A Medical Mystery
Of Epidemic Proportions
Teacher information

Summary:

Students explore a case study about a cholera epidemic and conduct laboratory tests to:

- Identify the disease-causing organism.
- Determine why people who have had cholera or been vaccinated do not have immunity.
- Determine how the new type of cholera-causing bacteria could have evolved.

They also consider courses of action that could be used to prevent the spread of cholera.

Core concepts:

- Diseases may be caused by bacterial pathogens.
- The immune system recognizes pathogens and produces specific antibodies to destroy pathogens
- Genetic changes in pathogens may allow them to escape immune system detection.
- Actions can be taken to prevent the spread of pathogens.

Class time required:

2 forty minute class periods + homework

Preparing for class:

Provide for each student:

- 1 copy of student instructions, "*A Medical Mystery of Epidemic Proportions*"
- Goggles
- Scissors
- Colored pencils (optional)
- Gloves (optional)

Part 1**:** For each team of 2-4 students, prepare a ***Vibrio cholerae*** **Rapid-Test Kit** that includes the following items:

- 1 copy of "*Instructions for Vibrio cholerae* Rapid-Test Kit"
- 2 strips of 0-14 colorpHast EMD pH indicator strips. Order from VWR

- 2 small test tubes or microtubes - wide enough to dip the chromatography paper into (for example, 2 ml microtubes). Label one tube "Well Water Sample" and label the other tube "Diarrhea Sample". Fill both tubes with pH 10 buffer. Optional: Add a very small amount of chocolate syrup and cream to the "Diarrhea Sample" if you would like to increase the realism.

Part 2: For each team of 2-4 students, prepare an **Antibody Test Kit** that includes:

- 1 copy of *Instructions for Antibody Test Kit.*
- 1 small test tube (or microtube) labeled "*Vc* from Patient". Fill this tube with tap water.
- 1 small test tube (or microtube) labeled "Known *O1 Vc*". Fill this tube with a saturated solution of calcium chloride.
- 1 small test tube (or microtube) labeled "O1 Antibody". Fill this tube with a saturated solution of baking soda (sodium bicarbonate) that has been colored faint blue using food coloring.
- 3 plastic droppers (Label if you would like to recycle the droppers.)
- 1 plastic test strip, photocopied on a plastic transparency sheet

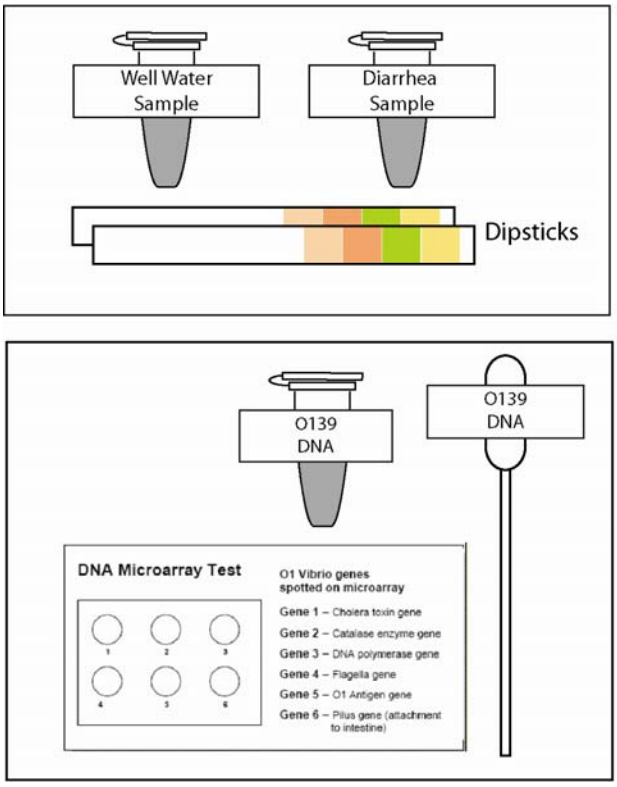
Part 3**:** For each team of 2-4 students, prepare a **DNA Microarray Test Kit** that includes:

- Colored copies of diagrams **A** and **B**
- DNA Microarray Test strip. Print or photocopy the test strip onto card stock paper and then apply a spot of 2% phenolphthalein to all circles except circle number 5.
- 1 small test tube (or microtube) of pH 10 buffer (or a 5% dilution of household ammonia) labeled "O139 DNA"
- 1 plastic dropper (labeled "O139 DNA" if you would like to recycle the droppers)

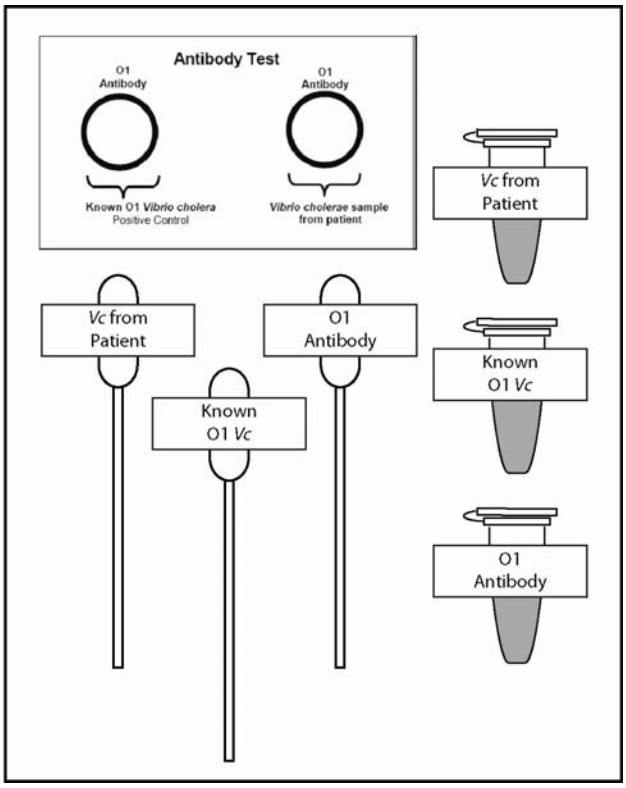
This project was generously funded by Science Education Partnership Award R25RR023285 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

Quick-Guide:

Part 1



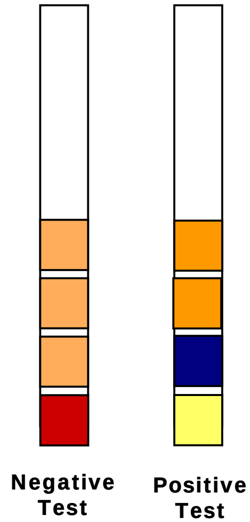
Part 2



Part 3

Instructions for
Vibrio cholerae Rapid-Test Kit

- Hold the dip stick by the white plastic end and dip the orange end into the sample for 1 second.
- Remove the dipstick and read the results immediately.
- If the results show a red square at the end of the dipstick, this indicates a negative test - *Vibrio cholerae* is <u>not present</u> in the sample.
- If the results show a dark blue square, this indicates a positive test -*Vibrio cholerae* is present in the sample.



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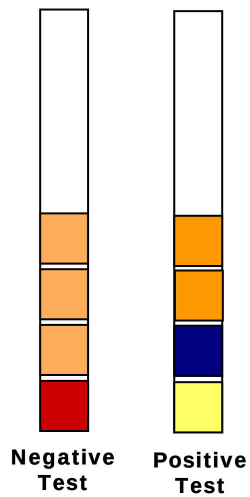

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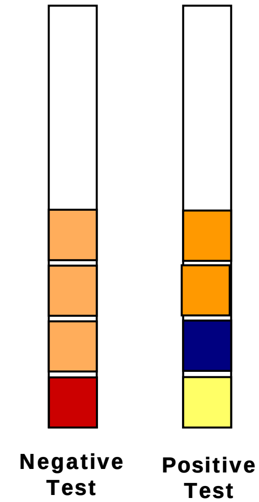

