ANTIGEN-ANTIBODY INTERACTION

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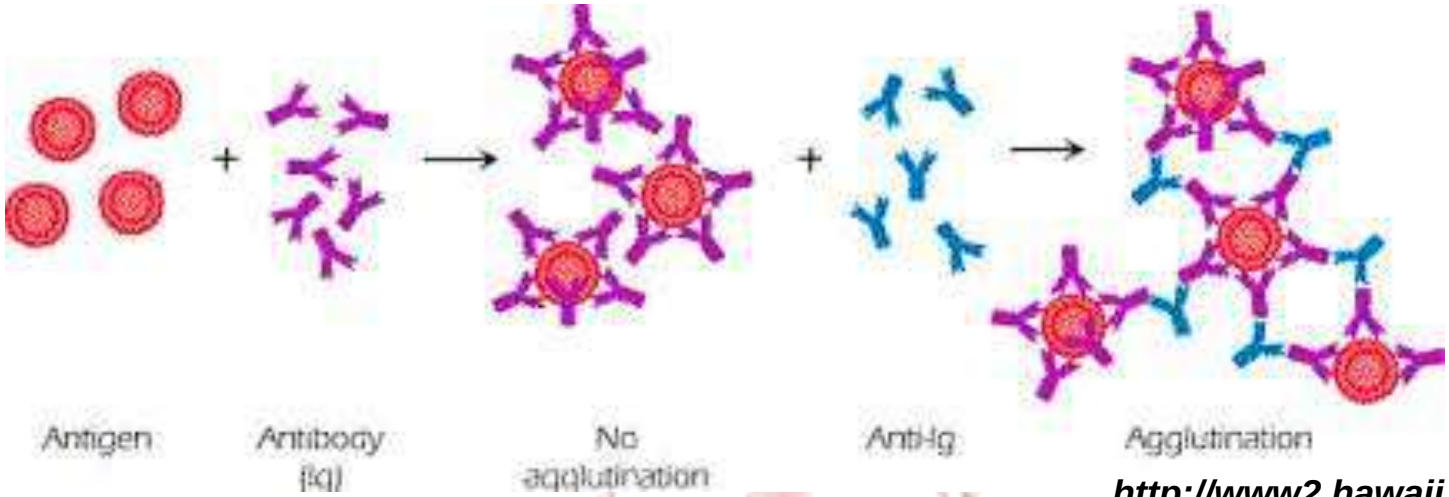
Introduction

- It is the specific interaction of antigens and antibodies.
- It is also called as Ag – Ab reaction.
- It is first described by Richard J. Goldberg at University of Wisconsin in 1952.
- It forms the basis of infectious disease diagnosis.
- It is the basis of modern study based on structural and functional aspects of different biomolecules.
- The Ag – Ab interaction is a three-step process, which are:
  - ***Primary stage:*** Formation of Ag – Ab complex.
  - ***Secondary Stage:*** Visible reaction, like precipitation, agglutination, etc.
  - ***Tertiary Stage:*** Destruction of Ag or its neutralization.

Ag – Ab interaction



http://www2.hawaii.edu/

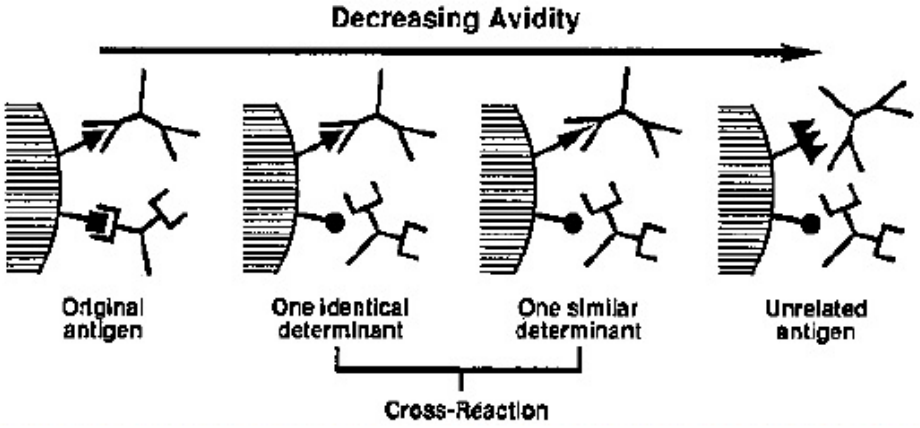
Depends on antigen affinity and avidity ⇨ **Antigen binding strength**

Antigen affinity : Measure of the binding strength at a single binding site. One epitope interacts with one paratope.

Antigen avidity: It is also called **functional affinity**. Measure of the total binding strength. One antibody may have 2 to 10 binding sites. Thus avidity vary from 2 to 10.

Characteristics

- Reaction is specific. An antigen interacts with its homologous antibody. However, cross reactions may occur due to antigenic similarity.
- Only the surface antigens participate in the interaction between antigen and antibodies.
- This interaction is strong but it can be reversible, since entire molecules of antigen and antibody react.



Antibody reacts with antigen having specific epitope ot sharing common or closely elated epitope. The avidity decreases with the decreasing structural closeness, untill it will no longer be detectable. The reactivity of the same antibody with several antigens is designed as cross-reaction (Roitt I. *Essential Immunology*, 4th ed. Blackwell, Oxford, 1980.)

Use of Ag – Ab interaction

- **In body**
  - It forms the basis of antibody mediated immune response against infectious antigens.
  - Tissue injury like hypersensitivity and autoimmunity.

- **In laboratory investigation**
  - Diagnosis of infection
  - Identification of infectious agents and difference between non-infectious antigen.
  - Quantification of antigen or antibody.

Types of Ag – Ab interaction

- Precipitation reaction
- Agglutination
- Neutralization
- Immunofluorescence
- Radioimmunoassay
- Enzyme-linked immunosorbent assay
- Immuno-electron microscopic test

Precipitation reaction

- It is the formation of insoluble precipitate in the presence of electrolytes at an optimal temperature and pH that usually sediments at the bottom of the tube.
- It is either:
  - **Sedimentation** – Settles down
  - **Flocculation** – Suspended as floccules
- It may occur in liquid media or in gels, such as agar, agarose, etc.
- Amount of precipitate is influenced by Ag/Ab ratio.
- The precipitation reaction is used in the identification of infectious agents, detection of Ag/Ab in diagnostic purpose, such as VDRL in syphilis, forensic application, and in standardization of toxins and anti-toxins.

