Designation: E2967 – 15

Standard Test Method for Assessing the Ability of Pre-wetted Towelettes to Remove and Transfer Bacterial Contamination on Hard, Non-Porous Environmental Surfaces Using the Wiperator[1]

This standard is issued under the fixed designation E2967; 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

'High-touch' environmental surfaces (HITES) are increasingly being recognized as potential vehicles for infectious agents (**1-3**).[2] Decontamination of such surfaces is almost always by either a disinfectant-spray-and-wipe procedure or, more commonly, by wiping with an applicator or towelette prewetted with a disinfectant (**4**). In either case, the microbicidal action of the disinfectant is combined with the physical action of wiping (mechanical removal). This standard, formulated after a critical review of available wipe test methods (**5-8**) and published standards, is based on the use of a mechanical device (the Wiperator; Appendix X1) designed to wipe HITES under controlled conditions and also to test the transfer of acquired microbial contamination to clean surfaces.

1. Scope

1.1 This standard is designed for use with a mechanized device (the Wiperator; Appendix X1) to test pre-wetted towelettes.

1.2 Two species of vegetative bacteria, one Gram-positive coccus (*Staphylococcus aureus*) and one Gram-negative bacillus (*Acinetobacter baumannii*), representing important nosocomial pathogens, are used to separately contaminate disks of magnetized and brushed stainless steel in order to test the towelettes for their relative ability to:

1.2.1 Decontaminate non-porous environmental surfaces experimentally-contaminated with vegetative bacteria; and

1.2.2 Transfer any acquired bacterial contamination on the towelettes to clean surfaces.

1.3 This test method is not meant for use with towelettes for decontamination of skin.

1.4 The values stated in SI units are to be regarded as the standard. The values given in parentheses, if any, are for information only.

1.5 This test method should be performed by persons with training in microbiology in facilities designed and equipped for work with infectious agents at the appropriate biosafety level.

1.6 It is the responsibility of the investigator to determine whether Good Laboratory Practice (GLP) regulations are required and to follow them where appropriate.

1.7 *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:*[3]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals

E2362 Practice for Evaluation of Pre-saturated or Impregnated Towelettes for Hard Surface Disinfection

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E2896 Test Method for Quantitative Petri Plate Method (QPM) for Determining the Effectiveness of Antimicrobial Towelettes

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

[3] 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.

2.2 *AOAC Standard:*[4]

AOAC Standards Method 961.02 Germicidal Spray Products as Disinfectants. Official Methods of Analysis

2.3 *CFR Standards:*[5]

40 CFR 160 Good Laboratory Practice Standards

21 CFR 58 Good Laboratory Practice Regulations

2.4 *Other Reference:*

Organization for Economic Cooperation and Development (OECD) Guideline No. 187; 2013 Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard, Non-Porous Surfaces[6]

3. Terminology

3.1 For definitions of standard terms, please refer to Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *boss, n*—a Teflon cylinder designed to hold the test towelette in place with the help of an 'O' ring.

3.2.2 *eluate, n*—recovered eluent that may contain the test organism(s).

3.2.3 *eluent, n*—any liquid that is harmless to the test organism and that is added to a carrier to recover the organism(s) in or on it.

3.2.4 *microbicidal action, n*—the action of an agent leading to killing of or irreversible damage to microorganisms.

3.2.5 *nosocomial infection, n*—an infection acquired during stay in a healthcare setting.

3.2.6 *soil load, n*—a mixture of one or more organic and inorganic substances added to the suspension of the test organism to simulate the presence of bodily secretions, excretions, or other interfering substances. It presents the test substance with a challenge to overcome the chemical demand from the soil load and physical shielding of microbes that it may provide (**9**).

3.2.7 *stock culture, n*—frozen culture used to prepare test cultures.

3.2.8 *towelette, n*—a pre-wetted piece of paper or cloth of natural or synthetic fibers for use in environmental or personal hygiene.

4. Summary of Test Method

4.1 The Wiperator is designed to simulate the orbital action of wiping using prewetted towelettes. The pressure during contact, duration of wiping as well as the number of wiping strokes in a given test can be preset, allowing for greater precision and reproducibility. The device can also test the transfer of microbial contamination on a used towelette to a clean surface being wiped. Disks of brushed stainless steel are used in this standard method as prototypical non-porous environmental surfaces. Each disk receives at its center 10 µL of the test organism, and the inoculum is dried. The disk is then placed in one of the two recessed areas on the Wiperator's carrier platform. A 4 cm^2 piece of control or test towelette is cut and mounted on a boss for placement in the Wiperator's spindle (Appendix X1). The platform with the inoculated disk is raised to contact the towelette and activate wiping. To test for transfer, a clean carrier in the second recessed area of the platform is brought under the towelette and wiped. The carriers are separately transferred to a vial with an eluent containing a validated neutralizer. The eluates are then assayed and $\log_{10}$ CFU removed or transferred are calculated.

