Designation: D7391 – 20

Standard Test Method for Categorization and Quantification of Airborne Fungal Structures in an Inertial Impaction Sample by Optical Microscopy[1]

This standard is issued under the fixed designation D7391; 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 a procedure that uses direct microscopy to analyze the deposit on an inertial impaction sample.

1.2 This test method describes procedures for categorizing and enumerating fungal structures by morphological type. Typically, categories may be as small as genus (for example, *Cladosporium*) or as large as phylum (for example, basidiospores).

1.3 This test method contains two procedures for enumerating fungal structures: one for slit impaction samples and one for circular impaction samples. This test method is applicable for impaction air samples, for which a known volume of air (at a rate as recommended by the manufacturer) has been drawn, and is also applicable for blank impaction samples.

1.4 Enumeration results are presented in fungal structures/sample (fs/sample) and fungal structures/m^3 (fs/m^3).

1.5 The range of enumeration results that can be determined with this test method depends on the size of the spores on the sample trace, the amount of particulate matter on the sample trace, the percentage of the sample trace counted, and the volume of air sampled.

1.6 This test method addresses only the analysis of samples. The sampling process and interpretation of results is outside the scope of this test method.

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

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]

D1193 Specification for Reagent Water

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

3. Terminology

3.1 *ASTM Definitions (see the ASTM Online Dictionary of Engineering Science and Technology*[3]):

3.1.1 *numerical aperture.*

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *circular impaction sample, n*—a sample of airborne particulate matter collected by means of a device that draws air through a round aperture at a specified rate, impacting the particles suspended in the air onto an adhesive medium, resulting in a circular area of deposition. A circular impaction sample may be collected by means of a cassette manufactured for that purpose, or by means of a sampling device that requires slides to be pre-coated with impaction medium.

3.2.2 *debris rating, n*—a distinct value assigned to an impactor sample based on the percentage of the sample area potentially obscured by particulate matter, and ranging from 0 to 5.

3.2.3 *field blank, n*—a sample slide or cassette carried to the sampling site, exposed to sampling conditions (for example,

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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.

[3] *ASTM Online Dictionary of Engineering Science and Technology* (Stock #: DEFONLINE) is available on the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org.

seals opened), returned to the laboratory, treated as a sample, and carried through all steps of the analysis.

3.2.4 *fungal structure (sing.), n*—a collective term for fragments or groups of fragments from fungi, including but not limited to conidia, conidiophores, and hyphae and spores.

3.2.5 *fungus (s), fungi, (pl.), n*—eukaryotic, heterotrophic, absorptive organisms that usually develop a rather diffuse, branched, tubular body (for example, network of hyphae) and usually reproduce by means of spores.[4] The terms 'mold' and 'mildew' are frequently used by laypersons when referring to various fungal colonization.

3.2.6 *hyaline, adj*—colorless.

3.2.7 *impaction medium, n*—a substance applied to a microscope slide used to collect (or capture) particulate matter during sampling.

3.2.8 *impaction sample, n*—a sample taken using impaction, for example, slit impaction sample, circular impaction sample.

3.2.9 *inertial impactor, n*—a device for collecting particles separated from an air stream by inertia to force an impact onto an adhesive surface. Inertial impactors are available in many designs, including those having a slit jet, yielding a rectangular sample trace, and a circular jet, yielding a circular sample trace.

3.2.10 *magnification/resolution combination 1, n*— ~150–400× total magnification and a point to point resolution of 0.7 µm or better, as checked by a resolution check slide.

3.2.11 *magnification/resolution combination 2, n*— ~400× or greater total magnification and a point to point resolution of 0.5 µm or better, as checked by a resolution check slide.

3.2.12 *minimum reporting limit (fs/sample); minimum reporting limit (fs/m^3), n*—the lowest result to be reported for total spores or any spore category. Since both fs/sample and fs/m^3 are reported, there are two minimum reporting limits.

3.2.13 *morphology, n*—the form and structure of an organism or any of its parts; for fungi, the shape, form, ornamentation, or combination thereof.

