



A Medical Mystery of Epidemic Proportions

Overview:

This collection of activities uses a cholera epidemic to illustrate concepts of biology including homeostasis, immunology, microbial evolution, and ecology. The activities place a focus on hands-on laboratory activities and skills integrated around the topic of cholera. They also illustrate how “new” technologies can be used to further explore and understand “old” questions in science.

Three of the activities (indicated by *) are key parts of a problem based learning case about the evolution of a new strain of cholera-causing bacterium, *Vibrio cholerae*. The other activities provide additional perspective on the biology of cholera.

Activity Title (Estimated time required)	Students will...	Purpose
*An Epidemic? (40 minutes)	Use a diagnostic test to identify the cause of an epidemic and then develop a plan for handling the cholera epidemic	Engage, assess prior knowledge and consider the impact of a pathogen on a community.
Educating the Villagers (40 minutes or homework)	Read a CDC fact sheet on cholera and summarize the information in words and diagrams.	Communicate background information on cholera and <i>Vibrio cholerae</i> bacteria.
How Do <i>Vibrio cholerae</i> Affect the Body? (40 minutes)	Use membrane models of intestines to explain the effect of <i>Vibrio cholerae</i> on water balance.	Apply understanding of cell transport and homeostasis.
* Why Aren't the Adults Immune to Cholera? (40 minutes)	Use antibody testing and manipulative models to explain why adults aren't immune to cholera.	Apply understanding of antibodies, antigens, and immunity.
* How Did O139 Evolve? (40 minutes)	Use microarray technology to test two hypotheses about the origin of the new <i>Vibrio cholerae</i> pathogen.	Apply understanding of evidences for evolutionary relationships.
Could a Cholera Epidemic Happen Here? (20 minutes or homework)	Imagine events that might influence the evolution of <i>V. cholerae</i> or its ability to cause disease.	Consider evolution and human actions as having implications for the future.
Evolution and Cholera (20 minutes)	View a PBS video and read about cholera evolution.	Apply understanding of natural selection.
Climate Change and Cholera (40 minutes)	Model the effect of climate change on ecosystems and food webs.	Apply understanding of ecosystem interactions.

Correlation with New York State Learning Standards:

Note: * indicate major understandings for the parts of a problem based learning case about the evolution of a new strain of cholera-causing *Vibrio cholerae*. Other major understandings correlate with the extension activities provided with this case.

Standard 1

- * 1.1b Learning about the historical development of scientific concepts or about individuals who have contributed to scientific knowledge provides a better understanding of scientific inquiry and the relationship between science and society.
- * 1.2a Inquiry involves asking questions and locating, interpreting, and processing information from a variety of sources.
- * 3.1a Interpretation of data leads to development of additional hypotheses, the formulation of generalizations, or explanations of natural phenomena.

Standard 4

- 1.1a Populations can be categorized by the function they serve. Food webs identify the relationships between producers, consumers, and decomposers carrying out either autotrophic or heterotrophic nutrition.
- 1.1b Every population is linked, directly or indirectly, with many others in an ecosystem. Disruptions in the numbers and types of species and environmental changes can upset ecosystem stability.
- * 1.2d If there is a disruption in any human system, there may be a corresponding imbalance in homeostasis.
- 1.2g Each cell is covered by a membrane that performs a number of important functions for the cell. The processes of diffusion and active transport are important in the movement of materials into and out of the cell.
- 2.1 g Cells store and use coded information. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.
- 2.1h Genes are segments of DNA molecules. Any alteration of the DNA sequence is a mutation. Usually, an altered gene will be passed on to every cell that develops from it.
- 3.1b New inheritable characteristics can result from new combinations of existing genes or from mutations of genes in reproductive cells.
- * 3.1e Natural selection and its evolutionary consequences provide a scientific explanation for the fossil record of ancient life forms, as well as for the molecular and structural similarities observed among the diverse species of living organisms.
- 3.1g Some characteristics give individuals an advantage over others in surviving and reproducing, and the advantaged offspring, in turn, are more likely than others to survive and reproduce. The proportion of individuals that have advantageous characteristics will increase.
- * 5.2a Homeostasis in an organism is constantly threatened. Failure to respond effectively can result in disease or death.
- * 5.2b Viruses, bacteria, fungi, and other parasites can infect plants and animals and interfere with normal life functions.

- * 5.2c The immune system protects against antigens associated with pathogenic organisms or foreign substances and some cancer cells.
- * 5.2d Some white blood cells engulf invaders. Others produce antibodies that attack them or mark them for killing. Some specialized white blood cells remain, able to fight off subsequent invaders of the same kind.
- * 5.2e Vaccinations use weakened microbes (or parts of them) to stimulate the immune system to react. This reaction prepares the body to fight subsequent invasions by the same microbes.
- * 5.2j Biological research generates knowledge used to design ways of diagnosing, preventing, treating, controlling, or curing diseases of plants or animals.
- 6.1g Relationships between organisms may be negative, neutral, or positive. Some organisms may interact with another in several ways. They may be in a producer/consumer, predator/prey, or parasite/host relationship; or one organism may cause disease in, scavenge, or decompose another.
- 7.1c Human beings are part of the Earth's ecosystems. Human activities can, deliberately or inadvertently, alter the equilibrium in ecosystems. Humans modify ecosystems as a result of population growth, consumption, and technology.

Laboratory Skills

- Uses a dichotomous key
- Follows directions to correctly use and interpret chemical indicators
- Collects, organizes, and analyzes data
- Analyzes results from observations/expressed data
- Formulates an appropriate conclusion or generalization from the results of an experiment.

An Epidemic?

Summary:

Students read a scenario about people affected by an outbreak of diarrheal disease. They use information from a booklet and from a simulated laboratory test to determine what actions that should be taken to deal with this outbreak.

Objectives:

Students will...

- Develop a list of facts and questions based on the scenario
- Apply information from a booklet to determine a plan of action for dealing with a disease outbreak
- Use a simulated rapid test kit to determine the cause of the outbreak
- Use a dichotomous key to determine treatments necessary for patients with specific symptoms

Preparing for class:

- Preview the graphics provided in Slides 1 and 2 on the *Medical Mystery* slide show. Consider previewing the entire slide show to familiarize yourself with the slides that correlate with other parts of the *Medical Mystery of Epidemic Proportions*.

NOTE: To open the Medical Mystery slide show, you will need to have Adobe FLASH Player installed on your computer.

To download FLASH player, go to

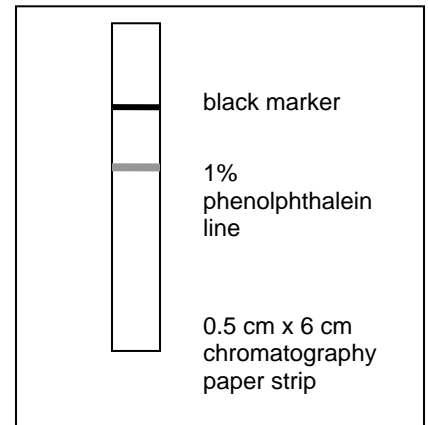
http://www.adobe.com/shockwave/download/download.cgi?P1_Prod_Version=ShockwaveFlash

Once FLASH Player is installed on your computer:

- Click on the “Medical Mystery” file.
- Click on “Select program from list”
- Select “Open with Internet Explorer”
- Click on beige bar at the top to “Allow Blocked Content”
- Click to allow blocked content.

- For each student, make one copy of the following handouts:
 - *An Epidemic?*
 - “*First Steps for Managing an Outbreak of Acute Diarrhea.*”
- Provide goggles for each team member. Gloves may also be provided but are optional.
- For each team of 2-4 students, prepare a “*Vibrio cholerae* Rapid-Test Kit” that includes:
 - 1 copy of the instructions “Using the *Vibrio cholerae* Rapid-Test Kit”

- 2 strips of heavy chromatography paper (3 MM chromatography paper VWR catalog #21427-456). Strips should be about 0.5 cm X 6 cm. Use a black permanent marker to draw a thin line across each strip (1.5 cm from the top). Use a fine paint brush to apply a thin line of 1% phenolphthalein indicator approximately in the middle of each strip (3 cm from the top).
- 2 small test tubes - wide enough to dip the chromatography paper into (for example, 2 ml microtubes). Label one tube “River Water Sample” and label the other tube “Diarrhea Sample”. Fill both tubes with pH 10 buffer or colorless household ammonia. Optional: Add a very small amount of chocolate syrup and cream to the “Diarrhea Sample” if you would like to increase the realism.



- Optional: Assemble of collection of the supplies available to the characters in the scenario: booklet, rapid-test kit, 1 lb bag of salt, 5 lb bag of sugar, 1 gallon bottle of chlorine, 2 boxes of gloves, 1 bottle of antibiotic tablets.

In the classroom:

1. Explain that students will be working to solve a medical mystery case that is based on events that occurred during the 1990's. The simulated case and experiments have been designed to introduce the process of scientific inquiry but do not represent a historical account of the actual events.
2. Distribute 1 copy of *An Epidemic?* to each student.
3. Ask four students to play the roles of Narrator, Alex, Daphne, and Ming.
4. Read the scenario aloud to the class. Consider showing slide 1 that shows the village and the people affected by the outbreak.
5. Students work individually to complete #1 and #2 on their handout. They make lists of the things they know and the things that they would like to know about the scenario.
6. Students share things on their lists with the class. As they do, the teacher or a student recorder should make posters (or lists on the board). Consider asking students to add to these posters (lists) after you have completed all of the *Medical Mystery* activities that you selected to implement with your classes.
7. Organize students into teams of 2-4 students.
8. Consider showing slide 2 that shows villagers affected by the outbreak. Distribute 1 copy of *First Steps for Managing an Outbreak of Acute Diarrhea* to each student.
9. Distribute one “*Vibrio cholerae* Rapid-Test Kit” to each team of students.
10. Students work in teams to complete #3 through # 6 on the *An Epidemic?* handout.
11. Allow time for debriefing on what students know and want to know about the medical mystery.

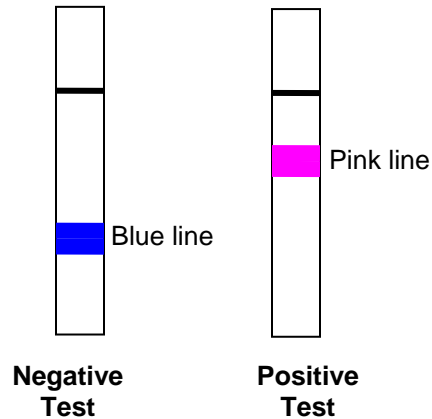
Additional resources:

- Consider extensions in which students:
 - Learn more about cholera by using the *Educating the Villagers* activity. This activity can be done as homework or as a class activity.
 - Learn how *Vibrio cholerae* infection disrupts homeostasis by using the “*How Do Vibrio cholerae Affect the Body*” activity. This activity includes animations and laboratory models that illustrate how the cholera toxin disrupts active transport and osmosis in the intestine.
- For specific information on the actual case that is simulated in this curriculum unit see:
Emergence and evolution of *Vibrio cholerae* O139. Shah M Faruque, David A Sack, R. Bradley Sack, Rita R. Colwell, Yoshifumi Takeda, and G. Balakrish Nair *Proceeding of the National Academy of Sciences*. February 4, 2003 Vol 100 no. 3
<http://www.pnas.org/cgi/content/abstract/100/3/1304>
- Recommended reading for teachers and students:
Ghost Map by Steven Johnson explores the history of cholera epidemiology and the threat that cholera may pose for the future of large cities. For further information see <http://www.theghostmap.com/>

Put one color copy in each *Vibrio cholerae* Rapid-Test Kit lab kit

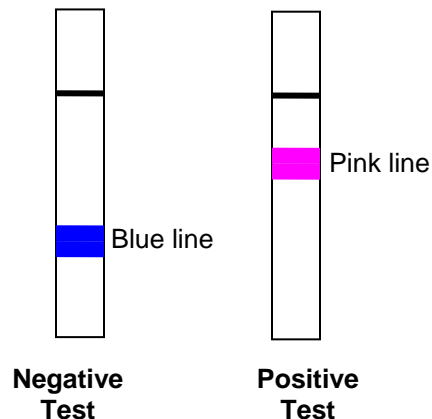
Using the *Vibrio cholerae* Rapid-Test Kit

1. Hold the dip stick by the end with a black line and place the other end into sample in the tube.
2. After one minutes, remove the dipstick and read the results.
3. If the results show one blue line, this indicates a negative test - *Vibrio cholerae* is not present in the sample.
4. If the results show one pink line, this indicates a positive test - *Vibrio cholerae* is present in the sample.



Using the *Vibrio cholerae* Rapid-Test Kit

5. Hold the dip stick by the end with a black line and place the other end into sample in the tube.
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8. If the results show one pink line, this indicates a positive test - *Vibrio cholerae* is present in the sample.



4. **Take action to maintain or restore patients' water balance. Use Oral Rehydration Solution to prevent or treat dehydration.**

For all patients: Monitor the patient for signs of dehydration regularly during the first six hours. Continue treatment until diarrhea stops and patient is rehydrated.

Oral Rehydration Solution (ORS) is more than just water. It contains low concentrations of sugar and salt to replace nutrients lost due to diarrhea.

If ORS packets are available, dilute one packet in one liter of boiled or treated water.

If ORS packets are not available, add the following to one liter of boiled or treated water:

- 2 teaspoons (small spoon) of salt
- 8 teaspoons (small spoon) of sugar

80% of diarrhea cases can be treated using only Oral Rehydration Solution (ORS)

First Steps for Managing an Outbreak of Acute Diarrhea



Is 1. Is this the beginning of an outbreak?

You might be facing an outbreak if you have seen an unusual number of acute diarrhea cases and the patients:

- Have similar symptoms (watery or bloody diarrhea).
- They live in the same area, eat the same food, or share a common water source.
- There is an outbreak in a neighboring community.

2. Is the patient suffering from cholera or shigella?

Acute diarrhea is a common symptom. If patients have more than three stools per day, it is important to differentiate between shigella or cholera.

Do a preliminary diagnosis based on:

- Patient and family members' symptoms

Symptom	Cholera	Shigella
Stools	Watery	Bloody
Fever	No	Yes
Abdominal Cramps	Yes	Yes
Vomiting	Yes	No
Rectal Pain	No	Yes

- The results of Cholera Rapid-Test
- **Send diarrhea samples to WHO (World Health Organization) laboratory for further testing.**

Cholera is an acute 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* produce a potent toxin that causes a large amount of painless, watery diarrhea that can quickly lead to severe dehydration and death if treatment is not promptly given. Vomiting also occurs in most patients.

