Using Biotechnology to Diagnose HIV/AIDS

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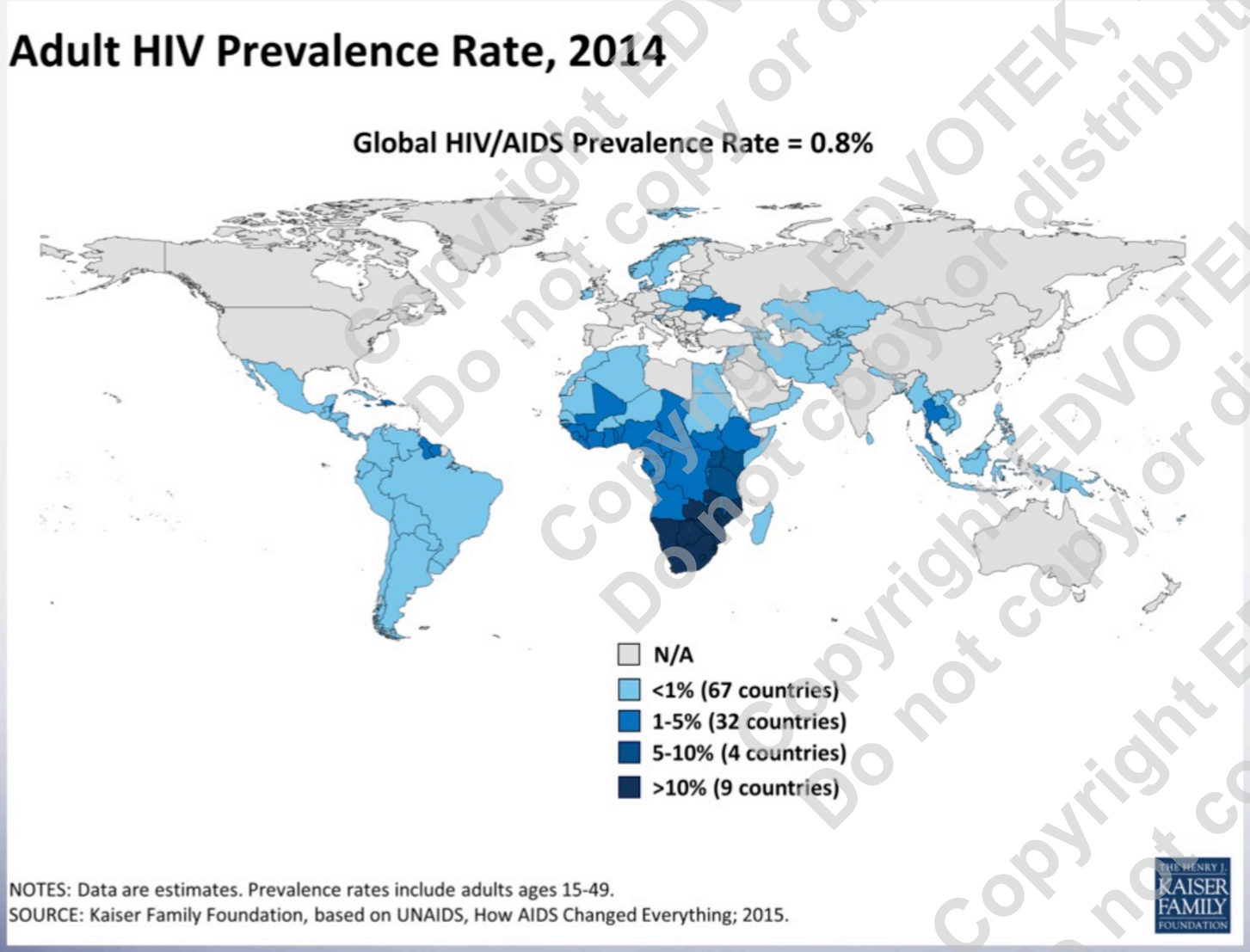
HIV affects millions of people around the world



- HIV/AIDS have become one of the most serious public health challenges
- There are approximately 36.9 million people living with HIV worldwide
- Many people do not have access to care or treatments, and HIV-positive patients are frequently unaware of their infectious status.

