Microbiology Lab Experiment Changes

Title: **Growth curve of *E.coli***

Objectives:

- Know the different phases of a standard growth curve
- Understand and use a ***direct*** measurement of growth using Serial dilutions (ex 6-2 & 1-6) and Standard Plate Count
- Understand and use an ***indirect*** measurement of growth using optical density

Introduction: Most bacterial species divide by binary fission and growth of bacteria is indicated by an increase in population size. Rate of increase in number of cells is dependent on optimal growth conditions and nutrient supply. Phases of growth cycle can be observed by plotting the increase in cell numbers over time in a closed system. To learn about different phases of bacterial growth, sterile broth was inoculated with *E. coli* and incubated at 37ºC in a rotary shaker. For this experiment, the broth culture of *E. coli* was sampled at various time points from the time of inoculation. Each group will use two values for measurement of growth at a specific time point and collect class data. To compare growth kinetics by a direct and an indirect method, each group will use class data and plot 2 graphs: (i) cfu/ml vs. time (connect the data points) on a semilog graph paper and (ii) Optical density vs. time (best fit line).

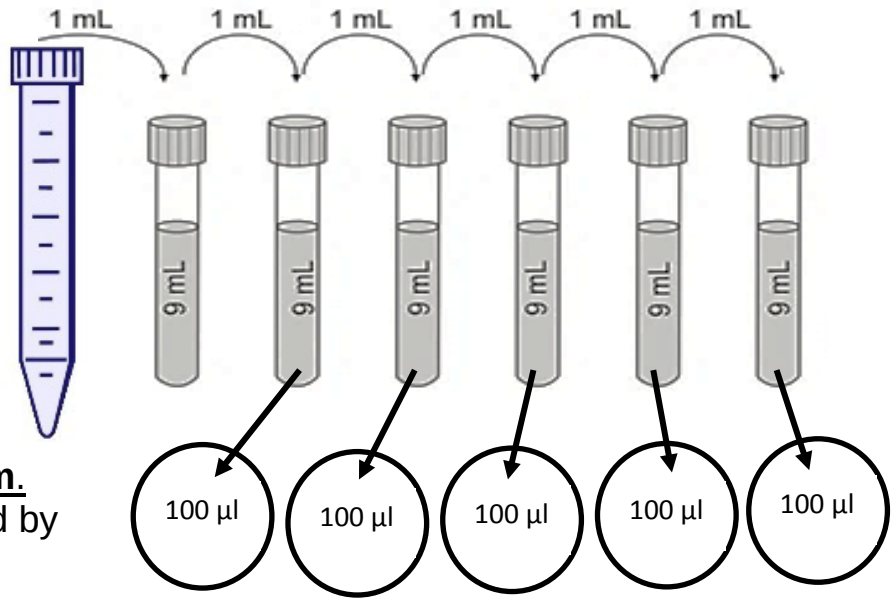
Live Organisms: *Escherichia coli* (broth culture)

Procedure: Procedure (Work in groups)

DIRECT METHOD:

Obtain (i) broth culture of *E.coli*
(ii) 5 sterile TSA plates
and (iii) six 9-mL water blanks.

a. Label six (9-ml water) tubes 1-6. Use **<u>vortex</u>** to mix the broth culture and aseptically take out 1.0ml of the culture. Carry out a **<u>10-fold serial dilution according to the diagram</u>**. Follow the procedure demonstrated by your instructor.



b. Pipette bacteria (0.1ml) from each dilution (tubes 2-6) onto labeled plates and spread using a sterilized spreader as demonstrated by instructor. Tape your set of 5 plates/grp. and place it in the bin for incubation. Discard dilution tubes in the wire basket.

INDIRECT METHOD:

c. Vortex the broth culture to have a homogeneous suspension of cells. Calibrate the spectrophotometer @ 600nm, using sterile TSB, ***before*** you read optical density (OD) for your culture. Use 2.0ml the broth culture in a disposable plastic cuvette and read OD. Record the OD for your group in the table.

CLASS DATA

Group #	Tube #	Time (hrs.)	OD of culture@ 600nm	Number of colonies	Dilution Factor	cfu/ml from plates	cfu/ml from web site
1	T_0	0					
2	T_2	2					
3	T_4	4					
4	T_6	6					
5	T_8	8					
6	T_{10}	10					

Next class, find the countable plate, determine dilution factors and calculate cfu/ml for **your** time group. To construct growth curve, record class data and use class data for both OD, calculate cfu/ml to plot over time. Go to computer at front of class and enter your OD to calculate cfu/ml. (http://www.genomics.agilent.com/biocalculators/calcODBacterial.jsp)

Take Home Lesson: If given the dilution, you should be able to determine the dilution factor. If given the number of colonies on a plate, you should be able to calculate the colony forming units (CFUs) per ml using the dilution factors (DF). **Remember only colony counts between 30 and 300 are usable**. If more than one plate has countable colonies, take an average. Numbers without units are meaningless!