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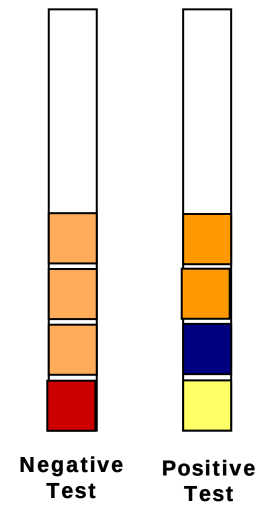
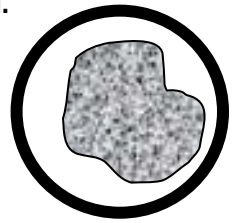


Instructions for Antibody Test Kit:

1. Place 2 drops of the **O1 Antibody** solution into both circles on the plastic test strip.
2. Add 2 drops of the **Known O1 Vc** (Positive Control) to the left circle on the plastic test strip.
3. Add 2 drops of the **Vc from Patient** to the right circle on the plastic test strip.

If antibodies bind to the antigens on the surface of the *Vibrio cholerae*, there should be visible **clumping.**



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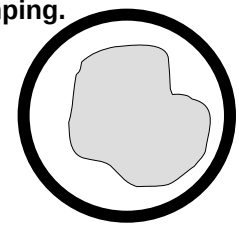

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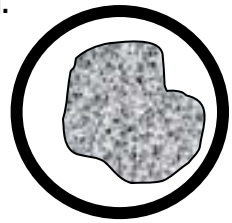

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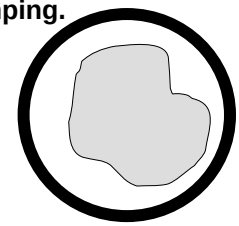
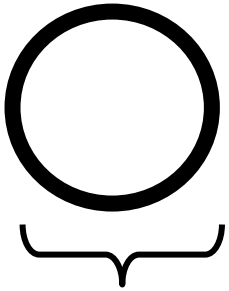


Duplicate on plastic transparency sheets and cut out on solid lines:

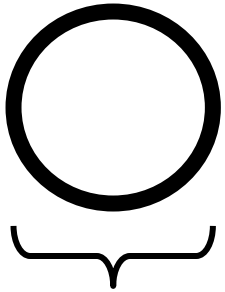
Antibody Test

O1 Antibody Solution



Known O1 *Vibrio cholerae*
Positive Control

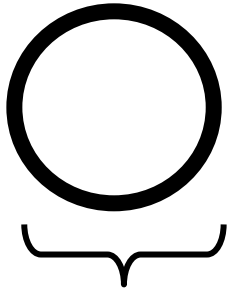
O1 Antibody Solution



***Vibrio cholerae* sample from patient**

Antibody Test

O1 Antibody Solution



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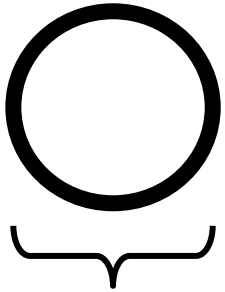

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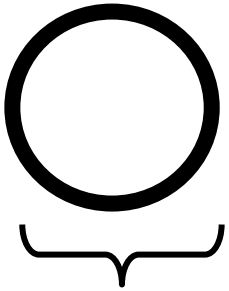

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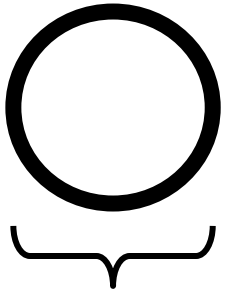

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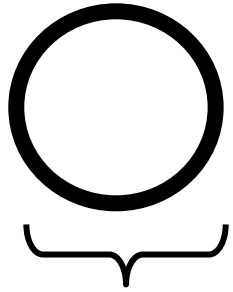

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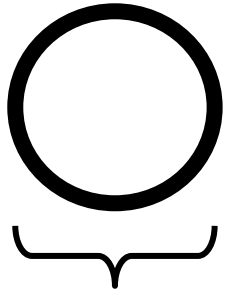

