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Standard Test Method for Evaluation of First Aid Antiseptic Drug Products[1]

This standard is issued under the fixed designation E1589; 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 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 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects. (See CFR Parts 50 and 56.)

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

1.5 *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:*[2]

D1193 Specification for Reagent Water

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

2.2 *Federal Standards:*[3]

CFR Parts 50 and 56

3. Terminology

3.1 *active ingredient, n*—a substance performing a function defined by this method.

3.2 *neutralization, n*—a process which results in quenching or inactivating inactivation of the antimicrobial activity of a formulation. This may be achieved with dilution of the formulation, or with the use of chemical agents, called neutralizers.

3.3 *neutralizer, n*—a procedure or chemical agent used to inactivate, neutralize, or quench the microbiocidal properties of an antimicrobial agent.

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

3.5 *sampling fluid, n*—a recovery fluid that may or may not contain a neutralizer to inactivate the active ingredients in test and internal reference formulations.

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

3.7 *transient microorganisms, n*—microorganisms that contaminate but do not normally permanently colonize the skin.

4. Summary of Test Method

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

4.1.1 *Effect of the Test Formulation to Reduce an Artificially Enhanced Skin Microbial Flora*—The forearms of subjects are occluded for 48 h prior to application of the test formulation 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 formulation is 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.

4.1.2 *Effect of the Test Formulation 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 microorganisms that develop under the dressings are compared 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.

4.2 The principal of the test is that the microflora of forearm skin is sparse. The impermeable dressing will increase surface

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[2] 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.

[3] Available from U.S. Government Publishing Office, 732 N. Capitol St., NW, Washington DC, 20401-0001, http://www.gpo.gov.

moisture by preventing diffusional water loss and thus expand transient resident skin microorganisms population. A significant antimicrobial effect by the test agent will be reflected by significantly lower population recovered from the nontreated site.

5. Significance and Use

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

5.1.1 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.

6. Apparatus

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

6.2 *Incubator*—Any incubator capable of maintaining a temperature of 35 ± 2°C.

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

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

7. Reagents and Materials

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

NOTE 1—Presterilized/disposable bacteriological pipettes are available from most laboratory supply houses.

7.2 *Water Dilution Bottles*—Any sterilizable container having a 150 to 200 mL capacity and tight closure.

7.3 *Scrubbing Cups*—Sterile cylinders, (Recommended height approximately 2.5 cm, inside diameter 2–3 cm.

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

7.5 *Test Formulation*—With directions for use.

7.6 *Occlusive Plastic Wrap*—Used to occlude skin sites.

7.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4 and 1.0 g octylphenoxypolyethoxyethanol (see Note 2) in 1 L of distilled water or higher purity water that meets or exceeds, Specification D1193, Type III or better. Include in this formulation a neutralizer specific for the antimicrobial in the test formulation (see Test Methods E1054) if appropriate. Adjust to pH 7.8 ± 0.1. Dispense appropriate volumes and sterilize .

NOTE 2—Also known as Triton X-100.

7.8 *Dilution Fluid*—Butterfield's phosphate buffered water[4] adjusted to pH 7.2, and containing an antimicrobial activator specific for the test formulation. (See Test Methods E1054.)

7.9 *Plating Medium*—Soybean-casein digest agar medium[5] or commercial equivalent.

7.10 *Personal Hygiene Kit*—contents may include various non-antimicrobial formulations such as shampoo, hand soap, non-aerosol deodorant, and gloves at the discretion of the investigator.

7.11 *Adhesive Tape*—surgical or other appropriate adhesive tape.

8. Procedure

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

8.1.1 *Number of Subjects*—Sample size calculations should be done to determine the number of subjects 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, its expected reduction from baseline). The minimum number of subjects (n) required for each test formulation can be estimated from the following equation:

$$n > S^2\left(\frac{(Z_{\alpha/2}+Z_{\beta})^2}{D^2}\right) \quad (1)$$

S_2 = estimate of variance (of reductions from baseline based on in-house data pool),
$(Z_{\alpha/2}$ = cumulative probability of the standard normal distribution, = 1.96 for α=0.05,
Z_{β} = power of the test = 0.842 for β = 0.80, and
D = expected efficacy (expected reduction from baseline).

NOTE 3—Experience has shown that a range from 12–18 subjects provides acceptable data.

