Designation: E 1883 – ~~97~~02

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 *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.*

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

2. Referenced Documents

2.1 *ASTM Standards:*

[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~~ Oct. 10, ~~1997~~. 2002. Published January 2003. Originally approved in 1997. Last previous edition approved in 1997 as E1883–97.

D 1193 Specification for Reagent Water[2]

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[3]

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. Available from U.S. Government Printing Office, Superintendent of Documents, Washington, DC 20402.[4]

3. Terminology

3.1 *Definitions:*

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

3.1.67 *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.[5,6]

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 ~~flora~~ 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~~,~~[5,7]. 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.

~~5.2 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.[6]~~

---

[2] *Annual Book of ASTM Standards*, Vol 11.01.

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

~~[4] Cade, A.R., "A Method for Testing Degerming Efficacy~~

[4] Available from U.S. Government Printing Office, Superintendent of ~~Hexachlorophene Soaps," *Journal of the Society of Cosmetic Chemistry* , Vol. 2: 1951, pp 181–291.~~ Documents, Washington D.C.

~~[5] Roth, R.R., Williams, D.J., "Microbial Ecology~~

[5] Cade, A.R., "A Method for Testing Degerming Efficacy of ~~the Skin"~~ Hexachlorophene Soaps," ~~*Annual Review*~~*Journal of ~~Microbiology,~~ the Society of Cosmetic Chemistry, Vol. ~~42: 1988, pp. 441–64.~~ 2: 1951, pp 181–291.*

~~[6] Title 21, Code~~

[6] Price, P.B., "The bacteriology of ~~Regulations (CFR), Part 50, Protection~~ normal skin: a new quantitative test applied to a study of ~~Human Subjects: Informed Consent Verification, Available from U.S. Government Printing Office, Superintendent~~ the bacterial flora and disinfectant action of ~~Documents, Washington, DC 20402.~~ mechanical cleansing," Journal Infection Control, Vol. 63:1938, pp 301–318.

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

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

6. Apparatus

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

6.2 *Incubator*—Any incubator ~~capable of~~ that can maintaining a temperature of ~~35 ± 2°C~~ 30-35 °C may be used.

6.3 *Sterilizer*—Any suitable steam sterilizer ~~capable of producing~~ that can produce the conditions of ~~sterilization.~~ 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.[8]

7.2 *Test Tubes*, or equivalent.

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

7.4 *Dilution Fluid*—Butterfield's phosphate buffer,[9] or ~~equivalent, containing an antimicrobial inactivator specific for the test formulation. (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~~ other suitable diluent, adjusted to pH 7.2 ± 0.1 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).~~ effective neutralizer if required. Adjust ~~to~~ pH ~~7.2, dispense in appropriate volumes, and sterilize at 121°C.~~ with 0.1 N HCl or 0.1 N Na OH (See Test Methods E 1054.)

7.5 *Plating Medium*—Soybean-~~c~~Casein ~~d~~ Digest ~~a~~ Agar Medium[10], or commercially available equivalent with appropriate neautralizers if needed.

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

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

7.7 *Sterile High Purity Water*—Specification D 1193, Type III, or better.

8. ~~Panelists~~ Subjects

8.1 *Number of ~~Panelists~~*— ~~The~~ subjects—Sample size calculations should be done to determine the number of ~~p~~ subjects necessary to find statistically significant differences (reductions) from baseline. The number of subjects required depends ~~upon~~ on the ~~level of~~ statistical ~~significance~~ confidence required for the ~~test~~ expected results, the variability encountered in the ~~study,~~ data collection (for example, variability in reductions from baseline), and the ~~relative~~ expected efficacy of the ~~antibacterial agent being evaluated. Panelists are selected~~ test product (for example, its expected reduction from ~~a group~~ baseline). This number of ~~adults who agree to~~ subjects (n) can be estimated from the following ~~test restrictions and for whom an initial estimate~~ equation:

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

Where:

S^2 = estimate of ~~the baseline population has been established. This group,~~ variance (of reduction from ~~which~~ baseline based on in-house data pool),

$Z_{\alpha/2}$ = cumulative probability of the ~~test panelist are to be selected, should be approximately 1.5 times larger than~~ standard normal distribution,
=1.96 for α=0.05,

Z_{β} = power of the ~~proposed test panel.~~ 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 ~~panelists.~~ 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] ~~Butterfield's Phosphate Buffer, *Journal of the Association of Official Analytical Chemist*, Vol. 22 625, 1949.~~

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

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

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

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

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, perfumes, colognes, etc. and rubber gloves for dish washing, gardening, painting, pumping gasoline, etc.

