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.

Designation: E723 – 13 (Reapproved 2019)

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

This standard is issued under the fixed designation E723; 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 practice 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 E875. This practice should be performed by persons who have had basic microbiological training.

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

1.3 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* (See 40 CFR Part 160.)

1.4 *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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

E640 Test Method for Preservatives in Water-Containing Cosmetics

E875 Practice for Evaluation of Fungal Control Agents as Preservatives for Aqueous-Based Products Used in the Paper Industry

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1326 Guide for Evaluating Non-culture Microbiological Tests

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

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Other Standards:*

40 CFR Part 160 Good Laboratory Practice Standards[3]

3. Terminology

3.1 For definitions of terms related to this practice, see Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *antimicrobial, n*—chemical or physical agent that kills or inactivates microorganisms or suppresses their growth or reproduction.

3.2.2 *bactericide, n*—a physical or chemical agent that kills bacteria, but not necessarily bacterial spores.

3.2.3 *biocide, n*—a physical or chemical agent that kills organisms.

3.2.4 *microbicide, n*—a physical or chemical agent that kills microorganisms.

3.2.5 *preservatives, n*—chemical 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 appropriate bacterial inoculums followed by addition of a bactericide that will reduce populations of bacteria and prevent the growth of survivors for a specified period of time. Bacterial numbers in the sample are determined at various time periods and compared to a contol without any bactericide. The proper level of antimicrobial is one that reduces and keeps the organisms to an acceptable level in the test material.

[1] This 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.

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[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.

[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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5. Significance and Use

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

NOTE 1—Control of fungal spoilage of similar products can be evaluated by Test Method E875.

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

6. Apparatus

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

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, such 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 ensure 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.

6.8 *Incubators,* capable of maintaining temperatures of 28 to 70 ±2 °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 Meter*—Any suitable for standardizing the pH of the culture medium. Non-bleeding pH test strips may be used 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 D1193, 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 ensure 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. It is best to use organisms that are known to cause spoilage of the material to be preserved. Alternatively, bacteria listed in Test Method E640 may be used. 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 1×10^5 to 1×10^6 CFU/mL.

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 depend upon the microbicide and its recommended concentrations. Record the pH of all samples at the beginning of the experiment.

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