
Biotechnology
CAPTIVATING SCIENCE EDUCATION
Explorer™
BIO-RAD
Professional Development

Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

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ELISA Immuno Exlorer™
HIV/AIDS Diagnostic Tool

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ELISA Immuno Explorer™

HIV/AIDS Diagnostic Tool

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Why Teach ELISA and HIV?

- **Hands-on Immunology**
- **Tangible results**
- **Laboratory extensions**
- **Real-world connections**
- **Link to careers and industry**
- **Standards-based:**
  One lesson integrates multiple standards
  - **Health sciences**
  - **Immunology**
  - **Biodefense**
  - **Immune response –** antibody/antigen interactions
  - **Disease –** infection, detection, transmission

Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

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Scientific Inquiry

- Tapping nature's tool kit to solve human problems
- Use of immunodetection to hunt for proteins
- Use of positive and negative experimental controls
- Interpretation of experimental results

Chemistry of Life

- Enzyme-substrate interactions
- Protein structure and function
- Properties of antigens and antibodies

Genetics

- DNA > RNA > protein — antibodies
- Antibody structure and function
- Antibody production via genetic recombination

Evolution

- Animal immune systems response
- HIV mutation and evolution
- Viral drug resistance
- Biowarfare in nature

Cell and Molecular Biology

- Immune response
- Manufacturing antibodies
- Virology and immunology

Environmental and Health Science

- HIV, mad cow disease, and bird flu testing
- Epidemiology and biodefense
- Drug, pregnancy, and GMOs testing
- Soil, water, air testing

ELISA Immuno Explorer Kit Advantages

- **Lab completed in a 45 min period**
- **Supplies for 48 students** (12 workstations)
- **Comprehensive and flexible curriculum**
- **Compelling real-world links**
- **Striking results**
- **Cost effective**
- **Classroom Safe**

Workshop Time Line

- **Introduction**
- **Human Immunodeficiency Virus**
- **ELISA-HIV Test**
- **Ways the ELISA-Immuno Explorer Kit can be used**

Human Immunodeficiency Virus (HIV)


HIV-1

- First diagnosed in 1981
- Over 20 million deaths worldwide, over a half million in the United States
- Over 40 million currently infected, over a million in the United States
- Half of all new infections are in people younger than 25
- Education has been effective in limiting the spread of HIV/AIDS

HIV Biology

What do we know?


HIV-1

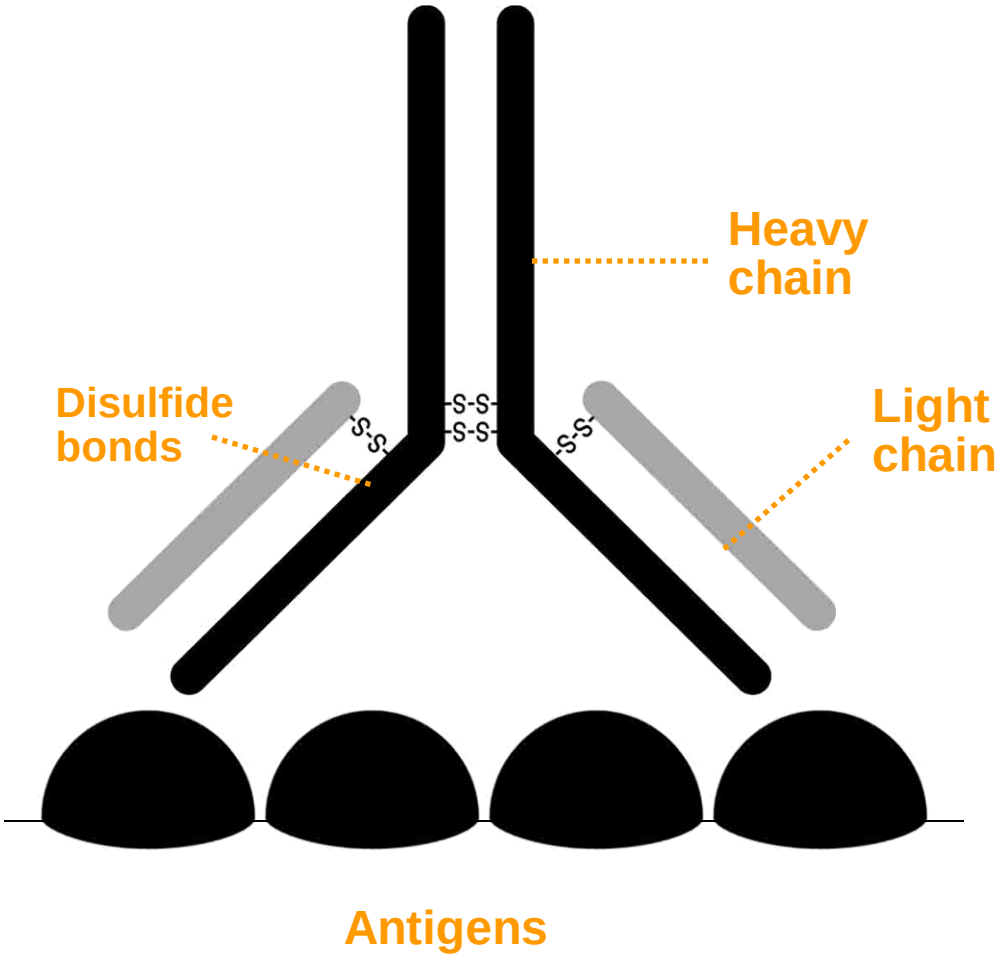
- HIV is an RNA Retrovirus
- Transmitted by exchange of body fluids, sharing needles, or blood transfusion
- Infects T-Cells in the immune system and thus destroys the immune system
- Flu-like symptoms within 1-2 months followed by latent period of up to 10 years
- HIV may have spread from an animal host to humans
- Treated but not cured by drugs which inhibit the action of HIV enzymes
- High error rate of replication (1/2000 nucleotides)

