



Designation: E 1174 – 9400

## Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel or Consumer Handwash Formulations<sup>1</sup>

This standard is issued under the fixed designation E 1174; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

### 1. Scope

1.1 This test method is designed to determine the ability effectiveness of an antimicrobial handwashing agent to give agents for the reduction of transient microbial flora (contaminants) when used in a handwashing procedure.

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 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.* For more specific precautionary statements see Note 1 and Note 2. 1.

1.5 This method may be used to evaluate topical antimicrobial handwash formulations.

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<sup>1</sup> This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 Antimicrobial Agents.

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1.6 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.<sup>2</sup>

## 2. Referenced Documents

### 2.1 ASTM Standards:

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products<sup>3</sup>

## 3. Terminology

### 3.1 Definitions:

3.1.1 *test organism*—an applied inoculum of an organism that has characteristics which allow it to be readily identified. The test organism is used to simulate a transient topical microbial contaminant. It may also be referred to as a marker organism, bacterial simulant, or bacterial contaminant.

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

3.1.3 *transient microorganisms*—organisms from the environment that contaminate but do not normally colonize the skin.

3.1.4 *active ingredient*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.1.5 *test formulation*—a formulation which incorporates antimicrobial ingredient(s).

3.1.6 *neutralization*—a process which results in quenching the antimicrobial activity of a test material. This may be achieved through dilution of the test material(s) to reduce the antimicrobial activity, or through the use of chemical agents, called neutralizers, to eliminate antibacterial activity.

3.1.7 *cleansing wash*—a non-antimicrobial wash intended to remove gross soil or residues from the hands of the panelists prior to the conduct of the study and as noted throughout the study. This may also be referred to as a cosmetic wash.

3.1.8 *healthcare personnel handwash*—a cleanser or waterless agent intended to reduce transient bacteria on the hands.

## 4. Summary of Test Method

34.1 This test method is conducted on a group of volunteer panelists who have refrained from using topical antimicrobial formulations for at least one week prior to the initiation of the test. Activity of the test material is measured by comparing the number of a marker bacteria test organisms recovered from artificially contaminated hands after use of the a handwashing formulation to the number recovered from contaminated unwashed hands. This hands not exposed to the test formulation. The method presents describes specific procedures to be followed using *Serratia marcescens*, a species of bacteria which produces a red pigment color on an agar surface as the contaminant bacteria test organism. The activity of the formulation is test material may be measured following 1, 3, 5, 7, a single wash and 10 handwashings multiple washes in a single clay using a neutralization recovery method.

34.2 An alternative test organism that is an acceptable marker is *Escherichia coli*. If this organism is used, culture Culture media and incubation conditions appropriate for this organism should be used. employed. The investigator should also be aware that there may be health risks associated with the use of this organism and precautions similar to those referenced in Note 1 should be undertaken.

## 4. Significance and Use

4.1 The procedure should be used to test the degerming effectiveness of antimicrobial hand washing agents, used by health care personnel, that are intended for frequent use and that are intended to reduce the level of contamination acquired through contact with contaminated objects or people.

4.2 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.<sup>3</sup>

<sup>2</sup> Annual Book of ASTM Standards Federal Register, Vol 11:05, 46, No. 17, Jan. 27, 1991.

<sup>3</sup> See Federal Register

<sup>3</sup> Annual Book of ASTM Standards, Vol 46, No. 17, Jan. 27, 1981; 11.04.

## 5. Significance and Use

5.1 The procedure may be used to test the effectiveness of antimicrobial handwashing agents. The test formulations may be designed for frequent use to reduce the transient bacterial flora on hands.

## 6. Apparatus

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

56.2 *Incubator*—Any incubator capable of maintaining a the following temperature of: *S. marcescens* ( $25 \pm 2^\circ\text{C}$  may be used.  $2^\circ\text{C}$ ) or *E. coli* ( $35 \pm 2^\circ\text{C}$ ). This temperature is required to assure ensure pigment production by the for *Serratia-S. marcescens*.

