THE **BIOTECHNOLOGY**
EDUCATION COMPANY®

LESSON PLAN:

Outbreak!

Learning about Zika Virus Transmission and Testing

Grade level: 9-12, College
Subject area: Biotechnology

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LP03.160914

General Overview:

LEARNING OBJECTIVES:

In this lesson, students will explore transmission and diagnosis of infectious diseases using the recent Zika outbreak as a model. First, students will use a simple model to simulate the spread of an infectious disease through a population. Next, they will use the Enzyme Linked Immunosorbent Assay (ELISA) to test patient samples for Zika. The results will be summarized in a laboratory report.

ESSENTIAL KEY QUESTIONS:

- What is an epidemic?
- What is a pandemic?
- How do infectious diseases spread through a population?
- How can we test for infectious diseases?

CONTENT STANDARDS:

NGSS Alignment:

HS-LS1: From Molecules to Organisms: Structures and Processes

Science and Engineering Practices:
- Asking Questions
- Developing and Using Models
- Scientific Investigations Use a Variety of Methods
- Analyzing and Interpreting Data

Crosscutting Concepts:
- Systems and System Models
- Cause and Effect
- Structure and Function
- Scale, Proportion, and Quantity

Common Core State Standards:

ELA/Literacy:
- RST.11-12.1
- RST.11-12.9
- WHST.9-12.9

Lesson Introduction:

Every summer, we prepare ourselves for warm weather, sunshine, and lots of outdoor activities. Unfortunately, the summer is also mosquito season! These small insects are both a nuisance and a public health concern as infectious diseases can be transmitted through their bite. This summer Zika Virus, a mosquito-borne contagious disease, has the public health community concerned. Scientists first identified Zika in Uganda in 1947, and sporadic Zika infections have occurred in Africa and parts of Asia since the 1950's. However, the 2015-16 pandemic that began in Brazil and quickly spread through the Americas has brought widespread attention to this virus due to its shocking consequences.

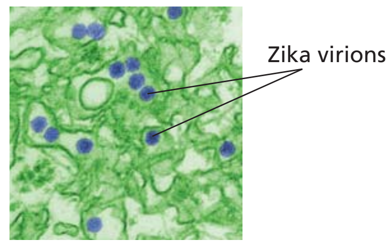

Figure 1: Transmission electron micrograph (TEM) of Zika virus.

Zika virus is a *Flavivirus* related to dengue, yellow fever, and West Nile. Each virion is roughly spherical in shape, measuring about 40 nanometers in diameter (Figure 1). The viral capsid proteins surround and protect the 10 kb strand of RNA that makes up the Zika genome. An envelope composed of viral glycoproteins and a host-derived membrane surrounds the capsid. The glycoproteins allow the virus to target and invade cells during an infection.

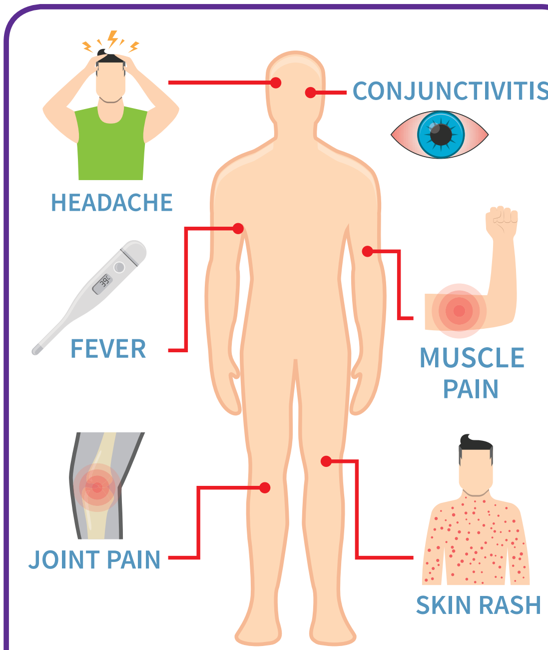

Figure 2: Symptoms of Zika.

