Charting New Horizons in Education

Lab part 2

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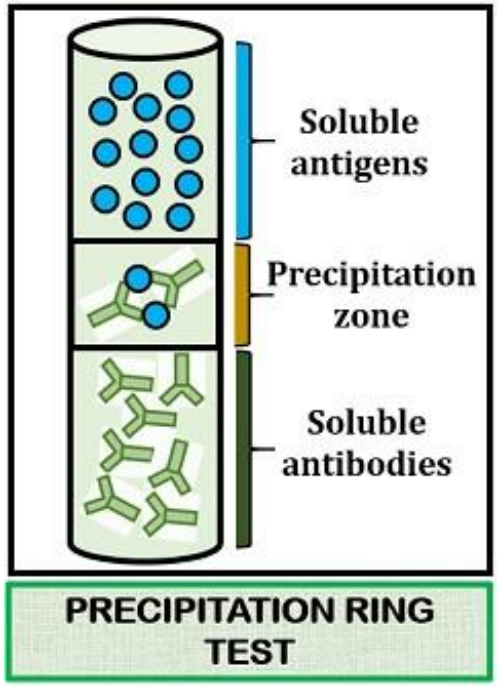
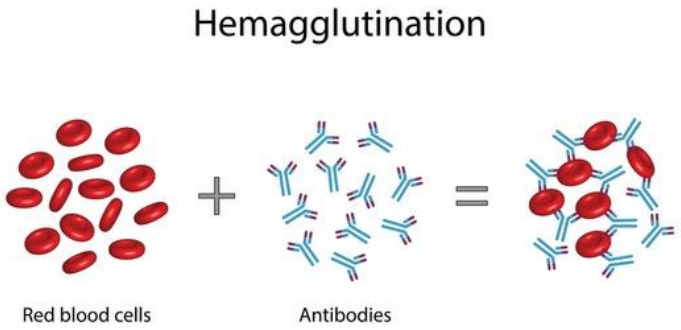
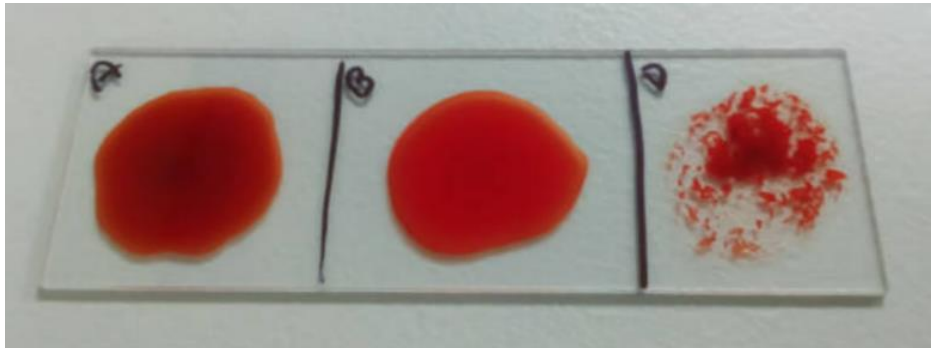
Precipitation vs Agglutination

- **Precipitation in Ag-Ab Binding**: Precipitation reactions are based on the interaction of **antibodies and antigens**. Here, two soluble substances—an antigen and an antibody—come together to form an **insoluble product** (precipitate) that appears as a **line** between two solutions.



- **Agglutination in Ag-Ab Binding**: Agglutination is the visible aggregation of **antigens and antibodies**. This reaction applies to **cell-bound antigens** (on RBCs or particles) that bind to antibodies. The endpoint is observed as **clumps** formed from the antigen-antibody complex.





MCQ

In agglutination reactions, the antigen is a _____ and in precipitation reactions, the antigen is a _____.

a) Bound to cell/soluble molecule
b) Soluble molecule/bound to cell
c) Bacterium/virus
d) Protein/carbohydrate
e) Virus/bacterium

Answer:
a) Bound to cell/soluble molecule

1) Precipitation reaction

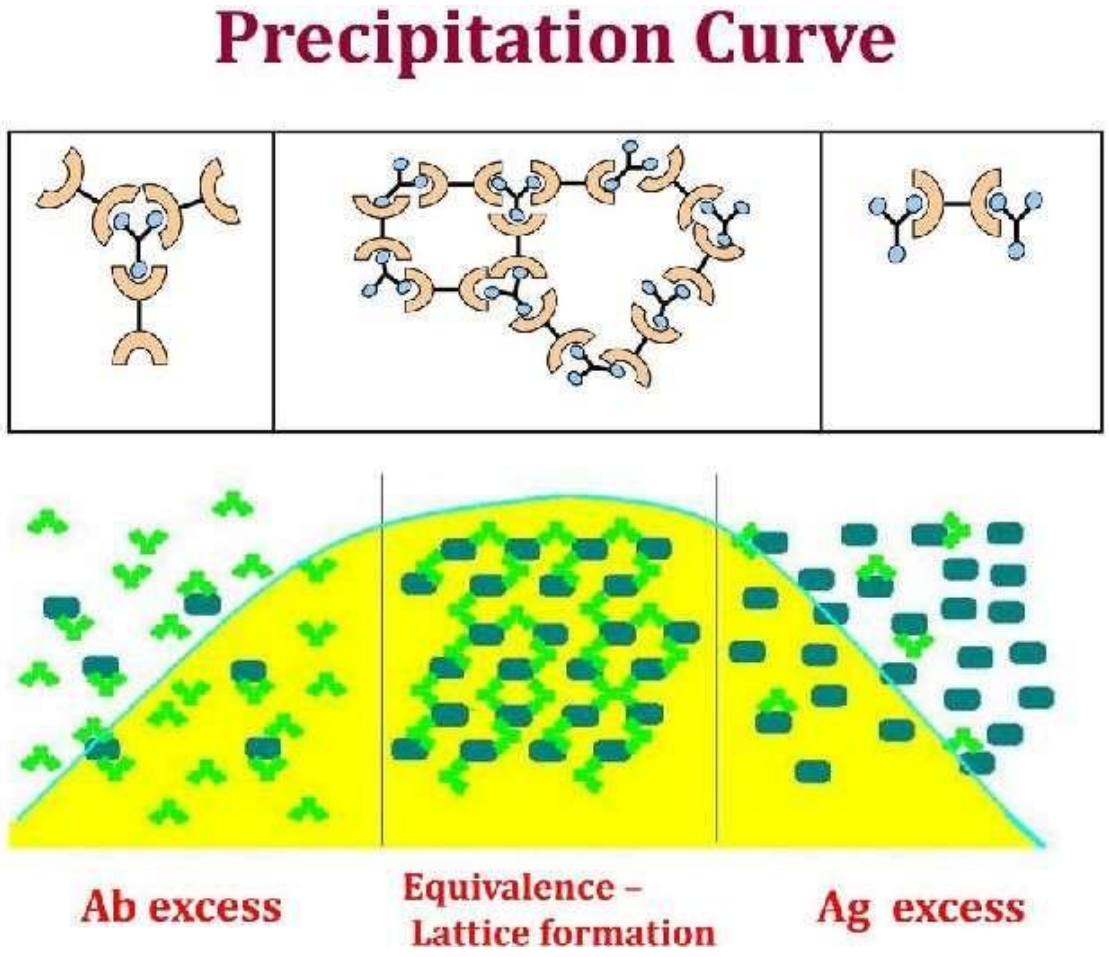
•**Amount of Precipitate**: Influenced by the proportions of antigen (Ag) and antibody (Ab).

- **Maximum Precipitation**: Occurs when Ag and Ab are present in **optimal or equivalent proportions**.
- **Lattice Formation**: Precipitation occurs when a **lattice network** (line or lattice) is formed.

