Designation: D7907 – 14

Standard Test Methods for Determination of Bactericidal Efficacy on the Surface of Medical Examination Gloves[1]

This standard is issued under the fixed designation D7907; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 The methods herein specify two analytical tests for quantitatively evaluating surface bactericidal efficacy of medical examination gloves incorporated with bactericidal properties. They may be used for the determination of bactericidal activity on either the outer or inner glove surface. The methods incorporate bacterial challenges in two different formats: Method (A) a saline or buffered saline solution, and Method (B) a saline or buffered saline solution containing an organic load. Each method represents a different means of microbial contamination that can be expected in the healthcare environment.

1.2 Methods described herein are not appropriate for virucidal, fungicidal, tuberculocidal or sporicidal evaluations as each of these categories require unique culture techniques and testing conditions. Results of the test methods described in this document are limited to bactericidal efficacy against vegetative bacteria.

1.3 A more expansive glove description, such as broad spectrum antimicrobial efficacy, would require testing of a broader list of microbial species than vegetative bacteria alone. It is recommended that interested manufacturers discuss species and strain selections with appropriate regulatory agencies before testing is commenced.

1.4 Testing is to be performed by individuals trained in microbiological techniques under appropriate controlled conditions to ensure integrity of results and personnel safety.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *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. Most regulatory agencies require compliance with Biocompatibility guidelines under ISO 10993.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D7160 Practice for Determination of Expiration Dating for Medical Gloves

E178 Practice for Dealing With Outlying Observations

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

2.2 *Other References:*

U.S. Pharmacopeia General Notices in U.S. Pharmacopeia 26th rev. Rockville, MD: U.S. Pharmacopeia; 2003. 2nd Supplement

3. Terminology

3.1 *Definitions:*

3.1.1 *active side, n*—refers to the inside or outside surface of the glove on which the antimicrobial agent has been placed. This may be both sides if the active agent is placed on both the inside and outside surfaces or if the actives agent is incorporated during compounding and is expressed onto both surfaces.

3.1.2 *aliquot, n*—a portion of a total amount of a solution or suspension.

3.1.3 *bactericide (or bacteriocide), n*—a substance that kills or destroys bacteria.

3.1.4 *bacteriostat, n*—chemical or biological material that stops the growth (reproduction) of bacteria, but does NOT kill them.

3.1.5 *challenge inoculum, n*—the viable microorganisms used to contaminate a specimen, device, or surface, often expressed as to number and type.

3.1.6 *colony forming unit (CFU), n*—a bacterial colony presumed to have originated from a single bacterium.

[1] This test method is under the jurisdiction of ASTM Committee D11 on Rubber and is the direct responsibility of Subcommittee D11.40 on Consumer Rubber Products.

Current edition approved May 1, 2014. Published May 2014. DOI: 10.1520/D7907-14.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3.1.7 *control glove, n*—the same glove formulation as the test glove in every aspect, but without the addition of the bactericidal agent(s).

3.1.8 *coverslip (cover glass), n*—a small, very thin piece of glass used to cover a specimen or smear on a microscope slide or, in this case, to cover the bacterial inoculum on the surface of the glove specimen being tested.

3.1.9 *fastidious bacteria, n*—bacteria that are difficult to grow and keep stable often requiring complex nutritional or environmental factors to survive.

3.1.10 *inoculated, n*—indicates that the challenge inoculum has been delivered to the test specimen or control.

3.1.11 *kill rate, n*—the rate at which microorganisms are killed.

3.1.12 *minimum effective concentration (MEC), n*—the lowest concentration of antibacterial agent per surface unit area which still kills the specified number of microorganisms in the time stated.

3.1.13 *naïve test specimen, n*—not yet contaminated with the target bacteria.

3.1.14 *neutralization, n*—the process for inactivating or quenching the activity of a microbiocide, often achieved through physical (for example filtration or dilution) or chemical means.

3.1.15 *nosocomial, n*—pertaining to or from a hospital or healthcare facility.

3.1.16 *nutrient media agar, n*—a nutritious bacterial growth medium appropriate for growth requirements of target bacteria to which agar has been added for solidification.

3.1.17 *organic load, n*—amount of organic substances present.