The Zika infection is often asymptomatic in healthy adults – it is believed 75% of cases are not diagnosed. Symptoms of Zika last 3-7 days and include fever, joint pain, conjunctivitis (or "pink eye"), and a rash (Figure 2). In a few patients, Zika infections have been linked to Guillain-Barre Syndrome, an autoimmune condition that results in nerve dysfunction and muscle weakness. The most devastating effect of Zika infection is in pregnant women, in which the virus causes defects in the developing fetus such as brain damage and microcephaly (small heads and reduced amount of brain tissue). At this time, there are no specific treatments for Zika. Most infections are treated with rest, fluids, and a fever-reducing drug like acetaminophen or ibuprofen. Pregnant women are carefully monitored to determine whether the virus is affecting fetal development.

A vaccine for Zika virus is in development as of August 2016. Until it is available, the best way to prevent infection is to prevent mosquito bites. As mosquitos transmit the virus, controlling the mosquito population can limit the spread of the disease. Individuals can protect themselves from mosquito bites by wearing proper clothing and insect repellent. Since Zika is transmitted through blood and body fluids, transmission of the disease can also occur through blood transfusions or intimate contact with a partner.

For current information on the Zika outbreak, be sure to visit the Center for Disease Control (CDC) website at **https://www.cdc.gov/zika.**

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USING IMMUNOASSAYS TO DETECT ZIKA

As a general rule, symptoms of Zika are enough to warrant its diagnosis if an individual has traveled to an area with an active outbreak. Medical professionals use an immunoassay to identify Zika infections in patients with severe symptoms, or in pregnant women who may have been exposed to the virus. Positive results are further confirmed using PCR to detect the viral genome.

In this simulation of Zika testing, we will be using the Enzyme-Linked ImmunoSorbent Assay (or ELISA) to detect the virus in patient samples. The ELISA is an immunoassay that uses antibodies to recognize an antigen of interest in a complex sample (summarized in Figure 3). It is often used as a preliminary screening test because it is simple and fast to perform.

In brief, the patient sample is added to the wells of a plastic plate, where it non-specifically adheres to the wells through hydrophobic and electrostatic interactions. After washing away any excess sample, the wells are "blocked" with a protein-containing buffer to prevent non-specific interactions between the antibody and the plastic wells. Next, the primary antibody is added to the wells. This "primary" antibody will recognize and bind to the virion's coat proteins. After an incubation period, the wells are washed to remove any primary antibody that did not bind. The secondary antibody is added to the wells where it recognizes and binds to the primary. Excess antibody is removed from the wells by washing several times with buffer. If the secondary antibody has bound to the primary antibody, it will remain in the well.

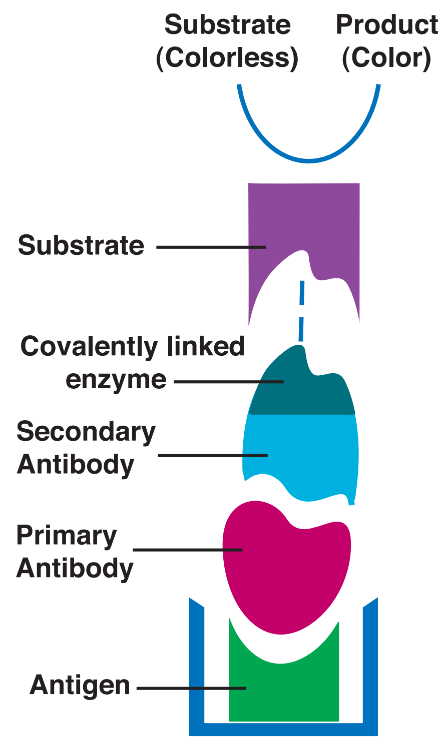

Figure 3: Schematic of traditional ELISA with primary and secondary antibodies.

The secondary antibody has been linked covalently to an enzyme that allows us to detect the antibody-antigen interactions. A clear, colorless substrate solution is added to each of the wells. In wells where the secondary antibody is present, the enzymatic reaction changes the substrate solution from clear to brown. Since the enzyme has a high catalytic activity, the ELISA can detect even the smallest amount of antigen.