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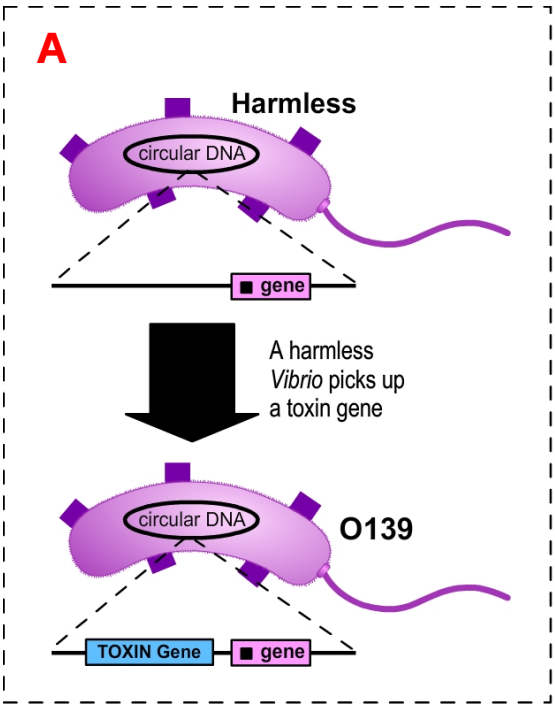
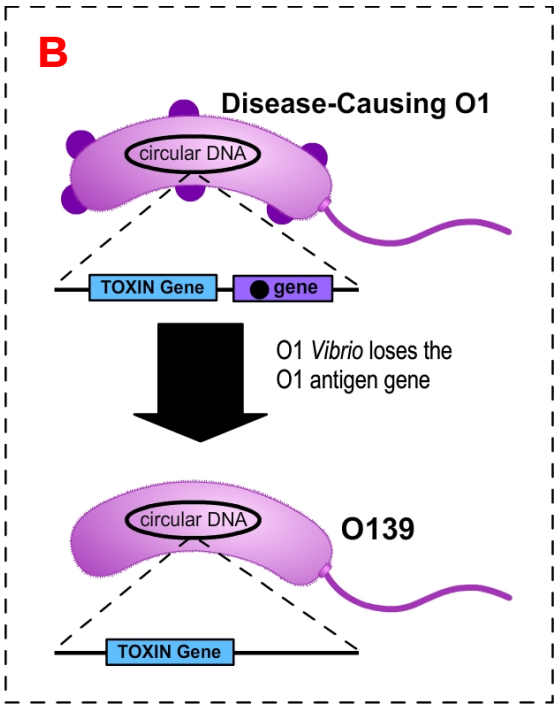
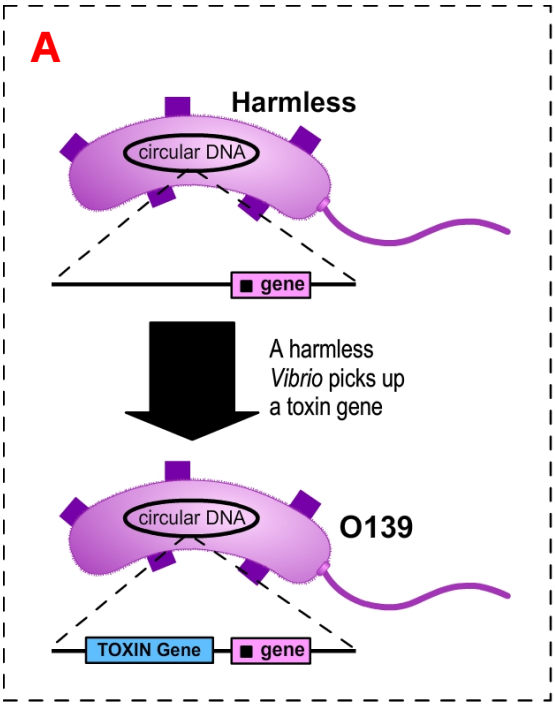
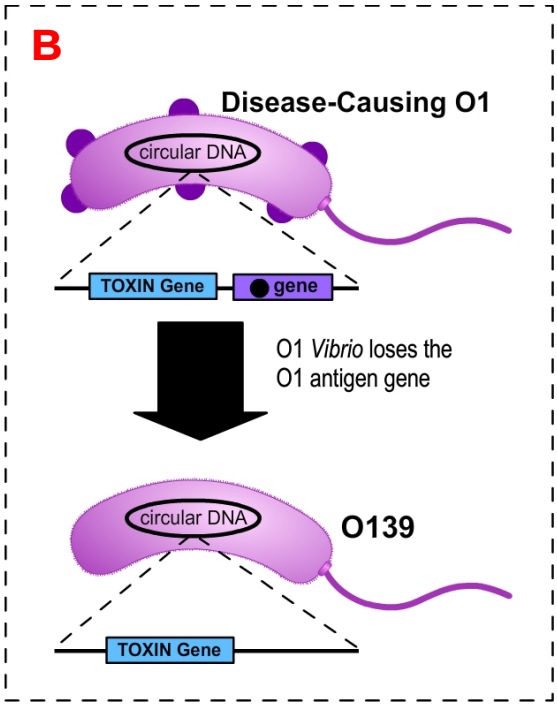
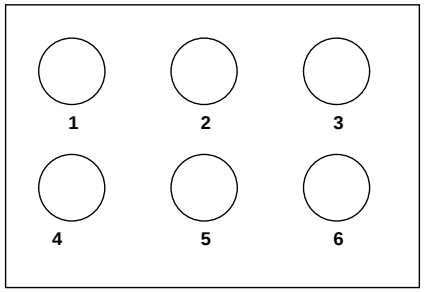








