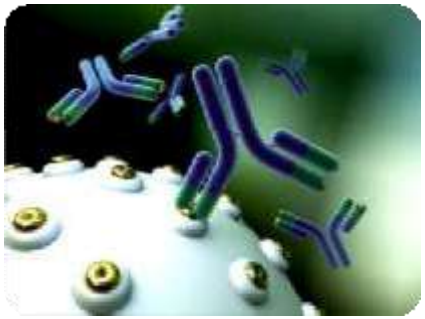
Dr Sanjesh G Rathi
Associate
Professor
SIPS

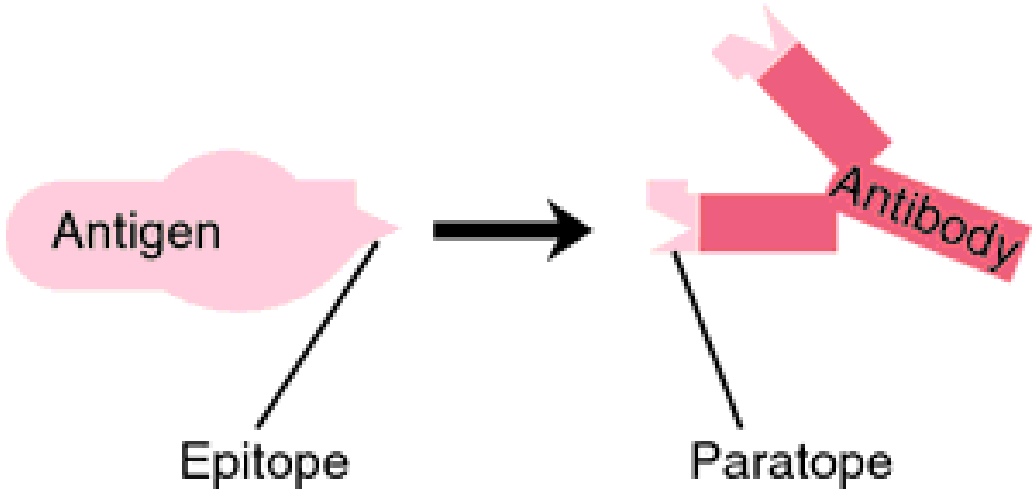
Antigen-Antibody System



Antigen

- An antigen is a substance which when introduced into a body evokes an immune response to produce a specific antibody with which it reacts specifically.
- Most of antigens are proteins but some are carbohydrates,lipids or nucleic acids.
- It can be classified as-
  - Complete antigen
  - Incomplete antigen (Haptens)

- **Epitope** is the smallest unit of antigenicity.
- The combining site on the antibody molecule, corresponding to the epitope is called the **Paratope**.

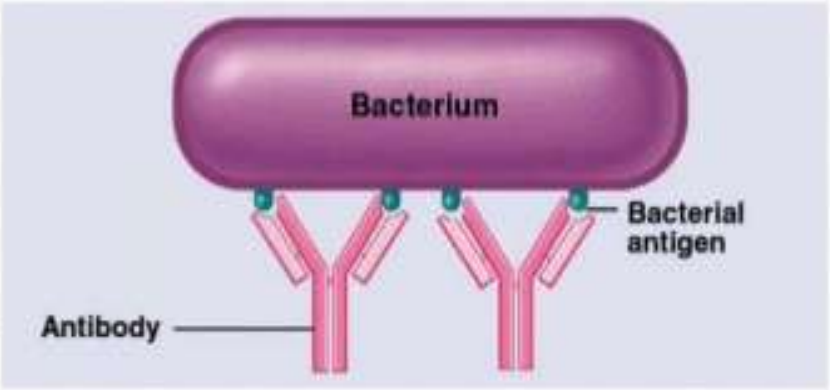


Antigens Characteristics

- **Foreignness:** Molecules recognized as "self" are not immunogenic
- **Molecular Size:** Small foreign molecule with molecular weight below 10,000 (hapten) weakly immunogenic & must be coupled to carrier molecule to be antigenic
  - Once antibodies are formed they recognize hapten

Antigen Characteristics

- **Chemical–Structural Complexity:** Amino acid homopolymers less immunogenic than heteropolymers (containing two or three different amino acids)



Antigens Characteristics

- **Antigenic Determinants (Epitopes)** :Small chemical groups on antigen molecule that can elicit immunological response & react with antibody
- **Dosage , Route & Timing of Antigen administration:** These factors affect immunogenicity

Antibody

- These are substances which are formed in the serum and tissue fluids in response to an antigen and react with that antigen specifically and in some observable manner.
- Chemically they are globulins, hence they are named immunoglobulins.
- They constitute about 20 – 25% of the total serum proteins and are mainly synthesized by plasma cells.

