Designation: F2101 – 23

Standard Test Method for Evaluating the Bacterial Filtration Efficiency (BFE) of Medical Face Mask Materials, Using a Biological Aerosol of *Staphylococcus aureus*[1]

This standard is issued under the fixed designation F2101; 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 healthcare profession involved in treating and caring for individuals injured or sick, as well as the patient, can be exposed to biological aerosols capable of transmitting disease. These diseases, which may be caused by a variety of microorganisms, can pose significant risks to life and health. Since engineering controls cannot eliminate all possible exposures, attention is placed on reducing the potential of airborne exposure through the use of medical face masks.

1. Scope

1.1 This test method is used to measure the bacterial filtration efficiency (BFE) of medical face mask materials, employing a ratio of the upstream bacterial challenge to downstream residual concentration to determine filtration efficiency of medical face mask materials.

1.2 This test method is a quantitative method that allows filtration efficiency for medical face mask materials to be determined. The maximum filtration efficiency that can be determined by this method is 99.9 %.

1.3 This test method does not apply to all forms or conditions of biological aerosol exposure. Users of the test method should review modes for worker exposure and assess the appropriateness of the method for their specific applications.

1.4 This test method evaluates medical face mask materials as an item of protective clothing but does not evaluate materials for regulatory approval as respirators. If respiratory protection for the wearer is needed, a NIOSH-certified respirator should be used. Relatively high bacterial filtration efficiency measurements for a particular medical face mask material do not ensure that the wearer will be protected from biological aerosols, since this test method primarily evaluates the performance of the composite materials used in the construction of the medical face mask and not its design, fit, or facial-sealing properties.

1.5 *Units*—The values stated in SI units or inch-pound units are to be regarded separately as standard. The values stated in each system may not be exact equivalents; therefore, each system shall be used independently of the other. Combining values from the two systems may result in nonconformance of the standard.

1.6 This test method does not address breathability of the medical face mask materials or any other properties affecting the ease of breathing through the medical face mask material.

1.7 This test method may also be used to measure the bacterial filtration efficiency (BFE) of other porous medical products such as surgical gowns, surgical drapes, and sterile barrier systems.

1.8 *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 determine the applicability of regulatory limitations prior to use.*

1.9 *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]

[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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[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.

E171/E171M Practice for Conditioning and Testing Flexible Barrier Packaging
E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods
E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method
F1494 Terminology Relating to Protective Clothing

2.2 *ANSI/ASQ Standard:*[3]
ANSI/ASQ Z1.4 Sampling Procedures and Tables for Inspection by Attributes

2.3 *ISO Standard:*[4]
ISO 2859-1 Sampling Plans for Inspection by Attributes

2.4 *Military Standard:*[5]
MIL-STD 36954C (1973) Military Specification: Mask, Surgical, Disposable

3. Terminology

3.1 *Definitions:*

3.1.1 *aerosol, n*—a suspension of solid or liquid particles in a gas.

3.1.2 *agar, n*—a semi-solid culture medium used to support the growth of bacteria and other microorganisms.

3.1.3 *airborne exposure pathways, n*—inhalation routes of exposure to the medical face mask wearer.

3.1.4 *bacterial filtration efficiency (BFE), n*—the effectiveness of a medical face mask material in preventing the passage of aerosolized bacteria, expressed in the percentage of a known quantity that does not pass the medical face mask material at a given aerosol flow rate.

3.1.5 *biological aerosol, n*—a suspension of particles containing biological agents which have been dispersed in a gas.

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.

3.1.7 *body fluid, n*—any liquid produced, secreted, or excreted by the human body.

3.1.8 *medical face mask, n*—an item of protective clothing designed to protect portions of the wearer's face, including the mucous membrane areas of the wearer's nose and mouth, from contact with blood and other body fluids during medical procedures.

3.1.8.1 *Discussion*—Medical face masks also function to partly limit the spread of biological contamination from the mask wearer (health care provider) to the patient.

3.1.9 *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.

3.2 For definitions of other protective clothing-related terms used in this test method, refer to Terminology F1494.

4. Summary of Test Method

4.1 The medical face mask material is clamped between a six-stage cascade impactor and an aerosol chamber. The bacterial aerosol is introduced into the aerosol chamber using a nebulizer and a culture suspension of *Staphylococcus aureus*. The aerosol is drawn through the medical face mask material using a vacuum attached to the cascade impactor. The six-stage cascade impactor uses six agar plates to collect aerosol droplets which penetrate the medical face mask material. Control samples are collected with no test specimen clamped in the test apparatus to determine the upstream aerosol counts.

