



Designation: F 1671 – 97**b03**

Standard Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Blood-Borne Pathogens Using Phi-X174 Bacteriophage Penetration as a Test System¹

This standard is issued under the fixed designation F 1671; 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

Workers, primarily those in the health care profession, involved in treating and caring for individuals injured or sick, can be exposed to biological liquids capable of transmitting disease. These diseases, which may be caused by a variety of microorganisms, can pose significant risks to life and health. This is especially true of blood-borne viruses which cause Hepatitis [Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV)] and Acquired Immune Deficiency Syndrome (AIDS) [Human Immunodeficiency Virus (HIV)]. Since engineering controls can not eliminate all possible exposures, attention is placed on reducing the potential of direct skin contact through the use of protective clothing that resists penetration (29 CFR Part 1910.1030). This test method was developed to assess the effectiveness of materials used in protective clothing for protecting the wearer against contact with blood-borne pathogens using a surrogate microbe suspended in a body fluid simulant under conditions of continuous contact.

1. Scope

1.1 This test method is used to measure the resistance of materials used in protective clothing to penetration by blood-borne pathogens using a surrogate microbe under conditions of continuous liquid contact. Protective clothing material *pass/fail* determinations are based on the detection of viral penetration.

1.1.1 This test method is not always effective in testing protective clothing materials having thick, inner liners which readily absorb the liquid assay fluid.

1.2 This test method does not apply to all forms or conditions of blood-borne pathogen exposure. Users of the test method should review modes for worker/clothing exposure and assess the appropriateness of this test method for their specific applications.

1.3 This test method has been specifically defined for modeling the viral penetration of Hepatitis (B and C) and Human Immunodeficiency Viruses transmitted in blood and other potentially infectious body fluids. Inferences for protection from other pathogens must be assessed on a case-by-case basis.

1.4 This test method addresses only the performance of materials or certain material constructions (for example, seams) used in protective clothing and determined to be viral resistant. This test method does not address the design, overall construction and components, or interfaces of garments or other factors which may affect the overall protection offered by the protective clothing.

1.5 The values stated in SI units or in other units shall be regarded separately as standard. The values stated in each system must be used independently of the other, without combining values in any way.

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.*

2. Referenced Documents

2.1 ASTM Standards:

D 1331 Test Methods for Surface and Interfacial Tension of Solutions of Surface Active Agents²

¹ This test method is under the jurisdiction of ASTM Committee F-23 on Protective Clothing and is the direct responsibility of Subcommittee F23.40 on Biological Hazards.

Current edition approved ~~Dec. 1997~~ August 10, 1997; 2003. Published ~~February 1998~~ October 2003. Originally published as F 1671 – 95 (formerly ES – 22); approved in 1995. Last previous edition approved in 1997 as F 1671 – 97**a**b.

- D 1777 Method for Measuring Thickness of Textile Materials³
 D 3776 Test Method for Mass Per Unit Area (Weight) of Fabric⁴
 D 3862 Test Method for Retention Characteristics of 0.2 µm Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality⁵
 E 105 Practice for Probability Sampling of Materials⁶
 E 171 Specification for Standard Atmospheres for Conditioning and Testing Flexible Barrier Materials⁷
 F 903 Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Liquids⁸
 F 1670 Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Synthetic Blood⁸
 2.2 *Military Standard:*
 MIL-STD-105 Sampling Procedures and Tables for Inspection by Attributes⁹
 2.3 *ANSI/ASQC Standard:*
 ANSI/ASQC Z1.4 Sampling Procedures and Tables for Inspection by Attributes¹⁰
 2.4 *ISO Standard:*
 ISO 2859-1 Sampling Plans for Inspection by Attributes^{11,12}
 2.5 *OSHA Standard:*
 29 CFR Part 1910.1030 “Occupational Exposure to Bloodborne Pathogens: Final Rule,” *Federal Register*, Vol 56, No. 235, Dec. 6, 1991, pp. 64175-64182.

3. Terminology

3.1 Definitions:

- 3.1.1 *agar, n*—a semisolid culture medium used to support the growth of bacteria and other micro-organisms.
 3.1.2 *aseptic, adj*—sterile, free from viable microbiological contamination.
 3.1.3 *assay, n*—analysis of a mixture to determine the presence or concentration of a particular component.
 3.1.3.1 *Discussion*—In this test method, the component being analyzed is a microorganism, Phi-X174 Bacteriophage.
 3.1.4 *assay fluid, n*—a sterile liquid used to wash the test specimen surface to determine microbiological penetration.
 3.1.4.1 *Discussion*—In this test method, the assay fluid is bacteriophage nutrient broth and the microorganism is the Phi-X174 Bacteriophage. The assay fluid is used to wash the Phi-X174 Bacteriophage from the normal inside surface of the test specimen.
 3.1.5 *bacteriophage, n*—a type of virus which infects bacteria.
 3.1.5.1 *Discussion*—In this test method, the bacteriophage is Phi-X174. The Phi-X174 Bacteriophage is not pathogenic to humans, but serves to simulate viruses that are pathogenic to humans.
 3.1.6 *blood-borne pathogen, n*—an infectious bacterium or virus, or other disease inducing microbe carried in blood or other potentially infectious body fluids.
 3.1.6.1 *Discussion*—For the purpose of this test method, the primary blood-borne pathogens include Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Human Immunodeficiency Virus (HIV). Other microorganisms must be considered on a case-by-case basis.
 3.1.7 *body fluid, n*—any liquid produced, secreted, or excreted by the human body.
 3.1.7.1 *Discussion*—In this test method, body fluids include those liquids potentially infected with blood-borne pathogens, including, but not limited to, blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids (see section 29 CFR Part 1910.1030).
 3.1.8 *body fluid simulant, n*—a liquid which is used to act as a model for human body liquids.
 3.1.8.1 *Discussion*—In this test method, the body fluid simulant is bacteriophage nutrient broth, which is intended as a model for human body liquids as it approximates the lower end of the surface tension range for blood and body fluids (excluding saliva), 0.042 ± 0.002 N/m.
 3.1.9 *challenge suspension, n*—a liquid containing an agent that is used to test the penetration resistance of materials.
 3.1.9.1 *Discussion*—In this test method, the challenge suspension is the bacteriophage challenge suspension; a nutrient broth containing the Phi-X174 Bacteriophage.

² *Annual Book of ASTM Standards*, Vol 15.04.

³ *Annual Book of ASTM Standards*, Vol 07.01.

⁴ *Annual Book of ASTM Standards*, Vol 07.02.

⁵ *Annual Book of ASTM Standards*, Vol 11.02.

