



Standard Test Method for Evaluation of First Aid Antiseptic Drug Products¹

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1. Scope

1.1 The tests described in this test method are designed to evaluate antimicrobial agents in formulations intended for use as first aid antiseptic products for their ability to reduce or suppress the growth, or both, of the skin microflora.

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 distance, in which case inches are used and metric units follow in parenthesis.

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.*

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 Methods

3.1 These test methods describe standard *in vivo* techniques to determine the following:

3.1.1 *Effect of the Test Agent to Reduce An Artificially Enhanced Skin Microbial Flora*—The forearms of subjects are occluded for 48 h prior to application of the test agents to increase the microbial population on the skin of the volar forearm surface. At treatment the occlusion material is removed and the skin is allowed to dry, the test agents are then applied to selected sites. At a pre-determined time(s) following application, the sites are microbiologically sampled and the samples plated for total aerobic bacteria count. The counts obtained from the treated sites are compared to counts obtained from untreated occluded sites.

3.1.2 *Effect of the Test Agent to Suppress the Growth of Normal Skin Flora When Applied As a Dressing*—The dressings are applied to the forearm for 24 h. The density of the resident organisms that develop under the dressings are com-

pared to the population that develops on a similar untreated occluded site. Following 24 h of occlusion, the sites are microbiologically sampled and the samples plated for total aerobic bacteria count.

3.2 The principal of the test is that the microflora of forearm skin is sparse. The impermeable dressing will increase surface moisture by preventing diffusional water loss and thus expand transient resident skin microorganisms. A significant effect by the test agent will be reflected by significantly lower population on the treated site.

4. Significance and Use

4.1 The procedures in this test method should be used to evaluate *in vivo* the antimicrobial activity of drug products applied topically to the skin that are intended to help prevent infection in minor cuts, scrapes and burns.

4.1.1 This test method is applicable for testing liquids, ointments, powders, films, or dressing containing or impregnated with an antimicrobial for their effect to reduce an enhanced skin microflora or their effects to suppress the growth of the skin flora, or both.

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 $35 \pm 2^\circ\text{C}$ may be used.

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

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

6. Reagents and Materials

6.1 *Bacteriological Pipette*—5.0 and 2.2 mL or 1.1 mL capacity.³

6.2 *Water Dilution Bottles*—Any sterilizable glass container having a 150 to 200 mL capacity and tight closure may be used.⁴

6.3 *Scrubbing Cups*—Sterile glass cylinders, height approximately 2.5 cm, inside diameter 2.54 cm.

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.

³ Presterilized/disposable bacteriological pipettes are available from most laboratory supply houses.

⁴ Milk dilution bottles of 150 mL capacity having a screw cap closure are available from Corning Glass Co., Kimble Glass Co. or most local laboratory supply houses.

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 *Polyvinylidene-chloride Wrap*⁵—Used to occlude skin sites.

6.7 *Sampling Solution*—Dissolve 0.4 g KH₂PO₄, 10.1 g Na₂HPO₄ and 1.0 g isocetylphenoxyethoxyethanol⁶ in 1 L of distilled water. Include in this formulation an activator specific for the antimicrobial in the test formulation (see Practices E 1054). Adjust to pH 7.8. Dispense in 100 mL volumes and sterilize for 20 min at 121°C.

6.8 *Dilution Fluid*—Butterfield's⁷ phosphate buffered water adjusted to pH 7.2, and containing an antimicrobial activator specific for the test formulation. (See Practices E 1054.)

6.9 *Plating Medium*—Soybean-casein digest agar.⁸

7. Procedure

7.1 *Reduction of Microbial Flora by Products That Are Not Applied Under Dressings.*

7.1.1 *Number of Subjects*—At least 16 subjects that meet eligibility requirements are to be enrolled in the study. At least 12 subjects are expected to complete the study.

7.1.2 *Subject Inclusion Criteria:*

7.1.2.1 Subjects are to be male or female and between the ages of 18 and 65 years.

7.1.2.2 Subjects who are not receiving antibiotics.

7.1.2.3 Subjects' hands and forearms are to be free of dermatoses, lesions, or other skin disorders.

7.1.2.4 Subjects who are in general good health as evidenced by history and limited medical examination.

7.1.3 *Subject Exclusion Criteria:*

7.1.3.1 Subjects who will be exposed to antimicrobial agents, medicated soaps, medicated shampoos or medicated lotions during the one week wash period or test period.

7.1.3.2 Subjects whose hands or forearms will be exposed to strong detergents, solvents or other irritants during the one week pre-wash period or test period.

