Enzyme immunoassay (HRPO)

Presented By:
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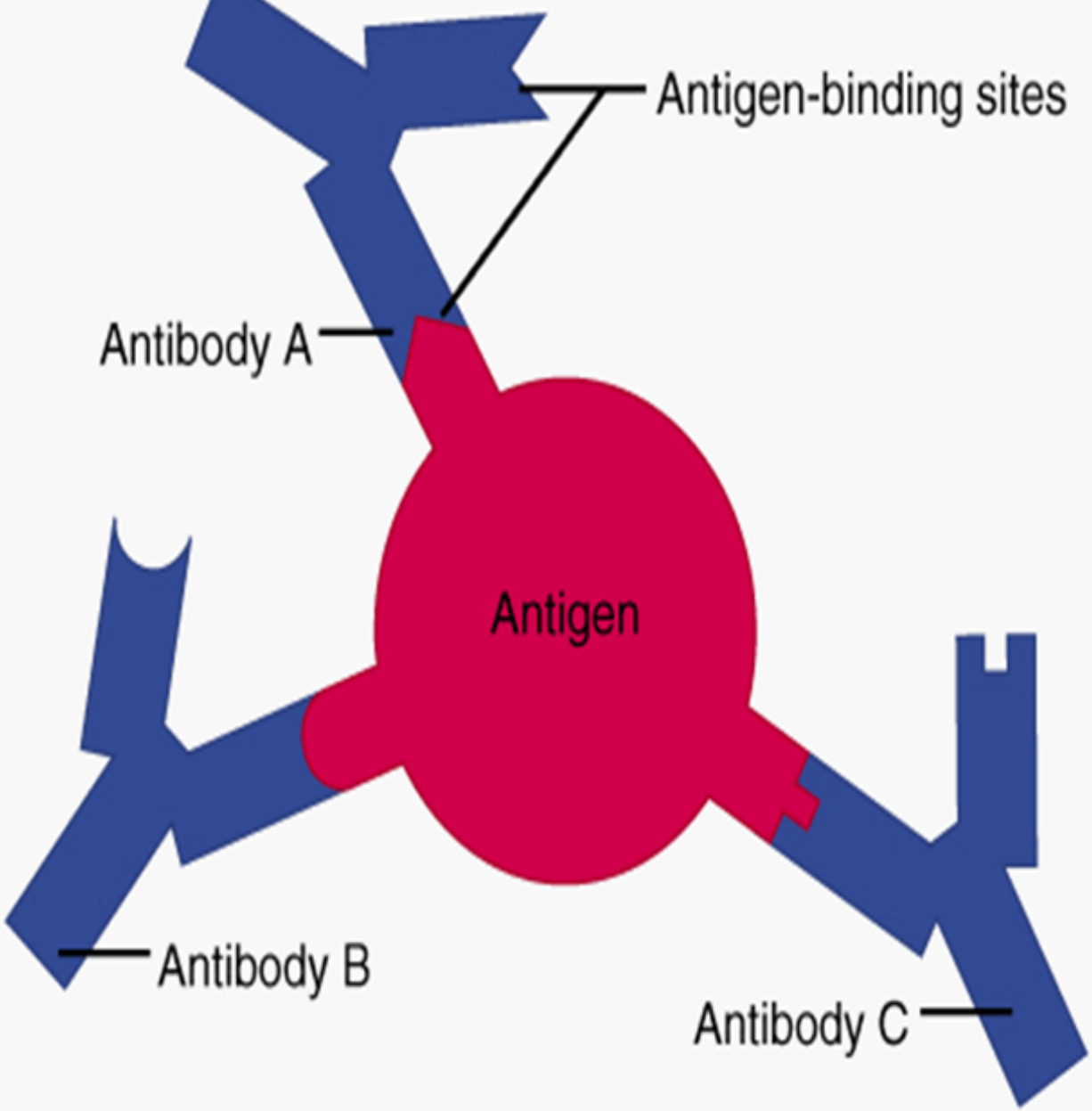
Serology Assay