4.2 The agar plates from the cascade impactor are incubated for 48 h and counted to determine the number of viable particles collected. The ratio of the upstream counts to the downstream counts collected for the test specimen are calculated and reported as a percent bacterial filtration efficiency.

5. Significance and Use

5.1 This test method offers a procedure for evaluation of medical face mask materials for bacterial filtration efficiency. This test method does not define acceptable levels of bacterial filtration efficiency. Therefore, when using this test method it is necessary to describe the specific condition under which testing is conducted.

5.2 This test method has been specifically designed for measuring bacterial filtration efficiency of medical face masks, using *Staphylococcus aureus* as the challenge organism. The use of *S. aureus* is based on its clinical relevance as a leading cause of nosocomial infections.

5.3 This test method has been designed to introduce a bacterial aerosol challenge to the test specimens at a flow rate of 28.3 L/mm (1 ft^3/min). This flow rate is within the range of normal respiration and within the limitations of the cascade impactor.

5.4 Unless otherwise specified, the testing shall be performed with the inside of the medical face mask in contact with the bacterial challenge. Testing may be performed with the aerosol challenge directed through either the face side or liner side of the test specimen, thereby allowing evaluation of filtration efficiencies which relate to both patient-generated aerosols and wearer-generated aerosols.

5.5 Degradation by physical, chemical, and thermal stresses could negatively impact the performance of the medical face mask material. The integrity of the material can also be compromised during use by such effects as flexing and abrasion, or by wetting with contaminants such as alcohol and perspiration. Testing without these stresses could lead to a false sense of security. If these conditions are of concern, evaluate the performance of the medical face mask material for bacterial filtration efficiency following an appropriate pretreatment technique representative of the expected conditions of use. Consider preconditioning to assess the impact of storage conditions and shelf life for disposable products, and the effects of laundering and sterilization for reusable products.

[3] Available from American Society for Quality (ASQ), 600 N. Plankinton Ave., Milwaukee, WI 53203, http://www.asq.org.
[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.
[5] Available from Standardization Documents Order Desk, Bldg. 4 Section D, 700 Robbins Ave., Philadelphia, PA 19111-5094, Attn: NPODS.

5.6 If this procedure is used for quality control, perform proper statistical design and analysis of larger data sets. This type of analysis includes, but is not limited to, the number of individual specimens tested, the average percent bacterial filtration efficiency, and standard deviation. Data reported in this way help to establish confidence limits concerning product performance. Examples of acceptable sampling plans are found in references such as ANSI/ASQ Z1.4 and ISO 2859-1.

6. Apparatus and Materials

6.1 *Apparatus:*

6.1.1 *Autoclave,* capable of maintaining 121 to 123 °C.

6.1.2 *Incubator,* capable of maintaining 37 ± 2 °C.

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

6.1.4 *Vortex Mixer,* capable of mixing the contents of 16 mm by 150 mm test tubes.

6.1.5 *Orbital Shaker,* capable of achieving 100 to 250 rpm.

6.1.6 *Refrigerator,* capable of maintaining 2 to 8 °C.

6.1.7 *Six-Stage Viable Particle Cascade Impactor.*

6.1.7.1 The use of plastic petri dishes is permitted but their dimensions should be as close as possible to the dimensions of the glass dishes for which the impactor is designed. The agar-to-grid distance for each stage of the impactor is also very important. The volume of agar should be determined according to the type of petri dishes selected. A volume of 27 mL is generally recommended for the glass dishes offered with the Andersen impactor. The volume used with the selected petri dishes should give an agar-to-grid distance comparable to that obtained with a reference glass dish filled with 27 mL of agar or the volume mentioned by the supplier.

6.1.8 *Vacuum Pump,* capable of 57 L/m (2 ft^3/mm).

6.1.9 *Air Pump/Compressor,* capable of 15 psig minimum.

6.1.10 *Peristaltic Pump,* capable of delivering 0.01 mL/min.

