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Laboratory Guidebook
Notice of Change

Chapter new, **revised,** or archived: MLG Appendix 2.05

Title: Most Probable Number Procedure and Tables

Effective Date: 06/29/14

Description and purpose of change(s):

The confirmation procedure for inconclusive screen tests for MPN tubes was added.

The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use. Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled "FSIS Guidance for Evaluating Test Kit Performance" available on the FSIS website.

APP 2 Procedure

APP 2.1 Introduction

APP 2.1.1 For the convenience of analysts using the procedures in this Guidebook, tables of Most Probable Numbers (MPN) are provided in this appendix.

APP 2.1.2 MPN is a procedure to estimate the population density of viable microorganisms in a test sample. It is based upon the application of the theory of probability to the numbers of observed positive growth responses to a standard dilution series of sample inoculum placed into a set number of culture media tubes. Positive growth response after incubation may be indicated by such observations as gas production in fermentation tubes or visible turbidity in broth tubes, depending upon the type of media employed. The sample should be diluted in such a manner that higher dilutions of the sample will result in fewer positive culture tubes in the series. The number of sample dilutions to be prepared is generally based on the expected population contained within the sample. If particularly high microbial populations are expected, the sample must be diluted to a range where the MPN can be obtained. Most reliable results occur when all tubes at the lower dilution are positive and all tubes at the higher dilution are negative. Generally tenfold serial dilutions are used in either a 3, 5 or 10 tube MPN series. When a higher number of tubes are inoculated in the series, the confidence limits of the MPN are narrowed. For particularly high microbial populations, the values obtained by MPN are generally not considered to be as precise as population numbers derived from direct plating methods; however, it should be emphasized that MPN values are only estimates while plate counts are direct counts of living organisms expressed in cfu/ml. MPN values are, however, particularly useful when low concentrations of organisms (<100/g) are encountered in such materials as milk, food, water and soil where particulate matter of the matrix may interfere with obtaining accurate colony counts.

APP 2.1.3 In application of probability theory to the determination of MPN values, it should be kept in mind that the following assumptions are generally considered to be accepted: (a) the organisms are randomly and evenly distributed throughout the sample, (b) the organisms exist as single entities, not as chains, pairs or clusters and they do not repel one another, (c) the proper growth medium, temperature and incubation conditions have been selected to allow even a single viable cell in an inoculum to produce detectable growth and (d) the population does not contain viable, sub-lethally injured organisms that are incapable of growth in the culture medium used.

APP 2.1.4 The following 4 tables present MPN values and corresponding 95% confidence limits for a 3 tube test series using 4 different, commonly used sets of inoculum quantities that should be particularly useful relative to performing the microbiological analyses previously described in various chapters of this Guidebook. These MPN tables may be considered to be abbreviated since not all possible combinations of positive and negative tubes within a series are presented. Those combinations that occur often enough to have statistical significance are included, while those that are improbable have been omitted. If laboratory analyses produce combinations that are not included in the tables, then one should repeat the test on another portion of the same lot of sample (assuming the microbiological integrity of the sample has not been compromised) as a possible performance error or contamination is indicated. If this is not possible and an MPN is imperative, then more complete tables should be consulted from other reference sources or the MPN can be calculated by equation (other reference sources) on the basis of the observed results.

APP 2.1.5 On occasions when more than three dilutions of a sample are used in a decimal series of dilutions of a 3-tube MPN determination; the following guidelines should be followed. Results from only three consecutive dilutions are used to determine the MPN. If one or more dilutions have all tubes positive, select the highest dilution (smallest sample quantity) with positive results in all tubes and the next two higher dilutions, as shown in examples a and b below. When none of the dilutions yield all tubes positive, select the three lowest dilutions for which the middle dilution contains the positive result, as shown in example c below. If a positive result occurs in a higher unselected dilution, add the number of positive tubes in this dilution to the results of the highest dilution of the three selected, as shown in example d below. When all dilutions tested yield all tubes positive, select the three highest dilutions (example e below). For additional information on MPN estimations, consult the APHA's Compendium of Methods for the Microbiological Examination of Foods (3rd edition, 1992, chapter 6).

