

AL-Mustaqbal University Collage.
Department of Pathological Analysis Technique.
Subject: - Advanced laboratory techniques.
Lecture-No. 13.
INTRODUCTION TO SEROLOGY.



INTRODUCTION TO SEROLOGY

• **Serology**: - is the scientific study of blood serum and other bodily fluids in vitro.

In practice, the term usually refers to the diagnostic identification of antibodies in the serum. Such antibodies are typically formed in response to an infection (against a given microorganism), against other foreign proteins (in response, for example, to a mismatched blood transfusion), or to one's own proteins (in instances of autoimmune disease).

• Serological tests may be performed for diagnostic purposes when an infection is suspected and in many other situations, such as checking an individual's blood type.

Serology blood tests help to diagnose patients with certain immune deficiencies associated with the lack of antibodies.

• There are several serology techniques that can be used depending on the antibodies being studied. These include:

• Agglutination. “Haemagglutination (HA), Haemagglutination Inhibition Test (HAI)”.

• Precipitation.

• Neutralization.

• Complement Fixation (CFT).

• Labeled immunoassay:

• Radioimmunoassay (RIA) (rarely used nowadays).

• Enzyme linked immunosorbent assay (ELISA).

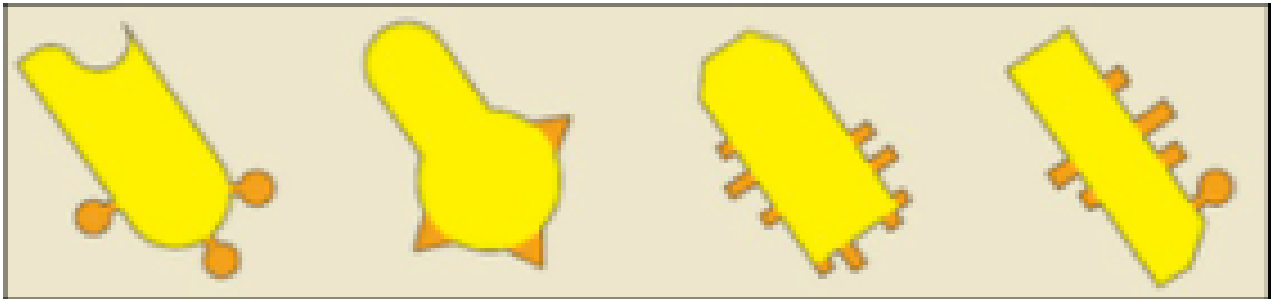
• Immunofluorescence (IF).

• Some serological tests are not limited to blood serum, but can also be performed on other bodily fluids such as semen and saliva, which have (roughly) similar properties to serum.

• All tests of serology are depending on Antigen-Antibody Reaction.

Antigen: -

Is a substance that stimulates antibody formation and has the ability to bind to an antibody.