•**Phenomena**:

- **Prozone Phenomenon**: Occurs when there is an **excess of antibodies**, preventing lattice network formation.
- **Postzone Phenomenon**: Occurs when there is an **excess of antigens**, also preventing lattice network formation.

•**Detectability**: For precipitation reactions to be detectable, they must occur within the **zone of equivalence** (where Ag and Ab are in optimal proportions).



MCQ

- Prozone is:

a) Zone of antigen excess
b) Zone of equivalence
c) Zone of antibody excess
d) Zone of complement excess
e) Zone of RBC excess

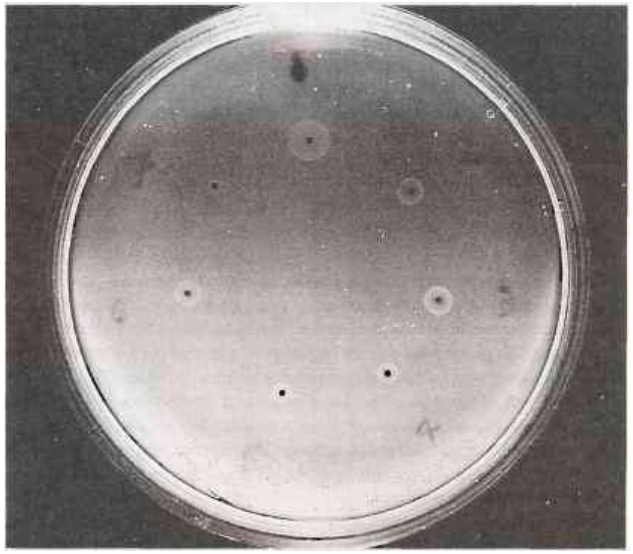
Answer: c) Zone of antibody excess

- Antigen-antibody precipitation is maximally seen in which of the following?

a) Excess of antibody
b) Excess of antigen
c) Equivalence of antibody and antigen
d) Antigen-Hapten interaction
e) Excess of both antigen and antibody

Answer: c) Equivalence of antibody and antigen

1- Single Radial Diffusion (Radial Immunodiffusion)

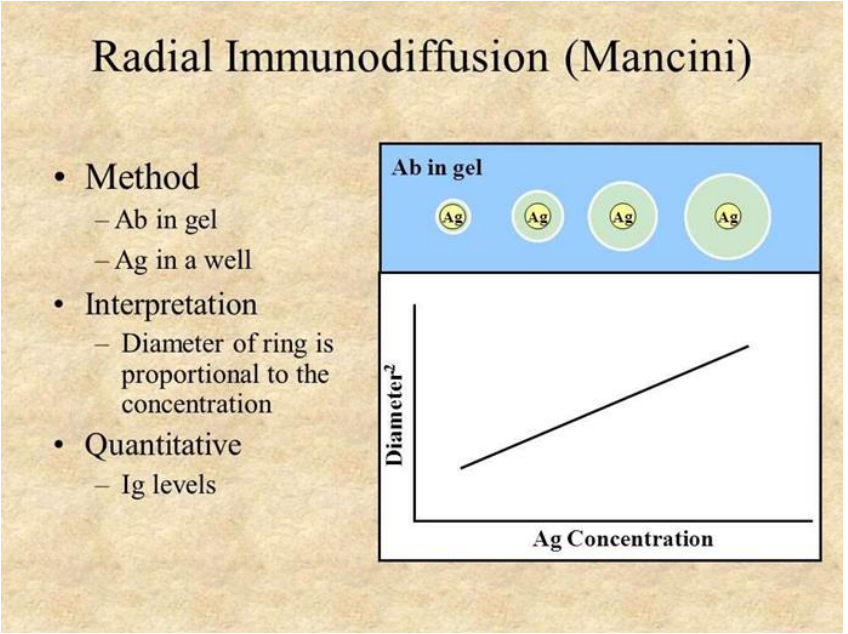


•**Procedure**:

- **Antibody (Ab)** is incorporated into an **agar gel** on a slide.
- **Antigen (Ag)** is added to small wells in the agar.
- Ag **diffuses radially** from the well, forming a **precipitation ring** around it.
- The **diameter of the halo** is used to estimate the concentration of the antigen.

•**Applications**:

- Estimation of **immunoglobulin (Ig) levels** in serum.
- Screening for **antibodies against viruses** (e.g., Influenza) or **bacteria**.



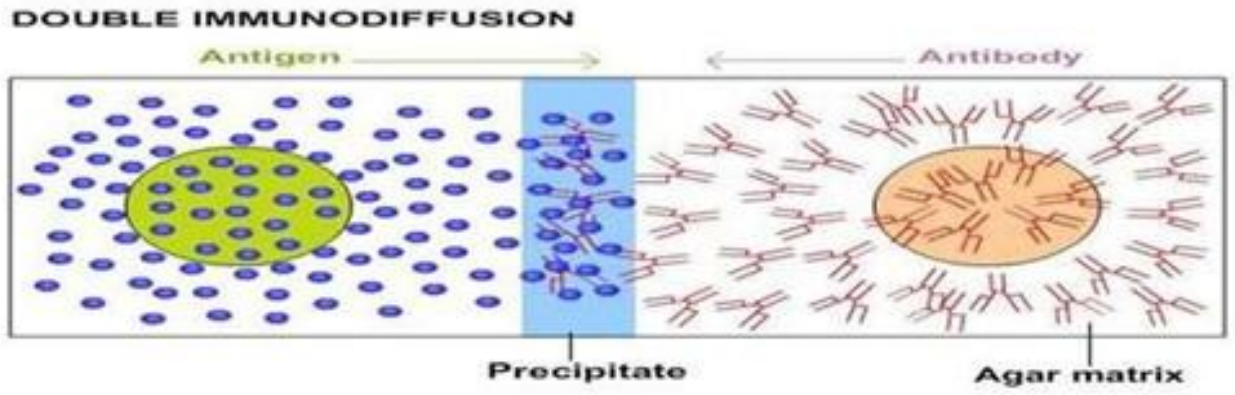
2- Double immunodiffusion

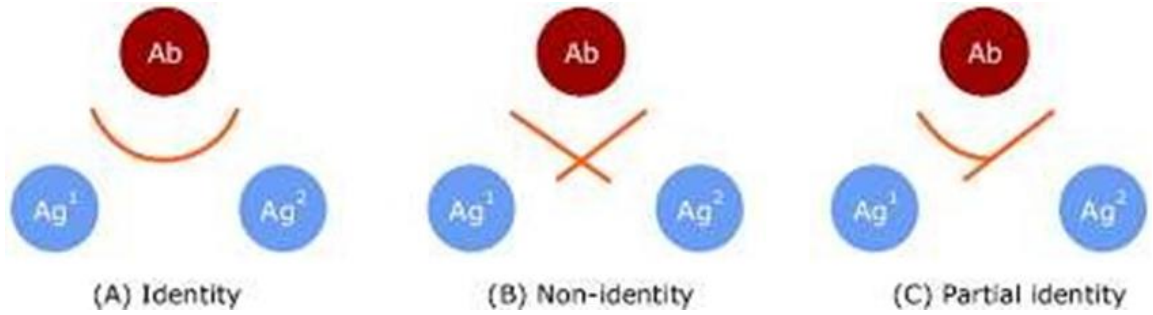
•**Process**:

- Diffusion of **antibody** and **antigen** occurs toward each other in an **agarose gel**.
- A **line of precipitate** forms if the antibody binds to the antigen.

