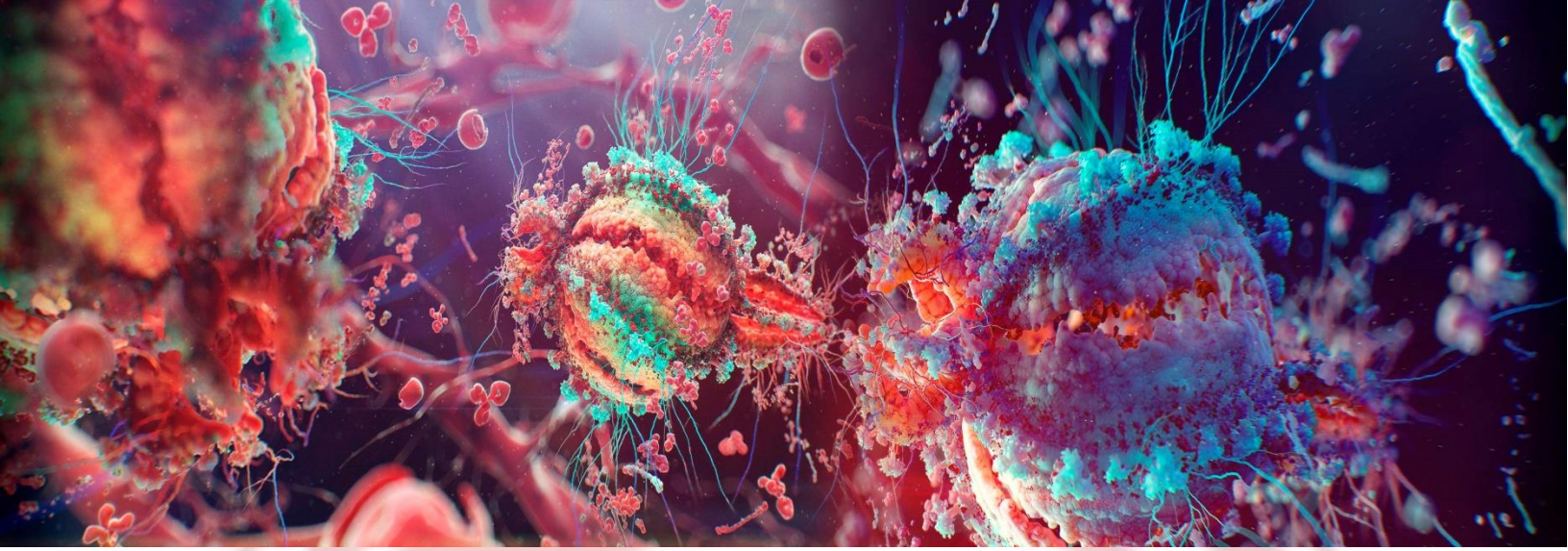


Immunology

Lecture (5)

Done by:
Ala Alrwashdeh

Today we will talk about Antigen-Antibody interaction

Antigen: Any chemical that creates an immune response, most are proteins or large polysaccharides
There are 2 types of antigen :
1-Microbes: Capsules, cell walls, toxins, and viral capsids.
2-Non microbes: Pollen, and egg white.

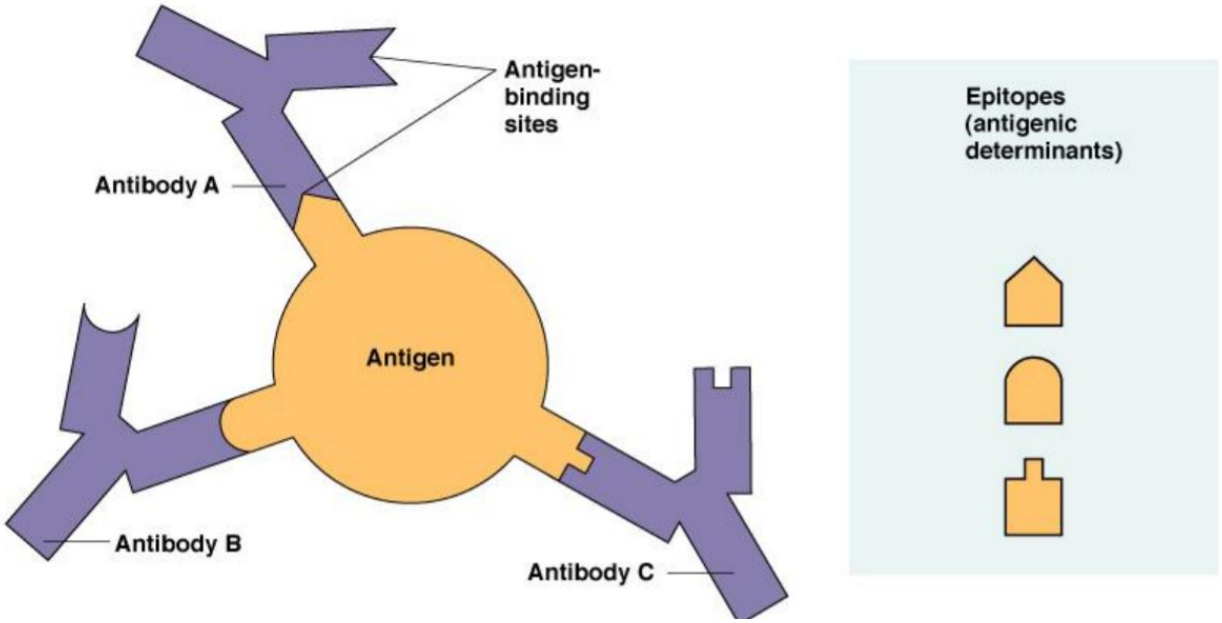
Antibodies: Immunoglobulins that recognize and bind to a particular antigen with high specificity and made in response to exposure to the antigen

Epitope: Small part of an antigen that interacts with an antibody (10-12 amino acids). Any given antigen may have several epitopes. Each epitope is recognized by a different antibody.