Shigella is an acute intestinal infection caused by ingestion of food or water contaminated by a genus of bacteria *Shigella*. *Shigella* bacteria are the major cause of diarrhea with blood and mucus in the stools. In the body, they invade and destroy the cells lining the small intestine, causing mucosal ulceration and bloody diarrhea. Other symptoms include fever, abdominal cramps, and rectal pain. Most patients recover within seven days. Shigella can be treated with antibiotics, although some strains have developed drug resistance.

3. Prompt treatment is critical for the survival of patients with cholera or shigella.

Use this key to determine the severity of dehydration and treatment needed for each patient.

Key for Treatment Planning:

<p>1. Does the patient have two or more of the following symptoms?</p> <ul style="list-style-type: none"> • Frequent diarrhea • Sunken eyes • Absence of tears • Dry mouth and tongue • Thirsty and drinks eagerly • Skin pinch goes back slowly 	<p>If NO, go to 2A If YES, go to 2B</p>
<p>2A. The patient is not dehydrated. Prevent dehydration by having the patient drink Oral Rehydration Solution after each stool. Give antibiotics for shigella. No antibiotics needed for cholera.</p>	
<p>2B. The patient is dehydrated. Does the patient have one or more of the following symptoms?</p> <ul style="list-style-type: none"> • Lethargic, unconscious, floppy • Unable to drink • Pulse is weak • Skin pinch goes back slowly 	<p>If NO, go to 3A If YES, go to 3B</p>
<p>3A. The patient is moderately dehydrated. Treat dehydration by having the patient drink Oral Rehydration Solution (according to the information provided on the back of the packet). Give antibiotics for shigella. No antibiotics are needed for cholera.</p>	
<p>3B. The patient is severely dehydrated. Treat dehydration by using an IV (intravenous) drip. In case this is not possible, rehydrate with 100 mL/kg oral rehydration solution in a three hour period. Give antibiotics for cholera or shigella.</p>	

An Epidemic?

Location: A remote village in India where extreme poverty and lack of transportation makes obtaining basic medical care difficult.

You are a member of a volunteer team of ecologists sent to survey water supplies in the area for disease causing pathogens.

Now, you find yourself facing something more serious than lack of clean water.



- Alex: We have a serious problem! I've seen at least 80 cases of severe diarrhea and vomiting today.
- Daphne: Some of the villagers that I've seen were losing what looked like a quart of watery diarrhea an hour. Two of them died from severe dehydration.
- Ming: We need an emergency action plan! It will be several days until we can get a medical team here to help us.
- Alex: I found a booklet called "First Steps for Managing an Outbreak of Acute Diarrhea". The only items we have in our medical supply kit are a *Vibrio cholerae* Rapid-Test Kit, a gallon of chlorine disinfectant, two boxes of gloves and a bottle of antibiotic tablets.

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1. List 5 things that you know about this problem and what could be causing it.

_____ ***Student answers will vary and may include prior knowledge.*** _____

2. List 5 things you would like to know to help the people in the village.

_____ ***Student answers will vary.*** _____

3. Based on the information in the booklet, “**First Steps for Managing an Outbreak of Acute Diarrhea**”, do you think this is the beginning of an outbreak? Why or why not?

Yes because an unusual number of acute diarrhea cases and the patients:

- **Have similar symptoms (watery or bloody diarrhea).**
- **They are live in the same area, eat the same food, or share a common water source.**

4. According to the booklet, what are the next two things that you should do?

Possible actions include:

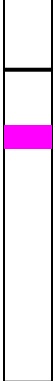

Do a preliminary diagnosis to determine if this is a cholera or shigella outbreak.

Do a Cholera Rapid Test.

Send diarrhea samples to WHO.

Begin treatment promptly.

5. Use the **Vibrio cholerae Rapid-Test Kit** to test the water sample and diarrhea samples.

Well Water Sample	Diarrhea Sample	Laboratory Results - Vibrio cholerae Rapid-Test Kit
		Conclusions: _____ _____ The samples contain cholera bacteria _____ _____ _____

6. Use the key in the number 3 of the booklet “First Steps for Managing an Outbreak of Acute Diarrhea” to determine what action should you take to help each of these patients.

- Patient 1 symptoms: frequent diarrhea, vomiting, weak pulse, sunken eyes, and lethargic.

The patient is severely dehydrated. Treat dehydration by using an IV (intravenous) drip. In case this is not possible, rehydrate with 100 mL/kg oral rehydration solution in a three hour period. Give antibiotics for cholera or shigella.

- Patient 2 symptoms: frequent diarrhea, vomiting, sunken eyes, dry mouth and tongue, but is alert and thirsty.

The patient is moderately dehydrated. Treat dehydration by having the patient drink Oral Rehydration Solution (according to the information provided on the back of the packet). Give antibiotics for shigella. No antibiotics are needed for cholera.

7. What actions could you take to prevent the spread of this pathogen to other people in the region?

Student answers may vary but may include: keep patients in isolation, provide clean water supplies, prevent food and water contamination.

Educating the Villagers

Summary:

Students read a CDC (Center for Disease Control) fact sheet on cholera. They summarize the reading by answering questions and creating illustrations. Teachers may opt to have students do all questions and illustrations, or small teams of students may be assigned to create posters that answers assigned questions.

Objectives:

- Students will read for information to answer a series of questions about cholera.
- Students will apply what they learn to illustrate key information from the reading.

Preparing for class:

- Make 1 copy of *Educating the Villagers* and 1 copy of *Cholera Fact Sheet* per student
- Optional: 1 copy of Daphne's journal for each student.
- Poster paper and markers for class posters

In the classroom:

1. This activity may be done for homework or as a class activity.
2. Distribute 1 copy of *Educating the Villagers* and 1 copy of *Cholera Fact Sheet* to each student.
3. Ask selected students to read the scenario out loud to the class.
4. (optional) Distribute copies of *Daphne's Journal* to each student.
5. Explain the importance of communicating information to people who may have a limited understanding of how waterborne diseases are spread.
6. Explain that when you read for information, it is helpful to read the question first and then look for the answer in the reading.
7. Students follow the instructions in the *Educating the Villagers* handout.

Alternative: Assign one question to each student (or team of students). Ask students to create a class poster that answers their assigned question and illustrates the answer. Post these in the classroom.

Additional resources:

For general information on cholera see:

- World Health Organization: <http://www.who.int/topics/cholera/en/>

- Center for Disease Control and Prevention: http://www.cdc.gov/nczved/dfbmd/disease_listing/cholera_gi.html
- Mayo Clinic: <http://www.mayoclinic.com/health/cholera/DS00579/>
- emedicine from WebMd: <http://www.emedicine.com/med/topic351.htm>
- Medical Ecology: <http://www.medicalecology.org/water/cholera/cholera.htm>
- NASA: http://outreach.eos.nasa.gov/EOSDIS_CD-03/docs/enso_cholera.htm
- PBS: *Cholera Domesticating Disease*
http://www.pbs.org/wgbh/evolution/library/10/4/1_104_01.html
- Medical Geography and Cholera in Peru:
http://www.colorado.edu/geography/gcraft/warmup/cholera/cholera_f.html
- Drinking water and human health: <http://www.drinking-water.org/flash/splash.html>



Cholera Fact Sheet

Cholera has been very rare in industrialized nations for the last 100 years; however, the disease is still common today in other parts of the world, including the Indian subcontinent and sub-Saharan Africa. Although cholera can be life-threatening, it is easily prevented and treated. In the United States, because of advanced water and sanitation systems, cholera is not a major threat; however, everyone, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it.

Cholera is an acute, diarrheal illness. The illness is often mild or without symptoms, but sometimes it can be severe. Approximately one in 20 infected persons has severe disease characterized by profuse watery diarrhea, vomiting, and leg cramps. In these persons, rapid loss of body fluids leads to dehydration and shock. Without treatment, death can occur within hours. In a severe case of cholera, a patient may lose up to 6 liters of water per day. This is serious because an adult body contains about 50 liters of water with 3.5 liters of that water in their blood.

Cholera is caused by infection of the intestine with a pathogen: the bacterium *Vibrio cholerae*. The bacterium attach to the walls of the small intestine where they secrete a potent toxin. This toxin causes the cells lining the intestine to secrete excessive amounts of salt into the intestinal lumen. The high salt concentration in the intestine leads to osmotic water loss and diarrhea. The rapid loss of body fluids leads to dehydration and shock. Without treatment, death can occur within hours.

A person may get cholera by drinking water or eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person. The disease can spread rapidly in areas with inadequate treatment of sewage and drinking water.

The cholera bacterium may also live in the environment in rivers and coastal waters. Shellfish eaten raw have been a source of cholera, and a few persons in the United States have contracted cholera after eating raw or undercooked shellfish from the Gulf of Mexico. The disease is not likely to spread directly from one person to another; therefore, casual contact with an infected person is not a risk for becoming ill.

Although signs and symptoms of severe cholera may be unmistakable in endemic areas, the only way to confirm a diagnosis is to identify the bacteria in a stool sample. The sample is grown on a selective medium then Gram stained. *Vibrios* are Gram negative and rod-shaped. But because cholera requires immediate treatment and because all cases of watery diarrhea are treated in the same way, doctors are likely to begin rehydration before a definitive diagnosis is made.

Rapid cholera dipstick tests are now available, enabling health care providers in remote areas to confirm diagnosis of cholera earlier. Quicker confirmation helps to decrease death rates at the start of cholera outbreaks and leads to earlier public health interventions for outbreak control.

In the United States, cholera was prevalent in the 1800s but has been virtually eliminated by modern sewage and water treatment systems. However, travelers to parts of Africa, Asia, or Latin America where epidemic cholera is occurring may be exposed to the cholera bacterium. In addition, food borne outbreaks have been caused by contaminated seafood brought into this country by travelers.

The risk for cholera is very low for travelers visiting areas with epidemic cholera if simple precautions are observed. All travelers to areas where cholera has occurred should observe the following recommendations:

- Drink only water that has been boiled or treated with chlorine or iodine. Other safe beverages include tea and coffee made with boiled water and carbonated, bottled beverages with no ice.
- Eat only foods that have been thoroughly cooked and are still hot, or fruit that you have peeled yourself.
- Avoid undercooked or raw fish or shellfish.
- Make sure all vegetables are cooked and avoid salads.
- Avoid foods and beverages from street vendors.

A recently developed oral vaccine for cholera is licensed and available in other countries. The vaccine appears to provide somewhat better immunity and have fewer adverse effects than the previously available vaccine. However, CDC does not recommend cholera vaccines for most travelers, nor is the vaccine available in the United States.

Cholera can be simply and successfully treated by immediate replacement of the fluid and salts lost through diarrhea. Patients can be treated with oral rehydration solution, a prepackaged mixture of sugar and salts to be mixed with water and drunk in large amounts. This solution is used throughout the world to treat diarrhea. Severe cases also require intravenous fluid replacement. With prompt rehydration, fewer than 1% of cholera patients die.

Antibiotics shorten the course and diminish the severity of the illness, but they are not as important as rehydration. Persons who develop severe diarrhea and vomiting in countries where cholera occurs should seek medical attention promptly.

Predicting how long a Cholera epidemic will last is difficult. The cholera epidemic in Africa has lasted more than 30 years. In areas with inadequate sanitation, a cholera epidemic cannot be stopped immediately, and, although far fewer cases have been reported from Latin America and Asia in recent years, there are no signs that the global Cholera pandemic will end soon.

Major improvements in sewage and water treatment systems are needed in many countries to prevent future epidemic cholera. International public health organizations are working to identify and investigate cholera outbreaks and design and implement preventive measures. In addition, they provide information on diagnosis, treatment, and prevention of cholera to public health officials and educate the public about effective preventive measures.

Adapted from: http://www.cdc.gov/nczved/dfbmd/disease_listing/cholera_gi.html

Daphne's Journal

Stricken villagers are curled up in fetal positions beneath the shade of the shade of a tree, which shields the sick from a searing midday sun. They are waiting to be admitted to an already crowded hospital tent.

Torrential rains that revived parched maize fields and replenished a nearly empty water tank serving the 2,000 villagers seemed like a godsend on Saturday. By Sunday, the restorative rainwater had turned lethal.



We think that the rain's runoff carried human waste from the fields -- where farmers squat and defecate -- and contaminated the stream that feeds the village's water-intake pipes. The result has been an outbreak of cholera. It's like they're drinking poison being piped up from the river.

One girl, weak and malnourished, died of the acute diarrheal disease Sunday shortly after curling up beneath a tree.

The hospital tent's 110 beds are full yet the cholera victims keep coming, more than 90 of them by Monday, carried by relatives or shuffling on weak legs until they collapse in the dirt to wait.

Cholera beds consist of sheets of plywood propped up on bricks. A hubcap-sized hole is cut in the middle of the wood. A bucket sits under the hole. Another bucket rests on the cement floor next to the victim's head.

The patients are too weak to speak. Vacant eyes loll back in ashen faces fixed in mute terror. Relatives hover, helpless. Groans and muffled cries fill the crowded, fetid rooms, punctuated by the splash of vomit and diarrhea streaming into plastic buckets.

The medical team brought boxes of IV bags and antibiotics provided by American donors. Three dozen villagers of all ages fortunate enough to receive

care cram two makeshift cholera wards. Volunteers try to keep up with the foul mess, dragging mops and squeegees across the cement floor.

If you don't get an IV started to rehydrate them immediately, they can die quickly. The only physician struggles to find a vein on the reed-thin arm of a 2-year-old boy. The doctor's tiny needle finally finds its mark so that it can deliver life-saving IV fluids.

The polluted stream is a short hike from a scattering of huts through shoulder-high fields of maize and row after row of leafy tobacco. A thick, black snake slithers between the raised beds. On a flat, low-lying area, the village's water supply intake pipes jut a few yards from shore into a slow-moving, swampy pocket of the stream.

A new well could be drilled or the intake pipes re-routed to a pond on a nearby hillside with powerful pumps, but these options are too expensive unless the government steps in. Another solution would undercut a long-held practice among farmers in India, who consider their fields open-air toilets. If every farmer built a latrine, it could solve the cholera problem.

In the meantime, villagers can use a temporary supply of clean water provided by the medical team. The young girl was the only cholera fatality in the village. With IVs, food and antibiotics, many people recovered rapidly. By Tuesday, the number of cholera patients falls to about 50, roughly half of those stricken the day before.

Modified from: <http://timesunion.com/fourthworld/affliction/graphics/story3.jpg>

Educating the Villagers

The medical emergency team from the World Health Organization has arrived. They have offered to take your team back to the airport so that you can return home.

Daphne: The medical team is so busy treating patients that they don't have time to make the villagers aware of what they can do to keep this epidemic from spreading further. I'd like to stay and help out.

Ming: I'd like to stay too. I know there's a language barrier, but I'd like to try to help people understand what's causing this epidemic and what needs to be done to stop it.

Alex: The medical team gave me a CDC Fact Sheet to distribute but I think many people will need simple, easy to understand answers to their questions.

How could you answer the villagers' questions about cholera? Include both brief written information and diagrams.

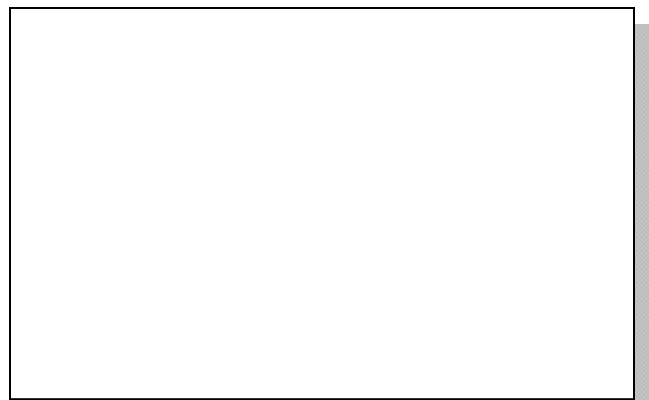
1. What is cholera?

_____ ***Cholera is an acute diarrheal disease.*** _____



2. What type of pathogen causes cholera?

_____ ***The bacterium Vibrio cholerae*** _____



3. How do you get cholera?

___ **By drinking water or eating food contaminated with the *Vibrio cholerae* bacteria** _____



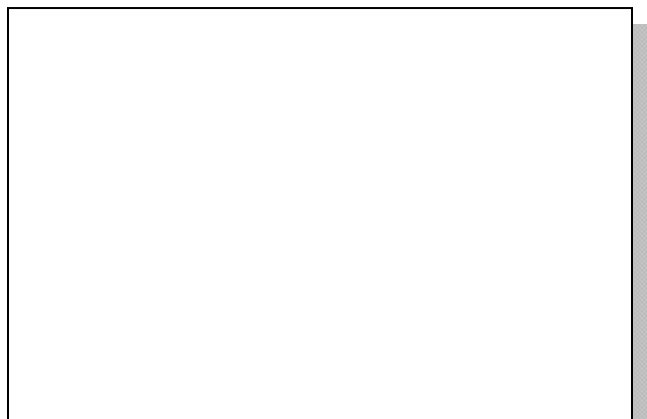
4. What are the symptoms of cholera?

___ **Watery diarrhea, vomiting, leg cramps.** _____



5. Why is cholera so deadly?

___ **Rapid loss of body fluids leads to dehydration and shock.** _____



6. How can cholera be prevented?

___ Avoid consuming contaminated water or food. Provide proper sanitation to prevent contamination of water supplies.

A large, empty rectangular box with a thin black border, positioned to the right of the text for question 6. It is intended for the student to write their answer.

7. How can cholera be treated?

___ By immediate replacement of fluids and salts lost through diarrhea. This can be done by drinking oral rehydration fluids or through intravenous fluid replacement.

A large, empty rectangular box with a thin black border, positioned to the right of the text for question 7. It is intended for the student to write their answer.

How do *Vibrio cholerae* Affect the Body?

Summary:

This activity was designed to show students that disrupting water and salt balance in the body can have serious health consequences. Students explore how cholera toxin affects cell transport and create a model “intestine” to illustrate why defective salt transport results in massive osmosis and diarrhea.

Objectives:

Students will...

- View graphics and animations that illustrates the effects of *Vibrio cholerae* on the human body. This animation shows how active transport maintains salt balance in a healthy individual. It then shows how cholera toxin interferes with the active transport of salt—resulting in a high concentration of salt in the intestine and osmotic diarrhea
- Observe the effects of normal and high salt concentrations in model “intestines” made from membrane.
- Apply their understanding of osmosis to explain their observations of the model.
- Observe graphics and animations that illustrate the effects of dehydration on the cells in the body.
- Observe graphics and animations that illustrate how rehydration solution can be used to restore normal water balance for cells.
- Apply what they learned about dehydration to other causes (exercise, heat, diarrhea, vomiting) and recommend treatments to restore salt and water balance.

Preparing for class:

1. Arrange for access to a computer and computer projector.
2. Preview the animations and graphics provided in Slides 3 through 14 on the *Medical Mystery* slide show.
3. Prepare 1 copy of *How do Vibrio cholerae Affect the Body?* per student.
4. For each team of 2-4 students, prepare a lab kit that contains:
 - 2 pieces of dialysis tubing—9-10 inches per piece Note: Serpent Skin ordered from Educational Innovations is an inexpensive alternative to dialysis tubing.
<http://www.teachersource.com/BiologyLifeScience/LifeScience/SerpentSkinTubing.aspx>
 - 1 coffee stirrer or stirring rod
 - 1 clear plastic cup (10 or 12 oz. tall form recommended)
 - 2 graduated 1 mL plastic pipets (droppers)
 - 1 small test tube (or a 1.5 ml microtube) labeled “LOW Salt.” Add approximately 1 ml of SUCROSE (table sugar) then add one drop of green food coloring to the tube.

Note: Sucrose is used in this experiment because it results in a faster osmotic response and reduces class time needed for this experiment.

- 1 large test tube (or a 15 ml conical tube) labeled “HIGH Salt.” Fill this tube with approximately 10 ml of SUCROSE (table sugar) then add 5 drops of green food coloring to the tube. Note: Sucrose is used in this experiment because it results in a faster osmotic response and reduces class time needed for this experiment.

In the classroom:

1. Distribute one copy of *How do Vibrio cholerae Affect the Body?* to each student.
2. Select students to read the scenario aloud to the class.
3. Read the first part of the scenario.
4. Show slides 3 through 7 of the *Medical Mystery* slide show that illustrate how *Vibrio cholerae* affects the body.
5. Read the second part of the scenario. Show slide 8 that illustrates Daphne’s experiment.
6. Students complete “Daphne’s Experiment.”
7. Ask students to summarize their observations and conclusions from the experiment. Show slide 9 to discuss the results of Daphne’s experiment.
8. Read the third part of the scenario.
9. Show slides 10 through 11 of the *Medical Mystery* slide show that illustrate what happens to body cells in a concentrated salt solution.
10. Read the last part of the scenario.
11. Show slides 12 through 14 of the *Medical Mystery* slide show that illustrate what happens to body cells placed in a rehydration solution.
12. Relate this activity to the importance of treating dehydration due to excessive exercise, heat exposure, vomiting, or diarrhea that may occur in their lives. Point out that this is most important for young children.

Optional: If you only have a single period class, you may wish to have students set up “Daphne’s Experiment” first - the day before or at the beginning of class. Students can then read the scenario and view slide show to give the fluid ample time to rise in the tubes.

How do *Vibrio cholerae* affect the body?

Alex: I don't understand how the *Vibrio cholerae* bacteria cause diarrhea.

Daphne: That's easy. There's an animation that shows what happens. If you look carefully you can see that the bacteria attach to the walls of the small intestine. They secrete a toxin that causes active transport carriers in the cells lining the intestine to pump excessive amounts of salt into the intestine.

View the slide show that illustrates how *Vibrio cholerae* affect the cells lining the intestine. (slides 3 through 7)

1. What type of transport (active or passive) is used to maintain the proper concentration of salt in the body? How can you tell?

Active transport. The carrier molecules in the membrane only turn when ATP energy is provided.

2. How does the cholera toxin secreted by *Vibrio cholerae* affect the transport of salt through the cell membranes of the cells lining the intestine?

It causes the transport proteins in the membrane to only pump salt out of the cells.

3. How does the cholera toxin affect the salt concentration in the body and in the intestine?

It increases the concentration of salt in the intestine and decreases the amount of salt in the body.

Ming: Great animation. But why does having a high concentration of salt in the intestine cause diarrhea?

Daphne: I can show you an osmosis experiment we did when I was in high school. It shows what happens when there is a high concentration of salt in the intestine.

Do "Daphne's experiment" using the instructions and materials in your laboratory kit. Answer the questions on the next page before you read the remainder of this scenario. (slide 8)

4. Look up the definition for the term “osmosis” in your textbook. Write the definition below.

Osmosis is the diffusion of water through a semi-permeable membrane.

5. Adding a solute (dissolved substance like salt) to a solution decreases the water concentration in the solution. Which solution has the highest water concentration—one with a high salt concentration or one with a low salt concentration?

One with a low salt concentration

6. Apply your understanding of osmosis. Predict what will happen to the level of the solution in the bag representing the normal person’s intestine. Explain your prediction.

The level of fluid should stay the same because the concentration of water inside and outside the bag are the same.

7. Apply your understanding of osmosis. Predict what will happen to the level of the solution in the bag representing the cholera patient’s intestine. Explain your prediction.

The level of fluid in the tube should rise the same because the concentration of water outside the bag is higher than the concentration of water inside the bag.

8. Observe and record the results of your experiment after one hour (or overnight). How is the “patient’s intestine” different from the “normal person’s intestine?”

The level of water rose in the patient’s intestine and stayed the same in the normal person’s intestine.

9. Use the definition of osmosis to explain why the secretion of salt into the intestine causes a cholera patient to produce large amounts of watery diarrhea.

Water diffuses into the intestine because the concentration of water in the patient’s body is higher than the concentration of water in the patient’s intestine.

Ming: Ok, so I get this osmosis in the intestine stuff. But why would having severe diarrhea be so deadly?

Daphne: I read that in a severe case of cholera, a patient can lose up to 6 liters of water per day. This is serious because an adult body contains about 50 liters of water with 3.5 liters of that water in their blood.

Alex: Losing all that water in the diarrhea makes the body severely dehydrated. That means that the body fluids have a low concentration of water. Imagine what happens when all the cells in the body are in a low water concentration environment.

View the slide show that illustrates what happens to body cells in a cholera patient. (slides 10 through 11)

10. What happens to body cells when the concentration of water in the body fluids decreases?

They shrink

Ming: Wow, those cells in the concentrated salt solution are really dehydrated. It's scary to think that's what happens to all of the cells in someone's body when they have severe diarrhea.

Alex: Severe dehydration can lead to serious complications—seizures, kidney failure, and coma. But shock is the most serious complication. When a person's blood pressure is very low, the oxygen supply to all body tissues is decreased. Untreated severe shock can cause death in a matter of minutes.

11. Explain why dehydration can have serious consequences for the patients.

It can lead to seizures, kidney failure, coma, or shock

Daphne: Sounds like restoring water balance for cholera patients is really important. That CDC fact sheet says cholera should be treated by giving patients oral rehydration solution—a mixture of water with a just a little bit of sugar and salt in it.

Alex: Do you think that adding rehydration solution will really make the cells go to back to normal?

View the slide show that illustrates what happens when rehydration solution is added to dehydrated body cells. (slides 12 through 14)

12. What happens when the cells are treated with rehydration solution?

They swell or they return to their normal size and shape

13. Why is it important that the rehydration solution contain salt and glucose?

To restore the normal levels of salt and glucose in the body fluids.

Ming: You know, cholera isn't the only thing that can result in dehydration. One guy on our track team ignored the coach's advice about staying hydrated during excessive exercise or on a hot day. He almost died.

Daphne: And there are other diseases besides cholera that can cause vomiting or diarrhea that results in serious dehydration.

Alex: People need to understand that it's very important to maintain proper water balance. It's important to treat dehydration due to any cause!

14. What patient symptoms do you think might indicate dangerous dehydration?

Student answers will vary but may include: excessive vomiting or diarrhea, rapid weight lost, shrunken skin/eyes, etc.

15. What should you do to treat dehydration and restore normal salt and water balance?

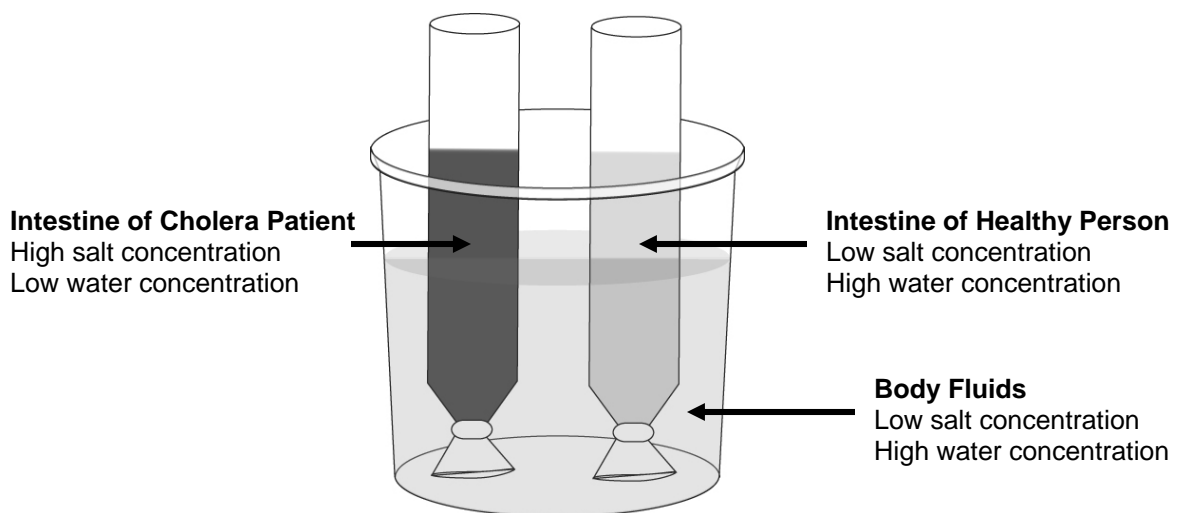
Have the patient drink oral rehydration solution to replace essential water, salts, and glucose. Seek medical attention is dehydration becomes severe.

16. What ingredients do you think are in products like sports drinks or products used to treat dehydration?

Water, sugar, and salts

Daphne's Experiment

1. Make a LOW salt concentration solution by filling the plastic cup approximately $\frac{3}{4}$ full with hot tap water. Add the small tube of colored salt (labeled "LOW Salt") and stir.
Note: This solution represents body fluids (such as blood and tissue fluids) that normally have a low salt and high water concentration.
2. Create a model of a NORMAL person's intestine.
 - Close the end of one piece of membrane tubing by tying a knot near the end as shown in the diagram below.
 - Use a graduated dropper to fill the membrane bag with 10 ml of LOW salt concentration solution.
 - Stand the tubing in the plastic cup of LOW salt concentration solution. The contents of this cup represent the normal body fluids.
3. Make a HIGH salt concentration solution by filling the large plastic tube of colored salt (labeled "HIGH salt") with hot tap water. Shake vigorously to mix thoroughly. *Note: This solution represents the fluids in the intestine of a cholera patient. Remember that the cholera toxin causes patients to secrete a large amount of salt into the intestine.*
4. Create a model of a CHOLERA PATIENT'S intestine.
 - Close the end of the other piece of membrane tubing by tying a knot near the end as shown in the diagram below.
 - Use a graduated dropper to fill this piece of membrane bag with 10 ml of HIGH salt concentration solution.
 - Stand the tubing in the plastic cup of LOW salt concentration solution. The contents of this cup represent the normal body fluids.
5. Adjust the level of the fluids in the two membrane bags so that they are at approximately the same height. Do this by removing small amounts of fluid from the tube where the level is too high. Allow this to for at least 30 minutes.