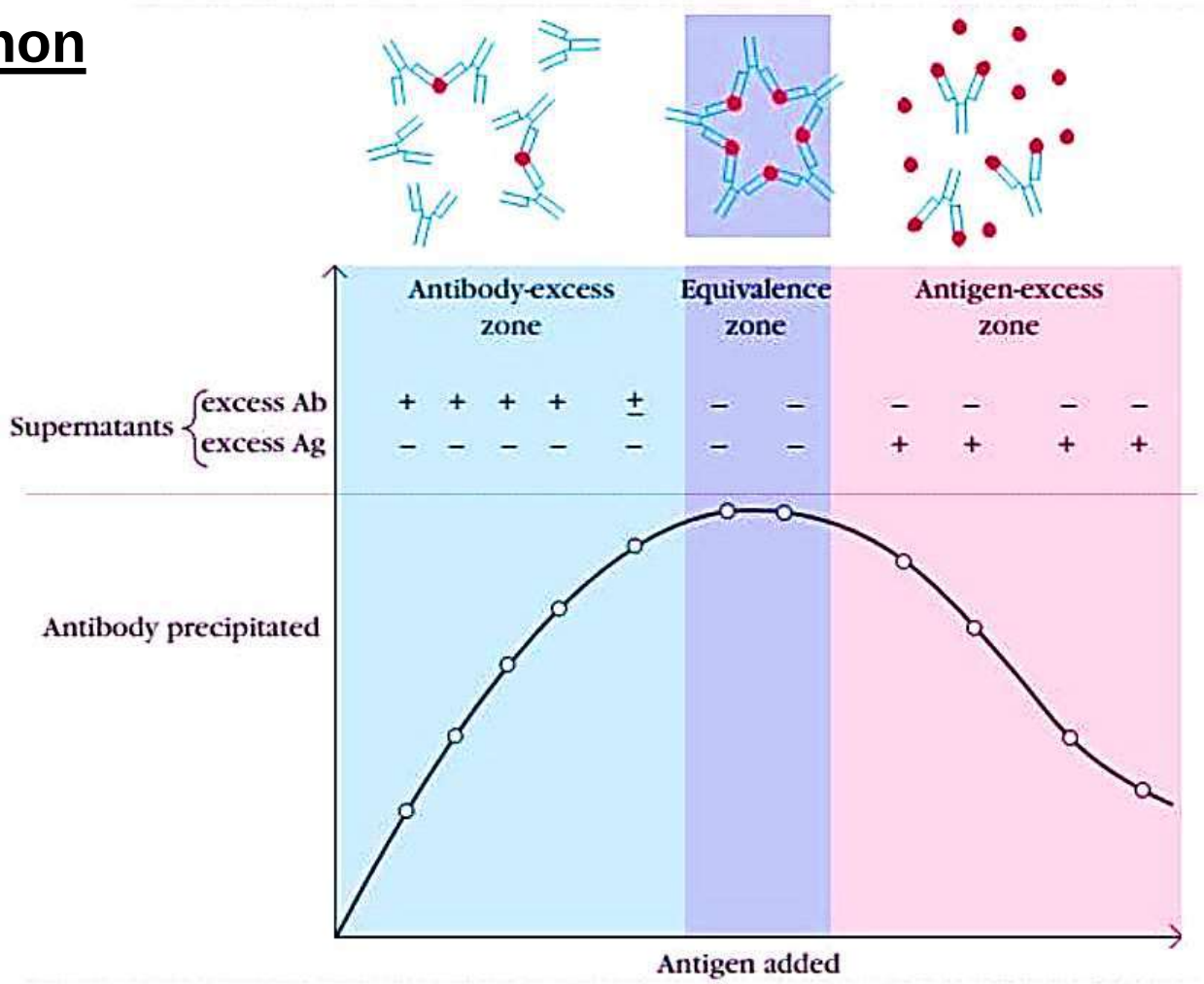
Precipitation reaction

Zone phenomenon

- It is a phenomenon in which visible agglutination and precipitation do not occur in mixtures of specific antigen and antibody because of antibody excess.
- It is based on Ag/Ab ratio. It can be classified in 3 zones, which are:
  - **Prozone:** Antibody is in excess. False negative precipitation may occur.
  - **Zone of equivalence:** Equal proportion of antigen and antibody, i.e. optimal proportion. It is most rapid reaction.
  - **Postzone:** Antigen is in excess. Precipitation is again weak or even absent.

Precipitation reaction

Zone phenomenon



Precipitation reaction

Types of precipitation reaction

1. **Simple precipitation test**

- **Precipitin ring test:** It is performed by using a standard Ag solution in the bottom of the tube and a serial dilution of Ab in the top of the tube. Glycerol in the solution prevents mixing resulting in the formation of ring at the interface.
- **Flocculation test:** Floccule is formed upon Ag-Ab mixing. It is of two kinds:
  - **Slide test:** In this test a drop of each of the Ag and the Ab are added on a slide, then both Ag and Ab are mixed by shaking. The reaction observed in the form of floccules formation, e.g. VDRL test for syphilis.
  - **Tube test:** Upon mixing of specific Abs with antigen, floccules are formed that can be seen with the naked eye, e.g. Kahn Flocculation test for syphilis.

Precipitation reaction

Types of precipitation reaction

2. **Immunodiffusion test:**
   - **Oudin method:** Carried out in gel; a single diffusion test in one direction. Sample containing Ag is placed into the test tube which diffuses into the gel and form line of precipitate where and antibody met in appropriate concentration.
   - **Okley-fulthorpe variation of Oudin: it** is double diffusion in one direction. Antibody is incorporated into gel in a test tube, a plane gel is solidified above this gel on which a sample containing Ag is placed. Both Ag and Ab diffuse into the plane gel and form visible precipitate where they met in appropriate concentration.
   - **Radial immunodiffusion:** It is single diffusion test in two dimension. It is quantitative test used to determine concentration of Ag or Ab in sample. In this test, wells are made on agar gel by using template and sample containing Ags is placed into the well. Specific Ab is added into gel and a visible ring of precipitate is formed around the well where antibody and antigen met in appropriate concentration.