3.1.18 *organic substance (organic load), n*—occurs in various forms (serum, protein, blood) and may interfere with the microbicidal activity of a bactericidal agent by reacting with or physically blocking the activity of the agent resulting in one that is less effective.

3.1.19 *outlier, n*—an outlier is an observation or subset of observations that appears to be inconsistent with the remainder of the data.

3.1.20 *recovery broth, n*—a broth, usually containing a neutralizer that preserves and protects surviving microorganisms after they have been subjected to antimicrobial treatment. As it is also used to extract the surviving bacteria from the test surface, the recovery broth may also be referred to as extraction broth or media.

3.1.21 *target bacteria, n*—term often used to refer to the specific bacteria used in the challenge inoculum for the test. Also referred to as the challenge bacteria.

3.1.22 *titer, n*—concentration of bacteria; the number of bacteria per specified aliquot.

3.1.23 *vegetative bacteria, n*—bacteria that are in the growth and reproductive phase; not in sporulated form.

3.1.24 *viable, n*—capable of living, developing, or germinating under favorable conditions.

4. Significance and Use

4.1 In the course of patient care, gloves of healthcare providers are often contaminated with microorganisms. This may occur when they come in direct or indirect contact with contaminated skin, oozing wounds, respiratory droplets, blood, amniotic fluid, saliva, or other potentially infectious materials (OPIM). It has been demonstrated that several bacteria known to be nosocomial pathogens, can survive for days, weeks and even months on surfaces that are touched by gloved hands. The presence of an effective antibacterial treatment on or in the glove that can rapidly reduce the number of viable bacteria on its surface, may also decrease the number of bacteria transferred from a contaminated source (reservoir) to a vulnerable patient or naïve site. These test methods enable assessment of bactericidal efficacy against a broad spectrum of bacteria and conditions, providing a means of efficacy comparisons for manufacturers, purchasers and users.

4.2 Four specific bacteria are listed to enable inter- and intra- laboratory test calibration and to provide common targets against which to compare antibacterial efficacy among products.

4.3 Manufacturers may additionally develop their own list of bacteria against which they will evaluate their products reflecting the circumstances in which their product will be used, the requirements set forth by the various agencies with which they are regulated, and the claims they are seeking. Alterations in test parameters must be validated and documented.

5. Interferences

5.1 Interfering substances can be bactericide-specific and can alter the results of this test. As they may impact bactericidal efficacy in actual use, it is critical that potentially interfering substances such as organic loads, ubiquitous in hospitals, be incorporated as part of the test challenge to determine impact.

5.2 Other potentially interfering substances should be assessed depending on the anticipated use and exposure of the manufacturer's glove.

6. Apparatus

6.1 *Analytical balance.*

6.2 *Bunsen burner or inoculation loop incinerator.*

6.3 *Colony counter.*

6.4 *Conical centrifuge tubes.*

6.5 *Coverslips,* non-coated Borosilicate glass size 18mm × 18mm – sterile.

6.6 *Forceps.*

6.7 *Freezer,* at –20 to –10°C.

6.8 *Glass rods,* bent (a.k.a. hockey sticks).

6.9 *Glass tubes (vials),* 20 mL.

6.10 *Incubator,* capable of temperature and environment required for test bacterium.

6.11 *Vertical Laminar Flow Cabinet (or Bio-safety hood).*

6.12 *McFarland Turbidity Standard 0.5 (or equivalent).*

6.13 *Miscellaneous Laboratory Ware.*

6.14 *Nephelometer (photometer)* or other means of turbidity determination.

6.15 *Plastic vials.*

6.16 *Petri plates* (sterile).

6.17 *pH Meter.*

6.18 *Pipettes-air displacement,* Eppendorf or equivalent, 50 to 1000 microliters with corresponding sterile disposable tips.

6.19 *Punch die or scissors* for specimen preparation.

6.20 *Refrigerator* at 2 to 8°C.

6.21 *Saline* (sterile normal/physiological saline).

6.22 *Spectrophotometer.*

6.23 *Sterile centrifuge tubes,* with caps, 50 mL.

6.24 *Sterile dispenser,* 10 mL delivery capacity.

6.25 *Sterile disposable gloves,* devoid of antibacterial agents.

