

Teacher Guide: How is Yogurt Made?

Class time: 30 to 50 minutes during two class periods. (Plus two to three days between class periods for the milk to solidify.)

Purpose: Students can grow safe yogurt bacteria in sterilized milk, observe that the bacteria solidify the milk into new yogurt and test how antibiotics affect the bacteria.

Materials:

- Student instructions: [Blackline Master 3](#)
- Small boxes of Parmalat or Li'l Milk ultra-high-temperature (UHT) pasteurized milk (typically sold in the non-refrigerated baking aisle of the grocery store)
- Cup(s) of yogurt (plain yogurt is simplest)
- Tube(s) of triple antibiotic ointment
- 15 ml graduated sterile plastic test tubes
- Lab markers (preferably alcohol-resistant)
- Paper towels
- 70% ethyl or isopropyl alcohol in squirt bottles (rubbing alcohol)
- Non-latex gloves
- Safety goggles
- Lab coats or lab aprons
- Alcohol burners or Bunsen burners
- Flame-sterilizable inoculation needles or loops (straightened paper clips taped to pencils can also be used as long as the paper clips have no plastic coating)
- Water bath (optional): Styrofoam cooler, aquarium heater, thermometer, plastic test tube racks, paper clips, nearby sink

Notes to the teacher: Yogurt contains harmless live bacteria (typically *Lactobacillus* and non-pathogen *Streptococcus* species). When a drop of these bacteria is added to fresh milk, they can acidify and solidify the milk to make new yogurt.

Triple antibiotic ointment contains three antibiotics (bacitracin, neomycin and polymyxin B). When a small amount of antibiotic ointment is added to fresh milk along with the yogurt bacteria, the antibiotics will kill the bacteria and prevent the milk from solidifying into new yogurt. This lab activity is a good demonstration to show that antibiotics kill bacteria.

This activity is also a good opportunity to discuss and demonstrate the importance of using sterile techniques in science experiments. In this experiment, we want only yogurt bacteria to grow in the milk,

not other bacteria from the classroom, students' hands or elsewhere. To minimize or avoid introducing other bacteria, students should:

- Wear gloves, sterilize gloves with alcohol by pouring some alcohol on the gloves and rubbing it around the gloves. After sterilizing the gloves with alcohol, students should avoid touching anything with their gloves that is not absolutely essential for the experiment.
- Use UHT milk, in which all of the bacteria have already been killed by high heat (unlike in regular milk). Because of this, UHT milk can be stored at room temperature (until it is opened and is exposed to new bacteria from the environment).
- Use sterile test tubes, keep them tightly capped except when adding something to them and be very careful handling the test tube caps.
- Sterilize an inoculation needle by passing its end through a flame for a few seconds, then let it cool in the air for a few seconds before using it to transfer a drop of yogurt or antibiotic ointment.
- Use separate inoculation needles for yogurt and antibiotic, and wipe and flame them between uses to avoid cross-contamination.

The yogurt bacteria will solidify a tube of milk overnight at 37° C, or in two to three days at room temperature. If you have a heated water bath, incubator or a warm room (like a boiler room), you can put the sealed tubes there to incubate. If you would like to create a water bath, you can easily make one by filling a large Styrofoam cooler with water, dropping an aquarium heater and a thermometer into it and adjusting the thermostat on the aquarium heater until it heats and maintains the water at around 37° C. Using paper clips like hooks, you can attach plastic test tube racks to the inner walls of the cooler, so that the racks will keep test tubes mostly immersed in the water with just their tops sticking out of the water.

Please make sure students follow all applicable safety rules in working with flames and using inoculation needles. Students should not consume any of the yogurt or milk before, during or after the experiment.

If you have enough time, your students can dilute a drop of yogurt in water, streak it on a microscope slide, Gram's stain the slide and observe the yogurt bacteria at 400x or 1000x under a microscope. *Lactobacillus* bacteria look like chains of tiny sausages linked end-to-end, and *Streptococcus* bacteria look like chains of tiny spheres. Both types of bacteria are gram-positive and should appear purple when Gram's stained. Students could test for bacteria from a cup of fresh yogurt and/or from their milk tubes after the samples have been incubated.

