Teacher's Guide

How Quickly Do Bacteria Grow?

Purpose: Students will relate real-world applications to mathematical concepts by monitoring bacterial growth over one week and calculating the rate of growth. Students will calculate surface area, draw graphs, and approximate bacteria and nanobe populations. Before starting this lab, the student should understand how to:

- calculate the surface area of a circle
- draw and label a graph
- define π, circumference, and radius

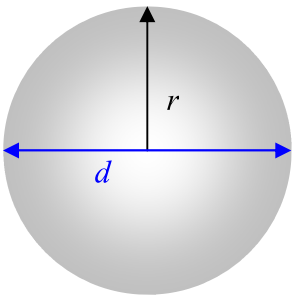
This activity works well after students have covered linear functions—students expecting a linear graph may find the nonlinear (exponential) relationship as a pleasant surprise.

Level Middle school (Biology, Pre-algebra, Algebra)

Time required One week total: 1 hour the first class; 30 minutes for the next 3 class meetings, about 5 minutes per student for presentations. Recommendation: Students inoculate their Petri dishes on Monday, and collect data on either Tue/Thurs/Mon OR Wed/Fri/Mon.

Teacher Background Nanobes are recently discovered bacteria that can be as small as 20 nm in size. A typical surface area for an average bacterium is about 9 μm^2 or 9,000 nm^2 (1,500 nm × 6,000 nm). A typcial surface area for a nanobe is approximately 400 nm^2 (20 nm × 20 nm).

The diagram below shows how to measure the diameter of a circle, and how to calculate the circumference and the surface area of a circle.



Diameter of a circle = $d = 2r$

Radius of a circle = $r = \frac{d}{2}$

Surface area of a circle = πr^2

Circumference of a circle = $2\pi r$

Pi = $\pi = 3.1459\ldots$

The existence of nanobes is somewhat controversial in the scientific literature. Many use the terms nanonobes and nanobacteria interchangeably while others note a distinction between the two. Nanobacteria are considered to be the smallest cell-walled organisms. They are in the nanometer size range and are approximately $1/10^{th}$ the size of bacteria (~1-2µm) . Nanobes are very small filamental structures found in organisms (debated) and rocks. They are 20nm or less

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in size which is similar to the size of nanobacteria. Many believe that nanobes are an unknown "life" form and if this proves correct then it will alter our view of the scale of living organisms.

Materials for each group of 2–3 students

- sterile Petri dish with nutrient agar (a standard size is 8.8 cm in diameter)
- sterile cotton-tipped applicator or swab (6 inch sticks work best)
- disposable gloves
- scotch tape
- permanent fine-tipped ink marker
- metric ruler
- paper, pencils, graph paper
- incubator *(optional)*, made from:
  - a styrofoam cooler (18"H × 24"W × 12"D)
  - desk lamp with incandescent bulb (about 15 watts)
  - extension cord
- digital camera *(optional)* and color printer *(optional)* for recording bacterial growth

Safety Information:

Students should wear gloves to ensure that no bacteria is transferred from their fingertips to the swab sample. Alert students that pathogenic bacteria (germs) are everywhere and to practice good hygiene. The bacteria collected will be no more pathogenic than what students are exposed to everyday, but encourage students not to touch their mouth, nose, ears or eyes.

Advance Preparation

1. OBTAIN STERILIZED PETRI DISHES CONTAINING NUTRIENT AGAR
   Use Petri dishes that are already sterilized and contain nutrient agar. Either borrow them from the school science biology department or order them from a biological supply store or an educational supply warehouse. Possible sources for ordering nutrient agar media kits is:

   Carolina Biological
   1-800-334-5551
   http://www.carolina.com/product/long+life+medium+kit+nutrient+agar.do?keyword=nutrient+agar&sortby=bestMatches

   Flinn Scientific
   1-800-452-1261
   http://www.flinnsci.com/store/Scripts/prodView.asp?idProduct=18504

