Designation: ~~E1259 – 10~~ E1259 – 16

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, D6751, and D7467), 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 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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 Leaded Aviation Gasolines
D975 Specification for Diesel Fuel Oils
D1655 Specification for Aviation Turbine Fuels
D2069 Specification for Marine Fuels (Withdrawn 2003)[3]
D2880 Specification for Gas Turbine Fuel Oils
D3699 Specification for Kerosine
D4814 Specification for Automotive Spark-Ignition Engine Fuel
D5465 Practice for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods
D6227 Specification for Unleaded Aviation Gasoline Containing a Non-hydrocarbon Component
D6293 Test Method for Oxygenates and Paraffin, Olefin, Naphthene, Aromatic(O-PONA) Hydrocarbon Types in Low-Olefin Spark Ignition Engine Fuels by Gas Chromatography (Withdrawn 2009)[3]
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
D7463 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Fuel, Fuel/Water Mixtures, and Fuel Associated Water

[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.

Current edition approved ~~Oct. 1, 2010~~April 1, 2016. Published ~~October 2010~~May 2016. Originally approved in 1988. Last previous edition approved in ~~2005~~2010 as ~~E1259 – 05.~~E1259 – 10. DOI: ~~10.1520/E1259-10.~~10.1520/E1259-16.

[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] The last approved version of this historical standard is referenced on www.astm.org.

D7464 Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing
D7467 Specification for Diesel Fuel Oil, Biodiesel Blend (B6 to B20)
D7687 Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel-Associated Water with Sample Concentration by Filtration
D7978 Test Method for Determination of the Viable Aerobic Microbial Content of Fuels and Associated Water—Thixotropic Gel Culture Method
E1259 Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C
E1326 Guide for Evaluating Non-culture Microbiological Tests

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.

[4] Item No. 21204, available from NACE International (NACE), 1440 South Creek Dr., Houston, TX 77084-4906, http://www.nace.org.
[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:~~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.~~

4.1.1 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.

4.1.2 At each sampling time interval, treated and untreated aliquots are checked for the treated population survival. Microbiological testing is coupled with gross observations of each system for biofilm formation and interfacial growth.

4.1.3 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.

4.1.4 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.

NOTE 1—Jar capacity should be determined based on the test plan designed fuel to water ratio and the expected sample volume size needed for weekly testing (9.5 and 9.9).

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.*

NOTE 2—Representative fuel samples from each product grade are available from all petroleum refiners.

7.5 *Synthetic Bottom Water.*

NOTE 3—In order to promote microbial growth of the inoculum when using the fuel as the sole source of organic nutrients, synthetic bottom water may contain various inorganic nutrients. An example, of a commonly used synthetic bottom water is Bushnell-Haas Mineral Salts medium (BHMSS).[6] with the concentration adjusted to simulate a particular type of bottoms-water (marine, brackish, fresh, etc.).

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.*

NOTE 4—Items 7.5 – 7.12 are available from a variety of media manufacturers and chemical supply companies.

8. Inoculum

8.1 *Inoculum Selection:*

8.1.1 Depending on the objectives of a test plan, one or more characterized cultures (for example: bacterium, yeast and mold) can be selected or microbially contaminated bottoms-water collected from a fuel system can be used.

[6] Bushnell, L.D. and Haas, H.F. 1941. The utilization of certain hydrocarbons by microorganisms. *J. Bacteriol.* 41: 653- 673.