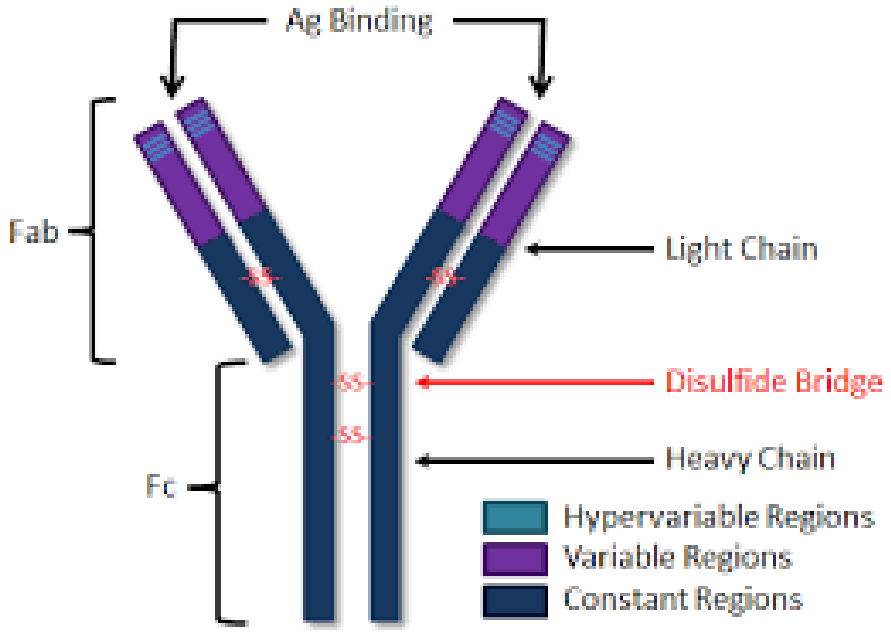
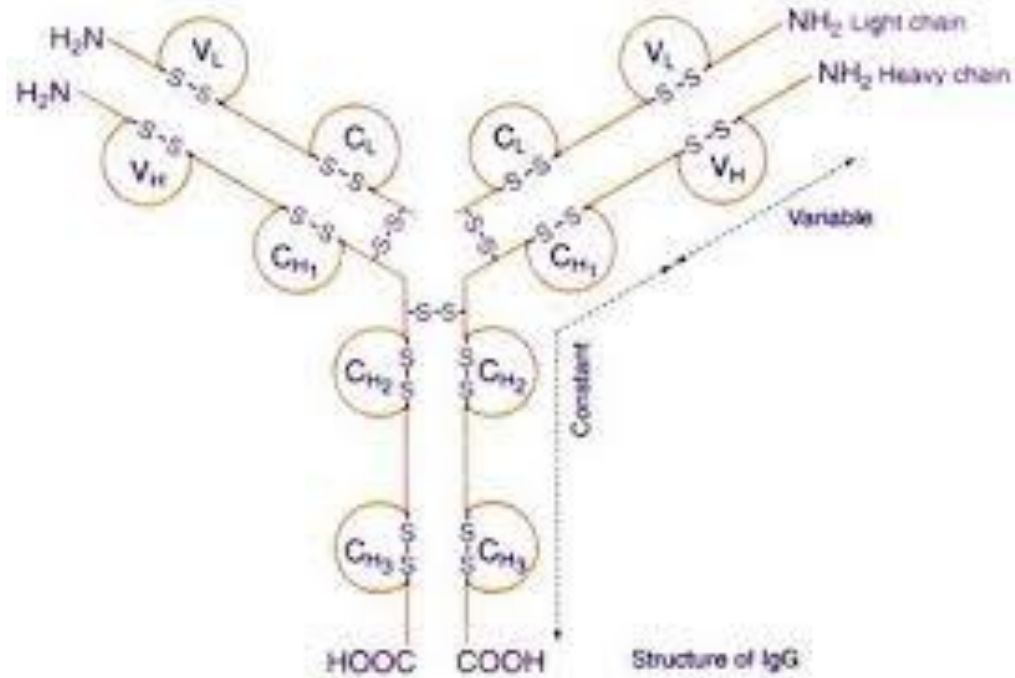
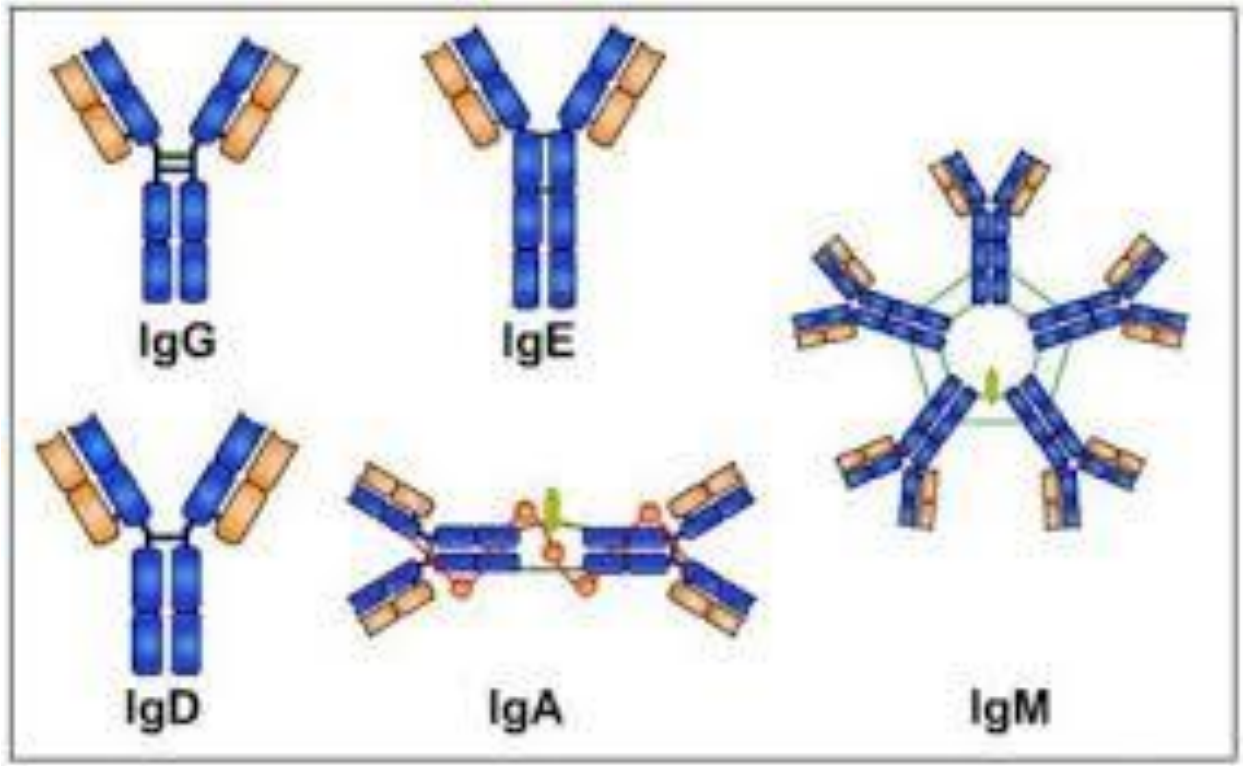