ELISA

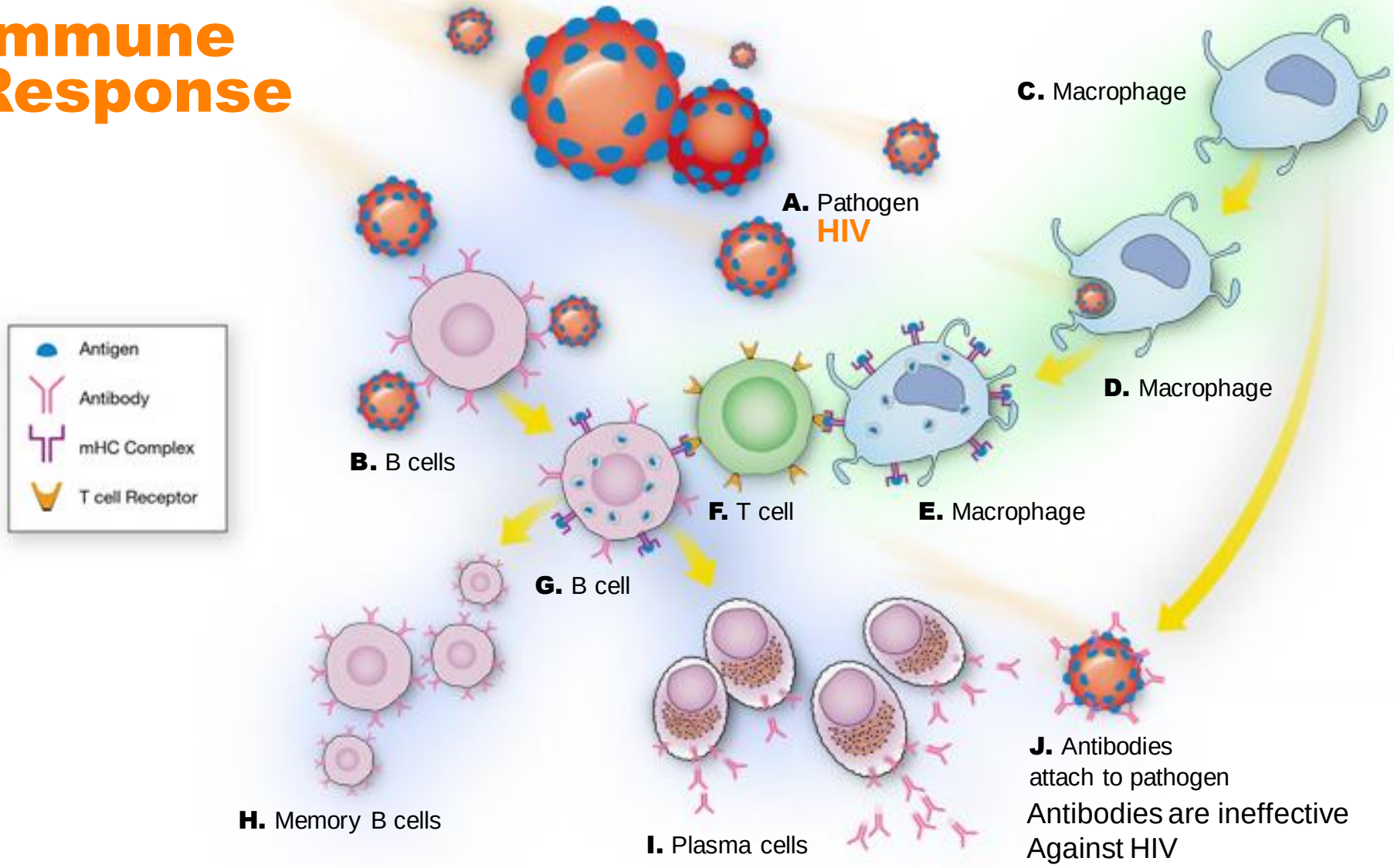
Enzyme-**L**inked
Immuno**s**orbant
Assay

