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.

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

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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?

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

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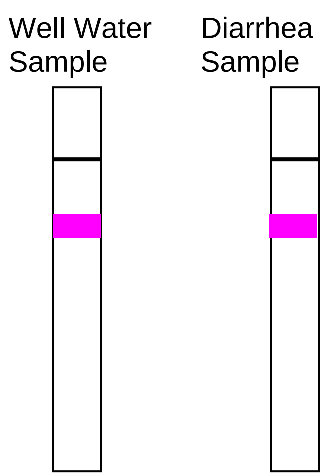
5. Use the ***Vibrio cholerae*** **Rapid-Test Kit** to test the water sample and diarrhea samples.



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

Conclusions: ________________________________

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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.

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

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

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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 <u>low</u> 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 <u>not</u> 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 <u>only</u> 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:

1. Does the patient have two or more of the following symptoms? • Frequent diarrhea • Sunken eyes • Absence of tears • Dry mouth and tongue • Thirsty and drinks eagerly • Skin pinch goes back slowly	If NO, go to 2A If YES, go to 2B
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.	
2B. The patient is dehydrated. Does the patient have one or more of the following symptoms? • Lethargic, unconscious, floppy • Unable to drink • Pulse is weak • Skin pinch goes back slowly	If NO, go to 3A If YES, go to 3B
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.	
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.	

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?

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2. What type of pathogen causes cholera?

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3. How do you get cholera?

4. What are the symptoms of cholera?

5. Why is cholera so deadly?

6. How can cholera be prevented?

7. How can cholera be treated?

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

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?

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   ____

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?

   ____

   ____

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

   ____

   ____

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.

____

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?

____

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.

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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.

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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?”

____

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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.

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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?

____

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.

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

____

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?

____

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

____

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!

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

____

____

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

____

____

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

____

Daphne's Experiment

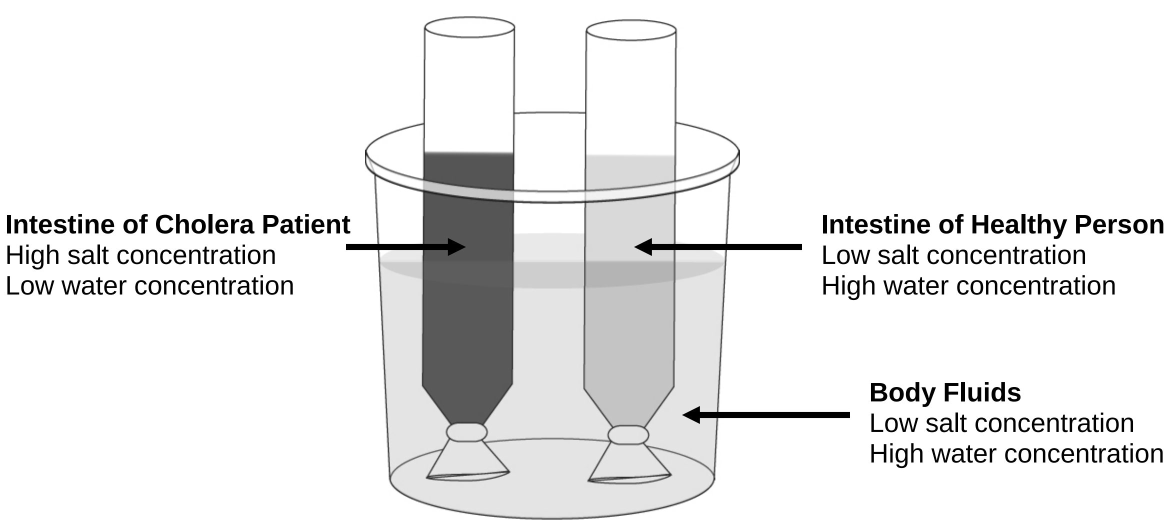
1. Make a LOW salt concentration solution by filling the plastic cup approximately ¾ full with hot tap water. Add the small tube of colored salt (labeled "LOW Salt") and stir.
   Note: This solution represents body fluids (such as blood and tissue fluids) that normally have a low salt and high water 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 <u>at least</u> 30 minutes.



