**Kit Contents**

- 20 plastic petri dishes
- 2 bottles of nutrient agar
- 1 bottle iodine solution
- 1 piece of blotter paper
- 10 packs of sterile swabs
- 4 disposal bags

**Introduction**

**Bacteria** are single-celled microorganisms that do not have a distinct cell nucleus enclosed by a membrane, unlike most other cells. Instead, the genetic material (a single strand of DNA) floats in a tangle in the interior of the cell. Microorganisms like this are called prokaryotic organisms. Bacteria are classified by their shape. There are three basic bacteria shapes: sphere, rod, and spiral. **Spherical** (cocci) bacteria are round or oval in shape. **Rod** (bacilli) ones are longer and look like a rectangle or long oval. **Spiral** bacteria have long bodies with a twist that forms a spiral pattern when connected to each other.

Bacteria reproduce most often by **binary fission**: a single parent bacterium divides to form two independent bacteria. This type of reproduction is called asexual because there is no exchange or combination of genetic material between two organisms. Fission occurs rapidly in as little as 20 minutes. Under perfect conditions a single bacterium could grow into over one billion bacteria in only 10 hours!

Some bacteria can also reproduce asexually by forming thick-walled **endospores** that are very resistant to conditions of extended heat, cold, or dryness. An endospore is formed within the cell body of a bacterium. Usually a bacterium forms only one endospore, which will produce only a single bacterium. Endospores are difficult to kill except by strong chemicals or high heat.

Generally when people think of “bacteria,” they think of harmful germs. Many bacteria are disease-causing **pathogenic** bacteria. However, “good” bacteria are an essential aid to our digestion process, and organic materials such as dead trees would not be broken down into dirt if not for bacteria.

Scientists grow **bacteria cultures** when they want to study bacteria in a lab. The bacteria are grown in a sterile **petri dish** containing **agar**. Agar is a gelatinous material extracted from seaweed that forms a moist surface favorable for bacteria reproduction. In addition, nutrients are added to the agar to provide a food source for the bacteria culture. The **nutrient agar** in this kit is a general-purpose media for growing a wide range of bacteria. A petri dish filled with agar is called a **culture dish**.

Bacteria grow in **colonies**, groups of thousands of individual bacterium. You should be able to tell different colonies of bacteria apart by their shape, texture, and color. In order to study a certain kind of bacteria, the bacteria from one colony are **isolated**: a sample of the bacteria from an individual colony is transferred to a new sterile culture dish. The new culture will contain only the isolated bacteria.

**Safety**

The bacteria you will grow in these experiments are types that are normally present in your house, but you are culturing them in greater numbers than usual, and this can be hazardous. Follow these safety guidelines:

1. Always wash your hands thoroughly **before and after** handling the culture dishes.
2. Minimize the time you leave the covers off your culture dishes. This helps prevent contamination of the cultures and also limits your exposure to the bacteria. In particular, be careful not to breathe on an open culture or to breathe the air directly above an open culture.

3. When you have finished studying a culture, pour some household bleach into the dish, then put the lid on, seal the dish in a plastic bag, and throw it away.

Preparing Culture Dishes
Before you can grow bacteria, you'll need to prepare sterile culture dishes with agar. Each bottle of nutrient agar in this kit should fill about 10 petri dishes.

1. Melt the agar in a hot water bath, so that it can be poured.
   
   • **Water Bath Method** - Loosen the agar bottle cap, but do not remove it completely. Place the bottle in hot water at 170-190°F until the agar is liquid. To prevent the bottle from tipping, keep the water level even with the agar level.
   
   • **Caution:** handle hot bottles with a potholder or heat-resistant glove.

2. Let the agar cool to 110-120°F (when the bottle still feels warm, but not too hot to touch) before pouring into petri dishes.

3. Remove the cap completely and use an alcohol prep pad to sterilize the mouth of the bottle.

4. Slide open the cover on a petri dish just enough to pour agar into the dish. Pour 10-13 ml agar into the dish, enough to cover the bottom. Don’t let the bottle mouth touch the dish when pouring agar. Cover the dish immediately to prevent contamination and tilt it back and forth gently until the agar coats the entire bottom. Fill additional dishes until the agar bottle is empty.

5. Let the culture dishes stand one hour for the agar to solidify before using them. Once the agar has solidified, turn the dishes upside down until needed.

Preparing Sensitivity Squares
For some experiments you'll need to use blotter paper to make sensitivity squares to study the effect of iodine, as well as household chemicals on bacteria cultures.

Cut a strip of blotter paper 3/8" wide, then cut the strip into individual squares that are 3/8" long. Label the squares with permanent ink and then soak them with a few drops of iodine, or household chemicals like bleach, dishwashing or hand soap, hand sanitizer, liquid laundry soap, hydrogen peroxide, isopropyl or rubbing alcohol, etc. Handle the squares with tweezers and carefully wipe off any excess liquid before placing them in a bacteria culture.

**Experiment 1 – Bacteria in the Air**
Bacteria of various types are in the air, both indoors and out. In this experiment you'll "capture" some and grow cultures of them. You'll also test iodine, and household chemicals to see which ones prevent the growth of bacteria. Use two culture dishes for this experiment.

1. Decide if you want to collect your bacteria indoors or out. Possible locations are in the garden, near an open window, or in the bathroom.

2. Take the covers off the culture dishes and set them in the location you chose. Place the covers open-side-down on a clean surface. Leave the culture dishes exposed for about an hour and then cover them.

3. Label each dish: for example, "Garden Air 1" and "Garden Air 2."

4. Store both dishes in a dark place like a closet where they will be undisturbed for a few days.

5. After 3-7 days observe the bacteria growth in each dish, leaving the covers on. Each dot is a bacteria colony. Take notes of your observations and make drawings.

6. Take 2-4 of the sensitivity squares, label them (e.g. "I" for iodine), and soak each with a few drops of a chemical you wish to test for anti-bacterial properties. Wipe off excess liquid, and then use tweezers to set the squares over bacteria in one of the cultures. Also include a plain square of blotter paper to see if that has any effect. If different bacteria are growing in the culture, try to put all the squares on the same kind. This will lessen the variables, since one chemical might affect two kinds of bacteria differently.

7. The second culture dish is your "control." It will show you what an air bacteria culture looks like without any chemicals. After a few more days, observe both cultures again. Here are some questions you might try to answer:

   • In the control culture, how many bacteria colonies do you see? Do any have a different appearance, texture, or color, indicating a different type of bacteria? How much of the dish is covered with bacteria?
In the sensitivity square test culture, how many colonies do you see? Have the bacteria covered this dish as much as in the control culture? What effect did the chemicals have on the bacteria growth under and around the sensitivity squares?

Evaluate your observations and decide what conclusions to draw from your experiment results. For example, did one chemical work better than the others at killing bacteria? Is one colony dominant? What variables might have affected the results?

**Experiment 2 – Bacteria on Surfaces**
Bacteria are also present on every surface, indoors and out, that is not sterilized. You can find some on the kitchen counter, sink, floor, dishes, bathtub, wall, toilet, books, etc. In this experiment, you'll compare bacteria cultures that you grow from several different surfaces around the house. You will need four culture dishes for this experiment.

1. Decide on three different surfaces that you want to collect bacteria from. Choose one to study the effects of iodine, and household cleaners on. You will make two cultures from this surface and one from each of the others.

2. Label the covers of the four culture dishes for each location (e.g. Kitchen Counter 1, Kitchen Counter 2, Bathroom Sink, Dog's Dish).

3. Take a pack of sterile swabs and remove one, being careful not to contaminate the tip by touching it. Rub the swab over a surface in a zigzag pattern to collect some of the bacteria.

4. Remove the cover from the labeled culture dish and lightly rub the swab back and forth over the agar in the dish as illustrated. (You may want to turn the dish a quarter turn and zigzag again for maximum coverage.) This zigzag technique effectively transfers some of the bacteria from the surface to the culture dish. Cover the dish immediately.

5. Repeat steps 3 and 4 on the other surfaces. Use a new sterile swab for each culture. Remember to make two cultures from the surface you selected for the household cleaner test.

6. Put the dishes in the dark at room temperature.

7. After 3-7 days, bacteria will grow where you swabbed. Add sensitivity squares as you did in Experiment 1 to test different chemicals or household cleaners.

8. After a few more days, observe the bacteria growth in each dish, leaving the covers on. Again, take notes of your observations and make drawings.

- In each culture without the sensitivity squares, how many bacteria colonies are visible? Sketch each dish to show the size, shape, texture, and color of each colony.

- In the sensitivity square test culture, note the various bacteria colonies. Sketch each culture and the effects of each chemical or household cleaner. Compare this dish to the control culture from the same surface.

Evaluate your observations and decide if there are conclusions you can draw from your results. For example, which surface had the greatest number of different bacteria colonies? Which one had the largest amount of bacteria growth? Considering any variables, which household cleaners were most effective? What additional experiments would help you confirm your conclusions from this experiment?

**Experiment 3 – Bacteria on Hands**
Bacteria live on you, too: on your hands, feet, skin, hair, etc. An easy way to study these bacteria is with cultures made from the palm of your hand. You will need two culture dishes for this experiment.

1. Rub a sterile swab over the palm of one hand in a zigzag pattern to collect some bacteria.

2. Remove the cover from a culture dish and lightly rub the swab back and forth on the agar as you did in Experiment 2. Cover the dish immediately.

3. Repeat steps 1 and 2 to make a second culture from the same palm. Use a new sterile swab.

4. Store the dishes in a closet.

5. After 3-7 days, bacteria will grow where you swabbed. Use tweezers to set four labeled sensitivity squares, each damp with a different soap or hand cleaner, over the bacteria growth in one of the dishes. Add a plain square as well, and try to put the squares on the same kind of bacteria to lessen the number of variables.

6. In a few days, take the culture dishes and observe the results.

- In the control culture, note how many bacteria colonies are visible. Sketch each culture, including the size, shape, texture, and colors of each colony.

- In the sensitivity square test culture, note the various bacteria colonies. Sketch each culture and...
the effect of each chemical or hand cleaner on the surrounding bacteria colonies. Compare this culture to the control culture and note the differences.

Evaluate your observations and decide if there are conclusions you can draw from your experiment results. For example, how did the predominant bacteria colony look? Which hand cleaners were most effective? What additional experiments would help you confirm your conclusions from this experiment?

Experiment 4 – Isolating Bacteria

Individual colonies of bacteria can be isolated for further study. This is accomplished by taking a few bacteria from one colony and using them to inoculate a new culture dish. In this experiment you will use the control culture from the last experiment to grow two isolated colonies. You’ll need two culture dishes for this experiment.

1. Examine the control culture from Experiment 3 or one of the cultures without sensitivity squares from Experiment 2. Try to find two different-looking bacteria colonies that are growing by themselves with minimal contact from adjacent colonies.

2. Touch a sterile swab to one of the colonies, then remove the cover from a culture dish and lightly rub the swab back and forth over the agar. Cover the dish as soon as you’re done.

3. Repeat step 2 with the second colony you selected. Use a new sterile swab for this culture.

4. Store the dishes in a dark place like a closet where they will be undisturbed for a few days.

5. After 3-7 days, take the culture dishes and carefully observe the bacteria growth in each dish, leaving the covers on. If you isolated one type of bacteria in each culture you should have colonies of only that type growing.

Evaluate your results. Were you able to isolate only one type of bacteria in each culture? If not, what additional steps might you take with these cultures to further isolate individual bacteria types?

Additional Experiments

At this point you might have unused culture dishes still available for additional experiments. This is an opportunity to take what you have learned so far and design experiments that answer more questions that you have about bacteria. Here are some ideas:

1. Study the bacteria that grow inside your mouth. Swab the inside of your mouth with a sterile swab and then inoculate a culture dish.

2. Study the effect of mouthwashes by making two cultures using swabs from the inside of your mouth. Use sensitivity squares soaked in different mouthwashes to see the effect of each mouthwash on bacteria. Take "before and after" samples as well: swab your mouth before using mouthwash and right after. Is there a difference?

3. Compare the bacteria that grow in your mouth with the bacteria that grow in the mouth of one or more pets.

4. Study different disinfectants by cleaning a different section of a kitchen or bathroom counter with each. Then use swabs to take samples of any remaining bacteria and inoculate culture dishes.

5. Test the antibacterial effects of natural substances like garlic, tea tree oil, or red pepper.

6. Study the bacteria in soil. Take a teaspoon of soil from different places in the garden or from different houseplants near the roots. Add a tablespoon of water to each and mix them well. When the soil settles, dip a clean swab into the water layer and swab a culture dish. Repeat with each soil type. Nitrogen-fixing bacteria (usually found around bean plants) convert nitrogen to a form that plants can take in through the soil.

7. Study the bacteria in an aquarium or pond. Dip a swab in the water and then inoculate a culture dish.

8. Most bacteria will be viable for a long time if they are transferred to fresh culture dishes every 2-3 weeks. Compare the lengths of time you are able to keep different isolated bacteria cultures alive.

9. Do bacteria grow in your shoes? Is there a difference in bacteria growth between fabric shoes and leather? Do foot powders work to cut down on bacteria?

10. Most non-pathogenic bacteria grow well at room temperature. Compare growth in a culture kept in the refrigerator versus one in a closet.

There are many more experiments that you can do with bacteria. The possibilities are endless!