

SERIAL DILUTION, PIPETTING PRACTICE

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23June89, 18July94, 17July 95, 18July 97, 1July 98, 1July 99, 2Jan 02, 28June 02, 24July 06, 1July09, 16July10, 15July11
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Yeast_Plate_Count/Serial_Dilutions.htm

Serial dilutions are performed in a *series* of repeated dilution steps. They are regularly used in microbiology when initial concentrations of bacteria are much too high to perform a plate count, or for producing a series of regular dilutions as in titrating serum. It involves a sequential series of dilutions performed as follows: equal measured volumes of **diluent** are placed in each of a labeled series of test tubes ("dilution blanks"), often with a repeating pipet. A small **aliquot** of the specimen sample is placed in the first tube and mixed. A small aliquot of that dilution is removed with a fresh pipet and added to the second tube. The second tube is then mixed, and an aliquot from it is transferred to the third tube in like manner. The process is continued until the series of dilutions has been completed (*i.e.*, a **serial dilution**). Notice that the concentration decreases exponentially as the dilution series progresses. In the following exercise, the relative concentrations are 1, 2, 4, 8, and 16. Dilutions of antibodies or serum for titrating are prepared in much the same fashion.

See the handout on *Dilutions and Sample Problems* for in-depth explanation of dilutions and sample problems. The handout on *Sterile Technique: Delivery with Pipets* describes pipette use.

Illustrate the following dilution process in your notebook with labeled tubes and all volumes involved so that you fully understand what you will be doing before you begin the exercise.

EQUIPMENT AND SUPPLIES *per table* of two students, each performing expt:

ten 16 x 150 mm tubes	2 pipet bulbs
two test tube racks (larger, fingered)	one vortex mixer
eight 5 mL pipettes in 1000 mL beaker	one spectrophotometer, warmed up
0.0005 % methylene blue ^{1,2} ; $A_{609} \approx 1.00$	two cuvettes in plastic test tube rack:
10 mL grad cylinder	Blank with 3 mL dH ₂ O (marked "B")
wax pencil	Sample (marked "S")
repipet with dH ₂ O, set for 3.00 mL	wipettes
	used pipet receptacle (plastic is best)

PREPARATIONS:

1. Label the test tubes 16x, 8x, 4x, 2x and 1x to indicate the relative concentration of dye they will contain. Add ~ 7 mL of the stock solution of methylene blue to "16x." Make dilution blanks: aliquot 3.00 mL of dH₂O into the other four tubes with a repipet.

DILUTIONS:

2. Transfer 3.00 mL of the initial methylene blue solution from #16 into tube #8, vortex to mix well. (You should be leaving ~ 4 mL in tube #16.)

When dilutions are done, 3 mL should remain in each of these tubes, except the last:

3. Using a clean pipet, withdraw 3.00 mL from tube #8, add it to #4. Mix as before.
4. Using a clean pipet, withdraw 3.00 mL from tube #4, add it to #2. Mix as before.
5. Using a clean pipet, withdraw 3.00 mL from tube #2, add it to #1. Mix as before.

READ AND PLOT THE ABSORBENCY OF THE DILUTION SERIES:

6. Read the A_{609} of each dilution, and plot on a graph with the relative concentration of methylene blue (indicated by the tube number) as the ordinate (X axis) and absorbency being the abscissa (Y axis). Use the blank tube (zero methylene blue and $A_{609} = 0.000$) as your first (zero) point. (A "lag" in the graph at low concentrations is commonly seen.)

¹ Dilute a stock solution of methylene blue (0.3%): Add 0.166 mL into 100 mL in dH₂O. Read its A_{609} . It should read ~1.000. Adjust up to 1.000 if the reading is below 0.900.

² Alternatively, dilute Hucker's Stain: 30 uL into 150 mL water, read at 590 nm. (Lag was worse.)

7/17/95

- 1) Had trouble with initial dilution, thought it should be 1:100, required closer to 1:600 dilution to equal A_{609} of 1.000 (0.99).
- 2) The serial dilution did not generate a linear curve, do not know why, perhaps some reaction of the methylene blue with water as it is diluted? Diluting 1:2 generated a lower OD than expected
- 3) Need to have *all* spectrophotometers warmed up.
- 4) Need to have vortex for *each* desk.
- 5) Emphasize the importance of following directions in protocol--dilution is easily messed up, pipets dirtied, MB wasted.