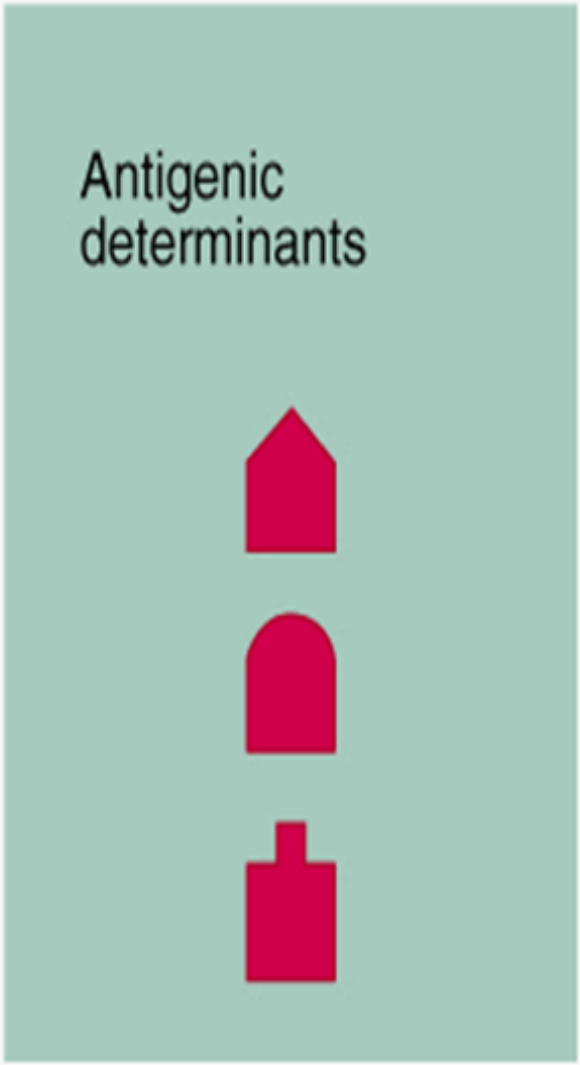
- Serological or immunological assays are used for the detection of foreign particleor viruses.

- Serology is the scientific study of serum and other fluids which are used to identify antibodies and antigens in the test samples. Such antibodies are formed in response to an infection.

Antigen (Ag)

- Any substance which evokes the production of antibodies is called an antigens and includes proteins, polysaccharides, lipids, carbohydrates, nucleic acids, enzymes, toxins etc.
- Each antigen is made up of distinct sub-regions.
- These regions stimulate the antigenic response and are the regions to which antibodies get attached. These regions are called antigenic determinants or Epitopes.
- Epitopes, are the actual stimulus for the production of particular antibody and are the combining sites of antibody.





Basic structure of Antigen

Antigen-activity

Immunogenicity of the antigen:

- When antigen stimulate the production of the antibody that will specifically react with antigen is known as immunogenicity of the antigen.

Antigenicity of the molecule:

- The ability of the antigen to combine with the specific antibody produced is called antigenicity of the molecule
- The specific regions of the antigen that induce & interact with specific antibody is called Epitopes.

Epitope

- **Epitope or antigenic determinant site-** It is the part of an antigen that is recognized, specifically by antibodies.
- Paratope- The part of an antibody that recognizes the epitope is called a paratope.

Antibody (Ab)

- A specific protein formed in the blood or fluid in response to injection of an antigen is called Ab.
- Ab are responsible for specific recognition and elimination (neutralization) of antigens
- Antibodies are proteins termed as γ globulin are built of two types of chains-
- Heavy chain (H)- molecular wt. of 50,000 to 70,000 and
- Light chain (L)- mol. Mt. of 23,000,
- These chains occur in pairs and linked to one another by disulphide bond.

