Cihan University/ Sulaimaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 5: Enzyme-linked Immunosorbent Assay (ELISA Techniques)

2023- 2024

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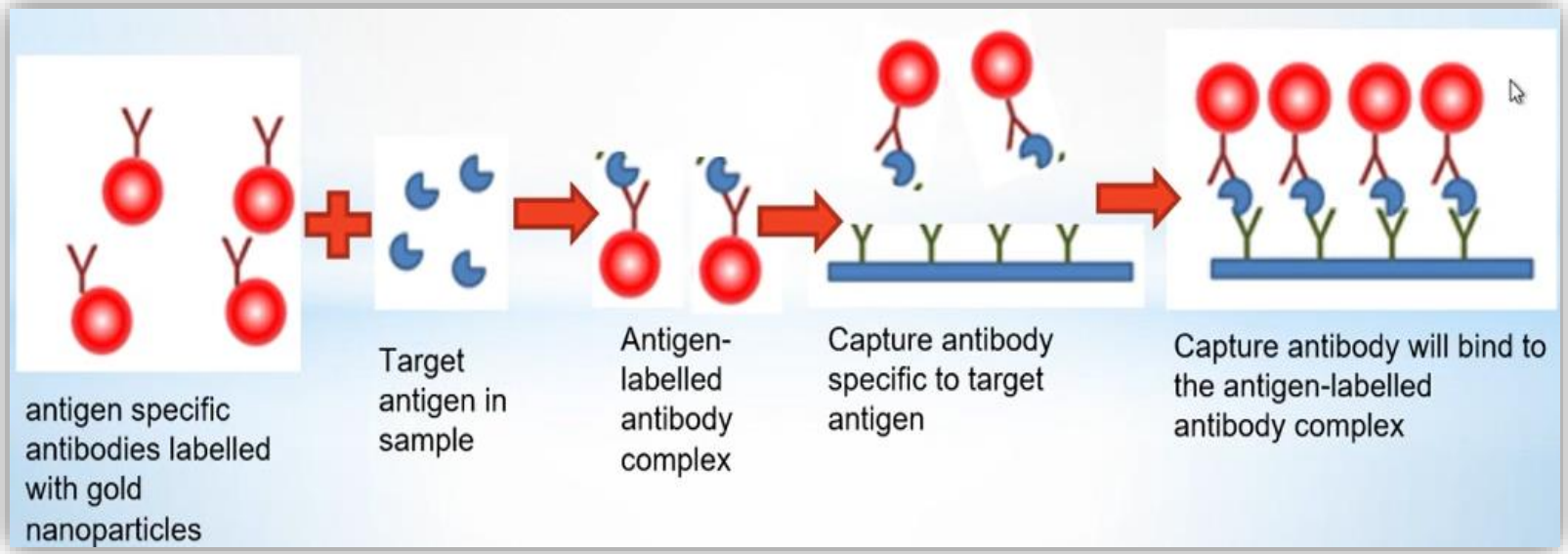
5- Antibody can be labeled:

1- Small Particles

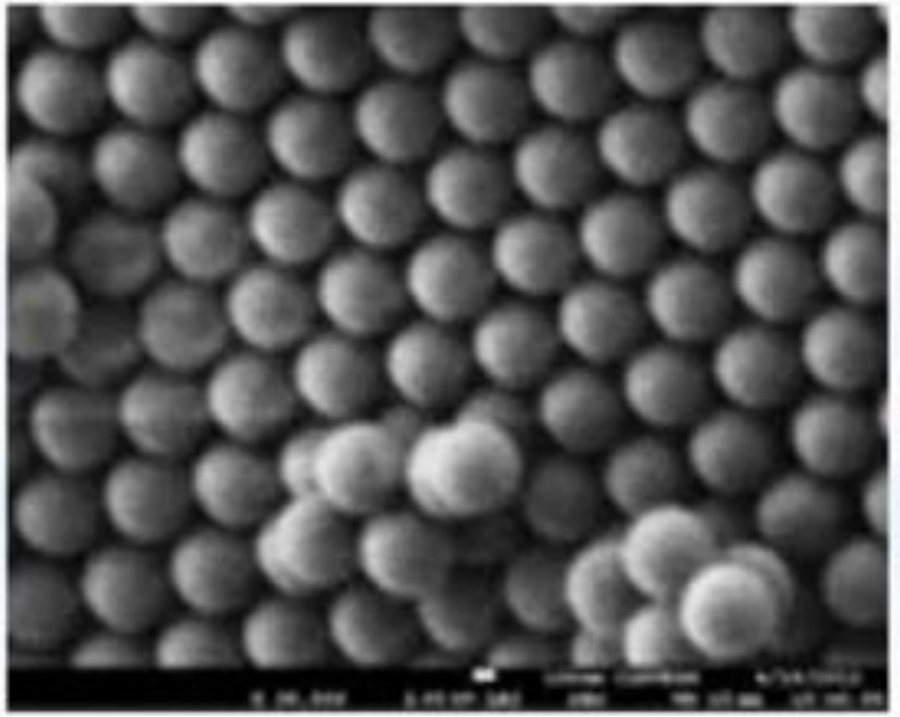
- The process of biotin labeling is known as biotinylation. Protein biotinylation involves covalently attaching biotin molecules to other biomolecules, such as antibodies, peptides, proteins, nucleic acids (DNA and RNA), oligonucleotides, and others.
- Biotin- Used in Lateral Flow Immunoassay, Western Blot, ELISAs, Flow Cytometry.
- Nanoparticles- Lateral Flow Immunoassay, Western Blot, ELISA.



E.g. Rapid strip Test. Red line is formed by accumulation of gold nanoparticles

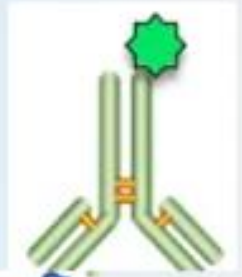
2- Particles

- Microspheres- Polystyrene latex- based microsphere, magnetic beads.
- Use in Lateral Flaw Immunoassay, Latex Agglutination Test, Fluorescence microsphere immunoassays (Luminex), Magnetic ELISA, Magnetic Immunoassays.



Microsphere. SEM image.

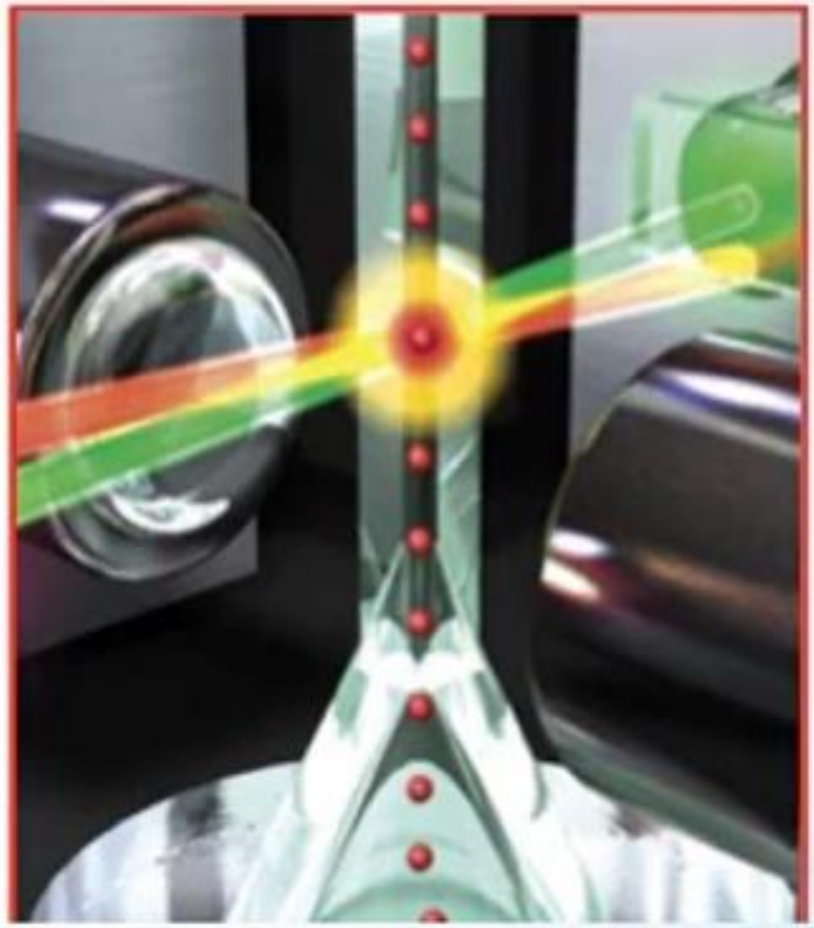
Florescent Microsphere Immunoassays (Luminex)



