Cihan University/ Sulaimaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 6: Enzyme-linked Immunosorbent Assay (ELISA Techniques)

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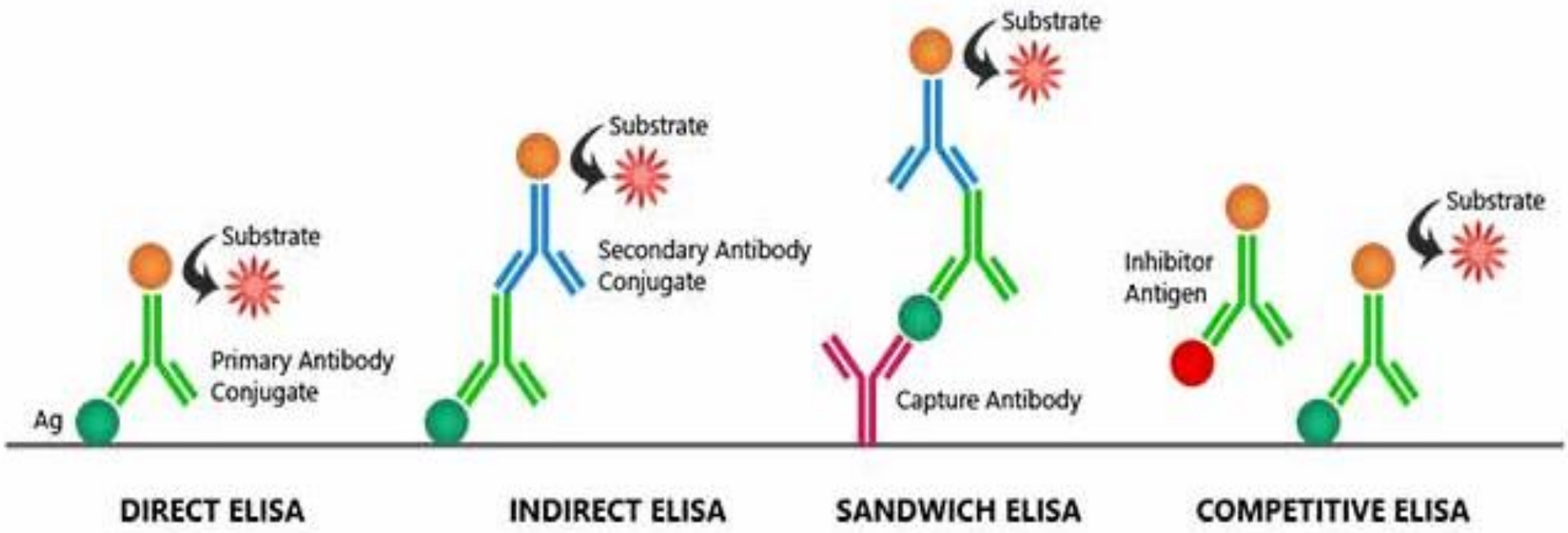
Enzyme Immunoassays (EIAs)

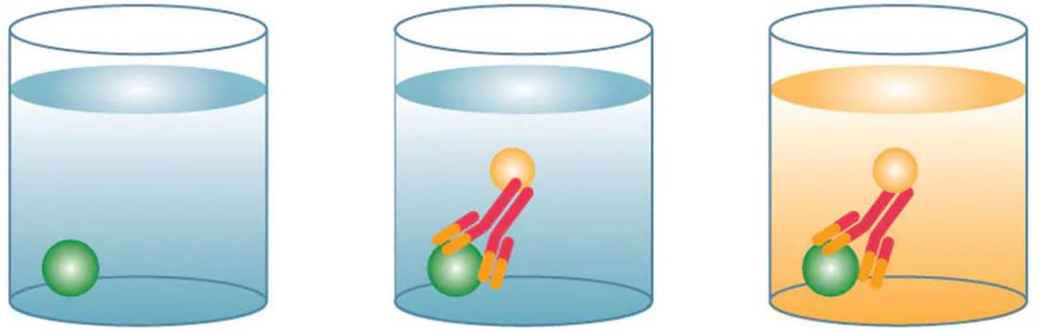
- Using enzymes as labels, were developed as alternatives to RIA.
- Enzymes are naturally occurring molecules that catalyze biochemical reactions. They can convert reagent substrates to produce chemically modified products that can be detected.
- Substances produced and detected in this method include colored or visible light, ultraviolet (UV) light, fluorescent light, and luminescence.
- These methods have sufficient analytical sensitivity for many clinical tests and eliminate the concerns of disposal problems or health hazards associated with radioactive isotopes.
- Because one molecule of enzyme can generate many molecules of product, the addition of an enzyme label further improves analytical sensitivity.
- Common enzymes used as labels in EIA include **horseradish peroxidase (HRP)**, **alkaline phosphatase (ALP)**, **β-D galactosidase**, and **glucose-6-phosphate dehydrogenase (G6PDH)**.