APP 2.2 Sample Preparation for Most Probable Number (MPN)

A series of 3 sequential MPN tubes may be inoculated directly from the primary dilution of the sample-culture medium homogenate if a low level of organisms is expected. Prepare the sample in the initial culture medium as directed for the analysis. Set up 3 empty tubes and 6 tubes that contain 9 ml of the appropriate culture medium. Add 10 mL of the homogenate to each of 3 empty tubes, 1 mL of the homogenate to each of 3 tubes of culture medium, and 0.1 mL to each of the last 3 tubes of culture medium. This series of tubes represents 1.0, 0.1 and 0.01 g of sample. The remainder of the sample homogenate should also be cultured.

Alternatively, prepare serial 10-fold dilutions of the sample in the appropriate culture medium and follow the outline below. This is the preferred method if it is anticipated that the level of organisms may exceed 10 growth units per gram.

Volume of Original Sample	Source of Inoculum	Quantity of Inoculum	Quantity of Culture Medium	Number of Tubes
10 mL	Sample	10.0 mL	90 mL	3
1.0 mL	Sample	1.0 mL	9.0 mL	3
0.1 mL	Dilution Tube 10-1	1.0 mL	9.0 mL	3
0.01 mL	Dilution Tube 10-2	1.0 mL	9.0 mL	3
0.001 mL	Dilution Tube 10-3	1.0 mL	9.0 mL	3
0.0001 mL	Dilution Tube 10-4	1.0 mL	9.0 mL	3

For FSIS laboratories to increase sensitivity in samples expected to have a low level of the organism of interest, perform the following procedure to prepare most product samples for pathogen MPN analysis.

Start with a 65 ± 2 g test portion (representative of the entire sample) taken from the same sample lot. Add 585 ml enrichment broth and stomach for 2 minutes.

Set up a 3-tube 5-dilution MPN (i.e., 15 individual dilutions for each sample) representing 10, 1, 0.1, 0.01, and 0.001 g of sample. Three containers will hold 100 ml homogenate each to represent the 10 g sample dilution. Three containers will hold 10 ml homogenate each to represent the 1 g sample dilution. Three containers will hold 1 ml homogenate plus 9 ml enrichment broth each to represent the 0.1 g sample dilution. Three tubes will contain 0.1 ml homogenate plus 9.9 ml enrichment broth each to represent the 0.01 g sample dilution; and add 0.1 homogenate to 9.9 ml enrichment, vortex, and add 1.0 ml from that dilution to each of three tubes containing 9.0 ml enrichment for the 0.001 g sample dilution.

APP 2.3 Completion of the MPN

After preparing all MPN dilutions, refer to the appropriate MLG chapter to complete analysis for each dilution and the remainder of the sample homogenate. The MPN combination used to derive the MPN Index from the appropriate table(s) will be determined from the screen test results. Confirm inconclusive screen results culturally up to the serology step for *E. coli* O157:H7 and non-O157 STECs, up to the β-hemolysis step for *L. monocytogenes,* or up to TSI and LIA slants for *Salmonella*.

APP 2.4 Example calculations

	Sample quantities (g or ml)[1]					Reported positive values	MPN estimate/ g or ml
Example	10	1	0.1	0.01	0.001		
a	3/3[2]	**3/3**	**2/3**	**0/3**	0/3	3-2-0	9.3
b	3/3	3/3	**3/3**	**2/3**	**0/3**	3-2-0	93.
c	0/3	**0/3**	**1/3**	**0/3**	0/3	0-1-0	0.31
d	3/3	**3/3**	**2/3**	**1/3**	1/3	3-2-2	21.
e	3/3	3/3	**3/3**	**3/3**	**3/3**	3-3-3	>1100

[1] The analyst should make sure that ALL sample dilution factors (including the preparation of any sample homogenate) are correctly applied in calculating the actual sample quantities subjected to MPN analysis.

[b2]Numerator = **No. positive tubes**

Denominator **No. tubes inoculated**

APP 2.5 Reference(s):

Blodgett, R. FDA Bacteriological Analytical Manual Online, Appendix 2, Most Probable Number Determination from Serial Dilutions, 2006.

Swanson, K.M.J, R. L. Petran and J. H. Hanlin. Chapter 6, Culture Methods for Enumeration of Microorganisms in "Compendium of Methods for the Microbiological Examination of Foods" Fourth Edition, 2001. Edited by F. P. Downes and K. Ito. Published by American Public Health Association.

Table 1. MPN Index and 95% Confidence Limits for Various Combinations of Positive Tubes in a 3 Tube Dilution Series Using Inoculum Quantities of 10, 1 and 0.1 g (ml).

Combination of Positives	MPN Index per g (ml)	95% Confidence Limits Lower	Upper
0-0-0	<0.03	---	0.095
0-0-1	0.030	0.0015	0.096
0-1-0	0.030	0.0015	0.11
0-1-1	0.061	0.012	0.18
0-2-0	0.062	0.012	0.18
0-3-0	0.094	0.036	0.38
1-0-0	0.036	0.0017	0.18
1-0-1	0.072	0.013	0.18
1-0-2	0.11	0.036	0.38
1-1-0	0.074	0.013	0.20
1-1-1	0.11	0.036	0.38
1-2-0	0.11	0.036	0.42
1-2-1	0.15	0.045	0.42
1-3-0	0.16	0.045	0.42
2-0-0	0.092	0.014	0.38
2-0-1	0.14	0.036	0.42
2-0-2	0.02	0.045	0.42
2-1-0	0.15	0.037	0.42
2-1-1	0.20	0.045	0.42
2-1-2	0.27	0.087	0.94
2-2-0	0.21	0.045	0.42
2-2-1	0.28	0.087	0.94
2-2-2	0.35	0.087	0.94
2-3-0	0.29	0.087	0.94
2-3-1	0.36	0.087	0.94
3-0-0	0.23	0.046	0.94
3-0-1	0.38	0.087	1.1
3-0-2	0.64	0.17	1.8
3-1-0	0.43	0.09	1.8
3-1-1	0.75	0.17	2.0
3-1-2	1.2	0.37	4.2
3-1-3	1.6	0.40	4.2
3-2-0	0.93	0.18	4.2
3-2-1	1.5	0.37	4.2
3-2-2	2.1	0.40	4.3
3-2-3	2.9	0.90	10.
3-3-0	2.4	0.42	10.
3-3-1	4.6	0.90	20.
3-3-2	11.	1.8	41.
3-3-3	>11.	4.2	---

Table 2. MPN Index and 95% Confidence Limits for Various Combinations of Positive Tubes in a 3 Tube Dilution Series Using Inoculum Quantities of 1, 0.1 and 0.01 g (ml).

Combination of Positives	MPN Index per g (ml)	95% Confidence Limits Lower	Upper
0-0-0	<0.3	---	0.95
0-0-1	0.30	0.015	0.96
0-1-0	0.30	0.015	1.1
0-1-1	0.61	0.12	1.8
0-2-0	0.62	0.12	1.8
0-3-0	0.94	0.36	3.8
1-0-0	0.36	0.017	1.8
1-0-1	0.72	0.13	1.8
1-0-2	1.1	0.36	3.8
1-1-0	0.74	0.13	2.0
1-1-1	1.1	0.36	3.8
1-2-0	1.1	0.36	4.2
1-2-1	1.5	0.45	4.2
1-3-0	1.6	0.45	4.2
2-0-0	0.92	0.14	3.8
2-0-1	1.4	0.36	4.2
2-0-2	2.0	0.45	4.2
2-1-0	1.5	0.37	4.2
2-1-1	2.0	0.45	4.2
2-1-2	2.7	0.87	9.4
2-2-0	2.1	0.45	4.2
2-2-1	2.8	0.87	9.4
2-2-2	3.5	0.87	9.4
2-3-0	2.9	0.87	9.4
2-3-1	3.6	0.87	9.4
3-0-0	2.3	0.46	9.4
3-0-1	3.8	0.87	11.
3-0-2	6.4	1.7	18.
3-1-0	4.3	0.90	18.
3-1-1	7.5	1.7	20.
3-1-2	12.	3.7	42.
3-1-3	16.	4.0	42.
3-2-0	9.3	1.8	42.
3-2-1	15.	3.7	42.
3-2-2	21.	4.0	43.
3-2-3	29.	9.0	100.
3-3-0	24.	4.2	100.
3-3-1	46.	9.0	200.
3-3-2	110.	18.	410.
3-3-3	>110.	42.	---

Table 3. MPN Index and 95% Confidence Limits for Various Combinations of Positive Tubes in a 3 Tube Dilution Series Using Inoculum Quantities of 0.1, 0.01 and 0.001 g (ml).