Detection antibody
(specific antibody labelled
with fluorescent reporter)

Target antigen

Microsphere with specific antibody

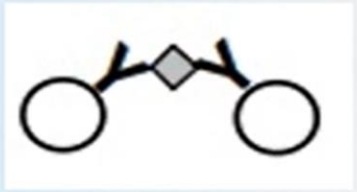


Green laser to count
positive / negative

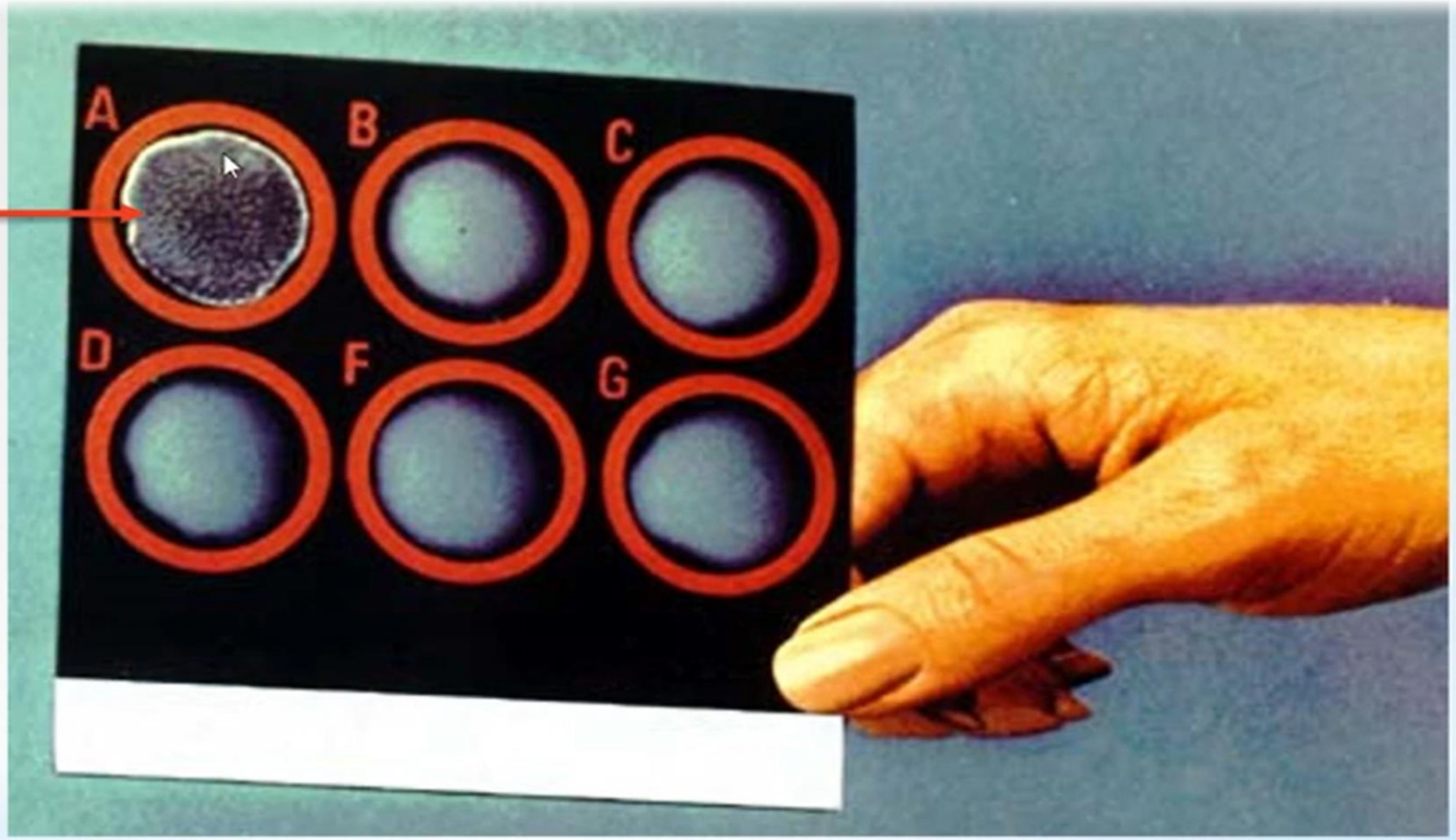
Red laser to
identify bead
(specific for
target antigen)

