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9221 C. Estimation of Bacterial Density

1. Precision of the Multiple-Tube Fermentation Test

The multiple-tube fermentation test is not very precise unless many sample portions are examined, so use caution when interpreting the sanitary significance of any single coliform result. Precision improves greatly when several samples from a given sampling point are estimated separately and their geometric mean is calculated.

Although most probable number (MPN) tables and calculations are described for use in the coliform test, they also can be used to determine the MPN of any organism so long as suitable test media are available. Online MPN calculators are available, but until a calculator's accuracy has been verified, confirm its results using an MPN table in this section.

2. Use of Tables to Determine MPN

Record the coliform concentration as MPN/100 mL. The MPN values for a variety of positive and negative tube combinations are given in Table 9221:2, Table 9221:3 and Table 9221:4. The sample volumes indicated in Tables 9221:2 and 3 are chosen especially for drinking water examinations. Table 9221:4 illustrates MPN values for combinations of positive and negative results when five 10-mL, five 1.0-mL, and five 0.1-mL sample-portion volumes of nonpotable water are tested. If the sample-portion volumes tested are identical to those found in the tables, then report the value corresponding to the appropriate combination of positive and negative results as the MPN/100 mL. However, if the series of decimal dilutions is different, then select the MPN value in Table 9221:4 that corresponds to the combination of positive results and calculate the actual MPN using the following formula:

$$\text{MPN/100 mL} = (\text{Table MPN/100 mL}) \times 10/V$$

where:

V = volume of sample portion at the lowest selected dilution.

If the decimal series[1] includes more than 3 dilutions, use the following guidelines to select the 3 most appropriate dilutions and then use Table 9221:4 and the equation above to calculate the MPN. See Table 9221:5, which provides several examples (A-G) of combinations of positives. First, remove the highest dilution (smallest sample volume) if it has all negative tubes and at least one remaining dilution has a negative tube. Next, remove the lowest dilution (largest sample volume) if it has all positive tubes and at least one remaining dilution has a positive tube. According to these guidelines, the 3 dilutions in Example A are selected by removal of the highest (0.001-mL) and the lowest (10-mL) dilutions.

If the lowest dilution does not have all positive tubes, and several of the highest dilutions have all negative tubes, then remove the highest negative dilutions (Example B).

More than 3 dilutions may remain after removal of the lowest dilution with all positive tubes and high dilutions with all negative tubes. In this case, if the highest dilution with *all* positive tubes is within 2 dilutions of the highest dilution with *any* positive tubes, then use the highest dilution with *any* positive tubes and the 2 immediately lower dilutions. In Example C, the highest dilution with all positive tubes is 0.1 mL, which is within 2 dilutions of 0.001 mL, which has 1 positive tube. In Example D, the highest

Table 9221:2. MPN Index and 95% Confidence Limits for All Combinations of Positive and Negative Results When Five 20-mL Portions Are Used

No. of Tubes Giving Positive Reaction Out of 5 (20 mL Each)	MPN Index/ 100 mL	95% Confidence Limits (Exact)	
		Lower	Upper
0	<1.1	–	3.5
1	1.1	0.051	5.4
2	2.6	0.40	8.4
3	4.6	1.0	13
4	8.0	2.1	23
5	>8.0	3.4	–

Table 9221:3. MPN Index and 95% Confidence Limits for All Combinations of Positive and Negative Results When Ten 10-mL Portions Are Used

No. of Tubes Giving Positive Reaction Out of 10 (10 mL Each)	MPN index/ 100 mL	95% Confidence Limits (Exact)	
		Lower	Upper
0	<1.1	–	3.4
1	1.1	0.051	5.9
2	2.2	0.37	8.2
3	3.6	0.91	9.7
4	5.1	1.6	13
5	6.9	2.5	15
6	9.2	3.3	19
7	12	4.8	24
8	16	5.8	34
9	23	8.1	53
10	>23	13	–