LAB 8
SERIAL DILUTION, POUR PLATES, AND ENUMERATION OF BACTERIA

Introduction

The number of bacteria in a small sample can be startling; for example, there are approximately 10^7 to 10^{10} bacteria in every gram of human feces! (That's 10,000,000 to 10,000,000,000 bacteria per g)

Direct microscopic counts of bacteria are impossible when the concentration is so high, therefore, dilution of the sample is necessary.

In this lab, a serial dilution will be made of a sample of bacteria, and then those dilutions will be used to culture bacteria in order to estimate their numbers.

Part I: Serial Dilution

To begin, you must know how to calculate dilution. The dilution of a sample in a diluent (the liquid used to dilute the sample) can be calculated as:

$$Dilution = \frac{vol.\ sample}{vol.\ sample + vol.\ diluent}$$

For example, if **1 mL** of a sample was diluted by adding it to **9 mL** of water (diluent), then:

$$Dilution = \frac{1}{1+9} = 1/10 = 10^{-1}$$

In microbiology, dilutions are usually reported as exponents. For example:

$1/10 = \mathbf{10^{-1}}$ $\qquad$ $1/100 = \mathbf{10^{-2}}$ $\qquad$ $1/1000 = \mathbf{10^{-3}}$ $\quad$ $1/10000 = \mathbf{10^{-4}}$ $\qquad\qquad$ and so on…

** You need to know the above equation, how to use it, and how to express dilutions as exponents!

A **serial dilution** is the dilution of a sample, in 10-fold dilutions. As shown in the illustration below, it begins when 1 mL of the bacterial sample is added to 9 mL, and it is mixed together (creating a 10^{-1} dilution). Then, 1 mL from that mixture is added to 9 mL, and it is mixed together (a 10^{-2} dilution). That procedure is repeated for as many dilutions as needed.
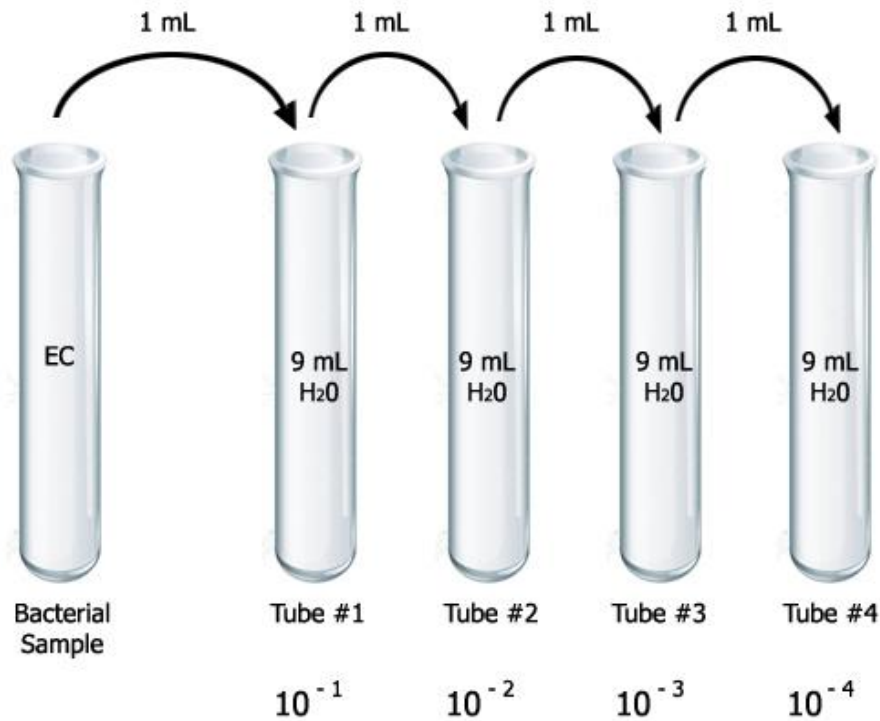
Materials and Methods:

- Bacterial sample (in a liquid medium in a test tube)
- Sterile pipette tips and pipettors
- 4 tubes containing 9 mL of sterile water each

Each group will create a serial dilution as shown in the illustration above.

