

Antigen - Antibody

Presented by -

Deepak Kumar Kashyap

Zoology department

D. P. Vipra college

Bilaspur

ANTIGEN-ANTIBODY INTERACTIONS

S Y N O P S I S

- ❑ INTRODUCTION
- ❑ DEFINITION
- ❑ FEATURES
- ❑ TYPES
- ❑ MECHANISM
- ❑ APPLICATION
- ❑ CONCLUSION
- ❑ REFERENCES

ANTIGEN-ANTIBODY INTERACTIONS

- ✓ **Antigen-antibody reaction** is the basis of **humoral immunity** or antibody mediated **immune response**.
- ✓ The noncovalent interactions that form the basis of antigen -antibody (Ag-Ab) binding include **hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals interactions**.
- ✓ Antigen-antibody interactions depend on four types of noncovalent interactions: hydrogen bonds, ionic bonds, hydrophobic interactions, and vander Waals interactions.

ANTIGEN-ANTIBODY INTERACTIONS

❑ ANTIGEN – ANTIBODY REACTION -

- An antibody combines specifically with the corresponding antigen or hapten in a manner which is very similar to the binding of a enzyme to its substrate and involving hydrophobic and ionic interaction.



FIG :- 1 ANTIGEN – ANTIBODY REACTION

ANTIGEN-ANTIBODY INTERACTIONS

- ❑ “The interaction between antigen & antibody is called antigen– antibody reaction. It is abbreviated as **Ag-Ab reaction.**”

ANTIGEN-ANTIBODY INTERACTIONS

- 1. IMMUNE COMPLEX**
- 2. SPECIFICITY OF Ag- Ab REACTION**
- 3. BINDING SITES OF ANTIGEN-ANTIBODY**
- 4. BINDING FORCES OF ANTIGEN & ANTIBODY**
- 5 . AVIDITY**
- 6. BONUS EFFECT**
- 7. CROSS -REACTION**

ANTIGEN-ANTIBODY INTERACTIONS

1. IMMUNE COMPLEX

- When antigen & antibody are brought together , the antibody binds with the antigen to form a complex molecules called **immune complex** or Ag- Ab complex.
- **Ag + Ab** \longrightarrow **Ag – Ab COMPLEX**

FIG :- 2 ANTIGEN – ANTIBODY REACTION

ANTIGEN-ANTIBODY INTERACTIONS

2. BINDING SITES OF ANTIGEN AND ANTIBODY

- In antigen-antibody reaction the antibody attached with the antigen.
- The part of the antigen which combines with the antibody is called **epitope** or antigenic determinates.
- An antigen may contain 10 to 50 antigenic determinants.
- Some time it may go up to 200.
- The part of the antibody which combines with the antigen is called **paratope** or antigen binding site.
- Most of the antibodies are bivalent having two binding sites.
- But the antibody IgM is multivalent having 5 to 10 binding sites.

