EDVOTEK WORKSHOP:

Outbreak! Zika Testing Using the Enzyme-Linked Immunosorbent Assay (ELISA)

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Outbreak! Zika Testing Using ELISA

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.

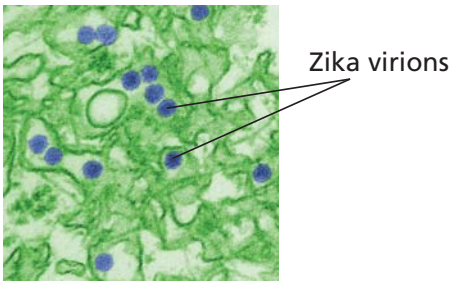

Figure 1: Transmission electron micrograph (TEM) of Zika virus.

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.

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.

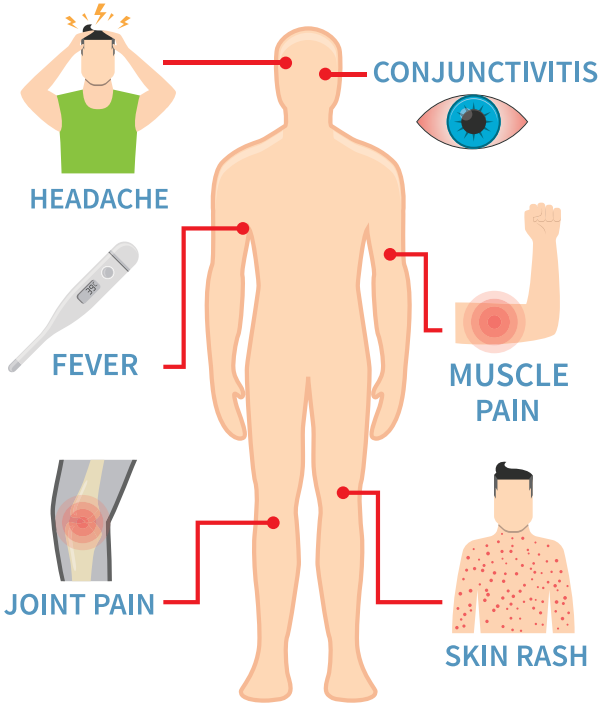

Figure 2: Symptoms of Zika.

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 mosquitoes 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.

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.

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.

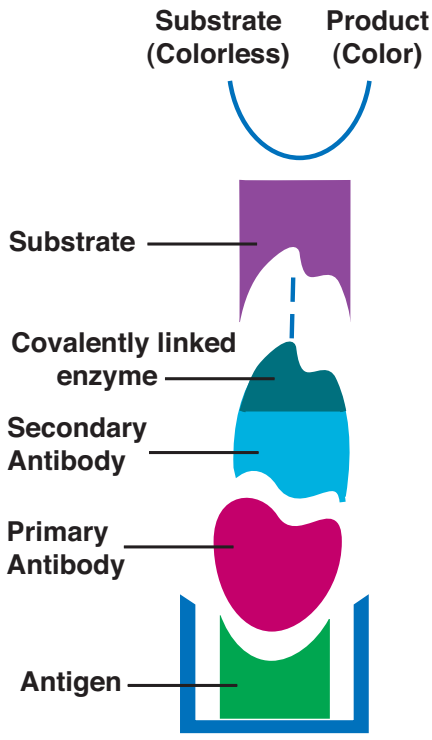
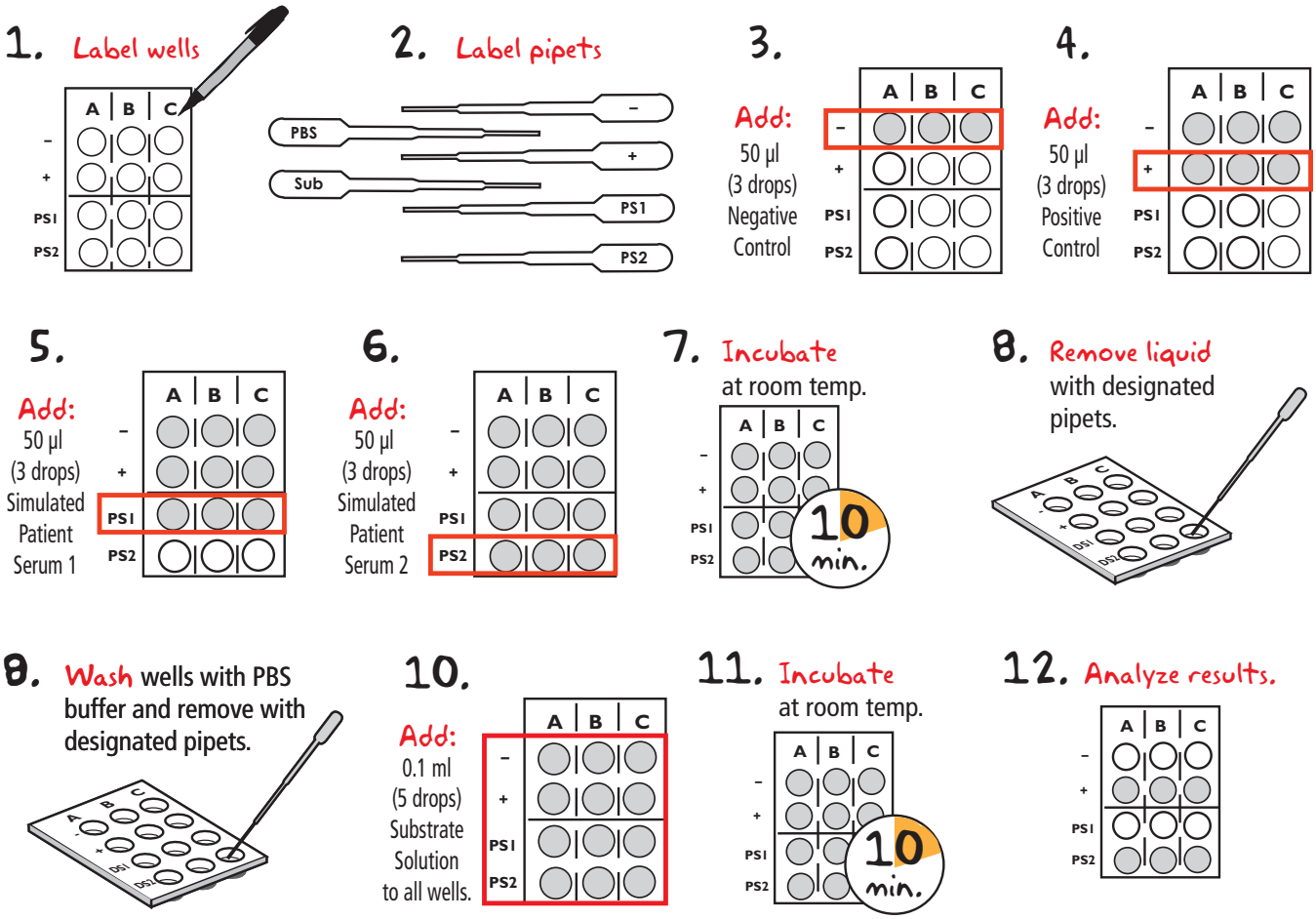


Figure 3: Schematic of traditional ELISA with primary and secondary antibodies.

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 room temp.
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 room temp.
12. **ANALYZE** the plate. If color is not fully developed after 10 minutes (step 11), incubate at room temp. for a longer period of time.

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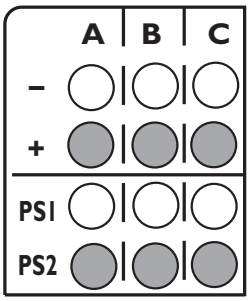
Experiment Results and Analysis



- 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.

Featured Experiment

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.

Tech Video youtube.com/EdvotekInc Watch us on YouTube

Video: The Enzyme Linked Immunosorbent Assay (ELISA)

Video: Measuring Liquids with an Adjustable Volume Micropipet

Video: Diluting a Concentrated Solution

Related Products

Cat. #269

Introduction to ELISA Reactions

For 10 Lab Groups. Your students will learn the basic principles of the Enzyme-linked mmunosorbent Assay (ELISA) in this precise and sensitive antibody-based detection kit. Experiment components do not contain human serum.

Cat. #278

Quantitative ELISA Laboratory Activity

For 6 Lab Groups. Now with NEW substrate! Antibodies are highly specific in their recognition of antigens. This ELISA experiment demonstrates the quantitation of varying concentrations of viral antigens as detected by the intensity of the color reaction due to the accumulation of products. This laboratory activity meets the requirements in the BSCS Blue Biology curriculum.

Cat. #273

Radial Immunodiffusion

For 10 Quantifications, 6 reactions each. Radial immunodiffusion quantitatively determines the level of an antigen. Antibody is incorporated into liquefied agar and allowed to gel. The antigen is added to small wells and radiates throughout the antibody-containing medium, leaving a precipitate throughout the gel. The amount of amount of diffusion is quantified.

Cat. # 594

EdvoPette™ Pipet Controller

The all-new EdvoPette™ Pipet Controller is a lightweight cordless pipetting controller ideally suited as an aliquoting tool for instructors and teaching assistants. It utilizes all standard serological pipets. The speed can be fine-tuned by applying varying finger pressure to the operating buttons.

Cat. #546

Incubation Oven

Features a digital temp. control with a range from Ambient +1°C to 60°C. Ideal for growing bacteria on agar plates at 37°C or for Southern and Western Blot analysis at 60°C. Includes two adjustable/removable shelves for increased capacity. Accepts bottles and flasks up to 2 L.

Cat. #5019

Mini EdvoRokr™

Features a tilt angle and optimized speed for gel blotting, washing and staining. With the tri-directional motion, the rocker provides thorough and gentle mixing ability. The 10.5" X 7.5" autoclavable plat mat can accept stackable platforms and are safe to use in cold rooms and incubators (4°C to 65°C) .

Related Products

Cat. #266

What's In My Lunch? Quantitative Milk Allergy ELISA

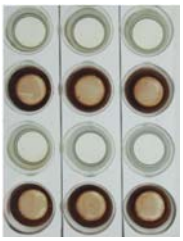
NEW

For 10 Lab Groups. Milk proteins are the most common food allergens in children. Accurate detection and labeling is vital to inform consumers about potentially dangerous foods. In this inquiry-based experiment, students will master the concepts behind the enzyme-linked immunosorbent assay (ELISA). Students will perform an ELISA to detect the presence and measure the concentration of whey protein in various food products.

Cat. #271

Simulation of HIV Detection by ELISA

For 10 Lab Groups. An HIV test detects HIV infection indirectly using an ELISA test against HIV antibodies in the blood. The test works by taking antibodies from the patient's blood and adding them to a microtiter plate coated with HIV antigen. If HIV antibodies are present in the blood, they will bind to the antigens on the plate. This binding is detected with an enzyme-linked secondary antibody that causes a color change upon addition of substrate. In this experiment, your students will perform an ELISA test by coating micotiter plate wells with simulated HIV antigen and then test simulated donor serum for anti-HIV antibodies.

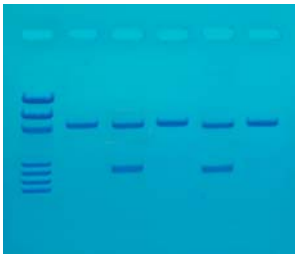


Cat. #122

Detection of the Influenza Virus

NEW

For 8 Lab Groups. The influenza virus, or "the flu," is a common contagious disease that affects the respiratory system. In this simulation, students will perform two common tests (RIDT, RT-PCR) used to diagnose the flu in a clinical setting.



Cat. #279

Immunology of Pregnancy Tests

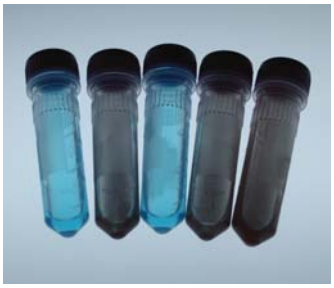
For 10 Lab Groups. One of the most commonly used over-the-counter diagnostic tests is the pregnancy test, based on the Enzyme-linked Immunosorbent Assay (ELISA). The experimental concepts and methodology involved with the ELISA will be introduced in the context of testing for pregnancy. None of the components have been prepared from human sources.

Cat. #280

Detecting the Silent Killer: Clinical Diagnosis of Diabetes

NEW

For 10 Lab Groups. Over 380 million people worldwide are afflicted by diabetes mellitus, a chronic disease that leads to high blood sugar. Due to genetic predisposition and high-calorie, low-activity lifestyles, that number continues to grow. Without early detection and treatment of diabetes, severe medical complications can occur. In this simulation, students will diagnose diabetes in three patients using the urine glucose test and Enzyme-linked Immunosorbent Assay (ELISA).



Cat. #274

In Search of the "Kissing Disease"

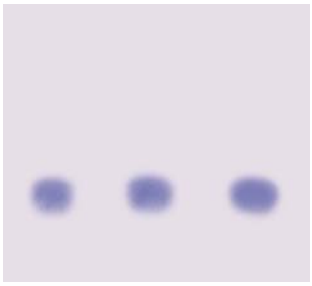
For 10 Lab Groups. Infectious mononucleosis is commonly known as the "kissing disease". The causative agent is Epstein-Barr virus (EBV) which can be transmitted through saliva during kissing. In this experiment, students search for the presence of EBV using the ELISA reaction to detect specific viral proteins.

Related Products

Cat. #275

HIV Detection by Simulated Western Blot

For 6 Blots. The second assay used to confirm a positive HIV ELISA result is the Western Blot. Students separate protein samples from hypothetical patients on agarose gels, transfer the samples to a membrane and detect the simulated HIV proteins. This kit is an introductory level experiment. For a comprehensive advanced course, we recommend Cat. #317.



Cat. #317

Western Blot Analysis

For 6 Blots. In Western blot analysis, protein identification is based on antibody and antigen reactions. Proteins are separated on a polyacrylamide gels and are transferred (blotted) to a nylon membrane. The membrane is exposed to solutions containing primary antibody, followed by a secondary antibody coupled to an enzyme. The membrane is then soaked in a substrate solution to develop the color reaction, which results in identification of the antigen protein band. The molecular weights of the visible bands are measured using prestained protein markers of known molecular weight. This kit does not require an electrotransfer apparatus.