Structure

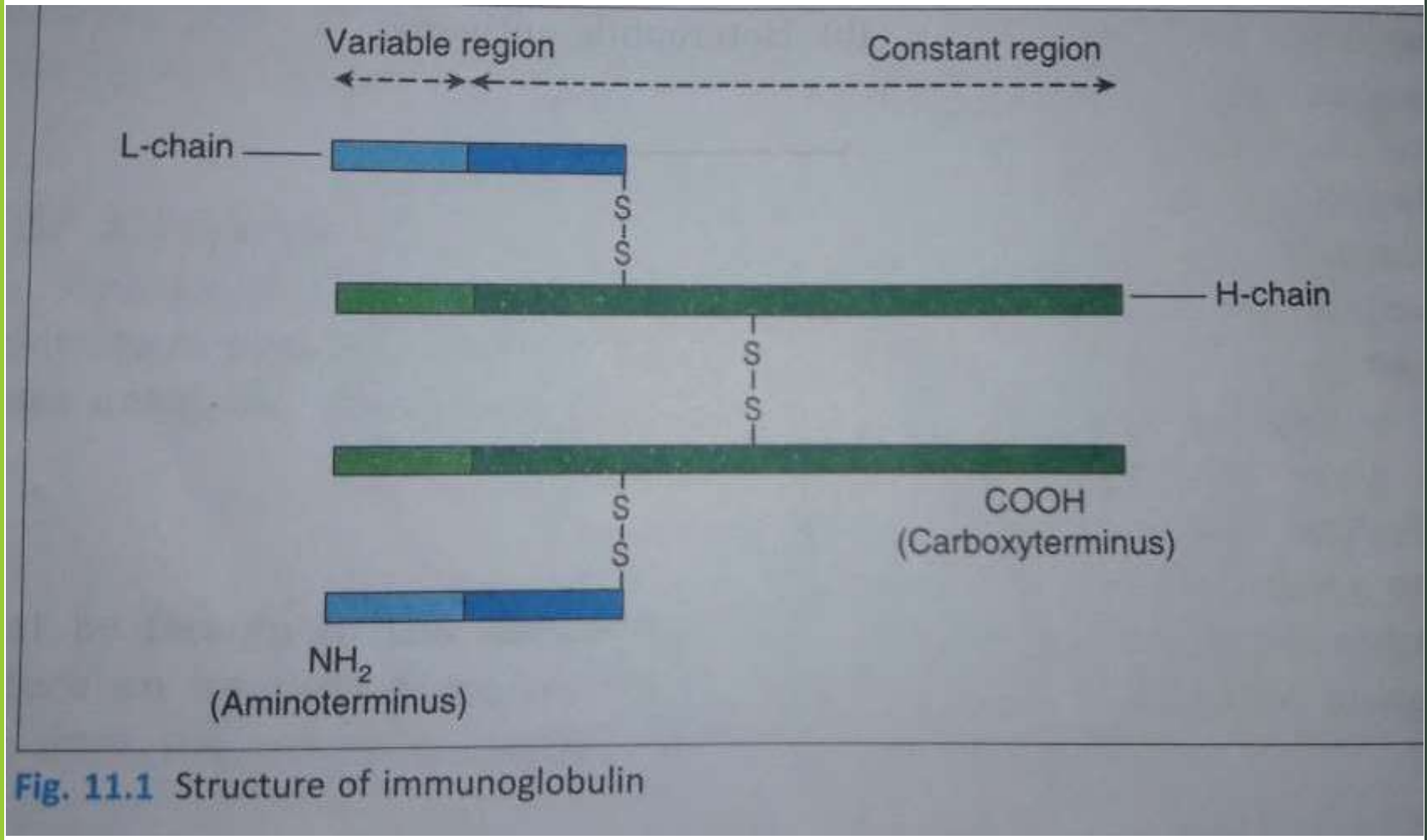


Fig. 11.1 Structure of immunoglobulin

Table 11.1: Properties of Immunoglobulin Classes

Property	IgG	IgA	IgM	IgD	IgE
Molecular weight	150,000	160,000	900,000	180,000	190,000
Sedimentation coefficient(S)	7	7	19	7	8
Heavy chain	Gamma	Alpha	Mu	Delta	Epsilon
Light chain	K or L	K or L	K or L	K or L	K or L
Serum concentration (mg/ml)	12	2	1.2	0.03	0.00004
Placental transport	+	–	–	–	–
Half life	23 days	6–8 days	5 days	3 days	2–3days
Intravascular distribution(%)	45	42	80	75	50
Present in milk	+	+	–	–	–

Role of Different Immunoglobulin Classes

IgG – protects the body fluids
IgA – protects the body surfaces
IgM – protects the blood stream
IgE – mediates reaginic hypersensitivity

Antigen Antibody Reactions

- The antigens and antibodies combine specifically with each other. This interaction between them is called Antigen – Antibody reaction.
- It may be abbreviated as Ag – Ab reaction.
- The first correct description of the antigen-antibody reaction was given by Richard J. Goldberg at the University of Wisconsin in 1952.

- These reactions form the basis for detection of infectious disease causing agents and also some non specific antigens like enzymes.
- The reactions between Ag and Ab occur in three stages.
  - In first or *primary stage* the reaction involves formation of Ag-Ab complex.
