

# **ELISA (HIV) Lab**



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**This simulated HIV ELISA was developed with the support of the  
Southern California Biotechnology Center Miramar College.**

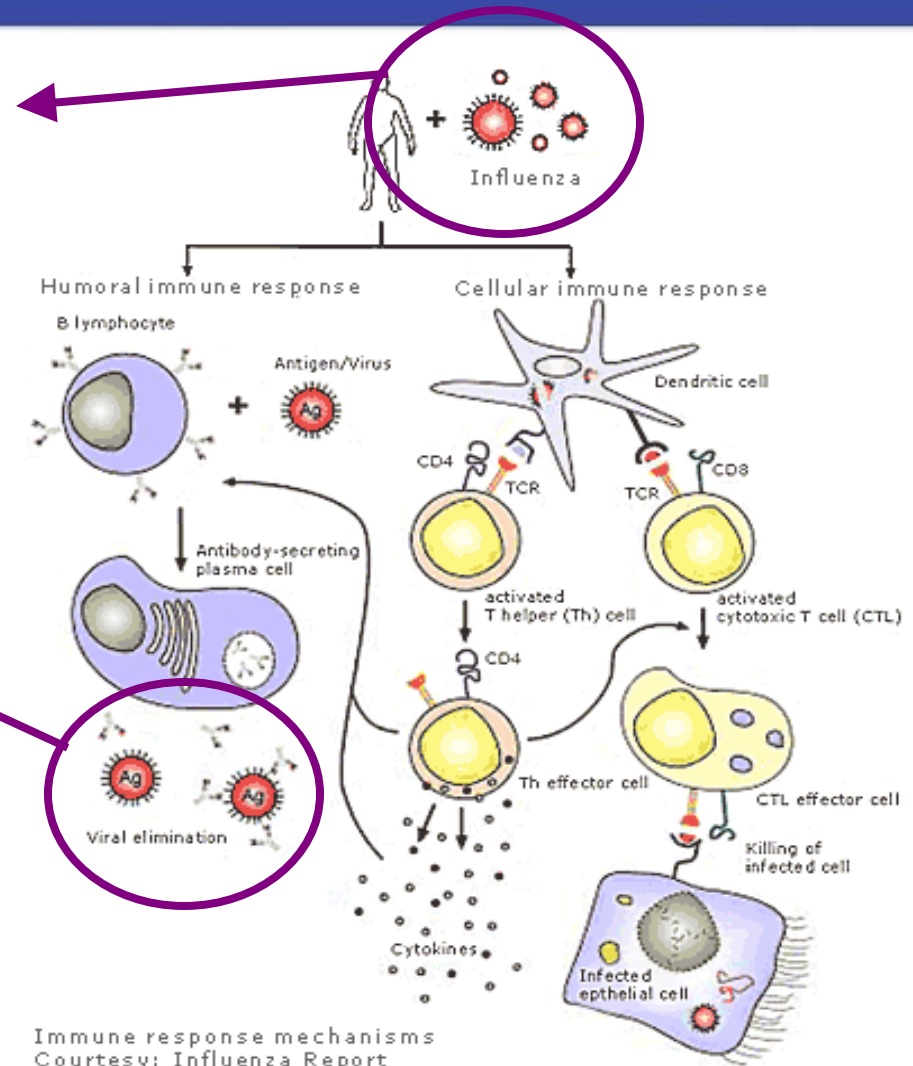


# Background

- **Enzyme-Linked ImmunoSorbent Assay**
- Immunology
  - Detecting the presence of antibodies or antigens
  - Uses the binding of antigens and antibodies to see a change, such as **color**
- **Uses**
  - Pregnancy
  - HIV
  - West Nile
  - Hepatitis
  - Protein recognition