ANTIGEN-ANTIBODY INTERACTIONS

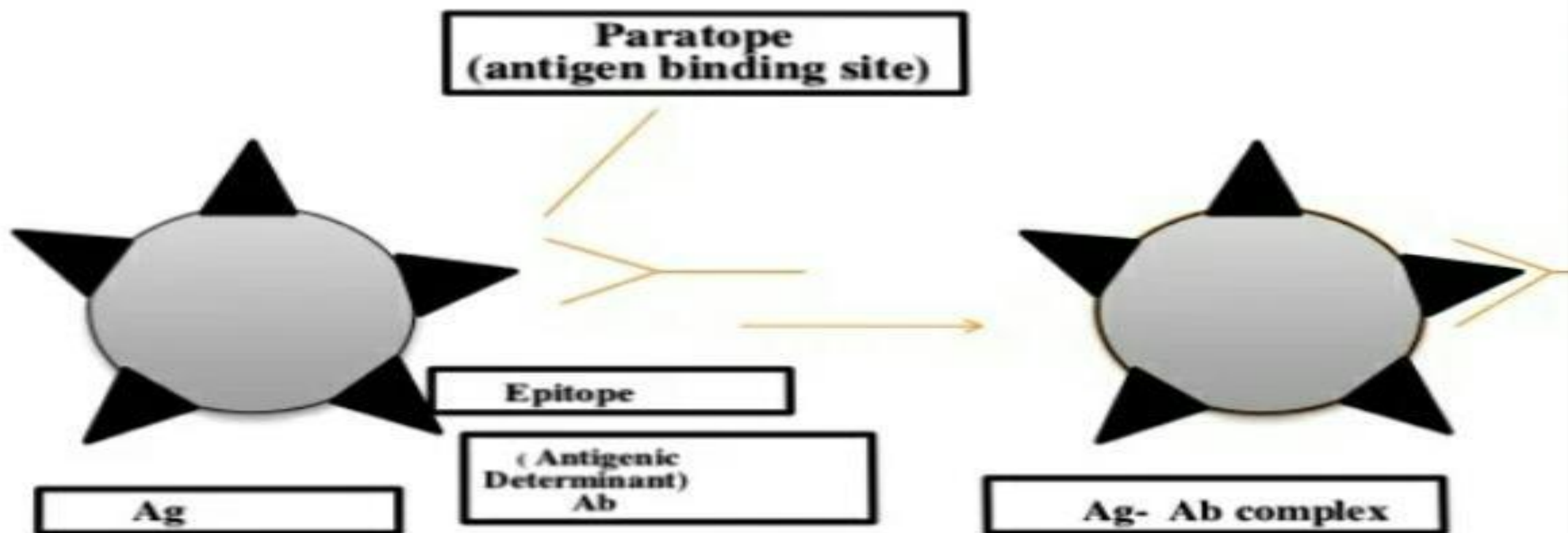


FIG:- 3 ANTIGEN AND ANTIBODY TO SHOW EPITOPE & PARATOPE

ANTIGEN-ANTIBODY INTERACTIONS

3. AVIDITY

- Avidity refer to be capacity of an antiserum containing to combine with the whole antigen that stimulated the production of antibodies.



- Where $n \text{ Ab}$ =number of antibodies
- $m \text{ Ag}$ =Antigenic determinants
- A multivalent antigen has many type of antigenic determinant stimulates the production of a particular antibody .

ANTIGEN-ANTIBODY INTERACTIONS

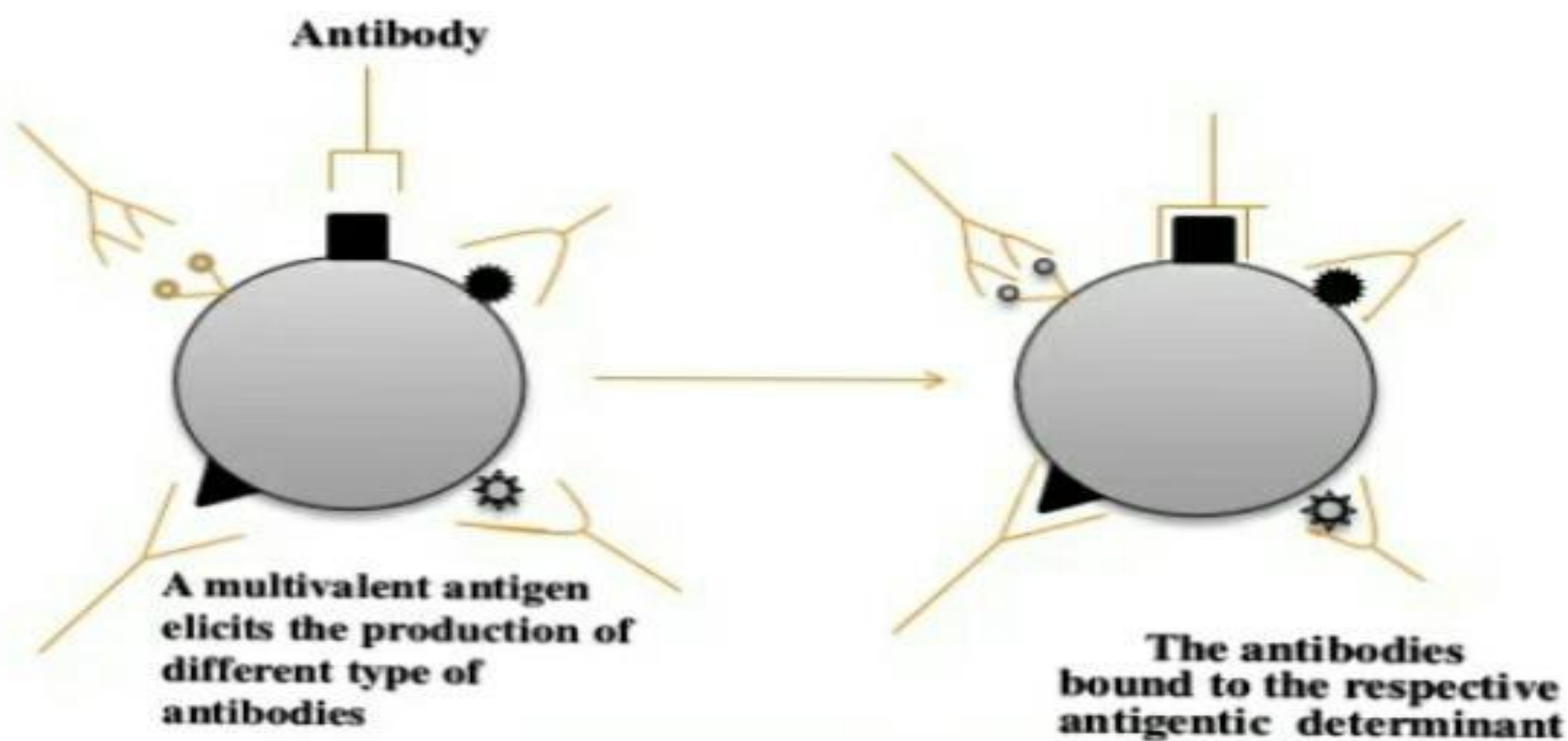


FIG :- 4 AVIDITY

4. CROSS REACTION

- An antiserum raised against a given antigen may sometimes react with another closely related antigen.
- This reaction is called cross reaction. & the antigen which produce the cross reaction is called **cross reactive antigen**.
- The **cross reaction** is due to the presence one or more identical antigenic determinants on the related antigen.