In this investigation, students will test two patients for Zika using a fast and easy ELISA. This test looks for the presence of Zika-specific antibodies in the patient samples. A color change from clear to brown is a positive result indicating that the patient has been infected with Zika. The CDC will investigate positive test results to prevent spread of the disease.

Pre-Lab Assessment

Before performing the exercise, be sure that students are comfortable with the following topics:

1. Disease can be transmitted by direct or indirect contact.
   a. Direct transmission occurs when an infected person contacts a vulnerable person.
   b. Indirect transmission occurs when disease is spread through the air, water, contaminated objects, or animal/insect vectors.

2. An endemic disease is always found in a specific area.
   a. Chicken Pox is endemic in the United States, although the number of cases has dropped dramatically due to vaccination efforts.
   b. Malaria is endemic in many parts of Africa. Infection can be prevented by avoiding mosquito bites and by taking anti-malarial drugs.

3. An epidemic occurs when a specific infectious disease spreads to more people than usual in a short amount of time. Most times, epidemics occur in a specific geographic locality.
   a. An epidemic of severe acute respiratory syndrome (SARS) struck China in 2002. Although it did spread worldwide, the majority of cases occurred in a limited geographic area. Epidemiologists and health care professionals were able to quickly contain the disease.

4. A pandemic occurs when a specific infectious disease spreads across a large geographic region, such as a continent. Pandemics can be worldwide as well.
   a. Every few years, a new strain of the Influenza virus evolves and spreads worldwide because there is little to no immunity against the disease. For example, the H1N1 flu virus was responsible for a pandemic in 2009 that may have affected around 200 million people worldwide.

5. Zika virus disease is caused by Zika virus, a *Flavivirus* related to dengue, yellow fever, and West Nile.
   a. Zika was first identified in Uganda in 1947.
   b. Sporadic Zika infections have occurred in Africa and parts of Asia since the 1950's.
   c. In 2016, the World Health Organization (WHO) declared that the Zika outbreak in the Brazil and the Americas was a major public health concern

6. Zika is spread by indirect transmission from mosquitos, or through contact with fluids from an infected person (intimate contact, blood transfusion).
   a. Approximately 75% of cases of Zika virus disease are asymptomatic.
   b. Symptoms of Zika last 3-7 days and include fever, joint pain, conjunctivitis (or "pink eye"), and a rash.
   c. In adults, Zika has been linked to neurological disorders like Guillain-Barre Syndrome, an autoimmune condition that results in nerve dysfunction and muscle weakness.
   d. In pregnant women, Zika has been linked to defects in the developing fetus such as brain damage and microcephaly (small heads and reduced amount of brain tissue).

7. Testing for Zika is performed by trained clinicians.
   a. An immunoassay is used to identify Zika in patients with severe symptoms or in pregnant women who may have been exposed.
   b. Positive results are confirmed using PCR.
   c. Once confirmed, any active Zika infections are reported to the CDC to prevent the spread of disease.

Activity #1: How Does an Infection Spread?

This activity will be performed as a class.

REQUIRED MATERIALS:

- EDVOTEK® Kit #S-68 – What is an Epidemic and How Does an Infection Spread? (Provides enough reagents for 20 students to perform this simulation.)
- Distilled or Deionized Water
- Laboratory Glassware
- Elastic Bands or Tape
- Personal Protective Equipment

NOTE: This exercise differs slightly from the literature included with Kit #S-68. PLEASE REVIEW THE LESSON before performing.

Cat. #S-68

What is an Epidemic and How Does an Infection Spread?

For 10 Lab Groups. Infectious agents such as bacteria & viruses can spread rapidly through a population and cause widespread disease and death. In this experiment, your students will use colored solutions to simulate the spreading of a disease in the classroom.

PRELAB PREPARATION:

1. Print out the instruction cards on page 9. Using scissors, separate the cards. You should have one "mosquito" card and 19 "host" cards. (***NOTE: Add one additional mosquito card if performing the experiment with 30+ students***).
   a. One student will be an "infected mosquito", the others will be "humans".
   b. The mosquito must always choose a partner for fluid exchange.
   c. The humans will choose a partner for fluid exchange. At each turn, the human may choose to swap fluids with their partner, or may choose to cap their tube for a turn to simulate "bug spray" or other protective measures.