5. Significance and Use

5.1 Microbial decontamination of environmental surfaces by wiping is subject to many variables (**4**), and failure to standardize them properly during testing of towelettes may give inconsistent test data. (See Practice E2362 and Test Method E2896.) In particular, precise control of the pressure applied during wiping, the normally brief wiping times of a few seconds as well as the style and number of wiping strokes are difficult without a programmable mechanical device. The Wiperator has been designed and tested with these crucial factors in mind. The method described here is to assess the role of wiping in ridding non-porous environmental surfaces of bacterial contamination using prewetted towelettes, and also to determine if the used towelette can transfer viable contamination to clean surfaces on contact.

6. Equipment and Apparatus

6.1 *Bunsen Burner or an Incinerator,* for sterilization of inoculating loops.

6.2 *Centrifuge,* for sedimentation of the cells of the test organism(s).

6.3 *Colony Counter,* for example, Quebec Colony Counter.

6.4 *Disks,* 1 cm diameter and 0.7 mm thick made from sheets of brushed and magnetized stainless steel (E2197; OECD 2013); further specifications on the disks and examples for their commercial sources are given in Appendix X2.

6.5 *Dissecting Microscope,* 20× magnification.

6.6 *Filter Sterilization System,* a membrane filtration system (0.22-µm pore diameter) for sterilization of heat-sensitive media and solutions.

6.7 *Incubator,* to maintain an incubation temperature of 36±1°C.

6.8 *Inoculating Loop,* for transfer and spread-plating of bacterial suspensions.

6.9 *Hot Air Oven,* for drying of labware.

6.10 *Laminar Flow Biosafety Cabinet (Class II),* for handling of microbial cultures at biosafety level 2 (BSL-2); procedures for the proper maintenance and use of such cabinets are given in Ref (**10, 11**).

6.11 *Positive Displacement Pipette,* a pipette and pipette tips that can accurately dispense 10 µL volumes.

[4] Available from AOAC International, 481 North Frederick Ave., Suite 500, Gaithersburg, Maryland 20877-2417, http://www.aoac.org.

[5] 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.

[6] Available from OECD Environment Directorate, Environment, Health & Safety Div., 2 rue André-Pascal 75775, Cedex 16, Paris, France (www.oecd.org/ehs/).

6.12 *Freezer,* at –20±2°C, to store soil load or other items requiring storage in a frozen state.

6.13 *Freezer,* at –80±2°C for storage of bacterial stock cultures.

6.14 *Refrigerator,* at 4±2°C for storage of prepared culture media, reagents and agar plates.

6.15 *Metallic Forceps,* straight or curved, *(a)* with smooth flat tips for handling membrane filters, and *(b)* appropriate for manipulating the metal disks.

6.16 *Sterilizer,* any steam sterilizer suitable for processing culture media and reagents.

6.17 *Vortex Mixer,* for uniform resuspension of bacterial cells and to elute bacteria from the disks.

6.18 *Wiperator*—Appendix X1 describes this device with its accessories[7] and also gives use instructions.

6.19 *Vacuum Source,* a vacuum pump; access to an in-house vacuum line or a water faucet vacuum apparatus is required to pull the samples through the membrane filters.

7. Materials and Supplies[8]

7.1 *Bovine Serum Albumin (BSA).*

7.2 *Cryovials,* 2-mL capacity.

7.3 *Deionized Distilled Water (DDW),* or water of equivalent quality, for making reagent solutions and culture media; can be produced in-house or obtained from a reputable commercial source (D1129; D1193).

7.4 *Glass Beads (solid borosilicate),* 3 mm diameter.

7.5 *Glycerol.*

7.6 *J-Cloth Reusable Towels (unmedicated),*[9] or equivalent material for use as a control towelette.

7.7 *Membrane Filters (polyethylsulfone),* 33 mm diameter (0.22 µm pore diameter) for use with a syringe.

7.8 *Personal Protective Equipment,* including gloves, labcoat and safety glasses.

7.9 *Pipettors with Sterile Tips,* including one 10-µL positive-displacement pipettor.

7.10 *Tryptic-soy Agar (TSA),* in 100 mm diameter plastic Petri plates.

7.11 *Polypropylene Test Tubes,* 15 mL capacity.

7.12 *Sodium Chloride (NaCl).*

7.13 *Tryptic-soy Agar (TSA).*

7.14 *Tryptic-soy Broth (TSB).*

7.15 *Tryptone.*

7.16 *Wide-mouth Nalgene Vials with Screw Caps,* 30 mL capacity.[10]

7.17 *Test Organisms—Staphylococcus aureus* (ATCC 6538) and *Acinetobacter baumannii* (ATCC 19568).

NOTE 1—Ensure that *S. aureus* for subculture and any subsequent use produces golden-colored colonies on TSA.

8. Methods

8.1 *Preparation of Media and Reagents*—All autoclaving to be in accordance with manufacturer's instructions or as specified in the standard operating procedures of the laboratory:

8.1.1 *Recovery Medium*—TSA in 100 mm Petri plates prepared in-house or obtained from a reputable commercial source; store the plates at 4±2°C and use within three months from the date of preparation or within the shelf-life indicated by the manufacturer.