Why aren't the adults immune to cholera?

Summary:

Students use models and simulated antibody testing to develop an explanation for why the adults who had cholera or were vaccinated for cholera are not immune during this epidemic.

Objectives:

Students will...

- Examine models of *Vibrio cholerae* and explain how the types differ.
- Use a model antibody and explain why people exposed to the O1 type of *Vibrio cholerae* should be immune to *Vibrio cholerae* O1 but not to other types of *Vibrio cholerae*.
- Use a simulated antibody agglutination test to determine that the cholera outbreak is not caused by any of the known types of *Vibrio cholerae*.
- Explain how a vaccine is made and how a vaccine leads to immunity.
- Explain why people who had been vaccinated for the O1 type *Vibrio cholerae* would not be immune to the new type of *Vibrio cholerae*.

Preparing for class:

1. Preview slides 15 through 18 and consider using these slides to explain why people:
 - ARE NOT immune to cholera during their first exposure to the pathogen
 - ARE immune to cholera during their second exposure to the pathogen
2. Make 1 copy of *Why aren't the adults immune to cholera?* per student.
3. For each team of 2-4 students, make 1 set of *Models of antibodies and Vibrio cholerae bacteria*. Distribute in sets in plastic bags or envelopes.
4. For each team of 2-4 students, prepare a laboratory kit for the antibody agglutination testing that includes:
 - Laminated *Instructions for Antibody Agglutination Test Kit*.
 - 1 small test tube (or microtube) labeled "*Vibrio cholerae Sample*". Fill this tube with tap water.
 - 1 small test tube (or microtube) labeled "*O1 Vc Positive Control*". 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 small test tubes (or microtubes) labeled "*O2-O46 Antibodies*", "*O47-O92 Antibodies*", and "*O93-O138 Antibodies*". Fill each of these tubes with tap water that

has been colored faint red, yellow, or green using food coloring (a different color for each type of antibody).

- 6 plastic droppers (Label if you would like to recycle the droppers).
- 1 plastic test strip, photocopied on a plastic transparency sheet

In the classroom:

1. Distribute 1 copy of *Why aren't the adults immune to cholera?* to each student.
2. Select students to read the scenario.
3. Read the scenario aloud to the class.
4. Consider using slides 15 through 18 to explain why people:
 - ARE NOT immune to cholera during their first exposure to the pathogen
 - ARE immune to cholera during their second exposure to the pathogen
5. Distribute to each team of 2-4 students:
 - 1 bag of models of antibodies and different types of *Vibrio cholerae* bacteria
 - 1 antibody agglutination test kit
6. Students complete the activities and related questions.
7. Debrief at the end of class by asking students to explain why the adults are not immune to cholera.

SAFETY - Wear goggles!

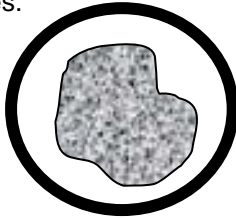
Instructions for Antibody Agglutination Test Kit:

Be careful to use a different dropper for each antibody and for each sample.

1. Place one drop of the appropriate antibody solutions (O1, O2-O46, O47-O92, or O93-O138) into the labeled circles on the plastic test strip. *Note: Some of the tubes contain a mixture of antibodies to reduce the amount of work needed for this experiment.*
2. Add one drop of the O1 VC Positive Control sample to the first well on the left. This sample contains known O1 *Vibrio cholerae*.
3. Add one drop of the *Vibrio cholerae* sample to the four wells on the right. This sample was collected from a patient from the village where the epidemic was occurring.
4. Observe the reactions in each of the circles to determine whether agglutination has occurred.

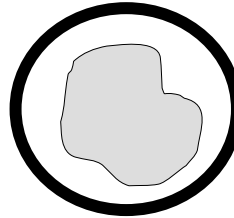
Agglutination

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



NO Agglutination

If there are no clumps, there is no interaction of the *Vibrio cholerae* and the antibodies in that well.



Instructions for Antibody Agglutination Test Kit:

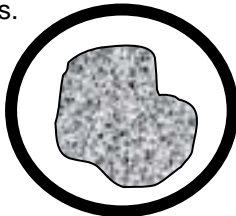
SAFETY - Wear goggles!

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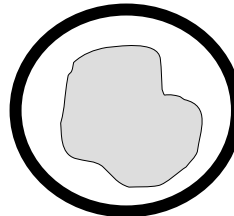
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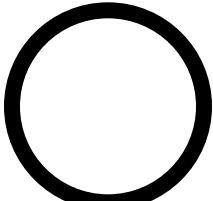
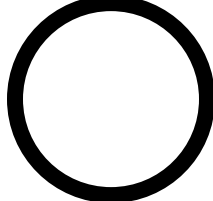
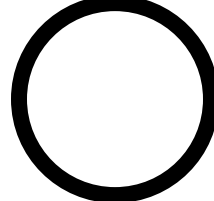
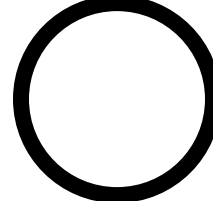
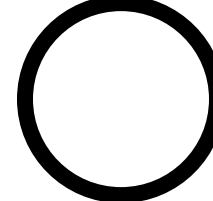

NO Agglutination

If there are no clumps, there is no interaction of the *Vibrio cholerae* and the antibodies in that well.



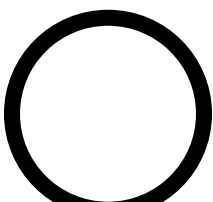
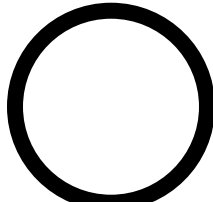
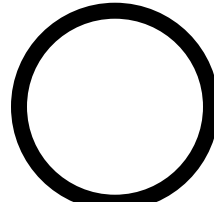
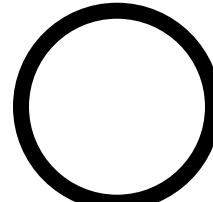
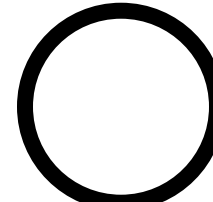

Duplicate on plastic transparency sheets and cut out on solid lines:

Laboratory Observations

O1 Antibody	O1 Antibody	O2 – 046 Antibodies	O47 – 092 Antibodies	O93 – O138 Antibodies
				
O1 Vc Positive Control				
	<i>Vibrio cholerae</i> sample from patient			

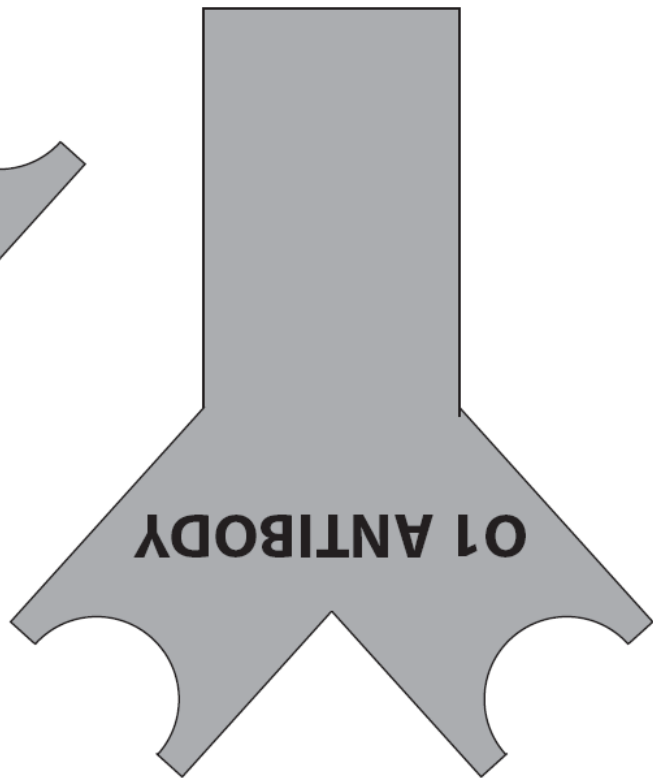
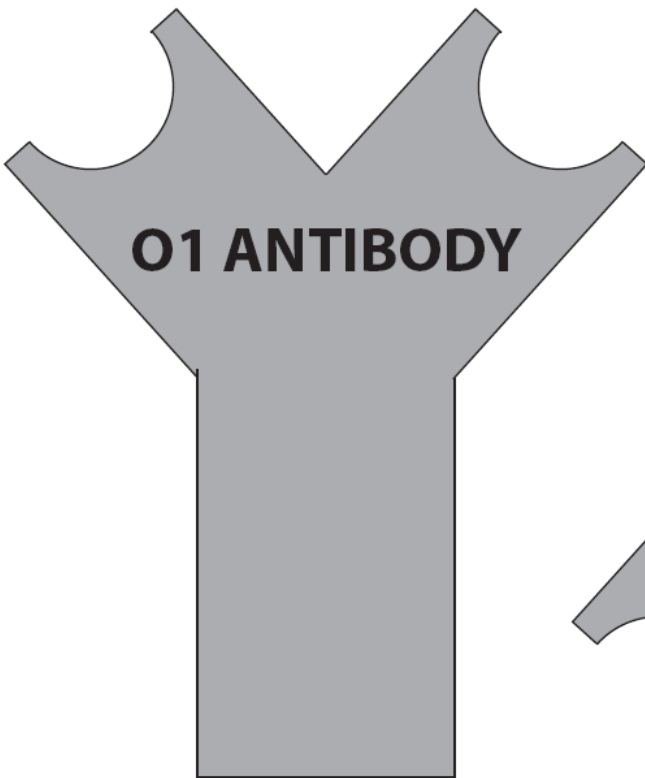
Conclusions:

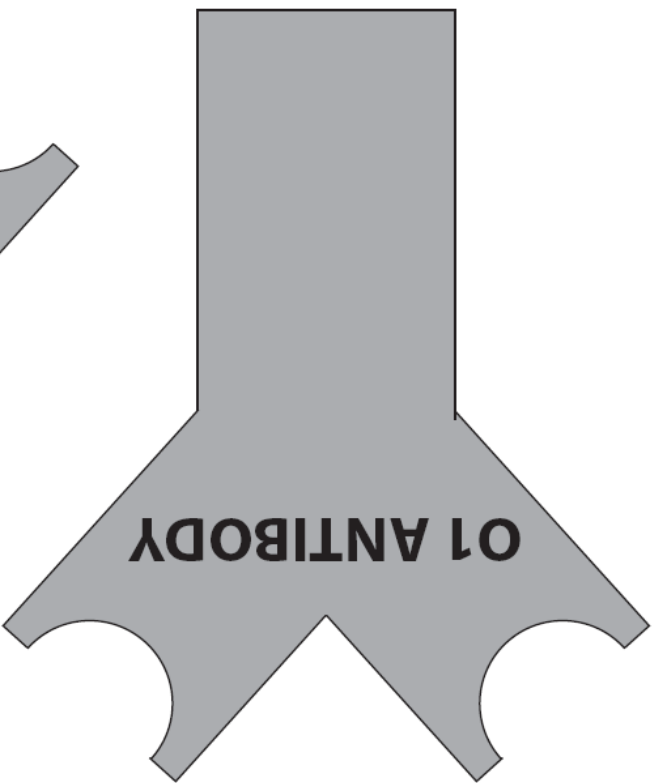
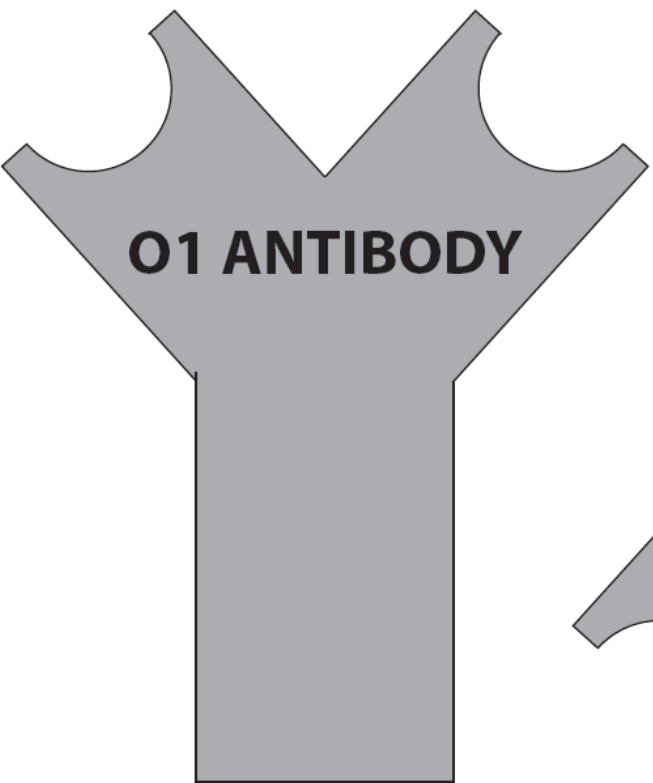
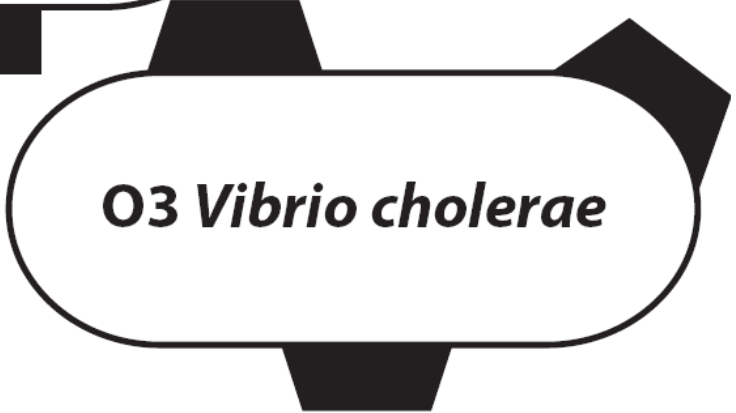
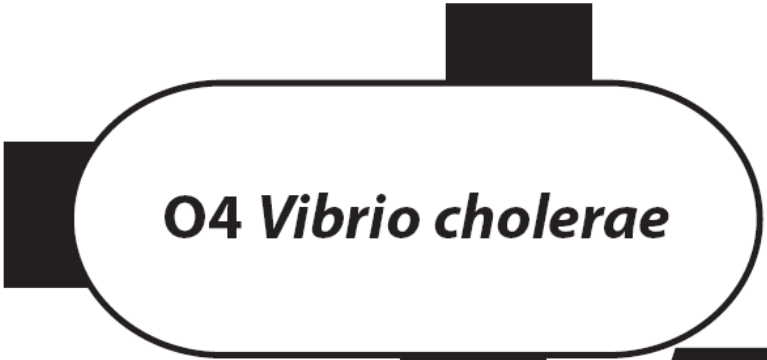
Laboratory Observations

O1 Antibody	O1 Antibody	O2 – 046 Antibodies	O47 – 092 Antibodies	O93 – O138 Antibodies
				
O1 Vc Positive Control				
	<i>Vibrio cholerae</i> sample from patient			

Conclusions:

Models of antibodies and *Vibrio cholerae* bacteria





Why aren't the adults immune to cholera?