Example of Latex Agglutination Test

Agglutination



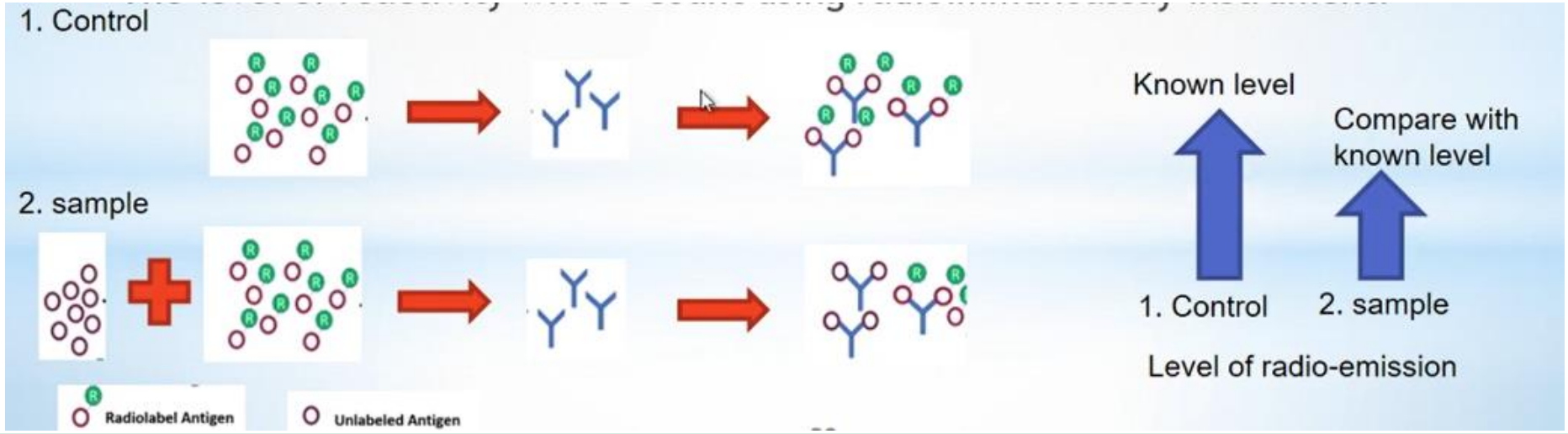
Particle agglutination (clumping of microspheres, to look like curdled milk)



Circle A shows a positive test (agglutination of the latex particles.) The other circles B-E show negative tests (no agglutination of the latex particles).