Precipitation reaction

Types of precipitation reaction

2. **Immunodiffusion test:**
   - **Ouchterlony method:** It is double diffusion test in two dimension. In this method, wells are cut an agar gel using a template. Serum containing antibody is placed into central well and different antigens are placed into surrounding well. Both antigen and antibody diffuse from the well and form visible precipitate. This technique is used to identify relationship between two antigens. Three different patterns can be observed:
     - If two antigens are similar, line of precipitate fused.
     - If two antigen are non-identical, line of precipitate crossed
     - If two antigen are partially identical, spur formation of precipitate occurs.

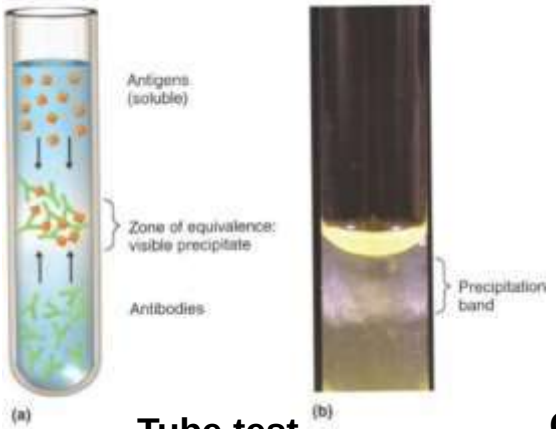
Precipitation reaction

Types of precipitation reaction

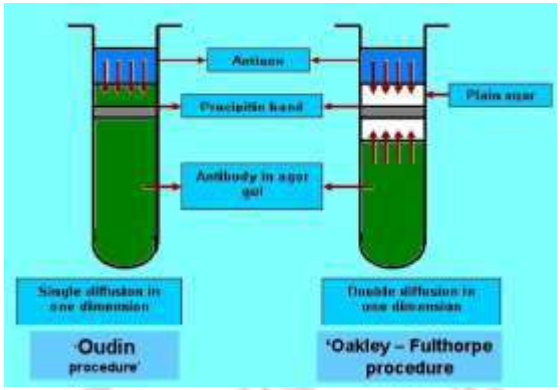
2. **Immunodiffusion test:**
   - **Immunoelectrophoresis**: it is of two kinds:
     - ***Rocket electrophoresis***: It is a quantitative test and is used to measure concentration of antigen or antibody in sample. Antibody is incorporated in gel and sample containing antigen is placed in well. Then electrophoresis is carried out. Antigen and antibody combine to from rocket shaped precipitate band where length is directly proportional to concentration of antigen.
     - ***Counter current immunoelectrophoresis:*** Agar gel is taken and two wells are made at opposite end of gel. Sample containing antigen is placed in one well and specific antibody is placed in another well. During electrophoresis negatively charged antigen migrate toward anode and positively charged antibody migrate towards cathode. Antigen and antibody combine to form line of precipitate where they met in appropriate concentration. This technique is rapid and more sensitive than simple immune-diffusion method. pH of gel should be 8.6 at which antibodies have positively charged and antigen have negative charge

Precipitation reaction

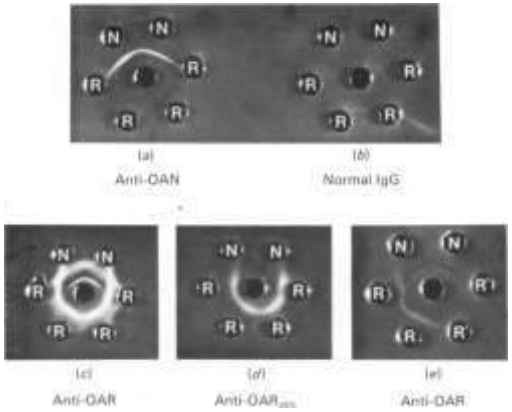
Types of precipitation reaction



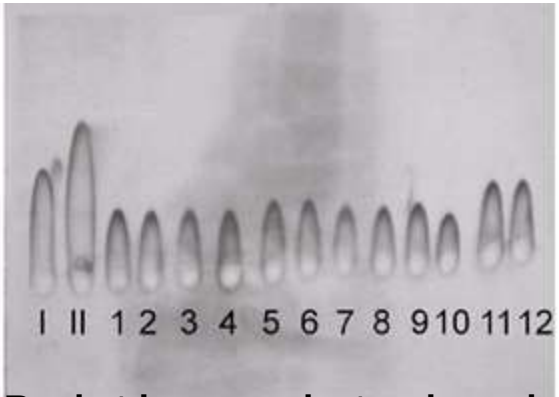
Tube test



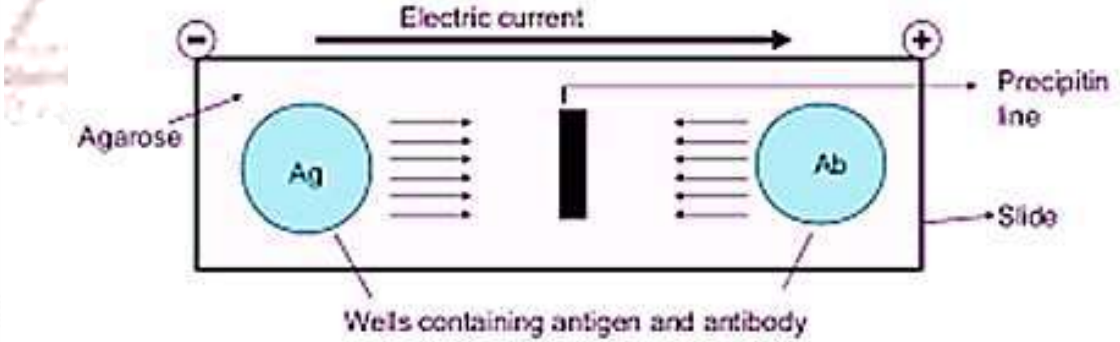
Okley-fulthorpe variation of Oudin



Radial immunodiffusion



Rocket immunoelectrophoresis



Counter current immunoelectrophoresis

Agglutination reaction

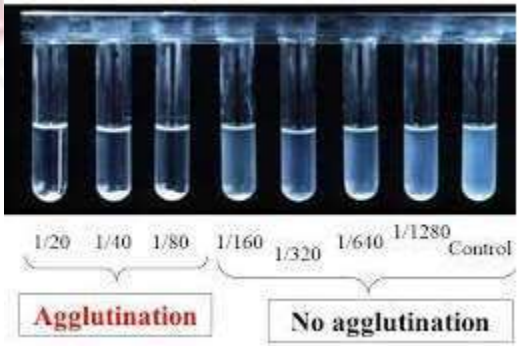
- It is a type of antigen antibody reaction in which a particulate antigen combines with its antibody in the presence of electrolytes at an optimal temperature and pH resulting in visible clumping of particles.
- It is more sensitive test than the precipitation reaction for the detection of antibodies.
- The reaction take place better with IgM antibody.
- Same zonal principle applies for agglutination reaction also.
- Incomplete or monovalent antibodies doesn't cause agglutination.
- Positive reaction is followed by clumping together of antigen.
- It is also applied in detection of various antigen for diagnostic purposes, and in study.

Agglutination reaction

Types of agglutination test

- **Slide agglutination test:** It is specific test developed to detect oxidase-positive gonococcal colony based on the non-immune reactivity between Fc protion of IgG and staphylococcal protein (SP) A. Antigen is placed on slide where antibody is mixed to agglutinate. It is also done in Widal test (for typhoid fever and parathyphoid fever).
- **Tube agglutination test:** It is specially done to perform Widal test to detect Enteric fever (typhoid fever and parathyphoid fever). In this test, one drop of Widal test antigen suspension (O, H, AH, and BH) from the reagent vials are added to test tubes (1 to 8) containing control and samples, mix well and incubate at 37 ºC overnight.

Slide agglutination test



Tube agglutination test

Agglutination reaction

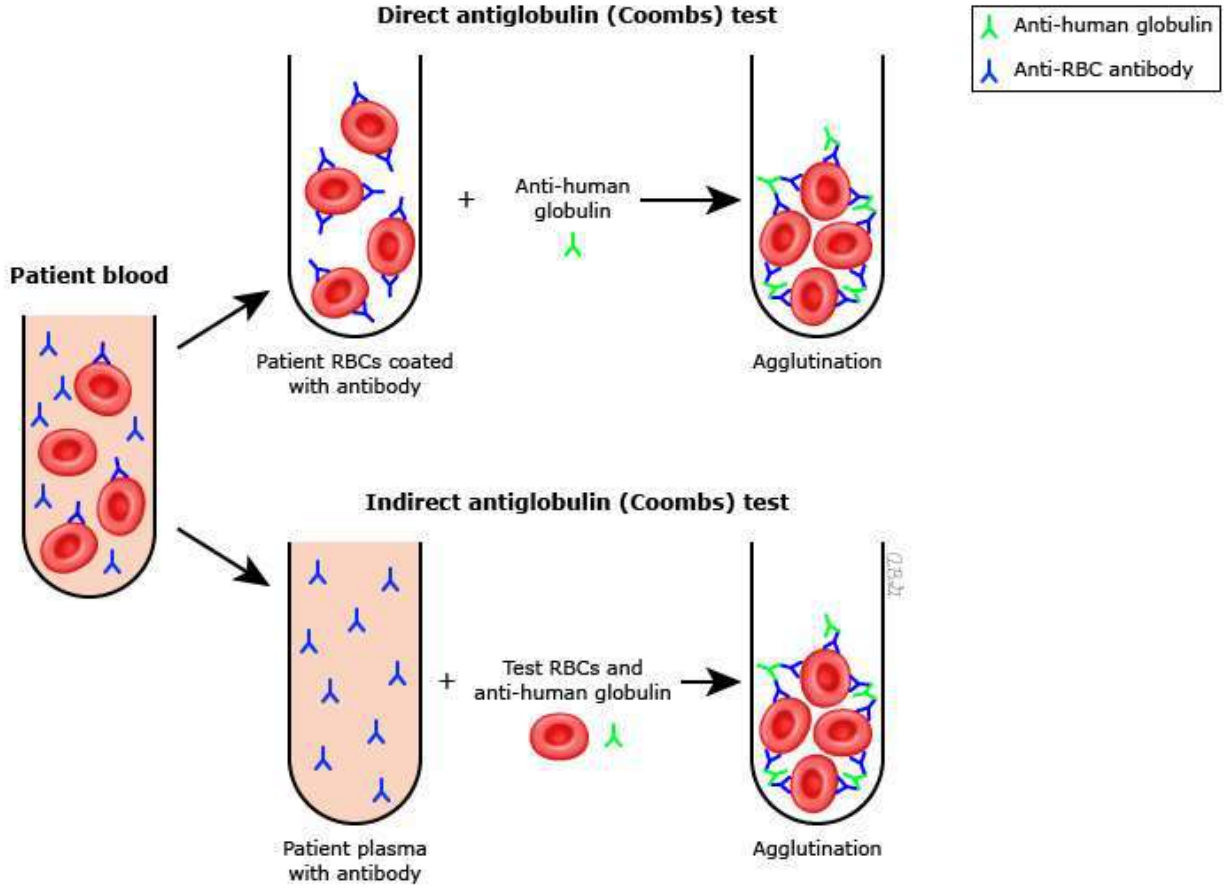
Types of agglutination test

- Coombs test: It is a test performed to find certain antibodies that attack RBCs. It can be done both by slide or tube. Tube is preferred. It is also called as antiglobulin testing. It is of two types:
  - ***Direct coombs test:*** It is done on a sample of RBCs from the body to detect antibodies that are already attached to RBCs. It is also called direct antibody test (DAT) generally done to detect any blood abnormalities like hemolytic anemia , erythroblastosis fetalis in newborn or autoimmune disorder , like lupus, etc.
  - ***Indirect Coombs test:*** It is done on a sample of the liquid part of the blood, i.e., on serum having dissociated antibodies. It is done to find antibodies in a recipient's or donor's blood before a transfusion.
- A positive Coombs test means there is disorder in the body.

It was originally devised by Coombs, Mourant and Race in 1945 for detection of incomplete Rh antibodies.

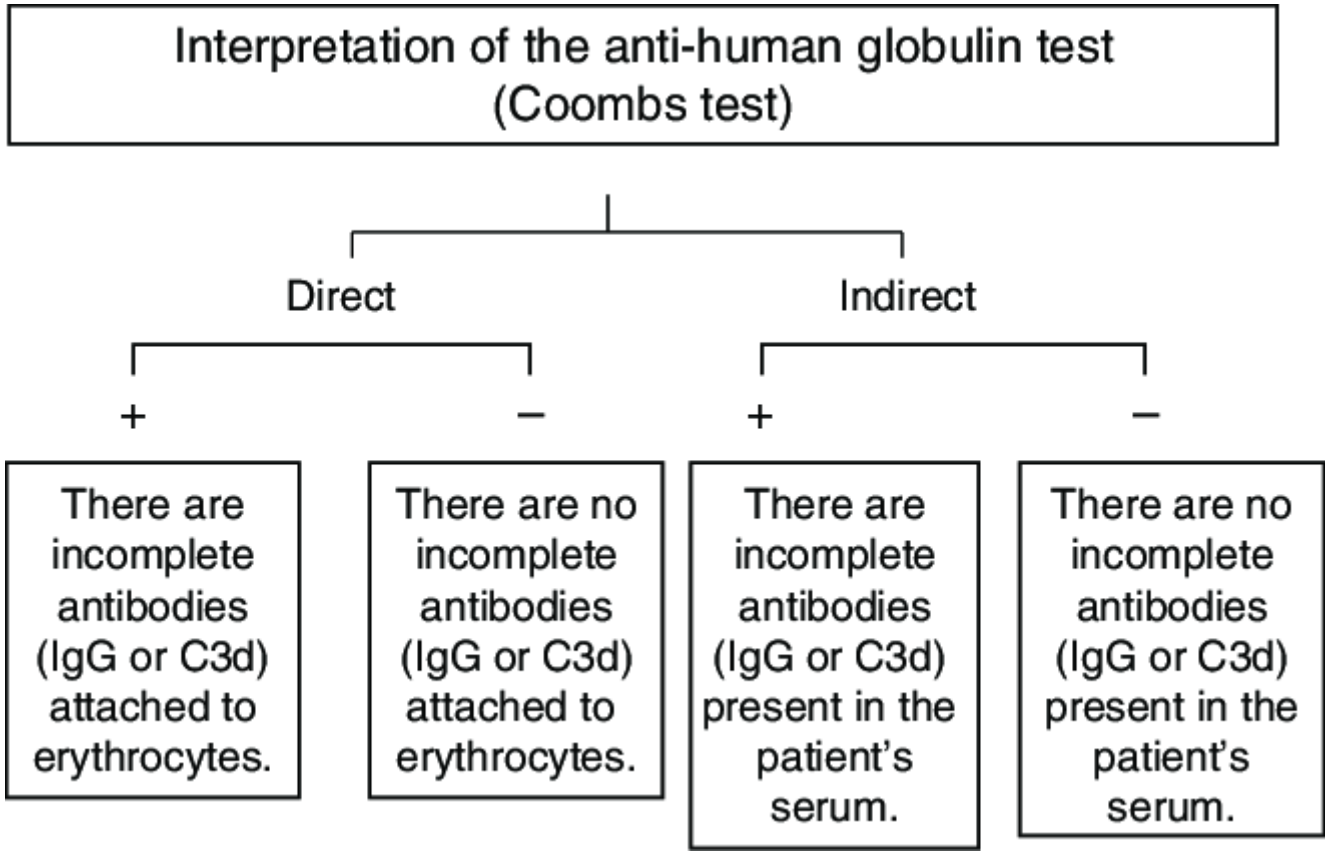
Agglutination reaction

Types of agglutination test



Agglutination reaction

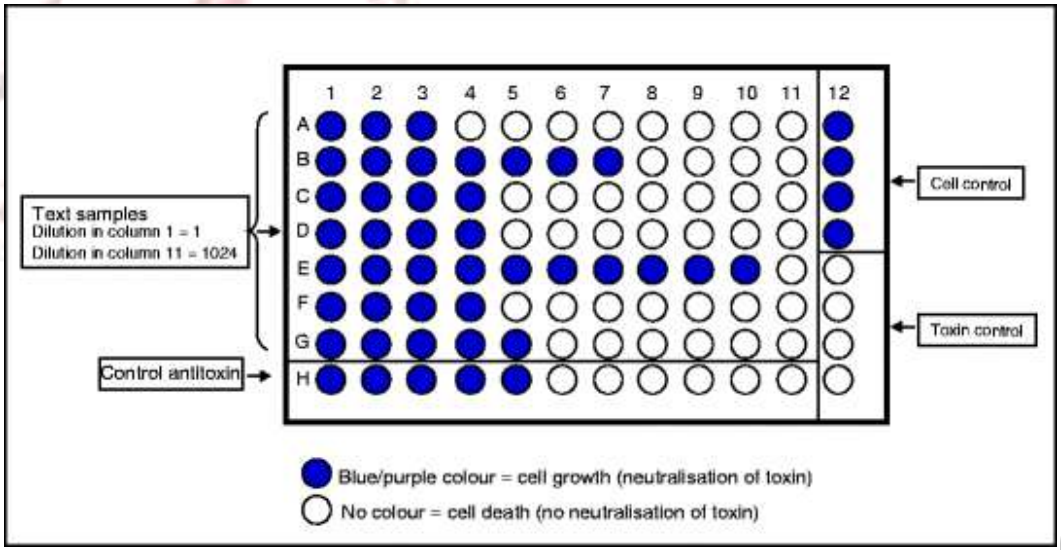
Types of agglutination test



Rediscovering Coombs test: DOI:10.1016/j.rmu.2016.07.001

Neutralization test

- The neutralization test measures the ability of the patient's antibody to neutralize antigen.
- It is considered a gold standard for the assessment of protective antibody.
- It can be
  - Virus neutralization
  - Bacterial neutralization
  - Toxin neutralization – can be measured in vivo and in vitro.
- Example is Shick test – Dipthera, Anti-streptolysin 'O' (ASO) test

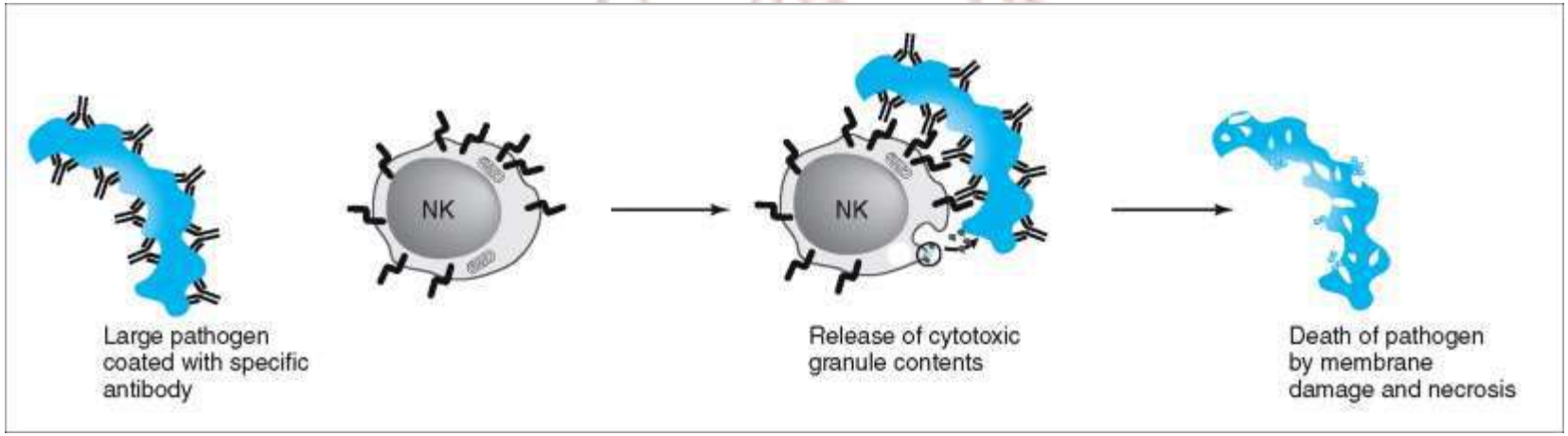


Opsonization

- It is a kind of antigen-antibody interaction that involves coating of pathogens with antibodies in order to increase their susceptibility to ingestion of phagocytes. The serum factors which binds to antigens are known as opsonin.
- It was first given by Wright and Douglas in 1903.

Opsonic index

It is a numerical measure of potency of a given serum to opsonize foreign material, such as bacteria.

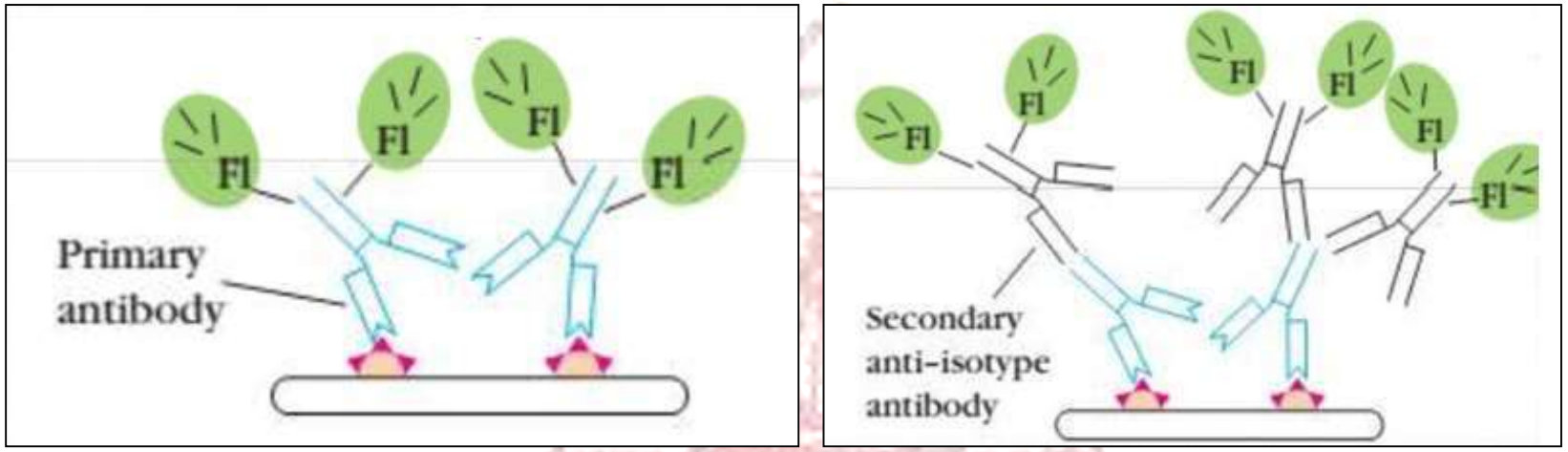


Encyclopedia of Human Nutrition (Second Edition), 2005

Immunofluorescence

- It is a type of immunohistochemical technique that utilizes fluorophores (a fluorescent chemical that emits different wavelength of light upon photon excitation) to visualize various cellular antigens.
- **Coons et al.** demonstrated that antibody labeled with fluorescent dyes can be used to locate and identify antigen.
- Commonly used fluorescent dyes are fluorescin isothiocyanate (blue green) and lissamine rhodamine (orange red).
- It can be of two types; Direct or indirect immunofluorescense.
- ***Direct immunofluorescence test:*** Specific antibody labeled with fluorophore used to detect unknown antigen – used to detect under fluorescent microscope, e.g. bacteria, virus or other antigens in blood, CSF, urine, faeces, tissues, etc. Rabies detection in brain is done by this method.
- **Indirect immunofluorescence test:** In this method, known antigen is fixed on a slide where coating with unknown antibody is done. After that antisera to primary antibody labeled with fluorophore is applied that produce light.

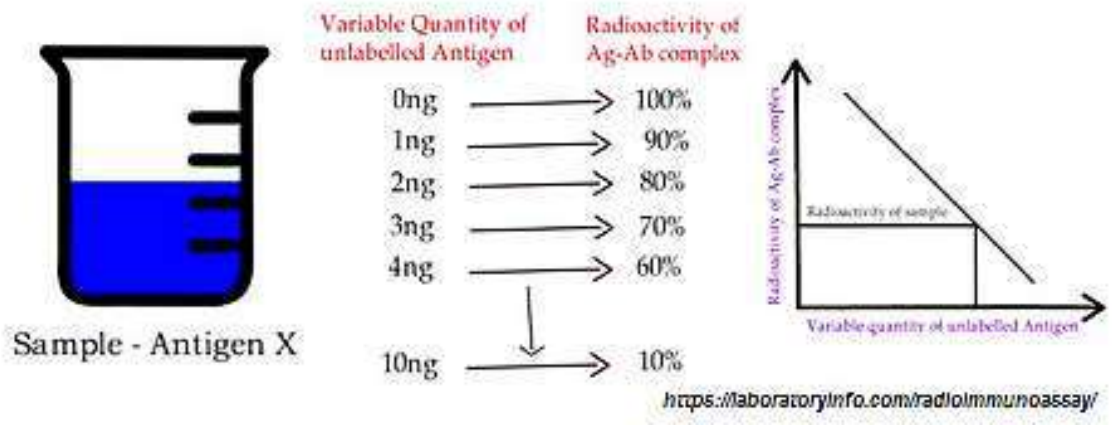
Immunofluorescence



Direct immunofluorescence **Indirect immunofluorescence**

Radioimmunoassay (RIA)

- It was developed by Solomon Berson and Rosalyn Yalow in 1950s - To know the levels of insulin - anti-insulin complexes in diabetics.
- It is a type of immunoassay that uses radiolabeled antigen or antibody in a stepwise formation of immune complexes.
- It is very sensitive method used to measure concentrations of antigens, e.g. hormone level, enzyme concentration, etc.
- Its principle is competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody – radioactivity is measured by gamma counter.



Enzyme-linked Immunoassay (ELISA)

- It is a biochemical procedure in which a signal produced by an enzymatic reaction is used to detect and quantify the amount of a specific substance in a solution.
- Very sensitive, requires only microlitre quantities of test antigens.
- Its principle is similar to that of immunofluorescence.
- It is usually performed in 96-well plate to detect antigens, antibodies, hormones, drugs, etc.
- 96-well plate is made of cellulose, polystyrene, polyvinyl or polycarbonate which may be round bottom or flat bottom tube. Capacity of each well is 400 µL.
- It may be of 3 types:
  - Direct ELISA
  - Indirect ELISA
  - Sandwich ELISA

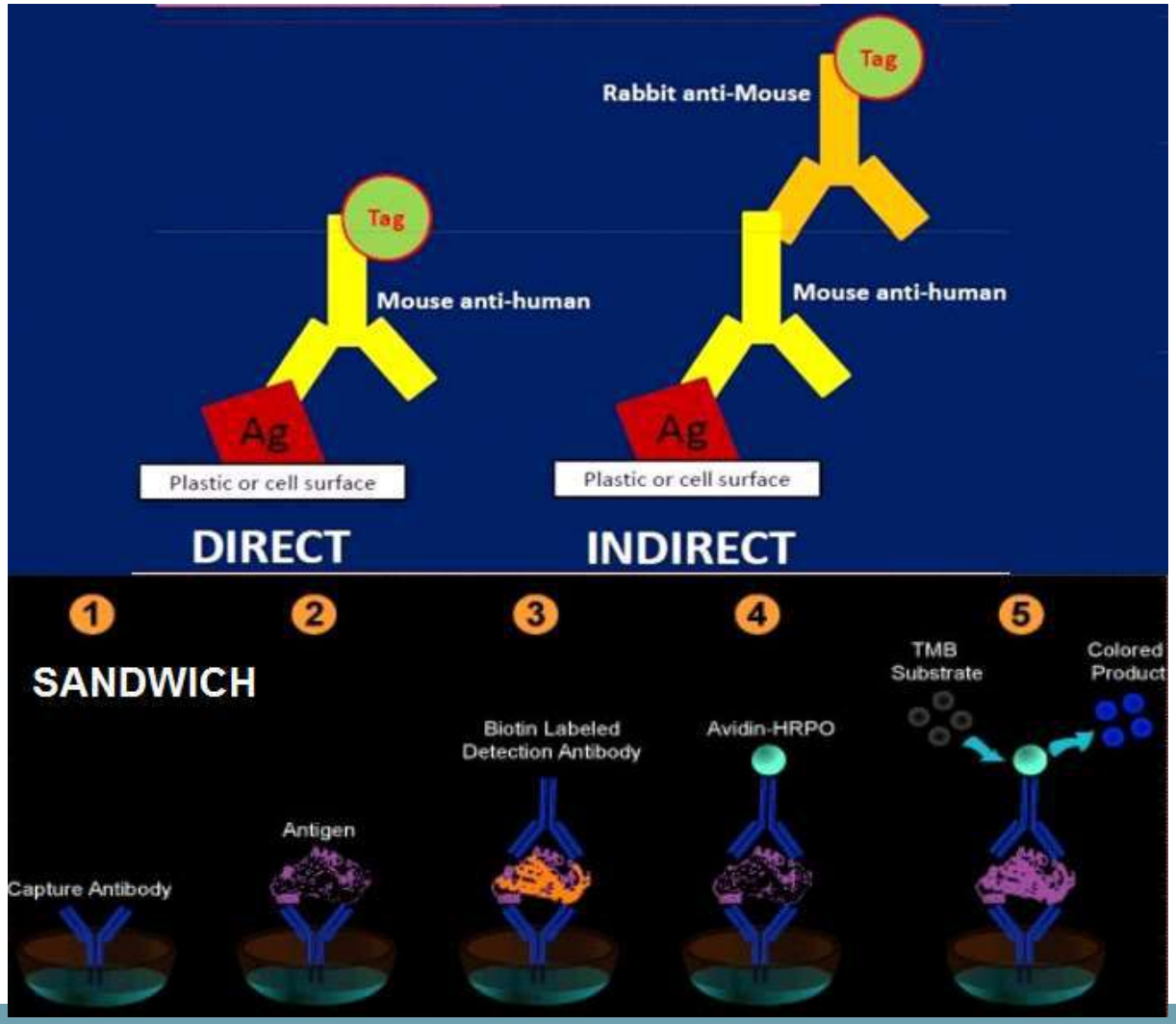
Non-competitive ELISA

  - Competitive ELISA
  - Ogives ELISA

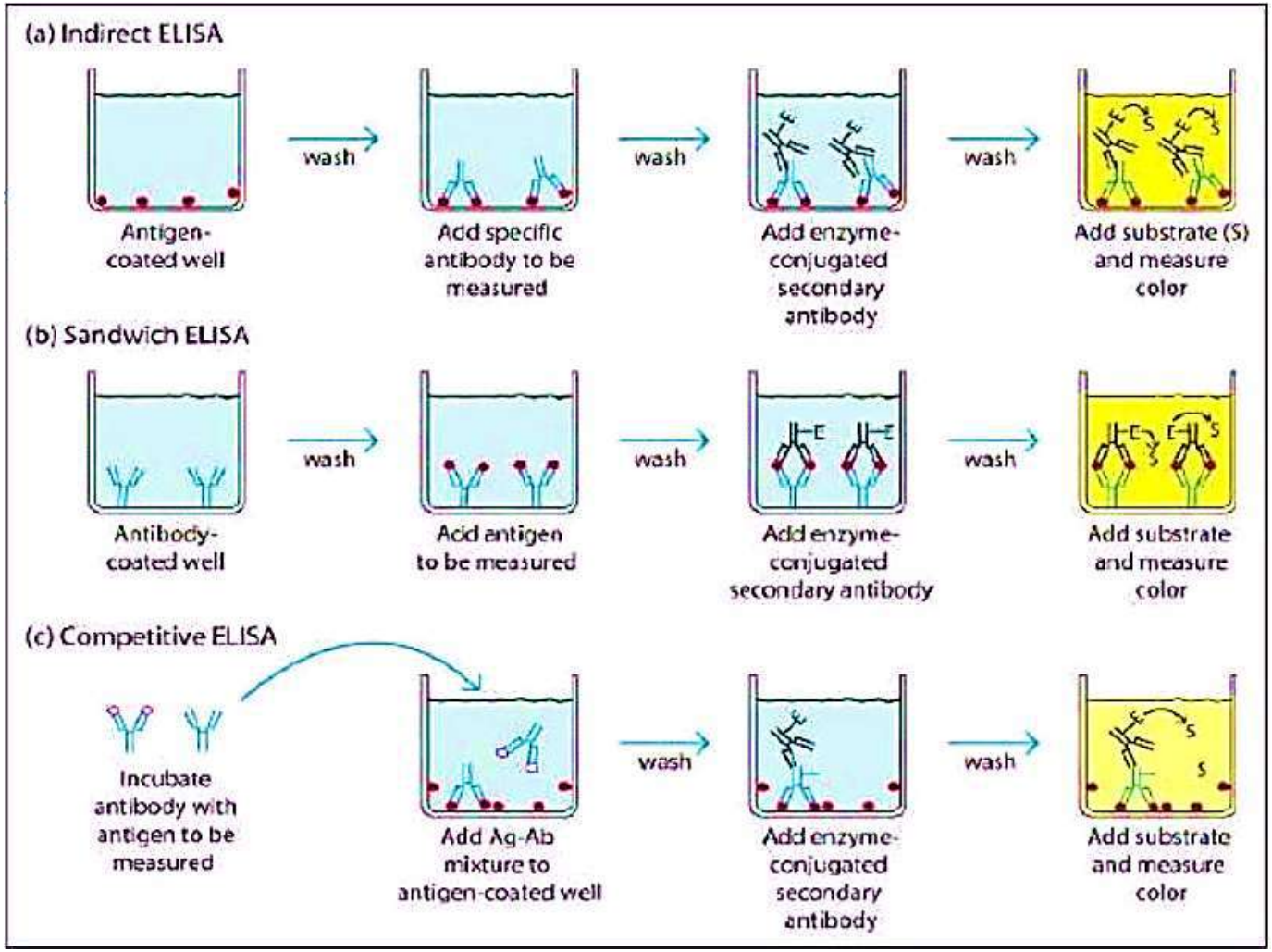
Enzyme-linked Immunoassay (ELISA)

- **Direct ELISA:** In this method, labeled primary antibody is used that directly reacts with the pre-coated (immobilized) antigen in the 96-well plate. It is not widely used but common for immunohistochemical staining of tissues and cells.
- **Indirect ELISA:** In this method, unlabeled primary antibody is applied to pre-coated or immobilized antigen and then labeled secondary antibody, which is specific to the primary antibody is used.
- **Sandwich ELISA:** In this method, 96-well plate is pre-coated with capture antibody specific to the antigen and sample containing antigens is added. Thereafter another labeled antibody called detection antibody specific to the antigen is used.
- **Competitive ELISA:** In this method, antibody is first incubated in solution with a sample containing antigen to form Ag-Ab mixture. Ag-Ab mixture is then added to an antigen coated microtiter well (96-well plate). Thereafter labeled secondary antibody is added to produce results.

Enzyme-linked Immunoassay (ELISA)

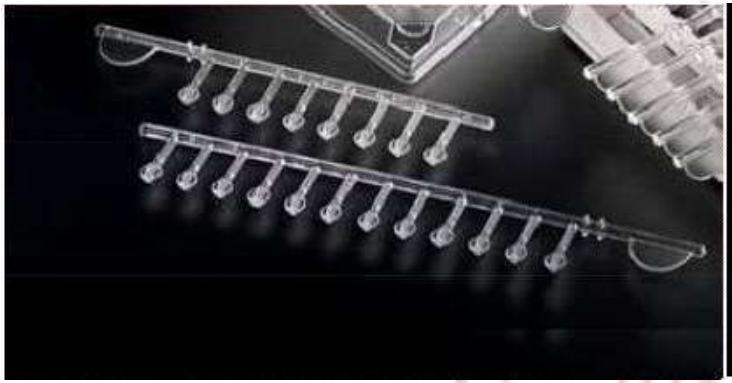


Enzyme-linked Immunoassay (ELISA)



Enzyme-linked Immunoassay (ELISA)

- **OGIVE ELISA (Multiple and portable ELISA):** It is a comparatively newer technique that uses a solid phase made up of an immuosorbent polystyrene rod with 8-12 protruding ogives.



Results

ELISA is utilized for obtaining different results, which are:

- **Quantitative:** Result can be interpreted on standard curve.
- **Qualitative:** It can also be done to detect the presence of specific antigen.
- **Semi-quantitative:** It can be used to compare the relative levels of antigen.

Further reading

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- Abbas A.K., Litchman A.H., Pillai S. 2022. Cellular and Molecular Immunology 10th Edition. Elsevier Inc., Philadelphia, USA.
- Punt J., Stanfor S., Jones P., Owen J. 2019. Kuby Immunology 8th Edition. W. H. Freeman, New York, USA.
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