



Designation: E 600 – 91

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—Bacterial Slime¹

This standard is issued under the fixed designation E 600; 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 bacterial slime in paper mill systems and their counterparts.

NOTE 1—A knowledge of microbiological techniques is required for these procedures.

1.2 *This standard does not purport to address all of the safety problems, 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:

D 4454 Test Method for Simultaneous Enumeration of Total and Respiring Bacteria in Aquatic Systems by Microscopy²

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products³

3. Significance and Use

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

4. Apparatus

4.1 *Balance*, with a platform to accommodate the bottles being used in this test. This balance should be sensitive to 0.1 g at a load of 200 g. An analytical balance sensitive to 0.1 mg should be employed to weigh the candidate preservative to be used in the preparation of the stock solutions.

4.2 *Bottles*—Borosilicate glass milk dilution bottles or other suitable containers fitted either with screw caps or Escher rubber stoppers. These bottles are used for water blanks and aqueous-based samples.

4.3 *Colony Counter*, such as the Quebec, Buck, and Wolffhuegel or equivalent. A hand tally for the recording of the bacterial count is recommended.

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

4.5 *Closures*, plastic caps, cotton (for plugs).

4.6 *Disintegrators*, standard laboratory blenders have been found suitable for this purpose.

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

4.8 *Incubators*, capable of maintaining temperatures of 30 to 37 $\pm$ 1°C.

4.9 *Petri Dishes*, 100 by 15 mm sterile plastic or borosilicate glass petri dishes.

4.10 *pH Meter*, sensitive to 0.05 pH.

4.11 *Pipets*—1.1 or 2.2-mL milk dilution type, 1.0-mL graduated in 0.01 mL, 10-mL graduated in 0.1 mL, and appropriately calibrated pipettors may be used. Serological pipets and pipettors should not be used for highly viscous materials.

4.12 *Sterilizers*—Steam sterilizer and hot-air oven.

4.13 Microbiological filter such as Millipore or Gelman.

5. Reagents and Materials

5.1 *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 Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.⁴ Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the accuracy of the determination.

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

5.3 *Aluminum Sulfate* ($\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$)—Prepare a 0.4 % solution of the hydrated aluminum sulfate in water and sterilize. Loss of water during sterilization is made up by adding sterile water.

5.4 *Citrate-Phosphate Buffer as an Alternative for Aluminum Sulfate*:

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

5.4.2 Mix 214.4 mL of Solution A with 185.6 mL of Solution B. Sterilize by filtration. If this buffer inactivates (neutralizes) the biocide under test, select an appropriate alternative buffer (see Practices E 1054).

5.5 *Pulp*—A groundwood pulp typical of current produc-

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

Current edition approved July 15, 1991. Published September 1991. Originally published as E 600 – 77. Last previous edition E 600 – 85.

² *Annual Book of ASTM Standards*, Vol 11.02.

³ *Annual Book of ASTM Standards*, Vol 11.04.

⁴ "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."

tion techniques that has been produced when no slimicide was used in the pulp system for 24 h.⁵ 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.

5.6 *Rosin Size*⁶—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.

5.7 *Culture Medium*—Standard dehydrated tryptone glucose extract agar or equivalent is recommended.

5.8 Phosphate buffer pH 7.2 (see Test Method D 4454).

6. Test Organisms

6.1 *Enterobacter aerogenes* (ATCC 13048) and *Pseudomonas aeruginosa* (ATCC 15442)⁷—Maintain these organisms on tryptone glucose extract agar at 32°C.

7. Preparation of Inoculum

7.1 Grow the bacterial cultures on tryptone glucose extract agar at 32°C. After 24 h of incubation, add 1 mL of sterile water to the surface of the slant. Gently scrape and suspend the organisms in the liquid. Transfer this volume to a tryptone glucose extract agar milk dilution bottle flat and incubate at 32°C for 24 h. The organisms harvested from this bottle slant will serve as inoculum for the pulp cultures. To obtain this inoculum, add 10 mL of sterile water to the bottle flat from a 99-mL sterile water blank. Disperse the growth on the flat throughout the water by gently moving it over the surface of the nutrient medium. Transfer 5 mL of this suspension to the remaining 89 mL of sterile water in the water blank. After addition, shake the contents of the water blank vigorously. Add 1 mL of this inoculum to each bottle containing the pulp substrate in the test. This inoculum can also be cultured on a tryptic glucose agar petrie plate and removed in similar fashion.

8. Pulp Cultures

8.1 Prepare a slurry of groundwood pulp by disintegrating 25 g of air-dry groundwood pulp in 976 mL of 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 water and mix thoroughly by swirling 20 times. 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 or magnetic 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 is to be run at 37°C, it is desirable to have the

bottles as near this temperature as practicable throughout the test.

NOTE 2—For site-specific evaluations, water of comparable hardness should be considered instead of distilled water.

8.2 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. Therefore, it is necessary to arrange each series so that the time interval does not increase or decrease throughout the series. Moreover, the aliquots of the biocides to be tested in the series shall be added at specific intervals.

8.3 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.

8.3.1 Add the amount of sterile 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.

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

8.3.3 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 no more than 1.0 mL of the stock solution be added to each bottle of pulp substrate.

8.3.4 Add sufficient sterile 0.4 % aluminum 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 level at which it is run. The inoculum and recovery medium should be at the same pH as the test to prevent shock to the organisms.

8.3.5 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. There shall be 2 000 000 to 10 000 000 cells/mL in the test pulp cultures. After the final addition of the test organism, place the inoculated bottles into an incubator at a temperature of 37°C and allow to stand for 3 h from the time the first pulp culture was inoculated. If desired, additional contact times may be included in the test. However, the 3-h contact period shall always be included in the test. Just prior to the addition of the biocide series and after the addition of the inoculum of the test organism, plate the control flask for its bacterial count.

8.3.5.1 After incubation, vigorously shake the pulp culture and immediately plate it in tryptone glucose extract agar in accordance with standard dilution plating techniques. Make standard dilutions in phosphate buffer. Neutralization of biocide at end of contact period should be demonstrated (see Practices E 1054). It is important that the pulp cultures be plated in the same order as they were inoculated, thus ensuring a uniform contact time. After the medium has solidified in the petri dishes, incubate them at 37°C for 48 h.

8.3.5.2 At the completion of the incubation period, count

⁵ Aspen Groundwood Pulp, available from Niagara of WI Paper Corporation, Niagara, WI 54151.

⁶ Rosin size Pexol 200, available from the Papermarkers' Chemical Dept. of Hercules, Inc., Wilmington, DE, has been found suitable for this purpose.

⁷ This strain will be submitted to the American Type Culture Collection.