BLUE'S THE CLUE

Time: One 45-minute class period, plus observation time over the next 2 to 3 days

LAB AT A GLANCE

This experiment introduces students to the effect of temperature on reducing and controlling the growth of bacteria. The video will be used to introduce food processing and transportation. Students will use pasteurized and ultra high temperature (UHT) milk, and observe how different temperatures (heat, room temperature, chilling, and freezing) affect the growth of spoilage bacteria. They will also learn about the importance of pasteurization in keeping food safe.

FOOD SAFETY CONNECTION

By learning about the effect of temperature on bacterial growth, students will be able to relate these findings to how they prepare and store food at home to help reduce bacterial growth.

GETTING STARTED

ADVANCE PREPARATION

- Order methylene blue.

  Note: This experiment was designed using methylene blue chloride 1% (Educational Reagent Aqueous Solution from Fisher Scientific — catalog #S71326).
- Mix 1 ml of methylene blue 1% solution in 25 ml of water.
- Sterilize the test tubes, test-tube caps, pipettes, and pipette bulbs (see page 9).
- Purchase pasteurized whole milk and ultra high temperature (shelf stable) whole milk. (Shelf stable milk can usually be found in the juice aisle. Ask your store manager to order it if it isn't available in your supermarket.)
- Place all the equipment on a lab table.
- Photocopy pages 31–33 (Pasteurization), page 41 (Shelf Stable), and page 46 (Ultra High Pressure Treatment) of the *Food Safety A to Z Reference Guide*.
- Photocopy the *Blue's the Clue Data Table* (page 41) for each team.

ABOUT UHT AND PASTEURIZED MILK

UHT milk is heated to at least 280° F (138° C) for 1 or 2 seconds, then packaged in sterile, airtight containers. Because of the high heat and special packaging, UHT milk contains fewer bacteria than conventionally pasteurized milk, and can be stored without refrigeration for up to 90 days. After opening, spoilage time for UHT milk is similar to that of conventionally pasteurized milk. Therefore, after opening, it should be refrigerated just like pasteurized milk.

Pasteurized milk is heated to at least 161° F (72° C) for 15 seconds. This process kills the pathogenic bacteria found in milk; however, it may not kill all the spoilage bacteria.

MATERIALS

For the Class

- 3 to 6 test-tube racks, depending on the number of teams. Teams can share test-tube racks.
- Refrigerator with freezer compartment, if possible
- *Food Safety A to Z Reference Guide*, (see pages above).
- *Dr. X and the Quest for Food Safety* video/DVD, Module 3 — Processing and Transportation

For Each Team of 3 to 4 Students

- 60 ml of pasteurized, whole milk (10 ml/test tube)
- 60 ml of ultra high temperature (shelf stable) whole milk (10 ml/test tube)
- Methylene blue dilute solution (1 drop per test tube)
- 6 sterile test tubes
- 6 sterile test-tube caps or aluminum foil to cover the test tubes
- Two sterile 10 ml pipettes
- One or two sterile 5 ml pipettes or eye droppers
- Sterile pipette bulbs or pipette aids
- Permanent marker to label test tubes
- *Blue's the Clue Data Table*

INTRODUCTION

Explain to students that later in Module 3, they'll learn more about irradiation and ultra high pressure treatment, but in this activity, they'll focus on pasteurization. Now ask students:

- ***Have you ever wondered why your parents are always asking you to put the milk back in the refrigerator? What might happen to that milk if it's left out at room temperature overnight?***
- ***In the video Module 1 — Understanding Bacteria, Dr. X talked about the Danger Zone. What precautions did he give about the "Zone"? What might be present in milk that has been left in the Danger Zone for more than 2 hours?***

SAFETY FIRST

- DO NOT DRINK THE MILK USED IN THE LAB.
- Never pipette by mouth. Always use a pipette bulb or aid.
- Wash test tubes and other materials in hot, soapy water after the lab.
- Before leaving the lab, wash your hands with hot, soapy water.
  Caution: Be careful not to spill methylene blue on the countertops or clothes; it may stain.

