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# Standard Test Method for Determination of Effectiveness of Cleaning Processes for Reusable Medical Instruments Using a Microbiologic Method (Simulated Use Test)[1]

This standard is issued under the fixed designation E 2314; 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.

## INTRODUCTION

Cleaning is acknowledged as the critical first step in the reprocessing of reusable medical instruments. A test method to examine the efficacy and reproducibility of cleaning procedures would be valuable in optimizing decontamination of medical instruments, as well as increasing the margin of safety of subsequent disinfection and sterilization procedures. This test method is a means of determining the efficacy of the instrument manufacturer's cleaning instructions. In this simulated use test cleaning steps are performed with the instruments in a controlled laboratory environment. Within this environment, various parameters may be exaggerated to create worst-case conditions for the test. Among these are the amount or type of organic soil or micro-organisms contaminating the instruments.

The test method was developed primarily for large medical instruments or instruments with internal channels or recesses (for example, flexible endoscopes) but may be used for any resuable medical instruments. It employs both direct inoculation and sampling methods for external surfaces and indirect inoculation and sampling methods for less accessible internal channels.

Cleaning is defined as the removal of foreign materials, most often mixtures of organic soil (for example, protein) and microorganisms, from medical instruments. Bacterial endospores are the preferred microorganisms in this simulated test because they would be more resistant to the potential microbiocidal effects of the cleaning processes and solutions. This method examines the reduction in the number of spores as a tracer of foreign materials and not necessarily the reduction in organic soil directly.

This test may be designed to either examine the efficacy of a complete cleaning cycle consisting of several integrated steps or individual cleaning step such as precleaning, manual cleaning, automated cleaning or rinsing.

## 1. Scope

1.1 This test method is written principally for large medical instruments or instruments with internal channels or recesses (for example, flexible endoscopes) but may be used for any resuable medical instruments.

1.2 This test method describes a procedure for testing the efficacy of a cleaning process for reusable medical instruments artificially contaminated with mixtures of microorganisms and simulated soil.

1.3 The test method utilizes bacterial spores as tracers for foreign materials and quantifies their removal as a means of determining the efficacy of a cleaning process.

1.4 The test method is designed for use by manufacturers of medical instruments and devices. However, it may also be employed by other individuals who have a knowledge of the instruments, techniques and access to appropriate facilities.

1.5 Worst-case conditions can be represented by exaggerating a specific test parameter or otherwise intentionally simulating an extreme condition such as performing the test without cleaning solutions or utilizing instruments which are not new.

1.6 The test procedure is devised to determine the efficacy of a cleaning process as applied to a particular instrument or group of instruments by simulating actual use situations.

[1] 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 on Antimicrobial Agents.

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1.7 The test procedure may be performed on test instruments using a complete cleaning cycle or be limited to particular phases of the cycle such as precleaning, manual cleaning, automated cleaning, or rinsing.

1.8 The test procedure is normally performed on a number of external and internal sites, but it may be restricted to one particular site on the instrument.

1.9 A knowledge of microbiological and aseptic techniques and familiarity with the instruments is required to conduct these procedures.

NOTE 1—Because contamination of the surfaces of instruments may occur as a result of rinsing with tap water, bacteria-free water should be used for all rinsing when a water rinse step is part of the cleaning directions.

NOTE 2—Test methods to determine the effectiveness of cleaning medical instruments has only recently been actively debated, and research efforts are in their infancy. Because published experimental results are scarce, it is premature to dictate experimental reagents, conditions or acceptance criteria.

NOTE 3—The total elimination of the target organisms is not the goal of cleaning. Therefore, there will almost always be a number of microorganisms surviving on the test instruments unless one of the solutions or processes disinfects or sterilizes the test instrument. The results of various clinical and laboratory tests suggest that cleaning processes alone can produce a $10^2$ to $10^4$ $\log_{10}$ reduction in bioburden. The exact reduction will depend upon the precise experimental conditions. The criteria for judging cleanliness should be determined and recorded before initiation of the test procedure.

NOTE 4—This test protocol employs target spores as indicators or tracers for foreign materials and monitors their removal by the cleaning process. It is certainly possible that other particulate target materials, such as microbeads (latex beads) could be used in place of microbes. These alternate approaches would be more practical in those circumstances where microbiological expertise is limited.