ANTIGEN-ANTIBODY INTERACTIONS

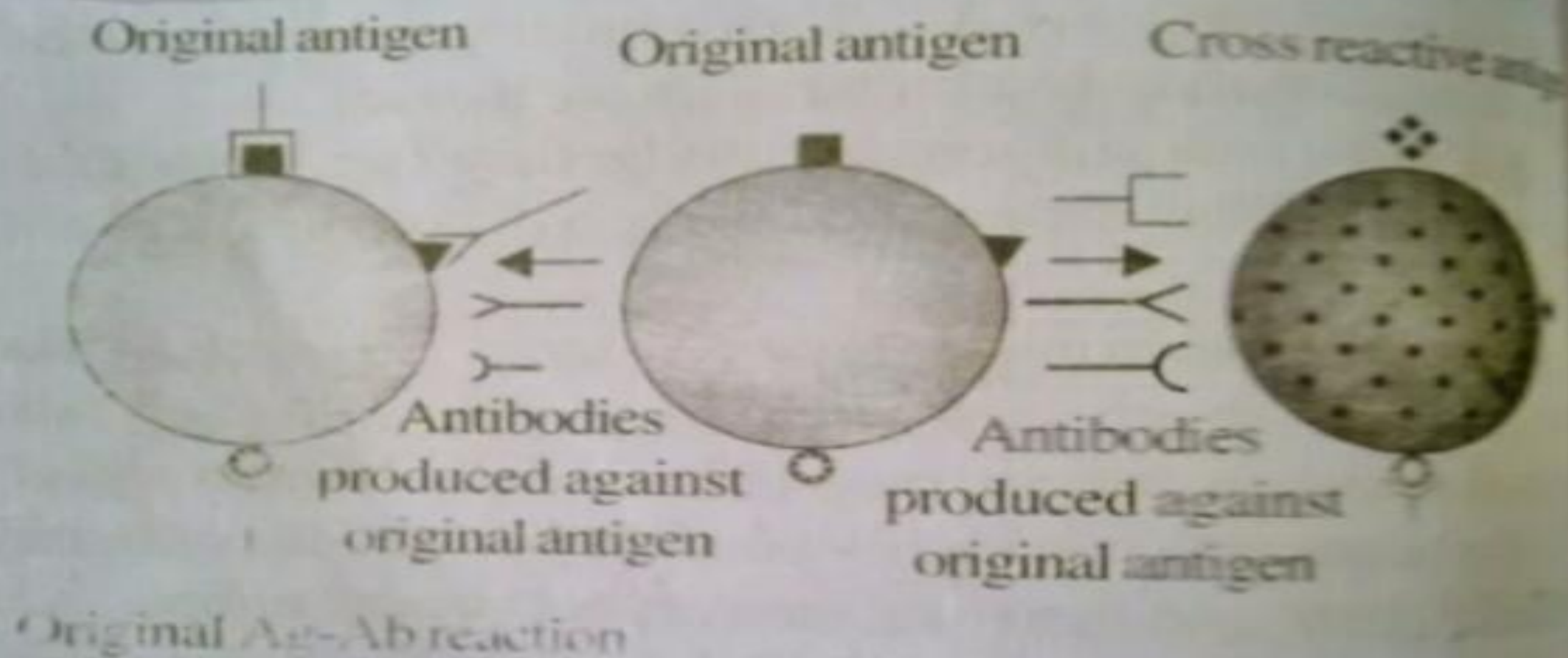


FIG: - 5 CROSS-REACTION

ANTIGEN-ANTIBODY INTERACTIONS

1. PRECIPITATION

- ✓ Precipitation refers to an *antigen – antibody reaction* between a soluble antigens & its antibody resulting in the formation of insoluble Precipitate. the antibody causing Precipitation is called **Precipitation**.

❑ MECHANISM

- ✓ Precipitation is due to the formation of *antigen – antibody complex*.
- ✓ The *antigen* is multivalent & the antibody is bivalent.
- ✓ As each antibody is a bivalent molecules, it can bridge two multivalent antigen molecules.
- ✓ This bridge leads to the formation of a lattice which forms the Precipitate.
- ✓ When antigen & antibody are in optimal concentration, the Precipitation

ANTIGEN-ANTIBODY INTERACTIONS

❑ PRECIPITATION TEST –

- ✓ precipitin test is a test of antigen – antibody reaction.
- ✓ precipitin reaction can be carried out by a classical experiment .
- ✓ A set of 5 or more reaction tubes are arranged serially & are marked as A, B , C, D, E .
- ✓ A constant volume of antiserum is added to each tubes.
- ✓ the antigen is added in increasing volume from tube A to E.
- ✓ antigen & antibody react together resulting in precipitation.
- ✓ The amount of precipitate formed is determined by the proportion of antigen & antibody.
- ✓ when the amount of precipitate formed in different tubes is plotted on a graph paper a curve is obtained. this curve is called precipitin curve.

