



## Standard Test Method for Evaluation of Antimicrobials in Distillate Fuels (Based on Preliminary Screening and Compatibility)<sup>1</sup>

This standard is issued under the fixed designation E 1259; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

### 1. Scope

1.1 This test method is designed to evaluate antimicrobial agents for the prevention of microbial-induced deterioration of distillate fuels (as defined by Specification D 396 as fuel) or system deterioration, or both.

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

1.2 It is the responsibility of the investigator to determine whether Good Laboratory Practice (GLP) is required and to follow them where appropriate (40 CFR, 160) or as revised.

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 and health practices and determine the applicability of regulatory limitations prior to use. See caution statement, Note 2, in Section 8.*

### 2. Referenced Documents

#### 2.1 ASTM Standards:

D 396 Specification for Fuel Oils<sup>2</sup>

D 4054 Practice for Evaluating the Compatibility of Additives with Aviation-Turbine Fuels and Aircraft Fuel System Materials<sup>3</sup>

#### 2.2 Federal Standard:

40 CFR, Part 160, Good Laboratory Practice Standards<sup>4</sup>

### 3. Summary of Test Method

3.1 This test method is conducted on a reference fuel for determining antimicrobial efficacy under well-defined conditions that include specific inocula *Pseudomonas aeruginosa*, American Type Culture Collection, (ATCC) No. 33988, *Hor-moconis resiniae*, ATCC No. 20495, and *Yarrowia tropicalis* (formerly *Candida tropicalis*, ATCC No. 18138, water/fuel ratios, and time of containment. It is designed for destructive sampling at regular intervals during bottom water buildup. This

test method allows for impact of fuel/water partitioning and time on the antimicrobial agent as well as the effect of continual rechallenge. Every 2 weeks, water phase is increased by 0.25 % while concomitantly a paired system is destructively tested. Thus, at 4 weeks, there is an equivalent 0.5 %, at 6 weeks 0.75 %, and at 8 weeks 1.0 %. At each sampling time interval, treated and untreated aliquots are checked for the three types of organisms in the initial inoculum. These counts are coupled with gross observations of each system for biofilm formation and interfacial growth.

### 4. Significance and Use

4.1 The procedure should be used to evaluate the relative efficacy of microbicides in distillate fuels. The effect of environmental conditions including a variety of fuel additives, metal surfaces, and climatology are variables that can be included in specific tests using this protocol.

### 5. Apparatus

5.1 *Colony Counter*—Any of several types, for example, a Quebec Colony Counter may be used.

5.2 *Incubator*—Any incubator capable of maintaining temperature of 30 to 35  $\pm$  2°C may be used.

5.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterility is acceptable.

5.4 *Separatory Funnels*—Eight 1-L funnels.

5.5 *Ring Stand*, suitable for supporting separatory funnel.

5.6 *Vortex*—Mixer.

### 6. Reagents and Materials

6.1 *Petri Dishes*—100 by 15 mm required for performing standard plate count.

6.2 *Bacteriological Pipets*—10.0 mL and 1.1, or 2.2 mL capacity.

6.3 *Water Dilution Bottles*—Any sterilizable glass container having a 150–200 mL capacity and tight closure may be used.

6.4 *Distillate Fuel*.<sup>5</sup>

6.5 *Synthetic Bottom Water*.<sup>6</sup>

6.6 *Soy Peptone Casein Digest Agar*.

6.7 *Sabouraud Dextrose Agar*.

<sup>5</sup> I-H CAT diesel fuel is available from Howell Hydrocarbons Inc., San Antonio, TX 78223-3531.

<sup>6</sup> Items 6.5-6.12 are available from a variety of media manufacturers and chemical supply companies.

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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<sup>2</sup> *Annual Book of ASTM Standards*, Vol 05.01.

<sup>3</sup> *Annual Book of ASTM Standards*, Vol 05.02.

<sup>4</sup> Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.