INQUIRY & INVESTIGATION

Using a Simple *Escherichia coli* Growth Curve Model to Teach the Scientific Method

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Abstract

The challenge of teaching in the sciences is not only conveying knowledge in the discipline, but also developing essential critical thinking, data analysis, and scientific writing skills. I outline an exercise that can be done easily as part of a microbiology laboratory course. It teaches the nature of the research process, from asking questions and developing a testable hypothesis to writing a scientific paper, as well as the concepts of bacterial growth and two classic techniques for measuring bacterial growth, spectrophotometry, and standard plate method.

Key Words: Escherichia coli*; growth curve; standard plate method; spectrophotometry; scientific method.*

○ Introduction

The biology department at Chestnut Hill College has endeavored to incorporate the research process into all of its undergraduate biology courses, beginning long before the recommendations in *Vision and Change in Undergraduate Biology Education* (AAAS, 2011). In most undergraduate microbiology courses, the laboratory portion is very technique oriented (e.g., Gram staining, identification of bacteria, testing for antimicrobial resistances). While all these concepts and techniques are important to teach, they do not necessarily utilize the research process. The challenge of teaching in the sciences is not only conveying knowledge in the discipline, but also developing essential critical thinking, data analysis, and scientific writing skills. Therefore, I have designed a laboratory exercise that teaches the simple concepts of how bacteria grow and how bacterial growth can be measured, while also emphasizing the development of a hypothesis, research design, data analysis, and the writing of a research paper (see Table 1 for overview).

> Escherichia coli *is an ideal microorganism for undergraduate research projects.*

Escherichia coli is an ideal microorganism for undergraduate research projects. It has a simple, completely sequenced genome and a rapid growth rate under optimal conditions; is easy to handle and cultivate; and is relatively harmless (Zimmer, 2008). Therefore, in a 3-hour laboratory session, when an overnight culture of *E. coli* is heavily inoculated into media already at the appropriate temperatures, it is possible to obtain a complete growth curve. (My PowerPoint presentation for this lab is available at https://mckernanmicrobiology.wikispaces.com.)

The objectives of the laboratory exercise are as follows:

- **To review and practice scientific inquiry.** I walk the class through the steps of scientific inquiry. We discuss the terms "hypothesis" and "theory"; the difference between "discovery science" and "hypothetico-deductive science"; the importance of a clearly defined purpose for a research study; and the effect of culture conditions on bacterial growth and the importance of studying bacterial growth.
- **To use spectrophotometry and standard plate method to determine the doubling time of *E. coli* during the exponential phase of growth.** Most students have taken a chemistry course and used spectrometry to measure pigmented solutions, but here bacteria are measured as particles in solution. As they take optical density readings, they very quickly see a growth curve developing. The standard plate method invites review of serial dilutions, the concept of colony-forming units, and how to determine the concentration of microorganisms. Using two methods gives the students an opportunity to discuss how different methods can be used to measure the same parameter – in this case, doubling time. But it is also important to know what the different methods really measure and how that might affect interpretation of the data, and that there are advantages and disadvantages of methods.
- **To use Excel in graphing and calculating doubling time.** The concepts of independent and dependent variables and the construction of tables and graphs are reviewed. Most students at this level have used Excel to make tables and graph data. But changing the y-axis to a logarithmic scale, adding an exponential line, and using the equation for the line to calculate

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Table 1. Overview of a laboratory exercise on bacterial growth.

Session 1	Discussion (20–30 minutes) of the activity's importance; forming a hypothesis; and explanation of procedures and their purpose. Optical density (OD) readings taken for 140–180 minutes. Two samples taken at ~40 minutes and at 80–100 minutes for standard plating method. Serial dilutions prepared and plates inoculated.
Session 2	Go over the growth-curve graphs that students prepared from OD data collected in session 1. Count colonies on plates prepared in session 1. Calculate colony-forming units/mL from data. Explanation of how doubling time is calculated from each method. Review the parts of a scientific paper and the rubric for grading.
First Draft and Final Draft	

doubling time is new to many students. Analyzing the raw data, optical density readings, and colony forming units/mL to determine doubling time is challenging and a highlight of this laboratory exercise.