TIME TO TUNE IN . . . Module 3 — Processing and Transportation

Introduce the video by explaining that on our next stop along the Farm-to-Table-Continuum, students are going to learn about Processing. Dr. X will beam them into the research lab of one of his scientist friends who looks at new ways to reduce the bacteria in our food through processing. Here are some things to think about while they watch the video:

- ***What do cows, astronauts, and elephants have to do with food safety and food processing?***
- ***What is pasteurization?***
- ***How can an egg be pasteurized in the shell without cooking it?***
- ***How can some types of milk stay safe without being refrigerated?***
- ***What process keeps food safe in outer space?***

Show video/DVD Module 3 — Processing and Transportation (Time: 7 minutes).

INSTANT REPLAY Time to review and summarize.

1. ***What's the relevance of cows, astronauts, and elephants to food safety and food processing?*** (Cows refer to pasteurization, astronauts refer to irradiation, and elephants refer to ultra high pressure treatment.)
2. ***What is pasteurization?*** (Pasteurization uses heat to kill harmful bacteria in foods.)
3. ***What is the time/temperature relationship?*** (Pasteurized milk is heated for a longer time at a lower temperature, and UHT milk is heated for less time at a higher temperature.)
4. ***How can an egg be pasteurized in the shell without cooking the egg or breaking the shell?*** (Manufacturers use a time/temperature relationship to pasteurize eggs in the shell without cooking them. Heating eggs above 140° F [60° C] will cook them. Thus, using a lower temperature of 130° F [54° C] for a long time, 45 minutes, kills bacteria without cooking.)
5. ***How can some types of milk stay fresh and safe without being refrigerated?*** (UHT milk contains fewer bacteria than conventionally pasteurized milk because it's heated to a higher temperature. It's also packaged in sterile, airtight containers. Therefore, UHT milk can be stored without refrigeration for up to 90 days.)
6. ***So . . . what prevents astronauts from getting foodborne illness in outer space?*** (Irradiation of their food)

BLUE'S THE CLUE

PROCEDURE

LAB 1 Design and Conduct Experiment

1. Ask students to form teams of 3 or 4 and encourage each team to develop a hypothesis on how temperature affects bacterial growth. Then ask them to design an experiment to test their hypothesis.
2. Introduce the three materials teams must use for their experiment: regular pasteurized milk, ultra high temperature (shelf stable) milk, and methylene blue.
3. Ask: ***How might you use methylene blue to help with your experiment?*** Students can research methylene blue and discover that it's an indicator dye used to determine the presence of bacteria in milk. Tell them they can use any of the other materials on the lab table. Also, there's a refrigerator and freezer they can use.
4. Let teams discuss their hypotheses and experimental designs for 10 to 15 minutes. Then, begin posing the following questions to help students design well-thought-out experiments:
   - ***What are some ways you could test the effect of temperature on bacteria? What did you learn about the effect of temperature on bacteria in Module 1 — Understanding Bacteria of the video/DVD?*** (Heating is a way to kill bacteria, whereas chilling and freezing are ways to retard the growth of bacteria.)
   - Explain that one container of milk came from the refrigerated dairy case of the supermarket and the other from an unrefrigerated shelf. Let students examine each container.
   - ***What's an important difference between the two milk products? Is there any information on the labels that relates to our question about the effect of temperature on bacterial growth?*** (Students should discover that one is pasteurized and the other is treated using ultra high temperature.)
   - ***What are the similarities and differences between pasteurized and ultra high temperature treatments?*** (Both pasteurization and ultra high temperature use heat to kill bacteria. Ultra high temperature methods use higher temperatures than regular pasteurization. Also, products treated at ultra high temperatures are packaged in special airtight containers to prevent bacteria from getting into the product.)
   - ***Could there be differences in the growth of bacteria between the two milks? What do you think the differences might be?*** (The regular pasteurized milk should show bacterial growth sooner than the UHT milk because the pasteurized milk has more bacteria in it.)
   - ***Should you consider these differences when you design your experiments? Why?*** (Yes, both milks should be tested in all conditions.)
   - ***How can you tell if bacteria are growing in the test samples?*** (Add methylene blue to each sample. If bacteria are growing, the methylene blue will become colorless and the milk will change from blue to white. This is not immediate, but happens over time.)
5. Have each group present their hypothesis and experimental design to the class. Encourage students to discuss the merits of each suggested test. (One effective experimental design is to test pasteurized milk and UHT milk at three temperatures — room temperature, chilled, and frozen.)
6. After the group discussions, give the teams time to revise their hypotheses and experimental designs.
7. Let teams conduct experiments according to their designs. ***Note:*** The test tubes must be checked each day after the experiment is conducted. Since the color change happens over time, you could miss important findings if you don't check every day.