56.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization is acceptable.

56.4 *Timer* (Stop-clock)—One that can be read for minutes and seconds.

56.5 *Handwashing Sink*—A sink of sufficient size to permit panelists to wash without touching hands to sink surface or other panelists.

56.5.1 *Water faucet(s) to*—To be located above the sink at a height which permits the hands to be held higher than the elbow during the washing procedure.

56.6 *Tap Water Temperature Regulator and Temperature Monitor*—To monitor and regulate water temperature of  $40 \pm 2^\circ\text{C}$ .

## 67. Reagents and Materials

67.1 *Bacteriological Pipettes*—10.0 and 2.2-mL or 1.1-mL capacity.<sup>4</sup>

67.2 *Water Dilution Bottles*—Any sterilizable glass container having a 150–200 mL capacity and tight closures may be used.<sup>5</sup>

67.3 *Erlenmeyer Flask*—2-L capacity for culturing test organism.

6.4 *Baseline Control Soap*, a liquid castile soap

7.4 *Cleansing Wash*—A mild, non-antimicrobial solid or other liquid soap containing no antimicrobial.

6.5 soap. (The investigator may choose to use the product vehicle.)

7.5 *Test Formulation Material*—Directions for use of the test formulation should material may be included if available. utilized. If there directions are not any available, use directions provided in this test method (see 9.5).

6.6 method.

7.6 *Gloves*—Loose-fitting gloves of latex, —Loose-fitting, unlined, powder-free gloves which possess ngo antimicrobial properties, or equivalent.<sup>6</sup> (Plastic bags with low bioburden may be used in place of gloves.)

67.7 *Sampling Solution*—Dissolve 0.4 g  $\text{KH}_2\text{PO}_4$ , 10.1 g  $\text{Na}_2\text{HPO}_4$  and 1.0 g isooctylphenoxy polyethoxyethanol<sup>7</sup> and with appropriately validated neutralizers in 1-L distilled water. Adjust pH to 7.8 with 0.1 N HCl or 0.1 N NaOH. Dispense in 75-mL volumes and sterilize for 20 min so that final volume after sterilization is 75 mL, sterilized at  $121^\circ\text{C}$ .<sup>8</sup>

67.8 *Dilution Fluid*—Butterfield's sterile phosphate buffered water—Sterile Butterfield's Buffer<sup>9</sup> or other suitable diluent, adjusted to pH 7.2 with suitable inactivator effective neutralizer for the antimicrobial test material. Adjust pH with 0.1 N HCl or 0.1 N NaOH. (See Test Methods E 1054.)

67.9 *Agar*—Contains soybean casein—Soybean casein digest agar<sup>10</sup> plus suitable inactivator.

6.10 agar, or other solid media appropriately validated to support growth of the test organism with appropriate neutralizers if needed.

7.10 *Broth*—Soybean casein digest: 1000 mL per 2-L flask.

## 7. digest broth or other liquid media appropriate to support growth of the test organism.

## 8. Test Organism

78.1 *Serratia marcescens* (ATCC No. 14756) is to be used as a marker the test organism. This is a strain having stable pigmentation at  $25^\circ\text{C}$ .

<sup>4</sup> Presterilized/disposable bacteriological pipettes are available from most local laboratory supply houses.

<sup>5</sup> Milk dilution bottles of 160-mL capacity having a screw-cap closure are available from Corning Glass Co., Kimble Glass Co. or most local laboratory supply houses.

<sup>6</sup> A suitable glove would be Pharmaseal 8873C, (sterile) Flexam Latex Procedure Glove from American Pharmaseal Laboratories, Glendale, CA 91209. A zone of inhibition test such as AATCC Test Method 90-1965 may be used to evaluate antimicrobial properties of gloves; AATCC Test Methods, American Association of Textile Chemists and Colorist, 1968 Technical Manual, Section B-475.

