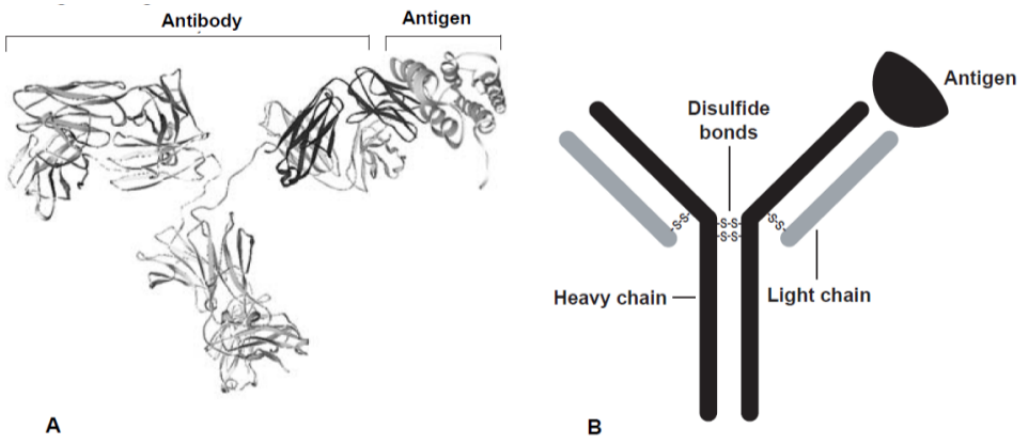
Bioterrorism Activity

Introduction

You are about to perform an experiment in which you will share simulated “body fluids” with your classmates. After sharing, you will perform an enzyme-linked immunosorbent assay or ELISA to determine if you have been exposed to a contagious “disease”. The ELISA uses antibodies to detect the presence of a disease agent, (for example, viruses, bacteria, or parasites) in your blood or other body fluid. You will then track the disease back to its source.

When you are exposed to a disease agent, your body mounts an immune response. Molecules that cause your body to mount an immune response are called antigens, and may include components of infectious agents like bacteria, viruses, and fungi. Within days, millions of antibodies — proteins that recognize the antigen and bind very tightly to it — are circulating in your bloodstream. Like magic bullets, antibodies seek out and attach themselves to their target antigens, flagging the invaders for destruction by other cells of the immune system.

Over 100 years ago, biologists found that animals’ immune systems respond to invasion by “foreign entities”, or antigens. Today, antibodies have become vital scientific tools, used in biotechnology research and to diagnose and treat disease. The number of different antibodies circulating in the blood has been estimated to be between 10^6 and 10^{11}, so there is usually an antibody ready to deal with any antigen. In fact, antibodies make up to 15% of your total blood serum protein. Antibodies are very specific; each antibody recognizes only a single antigen.



A) Structure of IgG bound to the HIV capsid protein p24 as determined by X-ray crystallography (Harris et al. 1998, Momany et al. 1996). These structures can be downloaded from the Protein Data Bank (www.pdb.ufmg.br, (Berman et al. 2000) using the PDB identification codes 1IGY and 1AFV and manipulated using free online software such as Rasmol and Protein Explorer. **B)** A commonly used representation of an antibody bound to an antigen.

How Are Antibodies Made?

Scientists have learned to use the immune response of animals to make antibodies that can be used as tools to detect and diagnose diseases. The study of the immune system is called “immunology”. Animals such as chickens, goats, rabbits, and sheep can be injected with an antigen and, after a period of time, their serum will contain antibodies that specifically recognize that antigen. If the antigen was a disease agent, the antibodies can be used to develop diagnostic tests for the disease. In an immunoassay, the antibodies used to recognize antigens like disease agents are called primary antibodies; primary antibodies confer specificity to the assay.

Other kinds of antibody tools, called secondary antibodies, are made in the same way. In an immunoassay, secondary antibodies recognize and bind to the primary antibodies, which are antibodies

from another species. Secondary antibodies are prepared by injecting antibodies made in one species into another species. It turns out that antibodies from different species are different enough from each other that they will be recognized as foreign proteins and provoke an immune response. For example, to make a secondary antibody that will recognize a human primary antibody, human antibodies can be injected into an animal like a rabbit. After the rabbit mounts an immune response, the rabbit serum will contain antibodies that recognize and bind to human antibodies. The secondary antibodies used in this experiment are conjugated to the enzyme horseradish peroxidase (HRP) which produces a blue color in the presence of its substrate, TMB. These antibody and enzyme tools are the basis for the ELISA.