7.1.3.3 Anyone not willing to fulfill the requirements of the protocol.

7.1.4 *Subject Instructions*—Subjects are to refrain from using any product containing an antimicrobial agent for at least one week prior to the test. Kits containing non-medicated bar soap, shampoo, and roll-on antiperspirant for use during this time are provided. Subjects are instructed not to use medicated creams, ointments, or take antibiotics. Swimming is not permitted. Subjects are instructed not to shower in the 48 h period proceeding the sampling period; however, a sponge bath is permitted. These instructions are to be given to each subject verbally and in written form.

7.1.5 *Test Site Occlusion*—Following a two-week wash-out period, the forearms of each of the twelve volunteers are

occluded with polyvinylidene-chloride wrap patches. The polyvinylidene-chloride wrap is cut to fashion a patch that will cover the volar aspect of the forearm, approximately 8 to 10 cm by 18 to 20 cm. A patch is placed in the mid-volar surface of each arm and anchored with tape.

7.1.6 *Treatment Procedure*—A test product treatment and two control sites, no treatment and vehicle control (formula without the active), are located on the volar aspect of each forearm, each site measures 3.5 cm by 3.5 cm. The assignment of the treatment and two controls to the three sites on each forearm is made according to a predetermined randomization scheme. Approximately 48 h following occlusion, individual sites are exposed 10 min prior to treatment, to allow the area to air dry. The test formulation and vehicle control sites are treated with the appropriate material according to the product label instructions. The treated test formulation sites and vehicle control sites are to be sampled after a 30 min exposure period. (Other appropriate time intervals may be used.) The untreated control sites are sampled without treatment at the same post-treatment time interval.

7.1.7 *Bacterial Sampling (Cup Scrubbing Method):*

7.1.7.1 Delineate the area to be sampled (5.07 cm²) by using a sterile glass sampling cylinder of 2.54 cm inside diameter. Firmly hold it against the skin during sampling to ensure that the washing fluid does not leak from the sampling site.

7.1.7.2 Three mL of sterile washing fluid (0.1 % Triton X-100 in 0.075 M phosphate buffer, pH 7.9) containing an inactivator for the antimicrobial agent is pipetted into the cup, and the entire area is scrubbed with moderate pressure for 1 min using a sterile rubber "policeman."

7.1.7.3 Remove the wash fluid with a sterile pipette and retained.

7.1.7.4 Repeat the scrubbing procedure (see 7.1.7.2) and pool the fluids from the two washes.

7.1.7.5 Dilution fluid is Butterfield's phosphate buffer containing an inactivator for the antimicrobial agent pH 7.2.

7.1.7.6 Plating medium and volume: soybean casein digest agar—Pour plate 1 mL of test sample using about 20 mL of the molten agar at about 45°C.

7.1.7.7 Promptly after the collection of the washing fluid, aliquots are plated undiluted, 10⁻¹ thru 10⁻⁴ for all samples.

7.1.7.8 All plating is in duplicate, and plates are incubated aerobically at 35°C ± 2°C for 48 ± 4 h before counting.

7.1.8 *Analysis of Data*—Transform counts from each test site on each arm to log₁₀ and use the log₁₀ counts for statistical evaluation. Evaluate differences between treatments and perform an overall analysis to test the null hypothesis of no difference among treatments. Test treatment differences for statistical significance at the 95 % confidence level, using appropriate parametric or non-parametric procedures, or both.

7.2 *Suppression of Growth Under a Dressing:*

7.2.1 *Number of Subjects*—At least 13 subjects that meet eligibility requirements will be enrolled in the study. At least ten subjects are expected to complete the study.

7.2.2 *Eligibility of Test Subjects*—Same as 7.1.2 and 7.1.3.

7.2.3 *Subject Instructions*—Same as 7.1.4.

7.2.4 *Treatment Procedure:*

7.2.4.1 *Application Sites*—A test product treatment and two

⁵ Saran Wrap® sold by Dow brands, Incorp., P.O. Box 68511, Indianapolis, IN, has been found suitable for this purpose.

⁶ Triton X-100, is available from Rohm and Haas Co., Philadelphia, PA.

⁷ Butterfield's Phosphate Buffer, *Journal of the Association of Official Analytical Chemists*, Vol 22, No. 625, 1939.

⁸ U.S. Pharmacopeia, XXII: United States Pharmacopeial Convention, Inc., Rockville, MD, see Chapter entitled "Microbial Limits Test," 1985.