<sup>7</sup> Peterson, A. F., "The Microbiology of the Hands: Evaluating the Effects of Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, pp. 125–130, 1973.

<sup>8</sup> Triton X-100, Rohm and Haas Co., Philadelphia, PA.

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<sup>8</sup> Peterson, A.F., "The Microbiology of the Hands: Evaluating the Effects of the Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, pp. 125–130, 1973.

<sup>9</sup> Butterfield's Phosphate Buffer.

<sup>9</sup> Horowitz, W. (Ed.) 1980. *Journal Official Methods of the Association Analysis of Official Analytical Chemists the AOAC*, Vol 22, No. 625, 1939. 13th Ed., Sec. 46.013 (m), p. 825. Assoc. of Off. Anal. Chemists, Washington, D.C. 1018 pp.

8.2 *Escherichia coli* (ATCC 11229) is an alternative test organism. When *E. coli* is used, the plating agar should include a suitable indicator (e.g. MUG<sup>10</sup>).

NOTE 1—**Warning:** The application of microorganisms to the skin may involve a health risk. Prior to applying the *S. marcescens* strain test organism to the skin, the antibiotic sensitivity profile of the strain should be determined. If the strain is not sensitive susceptible to G gentamycin, do not use it. If an infection occurs, the antibiotic sensitivity profile should be made available to the attending clinician.

Following the panelist subject's last contamination and wash with the test formulation, the panelist's subject's hands are to be sanitized by scrubbing with a 70-% eth isopropanol solution or equivalent. The purpose of this alcohol scrub is to destroy any residual *S. marcescens*.

~~7.2— test organisms on the skin.~~

~~8.3 Preparation of Marker Culture Test Organism Suspension—From stock~~

~~8.3.1 *S. marcescens*—A homogeneous culture is used to inoculate the hands. The stock culture should be at least two 24 hour broth transfers from the original ATCC culture, but there should be no more than 5 transfers removed from the ATCC culture. From the stock culture of *Serratia marcescens* ATCC No. 14756 in 2-L flask containing 1000 mL (ATCC 14756) inoculate the appropriate volume of *S* soybean-casein digest broth (67.10) with 0.1 milliliter of stock culture of *S. marcescens*/100mLs of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 h at 25°C ± 2°C. *S* Broth should develop a red pigment.~~

~~8.3.2 *E. coli*—A homogeneous culture is used to inoculate the hands, the stock culture should be at least two 24 hour broth transfers from the original ATCC culture, but no more than 5 transfers removed from the ATCC culture. From the stock culture of *Escherichia coli* (ATCC 11229) inoculate the appropriate volume of soybean-casein digest broth (7.10) with 0.1 milliliter of stock culture/100mLs of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 hours at 35 ± 2°C.~~

~~8.4 Swirl or shake the suspension before the withdrawal of each aliquot withdrawal: aliquot. Assay the suspension for number of organisms by membrane filtration technique or surface inoculation at the beginning and end of the use period. Do not use a suspension for more than 8-h.~~

## 8. Panelists

~~8.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatosis, open wounds, hangnail or other skin disorders that hours. The suspension may affect the integrity of the test and such that 12 subjects complete the study.~~

~~8.2 Instruct the volunteers to avoid contact with antimicrobials (other not vary more than the test formulation) for the duration of the test and for at least one week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions and soaps, also such materials as acids, bases and solvents. Bathing in biocide treated pools, hot tubs, spas, etc., should be avoided. Volunteers are to be provided with a kit of non-antimicrobial personal care products for exclusive use during the test and rubber gloves to be worn when contact with antimicrobials cannot be avoided. ± 0.5 log<sub>10</sub> cfu/mL over an 8 hour period.~~

## 9. Subjects

~~9.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatosis, open wounds, hangnails, or other skin disorders.~~

