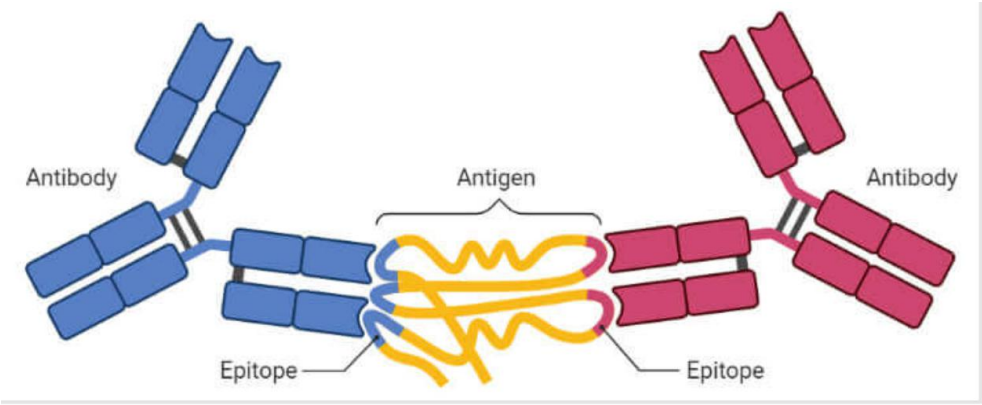
Antigen-Antibody Interaction

Antigen-Antibody (Ag-Ab) Interaction is a biochemical reaction between antibodies and specific antigens when they come closer to a distance of several nanometers. It is the binding of paratopes of antibodies to specific antigens on their epitopes that initiates a series of immunological responses to act against the respective antigens for their removal or destruction.

Antigen + Antibody ⇄ Ag-Ab complex → Immune Response



Antigen-Antibody (Ag-Ab) Interaction

➢ Antigen

(Anti= against; gen=thing that produces or causes)

Any foreign substances that when entering our body sometimes self-elicit a series of immune responses and are precisely called **immunogens**. Whereas some of them don't directly elicit an immune response but require the help of some other molecules (carrier proteins) to do so and are called **hapten**. The immunogens and hapten are collectively called **antigens**.

– They can be proteins, peptides, lipids, or, polysaccharides.

– Antibody binding site is called an **epitope**.

– Abbreviation: "**Ag**"

➢ Antibody

An antibody is simply the component produced by the immune system in response to antigens. So basically antigens are the generator of antibodies. They interact with each other to induce an immune response.

– Also called immunoglobulins (**Ig**)

– Y-shaped

– Glycoproteins

– Produced by plasma **B-cells**

– Antigen binding site is called **paratope**.

– Types: **IgG**, **IgA**, **IgM**, **IgE**, **IgD**

Properties of Antigen-Antibody Interaction

– Highly specific reaction

– Occurs in an observable manner

– Non-covalent interaction (Van der Waals forces, Ionic bonds, Hydrogen bonds, Hydrophobic interactions)

– No denaturation of antibodies and antigens

– Reversible

Stages of Antigen-Antibody Interaction

1. **Primary Stage:** It is the initial interaction between antigens and antibodies.

– Rapid

– With visible effects

Factors affecting Antigen-Antibody Interaction

Many factors affect Ag-Ab reactions. Some of the common factors are:

1. **Temperature:** It depends on the chemical nature of epitopes, paratopes, and, bonds involved. Eg. hydrogen bonds are stable at low temperatures and hydrophobic bonds are stable at high temperatures.

2. **pH:** Optimal pH range is 6.5 to 8.5. Extreme pH values change the conformation of antibodies and inhibit the reaction.
3. **Ionic strength:** The effect of ionic is important in blood group serology. Here the reaction is significantly influenced by sodium and chloride ions. In normal saline solution, Na^+ and Cl^- cluster around the complex and partially neutralize charges, potentially interfering with antibody binding to antigen. This could be problematic when low-affinity antibodies are used.

4. **Concentrations of Ag and Ab:** Increase in the concentration of antigen and antibody enhances the reaction.

5. **No. of antigen-binding sites:** More the no. of antigen-binding sites on the antibody, the more the chances of interaction. For eg., IgM is a pentamer and hence has 10 binding sites whereas IgG is a monomer and hence has only 2 binding sites so IgM will bind more efficiently with antigens.

6. **Structural arrangement:** If the structure of epitope and paratope is such that they could fit well as lock and key then it enhances the interaction between antigen and antibody.

Strength of Ag-Ab interactions

- **Affinity:** It is the strength with which one antigen binds on a single antigen-binding site on an antibody.
  - Low-affinity Ab: Bind Ag weakly and dissociates readily.
  - High-affinity Ab: Bind Ag tightly and remain bound longer.

- **Avidity:** It is a broader term than affinity. It is a measure of the overall strength of the Ag-Ab complex. It depends on:
  - The affinity of the antibody
  - Valency(no. of binding sites) of antibody and antigen
  - And the structural arrangement of epitopes and paratopes.

- **Cross-reactivity:** It refers to the ability of an antibody to bind to similar epitopes of different antigens.