1.10 *This standard may involve hazardous materials, operations, and equipment. 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:*

D 1193 Specifications for Reagent Water

E 1054 Practices for Evaluation of Inactivators of Antimicrobial Agents used in Disinfectant, Sanitizer, Antiseptic or Preserved Products

E 1766 Test Method for Determination of Efficacy of Sterilization Processes for Reusable Medical Devices

2.2 *Other Source:*

AAMI, TIR No. 30 A Compendium of Processes, Materials, Test Methods, and Acceptance Criteria for Cleaning Reusable Medical Devices[2]

## 3. Terminology

3.1 *Definitions:*

3.1.1 *accessible location*—a location on a reusable medical instrument(s) that may be contacted by bioburden, soil and cleaning agents.

3.1.2 *automated cleaning*—the removal of foreign material from medical instruments by means of a machine.

3.1.3 *bioburden*—the number and types of viable microorganisms that contaminate an instrument.

3.1.4 *CFU*—colony forming units.

3.1.5 *cleaning*—the removal of foreign materials, including organic soil (for example, protein) and microorganisms from medical instruments.

3.1.6 *cleaning solution*—a solution used to aid in the removal of foreign matter from medical instruments.

3.1.7 *manual cleaning*—the removal of foreign material from a medical instrument without the aid of a machine.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *cleaning efficacy*—the efficacy of cleaning may be calculated as the log reduction of viable microorganisms recovered from the test instruments as compared to the control instruments.

3.2.2 *control instruments*—reusable medical instruments which are inoculated but not subjected to the Test Cycle.

3.2.2.1 *control instrument recovery*—the quantity of inoculum that can be recovered from the accessible locations (for example, external surface sites and lumens, if any) of the control instruments.

3.2.3 *neutralizer*—a reagent used to stop the antimicrobial activity of residual cleaning agent(s) that may be present on test instruments and eluted along with the target microorganisms. (See Practices E 1054 for recommended neutralizers.)

3.2.4 *reusable medical instrument*—any medical instrument that is claimed by the manufacturer to be usable after reprocessing.

3.2.5 *test cycle*—a cleaning process that utilizes all of the parameters selected by the tester.

3.2.6 *test instruments*—reusable medical instruments which are inoculated and subjected to the Test Cycle. These instruments are used to determine the efficacy of the cleaning process.

3.2.6.1 *test instrument recovery*—the quantity of inoculum that can be recovered from the accessible locations (for example, external surface sites and lumens, if any) of the test instruments.

3.2.7 *test soil*—a formulation of organic materials used in testing the efficacy of cleaning.

3.2.8 *worst-case*—the intentional exaggeration of one or more parameters of a test compared to the normal condition. For example, this could include exaggerated soil load or deletion of cleaning steps.

## 4. Summary of Test Method

4.1 This test method is performed by inoculating interior or exterior surfaces, or both, of reusable medical instruments.

4.2 Both control instruments and test instruments are used in this test method. Prior to inoculation, all instruments are cleaned and reprocessed. An inoculum with high numbers of target microorganisms suspended in test soil is applied to both control and test instruments.

[2] Avaiable from, Association for the Advancement of Medical Instrumentation, (AAMI), 1110 North Glebe Road, Suite 220, Arlington, VA 22201-4795

4.3 It is impractical to determine inoculum recovery by immersion of large medical instruments or instruments with internal channels or recesses (for example, flexible endoscopes) in elution fluid because of their complexity, size, or deleterious effects from immersion. Therefore, rinsing or swabbing techniques are used to recover target microorganisms from these types of inoculated instruments.

4.4 Control instruments are used to determine the number of organisms which can be recovered from the instruments. At least two control instruments are inoculated in the same manner as the test instruments, however cleaning is not performed. An appropriate recovery method (see 4.3) is then used to determine the level of inoculum recoverable from the instruments. At least $10^6$ CFU recoverable per instrument are required. For an instrument with lumens, the total number of organisms recovered from both inside and outside surfaces of the instrument will be defined as the control instrument recovery.

