Bacterial Count

3-TURBIDIMETRY DETERMINATIONS:

When it is necessary to make bacteriological counts on large numbers of cultures, the quantitative plate count method becomes a rather cumbersome tool. A much faster method is to measure the turbidity of the culture with a spectrophotometer and translate this into the number of organisms.

To accomplish this, however, the plate count must be used to establish the count for one culture of known turbidity. To understand how a spectrophotometer works, it is necessary, first, to recognize the fact that a culture of bacteria acts as a colloidal suspension, which will intercept the light as it passes through. Within certain limits the amount of light that is absorbed is directly proportional to the concentration of cells. The light that passes through the culture activates a phototube, which, in turn, registers **percent transmittance (% T)** on a galvanometer. The higher the percent transmittance, the fewer are the cells in suspension.

There should be a direct proportional relationship between the concentration of bacterial cells and the absorbance (optical density, O.D.) of the culture. To demonstrate this principle, you will measure the %T of various dilutions of the culture provided to you.

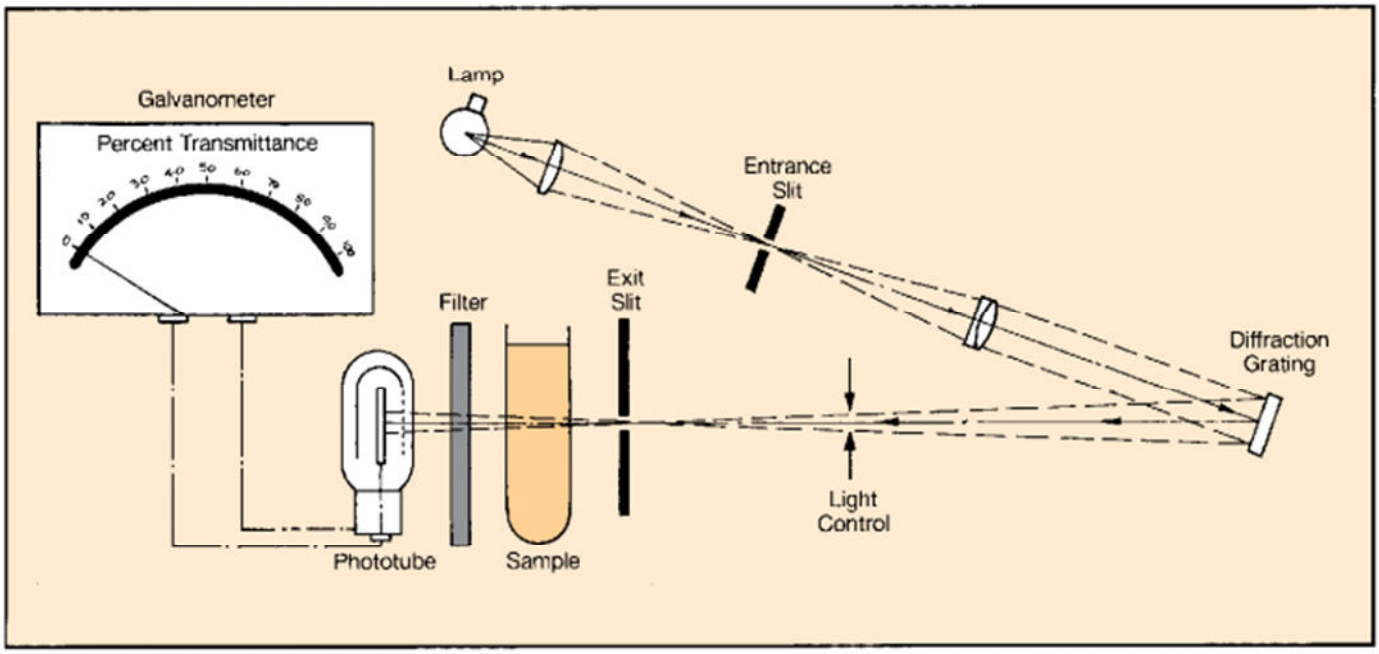


Figure 5 Schematic of a spectrophotometer

Procedure

1. Calibrate the spectrophotometer :
   a. Rinse the cuvette several times with distilled water to get it clean before using.
   b. Keep the lower part of the cuvette spotlessly clean by keeping it free of liquids, smudges, and fingerprints.
   c. Insert the cuvette into the sample holder with its index line registered with the index line on the holder.
2. Label a cuvette 1:1 (near top of tube) and four test tubes 1:2, 1:4, 1:8, and 1:16. These tubes will be used for the serial dilutions shown in figure-3. With a 5 ml pipette, dispense 4 ml of sterile nutrient broth into tubes 1:2, 1:4, 1:8, and 1:16.