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? ___________

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?

 ____

3. Which type of *Vibrio cholerae* was known to cause epidemic diarrhea? ______________

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.

 ____

 ____

 ____

 - Explain why people who were infected by another type of *Vibrio cholerae* would <u>not</u> be immune.

 ____

 ____

 ____

5. Why does an antibody bind to antigens on the surface of one kind *Vibrio cholerae* and not bind to other kinds of antigens?

 ____

 ____

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.

 ____

7. Describe the relationship between the O1 cholera vaccine and white blood cell activity.

 ____

 ____

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

____

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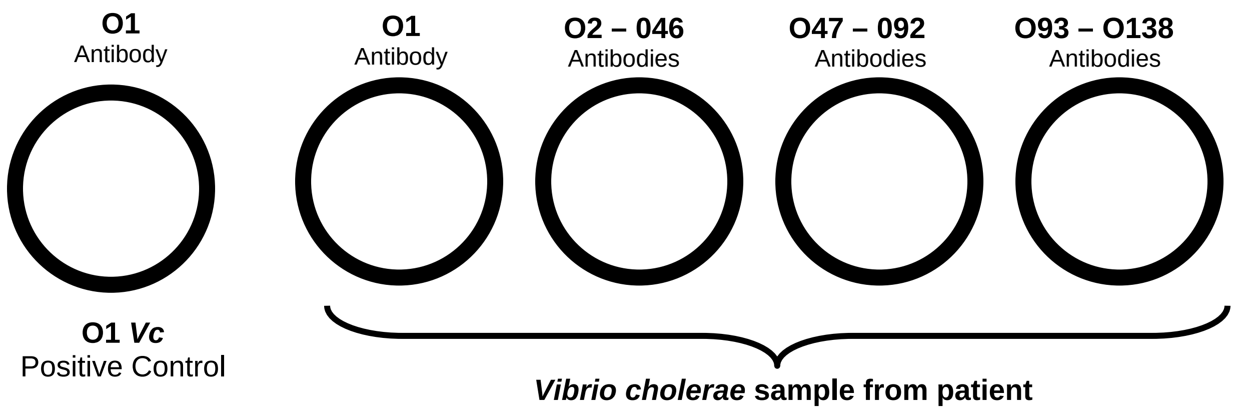
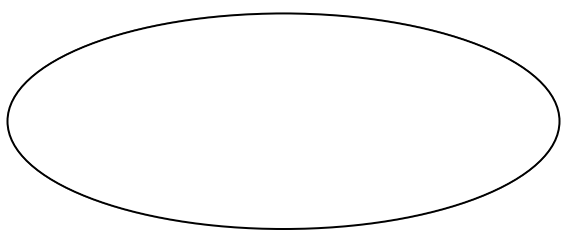
9. Use the Antibody Agglutination Test Kit to test the *Vibrio cholerae* sample from this cholera outbreak.

Laboratory Observations



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



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

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

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

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1. Observe the diagrams on the graphic sheet.

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

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     ____

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

     ____

     ____

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 0139 *Vibio*'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?

____

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?

____

____

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

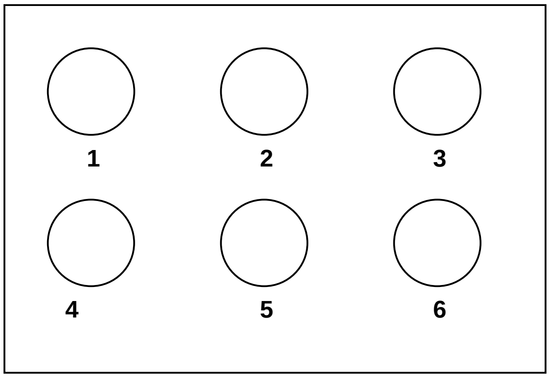
____

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

____

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 <u>red</u> tag.
- DNA from *Vibrio cholerae* O139 was labeled with a <u>green</u> tag.
- A <u>yellow</u> 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? ______________________________

8. Which gene is present in O1 but not in O139? ______

   What is the function of this gene?