8.1.2 *Culture Broth*—Prepare 400 mL of TSB according to the manufacturer's instructions and autoclave-sterilize; aliquot 10 mL into each 15-mL test tube and store at 4±2°C and use within three months from the date of preparation or within the shelf-life indicated by the manufacturer.

8.1.3 *Diluent*—Tryptone-sodium chloride (TSC) broth. Prepare by adding 1 g Tryptone and 8.5 g of NaCl to 1 L of DDW and autoclave sterilize.

8.1.4 *Eluent with Neutralizer*—Prepare in 1 L of TSC broth by adding:

8.1.4.1 Saponin 30 g/L.

8.1.4.2 L-histidine 1 g/L.

8.1.4.3 Polysorbate-80 30 g/L.

8.1.4.4 Asolectin from soybean 3 g/L.

8.1.4.5 Sodium thiosulfate (Fisher) 5 g/L

8.1.5 Store at 4±2°C and use within three months from the date of preparation.

NOTE 2—A different neutralizer may be used depending on the type(s) and level(s) of the active ingredient(s) in the towelette under test (E1054). However, the use of any such neutralizer must first be properly validated.

8.1.6 *Soil Load:*

8.1.6.1 Add 3.0 mg of BSA to 100 mL of TSC.

8.1.6.2 Pass the solution through a 0.22-µm pore diam. membrane filter mounted on a syringe, aliquot, and store at 4±2°C and use within three months from the date of preparation; it can be stored for up to one year at –20±2°C.

NOTE 3—A different soil load such as the one given in E2197 or the OECD guideline may be used instead.

8.2 *Preparation of Stock or Frozen Bacterial Stocks:*

8.2.1 Spread with an inoculating loop the bacterial culture over a plate of TSA and incubate for 18±2 h at 36±1°C.

[7] The sole source of supply of the Wiperator known to Subcommittee E35.15 at this time is Filtaflex Ltd. (www.filtaflex.ca), Almonte, Ontario, Canada. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend. Alternatively, the user may build the apparatus with assistance from Filtaflex.

[8] If a specific source is not indicated, that item may be purchased from any reputable scientific supplier.

[9] The J-Cloth brand Reusable Towels, which can be autoclave sterilized, are widely available in hardware stores in North America and elsewhere. They are composed of cellulosic fibers from wood pulp and biodegradable. Manufacturer: Associated Brands; Universal Product Code: 0 58354 51071 3. Address: Mississauga, ON, Canada; http://www.associatedbrands.com/our-products/brands/

[10] The sole source of supply of Nalgene vials (Catalog #2118-0001) known to the committee at this time is Nalge Nunc International, 75 Panorama Creek Dr., Rochester, N.Y. 14625-2385. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

8.2.2 Using an inoculating loop, pick up one discrete colony from the plate and place it in 10 mL of TSB. Incubate the broth for 18±2 h at 36±1°C.

8.2.3 Add 100 µL of the broth culture to 100 mL of TSB containing glycerol at a final conc. of 10%. Incubate the suspension at 36±1°C for 4±0.5 h.

8.2.4 Aliquot 1.0 mL volumes into cryovials for storage at –80±2°C.

8.2.5 Use the frozen stocks within two years from the date of their preparation.

8.3 *Preparation of Working Bacterial Cultures:*

8.3.1 Place 100 µL of a thawed suspension of *S. aureus* or *A. baumannii* inoculum into 10 mL of TSB. Incubate at 36±1°C for 18±2 h for each day's experimentation.

8.3.2 Centrifuge the 10-mL suspension at 3,000xg for 20 min and resuspend the pellet in 5 mL TSC.

8.4 *Stainless Steel Disk Carrier Preparation and Sterilization:*

8.4.1 Soak disks in a non-ionic detergent solution[11] for at least one hour to degrease prior to rinsing and sterilization; avoid extended soaking to minimize risk of corrosion.

8.4.2 Rinse the disks in DDW for five minutes to remove detergent and grease.

8.4.3 Inspect each disk under a dissecting microscope and select only those with uniform striations while also ensuring that the disks are free of rust and any other manufacturing defects.

8.4.4 In case there is a noticeable difference in the depth of striations on the two sides of the disk, use the side with deeper striations as the working surface.

8.4.5 Place up to 20 clean disks in a Nalgene vial, loosely replace its screw cap, and sterilize by autoclaving (20 minutes at 121°C).

8.5 *Inoculum Preparation:*

8.5.1 Add 0.5 mL of 10× soil load (8.1.5) to 4.5 mL of an 18±2-hour bacterial culture (8.3.2). Do not store such an inoculum but prepare one fresh on each day of testing.

8.6 *Inoculation of Stainless Steel Disks:*

8.6.1 Vortex the test bacterial suspension with the soil load for 30 sec to homogenize it.

8.6.2 Working in an operating biological safety cabinet, individually transfer with a sterile pair of forceps 20 of the sterilized disks to a sterile plastic Petri plate. Use a calibrated positive-displacement pipette to transfer 10 µL of the bacterial inoculum to the center of each disk; *do not spread the inoculum to avoid operator variability and also to keep the thickness of the inoculum the same on all disks*. For consistency, the same pipette tip should be used when inoculating a given batch of disks.