The antibody interacts with the antigen, but because the antigen is larger than the antibody so the antibody binds to small regions in the antigen (epitopes). Each antigen may have more than one epitope, so every epitope has its own antibody.

So, one antigen can bind with many antibodies.

All antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Molecules that stimulate immune responses are called immunogens.



Antibody-Antigen interaction

The interaction of the antibody with an antigen causes a change in the shape of the antibody, (we mean by this that there is a difference in the shape of antibody before and after the interaction with the antigen.

May cause the exposure of another site which then is responsible for the various reactions elicited by the antibody to destroy the foreign substance.

The interaction of antibodies and antigens may produce a network type complex, for this, we can find many antibodies (with different functions) binds with the same antigen.

Nature of Antigen-Antibody Reaction

We have three notions about the way of Antigen-Antibody reaction.

1-Lock and Key Concept: The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains.
Here we can imagine a locked door and we need a key to open it, so we need to ask ourselves how many keys can open this door? The answer is one key.

If you remember from the last two lectures that we said the antibody binding site is between light and heavy chains, so this theory says that we have amino acids in the binding site and it's complementary to the amino acids of antigen, so the antigen-antibody occurs in this way.

The Lock and Key Concept suppose that this reaction is an irreversible reaction, but that is wrong because this reaction is a reversible reaction.
so we need another theory to explain this reaction.

2-Non-covalent Bonds: The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds.

hydrogen bonds: a primarily electrostatic force of attraction between a hydrogen (H) atom which is covalently bound to a more electronegative atom or group.

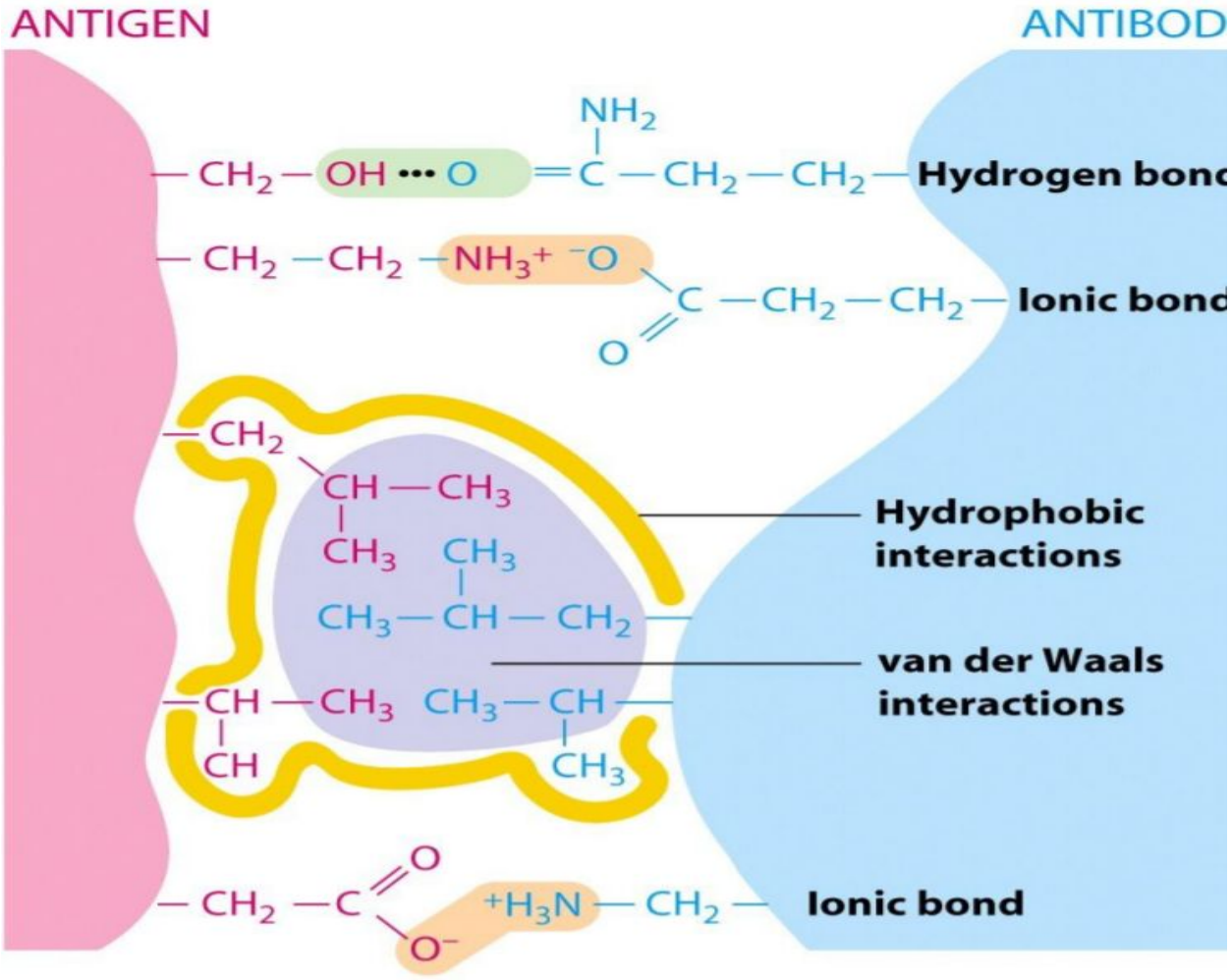
electrostatic bonds: a type of chemical bonding that involves the electrostatic attraction between oppositely charged ions.