4.5 After the Test Cycle has been completed using the test instruments, the inoculated target microorganisms remaining on these instruments are recovered using the same elution, recovery and quantitation procedures used to determine the number of target microorganisms on the control instruments. By comparing this test instrument recovery to the control instrument recovery, the efficacy of the cleaning process may be calculated.

## 5. Significance and Use

5.1 This method is designed to evaluate the effectiveness of cleaning reusable medical instruments using a specified cleaning process.

5.2 This method may be used to determine the effectiveness of cleaning processes of recesses, hinged sites, lumina, or other difficult-to-reprocess areas of reusable medical instruments.

5.3 This method may also be used to verify the claims for any portion of the cleaning cycle.

5.4 The recovery of surviving microorganisms may be accomplished using swabbing, rinsing, or total immersion of instruments.

5.5 The efficacy of the elution methods or loss of the applied inoculum may be assessed by recovery of target organisms from control instruments that have not been subjected to the cleaning process.

## 6. Apparatus

6.1 *Syringes*, 10 to 50 mL, sterile.

6.2 *Sterile Cotton or Dacron Swabs.*

6.3 *Sterile Petri Dishes*.

6.4 *Sterile Tubes*, to hold 10 mL.

6.5 *Sterile Bottles*, to hold 50 mL and sterile flasks to hold 250 to 500 mL.

6.6 *Sterilization Device*, for the medical instruments being examined. Alternatively, supplies for high level disinfection recommended by the instrument manufacturer.

6.7 *Water Bath*, which can maintain temperature from 20 to 50 ± 2°C.

6.8 *Incubator(s)*, which maintain 37 ± 2°C (for *B. atrophaeus*, formerly known as *Bacillus subtilis* subspecies *niger*) or temperature appropriate for selected target organism.

6.9 *Membrane Filters*, 0.45 µm, and filter supports for the filters.

6.10 *Colony Counter*.

6.11 *Disposable Plastic Pipettes*, various sizes.

6.12 *Reusable Medical Instruments*, reprocessed prior to each use.

6.13 *Cleaning Devices*, accessories or apparatus to be used in the Test Cycle and/or for reprocessing between uses as specified by the manufacturer of the test instrument.

6.14 *Vortex Mixer and/or Sonicator*.

6.15 *Vacuum Pump*.

6.16 *Shaker and/or Stirrer*.

## 7. Reagents

7.1 *Media*:

7.1.1 *Sterile USP Fluid D (Elution Fluid)*, containing polysorbate 80. Alternatively, sterile elution fluid solution containing 0.4 g $KH_2PO_4$, 10.1 g $Na_2HPO_4$, and 1.0 g isooctylphenoxypolyethoxy ethanol (Triton X-100) prepared in 1 L of Type III or better ASTM water adjusted to pH 7.8. Neutralizers appropriate for the cleaning solution may be added to either of these solutions.

7.1.2 *Soybean-Casein Digest Broth, USP*, with and without appropriate neutralizers for the specific test cleaning chemical(s) in the cleaning solution.

7.1.3 *Soybean-Casein Digest Agar, USP*, single or double strength with and without appropriate neutralizers in 10 to 50 mL tubes or bottles tempered to 48 ± 2°C.

7.1.4 *Sterile Saline or Phosphate Buffer*, for rinsing membrane filters.

7.2 *Target Organisms*—Standardized suspensions of *Bacillus atrophaeus* endospores (ATCC 9372) containing nominally $10^8$ CFU/mL should be used. Standardized bacterial spore suspensions are commercially available. The origin of the spore strain, production, storage, and expiration dates should be identified. Bacterial endospores are preferred as the target strain because they would be more resistant to potential microbiocidal effects of the cleaning solutions. If other microorganisms are used, appropriate changes in growth media and conditions should be made (also see requirements for Control Experiments in 9.1.4.1).

7.3 *Type III or better ASTM Water*, for making broth and elution fluids (see Specification D 1193).

7.4 *Rinse Water*—Water prepared by either steam sterilization or by 0.2 µm filtration (when a water-rinse step is part of the cleaning process).

7.5 *Test Soil*—Soil consisting of serum or solutions of serum proteins. These may be used alone or combined with other types of organic soil.

7.6 *Cleaning Solution*—The cleaning solution used in the Test Cycle.

