Designation: D7658 – 17

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 and health 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 Sampling and Analysis of Mold.

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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 um or better; magnification/resolution combination 2 shall be ~400× or greater total magnification and a point to point resolution of 0.5 um 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 um 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*—cCheck 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

the sample, and a cover slip is placed at such an angle that bubbles are minimized. This technique can be used regardless of whether the tape lift sample was submitted sample side up or down, and regardless of what the tape was affixed to when submitted.

12.2 *Sample Evaluation:*

12.2.1 Place prepared slide on the stage of the microscope. Center the sample deposit over the light source.

12.2.2 Align/adjust microscope following the manufacturer's instructions.

12.2.3 Using magnification/resolution 1, examine entire sample preparation to detect fungal matter.

12.2.4 If no fungal matter is detected at magnification/resolution 1, switch to magnification/resolution 2. Examine a minimum of 20 fields of view if fungal material is not detected.

12.2.5 If no fungal structures were detected, record lack of detection.

12.2.6 If fungal matter is identified at low magnification, switch to magnification/resolution 2 and relocate the area for identification and determination of fungal loading.

12.2.7 Determine and record each fungal type as encountered.

12.2.7.1 The minimum categories to be reported are:

(1) Alternaria,
(2) ascospores (undifferentiated),
(3) Aspergillus/Penicillium-like,
(4) basidiospores (undifferentiated),
(5) Chaetomium,
(6) Cladosporium,
(7) Curvularia,
(8) Drechslera/Biopolaris-like,
(9) smuts/*Myxomycetes/Periconia*,
(10) Stachybotrys/Memnoniella,
(11) Ulocladium, and
(12) hyphal fragments.

NOTE 3—Depending on the fungal type and the scan magnification, it may be necessary to employ greater magnification or oil immersion, or both, for identification.

12.2.8 Record the presence of hyphae, fruiting bodies, or clumps and chains of spores for each fungal type detected, or combination thereof.

12.2.9 Determine the fungal loading category. (Fungal loading categoriesdefined in 12.2.10.1.)

12.2.10 Determine and record the non-fungal loading category of the sample. (Non-fungal loading categories defined in 12.2.10.2.)

12.2.10.1 *Fungal Loading Categories:*

(1) The loading of fungal material is reported using a scale of Categories 0–5. Category 0 is assigned when no fungal material is observed. Category 1 is assigned when the fungal material loading covers less than 5 % of a representative field of view. Category 2 is assigned when the fungal material loading covers between approximately 5 % and 25 % of a representative field of view. Category 3 is assigned when the fungal material loading covers between approximately 25 % and 75 % of a representative field of view. Category 4 is assigned when the fungal material loading covers between approximately 75 % and 90 % of a representative field of view. Category 5 is assigned when the fungal material loading covers greater than approximately 90 % of a representative field of view. Refer to the visual representations of particle loading categories (Fig. 1).

12.2.10.2 *Non-Fungal Particle Loading Categories:*

(1) The loading of non-fungal background debris is reported by using a scale of Categories 0–5. Particle Category 0 is assigned when no background debris is observed. Particle Category 1 is assigned when debris loading covers less than 5 % of a representative field of view. Particle Category 2 is assigned when debris loading covers between approximately 5 % and 25 % of a representative field of view. Particle Category 3 is assigned when debris loading covers between approximately 25 % and 75 % of a representative field of view. Particle Category 4 is assigned when a debris loading covers between approximately 75 % and 90 % of a representative field of view. Particle Category 5 is assigned when debris loading covers greater than approximately 90 % of a representative field of view. Refer to the visual representations of particle loading categories (Fig. 1).

13. Quality Assurance/Quality Control

13.1 Establish and maintain a quality assurance/quality control system for this analysis.

NOTE 4—Accreditation bodies may have specific QA/QC requirements.

13.2 *Contamination Control:*

13.2.1 *Housekeeping*—Keep preparation and analysis areas clean, for example, routinely wet-wipe to minimize transfer of lab dust to samples.

13.2.2 *Process/Medium Blank*—At a defined frequency, place in the sample preparation area during sample preparation a piece of clear adhesive tape mounted tacky side up onto a glass slide. When the sample batch has been prepared, place a drop of mounting medium on the adhesive tape followed by a cover slip, to create a process blank that includes all glass and liquid components of a typical sample. Analyze in the same manner as a sample. Establish acceptance criteria for such blanks.

13.2.3 *Cross Contamination*—No more than one sample may be prepared at a time.