Van der Waals forces: a distance-dependent interaction between atoms or molecules. Unlike ionic or covalent bonds, these attractions do not result from a chemical electronic bond; they are comparatively weak and therefore more susceptible to disturbance. The van der Waals force quickly vanishes at longer distances between interacting molecules.

hydrophobic bonds: between hydrophobic substances.

All of these bonds are weak and reversible bonds.

3-Reversibility: Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible

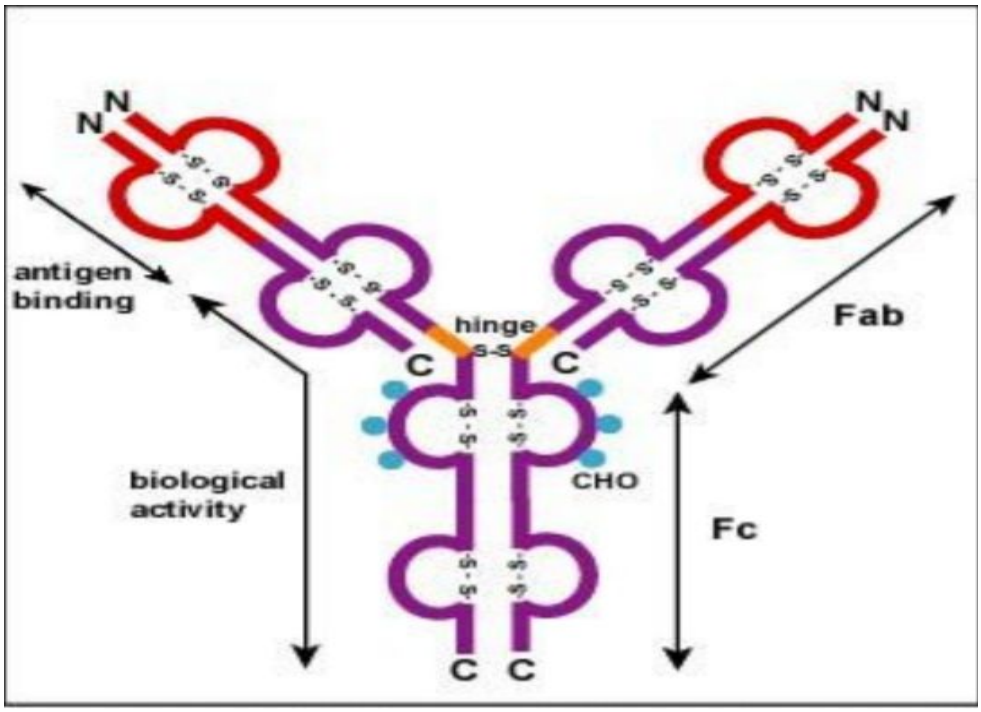


Antigen-antibody binding site

The Fab portion of the antibody has the complementarity-determining regions (red) providing specificity for binding an epitope of an antigen.

Every antibody has its own Fab which binds with one epitope.

The Fc portion (purple) directs the biological activity of the antibody.
Now we know why there are differences in functions in the different types of antibodies because each type of antibodies (IgA, IgD, IgE, IgG, IgM) has it own FC portion.



(S-S = disulfide bond; N = amino terminal of glycoprotein; C = carboxy terminal of glycoprotein; CHO = carbohydrate.)