⁶ *Annual Book of ASTM Standards*, Vol 14.02.

⁷ *Annual Book of ASTM Standards*, Vol 15.09.

⁸ *Annual Book of ASTM Standards*, Vol 11.03.

⁹ Available from Standardization Documents Order Desk, Bldg. 4 Section D, 700 Robbins Ave., Philadelphia, PA 19111-5094, Attn: NPODS.

¹⁰ Available from American Society for Quality Control, 611 E. Wisconsin (ASQ), 600 N. Plankinton Ave., Milwaukee, WI 53202.

¹¹ Available from American National Standards Institute (ANSI), 25 W. 42nd St., 4th Floor, New York, NY 10036.

¹² This test method is under the jurisdiction of ASTM Committee F23 on Protective Clothing and is the direct responsibility of Subcommittee F23.40 on Biological Hazards.

Current edition approved August 10, 2003. Published October 2003. Originally approved in 1995. Last previous edition approved in 1997 as F 1671 – 97b.

3.1.10 *lawn*, *n*—as in microbiology, a cloudy, uniform growth of bacteria in a thin layer of top agar in a petri dish.

3.1.10.1 *Discussion*—In this test method, *E. coli C.* has been selected as the bacterium used to produce the lawn.

3.1.11 *lysis*, *n*—the disintegration or destruction of whole bacterial cells.

3.1.11.1 *Discussion*—In this test method, the lysis of the host bacteria, *E. coli C.*, is caused by Phi-X174 Bacteriophage.

3.1.12 *medium (plural, media)*, *n*—a nutrient system for the cultivation of cells or organisms, and especially bacteria.

3.1.12.1 *Discussion*—In this test method, the term media is used to describe mixtures compounded to support the growth of specific microorganisms; for example, bacteriophage nutrient broth, top agar.

3.1.13 *morphology*, *n*—the form and structure of a particular organism.

3.1.14 *nutrient broth*, *n*—a liquid medium.

3.1.14.1 *Discussion*—In this test method, the nutrient broth is the bacteriophage nutrient broth which is used to culture the host bacteria, *E. coli C.*, and to aid in manipulating the Phi-X174 Bacteriophage through the various stages of the procedure, such as; suspending the Phi-X174 Bacteriophage for challenging the test material in the penetration cell, assaying the normal inside test material surface and, if required, making dilutions of the assay fluid for plating.

3.1.15 *penetration*, *n*—~~the flow movement of a liquid matter~~ through closures, porous materials, seams, and pinholes or other imperfections in a protective clothing material on a nonmolecular level.

3.1.15.1 *Discussion*—For this test method, the specific matter is a bacteriophage challenge suspension containing nutrient broth with Phi-X174 Bacteriophage.

3.1.16 *plaque*, *n*—as in virology, a visible, clear area, which is theoretically the result of the infection and lysis of host cells by a single viable virus.

3.1.16.1 *Discussion*—In this test method, the term plaque is used to describe a visible, clear area, in the lawn of *E. coli C.* in top agar, which is theoretically the result of a single viable Phi-X174, where the bacteria have been destroyed by bacteriophage infection and lysis.

3.1.17 *plaque forming unit (PFU)*, *n*—a virus particle capable of producing plaques by infecting and lysing bacteria in a lawn in top agar.

3.1.18 *plate*, *n*—as in microbiology, a Petri dish containing culture medium.

3.1.19 *protective clothing*, *n*—apparel used for the purpose of protecting parts of the body from contact with a potential hazard.

3.1.19.1 *Discussion*—In this test method, materials used in protective clothing are being evaluated. The potential hazard of contact with blood-borne pathogens is being simulated.

3.1.20 *sterile*, *adj*—free from viable microorganisms.

3.1.21 *surrogate microbe*, *n*—a microorganism which is used to act as a simulant for other microorganisms which are pathogenic to humans.

3.1.21.1 *Discussion*—In this test method, the surrogate microbe is the Phi-X174 Bacteriophage, intended as a model for HCV and to simulate both HBV and HIV.

3.1.22 *titer*, *n*—the quantity of a substance required to react with, or to correspond to, a given amount of another substance.

3.1.22.1 *Discussion*—In this test method, titer is used to describe the concentration of viable bacteriophage as measured in plaque forming units per millilitre (PFU/mL).

3.1.23 *viral penetration*, *n*—the penetration of a material by a virus.

3.1.23.1 *Discussion*—In this test method, viral penetration is used to describe the physical translocation of the Phi-X174 Bacteriophage through closures, seams, pores, and pinholes or other imperfections in materials used in protective clothing.

3.1.24 *viral resistant*, *adj*—referring to materials which impede viral penetration under specified laboratory test conditions and detection methods.

3.1.24.1 *Discussion*—In this test method, protective clothing materials which demonstrate *pass* results are considered to be resistant to viral penetration.

3.1.25 *virus*, *n*—a minute infectious agent, which lacks independent metabolism and is only able to replicate within a living host cell.

4. Summary of Test Method

4.1 A specimen is subjected to a nutrient broth containing a virus for a specified time and pressure sequence.

4.2 Visual detection of penetration is supplemented with an assay procedure that will detect viable viruses which penetrate the material even when liquid penetration is not visible. Any evidence of viral penetration for a test specimen constitutes failure.

5. Significance and Use

5.1 This test method is based on Test Method F 903 for measuring resistance of chemical protective clothing materials to penetration by liquids. This test method is normally used to evaluate specimens from individual finished items of protective clothing and individual samples of materials that are candidates for items of protective clothing.

5.1.1 Finished items of protective clothing include gloves, arm shields, aprons, gowns, coveralls, hoods, and boots.

5.1.2 The phrase “specimens from finished items” encompasses seamed and other discontinuous regions, as well as the usual continuous regions of protective clothing items.

5.2 It is known that body fluids penetrating protective clothing materials are likely to carry microbiological contaminants;

TABLE 1 Specimen Exposure Procedures

Procedure	Pressure/Time Sequence and Retaining Screen Options
A	0 kPa (0 psig) for 5 min, followed by 13.8 kPa (2 psig) for 1 min, followed by 0 kPa (0 psig) for 54 min.
A	0 kPa [0 psig] for 5 min, followed by 13.8 kPa [2 psig] for 1 min, followed by 0 kPa [0 psig] for 54 min. A retaining screen is not used to support the specimen.
B	0 kPa (0 psig) for 5 min, followed by 13.8 kPa (2 psig) for 1 min, followed by 0 kPa (0 psig) for 54 min.
B	0 kPa [0 psig] for 5 min, followed by 13.8 kPa [2 psig] for 1 min, followed by 0 kPa [0 psig] for 54 min. A retaining screen is used to support the specimen. The type must be specified.

however, visual detection methods are not sensitive enough to detect minute amounts of liquid containing microorganisms (1,2,3).¹³ This test method uses media containing Phi-X174 Bacteriophage. The visual detection technique of this test method is supplemented with a biologically based assay capable of detecting virus under the specified test conditions.

