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Standard Guide for Interlaboratory Studies for Microbiological Test Methods[1]

This standard is issued under the fixed designation D7847; 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.

ε[1] NOTE—Subsection 1.2 and Section 4 were corrected editorially in March 2015.

INTRODUCTION

Microbiological parameters present a number of unique challenges relative to chemical and physical test methods apropos of the development of precision and bias terms. A number of these challenges are discussed in Guide E1326. As a working group (WG) we first grappled directly with some of these issues during the development of Practice D6974. The drafts balloted at the D02.14 subcommittee level in February and June 2002, were balloted with the document identified as a Method. Moreover, the proposed Method was drafted as a harmonized document with the Energy Institute's (EI) Method IP 385. When the item was balloted at D02 level, members of D02.94 compelled us to change the title from Method to Practice. The argument was that ASTM Methods list single series of steps that lead to a measurable result (a bit of data; quantitative, semi-quantitative or qualitative). Because D6974 provides for the selection of different sample volumes (based on the estimated culturable population density) and different growth media (based on the sub-population to be quantified), it would only be accepted as an ASTM Practice; not a Method. This issue of performing interlaboratory studies for culture methods will be discussed below.

Since Practice D6974 was approved, two microbiological Methods have been approved by ASTM: Method D7463 and Method D7687. Although both methods measure adenosine triphosphate (ATP) in fuel and fuel-associated water samples the method of obtaining the sample differs; ASTM D7463 uses a liquid to liquid extraction whereas ASTM D7687 uses filtration.

Because these methods measure the concentration of a biomarker molecule, the issues that are relevant to ILS are similar to, but somewhat different than those that affect ILS for culture methods. Beckers[2] investigated microbiological test method ILS, but advised several measures that are either impractical for or not relevant to the methods that have been developed within D02: *(1)* Freeze inoculated samples after dispensing into portions for shipment to participating labs; *(2)* Use a single organisms challenge; *(3)* Add the challenge microbe to a sample matrix in which it is likely to proliferate.

This guide will list key issues that must be addressed when designing ILS for Methods intended to measure the microbial properties of fuels and fuel-associated waters.

1. Scope

1.1 Microbiological test methods present challenges that are unique relative to chemical or physical parameters, because microbes proliferate, die off and continue to be metabolically active in samples after those samples have been drawn from their source.

1.1.1 Microbial activity depends on the presence of available water. Consequently, the detection and quantification of microbial contamination in fuels and lubricants is made more complicated by the general absence of available water from these fluids.

1.1.2 Detectability depends on the physiological state and taxonomic profile of microbes in samples. These two parameters are affected by various factors that are discussed in this guide, and contribute to microbial data variability.

1.2 This guide addresses the unique considerations that must be accounted for in the design and execution of interlaboratory studies intended to determine the precision of microbiological test methods designed to quantify microbial

[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 and Cleanliness of Liquid Fuels.

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[2] Beckers, H. J., "Precision Testing of Standardized Microbiological Methods," *Journal of Testing and Evaluation*, JTEVA, Vol. 14, No. 6, November 1986, pp. 318-320.

contamination in fuels, lubricants and similar low water-content (water activity <0.8) fluids.

1.3 *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:*[3]

D156 Test Method for Saybolt Color of Petroleum Products (Saybolt Chromometer Method)
D1129 Terminology Relating to Water
D4012 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Water
D4175 Terminology Relating to Petroleum, Petroleum Products, and Lubricants
D6300 Practice for Determination of Precision and Bias Data for Use in Test Methods for Petroleum Products and Lubricants
D6469 Guide for Microbial Contamination in Fuels and Fuel Systems
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
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, Fuel/Water Mixtures, and Fuel-Associated Water with Sample Concentration by Filtration
E1259 Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C
E1326 Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria
E1601 Practice for Conducting an Interlaboratory Study to Evaluate the Performance of an Analytical Method
E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Energy Institute Standard:*[4]

IP 385 Viable aerobic microbial content of fuels and fuel components boiling below 90°C—Filtration and culture method

3. Terminology

3.1 For definition of terms used in this guide refer to Terminologies D1129, D4175 and E2756, and Guide D6469.

3.2 *Definitions:*

3.2.1 *free water, n*—water in excess of that soluble in the sample and appearing in the sample as a haze or cloudiness, as droplets, or as a separated phase or layer. **D156**

3.2.2 *specific concentration, n*—the fraction of a cell constituent as determined on a per cell basis.

3.2.2.1 *Discussion*—The specific concentration can be expressed as weight to weight, weight to volume or volume to volume basis. Enzymes are commonly reported in terms of their activity relative to a reference standard.

3.3 *Acronyms:*

3.3.1 *ATP*—adenosine triphosphate

3.3.2 *DNA*—deoxyribonucleic acid

3.3.3 *ILS*—interlaboratory study

3.3.4 *RNA*—ribonucleic acid

4. Determining Precision and Bias

4.1 *Bias Testing:*

4.1.1 There are no generally accepted reference standards for microbial cell constituents or for culture enumeration by viability test methods.

4.1.2 Consequently, bias cannot be determined for non-culture methods.

4.1.3 Data obtained from testing an accepted non-culture parameter or culture method can be compared against data obtained using a proposed new method.

4.1.3.1 Such comparisons are useful for benchmarking newly measure parameters against historically measure ones.

4.1.3.2 Because bioburden is not a condition of state and because individual microbial parameters respond to sources of variation differently, comparison of a new method's test results against those of a preexisting method cannot be used to determine the bias of either method.

4.2 *Precision Testing:*

4.2.1 *Repeatability Testing:*

4.2.1.1 *Sample Heterogeneity:*

(1) Unlike chemical and physical characteristics which are generally uniform throughout a well-mixed sample, microbes are discrete bodies that are dispersed in the medium.

(2) In contrast to inanimate particles, microbes typically form aggregates in which individual cells are bound to one another within a polymeric matrix that is difficult to remove without also damaging cells.

(3) Microbes are similar to inanimate particles in that their settling rate within a medium follows Stoke's law.

(4) Heterogeneous distribution of microbes within a medium is likely to be a significant source of variability relative to other factors affecting test method repeatability.[5]

(5) Microbes require free-water in order to be metabolically active (see 1.2).

(a) In a given fuel system, microbial population densities tend to be greatest at interfaces; particularly the fuel-water and fuel-system-surface interfaces.

[3] 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.

[4] Available from Energy Institute, 61 New Cavendish St., London, WIG 7AR, U.K., http://www.energyinst.org.uk.

[5] Passman, F. J., English, E., Lindhardt, C., "Using Adenosine Triphosphate Concentration as a Measure of Fuel Treatment Microbicide Performance," Morris, R. E., Ed., Proceedings of the 10th International Conference on the Stability and Handling of Liquid Fuels, Oct. 7-11, 2007, Tucson, AZ. Available at www.iash.net.