Antibody Binding Variations

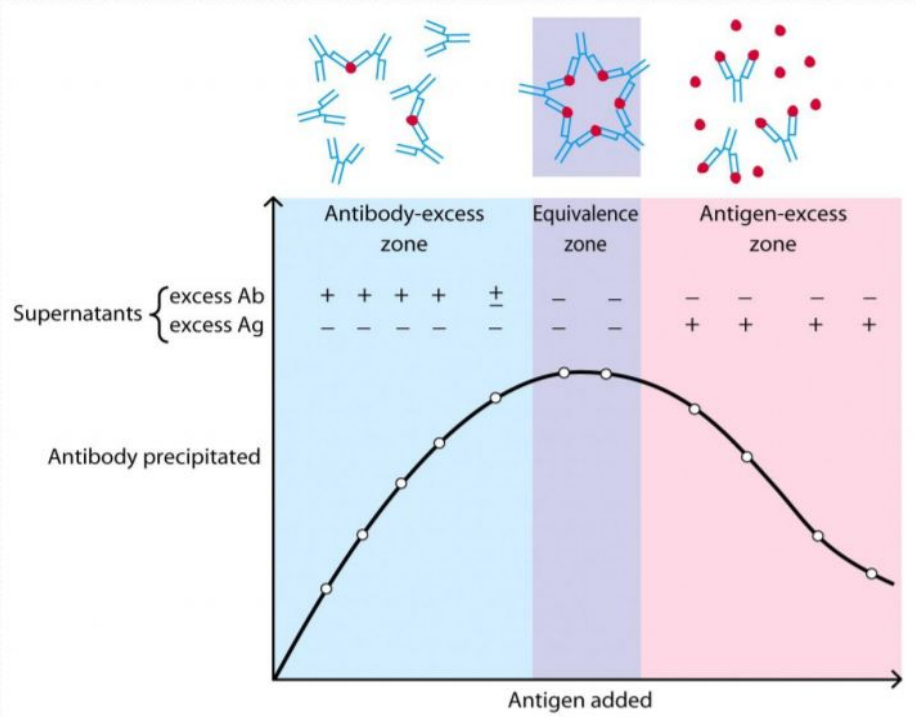
The various genes the cell splices together determine the order of amino acids of the Fab portion of both the light and heavy chain; the amino acid sequence determines the final 3-dimensional shape.

B lymphocyte differentiates to plasma cell which synthesizes antibody, so B lymphocyte considers how antibody structure is according to the amino acids in the epitopes in the antigens.

Therefore, different antibody molecules produced by different B-lymphocytes will have different orders of amino acids at the tips of the Fab to give them unique shapes for the binding epitope.
We should know that each b lymphocyte synthesizes one type of antibody, so if this lymphocyte synthesizes antibody X it can't synthesize antibody Z.

The antigen-binding site is large enough to hold an epitope of about 5-7 amino acids or 3-4 sugar residues.

Antigen-Antibody Binding Equilibrium



In this picture, we have 3 zones:

1-Antibody-excess zone

In this zone, we have antibodies more than antigens so the antibodies will compete with each other to bind with antigens so this will lead to the (on-off) situation and this means that antigen bind with antibody and broke this bindion to bind with another antibody and so on, this will make the bond between antibody and antigen a weak bond.

2- Equivalence zone

In this zone, we have the same number of antibodies and antigens, so each antibody will bind with one antigen only, so this bond is strong.

3-Antigen-excess

In this zone, we have antigens more than antibodies, but in this case, the antibody binds with antigen but he broke this binding when he sees another antigen (antibodies like men, he always look at other women), this will lead to the (on-off) situation, this bond is weak.

Affinity

Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody.

We should have attractive forces more than repulsive forces.

Affinity is the equilibrium constant that describes the Ag-Ab reaction as illustrated. Most antibodies have a high affinity for their antigens.

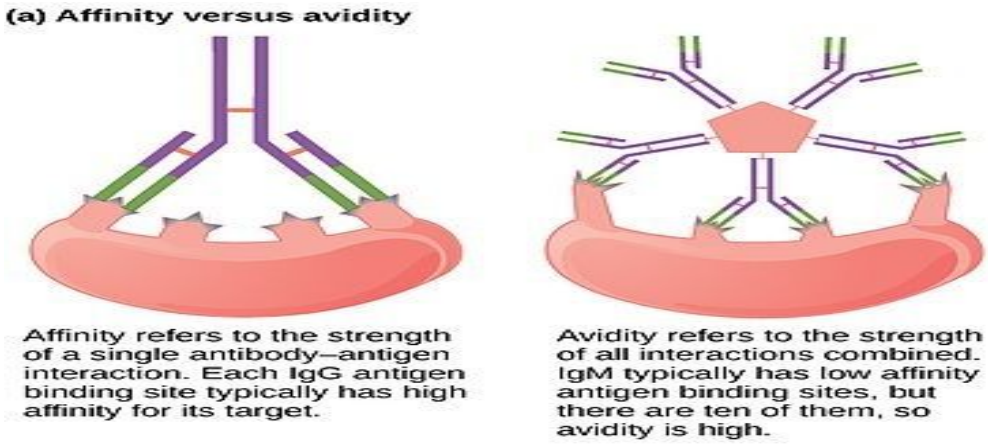
Avidity

Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies.

Affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.

Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities

We use avidity when antigen and antibody bind in more than 1 place, affinity when antigen and antibody bind in one place.
So, avidity is much stronger than affinity.



Specificity

Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen.

Each antibody always binds with one antibody only.

In general, there is a high degree of specificity in Ag-Ab reactions. Antibodies can distinguish differences in :

- the primary structure of an antigen
- isomeric forms of an antigen
- the secondary and tertiary structure of an antigen

Cross-Reactivity

Cross-reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen.

Cross-reactions arise because the cross-reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).

So, here we oppose what we have said in the specificity, we said that each antibody binds with one antigen only, but here he can bind with many antigens why? Because many antigens can share the same epitope.

Visualizing Antigen-Antibody Reactions

- Agglutination
- Precipitation
- Complement fixation
- Fluorescent antibody tests
- ELISA and RIA
- Western Blot

From here I recommend to listen to doctor's record.

1.Agglutination Testing

Agglutination: antigens are whole cells such as red blood cells or bacteria with determinant groups on the surface.