4. Shake the culture of *E. coli* vigorously to suspend the organisms, and with the same 5 ml pipette, transfer 4 ml to the 1:1 cuvette and 4 ml to the 1:2 test tube.

5. Mix the contents in the 1:2 tube by drawing the mixture up into the pipette and discharging it into the tube three times.

6. Transfer 4 ml from the 1:2 tube to the 1:4 tube, mix three times, and go on to the other tubes in a similar manner. Tube 1:16 will have 8 ml of diluted organisms.

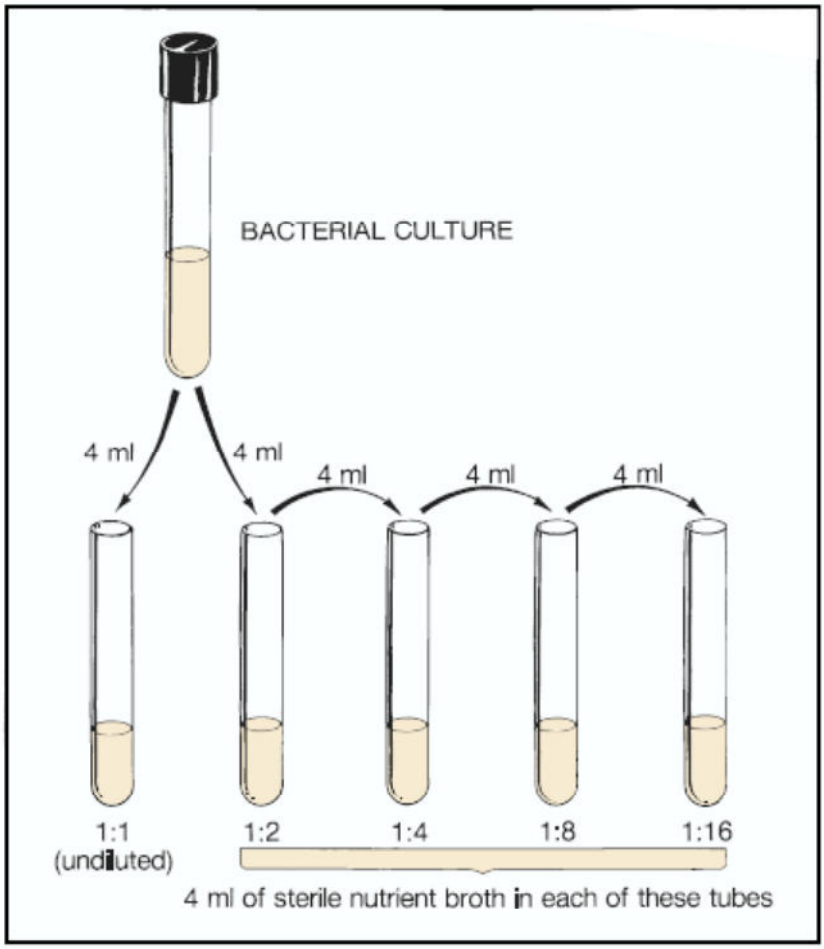


Figure .6 Dilution procedure for cuvettes

7. Measure the percent transmittance of each of the five tubes, starting with the 1:16 tube first. The contents of each of the test tubes must be transferred to a cuvette for measurement. Be sure to close the lid on the sample holder when making measurements. A single cuvette can be used for all the measurements.

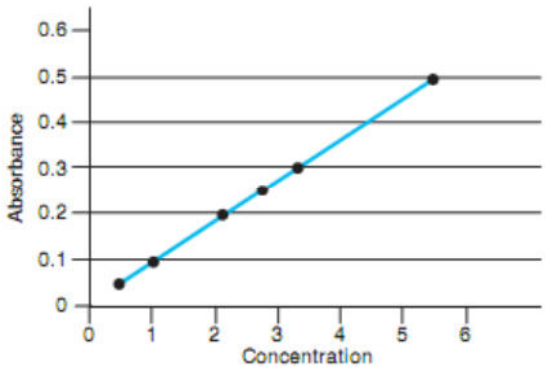
8. Convert the percent transmittance values to optical density (O.D.) using the following formula: O.D. = 2 - log of percent transmittance.
Example: If the percent transmittance of one of your dilutions is 53.5, you would solve the problem in this way: O.D. = 2- log of 53.5
= 2 - 1.7284
= 0.272

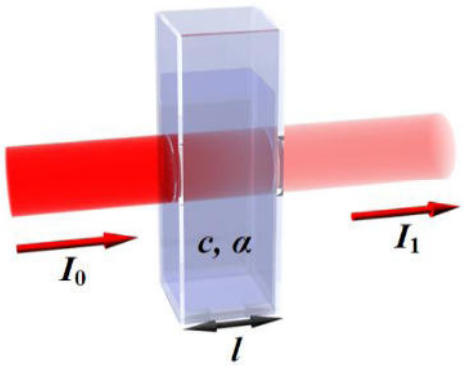
9. Record your observations and calculated bacterial counts per milliliter in the following table.

