



Designation: E 599 – 89

AMERICAN SOCIETY FOR TESTING AND MATERIALS
1916 Race St., Philadelphia, Pa. 19103

Reprinted from the Annual Book of ASTM Standards, Copyright ASTM
If not listed in the current combined index, will appear in the next edition.

Standard Test Method for Efficacy of Slimicides for the Paper Industry—Fungal Slime¹

This standard is issued under the fixed designation E 599; 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 covers the determination of the efficacy of slimicides for the control of fungal slime in paper mill systems and their counterparts.

1.2 *This standard may involve hazardous materials, operations, and equipment. This standard does not purport to address all of the safety problems 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. Significance and Use

2.1 This test method shall be used to determine if a slime control agent has application in the paper industry.

3. Apparatus

3.1 *Balance*, a balance with a platform to accommodate a 180-ml dilution bottle. This balance shall be sensitive to 0.1 g. An analytical balance sensitive to 0.1 mg shall be employed to weigh the candidate slime control agent to be used in the preparation of the stock solutions.

3.2 *Bottles*—Borosilicate glass milk dilution bottles fitted either with screw caps or Escher rubber stoppers are needed. These bottles shall be for water blanks and pulp cultures.

3.3 *Cotton*, for plugs.

3.4 *Culture Tubes*, recommended size 15 by 125 mm or 18 by 150 mm without lip, preferably of borosilicate glass.

3.5 *Disintegrators*.²

3.6 *Flaming Equipment*—Depending upon circumstances, either an alcohol lamp or a bunsen burner may be used to flame inoculating needles and other equipment.

3.7 *Incubators*—Reliable incubators that will meet temperatures of 30 to 37 ± 1°C to provide proper incubation temperatures shall be used.

3.8 *pH Meter*—Any reliable pH meter is suitable to standardize the pH of the culture.

3.9 *Pipets*, 1.1-mL milk dilution type, 1.0-mL graduated in 0.01 mL, and 10-mL graduated in 0.1 mL.

3.10 *Sterilizers*, steam sterilizer and hot-air oven.

4. Reagents and Materials

4.1 *Purity of Reagents*—Reagent chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Com-

mittee on Analytical Reagents of the American Chemical Society, where such specifications are available.³ 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.

4.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean distilled water or water of equal purity.

4.3 *Aluminum Sulfate (Alum)* (Al₂(SO₄)₃·18H₂O)—Prepare a 0.4 % solution of the hydrated aluminum in distilled water and sterilize in an autoclave. Any loss of water during sterilization is made up by adding sterile distilled water.

4.4 *Citrate-Phosphate Buffer as an Alternative for Alum*:

4.4.1 Prepare a 0.2 M solution of disodium phosphate, heptahydrate (Solution A). Prepare a 0.1 M solution of citric acid anhydrous (Solution B).

4.4.2 Mix 214.4 ml of Solution A with 185.6 mL of Solution B. Sterilize by filtration. If this buffer has a tendency to chelate the biocide under test select an appropriate alternative buffer.

4.5 *Pulp*—A groundwood pulp typical of current production techniques that has been produced when no slimicide was used in the pulp system for 24 h is needed.⁴ If untreated pulp is unavailable, thoroughly disperse and wash the pulp three times with water to remove any residual biocide that may inhibit the growth of the test organisms. Filter and air dry prior to use.

4.6 *Rosin Size*⁵—Prepare a 1 % solution of rosin size in a freshly boiled, warm, distilled water, sterilize, and compensate for loss during sterilization by adding sterile water.

4.7 *Culture Medium*—Sabouraud dextrose agar or potato dextrose agar are recommended. Adjust pH of recovery medium to pH of the test system.

5. Test Organisms

5.1 *Aspergillus niger* (IPC-144) and *Chaetomium globosum* ATCC-6205—Maintain these organisms on either Sabouraud or potato dextrose agar at 30°C.

6. Preparation of Inoculum

6.1 Grow the fungi on Sabouraud agar at 30°C. After 7

¹ This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antibacterial and Antiviral Control Agents.

Current edition approved June 30, 1989. Published August 1989. Originally published as E 599 – 77. Last previous edition E 599 – 84.

² The Waring blender has been found suitable for this purpose.

³ "Reagent Chemicals, American Chemical Society Specifications," American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, and the "United States Pharmacopeia."

⁴ Aspen Groundwood Pulp available from Niagra of WI Paper Corporation, Niagra, WI, 54151, has been found suitable for this purpose.