7.7 *Neutralizers (as appropriate)*—Chemical inactivators which interrupt the killing action of the cleaning agent. (See Practices E 1054 for recommended neutralizers.)

## 8. Procedure

8.1 Before use, all selected reusable medical instruments must be reprocessed according to manufacturer's instructions.

If an instrument is to be tested repeatedly, it must be reprocessed between each test.

8.2 *Inoculation of Instruments*:

8.2.1 This procedure describes the use of *Bacillus atrophaeus* endospores. Soybean casein digest media are appropriate for culture of this and related *Bacillus* endospores and may also be used to resuspend commercial spore suspensions.

8.2.2 Spore suspensions are to be mixed with the test soil for inoculation of the instruments. The spore count may be estimated spectrophotometrically or by using routine plating procedures with appropriate agar media. It is recommended that the suspension contain approximately $10^8$ CFU/mL.

8.2.3 Mix the spore suspension with a predetermined volume of a solution containing test soil.

8.2.3.1 Control experiments should confirm that the test soil solution does not inhibit the growth of the target spores. (Evaluation may be done using the procedures in Practices E 1054).

8.2.4 In a preliminary experiment, confirm that the inoculum (containing test soil) will supply sufficient numbers of spores so that $10^6$ CFU or a greater number of spores to be recovered from a control instrument. (It is anticipated that some fraction of the applied inoculum will be lost in the process of inoculation and therefore will not be recovered.)

8.2.5 In a preliminary experiment, confirm that it is possible to obtain consistent recoveries from control instruments.

8.2.6 The location of all inoculated sites must be documented (see 9.1.3.2). These sites should include the most difficult to access external sites and all internal channels.

8.2.7 Inoculate the exterior surface of instruments with a micropipette or swabs saturated with the inoculum. Inoculate the internal surfaces and lumens of endoscopes and similar instruments with a needleless hypodermic syringe. The inoculum volume may range from the void volume of the channel to 10 mL applied to the channel opening. If necessary to obtain a higher number of spores adhering to the surfaces, a fresh inoculum can be reapplied.

8.2.8 The inoculum should be applied to locations demonstrated or suspected to be difficult to clean. Emphasis should be on those sites which would be most heavily contaminated during clinical use.

8.2.8.1 Moving parts that are inoculated (for example, hinges) should be actuated after inoculation.

8.2.9 The location of and rationale for selecting inoculated sites must be documented (see 9.1.3.2).

8.2.10 After inoculation, instruments are positioned to facilitate drainage for at least 30 min at ambient temperature.

8.3 *Control Instruments*—The total number of recoverable target spores on the control instruments is determined by using the elution, recovery and quantitation techniques described in 8.5 on the control instruments. In order for the test to be valid, an average of $10^6$ target organisms must be recovered from control instruments. A minimum of two control instruments should be evaluated. Either one replicate with two instruments or two replicates with one instrument may be performed.

8.4 *Test Instruments*—The efficacy of the cleaning process is judged by determining the reduction in bioburden on the test instruments subjected to the cleaning process as compared to the control instruments. Enumerate the surviving target organisms with the elution and recovery techniques described in 8.5 (also see 8.8, Replication of Test).

8.4.1 If the effectiveness of a complete cleaning cycle with all elements is required, perform a complete Test Cycle using the instrument manufacturer's and/or the automated cleaner manufacturer's directions for cleaning of the test instruments. The contribution of any single phase of the cleaning cycle may be evaluated by performing either the precleaning, manual cleaning, automated cleaning, and rinsing, or other contributory steps separately or in combination.

8.4.2 Cleaning solutions, accessory devices, automated or mechanical cleaning equipment, as well as deviations from routine processing instructions may also be tested.

8.4.3 *Cleaning Agent Controls*—Tests shall be conducted to assess the sporicidal (antimicrobial) activity of the cleaning agent. Those that are clearly sporicidal (antimicrobial) as employed in the Test Cycle may not be appropriate for this test method.

8.4.3.1 A cleaning agent with some sporicidal activity or ability to prevent the growth of surviving bacterial spores may still be used if it is possible to interrupt the killing activity of the sporicide at the conclusion of the test cycle through the use of neutralizers (inactivators). Evaluation of the neutralizer may be done using the procedures in Practices E 1054.