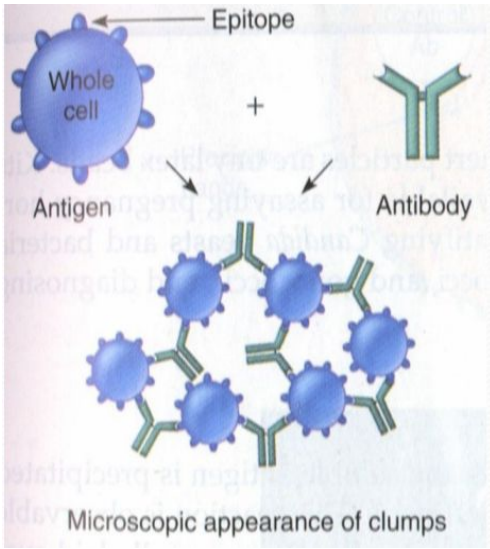
In this test, we use the antibody to find antigen, so if we want to find a specific type of bacteria, we use an antibody which binds with the epitope of the antigen of the bacteria, if we see agglutination that means that there are a bacteria.

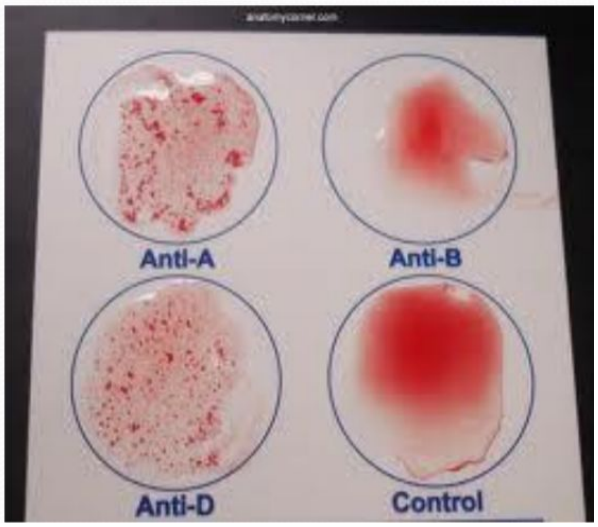
Antibodies cross-link the antigens to form visible clumps.

Performed routinely to determine ABO and Rh blood types.

Widal test: tube agglutination test for diagnosing salmonella and undulant fever.
Here we try to detect AB, so we take the disease Ag and bind it with the patient's sample.

Latex agglutination tests: tiny latex beads with antigens affixed.
tiny latex beads(a plastic piece with red or blue colour) with virus antigens then we added the serum, if there is Ab in serum it will bind to the virus, then the beads will agglutinate.

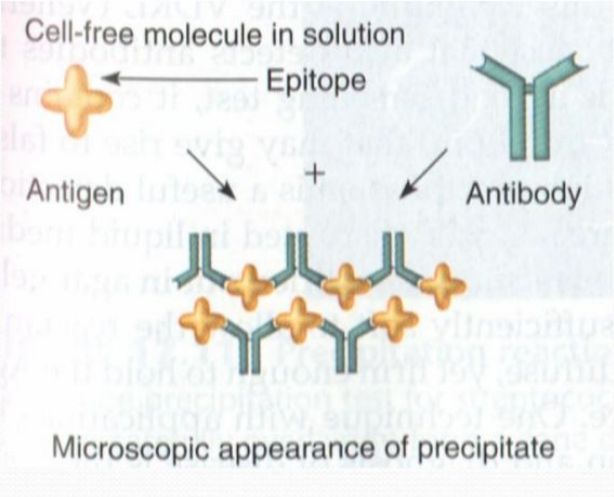


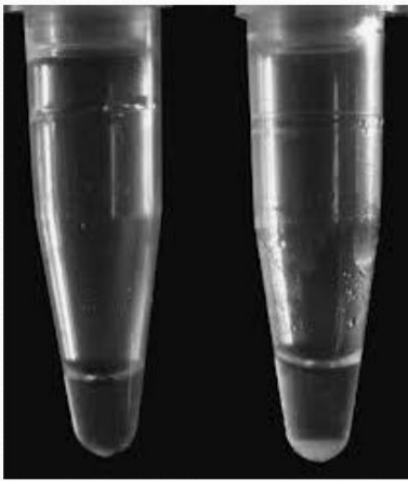


2.Precipitation Tests

Precipitation is the interaction of a soluble Ag with a soluble Ab to form an insoluble complex, The complex formed is an aggregate of Ag and Ab, Reaction is observable as a cloudy or opaque zone at the point of contact.