2. INCUBATOR PREPARATION *(OPTIONAL)*
   Bacteria will grow at room temperature (about 26ºC or 78ºF), but an incubator may speed growth. To make an incubator, buy a styrofoam cooler from a convenience store. Also purchase a corded light socket with an on/off switch. Trace a circle the diameter of the socket onto the middle of the cooler lid and then cut the circle out using a knife or box cutter. Place the socket with light bulb in the hole to increase the temperature in the cooler. Ideally, the temperature should be close to 30ºC (86ºF), but it's not critical. You may add a thermometer

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to monitor the temperature in the incubator. Instructions for more elaborate incubators can be found at the following websites or Google home made incubators.:

http://www.herpcam.com/incubator.htm
http://www.ogpbb.com/accessories/incubators/

Resources

- The following website, "Bacteria Growing Experiments in Petri Plates" by Science Company, provides some background information about growing bacteria in Petri dishes and explains 3 bacteria growing experiments:
  http://www.sciencecompany.com/sci-exper/Petridishes.htm
- The following website is a QuickTime video showing the technique on inoculating a Petri dish:
  http://www.csun.edu/scied/2-longitudinal/growth/Copy%20of%20videos/petriMovie.MOV

Directions for the Activity

Teaching Strategies: This activity works best in groups of 2–3 students. *Before* beginning the lab activity, ask the students the questions in the *Guided Dialog* section. Then explain the importance of using sterile procedures to prevent contamination of the Petri dish and review how to inoculate the Petri dishes with samples.

Procedure: On Monday, students will swipe an object of their choice with a cotton swab and inoculate nutrient agar Petri dishes with bacteria on the cotton swab. Then, they will measure the amount of surface area covered with bacteria on the Petri dish at 24, 72, and 168 hours for a Tue/Thurs/Mon class schedule or at 48, 96, and 168 hours for a Wed/Fri/Mon class schedule. (These measurement times work well for the modified block schedule, but can be altered for any class schedule.) Bacteria do not grow in perfect circles or other shapes. Have student groups discuss the best way to estimate how much of the Petri dish surface is covered.

The students will enter these data in a table and create a graph. If possible, have students enter their data into an Excel spreadsheet to produce their graphs and predict growth outcomes. Groups should design their own data table and graph. The graph will NOT be linear; it should be an exponential curve. Some students may expect a typical linear relationship and then discover a different relationship.

Students will calculate the approximate number of bacteria and, imagining that there were nanobes present, how many nanobes would be on their Petri dish. Depending on time and resources, you can have students either research the sizes of bacteria and nanobes or you can just give them an average number for each to use (see *Teacher Background* above for the average number for each).

Finally, have students present a PowerPoint or poster presentation, which should include:

- drawings or pictures (if a digital camera is available) of what the Petri dish looked like at each measurement
- how they calculated bacterial and nanobe growth
- how they calculated the two populations (bacteria and nanobe)
- a conclusion of what they learned

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Rev: 09/09

Guided dialog

Inoculation Day

Before beginning the lab, ask students questions to provoke thought and review what they already know. For example:

1. What are germs? *Germs are microscopic organisms that cause sickness or disease.*
2. What kinds of organisms are germs? *Yeast, viruses, fungi, and bacteria are organisms that can cause sickness or disease.*
3. How big are germs? *Viruses are 20–400 nanometers. Most bacteria are 1–6 micrometers (called, "microns").*
4. Can we see individual germs with our eyes? *No, germs are way too small to see with your eyes. The students can see colonies of bacteria but not single cells with their eyes.*
5. Where are germs found? *Everywhere! On people, desks, pens, doorknobs, cell phones. Everywhere!*

First Day of Measurement

Review with students how to measure the diameter of a circle (see *Teacher Background* on previous page).

First Day of Analysis

Review with students how to calculate the circumference and the surface area of a circle (see *Teacher Background* on previous page).

List any last minute details that the students must remember, including reiterating all safety precautions. Now, begin the lab.

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Rev: 09/09

Student Worksheet
(with answers)

How Quickly Does Bacteria Grow?

Safety

Wear gloves to ensure that no bacteria are transferred from your fingertips to the swab sample. The bacteria collected today will be no more pathogenic than what you are exposed to everyday, but do not touch your mouth, nose, ears or eyes during this lab activity.