   ____

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.

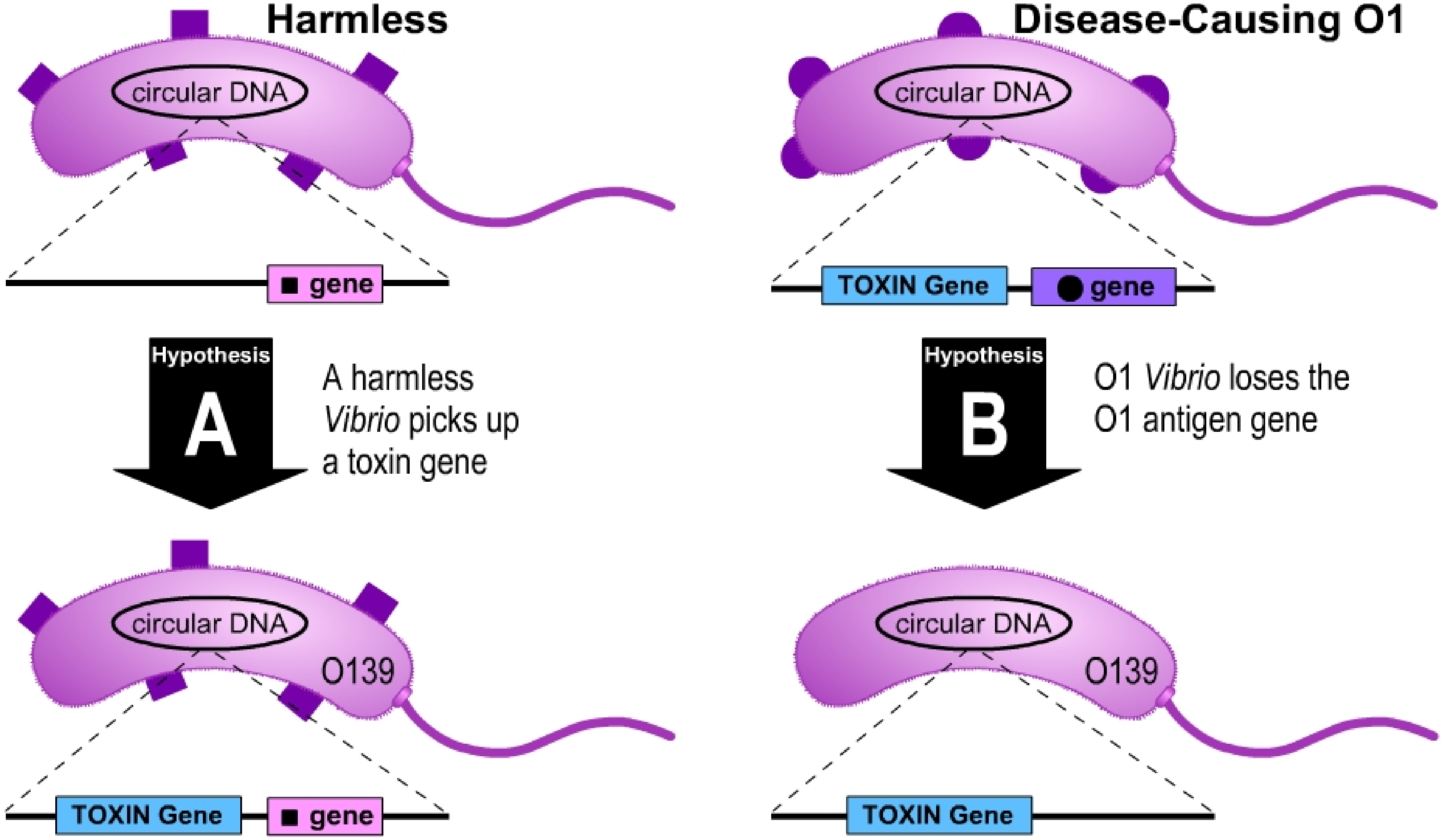
   ____________________________'s Hypothesis

   Because:

   ____

   ____

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



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?

