

## Lab)

### **Estimation or Determination of Microbial growth**

The quantitative determination of microbial growth (Bacteria, molds, yeasts) is required for many aims.

Two methods are most widely used for determination of microbial numbers are the standard or viable plate count method and spectrophotometric (turbidity) measurement method.

The standard plate count method is an estimation of the cells density, (only the living microbial cells).

The spectrophotometric measurement is based on turbidity of the microbial cells alive or dead. This method is not convenient for estimation of the molds growth, because the formation of unhemogenuously distribution of mycelia or hyphal cells in liquid growth culture. The growth in this case could be determined by viable plate count method.

The increment in the cells number or in the turbidity in a definite period indicates that the growth is occurred.

A) Viable plate count method.

This method is based on the number of distinct growing colonies on the agar plate .The favorite colonies number is suggested between 25-250 or 30-300 colonies.

Fewer than 25 colonies are not acceptable for statistical reasons , and more than 250 or 300 colonies are also not accepted because the colonies are too closed or overlapped to each other .In this case it cannot be distinguished as a distinct colony-forming units (CFUs).Depending upon the assumption that each viable cell is separated for all others and will develop into a single colony (CFU)thus the number of colonic represents the number of cells, that can grow under growth conditions .For the above reasons the sample of the liquid growth culture must be diluted with sterile saline or phosphate buffer until the suspected cells number is enough to count.

#### Materials

24 hour tryptic-soy broth culture of bacteria (nonpathogenic) 4-99 ml bottles or 10 tubes filled with sterile saline or phosphate buffer

1 ml graduated pipettes with pippeton

6 petri-dishes

6 agar tubes (tryptone glucose yeast agar)

48-50°c water bath

Boiling water bath

Burner

Spectrophotometer

4 tubes of tryptic soy broth

### **Procedures**

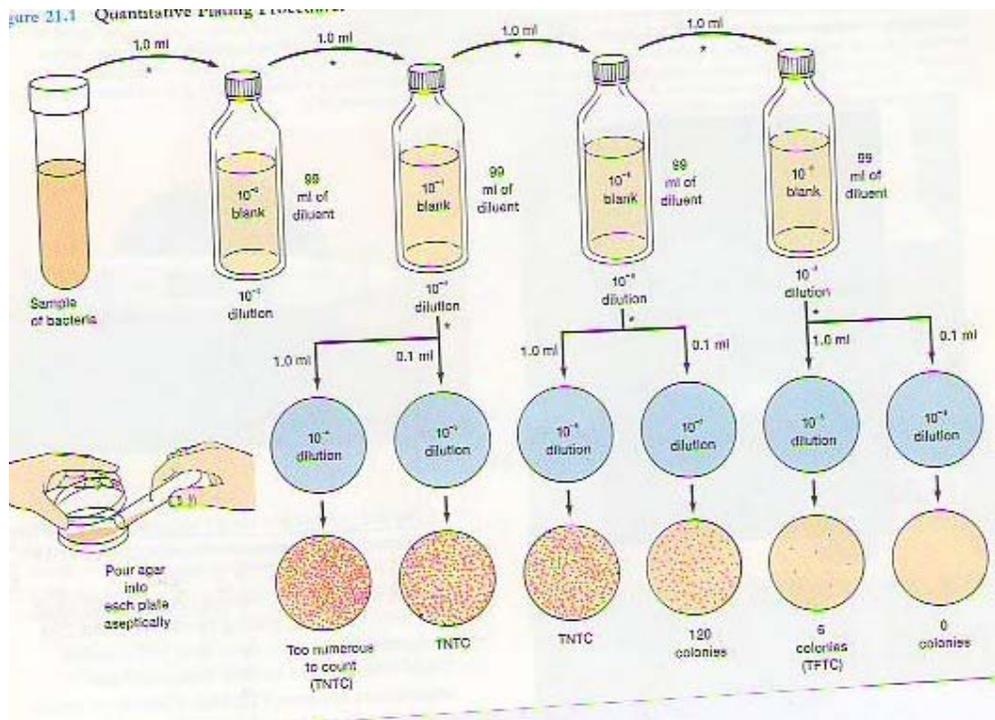
1- Label the bottom of the six Petri-dishes with following dilutions:  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$  to  $10^{-9}$ .

