

Antigen- Antibody Interactions

CBCSZ-203: Immunology

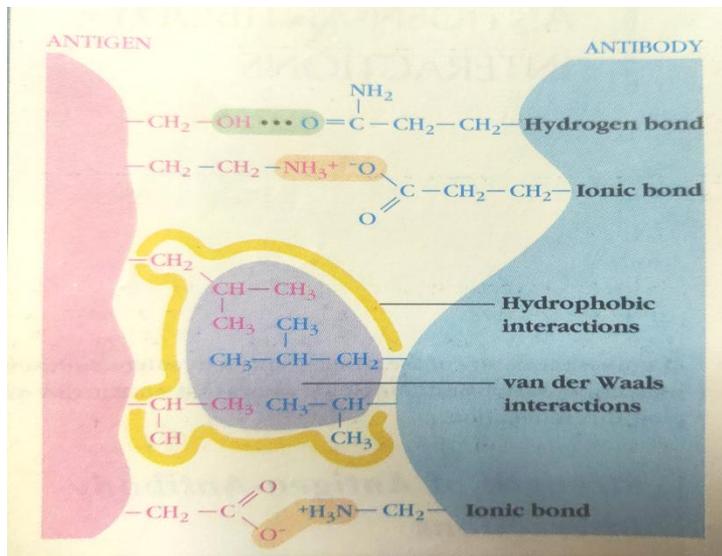


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- The antigen-antibody interaction involves noncovalent interaction between epitope of antigen and hypervariable region of antibody, which are highly complementary to each other.
- Interactions include hydrogen bonds, ionic bonds, hydrophobic interactions and van der Waals interactions.



Non covalent forces in antigen- antibody interaction

■ The strength of noncovalent interaction between single antigen binding site of antibody and single epitope is called **antibody affinity**.



Where k_1 is association rate constant and k_{-1} is dissociation constant

$k_1/k_{-1}=k_a$, is equilibrium constant and measure of affinity.

$K_a = \frac{\text{molar concentration of bound Ag-Ab}}{\text{molar concentration of unbound Ag and Ab}}$

$1/k_a = k_d$, is equilibrium constant for dissociation, a quantitative indicator of stability of Ag-Ab complex

- The strength between multiple binding site of antibody and multiple epitopes is called **antibody avidity**.
- **Cross reactivity:** Ag-Ab interactions are highly specific but sometimes antibody obtained by one antigen, can cross-react with an unrelated antigen. The cross reactivity occurs when two antigen share an identical epitope or if antibodies which are specific for one epitope, bind with unrelated epitope.

Precipitation Reactions

■ In aqueous solution, antibody interact with soluble antigen and form a visible precipitate. For this reaction antibody should be bivalent and antigen must be bivalent or polyvalent.

■ Precipitation reactions can be performed in fluid as well as in gel.

■ Precipitation reaction in fluid:

To observe the precipitation in fluid, a constant amount of antibody placed in a series of tubes and add antigen in increasing amount. Then centrifuge the tubes, collect the pellet and measure the amount of precipitate and plot the amount of precipitate against the concentration of antigen (precipitation curve). The maximum precipitation is occurred when the ratio of antibody to antigen is optimal called **equivalence zone**.

■ Precipitation reaction in gel (immunodiffusion)

Antigen and antibody diffuse together in agar gel and formed a visible line of precipitate.

Types of immunodiffusion

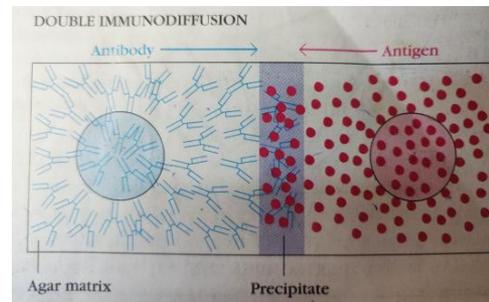
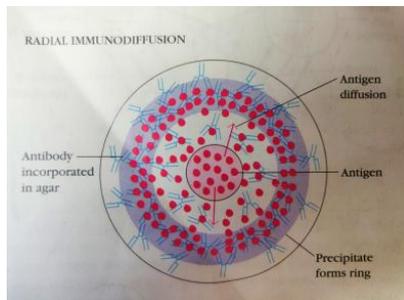
- (1) Radial immunodiffusion (Mancini method)
- (2) Double immunodiffusion (Ouchterlony method)

Radial immunodiffusion

An antigen sample is placed in a well and allowed to diffuse in antiserum containing gel and finally a precipitation ring is formed around the well. The area of ring is proportional to the concentration of antigen.