8.1.2 *Subject Inclusion Criteria:*

8.1.2.1 Individuals between the ages of 18 and 65 years being preferably both male and female,

8.1.2.2 Hands and forearms free of dermatoses, lesions, open wounds hangnails, or other skin disorders, and

8.1.2.3 Are in general good health as evidenced by history and limited medical examination.

8.1.3 *Subject Exclusion Criteria:*

8.1.3.1 Exposure to antimicrobial agents, medicated soaps, medicated shampoos or medicated lotions during the two week washout period or test period,

8.1.3.2 Exposure of hands or forearms to strong detergents, solvents or other irritants during the two week pre-wash period or test period,

8.1.3.3 Currently receiving a typical or systemic antibiotic, and

8.1.3.4 Not willing to fulfill the requirements of the protocol.

8.1.4 *Subject Instructions*—Subjects are to refrain from using any product containing an antimicrobial agent for at least two weeks prior to the test. Kits containing non-medicated bar

[4] Downes, F.P. and K. Ito, *Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Association, Washington, DC, 2001, p. 637 and p. 643.

[5] U.S. Pharmacopeia 2567, United States Pharmacopeial Convention, Inc., Rockville, MD,200234, see Chapter entitled "Microbial Limits Test."

soap, shampoo, and roll-on or stick antiperspirant for use during this time are provided. Subjects are instructed not to use medicated creams, ointments, or take antibiotics. Bathing in biocide treated pools, hot tubs, and spas is not permitted. Subjects are instructed not to shower in the 48 h period proceeding the sampling period; however, a sponge bath excluding the test areas is permitted.

8.1.5 *Test Site Occlusion*—Following a two-week wash-out period, the forearms of each of the human subjects are occluded with occlusive plastic wrap patches. The occlusive plastic 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 adhesive tape.

8.1.6 *Treatment Procedure*—A test formulation 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 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 to air prior to treatment, until visibly 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.

8.1.7 *Bacterial Sampling (Cup Scrubbing Method):*

8.1.7.1 Delineate the area to be sampled by a sterile sampling cylinder. Firmly hold it against the skin during sampling to ensure that the washing fluid does not leak from the sampling site.

8.1.7.2 Three mL of sterile sampling fluid is pipeted into the cup, and the entire area is scrubbed with moderate pressure for one minute using a sterile rubber "policeman."

8.1.7.3 The sampling fluid is removed with a sterile pipette and retained.

8.1.7.4 The scrubbing procedure is repeated and the fluids from the two washes are pooled.

8.1.7.5 Dilution fluid is Butterfield's phosphate buffer that contains a neutralizer for the antimicrobial agent.

8.1.7.6 Plating medium: Soybean casein digest agar using standard pour or spread plate procedure may be used.

8.1.7.7 Promptly after the collection of the washing fluid, aliquots are plated 10^0 through 10^{-4} for all samples.

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

8.1.8 *Analysis of Data*—Transform counts from each test site on each arm to $\log_{10}$ and use the $\log_{10}$ 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.

8.2 *Suppression of Growth Under a Dressing:*

8.2.1 *Number of Subjects*—Same as 8.1.1.

NOTE 4—Experience has shown that at least 10 subjects provides acceptable data.

8.2.2 *Eligibility of Test Subjects*—Same as 8.1.2 and 8.1.3.

8.2.3 *Subject Instructions*—Same as 8.1.4.

8.2.4 *Treatment Procedure:*

8.2.4.1 *Application Sites*—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. Following a two-week washout period, application of the test product and controls to the arms are made according to a predetermined randomization.

8.2.4.2 *Application of Liquid, Ointment and Powders*—One mL or 1 g of the test material is spread over each 25 cm^2 site. The product treated site, vehicle control site, and the untreated control site are immediately covered with a 25 cm^2 of occlusive wrap. Each site is occlusively sealed with occlusive wrap and tape. A strip of adhesive tape is placed between each site to prevent translocation of the test agents and the microorganisms from one site to another.

8.2.4.3 *Application of Impregnated Dressings*—Apply dressings according to label directions.

8.2.4.4 Each site is quantitatively sampled after 24 h of occlusion using the procedure described in 8.1.6.

8.2.5 *Analysis of Data*—Same as 8.1.8.

9. Neutralizer Validation

9.1 When neutralizers are added to culture media or diluting fluids, or both, validate the effectiveness of the systems according Test Methods E1054.

10. Precision and Bias

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

11. Keywords

11.1 antiseptic; bacteria; bandages; cup scrub; dressing; first aid; efficacy; first aid; impregnated dressing; occlusion; skin microflora