Where Is ELISA Used in the Real World?

With its rapid test results, the ELISA has had a major impact on many aspects of medicine and agriculture. ELISA is used for such diverse purposes as pregnancy tests, disease detection in people, animals, and plants, detecting illegal drug use, testing indoor air quality, and determining if food is labeled accurately. For new and emerging diseases like severe acute respiratory syndrome (SARS), one of the highest priorities of the US Centers for Disease Control (CDC) and the World Health Organization (WHO) has been to develop an ELISA that can quickly and easily verify whether patients have been exposed to the virus.

Why Do We Need Controls?

Positive and negative controls are critical to any diagnostic test. Control samples are necessary to be sure your ELISA is working correctly. A positive control is a sample known to be positive for the disease agent, and a negative control is a sample that does not contain the disease agent.

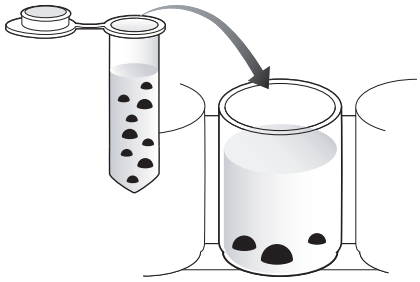
Your Task Today

You will be provided the tools and an experimental protocol to perform an ELISA. You will be given a simulated “body fluid” sample that you will share with your classmates. One or two of the samples in the class have been “infected”. You will also be provided with positive and negative control samples. Then you and your fellow students will assay your samples for the presence of the “disease agent” to track the spread of the disease through your class population.

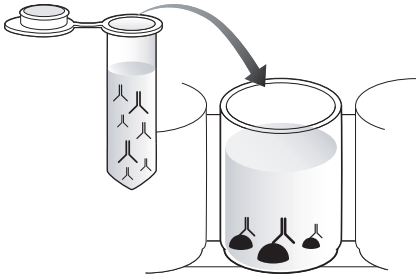
Step-by-Step Description of ELISA

The protocols in this kit rely on indirect antibody capture ELISA. The steps in this assay are:

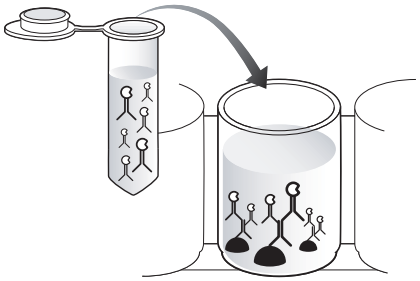
Step 1: Antigen is added to the wells of the microplate strip and incubated to allow binding, after which unbound antigen is washed from the wells with detergent. The detergent also serves as a blocking agent, binding to all unused protein binding sites in the wells and preventing nonspecific binding of antibody.



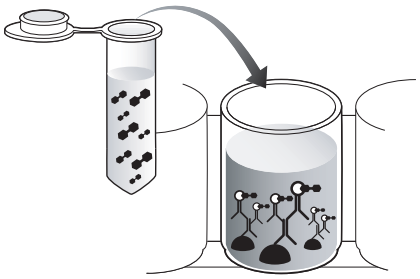
Step 2: Primary antibody solution is added to the wells and incubated to allow the antibody to bind to the antigen. Then unbound primary antibody is washed from the wells.

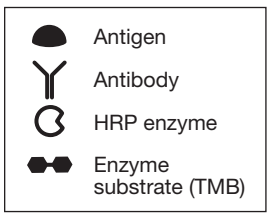


Step 3: Enzyme-labeled secondary antibody solution is added to the wells and incubated to allow the secondary antibody to bind to the primary antibody. Then unbound secondary antibody is washed from the wells.



Step 4: Chromogenic (color-producing) enzyme substrate is added to the wells and incubated to allow color to develop. Results of the assay are evaluated. Wells that remain colorless are negative and wells that turn blue are positive.





Bioterrorism Activity: Tracking Disease Outbreaks

Scenario:

The class has been on a field trip to the Smithsonian in Washington, D.C. While on the metro, it is possible that you have been exposed to smallpox, deliberately released in aerosol form. Smallpox virus, if not exposed to UV light, may survive for > 24 hours in cool, dry locations.

It is important to determine as soon as possible which students have been exposed. Vaccination within 2-3 days of exposure can prevent smallpox, but the vaccine is in short supply. Also, vaccination can have nasty side effects, so no one should be vaccinated unnecessarily. Vaccination of exposed individuals is essential to prevent further spread of the disease.