Double immunodiffusion

Antigen and antibody placed separately in well and diffuse toward each other. A precipitation line is formed when equivalence is reached.



Immunelectrophoresis

This is a combination of electrophoresis and double immunodiffusion. An antigen mixture is separated by electrophoresis into its components on the basis of charge. Then troughs are cut into the gel and antiserum is added to them. Antigen and antibody then diffuse toward each other and produce a precipitation line.

Agglutination reactions

The interaction of antigen and antibody in the formation of clumping is known as agglutination. Antibodies that involve such reactions are known as agglutinins.

Hemagglutination

This is performed for typing of ABO antigens. RBCs are mixed with antisera to A or B blood-group antigen on a clean slide. In the presence of antigen, agglutination occurs and forms a clump on slide. This method is used for matching blood types for transfusions.

Bacterial agglutination

In the response of bacterial infection, serum antibodies are formed. Serum from the infected person is serially diluted in a series of tubes and bacteria is added. The last tube showed visible agglutination. The agglutination titre of an antiserum is used to diagnose bacterial infection.

Passive agglutination

In this method, soluble antigen mixed with RBCs and treated with tannic acid/ chromium chloride (promote adsorption of antigen on surface of RBCs). Serum containing antibody then serially diluted into microtiter plate wells. Antigen coated RBCs added to each well. Agglutination is evaluated by the spread pattern of agglutinated RBCs on the bottom of well.

Radioimmunoassay (RIA)

In this technique, the radiolabeled antigen is mixed with antibody in that amount, which saturates the antigen binding sites of the antibody, then added the test sample containing unlabeled antigen of unknown concentration. Both type of antigen compete for binding and with increasing concentration of unlabeled antigen displaced the labeled antigen.

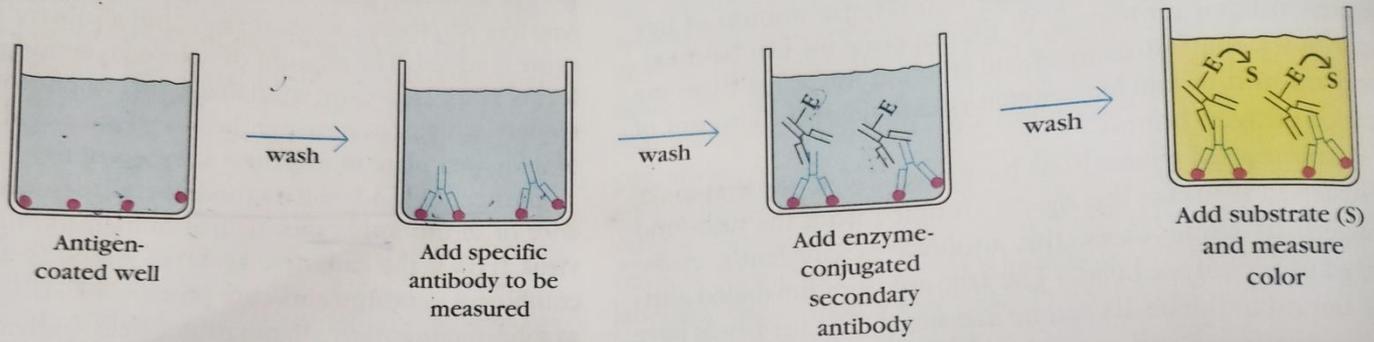
Enzyme-linked Immunosorbent Assay (ELISA)

Principle is similar to RIA but it is enzyme based technique. An enzyme (conjugated with an antibody) reacts with a substrate (chromogenic substrate) and generate a colored product.

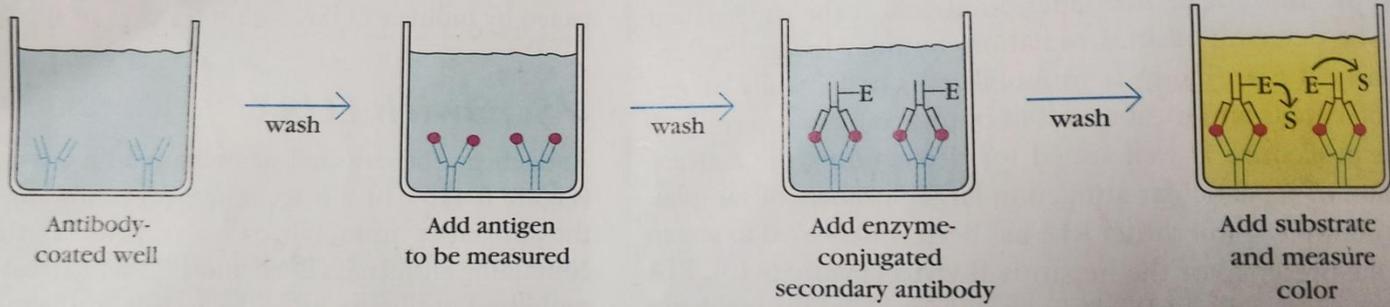
Types of ELISA

- (1) Indirect ELISA
- (2) Sandwich ELISA
- (3) Competitive ELISA

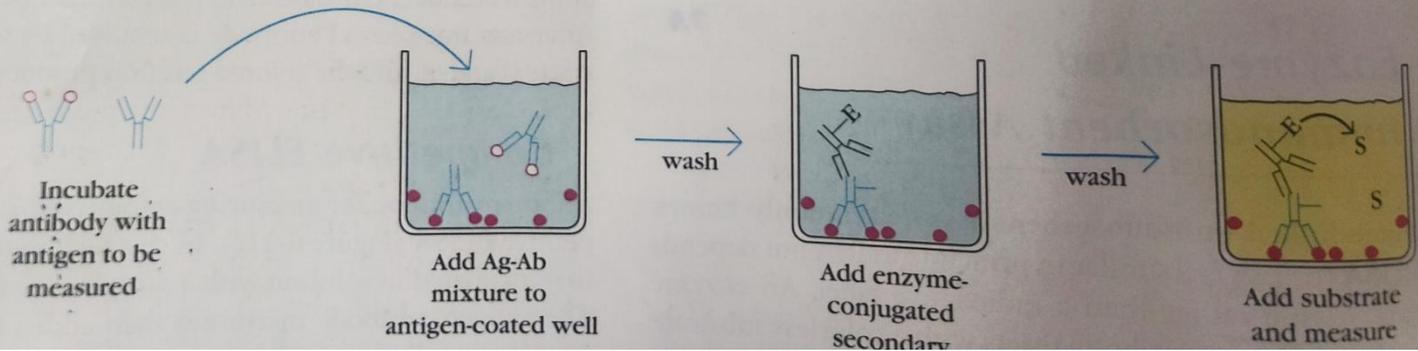
(a) Indirect ELISA



(b) Sandwich ELISA



(c) Competitive ELISA



Western blotting

This technique identifies a specific protein in a mixture of proteins. A protein mixture is separated by SDS-PAGE (polyacrylamide slab gel infused with a dissociating agent-sodium dodecyl sulfate). Protein bands are transferred to nitrocellulose membrane by electrophoresis and protein bands are recognized by flooding the membrane with radiolabeled/ enzyme linked antibody.

Immunofluorescence

In this technique the location of an antigen in tissue is determined by reaction with an antibody labelled with a fluorescent dye (fluorochrome). Fluorescent molecules absorb light of one wavelength (excitation) and emit light of another wavelength. The emitted light can be observed by fluorescence microscope. These fluorochromes can be conjugated with Fc region of an antibody.

Direct staining: Primary antibody is directly conjugated with fluorochrome.

Indirect staining: Unlabeled primary antibody is detected by additional fluorochrome labeled reagent.

References

A.G., Richard, J.K., Kindt, A.O. Barbara Kuby Immunology (fourth edition)

Sciencedirect.com