

Determination of Growth by Optical Density

This exercise is to accompany Exercises 6-1 and 6-3 (read only) in order to quantify the microbial population in a sample. Refer to Appendix E for background information and instructions on use of the Spectrophotometer.

Materials:

Spectrophotometer

Broth culture of *E. coli* (same culture used for Ex. 6-1)

Cuvettes (disposable)

Cuvette rack

5 ml Pipettes

Sterile uninoculated Tryptic Soy Broth (TSB)

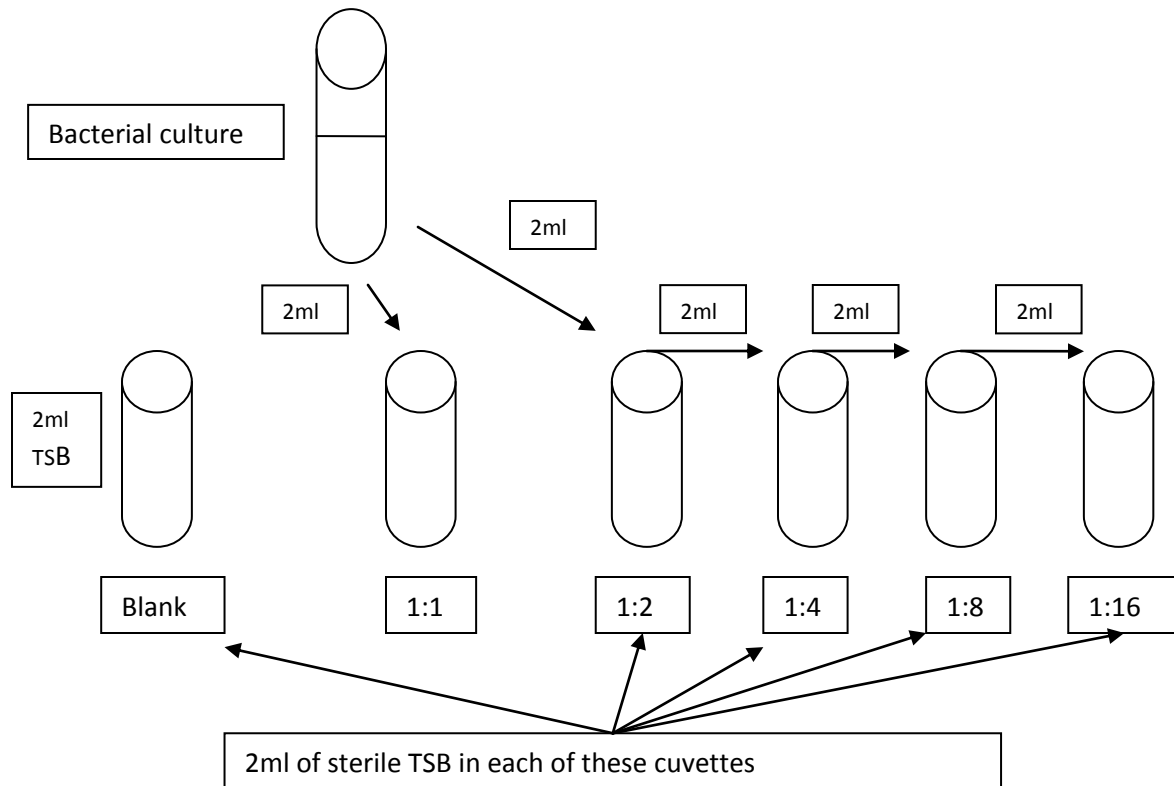
Kim wipes

Procedure

1. Calibrate the spectrophotometer using the procedure in Appendix E (it is also on the instrument). It is important to blank the spectrophotometer with the sterile uninoculated TSB to zero absorbance. This will ensure that any color in the broth is discounted when measuring the absorbance of the samples.
2. Follow the diagram below to set up the dilution series.
 - a. Using a 5 ml pipette, dispense 2ml of sterile NB into 6 cuvettes: blank, 1:1, 1:2, 1:4, 1:8, 1:16.
 - b. Mix the *E. coli* sample thoroughly to suspend the culture.
 - c. With the same pipette transfer 2ml of *E. coli* to the 1:1 cuvette, and 2ml to the 1:2 cuvette.
 - d. Mix the contents of the 1:2 cuvette by drawing the mixture up into the pipette and releasing back into the cuvette, repeat 2 times.
 - e. Complete the dilutions by transferring 2ml of sample from the 1:2 cuvette to the 1:4 tube, mix three times. Continue this for the remaining cuvettes (the 1:16 cuvette will end up with 4ml).
 - f. Use the blank cuvette to blank the spectrophotometer. Measure the optical density of each dilution. Record the O.D values in the table, plot them on a graph and answer the questions:
 1. Why is it necessary to perform a plate count in conjunction with the O.D. measurement? _____

 2. What is the correlation between the O.D. and cell number for your culture? _____

Dilution Procedure:



Fill out the following table:

Dilution:	O.D.
1:1	
1:2	
1:4	
1:8	
1:16	

Plot O.D. vs. Concentration of organisms.

Optical Density (O.D.)

