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# 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 D 6329; 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 *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 1193 Specification for Reagent Water[2]

D 1356 Terminology Relating to Sampling and Analysis of Atmospheres[3]

E 104 Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions[3]

2.2 *APHA Standards*[4]:

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 D 1356.

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.

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.

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[1] This guide is under the jurisdiction of ASTM Committee D-22 on Sampling and Analysis of Atmospheres and is the direct responsibility of Subcommittee D22.05 on Indoor Air.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.03.

[4] Available from American Public Health Association, 1015 15th St., NW, Washington, DC 20036.

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.

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 E 104). 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.

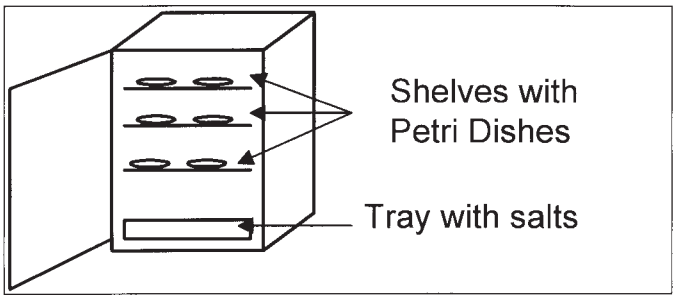


**FIG. 1 Schematic of Example Static Chamber**

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 the decontamination procedure as part of the Quality Assurance/Quality Control (QA/QC) plan.

5.4 Decontaminate the work area around the chambers routinely, especially before opening the chamber door. The chambers should be kept in a clean room, functionally Class 100 000 (M 6.5 or ISO 8) or better.

## 6. Reagents

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where

such specifications are available.[5] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as certified by Type II of Specification D 1193. It should conform to the Type A specifications for microbial classification.

6.3 *Microbiological Media*—Choose appropriate media depending upon the test microorganism selected. Commercially prepared media may be acceptable, but it may be necessary to prepare organism specific media. References should be consulted to determine the proper media for optimal growth of the test organism.

## 7. Characterization of Static Chamber

7.1 Characterize static chambers for all environmental parameters being measured before any material evaluations are performed. Chambers should be characterized for at least relative humidity and temperature. Take sufficient readings to ensure that the conditions will be maintained throughout the course of the experiment and will meet the QA/QC standards developed for a specific test.

7.1.1 *Equilibration*—Equilibrate disinfected chambers containing hygrometers before taking the first relative humidity reading. Place the hygrometers on a shelf for ease of reading through the walls of the chamber without opening the door. Take multiple sequential readings at appropriate intervals that were determined experimentally. The variation of the instrumentation must be determined and taken into consideration. For example, a minimum of four similar readings (± 5 %) over an 8 h period may be determined to demonstrate equilibrium.

7.1.2 *Recovery*—Determine the amount of time required for the chamber relative humidity to recover to test levels after opening the door for 1 to 2 min. This determination may be crucial, especially at the higher relative humidities. Exercise care to utilize hygrometers that have a rapid response time.

7.2 Check chamber relative humidity daily, and record readings depending on test length.

## 8. Sample Preparation

8.1 Specific details on the preparation of the samples will depend upon the characteristics of the material to be tested. Generally, replicate small pieces of the test material should be used. Depending upon the material, pieces as small as 4 × 4 cm may be used. Pieces should be placed on sterile petri dishes or other appropriate holders on the shelves in the chamber. Include controls and blanks within the QA/QC framework.

8.2 Common microbiological practice is to sterilize a surface or material before inoculation to ensure that the test organism is the only source being evaluated. Autoclaving is an extremely effective method if such a procedure does not alter the test material. Other methods, such as ionizing and nonionizing irradiation, ultraviolet, dry heat, and surface or vapor phase disinfection, are also acceptable if these methods do not harm the material and do not have residue effects or if all traces of the disinfectant can be removed prior to testing. Consult the test material manufacturer or conduct tests with the test material to determine the best method of sterilization. Specific details for decontamination depend upon the method selected and should be worked out before actual testing begins within the QA/QC framework.