5.3 Test Method F 1670, allows the screening of protective clothing materials for resistance to penetration with synthetic blood as a challenge liquid. Test Method F 1670 uses the same penetration test cell and technique, but exposes material specimens to synthetic blood with visual detection of liquid penetration. Materials passing Test Method F 1670 should then be tested against bacteriophage penetration using this test method to verify performance.

5.4 This test method has been specifically designed for measuring penetration of a surrogate microbe for Hepatitis (B and C) and the Human Immunodeficiency Viruses. The surrogate, Phi-X174 Bacteriophage, used in this test method is similar to HCV in size and shape but also serves as a surrogate for HBV and HIV. Inferences about protection from other pathogens must be assessed on a case-by-case basis.

5.5 Part of the protocol in Procedure A and B in Table 1, for exposing the protective clothing material specimens to the Phi-X174 Bacteriophage challenge suspension, involves pressurization of the penetration cell to 13.8 kPa ([2 psig]). This hydrostatic pressure has been documented to discriminate between protective clothing material performance and correlate with visual penetration results that are obtained with a human factors validation (4). Some studies, however, suggest that mechanical pressures exceeding 345 kPa ([50 psig]) can occur during actual clinical use (5, 6). Therefore, it is important to understand that this test method does not simulate all the physical stresses and pressures that might be exerted on protective clothing materials during actual use.

5.6 Medical protective clothing materials are intended to be a barrier to blood, body fluids, and other potentially infectious materials. Many factors can effect the wetting and penetration characteristics of body fluids, such as: surface tension; viscosity; and polarity of the fluids, as well as the structure and relative hydrophilicity or hydrophobicity of the materials. The surface tension range for blood and body fluids (excluding saliva) is approximately 0.042 to 0.060 N/m (7) . To help simulate the wetting characteristics of blood and body fluids, the surface tension of the Phi-X174 Bacteriophage challenge suspension is adjusted to approximate the lower end of this surface tension range. This is accomplished by adding surfactant to the Phi-X174 Bacteriophage nutrient broth. The resulting surface tension of the Phi-X174 Bacteriophage challenge suspension is approximately 0.042 ± 0.002 N/m.

5.7 Testing prior to degradation by physical, chemical, and thermal stresses which could negatively impact the performance of the protective material, could lead to a false sense of security. Additional tests should be considered that assess the impact of storage conditions and shelf life on disposable products and the impact of laundering and sterilization on reusable products. The integrity of the protective barrier may also be compromised during use by such effects as flexing and abrasion (8). Prewetting agents, such as alcohol, and contaminating agents, such as perspiration, may also compromise the integrity of the protective barrier. If these conditions are of concern, the performance of protective clothing materials should be evaluated for Phi-X174 Bacteriophage penetration following an appropriate preconditioning technique representative of the expected conditions of use.

5.8 This test method involves a sensitive assay procedure for determining protective clothing material resistance to penetration by a surrogate microbe. Because of the length of time required to complete this method, it may not be suitable for use as a material or protective clothing quality control or quality assurance procedure.

5.9 If this procedure is used for quality control or to support broad product claims concerning the viral resistant properties of materials used in protective clothing, proper statistical design and analysis of larger data sets than those specified in this test method should be performed.¹⁴ Examples of acceptable sampling plans can be found in MIL-STD-105, ANSI/ASQC Z1.4, and ISO 2859-1.

5.10 This test method requires a working knowledge of basic microbiological techniques (9) .

¹³ The boldface numbers in parentheses refer to the list of references at the end of this standard.

¹⁴ The U.S. Food and Drug Administration (FDA) has specific Acceptable Quality Level (AQL) requirements for the use of this test method. Manufacturers, distributors, and repackagers of surgical apparel should contact the FDA's Division of Small Manufacturers Assistance (DSMA).

6. Apparatus and Materials

6.1 Apparatus:

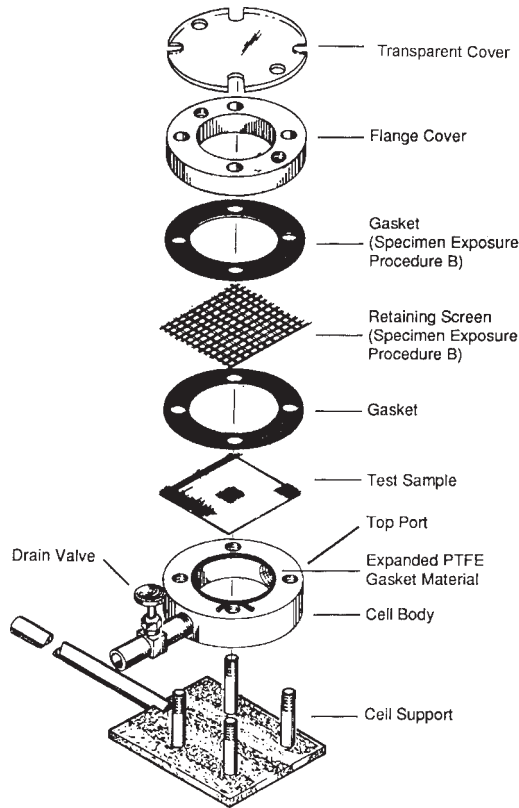


FIG. 1 Exploded View of the Penetration Test Cell with Retaining Screen

6.1.1 *Thickness Gage*, suitable for measuring thickness to the nearest 0.02 mm (or nearest 0.001 in.), as specified in Test Method D 1777, to determine the thickness of each protective clothing material specimen tested.

6.1.2 *Penetration Test Cell*,^{15,16} to restrain the test specimen during contact with the pressurized Phi-X174 Bacteriophage challenge suspension. In the test cell, the specimen acts as a partition separating the bacteriophage challenge suspension from the viewing side of the penetration cell. The capacity of the cell body is approximately 60 mL (2.0 oz) [2.0 oz] for the challenge suspension. A flange cover, with an open area to allow visual observation and assay of the specimen, and a transparent cover are included. The cell body has a top port for filling and a drain valve for draining the penetration test cell. Other items, such as a fitting to allow attachment of the air line to the top port in the cell body, gaskets, and the retaining screen will also be required. Specifications for the penetration cell are provided in Test Method F 903. Diagrams of the test cell and apparatus are provided in Figs. 1 and 2, respectively.

