

Standard Practice for Evaluation of Fungal Control Agents as Preservatives for Aqueous-Based Products Used in the Paper Industry¹

This standard is issued under the fixed designation E875; 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 Scope*

1.1 This laboratory practice is used to determine the efficacy of a fungal control agent to prevent spoilage of in-process aqueous-based products used in the paper industry.

1.2 For information on bacterial control agents, see Test Method E723.

1.3 It is the responsibility of the investigator to determine whether good laboratory practices (GLP) are required and to follow them when appropriate (see 40 CFR 160).

1.4 A knowledge of microbiological techniques is required for these procedures.

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

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 safety, health, and health environmental practices and determine the applicability of regulatory limitations prior to use.

<u>1.7 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.</u>

2. Referenced Documents

2.1 ASTM Standards:²

D1193 Specification for Reagent Water

E723 Practice for Evaluation of Antimicrobials as Preservatives for Aqueous-Based Products Used in the Paper Industry (Bacterial Spoilage)

E1839 Practice for Efficacy of Slimicides for the Paper Industry—Bacterial and Fungal Slime

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

*A Summary of Changes section appears at the end of this standard

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¹This practice is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

Current edition approved May 1, 2015Oct. 1, 2020. Published July 2015October 2020. Originally approved in 1982. Last previous edition approved in 20102015 as E875 - 10: E875 - 15. DOI: 10.1520/E0875-15.10.1520/E0875-20

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.

2.2 Federal Standard: 40 CFR 160 Good Laboratory Practice Standards³

3. Terminology

3.1 For definitions of terms used in this practice refer to Terminology E2756.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *fungal control agent, n*—an agent that either kills or prevents growth of fungi and either kills or prevents the germination of fungal spores. This term is applied to chemical biocidal or biostatic agents.

3.2.1.1 Discussion—

This term is applied to chemical biocidal or biostatic agents.

3.2.2 preservative, *n*—chemical agent used to prevent microbial spoilage of products due to microbial agent(s) added to a product to reduce or prevent microbial growth.

4. Summary of Practice

4.1 Aqueous material to be preserved is inoculated with an appropriate fungal innoculum followed by addition of a concentration of fungal control agent that will kill the fungi or prevent their growth for a desired period of time, or both. In addition, the agent will also prevent fungal spore germination. Fungal growth is determined by visible signs of deterioration in the test sample, and by obtaining fungal numbers and comparing them to a sample without any fungal control agent. The proper level of fungal control agent is one that prevents product deterioration and reduces and keeps the organisms to an acceptable level in the test material, as determined by the tester or user.

5. Significance and Use

5.1 This practice should be used to determine if a fungal control agent is effective to preserve pigment suspensions, dye solutions, pulp slurries, starch solutions, polymers, sizing agents, latex emulsions, and other specific aqueous-based materials used in the paper industry. Separate evaluations should be made on a representative type for each specific class of product to be preserved.

NOTE 1—Control of bacterial spoilage of similar products can be evaluated by Test Method E723.

NOTE 2-Slimicides for control of fungal or bacterial slime can be evaluated by Test Method E1839.

6. Apparatus

6.1 *Two Balances*— One should be sensitive to 0.1 g at a load of 200 g with a platform to accommodate bottles being used in the test. The second balance (analytical) should be sensitive to 0.1 mg and used for weighing test chemicals.

6.2 *Clean Sample Containers*, Containers (120 mL) with screw- cap lids are ideal for test aliquots. Other suitable containers include milk dilution bottle, 4 oz glass bottles, or sterile sampling bags.

6.3 *Flaming Equipment*—An alcohol lamp, bunsen burner, or electric device may be used to flame inoculating needles and other equipment.

6.4 *Incubators*—Incubators that control the temperature of the test $\pm 2^{\circ}$ C. Temperatures for test should be temperature at which the product will be stored.

6.5 Petri Dishes, 100 by 15-mm, plastic or borosilicate glass, sterile.

6.6 *pH Measurement*—Any pH meter is suitable to standardize the pH of the culture medium or to determine pH of samples. Nonbleeding test strips may be used for determining pH of test aliquots.

³ Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http:// www.access.gpo.gov.

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6.7 *Pipets*—1.0-mL graduated in 0.01 mL and 10-mL graduated in 0.1 mL. Serological pipets should not be used for highly viscous materials. Automatic pipettors may be used.

6.8 Pipetting Aid—rubber bulb or other device to eliminate mouth pipetting.

6.9 Sterilizers—pressurized steam sterilizer (121°C at 15 psi) or hot air oven capable of reaching 180 \pm 2°C for 2 \pm 0.2h.

6.10 Swabs—Sterile swabs (cotton or other appropriate fabric type) for aiding in removal of fungal spores from slants.

6.11 Sterile Funnel—Funnel with sterile glass wool for filtration of spore suspension.

6.12 Sterile Glass Beads-Glass beads (3-5 mm).

6.13 Tubes—Tubes for preparation of slanted media.

6.14 Milk Dilution Bottles, (100 mL).

7. Reagent and Materials

7.1 *Purity of Water*—Unless otherwise indicated, water shall be understood to mean distilled water or water of equal purity, as defined in Specification D1193, Type 3.

7.2 Freshly prepared test solutions of the fungal control agent shall be used in all tests. Some preservatives can be added with a micropipet.

7.3 *Test Materials*—Freshly prepared pigment slurries, adhesives, dye rosin, polymer, sizing solutions, and other classes of aqueous-based materials to be preserved should be used as the substrate.

7.4 *Culture Medium*—Dehydrated Sabouraud's Agar (maltose or dextrose) is recommended for fungi. A more selective medium may be used provided it is used in addition to Sabouraud. Results should indicate the data obtained with each medium.

7.4.1 Spore Suspending Medium and Container—Milk dilution bottles containing 100 mL Butterfield Buffer⁴ with solid glass beads, for preparing sterile spore suspensions.

7.4.2 Culture Media, slants of the selected agar.

8. Test Organisms

8.1 The test organisms selected may vary with the purpose of the test. If evaluating the basic effectiveness of a fungal control agent, the use of standard fungal cultures is recommended (see 8.2). If attempting to qualify a fungal control agent for a particularly difficult, or highly specific preservation application, the use of fungal spoiled product or selected fungal organisms isolated from the problem system, or similar systems, may be appropriate (see 8.3 and 8.4).

8.2 Standard fungal cultures suitable for this procedure include the following:

8.2.1 Aspergillus niger: ATCC 6275.

- 8.2.2 Penicillium pinophalum: ATCC 9644.
- 8.2.3 Trichoderma virens: ATCC 9645.

⁴ Butterfields Buffered Phosphate Diluent, Official Methods of Analysis of the Association of Official Analytical Chemists, K. Helrich, 15th ed., 1990, p. 429.