ELISA tests are based on immune system antibody molecules.

Antibody Structure



Immune Response



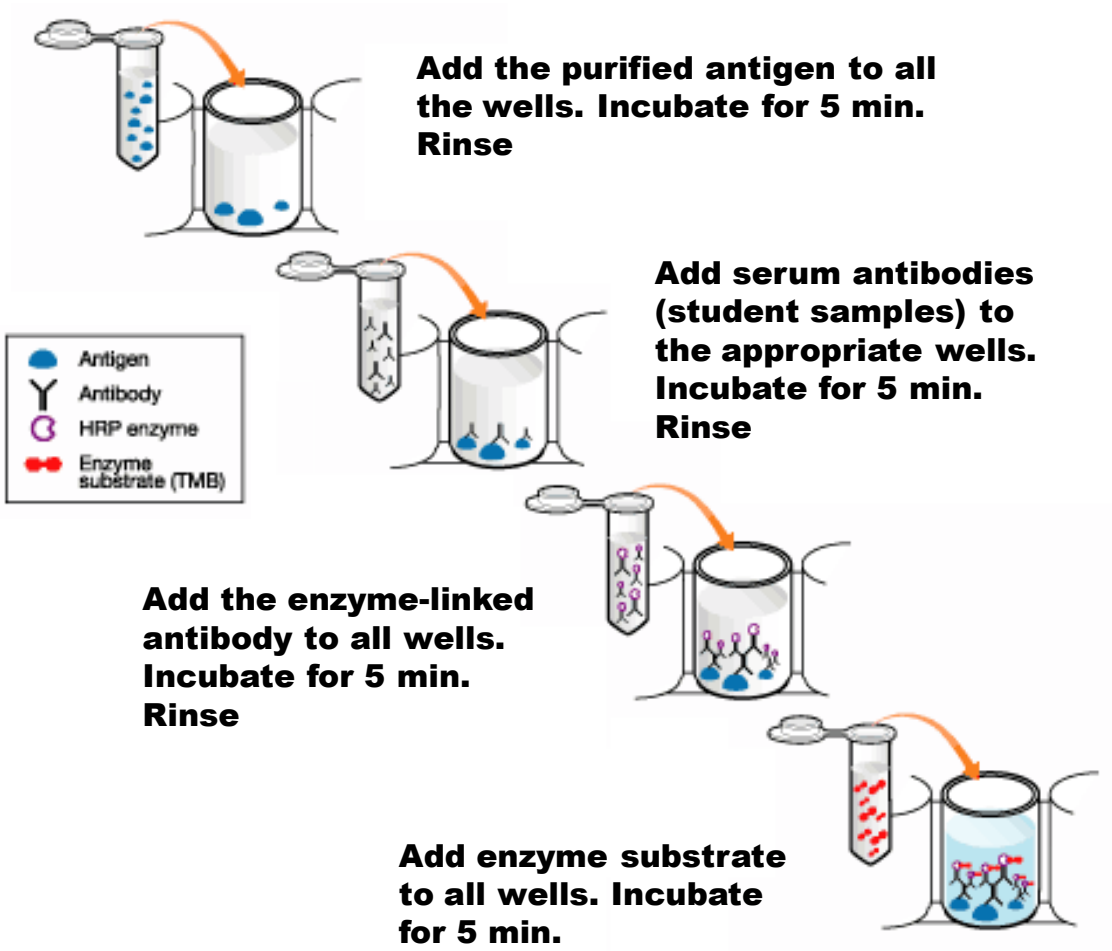
ELISA-HIV Test

Detecting Antibodies in Serum

Protocol III

- After 4-8 weeks of exposure to the HIV virus, the body will have produced a detectable level antibodies (immune response) against HIV
- ELISA (HIV-Test) detects the presence of serum antibodies against HIV protein antigens
- This is how HIV is detected in clinical laboratories
- Most common AIDS test