- **To write a science research paper.** The parts of a scientific paper are covered in detail (see https://mckernanmicrobiology.wikispaces.com); descriptions of what should be included in each section are posted for students to use as a guide in writing the paper. Also covered in detail are presentation of data in tables and graphs and the calculations for determining doubling time. Students submit a first draft, edit it, and turn in a final draft for grading.

I have taught this laboratory exercise for 6 years. The classes are usually composed of biology majors in their junior year, with an occasional sophomore or a few seniors. The students have always enjoyed it, and they frequently say in evaluations that it was the best lab of the semester. Students have often said that they like the lab because of its hands-on aspects: taking readings, seeing the growth curve emerge, pipetting, serial dilutions and plating, and the quantitative analysis of raw data to calculate doubling times. This exercise is usually run in the fourth week of the semester, so that students already have some practice with handling of bacterial cultures and aseptic technique. In the lecture portion of the course, the concepts of binary fission, four phases of bacterial growth, factors affecting bacterial growth, and techniques for measuring bacterial growth are covered. Therefore, the two techniques used to measure bacterial growth in this experiment – optical density measurement and standard plate method – have already been discussed.

In the first laboratory session, the process of scientific inquiry and the importance of studying bacterial growth are discussed. I lead the discussion toward why it might be important to study the effect of temperature on bacterial growth, and a hypothesis is developed for the growth of *E. coli* at 37°C and 25°C. The techniques of spectrophotometry and serial plate dilutions for measuring the growth rate of bacteria are established methodology for the microbiologists. It is also well documented that *E. coli* have a doubling time of 15–20 minutes under optimal conditions. I emphasize that an understanding of how this number was arrived at is important. It is necessary to determine what the doubling time is under our particular growth conditions. Also, science needs to be repeatable. Can we repeat the results observed by others? Why would we need to know that? What are optimal conditions? What if we wanted to determine a better growth medium for improved growth rates? We chose 37°C because it is human body temperature and *E. coli* is part of the normal flora of the gut; and 25°C because it is room temperature; but other temperatures could have been selected.

Next, the procedures are reviewed. The students inoculate their trypticase soy broth (TSB) tubes and learn how to use the spectrophotometer to measure optical density, which includes setting the wavelength, zeroing the instrument with an uninoculated broth tube, and then measuring the optical density of their freshly inoculated TSB tube. This measurement is their T = 0 minutes reading, and the experiment has begun. Using the standard plate method, two 1-mL samples are removed at two different time points in the experiment.

In the second session, "Parts of a Scientific Paper" are presented in a PowerPoint (https://mckernanmicrobiology.wikispaces.com). Then, as a class, the growth-curve graphs that students prepared from OD data collected in session 1 are evaluated. The students have varying degrees of expertise in Excel, and I have found that the majority of the class needs help with formatting their graphs. The data from the standard plate method are collected, and colony-forming units (CFU)/mL are calculated. An explanation of how doubling time is calculated from each method is covered (https://mckernanmicrobiology.wikispaces.com). Then the results are evaluated and the class brainstorms on whether the results confirm their hypothesis, and why or why not; whether the two methods gave comparable doubling times, and why or why not; the advantages and disadvantages of the two methods; and finally, how the procedures might be improved and what further study might include. Students find that the most difficult section of a scientific paper to write is the conclusion section, so I cover again what should be included in that section:

- Summary of the doubling times at the two temperatures and with both methods.
- Did you reach conclusions about the initial hypotheses?
- Are your results comparable to the conclusions of others?
- Are the two methods comparable? Why or why not?
- Identify sources of error and basic inadequacies of techniques.
- What improvements of methods and further steps are needed in research on the problem?