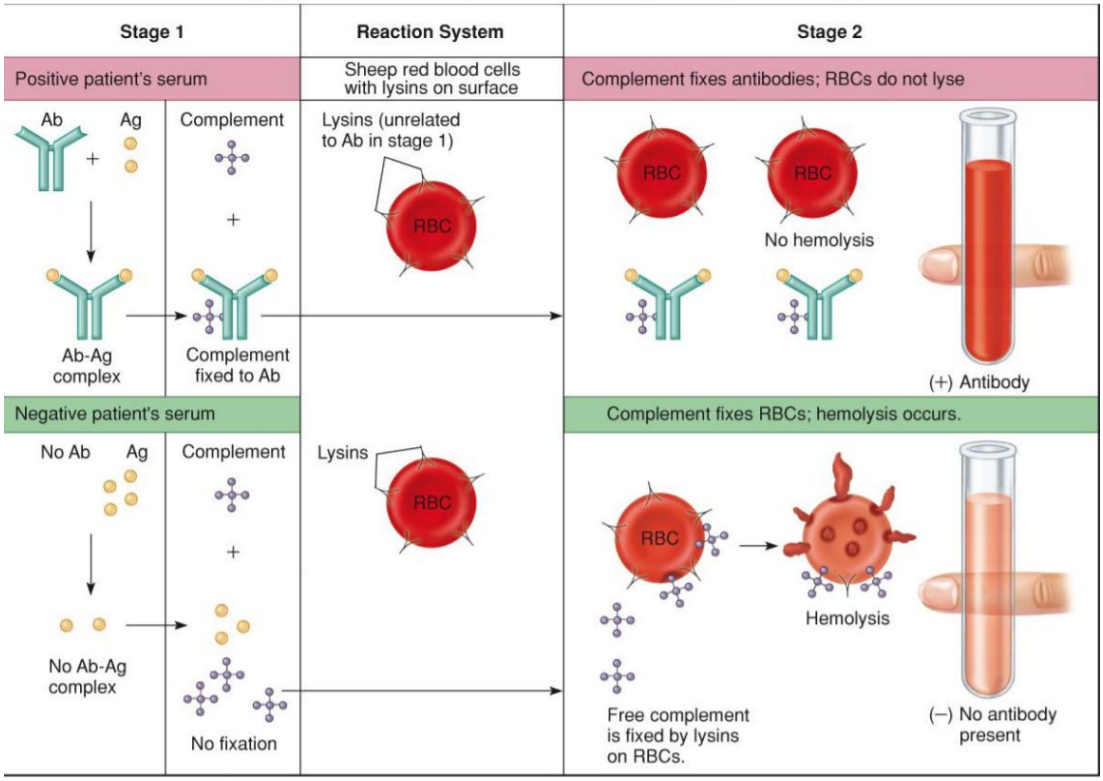
Example: VDRL (Venereal Disease Research Lab) test streptococcal group antigens testing.





