Designation: D7658 – 17 (Reapproved 2021)

Standard Test Method for Direct Microscopy of Fungal Structures from Tape[1]

This standard is issued under the fixed designation D7658; 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 uses optical microscopy for the detection, semi-quantification, and identification of fungal structures in tape lift preparations.

1.2 This test method describes the preparation techniques for tape-lift matrices, the procedure for confirming the presence of fungal structures, and the reporting of observed fungal structures

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]

D1193 Specification for Reagent Water

D1356 Terminology Relating to Sampling and Analysis of Atmospheres

3. Terminology

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

3.2 *Definitions of Terms Specific to This Standard:*

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

3.2.2 *magnification/resolution combination 1, n*— ~100–400× total magnification and a point to point resolution of 0.7 µm or better.

3.2.3 *magnification/resolution combination 2, n*— ~400× or greater total magnification and a point to point resolution of 0.5 µm or better.

3.2.4 *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 the sample.

3.2.5 *tape lift sample, n*—material lifted from a surface using clear, transparent, single sided, adhesive collection medium, typically tape or commercially available prepared slides.

4. Summary of Test Method

4.1 A tape lift sample is prepared.

4.2 The prepared sample is examined on an optical microscope for the presence, type and semi-quantification of fungal structures and reported.

5. Significance and Use

5.1 The significance of this test method is to standardize the analysis of the detection of removable fungal structures lifted from a surface with tape to improve consistency between laboratories and analysts.

5.2 This test method is intended to ensure consistent data to the end user.

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

5.4 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, optical acuity, and mental fatigue of the analyst.

5.5 This test method can be used to assess the presence and characteristics of fungal material on a surface.

6. Interferences

6.1 *Look-alike Non-fungal Particles*—Certain types of particles of non-fungal origin may resemble fungal structures.

[1] This test method 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.

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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.

These particles and artifacts may include air or plant resin, bubbles, starch, talc or cosmetic particles, or combustion products. Non-fungal reference slides (mounted similarly to tape-lift samples) should be examined by laboratory analysts to know how to differentiate 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.

6.2 *Particle Overloading*—High levels of non-fungal background particulate may obscure or cover fungal structures.

6.3 *Staining*—Fungal structures of different fungal species absorb stains at different rates, under or over-staining makes identification difficult. The problem can be minimized with careful control of stain concentrations.

NOTE 1—Staining, while optional, may help the analyst differentiate fungal structures from debris. Without staining, clear spores (especially small ones) may exhibit negative bias because the analyst has insufficient contrast to detect them while scanning.

7. Apparatus

7.1 *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 ~100–400× 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. Acceptable resolutions for combinations 1 and 2 shall be checked using a resolution check slide.

NOTE 2—It is recommended that at least one microscope or magnification system be available that is capable of magnification of ~1000× total magnification and a point to point resolution of 0.3 µm or better.

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

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

7.4 *Forceps,* for manipulating adhesive tape, cleaned to prevent cross contamination.

7.5 *Scalpel,* or other cutting tool, if needed for cutting tape, cleaned to prevent cross contamination.

8. Reagents and Materials

8.1 *Mounting medium (with or without stain),* for re-hydrating spores, optimal resolution, and securing the cover slip to the sample. For example, lactic acid, lacto-cotton blue stain, lacto-phenol-cotton blue stain, lacto-fuchsine stain, glycerin jelly (see Appendix X2 for examples of stain preparations).

8.2 *Microscope cover slips,* large enough to cover the tape preparation. For optimum performance, choose a cover slip thickness according to the recommendations of the microscope objective lens manufacturer.

8.3 *Microscope slides,* glass slides to be used when samples are not taken on commercially available lift-samples.

8.4 *Disinfectant,* for cleaning of forceps or scalpel.

9. Hazards

9.1 Components of re-hydrating liquids and stains may be corrosive or hazardous. Consult the appropriate Safety Data Sheet for any reagents used.

9.2 Sharp instruments used in sample preparation may cause injury if not handled with care. These sample instruments may, at times, be contaminated with biological material capable of introducing organisms to the user.

9.3 Samples shall be handled using good laboratory technique to minimize exposure.

10. Preparation of Apparatus

10.1 *Microscope Alignment/Adjustments/Lens Cleaning*—Follow the manufacturer's instructions.

11. Calibration and Standardization

11.1 *Graduation Spacing for Ocular Reticule:*

11.1.1 *Measuring Gradations on the Ocular Reticule*—For each magnification/resolution combination, verify the µm per graduation, using a stage micrometer, at the magnification(s) used for counting at least once per year, and after any service or repair to the microscope. The graduations are used to measure the size of spores as an aid to identification.

11.1.2 *Resolution Check*—Check the resolution of magnification/resolution combinations 1 and 2 at least annually and after servicing, as in accordance with the manufacturer's instructions for the resolution check slide used.

12. Procedure

12.1 *Preparation of Tape Lift Samples:*

12.1.1 If the tape lift was not submitted on clear tape or prepared adhesive slide, then the sample may not be analyzed using this test method.

12.1.2 Remove the tape lift from its container.

12.1.3 Mark each slide with a unique designation.

12.1.4 Mount sample in one of these three ways:

12.1.4.1 For samples submitted affixed to a microscope slide, gently lift one end of the tape from the slide with forceps and place a drop of mounting medium under the sample area. Additional manipulation of the sample may be necessary to attain uniform contact with the glass slide. Return the lifted portion of the tape to the slide taking care to minimize the amount of bubbles.

12.1.4.2 For samples submitted on a prepared adhesive slide, place a drop of mounting medium to the center of the sample. A cover slip is applied at such an angle that bubbles are minimized.

12.1.4.3 For all other submitted samples, cut a representative portion of a tape lift with scalpel and mount sample-side up on a microscope slide with forceps. Anchor on each side if needed. A drop of mounting medium is applied to the center of