Materials for observing bacteria under a microscope:

- Student instructions: [Blackline Master 4](#)
- Gram's stain (such as this [Gram's stain kit](#) from Home Science Tools)
- Pre-cleaned glass microscope slides
- Pre-cleaned thin glass cover slips
- Optional: Canada balsam or microscope slide mounting cement
- Microscopes with 400x (or 1000x and immersion oil)
- Squirt bottles with water

Student Guide: How is Yogurt Made?

Yogurt contains harmless live bacteria (typically *Lactobacillus* and non-pathogen *Streptococcus* species). You can observe how these bacteria affect milk, and you can test what happens when you add antibiotics. Throughout this lab it will be very important to practice good sterile techniques to avoid adding bacteria from the environment or from your skin to the experiment.

Notes: Wear gloves, goggles and other appropriate safety gear. To kill any environmental bacteria on your gloves, sterilize gloves with alcohol by pouring some alcohol on and rubbing it around the gloves until it dries. Do not touch anything with your gloves that you do not need to touch for this experiment. If necessary, alcohol your gloves again during the experiment.

The test tubes are sterile, and you should keep them tightly capped except when you need to add something to the tubes. When you remove the test tube cap, try to hold it with your alcohol-sterilized glove instead of setting it down on a surface that might have bacteria.

The box of ultrahigh-temperature (UHT) pasteurized milk is sterile. Any bacteria in the milk were killed when it was heated to a high temperature. This means the milk can be stored at room temperature until it is opened and exposed to environmental bacteria. (Normal milk still has some bacteria, so it must be refrigerated and will still go bad within a week or so as those bacteria multiply in the milk.)

Procedure:

1. Label four test tubes with your or your group's name and one each of the following labels:

-Yogurt	-Antibiotic
+Yogurt	-Antibiotic
-Yogurt	+Antibiotic
+Yogurt	+Antibiotic
2. Carefully remove the sterile straw from its plastic on the side of the UHT milk box and insert it into the box. The straw should form a tight seal as it passes through the foil opening. Carefully use the straw to put 10 ml of milk into each of the four test tubes (measure the amount added on the tube). If the straw dribbles milk on the table, clean up the mess with paper towels and ask your teacher for help.
3. You will use one inoculation needle to transfer yogurt bacteria and another to transfer antibiotic. To avoid cross-contamination, do not get the needles confused and wipe them off thoroughly between uses. Before each use of an inoculation needle, pass its tip through a flame for a few seconds to

kill any bacteria; then let it cool for a few seconds without setting it down (it might pick up more environmental bacteria from the table or other surfaces).

4. Dip a flame-sterilized inoculation needle into a freshly opened cup of yogurt, get a tiny blob of yogurt on the tip and add that yogurt blob to one of the +Yogurt tubes. Clean and flame sterilize the inoculation needle again and use it to add a tiny yogurt blob to the other +Yogurt tube.
5. Use another flame-sterilized inoculation needle to add a small blob of antibiotic ointment to one of the +Antibiotic tubes. To get most of the antibiotic off the needle, you may need to swish the needle around in the milk in the tube and/or gently scrape the needle against the inside wall of the tube. Clean and flame the inoculation needle again and use it to add a small antibiotic blob to the other +Antibiotic tube.
6. Seal the tubes tightly, and shake and swirl them around to mix their contents thoroughly. Give the tubes to your teacher to incubate at 37° C overnight, or at room temperature for two to three days.
7. What do you predict will happen to the milk in each of the four tubes after they have been incubated? Record your thoughts below.

