



# A Medical Mystery Of Epidemic Proportions

## Teacher information

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### Summary:

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

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

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

### Core concepts:

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

### Class time required:

2 forty minute class periods + homework

### Preparing for class:

Provide for each student:

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

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

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

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

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

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

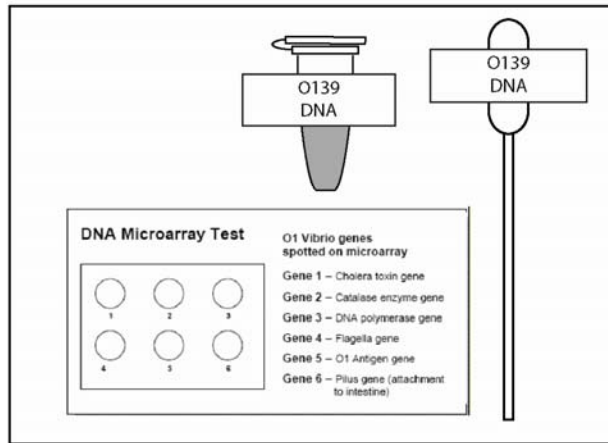
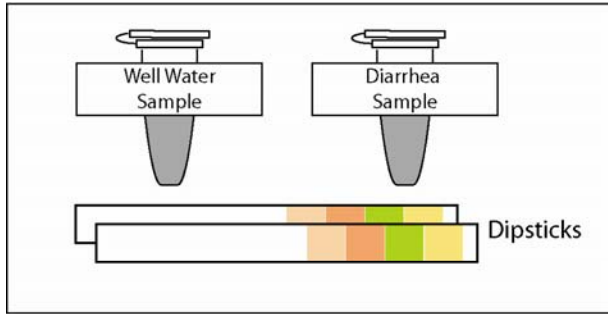
**Part 3:** For each team of 2-4 students, prepare a **DNA Microarray Test Kit** that includes:

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

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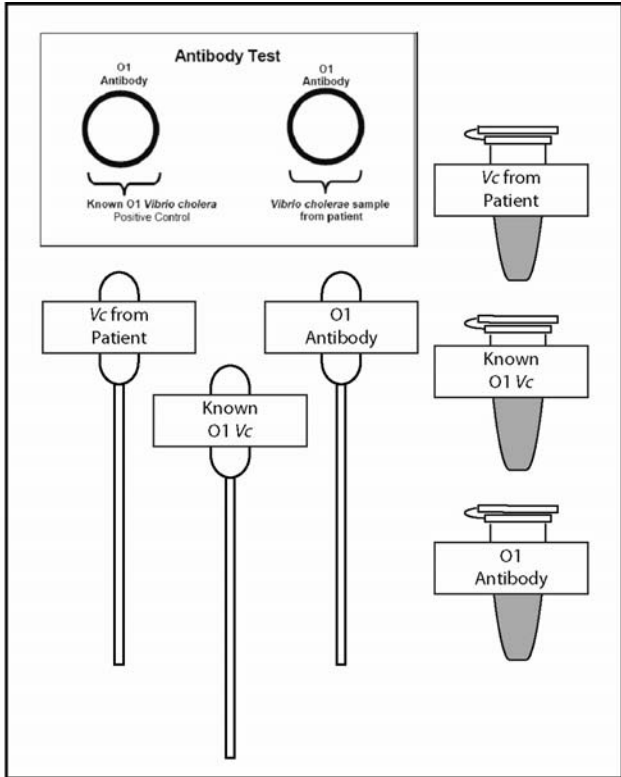
**Quick-Guide:**