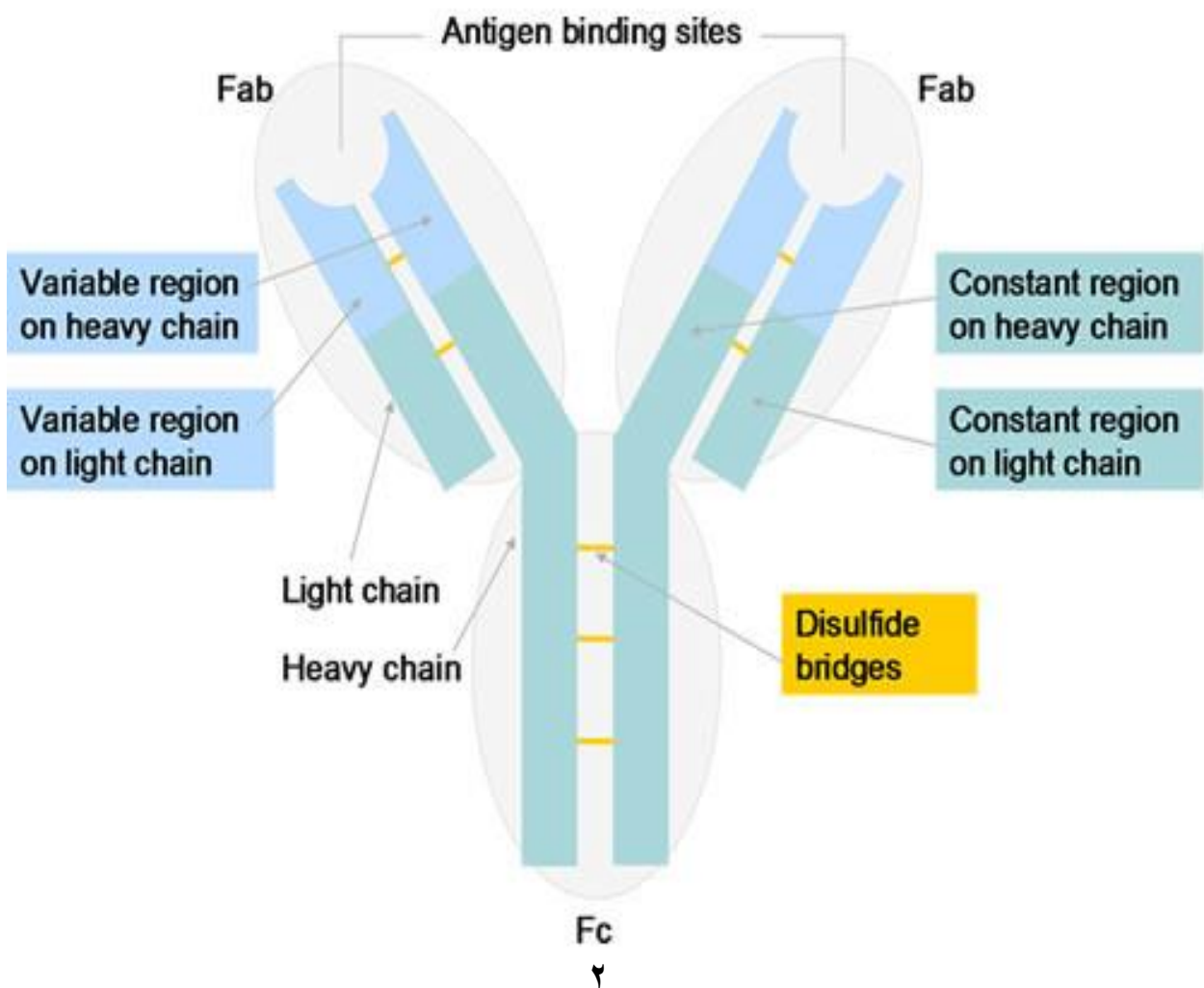


Antibody: -

Is specific glycoprotein's referred to as immunoglobulin's (Igs).



