Designation: E 723 – 97

Standard Test Method for Efficacy of Antimicrobials as Preservatives for Aqueous-Based Products Used in the Paper Industry (Bacterial Spoilage)[1]

This standard is issued under the fixed designation E 723; 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 laboratory test method is used to determine the efficacy of an antimicrobial for preventing bacterial spoilage of in-process aqueous-based products used in the paper industry. For information on fungal spoilage, see Test Method E 875. This test method should be performed by persons who have had basic microbiological training.

1.2 *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:*

D 1193 Specification for Reagent Water[2]

E 875 Test Method for Efficacy of Fungal Control Agents as Preservatives for Aqueous Based Products Used in the Paper Industry[3]

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[3]

E 1326 Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *antimicrobial, n*—chemical or physical agent that kills microorganisms.

3.1.2 *bactericide, n*—an agent that kills bacteria. This term is applied to chemical agents that kill bacteria but not necessarily bacterial spores.

3.1.3 *preservatives, n*—a chemical or physical agent used to prevent microbial spoilage of products.

4. Summary of Test Method

4.1 Aqueous material to be preserved is inoculated with an appropriate microbial inoculum followed by addition of a concentration of bactericide that will kill the microbes and prevent their growth for a desired period of time. Microbial numbers in the sample are determined at various time periods and compared to a contol without any biocide. The proper level of antimicrobial is one that reduces and keeps the organisms to an acceptable level in the test material.

5. Significance and Use

5.1 This test method should be used to determine if an antimicrobial prevents spoilage by bacteria and preserves pigment suspensions, dye solutions, pulp slurries, starch solutions, polymers, sizing agents, latex emulsions, and other aqueous-based materials used in the paper industry.

6. Apparatus

6.1 *Balance*—Two balances: one should be sensitive to 0.1 g at a load of 200 g and have a platform to accomodate bottles being used in the test. The second balance (analytical) should be sensitive to 0.1 mg and should be employed to weigh the candidate preservative to be used in the preparation of the stock solutions.

6.2 *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.

6.3 *Colony Counter*—Any one of several types may be used as the Quebec, Buck, and Wolfhuegel. A hand tally for the recording of the bacterial count is recommended if manual counting is done. Alternatively, an automated video colony counter may also be used.

6.4 *Culture Tube Closures*—Appropriate nontoxic closures should be selected.

6.5 *Culture Tubes*—Recommended size is 15 by 125 mm or 18 by 150 mm without lip, and preferably of borosilicate glass.

6.6 *Blender*—Any blender that will assure proper agitation and blending.

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

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[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 Agents.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

6.8 *Incubators*, capable of maintaining temperatures of 28 to 70 ± 1°C to provide proper incubation temperatures. Temperature should be consistent with the temperature of the product to be preserved.

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

6.10 *pH Measurement*—Any reliable pH meter is suitable to standardize the pH of the culture medium. Nonbleeding tests strips are recommended for samples.

6.11 *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.

6.12 *Sterilizers*, pressurized steam sterilizer or hot air oven capable of 180 ± 2°C for 2 ± 0.2 h.

7. Microbicides and Materials

7.1 Freshly prepared test solutions of the antimicrobial shall be used in all tests.

7.2 *Purity of Water*—All references to water as diluent or reagent shall mean distilled water or water of equal purity, unless otherwise noted (see Specification D 1193, Type III).

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

7.4 *Culture Medium*:

7.4.1 Standard dehydrated tryptone glucose extract agar or other medium that is known to recover organisms from the material to be tested is recommended.

7.4.2 For some substrates it may be necessary to add a small amount of nutrient material to ensure growth of the organisms in the material to be studied for preservation. For bacterial preservation studies, add 5.0 mL/L of 0.2 % nutrient broth to the test material to assure sufficient populations.

8. Test Organisms

8.1 The test organisms will vary with the material to be preserved and the purpose of the test. For specific materials that are contaminated, that material will serve as the inoculum. For general screening of activity or preventative evaluations, the inoculum may consist of a single or mixed culture of organisms that are known to cause problems in the material to be preserved. The viability of the microorganisms in the material to be tested should be verified prior to initiating the test.

