Microbiology Laboratory 9

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.

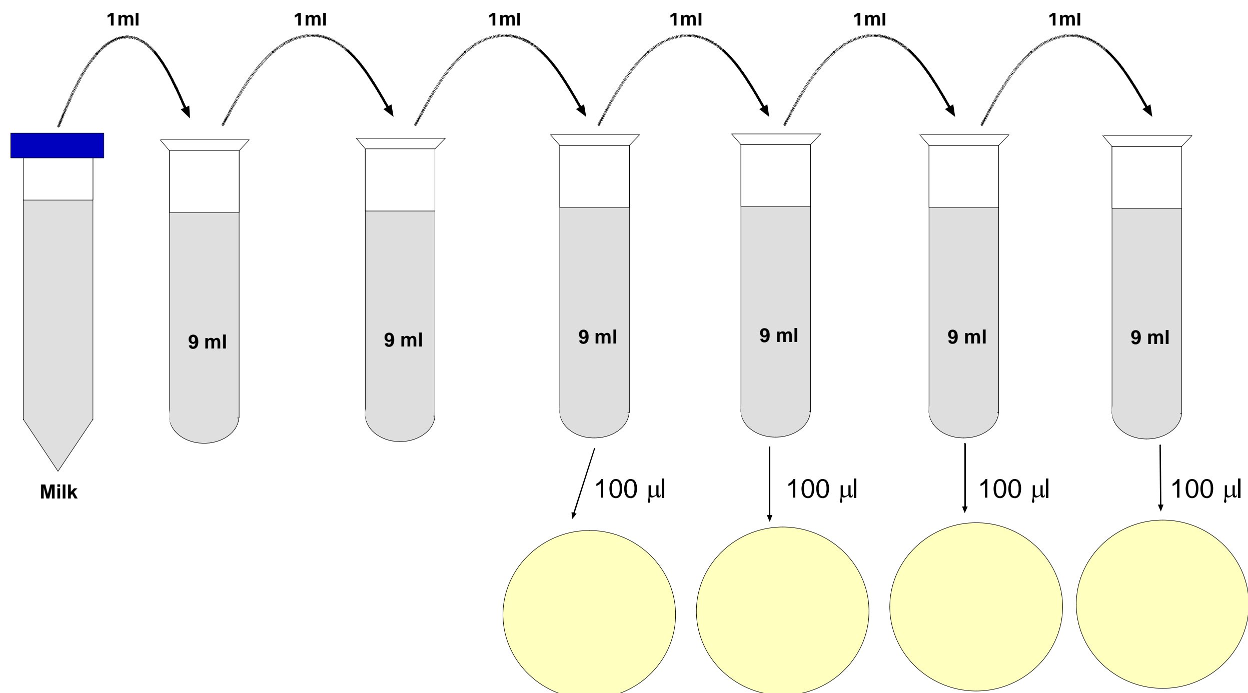
The most common uses of serial dilutions are to determine the concentration of cells or the concentration of a solute. It is helpful but not necessary to know an approximate concentration at the start of the experiment. In order to arrive at the desired concentration, use serial dilutions, instead of 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.



Blood Hemolysis

Background

Certain bacterial species produce extracellular enzymes that lyse red blood cells a process called hemolysis. The enzymes are called hemolysins or exotoxins. The hemolysins radially diffuse outwards from the colonies causing complete or partial destruction of the red cells (RBC). The destruction of the cells may or may not be associated with the complete denaturation of the hemoglobin protein within the cells. Obviously the ability to either partially or completely lyse RBCs, aids the bacteria to cause and prolong an infection

Hemolysis activity is observed when cultures are grown on a Blood Agar plate. In the US, "blood agar" is usually prepared from Tryptic Soy Agar enriched with 5% Sheep blood.

There are 3 or 4 types of hemolysis observed:

Alpha (α) hemolysis is the reduction of the red blood cell hemoglobin to methemoglobin in the medium surrounding the colony. This causes a **green or brown discoloration** in the medium surrounding the colony. The color can be equated with "bruising" the cells. Microscopic inspection of alpha-hemolyzed red blood cells shows that the cell membrane is intact, so it is not, in fact, true lysis. This type of hemolysis may be associated with the loss of potassium from the RBCs resulting in the discoloration.

Beta (β) hemolysis is defined as complete or true lysis of red blood cells. A **clear zone**, approaching the color and transparency of the base medium, surrounds the colony. The maximal activity of both the hemolysins (oxygen labile (SLO) and oxygen stable (SLS) hemolysins) of group A streptococci, is observed only in anaerobic conditions. As a result we will both streak and stab a blood agar plate. This will create an aerobic and anaerobic environment for the bacteria to grow.

Gamma (γ) hemolysis is somewhat self-contradictory. Gamma indicates the lack of hemolysis. There should be **no reaction** in the surrounding medium.

A possible fourth type of hemolysis is **Alpha prime or wide zone alpha hemolysis**. Here a small zone of intact RBCs is immediately adjacent to bacterial colony, with a clear zone of complete hemolysis surrounding the zone of intact RBCs.

Lab Assignment - Culture

1. Obtain a blood agar plate for every four bacteria (students).
2. Divide the plate into quarters by drawing lines on the bottom of the plate.
3. Inoculate each quadrant with a different bacteria (your "bug of the day") by . . .
   a. dragging your loop in a single straight line within the quadrant
   b. then immediately stab your loop into the agar (still within the same quadrant) one or two times
4. Label your plate and incubate where directed to by your instructor.

Serial Dilution Calculations Part 1

Calculate the Dilution Factors and the Concentrations and write the answers in the correct box below. Be sure you know how the numbers were obtained (you may see them again on a lab quiz).