

# Microbiology for the Health Sciences Laboratory

## Lab 5 - The Bacterial Growth Curve

### Introduction

Bacteria replicate exponentially by transverse binary fission in liquid or broth culture. The complete construction of a bacterial growth curve (i.e. an increase in cell numbers vs. time) requires measurement of population size at many intervals over an extended period of time. One can directly measure the increase in viable cells (cell counts of serial dilutions are the most accurate, but also the most tedious and time-consuming method), or one can indirectly measure increase in growth as a function of increasing turbidity in a liquid medium. Either method allows for construction of a typical microbial growth curve (with a lag, log, stationary and death phase) as well as the determination of the generation time (the time required for a bacterial population to double).

The purpose of this lab is to:

- 1) Use an indirect, spectrophotometric assay to determine the shape of a microbial growth curve;
- 2) Determine the generation time of a typical bacterium under ideal, defined conditions; and,
- 3) Evaluate the effects of antibiotics on microbial growth.

### A. Review the following for reference:

1. Text: Chapter 4 - Pages 82 to 83, 87 to 93, and 98 to 103.
2. Pay particular attention to the following: Phases of microbial growth in culture (Fig 4.6), and measuring growth via serial dilution (Fig 4.17) and turbidity (Fig 4.21).

### B. Do the following during lab:

#### 1. Growth Curve

- a. Basically, this lab requires much preparation, as well as 8-24 hours of actual measurement time, to determine the complete growth curve. Therefore, the following steps will have been completed before lab:
  - A broth cultures of *E. coli* were started Wednesday afternoon.
  - Fresh broth cultures of *E. coli* were started Thursday morning,
  - Growth flasks with 100 ml of Trypticase Soy Broth were inoculated with *E. coli* on Thursday about noon.
  - Growth Flasks were placed in a shaker at 37 C and rotated at 100 rpm (to provide O<sub>2</sub>).
  - Recordings of Optical Density were made every 15-20 minutes prior to lab.
  - Antibiotics will be/were added to flasks 2, 3 and 4 when the mo's are in the log phase of growth and the optical density is approximately 0.15.

#### b. Experimental design:

Flask	Inoculum	Treatment/Antibiotic	Test
#1	<i>E coli</i>	None	(+) Growth Control
#2	<i>E coli</i>	Streptomycin (0.1 mg/ml)	Effects of antibiotic
#3	<i>E coli</i>	Streptomycin (0.01 mg/ml)	Effects of antibiotic
#4	<i>E coli</i>	Vancomycin (0.1 mg/ml)	Effects of antibiotic
#5	None	None	(-) Growth Control

#### c. You will be responsible for the following:

- Measure and record optical density every 15-20 minutes in each of flasks #1 through #4 using flask #5 to 'blank' the spectrophotometer if needed.
- Add antibiotics to flasks #2, #3 and #4 when the OD reaches approximately 0.15.
- Continue to measure and record Optical Density every 15-20 minutes until 'done'.
- Note: Be extremely careful of the side arm flasks – they are fragile and very expensive!

#### 2. Last weeks lab:

- a. Complete observations on stained preparations from last week. Make sure that you view the slides prepared by other members of your group.
- b. Transfer an isolated colony of your throat mo to a broth tube. Do a gram stain on the isolated organism. You will use these cultures next week.

### C. Techniques

1. Use of the spectrophotometer (Spec 20) to measure growth:
  - Turn instrument on - Left hand knob
  - Adjust wavelength of light to 550 nm - Top knob
  - Set Transmittance to 0 % - Left hand knob
  - Place Control Flask in instrument and set OD to 0 - Right hand knob
  - Replace Control Flask with a Test Flask and read OD - On screen
  
2.
  - a. Make sure the side arm of the flask is clean and inserted into the Spec 20 *very carefully*.
  - b. Try to not let any stray light enter the cuvette area.
  - c. The culture must be in the log phase of growth in order to determine the Generation Time.
 

The log phase of growth is indicated by a straight line when growth is plotted on log paper.  
It is important to plot the data carefully because you will use the plotted data (not the recorded data) to determine the generation time.

**D. Results**

- Record data: optical density vs time on the table below
- Plot optical density vs. time (in min) for each growth flask using the log paper provided
- Label the phases of the growth curve and determine generation time (Flask 1) of *E coli*
- Discuss the significance of your observed experimental measurements

Time	Time		Flask 1	Flask 2	Flask 3	Flask 4
	Actual	Elapsed				
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						

2. Determination of Generation Time *During Log Phase of Growth* for Flask #1:
  - a. OD #1 (Choose a starting point) \_\_\_\_\_  
 Time #1 (Record time) \_\_\_\_\_
  - b. OD #2 (2 times OD#1) \_\_\_\_\_  
 Time #2 (Record time) \_\_\_\_\_  
 Time difference A = Time #2 minus Time #1 \_\_\_\_\_
  - c. OD #3 (2 times OD#2) \_\_\_\_\_  
 Time #3 (Record time) \_\_\_\_\_  
 Time difference B = Time #3 minus Time #2) \_\_\_\_\_
  - d. Average Generation Time = (Time difference A + Time difference B) / 2 = \_\_\_\_\_ minutes

## **E. Discussion and Conclusions**