NOTE 2—Volunteers may use antimicrobial containing antiperspirants and deodorants if they are the roll-on or stick type, and contact with the lower arms and hands is avoided.

8.4 *Selection of Test ~~Panelist~~ Subjects from Volunteer Group*—After the first baseline enumerations have been completed determine median bacterial count of each sampling wash (first and or fifth). The test panel should be comprised of the appropriate number of subjects with initial baseline bacterial counts closest to the median value. If the baseline sample are collected from both the first and fifth basins use only the median counts from the fifth basin sampling to select subjects.

9. Study Schedule

9.1 *Pre-Test Period*—A washout period of at least two weeks prior to the first baseline sampling is required. The subjects refrain from using or coming in contact with antimicrobial products during the washout period.

9.2 *Baseline Period*—Subjects will wash with ~~baseline control soap~~ bland cleansing formulation (see 7.6) as described below:

9.2.1 *Day 1 (Tuesday)*—Baseline bacterial counts of the first and or fifth basins of wash water are taken as described under "Bacterial Sampling".

9.2.2 *Days 2 and 3 (Wednesday and Thursday)*—Subjects continue to use baseline ~~control soap~~ bland cleansing formulation (see 7.6) *ad libitum* for all normal handwashing, showering and bathing.

9.2.3 *Day 4 (Friday)*—The appropriate number of subjects whose baseline counts were closest to the median are selected to continue in the study. The subjects continue to use baseline ~~control soap~~ bland cleansing formulation (see 7.6) for all washing until test period begins.

9.2.4 *Days 5 and 6 (Saturday and Sunday)*—Subjects continue to use baseline ~~control soap~~ bland cleansing formulation (see 6.6) *ad libitum.*

9.3 *Test Period*:

9.3.1 *Day 1 (Monday)*:

9.3.1.1 The second set of baseline counts are taken from the first and or fifth basin washes prior to use of the test product.

9.3.1.2 Subjects return all ~~baseline control soap~~ bland cleansing formulation (see 7.6) given them and are provided test product for exclusive use at home. Subjects are also provided a Home Use Record form to record all usages of the test product.

9.3.1.3 All subjects wash their hands with the assigned test ~~product~~ formulation under supervision at the laboratory three times. Washes are performed at least 1 h apart. Subjects use the test ~~product~~ formulation at home *ad libitum* for all handwashing, showering, and bathing.

9.3.2 *Day 2 through 5 (Tuesday through Friday)*—All subjects wash their hands three times daily under supervision on Tuesday through Friday. The washes are performed at least 1 h apart. Subjects use the test ~~product~~ formulation at home *ad libitum* for all handwashing, showering and bathing.

9.3.3 *Day 6 and 7 (Saturday and Sunday)*—Subjects use test product at home *ad libitum.*

9.3.4 *Day 8 through 11 (Monday through Thursday)*—All subjects wash their hands three times daily under supervision on Monday through Thursday. The washes are performed at least 1 h apart. Subjects use the test ~~product~~ formulation at home *ad libitum* for all handwashing, showering and bathing.

9.3.5 *Day 12 (Friday)*—Subjects wash with ~~baseline control soap~~ bland cleansing formulation (see 7.6) with bacterial counts made following the first and or fifth basin washes.

10. Procedure

10.1 *Supervised Wash Procedure With Test ~~Product~~ Formulation*—The following is a suggested procedure for a bar (solid) handwash ~~product.~~ formulation. The procedure for a liquid ~~product~~ test formulation or other form should specify the amount of ~~product with~~ test formulationwith which to wash and an appropriate wash time. The following timed, supervised handwashing procedure will be conducted three times daily.

