

Pipetteman and Titration exercises

There will be no report for this part of the lab exercise.

Materials needed:

Sharpie pens
Alcohol lamps
Glass dishes
Matches
Waste container- stand with autoclavable plastic bag
Bacterial spreaders
95% ethanol in small bottles – 1 per group
70% ethanol in squirt bottles – 1 per group
Paper towels
200 μ l sterile pipette tips – 1 box per group of 2
P200 pipetteman – 1 per student if possible
P20 pipetteman – 1 per student
Sterile eppendorf tubes in foil covered beakers – 4 per student
Eppendorf tube rack – 1 per group
Test tube rack – 1 per group
eppendorf tube with 100 μ l sterile saline – 1 per student
eppendorf tube with 10 μ l sterile saline – 1 per student
5 ml sterile saline (0.9%NaCl in H₂O) in a 15ml click lock tube – 1 per student
LB ampicillin (100 μ g/ml) bacterial plates – 6 per student
5ml overnight culture of *E. coli* (Ap^R strain) in 5 ml LB Amp (100 μ g/ml) 1 per student (grow in sterile click-lock tubes).

Pipetteman exercise:

Before you start: clean your bench top with 70% ethanol from the squirt bottle provided and wipe with paper towel. Dispose of the paper towel in the normal garbage. Do this at the beginning of every lab period to be sure your working area is free of contaminating bacteria and fungi.

Set your P200 pipetteman on 100 μ l. Put a sterile pipette tip on the end of the pipetteman. Pick up 100 μ l of saline solution and pipette it into a sterile eppendorf tube. Compare the amount to the 100 μ l eppendorf tube you have been given. Dispose of the used pipette tip in the waste container provided. Use a fresh tip and pick up the 100 μ l in the tube you have been given. Is your pipetteman picking up the entire volume?

Take your P20 pipetteman and set it to pick up 10 μ l. Repeat the exercise above. Use the P20 to pick up 10 μ l of saline and put it in a fresh eppendorf tube. Compare the volume to the 10 μ l tube you have been given. Pick up the 10 μ l volume and see if the entire volume gets picked up by your pipetteman. If there is a serious discrepancy tell your TA. Your pipettemen may be out of calibration.

Bacterial titration exercise:

You will be given an overnight culture of *E. coli*. This probably contains bacteria at a concentration over 10^8 per ml. Calculate the titer of the bacteria as follows: make serial dilutions, using sterile saline, of the starting culture to a final dilution of 10^{-6} . This is accomplished as follows: add 0.01 ml of cell culture to 0.99 ml sterile saline to make a 10^{-2} dilution. Then add 0.01 ml of the 10^{-2} dilution to 0.99 ml of sterile saline to make a 10^{-4} dilution. Then add 0.1 ml of the 10^{-4} dilution to 0.9 ml of sterile saline to make a 10^{-5} dilution. Finally, add 0.1 ml of the 10^{-5} dilution to 0.9 ml of sterile saline to make a 10^{-6} dilution. Mix each dilution thoroughly before making the next serial dilution. Use a new pipette tip for each dilution. Dispose of your used pipette tips in the container provided so they can be autoclaved when the lab is over.

Using a P200 pipette set at 100 with a sterile tip, deposit 0.1 ml of the 10^{-4} dilution of each strain onto an LBamp plate. Add 0.1 ml of the 10^{-5} dilution of each strain onto another LBamp plate. Add 0.1 ml of the 10^{-6} dilution of each strain onto another LBamp plate. Use the "flamed" glass spreader to sterily spread the bacterial suspension on the plates. These plates will be used to determine the titer (cell number) of each cell strain you used in the experiment. Label each plate with the type of media, your initials, the cell strain name, the dilution, the amount of the dilution plated, and the date. We will incubate the plates at 37 degrees overnight.

Day two: Come to the lab room and collect your plates. You should see individual colonies on at least one of them. Count the colonies on the plate. Look at the plate one dilution higher. There should be 10X more colonies. To calculate the titre of the original overnight culture, multiply the number of colonies by the degree of dilution. For example if you have 100 colonies on the 10^{-6} dilution plate you had $10^2 \times 10^6$ bacteria per ml (1.0×10^8) in the original culture.