____

____

____

____

____

____

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 <u>*you*</u> might want to ask about cholera or *Vibrio cholerae?*

   a. I would want to know…

____

____

____

____

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

____

____

____

____

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

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 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?*

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

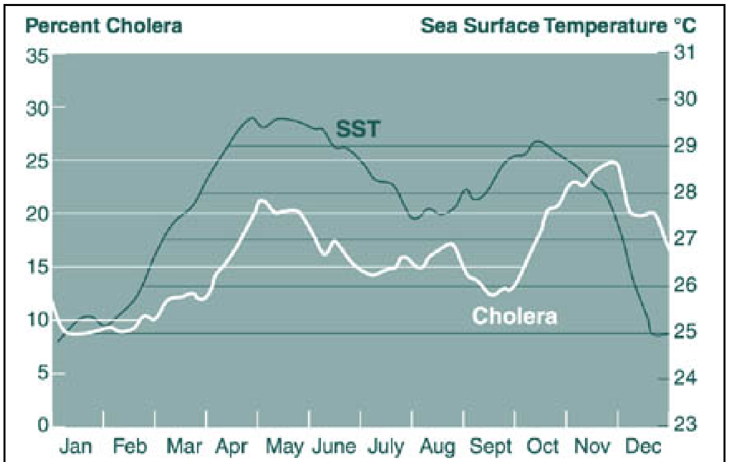
3. Why is improved sanitation the best defense against outbreaks of cholera and other pathogens, such as *E. coli* and *Shigella*, that cause diarrhea?

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 Nino, 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. *Vibio 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.



Source: http://www.gechs.org/aviso/08/

What likely happened during the El Nino 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.

   ____

   ____

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."

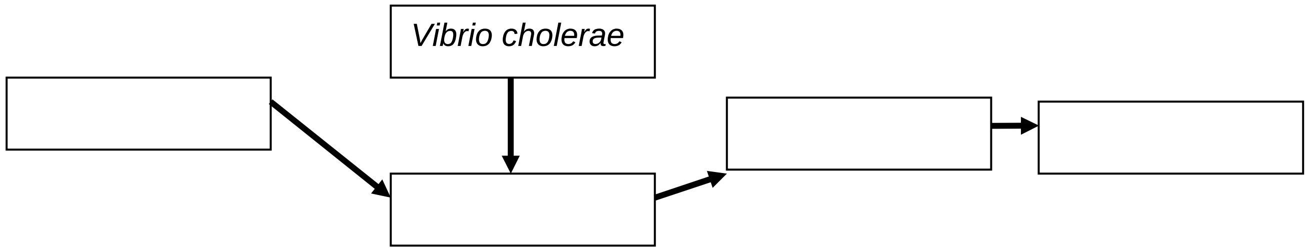
When the ocean water is **cool**, approximately how many spoonfuls of *Vibrio cholerae* bacteria does a human consume? _________ This concentration of Vibrio is <u>not</u> enough to cause serious disease because the *Vibrio* are likely to be killed by acids in the stomach.