2- Label the four bottles of saline or phosphate as follows  $10^{-2}$ ,  $10^{-4}$ ,  $10^{-6}$ ,  $10^{-8}$  or the tubes 10-1, 10-2, 10-3 up to 10-10.

3- Under aseptic conditions transfer 1 ml of liquid culture to the 99 ml saline the result is the first dilution step equal to 1:100 or  $10^{-2}$ . Then shake the bottle rapidly to serve the distribution of the bacterial cells and break up any cells clumps that may be present.

4- Immediately after shaking transfer 1 ml from  $10^{-2}$  bottle to the second 99 ml saline bottle to gain  $10^{-4}$  dilution.

5- Shake well and transfer to the third 99 ml saline bottle the dilution represents  $10^{-6}$  Repeat the process once more to gain  $10^{-8}$  dilution see the (fig. 31).



**Figure (1) viable plate count method.**

6- Shake the  $10^{-4}$  bottle and transfer 1 ml to one Petri-dish and 0.1 ml to another one Petri-dish Repeat this process 3-folds to gain 3-inoculated Petri-dishes for each dilution.

7- Repeat the above for each dilution  $10^{-6}$ ,  $10^{-8}$  see the (fig. 31)

by transfer in 1 ml and 0.1 ml you gain serial decimal dilution.

Pour the content of the agar tube, that method and cooled up to  $48-50^{\circ}\text{C}$  into each one Petri-dish and mix gently by moving the plate on the surface of the bench clock and anticlockwise, then left the Petri-plate to solidify the agar.

The plates inverted and incubated at favorite temperature ( $25-37^{\circ}\text{C}$ ) for 24-48 hours.

10-Observe the colonies growth on the plates and count the colonies with colony counter as demonstrates by instructor.

The plates with more than 250-300 colonies are designated too numerous to count (TNTC) or too much colonies (TMC) plates with less than 25-30 designated too few to count (TFTC).

11-Calculate the number of bacteria CFU as follows:

Number of bacteria in 1 ml of the original sample = the mean value of the colonies No. x 1/ dilution

i.e. No. of colonies in three plates of  $10^{-6}$  dilution 150, 140, 145

Mean value =  $(150+140+145)/3 = 145$

No. of bacteria =  $145 \times 1/10^{-6} = 145 \times 10^6 = 1.45 \times 10^8$

12-Record your results write a report.

Turbidimetric determination

This method is basically depended on the reciprocal relation between the amount of transmitted light and the suspension density of the bacterial cells in liquid culture.

The transmission of light in the visible spectrophotometer through the distilled water is 100% ,when the sample contains any suspended particles , these particles reflects the light beams .The reflected beams could be measured on the scale of the spectral at a definite wave length.

Therefore the amount of transmitted light decreases as the cell population (density) increases.

### Materials

24 – 48 hr. tryptic soy broth culture of E.coli

3- ml sterile tryptic soy broth tubes (6 tubs)

3-5 ml graduated pipettes with pipette.

Empty sterile tubes.

Spectrophotometer. (See figure 32a)

### Procedures

To make twofold dilution for standard curve (or serial decimal dilution) table 5 tubes the 1st is empty, the tubes No. 2,3,4,5 each one filled with 3-ml sterile tryptic soy broth.

From the original broth culture transfer with sterile pipette 3-ml to 1st tube and 3-ml to the tube NO.2 mix well and transfer 3 ml from tube No.2 to the tube No.3.