ELISA Procedures Overview



ELISA ANIMATION

Laboratory Quick Guide

Laboratory Quick Guide

ELISA Antibody Test

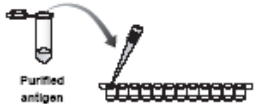
Student Workstation Checklist

One workstation serves 4 students.

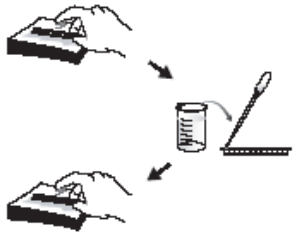
Item (Label)	Contents	Number	(✓)
Yellow tubes	Student test samples (0.25 ml)	4	❑
Violet tube (+)	Positive control (0.5 ml)	1	❑
Blue tube (–)	Negative control (0.5 ml)	1	❑
Green tube (AG)	Purified antigen (1.5 ml)	1	❑
Orange tube (BA)	Secondary antibody (1.5 ml)	1	❑
Brown tube (SUB)	Enzyme substrate (1.5 ml)	1	❑
12-well microplate strips		2	❑
50 µl fixed-volume micropipet or 20–200 µl adjustable micropipet		1	❑
Yellow tips		10–20	❑
Disposable plastic transfer pipet		1	❑
70–80 ml wash buffer in beaker	Phosphate buffered saline with 0.05% Tween 20	1	❑
Large stack of paper towels		2	❑
Black marking pen		1	❑

1. Label the yellow tubes (if necessary) to identify the samples being tested.
2. 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 to identify the samples being tested (3 wells each).

3. Use a fresh pipet tip to transfer 50 µl of purified antigen (AG) into all 12 wells of the microplate strip.
4. Wait 5 minutes for the antigen to bind to the plastic wells.



5. 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 splashing sample back into wells.
   b. Discard the top paper towel.
   c. Use your transfer pipet to fill each well with wash buffer, taking care not to spill over into neighboring wells. Note: the same transfer pipet is used for all washing steps.



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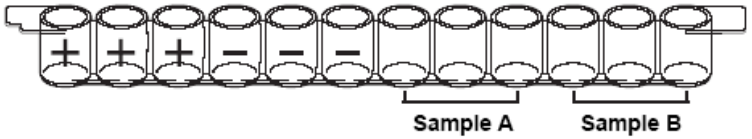
PROTOCOL III
ELISA Antibody Test

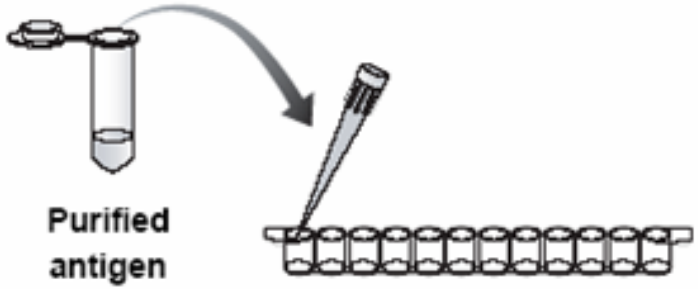
Step One

Label wells and add antigen

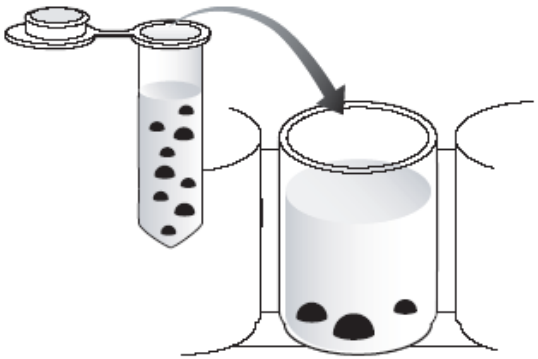
- **Obtain a test-sample**
- **Label the 12-well strip:**
  - **First 3 wells: positive controls "+"**
  - **Next 3 wells: negative controls "-"**
  - **Remaining wells to identify test-samples**



