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Standard Test Methods for Determination of Bactericidal Efficacy on the Surface of Medical Examination Gloves¹

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, health, and environmental practices and deter-*

mine the applicability of regulatory limitations prior to use. Most regulatory agencies require compliance with Biocompatibility guidelines under ISO 10993.

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

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 active 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.

¹ These test methods are under the jurisdiction of ASTM Committee D11 on Rubber and Rubber-like Materials and are the direct responsibility of Subcommittee D11.40 on Consumer Rubber Products.

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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.

3.1.5 *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.

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

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
<i>Staphylococcus aureus</i>	6538	Gram positive cocci Various human infections Sensitive to antibiotics normally used for treatment	BSL 2
<i>Enterococcus faecalis</i>	33186	Gram positive cocci Various human infections Sensitive to antibiotics normally used for treatment	BSL 2
<i>Pseudomonas aeruginosa</i>	9027	Gram negative rods Various human infections Sensitive to antibiotics normally used for treatment	BSL 2
<i>Klebsiella pneumoniae</i>	4352	Gram negative rods Various human infections Sensitive to antibiotics normally used for treatment	BSL 2