13.3 *Precision and Accuracy:*

13.3.1 *Analyst Training and Qualification*—Qualify an analyst to be competent to perform this test method by a combination of background and education, aerobiological and mycological training, experience, and performance on bulk samples of known/reference content (for example, reference slides). Analyst qualification should be continuing, through routine comparison with other analysts. For single-person organizations, such comparison would necessarily be interlaboratory exchange.

13.3.2 *Quality Assurance/Quality Control*—Reanalyze a minimum of 10 % of client samples. Set acceptance criteria.

14. Records

14.1 Record at least the following data for each sample:

14.1.1 Analyst (for example, initials on the worksheet),

14.1.2 Date of analysis.

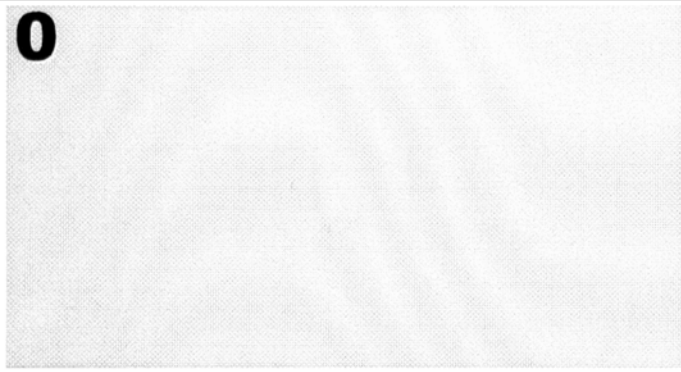
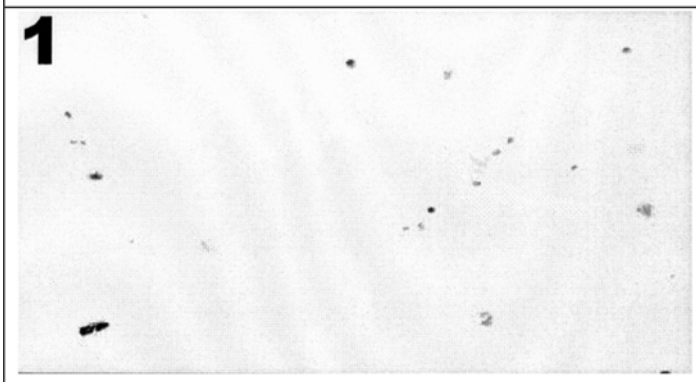
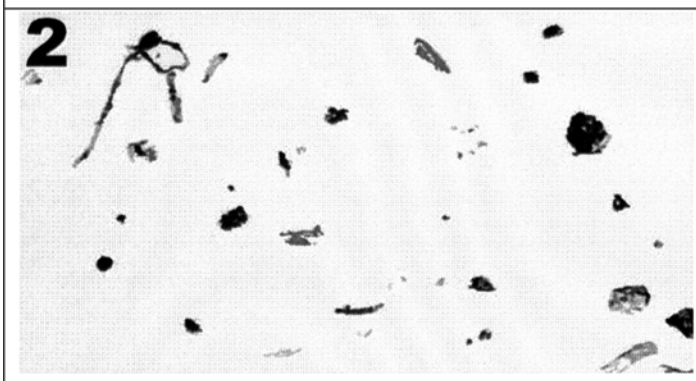
Particle Loading Category	Description
	No particulate matter observed
	Minimal (>not observed to approx. 5 %) particulate matter observed.
	Approx. 5 % to approx. 25 % of the field of view occluded with particulate matter.