DNA Microarray Test



O1 Vibrio genes spotted on microarray

Gene 1 – Cholera toxin gene

Gene 2 – Catalase enzyme gene

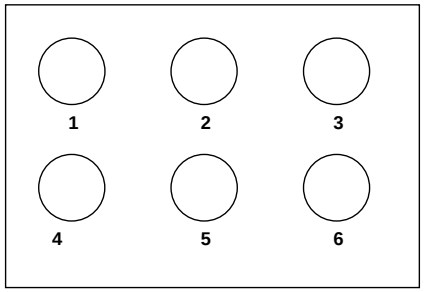
Gene 3 – DNA polymerase gene

Gene 4 – Flagella gene

Gene 5 – O1 Antigen gene

Gene 6 – Pilus gene (attachment to intestine)

DNA Microarray Test



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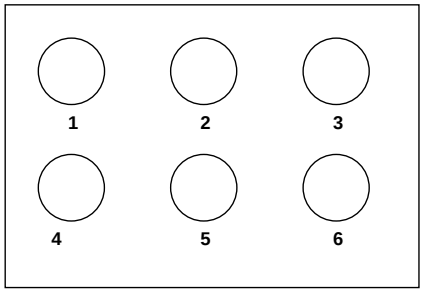

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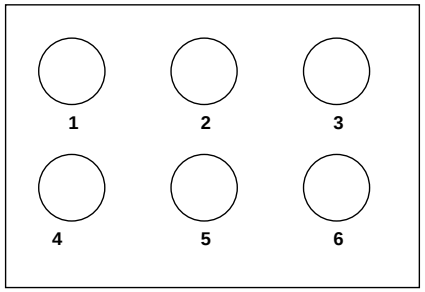

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Instructions for DNA Microarray Test

1. Place 1 drop of O139 DNA onto each circle on the DNA microarray.
2. If the O1 genes on the microarray and O139 genes that you added to the microarray are the same, they will bind to each other on the microarray and produce a pink color.
3. If the genes from O1 and 0139 are different, they will not bind to each other.

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A Medical Mystery
Of Epidemic Proportions – ***Teacher Answer Key***

Daphne's Blog - Sunday

I'm not sure my decision to be a Peace Corp volunteer was a good idea. I thought I was prepared for working in a village where extreme poverty and lack of transportation made obtaining food, water and medical care difficult. But, this has become really scary!

When I look out of my tent, I can see sick villagers curled up in the shade of a tree. They are waiting to be admitted to an already crowded hospital tent. One girl, weak and dehydrated, died of the severe diarrheal disease before she could get treatment.

We had a very heavy rainstorm here on Friday. I think that the rain's runoff carried human waste into the village's water supply. It's like the villagers are drinking poison. I'm afraid that this might be the beginning of a cholera epidemic. Here's what I found on the Internet about cholera.

> **Cholera** is an intestinal infection caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae.* It has a short incubation period, from less than one day to five days.
>
> *Vibrio cholerae* bacteria produce a potent toxin that causes a large amount of watery diarrhea that can quickly lead to severe dehydration and death if treatment is not promptly given.
>
> Cholera is usually transmitted through water or food that is contaminated by human feces containing *Vibrio cholerae* bacteria. Cholera outbreaks can occur in any part of the world where water supply, sanitation, food safety, and hygiene are inadequate.

