BIO208 Genetics Lab
Fundamental Biology Laboratory Skills (12 points)

EXPERIMENT OBJECTIVE: The objective of this experiment is for students to REVIEW the fundamental lab skills that are critical to their success in the field of biology.

BACKGROUND: The term “Aseptic” refers to free from contamination. It is critical to ensure that the only organism that is being cultivated on a plate is the one that you are studying. Students should monitor their plates closely at each stage of the experiment and record all results, any deviations or unexpected results. The student may be required to transfer broth culture to a slant, surface spread a culture on a plate and perform the streak plate technique to isolate individual colonies.

General precautions that you should take include:

- Wear appropriate protective apparel
- Wipe down your bench top with a disinfectant
- Work in the sterile zone around a Bunsen burner
- If you are working with a wire loop make sure to heat it to redness to destroy any microbes that are present and then cool it prior to introducing it in to your tube.
- Ensure that all your material is pre-sterilized
- At the end of your experiment dispose all material in the appropriate waste disposal containers.

There are several situations in biology that require you to work with very small concentrations and/or volumes. A dilution is when one substance (e.g. water or broth) is added to another to reduce the concentration of the first substance. The original substance being diluted is called the “stock” solution. Serial dilution is a method that allows scientists to work conveniently with small concentrations and volumes. This technique involves the removal of a small amount of an original solution to another container which is then brought up to the original volume using diluents typically broth, buffer or water. A very important feature is that every dilution is sequential, meaning that you withdraw a sample from the original tube only once. A 1:10 dilution means that you would add 1 ml of original to 9 ml of diluent. While it is common to use dilutions such as 1:10, 1:100, 1:1000 etc, it is equally feasible to make any other dilution (e.g.1:5 or 1:2.5 etc)

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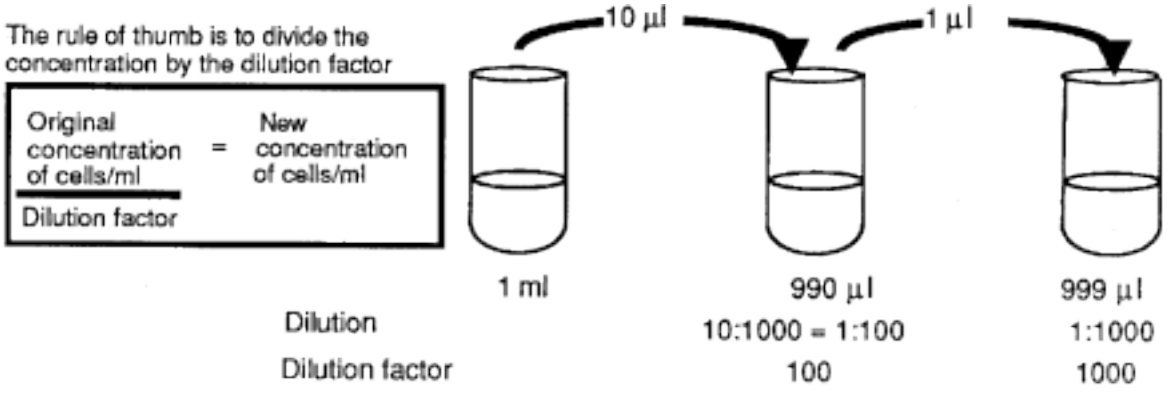
In the example below, if you have 1 mL of your original solution, and you remove10 µL and place it in a tube containing 990 µL of water or media you have made a 1:100 dilution. If the original solution contained 5 x 10^8 organisms or cells/mL, we now have a concentration of 5 x 10^6 cells/mL, because we have simply divided our concentration by the dilution factor, in this case 100. Now, if we want to dilute this by a factor of 1:1000, we must remove 1 µL of the second solution and place it in a tube containing 999 µL of media. We have now diluted our secondary concentration by 1000, and would then divide our concentration by 1000 to yield a 5 x 10^3 cells/mL.



Concentration (cells/mL)	5 x 10^8	5 x 10^6	5 x 10^3

Materials:

Staphylococcus aureus broth culture

6 Nutrient broth tubes

4 Nutrient agar plates

Sterile plastic loops

Sterile plastic spreaders

Sterile tips

Pipettors

Procedure: Students will be expected to have previously mastered all the techniques that are utilized in this lab. If you are unfamiliar with ANY of these techniques please let the Instructor know. Remember that you are working independently on this project and therefore you are solely responsible for the quality of the work submitted.

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DAY 1

1) Take a loopful of the *Staphylococcus aureus* culture provided and streak plate it on a nutrient agar plate to obtain isolated colonies.