About Methylene Blue

Methylene blue is an indicator dye that, in anaerobic conditions, becomes colorless and is reduced to leucomethylene. Methylene blue loses its color in the absence of oxygen because bacteria use up the oxygen present in the milk. The rate at which it loses its color is a relative measure of bacteria present in milk.

TIPS

- Carefully label all test tubes and test-tube racks.
- The methylene blue will mix better if the milk is added to the test tubes before the methylene blue. Mix thoroughly by lightly tapping the test tubes with your fingers.
- Gas will be produced, so don't close the test-tube caps tightly.

LAB 2 Observe and Record

Option: Students can use the *Blue's the Clue Data Table* to record their results.

1. Students should observe and record the time and any visual changes on day two of this lab activity. Ask: ***How did the data support or reject your hypothesis? What might happen if the chilled and frozen samples were left out at room temperature for several hours or overnight? Should we test them to find out?*** (Yes, let the chilled and frozen samples stand at room temperature until the following day. As they reach room temperature and remain in the Danger Zone for several hours, the bacteria will begin to grow. As this happens, the methylene blue will become colorless and the milk will change from blue to white. Observe and record the results.)
2. ***What might happen if the UHT samples were left out at room temperature for another day?*** (If you let the UHT samples sit out at room temperature for another day or more, the color will change to white. Observe and record the results.)

LAB 3 Observe, Record, and Report

1. Observe and record findings on the third day. Ask students: ***What happened to the frozen and chilled samples? What happened to the UHT samples?***
2. Give students 5 to 10 minutes to complete their *Data Table.*
3. Have teams present their findings to the class. They should report both positive and negative results and discuss ways they would improve their experimental design.
4. Remind students to include the relationship of their findings to food safety.

TIP To find the results you can expect from this experiment, see page 40.

F • A • Q

If bacteria in UHT milk don't grow rapidly, why do I have to keep the milk refrigerated after I open it?

Because there are fewer bacteria in UHT milk than in regular pasteurized milk, the spoilage bacteria in UHT milk take longer to grow. However, they will eventually multiply. You should always practice the safest precautions. Therefore, refrigerate the milk as soon as it is opened.

INSTANT REPLAY Time to review and summarize.

1. ***Were bacteria killed at the different temperatures? Why or why not? How could you tell?*** (No. Only heat kills bacteria. Room temperature isn't high enough to kill bacteria, and chilling and freezing do not kill bacteria, they just slow their growth. When the chilled and frozen milk reached room temperature, bacteria began to grow again.)
2. ***What's a basic difference between conventionally pasteurized and UHT milk?*** (UHT milk can be stored on a shelf without refrigeration for up to 90 days.)
3. ***Explain the importance of knowing about the Danger Zone in food safety.*** (Awareness of the Danger Zone helps people understand the importance of heating and chilling food, thus decreasing the amount of foodborne illness.)
4. ***What do chilling, freezing, and heating do to bacteria?*** (Chilling and freezing slow down the growth, but heating kills the bacteria.)