2. Using a permanent marker, label 20 test tubes sequentially from 1 to 20.

3. Prepare "infected" and "uninfected" solutions as described on page 10 of the Kit #S-68 literature.

4. Dispense 5 mL of the infected solution in one of the labeled tubes. This represents the Zika-infected mosquito. Tape one "mosquito" note to this tube. Make note of which number tube it is.

5. Dispense 5 mL of the uninfected solution into the remaining tubes. Tape one human note to each of these tubes.

6. Each student receives an instruction card, a plastic transfer pipet and a tube with 5 ml of a solution with a number and an instruction card. The solution represents "blood", and the instruction card describes how to exchange fluids.

STUDENT PROTOCOL:

1. Look at your instruction card and determine whether you are a human or a mosquito. The card contains the rules you will use to exchange "blood" with your classmates. Hide your card.

2. Circulate randomly around the room until the instructor calls "STOP".

3. Randomly select a nearby student to be "Contact 1". Register the code from Contact 1's tube in your notebook.
   a. Are one or both contacts using "bug spray"? If so, no fluid exchange occurs. Note this next to the contact number.
   b. Are both contacts exchanging fluids? The student with the lower number will remove 1 mL of "blood" from their tube and add it to the second student's tube. The second student will cap their tube, invert the tube to mix, then open the tube and add 1 mL of "blood" to the first student's tube. The second student will cap the tube and invert to mix.

4. Repeat Steps 2 and 3 again to randomly select a second student (Contact 2). The same rules apply as before.

5. Repeat Steps 2 and 3 again to randomly select a third student. At the end of the fluid exchange you should have the same volume that you started with (~5 mL). This represents your "self" and the other students with whom you exchanged "blood". Note that the proportions for the infecting agents are exaggerated to facilitate and to dramatize the transmission of an infection.

6. The teacher will add one drop of Phenol Red to your tube. If your solution turns pink then you have been infected with Zika. Make note of the color of your solution in your lab notebook.

ASSESSMENT:

After performing the exercise, the instructor will facilitate discussion between the students with the following questions. (The mosquito should remain anonymous, but should volunteer that his/her sample turned color.)

1) How many students have a pink color?

2) What percentage of students does that cover?

3) How can we determine who is the mosquito?

The class will then simulate the steps an epidemiologist may take to discover the source of an infection. Data is collected by entering information into the table describing all of the fluid exchanges. The infected individuals should be highlighted in one color. Use of "bug spray" during a fluid exchange should be highlighted in a different color. From this information, the students will work in small groups to determine the identity of the mosquito. Students may find that there is no clear candidate for the mosquito. After analyzing the data, each group should write a short report justifying their answer. The instructor then should share the identity of the mosquito.

Activity #1: Discussion Questions

Answers to Discussion Questions will vary depending on the experimental results.

1. What is the difference between indirect and direct disease transmission? After learning the identity of the mosquito, which humans received the disease through direct transmission? Indirect transmission?

2. How would the results change if the disease had a 50% chance of transmission between humans instead of a 100% chance of transmission?

3. How would the experiment differ if there were a vaccine for this disease?

DATA TABLE:

Student ID Number	Contact 1	Contact 2	Contact 3
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

You are THE MOSQUITO.

You must always exchange fluids with your partner UNLESS they are using “bug spray.”

You are HUMAN.

After choosing a partner for fluid exchange, you may:
1) Swap fluids
2) Cap your tube for a turn to simulate “bug spray” or other protective measures.

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Activity #2: Zika Testing Using the ELISA

This activity will be performed in groups of 2-4.

Cat. #267
Single Antibody ELISA Diagnostics

For 10 Lab Groups. Teach your students the ELISA technique in less than half the time of traditional ELISAs! This experiment eliminates the need for the primary and secondary antibody normally needed for ELISAs because the detection antibody has an enzyme linked to it directly. Simply add substrate to discover which patient is infected.