ANTIGEN-ANTIBODY INTERACTIONS

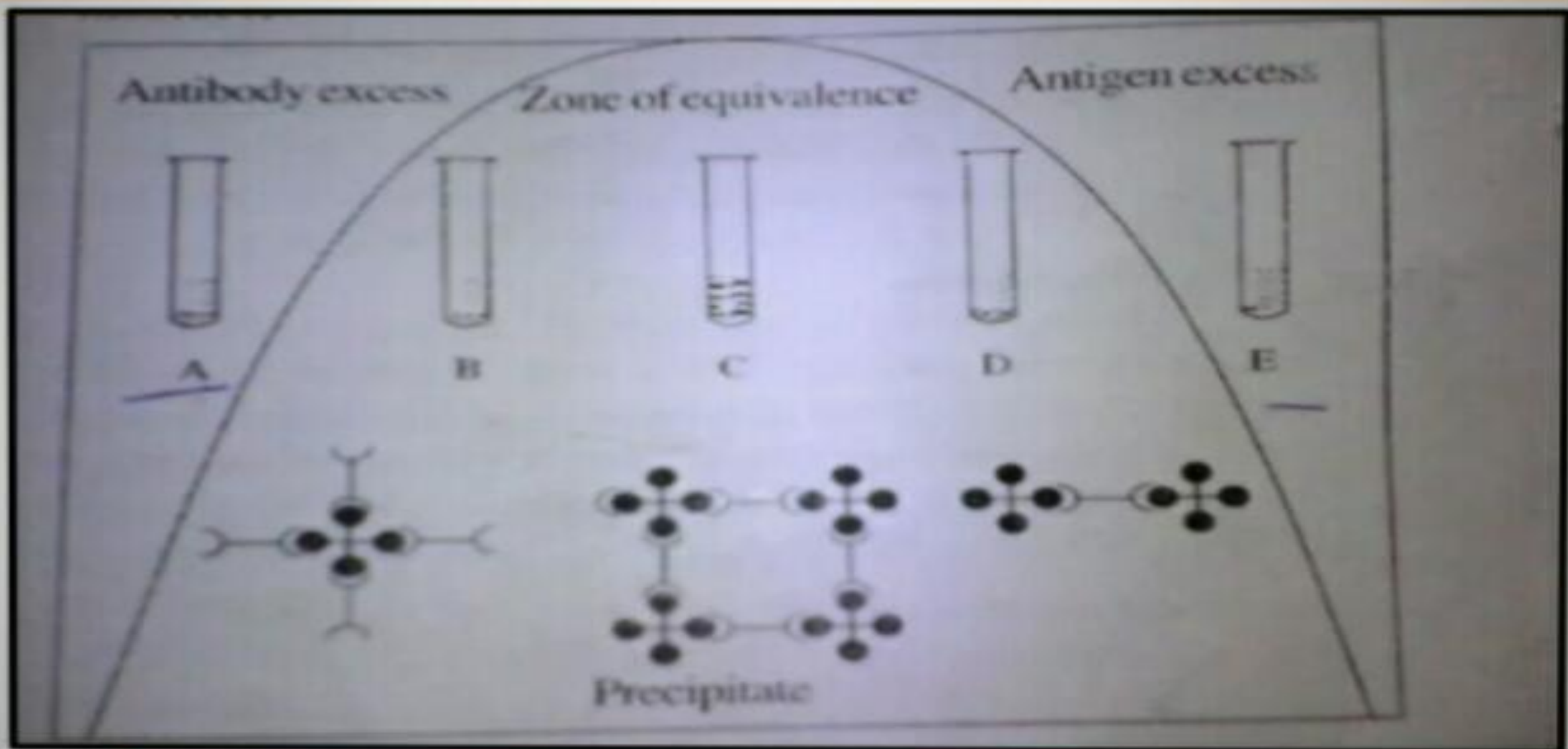


FIG : 6 PRECIPITATION TEST

ANTIGEN-ANTIBODY INTERACTIONS

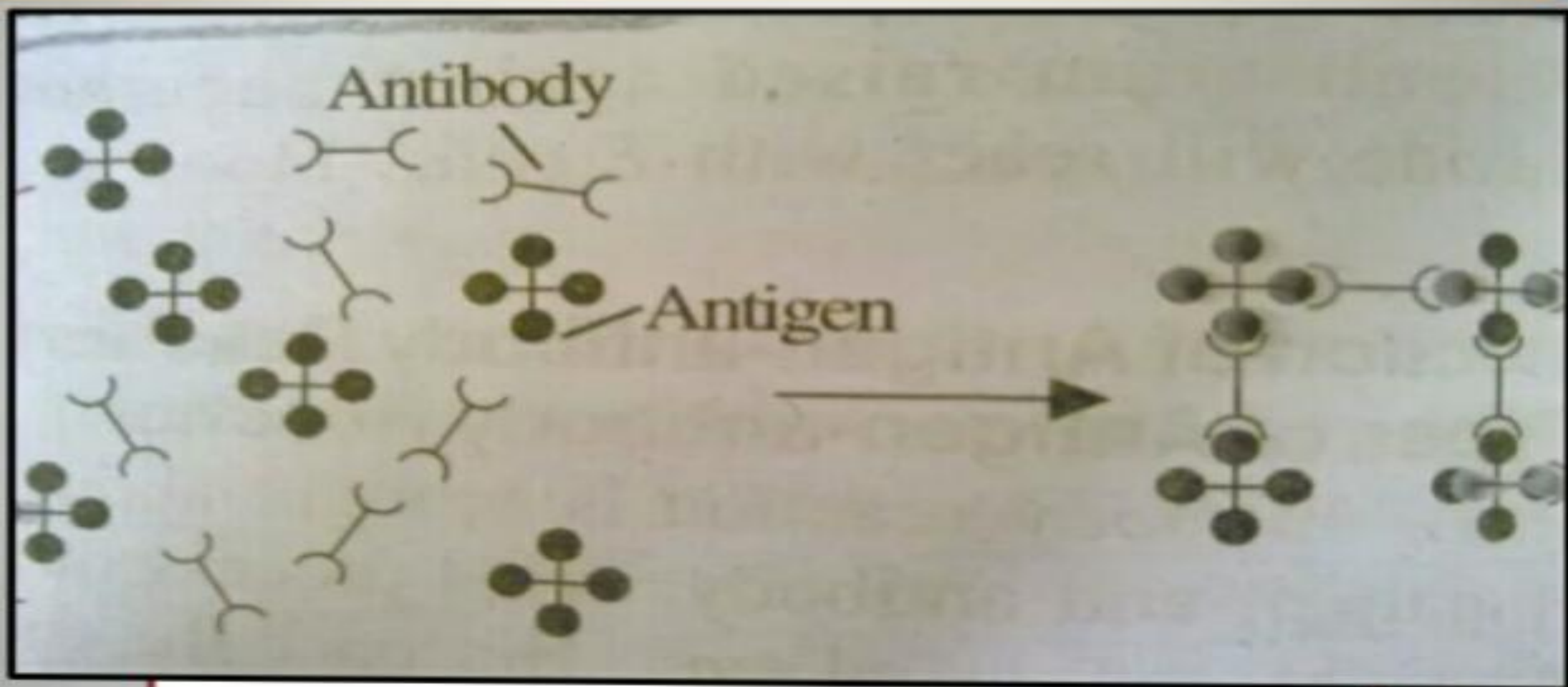


FIG:- 7 PRECIPITATION

ANTIGEN-ANTIBODY INTERACTIONS

❑ The precipitin curve shows 3 zones, namely :-

- Zone of antibody excess
- Zone of equivalence
- Zone of antigen excess

❑ APPLICATION

- Single immunodiffusion
- Double immunodiffusion
- Radio Immuno assay(RIA)
- Immuno electrophoresis
- Rocket immunodiffusion

ANTIGEN-ANTIBODY INTERACTIONS

❑ APPLICATION

✓ RADIOIMMUNOASSAY (RIA)-

- **Radioimmunoassay** is one of the most important techniques in the clinical biochemical fields for the quantitative analysis of hormones, and drugs .
- It combines the specificity of the immune reaction with the sensitivity of the radioisotope techniques.
- The most commonly used labels are **radioisotope** and **enzymes**.
- A variety of tests have been devised for the measurement of antigen and antibodies using such labeled reactants.

ANTIGEN-ANTIBODY INTERACTIONS

➤ APPLICATION

- This technique is also useful in diagnosing insulinomas, sex hormone sensitive tumors etc. and this facilitates proper treatment of the disease.
- Estimation of peptides steroids hormone, vitamins, drugs, antibodies nucleic acids, structural proteins hormone receptor proteins.