8.3 Equilibrate or bring to near equilibrium samples in the chamber before inoculation with the test organism. Equilibration time will depend upon both the material to be tested and the chamber relative humidity selected for the test. Determine equilibration times for each material prior to testing.

8.3.1 Ascertain equilibration by determining when the bulk moisture content of the material reaches a constant value. The use of a calibrated analytical balance is recommended.

8.3.2 Compute the bulk moisture content of the test material as follows:

$$MC = [(M_b - M_d) / M_d] \times 100 \quad (1)$$

where:

$MC$ = bulk moisture content (%),
$M_b$ = mass of the small piece (g), and
$M_d$ = mass of the small piece after drying (g).

$M_d$ may be determined either by oven drying at 105 to 110°C or desiccation to constant weight depending upon the test material. Time required for drying is determined experimentally. A sample can be considered dry when no significant weight change is detected in two consecutive weighings at least 1 h apart.

## 9. Selection of Test Organism

9.1 Selection of the appropriate test organisms is extremely important. Since growth requirements vary for different organisms, the selection process should include a justification for the particular organism or organisms chosen. Testing of materials with many different organisms from diverse groups is optimal. At a minimum, representative bacteria and fungi should both be tested. Initial tests should be performed with only one species of microorganism.

9.1.1 Criteria for organism selection are based on a number of factors. Appropriateness of the organism for the test material and the environment where the material is used are key factors.

9.1.2 Literature reviews or reference articles reporting on indoor field investigations are good sources of suggestions for potential test organisms.

9.2 Documentation of the test organism is critical. Standard strains from established culture collections may be selected or environmental organism, isolated from contaminated materials,

[5] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

may be used. Identification and verification of the identification and source of the organism must be included as part of the QA/QC plan.

## 10. Inoculation of Material with Test Organism

10.1 Small (30 to 40-mm square) pieces of material are usually sufficient for testing. Multiple replicates of the material should be run to minimize possible error and to demonstrate reproducibility.

10.2 *Determination of Amount of Inoculum*—The number of CFU of an organism used to inoculate each piece of material should be sufficiently high to provide an adequate challenge but at a level that is realistic to quantitate. Challenges ranging from 5000 to 7000 CFU per $cm^2$ have been reported.[6,7] Experimentally appropriate levels for each material are calculated based upon recovery during processing of the test sample, including dilution factors, amount plated, and minimum detection limit.

10.2.1 Calculate minimum detection limits (MDL) as follows:

$$MDL = (MCFU_p \div VP) \times DF \times TV \quad (2)$$

where:

$MDL$ = minimum detection limit,
$MCFU_p$ = minimum number of colonies that can be counted on one plate (1 CFU/plate),
$VP$ = aliquot (volume) of suspension spread on plate (ml),
$DF$ = dilution factor of original sample, and
$TV$ = total volume of diluent containing sample material (ml).

10.3 Inoculate organisms onto the test material as either a suspension or as a dry inoculum.

10.3.1 Dry inocula are at best qualitative or semi-quantitative and may be sufficient for visible growth/no growth evaluations.

10.3.2 Inoculation of materials with a known number of CFU suspended in a known volume of liquid carrier results in more reproducible data and quantification of growth. The suspension with an appropriate concentration of CFUs may be introduced into the samples as either an aerosol or pipetted directly onto the samples. Use a small inoculum, sufficient to deliver an adequate amount of CFU, while minimizing the amount of moisture added to the system. The small amount of moisture should be allowed to evaporate from the test material after inoculation. If large inocula volumes are desired, first calculate the moisture content of the material determined in 8.3 for equilibrated samples. Exercise care that the materials are kept in a sterile or clean environment, such as biological safety cabinet or High Efficiency Particulate Arrestor (HEPA) filtered area, while the moisture is evaporating.

10.3.2.1 Select an appropriate liquid carrier for the test organism with care. Attention should be paid to any potential nutrients in the diluent that could artificially enhance the ability of a material to support microbial growth throughout the course of the experiment. Use of sterile distilled and deionized water minimizes the amount of possible residual nutrient sources. Some microbial forms, such as vegetative organisms, may not survive in unbuffered water. Others, such as spores, are much hardier forms and are resistant to the osmotic pressure of unbuffered water.