8.2 To provide a uniformly inoculated substrate, the inoculum should be added to the entire quantity of the test substrate at one time, mixed thoroughly, and then dispensed into the separate test bottles.

8.3 The material under test should be inoculated with sufficient microorganisms either from pure cultures or contained in the spoiled material used as the inoculum to give a bacterial count of 1 000 000/mL or higher.

9. Procedure

9.1 Dispense 50-g aliquots (or any other suitable quantity) of the inoculated test material aseptically into sterile bottles (if necessary add nutrient). Treat the samples immediately with appropriate concentrations of the antimicrobial. Set up controls in duplicate. Note appropriate physical characteristics such as pH, color, odor, viscosity etc., of all test samples at this time.

9.1.1 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.

9.1.2 Add the desired volume of the stock solution of the antimicrobial to be tested to give the desired concentration in parts per million or percent. Stock solution of the antimicrobial should be of such strength so that the volume of antimicrobial solution added is no more than 1 % of the total volume of sample in each bottle. Do not add an antimicrobial to the control. Include in each test a minimum of five concentrations of the antimicrobial under test. Suggested antimicrobial concentrations are 50, 100, 200, 300, 400, and 600 ppm, or whatever range of concentration may be suitable for the material to be tested. Record the pH of all samples at the beginning of the experiment, using nonbleeding test strips.

9.1.3 Incubate all samples at 28 to 37°C (or other temperature at which the test material will be stored, such as 65°C for starch solutions) with the bottle capped tightly to avoid evaporation. The organisms being tested need to be viable and stable at the test temperature.

9.1.4 Determine concentration of viable organisms in the controls at the time of biocide addition and of all samples at periodic intervals after biocide addition (see Practices E 1054). This can be done with standard plate counts or other accepted alternative means of determining concentrations of organisms (see Guide E 1326). All plates or recovery medium should be incubated at the same temperature as the test.

9.1.5 Time intervals for determining the level of organisms remaining in the sample depend on the length of time the test material needs to be preserved in actual use and the acceptable level of contamination when the material is to be used. Thus, samples can be taken at 3 h, 8 h, 24 h, 48 h, 72 h, or weekly, depending on how rapidly and to what extent the inoculum needs to be killed.

9.1.6 If the material can be reinoculated while it is preserved or to determine the number of reinoculations a given preservative level will be able to handle, the samples should be reinoculated on a weekly or biweekly interval with ≥1 000 000 CFU/mL (see 8.3).

9.1.7 Typical test protocols range from biweekly inoculations with sampling 24 h postinoculation to weekly inoculations with sampling 7 days postinoculation. For comparative studies, these intervals and the inoculum must be constant. As in any lab test, it is difficult to duplicate the conditions that might exist in an actual production facility.

9.1.8 At each time interval (see 9.1.5) or after reinoculation (see 9.1.6), mix the sample thoroughly and immediately determine the level of microorganisms in the sample (see 9.1.4). The controls must maintain a high count throughout the study or show visual signs of deterioration. Either criterion can be used to indicate spoilage and the validity of the test.

10. Calculation

10.1 At each sampling time and at the end of each test, calculate the number of bacteria killed at each microbicide concentration tested as follows:

$$\% \text{ kill} = \frac{(\text{control plate count} - \text{test plate count})}{\text{control plate count}} \times 100$$

10.1.1 The percent kill at any given time is indicative of the effectiveness of the antimicrobial under test. The proper level of antimicrobial to use for the material being tested is the one that decreases the level of organisms to the acceptable level of organisms for the test material in an acceptable amount of time. Visual deterioration and other signs of degradation, such as changes in pH, color, odor, loss of viscosity, and so forth, should also be used to judge the degree of preservation obtained.

NOTE 1—Typically, the level of organisms should be reduced to less than 1000/mL in a 24 h period of time. This is equivalent to a 99.9 % kill when the inoculum provides 1 000 000/mL.

11. Precision and Bias

11.1 A precision and bias statement cannot be made.

12. Keywords

12.1 bacterial; bactericide; paper based products; preservative; pulp; spoilage