

MICROBIOLOGY 102 - VIRTUAL EXPERIMENT 15B SOLUTION TO THE "MPN" PROBLEM

A sample of lake water was diluted and inoculated into plates of **Plate Count Agar (PCA)** and tubes of **Lactose Lauryl Tryptose Broth (LLTB)** with Durham tubes. After incubation, the results were obtained as indicated on the following table. (Note: **Each** of the three columns of results on this table shows the observations for **two plates** and **three tubes**.)

dilution of lake water		10 ⁰	10 ⁻²	10 ⁻²
amount inoculated		0.1 ml	1.0 ml	0.1 ml
plated dilution		10 ⁻¹	10 ⁻²	10 ⁻³
dilution factor (inverse of p.d.)		10 ¹	10 ²	10 ³
colony count on PCA		too numerous	88 & 84	9 & 6
no. of LLTB tubes with:	growth	3	2	2
	gas bubble	3	2	1

- a. Determine the number of CFUs **per ml** of the lake water.

Using the formula from Appendix C:

$$\text{ave. no. of colonies} \times \text{dil. factor} = \text{CFU/ml of sample}$$

$$86 \quad \times \quad 10^2 \quad = \quad 8.6 \times 10^3$$

- b. Determine the presumptive, most probable number of coliforms **per ml** of the lake water. (Answer below.)

MINI MPN TABLE

number of positive tubes			MPN per inoculum of middle tubes
first set	middle set	last set	
3	3	3	>24
3	3	2	11.0
3	3	1	4.6
3	3	0	2.4
3	2	2	2.1
3	2	1	1.5
3	2	0	0.93
3	1	1	0.75
3	1	0	0.43
2	2	1	0.28
2	2	0	0.21
2	1	0	0.15
2	0	0	0.091
1	1	0	0.073
1	0	0	0.036
0	0	0	<0.036

- As the presumptive detection of coliforms is indicated by **growth and gas** in LLTB, we use the three values in the bottom row of the results table – i.e., **3 2 1**.
- Using the table on the right, **3 2 1 corresponds with 1.5** which is the average number of presumed coliforms inoculated into the **middle set of tubes** where each tube had received 1 ml of a 10⁻² dilution (a 10⁻² "plated dilution"). This is equivalent to **0.01 ml** of sample per tube. (Recall that the plated dilution is always equivalent to the amount of undiluted sample being plated.)
- Therefore, **1.5 coliforms in 0.01 ml = 1.5 X 10² coliforms/ml**. The answer is also achieved by taking **1.5 X the dilution factor of the middle set of tubes**.