6.1.3 *Retaining Screen*, a smooth finish plastic or metal square mesh screen meeting the following specifications is used for Procedure B from Table 1.

% open area	>50
Limits deflection of specimen to	<= 5.0 mm (0.2 in.)
Limits deflection of specimen to	<= 5.0 mm [0.2 in.]
Can be sterilized by either ethylene oxide or autoclaving	
Material of construction meets criteria in 15.4.	

6.1.4 *Air Pressure Source*, capable of providing air at 13.8 ± 1.38 kPa (2.0 ± 0.2 psig).

6.1.5 *Incubator*, capable of maintaining 35 to 37°C.

6.1.6 *Water Bath*, capable of maintaining 45 ± 2 °C.

6.1.7 *Analytical Balance*, capable of weighing 0.001 g.

6.1.8 *Vortex Mixer*.

¹⁵ The penetration test apparatus is available from Wilson Road Machine Shop, 1170 Wilson Rd., Rising Sun, MD 21911.

¹⁶ This is the sole source of supply of the apparatus known to the committee at this time. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,¹ which you may attend.

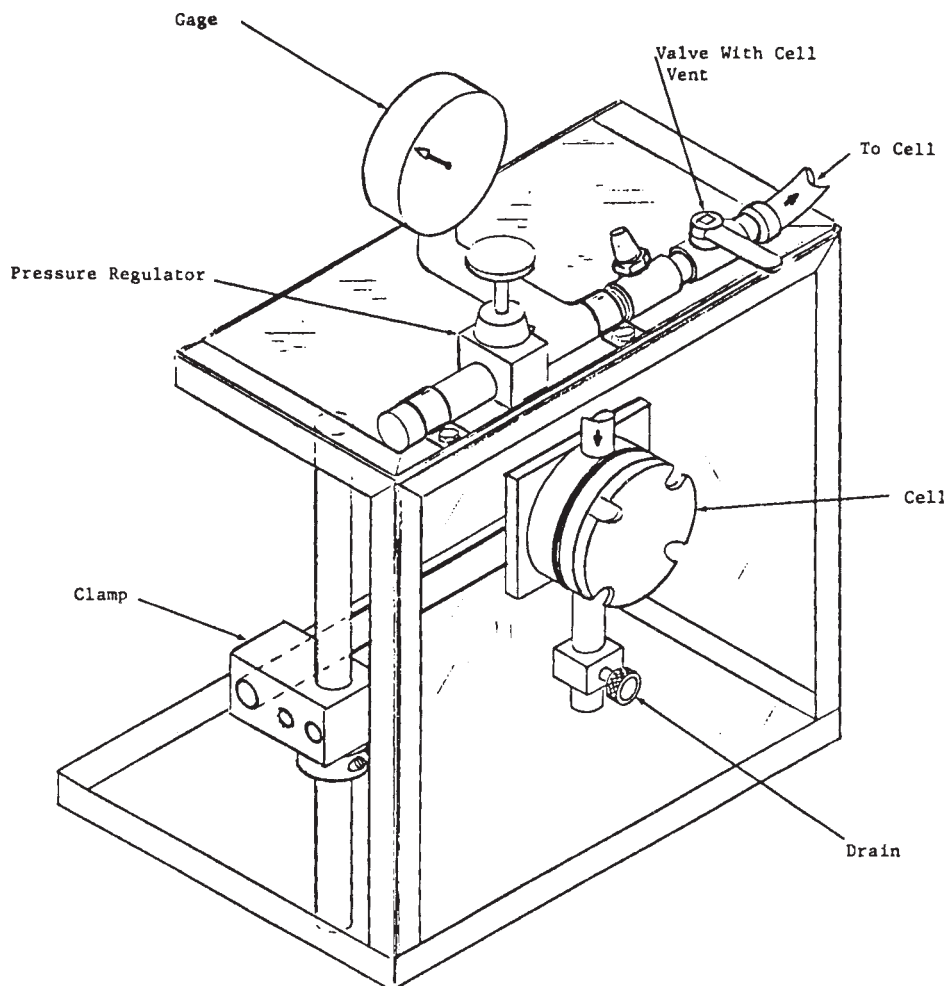


FIG. 2 Three Dimensional Side View of the Test Apparatus

6.1.9 Refrigerator, capable of maintaining 2 to 8°C.

6.1.10 Autoclave, capable of maintaining 121 to 123°C and 207 to 221 kPa absolute (30 [30 to 32 psia]).

6.1.11 Stopwatch or Electronic Timer .

6.1.12 Orbital Shaker.

6.1.13 pH Meter, sensitive to 0.1 pH units.

6.1.14 Inoculating Loop.

6.1.15 Torque Wrench, capable of 13.6 N·m ([120 in.-lb]).

6.1.16 Spectrophotometer, capable of 640 nm.

6.1.17 Centrifuge, capable of 10 000 × g.

6.2 Materials:

6.2.1 Petri Dishes, sterile 15 by 100 mm.

6.2.2 Pipettes, sterile 1, 5, and 10 mL.

6.2.3 Test Tubes, 13 by 100 mm.

6.2.4 Test Tube Rack, stainless.

6.2.5 Membrane Filters,^{16,17} sterile, 0.22 μm.

6.2.6 Bottles, sterile, glass, 100 to 500-mL capacity.

6.2.7 Micropipettes, capable of delivery of 2 μL, accurately and consistently.

7. Reagents

7.1 Bacteriophage, Phi-X174, ATCC 13706-B1.^{18,19 ,16}

¹⁷ Membrane filters shall be used which meet requirements of Test Method D 3862.

¹⁸ Cultures may be obtained from ATCC American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

¹⁹ Purified virus preparations may be obtained from Promega Corp., 2800 Woods Hollow Rd., Madison, WI 53711-5399.

NOTE 1—The surrogate microbe, Phi-X174 Bacteriophage, was selected as the most appropriate model for blood-borne pathogens because of its small size, spherical (icosahedral) morphology, environmental stability, nonhuman infectivity, high-assay sensitivity, rapid assay, and high titer. The Phi-X174 Bacteriophage has no envelope and is one of the smallest known viruses (0.027 μm in diameter). The Phi-X174 Bacteriophage is used at a challenge titer of at least 1.0×10^8 PFU/mL (plaque forming units per millilitre).

7.2 *Bacteria, E. coli C.*, ATCC 13706.^{18,16}

7.3 *Purified Water*, Q.S.