- **Using a new tip transfer 50ul of purified antigen (AG) into all 12 wells**
- **Wait 5 minutes for the antigen to bind**



Microplate Strips

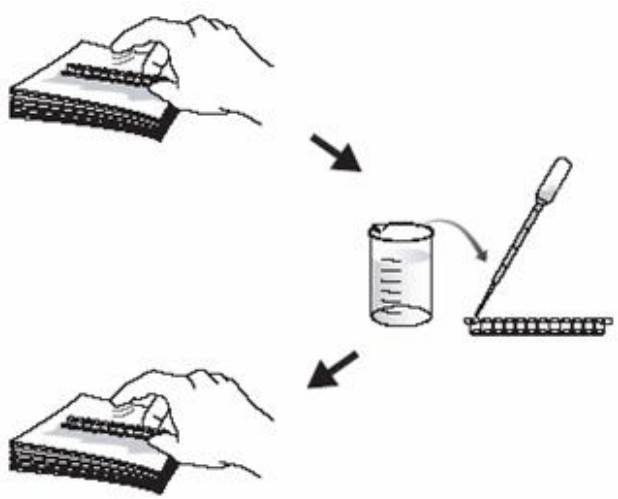


- **Microplate strips are made of polystyrene**
- **Hydrophobic side chains in amino acids bind to the polystyrene wells**

- **No coating is needed**

Step Two

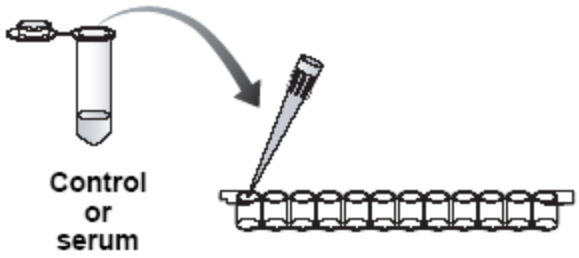
WASH



- Remove samples from wells by firmly tapping them on a paper towel
- Discard the top paper towel
- Using a disposable transfer pipette wash wells with wash buffer
- Remove wash buffer by firmly tapping the wells on a paper towel
- Discard the top paper towel
- Repeat wash step

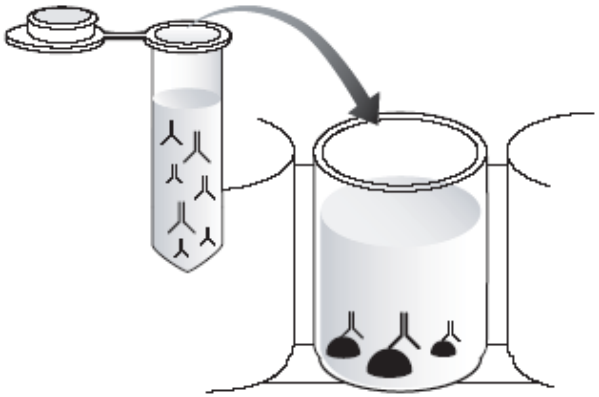
Step Three

Add controls and student serum samples



- Add 50 ul of positive control to 1st 3 wells
- Add 50 ul of negative control to 2nd 3 wells
- Add 50ul of student sample A which represents students serum sample to 3rd set of 3 wells
- Add 50ul of other student sample B which represents that student's serum sample to last 3 wells
- Samples are left in wells for 5 minutes.