- Radioimmunoassay has tremendous application in the diagnosis of hormonal disorders, cancers and therapeutic monitoring of drugs besides being useful in biomedical research.
- The most sensitive techniques for detecting antigen or antibody is radioimmunoassay (RIA).

ANTIGEN-ANTIBODY INTERACTIONS

2. AGGLUTINATION

- Agglutination is an antigen –antibody reaction where the antibody of serum causes the cellular antigen to adhere to one another to form clumps.
- It is the clumping of a particular antigen and its antibody.
- The antibody that cause agglutination are called agglutinins and particulate antigens aggregated are called agglutinogens.
- The particulate antigen include bacterial ,viruses ,RBC ,platelets lymphocytes ,etc.
- When red blood cells are agglutinated ,the reaction is called **Heamagglutination** .
- When bacterial cells are agglutinated ,the agglutination is called **Bacterial Agglutination**.

ANTIGEN-ANTIBODY INTERACTIONS

❑ MECHANISM OF AGGLUTINATION

- Agglutinations is brought about by the linking of antigen and antibodies.
- As most of the antibodies are bivalent ,an antibody can link two adjacent antigens.
- The IgM antibody is multivalent and it contains 5 Or 10 combining sites.
- Hence IgM antibody has the capacity to make clumps more effectively with a lesser number of molecules then that of IgM antibody molecule.
- The univalent antibodies (antibodies with a single combining site) cannot form clump or lattice and hence agglutination will not occur.

