Designation: D8068 – 19

# Standard Practice for Collection of Culturable Airborne Fungi or Bacteria on Agar Plates by Inertial Impaction Systems[1]

This standard is issued under the fixed designation D8068; 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

This practice was introduced to bring uniformity to the process of sample collection for culturable fungi or bacteria using inertial impaction samplers for analysis by culture and optical microscopy.

## 1. Scope

1.1 The purpose of this practice is to describe procedures for the collection of culturable airborne fungal spores or fragments or bacteria on agar plates using inertial impaction sampling techniques.

1.2 This practice does not include collection of culturable fungi or bacteria by devices not using agar plates.

1.3 This practice presumes that the user has a fundamental understanding of field investigative techniques related to the scientific process, and sampling plan development and implementation. It is important to establish the related hypothesis to be tested and the supporting analytical methodology needed in order to identify the sampling media to be used and the laboratory conditions for analysis.

1.4 This practice does not address the development of a formal hypothesis or the establishment of appropriate and defensible investigation and sampling objectives. It is presumed the investigator has the experience and knowledge base to address these issues.

1.5 This practice does not provide the user sufficient information to allow for interpretation of the analytical results from sample collection. It is the user's responsibility to seek or obtain the information and knowledge necessary to interpret the sample results reported by the laboratory.

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]

D1356 Terminology Relating to Sampling and Analysis of Atmospheres

D3195/D3195M Practice for Rotameter Calibration

D6044 Guide for Representative Sampling for Management of Waste and Contaminated Media

F1671 Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Blood-Borne Pathogens Using Phi-X174 Bacteriophage Penetration as a Test System

## 3. Terminology

3.1 *Definitions*—For definitions of terms used in this practice, see Terminology D1356.

3.1.1 *agar, n*—a semisolid culture medium used to support the growth of bacteria and other micro-organisms. **F1671**

3.1.2 *sample, n*—a portion of a population.

3.1.2.1 *Discussion*—A portion of material that is taken for testing or record purposes. **D6044**

3.1.3 *sample, representative, n*—a sample collected in such a manner that it reflects the characteristics of interest of a

[1] This practice 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 March 1, 2019. Published March 2019. Originally approved in 2018. Last previous edition approved in 2018 as D8068 – 18. DOI: 10.1520/D8068-19.

[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.

population (as defined) from which it is collected.

**adapted from D6044**

3.1.3.1 *Discussion*—A population in the context of this standard refers to culturable fungi- or bacteria colony-forming units that exist at the time and location of sampling.

3.1.4 *sampling train, n*—the assemblage of equipment necessary to sample atmospheres. **D1356**

3.1.4.1 *Discussion*—Complete assembly from the pump system through to the agar plate including any transport tubing and connectors.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *bacteria, n*—any of a class of microscopic prokaryotic organisms reproducing by fission or by spores.

3.2.2 *chain of custody (COC), n*—a document that provides for the traceable transfer of field samples to the analytical laboratory.

3.2.2.1 *Discussion*—The COC may or may not be combined with the Field Data Sheet.

3.2.3 *fungus (s), fungi (pl), n*—eukaryotic, heterotrophic, absorptive organisms that usually develop a rather diffuse, branched, tubular body (that is, network of hyphae) and usually reproduce by means of spores. **adapted from Kendrick[3]**

3.2.3.1 *Discussion*—The terms 'mold' and 'mildew' are frequently used by laypersons when referring to various fungal colonization.

3.2.4 *inertial impactor, n*—a device designed for the collection of particles that are separated from the air stream by inertia to force the impact onto an adhesive surface.

3.2.4.1 *Discussion*—Inertial impactors are available in many designs and include both 'multi-orifice' (also known as 'multiple-hole' or 'sieve plate') and 'slit to agar' (also known as rotating slit) samplers.

## 4. Significance and Use

4.1 This practice is intended for the collection of airborne particles on agar plates using inertial impaction for the purpose of culturing fungi or bacteria.

4.2 This practice is valuable when species level identification or quantity of culturable aerosolized fungi and bacteria are important factors for the indoor air quality investigation.

4.3 It is the responsibility of the user to assure that they are in compliance with all local, state, and federal regulations governing the inspection of buildings for fungal and bacterial colonization and the collection of associated samples.

4.4 This practice is intended to provide the user with a basic understanding of the equipment, materials, and instructions necessary to effectively collect air samples on agar plates using an inertial impactor.

4.5 This practice is intended to minimize systematic sampling variations between different data sets.

## 5. Preparation of Sampling Equipment

5.1 *Equipment List:*

5.1.1 *Sampling Train*—The combination of components from the pump or fan system through to the agar plate including any transport tubing, flow controller, and connectors. The configuration may be an integrated assembly or components that have been configured with an external pump.

NOTE 1—Rotary vane, diaphragm, linear magnetic, piston, and fan driven devices may have the open flow capacity for specific impactors; however, resistance to flow through the impactor can dramatically reduce flow rates. Care must be taken to select a pump and calibrator that are compatible with impactors to set and measure flow rates properly.

5.1.1.1 For external pump assemblies, use flexible tubing and connectors appropriate for secure connection of impactor to pump.

5.1.2 *Primary flow rate standard,* with a measuring range appropriate for the system and with a ±5 % tolerance of the desired flow rate.

5.1.3 *Secondary Flow Rate Standard*—Rotameter or other device used to check system performance in the field.

5.1.4 *Stop watch,* or other timing device capable of measuring time in increments of minutes and seconds (1 second resolution).

5.1.5 *Field Data Sheet*—Refer to 6.6.

5.1.6 *Agar plate.*

5.1.7 *Support stand (optional),* for consistent sample collection height.

5.1.8 70 % isopropanol technical grade or better.

5.1.9 Single use gloves to prevent contamination during sampling.

5.2 *Calibration:*

5.2.1 Calibrate or verify airflow through the assembly configuration that will be used in the field. For example, do not calibrate with one length of tubing and sample with a different length of tubing.

5.2.2 Use a primary or secondary flow rate standard to verify the airflow rate passing through the sampling assembly. Be aware that it is the airflow rate through the impactor, not the pump or fan that shall be calibrated.

5.2.3 Verify the secondary flow rate standard using a primary flow rate standard in accordance with the manufacturer's recommendations.

5.2.4 Integrated sampling assemblies may not be capable of being calibrated by the user. Follow manufacturer's instructions when user calibration is not possible.

5.2.5 Verify and record the airflow prior to and following field sampling. If the result of the post-field verification check is greater than ±10 % of pre-field verification, either (*1*) discard any samples collected, re-adjust the sampler as necessary, and re-sample; or (*2*) note any airflow rate variations in the field notes and any data interpreting documents, recording the magnitude of the change and averaging the airflow rates (Practice D3195/D3195M). Use the average airflow rate to calculate sample volume.

[3] Kendrick, B., *The Fifth Kingdom*, Focus Publishing / R. Pullins and Co, 2008.