7. Return the rice to the bag labeled "*Vibrio cholerae*."

8. Repeat steps 2 through 7 using the **warm ocean water** diagram.

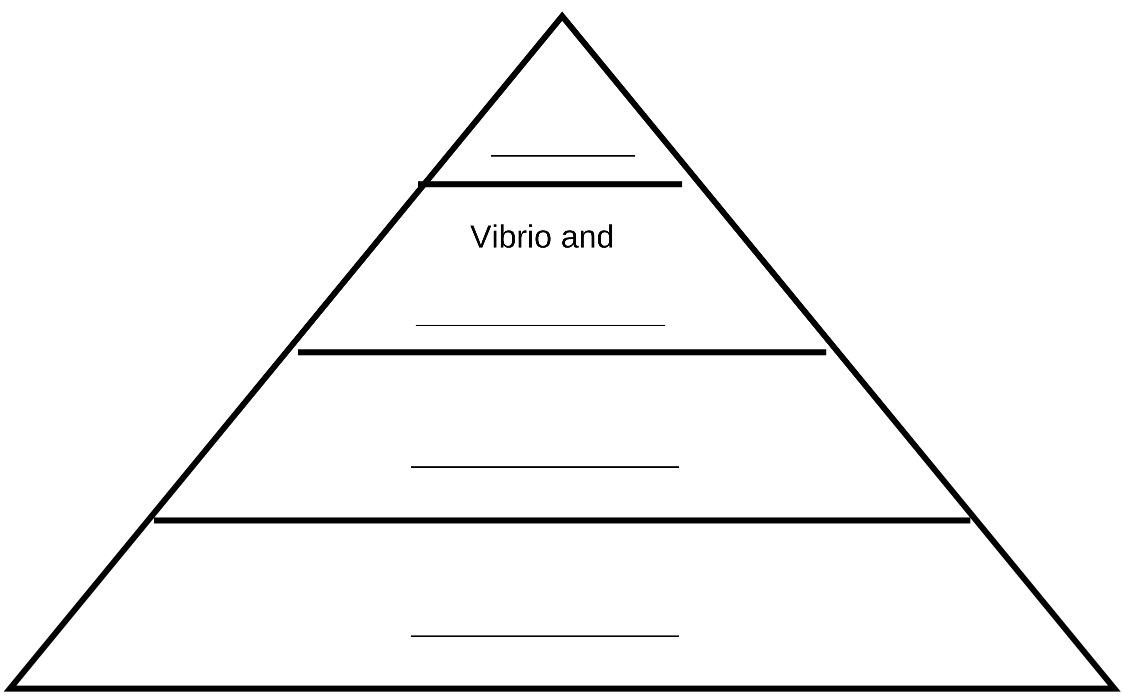
When the ocean water is **warm**, approximately how many spoonfuls of *Vibrio cholerae* bacteria does a human consume? _________ This concentration of Vibrio is enough to cause serious disease because some *Vibrio* are likely to escape being killed by acids in the stomach.

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? ____________________
    - heterotrophs? ____________________
    - parasites? ____________________
    - hosts? ____________________

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.