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HIV structure and activity

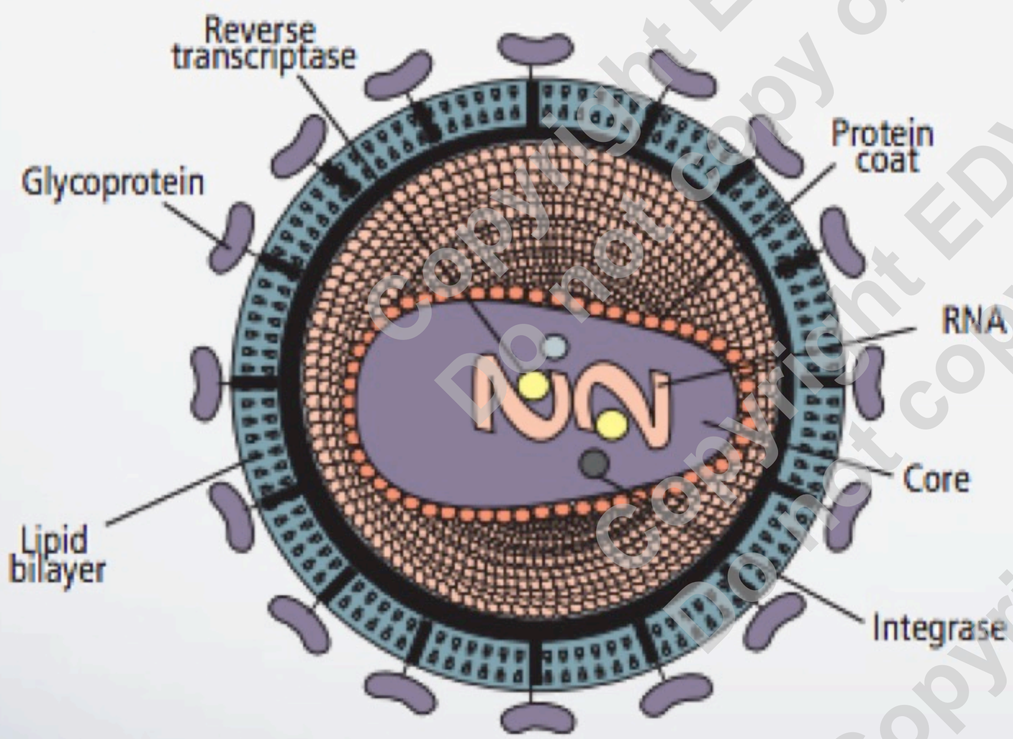

Figure 5: The structure of an HIV virus

- HIV is a lentivirus, a class of retrovirus, containing an RNA genome
- Untreated, HIV can lead to acquired immunodeficiency syndrome (AIDS)
- The HIV virus infects immune cells since as helper T cells (CD4+ cells), macrophages, and dendritic cells.
- Infection from patient-to-patient occurs through the transfer of bodily fluids.

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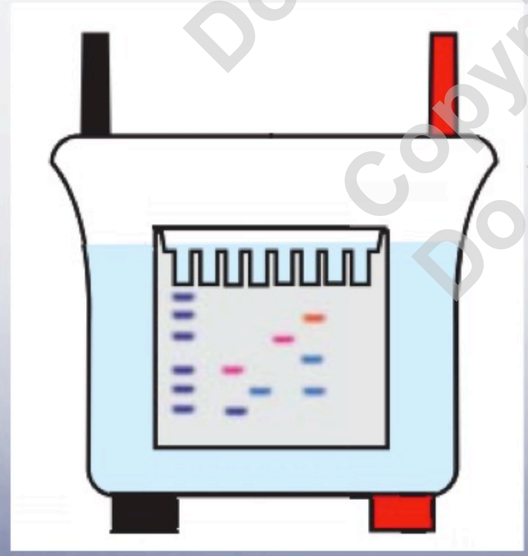
Using Biotechnology to Diagnose HIV/AIDS

1 **ELISA**

2 **SDS-PAGE**



- Early detection of HIV is important for treating the patient and preventing further spread
- Tests require high sensitivity and high specificity
- Current protocols often rely on two tests
  - Initial test using ELISA method
  - Confirmation by SDS-PAGE and Western Blot

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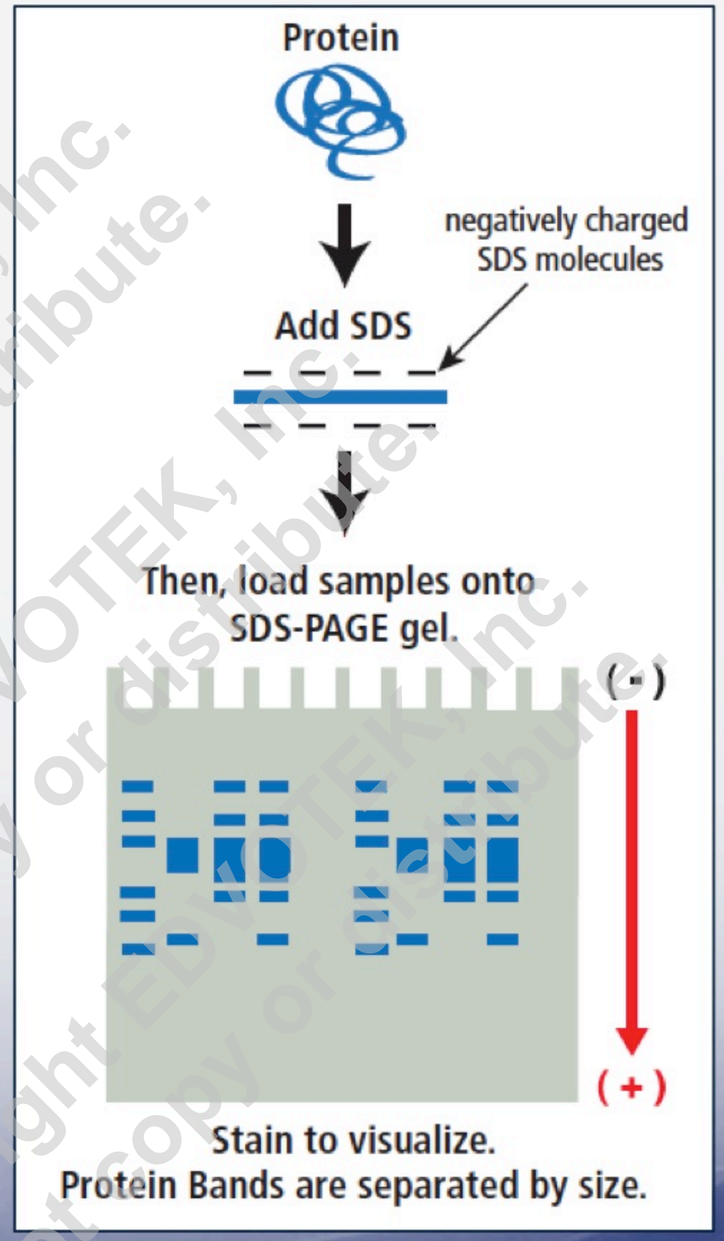
Simulation of HIV Detection by Protein Electrophoresis