Ag Binding
Fab
Light Chain
Disulfide Bridge
Heavy Chain
Fc
Hypervariable Regions
Variable Regions
Constant Regions

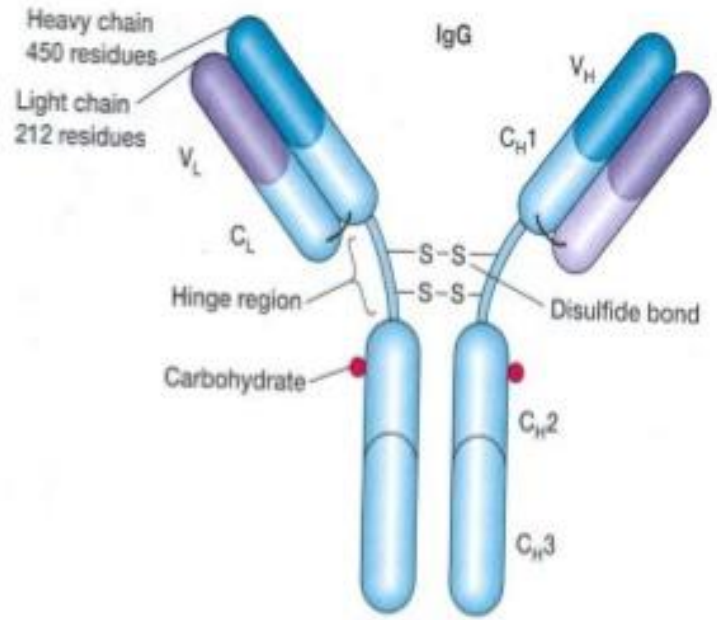


Structure of IgG


IgG
IgE
IgD
IgA
IgM

Immunoglobulin G (Ig G)

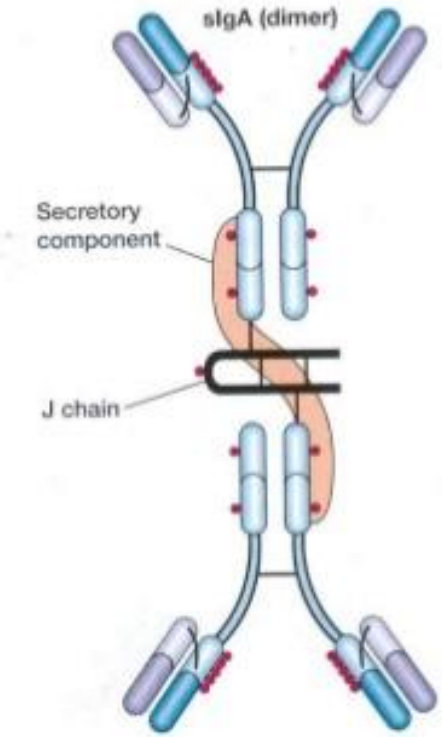
- Most abundant class in serum
- Constitutes 80% total immunoglobulin
- Present in blood, plasma and tissue fluids
- Contains less carbohydrate than other immunoglobulins
- It has a half life of 23 days: the longest of all of the immunoglobulin isotypes



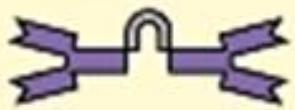
IgG (monomer) 	IgG is the most abundant of the circulating antibodies. It readily crosses the walls of blood vessels and enters tissue fluids. IgG also crosses the placenta and confers passive immunity on the fetus. IgG protects against bacteria, viruses, and toxins in the blood and lymph, and triggers action of the complement system.

