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Standard Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria[1]

This standard is issued under the fixed designation E 1326; 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 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 non-conventional 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]

D 3870 Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology[4]

D 4012 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Water

D 5245 Practice for Cleaning Laboratory Glassware, Plasticware, and Equipment Used in Microbiological Analyses

D 5465 Practice for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods

E 691~~Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method~~

~~E2250Method for Determination of Endotoxin Concentration in Water Miscible Metal Working Fluids~~ 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.

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[1] This guide is under the jurisdiction of ASTM Committee E35 on Pesticides 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] Withdrawn.

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 E 691 and D 3870. 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.

4.2 Nonconventional test users and potential users should employ this guide to evaluate results of the nonconventional test as compared to their present methods. Practices D 5245 and D 5465 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.

4.3 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.

4.4 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. Procedures

5.1 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 D 5465) 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 Method D 4012~~), Endotoxin Concentration (Method E2250), or the Most Probable Number (MPN) technique~~ **) or the Most Probable Number (MPN) technique (9) 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 assessing the accuracy and precision of a novel nonconventional method. No single method is universally applicable; consequently, it is imperative to determine the rationale for employing any given measurement procedure and to select a standard that will permit the determination of whether or not the nonconventional method achieves the objectives defined in the scope of the procedure.**

5.2 A knowledge of standard microbiological technique is required for this procedure. If that expertise is not currently available in-house, consult an outside testing laboratory. Many industrial microbiology laboratories are certified for the analysis of drinking water by the EPA or the state government (a listing of these laboratories can be obtained from the regional EPA office or the state government). There are also other microbiology laboratories that specialize in processing samples from different industries; these are often listed as "Laboratories—Testing" in the telephone book. It is important that this document be referenced when undertaking an evaluation with an outside laboratory.

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

	Water (**6**)	Dairy (**7**)	Environment (**8**)	Food (**9**)	Cosmetic (**9**)	Paper (**10**)	Pharmaceutical (**11**)
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)