REQUIRED MATERIALS:

- EDVOTEK® Kit #267 – Single Antibody ELISA Diagnostics
- Distilled or Deionized Water
- Laboratory Glassware
- 37°C Incubator
- Automatic Micropipets (5-50 uL) and tips (recommended)
- Personal Protective Equipment

In this investigation, students will test two patients for Zika using a fast and easy ELISA. This test looks for the presence of Zika-specific antibodies in the patient samples. A color change from clear to brown is a positive result indicating that the patient has been infected with Zika. The CDC will investigate positive test results to prevent spread of the disease.

PROCEDURE:

Before performing the experiment, be sure to emphasize good laboratory practices (proper personal protective equipment, exercise caution when using equipment, hand washing, etc.) Perform experiment as outlined in the lab packet (Available online at http://www.edvotek.com/site/pdf/267.pdf).

Before starting the experiment, students should:
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of the experiment.

During the experiment, students should:
- Record any observations in a lab notebook.

After the experiment, students should ask the following questions:
- Interpret the results – does the data support or contradict the hypothesis?
- If the experiment was repeated, what should change? Revise the hypothesis to reflect this change.

Be sure to have your students reflect upon these points in their lab notebooks. Students should also submit a formal lab report upon completion of the exercise.

Tech Video

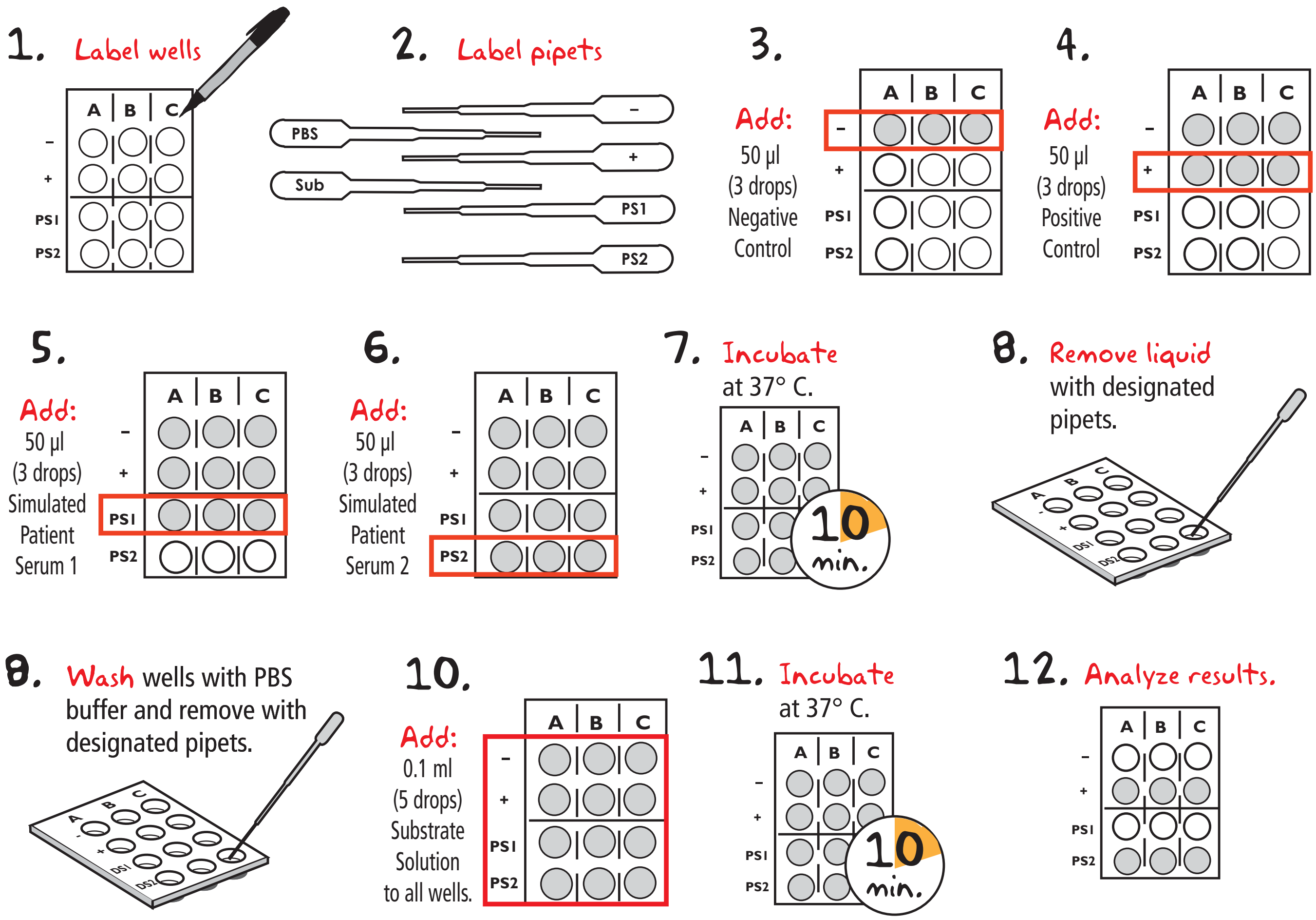
Watch us on YouTube

youtube.com/EdvotekInc

Video: The Enzyme Linked Immunosorbent Assay (ELISA)

RAPID SINGLE ANTIBODY BASED ELISA

Excerpts from Edvo-Kit 267

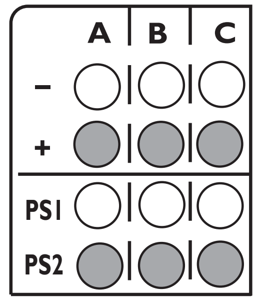


This is a direct ELISA where one antibody is used to which the enzyme is bound. The microtiter wells are pre-treated with the antigen. After the simulated patient serum sample is added to the wells, washed, and substrate is added, the conversion of the substrate to product results in the color formation for positive samples.

1. **LABEL** wells: "-", "+", "PS1", and "PS2" directly on the microtiter plate, or place the plate on a labeled sheet of paper. Put your initials or group number on the plate.
