Bacteria are single-celled prokaryotic organisms. As bacterial cells take in nutrients from their surroundings and convert it into cellular material, they grow to a point at which each cell divides into two cells. Each resulting cell grows and then divides in the same manner as the original cell. This method of reproduction is called **binary fission**. Under optimal conditions (e.g., sufficient nutrients and proper temperature) this process can occur quite rapidly.

When bacterial cells are first added to fresh medium, there may be a **lag phase** before growth begins. During the lag phase bacteria reorganize their cellular machinery and this results in a delay in growth. Once this occurs, cells divide rapidly in what is called the **exponential phase** of growth. In an old culture the cells have used all the nutrients and have stopped dividing. This phase when there is little change in the number of cells is called the **stationary phase**.

The **relative** number of cells in a broth culture can be quantified using spectrophotometry. As the number of cells increases, so does the turbidity (cloudiness) of the suspension. As turbidity increases, the amount of light able to be transmitted through the sample decreases, and the optical density (absorbance) of the sample increases. Therefore, an increase in absorbance (easily measured with a spectrophotometer) is indicative of an increase in the total number of bacteria in the culture.

Antibiotics are chemical substances (natural or synthetic) that are able to destroy or kill bacterial cells, or inhibit their growth. Most antibiotics act by inhibiting the formation of a particular type of macromolecule or structure in the microbial cell. Some types inhibit cell wall (peptidoglycan) synthesis, ultimately resulting in cell lysis. Other types inhibit protein or nucleic acid synthesis, effectively halting cell growth.

During this laboratory, you will use spectrophotometry to measure the growth (or **population density**) of broth cultures of *E. coli* before and after the addition of the common antibiotics penicillin and streptomycin. Cultures treated with an antibiotic will be compared to untreated control cultures. Prior to adding the antibiotic you should discuss the range of possible outcomes (i.e., develop a hypothesis). Can the data you collect be used to infer something about the mechanism of action of these two antibiotics?

**Materials**

You will work in teams comprised of sides of laboratory tables. Each team will require the following equipment and materials (some of which may be shared with the other group at your lab bench):

- Spec-200 spectrophotometer
- Vial w/ sterile H₂O
- 3 Nephelo culture flasks w/ 50 ml PC broth
- Sterile disposable pipettes and inoculating loops
- Overnight broth culture of *E. coli* B
- Phase-contrast microscope w/ slides
- Vial w/ antibiotic (25,000 I.U.)
- Marking pen and labeling tape
Procedure

Follow the procedure carefully as written below. Use of the Spectronic 200 spectrophotometer is described in the appendix at the end of the laboratory handout. You should read this section carefully before you begin the exercise.

Before inoculating your cultures, calibrate the Spec-200 as described in Appendix A. Label one Nephelo culture flask "CONTROL" and the other "TREATMENT".

$t = 0$ min: Using a sterile 1 ml pipette, inoculate 1.0 ml of an overnight culture of *E. coli* B into each of the two labeled Nephelo culture flasks. Immediately measure the O.D. (absorbance) of both cultures at 540 nm (Appendix A). Record your measurements in Table 1. As soon as you have measured the O.D., place the cultures in the 37°C incubator set at 200 rpm.

$t = 20$ min: Measure the O.D. of both cultures.

$t = 40$ min: Measure the O.D. of both cultures.

$t = 60$ min: Measure the O.D. of both cultures. Make microscopic observations of both cultures (Appendix B).

$t = 80$ min: Measure the O.D. of both cultures. As soon as you have measured the O.D., add 0.5 ml of a sterile aqueous solution of Penicillin or Streptomycin (as assigned by your instructor) at 25,000 international units to the TREATMENT flask, and 0.5 ml of sterile H₂O to the CONTROL flask. *(Why do this?)* Return your cultures to the incubator as quickly as possible.

$t = 100$ min: Measure the O.D. of both cultures.

$t = 120$ min: Measure the O.D. of both cultures.

