



Designation: D8070 – 23

Standard Test Method for Screening of Fuels and Fuel Associated Aqueous Specimens for Microbial Contamination by Lateral Flow Immunoassay¹

This standard is issued under the fixed designation D8070; 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 describes a procedure that can be used in the field or in a laboratory to detect antigens indicative of microbial contamination in liquid fuels, including those blended with synthesized hydrocarbons or biofuels, with kinematic viscosities (at 40 °C) of $\leq 24 \text{ mm}^2\text{s}^{-1}$ (for example, Specifications **D396**, **D975**, and **D1655**) and in fuel-associated water.

1.1.1 This test method has been validated by an ILS for a range of middle distillate fuels meeting Specification **D1655**, EN590, Specification **D975**, and ISO 8217:2012.

1.2 This test method semi-quantitatively assesses the concentration of specific antigens generated by commonly recovered, fuel-associated, aerobic microorganisms during active growth in fuel systems.

1.2.1 A proprietary formulation of antibodies and antibody mixtures is used to detect three types of microbial antigen contamination: antigens generally found in aerobic bacteria, antigens generally present in common fungi (yeast and molds), and an antigen that is characteristic of *Hormoconis resinae* (the fungus most commonly associated with fuel biodeterioration).

1.2.2 Although the antibodies and antibody mixtures are characteristic of diverse types of bacteria and fungi, it is unlikely that they are universal. Recognizing that for every microbe that has been isolated and characterized, it is likely that there are a billion that have not. Consequently, as is the case with all microbiological test methods, this test method does not purport to detect 100 % of the microbes present in a fuel or fuel-associated water sample.

1.3 For each of the three sets of antigen detected (*H. resinae*, common fungi, and aerobic bacteria), the test detects whether the antigen concentration present is within set ranges representing negligible, moderate, or heavy microbial contamination.

1.3.1 For fuel specimens, the antigen concentration ranges detected are $<150 \mu\text{g/L}$ (negligible), $150 \mu\text{g/L}$ to $750 \mu\text{g/L}$ (moderate), and $>750 \mu\text{g/L}$ (heavy).

1.3.2 For specimens of water associated with fuel, the antigen concentration ranges detected are $<33 \mu\text{g/mL}$ (negligible), $33 \mu\text{g/mL}$ to $166 \mu\text{g/mL}$ (moderate), and $>166 \mu\text{g/mL}$ (heavy).

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* For a specific hazard statement, see Section 8.

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*²

D396 Specification for Fuel Oils

D975 Specification for Diesel Fuel

D1129 Terminology Relating to Water

D1655 Specification for Aviation Turbine Fuels

D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants

D4176 Test Method for Free Water and Particulate Contamination in Distillate Fuels (Visual Inspection Procedures)

D6469 Guide for Microbial Contamination in Fuels and Fuel Systems

¹ This test method is under the jurisdiction of ASTM Committee **D02** on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of Subcommittee **D02.14** on Stability, Cleanliness and Compatibility of Liquid Fuels.

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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.

*A Summary of Changes section appears at the end of this standard

- D6974** Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures
- D7464** Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing
- D7687** Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel and Fuel-associated Water With Sample Concentration by Filtration
- D7847** Guide for Interlaboratory Studies for Microbiological Test Methods
- D7978** Test Method for Determination of the Viable Aerobic Microbial Content of Fuels and Associated Water—Thixotropic Gel Culture Method
- E1326** Guide for Evaluating Non-culture Microbiological Tests
- E2756** Terminology Relating to Antimicrobial and Antiviral Agents
- 2.2 *Other Standards:*
- BS EN590** Standard for Diesel Fuel³
- ISO 8217:2012** Petroleum products—Fuels (Class F)—Specifications of marine fuels⁴
- NATO Logistics Handbook** Chapter 15: Fuels, Oils, Lubricants and Petroleum Handling Equipment, Annex A: Aide Memoire on Fuels in NATO⁵
- IATA** Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks, Fifth Edition, 2015⁶

3. Terminology

3.1 Definitions:

3.1.1 For definitions of terms used in this test method, refer to Terminologies **D1129**, **D4175**, **E2756**, and Guide **E1326**.

