Designation: D6329 – 98 (Reapproved 2023)

Standard Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chambers[1]

This standard is issued under the fixed designation D6329; 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 Many different types of microorganisms (for example, bacteria, fungi, viruses, algae) can occupy indoor spaces. Materials that support microbial growth are potential indoor sources of biocontaminants (for example, spores and toxins) that can become airborne indoor biopollutants. This guide describes a simple, relatively cost effective approach to evaluating the ability of a variety of materials to support microbial growth using a small chamber method.

1.2 This guide is intended to assist groups in the development of specific test methods for a definite material or groups of materials.

1.3 Static chambers have certain limitations. Usually, only small samples of indoor materials can be evaluated. Care must be taken that these samples are representative of the materials being tested so that a true evaluation of the material is performed.

1.4 Static chambers provide controlled laboratory microenvironment conditions. These chambers are not intended to duplicate room conditions, and care must be taken when interpreting the results. Static chambers are not a substitute for dynamic chambers or field studies.

1.5 A variety of microorganisms, specifically bacteria and fungi, can be evaluated using these chambers. This guide is not intended to provide human health effect data. However, organisms of clinical interest, such as those described as potentially allergenic, may be studied using this approach.

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

1.7 *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.*

1.8 *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]

D1193 Specification for Reagent Water

D1356 Terminology Relating to Sampling and Analysis of Atmospheres

E104 Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions

2.2 *APHA Standards:*[3]

Standard Methods for the Examination of Water and Wastewater

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology D1356.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *amplification*—the act or result of increasing the quantity of microorganisms.

3.2.2 *CFU*—colony forming unit, which may arise from a single organism or multiple units, such as spores, in the case of the fungi.

3.2.3 *colony*—macroscopically visible growth.

3.2.4 *inoculation*—the act of introducing a microorganism (inoculum) into the test material.

[1] This guide is under the jurisdiction of ASTM Committee D22 on Air Quality and is the direct responsibility of Subcommittee D22.08 on Assessment, Sampling, and Analysis of Microorganisms.

Current edition approved Jan. 1, 2023. Published February 2023. Originally approved in 1998. Last previous edition approved in 2015 as D6329 – 98 (2015). DOI: 10.1520/D6329-98R23.

[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 American Public Health Association (APHA), 800 I St., NW, Washington, DC 20001, http://www.apha.org.

3.2.5 *inoculum*—viable test microorganism introduced onto a material by implanting a small amount on the surface or substrate.

3.2.6 *plate*—petri dish containing microbiological agar media on which microorganism are grown.

3.2.7 *static chamber*—a small chamber (enclosed space) with no internal forced air motion.

3.2.8 *susceptibility*—the vulnerability of a material or surface to colonization by microorganisms.

4. Significance and Use

4.1 The static chambers have several different applications:

4.1.1 The static chambers can be used to compare the susceptibility of different materials to the colonization and amplification of various microorganisms under defined conditions.

4.1.2 Chambers operated at high relative humidities may be used to perform worst case scenario screening tests on materials by providing an atmosphere where environmental conditions may be favorable for microbial growth.

4.1.3 Use of multiple chambers with different environmental parameters, such as a range of relative humidities, permits the evaluation of multiple microenvironments and allows investigation of materials under differing environmental conditions.

4.1.4 Drying requirements for wetted materials may also be investigated. This information may be relevant for determining material resistance to microbial growth after becoming wet. These conditions may simulate those where materials are subjected to water incursion through leaks as well as during remediation of a building after a fire.

4.1.5 Growth rates of microorganisms on the material may also be investigated. Once it has been established that organisms are able to grow on a particular material under defined conditions, investigations into the rate of organism growth may be performed. These evaluations provide base line information and can be used to evaluate methods to limit or contain amplification of microorganisms.

4.2 These techniques should be performed by personnel with training in microbiology. The individual must be competent in the use of sterile technique, which is critical to exclude external contamination of materials.

5. Apparatus

5.1 *Static Chamber*—Chambers should be relatively small and portable, contain three or four shelves, and be easily decontaminated. In addition, transparent walls are desirable because visual inspection of the test material and monitoring of instruments (that is, hygrometers) without opening the chamber is preferred. Fig. 1 is a schematic diagram of a possible static chamber. Acrylic desiccators are readily available, easily adaptable, and relatively inexpensive. Other options, such as glass, are also acceptable. Glass has the advantage of being autoclavable; however, it is frequently much less portable. The chamber door must provide ready access to the materials but should be airtight when closed.

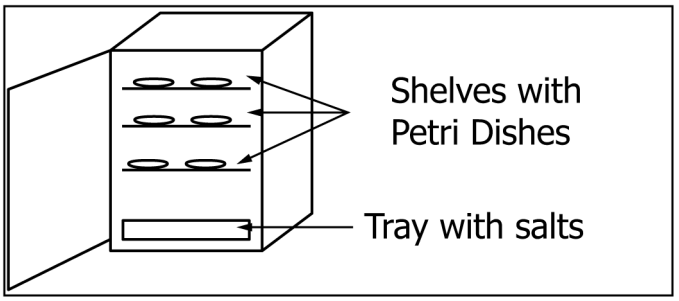

FIG. 1 Schematic of Example Static Chamber

5.1.1 *Relative Humidity*—Maintain humidities through the use of saturated salt solutions contained in trays on the bottom of the chambers (see Practice E104). It is essential that the chambers be tightly sealed so that the desired humidity will be maintained. Place hygrometers in the chambers for confirmation that humidities are being maintained, although saturated salt solutions are themselves standards. Exercise care that the salts selected for use in the chamber are not inhibitory to the test organisms.

5.1.2 *Temperature*—Control the temperature of the chambers. The chambers may be externally controlled through the use of constant temperature environments, such as a room or incubator. Chart recorders or other data logging devices are recommended to confirm maintenance of temperature. Controlled temperature is critical for two reasons. First, it can have a profound effect on the growth of microorganisms. Second, relative humidity is dependent upon temperature. The control limits may be defined by consulting a psychometric chart and determining the impact of temperature on a specific test RH.

5.1.3 Characterize instrumentation for evaluating other parameters if the instruments are to be employed during material testing. Conditions such as light need to be noted and controlled during the course of an experiment as these conditions may have an effect on the growth of the test organism. Light may be controlled externally by placing the chambers in a darkened room to remove light or in a continuously lighted room for a constant light source.

5.2 Provide ports, where needed, for the insertion of probes to monitor and record temperature and relative humidity, using externally located instrumentation as long as it is well sealed and contamination is avoided.

5.3 *Decontamination*—Decontaminate the chamber before initiating any analysis. Surface disinfection or vapor phase disinfection may be appropriate. Glass may be autoclaved. Follow the manufacturers' instructions, especially any safety precautions. If a chemical disinfectant is employed, clear the chambers of any residual disinfectant to prevent interference with the growth of the microorganisms on the material being evaluated. Thoroughly ventilate the chambers in a clean environment. Decontaminate the salt solutions. The method used is dependent upon the composition of the salts selected. Any instrumentation to be used during the evaluations, such as hygrometers, may be removed from the chambers during the decontamination procedure of the chamber surfaces and decontaminated separately; however, it is generally more effective for them to remain in the chambers. Verify the efficacy of