ANTIGEN-ANTIBODY INTERACTIONS

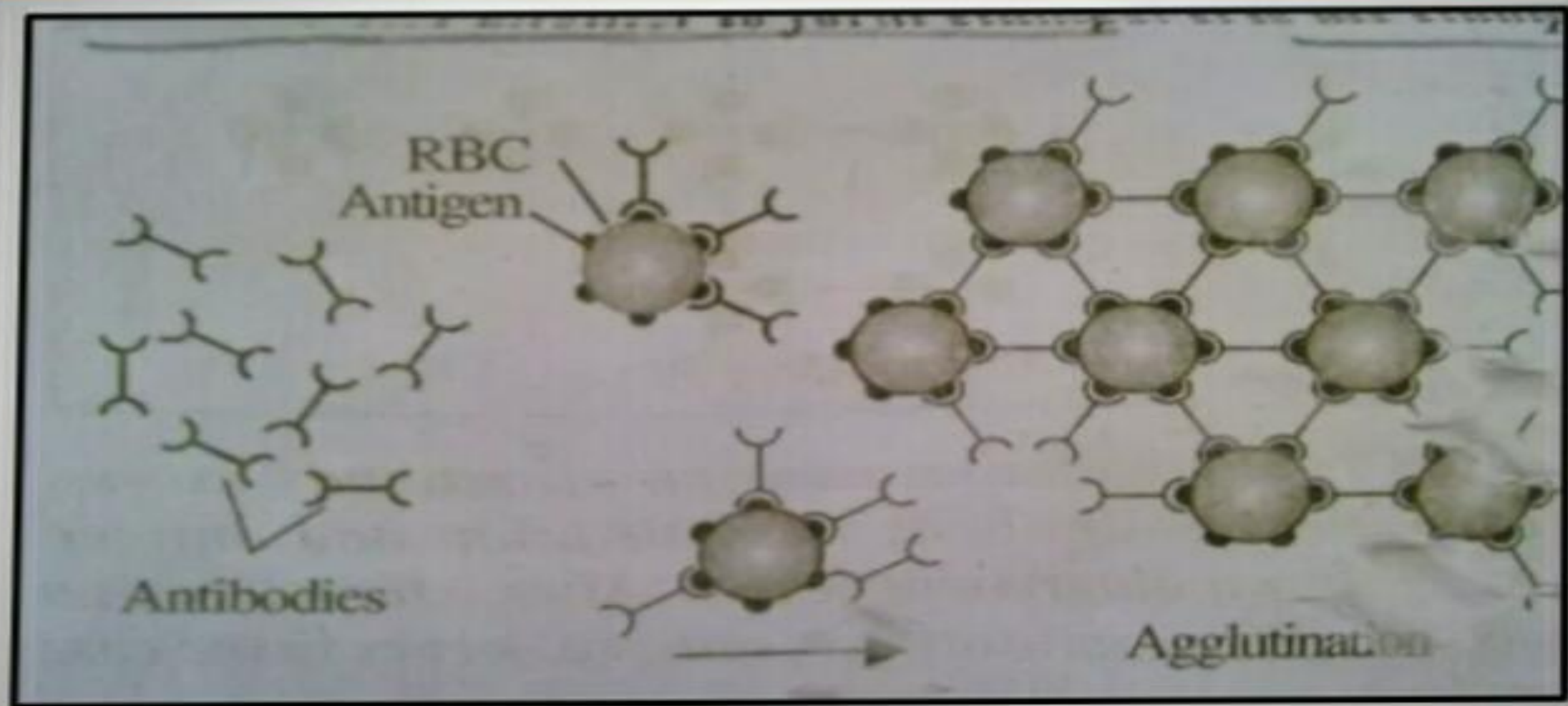


Fig :- 8 AGGLUTINATION

ANTIGEN-ANTIBODY INTERACTIONS

❑ AGGLUTINATION TEST:

Agglutination test refer to the examination of clump formation when particular antigen and its antibodies are combined.

- ABO blood group
- Rh blood group
- Widal test for typhoid
- Coomb's test for the identification of anti - Rh antibodies

ANTIGEN-ANTIBODY INTERACTIONS

❖ EXAMPLE

ABO BLOOD GROUP –

- The typing of blood , for ABO groups or Rh groups, involves agglutination reaction.
- For typing blood , a drop of the blood sample is mixed with a drop of antiserum A & another drop of the blood sample is mixed with a drop of antiserum B on a glass slide.
- If belongs to is clumped with antiserum A , the sample belongs to belongs to , if the sample is clumped with antiserum B , if the sample is clumped with both antiserum A & antiserum B , the blood sample belongs to belongs to group AB . If there is no agglutination the blood sample belongs to group O.

