



Standard Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology¹

This standard is issued under the fixed designation D 3870; 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 This practice deals with the performance characteristics of enumeration methods for microorganisms of health and sanitary significance. The performance characteristics cover membrane filter, pour plate, and spread-plate colony counting techniques. A performance characteristic is a quantitative, experimentally determined value that is used to assess the suitability of an analytical method for a given purpose. The performance characteristics dealt with here are specificity, including selectivity, recovery, upper counting range, and precision and lower counting range.

1.2 The purpose of establishing performance characteristics is to provide a set of uniform properties to describe bacterial enumeration techniques and selective media.

1.3 *This standard does not purport to address the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

- 2.1 *ASTM Standards:*
D 1129 Terminology Relating to Water²

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *lower limit of counting range*—that count below which the anticipated error becomes unacceptably large in relation to the count itself.

3.1.2 *precision*—the degree of agreement of repeated measurements of the same sample. The usual index of precision is the standard deviation.

3.1.3 *recovery*—the degree of agreement between the density of microorganisms obtained with a test method and the density obtained with an acceptable reference method.

3.1.4 *selectivity*—the ability of a method to encourage growth of the target organism while retarding development on nontarget organisms. In this way, overcrowding problems can be minimized.

3.1.5 *specificity*—the ability of a method to select and distinguish the microorganism under consideration from all others in the same environment.

3.1.6 *upper limit of counting range*—that point above which the reliability of the colony count on a single plate or membrane from a specified volume is affected by uncontrollable factors.

3.2 *Definitions*—For definitions of other terms used in this practice, refer to Terminology D 1129.

4. Significance and Use

4.1 Data on the performance characteristics are required to describe the acceptability of microbiological counting methods to the user.

4.2 Such data are used to determine the applicability of counting methods for research, monitoring, and regulatory purposes in order to assure uniformity and comparability of method results.

4.3 Living microorganisms are inherently more variable in numbers and in responses to test conditions, than chemical analytes. Hence, there is a need to establish criteria to assure that different microbiological methods are evaluated and characterized against a standard set of performance characteristics. These are herein established.

5. Statistical Procedures

5.1 Specificity and Selectivity:

5.1.1 Specificity is evaluated by selecting a representative number of target and nontarget colonies recovered from various aquatic environments. Multiple dilutions of a water sample are plated or filtered in triplicate from a sample or sample dilution that will provide noncrowded colonies. Incubate as directed. Examine *all* the colonies from no less than two plates or filters. Each plate must contain at least 30 presumptive target organisms. Perform sufficient biochemical tests on each colony to identify it as the target organism. Designate as false positives all colonies that do not verify as target types. Similarly, designate as undetected target all presumptive nontarget colonies that verify as target types.

5.1.2 The results of specificity testing are expressed as two individual terms; the error introduced by false positive colonies and the error resulting from undetected target colonies. Calculate the first term by dividing the number of false positive target colonies by the total presumptive target colony count. If there are no false positive colonies, this term will equal zero.

¹ This practice is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.24 on Water Microbiology.

Current edition approved June 15, 1991. Published October 1991. Originally published as D 3870 – 79. Last previous edition D 3870 – 79(1984) ^{ε1}.

² Annual Book of ASTM Standards, Vol 11.01.