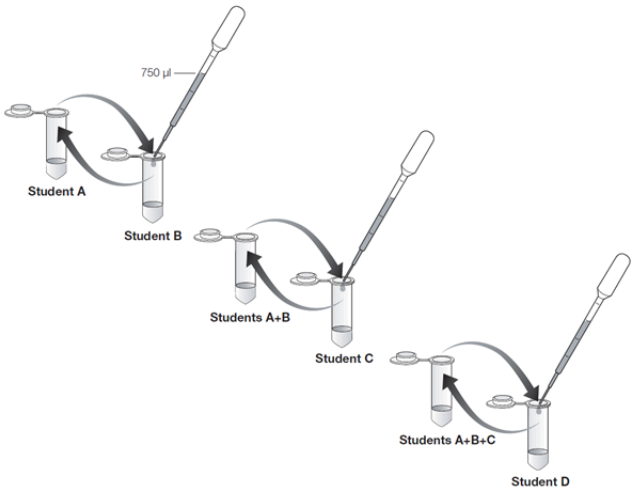
To determine which students have been exposed, perform an ELISA to detect the virus samples in their body fluid. Students who test positive for the virus should undergo immediate vaccination.

Name of pathogen	*Variola major*, smallpox virus
Type of organism	DNA virus in genus *Orthopoxvirus*
Infectious agent	Virus (only a few virus particles needed for infection)
Method of spread	Person to person via aerosols from infected individual. Can also be spread through infected clothing, bedding, etc. Intentional dissemination of aerosolized virus; if not exposed to UV light, virus may be viable for ε24 hours under optimal conditions.
Incubation	12–14 days
Symptoms	High fever, malaise, headache, backache, abdominal pain, rash
Infectivity	Most infectious at onset of rash; contagious until rash is totally gone.
Diagnosis	Initially by symptoms. Virus identification by electron microscopy. Identification of viral DNA by molecular biological methods. Development of ELISA against IgG, IgM, and antigen high priority with CDC and many companies (smallpox was eradicated before current immunoassays were developed).
Treatment	Postexposure vaccination within 2–3 days of exposure protects against the disease. Postexposure vaccination within 4–5 days of exposure may prevent a fatal outcome. After 4–5 days postexposure, supportive treatment only. Experimental treatment with antiviral drugs.
Mortality	Up to 30%
History as a pathogen	Used for biological warfare by British in North America in the 18th century. Army distributed blankets that had been used by smallpox patients. Mortality in some Native American tribes was 50%. In 1796, Jenner discovered cowpox vaccine effective against smallpox. Global immunization effort eradicated smallpox in 1977, and routine vaccination ceased. May have been weaponized in the Soviet Union in the 1980s

The table below gives an example of how a diagnostic test to detect smallpox virus in patient samples can be simulated. An ELISA to detect smallpox has not yet been developed, although it is a high priority with the CDC.

Tube Description	**Tube Color**	**Simulated Tube Contents**
Student samples	Yellow	Sample from patient's lesion
Positive control	Violet	Sample from lesion of chickenpox patient spiked with *variola* proteins
Negative control	Clear Blue	Sample from lesion of chickenpox patient
Primary antibody	Green	Anti-*variola* antibody from mouse
Secondary antibody	Orange	Anti-mouse immunoglobulin antibody conjugated to HRP
Enzyme Substrate	Brown	TMB

Bioterrorism Activity: Lab Protocol



1. Label a yellow tube and a plastic transfer pipet with your initials.
2. Use the pipet to transfer all your "bodily fluid" sample into the tube of another student. Gently mix the samples, then take back half of the shared sample (750 µl) to your own tube. Write down the name of the student next to "Sharing Partner #1".

yellow

3. When instructed to do so, repeat the sharing protocol two more times. Discard this transfer pipet after this step.

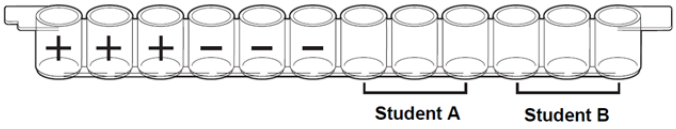
   Optional stopping point: Samples may be stored at 4°C overnight.