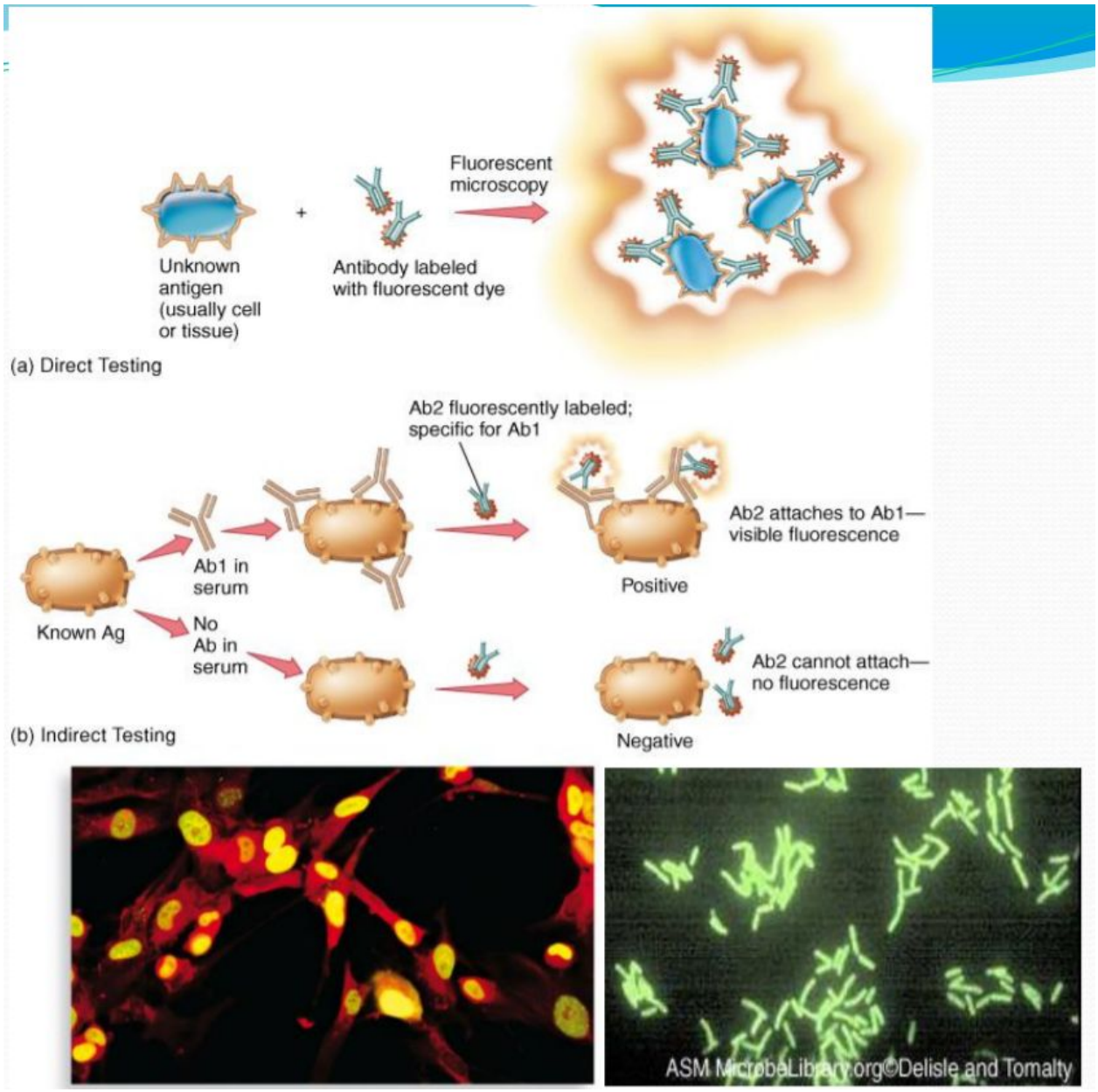
3. Complement fixation

Lysin or cytolysin: an antibody that requires complement to complete the lysis of its antigenic target cell.



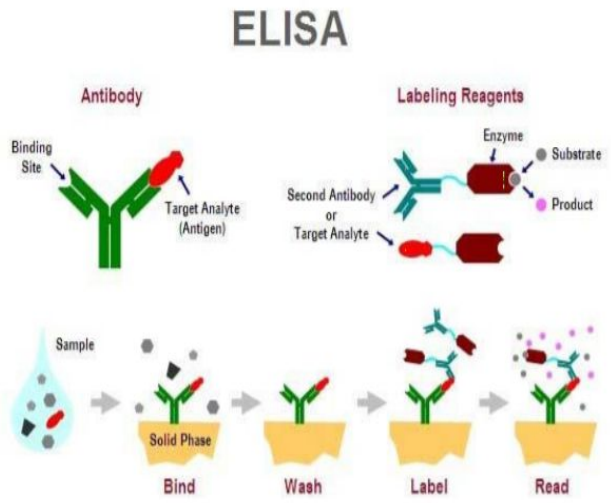
4-fluorescent antibodies and immunofluorescence testing

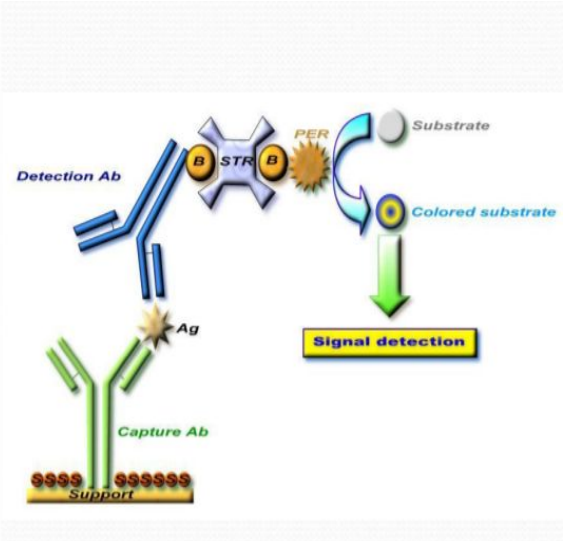
Direct testing: an unknown test specimen or antigen is fixed to a slide and exposed to a fluorescent antibody solution of known composition
Indirect testing: the fluorescent antibodies are antibodies made to react with the Fc region of another antibody



5.Radioimmunoassay (RIA) and Enzyme-Linked Immunosorbent Assay (ELISA)

Antibodies or antigens labelled with a radioactive isotope (RIA) or Enzyme (ELISA) used to pinpoint minute amounts of a corresponding antigen or antibody. Compare the amount of radioactivity present in a sample before and after incubation with a known, labelled antigen or antibody.