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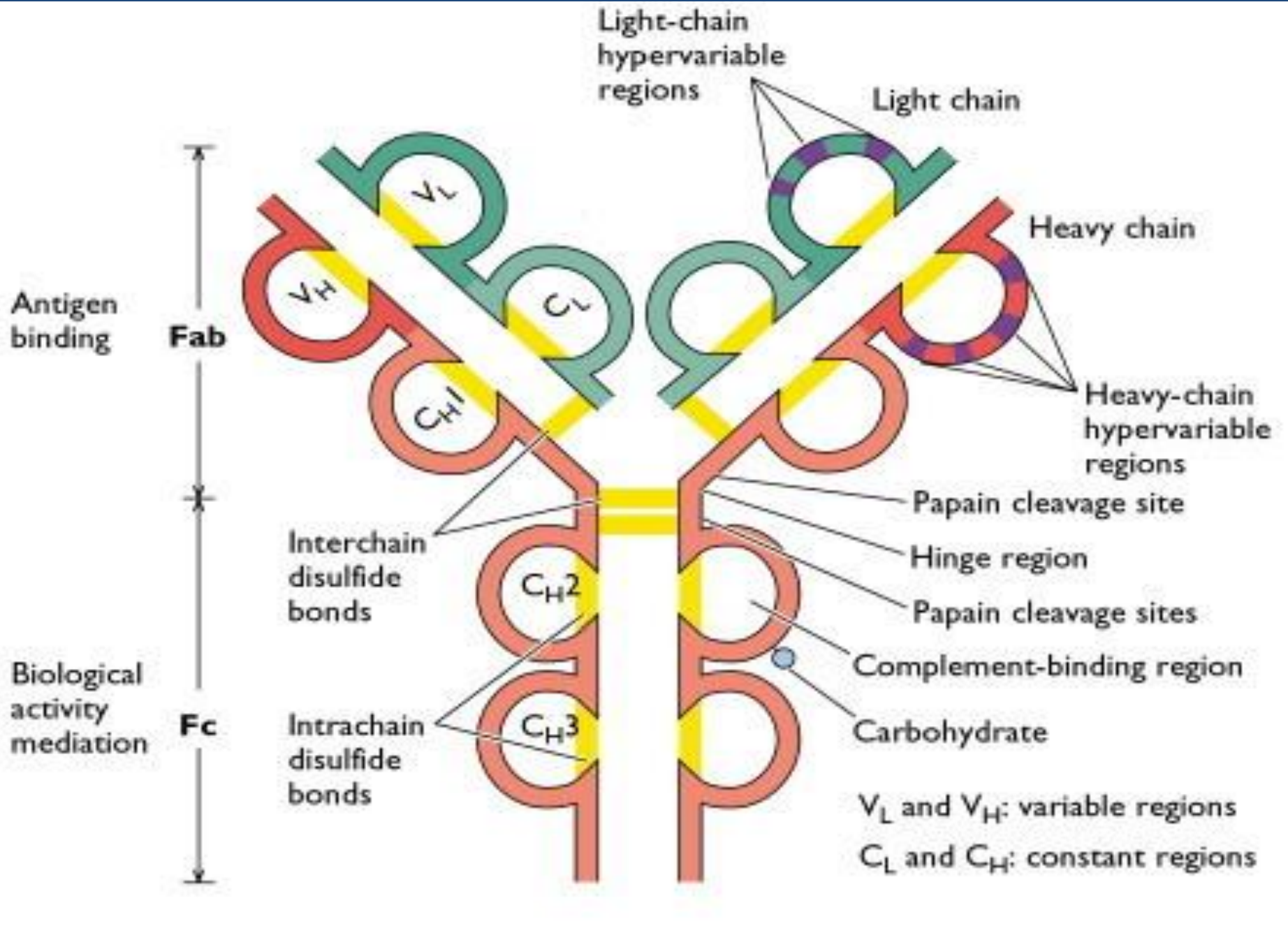
- The Ig monomer is a "Y"-shaped molecule that consists of four polypeptide chains.
- Two identical *heavy chains* and two identical *light chains* connected by disulfide bonds.
- Each chain is composed of structural domains called immunoglobulin domains.
- These domains contain about 70-110 amino acids and are classified into different categories (for example, variable or IgV, and constant or IgC) according to their size and function.

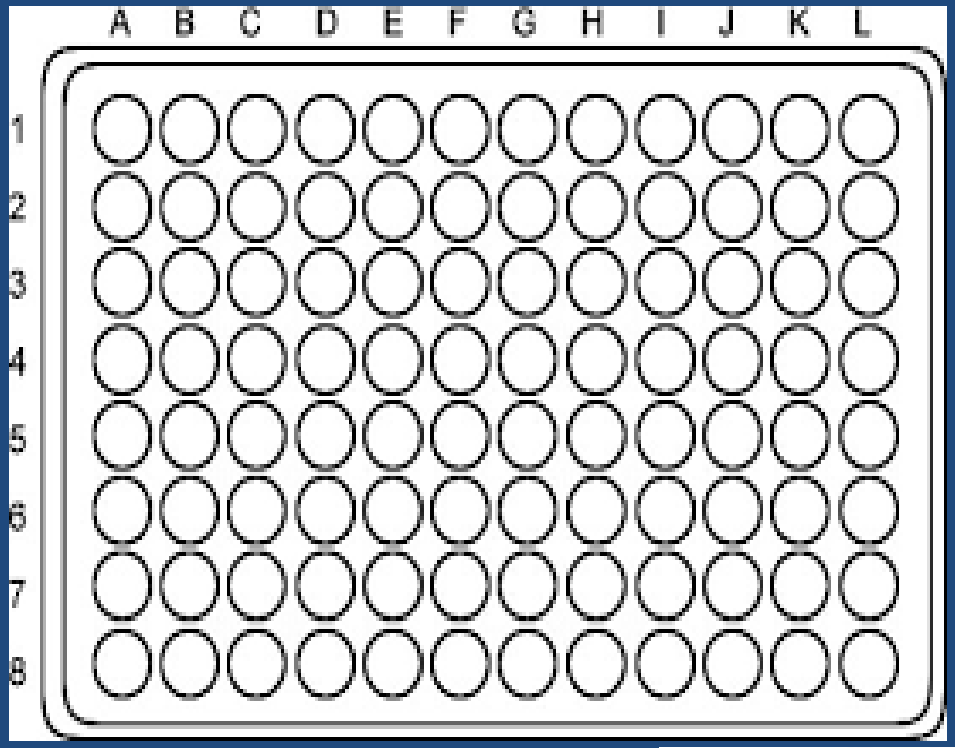


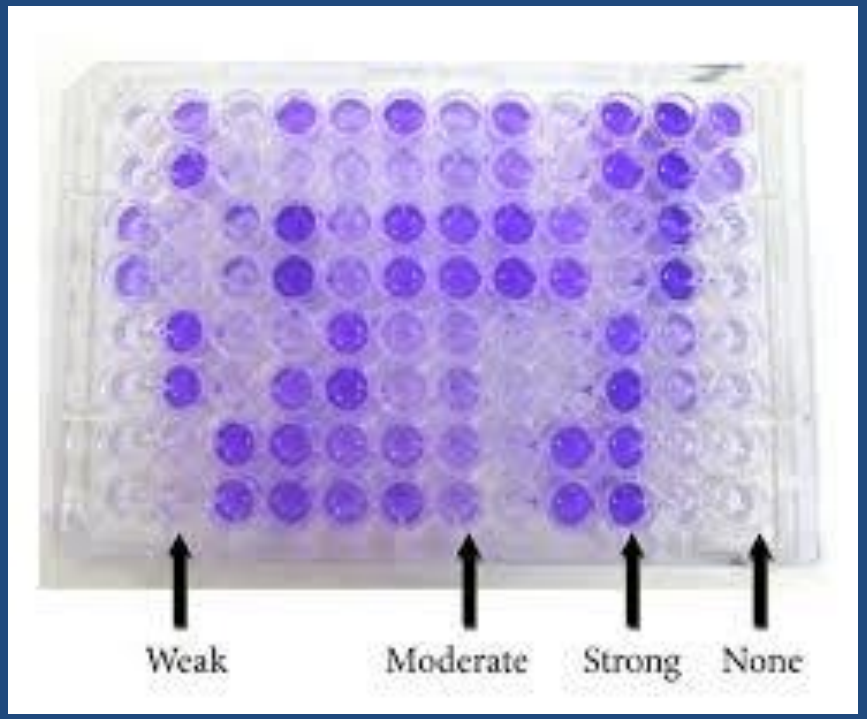
Basic Structure of Immunoglobulins

ELISA- Serology Assay

- The Enzyme-Linked Immuno-sorbent Assay (ELISA) is used for detection of viruses, insect vectors, seeds, and vegetative propagules in the given samples.
- ELISA was introduced by Clark and Adams (1977).

Microtitre Plate





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- ELISA is a solid phase immunoassay and usually done in microtitre plates made up of either polystyrene or polyvinyl chloride (PVC, flexible plates).
- Due to its adaptability, sensitivity, and economy in use of reagents, ELISA is used in a wide range of situations, especially to test a large number of samples in a relatively short period of time.
- ELISA fall into two broad categories: -

  (i) Direct ELISA (ii)Indirect ELISA

Direct ELISA or Double Ab Sandwich (DAS) ELISA

- In this assay, specific antibody is placed in the wells of a microtitre plate.
- The Ab is absorbed onto the walls of the plate.
- A test Ag is then added to each well.
- If the Ag reacts with the Ab, the Ag is retained when the well is washed to remove unbound Ag.
- An Ab-enzyme conjugated specific for the Ag is then added to each well.

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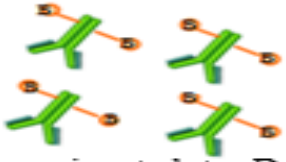
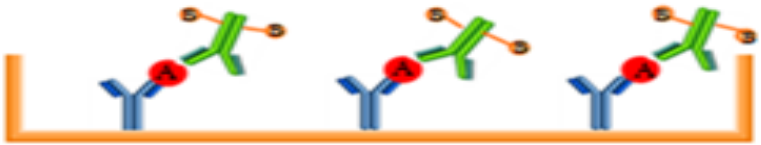
- The final complex is formed on an outer Ab-Enzyme, middle Ag, and inner Ab i.e. it is a layered (AbE-Ag-Ab) sandwich.
- A colorless substrate that the enzyme will convert to a coloured product is then added, and resulting product is quantitatively measured by optical density scanning of the plate.
- If the Ag has reacted with absorbed antibodies in the first step, the ELISA test is positive.
- If the Ag is not recognised by the absorbed Ab, the ELISA test is negative.

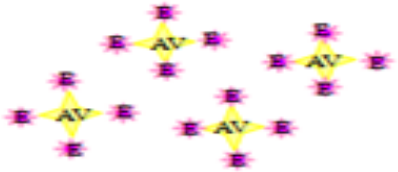
Capture Ab is pre-coated on the plate.

Adding standard & samples to wells. Anaylte present is bound by the immobilized Ab. Unbound materials are removed.

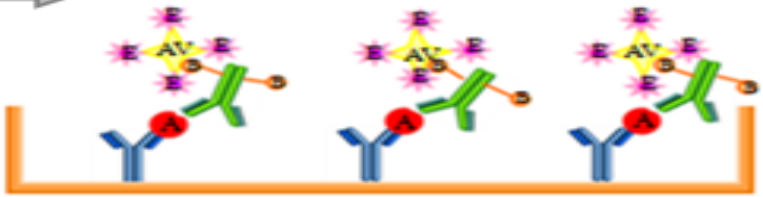


Biotin conjugated to Detection Ab(Detection reagent A) is added and binds to analyte absorbed on the plate. Usually multiple biotin molecules can conjugated to a antibody. Unbound ones are washed away.



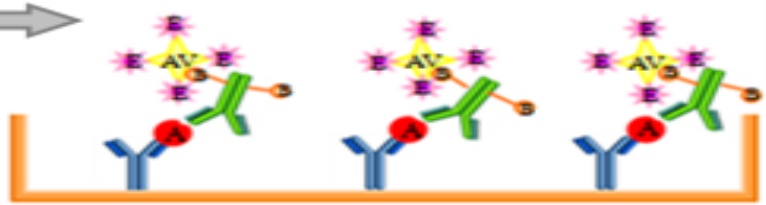


Avidin conjugated to HRP (Detection reagent B) is added and binds to biotin absorbed on the plate. Unbound ones are washed away.



Luminal Substrate

Add luminal substrate and incubate 10 minutes at 37oC. Then read relative light unit (RLU) value immediately.



Direct ELISA or Double Ab Sandwich (DAS) ELISA

Indirect ELISA or Direct Ag Coated (DAC) ELISA

- DAC ELISA detects Ab rather than Ag.
- In this assay, Ag is incubated and absorbed in the wells of a microtitre plate.
- Free Ag is washed away.
- The test antiserum is added, and if specific Ab is present, it binds to the Ag.
- Unbound Ab is washed away.
- The microtitre plate is incubated with desired Ag attached to their surface.

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- After allowing time Ab-Ag complex formation, the unbound Ab is washed away.
- An anti-antibody (anti gama globulin) that has been covalently coupled to an enzyme, such as horse radish peroxidase (HRPO), is added.
- The Ab-enzyme complex bind to the test Ab and unbound conjugate is washed away.

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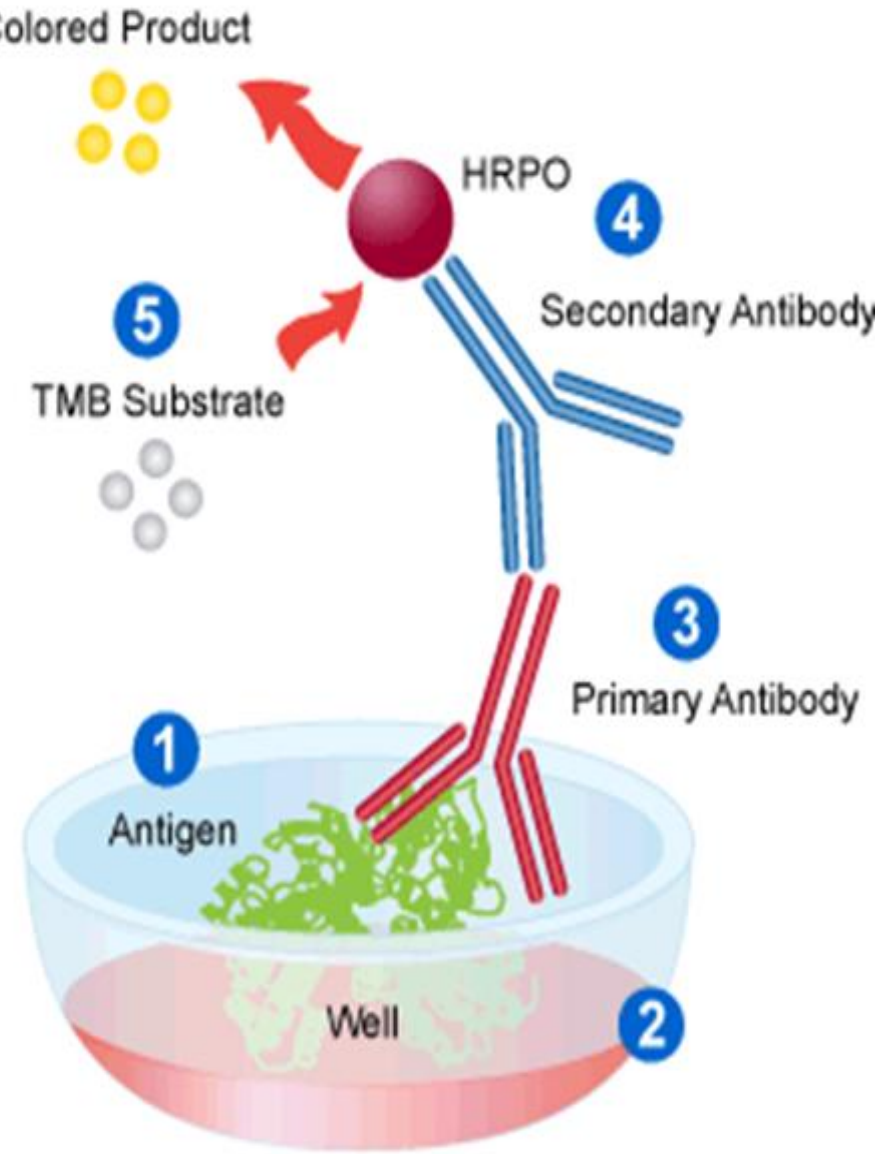
- The attached ligand is visualized by the addition of a chromogen.
- A chromogen is a colourless substrate acted on by the enzyme portion of the ligand to produce a coloured product.
- The amount of test Ab is quantized in the same way as an Ag in the DAS ELISA.

Indirect ELISA

1. Antigen/sample is added to plate.
2. Blocking buffer is added to block remaining protein-binding sites.
3. Next a suitable ***primary antibody*** is added.
4. A suitable ***secondary antibody – HRPO conjugate*** is then added which recognizes and binds to the primary antibody.
5. TMB substrate *(Leinco Prod. No. T118)* is added and is converted by HRPO to detectable form.



Indirect Ag Coated (DAC) ELISA or Indirect ELISA

Advantage of ELISA

High Sensitivity

- If trace amount (microgram or nanogram) of the Ag or Ab is present in the cell or subcellular part, the quantification can be done.

Strong Specificity

- Specificity of ELISA is due to the selectivity of the Ag or Ab.
- Actually, the binding only occurs in the epitope of an antigen or antigen-binding site of an antibody which is a complementary relationship so, the reaction between Ag or Ab shows a strong specifity.

Thank you