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Standard Test Method for Assessment of an Antibacterial Handwash Product by Multiple Basin Wash Technique[1]

This standard is issued under the fixed designation E1883; 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 This test method covers determining the effectiveness of an antibacterial handwash for reducing the level of aerobic bacterial flora on the hands, following an extended period of use.

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 this case, inches are used and metric units follow in parentheses.

1.4 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects. (Title 21 CFR, Part 50).

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

1.6 *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 *Other Standard:*[3]

Title 21 Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects: Informed Consent Verification & Part 56, Institutional Review Boards

3. Terminology

3.1 *Definitions:*

3.1.1 *active ingredient*—a substance performing a function defined by this method.

3.1.2 *active test formulation*—a formulation with an active ingredient.

3.1.3 *control test formulation*—a formulation without an active in this test method. Frequently a bland soap.

3.1.4 *neutralization*—a process that results in quenching or 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.

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

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

3.1.7 *test formulation*—a formulation containing an active ingredient.

3.1.8 *transient microorganisms*—microorganisms that contaminate but do not normally permanently colonize skin.

4. Summary of Test Method

4.1 This hand degerming protocol is a modification of the Cade Handwashing Procedure, that is a serial basin hand wash sampling technique.[4,5]

4.1.1 Two baseline bacterial counts will be determined for the hands and a post-usage count will be done after twelve days of antibacterial handwash usage. The samples are collected from basin wash water following one or more 60-s washes with a bar soap that does not contain an antimicrobial. At each sampling interval samples may be collected from the first and or fifth hand wash in a series of five washes.

[1] This test method 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] 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.

[4] Cade, A.R., "A Method for Testing Degerming Efficacy of Hexachlorophene Soaps," *Journal of the Society of Cosmetic Chemistry*, Vol. 2: 1951, pp 181–291.

[5] Price, P.B., "The bacteriology of normal skin: a new quantitative test applied to a study of the bacterial flora and disinfectant action of mechanical cleansing," Journal Infection Control, Vol. 63:1938, pp 301–318.

4.1.2 The data will be used to calculate the reduction of bacterial flora resulting from the use of the antibacterial test handwash product as described within the protocol. Reductions in bacterial populations calculated from baseline and post-treatment samples collected after the first basin wash are reflective of the antimicrobial test soaps ability to reduce the population of transient organisms on the hands. Reductions in bacterial populations calculated from baseline and post-treatment samples collected after the fifth basin wash are reflective of the antimicrobial test soaps ability to reduce the population of resident flora on the hands.

5. Significance and Use

5.1 This procedure should be used for *in vivo* evaluation of the performance of antibacterial handwash products that are intended to reduce the skin micro flora following repeated use. Activity against the combined transient and resident micro flora may be assessed. Historically counts from the first basin are considered to be transients.[4,6] The latter measurement is probably more meaningful as the resident population is more stable.

5.1.1 This test method is applicable for testing all forms of topical antimicrobial handwash formulations.

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used.

6.2 *Incubator*—Any incubator that can maintaining a temperature of 30 to 35°C may be used.

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

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

6.5 *Water Bath*—Any bath of appropriate size and capable of maintaining temperature at 45 ± 2°C.

6.6 *Wash Basins*—Sterile metal, plastic, or porcelain basins capable of containing approximately 3 L. Alternatively, non-sterile containers may be used if lined with a low bioburden plastic bag.

7. Materials and Reagents

7.1 *Bacteriological Pipettes,* 5.0 and 2.2 or 1.1 mL capacity.

7.2 *Test Tubes,* or equivalent.

7.3 *Test Formulation*—Directions for use of test formulation should be included if available.

7.4 *Dilution Fluid*—Butterfield's phosphate buffer,[7] or other suitable diluent, adjusted to pH 7.2 ± 0.1 with effective neutralizer if required. Adjust pH with 0.1 N HCl or 0.1 N Na OH (See Test Methods E1054.)

7.5 *Plating Medium*—Soybean-Casein Digest Agar Medium,[8] or commercially available equivalent with appropriate neautralizers if needed.

7.6 *Bland cleansing formulation,* a mild, non-antimicrobial solid or liquid cleanser. (The Investigator may choose to use the product vehicle.)

NOTE 1—This formulation should be used during the washout period and for all sample collection washes.

7.7 *Sterile High Purity Water*—Specification D1193, Type III, or better.

8. Subjects

8.1 *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). This number of subjects (n) can be estimated from the following equation:

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

Where:

S^2 = estimate of variance (of reduction 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).

8.2 *Recruiting*—Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatoses, open wounds, hangnails or other skin disorders that may affect the integrity of the test.

8.2.1 Individuals, such as hospital, nursing home, day-care center workers, or others who work in environments that may offer exposures to bacteria not expected in the general population should be excluded as subjects.

8.3 *Instruction of Volunteers*—Instruct the volunteers to avoid contact with antimicrobials (other than the test formulation) for the duration of the test and for at least two weeks prior to the first baseline sampling. This restriction includes shampoos, lotions and soaps, and such materials as acids, bases, and solvents. Bathing in biocide treated pools, hot tubs, and spas should be avoided.

8.3.1 Volunteers are to be provided with a kit of non-antimicrobial personal care products for exclusive use during the test and protective gloves to be worn when contact with antimicrobial can not be avoided. Polypropolyene gloves should be used for applying material such as makeup,

[6] Roth, R.R., Williams, D.J., "Microbial Ecology of the Skin" *Annual Review of Microbiology,* Vol. 42: 1988, pp. 441–64.

[7] Butterfield, C.T., "The selection of a Dilution Water for Bacteriological Examinations." J. bacteriol. 23: 355–368. 1931.

[8] *U. S. Pharmacopeia*, XXIII: United States Pharmacopeial Convention, Inc. Rockville, MD see chapter entitled "Microbial Limits Test," 1995.