Immunoglobulin A (Ig A)

- Constitutes 10-15 % of total immunoglobulins
- Present in milk, saliva, tears, mucous of respiratory tract, digestive tract and genitourinary tract.
- In serum exist as monomer
- In external secretions exist as dimer called secretory Immunoglobulin.
- Has ‘J’ chain and secretory piece.
- Half life: 6-8 days



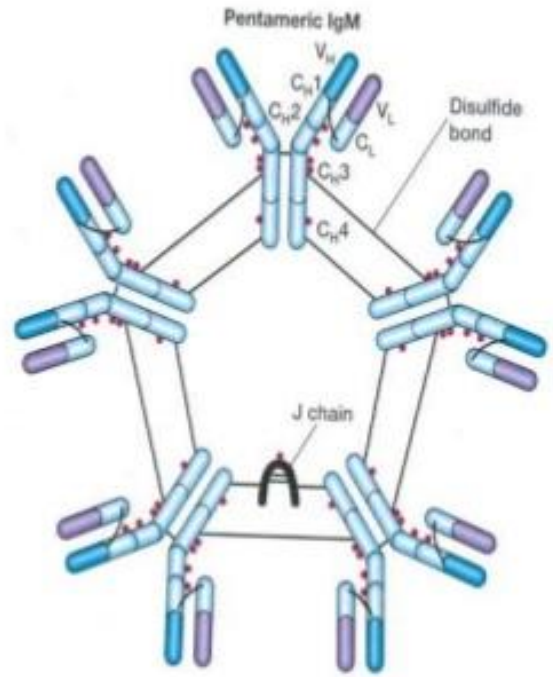
IgA
(dimer)



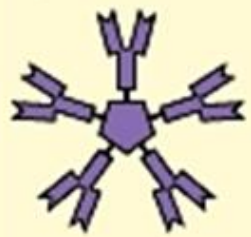
IgA is produced by cells in mucous membranes. The main function of IgA is to prevent the attachment of viruses and bacteria to epithelial surfaces. IgA is also found in many body secretions, such as saliva, perspiration, and tears. Its presence in the first milk produced helps protect the infant from gastrointestinal infections.

Immunoglobulin M (Ig M)

- Accounts for 5-10% of total serum proteins
- Polymer of five monomeric units (pentamer)
- Held together by disulfide bonds and ‘J’ chain
- Mol. Wt. of 900,000-10,00,000 (millionaire molecule)
- Half life: 5 days



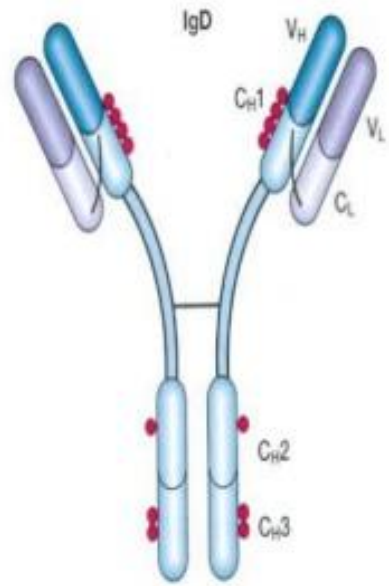
IgM
(pentamer)



IgMs are the first circulating antibodies to appear in response to an initial exposure to an antigen; their concentration in the blood then declines rapidly. Thus the presence of IgM usually indicates a current infection. IgM consists of five Y-shaped monomers arranged in a pentagonal structure. The numerous antigen-binding sites make it very effective in agglutinating antigens and in reactions involving complement. IgM is too large to cross the placenta and does not confer maternal immunity.

Immunoglobulin D (Ig D)

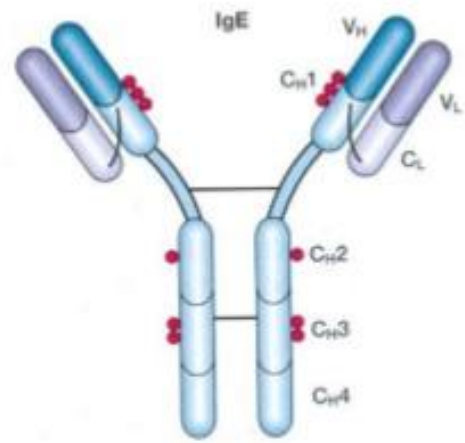
- Structure is similar to IgG
- Serum concentration 30 micrograms per ml
- Constitutes 0.2% of total immunoglobulins
- Half life: 3 days
- IgD together with IgM is major membrane bound immunoglobulin on unstimulated B lymphocytes-acts as recognition receptors for antigens



IgD (monomer) 	IgD antibodies do not activate the complement system and cannot cross the placenta. They are mostly found on the surfaces of B cells, probably functioning as antigen receptors that help initiate the differentiation of B cells into plasma cells and memory B cells.