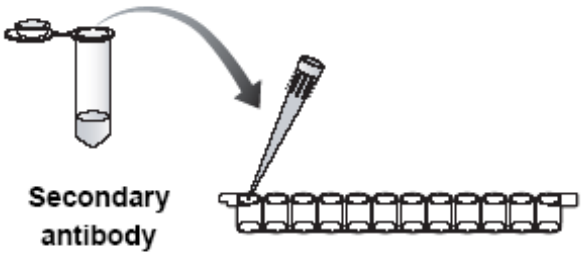
Wash Buffer

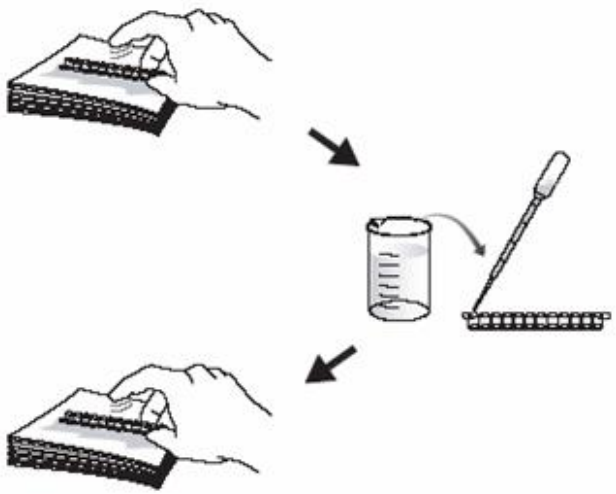


- **Wash buffer contains phosphate buffer saline (PBS) to keep antibodies in a stable environment that helps keep their structure**
- **Also contains Tween 20: a nonionic detergent removes non-specifically bound proteins and coats wells that acts as a blocking agent to reduce background**
- **Antibody will only bind to the simulated HIV antigen**

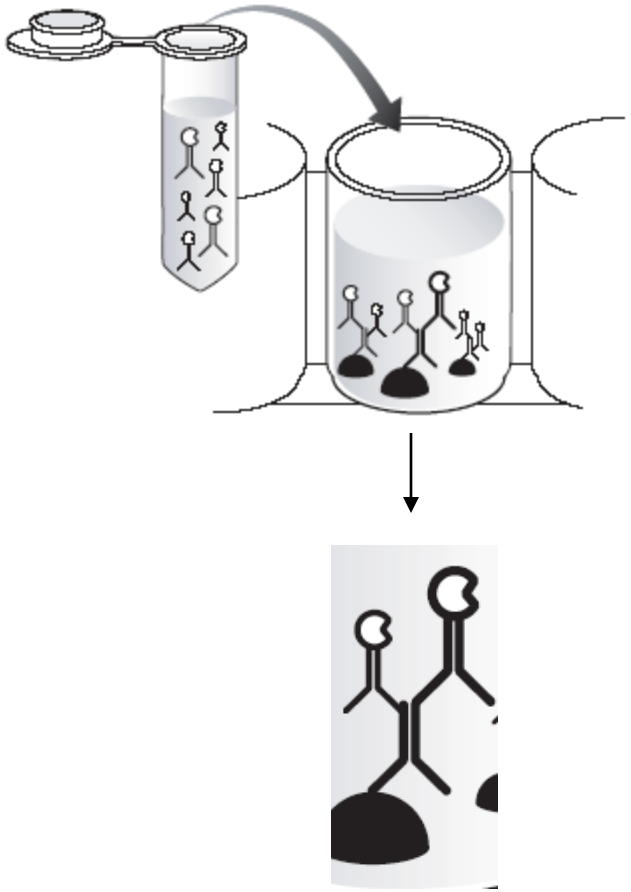
Step Three
Wash antibody and add enzyme-linked antibody

- **Wash the primary antibody from polystyrene wells as before**
- **WASH 2X**
- **Add 50ul of the enzyme-linked secondary antibody to each well**
- **Wait 5 minutes**