**Part 1**



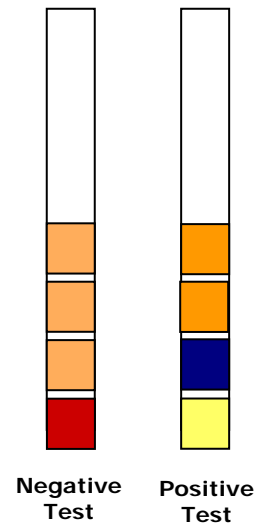
**Part 3**

**Part 2**



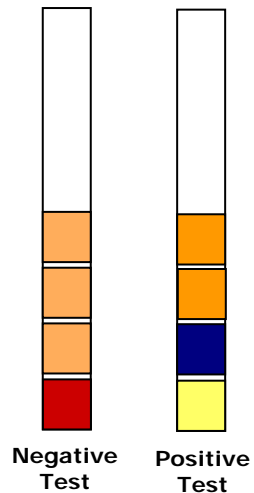
### Instructions for *Vibrio cholerae* Rapid-Test Kit

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



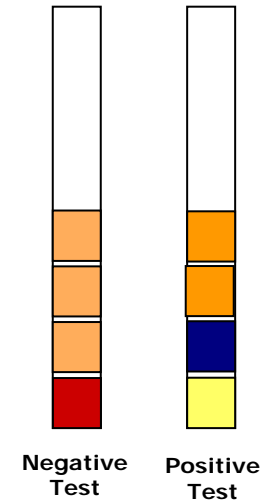
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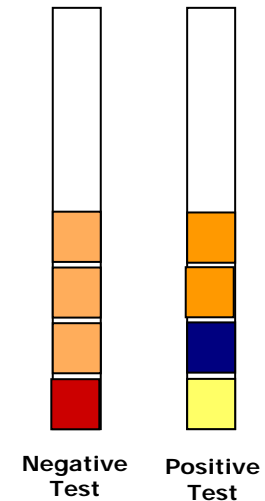
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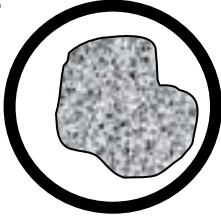
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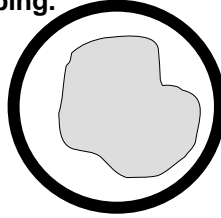
## Instructions for Antibody Test Kit:

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

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



If antibodies do NOT bind to the antigens on the surface of the *Vibrio cholerae*, there should be **no clumping**.