○ Materials

- Spectrophotometer: Spectronic 20D Set at 420 nm
- Tubes for spectrophotometer with 4 mL of trypticase soy broth, 4 per group

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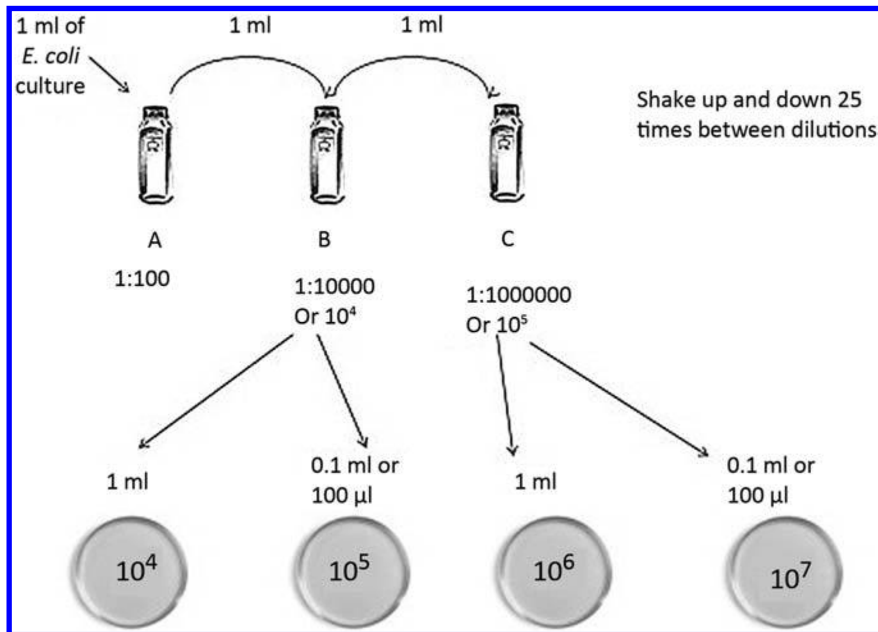

Figure 1. Serial dilution and plating procedure.

- *E. coli* overnight culture
- Trypticase soy agar (TSA) plates, 16 per group
- Water baths at 37°C and 25°C
- Dilution bottles with 99 mL sterile distilled water, 12 per group
- Sterile 1-mL pipets, micropipettes, and micropipette tips
- Sterile bent glass rods for spreading bacterial samples on TSA plates
- Plate spinners
- 70% and 95% ethanol
- Biohazard waste bags

○ Procedure

For this laboratory exercise, the students work in groups of four. Two members of the group work on generating the data for *E. coli* grown at 25°C, and two work on generating the data for *E. coli* grown at 37°C. Then the data are collated.

Spectrophotometry

Optical density (OD) measurement of bacterial cultures is a common technique for generation of the bacterial growth curve. The procedure used here was adapted from Kleyn et al. (2011). Sterile Spectronic 20 tubes containing 4 mL of trypticase soy broth are prepared and placed in water baths at 37°C and 25°C before lab begins. Each tube is inoculated 0.5 mL of an overnight *E. coli* culture. An uninoculated TSB Spectronic tube is used as a blank to zero the Spectronic 20D, which is set at 420 nm. Immediately after inoculation, the OD is read. Then tubes are returned to their appropriate water baths. Tubes are vortexed, and the OD readings are recorded every 20 minutes over the next 2.5 to 3 hours.