6.26 *Steam sterilizer.*

6.27 *Timer,* ± 2 second accuracy

6.28 *Turn Table (optional).*

6.29 *Vortex Mixer.*

7. Reagents and General Solutions

7.1 *Bovine Serum Albumin (BSA) powder.*

7.2 *Challenge bacteria,* shall be American Type Culture Collection (ATCC) strains identified for Comparison and Correlation verification (Table 1). Additional challenge bacteria may be selected from any of several worldwide official bacterial culture registries. To ensure vital bacteria that are not far removed from wild type characteristics, bacteria utilized in any phase of these studies should be no more than five passages from the original stock received. The method for doing this in a practical manner is described in Note 1.

7.3 *Deionized Distilled Water.*

7.4 *Ethyl alcohol,* 70 %.

7.5 *Nutrient media agar plates,* shall be appropriate for plate count recovery of the challenge (or target) bacteria.

7.6 *Neutralizer,* is necessary to block or inactivate the bactericide rapidly when the designated contact test time is completed. Utilize Test Methods E1054 for neutralization efficacy verification. Whatever method is used, the same method must be utilized for all test and control specimens in the session. Neutralizer shall not be toxic to the challenge bacteria.

7.7 *Purity of Reagents and Chemicals,* shall be Reagent grade for all tests.

7.8 *Phosphate Buffered Saline (PBS),* may be needed for fastidious bacteria if they die off in normal saline. PBS may be purchased commercially (for example Sterile Dulbecco's Phosphate Buffered Saline) or prepared in the laboratory using a similar formulation.

7.8.1 For example, the Association of Analytical Communities (AOAC) formulation:

7.8.1.1 In 800 mL distilled water:

Solution A: 8.0 g sodium chloride (NaCl)
0.2 g potassium chloride (KCl)
1.15 g sodium phosphate dibasic (Na_2HPO_4)
0.2 g potassium phosphate monobasic (KH_2PO_4)

7.8.1.2 In 100 mL distilled water:

Solution B: 0.1 g calcium chloride ($CaCl_2$)

7.8.1.3 In 100 mL distilled water:

Solution C: 0.1 g magnesium chloride ($MgCl_2$)

7.8.1.4 Prepare each solution separately, and then combine into one vessel.

7.8.1.5 While stirring, adjust to pH 6.9 to 7.1 using 1N NaOH. For most cells and culture conditions, the optimal pH of this solution after filtration is 7.0 to 7.4. To avoid fluctuations in pH, keep the vessel closed until the solution is filtered.

7.8.1.6 Sterilize the solution by filtration, using a 0.22 micron filter.

7.8.1.7 Sterile solutions should be dispensed aseptically into sterile containers and stored at 15°C to 30°C.

7.9 *Standard saline,* is 0.90 % w/v of sodium chloride (NaCl) in reagent grade water. Sterilized.

TABLE 1 Calibration Bacteria for Comparison Testing and Correlation Qualifications

NOTE 1—Although guidance for ordering and shipping ATCC strains is described on their website, anyone purchasing ATCC material is responsible for obtaining the permits. Information regarding the specific requirements for shipment to your location must be obtained from regional authorities. Contact information for ATCC is listed under references.

Identification	ATCC Strain	General Characteristics	Bio-safety Level
Staphylococcus aureus	6538	Gram positive cocci Various human infections Sensitive to antibiotics normally used for treatment	BSL 2
Enterococcus faecalis	33186	Gram positive cocci Various human infections Sensitive to antibiotics normally used for treatment	BSL 2
Pseudomonas aeruginosa	9027	Gram negative rods Various human infections Sensitive to antibiotics normally used for treatment	BSL 2
Klebsiella pneumoniae	4352	Gram negative rods Various human infections Sensitive to antibiotics normally used for treatment	BSL 2

7.10 *Bacteria*—There are two categories of bacteria used in this standard: Calibration Bacteria (four specific bacteria) and Efficacy Test Bacteria (those that may be selected by the manufacturer for testing).

7.10.1 *Calibration Bacteria*—Required test bacteria for assay calibration are listed in Table 1. Utilizing the same bacteria enables inter- and intra- laboratory test calibration as well as providing four common bacterial targets against which one may compare anti-bacterial efficacies among different products. These calibration bacteria must be tested when performing the test for any official comparison or submission purposes and should be performed, at least in part (ex: one gram negative and one gram positive bacteria) to validate internal competency and to ensure test-to-test correlation.