Recently, scientists have found bacteria that measure 20–400 nanometers in size. Originally thought to be viruses, these nanobacteria—also called *nanobes*—may be the smallest living organisms on Earth! These super-small bacteria resemble the fossilized structures found on meteorites that originated on Mars. So, not only do we find bacteria everywhere here on Earth, but maybe even in the vast distances in space!

Materials

- sterile Petri dishes with nutrient agar
- sterile cotton-tipped applicators or swabs
- disposable gloves
- scotch tape
- permanent fine-tipped ink marker
- metric ruler
- paper, pencils, graph paper
- incubator *(optional)*
- digital camera *(optional)*

Question Out of all the things you touch everyday, where would you find the most bacteria? Why?

Make a Prediction

Example prediction: I think that a doorknob would have more bacteria on it than anyplace else, because many people touch a doorknob with their hands.

Procedure

1. Decide with your group where you will collect your bacteria sample.
2. Take the *packaged* cotton swab and *closed* Petri dish to where you will collect the sample.
3. Rub the tip of the cotton swab across the area you are sampling. Twist the swab as you swipe it along the surface to help collect a good amount of bacteria onto the swab.
4. Open the Petri dish just enough to fit the tip of the swab into it. Gently rub and twist the swab at the center of your dish.
5. Immediately close the dish, seal it along the perimeter (edge of the lid) with clear tape.

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6. Label the tape with your name, date, time, and what you sampled. Throw the cotton swab into the trash.
7. Place your dish where instructed by the teacher to be stored until the next class. (One option is to place the dish in an incubator (such as, a styrofoam cooler with a lamp).

Record Your Observations

At the beginning of the next three classes, use a metric ruler to measure the size of the bacterial growth in the dish. Then, fill out the data table below for each observation.

#	**Date & Time**	**Observations** (drawing or picture)	**Size of Growth** (measure and describe)
1	*Wednesday, January 15, 2008; 10:00 a.m. (48 hours)*		*There is 1 circle. The diameter of the circle of bacterial growth is 0.4 cm.* *You will use this number to compute the surface area in the next table.*
2	*Friday, January 17, 2008; 10:00 a.m. (96 hours)*		*The circle has grown to a diameter of 1.2 cm.*
3	*Tuesday, January 22, 2008; 10:00 a.m. (168 hours)*		*The circle is now 3.2 cm in diameter.*

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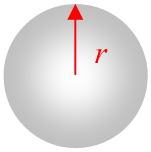
Rev: 09/09

Analyze the Results

1. Write the equation for the radius of a circle (radius of a circle = *r*). *r = d/2*

2. Draw a line on the circle below to indicate the radius of a circle.



3. Write the number for π (pi). π = *3.14159…* *(Students may use 3.14 in their calculations, but should round their answers up or down to the nearest significant digit.)*

4. Write the equation for the surface area of a circle. *surface area of a circle = πr^2*

5. Write the equation for the circumference of a circle. *circumference of a circle = $2\pi r$*

6. Calculate the surface area of bacterial growth using data from your data table.

Calculation of Bacterial Surface Area Growth

Hours of Growth	Calculate the surface area of bacterial growth. Show your calculations. Explain your work.
48 hrs.	*πr^2 = surface area of a circle* *The diameter of the circle from the table above is 0.4 cm at 48 hrs.* *Using the formula for the area of a circle, we get:* *$3.14 \times (0.4/2)^2 = 3.14 \times 0.2^2 =$ **$0.13\ cm^2$**.* *This is the surface area of the bacterial growth at 48 hrs.*
96 hrs.	*πr^2 = surface area of a circle* *The diameter of the circle from the table above is 1.2 cm at 96 hrs.* *Using the formula for the area of a circle, we get:* *$3.14 \times (1.2/2)^2 = 3.14 \times 0.6^2 =$ **$1.13\ cm^2$**.* *This is the surface area of the bacterial growth at 96 hrs.*
168 hrs.	*πr^2 = surface area of a circle* *The diameter of the circle from the table above is 3.2 cm at 168 hrs.* *Using the formula for the area of a circle, we get:* *$3.14 \times (3.2/2)^2 = 3.14 \times 1.6^2 =$ **$8.04\ cm^2$**.* *This is the surface area of the bacterial growth at 168 hrs.*