FIG. 1 Visual Representations of Particle Loading Categories

14.1.3 Reference to the microscope used (if multiple microscopes are present,)

14.1.4 Laboratory number or unique number for each sample,

14.1.5 Notes on sample conditions or analytical problems,

14.1.6 Magnification(s) used, and

14.1.7 Analytical results.

15. Report

15.1 The test report shall include at least the following:

15.1.1 Reference to this test method,

15.1.2 Laboratory identification, address, and telephone number,

15.1.3 Client identification and address,

15.1.4 Client sample identification,

15.1.5 Laboratory unique identification/laboratory number,

15.1.6 Date of sample receipt,

15.1.7 Condition of sample (for example, any problems with condition),

15.1.8 Date of analysis,

15.1.9 Date of report,

15.1.10 Analyst name,

15.1.11 Signature and printed name of person taking responsibility for the data in the report,

15.1.12 Significant modifications to this procedure, if any,

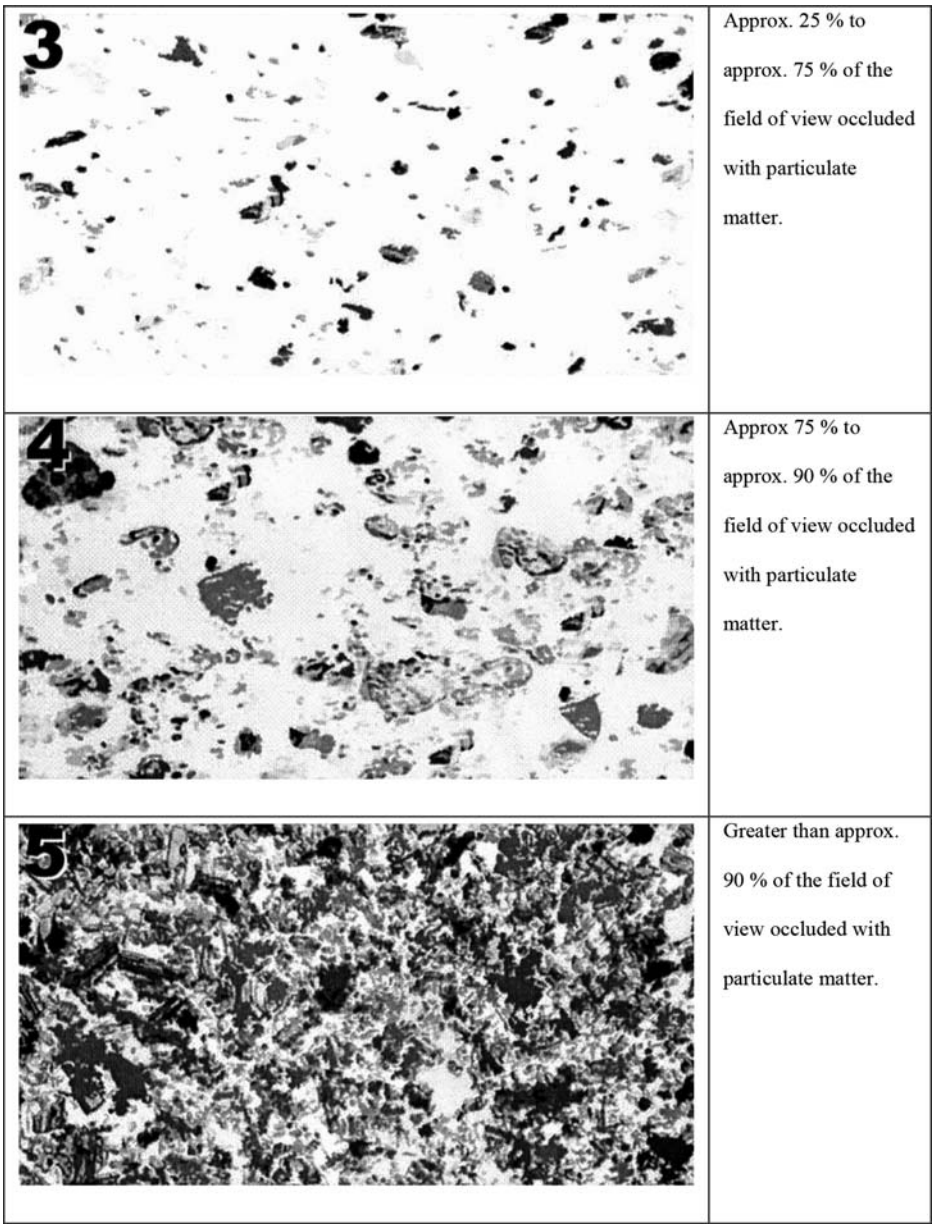


FIG. 1 Visual Representations of Particle Loading Categories *(continued)*

15.1.13 Page number and total number of pages in the report on each page or other mechanism for identifying each page as a part of the report and for indicating the end of the report,

15.1.14 Statement that the analysis only relates to the items tested,

15.1.15 Debris rating for each sample, and

15.1.16 Summary of any out of control situations connected to the analysis.

NOTE 5—The data contained in the report should be interpreted by the party that performed the on-site assessment from which the samples were collected and that has access to the data quality objectives used in the project for which the sample was collected (for example, notes on sample condition, substrate, loading, analytical problems, etc.).

16. Precision and Bias

16.1 Sources of negative bias include: spores being overlain or otherwise obscured by other particles, analyst missing spores during scanning, or the analyst mistaking spores for non-fungal particles, or combination thereof.