Repeat this process up to tube No.5 or more when it necessary .see (fig. 32b) discard the pipette. The 1st tube with original density of broth culture, 2nd diluted to 1/2, the 3rd 1/4, 4th 1/8, and the 5th to 1/16

Turn on the spectrophotometer by rotary knob B to the right.

Adjust the spectrophotometer as choose the desired wave length (550-600 nm) using knob D.

Place the cuvette in cuvette holder A , that contains just sterile broth and adjust the pointer to the right side at zero position of the scale using knob C. by this way spectrophotometer is standardized by blank that means the absorbance is zero (or the transmittance of 100%.)

Place the cuvette, which contain the diluted cells suspension in the cuvette chamber and read the absorbance. Repeat this step for the next dilutions and remember to mix the tubes in each step. Repeat the steps 5 between the experimental readings to confirm the scaling.

Record the results of the plats count method and the data of this method are used to draw a curve that represents the correlation between the optical density and cell number in each dilution. See (fig. 2c)

Figure 21.3 Twofold Serial Dilution for Standard Curve.

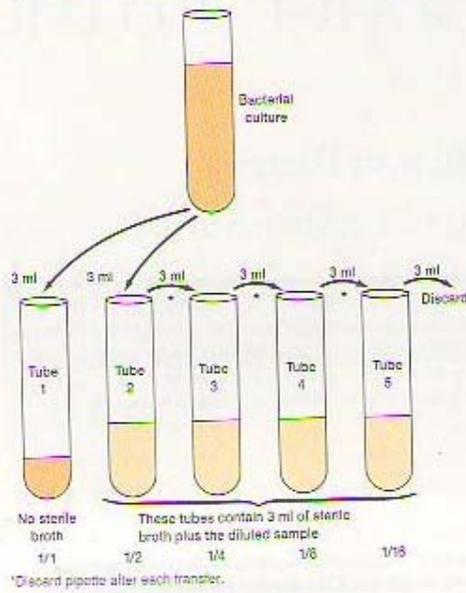


Figure (2b)

Figure 21.4 Typical Spectrophotometers. (a) The top photo illustrates an analog model where (A) is the sample holder; (B) the power switch/zero control knob; (C) the 100% T control knob; (D) the wavelength control knob. (b) The bottom photo is a digital model that features direct concentration readout.

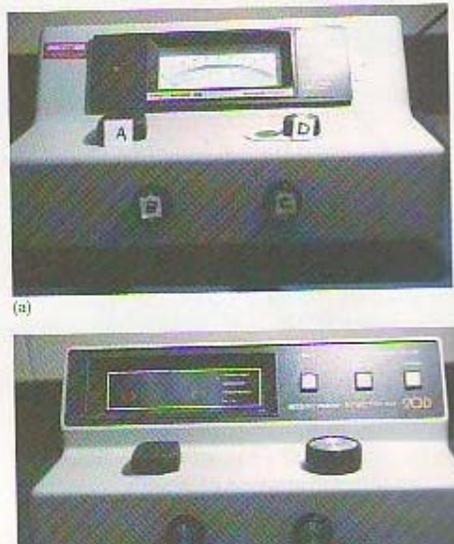


Figure (2a)

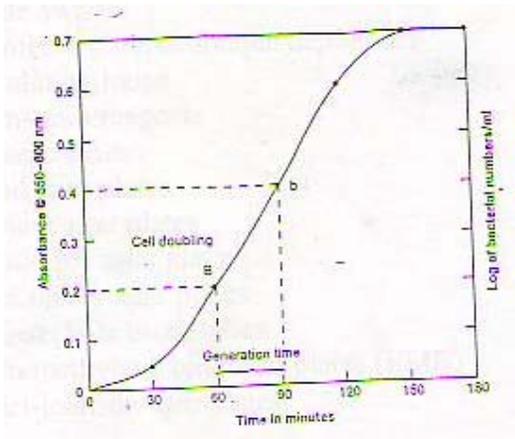


Figure (2c) Growth curve and indirect determination of generation time