Location: The laboratory tent of Dr. Bailey Denton, a microbiologist from WHO (World Health Organization).

Antibody – A protein produced by the immune system that fights infection

Antigen – Any substance that can trigger the immune system to make antibodies

Agglutination – Clumping that occurs when antibodies and antigens combine.

Dr. Denton: I'm really worried. You said that adult villagers who had cholera before or who had been vaccinated are now getting sick. That shouldn't be happening.

Daphne: Why not?

Dr. Denton: There is only one type of *Vibrio cholerae* bacteria that causes epidemic cholera - the O1 type. So those villagers should be making antibodies that destroy the O1 type of *Vibrio cholerae*..

Alex: What's an O1 type?

Dr. Denton: There are 138 known types of *Vibrio cholerae*. Most of them are harmless or non-pathogenic. One way *Vibrio cholerae* are identified or classified is by their O antigens. Each different type of *Vibrio cholerae* has a different O antigen on the outside.

Ming: So is this O1 or another type?

Alex: I don't know but we need to find out. If this is a new type of *Vibrio cholerae* that can cause epidemics, then we need to warn the medical team that they should be prepared for a LOT more cases of cholera.

Dr. Denton: Why don't you use this antibody agglutination test kit to see what type of *Vibrio cholerae* is in the diarrhea sample you brought? Mix the sample of your *Vibrio cholera* sample with the O1 antibody. If your sample is O1 *Vibrio cholerae*, the O1 antigens on bacteria surface should bind with the O1 antibody. That binding should cause the *Vibrio cholerae* to clump together, or "agglutinate."

Ming: And if it doesn't agglutinate?

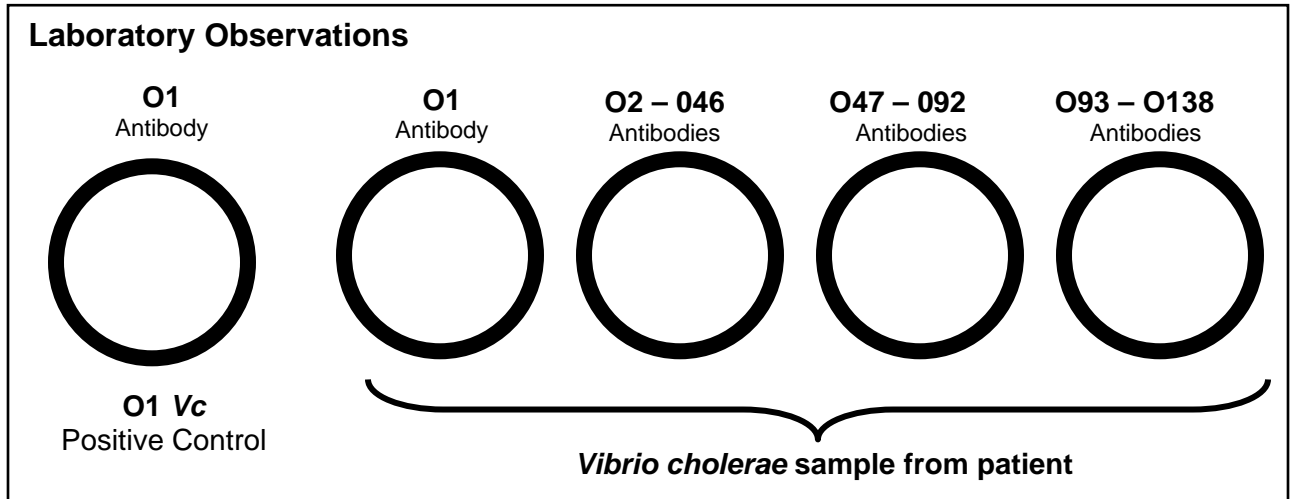
Dr. Denton: Then you can use the other three sets of antibodies to see if any of the other antibodies cause the *Vibrio cholerae* to agglutinate.

1. How many known types of *Vibrio cholerae* are there? **138**
2. Observe the paper models that show 4 types of *Vibrio cholerae*. How are the protein antigens present on the surface of the 4 types of *Vibrio cholerae* different from each other?
They have different shapes of antigens on their surface.
3. Which type of *Vibrio cholerae* was known to cause epidemic diarrhea? **O1**
4. Use the paper model of the O1 antibody produced by a person who had O1 cholera.
 - Explain why people who were infected by O1 *Vibrio cholerae* would be immune when they are exposed again to O1 *Vibrio cholerae*.
The O1 antibody has the right shape to combine with the antigens on the surface of the O1 Vibrio. The antibodies can destroy the bacteria to which they are attached.
 - Explain why people who were infected by another type of *Vibrio cholerae* would not be immune.
The O1 antibody does NOT have the right shape to combine with the antigens on the surface of the other types of Vibrio. The antibodies cannot destroy the bacteria unless they attach to them.
5. Why does an antibody bind to antigens on the surface of one kind *Vibrio cholerae* and not bind to other kinds of antigens?
Antibodies only bind to bacteria that have a matching antigen on their surface.
6. Some travelers get a vaccination that protects against O1 *Vibrio cholerae* infection before they visit areas where cholera epidemics are common. Identify one of the substances that could be included in an **O1** cholera vaccine.
Either weakened or killed O1 bacteria OR O1 antigens
7. Describe the relationship between the O1 cholera vaccine and white blood cell activity.
The antigens in the vaccine trigger the white blood cells to produce antibodies that destroy O1 bacteria.

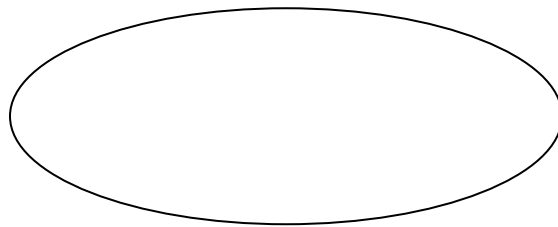
8. What should you observe in the antibody agglutination test if an antibody is able to bind to the *Vibrio* bacteria?

You should observe clumping (agglutination) of the bacteria.

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



10. Make a sketch to illustrate what the surface of the new *Vibrio cholerae* type might look like.



Accept diagram that shows bacteria that do not have circle-shaped antigens.

11. Explain why the adults and travelers who had been vaccinated for O1 type *Vibrio cholerae* are not immune during this cholera epidemic.

They make O1 antibodies and these do not have the correct shape to bind to the antigens on the surface of new kind of bacteria that is causing this outbreak.

12. What component of the new type of *Vibrio cholerae* pathogen might be used to make a vaccine?

Dead or weakened new type Vibrio or the new type of antigens from the surface of these bacteria.

How did O139 evolve?

Summary:

Students use models to illustrate two hypotheses for the evolution of the O139 *Vibrio cholerae*. An animation explains how today, DNA microarray technology can be used to study the genes present in bacteria. Students perform a simulated microarray test and analyze the results to determine which hypothesis is supported by their experiments. Teachers should explain that DNA microarray technology was not widely available in the 1990's (at the time of O139 emergence), and that other molecular techniques were used to distinguish different strains and provide evidence for the different hypothesis.

Objectives:

Students will...

1. Read for information used to model and illustrate two hypotheses for the evolution of O139 *Vibrio cholerae*.
2. View an animation that explains how microarray technology can be used to compare the DNA content of organisms.
3. Perform a simulated DNA microarray test using O1 and O139 *Vibrio cholerae* samples.
4. Analyze the results of the microarray tests to determine which hypothesis about the evolution of O139 *Vibrio cholerae* is supported by the molecular evidence.

Preparing for class:

1. Make 1 copy of *How did O139 evolve?* per student.
2. Make 1 “Two Hypotheses” graphic sheet per team of 2-4 students.
3. Preview slides 19 through 20. Be certain to click on the “Learn more about microarrays HERE” on slide 20.
4. Provide safety goggles for each student.
5. Prepare a microarray lab kit for each team of 2-4 students that contains:
 - 1 “Instructions for DNA Microarray Analysis Kit”
 - 1 plastic dropper
 - 1 microarray printed on high quality printer paper (vellum, or card stock)
 - 3 cotton swabs cut in half to make 6 swabs
 - 1 set of labeled small test tubes (or microtubes) as shown in the table on the following page:

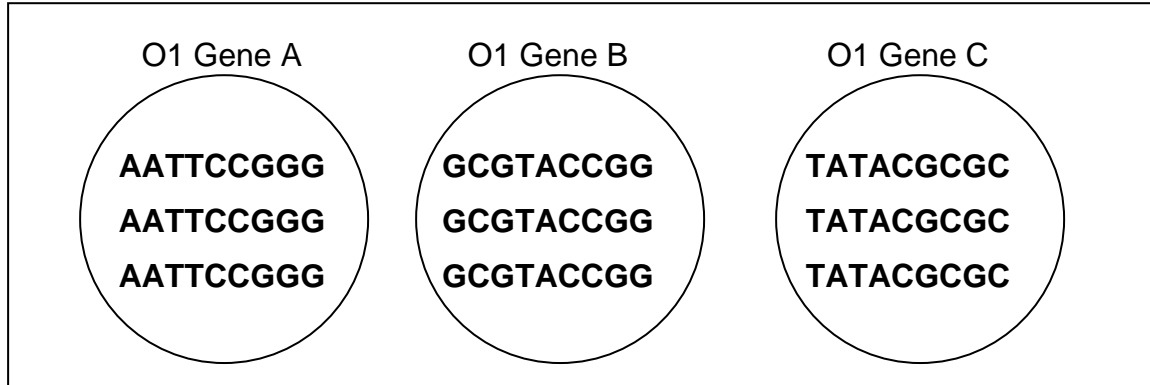
Tube Label	Contents
Gene 1	pH 7 buffer or Water
Gene 2	pH 7 buffer or Water
Gene 3	pH 7 buffer or Water
Gene 4	pH 7 buffer or Water
Gene 5	pH 2 buffer or White Vinegar
Gene 6	Water
O1 / O139DNA	0.05% methyl red

In the classroom:

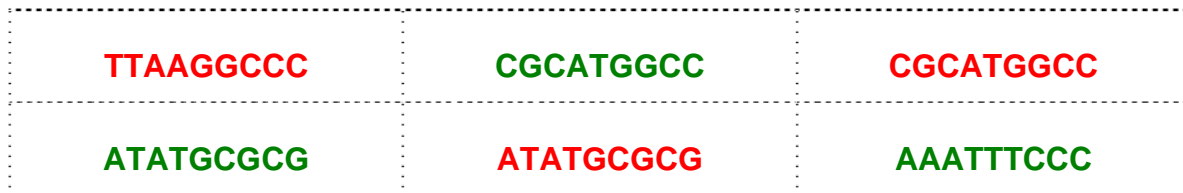
1. Distribute one copy of *How did O139 Evolve?* to each student.
2. Select students to read the scenario aloud to the class.
3. Read the first part of the scenario.
4. Distribute the graphic sheets that illustrate *Vibrio cholerae* evolution hypotheses to each student. Show slide 19.
5. Students answer question 1.
6. Read the second part of the scenario.
7. Show slide 20 -the simulated Dr. Dziejman web page and microarray animation. Be certain to click on the “Learn more about microarrays HERE” on slide 20.
8. OPTIONAL: Students can review what they learned about DNA microarrays by completing “How Does a DNA Microarray Work?” activity (see the following page). Note that this activity is not included in the student handout documents.
9. Students answer questions 2-5 on “How did O139 Evolve?”.
10. Distribute one *DNA Microarray Analysis Kit* to each team of students.
11. Students do the microarray analysis lab activity and answer the remaining questions.
12. Discuss the students’ answers to question 7.

How does a DNA Microarray Work?