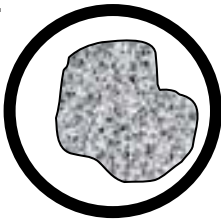


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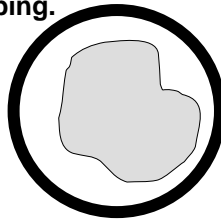
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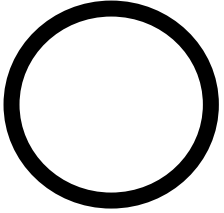
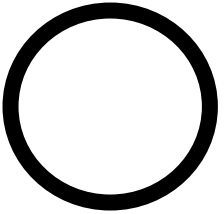
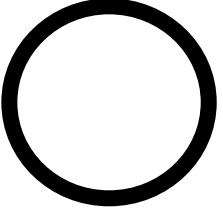
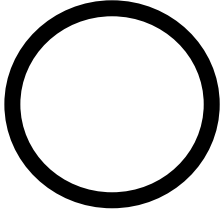
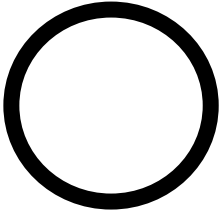
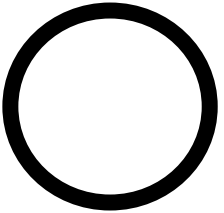
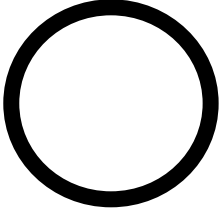
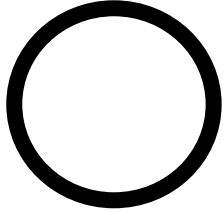
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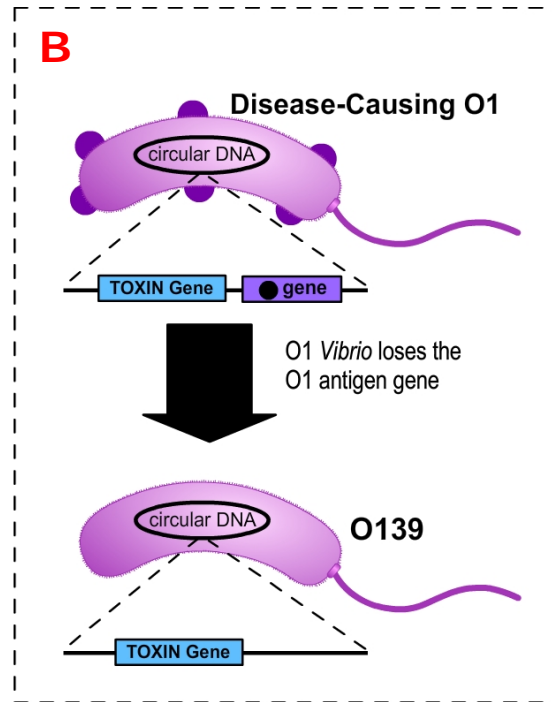
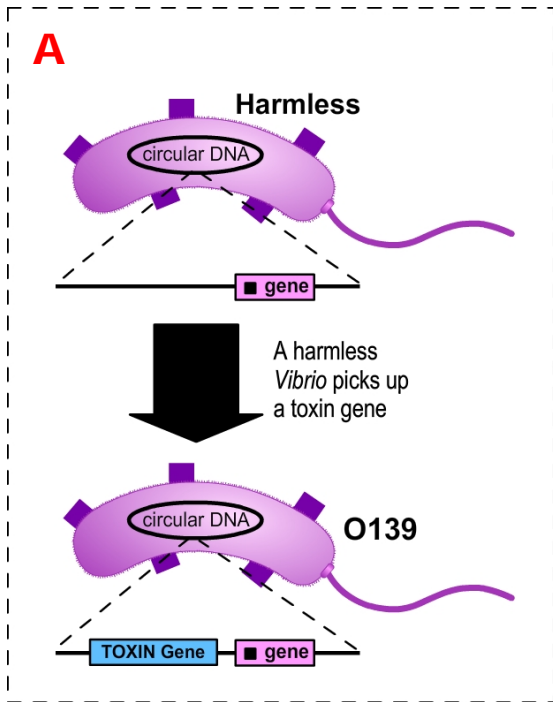
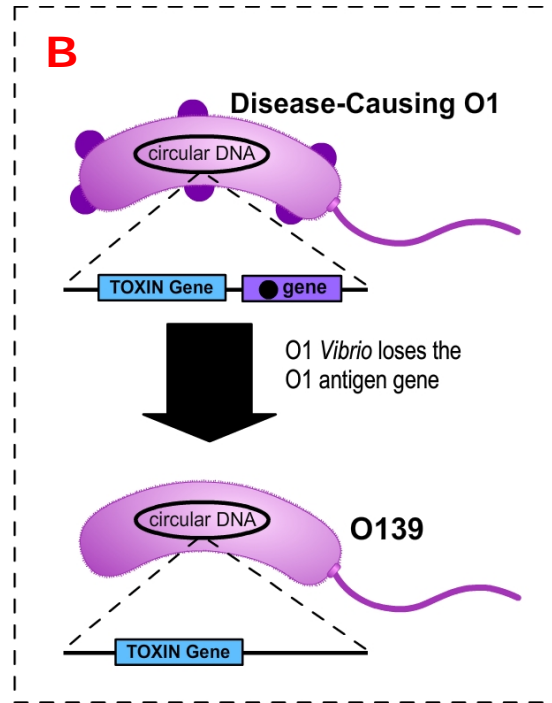
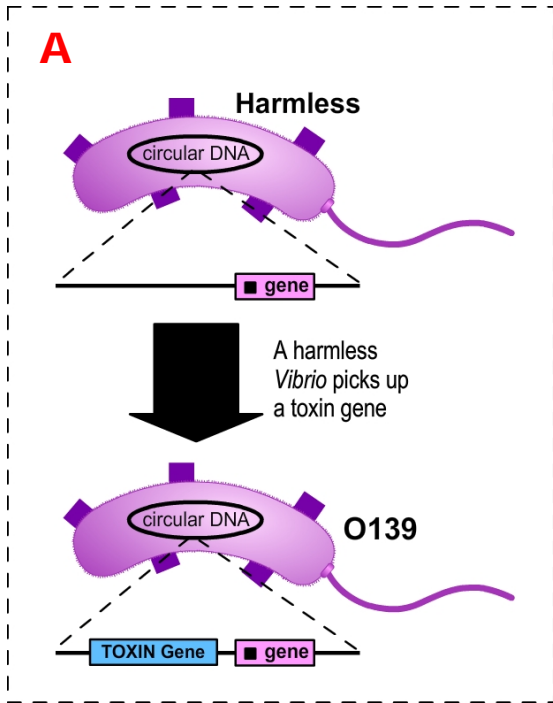