Combination of Positives	MPN Index per g (ml)	95% Confidence Limits Lower	Upper
0-0-0	<3.0	---	9.5
0-0-1	3.0	0.15	9.6
0-1-0	3.0	0.15	11.
0-1-1	6.1	1.2	18.
0-2-0	6.2	1.2	18.
0-3-0	9.4	3.6	38.
1-0-0	3.6	0.17	18.
1-0-1	7.2	1.3	18.
1-0-2	11.	3.6	38.
1-1-0	7.4	1.3	20.
1-1-1	11.	3.6	38.
1-2-0	11.	3.6	42.
1-2-1	15.	4.5	42.
1-3-0	16.	4.5	42.
2-0-0	9.2	1.4	38.
2-0-1	14.	3.6	42.
2-0-2	20.	4.5	42.
2-1-0	15.	3.7	42.
2-1-1	20.	4.5	42.
2-1-2	27.	8.7	94.
2-2-0	21.	4.5	42.
2-2-1	28.	8.7	94.
2-2-2	35.	8.7	94.
2-3-0	29.	8.7	94.
2-3-1	36.	8.7	94.
3-0-0	23.	4.6	94.
3-0-1	38.	8.7	110.
3-0-2	64.	17.	180.
3-1-0	43.	9.0	180.
3-1-1	75.	17.	200.
3-1-2	120.	37.	420.
3-1-3	160.	40.	420.
3-2-0	93.	18.	420.
3-2-1	150.	37.	420.
3-2-2	210.	40.	430.
3-2-3	290.	90.	1000.
3-3-0	240.	42.	1000.
3-3-1	460.	90.	2000.
3-3-2	1100.	180.	4100.
3-3-3	>1100.	420.	---

Table 4. MPN Index and 95% Confidence Limits for Various Combinations of Positive Tubes in a 3 Tube Dilution Series Using Inoculum Quantities of .01, 0.001 and 0.0001 g (ml).

Combination of Positives	MPN Index per g (ml)	95% Confidence Limits	
		Lower	Upper
0-0-0	<30.	---	95.
0-0-1	30.	1.5	96.
0-1-0	30.	1.5	110.
0-1-1	61.	12.	180.
0-2-0	62.	12.	180.
0-3-0	94.	36.	380.
1-0-0	36.	1.7	180.
1-0-1	72.	13.	180.
1-0-2	110.	36.	380.
1-1-0	74.	13.	200.
1-1-1	110.	36.	380.
1-2-0	110.	36.	420.
1-2-1	150.	45.	420.
1-3-0	160.	45.	420.
2-0-0	92.	14.	380.
2-0-1	140.	36.	420.
2-0-2	200.	45.	420.
2-1-0	150.	37.	420.
2-1-1	200.	45.	420.
2-1-2	270.	87.	940.
2-2-0	210.	45.	420.
2-2-1	280.	87.	940.
2-2-2	350.	87.	940.
2-3-0	290.	87.	940.
2-3-1	360.	87.	940.
3-0-0	230.	46.	940.
3-0-1	380.	87.	1100.
3-0-2	640.	170.	1800.
3-1-0	430.	90.	1800.
3-1-1	750.	170.	2000.
3-1-2	1200.	370.	4200.
3-1-3	1600.	400.	4200.
3-2-0	930.	180.	4200.
3-2-1	1500.	370.	4200.
3-2-2	2100.	400.	4300.
3-2-3	2900.	900.	10000.
3-3-0	2400.	420.	10000.
3-3-1	4600.	900.	20000.
3-3-2	11000.	1800.	41000.
3-3-3	>11000.	4200.	---