  - The *secondary stage* leads to visible events like precipitation, agglutination etc.
  - The *tertiary stage* includes destruction of Ag or its neutralization.

Its USES are

1. **In vivo**

- Forms basis of immunity against infectious diseases.

2. **In vitro**

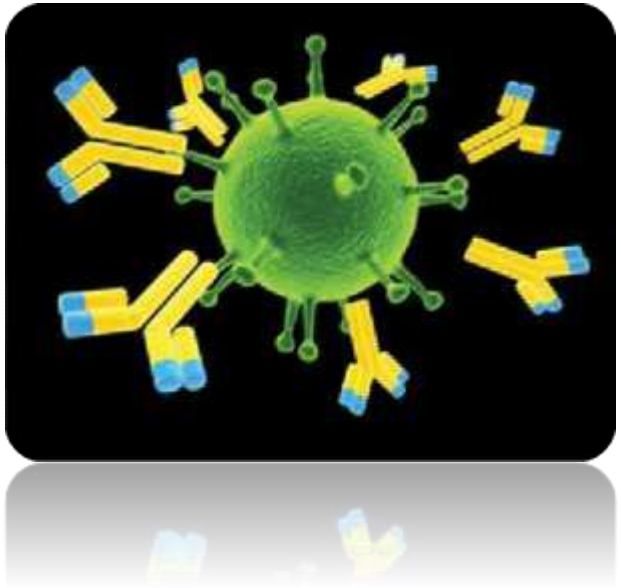
- For diagnosis of infections
- Helpful in epidemiological studies
- For identification of enzymes
- Detection and quantification of antigens or antibodies.

Characteristics

- Reaction is specific, an antigen combines only with its homologous antibody and vice-versa. However, cross reactions may occur due to antigenic similarity.
- Entire molecules of antigen and antibody react and not the fragments.
- Only the surface antigens participate in the antigen antibody reaction.
- The reaction is firm but reversible.

Types

- Precipitation reactions
- Agglutination
- Neutralization test
- Immunofluorescence
- Radioimmunoassay
- Enzyme linked immunosorbent assay
- Immunoelectronmicroscopic test



Precipitation reactions

- When a soluble antigen reacts with its antibody in the presence of electrolytes at an optimal temperature and pH, antigen antibody complex forms an insoluble precipitate that usually sediments at the bottom of the tube and it is called **precipitation**.

- Precipitation may occur in liquid media or in gels such as agar, agarose etc.

Applications

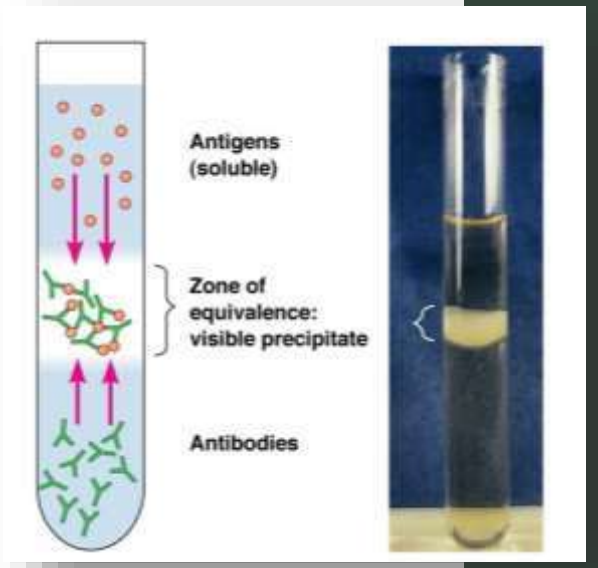
- Identification of bacteria. E.g., Lancefield grouping of streptococcus.
- Detection of antibody for diagnostic purposes. E.g., VDRL in syphilis
- Forensic application in identification of human blood and seminal stains
- To standardize toxins and antitoxins.

Types of precipitation reactions

- Ring test

e.g. C- reactive protein test

Streptococcal grouping by Lancefield technique

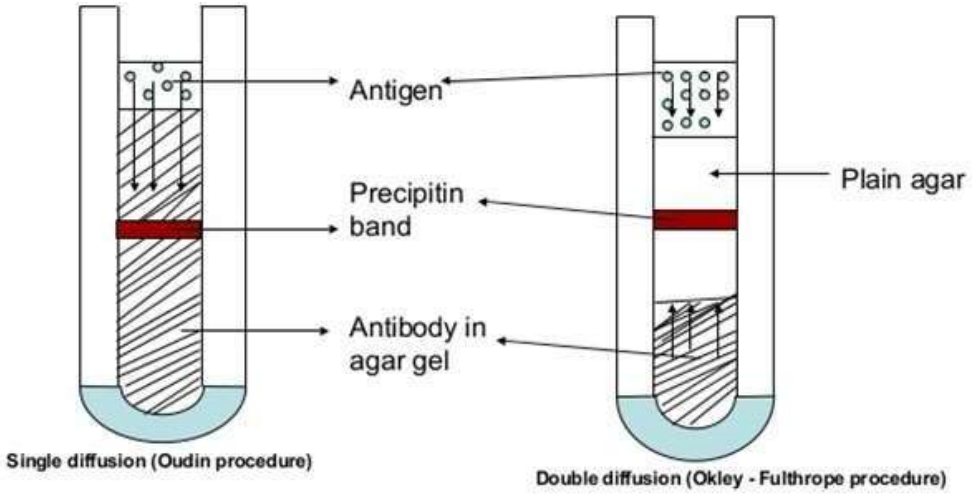


- Flocculation test

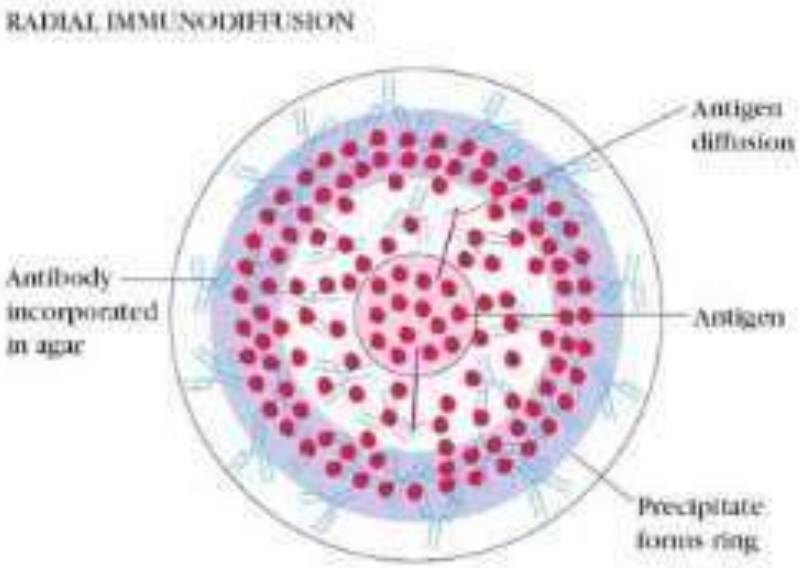
- Slide test. E.g., VDRL in syphilis

- Tube test. E,g., Kahn's test in syphilis

- Immunodiffusion test



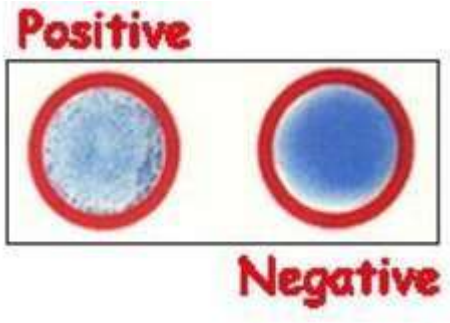
Single and double diffusion in one dimension



Agglutination

- It is an 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 than precipitation for detection of antibodies.
- The reactions take place better with IgM antibody.

Types



Slide Agglutination test

- Routine procedure to identify

bacterial stains. E.g., Salmonella species

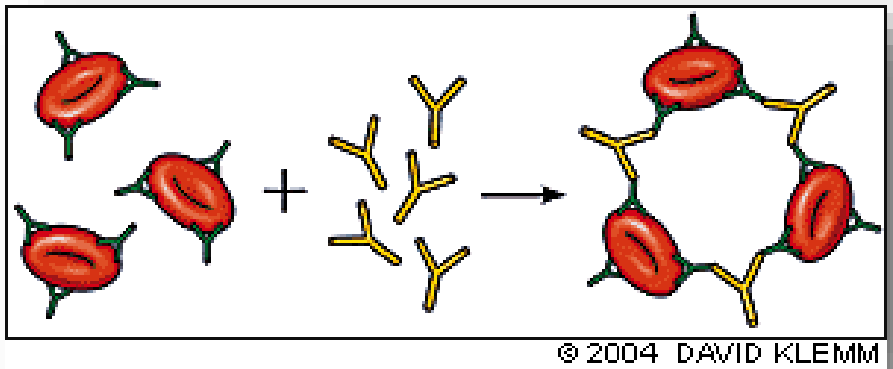
- Also used for blood grouping.

Tube Agglutination test

- Standard quantitative method for determination of antibodies.
- Routinely employed in diagnosis of typhoid, brucellosis and typhus fever

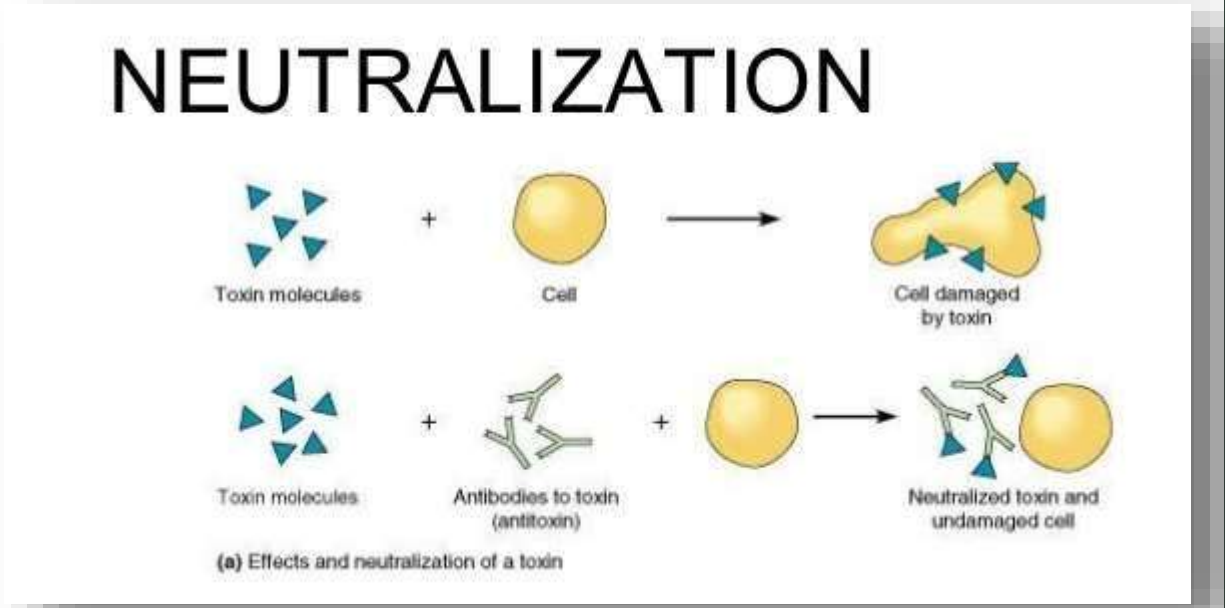
Coombs Test

- Originally devised by Coombs, Mourant and Race (1945) for detection of incomplete Rh antibodies.
- When sera containing incomplete anti-Rh antibodies are mixed with corresponding Rh-positive erythrocytes but no agglutination occurs.
- When such erythrocytes are treated with antiglobulin or COOMBS serum (rabbit antiserum with human gamma globulin), the cells are agglutinated.



Neutralization Test

- Bacterial exotoxins are capable of producing neutralizing antibodies (antitoxins) which play protective role in diseases such as diphtheria and tetanus.
- Toxin – antitoxin neutralization can be measured in vivo and in vitro.



In vivo tests:

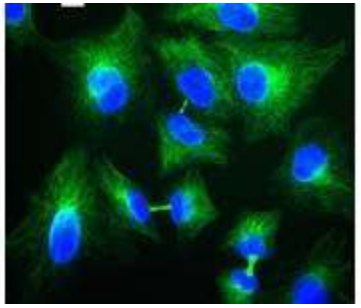
- Toxigenicity test. E.g., C. diphtheriae
- Shick test (similar test in human)

In vitro test:

- Antistreptolysin ‘O’ (ASO) test. E.g., Strep pyogenes
- Virus neutralization tests.

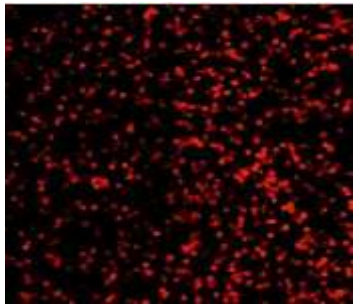
Immunofluorescence

- Fluorescence is the property of certain dyes which absorb rays of one particular wavelength (ultraviolet light) and emit rays with a different wavelength (visible light)



- Most commonly used dyes are:

1. Fluorescin isothiocyanate – blue green

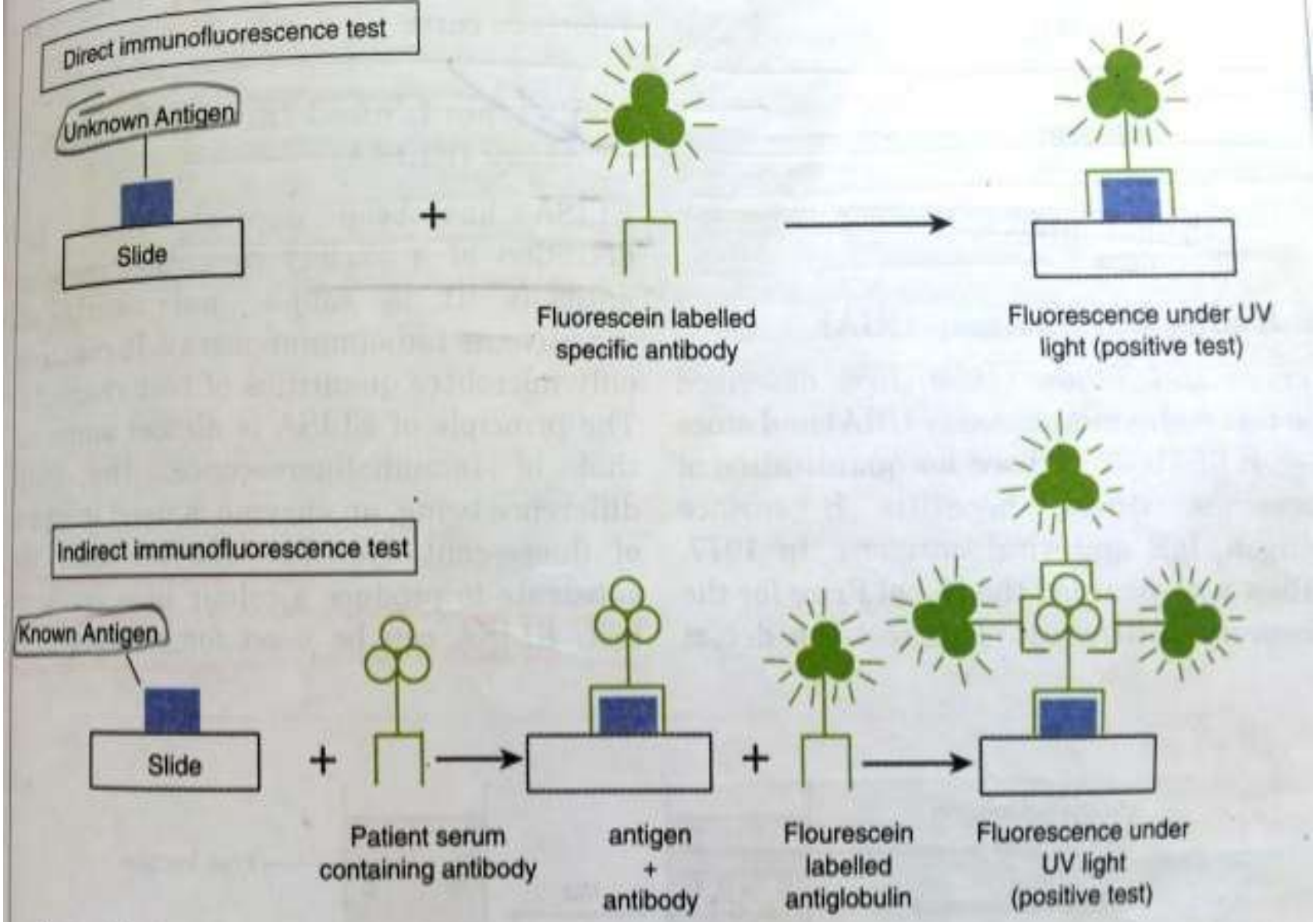


2. Lissamine rhodamine – orange red

- They are of two types:

1. Direct immunofluorescence

2. Indirect immunofluorescence



Example :

Treponema pallidum + Serum of syphilis patient (containing anti-treponemal antibodies which is globulin in nature) + Fluorescin labelled antiglobulin → Fluorescence (Positive)

Direct immunofluorescence

Uses:

- Commonly employed for detection of bacteria, viruses or other antigens in blood, urine, tissues and other specimens.
- Sensitive method to diagnosis Rabies.