2. **LABEL** pipets: "-", "+", "PS1", "PS2", "PBS", and "Sub". These are designated for adding samples and removing washes. **Save these pipets!**
3. **ADD** 50 µl or 3 drops of Negative Control to all three wells in the 1st Row.
4. **ADD** 50 µl or 3 drops of Positive Control to all three wells in the 2nd Row.
5. **ADD** 50 µl or 3 drops of Simulated Patient Serum 1 to all three wells in the 3rd Row.
6. **ADD** 50 µl or 3 drops of Simulated Patient Serum 2 in all three wells in the 4th Row.
7. **INCUBATE** for 10 minutes at 37° C.
8. **REMOVE** all liquid using the transfer pipet designated for each row.
9. **WASH** each well with PBS buffer by adding the PBS buffer until each well is almost full. The capacity of each well is approximately 0.2 ml. Do not allow the liquids to spill over into adjacent wells. Remove all the PBS from each of the wells with the transfer pipet designated for each row.
10. **ADD** 0.1 ml or 5 drops of the substrate solution to all of the wells.
11. **INCUBATE** for 10 minutes at 37° C.
12. **ANALYZE** the plate. If color is not fully developed after 10 minutes (step 11), incubate at 37° C for a longer period of time.

Activity #2: Results and Analysis

- Color should appear only in Rows 2 and 4.
- Row 1 is a negative control.
- Row 2 is a positive control.
- Row 3 is a negative patient sample.
- Row 4 is a positive patient sample.



RESULTS:
Patient 1 tested negative for Zika. No further testing is necessary.
Patient 2 tested positive for Zika. Positive results are confirmed by PCR. The results are reported to the CDC for further investigation.

Activity #2: Discussion Questions

1. **What is the ELISA and why is it used as a diagnostic test?**

   The ELISA is an immunoassay that uses antibodies to recognize an antigen of interest in a complex sample like blood. It is often used as a diagnostic test because it is simple and fast to perform.

2. **What are the next steps taken by a clinician getting a positive result for Zika by ELISA?**

   Positive ELISA results are further confirmed using a PCR test that detects the Zika genome. After PCR confirmation, the CDC will investigate the positive test results to prevent spread of the disease.

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