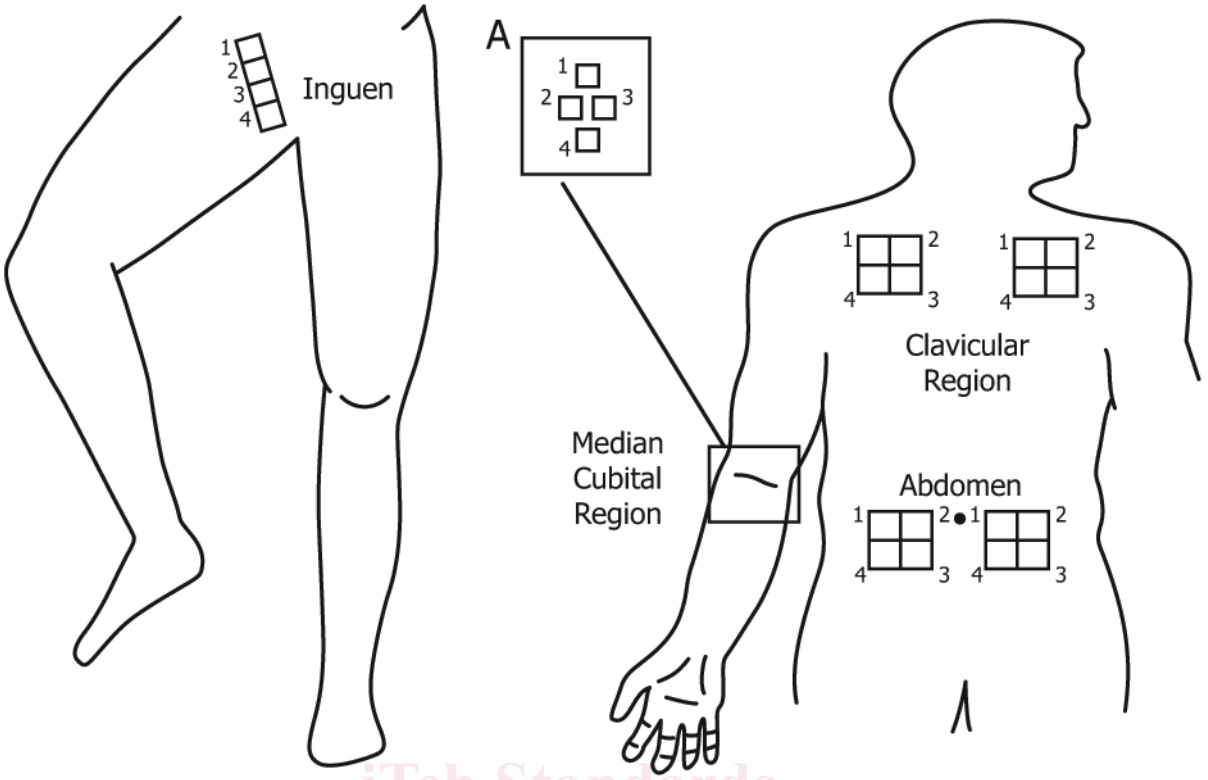

FIG. 1 Illustration of Approximate Sampling Locations on Treatment Sites: Inguen, Abdomen, Clavicular Region, and Median Cubital Region of Arm

inguina are delineated on the uppermost inner aspects of both thighs, centering the long axis of the template approximately 0.5 to 1.0 in. laterally along the inguinal crease, and marking the corners using a surgical skin marker. If, due to a subject's anatomy, the treatment site cannot be positioned as described, the site should be positioned as close as possible. In no instance should testing 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 (about 0.6 cm) between each of the areas.

8.1.2.1 Sampling areas are numbered from anterior to posterior, beginning with 1 and proceeding perineally to 4, and then are randomized to sampling for baseline and the three (or more) post-treatment sampling times (see Note 3).

NOTE 3—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 laterally from the inguinal crease onto the (dry) surface of the upper thigh. Hence, sampling areas must be confined to skin immediately adjacent to the inguinal crease where skin-to-skin contact provides the moist environment conducive to bacterial growth. Note that the large variance in the count data that results from randomization of the sampling areas likely will require testing of a relatively large number of subjects in order to demonstrate any 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 generally approximate ≤ 1 in (≤ 2.54 cm) in diameter.