10.1.1 Remove all jewelry from hands and wrists.

10.1.2 Moisten hands under warm ~~(38~~ (40 ± 2°C) running tap water.

10.1.3 Pick up the bar and wet it under the tap water. Using the bar, lather the hands for 30 s and then place the bar in the soap dish.

10.1.4 Wash the hands, the fingernail area, and the lower two-thirds of the forearms for 60 s.

10.1.5 Rinse the hands and the lower two-thirds of the forearms in running water for 30 s.

10.2 *Bacterial Sampling*—The handwashing technique used to collect samples to estimate the level of aerobic bacteria on the hands of each subject will be as follows:

10.2.1 Perform all sampling washes using ~~baseline~~ bland cleansing ~~wash~~ formulation (see 7.6) to the wrist according to the procedures described above (see 10.1).

10.2.2 The first and or fifth washings in this series will be performed in sterile basins containing one liter of sterile high purity water. The hands will be carefully washed only to the wrist then rinsed in the basin water. (Note: The second, third and fourth

washes if performed are executed under running tap water.)

10.2.3 Basin contents will be thoroughly mixed and a sample removed immediately for bacterial analysis.

10.3 *Bacterial Count Procedure*—The following aliquots of the basin contents will be plated to enumerate bacterial populations.

10.3.1 *Baseline Counts*—Using the pour plate technique, plate in triplicate 1.0 mL and 0.1 mL aliquots of a 1:10 dilution of the basin contents in soybean-casein digest agar medium or equivalent.

10.3.2 *Test Count*—Using the pour plate technique, plate in triplicate 1.0 mL aliquots of the basin contents; and, 1.0 mL and 0.1 mL aliquots of a 1:10 dilution of the basin contents in soybean-casein digest agar.

10.4 *Dilutions*—Sample dilutions are prepared in 9.0 mL blanks of Butterfield's phosphate buffer.

10.5 *Incubation*—All plates are incubated for ~~48 hours~~ ± 4 hrs at ~~35~~ 30 ± 2°C. After incubation, the colonies on the plates that are countable are enumerated with the aid of a colony counter. The total bacteria count per liter from the basin contents is calculated.

11. Neutralizer for the Test ~~Antibacterial Agent~~ Formulation

11.1 When neutralizers are added to culture media or dilution fluids, or both, validate the effectiveness of the systems according to Practice E 1054.

12. Analysis of Data

12.1 *Calculation of Bacterial Reduction*:

12.1.1 Bacterial counts per litre from the first and or fifth basin or each sampling day are determined and converted to $\log_{10}$ in order to minimize the effect of variability in counts obtained from different subjects. Data from the two baseline counts obtained from the first and or fifth basin samples are averaged for each individual. Geometric means of the data are calculated.

12.1.2 The following percent reductions may be calculated:

$$\textit{Percent Reduction Basin 1 Bacterial Population} = \frac{1 - (\textit{Geometric Mean of First} - \textit{Basin Counts Test Day})}{\textit{Geometric Mean of the 2 Baseline First Basin Counts}} \times 100 \quad (2)$$

$$\textit{Percent Reduction Basin 5 Bacterial Population} = \frac{1 - (\textit{Geometric Mean of Fifth} - \textit{Basin Counts Test Day})}{\textit{Geometric Mean of the 2 Baseline Fifth Basin Counts}} \times 100 \quad (3)$$

12.1.3 Estimate the standard deviation for each percent reduction value calculated above; using the assumption of a log normal distribution, construct 95 % confidence interval for the percent reduction value (*S*). Perform Student's independent t-tests to determine if there is a statistically significant bacterial reduction of the transient and or resident bacterial populations on test ~~product~~ formulation washed hands when compared to ~~baseline control soap~~ bland cleansing formulation (see 6.6) after twelve days of test ~~product~~ formulation use.

13. Precision and Bias

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

14. Keywords

14.1 antimicrobial; Cade; efficacy; handwash