~~9.2 Instruct subjects to avoid contact with antimicrobial products (other than the test material as dispensed for each test wash) for the duration of the test and for at least one week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions and soaps, also such materials as acids, bases and solvents. Bathing in biocide treated pools, hot tubs, or spas should be avoided. Subjects are to be provided with a kit of nonantimicrobial personal care products for exclusive use during the test and rubber gloves to be worn when contact with antimicrobial or harsh chemicals cannot be avoided.~~

## 10. Procedure

~~9.1 After panelists~~

~~10.1 After subjects have refrained from using antimicrobial formulations for at least 7 days, they perform a 30-s practice 30 second cleansing wash (7.4) in the same manner that is described for the test and control formulations, except that a solution of non-antimicrobial bland soap (see 6.4) is used. formulations. This procedure removes oil and dirt and familiarizes the panelists with the washing technique.~~

~~9.2 Contaminant Suspension and Hand~~

~~10.2 Hand Contamination—The contaminant is a—A liquid suspension of *Serratia marcescens* the test organism containing at least a minimum of 1 × 10<sup>8</sup> organisms per mL. (See Test Methods E 1054.) Five millilitres cfu/mL is used. See Table 1.~~

~~10.2.1 A 1.5mL aliquot of the contaminant culture are test organism suspension is dispensed 0 into the hands then subjects' cupped hands. This aliquot is rubbed over the entire surfaces of the hands; for 20 ± 5 s (front and back) not reaching above the~~

<sup>10</sup> United States Pharmacopeia, XXII: XXII: United States Pharmacopeial Convention, Inc., Rockville, MD, Chapter entitled "Microbial Limits Test." The MUG (4-methylumbelliferyl-β-D-gluconide) substrate is hydrolyzed by β-D-gluconidase to yield a fluorescent end product, 4-methylumbelliferone. β-D-gluconidase is possessed by *E. coli* (ATCC 11229). MUG is incorporated into the appropriate growth medium at 0.05 grams/L.

wrist. Application and spreading should involve about 45 s. The hands are then held still motionless away from the body and allowed to air dry for approximately 30 ± 5 s.

**TABLE 1 Hand Contamination with Test Organism Suspension**

Volume	Spread Time	Dry Time
1.5 mL	20 sec	30 sec
1.5 mL	20 sec	30 sec
1.5 mL	20 sec	90 sec

10.2.2 To continue the contamination of the hands, an additional 1.5mL aliquot of the test organism suspension is dispensed into the hands, distributed over the hands for 20 ± 5 seconds, and air dried for 30 ± 5 seconds.

10.2.3 To complete the contamination, a final 1.5mL aliquot of test organism suspension is dispensed into the hands, distributed over the hands for 20 ± 5 seconds, and air dried for 90 ± 5 seconds (Table 1).

NOTE 2—The hands may still be wet after the 90 seconds.

10.2.4 The total test organism suspension applied to the hands is 4.5 mL. Contamination may take approximately 5 minutes. This method of contamination minimizes the loss of test organism while spreading.

10.3 *Contamination Schedule*—The ~~panelist subjects'~~ hands are contaminated with the marker test organism prior to the baseline bacterial sample collection and prior to all each washing with the test material. Table 2 below illustrates a typical test. The number of repeated test washes may be reduced or eliminated at the discretion of the investigator.

**TABLE 2 Hand Contamination and Recovery Schedule**

Name	Contamination	Type of Wash	Recovery
Cleansing Wash	no	Cleansing Wash	no
Baseline	yes	no	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	no	Cleansing Wash	no
Test Wash 1	yes	Test Formulation	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	no	Cleansing Wash	no
Test Wash 2–10	yes	Test Formulation	no
Test Wash 11	yes	Test Formulation	Plate Recovered Sampling Solution with Neutralizer

9.10.4 *Baseline Recovery*—A baseline sample is taken after contamination to determine the number of marker organisms surviving on the hands. Bacterial sampling will follow the procedures outlined in 9.6.