3.2 Definitions:

3.2.1 *aerobic*, *adj*—(1) taking place in the presence of oxygen, (2) living or active in the presence of oxygen.

3.2.2 *antibody*, *n*—an immunoglobulin, a protein that is produced as a part of the immune response which is capable of specifically combining with the antigen.

3.2.2.1 *Discussion*—In the context of this test method, antibodies created for this purpose are utilized in conjunction with visual indicators to detect presence of microbial antigens.

3.2.3 *antigen*, *n*—a substance that stimulates the host to produce an immune response. In the context of this test method, specific antigens are detected as indicators of microbial contamination.

3.2.4 *buffer*, *n*—a compound or mixture that, when contained in solution, causes the solution to resist change in pH.

3.2.4.1 *Discussion*—Each buffer has a characteristic limited range of pH over which it is effective.

3.2.5 *colony*, *n*—a discreet visible aggregate of microorganisms that develops when a viable microorganism, or particle containing viable microorganisms, is introduced into a gel-based nutritive culture medium and reproduces there.

3.2.6 *colony forming unit (CFU)*, *n*—a viable microorganism or aggregate of viable microorganisms, which proliferate(s) in a culture medium to produce a viable colony.

3.2.7 *lateral flow device*, *n*—in immunology, an antibody-impregnated, porous medium through which an antigen-containing buffer is permitted to wick in order to bring the antigen into contact with the antibody.

3.2.7.1 *Discussion*—Typically, the antibody is linked to an indicator which produces a color reaction when antibody and antigen combine.

3.2.8 *metabolite*, *n*—a chemical substance produced by any of the many complex chemical and physical processes involved in the maintenance of life.

3.2.9 *microorganisms*, *n*—organisms too small to be seen with the naked eye, which generally include bacteria, protozoa, fungi, and microalgae (sometimes collectively called slime or microbial contamination).

3.2.9.1 *Discussion*—In the context of this test method, microorganisms are bacteria and fungi (yeasts and molds) that are capable of growth in fuels and associated aqueous-phase fluid.

3.3 Definitions of Terms Specific to This Standard:

3.3.1 *extraction fluid*, *n*—a mixture of buffer and surfactants used to extract antigens from the specimens.

3.3.2 *surfactant (surface active agent)*, *n*—a substance that affects the interfacial or surface tension of solutions even when present in very low concentrations.

3.4 Acronyms:

3.4.1 CFU—colony forming unit

3.4.2 FSII—fuel system icing inhibitor

3.4.3 LFD—lateral flow device

4. Summary of Test Method

4.1 Microbial contamination is detected using a series of antibodies immobilized onto a test tray in the form of three pairs of lateral flow devices (LFDs). These antibodies are used to detect antigens from common bacteria and fungi that proliferate in fuel tanks and systems.

4.1.1 The LFDs contain broad spectrum antibodies raised against cell components and materials generated during microbial growth on hydrocarbons (the antigens). These antibodies indicate the presence of *H. resinae*, other common fungi, and aerobic bacteria within concentration ranges described in **1.3**.

4.2 Fuel or aqueous specimens from fuel are mixed with an aqueous extraction fluid in an extraction bottle. The extraction fluid captures antigenic material present in the specimen.

4.3 Four drops of the separated extraction fluid are dispensed into the sample well of each of the six LFDs, which are arranged in three pairs.

³ Available from British Standards Institution (BSI), 389 Chiswick High Rd., London W4 4AL, U.K., <http://www.bsigroup.com>.

⁴ Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, <http://www.iso.org>.

⁵ Available from North Atlantic Treaty Organization (NATO) at <http://www.nato.int/docu/logi-en/1997/lo-15a.htm>.

⁶ Available from International Aviation Transport Association (IATA) at <http://store.iata.org>.