8.5 *Elution, Recovery, and Quantitation Techniques*:

8.5.1 In general, results can be reported for the entire instrument. However, it is also possible to elute and report individual sites such as an internal channel.

8.5.2 *External Surface Sites*—For external surface site of instruments, a sterile swab moistened with elution fluid is rubbed vigorously over the entire inoculated surface. Inoculated moving parts should be actuated during elution. This procedure is then repeated using a new moistened swab. Both swabs are placed in 10 mL of elution solution and mixed on a Vortex mixer or sonicated for 3 to 5 min to remove the spores from the swab. Immersion of the entire instrument in elution fluid in combination with sonication or agitation may be used as an alternative to swabbing for smaller instruments or devices.

8.5.2.1 The number of target spores recovered may be determined by preparation of 10-fold serial dilutions of a known volume of the recovered elution fluid, and then adding one mL samples of each dilution to 20 mL of molten agar (46 to 50°C) and poured into sterile Petri plates. Triplicate plates are prepared for each dilution, and the remaining volume of elution fluid recovered from the instrument is added to an equal volume of double-strength agar and poured into mini- or regular Petri plates. Allow agar to solidify and enumerate after incubation of the plates for 48 h or the time and temperature appropriate for enumeration of the selected target organism.

8.5.2.2 Alternatively, the number of target spores recovered from the recovered elution fluid may be assessed using membrane filtration. The appropriate dilution of the recovered elution fluid is placed on a pre-wetted (with sterile saline or phosphate buffer) filter and the vacuum turned on. The filter is washed with sterile saline or phosphate buffer (containing appropriate neutralizers when required). The membrane filter is

then placed onto the surface of the appropriate solid agar surface in a Petri plate. Enumerate after incubation for the appropriate time and temperature for the selected target organism.

8.5.3 *Internal Sites*—To recover target spores from a lumen or internal recess, aseptically irrigate with a volume of elution fluid equal to at least three times the void volume of the lumen. Repeat this irrigation with fresh solution three times, collecting all of the fluid in the same container. Mix the tube of recovered elution fluid with a Vortex mixer and prepare serial 10-fold dilutions. Subculture the elution fluid and enumerate as described above (see 8.5.2.1). Alternatively, dilutions may be cultured for survivors using membrane filtration described in 8.5.2.2.

8.5.4 After the incubation period, the number of colonies recovered from each instrument or test site(s) is determined by counting appropriate sets of triplicate plates and calculating the number of colony forming units (CFU) in the original sample of recovered elution fluid.

8.6 *Neutralization and Growth Inhibition Controls*—Tests shall be conducted to demonstrate that the neutralizer stops the antimicrobial action of the cleaning agent and is not inhibitory to the germination or outgrowth of the test spores. Evaluate using the procedures set forth in Practices E 1054.

8.7 *Comparative Quantitative Data*—The effectiveness of the cleaning cycle (or portions of it) may be evaluated by comparing the number of spores eluted from the control instruments (see 8.3) to the number eluted from the test instruments (see 8.4). The control instrument recovery must be a minimum of $10^6$ CFU per instrument. The number of organisms recovered from the test instrument will depend on the target organism, efficacy of the cleaning process, type of cleaning solution(s) used, and test instruments studied. The criteria for judging cleanliness should be recorded in the test report before initiation of the test procedure (see 9.1.3.7).

8.8 *Replication of Test*—The number of test instruments required should be determined by the size, complexity and intricacy of the instruments. For complex test instruments a minimum of five replicates should be evaluated. Either one replicate with five instruments or five replicates with one instrument, or any combination yielding five replicates may be performed.

## 9. Report

9.1 The report should contain the following minimal information:

9.1.1 *Apparatus*:

9.1.1.1 *Medical Instruments*—Identification of instrument(s) by manufacturer, name, model, and, if appropriate, serial number(s). Note if the instrument(s) are new or used.

9.1.1.2 *Accessory Cleaning Devices*, or apparatus used in the Test Cycle.

9.1.1.3 *Special Apparatus*—List any custom or special apparatus used in the performance of the test method.