•**Purpose**:

- This method is used to determine the presence of either an **antigen** or an **antibody**.
- Comparison of antigen



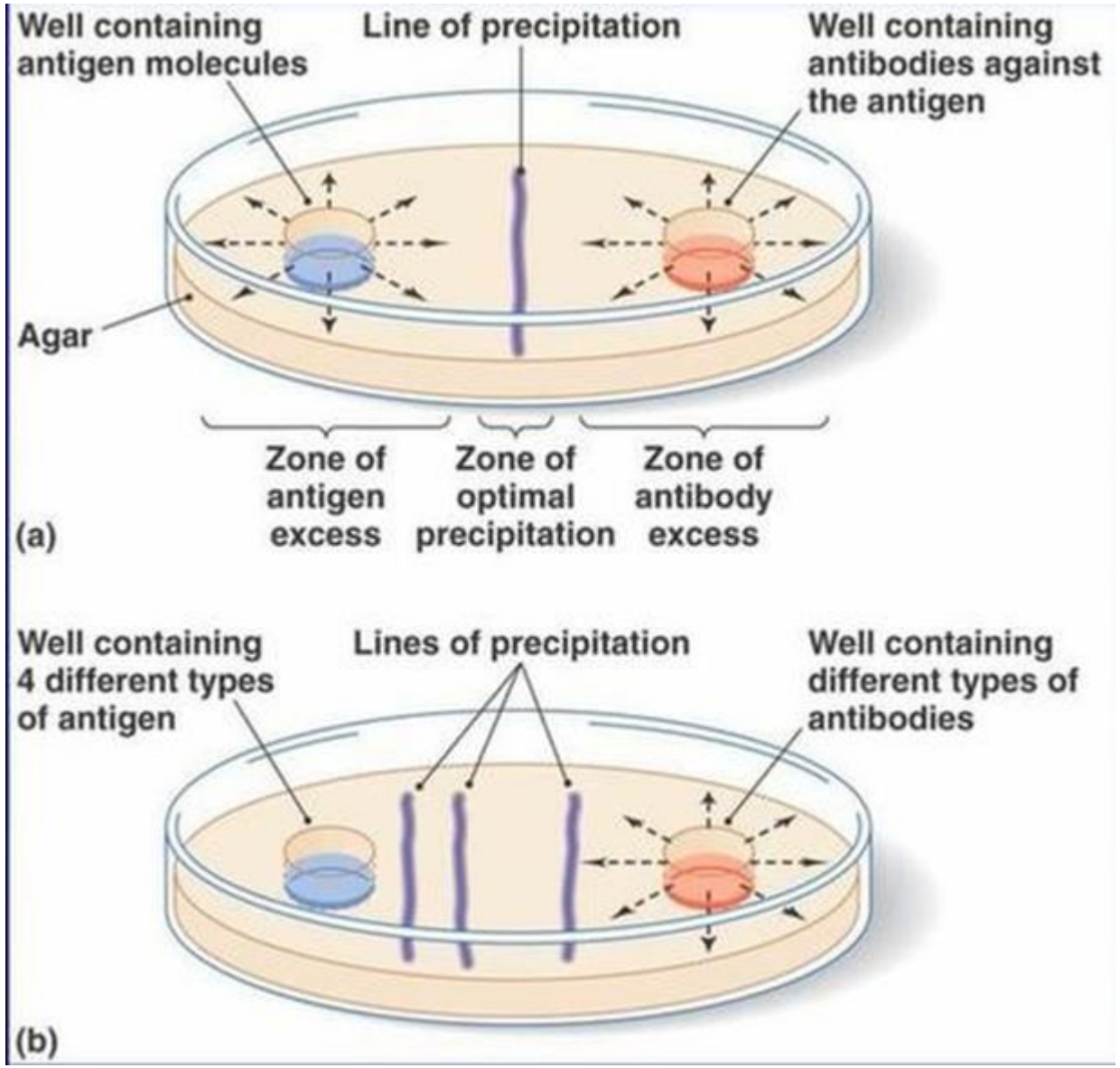


Ouchterlony results types to detect microbial antigens

Ouchterlony test

Ouchterlony test
The Ouchterlony Test is used for qualitative analysis, not for quantitative measurement.

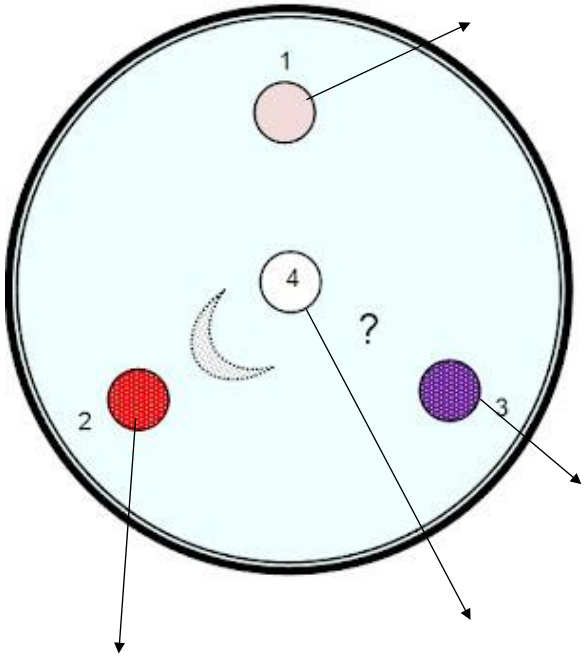
Mancini test
both qualitative and quantitative

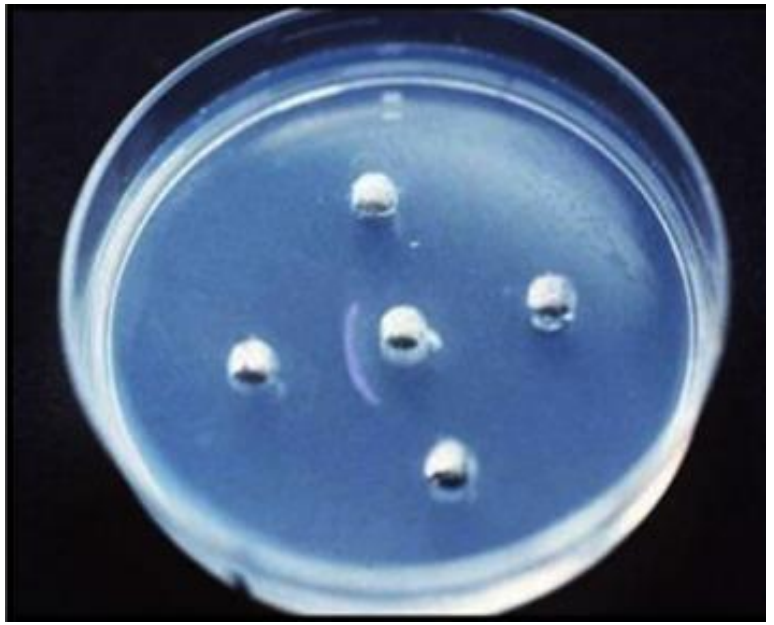


Ouchterlony test

4 → has a known antigen
1 and 2 and 3 unknown samples

After reaction;
only 2 has the Ab
absence of Ab in 1 and 3





MCQ

- The amount of various immunoglobulin classes can be measured by:

a) Double diffusion in one dimension
b) Single diffusion in radial dimension
c) Single diffusion in one dimension
d) Double diffusion in radial dimension
e) Double diffusion in three dimensions

Answer: b) Single diffusion in radial dimension

- If an antibody is uniformly distributed in a gel and an antigen is added to a well cut into the gel, the process is called:

a) Single diffusion
b) Double diffusion
c) Immunofixation
d) Retrodiffusion
e) Complement fixation

Answer: a) Single diffusion

MCQ

The Ouchterlony method of immunodiffusion analysis: Which one is true?

a) Is used to detect one antigen
b) Can directly compare the antigenic relatedness of two antigens
c) Is a standard quantitative assay
d) Requires use of radioactive antibodies
e) Measures only autoantibodies