⁵ Rosin size Pexol 200, available from the Papermakers' Chemical Dept. of Hercules, Inc., Wilmington, Del., has been found suitable for this purpose.

 E 599

days incubation, add 2 mL of sterile, distilled water to the surface of the slant. Gently scrape and suspend the organisms in the liquid. Transfer this volume to a Sabouraud agar milk dilution bottle flat and incubate at 30°C for 7 days. The organisms harvested from this bottle slant will serve as inoculum for the pulp cultures. To obtain this inoculum, add 10 mL of sterile, distilled water to the bottle flat from the 99-mL sterile water blank. Disperse the growth on the flat throughout the water by gently scraping the surface of the medium.

6.2 After the fungal spores and mycelium is dispersed in the liquid, transfer 5 mL of the suspension to the remaining 89 mL of sterile water in the water blank. After addition, shake the contents of the water blank vigorously until a uniform suspension results. Add 1 mL of this inoculum to each bottle containing the pulp substrate in the test.

7. Pulp Cultures

7.1 Prepare a slurry of groundwood pulp by disintegrating 25 g of air-dry groundwood pulp in 975 mL of distilled water. The time required for complete disintegration will vary from approximately 5 to 20 min depending upon the disintegration equipment employed and the condition of the pulp. Dilute this slurry with 1 L of distilled water and mix thoroughly by swirling 20 times. Enrich the pulp slurry with 0.5 % maltose (reagent grade) and 0.15 % Sabouraud powder (as used for liquid media). Then weigh 40 ± 0.1 -g portions of the pulp suspension into each of the desired number of borosilicate glass milk dilution bottles. Use only a pulp suspension prepared the same day. To ensure a uniform pulp suspension in each of the milk dilution bottles, constantly agitate the stock solution with a mechanical stirrer. Stopper or cap and sterilize the series of bottles. After sterilization, allow the bottles to cool to approximately 30°C and then weigh the bottles to the nearest 0.1 g. Since the final portion of the test shall be run at 30°C, it is desirable to have the bottles as near this temperature as practicable throughout the test.

7.2 The time interval between the preparation of the suspension of the test organism for inoculating the bottles and the addition of the suspension to each bottle may have some effect on the resistance of the organism. The proper aliquots of biocides shall be added to each bottle of substrate as promptly as possible.

7.3 Make the following additions aseptically to each bottle in the order named and shake vigorously after each addition, using 20 complete cycles in a vertical motion.

7.3.1 Add the amount of sterile distilled water that is necessary to bring the total weight of the contents of the bottle to 50.0 g after all other additions have been made.

7.3.2 Add 1 mL of sterile stock solution of rosin size.

7.3.3 Add the desired volume of the stock solution of the biocide indicated to give the desired concentration in parts per million. Do not add biocide to the negative control. Include in each test a reference biocide as a positive control that is known to have activity in this test. Include a minimum of five concentrations of the biocide under test and the reference biocide in each experiment. Run each test and reference biocide in duplicate. The biocide stock solution shall be of such strength so that no less than 0.1 mL or more than 1.0 mL of the stock solution be added to each bottle of pulp substrate.

7.3.4 Add sufficient sterile 0.4 % alum solution to provide a pH between 5.0 and 5.5. Determine the volume to be added by prior test of the pulp culture that has been prepared in the same manner as that described in this test method. If desired, additional pH levels may be investigated, but each test should indicate the pH and level at which it is run. The inoculum and recovery medium should be at the same pH of the test to prevent shock to the organisms.

7.3.4.1 When sterile citrate-phosphate buffer is used, add 5.0 mL to each bottle. The use of this buffer prevents the adverse effect that alum has on the growth of the test organism in some pulps.

7.3.5 Add 1 mL of an aqueous suspension of the test organism to each pulp culture. Prepare this suspension just before it is to be used.

7.3.6 After the final addition of the test organism, place the inoculated bottles into an incubator at a temperature of 30°C.

8. Interpretation of Results

8.1 Examine each bottle visually for the growth of a test organism after 7 and 14 days of incubation. Record the growth after each period of incubation on the basis of the following key:

- 0—no growth
- 1—scant or questionable
- 2—poor
- 3—good
- 4—excellent

This is a relative ranking based upon the fungal growth that develops in the controls. Effectiveness of the product under test should always be compared to the effectiveness of the reference biocides.

8.2 If there is any question regarding the growth of the test organism in the test series, repeat the test.

9. Precision and Bias

9.1 A precision and bias statement cannot be made for this standard at this time.

The American Society for Testing and Materials takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, 1916 Race St., Philadelphia, PA 19103.