9.5 *Wash in Section 12.*

## 11. Wash and Rinse Procedure—F

11.1 Conduct the test in accordance with the use directions for the test material. If test material directions are not available, the wash and rinse procedure described as follows ~~is for all washes with~~ should be used. Table 2 above shows the ~~test formulation~~ whether or not they are preceded by artificial contamination and recovery schedule for the hands. Five millilitres or an amount specified by the manufacturer overall study.

### 11.2 Liquid Formulations

11.2.1 Dispense 5 ml of the test formulation is dispensed onto the hands and rubbed over all surfaces, taking caution not to lose or dilute the substance. After the material is spread, a small amount of water is added from the tap and the into cupped hands. Spread over hands are completely lathered for 30 s. The and lower third<sup>1/3</sup> of the forearm is also washed. After completion of the wash, hands and forearms are rinsed under tap water at 40 ± 2°C for 30 s. A total of ten washes with the test formulation are involved. Bacterial samples are taken following the first, third, fifth, seventh, and tenth washes.

9.6 *Bacterial Sampling*—After specified washes, place rubber gloves (6.6) used for sampling on the right and left hand. Add 75 mL of sampling solution (6.7) to each glove and secure gloves above the wrist. After adding sampling solution, uniformly massage all surfaces of the hand for 1 min. After massaging aseptically sample the fluid of the glove. ~~forearms.~~

NOTE 2—**Caution:** No neutralizer 3—The 5 ml volume has been chosen for test purposes due to the antimicrobial requirement for washing hands and forearms.

11.2.2 Sparingly wet contaminated hands with 40 ± 2°C tap water.

11.2.3 Wash in a vigorous manner for 30 ± 5 seconds all surfaces of the ~~handwash formulation is included in hands and the sampling solution to inhibit the antimicrobial action once sampling is initiated. The 75 mL lower third of sampling fluid may be sufficient to dilute out the activity of antimicrobial, however, this forearm.~~ Caution should be demonstrated using a procedure such as described in Test Methods E 1054.

If neutralization is not accomplished by dilution include an antimicrobial inactivator specific for exercised to retain the test formulation being evaluated material in the sampling solution used hands. If the lather becomes too dry, a small amount of water may be added to e maintain lather.

11.2.4 Rinse thoroughly from fingertips to elbows under  $40 \pm 2^\circ\text{C}$  tap water for  $30 \pm 5$  seconds. Caution should be exercised to avoid contact with the bacterial samples sink and fixtures to eliminate recontamination from the hand following the final wash sink surfaces.

11.2.5 Subject's hands and forearms are lightly patted dry with test formulation.

A definite recommendation regarding paper toweling.

NOTE 4—After washes requiring sampling, the inclusion of an inactivator hands are not to be dried, but held upright until procedures in sampling solution (6.7) used 12.1 are performed.

### 11.3 Waterless Formulations<sup>11</sup>

11.3.1 Dispense 5 mL of test material into cupped hands.

NOTE 5—The 5 ml volume has been chosen for bacterial collection prior test purposes due to the final wash cannot be made. Two points should be considered in making a decision: (1) If an inactivator is included in requirement for washing hands and forearms.

11.3.2 Distribute test material over all sampling fluid, will residual inactivator on surfaces of the skin reduce hands and the efficacy lower third of the forearms. Continue rubbing in a vigorous manner for  $30 \pm 5$  seconds or until dry. Caution should be exercised to retain the test formulation material in subsequent washes the hands.

11.3.3 Subject's hands may be held upright and result in higher than expected bacterial counts? motionless prior to Bacterial Recovery (Section 12).

### 11.4 Solid Formulations

11.4.1 Sparingly wet contaminated hands and (2) Can samples collected without forearms with  $40 \pm 2^\circ\text{C}$  tap water.

11.4.2 Wet the product.

11.4.3 Rub the product between the hands and on the forearms for  $15 \pm 3$  seconds. Place product aside.

11.4.4 Lather lower third of forearms and hands for an additional  $30 \pm 5$  seconds. If the lather becomes too dry, a small amount of water may be processed quickly enough added to maintain lather.