10.4 To determine the recovery efficiency of the test organism from the test specimen, immediately after inoculation, assay triplicate test material samples.

## 11. Procedure

11.1 Inoculate sufficient replicates (at least three) of the test material with the test organism to ensure reproducibility. The number of replicates should be defined within the QA/QC plan. For tests with several test dates, multiple replicates of the material should be run for each test date. Quantitative evaluation of microbial growth usually involves the destruction of the material. Usually only one type of test material inoculated with one type of test organism may be placed in a chamber at one time, but multiple pieces of the same material inoculated with the same organism should be evaluated simultaneously.

11.2 After inoculation, carefully transport and place the pieces of material in the chamber. Avoidance of contamination by ambient air or physical contact is essential. This may be accomplished by covering the samples and carrying them on a clean, sterilized tray.

11.3 To ensure that the chamber or other samples do not become contaminated, do not replace them into the chamber after they have been removed. This will assist in ensuring that contamination does not occur. Only as many pieces as required for evaluation at each time should be removed.

## 12. Determination of Microbial Growth

12.1 Microbial growth on materials may be evaluated qualitatively or quantitatively.

12.2 Qualitative evaluation requires inspection of the test material for evidence of microbial growth. Inspection of the material may be microscopic or macroscopic. Scales for visual examination are available.[4,8] Most qualitative evaluations involve either measuring the diameter of the growth on the material or applying a subjective scale.

12.3 Quantitative evaluation yields an indication of the amount of growth within a specific time frame on a test material when compared with the level on the day of inoculation or day zero. Results are not subjective and offer a consistent method of evaluation that is not operator or technician dependent. Comparisons between different materials or different composition of the same material are possible.

12.3.1 For quantitative evaluation, replicate samples of the test material are removed from the static chamber and processed. For example, place the sample in a sterile container,

[6] Foarde, K., VanOsdell, D., and Chang, J., "Static Chamber Method for Evaluating the Ability of Indoor Materials to Support Microbial Growth," *Characterizing Sources of Indoor Air Pollution and Related Sink Effects, ASTM STP 1287*, B. Tichenor, ed., American Society for Testing and Materials, 1996, pp. 87–97.

[7] Foarde, K., VanOsdell, D., and Chang, J., "Evaluation of Fungal Growth of Fiberglass Duct Materials for Various Moisture, Soil, Use, and Temperature Conditions," *Indoor Air*, Vol 6 1996, pp. 83-92.

[8] Block, S.S., "Humidity Requirements for Mold Growth," *Applied Microbiology*, Vol 1, 1953, pp. 287-293.

and add sterile buffer or diluent to the material. Gently sonicate or vigorously shake the material/organism suspension to mechanically remove organisms from the test material. Selection of the appropriate buffer is important for separating organisms from test material sample and inhibiting possible clumping of the suspended organisms.

12.3.2 Dilution of the extraction buffer or diluent is required, if growth is suspected. A series of ten-fold dilutions are usually performed. Aliquots of the original material suspended in buffer, as well as the dilutions, are plated on appropriate media as determined in 6.3. Depending upon the test organism, the range of optimal counts is from 10 to 100 CFU per plate. The optimal maximum number of CFU is 100 per plate.[5]

12.3.3 Growth may be expressed as CFU per gram of material, per measured surface area of material, or per sample. The results should be compared with controls as well as original inocula to determine if growth of the organism has occurred. Calculate CFU/sample as follows:

$$CFU/\text{sample} = (\overline{CFU_p} \div VP) \times DF \times TV \quad (3)$$

where:

$\overline{CFU_p}$ = mean of *CFU* enumerated per plate,
*VP* = aliquot (volume) of suspension spread on plate (ml),
*DF* = dilution factor of original sample, and
*TV* = total volume of diluent containing sample material (ml).