Sharing Partner #1 _____

Sharing Partner #2 _____

Sharing Partner #3 _____

4. Label your 12-well strip. On each strip label the first 3 wells with a "+" for the positive controls and the next 3 wells with a "–" for the negative controls. Label the remaining wells with your and your lab partner's initials (3 wells each).



violet

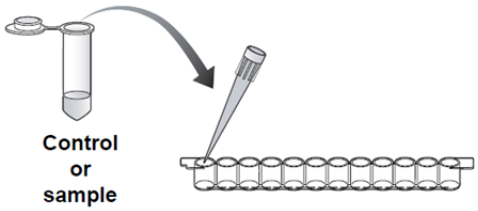
5. Use a fresh pipet tip to transfer 50 µl of the positive control (+) into the three "+" wells.

clear blue

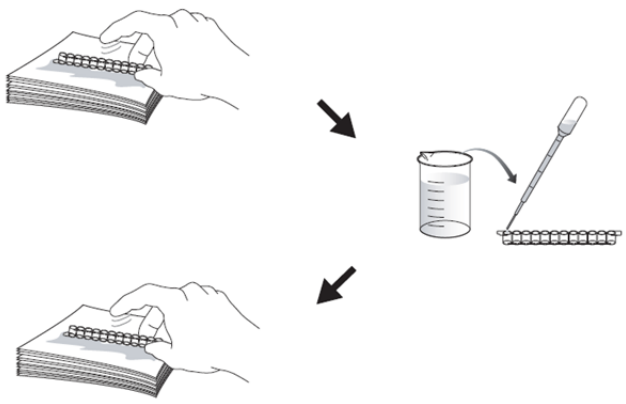
6. Use a fresh pipet tip to transfer 50 µl of the negative control (–) into the three "–" wells.

yellow

7. Transfer 50 µl of each of your team's samples from step 3 into the appropriately initialed three wells, using a fresh pipet tip for each sample.



8. Wait 3 minutes while all the proteins in the samples bind to the plastic wells.
9. WASH:
   a. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid samples splashing back into wells.
   b. Discard the top paper towel.
   c. Use a fresh transfer pipet to fill each well with wash buffer, taking care not to spill over into wells. Note: the same transfer pipet is used for all washing steps.
   d. Tip the microplate strip upside down onto the paper towels and tap.
   e. Discard the top 2–3 paper towels.

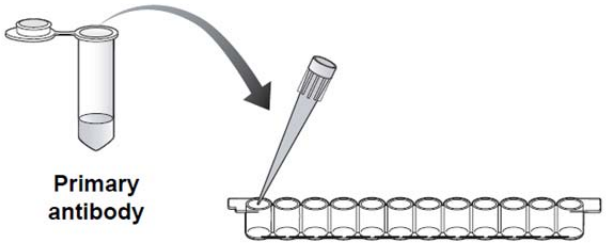


10. Repeat wash step 9.

WASH

green

11. Use a fresh pipet tip to transfer 50 µl of primary antibody (PA) into all 12 wells of the microplate strip.



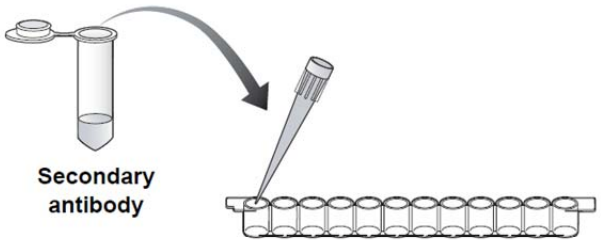
12. Wait 3 minutes for the antibodies to bind to their targets.

13. Wash the unbound primary antibody out of the wells by repeating all of wash step 9 **two** times.

WASH 2x

orange

14. Use a fresh pipet tip to transfer 50 µl of secondary antibody (SA) into all 12 wells of the microplate strip.



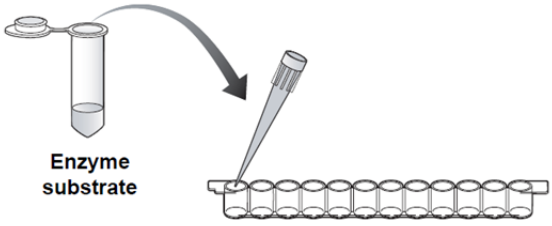
15. Wait 3 minutes for the antibodies to bind to their targets.

16. Wash the unbound secondary antibody out of the wells by repeating wash step 9 **three** times.

WASH 3x

brown

17. Use a fresh pipet tip to transfer 50 µl of enzyme substrate (SUB) into all 12 wells of the microplate strip.



18. Wait 3 minutes. Observe and record the results.