11.4.5 Rinse thoroughly from fingertips to elbows under  $40 \pm 2^\circ\text{C}$  tap water for  $30 \pm 5$  seconds. Caution should be exercised to avoid decreased bacterial counts due contact with the sink and fixtures to continued action of eliminate contamination from the sink surfaces.

11.4.6 Subject's hands and forearms are lightly patted dry with paper toweling.

### 11.5 Other Product Forms

11.5.1 Use standardized amount (e.g. weight, volume) of test formulation? Whatever material in accordance with use directions.

11.6 After washes requiring sampling, the decision, hands are not to be dried, but held upright until procedures in 12.1 are performed.

## 12. Bacterial Recovery

12.1 Within 5 minutes after specified washes (10.3), place gloves (7.6) used for sampling on the comparison hands. Add 75 mL of results across studies, sampling solution (7.7) with neutralizer to each glove and secure gloves above the investigator should indicate whether or not an inactivator has been included.

### 10. wrist.

12.2 Uniformly massage all surfaces of the hand for  $1 \text{ min} \pm 5$  seconds.

12.3 Aseptically retrieve a 3-5 mL sample of the fluid in the glove by pulling the glove away from the wrist, inserting a pipet into the finger region of the glove, and withdrawing the fluid.

12.4 The dilution and plating of the recovered sampling solution is completed within 30 minutes after sampling.

## 13. Enumeration of Bacteria in Sampling Solution

### 103.1 *S. marcescens*

13.1.1 Enumerate the *S. marcescens* in the recovered sampling solution (12.3) using standard microbiological techniques, such as membrane filter technique filtration or surface inoculation technique: spread plating. The pour plate technique is not appropriate recommended because subsurface *S. marcescens* colony forming units do may not exhibit the red pigment. Prepare sample pigment.

13.1.2 Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (67.8). Use soybean-casein digest agar (7.9) with suitable inactivator as recovery medium.

13.1.3 Incubate prepared plates  $48 \pm 4$  h at  $25 \pm 2^\circ\text{C}$ . Standard plate counting procedures are used to count only the red pigmented *S. marcescens*.

<sup>11</sup> This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 Antimicrobial Agents.

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13.2 E. coli

13.2.1 Enumerate the E. coli in the sampling solution using standard microbiological techniques, such as membrane filtration, pour or spread plating. Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.8). Use soybean-casein digest agar (7.9) with suitable inactivator and indicator (MUG<sup>10</sup>) as recovery medium.

13.2.2 Incubate prepared plates 48 ± 4 hour at 35 ± 2°C. Standard plate counting procedures are used to count only the fluorescent (MUG<sup>10</sup>) E. coli colonies. Fluorescent colonies are counted using long-wave UV light.

**14. Determination of Reduction**

14.1 Convert plate counts (cfu/hand) to log<sub>10</sub>. Average left and right hands.

14.2 Determine Log<sub>10</sub> Reductions at each sampling interval changes from baseline counts obtained with test material.

**12. recovery interval/wash using the following formula:**

$$\frac{\text{Log}_{10} \text{Reduction at Sampling Interval}}{\text{Log}_{10} \text{Baseline Recovery} - \text{Log}_{10} \text{Sampling Interval}} = \quad (1)$$

**15. Comparison of Test Material with a Control Material**

12.1 It Material

15.1 It may be desirable to compare the test material with a control material, other test formulations. If this is the case, an equivalent number of panelists should be assigned to the control product each formulation on a random basis. All test parameters will be equivalent for products, although the wash procedure for an established product may be different. Both products should be run concurrently.

12.2 Compare, at each sampling interval, changes from baseline counts obtained with test material to changes obtained with control material.

~~13.~~

**16. Precision and Bias**

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

**147. Keywords**

147.1 antimicrobial; contaminant; efficacy; handwash; healthcare; marker organism; simulant

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