2) Prepare six (1:10) serial dilutions of the *Staphylococcus aureus* culture provided. Make sure that you understand that the Dilution Factor in Table 1 below refers to the dilution of the "original" stock culture provided.

a) Set up six tubes labeled 1D-6D
b) Use Table I to determine how to prepare these serial dilutions. The appropriate volume of nutrient broth required to make the dilutions has been provided for you. All you will do is add 0.5 ml of the culture to the tubes as shown in Table I below.

Table I: Serial Dilutions of the given culture

Tube Label	Description of Sample	Volume transferred (ml)	Broth (ml)	Dilution Factor
1D	Dilution #1	0.5 ml of original culture sample	4.5	10
2D	Dilution #2	0.5 ml of #1D	4.5	100
3D	Dilution #3	0.5 ml of #2D	4.5	1000
4D	Dilution #4	0.5 ml of #3D	4.5	10,000
5D	Dilution #5	0.5 ml of #4D	4.5	100000
6D	Dilution #6	0.5 ml of #5D	4.5	1000000

3) Label three nutrient agar plates with your name and the dilution tube from which you will be obtaining the sample.

4) Aseptically transfer 100 µl from each of the tubes 4D, 5D and 6D to three nutrient agar plates. Using sterile spreaders carefully spread the culture evenly over the entire plate preferably till all the culture has been absorbed in to the plate. It is best to rotate the spreader very gently since the agar is very soft and it is very easy to gouge the surface.

5) Incubate your plates inverted in the 37℃ incubator. Remember that you are working with bacteria today but other model organisms you will use in this genetics class may have different growth requirements.

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Day 2

1) Remove your plates from the incubator and record your results in the worksheet provided. It is very important to be clear, and detail-oriented and to make sure to record any unexpected results.

2) You will be graded on the actual quality of your results, so make sure that the instructor initials your results before you leave class.

3) You must turn in your completed worksheet **one week** after you complete the experiment.

References:

Serial Dilutions made easy (1993) Jan Hilten and Carol Sanders

Access Excellence @ The National Health Museum Woodrow Wilson Biology Institute

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WORKSHEET TO BE SUBMITTED: (12 points).

NAME:

CLASS:

EXPERIMENT DATE:

SUBMITTED ON:

You are working independently on this project and therefore you are solely responsible for the quality of the work submitted. You MUST get the instructor to initial your results prior to leaving your lab, this will ensure that he/she has had the opportunity to check your plates.

OBJECTIVE (2 points): State the reason for doing the experiment. Include the major objective(s) for this lab along with a summary of what you learned or concluded from your results. This should be in paragraph form.

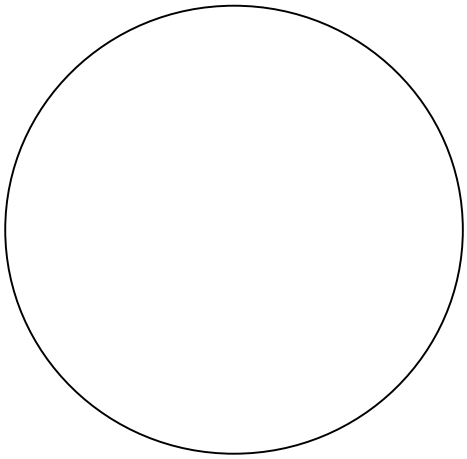
RESULTS:

I) STREAK PLATE TECHNIQUE: (2 points)

i) Did you get isolated colonies? Why is it important to get individual colonies?

ii) Colony description:

iii) Diagram :



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II) SERIAL DILUTION TECHNIQUE (8 points)

i) Did your experiment work as you expected? State YES or NO and explain. If you answered YES list the steps that you believe were critical to your success. If you answered NO list the steps that went wrong. (1.5 points)

ii) Record your results in the table below: Typically you count plates that have between 30-300 colonies. (2 points)

Tube Label	Description of Sample	Number of colonies obtained. CFU/100µl	Comments
4D	Dilution #4		
5D	Dilution #5		
6D	Dilution #6		

iii) Based on the data above calculate the concentration of bacteria (cells/mL) in the original culture provided. Use all plates that have “countable” colonies and report the average count. Make sure to take into account the volume of culture that you surface spread on the plate. (2 points)

iv) Based on the data above calculate the concentration of bacteria (cells/mL) in Tube 3D. (1 point)

v) You are given a culture that has $1x10^6$ CFU/ml. Write out a dilution scheme with a final volume of 1 ml that will allow you to obtain 50 colonies on a plate. Assume that you will be plating 100 uL of the final volume on the plate, as done in your experiment.

This is primarily a mathematical exercise, and you should recognize that if you were actually doing this experiment you would not be able to get **exactly** the number of colonies you want. (1.5 points)