Standard Plate Count

Standard plate count is one of the most common methods for determining bacterial numbers in a sample. The procedure used here was adapted from Benson (2013). Approximately 40 minutes into the experiment, 1 mL of the culture is removed for serial dilutions and plating as shown in Figure 1. After 40–60 minutes, another 1-mL sample is taken for serial dilutions and plating as shown in Figure 1 (these times were chosen because the culture was expected to be in the exponential phase of growth). The 1-mL samples are diluted by adding them to a sterile bottle (bottle A) containing 99 mL of water, resulting in a $1{:}10^2$ dilution. To disrupt clumps of bacterial cells, the bottle is shaken in a long arc motion 25 times. Then, in sequence: 1 mL from bottle A is transferred to bottle B, resulting in a $1{:}10^4$ dilution, and the bottle is shaken as before; 1 mL from bottle B is added to bottle C, resulting in a $1{:}10^6$ dilution, and this bottle is shaken as before; 1 mL from bottle B is pipetted on the plate labeled "10^4"; 0.1 mL from bottle B is pipetted on the plate labeled "10^5"; 1 mL from bottle C is pipetted on the plate labeled "10^6"; and 0.1 mL is pipetted onto the plate labeled "10^7". The samples are then spread over the surface of the agar by placing the plate onto the plate spinner, alcohol-flaming the bent glass rod, and using the rod to spread the bacteria while spinning the plate. Plates are incubated at 37°C for 24–48 hours, and the colonies are counted.

○ Examples of Results from Spectrophotometry

Calculation of Doubling Time from Spectrophotometric Data

Table 2 shows the spectrophotometric readings for *E. coli* grown at 25°C and 37°C; and Figure 2 is the graphic representation of the data from Table 2. (Tables 2, 3, and 4 and Figure 2 were taken from actual results obtained by students.) Figure 2A shows the lag, exponential, and stationary phases of growth at 25°C and 37°C. Figure 2B shows just the data points during the exponential phase of growth at each temperature and the resulting straight-line relation and equations that resulted. Because we are interested in determining the doubling time during the exponential phase of growth only, the data points for Figure 2B were chosen by determining the beginning and end of the exponential phase from Figure 2A. At 37°C, *E. coli*'s growth rate begins to plateau after 90 minutes; and at 25°C, *E. coli*'s growth rate begins to plateau after 141 minutes.

The equation for a linear line is $y = mx + b$, where b is the y intercept and m is the slope. The equation for an exponential line is $y = be^{mx}$, where b is the y intercept and m is the slope. Therefore, if y (optical density) is known, as in this case, the time (x) can be solved as $x = \ln(y/m)/b$. Now Excel will calculate the x value for a given y by programing with this equation: x = LN(y/0.0441)/0.028,

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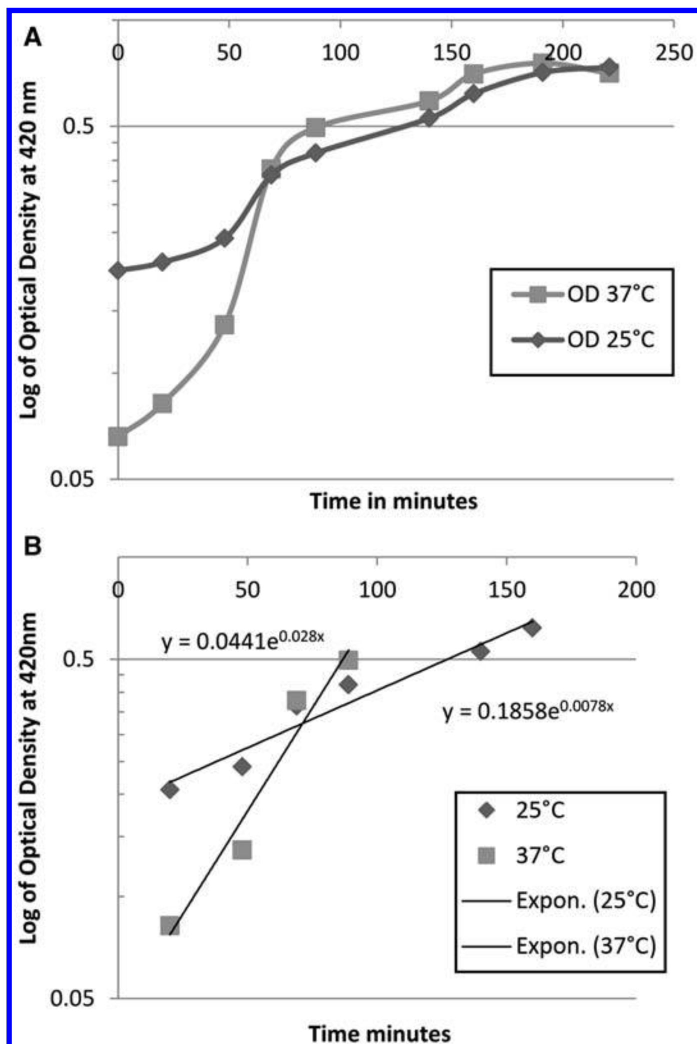