6.1.11 *Nebulizer,* capable of delivering a mean particle size of 3.0 µm ± 0.3 µm and a challenge level of 1700 to 3000 viable particles per test, as determined according to 12.3.

6.1.12 *Glass Aerosol Chamber,* 60 cm by 8 cm diameter tube.

6.1.13 *Colony Counter,* manual or automatic, capable of counting up to 400 colonies/plate.

6.1.14 *Timers,* capable of 0.1 s accuracy.

6.1.15 *Automatic Pipetor,* capable of delivering 1.0 mL ± 0.05 mL.

6.1.16 *Flow Meters,* capable of 28.3 L/min.

6.1.17 *Aerosol Condenser.*

6.1.18 *Pressure Gauge,* capable of 35 kPa ± 1 kPa accuracy.

6.1.19 *Air Regulator.*

6.2 *Materials:*

6.2.1 *Flasks,* 250 to 500 mL Erlenmeyer.

6.2.2 *Petri Dishes,* sterile, 15 by 100 mm.

6.2.3 *Pipettes,* 1 mL, 5 mL, and 10 mL.

6.2.4 *Test Tube Rack,* stainless.

6.2.5 *Bottles,* sterile, glass, 100 to 500 mL capacity.

6.2.6 *Inoculating Loop.*

6.2.7 *Stoppers/Closures,* of appropriate size to fit test tubes.

6.2.8 *Test Tubes,* 16 mm by 150 mm.

7. Reagents

7.1 *Tryptic Soy Agar (TSA).*[6]

7.2 *Tryptic Soy Broth (TSB).*[6]

7.3 *Peptone Water.*[6]

7.4 *Staphylococcus aureus,* ATCC #6538.

8. Hazards

8.1 Sterilize all apparatus and supplies which come into contact with the bacterial challenge suspension by autoclaving at 121 to 123 °C for a minimum of 15 min. Extreme care must be taken 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.

8.2 *Staphylococcus aureus* is common to the normal flora of the body, however, it is a leading cause of nosocomial infections and is a human pathogen. Technicians conducting the testing must have proper microbiological training. Gloves and other protective clothing equipment should be worn during testing to prevent contamination.

8.3 All aerosols must be contained to prevent exposure and reduce laboratory contamination.

9. Media Preparation

9.1 Prepare media using standard microbiological techniques.

9.2 Prepare agar plates for cascade impactor as specified by the manufacturer of the cascade impactor.

10. Test Specimen

10.1 Test specimens shall be taken from manufactured medical face masks, with all layers arranged in proper order.

11. Conditioning

11.1 Condition each specimen for a minimum of 4 h by exposure to a temperature of 21 ± 5 °C (70 ± 10 °F) and relative humidity of 85 ± 5 % as described in Practice E171/E171M using a controlled temperature and humidity chamber or space.

12. Preparation of the Bacterial Challenge

12.1 Inoculate an appropriate volume of tryptic soy broth and incubate with mild shaking at 37 ± 2 °C for 24 ± 2 h.

12.2 Dilute the culture in peptone water to achieve a concentration of approximately 5×10^5 CFU/mL.

12.3 The challenge delivery rate will be maintained at 1.7 to 3.5×10^3 viable particles per test. The challenge delivery rate is determined each day of testing and is based on the results of the positive control plates when the aerosol is collected in a

[6] The sole source of supply of the apparatus known to the committee at this time is Difco, Detroit, MI 48232. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

six-stage viable particle cascade impactor, with no test specimen clamped into the test system. The dilution of the challenge suspension will need to be adjusted to deliver the proper challenge level during testing.

13. Test Procedure

13.1 The aerosol challenge apparatus is outlined in Fig. 1.

13.2 Deliver the challenge to the nebulizer using a peristaltic or syringe pump. Connect tubing to nebulizer and peristaltic pump and into the challenge suspension; purge tubing and nebulizer of air bubbles.

NOTE 1—The peristaltic pump or syringe pump must be calibrated to deliver a consistent challenge volume throughout the testing interval.

13.3 Perform a positive control run without a test specimen clamped into the test system to determine the number of viable aerosol particles being generated. The mean particle size (MPS) of the aerosol will also be calculated from the results of these positive control plates.

13.4 Initiate the aerosol challenge by turning on the air pressure and pump connected to the nebulizer.

13.5 Immediately begin sampling the aerosol using the cascade impactor. Adjust the flow rate through the cascade impactor to 28.3 L/m.