- Simulation of patient analysis by SDS-PAGE
- SDS-PAGE is a technique used to separate proteins by size using an electric field
- Proteins will migrate through an acrylamide gel in a predictable manner



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What Do I Need to Perform SDS-PAGE Experiments?

Dual Protein electrophoresis LabStation™ - Cat. #151

- Vertical electrophoresis apparatus
- D.C. power source
- Micropipet or transfer pipet
- Polyacrylamide gels
- Electrophoresis Buffer
- Protein samples
- A way to visualize samples

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Preparing the SDS-PAGE gels for electrophoresis

We will be using pre-cast 12% SDS-PAGE gels for this experiment

1) Remove gel from packaging
2) Remove tape from bottom of gel
3) Gently remove comb
4) Insert gel into tank and submerge in buffer

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Loading our protein samples and running the gels!

Load 20 microliters of sample per well.

1. Standard protein marker
2. Positive Control
3. Patient Sample 1
4. Patient Sample 2
5. Patient Sample 3

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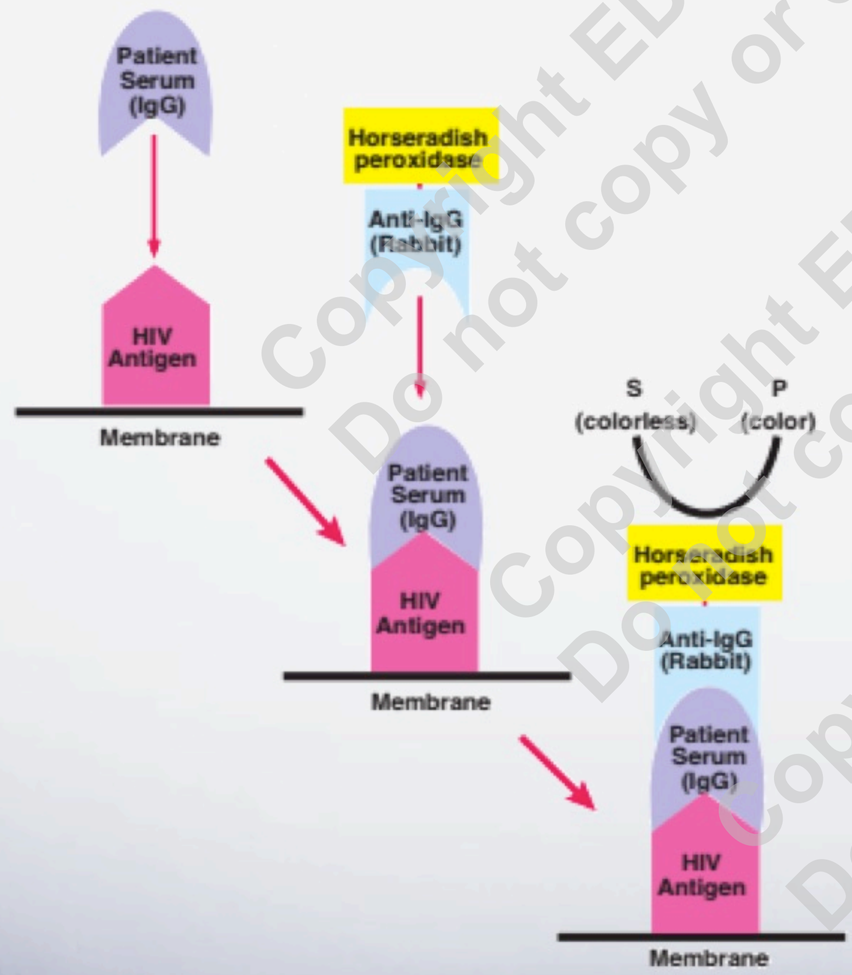
How does the HIV western blot work?



