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| **Group 10** |
| Describe the principles behind antibody antigen reactions and give at least 5 examples of how they are applied in the immunodiagnosis of named human diseases |
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| **7/24/2018** |
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Principles of antigen-antibody interaction

**Antigen-antibody interaction,** or **antigen-antibody reaction**, is a specific chemical interaction between [antibodies](https://en.wikipedia.org/wiki/Antibodies) produced by [B cells](https://en.wikipedia.org/wiki/B_cells) of the [white blood cells](https://en.wikipedia.org/wiki/White_blood_cells) and [antigens](https://en.wikipedia.org/wiki/Antigens) during [immune reaction](https://en.wikipedia.org/wiki/Immune_reaction). It is the fundamental reaction in the body by which the body is protected from complex foreign molecules, such as pathogens and their chemical toxins. In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex. The immune complex is then transported to cellular systems where it can be destroyed or deactivated.

There are several types of antibodies and antigens, and each antibody is capable of binding only to a specific antigen. The specificity of the binding is due to specific chemical constitution of each antibody. The [antigenic determinant](https://en.wikipedia.org/wiki/Antigenic_determinant) or epitope is recognized by the [paratope](https://en.wikipedia.org/wiki/Paratope" \o "Paratope) of the antibody, situated at the variable region of the polypeptide chain. The variable region in turn has hyper-variable regions which are unique [amino acid sequences](https://en.wikipedia.org/wiki/Amino_acid_sequence) in each antibody. Antigens are bound to antibodies through weak and noncovalent interactions such as [electrostatic interactions](https://en.wikipedia.org/wiki/Electrostatic_interactions), [hydrogen bonds](https://en.wikipedia.org/wiki/Hydrogen_bonds), [Van der Waals forces](https://en.wikipedia.org/wiki/Van_der_Waals_forces), and [hydrophobic interactions](https://en.wikipedia.org/wiki/Hydrophobic_interactions).

The principles of specificity and cross-reactivity of the antigen-antibody interaction are useful in clinical laboratory for diagnositic purposes. One basic application is determination of ABO blood group. It is also used as a molecular technique for infection with different pathogens, such as HIV, microbes, and helminth parasites.

## Types Of Antigen Antibody Reactions

**Agglutination Reactions:**

Aggregation of cells due to antibody binding is known as "Agglutination". The word agglutination is derived from Latin word agglutinate, means "to glue to."

In humus, binding of Abs pulls the antigen bearing cells close to each other resulting in the formation of clumps. The antibodies that cause agglutination of cells are called "agglutinins" and the antigens aggregated are called "agglutinates".

**Mechanism of Agglutination:**

The bivalent or multivalent antibodies can bind with two or more antigens at a time. When Ab binds to more than one antigen present on different cells, the individual antigen molecules brought close to each other, resulting in the formation of clumps.

Since IgM antibody has more number of antigen binding sites it is a more effective agglutinin. Agglutination of blood group antigens by IgM antibodies in mismatched blood transfusion is responsible for the blood transfusion reactions.

Agglutination test has wide application in clinical field. For example agglutination test is used to test blood groups, and infectious diseases such as typhoid, leptospirosis, malaria, trypanosomiasis, pneumonia etc.

**2. Precipitation and Flocculation Reaction:**

When an antibody binds to a soluble antigen, the antigen becomes insoluble and it may precipitate or float in the fluids. If Ag - Ab complex precipitates, it is referred as "precipitation reaction".

Some times the Ag-Ab complex may float instead of precipitation; in that case the reaction is called as "flocculation reaction" and the Ag-Ab complex is known as "floccule".

**Mechanism of Precipitation and Flocculation Reaction:**

Depending upon its valency a single antibody can bind to more than two antigens at a time. For example IgM Ab with valency 10 can bind to a maximum of 10 Ags at a time, if it happens to get suitable epitopes in its surroundings.

Since IgG antibodies valency is two they can bind to two antigens only. When a single antibody binds to more than one antigen a bridge between the soluble antigens develops and the grouped soluble antigens fail to dissolve in body fluids resulting their precipitation or flocculation.

However, the amount of precipitate and consequently it's' visibility directly depends on the quantities of both antigen and antibody and their ratio. When the Ag, Ab proportion is optimum the precipitate formation shows the maximum level. If the amount of antibody is excess than Ags, precipitation formation is not at maximum level and the serum is not saturated.

When antigen is in excess only, small antigen-antibody complexes are able to form and the small complexes are fairly soluble. Since the Ags are excess, the available Abs may not be sufficient to form a lattice, necessary for precipitation or flocculation.