Answer: b) Can directly compare the antigenic relatedness of two antigens

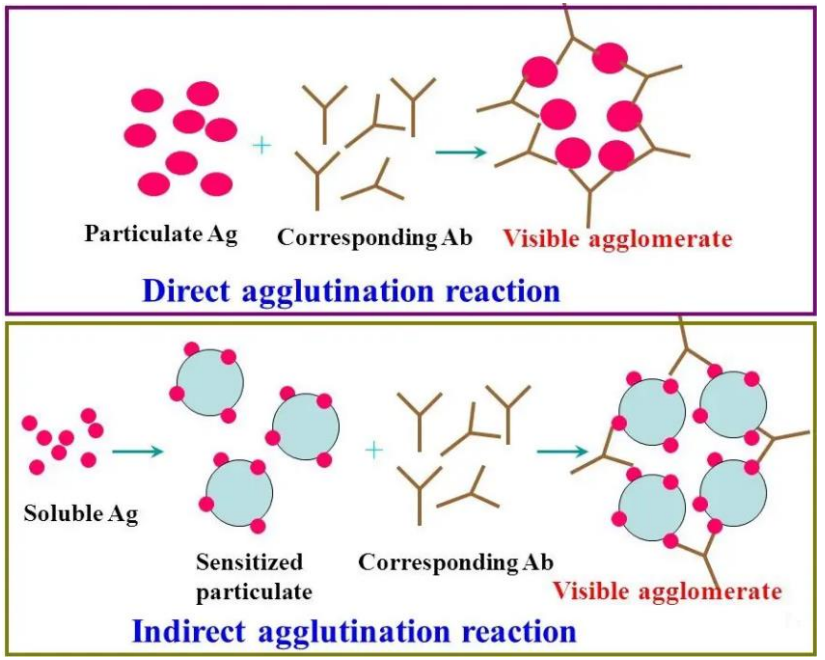
If an Ouchterlony immunodiffusion pattern shows an arc equidistant between antigens A and B, this indicates that the antigens:

a) Are identical
b) Are entirely different
c) Share a common epitope, with A being a more complex antigen
d) Share a common epitope, with B being a more complex antigen
e) Are partially identical

Answer: a) Are identical

B. Agglutination Reactions

- **Types of Agglutination Reactions**:
  - **Direct Agglutination**:
    - Commonly used in various diagnostic and serological assays, such as **blood typing** (hemagglutination), bacterial agglutination tests, and other immunological tests.
    - The primary goal is to detect the presence of specific antibodies in the patient's sample by directly mixing it with the target antigen.
    - In this reaction, antibodies in the serum cause the agglutination of red blood cells that express the corresponding antigen on their surface.
  - **Indirect Agglutination**:
    - In this reaction, red blood cells are coated with **soluble antigens**.
    - Antibodies in the serum cause agglutination when they bind to these antigen-coated cells

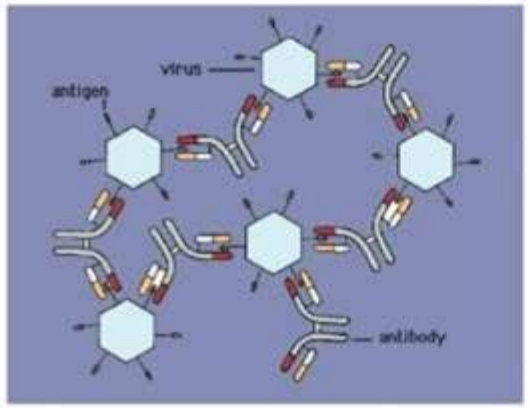


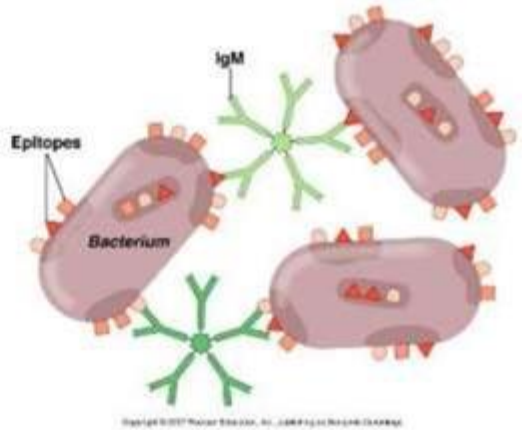
The general term **agglutinin** is used to describe **antibodies** that agglutinate **particulate antigens** (also known as **agglutinogens**).

When the antigen is an **erythrocyte**, the term **hemagglutination** is used

AGGLUTINATION

- Abs can bind and cross-link cells or particles aggregate formation
- Entrap microbial invaders
- IgM & IgA are the most suitable *(IgG in sufficient amounts can agglutinate cells)*





Direct agglutination test is divided into two classes

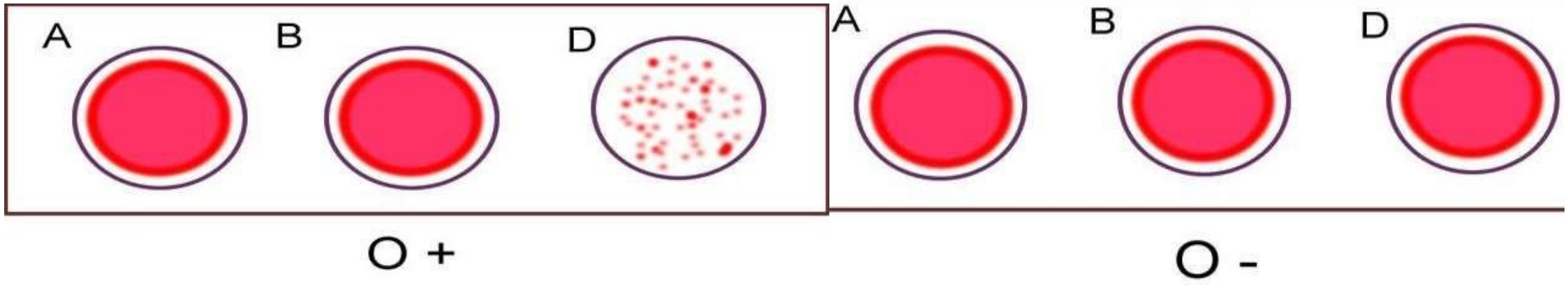
A. Slide Agglutination:
In this method, blood samples are mixed with **Anti-A**, **Anti-B**, and **Anti-D antibodies** on a slide to perform the agglutination.

•**Applications**:

- Used for **blood grouping**.
- Used for the **identification of bacteria** from clinical specimens.

•**Example**:

- Uses of **hemagglutination** include blood grouping and cross-matching. Antisera of the **IgM type** can be used in blood grouping. A smooth suspension of blood is placed on three slides, with a drop of antibody (Anti-A, Anti-B, and Anti-Rh) on each slide. Clumping of blood indicates the presence of the specific antibody antigen.
- It is also used in the identification and typing of microorganisms, such as **pneumococci**.

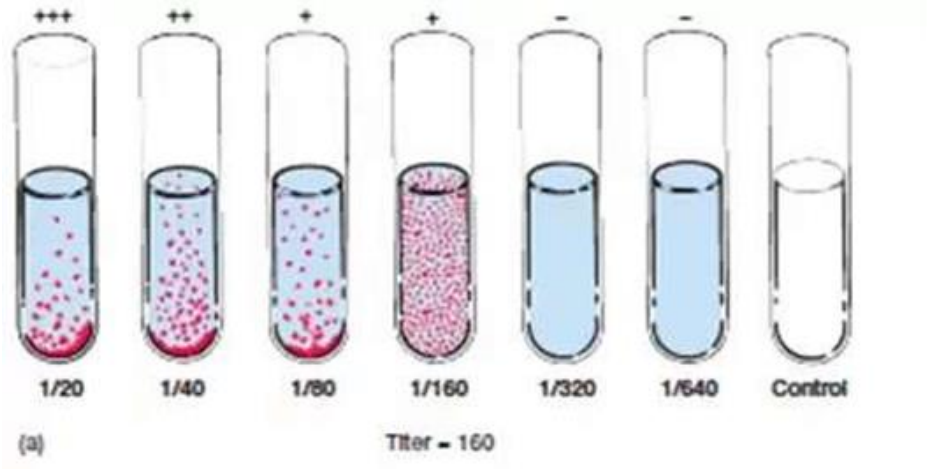


Direct agglutination test is divided into two classes

B. Tube Agglutination:
In this test, serial dilutions are made of an antibody sample (patient serum), and a constant amount of antigen is added. The last dilution that results in agglutination is determined and is called the **titer**. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination.