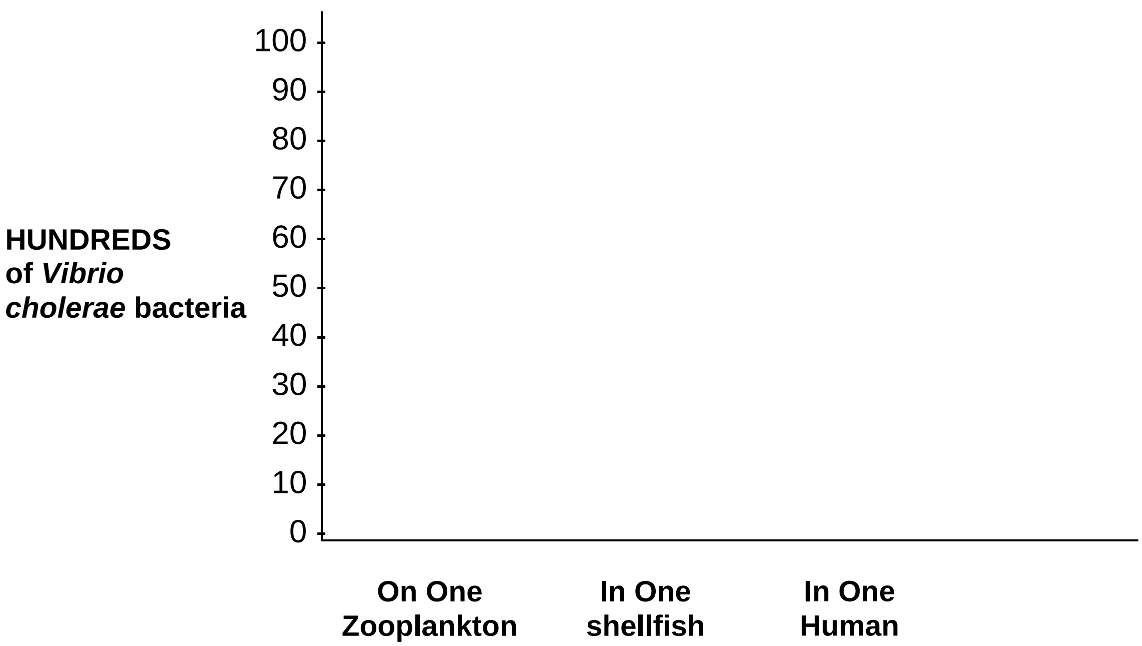


12. Explain why warmer ocean water temperatures could lead to a cholera epidemic.

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. ______________
- Calculate how many bacteria would be eaten by one human that ate ten shellfish. _______________

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.

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.