Figure 2. (A) Growth curve of *E. coli* grown at 25°C and 37°C. (B) Exponential phase of growth for *E. coli* at 25°C and 37°C.

Table 2. The growth of *E. coli* at 25°C and 37°C as measured by optical density (OD) at 420 nm.

$Time_{actual}$	Time (minutes)	OD_{420} 25°C	OD_{420} 37°C
12:30	0	0.195	0.066
12:50	20	0.206	0.082
1:18	48	0.241	0.137
1:59	69	0.364	0.378
2:19	89	0.42	0.496
3:10	140	0.527	0.59
3:30	160	0.618	0.703
4:01	191	0.71	0.755
4:21	221	0.735	0.707

obtained from the equation for the line at 37°C. My students usually need some help with entering the equation into Excel correctly.

Table 3. Number of colonies on each of the dilution plates for both times and incubation temperatures.

Dilution	Colony Counts: *E. coli* Grown at 25°C		Colony Counts: *E. coli* Grown at 37°C	
	At 40 minutes	At 100 minutes	At 45 minutes	At 100 minutes
10^4	352	TNC*	425	TNC*
10^5	42	138	57	554
10^6	5	30	19	61
10^7	2	2	11	7

*TNC-too numerous to count

Table 4. Doubling time in minutes of *E. coli* grown at 25°C and 37°C.

	Spectrophotometric Method	Standard Plate Method
25°C	88.87	34.78
37°C	24.76	21.20

Figure 3. Example of TSA plates after incubation.

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For the 37°C growth curve, I now ask the students to choose a y value that is on the line, so that this is an optical density that occurred during the exponential phase of growth. For example, y = 0.08. Then program Excel to calculate the x_1 value, which is the time in the growth curve where OD = 0.08. Now double the y value to 0.16 and again solve for x_2. Then subtract x_2 from x_1 to obtain the doubling time of *E. coli* at 37°C. From this data, the doubling for *E. coli* at 37°C is 24.76 minutes (see https://mckernanmicrobiology.wikispaces.com). Repeat this procedure for the 25°C growth curve. I ask the students to choose a y value that is on the line, so that this is an optical density that occurred during the exponential phase of growth. For example, y = 0.25. Then program Excel to calculate the x_1 value [x = LN(y/0.1858)/0.0078], which is the time in the growth curve where OD = 0.5. Now double the y value to 0.16 and again solve for x_2. Then subtract x_2 from x_1 to obtain the doubling time of *E. coli* at 25°C. From this data, the doubling for *E. coli* at 25°C is 88.87 minutes (see https://mckernanmicrobiology.wikispaces.com).