12.3.4 Changes in the numbers of CFU over time are quantitated. Compare the CFU from test day x to the CFU in the inocula or day 0 recovery results as follows:

$$\Delta CFU = CFU_{\text{day } x} - CFU_{\text{day } 0} \quad (4)$$

where:

$\Delta CFU$ = the change in CFU between a test day (*x*) and day *0*,
$CFU_{\text{day } x}$ = CFU on test day x, and
$CFU_{\text{day } 0}$ = CFU on day *0*.

12.3.5 The standard error of the means between the inocula or start date and the test date gives the statistical significance of the differences. The definition of significance should be defined within the Data Quality Objectives (DQO) of the QA/QC.

12.3.6 Several other measurement methods may be used for quantitative evaluation of test materials (for example, plate count, direct viable count, fluorescent antibody assay, and most probable number).

## 13. Quality Assurance/Quality Control

13.1 Static environmental chamber testing of materials should be conducted within the framework of a quality assurance project plan. This plan should contain the following:

13.1.1 *Project Description*—A brief description of the test material, how the material evaluation is to be performed, and the responsible individual(s).

13.1.2 *Data Quality Objectives*—A description of the parameter being measured, the reference method, precision, accuracy, and completeness.

13.2 *QA/QC Approaches and Activities*—The types of QA/QC activities that might be specified include establishment of a system of records/notebooks to ensure proper operation of equipment and recording of data, such as the following;

13.2.1 *Negative, Positive, and Comparison Controls*—The exact controls necessary are test dependent. For example, if evaluating the efficacy of a treatment, untreated controls of the same material need to be included for comparison;

13.2.2 *Sample Log Book*, to record receipt, storage, and disposition of materials;

13.2.3 *Instrument Maintenance Logs*, to document maintenance and repairs as well as calibration activities for all equipment;

13.2.4 *Materials Testing Log Books*, used to record all pertinent information for each test, including sample details, sample identification number, and test organism; and

13.2.5 Sample custody sheets and identification of the responsible personnel.

13.3 Quality control activities must be carried out by project staff in a routine, consistent manner to provide necessary feedback in operation of all measurements.

## 14. Report

14.1 The report will include the following:

14.1.1 Organism used and justification for selection;

14.1.2 Conditions in static chamber, including but not limited to temperature, relative humidity, and light; and

14.1.3 Results of qualitative or quantitative analysis, or both.

14.2 Qualitative results are subjective and may be reported according to a published scale or one developed by the testing laboratory. If the scale is developed by the testing laboratory, a detailed description of the scale should be included.

14.3 Quantitative results should be reported as CFU per sample, gram of sample, or measured surface area of sample.

14.4 QA/QC results will be reported that certify the quantity of the results compared to the DQOs.

## 15. Keywords

15.1 bacterial growth; building material; fungal growth; indoor air quality; indoor sinks; indoor sources; material susceptability; microorganisms; static chamber

# APPENDIX

**(Nonmandatory Information)**

## X1. POSSIBLE TEST FUNGI

### X1.1 Scope

X1.1.1 This list is limited to fungi that may used for testing. Many different test organisms are possible, and selection of the appropriate organism is part of the method development of a specific test method from this guide.

X1.1.2 This list is not intended to be complete, and it is the users responsibility to justify the organism selection as discussed in Section 9. It is also the users responsibility to identify the potentially toxigenic organisms.

### X1.2 Possible Test Fungi

X1.2.1 *Aspergillus versicolor*
X1.2.2 *Aspergillus flavus*
X1.2.3 *Aspergillus fumigatus*
X1.2.4 *Aspergillus glaucus*
X1.2.5 *Aspergillus niger*
X1.2.6 *Aspergillus terrus*
X1.2.7 *Stachybotrys atra*
X1.2.8 *Fusarium moniliforme*
X1.2.9 *Penicillium chrysogenum*
X1.2.10 *Penicillium brevicompactum*
X1.2.11 *Penicillium commune*
X1.2.12 *Penicillium expansum*
X1.2.13 *Penicillium viridicatum*
X1.2.14 *Cladosporium cladosporoides*
X1.2.15 *Cladosporium herbarum*
X1.2.16 *Cladosporium sphaerospermum*