Designation: ~~E1326 – 13~~ E1326 – 15

Standard Guide for Evaluating ~~Nonconventional~~Non-culture Microbiological Tests Used for Enumerating Bacteria[1]

This standard is issued under the fixed designation E1326; 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~~non-culture tests in determining the applicability of the test for processing different types of samples and evaluating the accuracy of the results. ~~Conventional~~ Culture test 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. Moreover any given culture test method typically recovers only a fraction of the total viable microbes present in a sample. It is these limitations that have recently led to the marketing of a variety of ~~non-conventional~~non-culture 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~~ Culture test methods estimate microbial population densities based on the ability of mircoorganisms in a sample to proliferate in or on a specified growth medium, under specified growth conditions. Non-culture test methods attempt to provide the same or complimentary information through the measurement of a different parameter. This guide is designed to assist investigators in assessing the accuracy and precision of ~~nonconventional~~non-culture methods intended for the determination of microbial population densities or activities.

1.3 It is recognized that the Heterotrophic Plate Count (HPC) 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~~ derivatives, the utilization of contaminated product components or genetic profile of the microbial population might be indicated. In evaluating ~~nonconventional tests,~~ non-culture tests, it is possible that the use of these alternative standard procedures ~~may~~might be the only means available for establishing correlation. In such cases, this guide can serve as a reference for those considerations.

1.4 ~~Since~~Because there are so many types of tests that could be considered ~~nonconventional,~~ non-culture based, 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~~E177 ~~Test Method~~ Practice for ~~Measurement of Cellular Adenosine Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel-Associated Water with Sample Concentration by Filtration~~Use of the Terms Precision and Bias in ASTM Test Methods

[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.

Current edition approved ~~Oct. 1, 2013~~May 1, 2015. Published ~~October 2013~~June 2015. Originally approved in 1990. Last previous edition approved in ~~2008~~2013 as ~~E1326 – 08.~~E1326 – 13. DOI: ~~10.1520/E1326-13.~~10.1520/E1326-15.

[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.

D7847 Guide for Interlaboratory Studies for Microbiological Test Methods
E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method
E2694E1601 Test Method for Measurement of Adenosine Triphosphate in Water-Miscible Metalworking FluidsPractice for Conducting an Interlaboratory Study to Evaluate the Performance of an Analytical Method
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.*Defintions:*
3.1.1 For definitions of terms used in this guide refer to Terminologies D1129, E2756, and E177.

3.2 *Abbreviations:*
3.2.1 *HPC*—Heterotrophic Plate Count

4. Summary of Guide

4.1 ASTM standard methods and practices are referenced for use by producers and users in order 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. a non-standard, non-culture test.

4.2 Recognizing that potential users of non-culture test methods might not have the resources with which or capabilities for evaluating the utility of non-standard, non-culture test methods, recommendations are provided to assist those users in identifying the capabilities that qualify microbiological laboratories to perform collaborative studies to evaluate those methods.

5. Significance and Use

5.1 This guide should be used by producers and potential producers of nonconventionalnon-culture tests to determine the accuracy, selectivity, specificity, and reproducibility of the tests, as defined in PracticesPractice E691 and D3870. Results of such studies should identify the limitations and indicate the utility or applicability of the nonconventionalnon-culture test, or both, for use on different types of samples.

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

5.3 Utilization of a non-culture test can reduce the time required to determine the microbiological status of the system and detect microbe that are not detected by culture testing. Consequently, non-culture tests can contribute to the improvement in the overall operating efficiency of microbial contamination condition monitoring and diagnostic efforts, and microbicide performance evaluations.

5.4 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 Detecting microbial contamination levels that exceed predetermined upper control limits indicates the need for an addition of an antimicrobial agent. agent or other corrective maintenance action. By accurately determining this in a shorter time period than by conventionalis possible than by culture methods, treatment with antimicrobial agents may circumvent more serious problems than if the treatment were postponed until conventionalculture results were available. If the antimicrobial treatment program reliesrelied on an inaccurate nonconventionalnon-culture test, then unnecessary loss of product and problems associated with inappropriate selection or improper dosing with antimicrobial agents would exist.

5.5 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 Practice E1601 provides guidance on the evaluation of analytical method performance. The guidance provided below amplifies the processes described in Practice E1601 as they apply to microbiological test methods.

6.2 ~~In order~~ Although the heterotrophic plate count (HPC) has been used historically 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~~ newly developed non-culture methods, and can ~~D5465) may be entirely satisfactory for this purpose~~ be an appropriate reference method in many cases (~~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~~ there are cases for ~~Table 1. When the Heterotrophic Plate Count is not a suitable refereed method, Adenosine Triphosphate Concentration (Test Methods~~ which HPC is not an appropriate refer ~~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 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.~~ method

6.2.1 The choice of referee method to use for validating a new or proposed non-culture method should be determined based on the parameter the new method purports to be measuring.

6.2.2 Several methods used for the HPC are listed in Table 1.

6.2.3 When none of the Table 1 variations of the HPC (Heterotrophic Plate Count) are suitable refereed methods, Adenosine Triphosphate Concentration (Test Method D4012) or the Most Probable Number (MPN) technique (7) may be more appropriate.

6.2.4 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 non-culture method.

6.2.5 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.

6.3 A knowledge of standard microbiological technique is required ~~for this procedure.~~ in order to conduct microbiological test method evaluations. 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.~~

6.3.1 Many industrial microbiology laboratories are certified for the analysis of drinking water by the EPA or the state government, or both (a listing of these laboratories can be obtained from the regional EPA office or the state government).

6.3.2 These and other independent microbiology laboratories often specialize in processing samples from different industries

6.3.3 Suitable microbiology laboratories are typically often listed as "Laboratories—Testing" in the telephone book or in directories such as the ASTM International Directory of Testing Laboratories[3]. 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 (~~54~~)	Dairy (~~65~~)	Environment (~~76~~)	Food (~~47~~)	Cosmetic (~~47~~)	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)