# Immunology Review

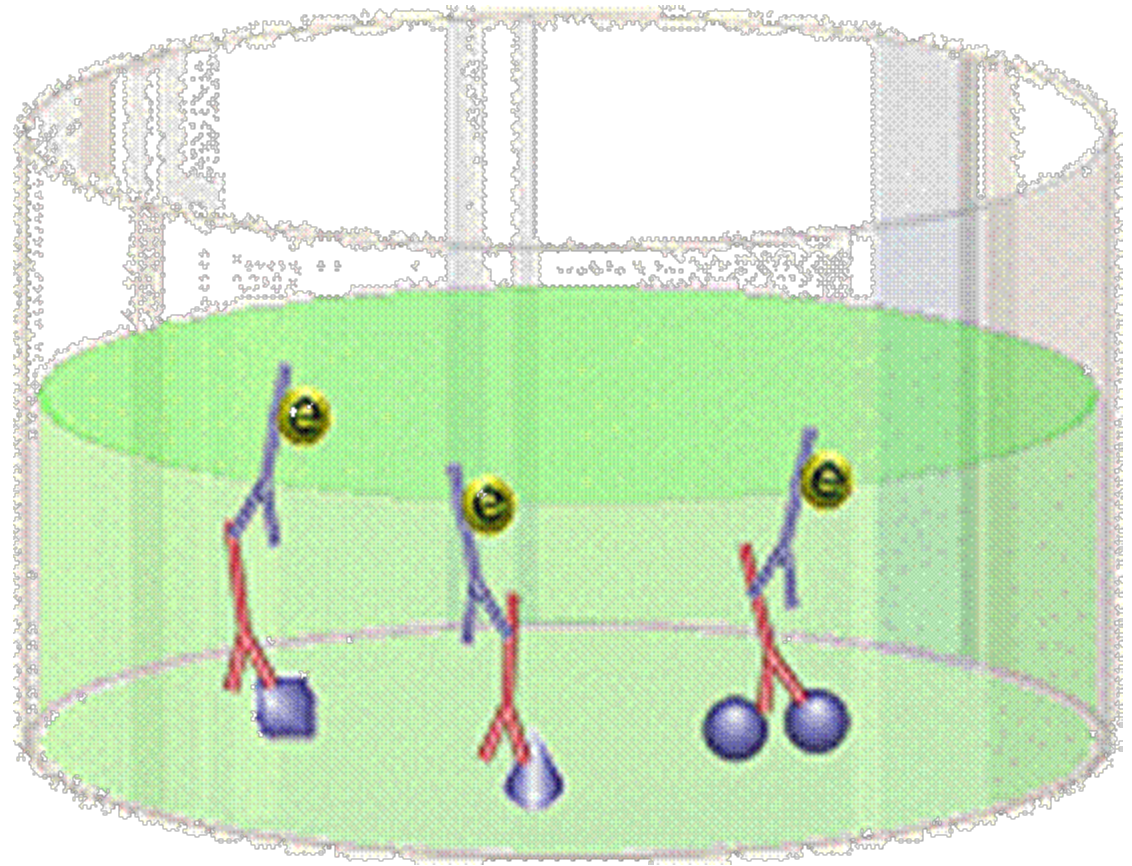
- **Antigen-foreign body** such as virus or bacterium that infects the body, in this lab the HIV virus
- **Antibody-** protein made by immune system in response to a antigen, in this lab what is being tested for





# How it Works

- [Click Here for Demo](#)





# The Scenario

- Work in a clinic
- Blood samples from Patient A and Patient B were collected
- Test for HIV antibodies