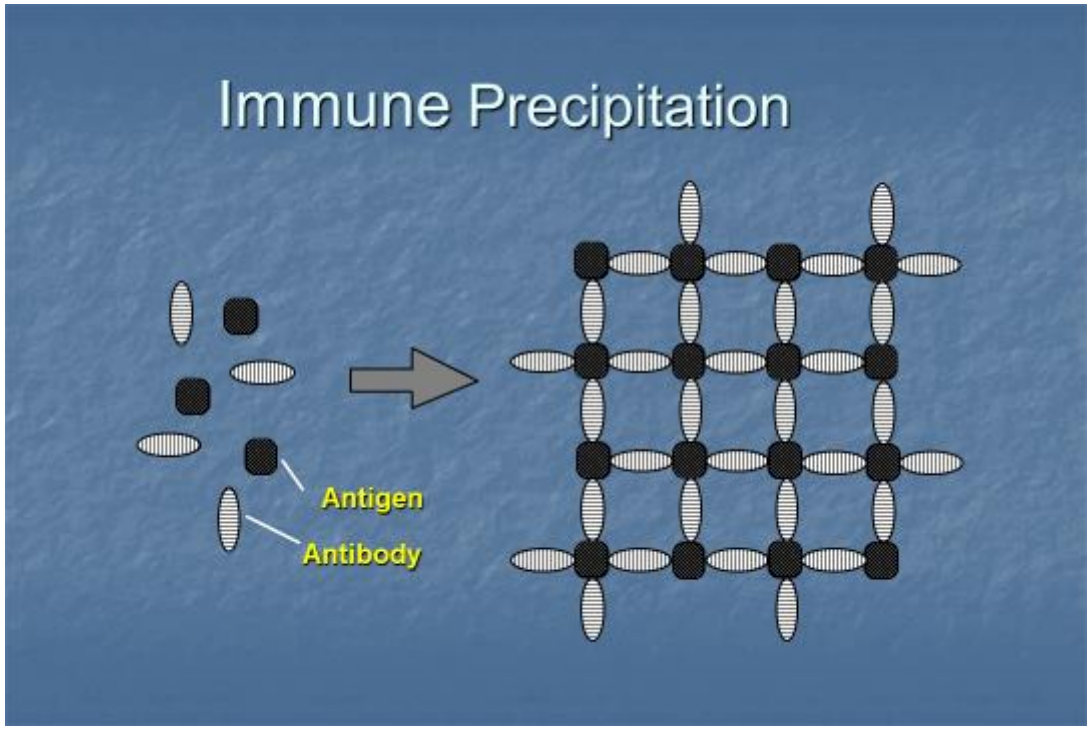
Immunoglobulin E (Ig E)

- Structure is similar to Ig G
- Has 4 constant region domains.
- Mol. Wt. 1,90,000
- Half life: 2 days
- Heat labile (inactivated at 56^0C in 1 hour)
- Normal serum concentration 0.3 ug/ml
- Mostly present extra cellularly
- Does not cross placenta

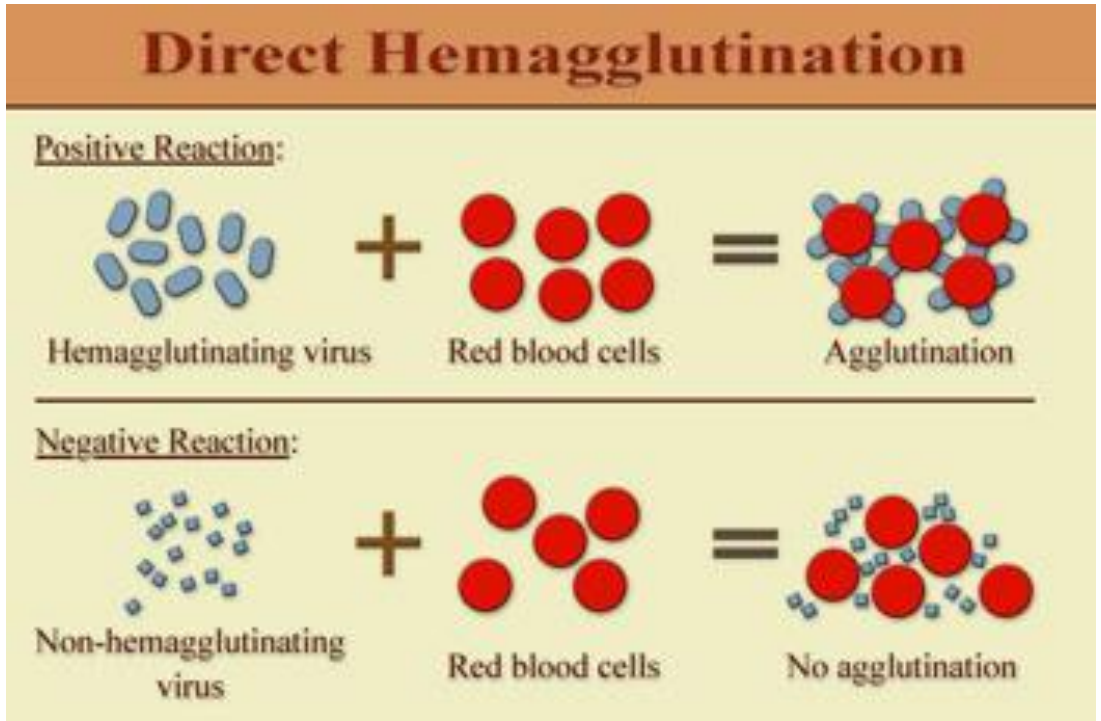


IgE
(monomer)

IgE molecules are slightly larger than IgG and represent only a small fraction of the antibodies in the blood. The tails attach to mast cells and basophils and, when triggered by an antigen, cause the cells to release histamine and other chemicals that cause an allergic reaction.


