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StandardGuide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria[1]

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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.

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

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

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

D7687 Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel-Associated Water with Sample Concentration by Filtration

D7847 Guide for Interlaboratory Studies for Microbiological Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E2694 Test Method for Measurement of Adenosine Triphosphate in Water-Miscible Metalworking Fluids

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 For definitions of terms used in this guide refer to Terminologies D1129 and E2756.

[1] This guide is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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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.

4. Summary of Guide

4.1 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.

5. Significance and Use

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

5.2 Nonconventional test users and potential users should employ this guide to evaluate results of the nonconventional test as compared to their present methods. Practices D5245 and D5465 should be reviewed in regards to the conventional microbiological methods employed. If conventional methods have not been used for monitoring the systems, then guidelines are included for obtaining microbiological expertise.

5.3 Utilization of a nonconventional test may reduce the time required to determine the microbiological status of the system and enable an improvement in the overall operating efficiency. In many cases, the findings of a significantly high level of bacteria indicates the need for an addition of an antimicrobial agent. By accurately determining this in a shorter time period than by conventional methods, treatment with antimicrobial agents may circumvent more serious problems than if the treatment were postponed until conventional results were available. If the antimicrobial treatment program relies on an inaccurate nonconventional test, then unnecessary loss of product and problems associated with inappropriate selection or improper dosing with antimicrobial agents would exist.

5.4 Since many methods based on entirely different chemical and microbiological principles are considered, it is not possible to establish a unique design and recommend a specific method of statistical analyses for the comparisons to be made. It is only possible to present guides that should be followed while performing the experiments. It is also recommended that a statistician be involved in the study.

5.5 There are various ways for categorizing microbiological test methods. One valid approach is to differentiate between methods intended to quantify a particular microbe from those intended to quantify overall bioburden.

5.5.1 Methods used to quantify a single microbe typically can be evaluated for precision (Practice E691). Even though it is unlikely that reference standards exist, often these methods can also be evaluated for bias relative to other methods used to detect the same microbe.

5.5.2 Methods used to quantify total populations are more problematic in terms of precision and bias testing. Guide D7847 addresses many of the factors that confound efforts to determine the precision of microbiological test methods used to quantify microbial contamination in fuels and fuel systems. Many of these issues are broadly relevant to the challenge of developing relevant precision terms for microbiological test methods used to quantify total bioburdens in industrial systems.

6. Procedures

6.1 In order to determine the utility of the nonconventional test, evaluate and compare the results to those obtained with a previously accepted standard method. The Heterotrophic Plate Count (Practice D5465) may be entirely satisfactory for this purpose (**3**); however, understand its limitations before it is used as the basis for evaluating methods that measure other parameters indicative of microbial life (metabolic activity, concentration of cell constituents, or whole cell numbers). Several methods used for the Heterotrophic Plate Count are listed in Table 1. When the Heterotrophic Plate Count is not a suitable refereed method, Adenosine Triphosphate Concentration (Test Methods D4012, E2694, and D7687) or the Most Probable Number (MPN) technique (**4**) may be more appropriate. Alternative standard enumeration methods or methods for measuring the rate of the appearance of derivatives or the rate of disappearance of components of the product in which the microbial contamination is being measured—where such phenomena are known to be correlated to microbial contamination levels—may also be used as referee methods for

TABLE 1 Comparison of Selected Heterotrophic Plate Count Procedures for Samples from Various Sources

	Water (5)	Dairy (6)	Environment (7)	Food (4)	Cosmetic (4)	Paper (8)	Pharmaceutical (9)
Media	TGE, SM, R2A or m-HPC	SM	SM or TGE	SM	ML	TGE	SCD
Dilution, H_2O	KH_2PO_4 + $MgCl_2$	KH_2PO_4	KH_2PO_4	KH_2PO_4	MLB	H_2O	KH_2PO_4
Incubation, °C	35 ± 0.5 20 or 28 (R2A)	32 ± 1	35 ± 0.5	35	30 ± 2	36 ± 0.5	30–35
Incubation, h	48 ± 3 72 ± 4 (bottled water) 72–168 (R2A medium)	48 ± 3	48	48 ± 2	48	48	48–72
Amount of Agar, mL	10–12 (Pour Plate) 15 (Spread Plates) 5 (Membrane Filter)	10–12	10+	12–15	Spread Plates	15–20	15–20

TGE = Tryptone Glucose Extract Agar
SM = Standard Methods Agar (Tryptone Glucose Yeast Agar)
ML = Modified Letheen Agar
MLB = Modified Letheen Broth
SCD = Soybean Casein Digest Agar
R2A = Low-Nutrient Media (which may not be available in dehydrated form)
m-HPC = Formerly called m-SPC Agar (used for membrane filtration)