Microbiology Laboratory 11

Serial Dilutions

Background

Many areas of science use serial dilutions in the preparations for different experiments. Serial dilutions are usually made in increments of 1000, 100 or 10. The concentration of the original solution and the desired concentration will determine how great the dilutions need to be and how many dilutions are required. Important also is the total volume of solution needed. If only small quantities of solutions are needed then greater numbers of dilutions are necessary.

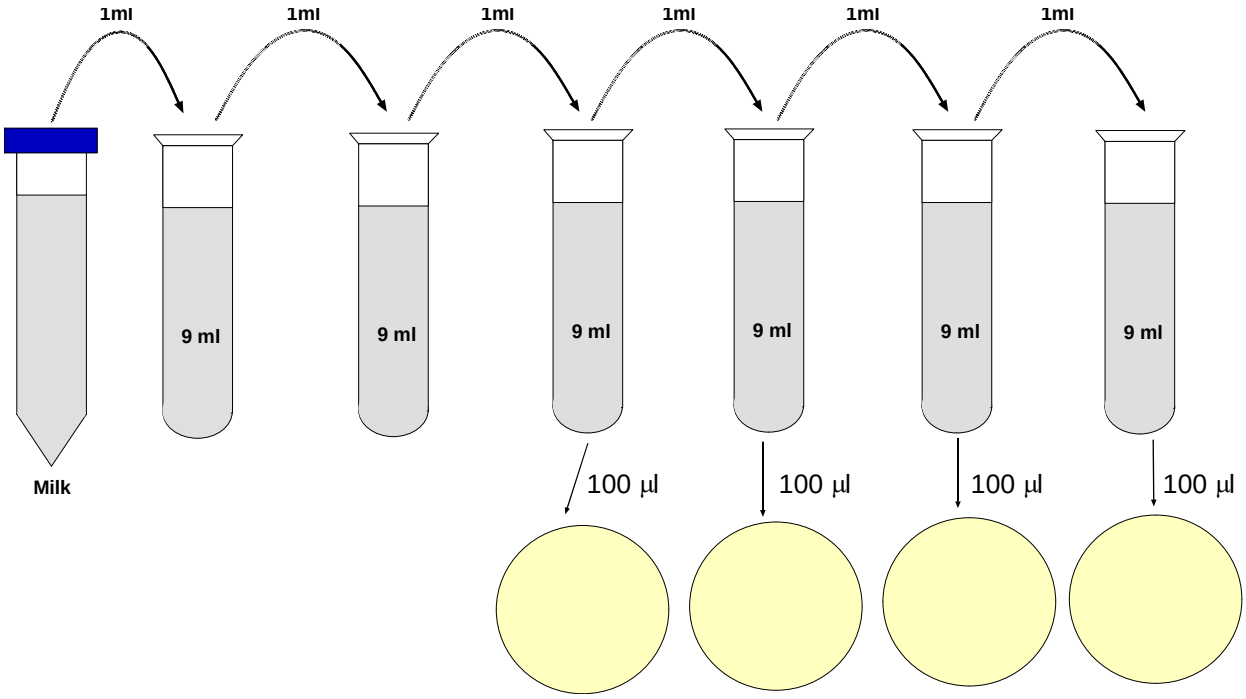
The most common uses of serial dilutions are to determine the concentration of cells or the concentration of a solute. It is helpful but not necessary to know an approximate concentration at the start of the experiment. In order to arrive at the desired concentration, use serial dilutions, instead of making one big dilution. This method is not only cost effective but, it also allows for small aliquots to be diluted instead of unnecessarily large quantities of materials.

This technique involves the removal of some of the original solution and then adding it to another container which contains a known amount of the same buffer as the original solution. This type of dilution describes the ratio of the solute to the final volume of the diluted solution.

In this lab we will be making a series of $^1/_{10}$ dilutions. The amount transferred divided by the total amount of the final solution is the dilution factor. So in this case when you transfer 1 ml of the solution to 9 ml of water, the final total volume is 10 ml. The dilution factor is the amount transferred (1 ml) divided by the total amount (10 ml) or $^1/_{10}$.

Lab Assignment - Dilutions

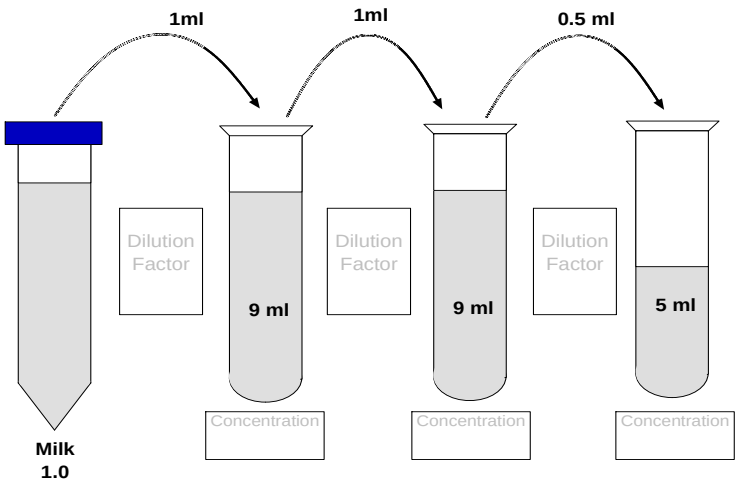
1. Obtain several tubes containing 9ml of sterile water.
2. Using a micropipetter, transfer 1ml of either pasteurized or unpasteurized milk to the first tube.
3. Using a different tip, transfer 1ml from your water + milk tube to a new water tube.
4. Continue in like manner till you have 5 or 6 tubes.
5. Transfer 100µl from the last four tubes to four separate NA plates.
6. Spread the transferred 100µl around the NA plate with a sterile hockey stick.
7. Once your plates have dried, label each plate (concentration), and incubate inverted at 37°C.



Serial Dilution Calculations Part 1

Calculate the Dilution Factors and the Concentrations. Write the answers in the correct box in the figure to the right.

Be sure you know how the numbers were obtained (you may see them again on a lab quiz).



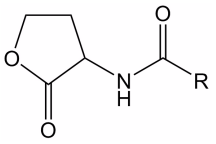
Quorum Sensing

Background

Many functions in bacteria are regulated by other bacteria in a process called autoinduction or quorum sensing (QS). In QS, the bacteria constantly produce small highly diffusible molecules which cross the plasma membrane to the external environment. At low levels these molecules produce no evident effect. As population density increases or as diffusion is constrained, as in a biofilm for example, the concentration of these molecules increases. At the correct concentration enough of the molecules will diffuse into other cells and be bound by special receptor proteins. Once the intracellular receptor proteins bind the diffusible molecule, they can then turn on or off certain genes. In this manner, prokaryotes can communicate with other prokaryotes.

Quorum sensing was first described as the mechanisms responsible for controlling bioluminescence in *Vibrio fisherii*, a Gram-negative marine bacteria. Initially, it was thought a quaint if interesting phenomenon. This view changed rapidly as other molecules were identified and were shown to be functioning in a great number of additional bacterial systems including virulence, motility, pigment production, and the development of normal biofilm architecture. One class of quorum sensing molecules that has been found to be expressed by several bacteria are the acyl-homoserine lactones (AHLs).

In this lab, we will use the Gram-negative bacterium, *Chromobacterium, violaceum*. This bacterium, can produce a water-insoluble purple pigment with antibacterial activity called violacein when properly induced by AHLs. We will use three strains of the bacterium.



The **wildtype** produces AHLs and responds to AHLs by producing the pigment, violacein.

The **reporter strain** can not produce any AHLs but does respond to AHLs produced by other bacteria (strains) by producing the pigment.

Lab Assignment - Culture

Working in pairs . . .

1. Inoculate a NA plate, as shown in the diagram to the right. Be careful that your streaks get close to each other but without touching.
2. Be sure to flame you loop between each streak.
3. Label your plate, and incubate inverted at 37°C.