BLUE'S THE CLUE

Here are the results you can expect from this experiment:

Room temperature samples

- The ***pasteurized milk*** will turn white by Lab 2 (day 2), indicating that there are some spoilage bacteria in the milk. At a temperature conducive to bacterial growth, they will multiply.
- The ***UHT milk*** will still be blue by Lab 2 (day 2). This is because the UHT milk has fewer spoilage bacteria than regular pasteurized milk. Thus, it takes longer to see any bacterial growth. Bacteria do not quickly multiply in the UHT milk.
- After leaving the UHT milk at room temperature for another day or two, the color will turn white, indicating that spoilage bacteria will ultimately grow in the UHT milk.

Chilled and frozen samples

- Both the pasteurized and UHT chilled and frozen milk samples will still be blue by Lab 2 (day 2), indicating that cold temperatures retard bacterial growth.
- After leaving the chilled and frozen samples at room temperature for another day or two, the color will change to white. This indicates that when the temperature rises into the Danger Zone (room temperature), bacteria can grow. It may take longer for the UHT milk to change to white because there are fewer spoilage bacteria in UHT milk than in regular pasteurized milk.

SUMMARY

Temperature affects the growth of bacteria. Heating kills bacteria and chilling or freezing retards the growth of bacteria. Pasteurization is the process of destroying *harmful* bacteria that could cause disease by applying heat to a food; however, some spoilage bacteria may still be present. Bacteria grow more quickly in regular pasteurized milk than in UHT milk because the latter uses higher temperatures, thus killing more bacteria. Also, UHT milk is sealed in sterile, airtight containers.

EXTENSIONS

- Test UHT milk that has an expiration date that has passed and UHT milk that has an expiration date in the future. See if the "expired" milk changes more quickly than the fresher milk.
- Try this experiment using a variety of milk forms: powdered, skim, 1%, 2%, etc.
- Relate your pathogen to this experiment and record the information in your food safety portfolio.

CAREER CONNECTION

See real-life scientists in action!

- **www.foodsafety.gov/~fsg/teach.html**
- *Food Safety A to Z Reference Guide*

RESOURCES

- ***Food Safety A to Z Reference Guide*** (See the following terms — Bacteria, Danger Zone, Methylene Blue, and Pasteurization.)
- ***Dr. X and the Quest for Food Safety*** video/DVD Module 3 – Processing and Transportation
- ***Laboratory Exercises for Microbiology***, by John P. Harley and Lansing M. Prescott, 4th Edition, WCB McGraw-Hill: Boston, 1999 (for additional information on methylene blue)
- **Web sites**

  Pasteurization — Dairy Science and Technology/University of Guelph, Canada
  www.foodsci.uoguelph.ca/dairyedu/pasteurization.html

  National Milk Producers Federation
  www.nmpf.org

SCILINKS® THE WORLD'S A CLICK AWAY

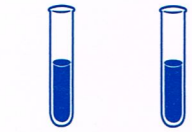
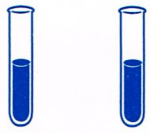
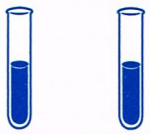
Keyword: Pasteurization
Go to: www.scilinks.org
Code: FS302

UP NEXT . . .

It's time for you to solve a mystery! In the next activity, we'll work in the lab to uncover all the juicy details.

BLUE'S THE CLUE DATA TABLE

Name _____ **Date** _____ **Class/Hour** _____

Day 1 Original Sample	Day 2 Describe Visual Changes	Day 3 Describe Visual Changes	Day 4 Describe Visual Changes
Room Temperature  Pasteurized Milk UHT Milk	Pasteurized: UHT:	Pasteurized: UHT:	Pasteurized: UHT:
Refrigerated  Pasteurized Milk UHT Milk	Pasteurized: UHT:	Pasteurized: UHT:	Pasteurized: UHT:
Frozen  Pasteurized Milk UHT Milk	Pasteurized: UHT:	Pasteurized: UHT:	Pasteurized: UHT:

1. How did the data support or reject your hypothesis?

2. What do you predict will happen if the chilled and frozen samples are left out at room temperature for another day?

3. What do you predict will happen if the UHT samples are left at room temperature for another day?

4. Explain the relationship of your findings to food safety.