8.6.3 Safely discard in a disinfectant solution any disk where the inoculum has run off the center.

8.6.4 Transfer the Petri plate containing the inoculated disks to a 36±1°C incubator for 30 minutes to dry the inocula; raise the Petri plate lid and place it off center to partially open the plate to expedite the drying.

NOTE 4—Alternatively, the inoculated disks may be dried in a desiccator (E2197).

8.7 *Preparation of the Test and Control Towelettes:*

8.7.1 Disinfect the area around the opening of the wipe container or bag with a paper towel soaked in 70% (v/v) ethanol.

8.7.2 Invert the test wipe container or bag and let rest for 5 seconds to help in uniform hydration of the wipes; invert again and wait for another 10 seconds; using sterile gloves, remove the wipe to be tested, discarding the first three towelettes. If towelettes require "activation," follow the manufacturer's instructions.

8.7.3 To minimize the evaporation of the liquid from the towelettes it is recommended that two staff members be recruited to perform the steps described in sections 8.7.4 to 8.7.6. Evaluate at least two lots of each towelette formulation. Evaluate three towelettes per lot. Excise and test one test square from each towelette.

8.7.4 To minimize any extraneous microbial contamination of the towelettes under test, wear sterile gloves and also use a sterile pair of scissors to cut out from the center of one test wipe a 4 cm × 4 cm square.

8.7.5 Mount a single layer of each towelette piece onto a boss with an O-ring and place the loaded boss in a sterile Petri plate to minimize evaporation and contamination.

8.7.6 For control (J-Cloth) wipes,[9] mount each sterile piece onto a boss and then soak into it 20 µL TSC diluent immediately before placing the boss into the Wiperator; repeat the procedure with the remaining control wipes.

NOTE 5—The level of liquid in most commercial towelettes is sufficiently small to avoid dripping during their normal handling and manipulations. In case dripping is a concern, excess liquid should be drained off prior to further processing of the towelette.

8.8 *Placement of Disks in Carrier Platforms:*

8.8.1 Make sure that the magnets are attached to the carrier platforms to retain the disks in place during the wiping process (Appendix X1; Fig. X1.3B).

8.8.2 In case sufficient numbers of presterilized carrier platforms are not at hand decontaminate them by flaming and let them cool down before placement in a sterile plastic Petri plate.

8.8.3 Using a sterile pair of forceps (6.15), place an uninoculated carrier (for transfer testing) first in the recessed slot on one side of the platform; place an inoculated carrier, contaminated side up, in the other slot.

8.9 *Testing for Removal/Inactivation of Microbial Contamination, as well as Transfer of Viable Organisms:*

8.9.1 Place a control- or test-towelette-loaded boss on the spindle of the Wiperator.

8.9.2 Holding the loaded platform with the contaminated disk proximal to the Wiperator, slide the platform up the ramp as far as it will go, and quickly raise it up to the boss to trigger the wiping action. Hold it in place for the required length of wiping.

[11] 7X Cleaning Solution: Manufacturer: MP Biomedicals Inc., Catalog Number: 09-76-670-94.

8.9.3 Remove the wiped disk from the platform and place it within 5±2 sec in a Nalgene vial with 1 g glass beads and 1 mL of eluent with the neutralizer.

8.9.4 Rotate the disk platform to bring the clean disk in contact with the towelette just used for wiping. Wipe again for 5 or 10 sec.

8.9.5 Remove the second disk from the platform and follow the procedure as in 8.9.3.

NOTE 6—The Wiperator is designed to increase the wiping time in increments of 5 sec to a maximum of 45 sec in a given test. However, all testing of the device thus far is based on wiping times of no longer than 10 sec to better simulate the field use of towelettes.

8.9.6 Vortex the contents of the vials for 30 sec.

8.9.7 Repeat procedure using the remaining towelettes to be tested.

8.9.8 Serially dilute the eluates and use the Miles-Misra plating technique **(12)** to inoculate appropriate dilutions onto plates of TSA (Appendix X3).

NOTE 7—Other means of sample assay, such as spread-plating or membrane filtration, may be used as long as they can adequately detect small numbers of CFU in as large a volume of eluate as possible.

8.9.9 Incubate the plates at 36±1°C and observe them for growth after 48±2 h of incubation.

8.9.10 Count and record the number of colonies on control and test plates to calculate $\log_{10}$ reductions in CFU attributable to wiping or $\log_{10}$ CFU transferred to a clean disk.

9. Controls

9.1 Run sterility controls on all media and reagents as well as on the soil load and the carriers.

9.2 *CFU in the Test Inoculum*—To ensure that the final inoculum (8.5.1) contains the required level of microbial challenge/10 µL to be placed on each disk, make 10-fold dilutions of it in TSC, plate the dilutions and incubate the plates at 36±1ºC for 18±2 h. Count the CFU.

9.3 *Input Control*—Place 10 µL of the test inoculum onto a disk, elute the inoculum from the disk within 20±2 sec by vortexing for 30 sec. Assay the eluate for CFU by incubating the plates at 36±1ºC for 18±2 h. This control is to determine the efficiency of recovery of the test bacteria from the disks in relation to the CFU in the test inoculum.