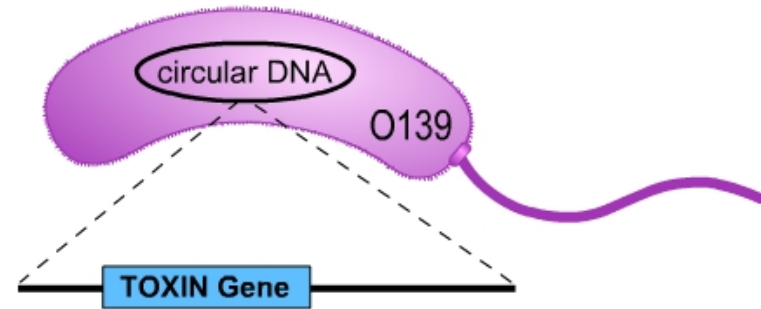
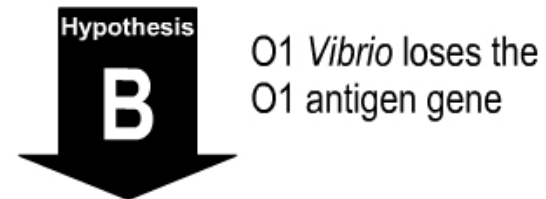
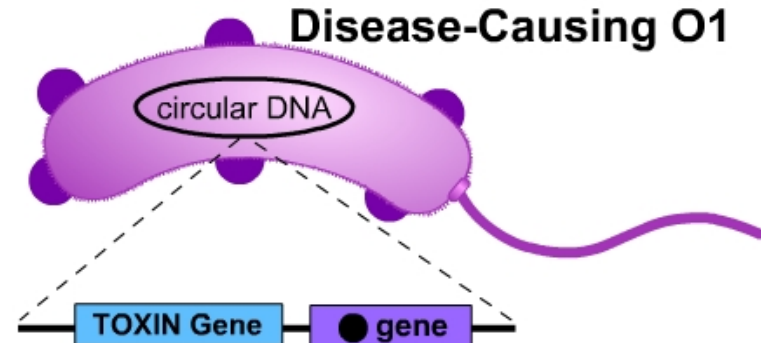
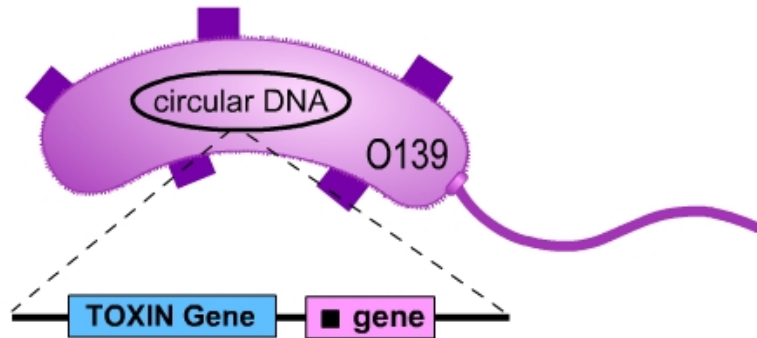
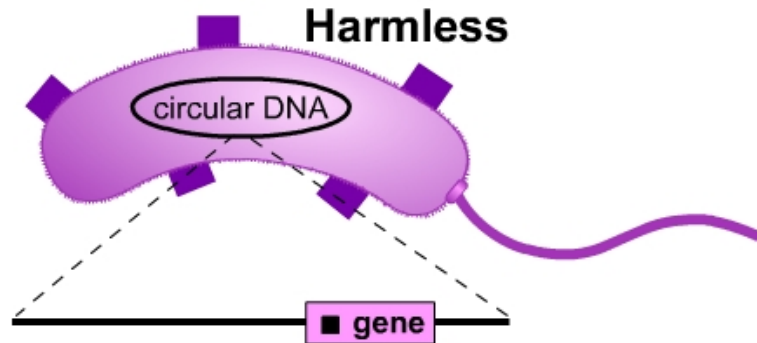
The diagram below shows an O1 DNA Microarray printed with genes from O1 *Vibrio cholerae*.



1. Cut along the dotted lines to separate the DNA molecules at the bottom of the page.
 - DNA from O1 Vibrio is labeled **red**.
 - DNA from O139 Vibrio is labeled **green**.
2. Illustrate would happen if the mixture of the O1 and O139 DNA molecules was applied to the microarray by taping the DNA pieces that you cut out onto the circles on the microarray that have genes with complementary (opposite) base sequences.
3. Which genes (A, B, and/or C) attach to the red DNA from O1 Vibrio? _____
4. Which genes (A, B, and/or C) attach to green DNA from O139 Vibrio? _____
5. If both red and green labeled DNA both attach to a gene spot on the microarray, a yellow color results. Which gene spots on the microarray would turn yellow? _____
6. Which genes (A, B, C) are present in both O1 and O139 Vibrio? _____
7. Which genes (A, B, C) are present in ONLY the O1 Vibrio? _____
8. Explain why did the green AAATTTCCC molecule did NOT attach to the microarray?



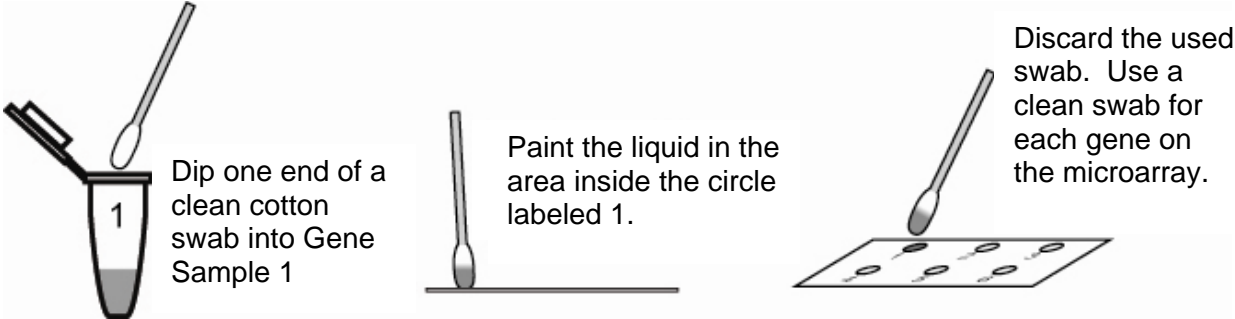
Two Possible Hypotheses for the Evolution of O139 *Vibrio Cholerae*



Safety: Wear goggles

Instructions for DNA Microarray Analysis Kit

1. “Print” your microarray by dotting genes 1-6 from **O1 *Vibrio cholerae*** to make your microarray. Follow the instructions illustrated below:

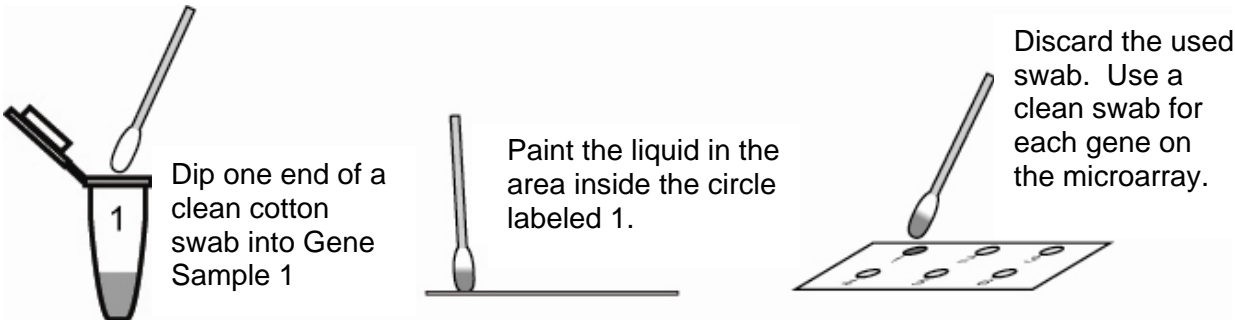


2. Use a clean cotton swab to “print” each of the remaining genes (2-6) on the microarray
 3. Use a dropper to apply one drop of the labeled **O1 / O139 DNA** mixture to each spot.
 4. Immediately record the colors that appear on each spot (red, green, or yellow).
-

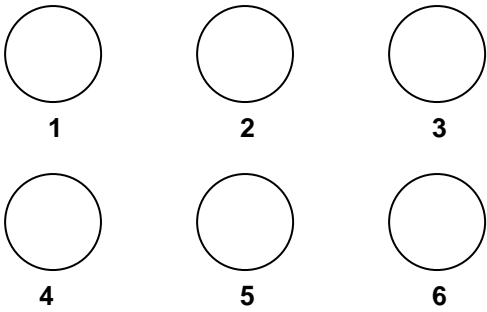
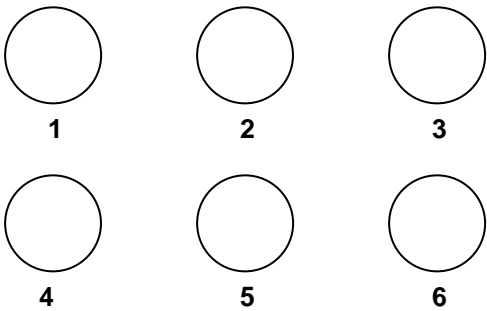
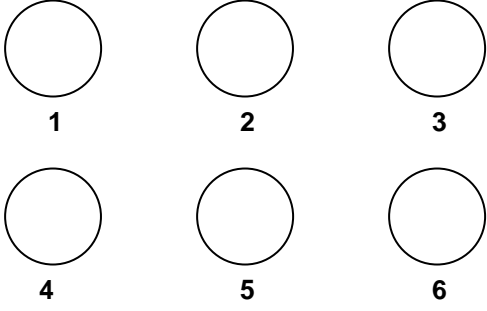
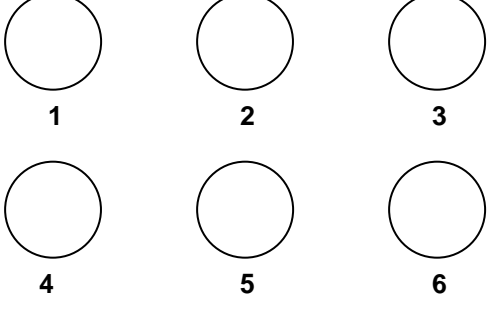
Safety: Wear goggles

Instructions for DNA Microarray Analysis Kit

1. “Print” your microarray by dotting genes 1-6 from **O1 *Vibrio cholerae*** to make your microarray. Follow the instructions illustrated below:



2. Use a clean cotton swab to “print” each of the remaining genes (2-6) on the microarray
3. Use a dropper to apply one drop of the labeled **O1 / O139 DNA** mixture to each spot.
4. Immediately record the colors that appear on each spot (red, green, or yellow).

	<p><i>Vibrio cholerae</i> O1 Genes spotted on the microarray</p> <ul style="list-style-type: none"> • Gene1: Cholera toxin gene • Gene 2: A 56 Surface protein gene • Gene 3: DNA polymerase gene • Gene 4: Flagella gene • Gene 5: O1 Antigen gene • Gene 6: Pilus gene (attachment to intestine)
	<p><i>Vibrio cholerae</i> O1 Genes spotted on the microarray</p> <ul style="list-style-type: none"> • Gene1: Cholera toxin gene • Gene 2: A 56 Surface protein gene • Gene 3: DNA polymerase gene • Gene 4: Flagella gene • Gene 5: O1 Antigen gene • Gene 6: Pilus gene (attachment to intestine)
	<p><i>Vibrio cholerae</i> O1 Genes spotted on the microarray</p> <ul style="list-style-type: none"> • Gene1: Cholera toxin gene • Gene 2: A 56 Surface protein gene • Gene 3: DNA polymerase gene • Gene 4: Flagella gene • Gene 5: O1 Antigen gene • Gene 6: Pilus gene (attachment to intestine)
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How did O139 evolve?

Location: A university campus in Rochester, New York

Ming: Wow! We really discovered a new type of *Vibrio cholerae*. Scientists are calling it “O139”!

Daphne: That explains why the adults who had cholera weren’t immune. Their O1 antibodies couldn’t recognize this new O139 *Vibrio cholerae*.

Alex: But where did O139 come from? How did this new O139 *Vibrio cholerae* evolve?

Ming: Do you think O139 could have evolved from a non-pathogenic harmless type of *Vibrio cholerae* that somehow 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.

Daphne: That’s possible. But I have a different hypothesis. I think that O139 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*.

Gene – A sequence of DNA that codes for a protein

Microarray – A grid of DNA genes arranged on a glass slide or silicone chip. A typical microarray contains 10,000-200,000 microscopic DNA spots

Evolution – The process of change over time in the heritable traits of a population of organisms.

Toxin - Poisonous substance

1. Observe the diagrams on the graphic sheet.

- Which O139 *Vibrio cholerae* diagram (A or B) best represents Ming’s hypothesis? Explain your choice.

Diagram A because it started out harmless and then got a cholera gene.

- Which O139 *Vibrio cholerae* diagram (A or B) best represents Daphne’s hypothesis? Explain your choice.

Diagram B because it started out as an O1 type and then lost the ability to make the O1 antigen.

- Alex: Both hypotheses seem reasonable to me. Maybe comparing the genes of the O1 *Vibrio* with the genes of our new O139 *Vibrio* might help us figure out which is the best hypothesis.
- Ming: I think I know how to do that. One of my friends, Dr. Michelle Dziejman (*JAY-MAN*) at the University of Rochester Medical Center is studying the *Vibrio cholerae* genes. She has made a **microarray** that is spotted with DNA from many different O1 *Vibrio* genes. She uses this microarray to compare the DNA of O1 *Vibrio* with the DNA from other types of *Vibrio*.
- Alex: Great! Ask Dr. Dziejman if we could use one of her O1 microarrays to compare the O139 *Vibrio*'s genes with the genes from O1 *Vibrio*. Maybe we can figure how the new O139 *Vibrio cholerae* evolved.
- Daphne: Before we meet with your friend, I'd like to know more about how these microarrays work.
- Ming: Dr. Dziejman has a great animation on her web site that shows how her microarray is used to compare the DNA from different organisms!
-

View the virtual website and animations to learn how a microarray works. (slide 20)

Dr. Dziejman's actual website can be found at
<http://www.urmc.rochester.edu/smd/mbi/faculty/Dziejman.htm>

2. What is printed on an O1 microarray?

Known genes from O1 *Vibrio cholerae*.

3. When a mixture of O1 and O139 DNA is poured onto the O1 microarray, DNA molecules in the mixture bind to some of the printed spots and do not bind to other printed spots. What causes DNA molecules to bind to some of the genes printed on the microarray?

The DNA only binds to spots that are complementary—opposite.

4. What does a yellow spot on the microarray mean?

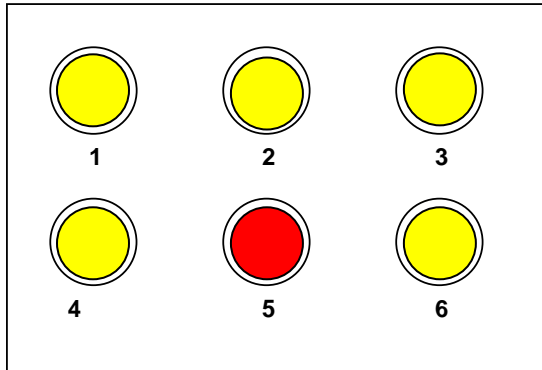
The gene at that spot is found in both O1 and O139 *Vibrio*.

5. What does a red spot on the microarray mean?

The gene at that spot is only found in O1 *Vibrio*.

6. Follow the instructions in the DNA Microarray Analysis Kit. Record your laboratory observations.

Laboratory Observations



Vibrio cholerae O1 Genes spotted on the microarray

- Gene1: Cholera toxin gene
- Gene 2: A 56 Surface protein gene
- Gene 3: DNA polymerase gene
- Gene 4: Flagella gene
- Gene 5: O1 Antigen gene
- Gene 6: Pilus gene (attachment to intestine)

Remember: You applied a mixture of O1 / O139 DNA to the microarray.

- DNA from *Vibrio cholerae* O1 was labeled with a red tag.
- DNA from *Vibrio cholerae* O139 was labeled with a green tag.
- A yellow spot on the microarray indicates that DNA from both *Vibrio cholerae* O1 and DNA from *Vibrio cholerae* O139 have bound to the microarray.