Precipitation Reaction

The reaction between soluble (small) antigens and soluble antibodies forms an insoluble precipitate. The proportion of Ag and Ab in the reaction must be equivalent for the reaction to occur.

– In the presence of electrolytes

– At specific pH and Temperature

– Antibodies involved are called **precipitins**

– Types:

1) In Agar (Immunodiffusion)- **Immunodiffusion** is a diagnostic test which involves diffusion through a substance such as agar, which is generally soft gel agar (2%) or agarose (2%), used for the detection of antibodies or antigen.

2) In Agar with an electric field **(Immunoelectrophoresis)**- Immunoelectrophoresis, also called gamma globulin electrophoresis, or immuno globulin electrophoresis, is a method of determining the blood levels of three maj or immunoglobulins: immunoglobulin M (IgM), immunoglobulin G (IgG), and im munoglobulin A (IgA). Immunoelectrophoresis aids in the diagnosis and evaluati on of the therapeutic response in many disease states affecting the immune syste m. Immunoelectrophoresis is also used frequently to diagnose multiple myeloma, a disease affecting the bone marrow

Agglutination Reaction

The reaction between insoluble (large) antigens and soluble antibodies leads to agglutination.

– Formation of visible clumps occurs.

– Occurs on the surface of the particle involved.

– Antibodies involved are called **agglutinins.**

***Note**: In the case of sensitivity agglutination reaction is more sensitive than precipitation reaction.*

Applications of Antigen-Antibody Interaction

1. Determination of blood groups i.e. blood typing.
2. Rapid diagnosis test kits used for pregnancy detection as well as detection of several diseases such as malaria, dengue, etc. are based on this principle. They require very little time for the tests.
3. Serological ascertainment of exposure to infectious agents.
4. Quantification of drugs, hormones, viral antigens, etc.
5. Detection of presence or absence of proteins in serum.
6. To study the characteristics of different immunodeficiency diseases.

ELISA

"ELISA is a plate based technique used to detect and quantify peptides, antibodies, proteins and hormones."

- ✓ ELISA is the basic assay technique, known as **enzyme-linked immunosorbent assay** that is carried out to detect and measure antibodies, hormones, peptides and proteins in the blood.
- ✓ Antibodies are blood proteins produced in response to a specific antigen.
- ✓ It helps to examine the presence of antibodies in the body, in case of certain infectious diseases.

Principle of ELISA

ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity. ELISA can provide a useful measurement of antigen-antibody concentration.

Types Of ELISA

ELISA tests can be classified into three types depending upon the different methods used for binding between antigen and antibodies, namely:

1. Indirect ELISA

- ❖ Indirect ELISA detects the presence of an antibody in a sample.
- ❖ The antigen is attached to the wells of the microtitre plate.

- A sample containing the antibodies is added to the antigen-coated wells for binding with the antigen.
- The free primary antibodies are washed away and the antigen-antibody complex is detected by adding a secondary antibody conjugated with an enzyme that can bind with the primary antibody.
- All the free secondary antibodies are washed away. A specific substrate is added which gives a coloured product.
- The absorbance of the coloured product is measured by spectrophotometry.

2. **Sandwich ELISA**

- Sandwich ELISA helps to detect the presence of antigen in a sample
- The microtitre well is coated by the antibody.
- The sample containing the antigen is added to the well and washed to remove free antigens.
- Then an enzyme-linked secondary antibody, which binds to another epitope on the antigen is added. The well is washed to remove any free secondary antibodies.
- The enzyme-specific substrate is added to the plate to form a coloured product, which can be measured.

3. Competitive ELISA

- Competitive ELISA helps to detect antigen concentration in a sample.
- The microtitre wells are coated with the antigen.
- Antibodies are incubated in a solution having the antigen.
- The solution of the antigen-antibody complex is added to the microtitre wells. The well is then washed to remove any unbound antibodies.
- More the concentration of antigen in the sample, lesser the free antibodies available to interact with the antigen, which is coated in the well.
- The enzyme-linked secondary antibody is added to detect the number of primary antibodies present in the well.
- The concentration is then determined by spectrophotometry.

Diseases That Can Be Diagnosed Using ELISA

ELISA can be used to detect some of these conditions: Ebola, Pernicious anaemia, AIDS, Rotavirus, Syphi, Zika virus, Carcinoma of the epithelial cells etc

Advantages of ELISA

Following are some of the advantages of the ELISA technique:

1. Results fetched from ELISA gives an accurate diagnosis of a particular disease since two antibodies are used.
2. Can be carried out for complex samples as the antigen is not required to get purified to detect.
3. It is highly responsive since direct and indirect analysis methods can be carried out.
4. It is a rapid test, yields results quickly.
5. Possible detection for ELISA ranges from the quantitative, semi-quantitative, standard curve, qualitative, calibration curve models etc.
6. Easier to perform and uncomplicated process as compared to other assays which require the presence of radioactive materials.

Applications of ELISA

1. The presence of antibodies and antigens in a sample can be determined

2. It is used in the food industry to detect any food allergens present

3. To determine the concentration of serum antibody in a virus test.

4. During a disease outbreak, to evaluate the spread of the disease, e.g. during recent COVID-19 outbreak, rapid testing kits are being used to determine presence of antibodies in the blood sample