7.4 *Nutrient Broth*.^{20,16}

7.5 *Calcium Chloride* (CaCl_2).

7.6 *Potassium Chloride* (KCl).

7.7 *Sodium Hydroxide* (NaOH), *N* solution.

7.8 *Surfactant*, polysorbate 80.^{21,16}

7.9 *Bacto-Agar*.^{20,16}

8. Hazards

8.1 Sterilize all apparatus and supplies which come into contact with the Phi-X Bacteriophage, before and after conducting this test method, by autoclaving from 121 to 123°C and from 207 to 221 kPa absolute (~~30~~ [30 to 32 psia]) for 15 min. Other methods of sterilization may be used provided there is no inhibition of the test organisms during the subsequent course of the test. Exercise extreme care to avoid contamination of the laboratory spaces by complete sterilization or high level disinfection of all apparatus and supplies. This will reduce the possibility of laboratory contamination.

8.1.1 If test specimens are sterilized, ensure that the method of sterilization chosen does not unintentionally affect the performance of the test specimen. Sterilize test specimens in accordance with manufacturer's recommendations.

8.2 Although there is no known hazard for human infection from Phi-X174 Bacteriophage, test operators should avoid direct contact with liquids containing the bacteriophage.

8.3 Either keep a transparent safety shield between the penetration cell and the observer or, alternatively, perform the tests behind the window in a safety hood.

9. Preparation of Media

9.1 *Bacteriophage Nutrient Broth (Phi-X)*:

9.1.1 Prepare bacteriophage nutrient broth using the following:

Nutrient broth	8.0 \pm 0.1 g
Potassium chloride	5.0 \pm 0.06 g
Calcium chloride	0.2 \pm 0.003 g
Purified water, Q.S. to	1000 \pm 12.5 mL
Surfactant	0.1 \pm 0.00125 mL (9.1.3)

9.1.2 Adjust the pH of the bacteriophage nutrient broth to 7.2-7.4 using 2.5 *N* sodium hydroxide.

9.1.3 Dilute 1 volume of 0.1 % surfactant with nine volumes of bacteriophage nutrient broth. To ensure adequate mixing, prior to sterilization, heat the bacteriophage nutrient broth while stirring in the surfactant. A final concentration of 0.01 % surfactant is recommended to adjust the final surface tension to 0.042 ± 0.002 N/m.

9.1.4 Sterilize the bacteriophage nutrient broth in the autoclave.

9.1.5 Measure the resulting surface tension of the sterile solution using Test Method D 1331. Do not use the bacteriophage nutrient broth unless the corrected surface tension is within the 0.042 ± 0.002 -N/m range.

9.2 *Bottom Agar (Phi-X)*:

9.2.1 Prepare bottom agar using the following:

Bacto-agar	15.0 \pm 0.19 g
Nutrient broth	8.0 \pm 0.1 g
Potassium chloride	5.0 \pm 0.06 g
Purified water, Q.S. to	1000 \pm 12.5 mL
Calcium chloride	1.0 \pm 0.0125 mL
(Add sterile calcium chloride after autoclaving the bottom agar, 9.2.2.)	

9.2.2 Prepare sterile calcium chloride by autoclaving a 1-*M* solution of calcium chloride in purified water.

9.2.3 Adjust the pH of the bottom agar to 7.2-7.4 using 2.5 *N* sodium hydroxide.

9.2.4 Sterilize the bottom agar in the autoclave.

9.3 *Top Agar (Phi-X)*:

9.3.1 Prepare the top agar using the following:

Bacto-agar	7.0 \pm 0.09 g
Nutrient broth	8.0 \pm 0.1 g
Potassium chloride	5.0 \pm 0.06 g

²⁰ Nutrient Broth and Bacto-Agar may be obtained from Difco, Detroit, MI 48232.

²¹ Surfactant type Polysorbate 80 is suitable for this use and may be obtained from Aldrich Chemical Co., Inc., 1001 West Saint Park Ave., Milwaukee, WI 53233.

Purified water, Q.S. to	1000 ± 12.5 mL
Calcium chloride	1.0 ± 0.0125 mL
(Add sterile calcium chloride after autoclaving the top agar, 9.3.2.)	

9.3.2 Prepare sterile calcium chloride by autoclaving a 1-M solution of calcium chloride in purified water.

9.3.3 Adjust the pH of the top agar to 7.2-7.4 using 2.5 N sodium hydroxide.

9.3.4 Sterilize the top agar in the autoclave.

10. Test Specimens and Controls

10.1 Test Specimens:

10.1.1 Specimens selected from single material samples or individual protective clothing items consist of either a single layer or a composite of multiple layers that is representative of an actual protective clothing material or construction with all layers arranged in proper order.

10.1.1.1 If, in the design of an item of protective clothing, different materials or thicknesses of materials are specified at different locations, select specimens from each location.

10.1.1.2 If, in the design of an item of protective clothing, seams are claimed to offer the same protection as the material, test additional specimens containing such seams. Prepare seam specimens such that the entire seam configuration (profile) fits within the penetration test cell inner diameter. Use polytetrafluoroethylene (PTFE) gasket materials between the cell body and test specimen and between the flange cover or retaining screen and specimen to help prevent leakage.^{22,16}

10.1.1.3 Ensure that there are no foreign substances on either side of the area of the material specimen actually tested (for example, it is known that some inks affect the bacteriophage) (10).

10.1.2 Each material specimen to be tested shall have a minimum dimension of 70 mm-([2.75 in.]). A75-mm-([3.0-in.]) square is preferred.

10.1.3 Test three specimens taken at random from each material, composite, area (in the case of a heterogeneous design), or other condition. When false positive failures are suspected, retest (15.2.3). Random samples shall be generated as described in Practice E 105.

10.1.4 It is possible that protective clothing materials incorporating an impervious layer between two fabric layers may be sensitive to false positive failures by wicking at the edges. Seal the edges of the test specimens to prevent *wicking* modes of failure. Seal test specimens with an adhesive, parafilm, paraffin wax, or adhesive backed foam prior to testing.

10.1.4.1 Seal only the edges of the test specimens, leaving the center 57-mm-([2.25-in.]) area open for testing. Do not allow sealants to intrude, block, or occlude the structure of the test specimen in the test area, as this will compromise the test procedure. Choose sealants and sealing methods that are compatible with the protective clothing materials and sterilization methods.

10.1.5 Sterilize test specimens if sterilization is part of the process used to prepare protective clothing, employing the subject materials, for use. Use a method of sterilization which does not unintentionally affect the performance of the test specimens. Sterilize test specimens in accordance with manufacturer's recommendations.

