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Standard Guide for Quantification of Microbial Contamination in Liquid Fuels and Fuel-Associated Water by Quantitative Polymerase Chain Reaction (qPCR)¹

This standard is issued under the fixed designation D8412; 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 guide covers procedures for using quantitative polymerase chain reaction (qPCR), a genomic tool, to detect, characterize and quantify nucleic acids associated with microbial DNA present in liquid fuels and fuel-associated water samples.

1.1.1 Water samples that may be used in testing include, but are not limited to, water associated with crude oil or liquid fuels in storage tanks, fuel tanks, or pipelines.

1.1.2 While the intent of this guide is to focus on the analysis of fuel-associated samples, the procedures described here are also relevant to the analysis of water used in hydrotesting of pipes and equipment, water injected into geological formations to maintain pressure and/or facilitate the recovery of hydrocarbons in oil and gas recovery, water co-produced during the production of oil and gas, water in fire protection sprinkler systems, potable water, industrial process water, and wastewater.

1.1.3 To test a fuel sample, the live and recently dead microorganisms must be separated from the fuel phase which can include any DNA fragments by using one of various methods such as filtration or any other microbial capturing methods.

1.1.4 Some of the protocol steps are universally required and are indicated by the use of the word *must*. Other protocol steps are testing-objective dependent. At those process steps, options are offered and the basis for choosing among them are explained.

1.2 The guide describes the application of quantitative polymerase chain reaction (qPCR) technology to determine total bioburden or total microbial population present in fuel-associated samples using universal primers that allow for the quantification of 16S and 18S ribosomal RNA genes that are

present in all prokaryotes (that is, bacteria and archaea) and eucaryotes (that is, mold and yeast collectively termed fungi), respectively.

1.3 This guide describes laboratory protocols. As described in Practice D7464, the qualitative and quantitative relationship between the laboratory results and actual microbial communities in the systems from which samples are collected is affected by the time delay and handling conditions between the time of sampling and time that testing is initiated.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard with the exception of the concept unit of gene copies/mL (that is, 16S or 18S gene copies/mL) to indicate the starting concentration of microbial DNA for the intended microbial targets (that is, bacteria, archaea, fungi).

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.*

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

- D1129 Terminology Relating to Water
- D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants
- D6469 Guide for Microbial Contamination in Fuels and Fuel Systems
- D6974 Practice for Enumeration of Viable Bacteria and

¹ This guide 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.