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Rev: 09/09

7. Calculate the bacteria and nanobe populations. First, convert bacterial surface areas from cm^2 to μm^2 or to nm^2. Remember:

$$1\ cm^2 = 10{,}000\ \mu m^2 = 10{,}000{,}000\ nm^2$$

surface area of a bacterium ~ 9 μm^2

surface area of a nanobe ~ 400 nm^2

Calculation of Bacteria and Nanobe Populations

Hours of Growth	Surface Area of Growth	Bacteria Population
48	*0.13 cm^2*	*0.13 cm^2 × 10,000 $\mu m^2/cm^2$ = 1,300 μm^2 1,300μm^2/9μm^2 ~ 144 bacteria*
96	*1.13 cm^2*	*1.13 cm^2 × 10,000 $\mu m^2/cm^2$ = 11,300 μm^2 11,300 μm^2/9 μm^2 ~ 1,256 bacteria*
168	*8.04 cm^2*	*8.04 cm^2 × 10,000 $\mu m^2/cm^2$ = 80,400 μm^2 80,400 μm^2/9μm^2 ~ 8,933 bacteria*
Hours of Growth	**Surface Area of Growth**	**Nanobe Population**
48	*1,300 μm^2*	*1,300 μm^2 × 1,000 $nm^2/\mu m^2$ = 1,300,000 nm^2 1,300,000 nm^2/400 nm^2 = 3,250 nanobes*
96	*11,300 μm^2*	*11,300 μm^2 × 1,000 $nm^2/\mu m^2$ = 11,300,000 nm^2 11,300,000 nm^2/400 nm^2 = 28,250 nanobes*
168	*80,400 μm^2*	*80,400 μm^2 × 1,000 $nm^2/\mu m^2$ = 80,400,000 nm^2 80,400,000 nm^2/400 nm^2 = 201,000 nanobes*

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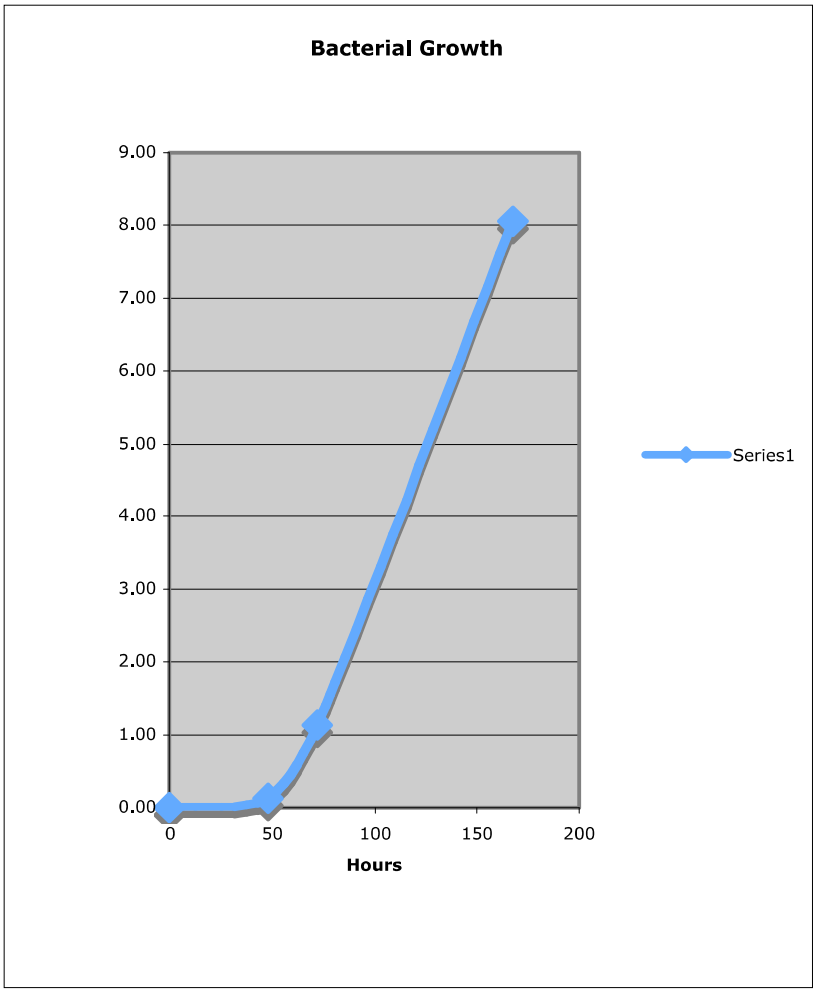
8. Using your results from the previous page, graph the growth of the bacteria versus time. Determine the best scale to use for graphing by looking at the ranges of your growth data. *(Students can use graph paper to graph the growth of the bacteria, and then attach their graph to this page.)*



