Lecture 7

I. Quantization of bacteria

A. This is an attempt to determine the number of bacteria per mL in a culture. This can be done either directly or indirectly.

1. Direct methods

a. _____

i. This means one actually counts each bacterial cell on a microscope slide.

Advantage: No overnight incubation is required.

Disadvantages: This takes a great deal of time and we _____ _____.

b. The _____ (SPC) method

i. A _____. Set aliquots of the final, dilute solution are spread on agar plates.

ii. Every cell that was spread onto the plate develops into a _____. These colonies can be counted and, since they originated from a single cell, they _____ _____that was spread onto the plate.

iii. The dilutions made from the original culture can then be taken into account and one can _____ _____ to determine the _____of the original culture.

Advantages: Only counts _____; can measure really low concentrations; doesn't require lots of instrumentation or training.

Disadvantages: Aggregates of bacteria will form _____ if not well dispersed; results are not immediate, as you have to wait for growth.

2. Indirect methods

a. Spectrophotometer: Measures _____

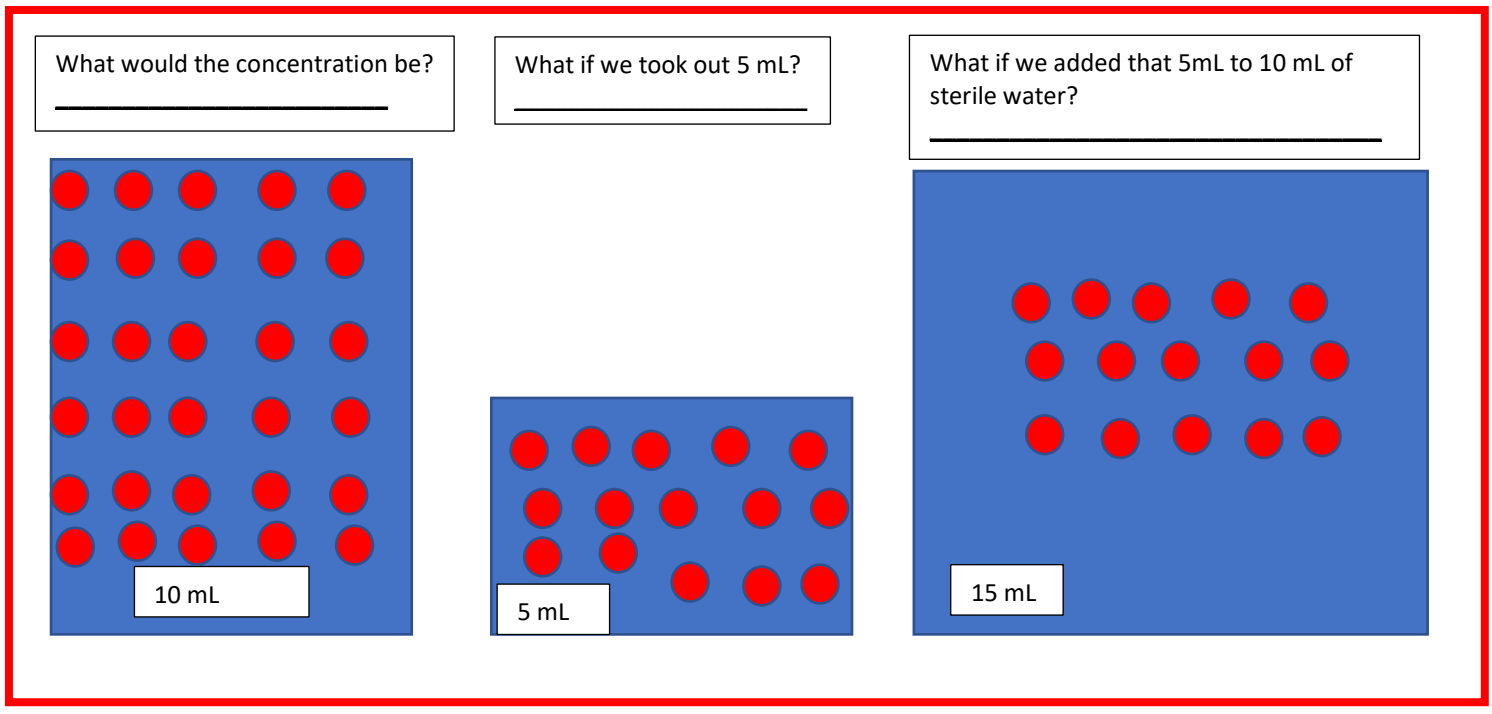
b. Measurement of _____.

c. Measurement of _____ products.