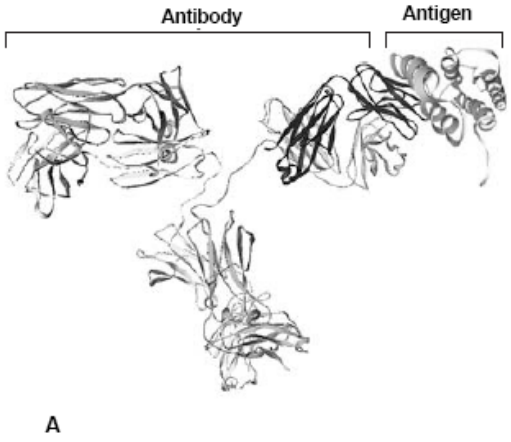


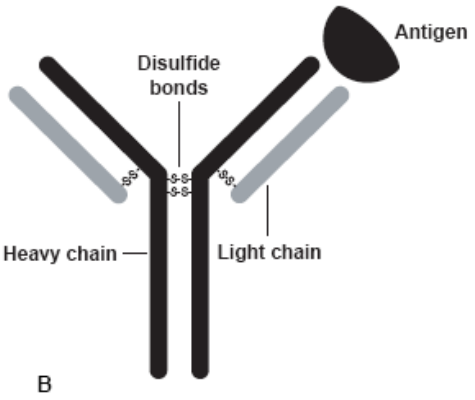


Antibody Specificity

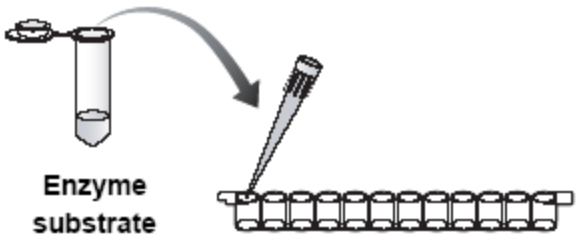


- **Secondary antibody (enzyme-linked antibody) will only bind to the primary antibody (serum antibody)**
- **Secondary antibody specifically recognizes the constant region of the primary antibody**
- **In which wells do you predict this is happening?**

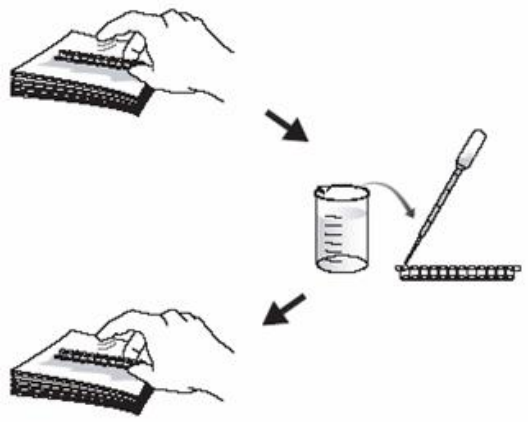




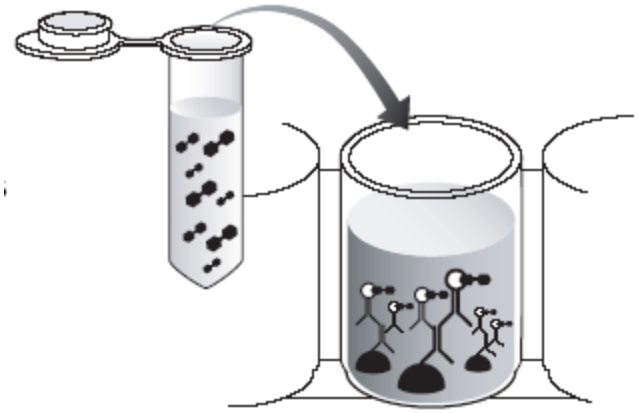
Step Four
Add enzyme substrate



- Wash the enzyme-linked secondary antibody from polystyrene wells as before
- Using a disposable transfer pipette wash wells with wash buffer
- WASH 3X
- Add 50ul of the enzyme substrate to each well
- Wait 5 minutes
- positive samples will begin to turn blue



What are the reagents?



Purified Antigen: Chicken gamma globulin

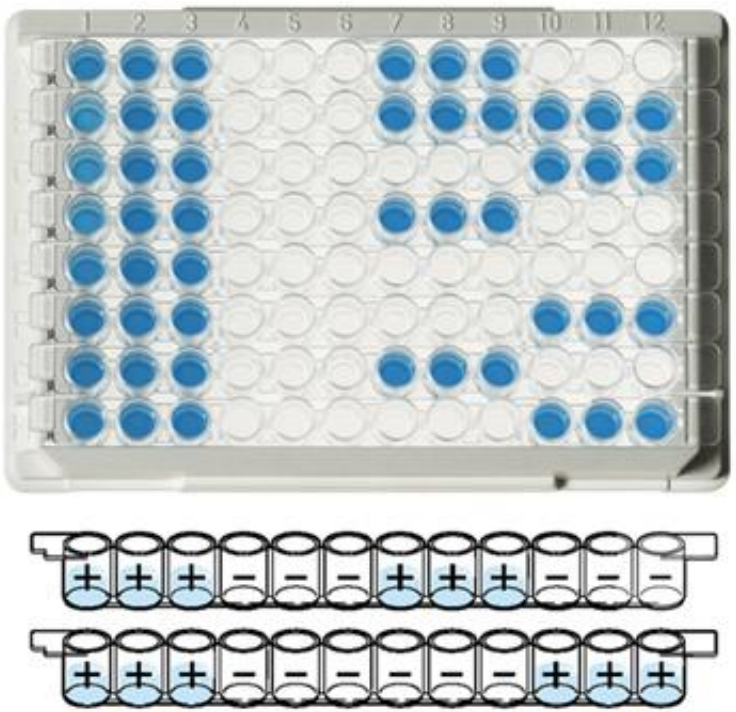
Primary antibody (Serum Samples): Polyclonal anti-chicken antibody made by rabbits

