Designation: E 979 – 91 (Reapproved 2004)

Standard Test Method for Evaluation of Antimicrobial Agents as Preservatives for Invert Emulsion and Other Water Containing Hydraulic Fluids[1]

This standard is issued under the fixed designation E 979; 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.

INTRODUCTION

Invert emulsion hydraulic fluids typically contain 60 % mineral oil and 40 % water (by volume). These fluids routinely are prepared using proprietary, oil-soluble, emulsifying agents, as well as other emulsifiable constituents. They are recommended for use where conditions indicate a low-cost, fire retardant product, compatible with water-based metal working fluids.

The high water content of these hydraulic fluids makes them susceptible to microbial attack. Uncontrolled microbial growth in these fluids can cause cartridge filter unit plugging, maladorous conditions, or general biodeterioration. Problem microorganisms associated with these fluids include bacteria and fungi.

The hydraulic system is essentially a closed one in which water of evaporation is added to maintain a fixed volume. The inclusion of an efficacious preservative in the water containing hydraulic fluids can prevent microbial growth and the resulting problems that follow.

1. Scope

1.1 This laboratory test method is designed to evaluate the utility and effectiveness of antimicrobial agents intended to control microbial growth in invert emulsions and other water containing hydraulic fluids.

NOTE 1—Procedures for preparation of water soluble hydraulic fluids and recovery of organisms appear in Method E 686.

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:* [2]

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

E 686 Method for Evaluation of Antimicrobial Agents in Aqueous Metal Working Fluids

3. Summary of Test Method

3.1 The antimicrobial agent to be evaluated is incorporated into an emulsion system by (*a*) addition to the aqueous phase employed in the preparation of the emulsion, (*b*) in doses to the formulated system, or (*c*) by other methods suitable for the test compound.

3.2 A heavy bacterial or fungal inoculum, or both, is then added.

3.3 The resulting mixture is aerated and passed over the surface of a simulated filter system for a minimum period of eight weeks either continuously or with shutdowns to simulate actual operations conditions.

3.4 The degree of microbial control is determined by periodic plate counts of the emulsion and visual observations for microbial fouling of the simulated filter surface.

NOTE 2—A knowledge of standard microbiological techniques is required for this procedure. It is also required that good laboratory practices be followed throughout these tests. This means appropriate containment for the microbiological systems being evaluated. The systems should be maintained in an enclosure so that during the aeration process the mists and aerosols generated do not contaminate the laboratory environment.

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternate 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.

4. Significance and Use

4.1 This procedure is designed to determine the effectiveness of antimicrobial agents intended for microbial control in invert emulsions and other water containing hydraulic fluids.

5. Apparatus

5.1 *Air Supply*—Any air source which is free from organic vapors, organic matter, or other objectionable material may be used.

NOTE 3—If desired, air may be sterilized as follows:

Pack two 150-mm long drying tubes (bulb type) loosely with glass wool in a series with neoprene stoppers, glass tubing, and neoprene tubing. Wrap loosely in aluminum foil and steam sterilize at 15 to 20 psi for 30 minutes. Cool to room temperature while still wrapped. In-line pre-sterilization air filters are available from most local laboratory supply houses.

Insert into air line with bulbs on upstream side. Average lifetime in continuous use is two weeks. Discard sooner if upstream filter becomes wet or contaminated with oil.

5.2 *Colony Counter*—Any one of several types may be used.

5.3 *Incubator*—Any cabinet capable of maintaining a temperature of 35°C ± 1°C may be used.

5.4 *Test Cabinet*—A large cabinet capable of maintaining a temperature of 35°C ± 1°C, able to house several two liter beakers, and into which an air line can be introduced.

5.5 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization is acceptable.

5.6 *Simulated Filters*:

5.6.1 *Strainer*, 3-in. epoxy coated, ¼-in. mesh gutter strainer.[3]

5.6.2 *Screen*, 16 by 18 in. fiberglass screening material.[4]

5.6.3 *Wire*, 20-gage, galvanized or stainless steel.

5.7 *Tubing*, ¼-in. ID Tygon.[5]

5.8 *T-Connectors*, ¼-in. polypropylene.

5.9 *Laboratory Blender*—Any standard adjustable speed laboratory blender having a 2-L capacity glass or metal container is satisfactory.