7.10.2 *Efficacy Test Bacteria*—Manufacturers wishing to test additional bacteria should do so based on the intended use of the glove, likely organisms that may be encountered, requirements set forth by the various agencies with which they are regulated, and the claims they are seeking. Alterations in test parameters must be validated and documented. Bacteria used in this test method shall be ATCC registered strains with no more than five transfers from the original ATCC vial as recommended in the U.S. Pharmacopeia 26th rev, 2nd supplement. This requirement guards against loss of vitality and virulence often noted after many transfers.

NOTE 1—This is readily feasible if the initial cultivation and harvest product from the original vial are divided into multiple vials and deep frozen or lyophilized. The second transfer should similarly be preserved. Refer to growth and long term preservation methods explained on the ATCC website.

7.10.3 There may be instances when primary isolates are also utilized for testing purposes. The same requirement of no more than five transfers applies as does the means to accomplish that requirement as described above.

8. Test Specimens

8.1 *Preparation of Test Specimens:*

8.1.1 Specimen shall be obtained from gloves that represent the final finished pre-shipment glove. This is appropriate for screening and quality assessments. For aging studies, time periods should be set to test real time unopened-package storage evaluations and for developing an associated stability curve following the parameters set forth in Practice D7160. Antibacterial efficacy should also be performed on opened boxes or packages of treated gloves to determine the effect of leaving them open over time. Rationale for the condition of the glove tested must be recorded.

8.1.2 Only one surface (inside or outside surface) of a test specimen shall be tested. If both the inside and the outside surfaces of a glove are to be evaluated, a separate set of specimens shall be prepared to evaluate the other surface. This avoids the difficulty of trying to inoculate both sides of the test specimen at the same time.

8.1.3 A test specimen with a surface area of 10.0 cm^2 ± 1.0 cm^2 shall be cut from the glove. The specimen with the required surface area may be prepared by cutting a square, rectangular or circular with scissors or with a punch die. Clean the edge of the cutting implement or punch device with alcohol before commencing specimen preparation on each new glove.

8.1.4 Identify the bio-active side of each test specimen such that it is easily identified throughout the preparation and challenge phase of the study and to ensure the bioactive side is the one tested. Similarly identify the same side surface of the control specimen (inside or outside facing surface of the glove). Validate the method of marking is not, in itself, bactericidal as are some markers.

8.1.5 Record the position on the glove from which the specimen was obtained.

8.1.6 Place prepared test specimen onto a sterile surface (for example, a Petri plate) with the bio-active side to be tested facing up.

8.1.7 *Control Specimen (CS):*

8.1.7.1 Control Specimen are identical to test specimen but without the antibacterial agents and are prepared in the same manner as the test specimen.

8.1.7.2 Place prepared Control Specimen onto sterile Petri plate with the surface to be tested facing up.

9. Preparation of Apparatus

9.1 Coverslips shall be sterile. They may be purchased sterile or sterilized by steam or dry heat sterilization, or be dipped in 70 % ethyl alcohol and flamed.

10. Calibration and Standardization

10.1 Micro-pipettors shall be calibrated.

10.2 Incubators, refrigerators and water baths shall be within calibration period and temperature recorded.

10.3 Timer must be calibrated.

11. Summary of Test Methods

11.1 The following test methods are designed to determine the level of bactericidal efficacy on the outside or inside surfaces of treated medical examination gloves under different exposure conditions.

11.2 *Test Method A*—To represent bacteria in a solution without organic loading, challenge bacteria are suspended in sterile standard or phosphate buffered saline. A 20 ± 0.1 µL aliquot of the bacterial challenge is inoculated onto a test specimen, dispersed and held in place by a sterile coverslip. This method ensures an even distribution across a defined surface area without unequal levels of absorption among specimens. The log reduction from the control specimen without an anti-bacterial agent, an uninoculated control specimen, and the inoculum titer, is determined per unit of time. Of the two test methods, Method A is the easiest to perform and pass. It may thus be the method most often used by manufacturers for initial screening of potential antibacterial agents.