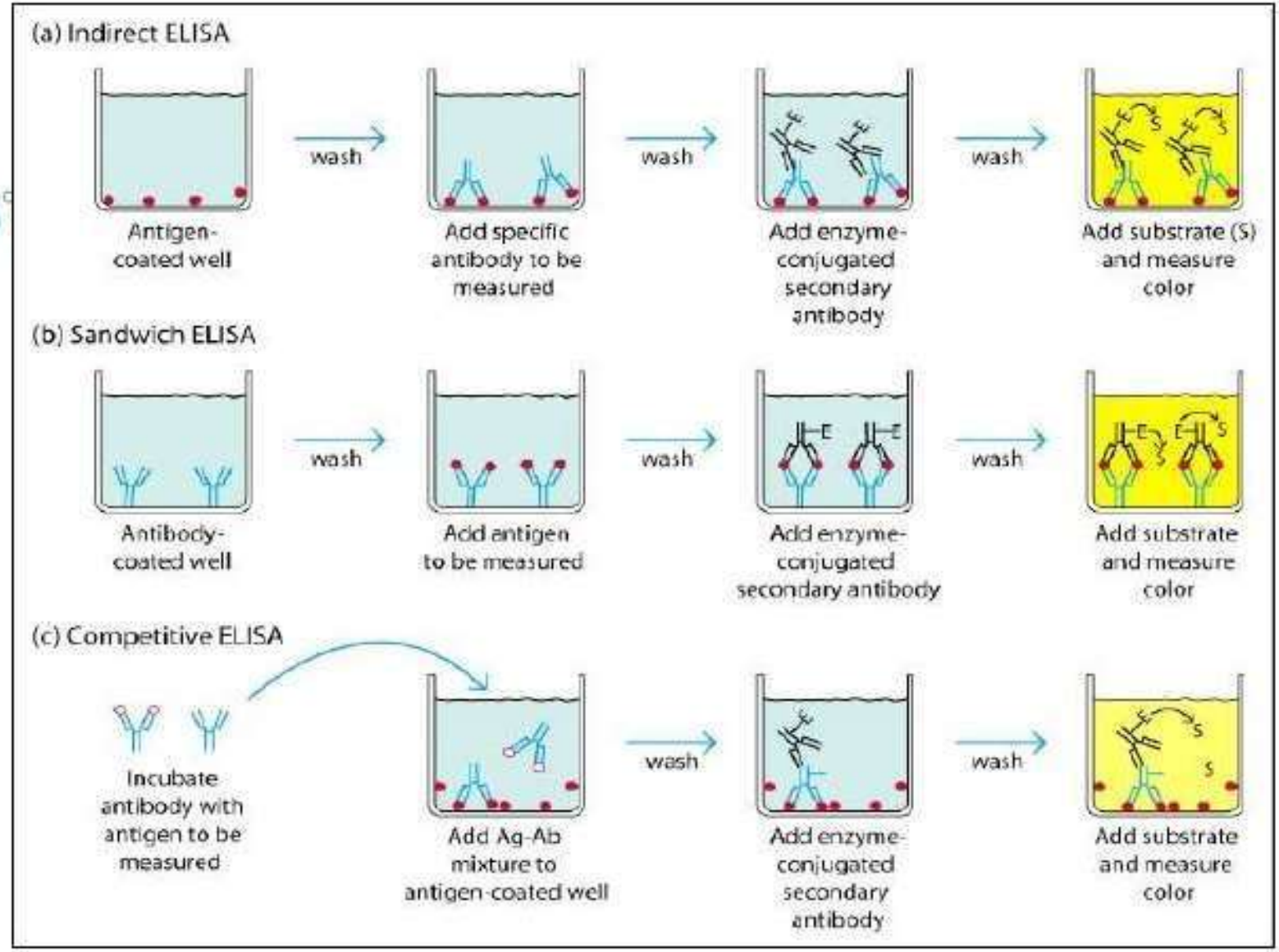
Disadvantage: Separate fluorescent labelled antibody has to be prepared for each antigen to be tested.

Indirect immunofluorescence

- A single antihuman globulin fluorescent conjugate can be employed for detection of antibody to any antigen
- This has overcome the disadvantage of direct immunofluorescence

ELISA

- Enzyme linked immunosorbent assay is a simple and a sensitive test.
- Requires only microlitre quantities of test reagents.
- The principle of ELISA is almost same as that of immunofluorescence, the only difference being, an enzyme is used instead of fluorescent dye.
- It can be used for detection of Antigen or Antibody.
- Types: Sandwich, Indirect, Competitive ELISA



Types of ELISA

Uses:

Detection of HIV antibodies in serum

Detection of mycobacterial antibodies in TB

Detection of Hepatitis B markers in serum

Detection of enterotoxin of E.coli in feces.

Immuno electron microscopic tests

1. Immunoelectron microscopy
2. Immuno Ferritin test
3. Immuno enzyme test

- Immuno electron microscopy: Viral particles are mixed with specific antisera and are observed under electron microscope. These are seen as clumps.

  Used in detection of Hepatitis A virus.

- Immuno ferritin test: Ferritin (electron dense substance) conjugated antibody is used to react with an antigen.

  Used in identification of Legionella species.

- Immuno enzyme test: Tissue sections are treated with peroxidase labelled antisera to detect the corresponding antigen and in viewed under electron microscope.

Conclusion

- Therefore we see the application of antigen antibody reactions in the diagnosis of diseases which can help in developments of varieties of diagnostic tests.
- In clinical practice, they help in:
  - Preventing destructive diseases.
  - Preventing progression of the diseases.
  - Identifying high risk patients
  - Target treatment of specific diseases
  - Monitor the effects of the treatment.

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- Textbook of Microbiology for dental students. Ananthnarayan and Paniker. 8th edition
- Essential Microbiology for Dentistry, 4th Edition. Lakshman Samaranayake.
- https://en.wikipedia.org/wiki/Antigen_antibody_interaction

Previous year questions

- Antigen and Antibody system. (10marks) (MDS Degree Examination May 2009).


Thank you