B. Determining concentration

1. Concentration is the ratio of the mass or volume of a substance (solute) to the mass or volume of the solvent or solution.

2. Often in microbiology we use concentration to determine amounts of cells, proteins, DNA, etc.



How it's done: Dilutions

Remember that the standard plate count, or SPC, method requires one to figure backwards to the original culture concentration, or titer, by taking into account the number of colonies on the plates and the dilutions that were made before an aliquot was spread onto a plate. There are two methods that can be used to determine the titer of original cultures. The following is an example of a set of dilutions that could be made on a culture before plating.

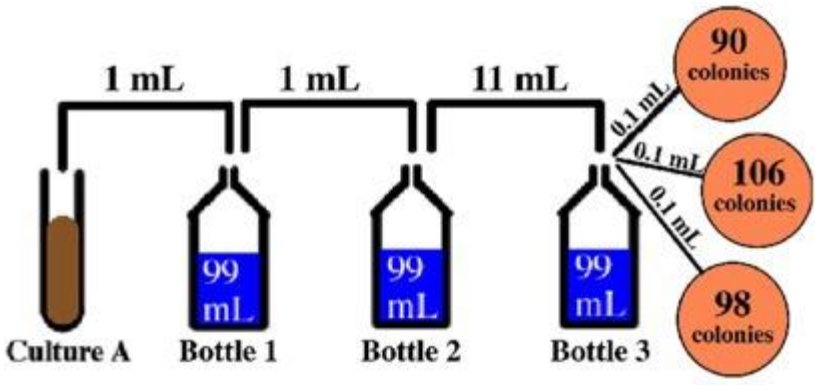
Note: An assignment you will be required to turn in deals with dilutions. See page 159 in the appendix for the assignment.

Countable Colonies

A plate with "countable colonies" has between 30 and 300 colonies. Less than 30 is Too Few To Count (TFTC). Over 300 is Too Numerous to Count (TNTC)

Method 1

First, consider that the number of colonies on each plate indicates the number of bacterial cells that were in the final 0.1 mL aliquot that was spread on the plate. Each of the three plates shown in the figure were spread with the same volume of liquid (0.1 mL) from Bottle 3, so we can average the number of colonies on the three plates. Note that we can only do this because all the plates have "countable colonies":



90 + 106 + 98 = 294 colonies
294 colonies / 3 plates = 98 colonies per plate

Remember this is also the average number of cells per 0.1 mL of fluid taken from Bottle 3. Thus, the concentration, or titer, of Bottle 3 can be calculated:

98 cells / 0.1 mL = 980 cells/mL in Bottle 3

In order to figure the titer of Bottle 2, we must recall lessons from General Chemistry and the equation $C_1V_1 = C_2V_2$, where C_1 is the concentration of the more concentrated solution, V_1 is the volume taken out of that solution, C_2 is the concentration of the more dilute solution, and V_2 is the final total volume of that solution. Let's manipulate this equation to apply to our current situation and allow us to calculate the concentration of

Bottle 2:

$$C_{\text{bottle 2}}\ V_{\text{removed from Bottle 2}} = C_{\text{Bottle 3}}\ V_{\text{final in Bottle 3}}$$

$$(C_{\text{Bottle 2}})(11\text{ mL}) = (980\text{ cells/mL})(110\text{ mL})$$

$$C_{\text{Bottle 2}} = 9.8 \times 10^3\text{ cells/mL}$$

We can use this same equation to determine the titer in Bottle 1 and finally in Culture A:

$$C_{\text{Bottle 1}}\ V_{\text{removed from Bottle 1}} = C_{\text{Bottle 2}}\ V_{\text{Final in Bottle 2}}$$

$$(C_{\text{Bottle 1}})(1\text{ mL}) = (9.8 \times 10^3\text{ cells/mL})(100\text{ mL})$$

$$C_{\text{Bottle 1}} = 9.8 \times 10^5\text{ cells/mL}$$

$$C_{\text{Culture A}}\ V_{\text{removed from Culture A}} = C_{\text{Bottle 1}}\ V_{\text{final in Bottle 1}}$$

$$(C_{\text{Culture A}})(1\text{ mL}) = (9.8 \times 10^5\text{ cells/mL})(100\text{ mL})$$