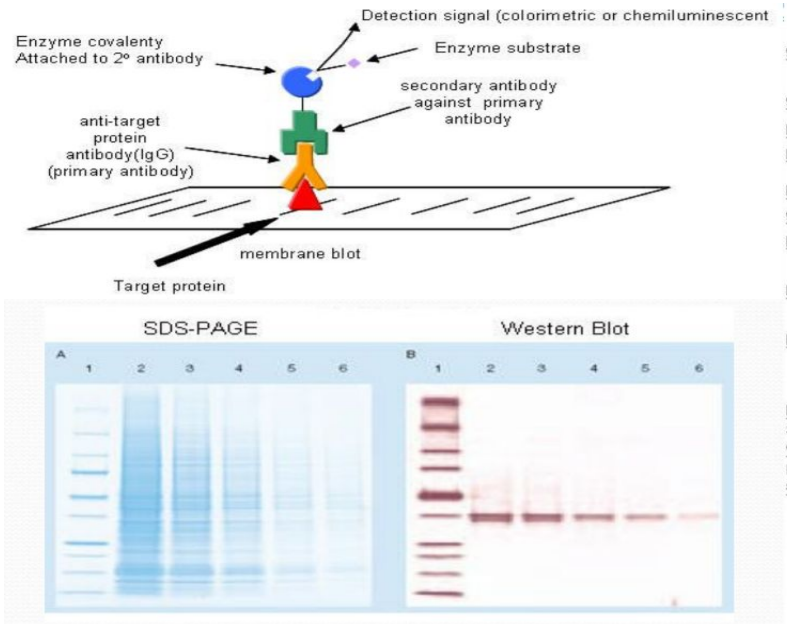


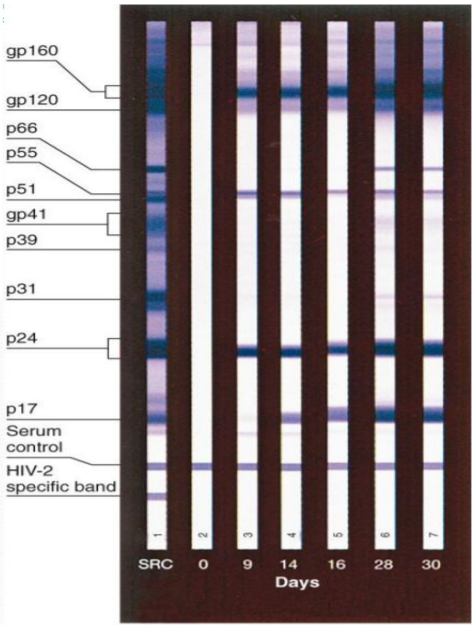
6-The Western Blot for Detecting Proteins

Test material is electrophoresed in a gel to separate out particular bands

Gel transferred to a special blotter that binds the reactants in place

Blot developed by incubating it with a solution of antigen or antibody labelled with radioactive, fluorescent, or luminescent labels.





7-Flow Cytometry

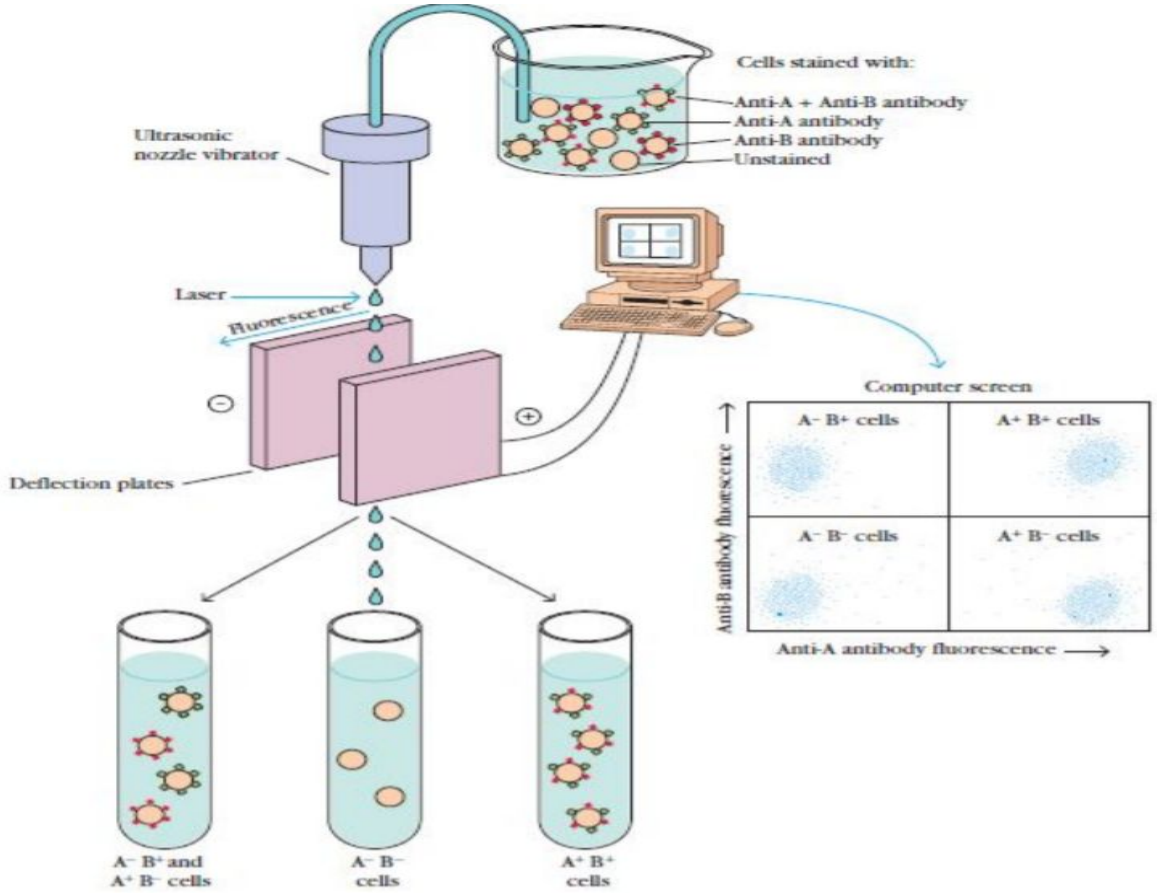
The flow cytometer was designed to automate the analysis and separation of cells stained with fluorescent antibody

The flow cytometer uses a laser beam and light detector to count single intact cells in suspension

Every time a cell passes the laser beam, light is deflected from the detector, and this interruption of the laser signal is recorded

Those cells having a fluorescently tagged antibody bound to their cell surface antigens are excited by the laser and emit light that is recorded by a second detector system located at a right angle to the laser beam

It has a large number of medical application for example in the classification and treatment of leukaemias.



Thank you