9.4 *Baseline Control*—Once the inocula on the disks are dry, individually elute three such disks within 5 minutes and titrate the eluates for CFU by incubating the plates at 36±1ºC for 18±2 h. Average the CFU counts from the three eluates. This value, which should not be less than $10^{5.5}$ and not more than $10^{6.5}$ CFU/disk, will represent the number of CFU surviving the inoculum-drying process and also will provide the basis for calculating the $\log_{10}$ values in CFU after wiping and transfer.

10. Neutralization Confirmation

10.1 For each type of towelette to be tested, a neutralization confirmation test must be performed against each species of test organism.

10.2 Wipe an uninoculated disk with the towelette under test, place the disk within 20±2 sec in 1 mL of the eluent with the neutralizer, and add 10 µL of test bacterial inoculum containing approximately 200–400 CFU.

10.3 For control, place an uninoculated disk in 1 mL of the eluent with the neutralizer and add 10 µL of test bacterial suspension containing approximately 200–400 CFU.

10.4 Hold the vials for 15 minutes at room temperature (22±2°C) and titrate the eluates for CFU. Effective neutralization of bactericidal activity in the eluates will be confirmed if the numbers of CFU in the test eluates are within 80–120% of the mean CFU value from the control eluates (OECD 2013; E1054).

11. Hazards and Precautions

11.1 All handling of infectious materials and chemicals and their disposal must comply with the local biosafety and chemical requirements.

11.2 All required Materials Safety Data Sheets (MSDS) must be available and readily accessible to all participating technical staff at the testing site.

11.3 A functioning fume hood must be available on-site for the handling of hazardous chemicals.

11.4 The procedures described here are technique-sensitive and require strict adherence to the protocols. Everyone involved in conducting the experimental work must receive prior training in the safe handling of infectious agents and hazardous chemicals.

11.5 Maintain a proper chain of custody for all items received for testing. Store all such items in a safe and secure location at the test facility with access by authorized personnel only.

12. Data/Records Management

12.1 Record all data promptly either as electronic files or on data sheets legibly, and in indelible ink.

12.2 Store all data in a secure place with controlled access.

13. Quality Control

13.1 For quality control purposes, the required information is documented on appropriate forms.

13.2 In the event contamination is detected in any of the media and reagents or a mistake is detected in sample dilutions/inoculations, the entire test must be repeated.

14. CFU ($\log_{10}$) Concentrations/Reductions per Experiment

14.1 *Calculations:*

$$CFU_x = \log_{10}\left(\frac{CFU \times Dilution}{Sample\ Volume\ (mL)}\right) \quad (1)$$

where:

CFU_x = $\log_{10}$ concentration of bacterial CFU/mL,

CFU = number of colony forming units recovered,

Dilution = a particular serial dilution plated, and

Sample Volume (mL) = volume plated/counted (example 5 × 20 µL = 0.1 mL).

$$LR = CFU_{baseline^{123}} - CFU_{test^{12}} \quad (2)$$

$$LR = \left(\frac{CFU_{baseline^1} + CFU_{baseline^2} + CFU_{baseline^3}}{3}\right) - \left(\frac{CFU_{test^1} + CFU_{test^2}}{2}\right) \quad (3)$$

where:

LR = mean $\log_{10}$ reduction,
$CFU_{baseline}$ = $\log_{10}$ CFU/mL of three baseline control replicates, and
CFU_{test} = $\log_{10}$ of CFU/mL of two test replicates.

14.1.1 *CFU (log_{10}) Reductions per Test Product:*

$$MLR = \left(\frac{LR^1 + LR^2 + LR^3}{3}\right) \quad (4)$$

where:

MLR = mean $\log_{10}$ reductions of 3 experiments.

14.1.2 *Inoculum CFU Concentration:*

$$CFU_{inoculum} = \log_{10}\left(\frac{CFU \times Dilution}{Sample\ Volume\ (mL)}\right) \quad (5)$$

where:

$CFU_{inoculum}$ = $\log_{10}$ concentration of bacterial CFU/mL of inoculum.

$$MCFU_{inoculum} = \left(\frac{CFU_{inoculum} + CFU_{inoculum} + CFU_{inoculum}}{3}\right) \quad (6)$$

where:

$MCFU_{inoculum}$ = mean $\log_{10}$ $CFU_{inoculum}$ of three replicates.

14.1.3 *Input Control:*

$$CFU_{disk\ eluate} = \log_{10}\left(\frac{CFU \times Dilution}{Sample\ Volume\ (mL)}\right) \quad (7)$$

where:

$CFU_{disk\ eluate}$ = $\log_{10}$ concentration of bacterial CFU/mL eluted from the stainless steel carriers immediately after application.

$$MCFU_{disk\ eluates} = \left(\frac{CFU_{disk\ eluates} + CFU_{disk\ eluates} + CFU_{disk\ eluates}}{3}\right) \quad (8)$$

where:

$MCFU_{disk\ eluates}$ = mean CFU/mL concentration of disk eluates.

$$CFU_{disk\ dry} = \log_{10}\left(\frac{CFU \times Dilution}{Sample\ volume\ (mL)}\right) \quad (9)$$

where:

$CFU_{disk\ dry}$ = $\log_{10}$ concentration of bacterial CFU/mL of dried inoculum on stainless steel carrier.

$$MCFU_{disk\ dry} = \left(\frac{CFU_{disk\ dry} + CFU_{disk\ dry} + CFU_{disk\ dry}}{3}\right) \quad (10)$$

where:

$MCFU_{disk\ dry}$ = mean CFU of disks after drying.

$$IC = M_{disk\ eluate} - M_{disk\ dry} \quad (11)$$

where:

IC = input control.