** Use a new pipette tip for each transfer! **
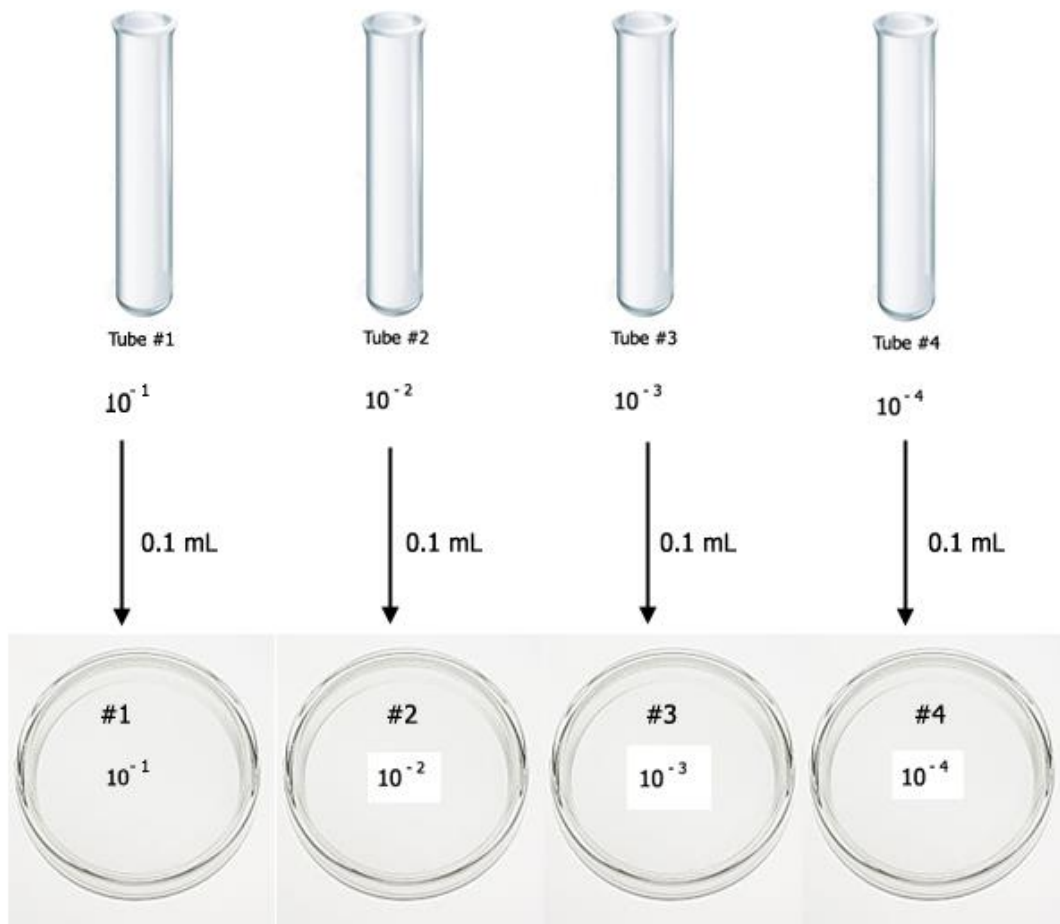
Label the tubes as #1, #2, #3, and #4.

Part II: Pour Plate Technique

Next, each group will prepare a pour plate for each of the tubes labeled #1, #2, #3, and #4.

Materials and Methods:

- Sterile pipette tips and pipettors
- The 4 tubes (tubes #1, #2, #3, and #4) containing diluted bacteria, from Part I
- 4 sterile Petri dishes (label them #2, #3, and #4)
- 4 tubes of melted Nutrient Agar (keep warm until use, so they don't solidify)

Each group will transfer **0.1 mL** (100 µL) of diluted bacteria from each tube into an <u>EMPTY</u> Petri dish, as shown below.

Tube #1	Tube #2	Tube #3	Tube #4
10^{-1}	10^{-2}	10^{-3}	10^{-4}
0.1 mL	0.1 mL	0.1 mL	0.1 mL
#1 10^{-1}	#2 10^{-2}	#3 10^{-3}	#4 10^{-4}

Once the diluted bacteria samples have been added to the Petri dishes, pour a melted Nutrient Agar into each Petri dish. Gently swirl the Nutrient Agar and diluted bacteria samples together, and let the Petri plate solidify. This is called the **pour plate** technique.

Incubate for 24 to 48 hours at 98.6°F.

Part II: Enumerating Bacterial Densities (the following week)

After incubation, count the number of bacterial colonies growing in Petri plates #1, #2, #3, and #4.
If there are more than 200 colonies on a Petri plate, stop counting and enter "TMTC" in the table below.
(TMTC stands for "Too Many To Count")

	Petri Plate #1	Petri Plate #2	Petri Plate #3	Petri Plate #4
Number of bacterial colonies				

Next, calculate the bacterial densitiy of the original bacterial sample. Because you counted colonies, and not individual bacterial cells, it will be expressed as **CFU / mL**, which stands for **Colony-Forming Units**. Calculate the CFU / mL, using the above data and the following equation:

$$CFU / mL = \text{\# colonies} \times \frac{1}{\text{dilution}} \times 10$$

For example, if you counted 21 colonies on Petri plate #3 (which was a 10^{-3} dilution) then:

$$CFU / mL = 21 \times \frac{1}{10^{-3}} \times 10$$

An easy way to calculate this is to convert the fraction into its reciprocal;

$1 / 10^{-3}$ is equal to 1000

So the calculation above is CFU / mL = 21 X 1000 X 10 = 210,000

When calculating your bacterial densities, you can use the following fraction conversions:

1 / 10^{-1} is equal to 10

1 / 10^{-2} is equal to 100

1 / 10^{-3} is equal to 1000

1 / 10^{-4} is equal to 10000

Use the space below to do your calculations:

Petri plate #1:

Petri plate #2:

Petri plate #3:

Petri plate #4:

Enter your results in the table below:

Original bacterial density (CFU / mL):

	Petri Plate #1	Petri Plate #2	Petri Plate #3	Petri Plate #4
CFU / mL				

Were the results (CFU / mL) similar among all Petri plates?   **Y   /   N**

If there was extreme variation among your estimates, remember possible sources of error, such as overlooking small colonies, mistakes in pipetting and dilution, and erroneous calculations.