



Designation: F1671/F1671M – 13

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 F1671/F1671M; 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.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the*

¹ This test method is under the jurisdiction of ASTM Committee F23 on Personal Protective Clothing and Equipment and is the direct responsibility of Subcommittee F23.40 on Biological.

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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

D1331 Test Methods for Surface and Interfacial Tension of Solutions of Paints, Solvents, Solutions of Surface-Active Agents, and Related Materials

D1777 Test Method for Thickness of Textile Materials

D3776 Test Methods for Mass Per Unit Area (Weight) of Fabric

D3862 Test Method for Retention Characteristics of 0.2- μ m Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality

E105 Practice for Probability Sampling of Materials

E171 Practice for Conditioning and Testing Flexible Barrier Packaging

F903 Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Liquids

F1670 Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Synthetic Blood

2.2 Military Standard:

MIL-STD-105 Sampling Procedures and Tables for Inspection by Attributes³

2.3 ANSI/ASQC Standard:

ANSI/ASQC Z1.4 Sampling Procedures and Tables for Inspection by Attributes⁴

2.4 ISO Standard:

ISO 2859-1 Sampling Plans for Inspection by Attributes⁵

2.5 OSHA Standard:

29 CFR Part 1910.1030 "Occupational Exposure to Blood-borne Pathogens: Final Rule," *Federal Register*, Vol 56, No. 235, Dec. 6, 1991, pp. 64175-64182.⁶

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.

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 Available from Standardization Documents Order Desk, DODSSP, Bldg. 4, Section D, 700 Robbins Ave., Philadelphia, PA 19111-5098, <http://dodssp.daps.dla.mil>.

⁴ Available from American Society for Quality (ASQ), 600 N. Plankinton Ave., Milwaukee, WI 53203, <http://www.asq.org>.

⁵ Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, <http://www.ansi.org>.

⁶ Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, <http://www.access.gpo.gov>.

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.

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 movement of matter through closures, porous materials, seams, and pinholes or other imperfections in protective clothing 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*—an item of clothing that is specifically designed and constructed for the intended purpose of isolating all or part of the body from a potential hazard; or, isolating the external environment from contamination by the wearer of the clothing.

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 **F903** 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; however, visual detection methods are not sensitive enough to detect minute amounts of liquid containing microorganisms (**1, 2, 3**).⁷ 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 **F1670**, allows the screening of protective clothing materials for resistance to penetration with synthetic blood as a challenge liquid. Test Method **F1670** 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 **F1670** 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

⁷ The boldface numbers in parentheses refer to the list of references at the end of this standard.

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.⁸ 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).

6. Apparatus and Materials

6.1 Apparatus:

6.1.1 *Thickness Gauge*, suitable for measuring thickness to the nearest 0.02 mm [or nearest 0.001 in.], as specified in Test Method **D1777**, to determine the thickness of each protective clothing material specimen tested. (Optional.)⁹

6.1.2 *Penetration Test Cell*,^{10,11} 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] 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 **F903**. 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.] |
| Can be sterilized by either ethylene oxide or autoclaving | |
| Material of construction meets criteria in 15.4 . | |

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 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. A retaining screen is used to support the specimen. The type must be specified. |

⁸ 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).

⁹ Thickness may be determined prior to performing the test procedure but is not required. The thickness data for the material may be available from the manufacturer. Manipulation or handling of the test specimens prior to testing increases the chance for contamination.

¹⁰ The sole source of supply of the penetration test apparatus known to the committee at this time is Wilson Road Machine Shop, 1170 Wilson Rd., Rising Sun, MD 21911.

¹¹ 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.

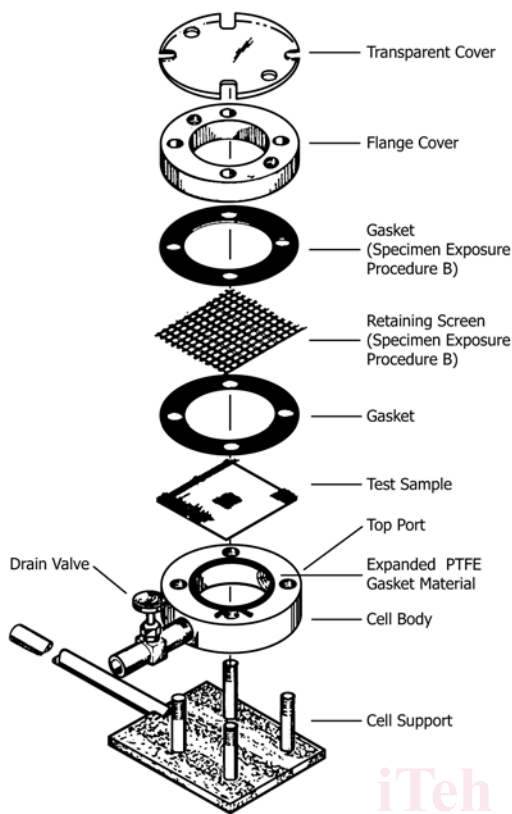


FIG. 1 Exploded View of the Penetration Test Cell with Retaining Screen

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 $45 \pm 2^\circ\text{C}$.

6.1.7 *Analytical Balance*, capable of weighing 0.001 g and suitable for measuring weight to the nearest 10 g/m^2 [0.1 oz/yd^2] in accordance with Test Method D3776. (Optional).¹²

6.1.8 *Vortex Mixer*.

