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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 E 1883; 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~~

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 *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]

D 1193 Specification for Reagent Water

E 1054~~Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products~~ Test Methods for Evaluation of Inactivators of Antimicrobial Agents

2.2 *Other Standard:*

Title 21 Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects: Informed Consent Verification & Part 56, Institutional Review Boards[3]

3. Terminology

3.1 *Definitions:*

3.1.1 *active ingredient*~~—a substance added to a formulation specifically for inhibition or inactivation of microorganisms.~~ —a substance performing a function definded 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*~~—inactivation of the antibacterial of the test material. This can be achieved through dilution of active test formulations to levels too low to have an antibacterial activity, or through the use of chemical agents, called neutralizers, to eliminate antibacterial activity.~~ —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.

~~[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. Current edition approved May 10, 1997. Published January 1998.~~

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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* ~~, Vol 11.01.~~volume information, refer to the standard's Document Summary page on the ASTM website.

~~[3] Annual Book of ASTM Standards, Vol 11.04.~~

[3] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

3.1.6

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

3.1.8 *transient microorganisms*—microorganisms from the environment 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 second 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.

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 floraorganisms 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] '. The latter measurement is probably more meaningful as the resident population is more stable.

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

5.2Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.[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 capable of maintaining a temperature of 35 ± 2°C may be used. —Any incubator that can maintaining a temperature of 30-35 °C may be used.

6.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization. —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 Materials*Test Formulation— Directions for use of test formulation should be included if available.

7.4 *Dilution Fluid*— Butterfield's phosphate buffer,or equivalent, containing an antimicrobial inactivator specific for the test formulation. (See Test Methods E1054[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 E 1054.)

7.4.1*Butterfield's Phosphate Buffer Stock Solution*—Dissolve 34 g of KH_2PO_4 in 500 mL of distilled water, adjust to pH 7.2 with approximately 75 mL of 1*N* NaOH, and dilute to 1 L. Store under refrigeration.

7.4.2*Dilution Fluid*— Dilute 1.25 mL of Butterfield's Phosphate Buffer stock solution to 1 L with distilled water (antimicrobial inactivator may be added prior to adding water). Adjust to pH 7.2, dispense in appropriate volumes, and sterilize at 121°C.

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

[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.

[6] Title 21, Code of Regulations (CFR), Part 50, Protection of Human Subjects: Informed Consent Verification, Available from U.S. Government Printing Office, Superintendent of Documents, Washington, DC 20402.

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

[7] Presterilized/disposable bacteriological pipettes are available from most laboratory supply houses.

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