**3. Complement Fixation:**

Antibody binding with antigen is not sufficient to remove the antigen from body. Hence Ag-Ab complex initiates activation and binding of complement system to it. Binding of complement system to Ag-Ab complex is known as complement fixation.

The activated complement enzymes continue further immune reaction that finally removes the foreign agent (antigen).

**Mechanism of Complement Fixation:**

Inactive enzymes of complement system get activated in a cascade manner in the presence of Ab- Ag complex, and bind to the pathogen to form membrane associated complex- MAC.

The complement fixation assay can be used to check presence of specific antibody or specific antigen in an individual's serum. It was widely used to diagnose infections, particularly microbial infections and rheumatic diseases that are not easily detected by culture methods.

But now new serological methods such as ELISA, PCR and DNA-based methods of pathogen detection are in use for clinical diagnosis.

Antibody structure

In an antibody, the [Fab (fragment, antigen-binding) region](https://en.wikipedia.org/wiki/Fab_region" \o "Fab region) is formed from the amino-terminal end of both the light and heavy chains of the [immunoglobulin](https://en.wikipedia.org/wiki/Immunoglobulin) polypeptide. This region, called the variable (V) domain, is composed of amino acid sequences that define each type of antibody and their binding affinity to an antigen. The combined sequence of variable light chain (VL) and variable heavy chain (VH) creates three hypervariable regions (HV1, HV2, and HV3). In VL these are roughly from residues 28 to 35, from 49 to 59, and from 92 to 103, respectively. HV3 is the most variable part. Thus these regions are the paratope, the binding site of an antigen. The rest of the V region between the hypervariable regions are called framework regions. Each V domain has four framework domains, namely FR1, FR2, FR3, and FR4.[

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## Properties

**Chemical basis of antigen-antibody interaction**

Antibodies bind antigens through weak chemical interactions, and bonding is essentially [non-covalent](https://en.wikipedia.org/wiki/Non-covalent). [Electrostatic interactions](https://en.wikipedia.org/wiki/Electrostatic_interaction), [hydrogen bonds](https://en.wikipedia.org/wiki/Hydrogen_bond), [van der Waals forces](https://en.wikipedia.org/wiki/Van_der_Waals_force), and [hydrophobic interactions](https://en.wikipedia.org/wiki/Hydrophobic_interaction) are all known to be involved depending on the interaction sites.[

**Affinity of the interaction**

Antigen and antibody interact through a high affinity binding much like lock and key. A dynamic equilibrium exists for the binding. For example, the reaction is a reversible one, and can be expressed as:

{\displaystyle {\ce {[Ab] + [Ag] <=> [AbAg]}}} [Ab] + [Ag] [AbAg]

where [Ab] is the [antibody](https://en.wikipedia.org/wiki/Antibody) concentration and [Ag] is the [antigen](https://en.wikipedia.org/wiki/Antigen) concentration, either in free ([Ab],[Ag]) or bound ([AbAg]) state.

The equilibrium association constant can therefore be represented as:

{\displaystyle K\_{a}={\frac {k\_{{\ce {on}}}}{k\_{{\ce {off}}}}}={\frac {{\ce {[AbAg]}}}{{\ce {[Ab][Ag]}}}}}Ka = ([AbAg])/( ([Ab] [Ag]))

where *K* is the [equilibrium constant](https://en.wikipedia.org/wiki/Equilibrium_constant).

Reciprocally the dissociation constant will be:

{\displaystyle K\_{d}={\frac {k\_{{\ce {off}}}}{k\_{{\ce {on}}}}}={\frac {{\ce {[Ab][Ag]}}}{{\ce {[AbAg]}}}}}Kd = {\displaystyle K\_{a}={\frac {k\_{{\ce {on}}}}{k\_{{\ce {off}}}}}={\frac {{\ce {[AbAg]}}}{{\ce {[Ab][Ag]}}} ([Ab][Ag])/( ([AbAg]))

However, these equations are applicable only to a single epitope binding, i.e. one antigen on one antibody. Since the antibody necessarily has two paratopes, and in many circumstances complex binding occurs, the multiple binding equilibrium can be summed up as:

{\displaystyle K\_{a}={\frac {k\_{{\ce {on}}}}{k\_{{\ce {off}}}}}={\frac {{\ce {[AbAg]}}}{{\ce {[Ab][Ag]}}}}={\frac {r}{c(n-r)}}}{\displaystyle K\_{a}={\frac {k\_{{\ce {on}}}}{k\_{{\ce {off}}}}}={\frac {{\ce {[AbAg]}}}{{\ce {[Ab][Ag]}}}}}Ka = ([AbAg])/( ([Ab] [Ag])) = r/c(n-r)

where, at equilibrium, c is the concentration of free ligand, r represents the ratio of the concentration of bound ligand to total antibody concentration and n is the maximum number of binding sites per antibody molecule (the antibody valence).