11.3 *Test Method B*—To simulate conditions of glove contamination by organic substances expected in a healthcare environment, test surfaces are first exposed to organic loading. This will present a challenge to many antibacterial treatments in that the protein in an organic load can neutralize several types of bactericides and convert others to bacteriostatic rather than bactericidal activity. After organic conditioning, the site is

challenged with a liquid suspension of target bacteria containing an organic load. The log reduction from the control specimen that did not contain an antibacterial agent as well as the log reduction from the challenge population recovered from the inoculum is determined per unit of time.

12. Method A Procedure: Determination of Antibacterial Efficacy Against Challenge Bacteria in a Sterile Normal or Phosphate Buffered Saline

12.1 *Method A Inoculum Preparation*—The inoculum is prepared by inoculating an agar plate with a pure culture of the appropriate challenge organism utilizing a standard streak isolation technique. Incubate plate at appropriate temperature for 18 to 24 h. Select 3 to 5 well isolated colonies with identical colony morphology. Touch the top of the first colony and inoculate a tube containing 4 to 5 mL of sterile saline or phosphate buffered saline. Flame the loop, cool tip in sterile portion of the agar, touch the surface of the next colony, and again inoculate the saline suspension. Repeat the process for each colony. Adjust the suspension to match a 0.5 McFarland turbidity standard. This results in a suspension containing approximately 10^8 CFU/mL. Alternatively, estimated microbial counts may be determined by a spectrophotometric reading or other turbidimetric means of determination; however, actual counts must be confirmed by standard plate count methods to ensure accurate log reduction calculations.

12.2 The prepared challenge inoculum can be held for up to 3 h on ice. If ice is determined to be lethal to fastidious test microorganisms, or interferes with the assay, room temperature may be used, but must be specified in documentation.

12.3 Study shall be conducted with appropriate safety considerations including use of a vertical laminar flow hood at the designated bio-safety level (BSL) specified for bacteria utilized in the assay (specified by the bacterial registry source).

12.4 Specified contact kill times: Both test and control specimen will be evaluated at contact times: 0, 5, 10, 20, and 30 min. A minimum of three replicates will be tested for each different bacteria and contact time period. These four exposure periods provide the basis by which one glove can be compared to any other glove. More time periods should be added as appropriate to the use of the glove and claims sought. For example, exposure times of less than 5 min would be appropriate for examination gloves used during care of a critically ill patient.

12.4.1 If utilizing this test method for internal use only, contact time periods may be altered to fit historical knowledge of product performance and the amount of information sought (for example, Go/no go or extended degradation curve) which may utilize fewer or additional points. This would be a test method modification and must be identified as such when referencing this method.

12.5 *Bacterial Challenge Testing*—Testing at each time interval shall be conducted in triplicate with the average of the three replicates utilized in calculations. Place a 20 ± 0.1 µL aliquot of the prepared inoculum suspension on the test specimen. This 20 µL challenge inoculum must contain a minimum titer of 10^6 target bacteria to ensure there is a sufficient number of viable bacteria to be able to calculate at least a 4 log kill reliably for a given time period. There are two ways the challenge inoculum may be delivered to the test specimen. Whichever is selected must be done for all specimens.

12.5.1 *Challenge Delivery Option (1)*—Deliver a 20 ± 0.1 µL aliquot of the prepared Method A Challenge Inoculum directly onto the test specimen surface. With forceps, immediately place a sterile coverslip on top of the delivered challenge droplet.

12.5.2 *Challenge Delivery Option (2)*—Place a 20 ± 0.1 µL aliquot of the prepared Method A Challenge Inoculum on the sterile coverslip. This may be accomplished while holding the coverslip with forceps near the test specimen or by resting the coverslip on the specimen while it is being inoculated. Using the forceps, rest the edge of the inoculated coverslip on the test specimen and invert it onto the test site so that the inoculum is trapped under the coverslip, against the test specimen.

12.6 When the specified contact time is completed:

12.6.1 *Neutralizer/Extraction*—Use sterile or flamed and cooled forceps to immediately place the specimen with its coverslip into a 50 mL sterile conical centrifuge tube containing 10 mL of qualified Neutralizer Solution and mix for 15 s using a vortex mixer. (Note: if the specimen sticks to the side of the tube, it should slide down when the broth consumes it during vortexing. If it does not, tap against the tube to dislodge the specimen back into the broth.) Avoid foaming as this kills injured organisms and introduces variability. The coverslip will break into pieces and sink to the bottom of the tube after agitation, while surviving bacteria will remain dispersed. Determine the appropriate neutralization for the remainder of the process (for example, standard, filtration, volume dilution) as described in Test Methods E1054.

12.6.2 *Enumeration*—Determine the number of recovered bacteria. Procedure for enumeration depends on neutralization method utilized from Test Methods E1054. Once that optimal recovery has been achieved, record the speed (rpm) or model vortex mixer with its power setting for use each time the vortex is utilized in the test.

12.6.2.1 *Standard Neutralization and Enumeration*—Remove a 1 ± 0.05 mL aliquot from the neutralization/extraction tube above the broken glass debris. Plate out for enumeration in duplicate by standard plate count method on appropriate recovery agar. Remove another 1 ± 0.05 mL from the neutralizer and perform standard serial dilutions in 9.0 ± 0.1 mL neutralizer solution plated out in duplicate. Invert plates and incubate as appropriate for the bacterium used (usually 24 to 48 h). To ensure sufficient bacteria for reliable calculations of very active bactericides, a backup filtration of the remaining fluid in the initial neutralization tube and first serial dilution tube may be prudent.

12.6.2.2 *Neutralization by Filtration Method*—Remove a 1 ± 0.05 mL aliquot from the neutralization/extraction tube and immediately perform serial dilution in 9.0 ± 0.1 mL sterile saline or phosphate buffered saline in duplicate. Filter each dilution through an appropriate pore size filter (no larger than 0.45 micron). Place filter right side up on appropriate recovery agar. Invert plate and incubate. To ensure sufficient bacteria for

reliable calculations of very active bactericides, a backup filtration of the remaining fluid in the initial neutralization tube may be prudent.

12.6.2.3 *Neutralization by Volume Method* in volume and solution validated to effectively neutralize bactericide activity as described in Test Methods E1054. Enumerate by standard plate count or filtration as described in 12.6.2.1 and 12.6.2.2 respectively.

12.7 *Inoculum Control (IC),* utilizes the same inoculum used in the Method A test. A 20 ± 1 µL aliquot of the Challenge Inoculum is serially diluted and plated out to represent the original titer. This should be done in triplicate at the beginning and end of the testing session to ensure die-off or reproduction over that amount of time does not impact results. For the test to be valid, microbial counts must be >90 % similar. Efficacy should be calculated using the mean between the two inoculum titer determinations.

12.7.1 Incubate plates at appropriate optimal temperature for target organism for 24 to 48 h as determined appropriate for most accurate enumeration CFUs.

12.8 *Extraction Control* for a defined contact time is performed by inoculating a control specimen covering the inoculum with a sterile coverslip and immediately placing the inoculated specimen and coverslip combination into the neutralizer/extraction solution and method selected. Vortex for 15 s at the same vortex power level used for the test specimen, serially dilute and plate out to obtain extraction count. Determine the percent extraction by comparing to the inoculum control. Perform three replicates and utilize the average for the extraction efficiency calculation. Extraction efficiency must be >80 %.

12.8.1 If less than 80 % of the challenge inoculum is recovered, consider use of a mild surfactant to facilitate extraction and/or extending the vortex time to 30 s. If a mild surfactant is selected, it must be shown to be non-toxic to target bacteria (document).

13. Method B Procedure: Determination of Antibacterial Efficacy Against Challenge Bacteria in Saline or Phosphate Buffered Saline Solution With Organic Load

13.1 *Method B Organic Load Challenge Suspension Preparation*—Grow test organism in appropriate broth medium as described in Method A above. Add 5 % (w/v) bovine serum albumin (BSA) powder to the sterile suspension saline or phosphate buffered saline solution prior to adding the bacteria. This solution simulates organic soiling that can occur in healthcare environments. Add bacterial colonies as specified in Method A. Adjust to a concentration of approximately 5.0×10^8 CFU/mL ± 0.5 log with organic load solution or more bacterial colonies as needed.

13.2 Study shall be conducted with appropriate safety considerations including use of a vertical laminar flow hood at the designated biosafety level specified for bacteria utilized in the assay (listed on the ATCC web site).

13.3 *Contact Kill Times*—Minimum exposure/evaluation time periods are identical to those specified in Method A: 0, 5, 10, 20, and 30 min for both test specimen and controls.