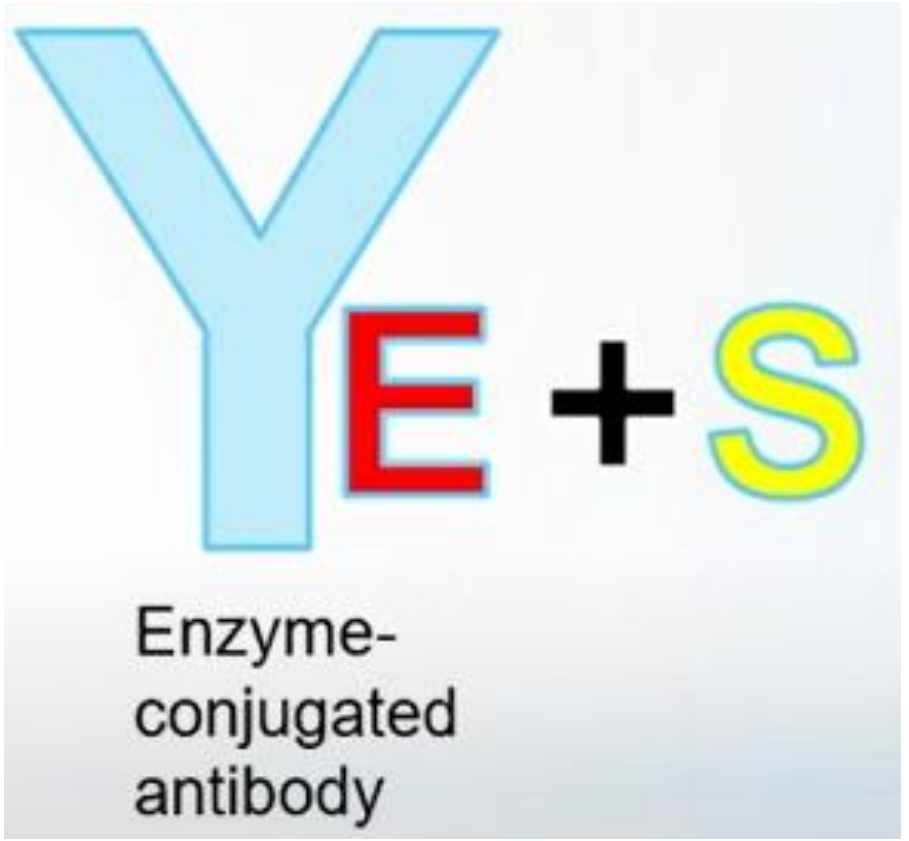
3- Isotope

- Radioisotopes- labelled antigen/ Antibody,
- Use in **Radioimmunoassay**,
- Is the **most sensitive** and **specific methods** of invitro assay techniques used to measure concentrations of antigen.
- Example: Radioisotopes- labelled target antigen+ sample (unknown specific antibody).
- The level of reactivity will be count using radioimmunoassay instrument.