15. Calculating $\log_{10}$ Reductions on Wiping

15.1 *Calculation:*

$$LR = ML_{Baseline} - ML_{Test} \quad (12)$$

where:

LR = $\log_{10}$ reduction.
$ML_{Baseline}$ = mean $\log_{10}$ CFU/mL of three baseline replicates, and
ML_{Test} = mean $\log_{10}$ of CFU/mL of three test replicates.

16. Precision and Bias

16.1 A precision and bias statement for this test method cannot be made at this time. To determine the bias, no material having an accepted reference value is currently available.

17. Keywords

17.1 *Acinetobacter baumannii*; decontamination; disinfection; environmental surfaces; infection control; microbial transfer; nosocomial pathogens; *Staphylococcus aureus*; towelette; Wiperator; wiping

APPENDIXES

(Nonmandatory Information)

X1. THE WIPERATOR MANUAL

Filtaflex Ltd., Almonte, Ontario, Canada; (613) 256-3066 (www.filtaflex.ca)

X1.1 Wiperator

X1.1.1 The Wiperator (Fig. X1.1) permits consistent testing of the efficiency of towelettes by allowing users to control the duration and pressure of test wipings on sample surfaces. During a test, a towelette executes an orbital motion of 10-mm diameter at 1 orbit/s, with its orientation changing by only 6° (similar to a towelette held in a human hand wiping a larger surface). A single wipe run is 5 s—by holding the sample in place, up to 9 consecutive runs (for a total wiping time of 45 s) can be made. The default weight on a towelette during wiping is 150 g and an accessory 150 g weight is supplied.

X1.2 Wipe Loader

X1.2.1 The Wiper Loader (Fig. X1.2A) allows you to wrap a towelette quickly and securely on a Boss.

X1.2.2 Before inserting it in the Wiperator, wrap the test towelette over the end of a Teflon Wipe Boss and secure it by a rubber O-ring (Fig. X1.2B).

X1.2.3 Two sizes of O-ring and 10 bosses supplied with the Wiperator should allow you to accommodate any towelette material thickness (J-Cloth shown) (Fig. X1.2C).

X1.3 Platform for Disks

X1.3.1 Each supplied platform (Fig. X1.3A) accommodates two 1 cm diameter disks of magnetized and brushed stainless steel. The disks drop into circular recesses and small magnets inserted at the back of the platform (Fig. X1.3B) will keep the disks in place during the wiping; two slots on either side of each recess allow you to use forceps to remove the disks for further processing.

X1.3.2 If the test calls for wiping and transfer, one disk (the Donor) on the platform is contaminated with the test organism and the other (the Recipient) is uncontaminated. At the start of the test, position the platform with the Donor away from the Wiperator to initiate the wiping. Then, rotate the platform to bring the Recipient into position for testing the transfer.

X1.3.3 The carrier platform must be decontaminated between uses either by autoclaving or by wiping with 70% (v/v) ethanol.

X1.4 Powering Up

X1.4.1 Plug the 12V DC adapter into the jack behind the Wiperator. Press the ON button gently and the LCD screen will immediately display “INTRODUCE SAMPLE”. There is no OFF button—the Wiperator will switch itself off after 10 min of inactivity.

NOTE X1.1—Except during runs, the Wiperator maintains the brass Wipe Spindle in its most forward position, for easy access. If you displace it (for example, introducing a Boss), it will either jerk to oppose the displacement, or make a full revolution, depending which way it is displaced. The movement cannot hurt, but may surprise you.