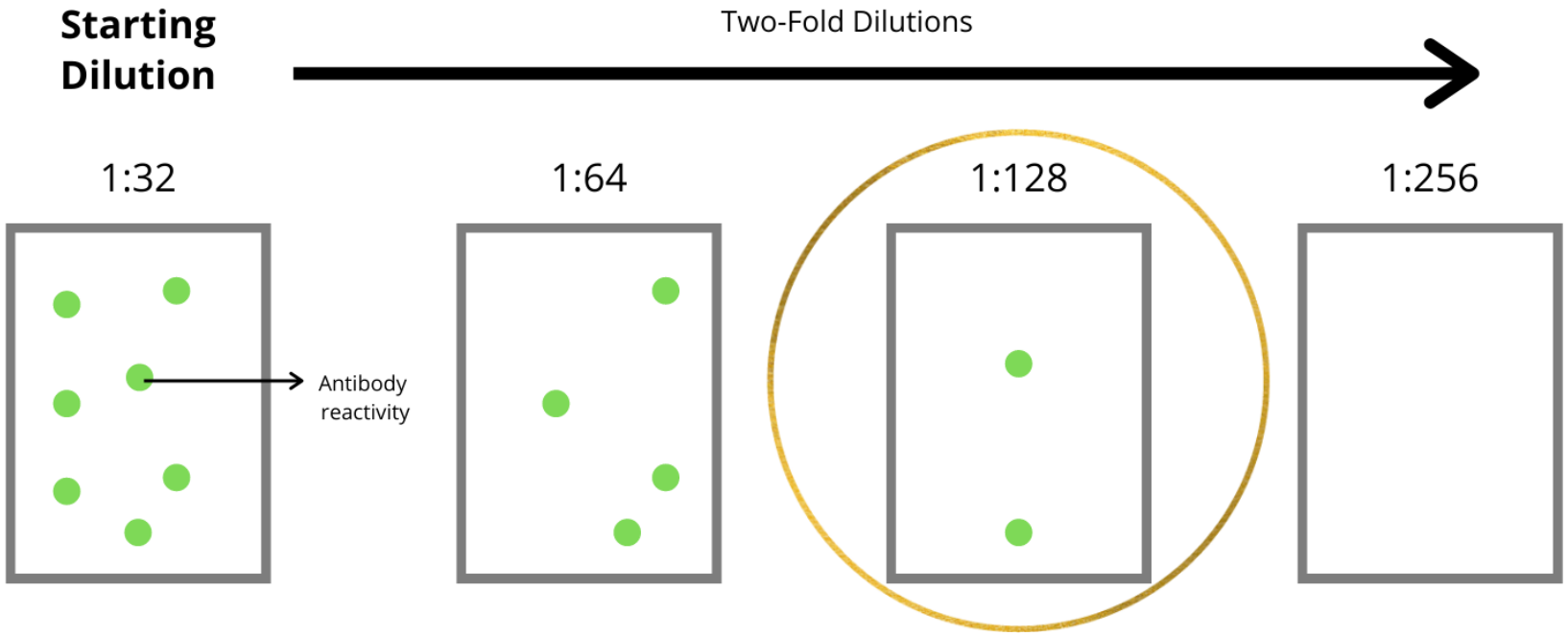
•**Applications**:

- Used for the **brucellosis test**.
- Used in the **Widal test** for the diagnosis of **typhoid fever** to detect specific antibodies. The antigens used in this procedure include **Salmonella O** (somatic) and **H** (flagellar) antigens



Titer

- **Titer**; The level of antibody in serum is expressed as the highest dilution of antibodies that gives a positive reaction with antigen. It can be diagnostic or prognostic.
- A high titer (highest dilution) suggests a strong immune response, while a low titer may indicate a weaker response.



MCQ

Antibody titer refers to the:

1.Absolute amount of specific antibody

2.Affinity of specific antibody

3.Avidity of specific antibody

4.Concentration of specific antigen

5.Highest dilution of antibody still able to give a positive result in a test system

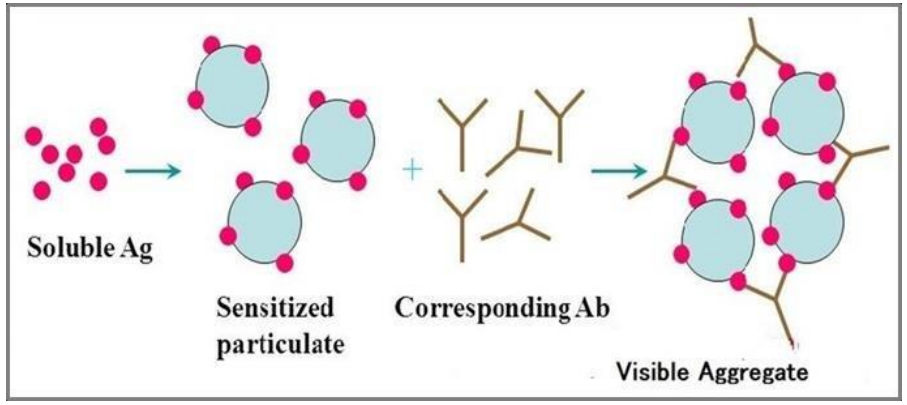
Answer:
5) Highest dilution of antibody still able to give a positive result in a test system

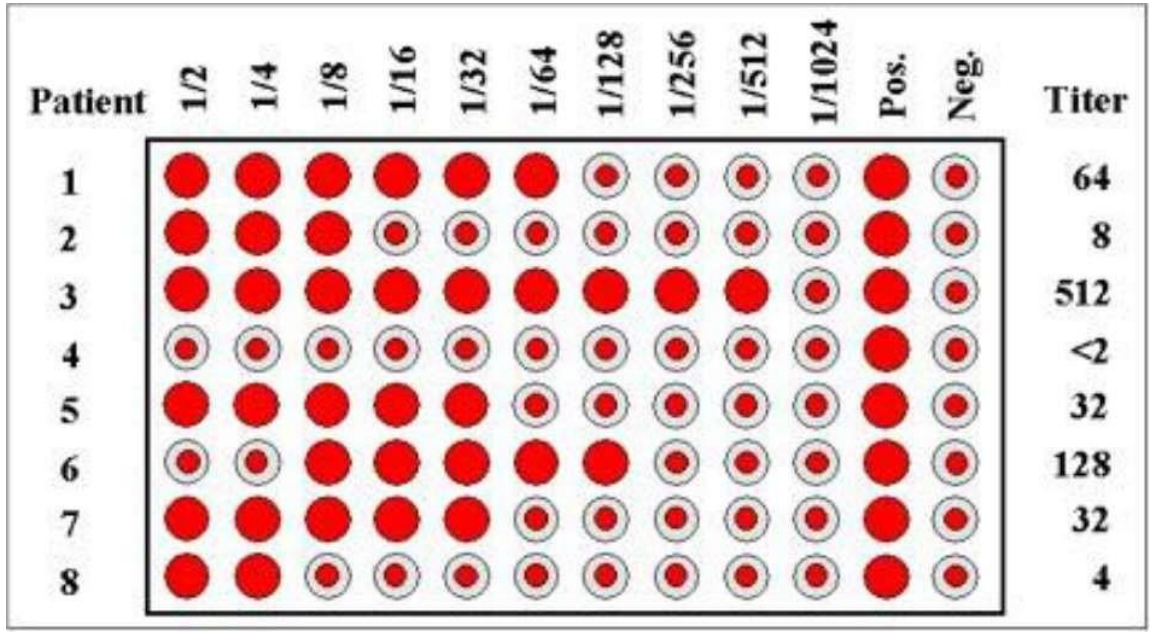
2. Indirect Agglutination (Passive Agglutination)

When a **soluble antigen** is used in an agglutination reaction, it is often coated on a **carrier particle**, and agglutination takes place on the surface of the carrier molecule. In the indirect agglutination test, **RBCs**, **latex**, or **bentonite**, etc., are used as carrier molecules.