$t = 140$ min: Measure the O.D. of both cultures. Make microscopic observations of both cultures.

$t = 160$ min: Measure the O.D. of both cultures. Be sure all data is entered into the class data sheet. Clean up as directed.

WHEN YOU ARE FINISHED WITH YOUR EXPERIMENT, PLEASE DO THE FOLLOWING:

1. Turn off the Spec-200.

2. Remove labels from your CONTROL and TREATMENT flasks. Place the flasks near the sink at the front of the lab.

3. Be sure that all materials are returned to the trays at each table. *Please leave things as you found them!*

4. Wash your hands thoroughly with soap and water.
Results

Table 1. Optical density (absorbance) and microscopic observations of CONTROL and TREATMENT cultures over a 160 minute time period.

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>ABSORBANCE @ 540 nm</th>
<th>MICROSCOPIC OBSERVATIONS @ 400 X</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>TREATMENT</td>
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<td>160</td>
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</tbody>
</table>
APPENDIX A
Use of the Spectronic-200 Spectrophotometer

Calibration of the Spec-200

1. Turn on the Spec-200 (switch is in back). When prompted, press “Enter” (unlabeled round button) and wait for initializing to complete. Make sure “Spec 200 Modern Interface” is selected, and press “Enter.”

2. Make sure the Application is set to “Live Display” and the Measurement Mode is set to “Abs” (Absorbance). Use the down arrow to select “GO” and press “Enter.”

3. Set the wavelength ($\lambda$) to 540 nm using either the forward/back arrow keys, or the $\lambda$ knob. (Note: for fine control, press down on the knob while turning.)

4. Tilt the Nephalo culture flask labeled “BLANK” so that the tube is completely filed with broth. Open the sample compartment and place the tube of the flask completely into the RIGHT sample holder. (Note: adjust the width of the sample holder if needed—it should hold the flask completely upright without being too tight.) Orient the flask so that the large cap points toward the right side of the machine. NOTE: You will not be able to close the lid with the flask inserted, but this will not adversely affect your readings.

5. Press the “0.00” button and wait for zeroing to complete. (By doing this, your experimental readings are made relative to the absorbance of the control tube.) After zeroing is complete, remove the “BLANK” and set it aside.

Measuring O.D. with the Spec-200

1. Make sure the caps on the flasks are tight.

2. As you did with the “BLANK” flask, place the tube of the flask labeled "CONTROL" completely into the RIGHT sample holder and record the absorbance reading.

3. Repeat Step 2 for the flask labeled "TREATMENT."

4. Loosen the caps and return the flasks to the incubator as quickly as possible.

APPENDIX B
Making Microscopic Observations

Using a sterilized inoculating loop, transfer a drop of culture to a clean glass slide and cover the drop with a coverslip. Observe the slide with the phase-contrast microscope using standard microscopic techniques. Although it is difficult to get an accurate estimate of bacterial numbers using this technique, you may be able to get an impression of the relative density of bacteria in the culture at any given time. You should also look carefully at the arrangement of the cells in each culture. Do the cells exist separately from one another? Do they form chains or clusters? Is there any difference before and after the addition of the antibiotic?
ASSIGNMENT

Results
Your instructor will provide you with raw data from multiple lab sessions. Following the guidelines discussed in "Working with Statistics" and the "Guide to Writing Scientific Papers," analyze and present this data in a meaningful and informative way.

Conclusions
Use your results to concisely address the following questions (i.e., be brief and to the point):

• In comparing the treated cultures to the control culture, what can you conclude about the effect of each antibiotic on the relative number of cells in suspension?
• What does your answer to the previous question suggest about the specific mechanism of action of penicillin? How about streptomycin? You are encouraged to compare your interpretations with outside sources (which should be cited appropriately).

Note that while your work should be suitable for INCLUSION in a scientific paper (in particular, the results and discussion sections), you are NOT required to write a full paper for this assignment!