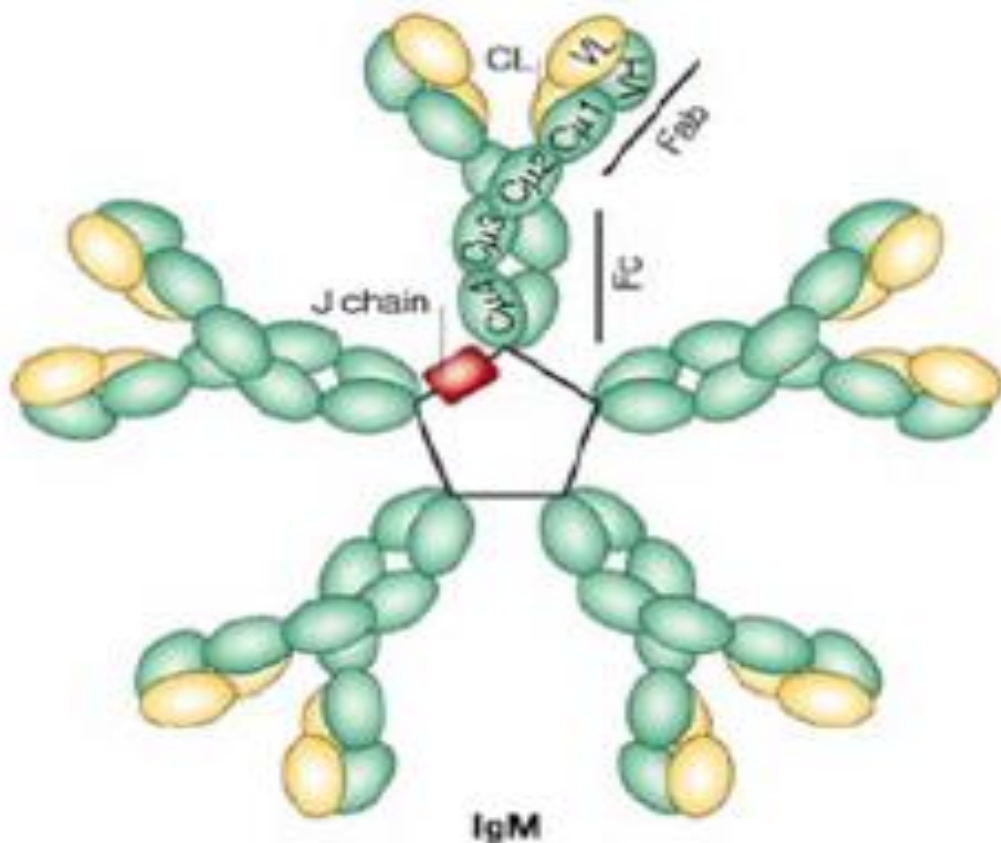
Antibody structure



SOME MAJOR CLASSES OF ANTIBODIES IN SEROLOGICAL DIAGNOSIS

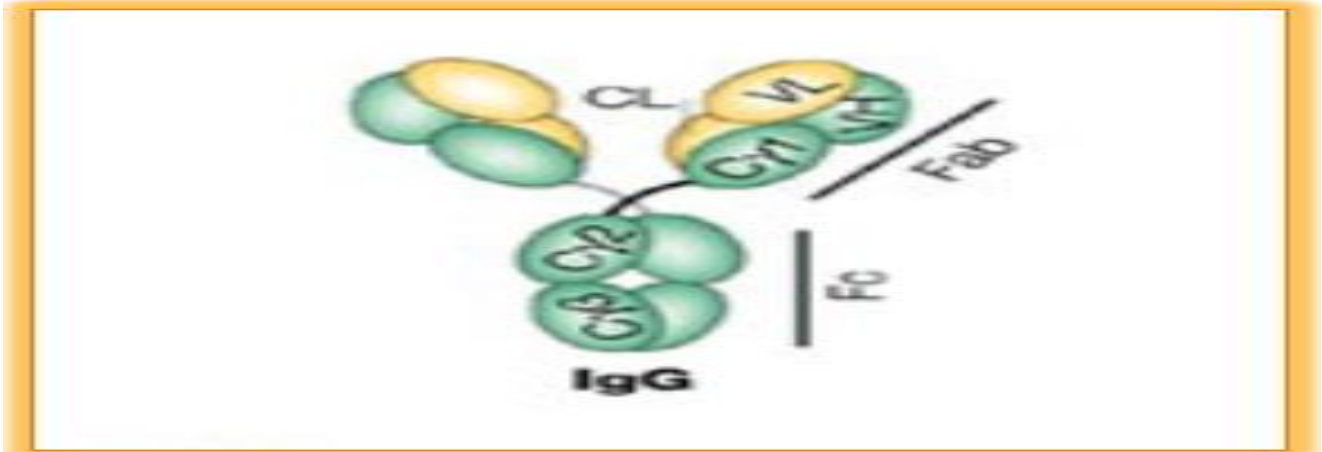
IgM:

- It is a basic antibody that is produced by B cells.
- It is the largest antibody in the human circulatory system.
- It is the first antibody appears in response to initial exposure to antigen.
- Demonstrating of IgM antibodies in a patient's serum indicates recent infection or in a neonate's serum indicates intrauterine infection (e.g. congenital rubella).
- It declines with convalescence.



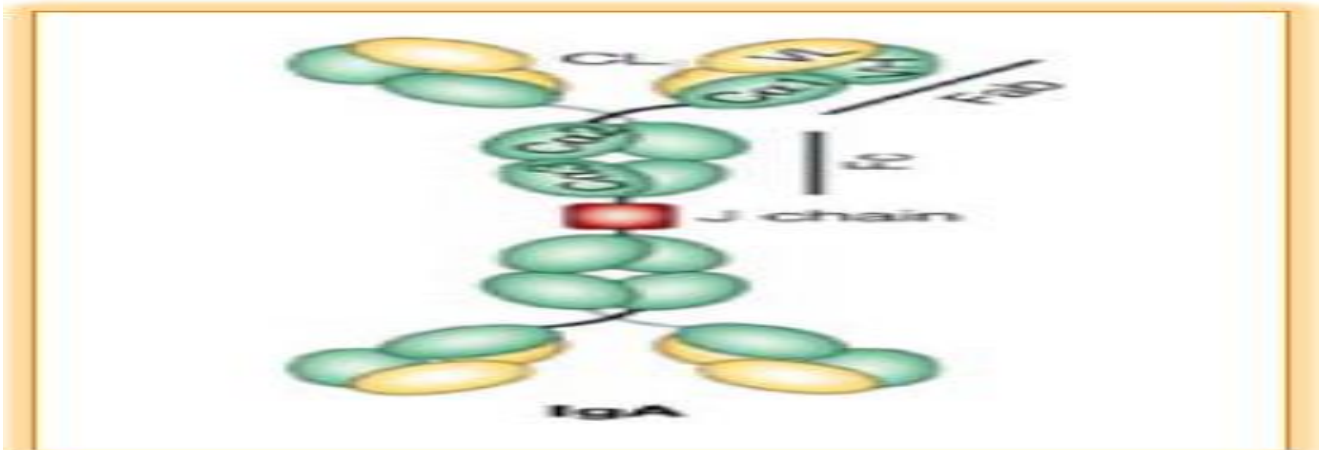
IgG:

- IgG antibodies are involved in predominantly the secondary immune response.
- Appear following infection or administration of vaccine.
- IgG is the only antibody that can pass through the human placenta.
- The IgG antibodies there after increase in titer and remain high during convalescence and for some time after ward.
- There are four IgG subclasses (IgG1, 2, 3, and 4) in humans, named in order of their abundance in serum.