•**Applications**:

- Used in the **Treponema pallidum hemagglutination assay (TPHA) for diagnosing syphilis**



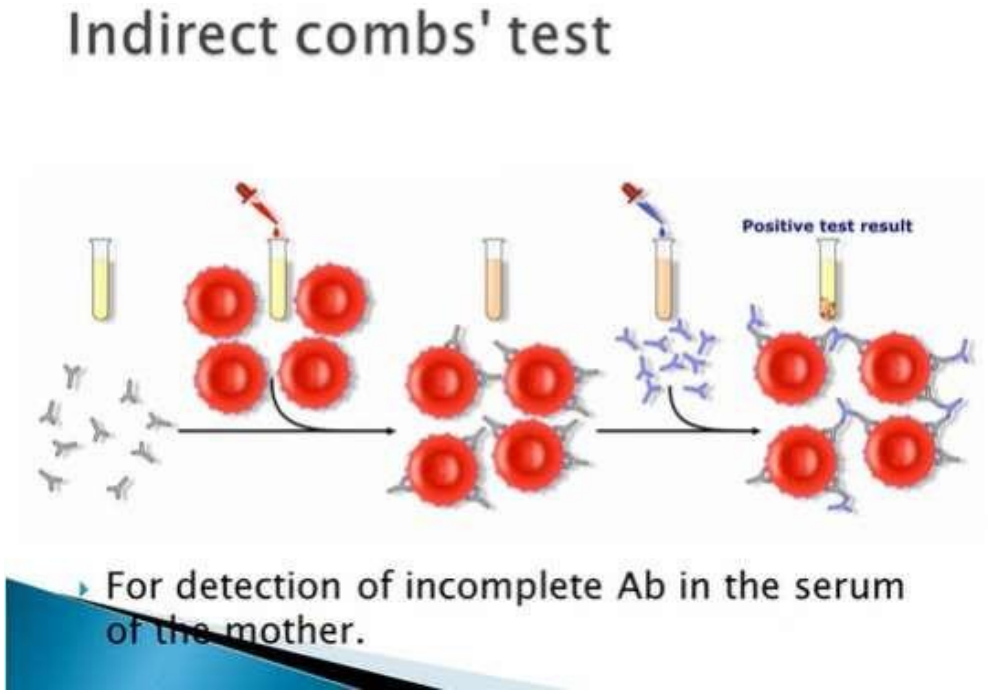


Indirect Coombs Test

Anti-Human Globulin (AHG): Also known as **Coombs reagent**, AHG is a type of antibody used in **immunohematology** and **blood bank testing**.

•**Indirect Coombs Test**: This test is utilized in **pre-transfusion testing** to determine if the patient's serum contains any antibodies that could react with the donor's red blood cells.

•**Importance**: This testing is essential to prevent **transfusion reactions** in patients. It is often relevant in conditions such as **autoimmune hemolytic anemia** or **hemolytic disease of the newborn**



MCQ

A serological test that uses red blood cells coated with exogenous antigens to detect patient antibodies against those exogenous antigens is called:

a) Latex agglutination
b) Hemagglutination
c) Neutralization
d) Complement fixation
e) Direct agglutination

Answer: b) Hemagglutination

When carrier particles are coated with an antigen that is not normally found on them, this is known as:

a) Direct agglutination
b) Passive agglutination
c) Reverse passive agglutination
d) Agglutination inhibition reaction
e) Complement fixation

Answer: b) Passive agglutination

MCQ

- The direct Coombs' test is designed to detect when people have a disease that causes them to:

a) Have an excessively high fever
b) Quit making antibodies
c) Make too many red blood cells
d) Produce antibodies that bind to their own red blood cells
e) Have tumor markers

Answer:
d) Produce antibodies that bind to their own red blood cells

- Which of the following is used in typing of microorganisms such as pneumococci?

a) Hemagglutination
b) Passive agglutination
c) Reverse passive agglutination
d) Direct agglutination
e) Latex agglutination

Answer:
d) Direct agglutination

MCQ

The immune technique used in the TAPH test for syphilis is:

a) Ouchterlony double diffusion
b) Radial immunodiffusion
c) Passive hemagglutination
d) Passive coaggutination
e) Direct Coombs' test

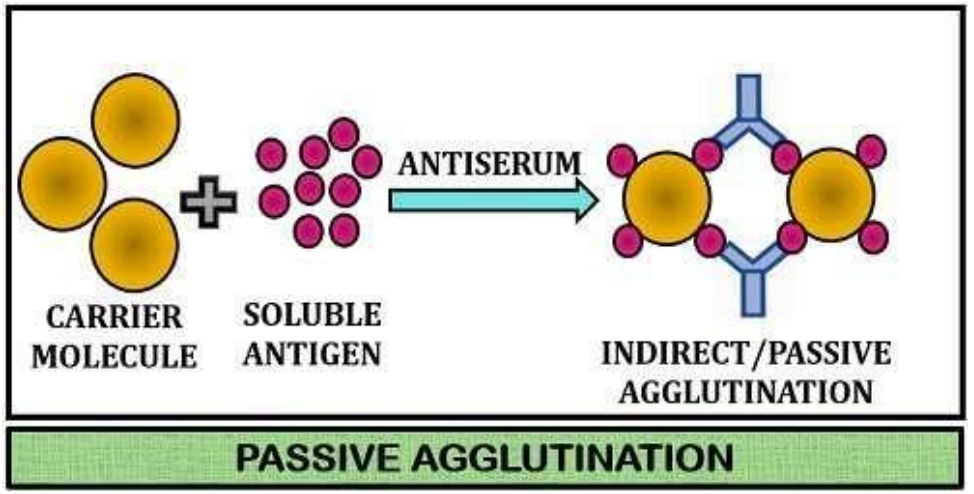
Answer:
c) Passive hemagglutination

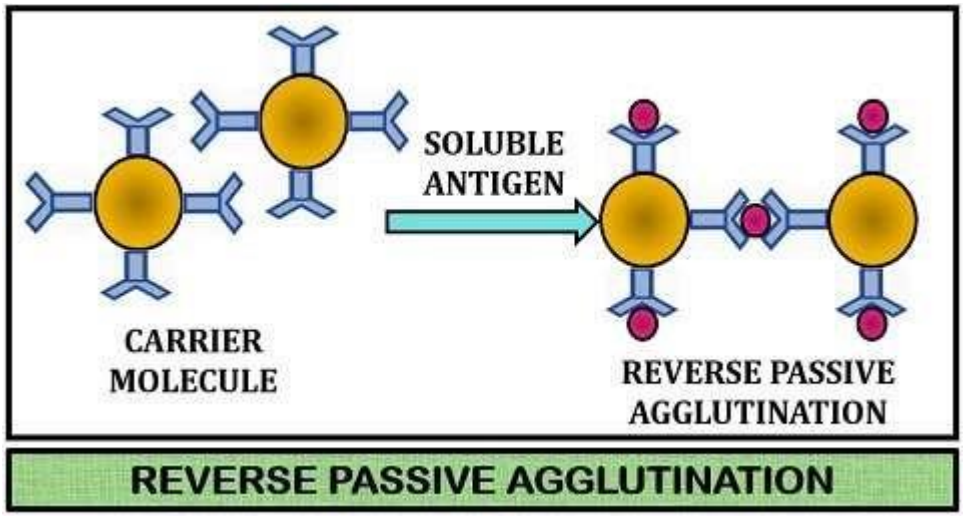
3. Reverse Passive Agglutination

•**Principle**: In this method, a **soluble antigen** binds to **antibodies** that are coated on **carrier particles**, resulting in agglutination.

•**Purpose**: This technique is used to detect **antigens**.

•**Example**: One application is in detecting **cholera toxin**



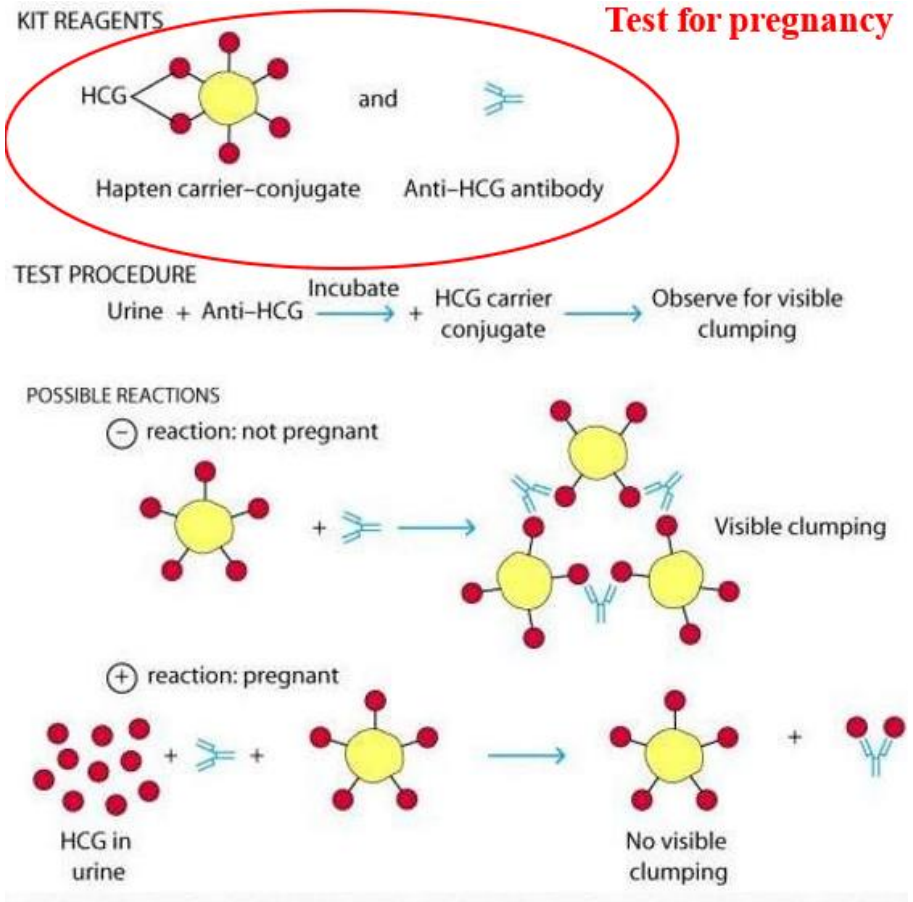


4. Agglutination Inhibition

•**Principle**: Agglutination inhibition reactions are based on competition between **particulate** and **soluble antigens** for limited antibody-combining sites. The **lack of agglutination** indicates a positive reaction.

•**Examples**:

- **RF**: Rheumatoid factor.
- **CRP**: C-reactive protein in inflammation tests



MCQ

In which of the following tests does the absence of agglutination indicate a positive result?

a) Hemagglutination
b) Passive agglutination
c) Reverse passive agglutination
d) Agglutination inhibition
e) Latex agglutination

Answer: d) Agglutination inhibition

- In the HCG latex agglutination test,

a) The antigen (HCG) is a natural particle
b) Antigen (HCG) molecules are artificially bound to particles
c) Antibody (anti-HCG) is attached to particles
d) The antigen-antibody reaction is competitive (no agglutination indicates a positive result)
e) Direct agglutination

Answer: d) The antigen-antibody reaction is competitive (no agglutination indicates a positive result)

MCQ

In the CRP latex agglutination test:

a) We test the presence of CRP in the patient
b) We test the presence of anti-CRP in the patient
c) It is direct agglutination
d) The antigen (CRP) is fixed on latex
e) The antigen (CRP) is a natural particle

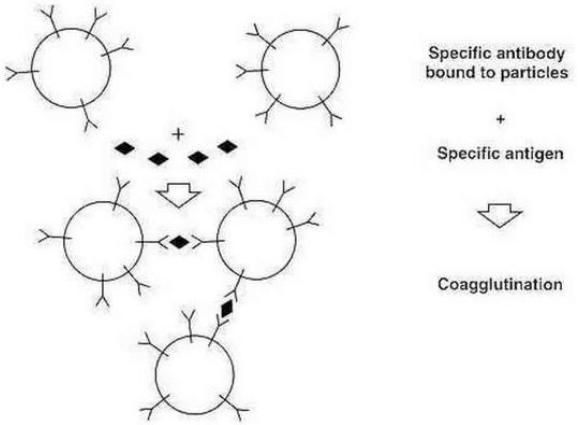
Answer:
A and D and E all are true???

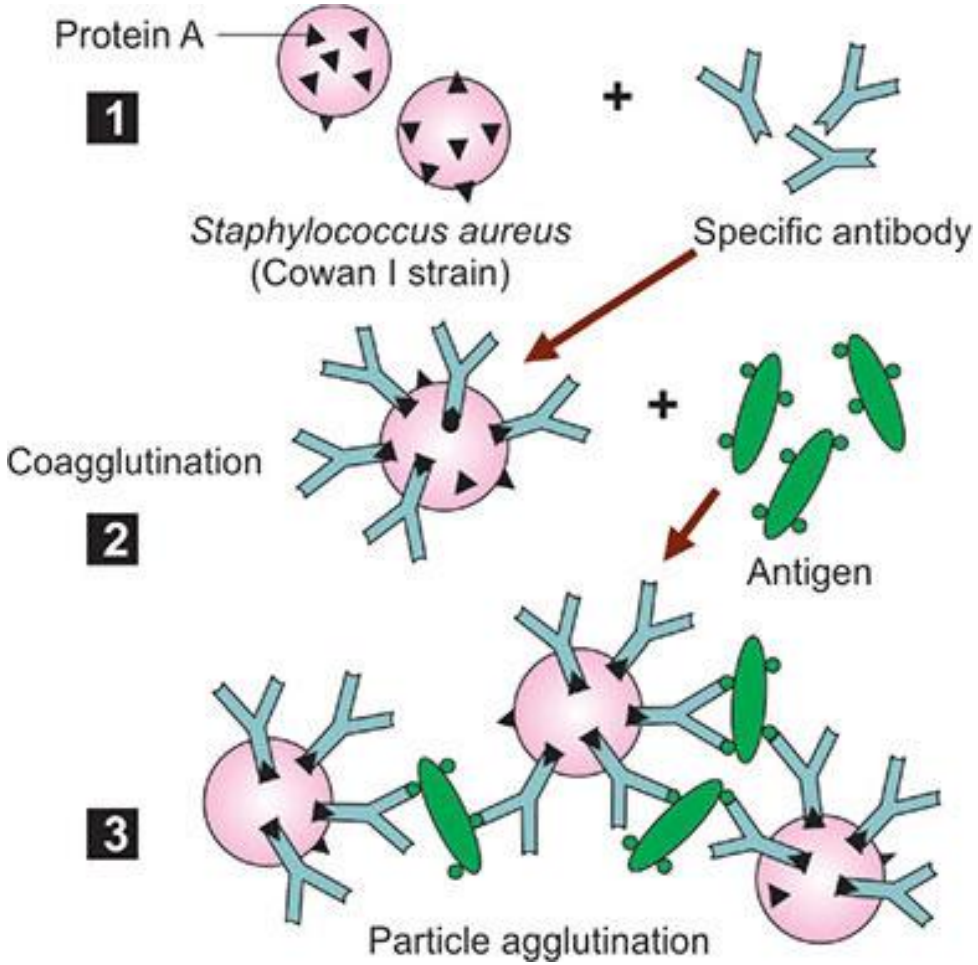
5. Coagglutination

Coagglutination (CoA) is similar to the **latex agglutination** technique for detecting antigens.