ANTIGEN-ANTIBODY INTERACTIONS

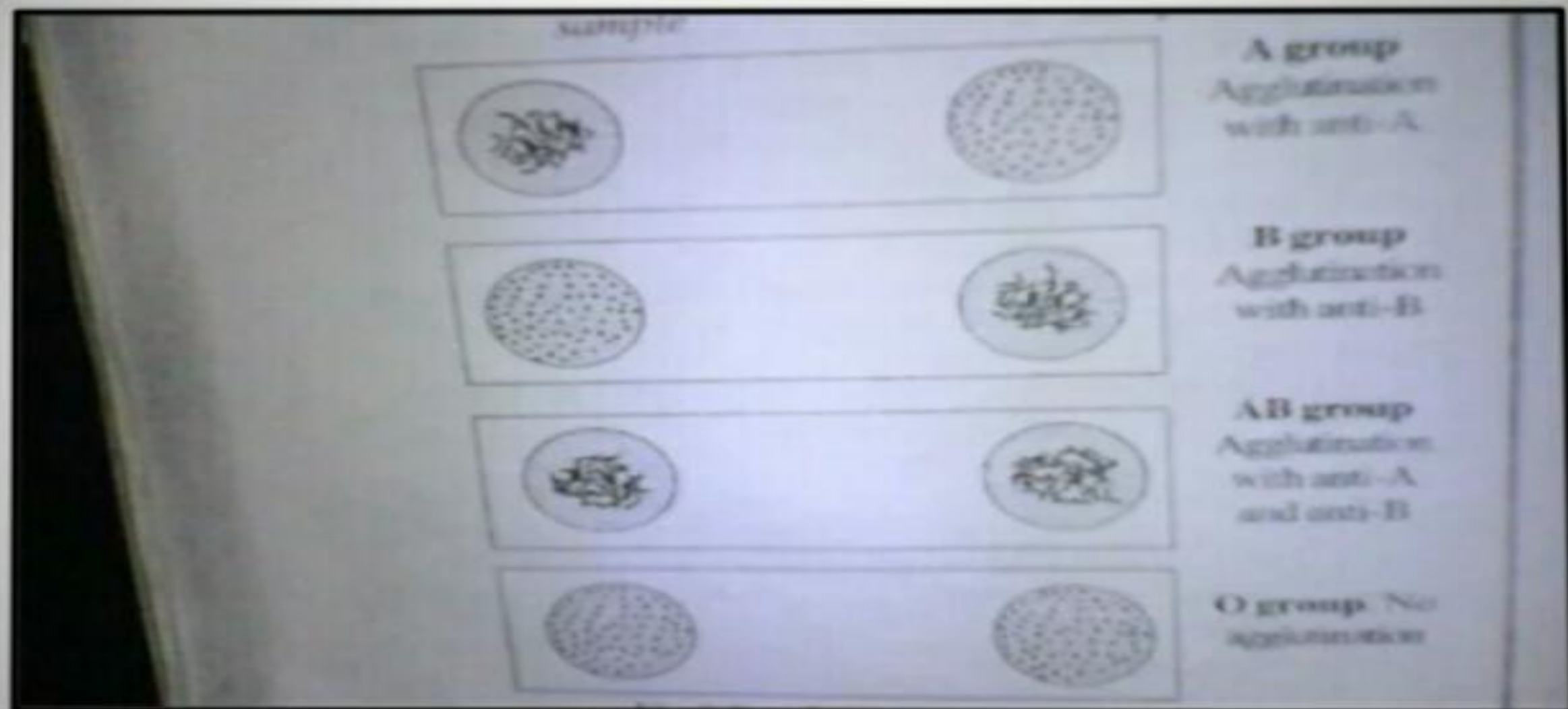


FIG :- 9 AGGLUTINATION

ANTIGEN-ANTIBODY INTERACTIONS

- **ELISA** also known as an **enzyme linked immunosorbent assay** is a biochemical Techniques used mainly in immunology to detected the Presence Of an **antibody** or an **antigen** in a sample.
- **Enzyme-linked immunosorbent assay, commonly known as ELISA or EIA**), is similar in principle to RIA but depends on an enzyme rather than a **radioactive label**.
- **ELISA** can also be used in toxicology as a rapid presumptive screen for certain classes of drug.

ANTIGEN-ANTIBODY INTERACTIONS

➤ Enzyme Linked Immunosorbent Assay (ELISA)

➤ Term Was Coined By *Engvall* and *Pearlmann* in **1971**

▪ Different Type

✓ Indirect ELISA

✓ Sandwich ELISA

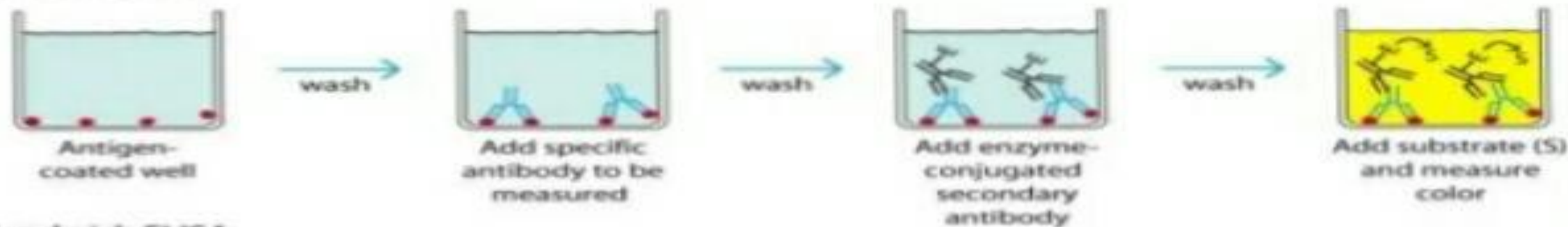
✓ Competitive ELISA

➤ **ELISA** used in the detection and quantization of several antigen as well as antibodies.

➤ Indirect **ELISA** method to detect the presence of serum antibody against HIV. The causative agent of AIDS.

ANTIGEN-ANTIBODY INTERACTIONS

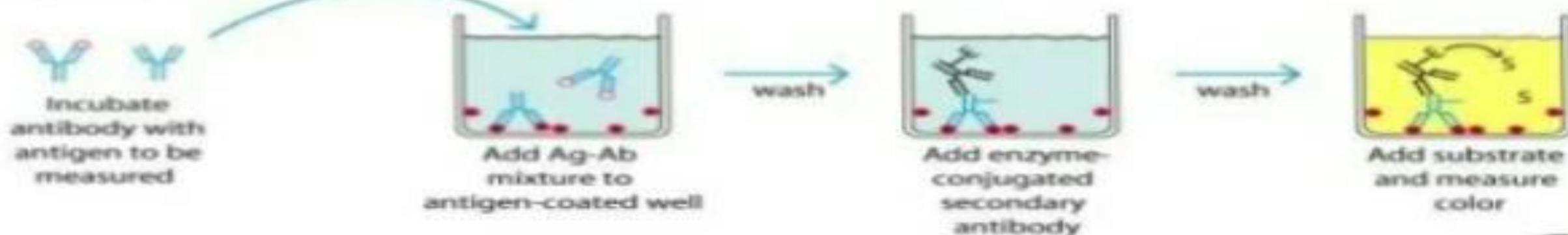
Indirect ELISA



Sandwich ELISA



Competitive ELISA



ANTIGEN-ANTIBODY INTERACTIONS

➤ Enzyme Linked Immunosorbent Assay (ELISA)

➤ Term Was Coined By *Engvall* and *Pearlmann* in 1971

• Different Type

✓ Indirect ELISA

✓ Sandwich ELISA

✓ Competitive ELISA

➤ **ELISA** used in the detection and quantization of several antigen as well as antibodies.

➤ Indirect **ELISA** method to detect the presence of serum antibody against HIV. The causative agent of AIDS.

ANTIGEN-ANTIBODY INTERACTIONS

❑ INDIRECT ELISA-

- Antibody can be detected or quantitatively determined with an indirect ELISA.
- Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well.
- After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary antibody (Ab2).
- Any free Ab2 then is washed away, and a substrate for the enzyme is added.
- The amount of colour reaction product that forms is measured by specialized spectrophotometric plate readers.

6.0 Indirect ELISA

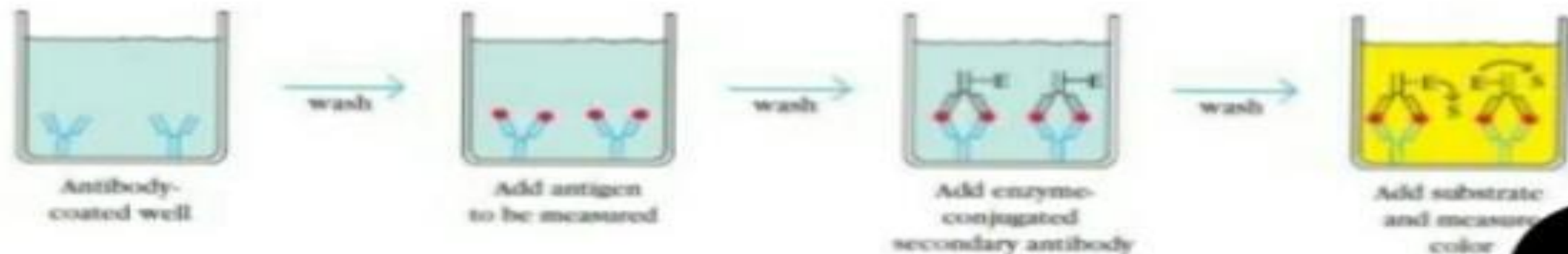


ANTIGEN-ANTIBODY INTERACTIONS

❑ SANDWICH ELISA-

- In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well.
- A sample containing antigen is added and allowed to react with the immobilized antibody.
- After the well is washed, a second enzyme-linked antibody specific for a different epitope on the Antigen is added and allowed to react with the bound antigen.
- After any free second antibody is removed by washing, substrate is added, and the colour reaction product is measured.

(b) Sandwich ELISA



ANTIGEN-ANTIBODY INTERACTIONS

❑ COMPETITIVE ELISA

- In this technique, antibody is first incubated in solution with a sample containing antigen.
- The antigen-antibody mixture is then added to an antigen coated microtiter well.
- The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well.
- In the competitive assay, however, the higher the concentration of antigen.

- ✓ **Antigen-antibody reaction** is the basis of humoral immunity or antibody mediated immune response.
- ✓ The noncovalent interactions that form the basis of antigen -antibody (Ag-Ab) binding include **hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals interactions.**
- ✓ **ELISA** also known as an enzyme linked immunosorbent assay is a biochemical Techniques used mainly in immunology to detected the Presence Of an **antibody** or an **antigen** in a sample.
- ✓ **Radioimmunoassay** is one of the most important techniques in the clinical biochemical fields for the quantitative analysis of hormones, and drugs .

BOOK NAME	AUTHER NAME	EDITION
IMMUNOLOGY	PROF. DULSY FATIMA	1 ST Edition
IMMUNOLOGY W.H.FREEMAN	JAINS KUBY	6 th Edition
MICROBIOLOGY	ANANTHANARAYAN & PANIKER'S	8 TH Edition-
INTERNET SOURCES &CLASSNOTES-	2013	

Thank you