8.1.2.3 The test formulation and control materials are then randomized bilaterally to the treatment sites.

8.1.3 Abdominal treatment sites are to be located within 5-by-5-in (12.7-by-12.7-cm) areas to the right and left of the umbilicus. 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 three different treatment exposure times, and the remaining quadrant is used for a baseline count. The test formulation and control materials are then randomized to the treatment sites, right and left, and baseline and the three post-treatment sampling times are randomized to the four sampling areas within each site.

8.2 *Vascular Precatheterization Skin Preparations:*

8.2.1 The skin sites selected for evaluation of the effectiveness of vascular 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. 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 8.1.2.1, Note 3, and Fig. 1).

8.2.3 The dry skin sites and sampling configurations used in testing vascular precatheterization preparations are illustrated in Fig. 1 and 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 s), 12 h, or 24 h, and the remaining sampling area is used for a baseline count. The test formulation and control materials 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 or phlebotomy. Bacterial baseline populations should be at least 1.0 $\log_{10}/cm^2$ greater than the detection limit of the sampling procedure. A suitable dry-skin area is the median cubital region of the arm.

8.3.2 The dry-skin site and sampling configuration used in testing preinjection 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 four sampling areas. The template is applied to the treatment site, and a surgical marker is used to demarcate the sampling areas. These are numbered 1 through 4 at outside corners, as illustrated for vascular precatheterization testing (Fig. 1, Detail A). Three sampling areas of the site are used for the treatment exposure of 30 s, and the remaining sampling area is used for a baseline count

8.3.3 The test formulation and control material are then randomized to the treatment sites, and baseline and post-treatment are randomized to the sampling areas.

9. Procedure

9.1 *Number of Subjects*—Because the purpose of the study is to demonstrate efficacy (defined as a significant reduction from baseline counts), sample size calculations should be done to determine the number of subjects per treatment group necessary to find statistically significant differences (reductions) from baseline. The number of subjects required depends on the statistical confidence required for the expected results, the variability encountered in the data collection (for example, variability in reductions from baseline), and the expected efficacy of the test product (for example, approximate reductions from baseline expected). This number of subjects per treatment group (n) can be estimated from the following equation (**4**):

$$n \geq S^2 \left[\frac{(Z_{a/2} + Z_\beta)^2}{D^2} \right]$$

where:

S^2 = estimate of variance (of reductions from baseline based on in-house data pool);
$Z_{a/2}$ = cumulative probability of the standard normal distribution = 1.96 for α = 0.05;
Z_β = power of the test = 0.842 for ß = 0.80; and
D = expected efficacy (expected reduction from baseline).

9.2 Recruit a sufficient number of healthy adult volunteers who have no visual evidence of dermatoses, open wounds, or other skin disorders that may affect the test.

9.3 *Pretest Period (14 days)*—Instruct volunteers selected as test subjects to avoid contact with antimicrobials for the duration of the pretest period and, other than test formulations, for the test periods. This restriction includes antiperspirants, deodorants, shampoos, lotions, bathing soaps, body powders, other hygienic products that contain antimicrobials, and such materials as acids, bases, and solvents. Subjects also are to refrain from wearing clothes that have been treated with antimicrobials or fabric softeners, and from bathing in biocide-treated pools, hot tubs, or spas.

9.3.1 Provide test subjects with a kit of nonantimicrobial personal hygiene products for exclusive use during the pretest and test periods. Subjects are not to shower or tub-bathe during the 24-h period prior to the application of test material or microbial sampling. The bathing restriction period may be lengthened, if desired, to increase bacterial populations.

9.3.2 If the skin sites selected for testing include areas that would require clipping of hair prior to surgery (for example, the abdominal and inguinal regions), hair from these sites should be clipped to reduce difficulties bandaging them. Clipping must be performed at least 48 h prior to microbial sampling for first baseline.

9.4 *Test Period:*

9.4.1 A baseline sample is to be collected at the time of testing. The sample should be taken from a sampling area predetermined by design (for example, on an inguinal site) or by random assignment to sampling areas within a treatment site.

9.4.2 *Treatment Application Procedure*—Immediately after taking the baseline sample, the treatment is applied according to label directions or as stated in the test protocol.

9.4.3 *Sampling Schedule*—According to the predetermined sampling design or randomization, samples of the prepped site are taken from the sampling areas when the specified post-treatment exposure times have elapsed.

NOTE 4—Between the time of treatment application and final sampling, subjects should avoid activities or positions that would cause untreated skin sites or clothing to contact treated sites. To allow the subjects some degree of mobility between the time of treatment and final sampling, the treated skin areas can be covered with a sterile semi-occlusive dressing (7.6 and 7.7). This material is applied in a manner so as to protect the treated skin site from contact with untreated skin, and such that air