- HIV proteins are separated by size on the polyacrylamide gel
- Once the gel has run the proteins are transferred onto a membrane
- Patient serum samples are prepared and incubated with membrane
  - HIV antibodies in serum will bind to the HIV antigens
- An enzyme-linked secondary antibody is used to reveal the presence of patient antibodies

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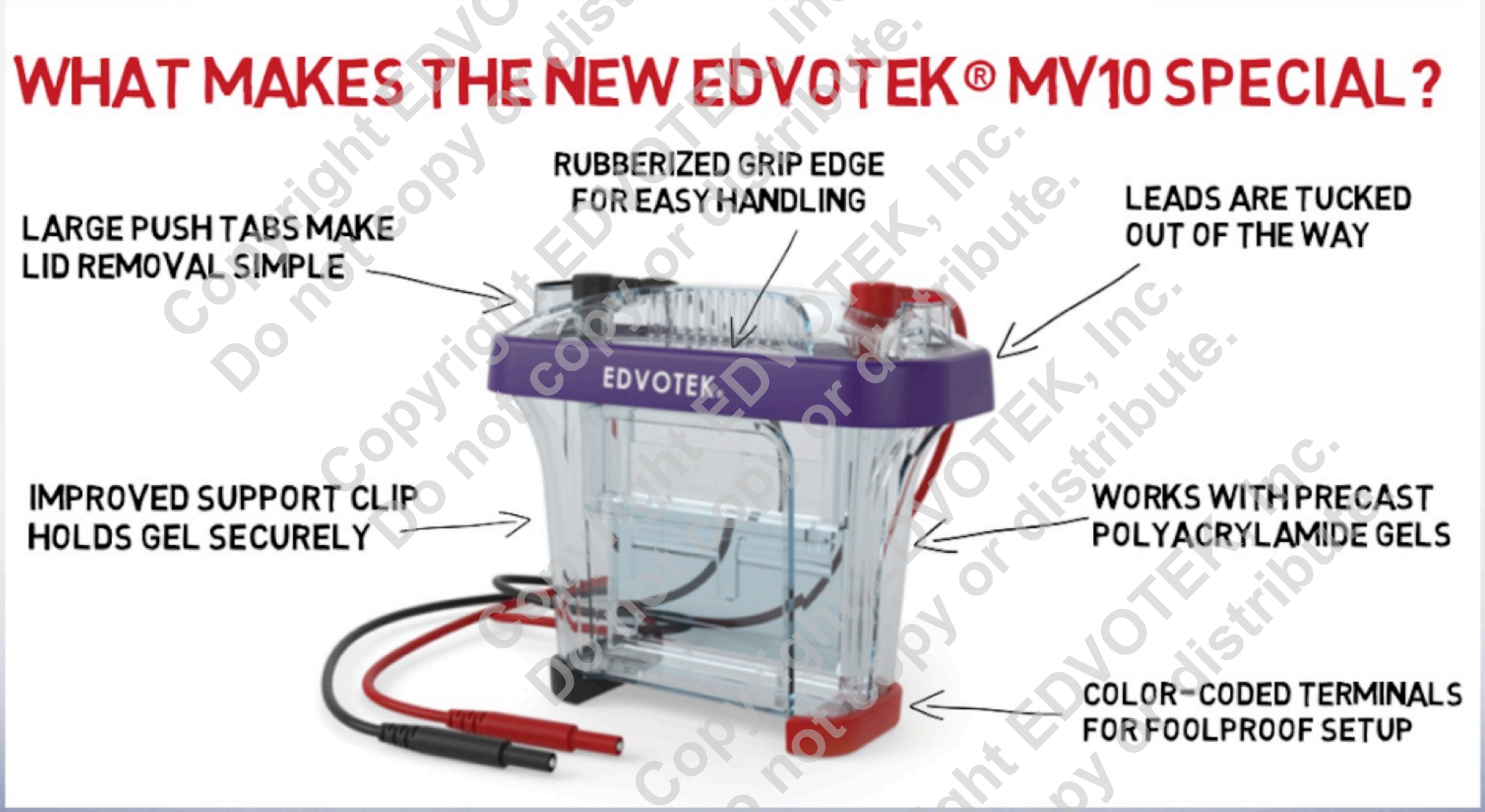
A protein electrophoresis chamber designed for the classroom



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Power Supplies Provide Current for Electrophoresis

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DuoSource™ 150
(75/150 V)

Cat. #5010
TetraSource™ 300
(30-300V)

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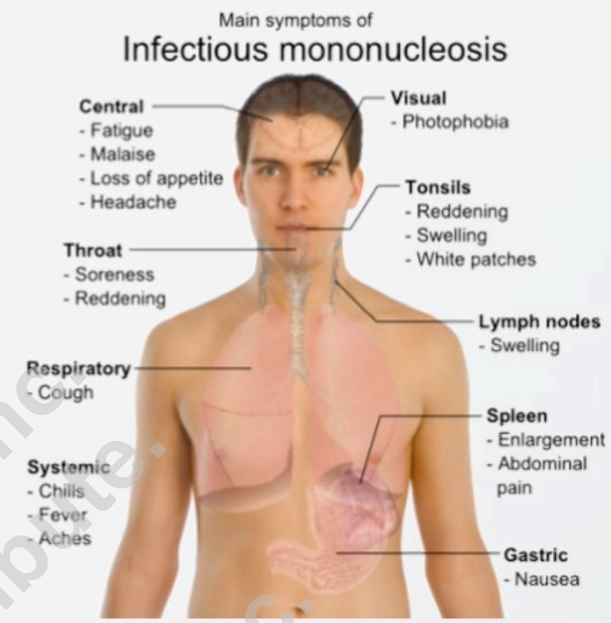
Enzyme Linked Immuno-Sorbent Assay (ELISA)

- Scientists use the ELISA to quantitatively detect the presence of molecules within a sample.
  - Allergen detection – milk, peanut, walnut, egg proteins
  - Hormones – Pregnancy tests
  - Toxicology – drug tests