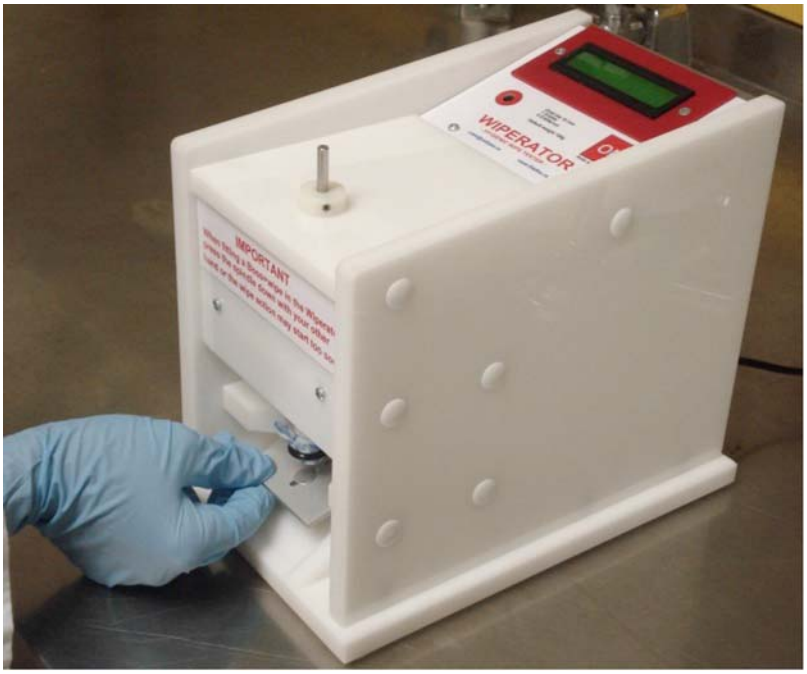

FIG. X1.1 Wiperator

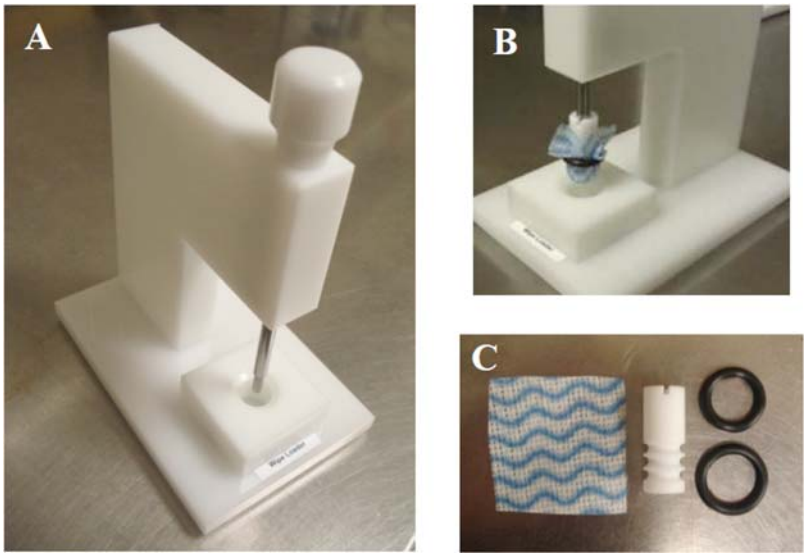

FIG. X1.2 Wipe Loader

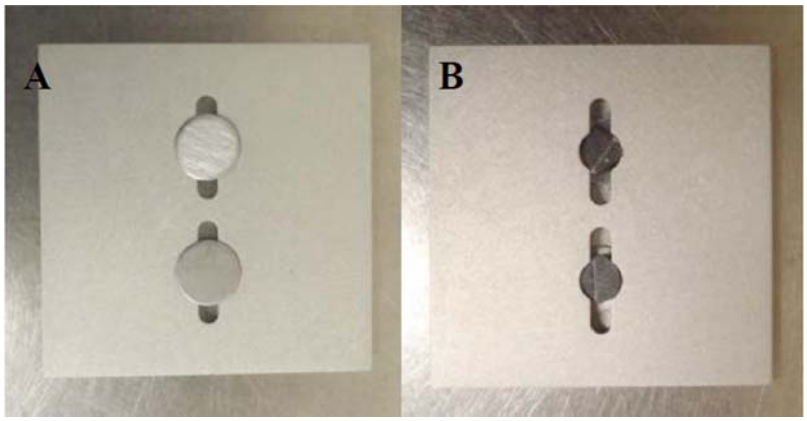

FIG. X1.3 Platform for Disks

X1.5 Introducing the Towelette-loaded Boss

X1.5.1 Slip the Boss up the brass Wipe Spindle and rotate it slightly to ensure its grooves engage the Restrainer Rod—it will be held there magnetically. Do not rotate the Boss once it has engaged, or the Wiperator may make a revolution (see Note X1.1).

NOTE X1.2—When sliding a Boss up the Spindle always press the upper Spindle down with your other hand to prevent it rising up—otherwise, the Wiperator may engage as though a sample has been introduced—this cannot hurt you, but it will waste time until the Wiperator recovers from the mistake.

X1.6 Introducing the Disk Platform for a Wipe Run

X1.6.1 In its "ready" state, the Wiperator's LCD displays "INTRODUCE SAMPLE." A Wipe orbits the outermost Sample Disk. Hold the disk platform by its edge and slide it up the Ramp as far as it will go. Quickly raise the platform up to the towelette-loaded boss to start a 5-sec run; the time-remaining is displayed on the LCD. At the end of 5 sec, wiping will pause for 1 sec, the Wiperator will chirp, and the LCD will display "RUN 1 TERMINATED." Quickly lower the Sample Carrier unless you wish to initiate another 5-sec run. The Wiperator will return to its "INTRODUCE SAMPLE" state.

X1.7 Repeat Runs

X1.7.1 If you keep the disk platform raised, the wipe process will begin for another 5 sec, and then pause for 1 sec. The LCD will now display "RUN 2 TERMINATED." You can perform up to 9 runs this way—after that the Wiperator will tell you "NO MORE..."