Immune Precipitation
Antigen
Antibody

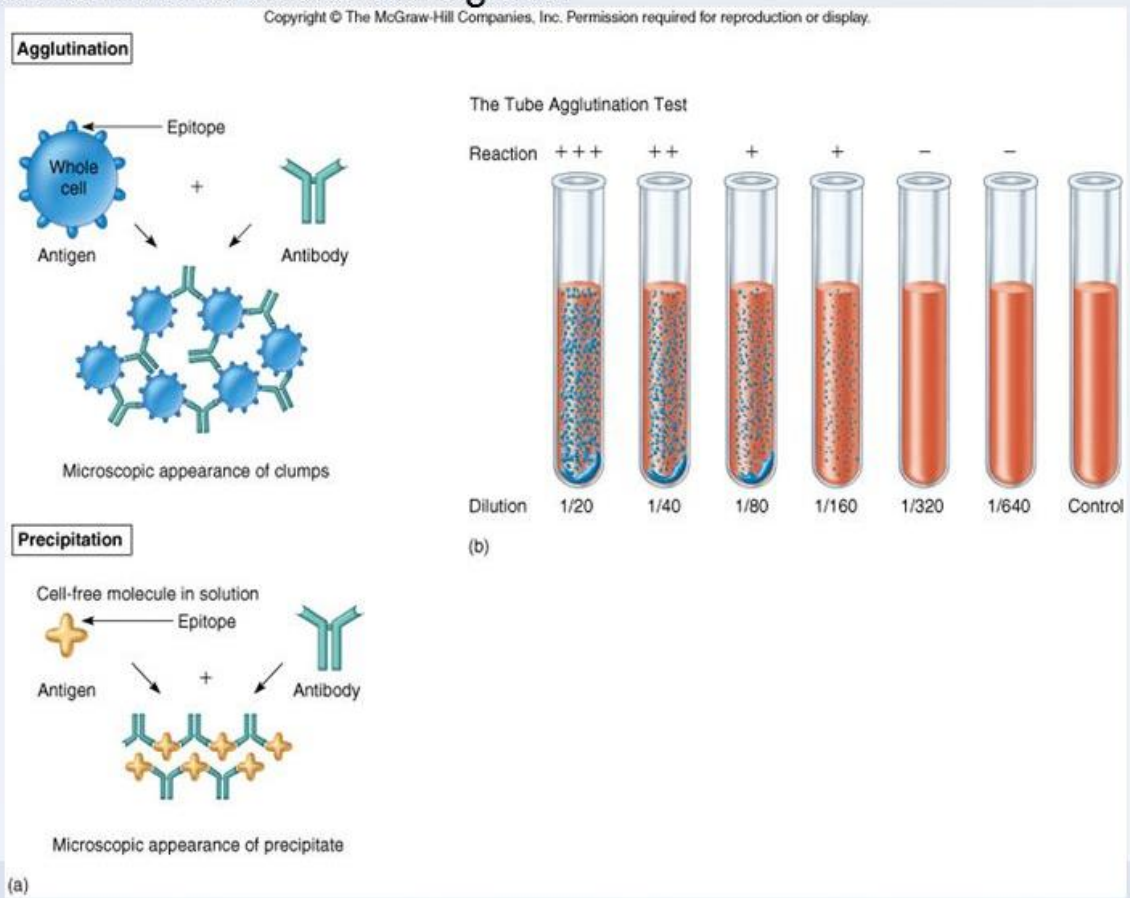
Direct Hemagglutination



- Agglutination reactions involve whole cell antigens, while precipitation reactions involve soluble antigens.

-

Cellular/molecular view of agglutination and Precipitation reactions that produce visible Ag-Ab complexes.



Widal test

- Detects O and H agglutinins for typhoid and paratyphoid bacilli.
- Two types of tubes are used
  - Dreyer's tube for H agglutination: narrow tube with conical bottom
  - Felix tube for O agglutination: short round bottomed tube
- H agglutination: loose, cottony agglutinates
- O agglutination: compact granular agglutinates.