1. What bacterial pathogen causes cholera?

   Vibrio cholerae

2. How do these bacteria lead to the death of people who are not given prompt treatment?

   The bacteria produce a potent toxin that causes a large amount of watery diarrhea that can lead to severe dehydration.

3. How is cholera spread from one person to another?

   Cholera is spread through water or food that is contaminated with human feces containing Vibrio cholerae bacteria.

Part 1: What is causing this disease outbreak?

Daphne's Blog - Monday

An emergency medical team arrived and set up a hospital tent. They brought boxes of IV bags and antibiotics provided by American donors. Prompt treatment of diarrheal disease with an IV fluid is critical! The patients can die very quickly if IV's aren't started immediately to replace the fluids that victims have lost.

This epidemic is horrible! There are many new patients today. The hospital beds are just cots with a hubcap-sized hole is cut in the middle. A bucket sits under the hole. Another bucket rests on the floor next to the victim's head. The patients are too weak to speak. Groans, sounds of vomit and diarrhea splashing into the buckets, and a horrible stench fill the crowded hospital. The hospital tent's 110 beds are full but the patients keep arriving.

There are many pathogens that could cause an outbreak of diarrheal disease. Luckily they have a *Vibrio cholerae* Rapid-Test Kit that can determine if this is outbreak is caused by *Vibrio cholerae* bacteria. If this is a cholera outbreak, it will be important to take quick action to prevent the spread of *Vibrio cholerae* bacteria to other villages.

1. Use the *Vibrio cholerae* Rapid-Test Kit to test a sample of well water from the village and a diarrhea sample collected from a patient.
2. Record your laboratory observations by coloring or labeling the pictures of the dipsticks.
3. Record your conclusions based on the Rapid-Test results. Does the test show that *Vibrio cholerae* are present?

Well Water Sample

Diarrhea Sample

***Vibrio cholerae* Rapid-Test Kit**
Laboratory Observations

Conclusions:

Both the well water sample and the diarrhea sample contain Vibrio cholerae bacteria. OR
The villagers have cholera.

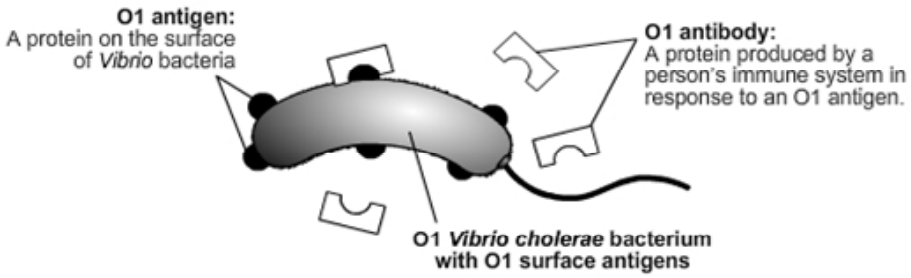
Part 2: Why are people who should be immune getting sick?

Daphne's Blog - Wednesday

Two members of the medical team have developed cholera. That shouldn't be happening because they both had cholera vaccinations. People who had cholera before are also getting sick. This is turning into a very scary medical mystery! Why are people who were vaccinated or who had cholera before getting sick?

According to the doctors, only one type of *Vibrio cholerae* bacteria causes epidemic cholera – this is known as the O1 type. People who have been infected with cholera in the past and people who had cholera vaccinations should be immune to the O1 type of cholera. Their immune system should be making O1 antibodies that bind with and destroy the O1 type of *Vibrio cholerae*.

Type O1 *Vibrio cholerae* have O1 antigens on their surface. When a person is exposed to O1 *Vibrio cholerae*, their immune system produces specific O1 antibodies. These antibodies bind to antigens and destroy O1 *Vibrio cholerae* antigens.



The emergency medical team is concerned that a new type of *Vibrio cholerae* bacteria might be causing this outbreak. If that's true, then the new type of *Vibrio cholerae* may not have O1 antigens. That would mean that the O1 antibodies cannot bind to and destroy the new type of *Vibrio cholerae*.

