To get partial credit, please show all your calculations. If you give only a number answer, and it's incorrect, you will get zero on that question.

1) Plate count lab: A) Report the number of colonies counted on each dilution plate. If a particular dilution was uncountable, then label as such. From the statistically meaningful plates, calculate the concentration of bacteria in the original sample. Specify which plate count is being used for the calculation; also include the Winogradsky column number the samples were taken from. B) Report the colonies counted on the fecal coliform plates, the volume of sample water filtered, the location of where the sample was collected and calculate the number of fecal coliforms per 100 ml of sample. Indicate if the water is safe to drink, safe to shellfish, or safe to swim in.

2) DAPI count lab: Report the average number of bacteria per grid counted under the microscope using the DAPI stain. From this number, calculate the bacterial concentration in the original sample. Specify the volume of sample you stained/filtered. Also specify which Winogradsky column the data are from. Note, the size of the grid in the microscope is approximately 100 µm x 100 µm with the 100x objective.

3) A) What did we use to preserve the bacterial samples in Tuesday’s lab for later counting? B) Why did we add a preservative?

4) A) What are two disadvantages of using Acridine Orange for direct bacterial counts? B) What cellular macromolecule(s) does Acridine Orange bind to?

5) Why are bacterial numbers measured by plate counts lower than bacterial numbers measured by direct counts?

6) A) What organism caused the largest outbreak of a waterborne disease in US history? B) Does the fecal coliform count measure the concentration of pathogenic organisms in a water sample? Explain your answer.

7) A) Assuming a bacterial concentration of $5.4 \times 10^6$ cells ml$^{-1}$ (this notation is the same as cells/ml) and that a cell has 20 fg C (f is femto, $10^{-15}$), what is the carbon concentration associated with the bacterial biomass? Please give your answer in µmol C l$^{-1}$. B) If we assume that the bacteria in (A) were growing at 1 d$^{-1}$, and had a growth efficiency of 20%, what would the rate (in µmol C l$^{-1}$ d$^{-1}$) of CO$_2$ production by bacteria be?

8) A) Mechanistically, it would be possible to stain bacteria with DAPI, then put a drop of the stained bacteria on a microscope slide and count them. Then why do we filter the sample in the DAPI method? B) Why do we run a blank along with the sample in the DAPI counts? C) If the blank was not zero, how would you correct your counts?

9) Why do we use fluorescence and not phase contrast to count bacteria in natural samples?

10) A) What benefit does immersion oil provide in light microscopy? B) What is the approximate size of the smallest object that can be resolved using light microscopy?