3.2.14 *mounting medium, n*—a liquid, for example, lactic acid or prepared stain, used to immerse the sample particulate matter and to attach a cover slip to an impaction sample.

3.2.15 *sample trace, n*—the area of particle deposition, that is, the deposit on a slit impaction sample resembling a narrow rectangle, or the circular deposit on a circular impaction sample.

3.2.16 *septum (s), septa (pl.), n*—a cell wall or partition.

3.2.17 *slide adherent, n*—an adhesive or liquid used to affix an impaction sample substrate to a microscope slide.

3.2.18 *slit impaction sample, n*—a sample of airborne particulate matter collected by means of a device that draws air through a linear aperture at a specified rate, impacting the particles suspended in the air onto an adhesive medium, resulting in a rectangular area of deposition. A slit impaction sample may be collected by means of a cassette manufactured for that purpose, or by means of a sampling device that requires slides to be pre-coated with impaction medium.

3.2.19 *spore category, n*—a grouping used for identification and quantifation of fungal structures. A spore category may contain a specific genus (for example, *Stachybotrys*), or it may represent a combination of genera (for example, *Aspergillus/Penicillium*-like).

3.2.20 *traverse, n*—a portion of analysis of an impactor sample consisting of one scan under the microscope from a sample-less portion of the impaction medium across the deposit to a corresponding sample-less portion of the impaction medium on the other side.

3.3 *Symbols:*

3.3.1 *fs*—fungal structure

3.3.2 *fs/m^3*—fungal structures per cubic metre

3.3.3 *m^3*—cubic metre

3.3.4 *mm*—millimetre

3.3.5 *µm*—micrometre

4. Summary of Test Method

4.1 Samples have been previously collected utilizing an impaction device operating at the device manufacturer's recommended sample flow rate. Each sample consists of an optically clear substrate coated with an adhesive and optically transparent medium onto which particles have been deposited through inertial impaction.

4.2 A sample is mounted to a microscope slide and examined by bright field microscopy using at least two magnification/resolution combinations.

4.3 Spores are differentiated from each other, other fungal structures, and from non-fungal material by color, size, shape, presence of a septum or septa, attachment scars, surface texture, etc., by means of a taxonomic comparison with standard reference texts or known standard samples, or both (see Section A1.1 for suggested references). The number of spores that match each spore category are then calculated in units of fungal structures per sample (fs/sample) and also fungal structures per cubic meter of air (fs/m^3).

5. Significance and Use

5.1 This test method is used to estimate and categorize the number and type of fungal structures present on an inertial impactor sample.

5.2 Fungal structures are identified and quantified regardless of whether they would or would not grow in culture.

5.3 It must be emphasized that the detector in this test method is the analyst, and therefore results are subjective, depending on the experience, training, qualification, and mental and optical fatigue of the analyst.

6. Interferences

6.1 *Differentiation of Fungal Genera/Species*—Because of the similar size and morphology of some fungal spores of different genera and the absence of growth structures and mycelia in airborne samples, differentiation by microscopic

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

examination alone is difficult and spores must be grouped into categories based strictly on morphology. In many cases, identification at the genus level is presumptive. For example, differentiation between *Aspergillus* and *Penicillium* using this test method is not typical, so a combined *Aspergillus*/*Penicillium*-like category is used. When differentiation between such genera is desired, a different test method must be used. Unequivocal identification of every spore in each category is not possible due to optical limitations, the atypical nature of some of the spores, overlapping morphology among different spore types, or combination thereof, and therefore, certain spores must be categorized as Miscellaneous/Unidentifiable.