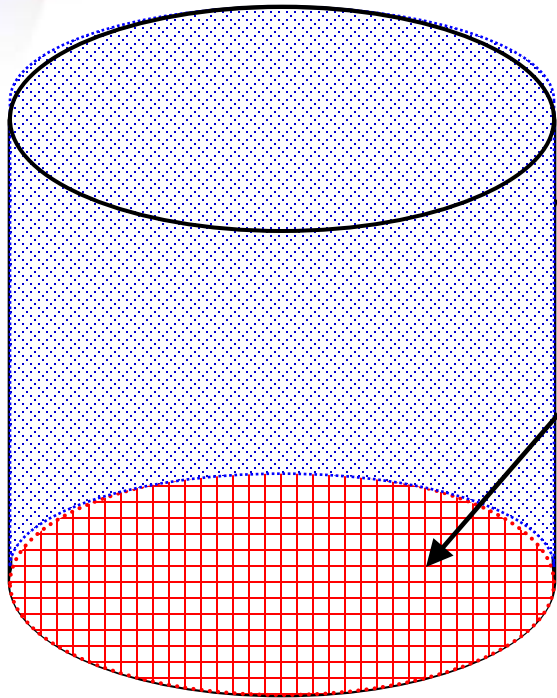
# Your Lab Station

- 4 8-well strips coated
- Rack with
  - Samples:
    - 1 + control (Green)
    - 1 – control (Yellow)
    - 1 Patient A (Pink)
    - 1 Patient B (Blue)
  - 2 2mL 2° antibody (Clear)
  - 2 2mL TMB (Amber)
- Squirt bottle with PBS
- P200 micropipette and tips





# What Has Been Done

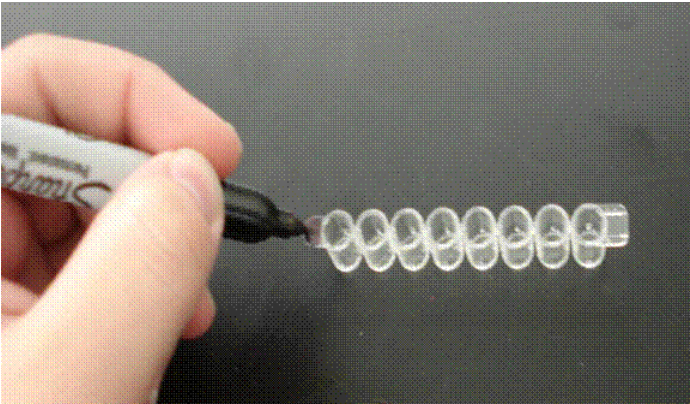


- Wells have been coated with HIV antigens (the **red**). This is NOT real HIV.
- Wells have been blocked so that no other proteins or antigens will get stuck (the **blue**)



# Before Adding the Samples

- Properly orient to the top of your strip
- Mix the contents of each solution by gently inverting the tubes
- **IMPORTANT:** Always change pipette tips between different samples.







# Adding the Sample

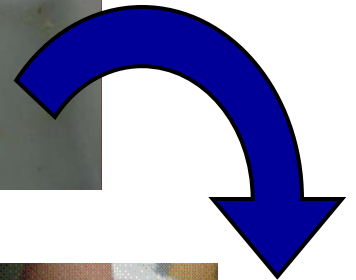
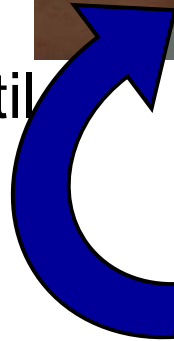
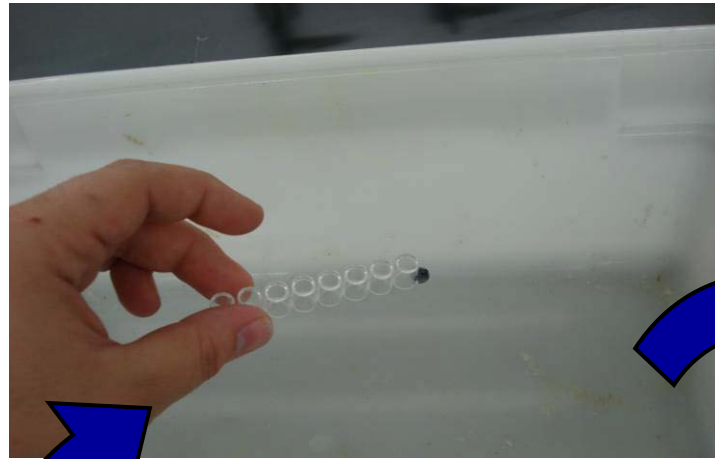
- Add 100  $\mu\text{L}$  of + control (**GREEN**) to wells 1 & 2
- Add 100  $\mu\text{L}$  of – control (**YELLOW**) to wells 3 & 4
- Add 100  $\mu\text{L}$  of Patient A (**PINK**) to wells 5 & 6
- Add 100  $\mu\text{L}$  of Patient B (**BLUE**) to wells 7 & 8
- Let sit 5 minutes





# Washing

- WHY?
  - To rinse away the other proteins and antibodies that are not binding in order to make sure there is not a false positive
- Empty wells by flicking until no more liquid leaves
- Blot dry on paper towels
- Fill wells to the top with PBS buffer
- Empty and blot as above
- Do this wash 4 times





# Add the 2° Antibody

- Add 100  $\mu\text{L}$  of the 2° antibody (CLEAR) to each well
- Empty wells and blot a 4 times (just as before)

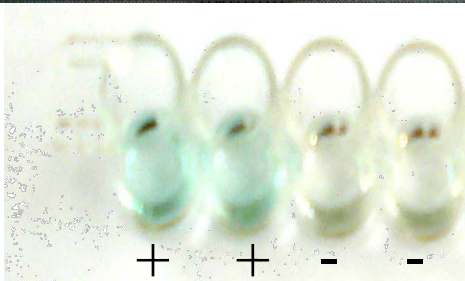




# Detecting the Results

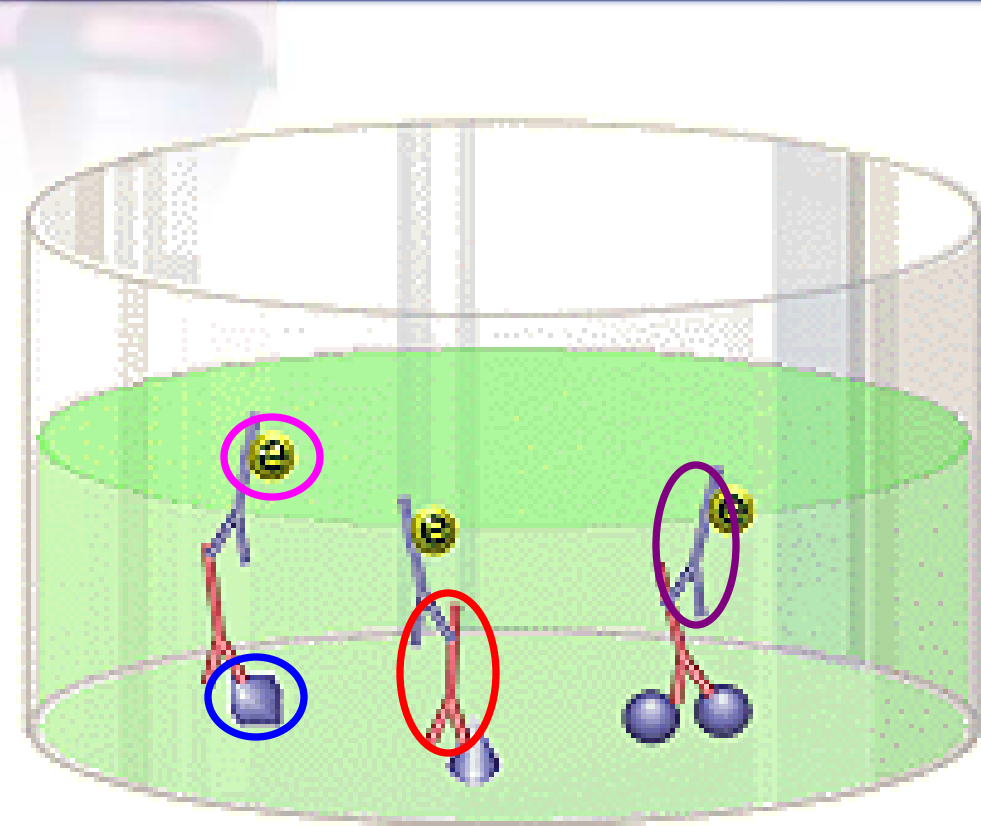


- Add 100  $\mu\text{L}$  of the TMB substrate (**AMBER**) to each well
- Wait for a few minutes
- A color change to blue indicates positive results
- Record your results and answer the questions





# What Happened?



- Substrate (TMB) making color change
- 2° antibody
- Antibody we are testing
- Antigen already coated and present