Microbiology Laboratory 9

Observations from Last Week's Lab

Radiation Exposure

First note what species of bacteria you use. Next note what exposure of radiation was required to kill the bacteria. It could be less than 15 seconds or greater than 2 minutes or some time in between. You are looking for a plate with very few or no colonies on the exposed half.

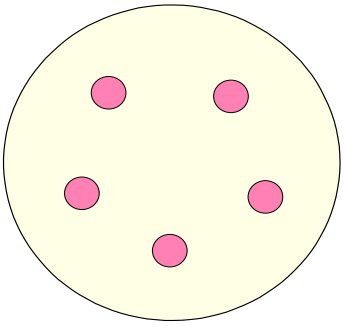
Filter Paper Disk diffusion or Kirby-Bauer Sensitivity testing

In this test paper disks containing specific concentrations of an antibiotic or an antimicrobial are placed on a lawn of bacteria on the agar surface. The compound in the disks diffuse out into the agar forming a concentration gradient. If the compound inhibits or kills the organism cultured in the lawn, there will be an area around the disk with no bacterial growth. This area of no growth is called the **zone of inhibition**. Next week after the plate has been incubated, students will measure across the entire zone, through the disk to assess the effectiveness of the compound on your organism.

Lab Assignment - Antibiotic testing

- Label the bottom of your plate so that you will be able to recognize next week.
- Transfer 100 µl of liquid culture to a labeled agar plate. Spread the culture around with an alcohol sterilized hockey stick.
- Let the agar surface dry before applying the disks.
- Bring your plate to the front to dispense the disks with an automatic dispenser.
- Remove the lid of the plate, and place the dispenser over the plate.
- Slide the arm on the side to dispense the disks.
- Remove the automatic dispenser and replace the lid of your plate.
- Back at your lab bench with a sterile forceps/hockey stick/tip tap each disk lightly to secure it to the medium.
- Invert your plate and place it in the 37°C incubator.

Lab Assignment - Antiseptic testing



- Label the bottom of your plate with some identifying mark that you will be able to recognize next week.
- Transfer 100 µl of liquid culture to a labeled agar plate. Spread the culture around with an alcohol sterilized hockey stick.
- Let the agar surface dry before applying the disks.
- Using alcohol sterilized forceps remove a sterile disk for the test tube aseptically.
- Dip the disk in your antiseptic of choice.
- Let any excess antiseptic drip off the disk then drag the disk along the inside side of the container to remove any additional excess.
- Place the disk on your agar surface with your lawn of bacteria.
- Place a total of four to five disks (each with a different antiseptic) in a circle on your plate.

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.

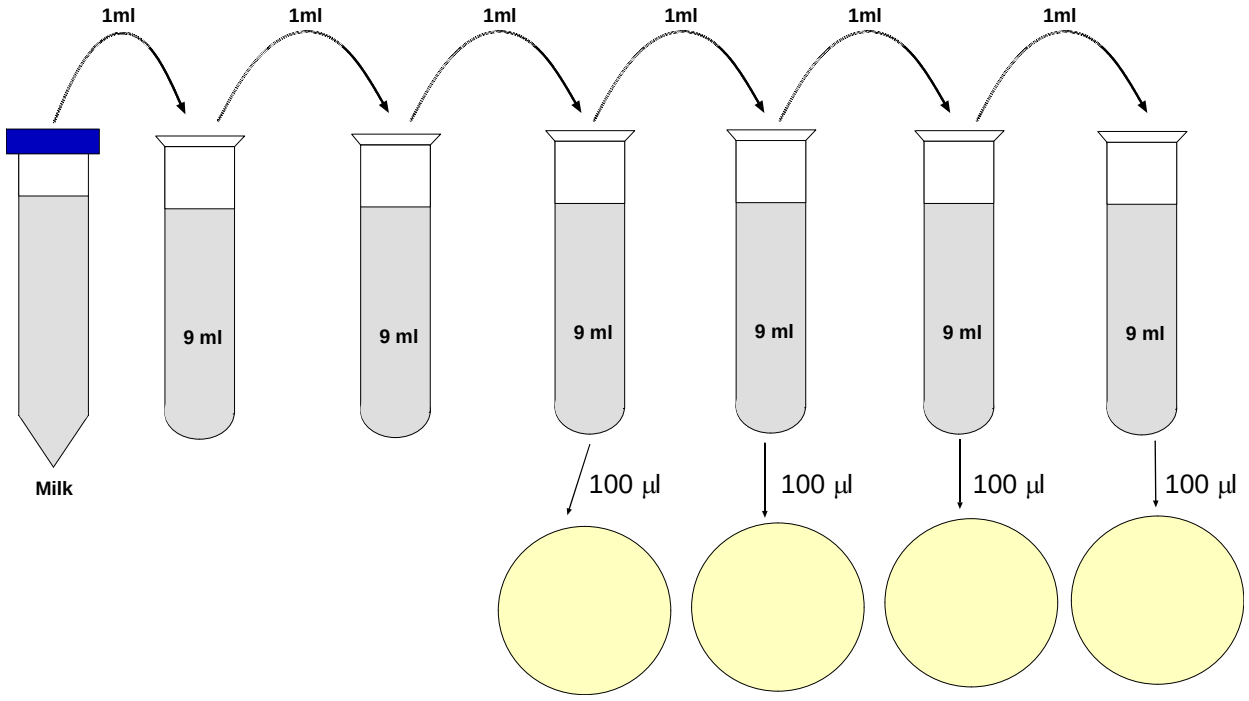
The most common uses of serial dilutions are to determine the concentration of cells or the concentration of a solute. It is helpful but not necessary to know an approximate concentration at the start of the experiment. In order to arrive at the desired concentration, use serial dilutions, instead of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