Tube 1: -Yogurt -Antibiotic

Tube 2: +Yogurt -Antibiotic

Tube 3: -Yogurt +Antibiotic

Tube 4: +Yogurt +Antibiotic

8. After the tubes have been incubated and you get them back from your teacher, keep the tubes sealed while closely observing the color and texture of the milk in each tube. Try slowly leaning the tubes to one side or the other. Try gently turning the tubes upside down. Record your observations for each tube:

Tube 1: -Yogurt -Antibiotic

Tube 2: +Yogurt -Antibiotic

Tube 3: -Yogurt +Antibiotic

Tube 4: +Yogurt +Antibiotic

Answer the following questions to analyze your results:

1. Did the results match your predictions for each test tube? Explain.

-
2. If the results matched your predictions, what do you think happened in each tube?
3. If the results did not match your predictions, what do you think happened that you did not expect?
What additional factors might have influenced the results?
4. How might the experiment have turned out differently if you had not practiced sterile techniques?
5. How might the experiment have turned out differently if you had used the same inoculation needle for transferring both yogurt and antibiotic, and did not clean or flame the needle between uses?

6. What variations of this experiment could you do to test other conditions, or a range of conditions?

7. Relatively speaking, how effective is antibiotic ointment on *Lactobacillus* and non-pathogen *Streptococcus*? What are the antibiotics in the ointment used in your experiment? Research to find out how each antibiotic kills *Lactobacillus* and non-pathogen *Streptococcus*. What other types of bacteria could these antibiotics be useful for?

8. Knowing what you do now, how do you feel about eating yogurt? Research and explain how yogurt is made. How does this relate to the experiment you just performed?

9. Research *Lactobacillus* and non-pathogen *Streptococcus* bacterial species. Are they gram-positive or gram-negative bacteria? What color would you expect them to turn if dyed with Gram's stain? In what environment do they thrive? How do they affect humans?

Student Guide: Gram Staining

Use the following set of instructions to stain your cultured bacteria, prepare a microscope slide and view it under a microscope. Write down your observations and explain how they compare with your prediction about the color of the Gram's stain for each type of bacteria.

Procedure:

1. Fill a 15-ml plastic test tube with 5 ml of water, add a drop of yogurt and put the cap on the tube. Seal the tube tightly, and shake and swirl it vigorously to mix it well.
2. Use a clean inoculation needle to put a drop from the tube on a clean microscope slide. If necessary, use the needle or the edge of another clean slide to smear the liquid into a very thin film.
3. Let the bacteria on the slide air dry for about two minutes, then hold the slide with forceps (tweezers) and move it back and forth through the alcohol lamp flame three to four times. Do not let the slide get too hot.
4. Put one to two drops of crystal violet stain on the sample and let it sit for 60 seconds.
5. Rinse off the stain with a gentle squirt from the water bottle.
6. Put one to three drops of Gram's iodine stain on the sample and let it sit for 60 seconds.
7. Rinse off the stain with a gentle squirt from the water bottle.
8. Tilt the slide and add ethyl alcohol one drop at a time so that the alcohol runs over the entire sample. Stop adding alcohol drops as soon as the liquid dripping off the slide becomes colorless. That might take about five seconds or so.
9. Rinse off the alcohol with a gentle squirt from the water bottle.
10. Put one to three drops of safranin stain on the sample and let it sit for 60 seconds.
11. Rinse off the stain with a gentle squirt from the water bottle.
12. Very gently blot (but do not rub) the slide with a paper towel to dry it.
13. Put a clean cover slip on the slide. If necessary, use another slide or a paper towel to very gently mash it down or move it into position. If instructed by your teacher, add a tiny drop of Canada balsam or microscope slide mounting cement before you put the cover slip on the slide.

14. Observe the slide under the microscope. Start at low power (40x), focus back and forth until you find a reddish-purple colored layer and then work your way up to higher powers, refocusing each time as necessary. (Be careful not to focus on other, non-colored layers that might just be dust, bubbles or scratches above or below the actual sample.)
15. Describe what bacterial shapes and colors you see. The bacteria will be tiny, even at 400x or 1000x magnification. You may also see clumps of protein, blobs of fat or other fairly large debris from the yogurt. If you see something photogenic, you can hold a cell phone camera up to the eyepiece and adjust the camera position and focus to take a photo.

Notes:

- If the bacteria are colorless, you may have used too little stain or left the stain on too briefly, or you may have rinsed with too much water or ethyl alcohol.
- If the bacteria are too dense, you can dilute a drop of yogurt with more water and make a new slide.
- If the bacteria are too scarce, you can dilute a drop of yogurt with less water and make a new slide.