Name: _____ Block: __ Date: _____

Serial Dilution Lab

Objective:

The quantity of bacteria present in a sample is an important part of the microbiology report. In this lab two different methods for determining bacterial quantities will be incorporated; serial dilution techniques and calibrated loops. The lab will begin by preparing a 0.5 McFarland broth of E.coli and continuing with 4 serial dilutions. The quantity of bacteria in each dilution will be verified by plating the broths to nutrient agar with a 0.001 ml inoculating loop.

Background:

Many laboratory procedures involve the use of dilutions. It is important to understand the concept of dilutions, since they are a hand tool used throughout all areas of the clinical laboratory. These dilutions have to be considered as they make a quantitative difference in what is going on. First, there are several terms used in expressing dilution:
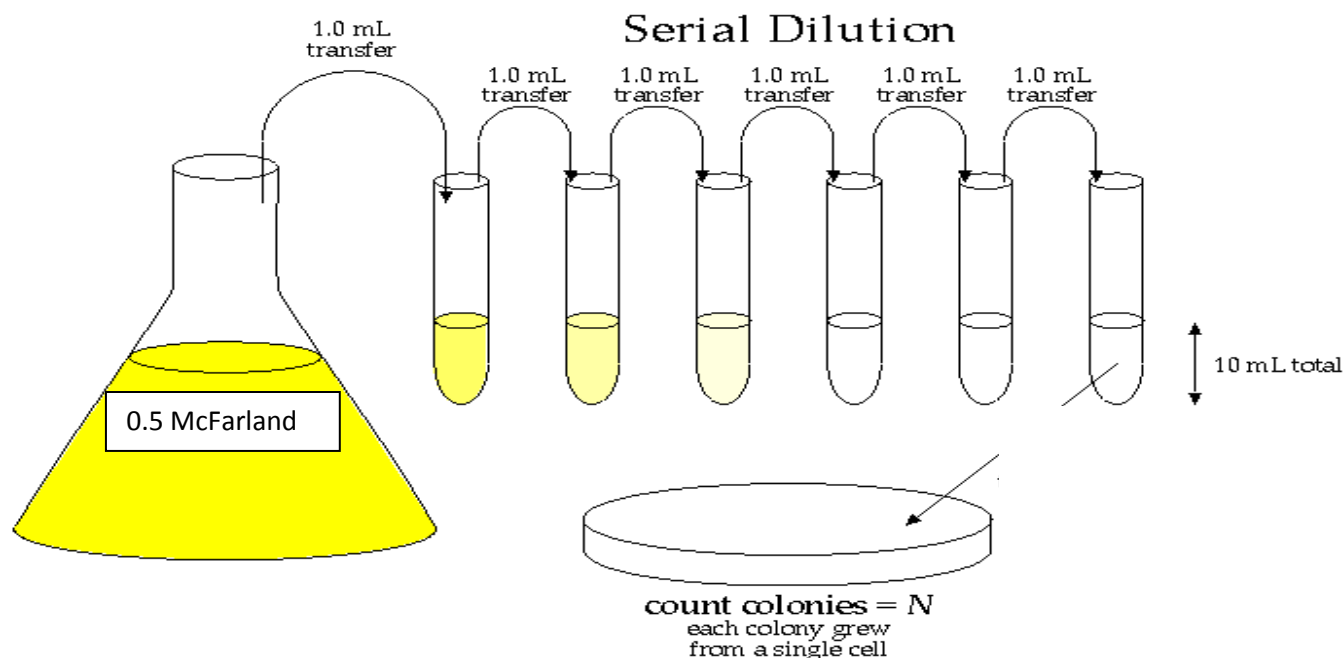
1. Dilution: - Dilutions are expressed as the ratio of the quantity of a desired solute (serum, urine, chemical solution, etc.) contained in a solvent (diluent).
2. "Diluted to" - This is essentially the same as "dilution." If 1 milliliter is diluted to 10 milliliters, enough diluent is added to the original volume to yield a final, total volume of 10 milliliters.
3. "Added to" - This expression is usually a hang-up since it is not the same as "diluted to." "Added to" refers to the volume of the solute added to a specified volume of solvent.
4. "Serial dilution" - This term is frequently used and refers to a "multiple" dilution problem. In other words, an initial dilution is made and then this dilution is used to make a second dilution, and so on.

Dilutions must be used carefully and the calculation of dilution factors must be done accurately, since an error may seriously affect a test result. Read the following material closely in order to understand the variety of ways dilutions are used.

Colony counts are an enumeration by direct count of viable, isolated bacterial or fungal cells or spores capable of growth on solid culture media. Each colony (i.e., microbial colony-forming unit) represents the progeny of a single mother cell in the original inoculum. The method is used routinely by environmental microbiologists for quantifying organisms in air, food, and water; by clinicians for measuring patients' microbial load; and in antimicrobial drug testing.

Materials:

Nutrient broth	0.001 ml inoculating loops	*E.coli* culture plate
5 test tubes	5 nutrient agar plates	1 sterile swab
4 graduated pipettes		

Serial Dilution

1.0 mL
transfer

1.0 mL
transfer

1.0 mL
transfer

1.0 mL
transfer

1.0 mL
transfer

1.0 mL
transfer

10 mL total

0.5 McFarland

count colonies = N
each colony grew
from a single cell

Procedure:

1. Prepare 50.0 ml of nutrient broth. Boil for 10 minutes.
2. Obtain 5 test tubes and label 1-5.
3. Deliver 10.0 ml of broth to tube #1 and 9.0 ml of broth to tubes #2 through #5.
4. Create a 0.5 McFarland dilution using *E.coli* in tube #1.
5. Using a graduated pipette obtain 1.0 ml of broth from tube #1 and deliver to tube #2. Use pipette to mix new dilution.
6. Transfer 1.0 ml of this new dilution from tube #2 to tube #3, again mix with pipette.
7. Transfer 1.0 ml of the dilution from tube #3 to tube #4, again mix pipette.
8. Obtain 5 nutrient agar plates, label each with the date, group number and tube #1 through tube #5.

9. Inoculate a nutrient agar plate from each tube (be sure to use a new loop for each plate). Follow the illustration below as a diagram for the streaking technique. When dipping the inoculating loop into the broth submerge only the loop under the surface of the broth. If the loop is submerged too deep it will gather more than the 0.001 ml of liquid.
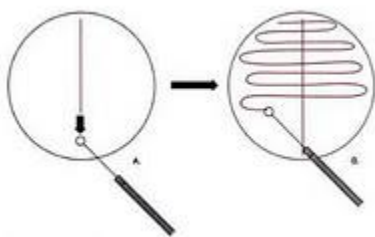
10. Incubate plates for 24 hours.
11. Record colony count on each plate using the following equation.

# of colonies	X	loop size	=	Colony Count
154		0.001 ml		
154		1000	=	154,000/ml

Data Table

	Tube #1	Tube #2	Tube #3	Tube #4	Tube #5
Cell Concentration	1.5X10^8 CFU/mL				
# of Colonies on plate					
Colony Count					

Discussion:
1. **What is the reduction factor between each tube?**

2. **What would the cell concentration be if you made tube #6?**

3. **Why is it helpful to perform the dilutions?**

4. **Explain why a colony count is equal to a living bacterial cell count.**

5. **The range in error should be no more than +/- 10 colonies. How did your colony counts compare with your cell concentrations for each tube?**