13.4 *Organic Load Pre-Conditioning*—All test and control specimens will be exposed to an organic loading step prior to challenge inoculation. Place 50 µL of sterile Buffered Dilution saline containing 5 % (w/v) BSA powder on the exposure area of the test and control specimens. Spread for 5 s with an alcohol flamed bent glass rod. After spreading, the BSA will be allowed to contact the surface for an additional 5-min organic load exposure. After the BSA organic load pre-exposure, testing with the bacterial challenge can proceed.

13.5 *Bacterial Challenge with Organic Load*—The challenge inoculum shall be a 20 ± 0.1 µL aliquot of the Challenge Suspension and shall consist of a minimum of 10^6 bacteria to enable the determination of at least a 4 log kill reliably.

13.5.1 *Challenge Delivery Option (1)*—Deliver the 20 ± 0.1 µL aliquot of the prepared Organic Load Challenge Inoculum directly onto the organic load pre-conditioned test specimen surface. With forceps, immediately place a sterile coverslip on top of the delivered challenge.

13.5.2 *Challenge Delivery Option (2)*—Place a 20 ± 0.1 µL aliquot of the prepared Organic Load Challenge Inoculum on the sterile coverslip. This may be accomplished while holding the coverslip with forceps near the test specimen or resting the coverslip on the specimen while it is being inoculated. Using the forceps, rest the edge of the inoculated coverslip on the test specimen and invert it onto the test site so that the inoculum is trapped under the coverslip, against the test specimen.

13.6 When the exposure time is completed, immediately follow the steps specified in Method A for recovery and enumeration of surviving bacteria depending on the method of neutralization selected.

13.7 Determine the inoculum titer and extraction efficacy as described in Method A.

13.8 Incubate at appropriate optimal temperature for the target organism for 24 to 48 h as determined appropriate for most accurate enumeration of the CFU.

14. Biocidal vs. Biostatic Antibacterial Activity

14.1 Biocidal vs. biostatic activity is a critical determinant when considering potential antimicrobial agents for use on medical gloves. Some antimicrobial agents switch from being bactericidal to becoming bacteriostatic after they have been in contact with specific substances (for example, organic matter). By testing products using both Method A and Method B you can determine whether or not an agent is bactericidal in circumstances normally encountered in the medical environment. To quench any further anti-bacterial activity in the test specimens described in Methods A and B, test specimens were immediately neutralized as soon as their exposure period was completed. Subsequent plating tells you whether or not the bacteria were killed. If they were killed, they will not grow. The test agent has been a successful bactericide. If they grow, the organisms were not killed. The antibacterial agent on the glove specimen was either ineffective or it was bacteriostatic, allowing bacteria to resuming growth and multiplication after no longer in contact with the bacteriostatic agent.

15. Calculation or Interpretation of Results

15.1 *Calculations*—Calculate the log and percent reduction of the bacteria resulting from contact with the specimen using the following formula. Results for the antibacterial efficacy shall be calculated as reduction of bacteria from the initial challenge inoculum (CI) and from the specimen control (SC), if one is used. Each is to be expressed in log (A, B) and percent (C, D) reduction as described in A–D below.

CI = Bacteria (CFU) in challenge inoculum (average between challenge inoculum titer at the beginning and the end of test session)
TS = CFU extracted from antibacterial treated test specimen

NOTE 2—It may not be possible to have a specimen control when evaluating products from the field. Therefore, your only control used for calculation would be the 0 time, inoculum challenge titer and must state as such when calculating the bactericidal efficacy.

A) Log reduction from challenge inoculum = CI – TS expressed in $\log_{10}$ scale (10^x)

B) Log reduction from control specimen titer = SC – TS expressed in $\log_{10}$ scale (10^x)

C) % bacterial reduction efficacy from challenge inoculum = [(CI – TS) ÷ CI] × 100

D) % bacterial reduction efficacy compared to control specimen = [(SC – TS) ÷ SC] × 100

15.2 *Data Presentation*—It is recommended that bacterial reduction data be presented in both chart and kill curve formats. This provides the evaluator discrete information in a readily discernible table format and integrated kill kinetics in trended graphic format. Both display modes together are preferred by many regulatory agencies. These are test methods, and as such, results are designed to enable comparisons of different antibacterial agents, application methods, and manufacturing processes. They are also intended to provide standardized data by which manufacturers, regulatory agencies and customers can assess the bactericidal performance on the surfaces of different gloves utilizing standardized methods.