To determine if this is an O1 type or a new type of *Vibrio cholerae*, you can do an antibody test that uses O1 antibodies. If the O1 *Vibrio cholerae* bacteria are present in the patient sample, the O1 antibodies should bind with the O1 antigens on the bacteria surface. The binding will cause the *Vibrio cholerae* to clump together.

1. Which type of *Vibrio cholerae* bacteria is known to cause cholera epidemics?

   O1 Type Vibrio cholerae

2. What is the difference between an O1 antigen and an O1 antibody?

   An O1 antigen is a protein on the surface of an O1 Vibrio cholerae bacterium. An O1 antibody is a protein made by an infected person's immune system.

3. Explain why people who were infected by O1 *Vibrio cholerae* before should not get sick when they are exposed to this pathogen again.

   They were exposed to the Vibrio cholerae before so their immune systems should be making O1 antibodies that destroy the Vibrio cholerae.

4. What results should you observe if O1 antibodies are mixed with O1 *Vibrio cholerae* bacteria?

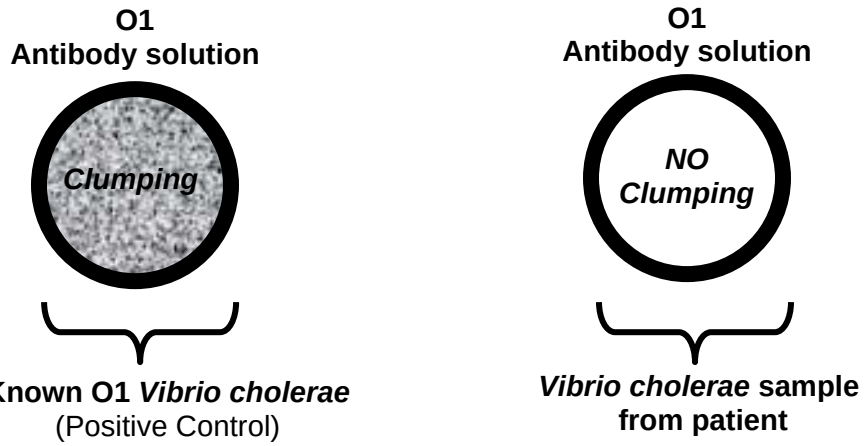
   There should be visible evidence of clumping.

5. Use the Antibody Test Kit to test the *Vibrio cholerae* sample from this cholera outbreak.

6. Record your laboratory observations indicating whether the samples clumped or did not clump.

7. Record your conclusions based on the results of the antibody testing

Antibody Testing Laboratory Observations



Conclusions:

The Vibrio cholerae sample from the patient does not have O1 antigens on its surface. This must be a new or different kind of Vibrio cholerae.

8. Why was it important to test a known sample of O1 *Vibrio cholerae* when using the antibody test kit?

   This is a control to be certain that the O1 antibodies are working properly.

9. Explain why the adults who had cholera in the past or who had been vaccinated for O1 type *Vibrio cholerae* are not immune during this cholera epidemic.

   Their O1 antibodies do not recognize this new pathogen OR the new kind of Vibrio does not have O1 antigens on its surface.

Part 3: How did O139 evolve?

Daphne's Blog - Friday

OK, one mystery has been solved. This cholera outbreak is caused by a **new** type of *Vibrio cholerae* called **O139**.

But now there is a new mystery. How did this new O139 *Vibrio cholerae* evolve? The scientists I've been talking to have two hypotheses.

Hypothesis 1: The new O139 *Vibrio cholerae* evolved from a non-pathogenic (harmless) type of *Vibrio cholerae* that picked up a gene for the cholera toxin. If that happened the harmless type could become a "killer" *Vibrio cholerae* that is not recognized by O1 antibodies.

Hypothesis 2: The new O139 *Vibrio cholerae* evolved from an O1 *Vibrio cholerae* that mutated and lost the ability to make the O1 antigen. If that happened, people's O1 antibodies also wouldn't be able to recognize the new O139 *Vibrio cholerae*.

