Designation: F1608 – 21

Standard Test Method for Microbial Ranking of Porous Packaging Materials (Exposure Chamber Method)[1]

This standard is issued under the fixed designation F1608; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is used to determine the passage of airborne bacteria through porous materials intended for use in packaging sterile medical devices. This test method is designed to test materials under conditions that result in the detectable passage of bacterial spores through the test material.

1.1.1 A round-robin study was conducted with eleven laboratories participating. Each laboratory tested duplicate samples of six commercially available porous materials to determine the Log Reduction Value (LRV) (see calculation in Section 12). Materials tested under the standard conditions described in this test method returned average values that range from LRV 1.7 to 4.3.

1.1.2 Results of this round-robin study indicate that caution should be used when comparing test data and ranking materials, especially when a small number of sample replicates are used. In addition, further collaborative work (such as described in Practice E691) should be conducted before this test method would be considered adequate for purposes of setting performance standards.

1.2 This test method requires manipulation of microorganisms and should be performed only by trained personnel. The U.S. Department of Health and Human Services publication *Biosafety in Microbiological and Biomedical Laboratories* (CDC/NIH-HHS Publication No. 84-8395) should be consulted for guidance.

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

1.4 *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.*

1.5 *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]

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 *Definitions:*

3.1.1 *porous packaging material, n*—a material used in medical packaging which is intended to provide an environmental and biological barrier, while allowing sufficient air flow to be used in gaseous sterilization methods (for example, ethylene oxide, steam, gas plasma).

4. Summary of Test Method

4.1 Samples of porous materials are subjected to an aerosol of *Bacillus atrophaeus* spores within an exposure chamber. Spores which pass through the porous sample are collected on membrane filters and enumerated. The LRV is calculated by comparing the logarithm of the number of spores passing through the porous material with the logarithm of the microbial challenge.

4.2 *Standard Set of Conditions*—This test method specifies a standard set of conditions for conducting the exposure chamber test method. A standard set of conditions is required to enable evaluation of materials between laboratories. The conditions stated in this test method were chosen for several reasons. First, it is difficult to maintain an aerosol of spores over long periods of time. (Also, if the spore challenge time is long, the cost of the test increases). Second, to determine the differences between materials, it is necessary to test the

[1] This test method is under the jurisdiction of ASTM Committee F02 on Primary Barrier Packaging and is the direct responsibility of Subcommittee F02.15 on Chemical/Safety Properties.

Current edition approved Oct. 1, 2021. Published November 2021. Originally approved in 1995. Last previous edition approved in 2016 as F1608 – 16. DOI: 10.1520/F1608-21.

[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.

materials under conditions which allow passage of bacterial spores. If a material does not allow any passage of spores, all that can be stated is that it has better resistance to penetration than the severity of the challenge conditions. Third, it is necessary to have a large spore challenge level to be able to detect the passage of spores through the entire range of commercially available porous packaging materials. The standard conditions stated in this test method are based upon these factors. (Additional information may be found in the References section). However, since many factors influence the determination of an appropriate porous material (outlined in 5.1.1 – 5.1.4), each user may modify these conditions (that is, bacterial challenge, time, flow rate) after first conducting studies at the specified standard conditions. The standard set of target parameters for conducting the test method are as follows:

4.2.1 *Flow Rate Through Sample*—2.8 L/min.

4.2.2 *Exposure Time*— 15 min.

4.2.3 *Target Microbial Challenge* —1×10^6 colony forming units (CFU)/sample port.

5. Significance and Use

5.1 The exposure-chamber method is a quantitative procedure for determining the microbial-barrier properties of porous materials under the conditions specified by the test. Data obtained from this test is useful in assessing the relative potential of a particular porous material in contributing to the loss of sterility to the contents of the package versus another porous material. This test method is not intended to predict the performance of a given material in a specific sterile-packaging application. The maintenance of sterility in a particular packaging application will depend on a number of factors, including, but not limited to the following:

5.1.1 The bacterial challenge (number and kinds of microorganisms) that the package will encounter in its distribution and use. This may be influenced by factors such as shipping methods, expected shelf life, geographic location, and storage conditions.

5.1.2 The package design, including factors such as adhesion between materials, the presence or absence of secondary and tertiary packaging, and the nature of the device within the package.

5.1.3 The rate and volume exchange of air that the porous package encounters during its distribution and shelf life. This can be influenced by factors including the free-air volume within the package and pressure changes occurring as a result of transportation, manipulation, weather, or mechanical influences (such as room door closures and HVAC systems).

5.1.4 The microstructure of a porous material which influences the relative ability to adsorb or entrap microorganisms, or both, under different air-flow conditions.

6. Apparatus

6.1 This procedure should be conducted in a microbiological laboratory by trained personnel. As a result, it is assumed that basic microbiological equipment and supplies for conducting routine microbiological manipulations (that is, standard plate counts, sterilization with an autoclave, and so forth) will be available.

6.2 *Exposure Chamber,* constructed primarily from acrylic sheeting and consists of two major sections, as illustrated in Fig. 1. The bottom section contains a six-place manifold connected to six flowmeters, one per port, containing hoses attached to six filtering units. The port to the manifold is attached to a vacuum source. A vacuum gauge is mounted between the manifold and the vacuum source. The upper chamber contains a fan for dispersion of the bacterial aerosol, a port for attachment of the nebulizer, a port for exhausting the chamber, and a plate for attachment of disposable or sterilizable filter units. The chamber may use disposable filter units or reusable filter units, or both.

7. Materials

7.1 *Bacillus atrophaeus* (ATCC9372), aqueous spore suspension in water.

7.2 *Soybean Casein Digest Agar/ Tryptic Soy Agar*—Bottles for pour plates and pre-poured plates (~25 mL in 100 by 15-mm plates) prepared commercially or in accordance with standard techniques.

7.3 *Sterile Cellulose Nitrate Filters,* 47 or 50-mm diameter, depending upon filter unit specification, 0.45-µm pore size.

7.4 *Sterile Bottle-Top Filter Units,* (Falcon-type 7104 or filter holders with funnel 310-4000 or equivalent).

7.5 *Glass Nebulizer.*

7.6 *Sterile Forceps.*

7.7 *Incubator,* 30 to 35°C.

7.8 *Disk Cutter,* 47 or 50-mm diameter, depending upon filter unit specification.

7.9 *Sterile Gloves.*

7.10 *Sterile Syringe,* 3-cm^3 with needle or micropipette.

7.11 *Sterile Pipettes,* to deliver 0.1, 1, 10, and 25 mL.

7.12 *Blender,* with sterile ½-pt jar(s).

7.13 *Vortex Mixer.*

7.14 *Vacuum Pump,* with air filter.

7.15 *NIST Traceable Calibrated Timer.*

7.16 *NIST Traceable Calibrated Flowmeters*—One pressure flowmeter with a range from 5 to 30 L/min; six vacuum flowmeters each with a range from 1.0 to 5.0 L/min.

7.17 *Sterile Petri Plates.*

7.18 *Sterile Water,* 100 and 9.9-mL aliquots, or other appropriate volumes for membrane grinding and dilutions.

7.19 *Hoses and Piping*— See Section 9 for lengths and diameters.

7.20 *Rubber Stoppers with Holes*—See Section 9 for sizes.

7.21 *Trap Jar.*

7.22 *NIST Traceable Calibrated Vacuum Gauge.*

7.23 *Compressed Air Source,* with air filter.

7.24 *Biocontainment Hood.*

7.25 *Chlorine Bleach,* or suitable sporocide.