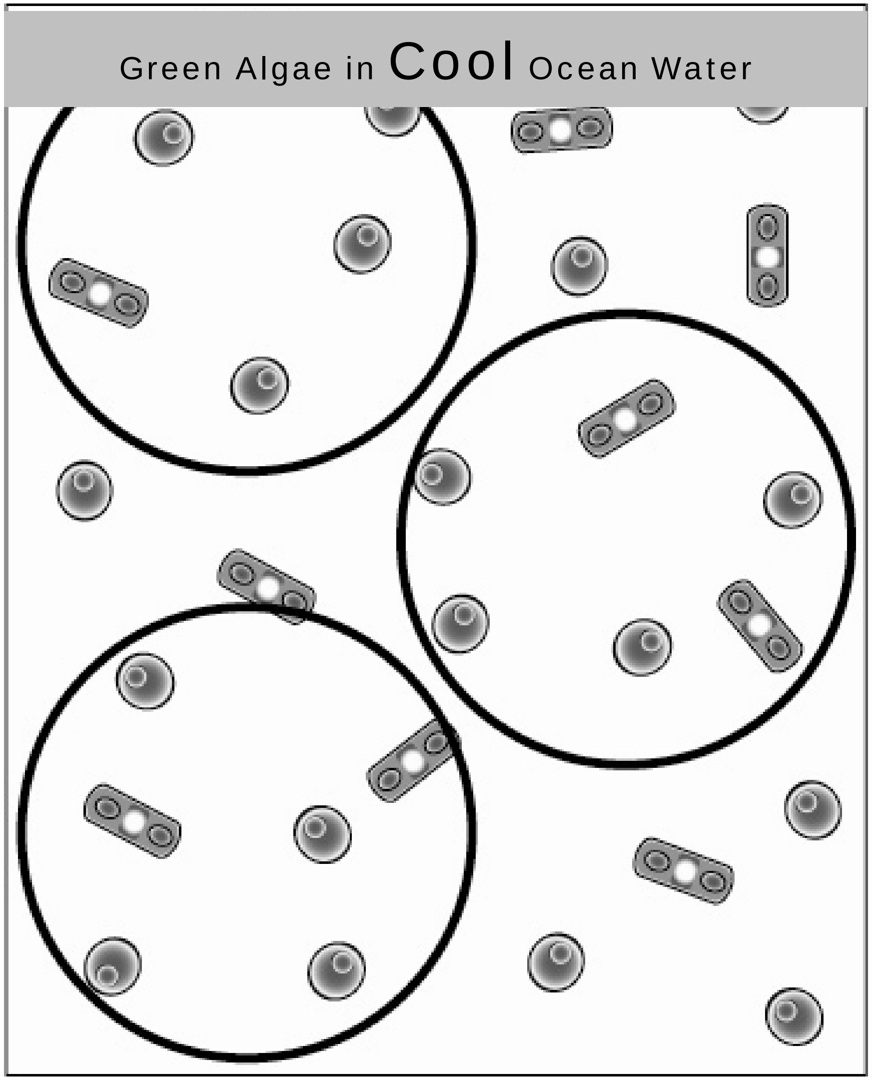
____

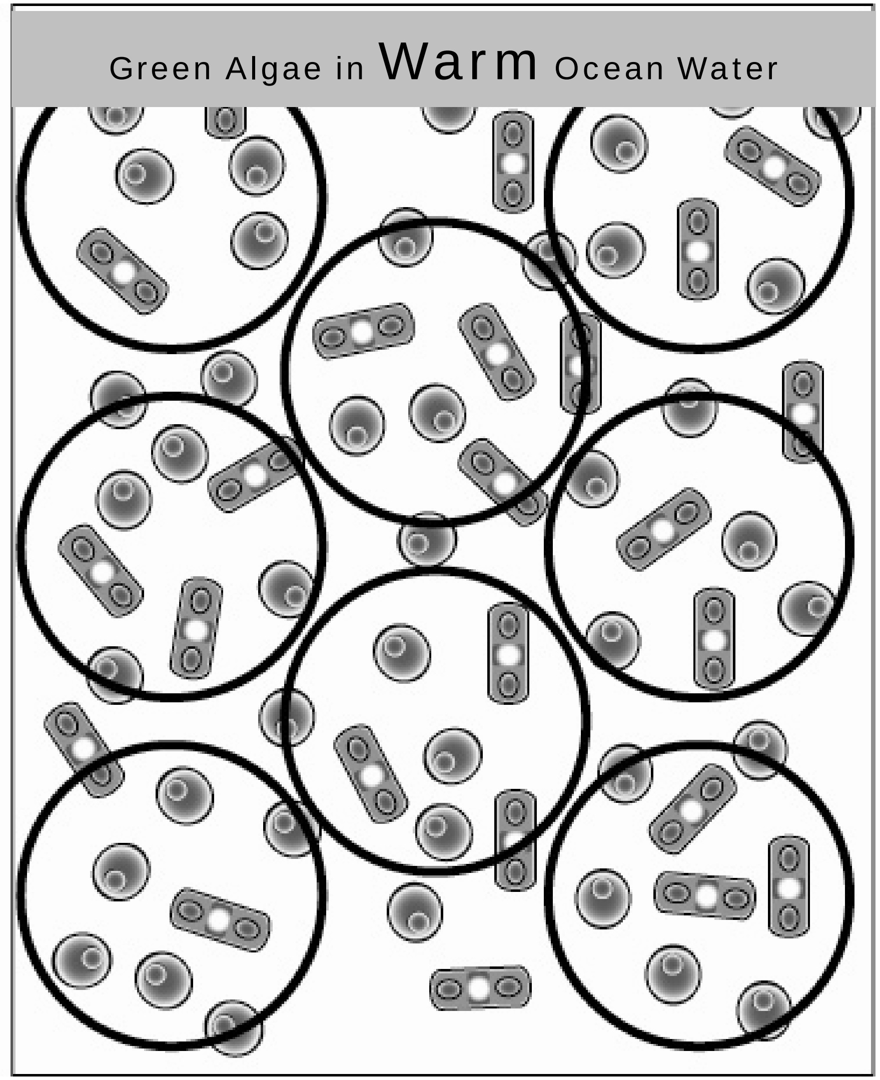
____

____

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 <u>you</u> could take to prevent climate changes that lead to cholera outbreaks.

- ____
- ____
- ____


Green Algae in Cool Ocean Water


Green Algae in Warm Ocean Water