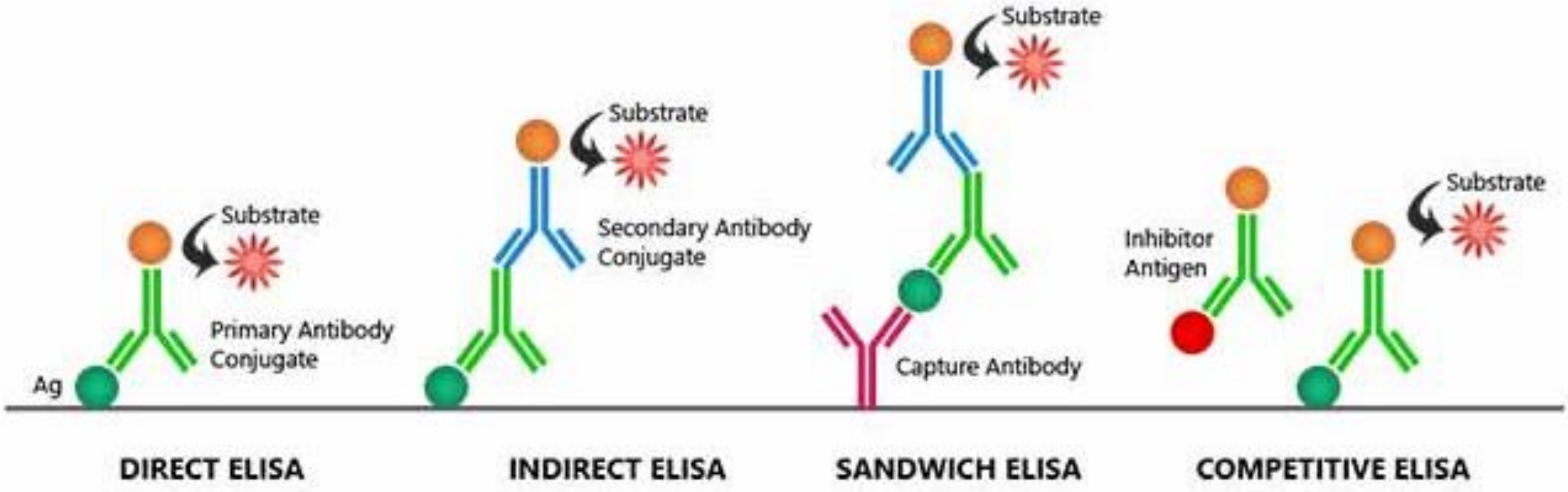


4- Enzymatic Protein

- Uses an enzyme linked to an antibody to detect antibody/ antigen.
- To use enzyme- labelled antibody, samples are incubated with a substrate that is catalyzed by the enzyme to produce a colored product (chromogenic assay) or light (chemiluminescent assay).
- Color producing assays are useful for ELISA, Lateral Flow Immunoassay, Western blots, and immunohistochemistry.
- While light- producing reactions are most frequently used in Western blot and chemiluminescence Immunoassay (CLIA).

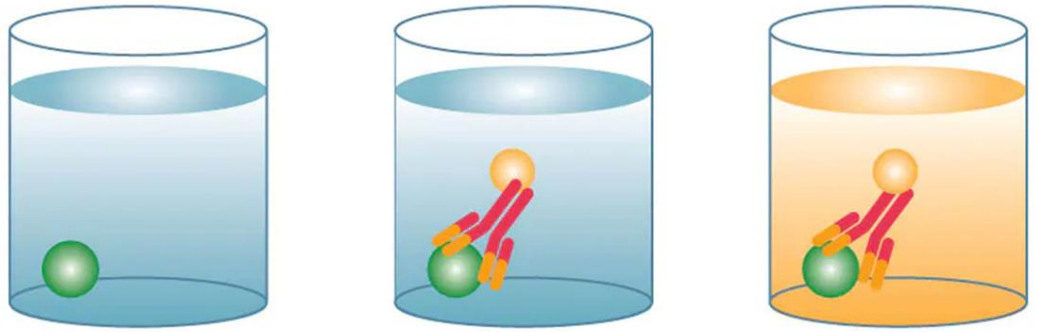


Types of ELISA



Direct ELISA

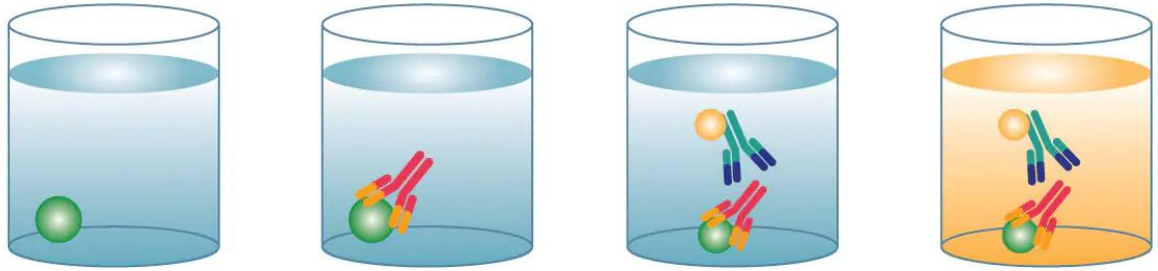
- In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein.
- Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample.
- Since only one antibody is used in a direct ELISA, 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.

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

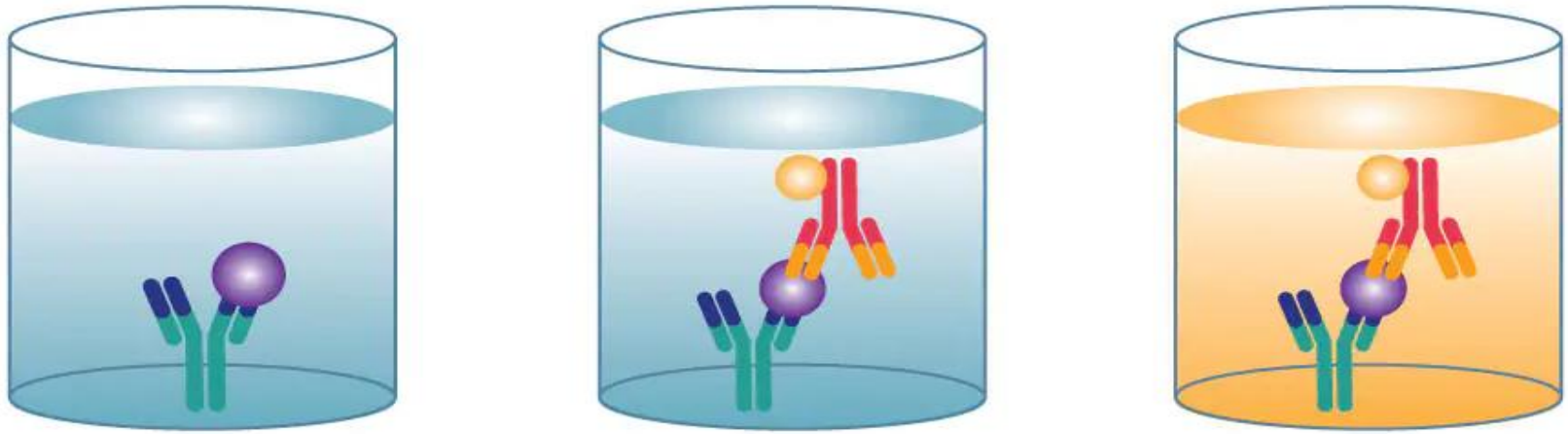


Sandwich ELISA

- 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.
- Sandwich ELISAs are highly specific, since two antibodies are required to bind to the protein of interest.
- When to Use: Determining analyte concentration in a biological sample.

Sandwich ELISA

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

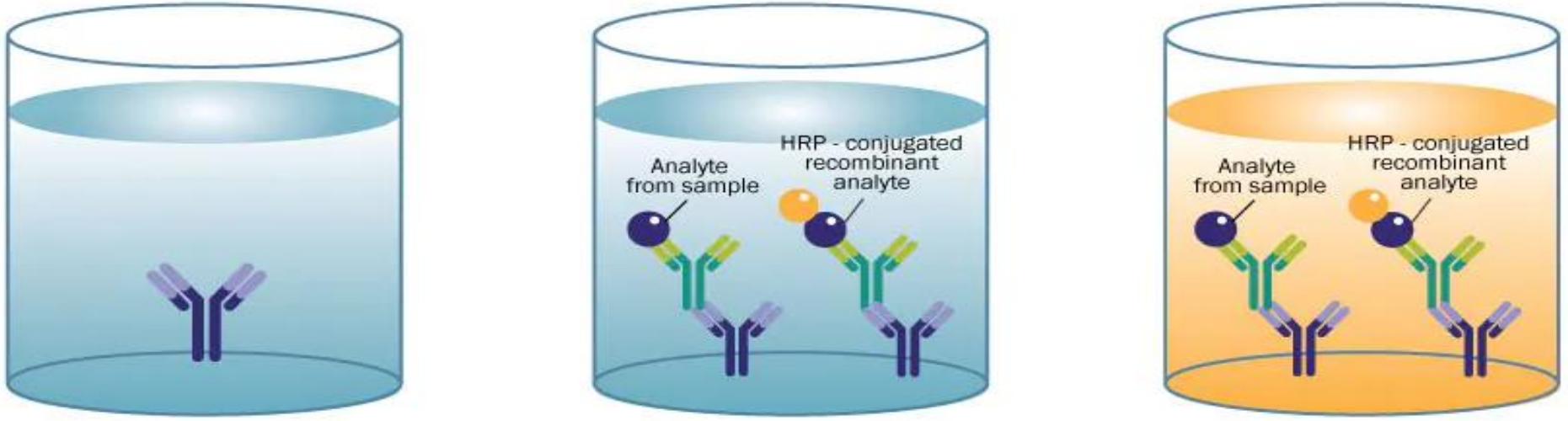


Competitive ELISA

- 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.

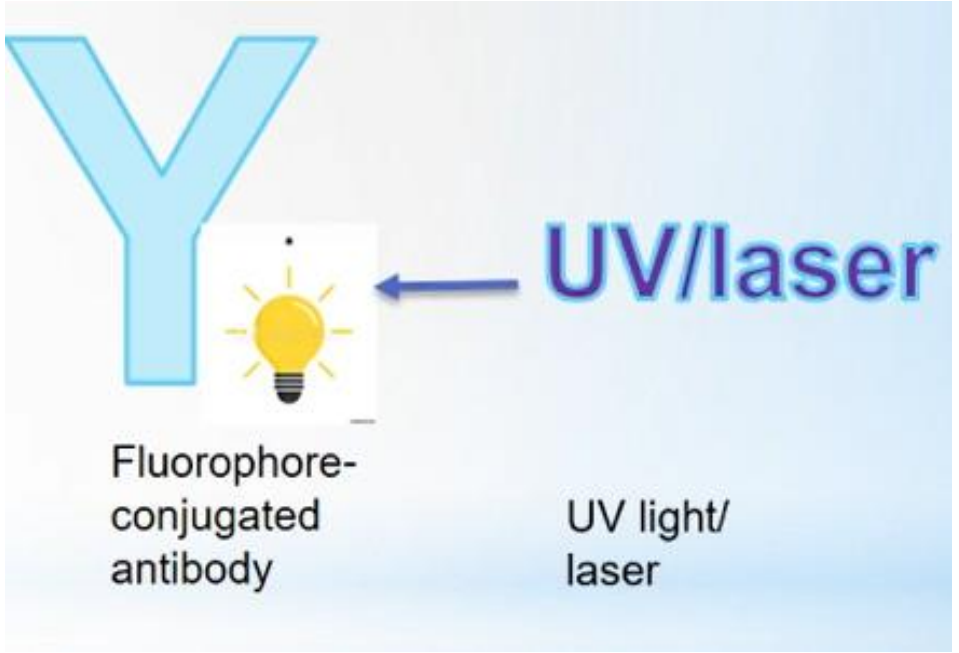
Competitive ELISA

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



5- Florescent dyes/ Fluorophore

- The use of fluorophore- labelled antibodies,
- Since fluorescent dyes are directly conjugated to the antibody, no enzyme/ substrate or binding interactions are required for detection.
- Therefore, the amount of fluorescent signal detected is directly proportional to the amount of target protein in the sample.
- Fluorescent labels are used in flow cytometry, western assays.
- Can be used for highly quantitate assays.



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