10.2 Controls:

10.2.1 Use the following controls concurrently with the testing of each protective clothing material.

10.2.1.1 *Aerosol/Airborne Contamination Controls*—Settle plates or other appropriate means may be utilized to determine background aerosol/airborne counts for the Phi-X174 Bacteriophage.

10.2.1.2 *Non-sterile Material Blank Control*—When non-sterile specimens are tested, an optional non-sterile material blank control may be included in the test program to demonstrate that the test materials are not contaminated with the Phi-X174 Bacteriophage prior to the test. Select this specimen from the same non-sterile materials that are being tested and subject it to the same handling and exposure procedures as the other test specimens, with the exception of using sterile nutrient broth with polysorbate 80 only as the challenge suspension.

10.2.1.3 *Negative Test Sample Controls*— These samples should be made of a heavy gage monolithic film.^{16,23}

10.2.1.4 *Positive Test Sample Controls*— These samples should be made of a microfiltration medium with a pore size that is slightly larger than the mean diameter of the Phi-X174 Bacteriophage, 0.027 μm.^{16,24}

11. Conditioning

11.1 Condition each protective clothing specimen for a minimum of 24 h by exposure to a temperature of 21 ± 5°C and a relative humidity of 30 to 80 % as described in Specification E 171.

11.2 Other preconditioning options than those prescribed in 11.1 may be necessary to assess possible degradation mechanisms of protective clothing materials (5.7).

12. Preparation of Bacteriophage Challenge Suspension

12.1 Using an inoculating loop, inoculate 10 to 25 mL of bacteriophage nutrient broth in a 250-mL Erlenmeyer flask with *E.*

²² PTFE gasket material is available from W. L. Gore and Associates, Inc., Industrial Sealant Group, Elkton, MD 21921.

²³ One type of negative test sample control is a medical packaging grade of polyester film. Polyester film may be obtained from the Du Pont Co., Wilmington, DE 19898.

²⁴ One type of positive control is a 0.04-μm microporous film. Microporous nylon film may be obtained from Meissner Filtration Products, 4181 Calle Tesoro, Camarillo, CA 93012.

coli C. Incubate the bacterial culture overnight at 35 to 37°C with shaking (200 to 250 rpm).

12.2 Prepare a 1:100 dilution of the overnight bacterial culture in 100 mL of fresh bacteriophage nutrient broth in a 1-L Erlenmeyer flask. Incubate the flask at 35 to 37°C with shaking (12.1). Grow bacterial culture to a density of 2 to 4×10^8 CFU/mL (about 3 h). This cell density corresponds to a 0.3 to 0.5 absorbance reading (at 640 nm), as measured on a spectrophotometer.

12.3 Inoculate the bacterial culture with 5 to 10 mL of the Phi-X174 Bacteriophage stock having a titer of 1.0×10^9 to 1.0×10^{10} PFU/mL. The ratio of bacteriophage to bacterial cells should be between 0.1 to 2.0.

12.4 Incubate the inoculated bacterial culture at 35 to 37°C with vigorous shaking for 1 to 5 h or until lysis is complete. Lysis is considered complete when the absorbance reading at 640 nm stops decreasing.

12.5 Centrifuge the culture for 20 min at $10\,000 \times g$ to remove large cell debris. Decant the supernatant into a clean tube.

12.6 Filter the bacteriophage-containing supernatant through a 0.22- μ m filter to purify the bacteriophage solution.

12.7 Determine the titer of the bacteriophage stock and store at 2 to 8°C. The phage titer obtained is typically in the range from 3.0 to 7.0×10^{10} PFU/mL.

12.8 Prepare the bacteriophage challenge suspension by diluting the phage stock (12.7) in the bacteriophage nutrient broth (9.1) to the required concentration (15.4.2). Verify the final concentration of the phage using the assay procedure (Section 14).

13. Test Procedure

13.1 Measure the thickness of each specimen to the nearest 0.02 mm (~~or~~ or nearest 0.001 in.) in accordance with Test Method D 1777.

13.2 Measure the weight of each specimen to the nearest 10 g/m² (~~[0.1 oz/yd²]~~ according to) in accordance with Test Method D 3776, Option C—Small Swatch of Fabric.

13.3 If elected, settle plates can be used as a type of aerosol/airborne contamination control. Place settle plates in strategic locations during the aseptic test specimen insertion, filling, testing, draining, and assay operations to help identify potential problems associated with aerosolized or airborne Phi-X174 Bacteriophage. Prepare settle plates as follows:

13.3.1 Dispense 2.5 mL of sterile molten top agar into sterile test tubes and hold the temperature of the top agar at $45 \pm 2^\circ\text{C}$. Prepare one test tube for each settle plate.

13.3.2 Add 0.2 to 0.3 mL of an overnight culture of *E. coli C*. to each top agar tube.

13.3.3 Vortex tubes well and pour contents over the surface of bottom agar plates.

NOTE 2—Refer to the Manual of Methods for General Bacteriology (9) for the preparation of bottom agar plates.

13.3.4 Allow agar to solidify. Settle plates may be used immediately.

13.3.5 After use, incubate settle plates with the assay plates for the test sample replicates and positive and negative controls.

13.4 When distortion of the test material is suspected of causing failure with Specimen Exposure Procedure A of this test method, use Specimen Exposure Procedure B. Specimen Exposure Procedure B involves the use of a retaining screen which is used when support of extensible or elastomeric materials is required.

13.4.1 Select Specimen Exposure Procedure A or B from Table 1.

13.5 Determine the compatibility of each test material (15.4).

13.6 Autoclave the penetration test cell at 121 to 123°C and 207 to 221-kPa absolute (~~30~~ [30 to 32 psia]) for 15 min. Allow the penetration cell to cool to room temperature.

13.7 With the sterile cell placed horizontally on the laboratory bench, insert the specimen in the penetration cell with the normal outside surface toward the cell reservoir which will be filled with the Phi-X174 Bacteriophage challenge suspension.

13.7.1 Assemble the sterile components of the cell as follows: place gaskets between the penetration cell and the test specimen; the specimen and the retaining screen (if used); and the retaining screen and the flange cover as shown in Fig. 1. Close the penetration cell by putting on the flange cover and the transparent cover. Polytetrafluoroethylene (PTFE) gasket material is recommended for use between the cell body and the test specimen to help prevent leakage.^{22,16}

NOTE 3—Sterile, clear plastic film may be used instead of the transparent cover.