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 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 $10\,000 \times g$.

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.

¹² The weight may be determined prior to performing the test procedure but is not required. The basis weight of the material may be available from the manufacturer. Manipulation or handling of the test specimens prior to testing increase the chance for contamination.

6.2.5 *Membrane Filters*,^{11,13} sterile, 0.22 μm .

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

6.2.7 *Micropipets*, capable of delivery of 2 μL , accurately and consistently.

7. Reagents

7.1 *Bacteriophage*, Phi-X174, ATCC 13706-B1.^{14,15,11}

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.^{14,11}

7.3 *Purified Water*, Q.S.

7.4 *Nutrient Broth*.^{16,11}

7.5 *Calcium Chloride* (CaCl_2).

7.6 *Potassium Chloride* (KCl).

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

7.8 *Surfactant*, polysorbate 80.^{17,11}

7.9 *Bacto-Agar*.^{16,11}

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 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.

¹³ Membrane filters shall be used which meet requirements of Test Method D3862.

¹⁴ Cultures may be obtained from ATCC American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

¹⁵ Purified virus preparations may be obtained from Promega Corp., 2800 Woods Hollow Rd., Madison, WI 53711-5399.

¹⁶ Nutrient Broth and Bacto-Agar may be obtained from Difco, Detroit, MI 48232.

¹⁷ 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.

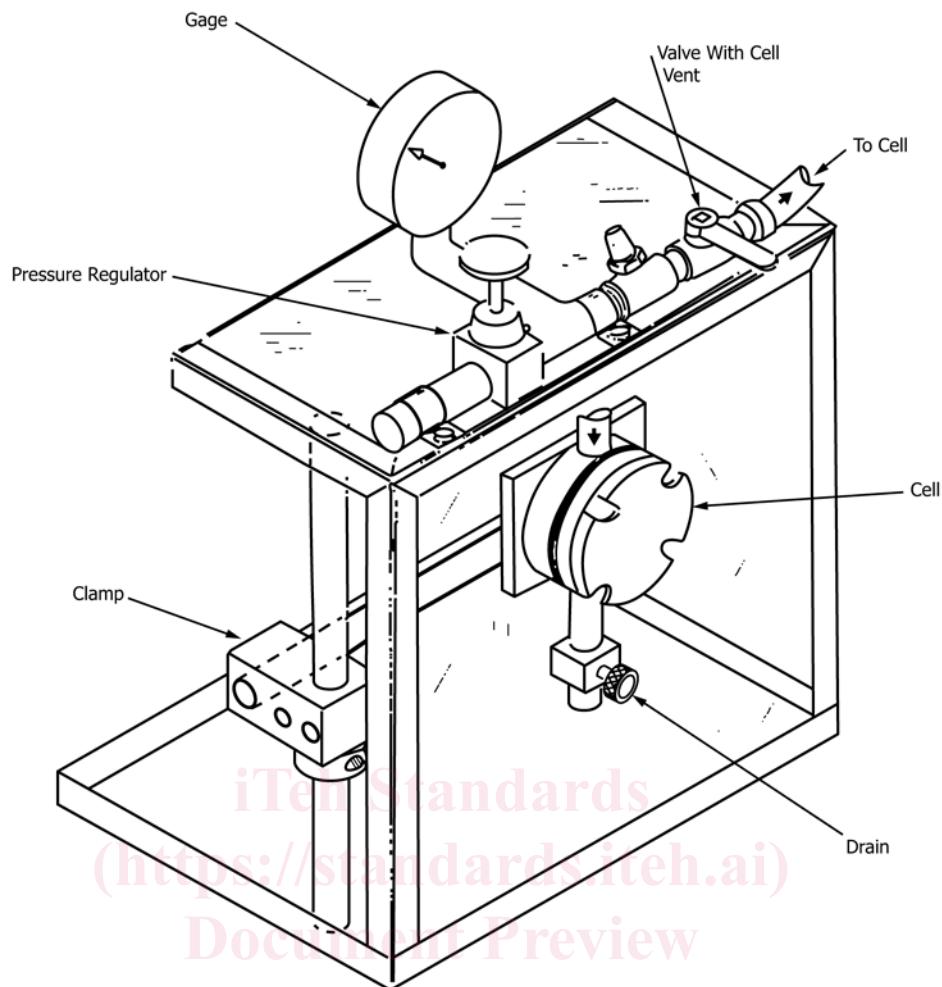


FIG. 2 Three Dimensional Side View of the Test Apparatus

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 ± 0.1 g |
| Potassium chloride | 5.0 ± 0.06 g |
| Calcium chloride | 0.2 ± 0.003 g |
| Purified water, Q.S. to | 1000 ± 12.5 mL |
| Surfactant | 0.1 ± 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 D1331. 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 ± 0.19 g |
| Nutrient broth | 8.0 ± 0.1 g |
| Potassium chloride | 5.0 ± 0.06 g |
| Purified water, Q.S. to | 1000 ± 12.5 mL |
| Calcium chloride | 1.0 ± 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 ± 0.09 g |
| Nutrient broth | 8.0 ± 0.1 g |
| Potassium chloride | 5.0 ± 0.06 g |
| 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.