To see which hypothesis is correct, the doctors have sent a sample of the new O139 Vibrio cholerae to the CDC (Center for Disease Control). Researchers at the CDC will use a DNA test called a "microarray" to compare the genes of the O1 Vibrio cholerae with the genes of the new O139 Vibrio cholerae.

1. Observe diagrams A and B on the colored graphic sheet in your lab kit.
   - Which diagram best illustrates Hypothesis 1? _***A***_

     Explain your answer:

     Because the first Vibrio was harmless and the diagram shows it getting a toxin gene.

   - Which diagram best illustrates Hypothesis 2? __***B***__

     Explain your answer:

     Because the first Vibrio was an O1 type and the diagram shows it losing the gene for the O1 surface antigen.

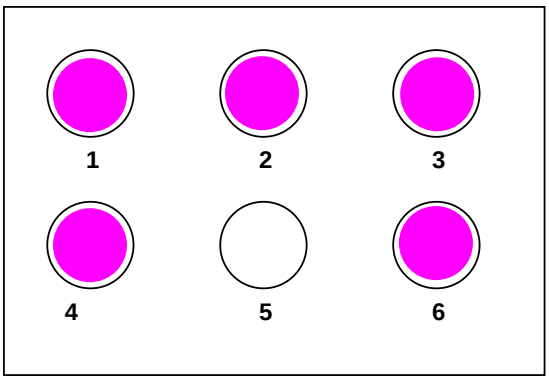
2. Your lab kit contains a simulated DNA microarray has been spotted with DNA from many different O1 *Vibrio cholerae* genes. Your kit also contains a tube of simulated DNA isolated from the O139 *Vibrio cholerae*.

3. Follow the instructions in the DNA Microarray Test Kit to test the O139 DNA sample.

4. Record your laboratory observations below by coloring in the DNA spots that are pink.

5. Record your conclusions from the microarray testing.

DNA Microarray Laboratory Observations



O1 *Vibrio cholerae* genes spotted on the microarray:

Gene 1 – Cholera toxin gene

Gene 2 – Catalase enzyme gene

Gene 3 – DNA polymerase gene

Gene 4 – Flagella gene

Gene 5 – O1 Antigen gene

Gene 6 – Pilus gene (attachment to intestine)

Conclusions:

O139 does not have an O1 antigen gene. All of its other genes are the same as the O1 genes.

6. Which hypothesis is supported by the results of your DNA microarray analysis?

- Hypothesis 1: O139 evolved from a harmless *Vibrio cholerae* that picked up a gene for the cholera toxin.
- Hypothesis 2: O139 evolved from an O1 *Vibrio cholerae* that lost the gene for the O1 surface antigen.

Hypothesis: ***2***

Support your answer with evidence from the DNA microarray testing.

Most of the O139 genes are like the O1 genes. The O139 DNA did not contain the gene for the O1 surface antigen.

7. Why is the evolution of a new type of *Vibrio cholerae* a serious health threat?

Because people who had cholera before or who were vaccinated can spread this disease to others. OR Because more people can get this type of cholera because they do not make antibodies against it. OR Because the current cholera vaccine won't protect people from O139.

Part 4: What can be done to prevent cholera epidemics?

Daphne's Blog - Sunday

The medical team says that we need to take action to prevent additional cases of cholera. They've asked me to work with people who live in this village and nearby villages to make them aware of what they could do to prevent the spread of the O139 *Vibrio cholerae*.

The immediate solutions seem so simple. Encourage villagers to use the temporary supply of clean water provided by the medical team. Drill wells that would provide clean water. Build latrines so that villagers wouldn't use their fields as open-air toilets. But these options are too expensive unless the government steps in.

Hopefully, in the future, scientists will develop a new vaccine that protects people from the O139 *Vibrio cholerae.*

1. What actions should villagers take to prevent O139 type *Vibrio cholerae* from causing future cholera epidemics?

   Student answers will vary but may include:

   - ***Keep the water from getting polluted.***
   - ***Do not drink contaminated food or water.***
   - ***Drill new wells to get clean water.***
   - ***Build latrines.***
   - ***Make a new vaccine that works for O139.***

2. Why are implementing these actions difficult in many areas of the world?

   Student answers but may include: poverty, lack of medical supplies, lack of food, lack of water, lack of education may prevent taking needed actions.