7. Which O1 genes are the same as O139 genes? 1,2,3,4, and 6
8. Which gene is present in O1 but not in O139? 5 What is the function of this gene? It is the gene for the O1 surface antigen
9. Whose hypothesis is supported by the results of your microarray analysis?
- Ming's hypothesis: O139 evolved from a harmless *Vibrio cholerae* that picked up a gene for the cholera toxin.
 - Daphne's hypothesis: O139 evolved from an O1 *Vibrio cholerae* that lost the gene for the O1 surface antigen.

Daphne's's Hypothesis

Because: ***The DNA from O1 and O139 is very similar. The only difference is that O139 does not make the O1 antigen.***

Could a cholera epidemic happen here?

Summary:

Students consider what might happen if evolution continues in *Vibrio cholerae* populations and human actions or natural events affect the ability of these bacteria to cause disease.

Objectives:

Students will...

- Read about factors that might influence the evolution of *Vibrio cholerae* in the future.
- Consider what their future might be like if *Vibrio cholerae* continue to evolve.
- Identify human actions that could promote *Vibrio cholerae* evolution.
- Identify a possible research question that might lead to a cholera prevention, treatment, or cure.

Prepare for class:

- Prepare 1 copy of *Could a cholera epidemic happen here?* per student

In the classroom

1. Distribute 1 copy of *Could a cholera epidemic happen here?* to each student.
2. Select student readers.
3. Students read the scenario aloud to the class.
4. Students could complete the activity in class or for homework.
5. Allow time for students to share their answers in small groups or with the entire class.

Could a cholera epidemic happen here?

Location: University of Rochester, in the laboratory of Dr. Michelle Dziejman

Ming: It looks like Daphne's hypothesis was correct. O139 evolved from an O1 *Vibrio cholerae* that lost the ability to make the O1 surface antigen.

Dr. Dziejman: That explains why O139 *Vibrio cholerae* could infect some people who had cholera before or who had been vaccinated for the O1 type of cholera. Those people would not be immune to O139 because their O1 antibodies don't recognize the new O139 type.

Daphne: That also means that the vaccine for the O1 type won't work for O139. I hope they can make a vaccine for O139!

Dr. Dziejman: The World Health Organization has reported a growing number of cases of O139 cholera in other countries. The O139 outbreak seems to be turning into a pandemic (a worldwide or widespread epidemic). Research has shown that travelers are carrying this to other countries. Several large cities with poor sanitation and inadequate water treatment have had massive cholera epidemics.

Daphne: If you think about it, the idea that of *Vibrio cholerae* bacteria evolving is really frightening. If these bacteria continue to evolve, you could end up with new types of *Vibrio cholerae* that could cause new epidemics anywhere in the world.

Alex: Scientists have discovered that *Vibrio cholerae* live on the shells of tiny water animals called zooplankton. The bacteria and the zooplankton can be found world-wide. Although it is rare, there have been a few cases of cholera caused by people in the United States eating contaminated shellfish.

Ming: One scientist has discovered a harmless type of O1 *Vibrio cholerae* with a surface covering that enables it to survive in chlorinated water. That means that in the future, that type of *Vibrio cholerae* may not be killed in water treatment facilities.

Daphne: And I saw a scientist talking on a news program about global warming. She said that climate change is increasing the number of environments with the warm temperatures needed for the growth of *Vibrio cholerae*.

Dr. Dziejman: My lab studies a new strain of *Vibrio cholerae* that causes disease without making the cholera toxin or the typical proteins necessary for the bacteria to attach to the intestine. We are trying to understand the different mechanisms that this particular strain uses to cause cholera.

Daphne: Wow! Imagine what could happen if *Vibrio cholerae* continues to evolve. Mutations, international travel, global warming, poverty, large cities, chlorine resistance, no vaccine. I guess it's not safe to assume that cholera will never affect people in the United States.

1. What are three things that scientists have discovered that could lead to cholera outbreaks in the United States?
 - ***The vaccine for the O1 type won't work for O139.***
 - ***Travelers are carrying this to other countries.***
 - ***Vibrio cholerae live on the shells of tiny water animals called zooplankton and can be carried through ocean waters.***
 - ***One harmless type of O1 Vibrio cholerae has a surface covering that enables it to survive in chlorinated water.***
 - ***Climate change is increasing the number of environments with the warm temperatures needed for the growth of Vibrio cholerae.***
 - ***A new type of Vibrio causes cholera without making the cholera toxin or the typical proteins necessary for the bacteria to attach to the intestine.***
2. If you were a scientist who was worried about a world-wide cholera epidemic (known as a pandemic), what is one research question that you might want to ask about cholera or *Vibrio cholerae*?

a. I would want to know...

Student answers will vary.

b. Because when I know the answer, I might be able to...

Student answers will vary but they should indicate why knowing the answer to the research question may help prevent cholera epidemics.

("Prevent, cure, or treat" diseases is NOT an acceptable answer. You must explain how or why.)

Evolution and cholera

Summary:

Students view a PBS video clip and read a brief article about cholera evolution. They apply their understanding of natural selection to answer questions about the impact of evolution on *Vibrio cholerae* populations.

Objectives:

- Students will view a video on the work of Paul Ewald, an evolutionary biologist.
- Students will apply their understanding of natural selection to answer questions about *Vibrio cholerae* evolution.

Prepare for class:

- Preview the PBS video segment from *Evolution: "The Evolutionary Arms Race"* at http://www.pbs.org/wgbh/evolution/library/10/4/1_104_01.html This video segment features the work of biologist Paul Ewald, who studies the evolution of virulence of disease organisms. The case study of the 1991 cholera epidemic in South America is the backdrop for this segment. Ewald describes how, over a few years time, society can steer the evolution of such pathogens toward becoming milder.
- Prepare 1 copy of *Evolution and cholera* per student

In the classroom

1. Distribute 1 copy of *Evolution and cholera* to each student.
2. Show the PBS video segment
3. Students could read the information on the handout and complete the activity in class or for homework.
4. Allow time for students to share their answers in small groups or with the entire class.

Evolution and cholera

View the video segment from *Evolution: "The Evolutionary Arms Race"* at http://www.pbs.org/wgbh/evolution/library/10/4/l_104_01.html

This video segment features the work of biologist Paul Ewald, who studies the evolution of virulence of disease organisms. The case study of the 1991 cholera epidemic in South America is the backdrop for this segment. Ewald describes how, over a few years time, society can steer the evolution of such pathogens toward becoming milder.

Some characteristics give individuals an advantage over others in surviving and reproducing, and their advantaged offspring, in turn, are more likely than others to survive and reproduce. The proportion of individuals that have advantageous characteristics will increase. Scientists are using this evolutionary concept to explain why some viruses and bacteria are highly virulent and life-threatening, while others reside in their hosts with few, if any, ill effects.

To understand these concepts, one has to look at infections from the point of view of the pathogens: The most successful pathogens are those that survive, reproduce, and are transmitted to as many new hosts as possible.

The *Vibrio cholerae* pathogens that cause cholera spread easily through bed sheets, clothes, and sewage-contaminated water. There are two possible strategies that a *Vibrio cholera* population can use to be successfully transmitted to many new human hosts.

Strategy 1: *Vibrio cholerae* can cause massive amounts of deadly diarrhea so that the bacteria are spread to new human hosts. The host may die, but the vibrios are likely to be transmitted to new hosts.

Strategy 2: *Vibrio cholerae* can cause a milder form of the disease for the host or the infection can be asymptomatic. The milder form of the bacteria can be transmitted to new hosts because they are shed by more people for longer periods of time.

For *Vibrio* populations in environments with poor sanitation, the first strategy is the most effective in spreading the disease to new hosts. Therefore, when water is contaminated with *Vibrio*, bacterial virulence remains high, and infected people suffer and die.

When water is purified and kept clean, the pathogen cannot be spread as readily through poor sanitation. Therefore, the second strategy is most effective in spreading the disease to new hosts. The pathogens are benefited by adaptations that make them less virulent (deadly) so that they can be carried by more people for longer periods of time. For example, in India during the 1950's and 1960's, a campaign to clean up water may have allowed a *Vibrio cholerae* strain that produced less severe disease to displace more virulent *Vibrio* strains in the environment..

Modified from http://www.pbs.org/wgbh/evolution/library/10/4/l_104_01.html

1. Why does an environment with poor sanitation select for more virulent *Vibrio*?

In environments with poor sanitation, Vibrio that can cause massive amounts of deadly diarrhea are more effective in spreading to new human hosts. The host may die, but the Vibrio are likely to contaminate water supplies and be transmitted to new hosts.

2. Why does an environment with good sanitation select for milder forms of *Vibrio*?

The pathogens are benefited by adaptations that make them less virulent (deadly) so that they can be carried by more people for longer periods of time

3. Why is improved sanitation the best defense against outbreaks of cholera and other pathogens, such as *E. coli* and *Shigella*, that cause diarrhea?

It promotes the evolution of less deadly Vibrio cholerae types. Cleaning up water supplies leads to the evolution of milder forms of cholera displacing the more dangerous Vibrio type.

Climate Change and cholera

Summary:

Students read an article about and model the effect of ocean temperature change on the risk of cholera epidemics.

Objectives:

Students will...

- Read an article about the effect of climate change on ecosystems that include *Vibrio cholerae*.
- Create a model and a food web that illustrates how warmer water temperatures and biological magnification can lead to a cholera epidemic.
- Explain how remote satellite sensing could be used to prevent cholera.

Preparing for class:

- Prepare 1 copy of *Climate change and cholera* per student.
- (optional) For advanced students consider printing “What cholera can teach us about climate change at <http://stanmed.stanford.edu/2007spring/cholera.html> /
- Prepare 1 kit of materials per team of 2-4 students that contains:
 - 1 copy of “Green Algae in Cool Ocean Water” and “Green Algae in Warm Ocean Water.”
 - 1 sandwich bag with $\frac{1}{2}$ to $\frac{3}{4}$ cup of rice labeled “Vibrio cholerae”
 - 1 plastic spoon
 - 8 small plastic condiment cups (1 oz.) labeled “Zooplankton”
 - 1 clear or translucent plastic cup (10 oz.) labeled “Shellfish”
 - 1 clear or translucent plastic cup (12 or 16 oz.) labeled “Human”

In the classroom:

1. Distribute 1 copy of *Climate change and cholera* to each student.
2. Allow time for students to read the article. This may be done for homework.
3. Students work in teams of 2-4 students to create a model of the food web described in the reading.
4. Students answer the questions in their handout.
5. Ask students to explain the concept of biological magnification.

Note: This modeling activity is modified from a lab activity designed by Todd Shuskey from Perry High School, Perry, NY.

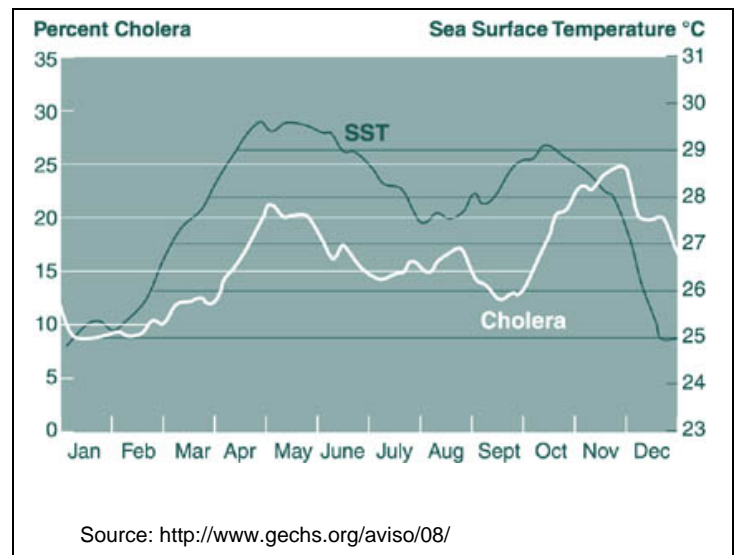
Climate change and cholera

Watching Peru's Oceans for Cholera Cues

Before 1991, no one in Peru could remember a cholera outbreak. Then, in a single day, it hit hard up and down the coast and took off from there, eventually killing thousands. A smaller epidemic occurred in 1998. Large outbreaks are often traced back to contaminated water supplies that are commonly associated with algal or zooplankton blooms.

Scientists believe that the Peru cholera epidemics were caused in part by a change in ocean (and sea) temperatures. Both the 1991 and 1998 epidemics were linked to El Niño, the periodic and unpredictable weather disruption that leads to warmer ocean currents. Warm ocean currents encourage the growth and spread of *Vibrio cholerae*, the bacteria that cause cholera. *Vibrio cholerae* often live and feed on the shells of zooplankton — microscopic animals that drift in the ocean. Warmer waters and a rich food source promote zooplankton population growth, thus increasing the chance of a cholera epidemic.

What likely happened during the El Niño cholera epidemics in the 1990s was this: The warmer ocean temperature increased the reproductive rate in green algae. There were more algae for zooplankton to feed on. The increase in zooplankton provided food and habitats for the *Vibrio cholerae* bacteria. Shellfish ate the infected zooplankton. People who ate raw, infected shellfish developed cholera. These people contaminated local water and food supplies with *Vibrio cholerae* causing the spread of cholera to others in the area.



Researchers are worried that global warming will increase the number of cholera epidemics in Peru and other parts of the world. Ecologists have set up surveillance systems to monitor ocean water temperature and detect cholera-causing bacteria in the ocean before an epidemic begins. If the sea temperature increases, cholera bacteria may grow faster and this could trigger a new epidemic. If researchers find cholera bacteria before an epidemic begins, they can at least warn people to cook fish thoroughly, boil drinking water, and keep their hands clean.

Modified from: <http://www.npr.org/templates/story/story.php?storyId=19344123>

1. State one conclusion that you can draw based on the graph provided with the reading passage.

Warmer ocean temperatures are correlated with increased cholera epidemics.

Now, use the model pieces in your kit to model the feeding relationships in **cool ocean water** and in **warm ocean water**.

2. The small spots on the diagrams of **cool ocean water** represent green algae that are a food source for zooplankton animals. Each large circle surrounds the green algae that one zooplankton animal needs to eat to survive. Place one zooplankton cup on each of the circles to represent the zooplankton eating the green algae.
3. *Vibrio cholerae* bacteria live and feed on the exoskeletons (outside coverings) of tiny zooplankton animals. Place 1 spoonful of rice in each zooplankton cup to represent the *Vibrio* bacteria living on the zooplankton.
4. Shellfish, like clams and oysters, eat the zooplankton. Place the zooplankton cups (with “*Vibrio*” rice) into the larger “shellfish” cup to represent the zooplankton being eaten by the shellfish.
5. Humans eat the shellfish. Place the shellfish cup and its contents into the large up labeled “human.”
6. Estimate the number of spoonfuls of “*Vibrio*” rice present in the “human.”