Cat. #279

- The ELISA is commonly used for medical diagnostics, as it is can be used to identify antigens in blood and other biological samples.
  - Disease detection – WNV, HIV, TB, Infectious Mononucleosis

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Antibodies Distinguish Between "Self" and "Non-self"

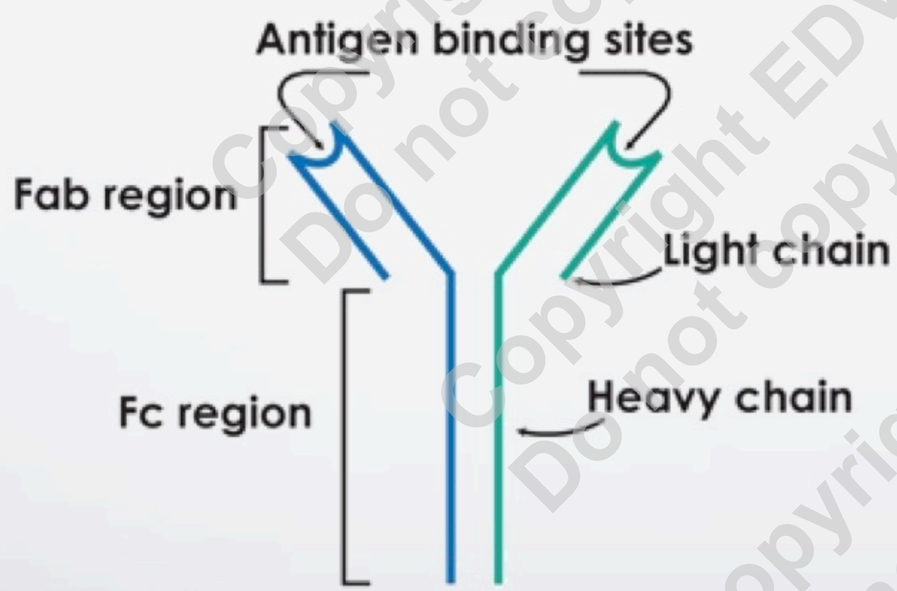

Figure 1:
General Structure of an Antibody

- Antibody molecules comprise four linked polypeptide chains: two "heavy chains" and two "light chains" that are connected by disulfide bonds.
- The amino acid sequence of the antigen binding site is variable, allowing each antibody to recognize a unique **epitope** (a particular location within an antigen).
- Antibodies can be **polyclonal** (heterogeneous mixture) or **monoclonal** (directed against a single epitope).

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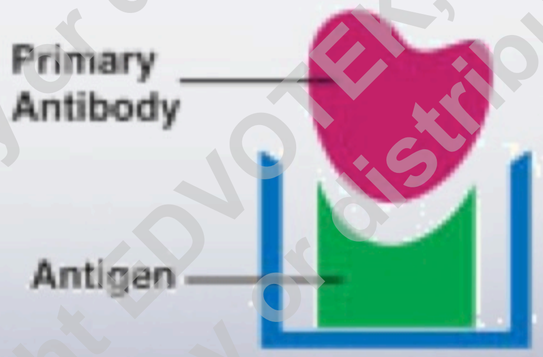
ELISAs Detect Specific Antigens in Complex Mixtures

- **Step 1:** The sample is added to the wells of the microtiter plate, where it adheres to the plastic through hydrophobic and electrostatic interactions.
- **Step 2:** After washing away any unadsorbed sample, the wells are "blocked" with a protein-containing buffer to prevent non-specific interactions.
- **Step 3**: The **primary** antibody is added to the wells, where it recognizes the antigen and binds through electrostatic interactions. Excess antibody is washed out of the wells.



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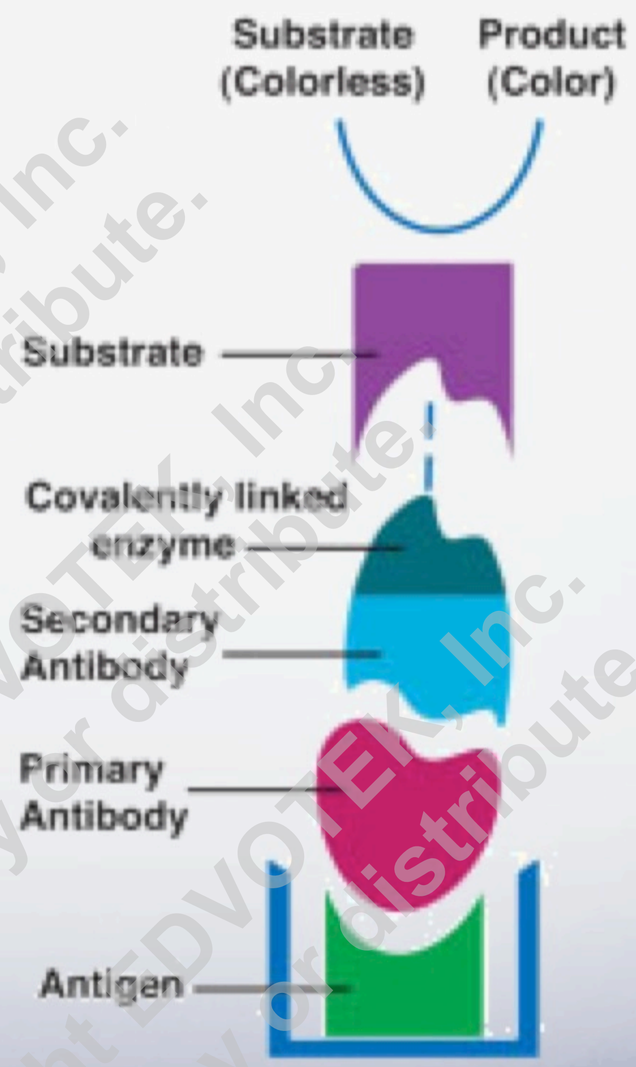
ELISAs Detect Specific Antigens in Complex Mixtures