16.2 Sources of positive bias include: analyst mistaking bubbles or other non-fungal particles for spores.

16.3 Precision data will be developed within the five-year time frame after this test method is published.

17. Keywords

17.1 fungal material; fungal particulate; fungi; fungus; indoor air quality; mold; mould; particulate; sampling; spore; surface sampling; tape-lift; tape

ANNEX

(Mandatory Information)

A1. REFERENCES FOR FUNGAL SPORE IDENTIFICATION

A1.1 Fungal spores and other structures should be identified by a combination of reference texts (for example, Lacey and West,[3] and Smith[4]) and microscopical mounts from known sources (for example, national culture collections). All analyses should be performed by a trained and qualified analyst (see 13.3.1) utilizing appropriate mycology reference materials.

[3] Lacey, M. E., and West, J. S., *The Air Spora: A Manual for Catching and Identifying Airborne Biological Particles*, Boston, MA: Springer, 2006.

[4] Smith, G. E., *Sampling and Identifying Allergenic Pollens and Molds – an Illustrated Identification Manual for Air Samplers*, San Antonio, TX: Blewstone Press, 1990.

APPENDIXES

(Nonmandatory Information)

X1. DIATOM RESOLUTION CHECK

X1.1 A diatom test slide[5] can be used to document the resolution of an optical system.

[5] The sole source of supply of diatom resolution check slides known to the committee at this time is K. D. Kemp at Microlife Services, Blautannen, Wickham Way, East Brent, Somerset, England TA9 4JB. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend.

X2. DIRECTIONS FOR PREPARATION OF COMMON STAINS

X2.1 *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. 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.

X2.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type II of Specification D1193.

X2.3 *Lacto-Cotton Blue Stain:*

glycerol	250 mL
85 % lactic acid	100 mL
cotton blue stock	3 mL
de-ionized water	50 mL

LCB Stain:

(1) Mix the water, lactic acid, and glycerol (in that order) for one hour on a stir plate.

(2) Once the solution is homogenous, add 3-ml of Cotton Blue Stock solution (recipe given below) to the above solution.

(3) Stir the entire mixture for an additional hour.

(4) Cover the flask while the mixture is stirring to insure against airborne contamination.

Cotton Blue Stock Solution:

85 % lactic acid	99 ml
aniline (cotton) blue crystals	0.1 g

(1) Add cotton blue crystals to lactic acid while stirring vigorously on a stir plate.

(2) Stir until cotton blue crystals are dissolved.

(3) Filter the solution (for example, through a #50 Whatmann 90-mm filter disc).

(4) After filtration has occurred, check the clarity of the stock dye solution by placing one drop of the dye on a clean microslide and examine at 400×. Dye should be dissolved, not suspended.

NOTE X2.1—It may be desirable to prepare to a lighter color by decreasing the amount of Cotton Blue Stock added to the stain.

X2.4 *Lacto-Fuchsin Stain:*

85 % (or higher) lactic acid	40 ml
acid fuchsin	0.04 gm

(1) Heat 40 mls of 85 % lactic acid to near boiling.

(2) Add 0.04 g of acid fuchsin and stir on hotplate until completely dissolved.

(3) Remove solution from heat.

(4) Aliquot into 4-ml screw cap vials with septated caps.

(5) Label as "Acid Fuchsin" and date.

X2.5 *Lacto-Phenol-Cotton Blue Stain:*

glycerol	30 ml
lactophenol cotton blue stain (prepared solution, VWR catalog # VW3427 or equivalent)	0.5 gm
de-ionized water	30 ml
phenol	1 gm

(1) Heat 30-ml de-ionized water to near boiling.

(2) Add 30 ml of glycerol; stir until mixed and solution is clear.

(3) Remove from heat.

(4) Add 1.0-gm phenol; stir until dissolved.

(5) Add 0.5 ml of lactophenol cotton blue stain

X2.6 *Levetin 89 – L-PVA Permanent Slide Mount:*

polyvinyl alcohol powder (PVA)	7.5 gm
distilled water	50 mL
85 % (or higher) lactic acid	22 mL
phenol	22 gm

(1) Heat the distilled water to 80°C in a water bath.

(2) Add the polyvinyl alcohol (PVA) powder to the distilled water while stirring.

(3) Continue stirring until the solution attains the viscosity of thick molasses. If there are a few lumps that do not dissolve, remove them.

(4) Add the lactic acid to the PVA solution and mix well.

(5) In a separate beaker, melt the phenol using a water bath.

(6) Add the phenol to the PVA solution and stir until homogeneous.

(7) Store in tightly closed small vials. Over time, the phenol will cause the medium to darken, but the clarity of mounts will be unaffected.

(8) Mounts may be examined immediately, but harden gradually over a few days.

NOTE X2.2—This medium is compatible with double-sided tape, acid fuchsin, and phenosafranin. This medium is not compatible with cotton blue stain.