13.8 Torque the bolts in the penetration cell to 13.6 N·m (~~[120 in.-lb]~~) each.

13.9 Mount the penetration cell in the test apparatus in a vertical position as shown in Fig. 2 (drain valve down) but do not connect the air line to the cell.

13.10 Close the drain valve.

13.11 Carefully fill the chamber of the penetration cell through the top port with approximately 60 mL of Phi-X174 Bacteriophage challenge suspension (a sterile syringe or funnel is useful). If liquid appears to penetrate through the test specimen at anytime during the test, terminate the test.

13.12 Observe for 5 min.

13.13 Connect the air line to the penetration cell.

13.14 Supply pressurized air through the top port of the penetration cell. Slowly raise the pressure to 13.8-kPa gage (~~2 psig~~) [2 psig] at a rate no faster than 3.5 kPa/s (~~[0.5 psig/s]~~).

13.15 Hold the pressure constant, 13.8 ± 1.38 kPa (~~2~~ [2 \pm 0.2 psig]) for 1 min, and monitor the viewing side of the specimen for the appearance of liquid.

13.16 Turn off the pressure and open the cell valve to the vent position.

13.17 If liquid penetration is not visible at this point, observe the specimen again after an additional 54 min.

13.18 At the end of the time period, open the drain valve and drain the penetration cell of the bacteriophage challenge suspension.

13.18.1 Dilute and assay the Phi-X174 Bacteriophage challenge suspension collected from at least the last penetration cell of each set of replicates after the test to be sure that there has been no loss of bacteriophage virulence during the test (15.3).

13.19 With the cell placed horizontally on the laboratory bench remove the transparent cover.

13.20 Immediately after removing the cover, slowly add 5.0 mL of sterile nutrient broth, with 0.01 % surfactant, onto the exposed surface of the specimen's normal inside surface. Gently swirl or rock the penetration cell for approximately 1 min to ensure contact of the assay fluid with the entire viewing surface of the test specimen. Remove the assay fluid as soon as possible with a sterile pipette to a sterile vial. Some materials absorb the assay fluid requiring a larger volume wash. If a larger volume is necessary, be sure to adjust the calculation of PFU/mL in the report.

13.21 Assay immediately in accordance with Section 14.

13.21.1 A longer period may lapse between collection of the assay fluid and the actual assay if stability of the bacteriophage in the assay fluid can be demonstrated.

13.22 Disassemble apparatus and clean the penetration cell. Disinfect the air lines periodically to prevent contamination.

13.22.1 Clean the penetration cell by rinsing with water and then autoclaving at 121 to 123°C and 207 to 221 kPa absolute (30 to 32 psia) for 15 min.

13.23 Test the remaining specimens and one negative control and one positive control for each test material.

14. Assay Procedure

14.1 Dispense 2.5 mL of sterile molten top agar into sterile test tubes and hold the temperature of the top agar at $45 \pm 2^\circ\text{C}$.

14.2 Prepare duplicate plates for each assay collected for each replicate and control.

14.3 Prepare inoculate tubes by adding 0.5 mL of the assay medium to a top agar tube immediately after removing the tube from the heat.

14.4 Add 100 μL of an overnight culture of *E. coli* C. to each of the inoculate tubes.

14.5 Vortex tubes well and pour over the surface of the bottom agar plates.

14.6 Allow agar to solidify and incubate at 35 to 37°C for (at least) 6 to 18 h.

14.7 Observe for the presence of plaques and interpret results as described in Section 15.

14.8 If quantitation is needed, and the total number of plaques is too great to count, prepare serial 1 to 10 dilutions in bacteriophage nutrient broth of the assay fluid and assay for phage as outlined in 14.1-14.7.

15. Interpretation of Results

15.1 *Controls:*

15.1.1 *Aerosol/Airborne Contamination Controls*—If any background counts (above zero) are observed, the test is considered invalid.

15.1.2 *Negative Test Sample Controls*— Negative test sample controls should pass the test with no detectable (<1 PFU/mL) transfer of the Phi-X174 Bacteriophage.

15.1.3 *Positive Test Sample Controls*— Positive test sample controls should fail the test.

15.1.4 *Non-sterile Material Blank Control*—Non-sterile material blank controls should pass the test with no detectable (<1 PFU/mL) Phi-X174 Bacteriophage in the assay titer.

15.2 *Passing or Failing Performance :*

15.2.1 This test method, as written, is intended to determine the viral resistant performance of single material samples or materials selected from individual protective clothing items under specific test conditions. Protective clothing material specimens that exhibit no detectable (<1 PFU/mL) Phi-X174 in the assay titer pass the test.

15.2.2 This test method can qualify the integrity of materials, support broad product claims, and be used as a quality control and quality assurance procedure when modified to include larger data sets with proper statistical design and analysis (5.9).

15.2.3 *False Positives*—The possibility of false positives resulting from contamination will be minimized by following standard microbiology regimes and proper aseptic techniques. Whenever there is any reasonable doubt, repeat the test using statistically valid sampling methods. Refer to 10.1.4 for sealing instructions.

15.2.4 While quantitative results can be obtained by this test method to provide a *pass* or *fail* determination, the number of PFU/mL must not be used for comparison between laboratories. The precision statement for this test method is solely based on the *pass/fail* determination for the materials tested.

15.3 *Loss of Phi-X174 Bacteriophage Challenge Titer* —Determine the titer of the bacteriophage challenge suspension before and after the test. Use the bacteriophage challenge suspension drained from the cells for the post-test titering (13.18.1). If the titer of the Phi-X174 Bacteriophage challenge suspension fell below 1×10^8 PFU/mL during the test, the test is invalid and must be repeated with an initial titer high enough to provide 1×10^8 PFU/mL at the conclusion of the 60-min test.

15.4 *Compatibility Testing:*

15.4.1 Some test materials may confound virus penetration test results thereby rendering the test invalid. Conditions that may confound virus penetration test results are: inactivation of the Phi-X174 Bacteriophage or the host bacterium, *E. coli* C., by

components contained in test materials or retaining screens (if used); binding of the Phi-X174 Bacteriophage to the test materials or retaining screens (if used); and desiccation of the Phi-X174 Bacteriophage (11). The compatibility of the test materials, the retaining screens (if used), and the microorganisms utilized in this test procedure shall be determined.

15.4.2 Evaluating if the test material (or retaining screen, if used) contains any inhibiting substance shall be determined by using the following procedure:

15.4.2.1 Test three specimens representing each material to be tested in Section 13.

15.4.2.2 With the sterile cell placed horizontally on the laboratory bench, insert the specimen in the penetration cell with the normal outside surface toward the cell reservoir.