Fig. 9.10 : Widal tube test showing bacterial agglutination

Fig. 9.12 : Somatic ('O') agglutination of *Salmonella* by O antiserum (examination of deposit by coverslip preparation) shown a polar attachment of bacterial cells

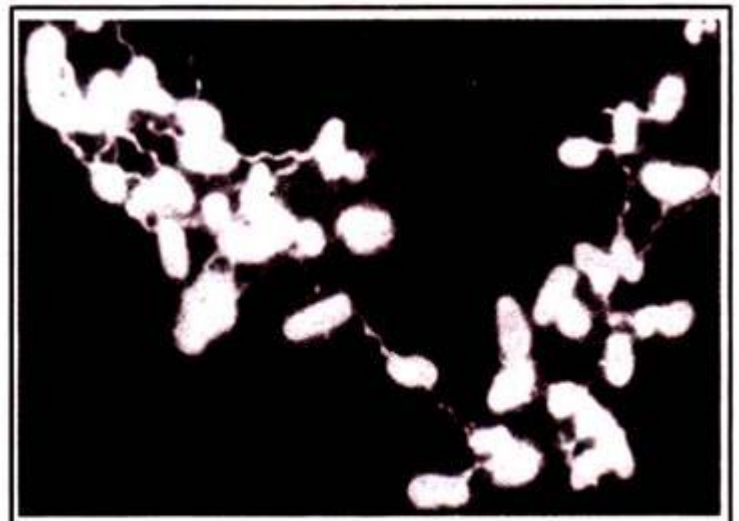


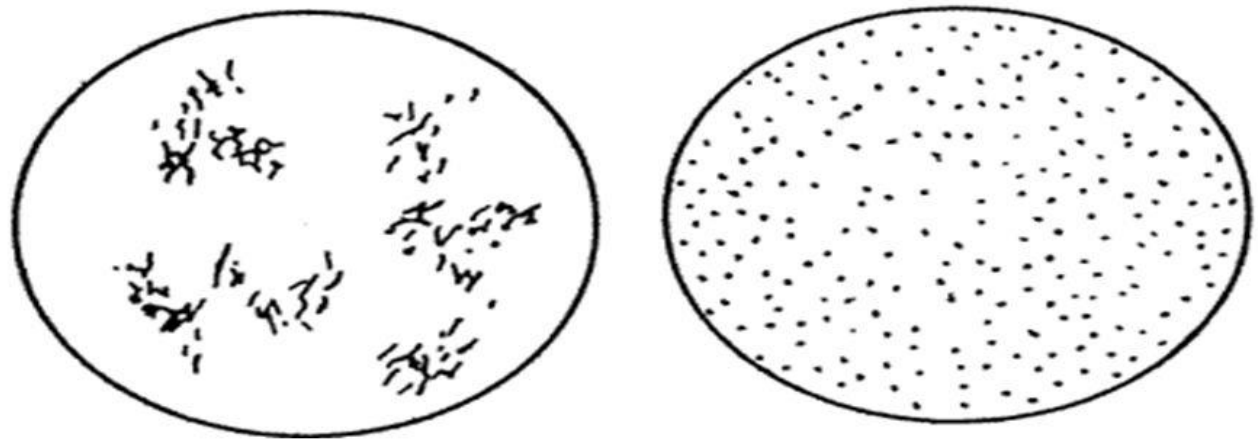
Fig. 9.11 : Flagellar agglutination of *Salmonella typhosa* by H antiserum : clumps of cells formed by intertwining of thickened flagellar structures (Electron microscopy)

- VDRL test
  - Serological test for syphilis widely used to test for primary and secondary syphilis
    - Performed on blood serum
  - VDRL = Venereal Disease Research Laboratory

VDRL Serological Procedure Principles

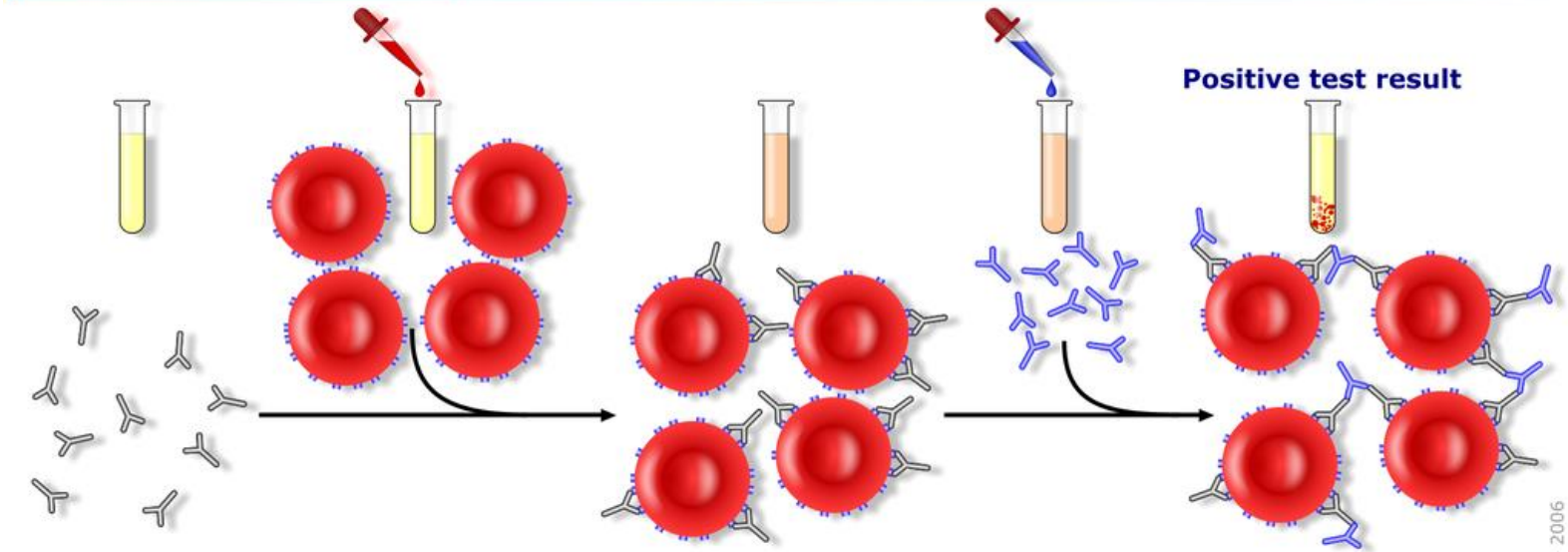
- VDRL Antigen is a nontreponemal antigen composed of cardiolipin cholesterol and lecithin. The nontreponemal tests measures anti-lipid antibodies, which are formed by the host in response to **lipids released from damaged host cells early in infection with T. pallidum,** and lipid-like material form the treponemal cell surface. During syphilis infection, an antibody-like substance called reagin can be detected in the patient's serum or CSF.

VDRL



- Each preparation of antigen suspension should first be examined by testing with known positive or negative serum controls.
- The antigen particles appear as short rod forms at magnification of about 100x. Aggregation of these particles into large or small clumps is interpreted as degrees of positivity
- Reactive on left, non-reactive on right

Indirect Coombs test / Indirect antiglobulin test



Recipient's serum is obtained, containing antibodies (Ig's).

Donor's blood sample is added to the tube with serum.

Recipient's Ig's that target the donor's red blood cells form antibody-antigen complexes.

Anti-human Ig's (*Coombs antibodies*) are added to the solution.

Agglutination of red blood cells occurs, because human Ig's are attached to red blood cells.

COMPLEMENT FIXATION TEST

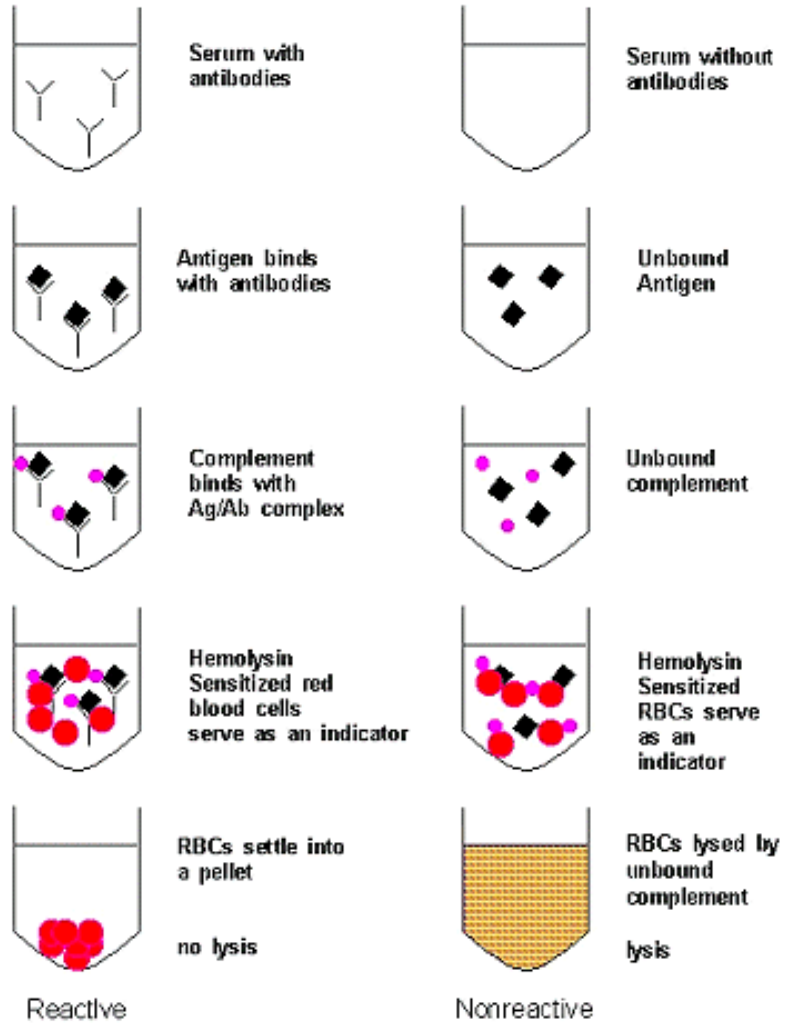
Principle

- Complement binds to Ag-Ab complex and gets absorbed during the combination of antigens and antibody.
- This property of antigen–antibody complex to fix the complement is used in complement fixation test for the identification of specific antibodies.

- If the complement is fixed on test system(Ag + Ab), then there will be **no lysis** of sheep erythrocytes, thus denoting a **positive test**.

- If the complement is available not bound to test system, it will be free to combine with indicator system resulting in **hemolysis** denoting a **negative test**.

Complement Fixation Test

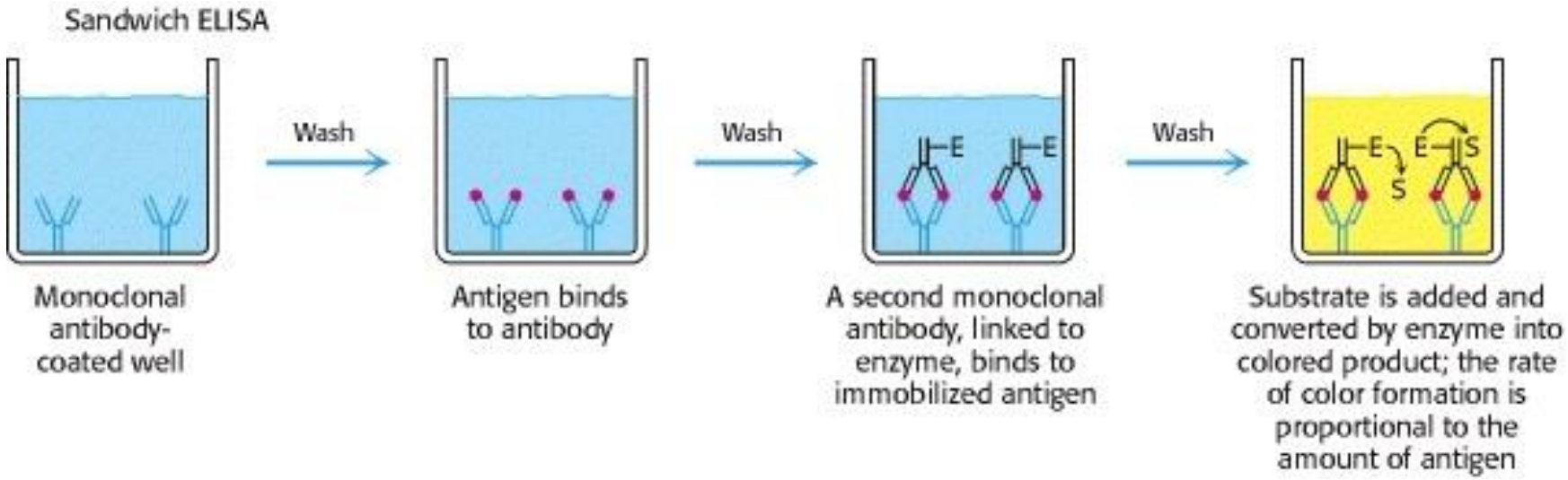


What is ELISA?

- Enzyme-Linked Immunosorbent Assay (ELISA) is biochemical assay technique used mainly in immunology.
- It is a plate-based assays designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones.
- First and most basic test to determine if an individual is positive for a selected pathogen, such as HIV.
- Dimension of ELISA plant:
  - 8 cm x 12 cm plastic plate which contains an 8 x 12 matrix of 96 wells, each of which are about 1 cm high and 0.7 cm in diameter.

BASIC PRINCIPLE OF ELISA

- Use an enzyme to detect the binding of antigen (Ag) antibody (Ab).
- The enzyme converts a colorless substrate (chromogen) to a colored product, indicating the presence of Ag : Ab binding.
- An ELISA can be used to detect either the presence of Antigens or antibodies in a sample depending how the test is designed.
- ELISA was dveloped in 1970 and became rapidly accepted


Sandwich ELISA
Wash
Wash
Wash
E
E
E
E
S
S
Monoclonal antibody-coated well
Antigen binds to antibody
A second monoclonal antibody, linked to enzyme, binds to immobilized antigen
Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of antigen

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.

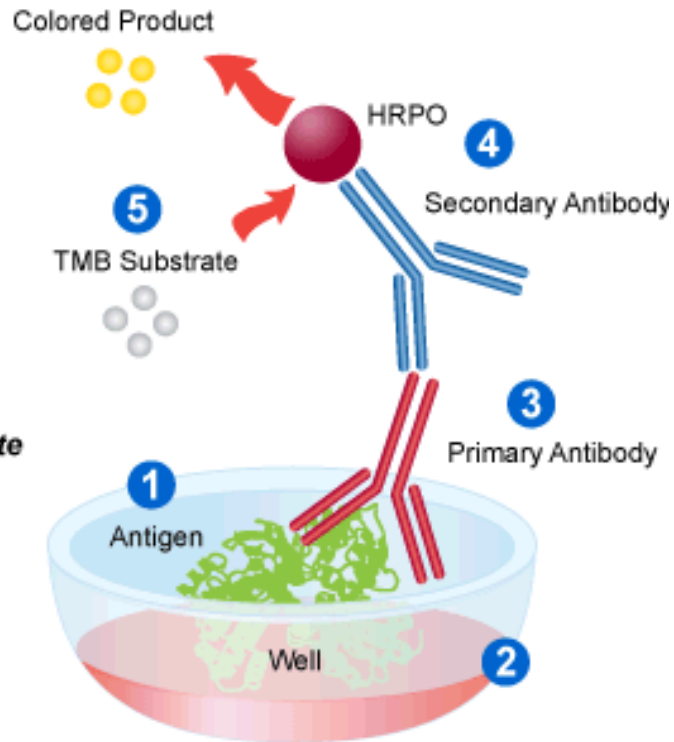


Diagram 1: *Illustration of Indirect ELISA method.*