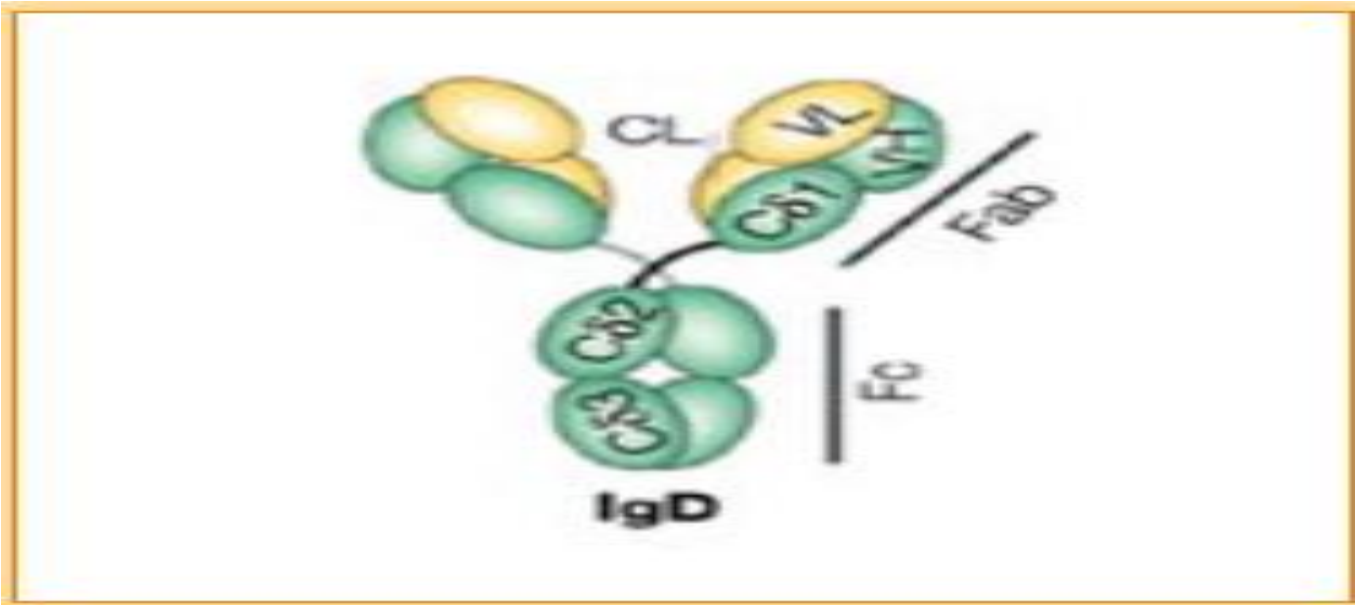
IgA:

- It is an antibody that plays a critical role in mucosal immunity.
- It has two subclasses (IgA1 and IgA2).
- IgA is the main immunoglobulin found in mucous secretions, including tears, saliva and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium.
- It is also found in small amounts in blood.
- IgA found in high concentration in breast milk then transferring the immunity to the newborn infant.



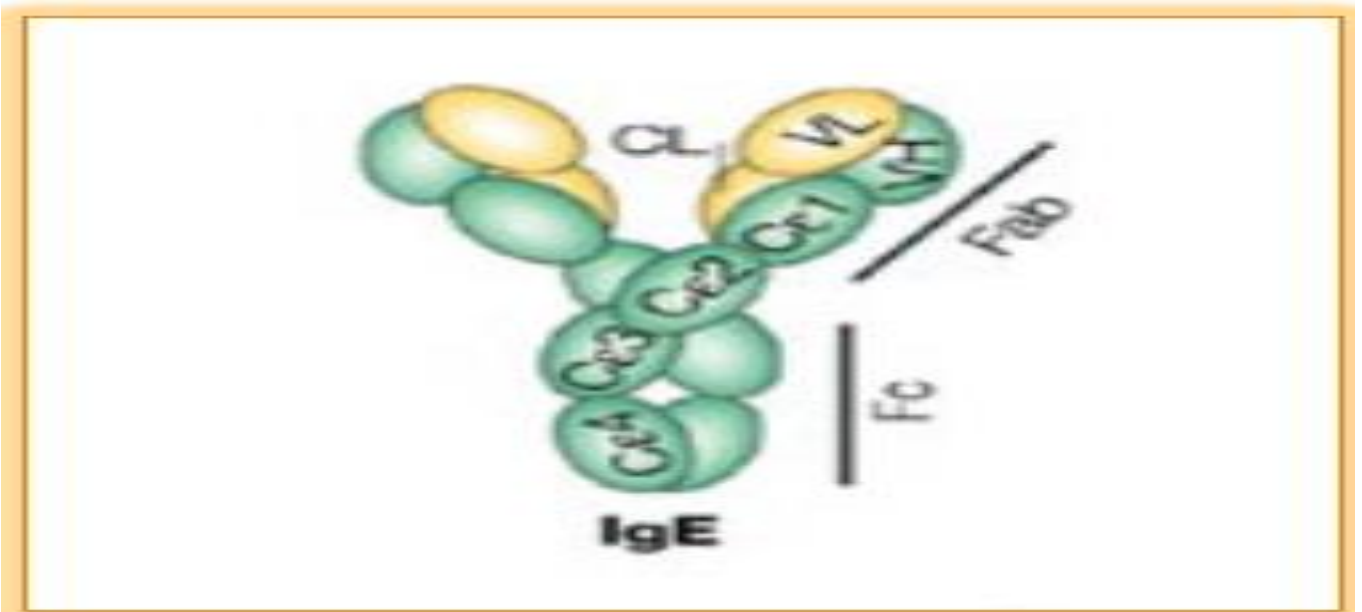
IgD:

- Found on B cell surfaces.



IgE:

- Its main function is immunity to parasites.
- It also plays an essential role in the allergy disorders.



SEROLOGICAL DIAGNOSIS

It is preferred to take paired sera (two serum sample) in which: -

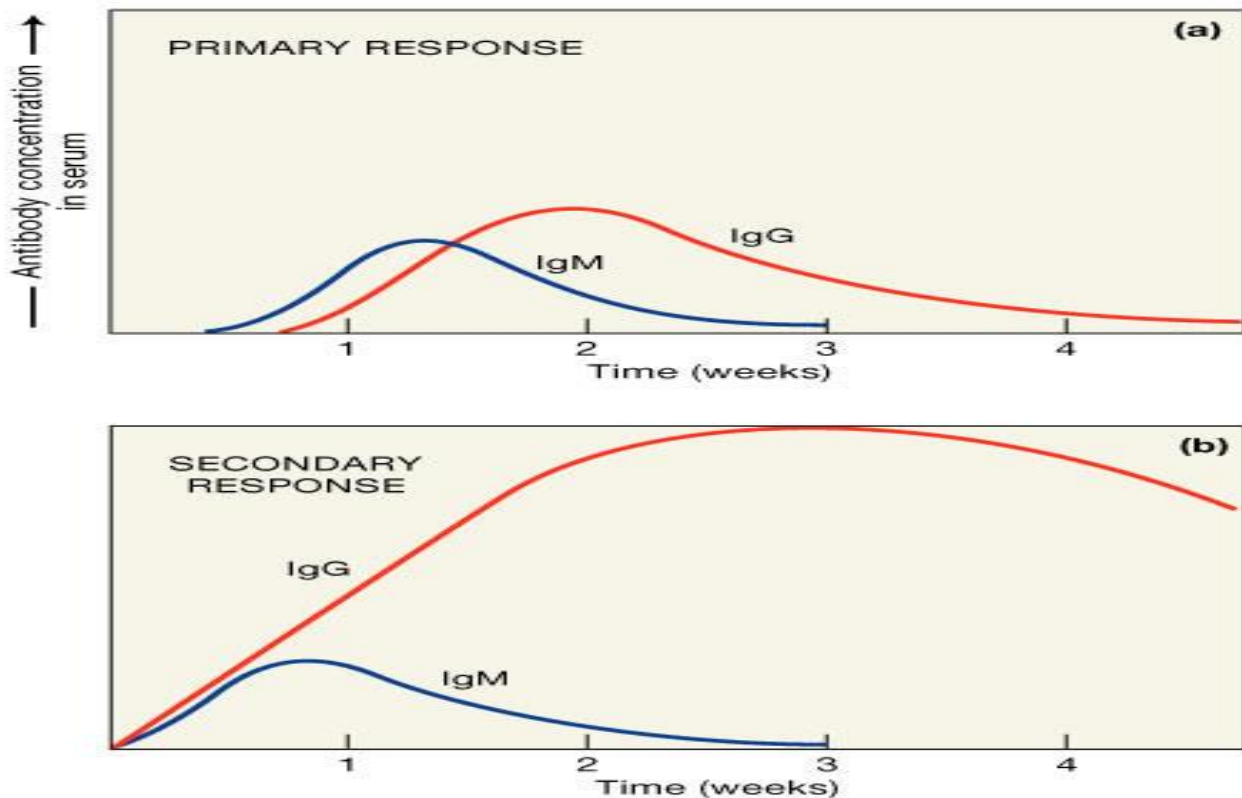
1.) 1st sample (Acute Serum): it is the serum taken as soon as the viral etiology is suspected, (usually at \approx 0-5 days "1st few days") after the onset of illness (Acute phase). Here Abs present in very low titer or not yet being produced.

2.) 2nd sample (Convalescent Serum): it is the serum sample taken at \approx 14-21 days after the onset of illness (convalescent phase). Antibodies are present in high titer.

Titration (quantification) of IgG Antibodies: -

The titer: - is a measure of antibodies concentration in serum sample.

It is essential in serological test (antibody detection techniques) because if IgG Abs detected in serum, this may indicate **immunization by vaccine** or **cured previous infection** as well as **recent infection**.



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Applications of serology:

- To test the immunity/past infection.
- Epidemiological studies.
- To test the efficacy of vaccines.

AGGLUTINATION & HAEMAGGLUTINATION

Agglutination is the clumping of particles.

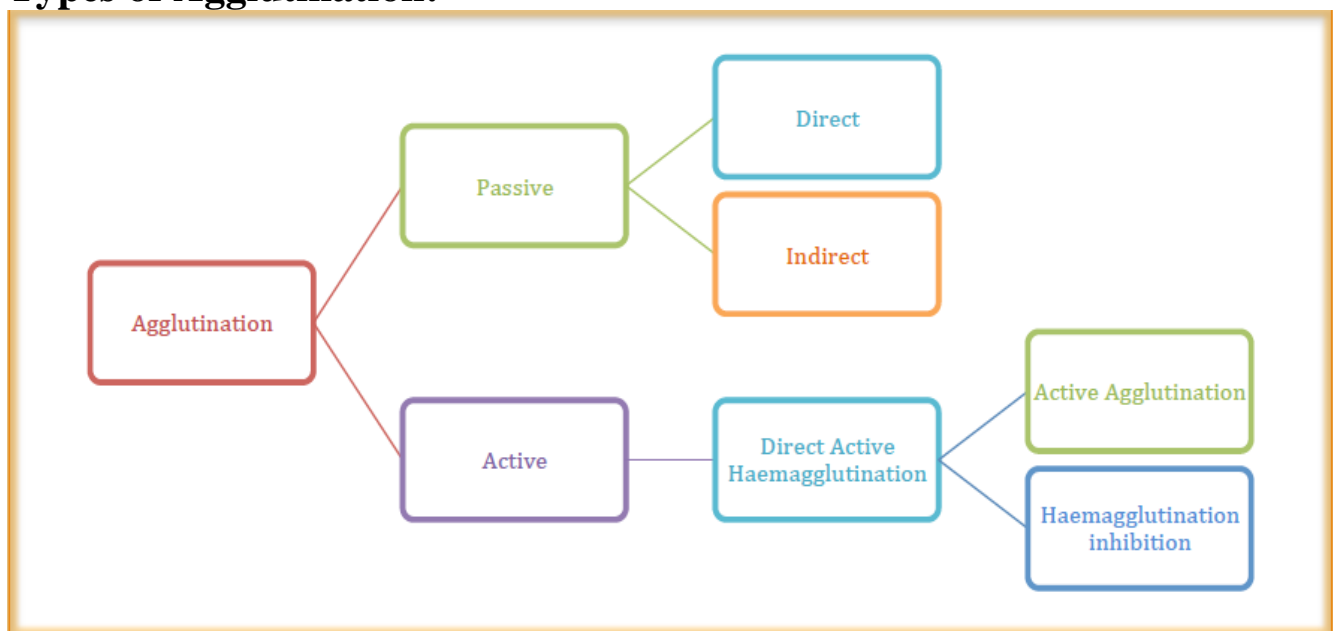
This occurs in biology in three main examples:

1. The clumping of cells such as bacteria, viruses or red blood cells in the presence of an antibody. The antibody or other molecule binds multiple particles and joins them, creating a large complex.
2. The coalescing of small particles that are suspended in a solution; these larger masses are then (usually) precipitated.
3. An allergic reaction type occurrence where cells become more compacted together to prevent foreign materials entering them. This is usually the result of an antigen in the vicinity of the cells.
4. Also occurs when people are given blood transfusions of the wrong blood group.

Agglutination in microbiology:

Agglutination is commonly used as a method of identifying specific bacterial or viral antigens or antibody, and in turn, the identity of such bacteria or viruses. Because the clumping reaction occurs quickly and is easy to produce, agglutination is an important technique in diagnosis. The result of agglutination is visible clumps that referred to “agglutinates”. By this technique the invisible Ag/Ab reaction becomes visible by naked eye.

Types of Agglutination: -



Passive agglutination: -

It is agglutination test in which the specific antibody or known antigen is attached (pre-coating or sensitization) to inert particles or cells.

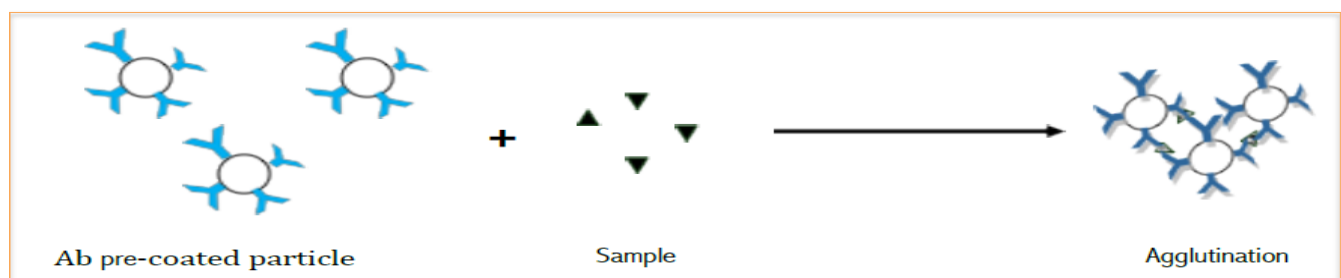
The carrier particles are used only to show that an Ag/Ab reaction has occurred.

• **The major substances and cells used as carriers in passive agglutination tests include:**

1. Latex particles (polystyrene or latex agglutination)
2. Carbon particles
3. Sensitized Red blood cells

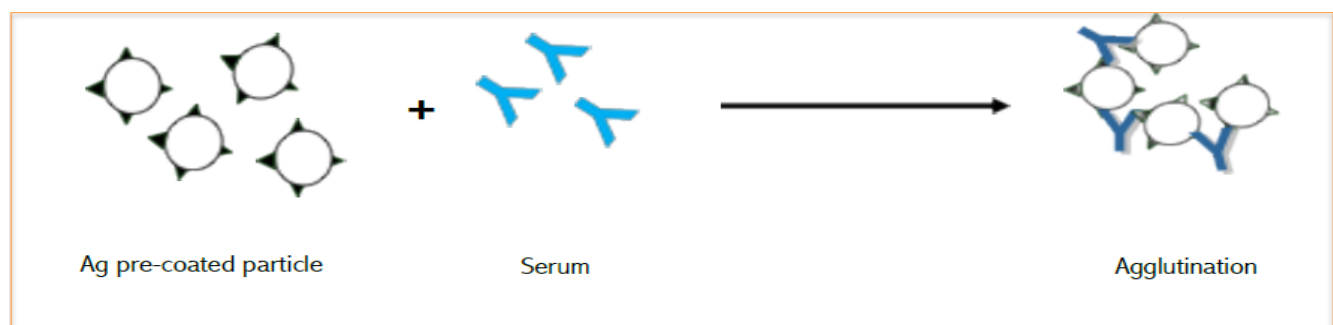
They have no role in Ag/Ab reaction "Inert particle"

The carrier particles are used only to show that an Ag/Ab reaction has occurred.



2. Indirect Passive Agglutination:

The inert particle is pre-coated with known Ag and then we add patient's serum which contains corresponding Ab.

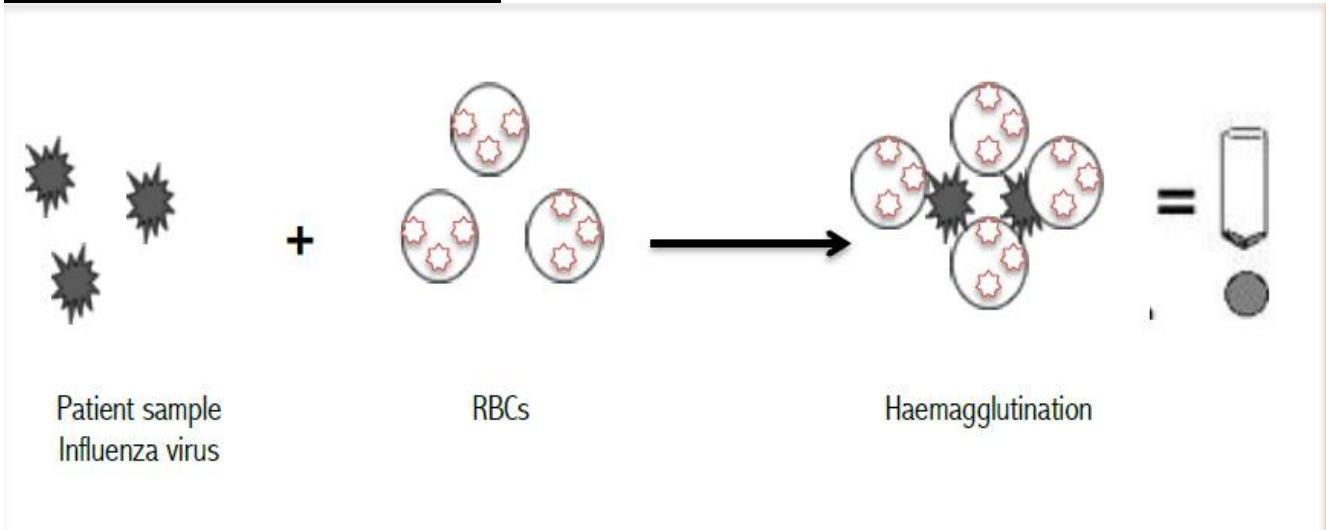


Active agglutination:

When the agglutination occurs to bacterial cell due to presence of antibody against that bacterial cell's antigen or to RBCs due to presence of antibody or virus (by viral surface proteins "hemagglutinins") that can attach to RBC's

natural self Ag i.e. no previous pre-coating or sensitization of RBCs in the lab; this is called Haemagglutination (Haem = RBC). RBC or bacterial cells only can show active agglutination. Neither latex nor carbon can be used because they contain no reactive self-Ag on their surfaces unlike RBC and bacteria.

Active haemagglutination:-

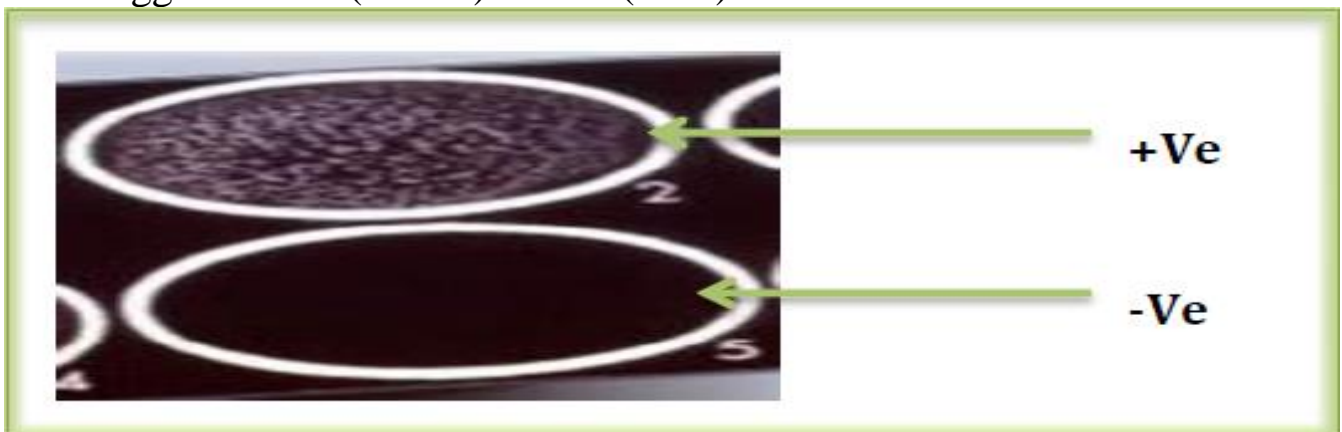


Results:

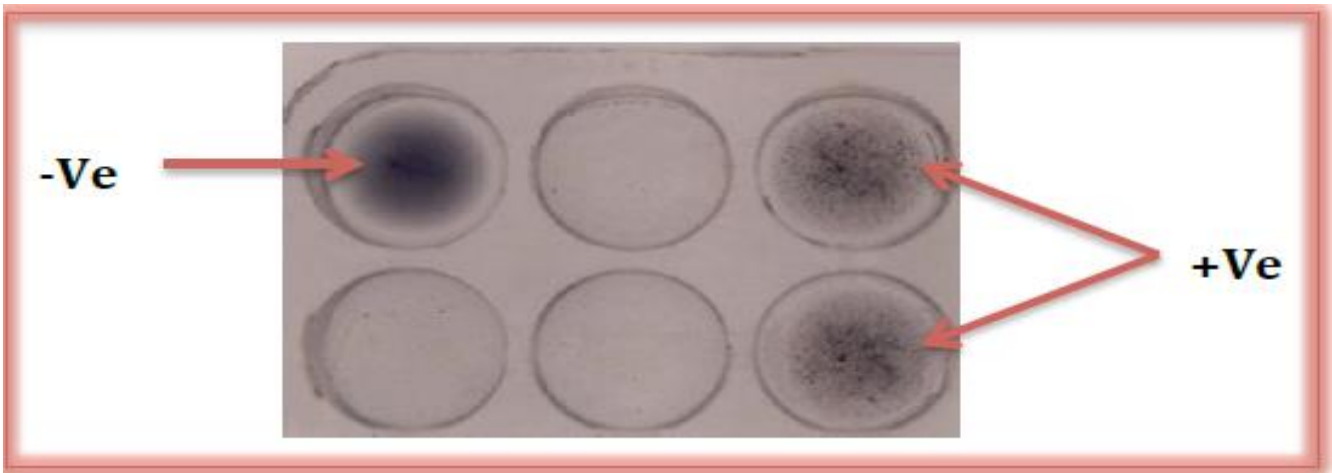
Agglutination tests can be performed:

- On slides (frequently used with latex and carbon).
- In tube (usually with haemagglutination).
- In micro-titration plates (usually with haemagglutination).

Latex agglutination (ASLO) results (slide):



Carbon agglutination (RPR) result (slide):



Haemagglutination result:

