

A “VIRTUAL EXPERIMENT” TO GO ALONG WITH EXPERIMENT 15 IN MICROBIOLOGY 102

This virtual experiment is like “Virtual Experiment 4” in that (1) it is meant to be an exercise in bacterial quantitation where the setup of each problem is like an experimental situation in a lab where this procedure takes place, and (2) it is not yet finalized such that it can be added to our Virtual Lab Manual (which has its own numbering system) where most of our virtual experiments presently appear.

The basis of the Most Probable Number (MPN) method of enumerating bacteria has its explanation in our web page at <http://www.jlindquist.net/generalmicro/102dil3.html>. Please read through this page, and note the examples given at the end and how they are worked out.

You can work through the following problem, and the answer will subsequently be given in an update on the website’s homepage. Note that this problem also includes a determination of “total” CFUs per ml which is generally run along with a quantitation of coliforms by the MPN Method.

A sample of lake water was diluted and inoculated into plates of **Plate Count Agar** (PCA) and tubes of **Lactose Lauryl Tryptose Broth** (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
For convenience, you can indicate the plated dilution or dilution factor here.				
colony count on PCA		too numerous	88 & 84	9 & 6
# of tubes showing	growth	3	2	2
	gas bubble	3	2	1

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

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

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