



Standard Test Method for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C¹

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 (ε) 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 microbially influenced deterioration of liquid fuels (as defined by Specification D 396, D 910, D 975, D 1655, D 2069, D 2880, D 3699, D 4818 and D 6227), system deterioration, or both.

1.2 Knowledge of microbiological techniques is required for these procedures.

1.3 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.

1.4 *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 396 Specification for Fuel Oils²
- D 910 Specification for Aviation Gasolines²
- D 975 Specification for Diesel Fuel Oils²
- D 1655 Specification for Aviation Turbine Fuels²
- D 2069 Specification for Marine Fuels²
- D 2880 Specification for Gas Turbine Fuels³
- D 3699 Specification for Kerosine³
- D 4814 Specification for Automotive Spark-Ignition Engine Fuel⁴
- D 6227 Specification for Grade 82 Unleaded Aviation Gasoline⁴
- D 6469 Guide to Microbial Contamination in Fuels and Fuel Systems⁴

2.2 Federal Standards:

- 40 CFR, Part 79, Fuels and Fuel Additives Registration Regulations⁵

40 CFR, Part 152, Pesticide Registration and Classification Procedures⁵

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *antimicrobials, n*—chemical agents that are cidal or static to microorganisms.

3.1.2 *microbial-induced deterioration, n*—decomposition/degradation of material (fuel) or making unsuitable for use, as a result of metabolic activity or the presence of microbes

3.1.3 *microbicide, n*—an agent that kills microbes: bacterial vegetative cells, fungal vegetative cells and spores, algae and protozoa. This term is applied to chemical agents that kill microbes.

4. Summary of Test Method

4.1 This test method is conducted on a reference fuel, and determines the antimicrobial efficacy under well-defined conditions that include specific inocula *Pseudomonas aeruginosa*, American Type Culture Collection, (ATCC) No. 33988, *Hormoconis resinae*, ATCC No. 20495, and *Yarrowia tropicalis* (formerly *Candida tropicalis*, ATCC No. 18138; as well as 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 increase in the water phase to 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.

5. Significance and Use

5.1 Guide D 6469 details the types of problems associated with uncontrolled microbial growth in fuels and fuel systems. Treatment with effective antimicrobial agents is one element of contamination control strategy.

5.2 The procedure should be used to evaluate the relative efficacy of microbicides in distillate fuels. The effect of environmental conditions, such as a variety of fuel additives,

¹ This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

Current edition approved April 10, 2001. Published June 2001. Originally published as E 1259 – 88. Last previous edition E 1259 – 94.

² *Annual Book of ASTM Standards*, Vol 05.01.

³ *Annual Book of ASTM Standards*, Vol 05.02.

⁴ *Annual Book of ASTM Standards*, Vol 05.03.

⁵ Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.