Designation: D 3870 – 91

Standard Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology[1]

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 Water[2]

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

[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.

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[2] Annual Book of ASTM Standards, Vol 11.01.

TABLE 1 Results from Example 2

	Test Medium Count [A]		Reference medium Count [A]	
	0 h	24 h	0 h	24 h
Strain 1	101 [A]	97	101	93
Strain 2	99	95	105	103
Strain 3	101	98	97	109
Strain 4	110	100	105	100
Strain 5	100	98	100	95
Average recovery	102	98	102	100

[A] Mean of 5 counts from replicate plates.

Calculations:

$$\text{Recovery (0 h)} = \frac{\text{test medium count}}{\text{reference medium count}} \times 100$$

$$= \frac{102(100)}{102} = 100\ \%$$

$$\text{Recovery (24 h)} = \frac{98(100)}{100} = 98\ \%$$

there are no false positive colonies, this term will equal zero. Calculate the second term by dividing the number of undetected target colonies by the sum of the verified target colonies and undetected target colonies. If there are no undetected target colonies, this term will equal zero. The specificity index is reported as two individual terms. The nearer each term is to zero, the more specific the method.

5.1.3 *Example 1*—The following results were obtained after examining five water samples from different aquatic environments:

Presumptive target colonies examined	320
Presumptive nontarget colonies examined	210
False positive colonies	32
Undetected target colonies	13

Indices of specificity:

$$\text{false positive error} = \frac{32}{320} = 0.1$$

$$\text{undetected target error} = \frac{13}{320 - 32 + 13}$$

$$= 0.043$$

Selectivity is evaluated using the presumptive target colonies generated to evaluate specificity (see 5.1.4) and a total of all countable colonies that developed during each analysis. The selectivity index can then be calculated as the ratio of these numbers.

5.1.4 *Example 2*—Using the data presented in 5.1.3:

Presumptive target colonies = 320

Total countable colonies = 320 + 210 = 530

$$\text{Index of selectivity: } \frac{320}{530} = 0.604$$

5.2 *Recovery*:

5.2.1 To determine the recovery of a test method, seed a water sample (filter sterilized stream, lake, or ocean water) with a laboratory culture of the target organism. Stress the seeded sample, for example, hold at 11°C for 24 h before performing the recovery assays. Enumerate the target organisms in the seeded sample with the test and reference methods before and after stressing the sample. Use at least five replicates at each dilution. Repeat this procedure with five or more strains of the target organism.

TABLE 2 Results from Example 4

LC	HC	LC	HC	LC	HC
8 to 48		12 to 64		21 to 98	
9 to 44		14 to 67		21 to 100	
9 to 48		14 to 70		21 to 102	
10 to 50		14 to 72		22 to 100	
10 to 51		14 to 72		23 to 96	
11 to 50		15 to 70		23 to 95	
11 to 52		17 to 80		24 to 97	
11 to 53		17 to 82		26 to 95 [A]	
11 to 53		17 to 83		28 to 97 [A]	
11 to 55		19 to 85		28 to 100 [A]	
12 to 55		19 to 90		28 to 99 [A]	
12 to 57		19 to 92		28 to 101 [A]	
12 to 58		19 to 93		30 to 103 [A]	
12 to 58		20 to 95		32 to 106 [A]	
12 to 58		20 to 95		36 to 110 [A]	
12 to 60		20 to 96			
12 to 63		20 to 98			

[A] The μ-test values are greater than 1.96 and therefore the expected (5 × LC) and observed (HC) counts are not members of the same distribution of means. The upper limit of the counting range for this technique would be 95 colonies.

5.2.2 Report the mean test method density as a percentage of the mean reference method density.

5.2.3 *Example 3*—The results in Table 1 were obtained with five strains of target organism assayed with a test method and a reference method before and after subjecting the seeded samples to a low temperature for 24 h.

5.3 *Upper Limit of Counting Range*:

5.3.1 The calculations that follow compare counts from dilutions of the same sample, therefore a Poisson distribution can be assumed.

5.3.2 Determination of the upper counting limit requires a sufficient number of natural samples from various aquatic environments. The number required depends only on the difficulty encountered in defining the limit. Each sample shall contain the highest countable number of target organisms in the largest volume that can be plated or filtered. Make an appropriate number of five-fold dilutions and determine the density of organisms in triplicate for each dilution. Incubate as required. Count the plates of two neighboring dilutions and record the results as high count (HC) and low count (LC). Do not count plates where the LC mean is less than eight colonies (see 5.3.2).

5.3.3 Report the results of this testing as an upper limit, below which the reliability of the method is not affected. Determine that limit by multiplying the lower mean count of each pair from a sample by 5. Using the μ-test formula given by Hald (1960),[3]

$$\mu = \frac{X_1 - X_2 - 1}{\sqrt{X_1 + X_2}} \tag{1}$$

determine if the LC × 5 and the HC are means from the same distribution. The expectation is that 5 × LC should equal HC. If:

$$\mu = \frac{|\,(5 \times LC) - HC - 1|}{\sqrt{(5 \times LC) + HC}} > 1.96 \tag{2}$$

[3] Hald, *Statistical Theory with Engineering Application*, John Wiley and Sons, Inc., New York, NY, 1960, p. 725.