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Standard Guide for Interlaboratory Studies for Microbiological Test Methods¹

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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, four microbiological test methods have been approved by ASTM: D7463, D7687, D7978, and D8070.

Because these methods measure the concentration of a biomarker molecule or microorganisms, the issues that are relevant to ILS are similar to, but somewhat different than those that affect ILS for culture methods. Beckers² investigated microbiological test method interlaboratory studies, 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 contamination in fuels, lubricants and similar low water-content (water activity <0.8) fluids.

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

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