Types of ELISA



Direct ELISA

- In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein.
- Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample.
- Since only one antibody is used in a direct ELISA, they are less specific than a sandwich ELISA.
- When to Use: Assessing antibody affinity and specificity.
- Investigating blocking/inhibitory interactions.

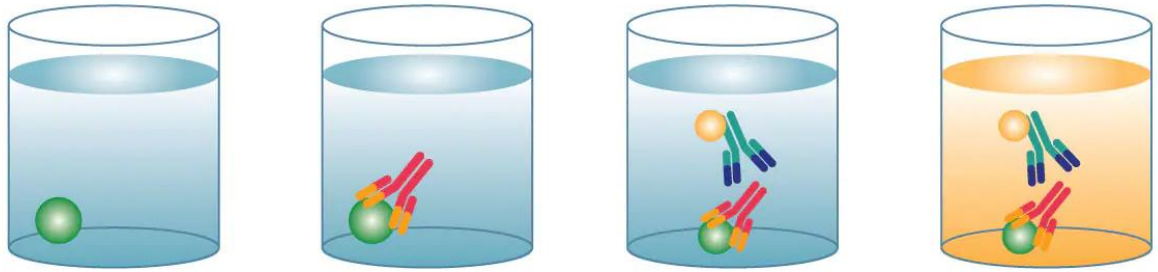


- **Advantages:**
  - ✓Fast and simple protocol
- **Disadvantages:**
  - ✓Less specific since you are only using 1 antibody.
  - ✓Potential for high background if all proteins from a sample are immobilized in well.

https://www.youtube.com/watch?v=pwBqkkjd80A

Indirect ELISA

- An indirect ELISA is similar to a direct ELISA in that an antigen is immobilized on a plate, but it includes an additional amplification detection step.
- First, an unconjugated primary detection antibody is added and binds to the specific antigen.
- A conjugated secondary antibody directed against the host species of the primary antibody is then added.
- Substrate then produces a signal proportional to the amount of antigen bound in the well.
- When to Use: Measuring endogenous antibodies.
- **Advantages:**
- Amplification using a secondary antibody



- **Disadvantages:**
- Potential for cross-reactivity caused by secondary antibody