○ Example of Results from Standard Plate Method

Calculation of Doubling Time from Standard Plate Method Data

In the second lab session, students count the number of colonies that have grown on TSA plates (see Figure 3 for an example of the plates) that were inoculated as described above. Typical results of colony counts are shown in Table 3. Multiple dilutions are plated at each time point and temperature, to (hopefully) ensure that one dilution plate will result in a number of colonies between 30 and 300; this has been shown to have the greatest accuracy. Using their data, I demonstrate how to calculate the CFU/mL for one time point at one temperature. Then I ask them to calculate the second point and check their work. Once the two time points have been calculated, we can use these numbers to calculate doubling time. For example (from Table 3), at 40 minutes and a temperature of 25°C, the 10^5 plate is best with 42 colonies.

In the culture at that point in our experiment, $42 \times 10^5 = 4.2 \times 10^6$ CFU/mL. Then, at 100 minutes, the 10^5 plate is best with 38 colonies (in the culture at that point in our experiment, $138 \times 10^5 = 1.38 \times 10^7$ CFU/mL). From these two data points, the magnitude of increase in culture can be calculated by dividing:

$$1.38 \times 10^7 \text{ CFU/mL} \div 4.2 \times 10^6 \text{ CFU/mL} = 3.45\text{-fold increase in 60 minutes}$$

So now the culture has increased 3.45-fold in 60 minutes (the time differs between collection of two samples), but we need to determine how many minutes it took the culture to double. To calculate the doubling time, set up a ratio and solve for X:

$$\frac{60 \text{ min}}{3.45} = \frac{X}{2}$$

X = 34.78 minutes = the time it takes for the culture to double at 25°C

Table 5. Grading rubric.

Total Points	**50**
Title: Reflects the paper's content and emphasis clearly and accurately. Includes dependent and independent variables and the organisms used.	**3**
[Abstract: Not necessary in this paper. But happy to evaluate.]	
Introduction	**10**
State research problem and hypothesis	
Literature review	
Importance or relevance of research project	
Methods	**7**
Research design	
Equipment and material How data were collected and procedures	
Results	**18**
Data collected, organized	
Construction of tables and figures	
Data analysis and calculations	
Discussion	**10**
Original hypothesis is addressed: supported or refuted?	
Appropriate interpretation of data	
Comparison to literature and related studies	
Design improvements and future work are included	
References	**2**

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Repeating these calculations for the CFU/mL data obtained for culture grown at 37°C (Table 3), the final result is doubling time = 21.4 minutes.

○ Summary of Results

The results presented above are actual results from a group of students. As shown in Table 4, *E. coli* has a faster doubling time at 37°C. There is general agreement between the two methods for the 37°C data. However, there is a large difference in doubling time obtained from the two methods for the 25°C data. So the challenge is helping students determine where sources of error could have occurred. One common source of error is the students' micropipetting skills – which is often obvious when examining the CFU results. Did the CFU data decrease by a factor of 10 with 10-fold dilutions? Another source of error may be that the two time points chosen for the standard plate method were not during the log phase of growth. Therefore, obvious improvements would include choosing more time points and doing replicates of the dilutions for the standard plate method.

○ Conclusions

This laboratory exercise uses two classic methods in microbiology for measuring bacterial growth. What I have done is combine them into an exercise that focuses on scientific inquiry, research design, data analysis, and scientific writing. Although developing the hypothesis is very straightforward in this exercise, it allows for a discussion of why one might choose that hypothesis and what evidence has led to developing it. This experiment is easily adaptable to asking other experimental questions, such as "How is growth rate affected by aerating or shaking the cultures, by changing the sugar source or using defined media with different amino acids, or by adding agents that might inhibit the growth of *E. coli*?"

The use of two different methods to measure the same parameter lends itself to evaluating the methods chosen as part of the design of the research project and how that can affect interpretation of data and conclusions. The presentation of data in tables and graphs and the use of computers to help with data analysis and interpretation are integral to the research process. The practice of writing a scientific paper advances students' scientific writing skills. These procedures also give students another opportunity to practice aseptic technique, handling of bacterial cultures, serial dilutions, pipetting, and micropipetting – and the feeling that they are really becoming scientists. Table 5 shows a grading rubic that can be used to evaluate a student's work.

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