Designation: E1259 – 05

Standard Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C[1]

This standard is issued under the fixed designation E1259; 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 practice is designed to evaluate antimicrobial agents for the prevention of microbially influenced deterioration of liquid fuels (as defined by Specification D396, D910, D975, D1655, D2069, D2880, D3699, D4814, D6227, and D6751), 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:*[2]

D396 Specification for Fuel Oils
D910 Specification for Aviation Gasolines
D975 Specification for Diesel Fuel Oils
D1655 Specification for Aviation Turbine Fuels
D2069 Specification for Marine Fuels[3]
D2880 Specification for Gas Turbine Fuel Oils
D3699 Specification for Kerosine
D4057 Practice for Manual Sampling of Petroleum and Petroleum Products
D4814 Specification for Automotive Spark-Ignition Engine Fuel
D5465 Practice for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods
D6227 Specification for Grade 82 Unleaded Aviation Gasoline
D6293 Test Method for Oxygenates and Paraffin, Olefin, Naphthene, Aromatic(O-PONA) Hydrocarbon Types in Low-Olefin Spark Ignition Engine Fuels by Gas Chromatography
D6469 Guide for Microbial Contamination in Fuels and Fuel Systems
D6729 Test Method for Determination of Individual Components in Spark Ignition Engine Fuels by 100 Metre Capillary High Resolution Gas Chromatography
D6733 Test Method for Determination of Individual Components in Spark Ignition Engine Fuels by 50-Metre Capillary High Resolution Gas Chromatography
D6751 Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels
D6974 Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures
E1326 Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria

2.2 *NACE Standard:*

TM0172 Determining Corrosive Properties of Cargoes in Petroleum Product Pipelines[4]

2.3 *Federal Standards:*

40 CFR, Part 79, Fuels and Fuel Additives Registration Regulations[5]
40 CFR, Part 152, Pesticide Registration and Classification Procedures[5]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *antimicrobial*, *n*—see *biocide*.

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

[1] This practice is under the jurisdiction of ASTM Committee E35 on Pesticides 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] Withdrawn. The last approved version of this historical standard is referenced on www.astm.org.

[4] Item No. 21204, available from NACE International, Houston TX.

[5] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.

3.1.2.1 *Discussion*—Biocides are further classified as bactericides (kill bacteria), fungicides (kill fungi), and microbicides (kill both bacterial and fungi). They are also referred to as *antimicrobials*.

3.1.3 *microbially-influenced deterioration*, *n*—decomposition/degradation of material (fuel) or making unsuitable for use, as a result of metabolic activity or the presence of microbes.

3.1.4 *microbicide*, *n*—see *biocide*.

3.1.5 *microcosm*, *n*—a miniature system used to model larger systems.

3.1.5.1 *Discussion*—It is generally impractical to evaluate microbicide performance in large fuel storage system capacities (> 24 000 m^3), consequently small volume (1.0 to 208 L capacity) microcosms are used as model systems.

4. Summary of Practice

4.1 This practice is conducted on a fuel representative of the grade to be treated, and determines the antimicrobial efficacy under well-defined conditions that may 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; or an uncharacterized inoculum from a microbially contaminated fuel system. Additionally, water/fuel ratios and containment time are also defined. This practice allows for impact of fuel/water partitioning and time, on the antimicrobial agent, as well as the effect of continual rechallenge. 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. The size of the test system, total volume of fluid, fuel to bottom-water ratio and test duration may vary depending on the specific objectives of the test. Before beginning any test plan intended to meet performance testing compliance requirements, confirm that the cognizant authority accepts the test protocol.

5. Significance and Use

5.1 Guide D6469 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 liquid fuels boiling below 390°C. The effect of environmental conditions, such as a variety of fuel additives, metal surfaces, and climatology, are variables that can be included in specific tests using this protocol.

5.3 This practice addresses product performance issues only. Regulatory Agencies restrict and control the use of both pesticides (in the U.S.: 40 CFR 152) and fuel additives (40 CFR 79). Regardless of performance in this method, antimicrobials must only be used in compliance with applicable regulations. Specific industries, for example, the aviation industry, may place further restrictions on chemicals used for fuel treatment.

6. Apparatus

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

6.2 *Drums; Steel*—208 L (55 gal) 16 ga. steel, open-head drum with removable 16 ga. lid fitted with 2.05 cm and 1.90 cm threaded ports for venting and sampling.

6.3 *Incubator*—Any incubator capable of maintaining temperature of 30 to 35°C may be used.

6.4 *Glass Jars*—1 L capacity, French square or similar configuration.

6.5 *Pails; Steel*—18.9 L (5 gal) steel, open-head pail with removable 16 ga. lid fitted with 2.05 cm and 1.90 cm threaded ports for venting and sampling.

6.6 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterility is acceptable. A pressurized filter sterilization apparatus of appropriate capacity to filter sterilize the test fuels and bottom-water used in the negative control microcosms. A 0.2 µm pore-size methyl cellulose or cellulose acetate membrane should be used as the filtration medium.

6.7 *Vortex*—Mixer.

7. Reagents and Materials

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

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

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

7.4 *Fuel.*[6]

7.5 *Synthetic Bottom Water.*[7]

7.6 *Soy Peptone Casein Digest Agar.*

7.7 *Sabouraud Dextrose Agar.*

7.8 *Agar, Bacteriological Grade.*

7.9 *Potassium Tellurite Solution*—sterile 1 %.

7.10 *Gentamicin Sulfate*—50 µg/mL.

7.11 *Plate Count Agar.*

7.12 *Potato Dextrose Agar.*

8. Inoculum

8.1 *Inoculum Preparation and Maintenance*:

8.1.1 *Inoculum Revitalization*—Cultures are *Pseudomonas aeruginosa*, ATCC No. 33988, *Hormoconis resinae*, ATCC No. 20495, and *Yarrowia tropicalis* (formerly *Candida tropicalis*), ATCC No. 18138. Obtain cultures from ATCC. Before initiating fuel antimicrobial tests, revitalize each of the three cultures in accordance with the instructions contained with each culture.

8.1.2 *Maintenance and Preparation of Inocula*—All three cultures are transferred from slants of a specified agar, (*a*) *Pseudomonas aeruginosa* (Plate Count Agar), (*b*) *Hormoconis resinae* Potato Dextrose Agar), and (*c*) *Yarrowia tropicali* (Potato Dextrose Agar) to synthetic bottom water medium in a

[6] Representative fuel samples from each product grade are available from all petroleum refiners.

[7] Items 7.5-7.12 are available from a variety of media manufacturers and chemical supply companies.