Draw Conclusions

1. When might it be useful to know how fast bacteria can grow at certain temperatures?

   Example answer: Knowing how to keep bacteria from growing would be very important when storing food either at home or in a restaurant. It would also be important for understanding how fast infectious bacteria grow to help prevent the spread of illness.

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2. Describe your graph. Did anything surprise you? Explain.

Example answer: I expected bacterial growth to have a linear graph, but I got a curved graph instead.

3. As more time goes by, more bacteria grow. What kind of function is this?

Example answer: This is a positive or direct function. As one variable increases, the other variable also increases.

4. Nanobacteria are so small they cannot be seen with a light microscope. Knowing that doctors identify what type of bacterial infection a patient has and what kind of medicine a patient needs by looking at the bacteria through a light microscope, what problems might doctors have if nanobacteria were to also cause infection?

Example answer: Doctors would not be able to identify the bacteria with optical microscopes and may not be able to prescribe the proper antibiotic. Medical labs may need to use more powerful microscopes to identify the nanobacteria.

Going Further Look at your graph.

a. How many bacteria do you think will be in your dish in 200 hours? Write your prediction.

Example prediction: I think there will be about 11,000 bacteria because the surface area would be about 10 cm^2 in about 200 hours.

b. What could you do to test and see if you are right?

Example answer: Continue to grow the bacteria longer and then take another measurement.

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c. If you let your bacterial population grow for 1 month, how would the bacterial growth in the Petri dish differ from growth in the location you took your sample from?

Example answer: Eventually, the bacteria in the Petri dish would run out of nutrients and the colonies would stop growing. On the doorknob, there is constant contact by people which would help keep the bacterial populations renewed.

Extra Credit Follow the steps you outlined in answer "b" above. If time allows, test to see whether your prediction in answer "c" is accurate.

Assessment

Each student will turn in their completed worksheet at the end of the entire lab so the teacher can assess the student's understanding of the lab and his/her ability to relate real-world applications to mathematical concepts. Points can be given for correctly:

- measuring the size of the bacterial growth in the Petri dish, and then correctly filling out the data table for each observation
- calculating the surface area of bacterial growth
- converting bacterial surface areas from cm^2 to μm^2 or to nm^2
- calculating the bacteria and nanobe populations
- drawing and plotting the graph
- explaining their conclusions

Students may also earn points for giving a PowerPoint or poster presentation displaying and describing their work and the conclusion(s) of what they learned.

Extra credit points can be given for correctly:

- answering the *Going Further* questions
- completing the *Extra Credit* work

For further information on nanobacteria and nanobes:

http://en.wikipedia.org/wiki/Nanobe
http://www.microscopy-uk.org.uk/index.html?http://www.microscopy-uk.org.uk/nanobes/nanoimages.html
http://serc.carleton.edu/microbelife/topics/nanobes/index.html

National Science Education Standards (Grades 5–8)

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard C: Life Science

- Diversity and adaptations of organisms

National Math Education Standards (Grades 6–8)

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Content Standard: Algebra

- Understand patterns, relations, and functions.
- Represent and analyze mathematical situations and structures using algebraic symbols.
- Use mathematical models to represent and understand quantitative relationships.

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