15.3 *Antimicrobial Performance Criteria* are not specified within this document as requirements and expectations will vary depending on regulatory agencies and the manufacturer's reasons for performing the test.

15.4 *Outliers*—There may occasionally be an individual result or subset of individual test results that are inconsistent with the remainder of the data. If an outlier is suspected, a determination may be made utilizing Practice E178. If an outlier status is confirmed, the data point (s) may be dropped from the calculations. Such determinations must be documented. In some cases, Practice E178 may not be conclusive. If this is the case, consider stating the situation in the report and calculate the data with and without the outlier.

16. Performance Criteria

16.1 Performance criteria are not set for this method. The test may be used for screening, quality monitoring or other purposes where manufacturers will set their own performance requirements. Alternatively, this test method may be used for regulatory submissions where performance requirements may be specified. Check with appropriate agency for any unique parameter or performance requirements.

17. Report

17.1 Challenge bacteria must be specified with ATCC strain and the number of transfers from original stock received from ATCC. If primary isolates are also utilized, all information shall be captured regarding conditions, dates, and circumstances regarding isolation and preservation of isolate.

17.2 Surface preparation and any conditioning must be fully described.

17.3 Test Method and challenge inoculum delivery method must be specified.

17.4 The inoculum challenge count at the beginning and end of the test session shall be assessed and recorded.

17.5 Contact times must be specified.

17.6 Neutralization formulation must be specified and its qualification performance according to Test Methods E1054 documented.

17.7 Glove size and lot identification of specimens must be specified and traceable to device history record (where appropriate) with associated manufacturing and process parameters accessible to manufacturer. The amount of antimicrobial agent that is actually present on the finally product must be specified along with the tolerance deviation for the manufacturer's own product. (This will not, of course, be possible when testing products from the field.)

17.8 All pre-conditioning parameters must be recorded including storage and aging conditions, pre-treatments, physical stress challenges, etc.

17.9 Report Data maintained must include:

17.9.1 Location on glove from which test specimen was obtained and the surface side tested (that is, inside or outside of glove).

17.9.2 Contact times must be specified.

17.9.3 Neutralization formulation must be specified and its qualification performance in the recovery broth verified according to Test Methods E1054, recorded and data maintained.

17.9.4 Specimen and surface preparation must be described.

17.9.5 Size and lot identification of specimens must be specified and traceability to device history record with all manufacturing and process parameters accessible.

17.9.6 All storage and aging conditions shall be specified where appropriate.

17.9.7 Raw data should be documented and maintained as is required by specific regulatory agencies if used for submissions.

18. Keywords

18.1 antibacterial; antimicrobial; bactericidal; bacteriocidal; biocidal; exam glove; examination glove; gloves; medical; medical glove; organic load; surface active

REFERENCES

(1) Official Methods of Analysis of AOAC International, 17th Edition, 2000

(2) ATCC American Type Culture Collection, P.O. Box 1549 Manassas, VA 20108, USA or website at: www.atcc.org

(3) AATCC American Association of Textile Chemists and Colorists, P.O Box 12215, Research Triangle Park, North Carolina, USA 27709 or website at: www.aatcc.org

(4) Draft Guidance for Industry and FDA Staff: Premarket Notification [510(k)] Submission for Medical Devices that include Antimicrobial Agents. July 19, 2007: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071380.htm

BIBLIOGRAPHY

(1) ASTM E691 Standard Practice for Conducting an Inter-laboratory Study to Determine the Precision of a Test Method

(2) ASTM E2149 Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions

(3) ASTM E2197 Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Viricidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides

(4) ASTM E2756 Standard Terminology Relating to Antimicrobial and Antiviral Agents

(5) JIS Z 2801 Japanese Industrial Standard test for Antimicrobial Activity and Efficacy

(6) ISO 22196 Measurement of Antibacterial Activity on Plastics Surfaces

(7) AATCC Test Method 147 Antibacterial Activity Assessment of Textile Materials

(8) Official Methods of Analysis AOAC International, 17th Edition, 2000, Chapter 6, Section 6.3.03A

(9) Clinical and Laboratory Standards Institute, Standard M7: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically