

Chapter 13- Lab
ELISA- AIDS Simulation Testing
(Flinn Scientific)

Purpose: Students will be conducting the ELISA test to determine which person's are infected with the HIV virus.

Materials:	well plate	Substrate Color Indicator	Unknown Sera 1
	pipet, fine tip	Simulated known HIV positive	Unknown Sera 2
	HIV Simulated Antigens	Simulated known HIV negative	Unknown Sera 3
	Secondary Simulated Antibody		Unknown Sera 4
			Unknown Sera 5

Procedure:

****Use different pipets to prevent cross contamination!!!**

Part 1- The ELISA Test

- 1 Using a pipet, place one drop of **HIV Antigen (HIV-A)** into the two wells in the **KNOWN** row.
- 2 Using a different pipet, place one drop of the **Known Positive Sera (Sr+)** into the well labeled "+" and let this drop set for 1 minute.
- 3 Using a different pipet, place one drop of the **Known Negative Sera (Sr-)** into the well labeled "-" and let this drop set for 1 minute.
- 4 Using a different pipet, add one drop of **Secondary Antibody (2°A)** to both the "+" and "-" wells.
- 5 Using a different pipet, add one drop of the **Substrate Indicator (Sub)** to both the "+" and "-" wells.
- 6 Observe any color changes and record your observations on the data sheet.

Part 2- Testing the Unknowns for the Presence of HIV

****The procedure for this part will be identical to Part 1 with the exception that after Step #1, you will be adding the Unknown Sera's 1-5 to each well numbered 1-5. You will NOT be adding Known Positive Sera or Known Negative Sera to any of the wells.**

- 1 Using a pipet, place one drop of **HIV Antigen (HIV-A)** into wells 1-5 in the **UNKNOWN** row.
- 2 Using a different pipet, place on drop of each **Unknown Sera (U1, U2, U3, U4, U5)** into the appropriate well number.
- 3 Using a different pipet, add one drop of **Secondary Antibody (2°A)** to wells 1-5.
- 4 Using a different pipet, add one drop of the **Substrate Indicator (Sub)** to wells 1-5.
- 5 Observe any color changes and record your observations on the data sheet in the Table in Part 2. For the Table, place a check in the box with the observed test result (positive or negative).

Data Sheet

Part 1

1. Describe a positive ELISA test result for this lab.
 2. Describe a negative ELISA test result for this lab.
 3. What was the purpose for running the ELISA test with a known HIV positive sera and a known HIV negative sera?
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Part 2

Table

Sera	Positive	Negative
1		
2		
3		
4		
5		

1. Which individuals, 1-5, should definitely be tested further (Western Blot Analysis) for HIV infection?
2. Would a person infected with HIV have a positive ELISA test the day after they became infected? Explain