13.6 Time the challenge suspension to be delivered to the nebulizer for 1 min.

13.7 Time the air pressure and cascade impactor to run for 2 min.

13.8 At the conclusion of the positive control run, remove plates from the cascade impactor. Label each plate with the corresponding stage number.

13.9 Place new agar plates into the cascade impactor and clamp the test specimen into the top of the cascade impactor, with either the inside or outside oriented toward the challenge as intended. The test area shall be approximately 40 cm^2. An alternative means of clamping the specimen into the apparatus may be used as long as the test area remains 40 cm^2. If the test area is different, note this deviation on the final report.

13.10 Initiate the aerosol challenge as outlined above.

13.11 Repeat the challenge procedure for each test specimen.

13.12 Repeat a positive control sample after completion of the test sample set.

13.13 Perform a negative control sample by collecting a 2-min sample of air from the aerosol chamber. No bacterial challenge should be pumped into the nebulizer during the collection of the negative control sample.

13.14 Incubate agar plates at 37 ± 2 °C for 24 to 52 h.

13.15 Count the bacterial colonies on each of the six-stage plates of the cascade impactor. The plate count values for stages 3 to 6 are converted into probable fit values using the positive hole conversion chart from the cascade impactor manual.

NOTE 2—Per the cascade impactor manual, positive hole correction is

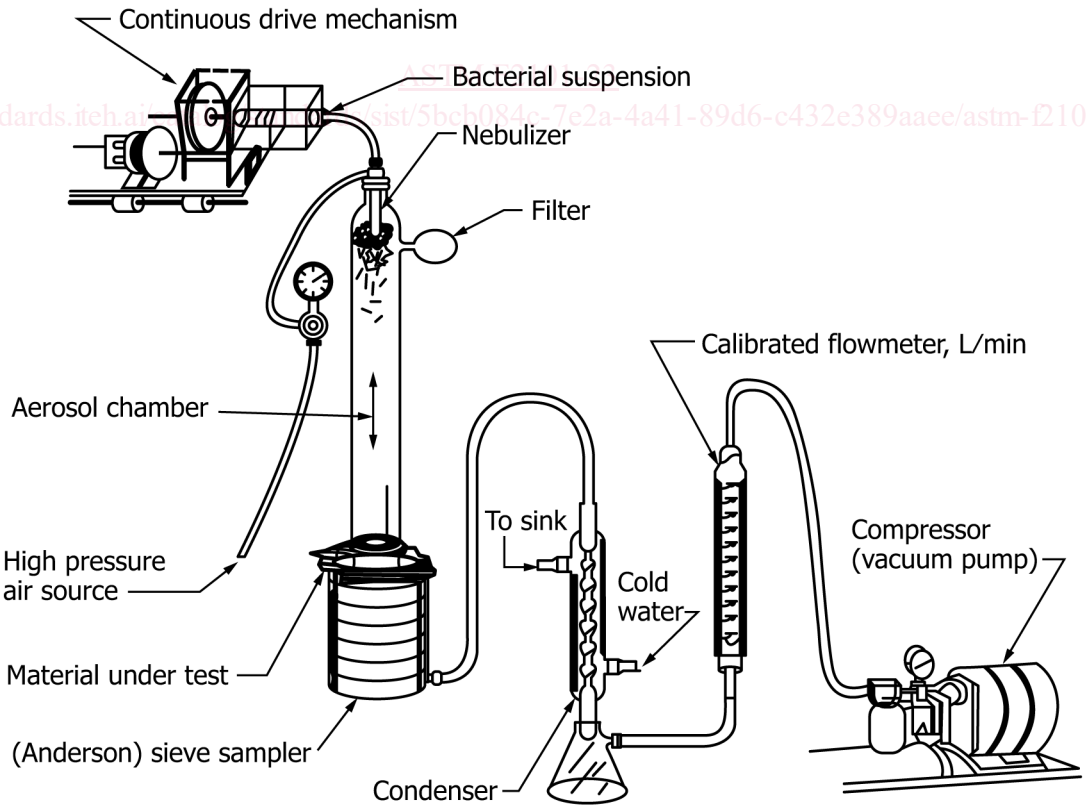

FIG. 1 Bacterial Filtration Efficiency Test Apparatus