# “Aaaaah…Chooo!”
# Transmission of Disease-Causing Microbes

## Science in the Real World
Microbes In Action

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# At A Glance

**Description**

This lesson gives the classroom teacher an easy way to show the ease with which human pathogens may spread without violating health standards. Germ transmission is shown by simulating a human sneeze or cough using a bacteria contaminated spray bottle and plates of nutrient agar.

**Time Requirements**

The activity will take two fifty-minute class periods. One fifty-minute class period will be used to set-up the lab and to answer day 1 analysis questions. The next day, a second fifty-minute class period will be used to record observations and answer the rest of the analysis questions.

**Curriculum Placement**

This exercise could be used as an introduction to the scientific method. It could also be used in a unit on the human body, or bacteria.

**Equipment**

Autoclave or sterilizer

**Materials (per class of 30)**

1- 50 ml flask of overnight *Bacillus subtilis* broth culture **or** 1 nutrient agar plate of *B. subtilis* and 50 ml sterile nutrient broth
36 nutrient agar plates
1 sterile spray bottle (must be capable of mist setting)
Marking pens
Meter stick
Masking tape
0.2% bleach solution
1 latex examination glove

# Aaaaah…Chooo
## Germ Transmission Lab

Background

For hundreds of years, as far back as ancient times, people have known that some diseases are contagious. You may have noticed certain 'bugs' going around your school. How do various flu bugs, strep throats, colds, and many other diseases travel? The most common means of disease transmission is through aerosols, such as coughs and sneezes. Other means include casual human contact (handshakes, kissing), sexual contact, and eating contaminated food.

What causes us to sneeze? A sneeze commonly occurs because the nerve endings that are found within the mucous membranes in are nose are irritated. This can happen because we have an illness, or something foreign to our nose has entered it, like bacteria, mold or dust. When these nerve endings are irritated, sneezing is a reflex that tries to rid our nose of these irritating objects.

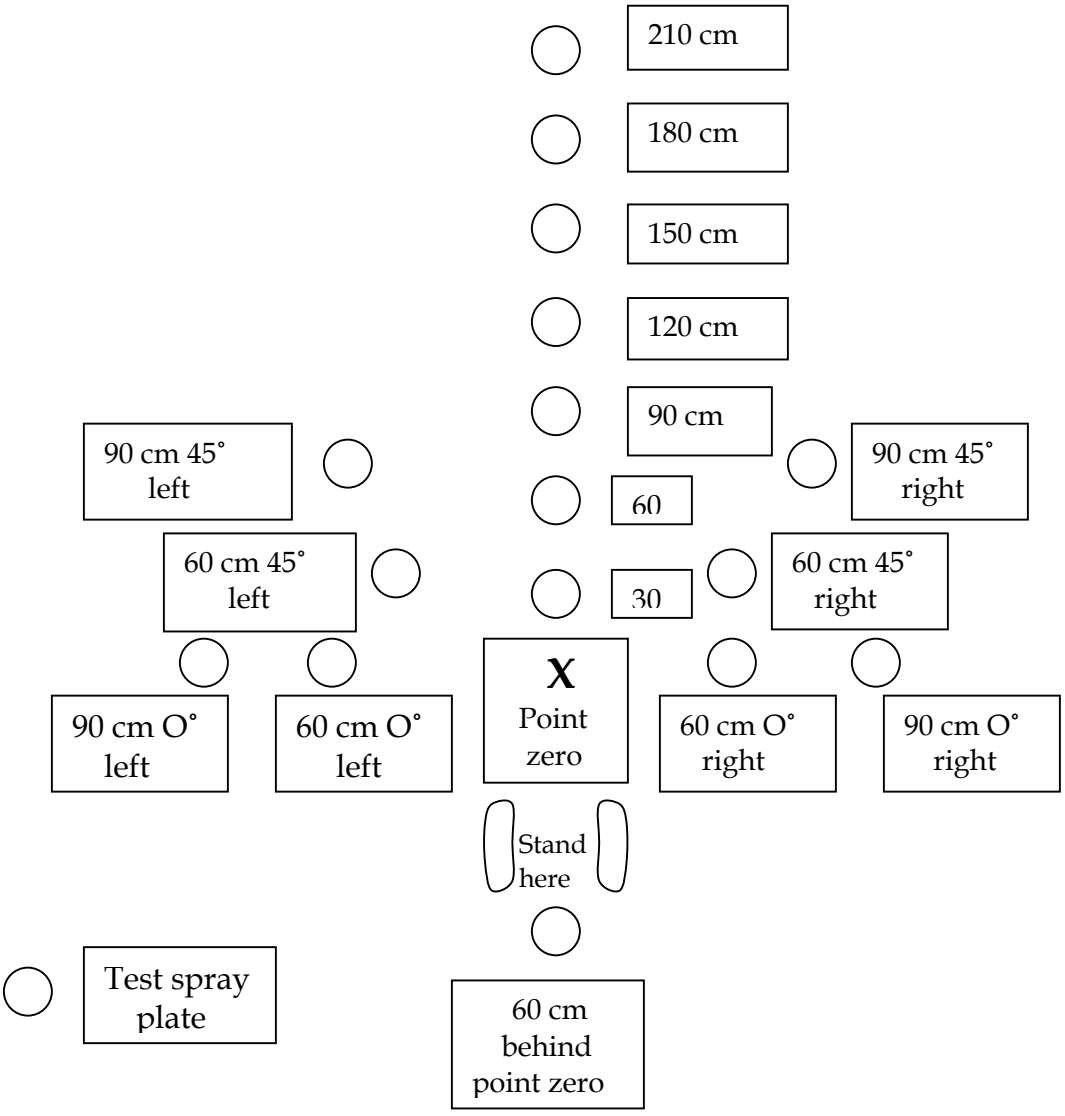
So how do we avoid passing our germs? Most of us have been chastised by our mothers to "cover your mouth" if you are going to cough or sneeze. Is this technique just an old wives tale, or something that can really prohibit the transmission of germs? This activity will test the theory of germ transmission via a sneeze and the "cover your mouth" idea.

**Purpose**

1. To learn just how far germs can travel as a result of a sneeze.
2. To practice using the terminology and steps of the scientific method
3. To observe and count bacterial colonies growing on nutrient agar.

# Procedure- Day 1, part 1

1. Your teacher will assign you to groups of two and give you one of the distances shown below to be responsible for.



2. Obtain one nutrient agar plate and label it, on the bottom near the edge with your name, the date, your class period and the placement that you were assigned.

3. Your teacher will select a few volunteers to map out the area for plate placement with masking tape. Once this is complete take your plate over and place it in the correct spot.

4. Stand along the side of the plates and wait for all of the groups to finish placing their plates.

5. When every plate has been placed in its position, your teacher will tell one team member to remove the lid from your plate and set it upside down next to the plate. **(The group with the plate that is 60 cm behind point zero must remove their lid <u>first</u>!)**
   Quickly, but calmly back out of the test area to the perimeter, making sure not to step on or over any plates.

6. Your teacher or a chosen student will stand with the spray bottle at point zero. This person will shake the spray bottle to make sure the bacteria is well mixed.

   They will then make one spray into the petri dish called the 'test spray plate' and close it.

   Finally, they will then make one complete spray, facing toward the center petri dishes, holding the spray bottle waist high.

7. Wait approximately 10 seconds for the mist to fall and then replace the lids on your plate. Be careful that you do not step over or on the plates. **(The group assigned 60 cm behind point zero must be the <u>last</u> to put on their lid!)**

8. Pick up your plate, invert it, and place it at room temperature for 24 hours.

## Procedure- Day 1, part 2

1. Repeat steps 2-5 above. When you label this dish add the word "glove", along with date, name, etc. (Remember that the group assigned to 60 cm behind point zero must be the first to remove their lid, and the last to put it back on!)

2. This time the teacher will place a gloved hand 5-7 cm in front of the spray bottle to simulate covering your mouth while sneezing, and make one complete spray facing toward the center petri dishes, holding the spray bottle waist high.

3. Repeat steps 7 & 8.

## Procedure- Day 2

1. Observe plates. *B subtilis* has large cream-colored colonies. Observe the test spray plate to determine the correct appearance of the bacterium.

2. When bacteria grow on the surface of nutrient agar in a petri dish, they grow in one of two ways:

    a. a lawn—this looks like a film of continuous bacteria growing on the surface of the agar. It represents so many bacteria that they grew together into one continuous surface.

    b. colonies—these look like small dots. Each dot started as one microscopic bacterial cell that has multiplied into many, many cells.

3. Count the number of colonies found on your plate. To make counting easier following the steps below:

    a. Draw 2 lines dividing your plate into 4 equal sections.

    c. Count the colonies in each section separately and add them together.

    d. Using your marker, place a dot over each colony as you count it (this will help you keep track of which ones you have counted) and write the total for that section on the dish.

    e. Add the numbers together to get the total number of colonies for your dish. Record this number in the appropriate data table. If the number is over 300, record >300.

4. Record the class data of all of the plates as they finish counting. Be sure to record the data from Part I (no glove) in data table 1, and the data from Part II (with glove) data in data table II.

Name _____

Date_____

# Aaaah Chooo!!!!!!!

## Day 1 Results & Analysis

1. Consider part 1 and form a hypothesis as to which plate you think will become the most affected. (Remember the If...then... format)

   Also, form a hypothesis as to which plate you think will be the least affected.

   Finally, predict how the gloved hand over the spray bottle will affect the growth of bacteria

2. Scientists would refer to one plate that was assigned, as a **negative control**. Which plate would this be? Why is it called a <u>negative</u> control?

3. Which one would be considered the **positive control?** Why?

4. Why are controls used?

5. Why is it necessary to close the plates quickly after the spray has occurred?

6. Why do the directions say to make sure the plate that is located 60 cm behind is the first to remove their lid and the last to put it back on.

## Data Table for Part 1- Without Glove

| Distance | Number of colonies |
|---|---|
| 30 cm center | |
| 60 cm center | |
| 90 cm center | |
| 120 cm center | |
| 150 cm center | |
| 180 cm center | |
| 210 cm center | |
| 60 cm 45˚angle right | |
| 90 cm 45˚ angle right | |
| 60 cm 45˚ angle left | |
| 90 cm 45˚ angle left | |
| 60 cm 0˚ angle right | |
| 90 cm 0˚ angle right | |
| 60 cm 0˚ angle left | |
| 90 cm 0˚ angle left | |
| 60 cm behind point zero | |

## Data Table for Part 2- With Glove

| Distance | Number of colonies |
|---|---|
| 30 cm center | |
| 60 cm center | |
| 90 cm center | |
| 120 cm center | |
| 150 cm center | |
| 180 cm center | |
| 210 cm center | |
| 60 cm 45˚angle right | |
| 90 cm 45˚ angle right | |
| 60 cm 45˚ angle left | |
| 90 cm 45˚ angle left | |
| 60 cm 0˚ angle right | |
| 90 cm 0˚ angle right | |
| 60 cm 0˚ angle left | |
| 90 cm 0˚ angle left | |
| 60 cm behind point zero | |

## Day 2 Results and Analysis

1. What was the furthest distance that the bacteria traveled in part 1?

   Part 2?

2. What happened when the glove was used to represent "covering your mouth" when you sneeze?

3. Did your plate have growth other than *B. subtilus*? How can you explain this?

4. Explain what this lab has taught you about germ transmission.

5. Design an additional experiment that could be used to show other forms of bacterial or viral germ transmissions.

# Teacher Guide

## Instructional Objectives

At the end of this activity, the students should be able to:

1. Demonstrate the method of scientific inquiry:
    a. stating a problem
    b. writing a hypothesis
    c. performing an experiment according to given directions
    d. gathering data
    e. analyzing data
    f. developing further investigations

2. Demonstrate the following laboratory skills:
    a. measuring
    b. counting

## Sources of Supplies

Carolina
2700 York Road
Burlington, NC 27215
(800) 334-5551

| Description | Stock Number | Quantity | Cost |
|---|---|---|---|
| Petri dishes | CE-74-1251 | 500 | $ 95.50 |
| Spray bottle | CE-66-5565 | 1 | $ 3.70 |
| Meter Sticks | CE-70-2620 | 12 | $ 4.75 |
| Masking tape | CE-64-4860 | 1 | $ 6.30 |
| *Bacillus subtilis* plate | CE-15-5156 | 1 | $ 9.00 |
| Nutrient Agar (dry) | CE-78-5320 | 100 g | $ 28.80 |
| Nutrient Broth (125ml) | CE-77-6380 | 1 | $ 6.00 |
| Sharpie Markers (Set of 4) | CE-64-4298 | 4 | $ 18.60 |

# Preparation

- At least one week before the lab (but no more than 3 weeks before), obtain a culture of *Bacillis subtilis*. Store culture in refrigerator.
- Prepare nutrient agar plates. One liter of medium will provide about 40 plates. Dissolve 23 grams of nutrient agar powder into 1 liter of distilled water (tap water will work). Prepare enough to use approximately 20 mL per plate. Prepare agar batches that only fill the flasks half way to prevent boil over. Once you have prepared the nutrient agar, cover the flasks with aluminum foil and place them in an autoclave or pressure cooker.

  Follow the instructions on your sterilizer to complete the sterilization process. Generally, you need 15 lbs. of pressure and a temperature of 121˚C for 20-25 minutes to achieve sterility.

  Once the materials have been sterilized, the nutrient agar must be poured into the plates before it solidifies. It is best to pour the agar when it has cooled enough to be held in your hand (45-50˚C), but still feels hot. Spread the plates out on the lab tables, lift the lid on one side like a clamshell. You may want to pour 20mL of water into a plate to give you an idea of how far to fill the plate. Once all of the plates have been poured, let them sit until the agar solidifies. Store the plates upside down until you are going to use them. If it is going to be several days, put the plates back into the sleeve that they came in and store in the refrigerator.

- To prepare the spray bottle "germs":

  1. Obtain a streak plate of *Bacillus subtilis*. Add three sterile loops of bacteria to 50 ml sterile nutrient broth. Incubate at room temperature ($37^{O}$C) for 24 hours.

  2. After 24 hours, clean spray bottle as usual, and then rinse with 0.2 % bleach solution.

  3. Immediately pour the nutrient broth culture in to the clean sprayer.

# Teacher's Hints and Troubleshooting

1. Make sure to prepare a fresh culture 24 hours in advance.

2. The number of plates listed is only a suggestion. Fewer plates may be used to lessen your prep time. It is good for the students, though, to see how far and in how many directions a sneeze can travel. Laying out the center row as 30, 90, 150, and 210 cm increments works well and saves 3 plates per class.

3. Make sure to mix the spray bottle thoroughly prior to spraying!

4. Also, make sure that your spray bottle has a good mist setting. If you must pump the handle a few times to get a good mist, bring a beaker to the test area and spray into it a few times before you complete the test spray.

5. The control is essential, there are many other types of bacteria in the air, and we are only concerned with *Bacillus*. A positive control has been incorporated by directly spraying a plate before the experiment (test spray plate). This will allow the students an opportunity to see what *Bacillus subtilis* looks like, which will help them to interpret their results.

6. If you want to save money on plates, consider doing the Part 2 with one class and Part 2 with another class and comparing the data.

7. You may want to have your students bring in bandanas, to wear over their faces during this activity. <u>It is not necessary</u>! *Bacillus* is not a pathogen.

8. If you have dry nutrient agar available you can make the needed 50 ml solution of nutrient broth instead of purchasing it. To prepare 50 mL of nutrient broth, weigh out 0.4 grams of nutrient broth powder. Add 50 mL of distilled water to a 100 mL beaker or flask. Dissolve the powder completely in the water. Cover with a cap or aluminum foil and place it in an autoclave or pressure cooker. Sterilize at 121˚ C for 20-25 minutes.

**Going further**: Have 9 students wear one glove for day 1 part 2. After you block the mist, shake hands with one student. (Make sure the mist on your glove is transferred to their fingers. Have that student shake hands with another, and continue through the entire class. As soon as students have been 'infected' have him or her gently press their fingers into a sterile nutrient agar plate. This will show them what happens if you don't wash your hands after sneezing.

## Day 1 Results & Analysis- Answer Key

1. Consider part 1 and form a hypothesis as to which plate you think will become the most affected. (Remember the If...then... format)

   ***Answers will vary***

   Also, form a hypothesis as to which plate you think will be the least affected.

   ***Answers will vary***

   Finally, predict how the gloved hand over the spray bottle will affect the growth of bacteria

   ***Answers will vary- should state that the number of bacterial colonies will decrease.***

2. Scientists would refer to one plate that was assigned, as a **negative control**. Which plate would this be? Why is it called a negative control?

   ***The plate behind point zero. It was not exposed to the bacteria from the bottle.***

9. Which one would be considered the **positive control?** Why?

   ***The test plate. It was directly infected with the bacteria.***

10. Why are controls used?

    ***To ensure that we know what we are looking at.***

11. Why is it necessary to close the plates quickly after the spray has occurred?

    ***There are microbes floating in the air. Quickly closing the lids helps to limit the number of these microbes landing on the plates.***

12. Why do the directions say to make sure the plate that is located 60 cm behind is the first to remove their lid and the last to put it back on?

    ***That plate shows us what microbes are in the air. Keeping that plate open the longest gives us a valid analysis of what is in the air.***

## Day 2 Results and Analysis

1. What was the furthest distance that the bacteria traveled in part 1?

   ***Answers will vary***

   Part 2?

   ***Answers will vary***

2. What happened when the glove was used to represent "covering your mouth" when you sneeze?

   ***Answers will vary- should state that the germs were not transferred as far, or as wide.***

3. Did your plate have growth other than *B. subtilus*? How can you explain this?

   ***Answers will vary- you may find some other bacteria and/or fungus will be floating around your school.***

4. Explain what this lab has taught you about germ transmission.

   ***Answers will vary***

5. Design an additional experiment that could be used to show other forms of bacterial or viral germ transmissions.

   ***Answers will vary***