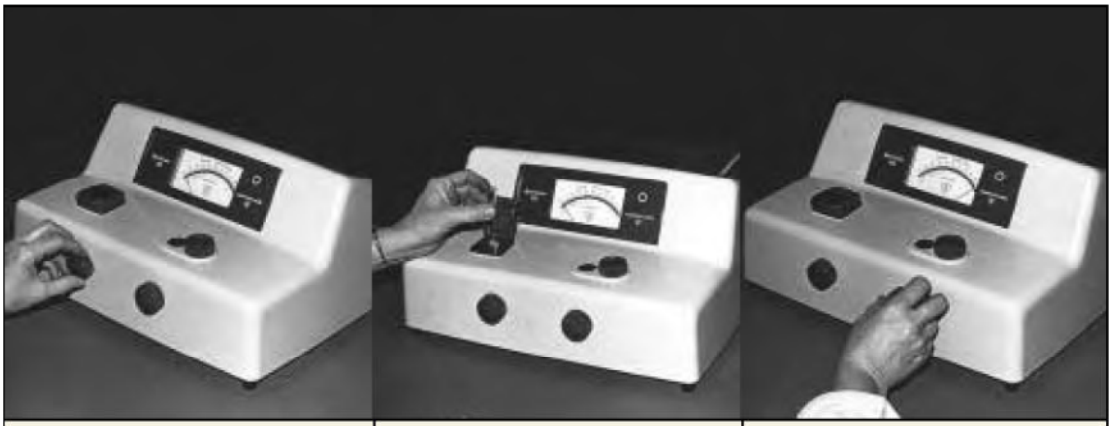
10. Record your data from the turbidimetry experiment in the following table.

11. Construct a cell biomass standard curve by plotting the absorbance on the *y*-axis and the colony-forming units per milliliter on the *x*-axis.

12. Plot the O.D. values on the graph of the Laboratory Report.







1 Turn on instrument by rotating zero control knob clockwise. Do this 20 minutes before measurements are to be made. Also, set wavelength knob (top of instrument) at 686 nanometers wavelength. Adjust the meter needle to zero by rotating zero control knob.

2 Insert a cuvette containing 3 ml of sterile nutrient broth into sample holder. The cover must be closed. Keep the index line of cuvette in line with index line on the sample holder. Refer to instructions 1a through 1e on page 97 concerning care of cuvette.

3 Adjust the meter to read 100% transmittance by rotating light–control knob. Remove cuvette of nutrient broth and close lid. If needle does not return to zero, readjust accordingly. Reinsert nutrient broth again to see if 100% transmittance still registers. If it has changed, readjust with light–control knob. Once meter is adjusted for 0 and 100%, transmittance, turbidity measurements can be made. Recheck calibration from time to time to make certain instrument is set properly.

Figure 23.7 Calibration procedure for the B & L Spectronic 2 on page 97

Review Questions

1. What is the difference between % *T* and absorbance?

2. Why is the viable plate count technique considered to be an indirect measurement of cell density, whereas the turbidimetry method is not a "count" at all?

3. Why is absorbance used in constructing a calibration curve instead of percent transmittance?

4. What is the purpose of constructing a calibration curve?

5. Why is it necessary to perform a plate count in conjunction with the turbidimetry procedure?

6. Give several reasons why it is necessary to shake the water blanks 25 times.

7. What is a CFU?

8. How would you prepare a series of dilutions to get a final dilution of 10^{-10}? Outline each step.

9. Why was 550 to 600 nm used in the spectroscopy portion of this experiment?

10. How would you define biomass?

11. What are several advantages to spectrophotometric determination of bacterial numbers? Several disadvantages?

Laboratory Report

Name: _____

Date: _____

Lab Section: _____

Determination of Bacterial Numbers

1. Record your observations and calculated bacterial counts per milliliter in the following table.

Petri Plate	Dilution	ml of Dilution Plated	Number of Colonies	Bacteria Count per ml of Sample*
1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____
4	_____	_____	_____	_____

*This value is also expressed as colony-forming units per milliliter (CFU/ml).

2. Record your data from the turbidimetry experiment in the following table.

Turbidity-Absorbance Standard Curve

Dilution	CFU/ml	% T	Absorbance
Undiluted	_____	_____	_____
1/2 dilution	_____	_____	_____
1/4 dilution	_____	_____	_____
1/8 dilution	_____	_____	_____
1/16 dilution	_____	_____	_____

3. Construct a cell biomass standard curve by plotting the absorbance on the *y*-axis and the colony-forming units per milliliter on the *x*-axis.