



Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG Appendix 2.02

Title: Most Probable Number Tables

Effective Date: 7/3/03

Description and purpose of change(s):

The Microbiology Laboratory Guidebook method chapters are currently under revision. The formatting is being changed to meet the requirements of the laboratory document control system. Tables have been updated to reflect changes in FDA BAM MPN tables. Additional instructions for tube preparation have been added.

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health and Science

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Revision: 02	Replaces: MLG Appendix, Most Probable Number Tables Volume 1 and 2, Rev.1	Effective: 7/3/03

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.

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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 original 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).

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APP 2.2 SAMPLE PREPARATION FOR MOST PROBABLE NUMBER (MPN)

A series of 3 sequential MPN tubes may be inoculated directly from the 1:10 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

APP 2.3 Completion of the MPN

After all MPN dilutions have been completed, refer to the MLG for each pathogen being analyzed to determine incubation times and temperatures and procedures for completion of the analyses.

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APP 2.4 Example calculations

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

$$\text{b}^2 \text{ Numerator} = \frac{\text{No. positive tubes}}{\text{Denominator}}$$

Denominator **No. tubes inoculated**

APP 2.5 Reference(s):

Richard P. Mageau, Most Probable Number Tables, Appendix Volume 1 & 2, Revision #1; 1/10/01, Microbiology Laboratory Guidebook, 3rd ed. USDA Food Safety Inspection Service.

Garthright, W.E., Bacteriological Analytical Manual Online, Appendix 2, Most Probable Number Determination from Serial Dilutions, USFDA, CFSAN, 2001.

APHA's Compendium of Methods for the Microbiological Examination of Foods (4th edition, 2001, chapter 6).

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.
3-0-0	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.	-