Secondary antibody (enzyme-linked): Polyclonal anti-rabbit antibody made by goats linked (conjugated) to horseradish peroxidase (HRP)

Enzyme substrate: 3,3',5,5' – tetramethylbenzidine (TMB) – a colorless solution that when oxidized by HRP turns blue

ELISA Kit Results

Clear Determination Of Positive And Negative Results



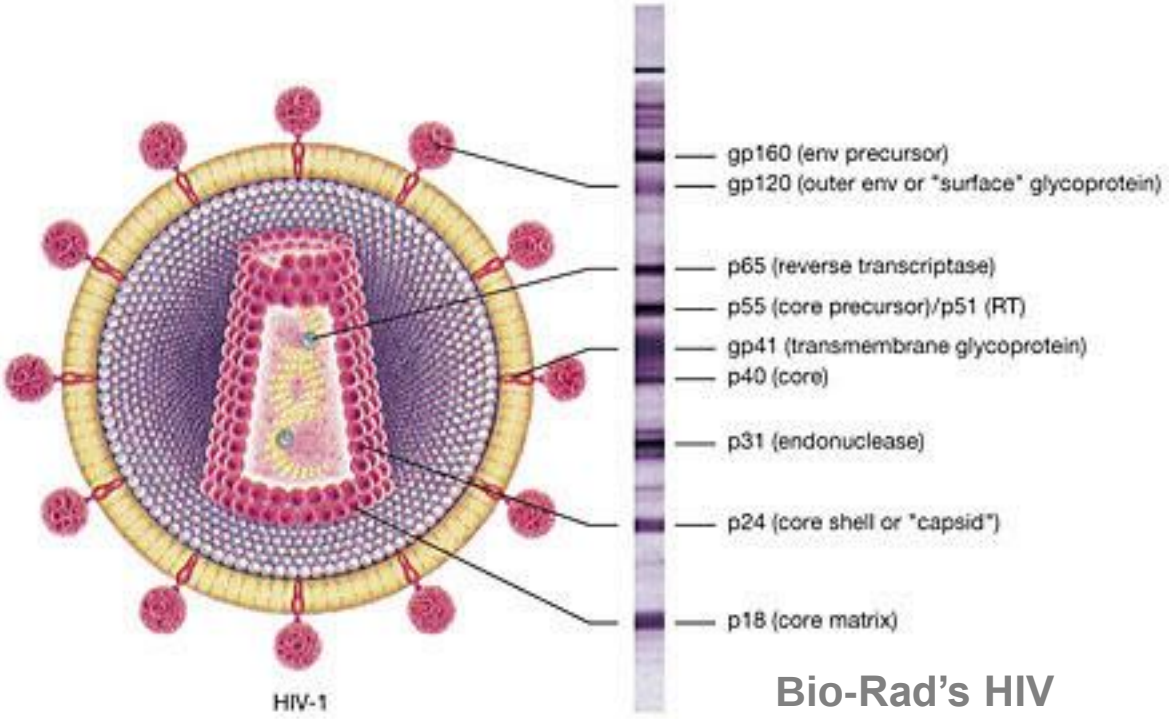
Ways The ELISA Kit Can Be Used

Protocol	Type of ELISA	Real-World Application	Objectives
I	Tracking outbreaks of disease	HIV, Bird Flu and West Nile viruses, common cold, cholera, smallpox, anthrax, and STDs	Epidemiology, disease spread, public health
II	Detecting antigens	Pregnancy, drug, GMO and allergen tests Air food and water testing HIV, smallpox, West Nile and Bird Flu viruses	Uses for antibodies in research, medicine, and consumer goods
III	Detecting antibodies in serum	HIV, Lyme disease, trichinosis, West Nile virus, and Bird Flu virus	Detecting exposure to disease causing agents

Bio-Rad HIV Clinical Diagnostic Kits

HIV can be detected by ELISA or western blot technology. (Both of which are developed using the basis of the mammalian immune system) ELISA tests are very quick. Western Blot tests are slower and more expensive and are used for confirmatory tests.

Bio-Rad's HIV-2 ELISA Kit



Bio-Rad's HIV Western Blot Kit