15.4.2.3 Assemble the sterile components of the cell as shown in Fig. 1. Refer to 13.7.1.

15.4.2.4 Torque the bolts in the penetration cell to 13.6 N·m ([120 in.-lb]) each.

15.4.2.5 With the cell remaining in the horizontal position on the laboratory bench, place a 2.0-μL aliquot of the Phi-X174 bacteriophage in bacteriophage nutrient broth, containing a total of 900 to 1200 PFU, near the middle of each piece of material.

15.4.2.6 Prepare a control by adding a 2.0-μL aliquot from the same suspension of Phi-X174 Bacteriophage in bacteriophage nutrient broth directly into 5.0 mL of sterile bacteriophage nutrient broth.

15.4.2.7 After 60 min, quantitatively assay in accordance with Section 14.

15.4.2.8 Calculate the ratio of the control assay titer to the test material assay titer, using Eq 1.

$$\text{ratio} = \frac{\text{control assay titer (PFU/mL)}}{\text{test material assay titer (PFU/mL)}} \quad (1)$$

The titer of the Phi-X174 Bacteriophage challenge suspension prepared in 12.8 and used for the test procedure in Section 13 shall be $2 (\pm 1) \times 10^8$ PFU/mL times the ratio calculated.

16. Report

16.1 State that the test was conducted as directed in Test Method F 1671, Specimen Exposure Procedure A or B.

16.2 Describe the material tested and method of sampling used.

16.2.1 Report if the material was taken from roll goods or garments. If the material was taken from either, report the type (fiber and coating compositions), supplier, lot number, and date of receipt of the material tested. If the material was taken from garments, report under subheadings for each material, composite, type of seam, or other conditions tested, and its position on the garment.

16.3 Report the following information:

16.3.1 Thickness of each material specimen and the average thickness of the materials tested.

16.3.2 Weight of each material specimen and the average weight of the materials tested.

16.3.3 Method of sterilization for material specimens, if used.

16.3.4 The ratio calculated during compatibility testing (15.4.2).

16.3.5 The results for all controls to ensure test validity. These results would include the number of PFU for each settle plate by location (15.1.1), and the number of PFU/mL of assay fluid for each negative control (15.1.2) positive control (15.1.3), and non-sterile material blank control, if used, (15.1.4), the starting and ending bacteriophage challenge titer (15.3.1).

16.3.6 The type and specification for the support screen, if used.

16.3.7 The *pass* or *fail* determination for each replicate specimen, each retest specimen, and if elected, the number of penetrating Phi-X174 in PFU/mL for each replicate and retest specimen.

16.3.7.1 Proper statistical design and analysis of larger data sets than those specified in this test method should be performed if this procedure is used for quality control or to support broad product claims. This type of analysis could include, but may not be limited to, the number of individual specimens tested, the average percent passing or failing, or both, with a standard deviation. If calculated, the average number of penetrating Phi-X174 in PFU/mL and a standard deviation may be used within an individual laboratory. Confidence limits may also be included.

16.3.8 Record if the test was terminated due to visible liquid penetration, as *yes* or *no*, for each replicate specimen, each retest specimen, and for each negative and positive control.

17. Precision and Bias

17.1 *Precision*—The precision of Procedures A and B from Table 1 of this test method was determined by interlaboratory testing using three materials, one negative control, and one positive control. The results of these interlaboratory tests showed total agreement for all materials and material replicates among all participating laboratories. The results of these interlaboratory tests are provided in the research report.²⁵

17.2 *Bias*—The procedures in this test method for measuring the viral penetration resistance of protective clothing materials have no bias because results are determined as either pass or fail.

18. Keywords

18.1 blood; blood-borne pathogens; body fluids; Phi-X174 Bacteriophage; protective clothing; viral penetration

²⁵ Available from ASTM Headquarters. Request RR:F23-1002.

REFERENCES

- (1) Shaddock, P. P., Tyler, D. S., Lyerly, H. K., et al, “Commercially Available Gowns Do Not Prevent Penetration by HIV-1,” *Surgical Forum*, American College of Surgeons, Vol XLI, 1990, pp. 77–80.
- (2) Kotilainen, H. R., Cyr, W. H., Truscott, W., et al, “Ability of the 1000 mL Water Leak Test for Medical Gloves to Detect Gloves with Potential for Virus Penetration,” *Performance of Protective Clothing: Fourth Volume, ASTM STP 1133*, McBriarty, J. P., and Henry, N. W., Eds., American Society for Testing and Materials, Philadelphia, PA, 1992.
- (3) Brown, P. L., “Protective Clothing for Health Care Workers: Liquidproofness versus Microbiological Resistance,” *Performance of Protective Clothing: Fourth Volume, ASTM STP 1133*, McBriarty, J. P., and Henry, N. W., eds., American Society for Testing and Materials, Philadelphia, PA, 1992.
- (4) McCullough, E. A., and Schoenberger, L. K., “Liquid Barrier Properties of Nine Surgical Gown Fabrics,” *INDA Journal of Nonwovens Research*, Vol 3, No. 3, Summer 1991, pp. 14–20.
- (5) Smith, J. W., and Nichols, R. L., “Barrier Efficiency of Surgical Gowns,” *Archives of Surgery*, Vol 126, June 1991, pp. 756–762.
- (6) Altman, K. W., et al, “Transmural Surgical Gown Pressure Measurements in the Operating Theater,” *American Journal of Infection Control*, Vol 19, 1991, pp. 147–155.
- (7) *Geigy Scientific Tables*, Volume 1—Units of Measurement, Body Fluids, Composition of Blood, Hematology, Somatometric Data, 1984, (Lentner, C., ed.) Medical Education Division, Ciba-Geigy Corp., West Caldwell, NJ.
- (8) Telford, G. L., and Quebbeman, E. J., “Assessing the Risk of Blood Exposure in the Operating Room,” *American Journal of Infection Control*, Vol 21, No. 6, December 1993, pp. 351–356.
- (9) *Manual of Methods for General Bacteriology*, American Society for Microbiology, Washington, DC 20006, 1981.
- (10) Lytle, C. D., Felten, R. P., and Truscott, W., “Dye To Use with Virus Challenge for Testing Barrier Materials,” *Applied and Environmental Microbiology*, Vol 57, No. 6, June 1991, pp. 1842–1843.
- (11) Lytle, C. D., Cyr, W. H., et al, “Important Factors for Testing Barrier Materials with Surrogate Viruses,” *Applied and Environmental Microbiology*, Vol 57, No. 9, September 1991, pp. 2549–2554.

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org).