https://www.youtube.com/watch?v=aLuJ66BVQhU

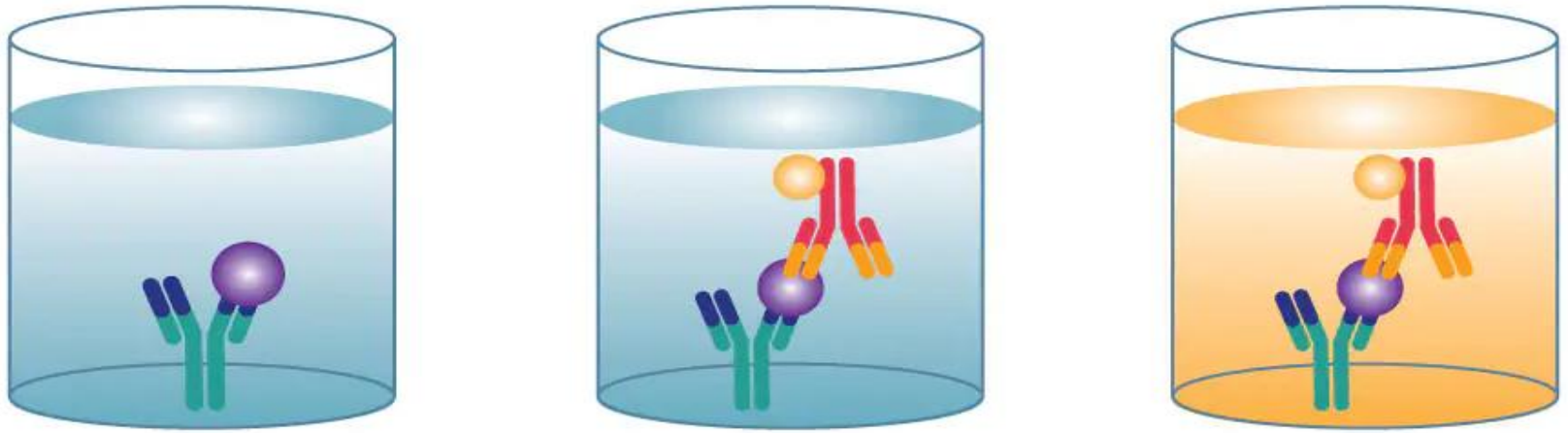
Sandwich ELISA

- Sandwich ELISAs are the most common type of ELISA.
- Two specific antibodies are used to sandwich the antigen, commonly referred to as matched antibody pairs.
- Capture antibody is coated on a microplate, sample is added, and the protein of interest binds and is immobilized on the plate.
- A conjugated-detection antibody is then added and binds to an additional epitope on the target protein.
- Substrate is added and produces a signal that is proportional to the amount of analyte present in the sample.
- It is highly specific, since **two antibodies** are required to bind to the protein of interest.
- When to Use: Determining analyte concentration in a biological sample.

https://www.youtube.com/watch?v=Gf259e8ch7c

Sandwich ELISA

- **Advantages:**
  - ✓Highest specificity and sensitivity.
  - ✓Compatible with complex sample matrices.
- **Disadvantages:**
  - ✓Longer protocol.
  - ✓Challenging to develop.



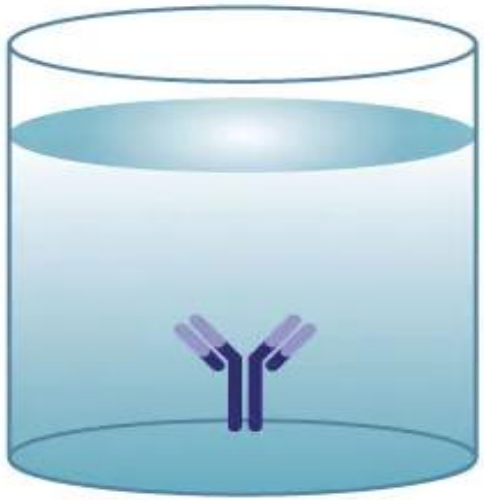
Competitive ELISA

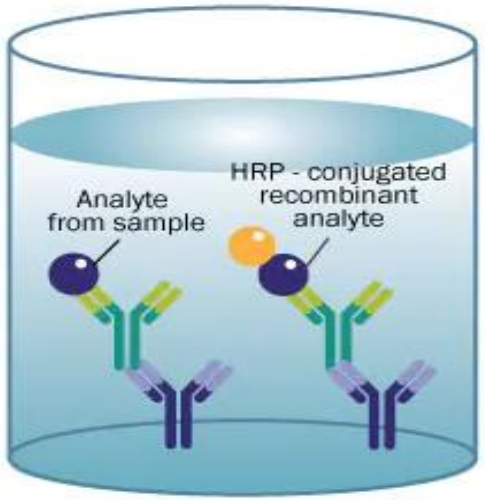
- Competitive ELISAs are commonly used for small molecules, when the protein of interest is too small to efficiently sandwich with two antibodies.
- Similar to a sandwich ELISA, a capture antibody is coated on a microplate.
- Instead of using a conjugated detection antibody, a conjugated antigen is used to complete for binding with the antigen present in the sample.
- The more antigen present in the sample, the less conjugated antigen will bind to the capture antibody.
- Substrate is added and the signal produced is **inversely proportional** to the amount of protein present in the sample.
- When to Use: Determining concentrations of a small molecules and hormones.

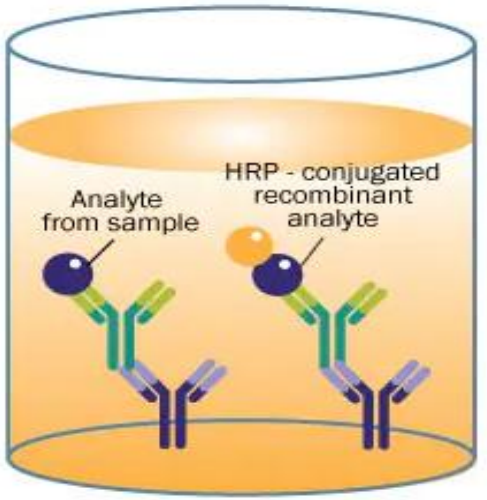
https://www.youtube.com/watch?v=21bCkmy7K7s

Competitive ELISA

- **Advantages:**
  - ✓Ability to quantitate small molecules.
- **Disadvantages:**
  - ✓Less specific since you are only using 1 antibody.
  - ✓Requires a conjugated antigen.







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