5.10 *Hypodermic Needle*, 16-gage needle.

5.11 *Microscope*, Brightfield microscope equipped with 40× and 100× objectives.

5.12 *Labware*:

5.12.1 *Culture Dishes*—100 mm by 15 mm sterile culture dishes made of glass or plastic are required for making standard plate counts.[6]

5.12.2 *Bacteriological Pipettes of* 1.1 *or 2.2-mL Capacity.*[7]

5.12.3 *Water Dilution Bottles*—Any sterilizable glass containers having a 150 to 200-mL capacity and tight closures may be used.[8]

5.12.4 *Two-Liter Borosilicate Glass Beakers.*

5.12.5 *Bent Glass Rod.*

5.12.6 *Screw Cap Culture Tubes*, autoclavable, 15 by 150 mm.

5.13 *Water Bath*—Maintain at 46°C ± 2°C to anneal agar based microbiological media.

5.14 *Aluminum Foil.*

6. Reagents and Materials

6.1 *Invert Emulsion Emulsifier.*[9]

6.2 *Paraffinic Mineral Oil.*

6.3 *Deionized or Distilled Water* (>2 MOHM quality)

6.4 *Gentamicin Sulfate.*[10]

6.5 *Arlacel 80.*[11]

6.6 *Tween 60.*[11]

6.7 *Phosphate Buffer—For serial dilutions.*

6.8 *Mineral oil, sterile.*

6.9 *Microbiological Media*—General retrieval media consistent with good microbiological practices are acceptable. Examples are as follows:

6.9.1 *Soybean-Casein Digest Agar*, U.S.P. XIX, Medium II.[12]

6.9.2 *Fluid Soybean-Casein Digest Medium*, U.S.P. XIX, Medium III.[12]

6.9.3 *Sabouraud Dextrose Agar*, U.S.P. XIX, Medium 20.[9]

6.9.4 *Sabouraud Dextrose Broth*, U.S.P. XIX, Medium 21.[9]

6.9.5 *American Petroleum Institute (API) agar,*[9] for enumeration of sulfate reducing bacteria.

6.10 *Inoculum*:

6.10.1 The inoculum may vary according to the users' requirements. It may be either undefined or defined.

6.10.1.1 An undefined inoculum may consist of microorganisms isolated from a "spoiled" invert emulsion hydraulic fluid which exhibits microbiologically induced phase generation, or which is known to have caused plugging of a hydraulic system filter due to microbial slime, and grown in a nutrient medium.

6.10.1.2 An undefined inoculum may consist of the following: (*1*) equal volumes of fluid soybean-casein digest and "spoiled" (see section 6.1.1.1) hydraulic fluid aerated at 35°C for 24 h (typically) until the bacterial count reaches 10^9 CFU/mL, (*2*) equal volumes of sabouraud dextrose broth and "spoiled" (see 6.10.1.1) hydraulic fluid aerated at 35°C for 24

[3] Gutter strainers available from Billy Penn Corp., Philadelphia, PA 19122, have been found suitable.

[4] Fiberglass mesh screening material (18 by 16) is available from any local hardware dealer.

[5] Tygon is available from most local laboratory supply houses.

[6] Presterilized and disposable plastic petri dishes are available from most local laboratory supply houses.

[7] Presterilized and disposable 1.1-mL bacteriological pipettes are available from most local laboratory supply houses.

[8] Milk dilution bottles of 160-mL capacity having screw-cap closures are available from Corning Glass Works, P.O. Box 5000, Corning, NY 14831, Owens Illinois Glass Co., P.O. Box 230, Vineland, NJ 08360, or most laboratory supply houses.

[9] A satisfactory emulsifier for the preparation of invert emulsion hydraulic fluids is Compound #5162 available from the Lubrizol Co., Wickliffe, OH.

[10] Gentamicin sulfate can be obtained as Garamycin Reagent Solution, available in two concentrations of 10 and 50 mg/mL, from the Schering Corp., Kenilworth, NJ 07033.

[11] Arlacel 80 and Tween 60 are available from the Specialty Chemicals Division, ICI American Inc., Wilmington, DE 19897.

[12] Available in dehydrated form from Baltimore Biological Laboratories, Cockeysville, MD; Difco Laboratories, Detroit, MI, or other laboratory media supply houses.