



Standard Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels— Filtration and Culture Procedures¹

This standard is issued under the fixed designation D6974; 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 covers a membrane filter (MF) procedure for the detection and enumeration of Heterotrophic bacteria (HPC) and fungi in liquid fuels with kinematic viscosities $\leq 24 \text{ mm}^2 \cdot \text{s}^{-1}$ at ambient temperature.

1.2 This quantitative practice is drawn largely from IP Method 385 and Test Method [D5259](#).

1.3 This test may be performed either in the field or in the laboratory.

1.4 The ability of individual microbes to form colonies on specific growth media depends on the taxonomy and physiological state of the microbes to be enumerated, the chemistry of the growth medium, and incubation conditions. Consequently, test results should not be interpreted as absolute values. Rather they should be used as part of a diagnostic or condition monitoring effort that includes other test parameters, in accordance with Guide [D6469](#).

1.5 This practice offers alternative options for delivering fuel sample microbes to the filter membrane, volumes or dilutions filtered, growth media used to cultivate fuel-borne microbes, and incubation temperatures. This flexibility is offered to facilitate diagnostic efforts. When this practice is used as part of a condition monitoring program, a single procedure should be used consistently.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *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.*

¹ This practice 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 and Cleanliness of Liquid Fuels.

Current edition approved July 1, 2016. Published July 2016. Originally approved in 2003. Last previous edition approved in 2013 as D6974 – 09 (2013) ^{ϵ 2}. DOI: 10.1520/D6974-16.

2. Referenced Documents

2.1 ASTM Standards:²

- [D1129 Terminology Relating to Water](#)
- [D1193 Specification for Reagent Water](#)
- [D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants](#)
- [D5259 Test Method for Isolation and Enumeration of Enterococci from Water by the Membrane Filter Procedure](#)
- [D6426 Test Method for Determining Filterability of Middle Distillate Fuel Oils](#)
- [D6469 Guide for Microbial Contamination in Fuels and Fuel Systems](#)
- [D7463 Test Method for Adenosine Triphosphate \(ATP\) Content of Microorganisms in Fuel, Fuel/Water Mixtures, and Fuel Associated Water](#)
- [D7464 Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing](#)
- [E1326 Guide for Evaluating Non-culture Microbiological Tests](#)
- [F1094 Test Methods for Microbiological Monitoring of Water Used for Processing Electron and Microelectronic Devices by Direct Pressure Tap Sampling Valve and by the Presterilized Plastic Bag Method](#)

2.2 Energy Institute Standards:³

- [IP 385 Determination of the Viable Aerobic Microbial Content of Fuels and Fuel Components Boiling Below 390 °C—Filtration and Culture Method](#)

3. Terminology

3.1 Definitions:

3.1.1 For definition of terms used in this method refer to Terminologies [D1129](#) and [D4175](#), and Guide [D6469](#).

3.1.2 *aseptic, adj*—sterile, free from viable microbiological contamination.

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 Available from Energy Institute, 61 New Cavendish St., London, W1G 7AR, U.K., <http://www.energyinst.org.uk>.

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