- **Step 4:** The **secondary** antibody, which recognizes the primary antibody, is added to the wells. If the antibody can bind with the antigen, it will remain in the well after the wells are washed.
- **Step 5:** The enzyme-linked secondary antibody allows us detect the presence of the antibody-antigen complex when the substrate is added to all the wells.
  - Chromogenic detection
  - Fluorogenic detection
  - The ELISA detects low antigen concentrations because each enzyme can produce many product molecules.



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The ELISA can be Quantitative or Qualitative

- Qualitative: "Yes or No?"
  - Results are determined by comparing to a known standard.
- Quantitative: "How much?"
  - A "standard curve" is generated using known concentrations of the target molecule
  - The experimental sample is assayed and the result is compared to standard curve

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Quantitative ELISA #278

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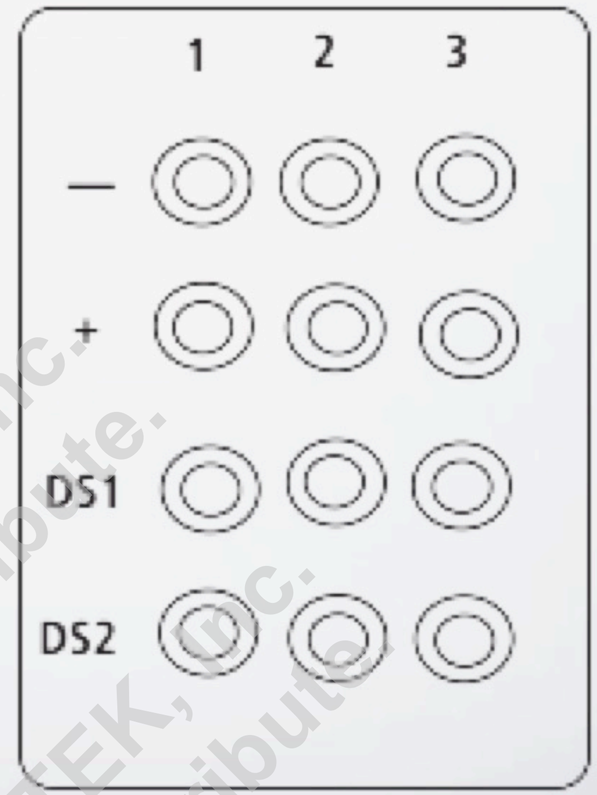
Simulation of HIV Detection by ELISA

LABEL MATERIALS:

1. Label wells: "-", "+", "DS1", and "DS2" 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: "-", "+", "DS1", "DS2", "PBS", and "Sub". These are designated for adding samples and removing washes - save these pipets!



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Simulation of HIV Detection by ELISA

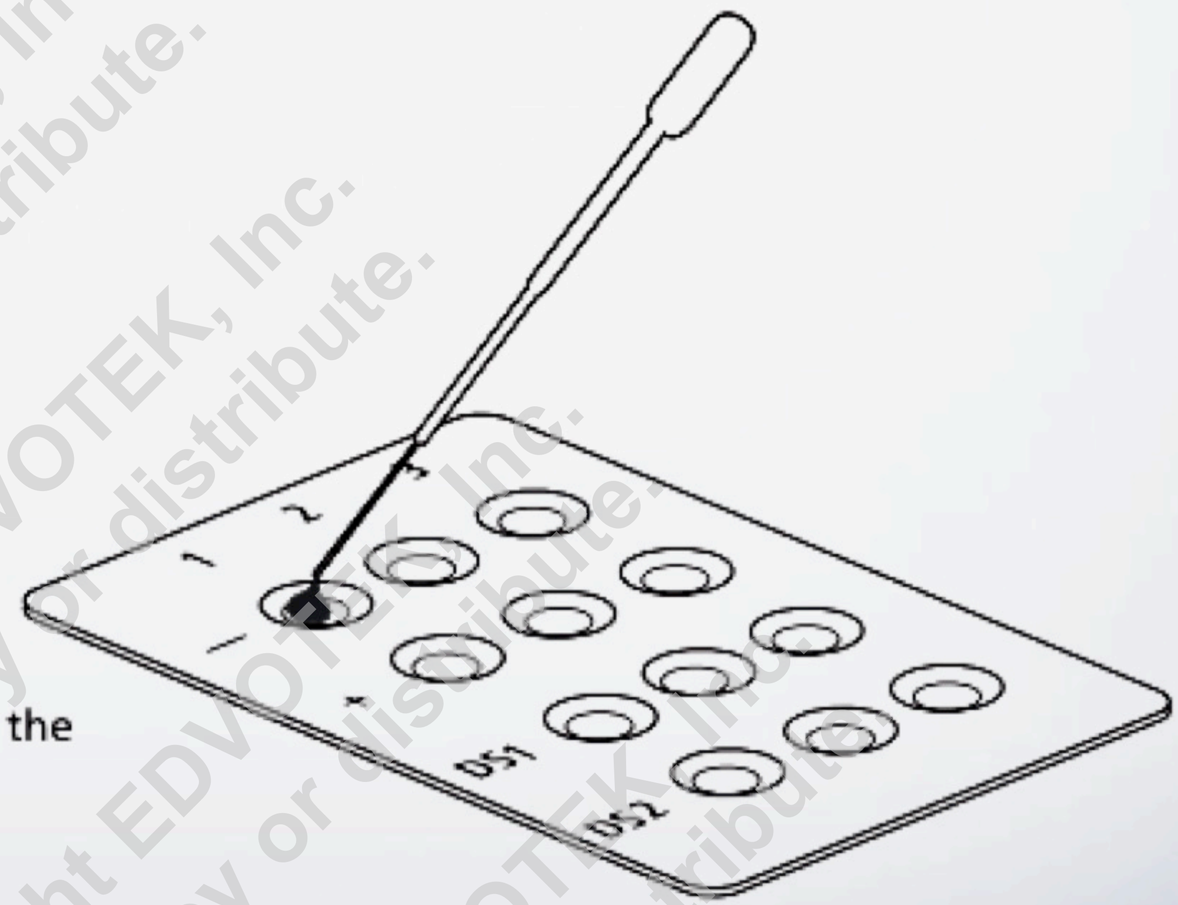
ANTIBODY CONTROLS AND SAMPLES:

3. Add 3 drops of Negative Control to all three wells in the 1st Row.

4. Add 3 drops of Positive Control to all three wells in the 2nd Row.

5. Add 3 drops of Donor Serum 1 to all three wells in the 3rd Row.

6. Add 3 drops of Donor Serum 2 in all three wells in the 4th Row.



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Simulation of HIV Detection by ELISA

7. Incubate for 10 minutes. 37°C

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.

SUBSTRATE:

10. Add 0.1 ml or 5 drops of the substrate solution to all of the wells.

10. Incubate for 10 minutes. 37°C

ANALYSIS:

12. Remove the plate for analysis.

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Simulation of HIV Detection by ELISA

1. Label the plate.
2. Label the pipets.
3. Add three drops of the Negative Control to the first row.
4. Add three drops of the Positive Control to the second row.
5. Add three drops of Patient Sample A to the third row.
6. Add three drops Patient Sample B to the fourth row.
7. Add three drops of Patient sample C to the fifth row.
8. Incubate for 10 minutes.
9. Remove liquid using the transfer pipet designated for each row.
10. Wash each well by adding the PBS buffer to each well until it is almost full (~ 8 drops). Remove all the PBS from the wells. Do not allow buffers to spill over into adjacent wells.
11. Add five drops of the substrate solution into each well
12. Incubate for 10 minutes.
13. Analyze your results.

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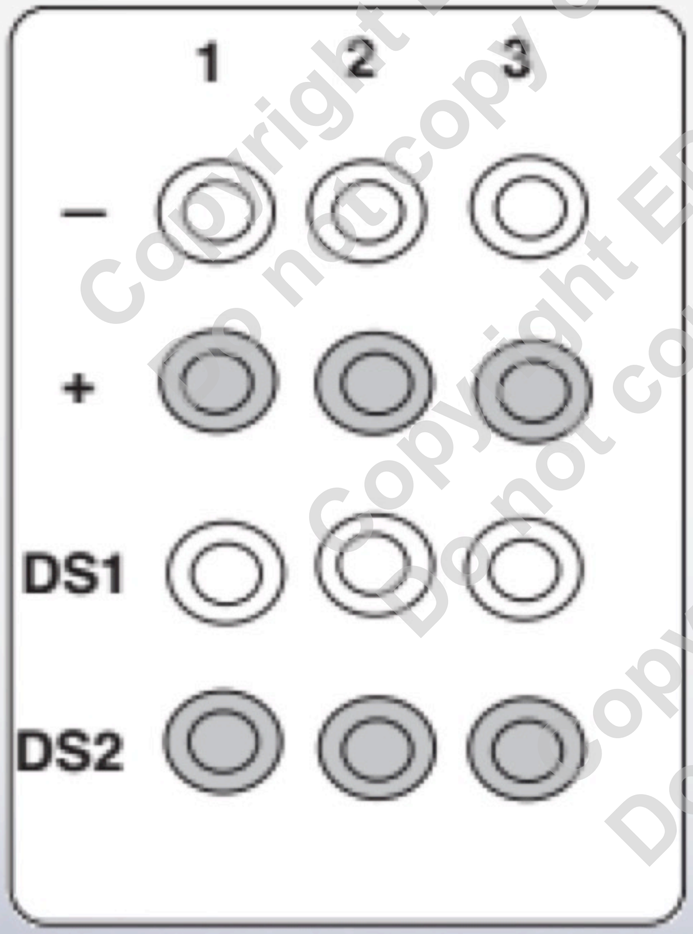
Identification of HIV with the ELISA



- Row A – Negative Control (-)
- Row B – Positive Control (+)
- Row C –Donor Serum 1 (DS1)
- Row D – Donor Serum 2 (DS2)

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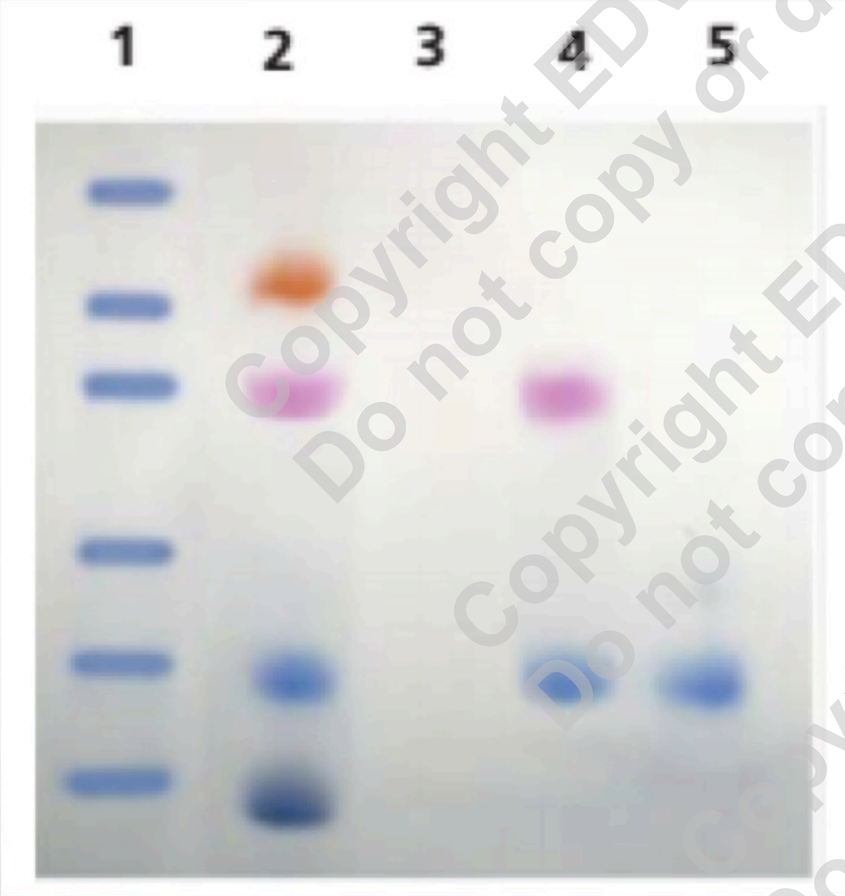
Analyzing the SDS-PAGE gels



- Carefully remove the gel from the electrophoresis chamber
- Using a spatula, gently pry the plastic plates apart to free the gel
- Gently transfer the gel into a plastic weigh-boat for analysis

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Analyzing the SDS-PAGE gels

Standard Protein Marker Molecular Weights
94,000 Da
67,000 Da
38,000 Da
30,000 Da
20,000 Da
14,000 Da

Lane	Sample Name	Number/Size of bands	Diagnosis
1	Standard Protein Marker	----------	----------
2	Positive Control	4 bands (72kD, 38kD, 20kD, 14kD)	HIV positive
3	Patient 1	0 bands	HIV negative
4	Patient 2	2 bands (38kD, 20kD)	HIV positive
5	Patient 3	1 band (20kD)	Inconclusive – should be retested by doctor

Protein Size	HIV Protein Name	Category	Protein Description
72,000 Da	p65	Enzyme	Reverse Transcriptase
38,000 Da	p41	Transmembrane	Envelope protein
20,000 Da	p24	Structural	Capsid protein
14,000 Da	p18	Structural	Matrix protein

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Using Biotechnology to Diagnose HIV/AIDS

- HIV is a major public health concern worldwide
- Early detection is important for treatment of the disease in positive patients
- Detection relies on a combination of screening (ELISA) and confirmatory (Western Blot) tests.
- ELISA tests rapidly screen patient samples for the presence of viral antigens
- Western blot tests are used to confirm the presence of a combination of vial proteins

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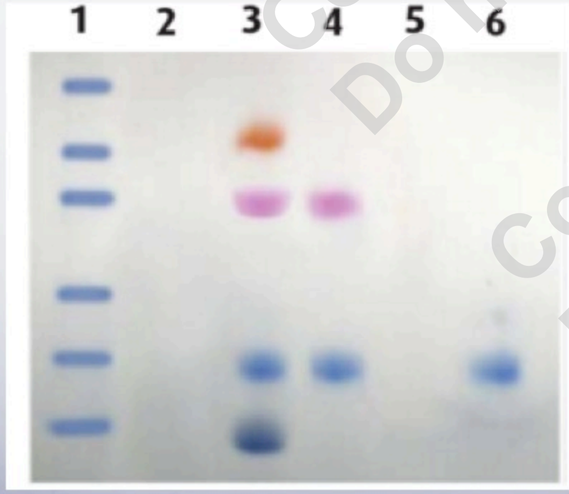
AIDS Kit I: Simulation of HIV Detection by ELISA (271)

Detecting the Silent killer: Clinical Diagnosis of Diabetes (280)



AIDS Kit III: Simulation of HIV Detection by Protein Electrophoresis (151)

Cancer Gene Detection (115)

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