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# Standard Test Method for Efficacy of Fungal Control Agents as Preservatives for Aqueous-Based Products Used in the Paper Industry<sup>1</sup>

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 ( $\varepsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This laboratory test method 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  $\frac{160}{160}$ ).

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

<del>1.5</del>

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

<u>1.6</u> 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.

## 2. Referenced Documents

2.1 ASTM Standards:<sup>2</sup>

D1193 Specification for Reagent Water E599Test Method for Efficacy of Slimicides for the Paper Industry--Fungal Slime E600Test Method for Efficacy of Slimicides for the Paper Industry--Bacterial Slime<sup>0</sup>

E723 Test Method for Efficacy of Antimicrobials as Preservatives for Aqueous-Based Products Used in the Paper Industry (Bacterial Spoilage) Test Method for Efficacy of Antimicrobials as Preservatives for Aqueous-Based Products Used in the Paper Industry (Bacterial Spoilage)

E1839 Test Method for Efficacy of Slimicides for the Paper IndustryBacterial and Fungal Slime

2.2 Federal Standard:

40 CFR 160 Good Laboratory Practice Standards<sup>3</sup> ASTM E875-10

3. Terminology 3. Ter

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *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.

3.1.2 preservative, n-chemical agent used to prevent microbial soilage of products due to microbial growth.

### 4. Summary of Test Method

4.1 Aqueous material to be preserved is inoculated with an appropriate fungal inoculum 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.

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<sup>&</sup>lt;sup>1</sup> This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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<sup>&</sup>lt;sup>2</sup> 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.

<sup>&</sup>lt;sup>3</sup> 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.

## 5. Significance and Use

5.1 This test method 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 Methods-Method E599E1839-and E600.

## 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, (120 mL containers 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.

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 or hot air oven capable of reaching  $180 \pm 2^{\circ}$ C for  $2 \pm 0.2$ h.

6.10 Swabs, sterile, for aiding in removal of fungal spores from slants.

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

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

6.13 *Tubes*, for preparation of slanted media.

6.14 Milk Dilution Bottles.

## 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 (maltrose 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 Media and Container—Milk dilution bottles containing 100 mL Butterfield Buffer<sup>4</sup> 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 spoiled product or 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.
- 8.2.4 Candida albicans: ATCC 10231.
- 8.2.5 Saccharomyces cerevisiae: ATCC 4111.

Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401. <sup>4</sup> Butterfields Buffered Phosphate Diluent, Official Methods of Analysis of the Association of Official Analytical Chemists, K. Helrich, 15th ed., 1990, p. 429.