6.2 *Look-Alike Non-Fungal Particles*—Certain types of particles of non-fungal origin may resemble fungal spores. These particles and artifacts may include air or plant resin bubbles, starch, talc, cosmetic particles, or combustion products. Standards (mounted similarly to impactor samples) should be examined by laboratory analysts to know how to identify such particles. Examination of suspect particles using optical conditions other than bright field microscopy (for example, polarized light microscopy, phase contrast microscopy, differential interference contrast) may be helpful whenever significant concentrations of look-alike particles are present. In some cases dust and debris can mimic the morphology of particles of interest. When look-alike particles are present in high concentration, accurately counting spores with similar morphology is difficult. When these conditions exist, they should be reported in the analysis notes section of the report.

6.3 *Particle Overloading*—High levels of particulate matter on an impaction sample will bias the analysis in two ways:

(1) Particle capture efficiency decreases, and

(2) Debris obscures or covers spores.

Both of these factors produce a negative bias.

6.4 *Staining*—Staining, while optional, may help the analyst differentiate spores from debris. Without staining, clear spores (especially small ones) may exhibit negative bias because the analyst has insufficient contrast to notice them while scanning. Also, because spores of different fungal species absorb stains at different rates, under or over-staining makes identification difficult. The problem can be eliminated by careful control of stain concentrations.

6.5 *Impaction Medium Stability and Clarity*—Chemicals present in some mounting media may affect the physical stability or clarity of the impaction medium. For instance:

(1) Samples collected on silicone grease medium should first be warmed on a hot plate at approximately 40°C to “fix” the sample in place, when using lacto-phenol cotton blue stain, and

(2) Slides and cassettes using methyl cellulose ester + solvent adhesive medium, which is stable in lacto-phenol cotton blue stain, will “fog” with Calberla’s stain due to the water and alcohol mixture; warming fogged slides may temporarily clear them.

The lab or analyst should develop through experimentation an impaction medium/mounting medium combination that will result in acceptable stability, clarity, and spore visibility.

6.6 *Uneven Impaction Medium Uniformity*—Uneven thickness may be present in greased slides, pre-coated slides and manufactured cassettes. The microscopist will compensate by adjusting the plane of focus. When grease is too thick, differentiating small spores from background artifacts (especially air bubbles) in the grease preparation becomes difficult. When grease is too thin, shrinkage and pooling may have occurred, causing particle loss during sampling.

7. Apparatus

7.1 *Marking pen,* for marking sample slides.

7.2 *Microscope or magnification system,* having a precision *x-y* mechanical stage. The microscope or magnification system used for analysis shall be capable of at least two magnification/resolution combinations as follows: magnification/resolution combination 1 shall be ~150–400× total magnification and a point to point resolution of 0.7 µm or better; magnification/resolution combination 2 shall be ~400× or greater total magnification and a point to point resolution of 0.5 µm or better. It is recommended that at least one microscope or magnification system in the lab be capable of magnification of ~1000× total magnification and a point to point resolution of 0.3 µm or better. That the resolution for combinations 1 and 2 is suitable is to be checked using a resolution check slide (see 13.2.3).

7.3 *Reference Slides*—a series of mounted field samples to be used as counting references. Analysts’ results from these slides are expected to be within laboratory acceptance limits to prove competence.

7.4 *Reticule,* width defining, an optical device in the light path of the microscope capable of being reproducibly set to define a traverse width no larger than 0.75× the diameter of the ocular field of view, and having graduations of an appropriate dimension to allow measurement of spore size, for example, Walton-Beckett reticule (round) or 100 divisions in 10 mm (linear or square). If a non-round reticule is used, procedures must be in place to ensure that the reticule is correctly positioned for each analysis.

7.5 *Stage micrometer,* traceable to the National Institute of Standards and Technology (NIST) or equivalent international standard.

7.6 *Resolution check slide,* a microscope slide on which calibrated distances, shapes, and line widths provide reliable and simple image resolution and shape identification performance of the microscopic and analyst at magnification. Examples include: a slide onto which a variety of diatoms have been mounted, including examples of *Stauroneis phoenicenteron* and *Pleurosigma angulatum*, a brightfield resolution test slide, or equivalent.

7.7 *Syringe or dropper,* for dispensing liquid during sample preparation.

8. Reagents and Materials

8.1 *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