The overall strength of the binding of an antibody to an antigen is termed its [avidity](https://en.wikipedia.org/wiki/Avidity) for that antigen. Since antibodies are bivalent or polyvalent, this is the sum of the strengths of individual antibody-antigen interactions. The strength of an individual interaction between a single binding site on an antibody and its target epitope is termed the affinity of that interaction.

Avidity and affinity can be judged by the [dissociation constant](https://en.wikipedia.org/wiki/Dissociation_constant) for the interactions they describe. The lower the dissociation constant, the higher the avidity or affinity, and the stronger the interaction.

## Immunodiagnosis of human disease based on antibody-antigen reactions

1. **Western blot.** The western blot (sometimes called the **protein immunoblot**) is a widely used [analytical technique](https://en.wikipedia.org/wiki/Analytical_technique) used in [molecular biology](https://en.wikipedia.org/wiki/Molecular_biology), [immunogenetics](https://en.wikipedia.org/wiki/Immunogenetics" \o "Immunogenetics) and other molecular biology disciplines to detect specific [proteins](https://en.wikipedia.org/wiki/Proteins) in a sample of tissue homogenate or extract.

[Synthetic](https://en.wikipedia.org/wiki/Synthetic_antibody) or [animal-derived](https://en.wikipedia.org/wiki/Polyclonal_antibodies) [antibodies](https://en.wikipedia.org/wiki/Antibody) are created that react with a specific target protein. The sample material undergoes protein denaturation, followed by gel electrophoresis. Next, the electrophoresis membrane is washed in a solution containing the specific antibody. The excess antibody is then washed off, and a secondary antibody that reacts with the first antibody is added.

A Western blot test may be used to diagnose [HIV](https://www.healthline.com/health/hiv-aids), which causes AIDS, Helicobacter pylori*infection*,Lyme disease

1. **Elisa Test** An [enzyme-linked immunosorbent assay](https://www.healthline.com/health/elisa-western-blot-tests-for-hiv), also called ELISA or EIA, is a test that detects and measures antibodies in your blood. This test can be used to determine if you have antibodies related to certain infectious conditions. Antibodies are proteins that your body produces in response to harmful substances called [antigens](https://www.healthline.com/health/cold-flu/fun-facts).

An ELISA test may be used to diagnose [**HIV**](https://www.healthline.com/health/hiv-aids)**, which causes AIDS**, [**pernicious anemia**](https://www.healthline.com/health/pernicious-anemia), [**squamous cell carcinoma**](https://www.healthline.com/health/squamous-cell-skin-cancer), **varicella-zoster virus**, which causes [chickenpox](https://www.healthline.com/health/chickenpox) and [shingles](https://www.healthline.com/health/shingles), [**Zika virus**](https://www.healthline.com/health-news/zika-infects-brain-cells-researchers-say)

1. **Fluorescence-activated cell sorting**

Fluorescence-activated cell sorting (FACS) is a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell.   
  
The cell suspension is entrained in the center of a narrow, rapidly flowing stream of liquid. The flow is arranged so that there is a large separation between cells relative to their diameter. A vibrating mechanism causes the stream of cells to break into individual droplets. The system is adjusted so that there is a low probability of more than one cell per droplet. An electrical charging ring is placed just at the point where the stream breaks into droplets. A charge is placed on the ring based on the immediately prior fluorescence intensity measurement, and the opposite charge is trapped on the droplet as it breaks from the stream. The charged droplets then fall through an electrostatic deflection system that diverts droplets into containers based upon their charge. The stream is then returned to neutral after the droplet breaks off.

**Fluorescence-activated cell sorting** may be used to diagnose **lymphomas** , **prenatal diagnosis of disorders**,

1. **Fluorescent Antibody Test**

Rapid visualization of bacteria from a clinical sample such as a throat swab or sputum can be achieved through **fluorescent antibody (FA) techniques** that attach a fluorescent marker (**fluorogen**) to the constant region of an antibody, resulting in a reporter molecule that is quick to use, easy to see or measure, and able to bind to target markers with high specificity. We can also label cells, allowing us to precisely quantify

particular subsets of cells or even purify these subsets for further research.As with the enzyme assays, FA methods may be direct, in which a labeled mAb binds an antigen, or indirect, in which secondary polyclonal antibodies bind patient antibodies that react to a prepared antigen.

**Fluorescence-activated cell sorting** may be used to diagnose **Syphilis,Pnuemonia,** **Legionnaires' disease,** **strep throat**