When the ocean water is **cool**, approximately how many spoonfuls of *Vibrio cholerae* bacteria does a human consume? _____

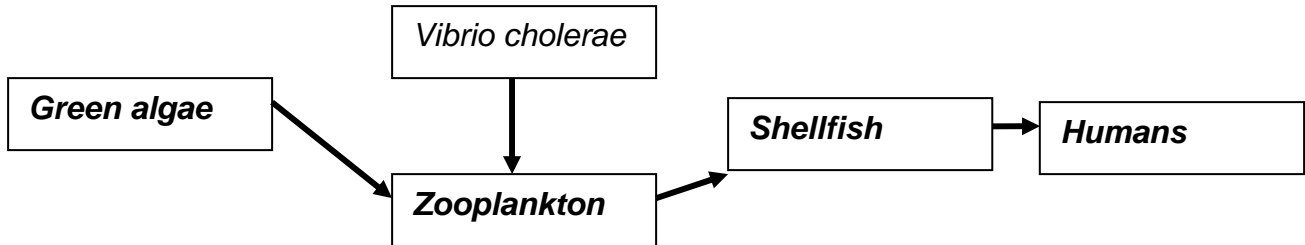
This concentration of *Vibrio* is not enough to cause serious disease because the *Vibrio* are likely to be killed by acids in the stomach.

7. Return the rice to the bag labeled “*Vibrio cholerae*.”
8. Repeat steps 2 through 7 using the **warm ocean water** diagram.

When the ocean water is **warm**, approximately how many spoonfuls of *Vibrio cholerae* bacteria does a human consume? _____

This concentration of *Vibrio* is enough to cause serious disease because some *Vibrio* are likely to escape being killed by acids in the stomach.

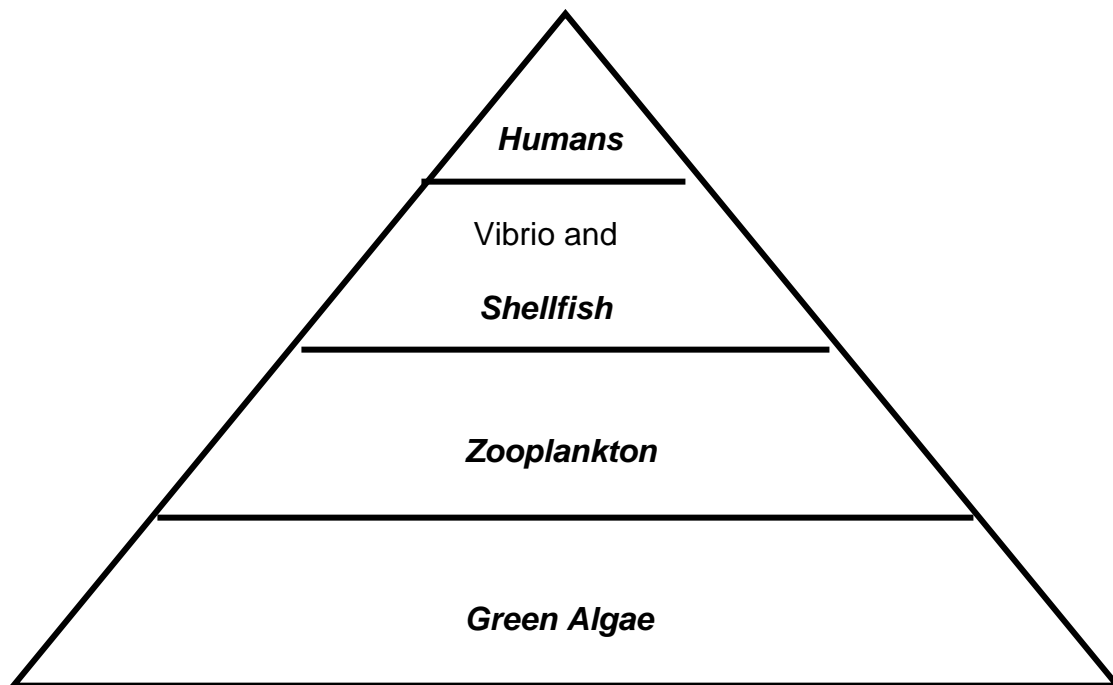
9. Fill in the boxes on the diagram below to show a food web that includes the following organisms: humans, zooplankton, green algae, and shellfish.



10. Which organisms in this food chain are

- autotrophs? **Green Algae**
- heterotrophs? **Vibrio, Zooplankton, Shellfish, and Humans**
- parasites? **Vibrio cholerae**
- hosts? **Zooplankton**

11. Complete the energy pyramid to represent the transfer of food energy in your model. Write the names of the appropriate organisms on the lines.



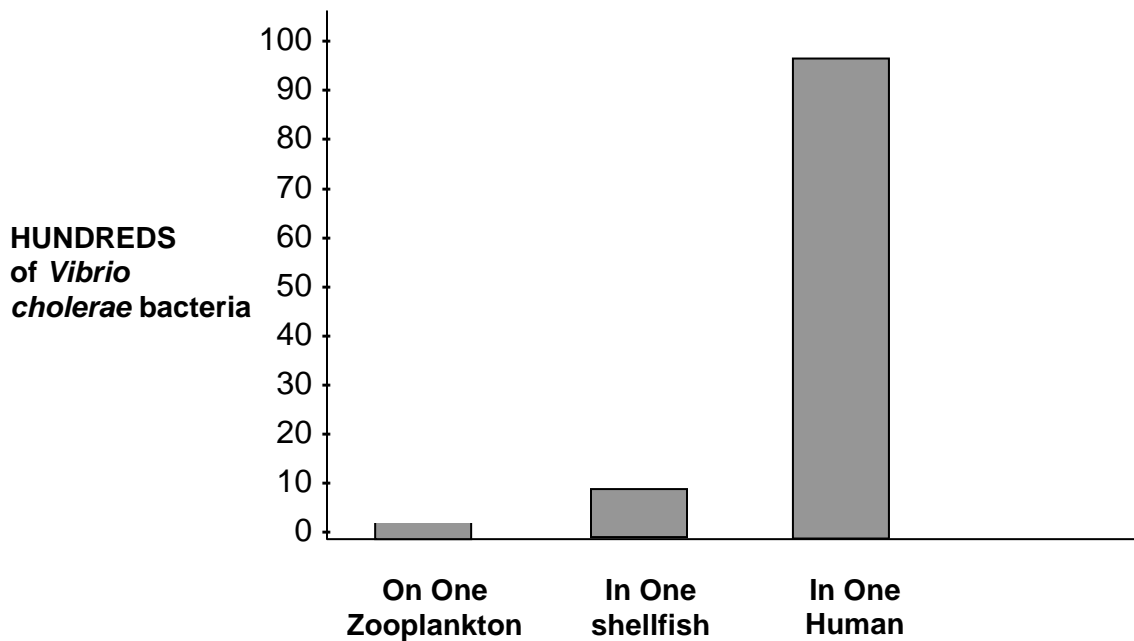
12. Explain why warmer ocean water temperatures could lead to a cholera epidemic.

The warmer ocean temperature increased the reproductive rate in green algae. There were more algae for zooplankton to feed on. The increase in zooplankton provided food and habitats for the Vibrio cholerae bacteria. Shellfish ate the infected zooplankton. People who ate raw, infected shellfish developed cholera.

13. Assume that each zooplankton has 100 *Vibrio cholerae* bacteria living on it.

- Calculate how many bacteria would be eaten by one shellfish that ate ten zooplankton. **1,000**
- Calculate how many bacteria would be eaten by one human that ate ten shellfish. **10,000**

14. Draw bars on the graph grid below to make a BAR graph that summarizes the information from question 13. Notice that the scale on the left is expressed in HUNDREDS of *Vibrio*.



15. **Biological magnification** is defined as a cumulative increase in the concentrations of a toxic substances or pathogens in successively higher levels of the food chain. Explain why the graph you constructed illustrates the concept of biological magnification.

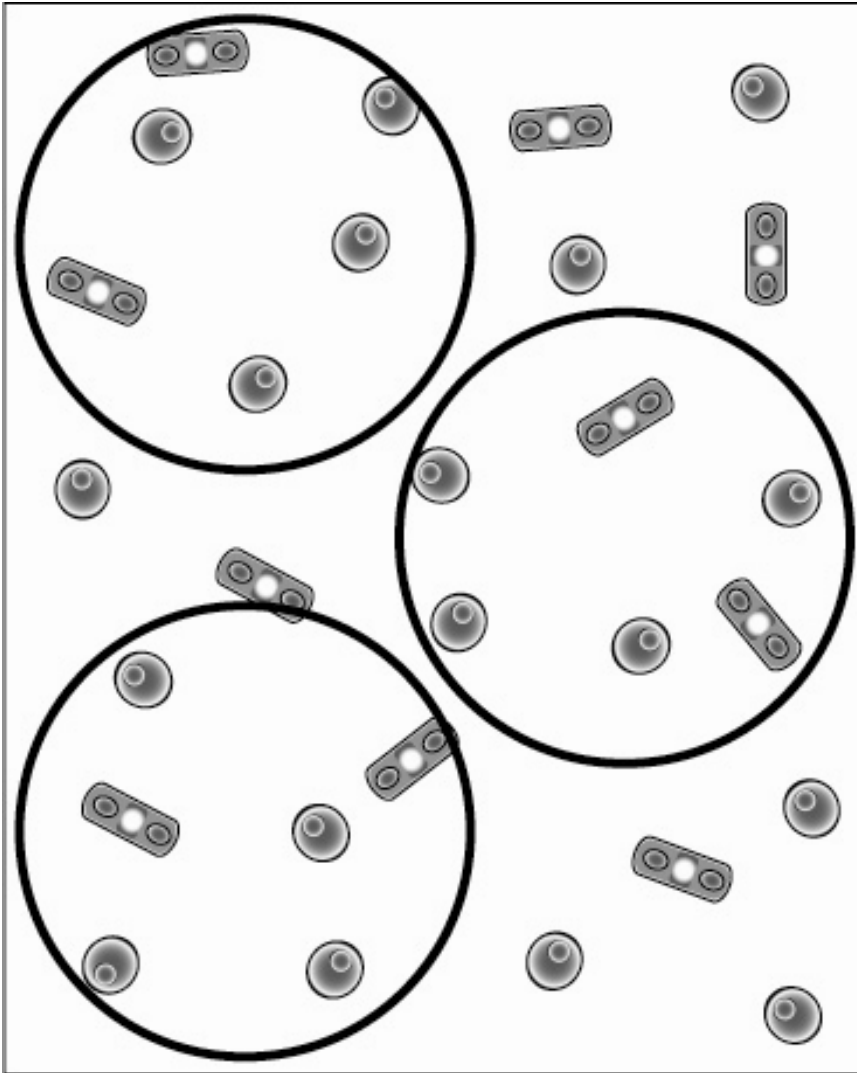
As you go from zooplankton (low on the food chain) to humans (high on the food chain) the number of Vibrio increases dramatically.

16. Researchers are now using satellite imaging to determine regions in the ocean where there is a high concentration of chlorophyll (green pigments involved in photosynthesis). Explain how this technique might provide information on likely sites for cholera outbreaks.

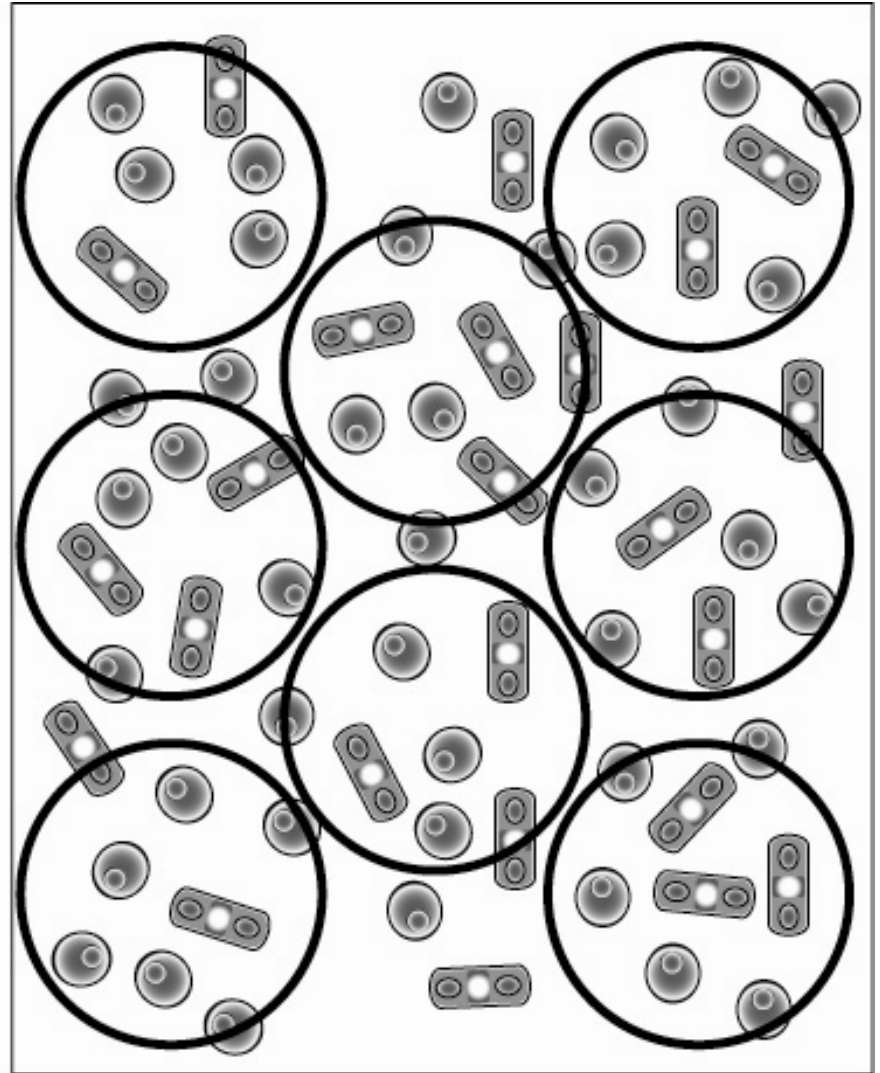
Green algae contain chlorophyll. High concentrations of chlorophyll would indicate a high concentration of green algae that are food for the zooplankton that carry Vibrio bacteria.

17. Researchers are worried that global warming will lead to rising ocean temperatures and an increase in the occurrence of cholera epidemics. Explain three actions that you could take to prevent climate changes that lead to cholera outbreaks.

- ***Reduce use of energy produced from fossil fuels,***
- ***Reduce destruction of plants***
- ***Reduce destruction of plant habitats by pollution***



Green Algae in **Cool** Ocean Water



Green Algae in **Warm** Ocean Water