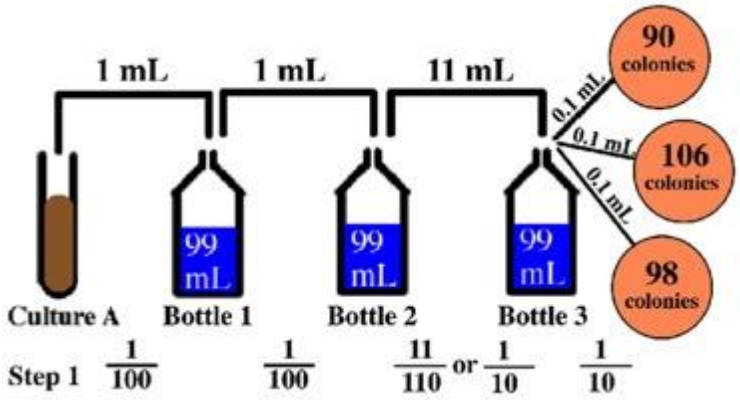
$$C_{\text{Culture A}} = 9.8 \times 10^7\text{ cells/mL}$$

Method 2:

This method uses more of a pictorial approach.

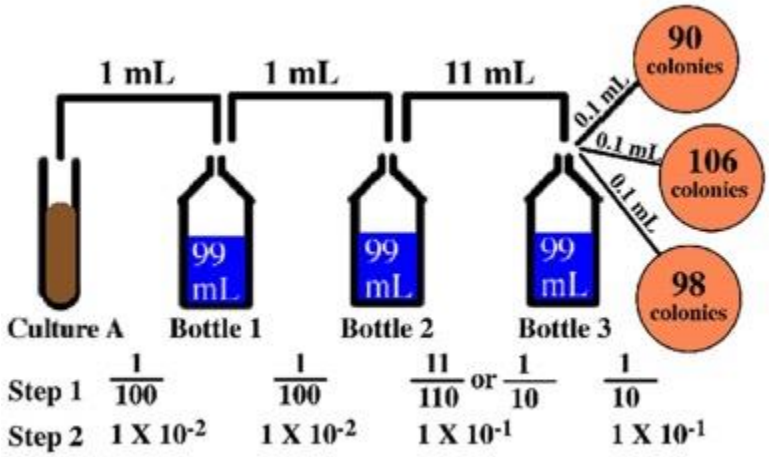
Step 1:

The first step of this method is to write each dilution as a fraction with the volume transferred out of the more concentrated solution over the total volume of the less concentrated solution. In the final transfer, 0.1 mL is transferred out of the more concentrated solution and put directly on the plate. This is a 1/10 dilution.



Step 2:

In the second step of this method, the fractions are converted to scientific notation.



Step 3:

In the third step, the product of the individual dilution factors is calculated to give the final dilution factor:

$$(1 \times 10^{-2})(1 \times 10^{-2})(1 \times 10^{-1})(1 \times 10^{-1}) = \text{Final dilution factor}$$

Step 4:

In microbiology, the reciprocal of the final dilution factor is called the plating factor. In step 4, the plating factor is calculated.

$$\text{Plating Factor (p.f.)} = 1/(1 \times 10^{-6})$$
$$\text{Plating Factor (p.f.)} = 1 \times 10^{6}$$

Step 5:

Finally, to determine the concentration of Culture A, the average number of colonies (as calculated in method 1) is multiplied by the plating factor.

$$\text{p.f. x average number of colonies = titer of original culture } (1 \times 10^{6})(98) = 9.8 \times 10^{7} \text{ bacteria/mL}$$

Please feel free to use either method to determine titer of original cultures. Method 1 is more concise and more clearly tracks units. In microbiology, method 2 is commonly used and, even if you select method 1, it will be necessary to be familiar with the term "plating factor."

Significant figures:

Experimentally, the number of significant figures that can be retained is determined by the accuracy of the measuring devices selected. Consider a 5 mL pipet shown in the figure above. Three significant figures could be retained when using this device because two figures can be read and the third is allowed to have uncertainty. Practice finding 4.55 mL on this pipet. In problems that are not encountered in the lab (such as the dilution problems assignment), retain the number of significant figures you are given.