X1.8 Platform Moved Error

X1.8.1 During any 5 sec run, the Wiperator will detect inadvertently lowering of the disk platform and possibly compromise the test. If you reposition the platform in less than 1 sec, the Wiperator will continue. Otherwise, it will stop for 15 sec, make long beeps, and display a "RUN ABORTED" message.

NOTE X1.3—Particularly when not using the extra 150 g weight, always lower the Sample Carrier quickly so that its momentum carries the mechanism completely down. Too slow a removal may cause a "RUN ABORTED" single rotation and its 15 sec pause.

X1.9 Specifications

X1.9.1 Length 235-mm, width 150-mm, height 210-mm, and weight 3.0 kg; 12V DC, 2.1-mm center + jack. Orbit speed is 1 revolution/sec (rps) ± 1%. Default mechanism weight

149±1g (actual weight during wipe will be dependent on weight of added Wipe). One extra 150 g weight is supplied.

X2. SPECIFICATIONS FOR DISK CARRIERS OF STAINLESS STEEL

X2.1 Ferritic Stainless Steels

X2.1.1 Consist of chromium (17%) and iron and essentially nickel-free. AISI Type 430 (European equivalent name X6Cr17 and number 1.4016) belongs to Group 2, which is the most widely used family of ferritic alloys. Ferritics are also easier to cut and work.

X2.2 Dimensions

X2.2.1 1 cm (0.39370 inch) in diameter; 0.7-mm (0.27559 inch) thick. AISI 430 - ASTM A240; Japanese Industrial Standard (JIS) G4305; EN 10088-2 No. 4 Finish (EN 10088-2 1J/2J). This is a ground unidirectional finish obtained with 150 grit abrasive (AISI).

X2.3 Passivation

X2.3.1 This is a soak in a mild acid bath for a few minutes to remove any impurities and accumulated debris from the disk surface.

X2.4 Tumbling

X2.4.1 To remove the punching burrs from the edges of the disks they are tumbled in a barrel together with ceramic chips and a cleanser.

X3. THE MILES AND MISRA TEST METHOD

X3.1 Introduction

X3.1.1 The Miles and Misra test method **(12)**, a simple, fast and economical method to measure the number of colony forming units (CFU) of bacteria in clinical and environmental samples, is used to assay the eluates from the carriers.

X3.2 Materials

X3.2.1 Tryptone-sodium chloride (TSC) + 0.1% polysorbate-80 for serial dilutions.

X3.2.2 Disposable plastic plates (100-mm diam.) with Trypticase soy agar (TSA) as recovery medium for the bacteria.

X3.2.3 Sterile 50-dropper Pasteur pipettes to deliver a 20-µL-volume per drop.

X3.3 Method

X3.3.1 Dry the surface of TSA plates by placing them in the incubator (36±1°C) for at least 1 h before use to remove any condensed water on the agar surface for rapid and even soaking of the inocula into the culture medium.

X3.3.2 Label the culture plates with a sample identifier and date of inoculation.

X3.3.3 Mark the outside bottom surface of each plate into three segments (Fig. X3.1) and label them as undiluted, -1, and -2 (or another appropriate dilution series).

X3.3.4 Prepare 10-fold serial dilutions of each test sample using TSC as diluent.

X3.3.5 Using a Pasteur pipette, place 5 discrete drops of a dilution separately onto the surface of the appropriate segment of the culture plate.

X3.3.6 Hold the plates at room temperature for 30±2 minutes in an upright position with the lids fully covered to let the inoculated drops soak into the agar.

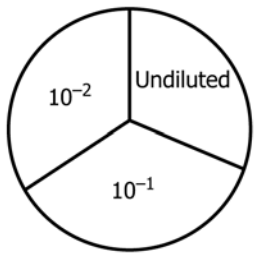

FIG. X3.1 Demarcation and Labeling of Petri Plate for Placement of Drops

X3.3.7 Incubate the plates in an inverted position at 36±1°C for 48±2 h.

X3.3.8 Count and record the number of CFU on the plates (Fig. X3.2).

X3.3.9 If counting from a dilution, then the total number of CFU on a segment X the dilution factor X 10 = CFU/mL of the sample.

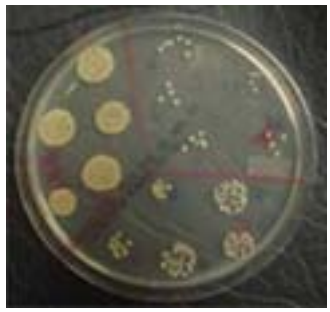

FIG. X3.2 Petri Plate Showing CFU of *Staphylococcus aureus* in the Drops from Undiluted Suspension and Two Serial 10-fold Dilutions

X3.3.10 To check for sterility of the recovery medium, incubate an uninoculated plate at 36±1°C for 48±2 h.

X3.3.11 To check the sterility of the culture broth, diluent (TSC) and soil load, place 25 drops of each on separate agar plates and incubate as above.

X3.3.12 All sterility control plates must be free from any detectable growth for the test to be valid.

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