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Standard Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria¹

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1. Scope

1.1 The purpose of this guide is to assist users and producers of nonconventional tests in determining the applicability of the test for processing different types of samples and evaluating the accuracy of the results. Conventional procedures such as the Heterotrophic (Standard) Plate Count, the Most Probable Number (MPN) method and the Spread Plate Count are widely cited and accepted for the enumeration of microorganisms. However, these methods have their limitations, such as performance time and degree of accuracy. It is these limitations that have recently led to the marketing of a variety of nonconventional procedures, test kits and instruments.

1.2 A conventional test is one that is widely accepted and published as a standard microbiological method or related procedure. A new, nonconventional test method will attempt to provide the same information through the measurement of a different parameter. This guide is designed to assist investigators in assessing the accuracy and precision of nonconventional methods intended for the determination of microbial population densities or activities.

1.3 It is recognized that the Heterotrophic Plate Count does not recover all microorganisms present in a product or a system (1, 2).² When this problem occurs during the characterization of a microbiological population, alternative standard enumeration procedures may be necessary, as in the case of sulfate-reducing bacteria. At other times, chemical methods that measure the rates of appearance of metabolic derivatives or the utilization of contaminated product components might be indicated. In evaluating nonconventional tests, the use of these alternative standard procedures may be the only means available for establishing correlation. In such cases, this guide can serve as a reference for those considerations.

1.4 Since there are so many types of tests that could be considered nonconventional, it is impossible to recommend a

specific test protocol with statistical analyses for evaluating the tests. Instead, this guide should assist in determining what types of tests should be considered to verify the utility and identify the limitations of the nonconventional test.

2. Referenced Documents

2.1 *ASTM Standards*:³

[D3870 Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology \(Withdrawn 2000\)](#)⁴

[D4012 Test Method for Adenosine Triphosphate \(ATP\) Content of Microorganisms in Water](#)

[D5245 Practice for Cleaning Laboratory Glassware, Plasticware, and Equipment Used in Microbiological Analyses](#)

[D5465 Practice for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods](#)

[E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method](#)

3. Summary of Guide

3.1 ASTM standard practices are referenced for use by producers and users to determine the potential utility of the nonconventional test. Users of tests who are unequipped for performing standard microbiological tests are given recommendations for seeking out microbiological laboratories that could perform collaborative studies to evaluate and verify the information generated with the nonconventional tests.

4. Significance and Use

4.1 This guide should be used by producers and potential producers of nonconventional tests to determine the accuracy, selectivity, specificity, and reproducibility of the tests, as defined in Practices [E691](#) and [D3870](#). Results of such studies should identify the limitations and indicate the utility or

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² The boldface numbers in parentheses refer to the list of references at the end of this guide.

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 The last approved version of this historical standard is referenced on www.astm.org.

applicability of the nonconventional test, or both, for use on different types of samples.

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