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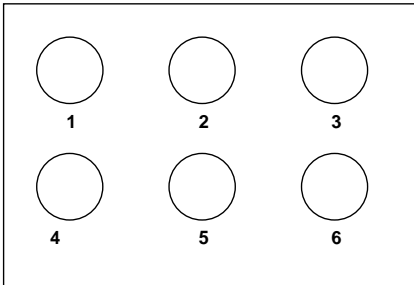


Duplicate on plastic transparency sheets and cut out on solid lines:

<p style="text-align: center;"><b>Antibody Test</b></p> <p>O1 Antibody Solution                      O1 Antibody Solution</p>  <p style="text-align: center;">Known O1 <i>Vibrio cholerae</i> Positive Control</p>  <p style="text-align: center;"><i>Vibrio cholerae</i> sample from patient</p>	<p style="text-align: center;"><b>Antibody Test</b></p> <p>O1 Antibody Solution                      O1 Antibody Solution</p>  <p style="text-align: center;">Known O1 <i>Vibrio cholerae</i> Positive Control</p>  <p style="text-align: center;"><i>Vibrio cholerae</i> sample from patient</p>
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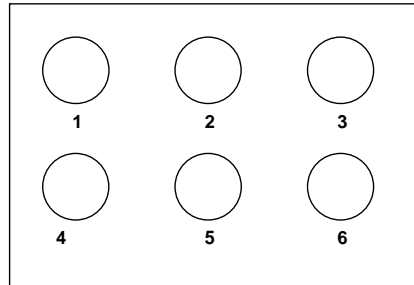
### DNA Microarray Test



#### O1 Vibrio genes spotted on microarray

- Gene 1** – Cholera toxin gene
- Gene 2** – Catalase enzyme gene
- Gene 3** – DNA polymerase gene
- Gene 4** – Flagella gene
- Gene 5** – O1 Antigen gene
- Gene 6** – Pilus gene (attachment to intestine)

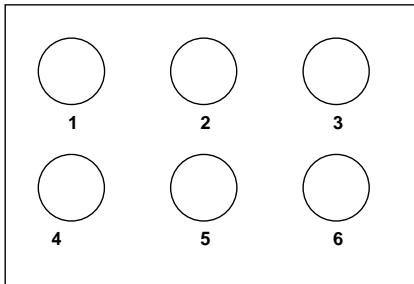
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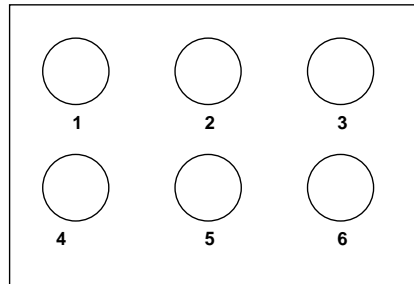
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### Instructions for DNA Microarray Test

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

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