ESTIMATION OF COLIFORM DENSITIES BY THE MPN METHOD

As the phrase “most probable number” implies, whatever value is obtained is just “most probable.” It also follows that the techniques of probability will be used to obtain this number. Consider the 10-mL portions (dilution) inoculated into the five replicate tubes.

If α is the expected number of bacteria per mL, the expected number of bacteria in the 10 mL (in one tube) is $\lambda = 10\alpha$. Thus, from the Poisson probability, for any one tube, the probability that there will be no bacteria ($X = y = 0$) is:

$$Prob(X = y = 0) = \frac{\lambda^{y}}{y!}e^{-\lambda} = \frac{(10\alpha)^{0}}{0!}e^{-10\alpha} = e^{-10\alpha}$$

Let q be the number of negative tubes and p be number of positive tubes in the five replicate tubes. (Note that the symbols q and p hold for any number of replicate tubes, not only 5 tubes.) These q negative results occur at the same time, so they are intersection events. Thus, from the probability of intersection events, Equation for the q negative results becomes:

$$Prob(X = y = 0) = (e^{-10\alpha})^{q}$$

The p positive tubes also are intersection events. If the probability of a negative result is $e{-}10\alpha$, the probability of a positive result is 1-$e^{-10\alpha}$ By analogy, the intersection probability of the p events is :

$$Prob(X = y \neq 0) = [1 - e^{-10\alpha}]^{p}$$

Of course, the q negative results and the p positive results are all occurring at the same time; they are also intersection events to each other. Combining the q and the p results constitutes the dilution experiment. Because the intersection of the q and the p events is the dilution, call the corresponding probability as $Prob(D)$, where D stands for dilution. Thus, from the intersection probabilities of the q and p events, the probability of the dilution is:

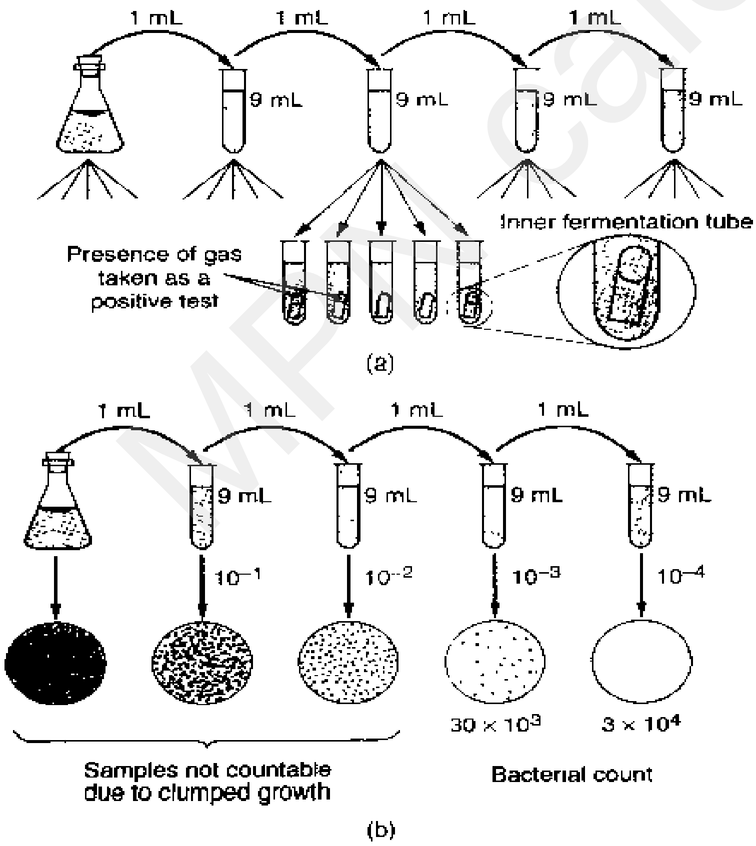
$$Prob(D) = (1 - e^{-10\alpha})^{p}(e^{-10\alpha})^{q}$$

Let the number of dilutions be equal to j number, where each dilution has a sample size of m mL (instead of the 10 mL). Each of these j dilutions will also be happening at the same time, and are, therefore, intersection events among themselves. Thus, in this situation, Equation becomes:

$$Prob(D) = \prod_{i=1}^{i=j}[(1 - e^{-m_i\alpha})^{p_i}(e^{-m_i\alpha})^{q_i}]$$

where $\prod$ is the symbol for the product factors. The product factors are used, because the j number of dilutions are intersection events. Now, we finally arrive at what specifically is the quantitative meaning of MPN; this is gleaned from above Equation . In this equation, for a given number of serial dilutions and respective values of q and p, the value of α that will make $Prob(D)$ a maximum is the most probable number of organisms. Thus, to get the MPN, the above equation may be differentiated with respect to α and the result equated to zero to solve for α. This scheme is, however, a formidable task. The easier way would be to program the equation in a computer. For a given number of serial dilutions and corresponding values of q and p, several values of α are inputted into the program. This will generate corresponding values of $Prob(D)$, along with the inputted values of α. The largest of these probability values then gives the value of α that represents the MPN. A table may then be prepared showing serial dilutions and the corresponding MPN.

Multiple-Tube Fermentation. The multiple-tube fermentation technique is based on the principle of dilution to extinction as illustrated on Fig. 2–33. Concentrations of total coliform bacteria are most often reported as the *most probable number per 100 mL* (MPN/100 mL). The MPN is based on the application of the Poisson distribution for extreme values to the analysis of the number of positive and negative results obtained when testing multiple portions of equal volume and in portions constituting a geometric series. It is emphasized that the MPN is not the absolute concentration of organisms that are present, but only a statistical estimate of that concentration. The



complete multiple-tube fermentation procedure for total coliform involves three test phases identified as the presumptive, confirmed, and completed test. A similar procedure is available for the fecal coliform group as well as for other bacterial groups (Standard Methods, 1998).

The MPN can be determined using the Poisson distribution directly, MPN tables derived from the Poisson distribution, or the Thomas equation.

The joint probability (based on the Poisson distribution) of obtaining a given result from a series of three dilutions is given by Eq. (2–74). It should be noted that Eq. (2–74) can be expanded to account for any number of serial dilutions.

$$y = \frac{1}{a}\left[(1 - e^{-n_1\lambda})^{p_1}(e^{-n_1\lambda})^{q_1}\right]\left[(1 - e^{-n_2\lambda})^{p_2}(e^{-n_2\lambda})^{q_2}\right]\left[(1 - e^{-n_3\lambda})^{p_3}(e^{-n_3\lambda})^{q_3}\right] \qquad (2\text{–}74)$$

where y = probability of occurrence of a given result
a = constant for a given set of conditions
n_1, n_2, n_3 = sample size in each dilution, mL
λ = coliform density, number/mL
p_1, p_2, p_3 = number of positive tubes in each sample dilution
q_1, q_2, q_3 = number of negative tubes in each sample dilution

When the Poisson equation or MPN tables are not available, the Thomas equation (Thomas, 1942) can be used to estimate the MPN.

$$\text{MPN/100 mL} = \frac{\text{number of positive tubes} \times 100}{\sqrt{\left(\begin{array}{c}\text{mL of sample in}\\ \text{negative tubes}\end{array}\right) \times \left(\begin{array}{c}\text{mL of sample in}\\ \text{all tubes}\end{array}\right)}} \qquad (2\text{–}75)$$

In applying the Thomas equation to situations in which some of the dilutions have all five tubes positive, the count of positive tubes should begin with the highest dilution in which at least one negative result has occurred. The application of the Thomas equation is illustrated in Example 2–13.

EXAMPLE 2–13 **Calculation of MPN Using Multiple-Tube Fermentation Test Results** The results of a coliform analysis using the multiple-tube fermentation test for the effluent from an intermittent sand filter (see Chap. 11) are as given below. Using these data, determine the coliform density (MPN/100 mL) using the Poisson equation, the Thomas equation, and the MPN tables given in Appendix E.

Size of portion, mL	Number positive	Number negative
1.0	4	1
0.1	3	2
0.01	2	3
0.001	0	5

Solution

1. Determine the MPN using the Poisson equation [Eq. (2–74)]. Substitute the appropriate values for n, p, and q and solve the Poisson equation by successive trials.

   $n_1 = 1.0$ $\quad p_1 = 4$ $\quad q_1 = 1$

   $n_2 = 0.1$ $\quad p_2 = 3$ $\quad q_2 = 2$

   $n_3 = 0.01$ $\quad p_3 = 2$ $\quad q_3 = 3$

   $n_4 = 0.001$ $\quad p_4 = 0$ $\quad q_4 = 5$

   a. Substitute the coefficient values in Eq. (2–74) and determine ya values for selected values of λ.

$$y = \frac{1}{a}[(1 - e^{-1.0\lambda})^4(e^{-1.0\lambda})^1][(1 - e^{-0.1\lambda})^3(e^{-0.1\lambda})^2][(1 - e^{-0.01\lambda})^2(e^{-0.01\lambda})^3][(1 - e^{-0.001\lambda})^0(e^{-0.001\lambda})^5]$$

λ	ya
3.80	3.6754×10^{-7}
3.84	3.6773×10^{-7}
3.85	3.6774×10^{-7}
3.86	3.6773×10^{-7}
3 90	3.6755×10^{-7}

   b. The maximum value of ya occurs for a λ value of 3.85 organisms per milliliter. Thus the MPN/100 mL is

   MPN/100 mL = 100 × 3.85 = 385

2. Determine the MPN using the Thomas equation [Eq. (2–75)].
   a. Number of positive tubes (4 + 3 + 2) = 9
   b. mL of sample in negative tubes = [(1 × 1.0) + (2 × 0.1) + (3 × 0.01) + (5 × 0.001)] = 1.235
   c. mL of sample in all tubes = [(5 × 1.0) + (5 × 0.1) + (5 × 0.01) + (5 × 0.001)] = 5.555

$$\text{MPN/100 mL} = \frac{9 \times 100}{\sqrt{(1.235) \times (5.555)}} = 344/100\text{ mL}$$

3. From Appendix G, eliminating the portion with no positive tubes, as outlined, the MPN/100 mL is 390.

Comment It should be noted that MPN tables were developed for use before the advent of the small hand-held scientific calculator as a means of computing the results from a multiple-tube fermentation test. With the use of the scientific calculator, the results from all of the serial dilutions can be considered.