9.1.2 *Reagents*:

9.1.2.1 *Target Organism*—Specify the genus, species, method used to identify, origin, production, storage, and expiration dates of the spore strain used for the inoculum.

9.1.2.2 *Test Soil*—Specify the composition, method of preparation and storage of the soil.

9.1.2.3 *Inoculum*—Specify the concentration of spores and soil in the final inoculum.

9.1.2.4 *Cleaning Agent(s)*—Identify the brand name(s) (if applicable), active ingredients, use dilution and special use conditions, if applicable.

9.1.2.5 *Neutralizers*—Identify the neutralizer(s), its final concentration and the solutions or media which contain neutralizer(s).

9.1.2.6 *Special Reagents*—List any custom or special reagents used in the performance of the test method.

9.1.3 *Procedure*:

9.1.3.1 *Preparation of Medical Instruments*—Describe the reprocessing method used to prepare instruments prior to use.

9.1.3.2 *Inoculation*—Describe internal and external sites inoculated. A labeled illustration identifying these sites may be helpful for complex instruments.

*(1)* List any special methods or custom devices used to inoculate the instruments.

*(2)* Provide the rationale used to identify the most difficult to clean sites.

9.1.3.3 *Worst-Case*—List any worst-case conditions employed and the rationale for their use.

9.1.3.4 *Test Cycle*—List all of the procedures, associated reagents and apparatus used as part of the Test Cycle.

9.1.3.5 *Elution*—List any special methods or custom devices used to elute the instruments.

9.1.3.6 *Replication*—List the number of instruments and number of replicates performed. Identify those instruments used as test and those used as control instruments.

9.1.3.7 *Cleaning Criteria*—State the criteria selected to determine the effectiveness of the cleaning process.

9.1.4 *Results*:

9.1.4.1 *Control Experiments*:

*(1) Neutralization Controls*—List the results of tests performed to demonstrate that neutralizers employed stop the antimicrobial action of the cleaning solution which may remain on the test instruments (see 8.6).

*(2) Growth Inhibition*—List results of tests to demonstrate that neutralizers employed are not inhibitory to growth of target spores (see 8.6).

*(3) Cleaning Agent Controls*—List the results of tests to examine the sporicidal activity of the cleaning agent (see 8.4.3).

*(4) Soil Controls*—List the results of tests performed to determine the effect of the soil on the target spores (see 8.2.3.1).

9.1.4.2 *Effectiveness of the Cleaning Process*:

*(1) Control Instrument Recovery*—List the results obtained with each control instrument.

*(2) Test Instrument Recovery*—List the results obtained with each test instrument.

*(3) Cleaning Efficacy*—List the value derived for the effectiveness of the cleaning process.

9.1.5 *Deviations*—Describe substantive deviations from the test method.

9.1.6 Statement that the test was conducted in accordance with ASTM Standard F 2314 Test Method for Determination of Effectiveness of Cleaning Processes for Reusable Medical Instruments Using a Microbiologic Method (Simulated Use Test).

## 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 cleaning; cleaning solution; endoscope; recovery and elution; reprocessing; reusable medical instrument

## REFERENCES


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### *Cleaning Efficacy*

(**2**) Hanson, P. J. V., Gor, D., Clarke, J. R., et al., "Contamination of Endoscopes Used in AIDS Patients," *Lancet*, 1989, pp. 86-88.

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### *Organic Soils*

(**5**) AAMI, *A Compendium of Processes, Materials, Test Methods, and Acceptance Criteria for Cleaning Reusable Medical Devices*, AAMI TIR No. 30, 2003.

### *Elution Fluid*

(**6**) *The United States Pharmacopoeia XXII*, Sterility Tests, Diluting and Rinsing Fluids, Rand McNally, Taunton, MA, 1990, p. 1484.

(**7**) Williamson, P., "Quantitative Estimation of Cutaneous Bacteria," *Skin Bacteria and Their Role in Infection*, Marbac, H. I. and Hildick-Smith, G., eds., McGraw-Hill, New York, 1965.

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(**8**) Williamson, P., "Quantitative Estimation of Cutaneous Bacteria," *Skin Bacteria and Their Role in Infection*, Marbac, H. I. and Hildick-Smith, G., eds., McGraw-Hill, New York, 1965.

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