•**Mechanism**: **Protein A**, a uniformly distributed cell wall component of **Staphylococcus aureus**, binds to the **Fc region** of most **IgG isotype antibodies**, leaving the **Fab region** free to interact with antigens present in the applied specimens.

•**Indicator**: The visible agglutination of the **S. aureus** particles indicates the **antigen-antibody reaction**.





C. Complement Fixation Test

The complement fixation test is an immunological medical test used to look for evidence of infection. It tests for the presence of either specific antibodies or specific antigens in a patient's serum.

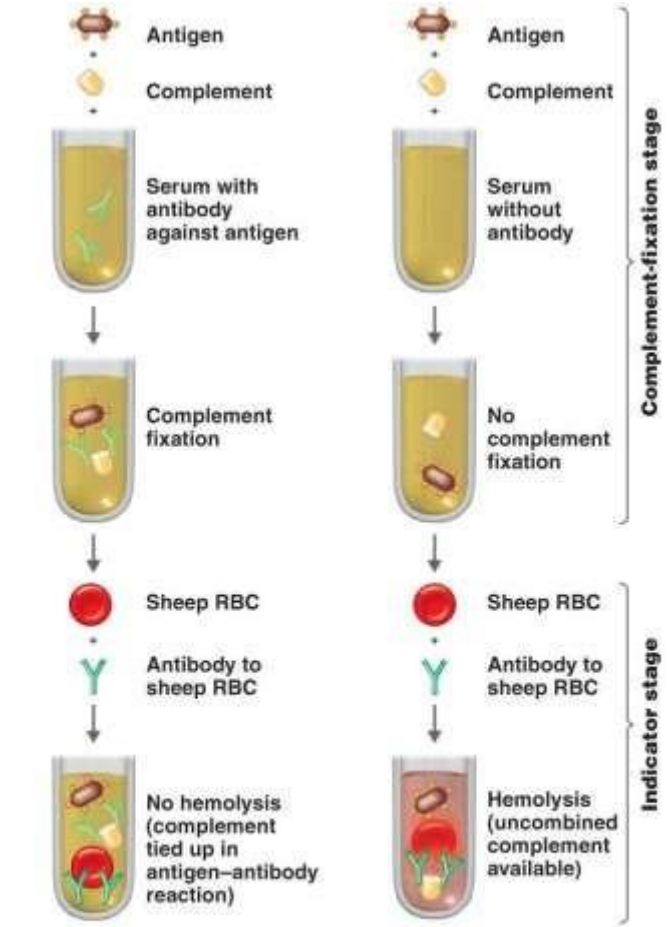
•**Complement**: This is a group of proteins that are normally present in blood serum and play a role in **immune defense**.

•**Procedure**:

- An **indicator system** is employed, which involves **sheep red blood cells (sRBC)** coated with antibodies that specifically bind to complement proteins (hemolysin), along with **anti-sRBC antibody** and complement. The specific antigen is added if looking for antibodies in serum, or specific antibodies are added if looking for antigens in serum.

•**Interpretation**:

- If either the antibody or antigen is present in the patient's serum, then the complement is **completely utilized**, and the **sRBCs are not lysed**.
- Conversely, if the antibody (or antigen) is absent, the complement is not used up, allowing it to bind to the anti-sRBC antibody, resulting in the **lysis of sRBCs**



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

Uses of Complement Fixation Test (CFT)

The complement fixation test is used to diagnose various infections, including:

- **Syphilis** (Wassermann reaction)
- **Gonorrhea**
- **Rickettsial infections**
- Screening for antibodies against a variety of possible pathogenic microbes, especially **viruses**

Complement fixation test(CFT)

Serum(? Abs)+ known Ag+C →Incubate for 1 hour, then add indicator system(Sheep RBCs+Ani-sheep RBCs).→Incubate for 1 hour

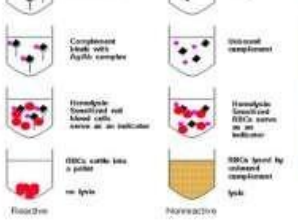
+ve = No lysis

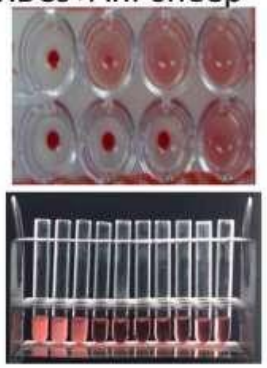
-ve = lysis

Uses:

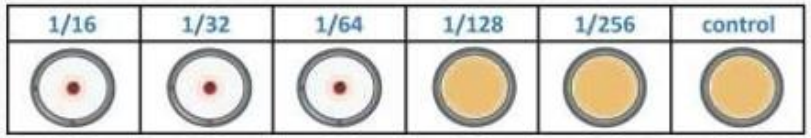
Diagnosis of:

Syphilis, Viral Infections, Gonorrhoea,....

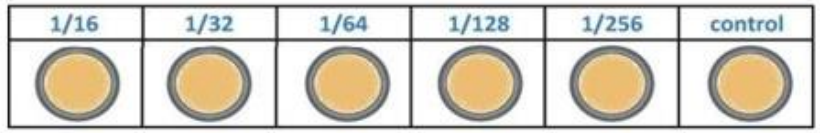




Positive case with titer 64



Negative case



MCQ

The Wasserman reaction is:

a) Tube flocculation test
b) Complement fixation
c) Slide agglutination test
d) Immunoassay
e) Precipitation reaction

Answer:
b) Complement fixation

All the following are agglutination reactions except:

a) Widal test
b) Brucella test
c) Wasserman reaction
d) Indirect Coombs'
e) Direct Coombs'

Answer:
c) Wasserman reaction

MCQ

Lysis of sheep red blood cells indicates:

a) The patient has the antibody being tested for
b) Presence of antigen in the patient
c) Presence of clotting factors in the patient
d) Presence of complement proteins in the patient
e) The patient does not have the antibody (negative)

Answer:
e) The patient does not have the antibody (negative)

Immunoelectrophoresis

1.When an **electric current** is applied to a slide layered with **gel**, the **antigen mixture** placed in **wells** is separated into individual **antigen components** according to their **charge** and **size**.

2.Following **electrophoresis**:

- The separated **antigens** are reacted with specific **antisera**, which are placed in **troughs** parallel to the **electrophoretic migration**.
- **Diffusion** is allowed to occur.

3.The **antiserum** present in the **trough** moves toward the **antigen components**, resulting in:

- The formation of separate **precipitin lines** within **18-24 hours**.
- Each **precipitin line** indicates a reaction between individual **proteins** and their corresponding **antibody**

Immunoelectrophoresis

Countercurrent Immunoelectrophoresis (CIE);
Procedure:

1.Wells are made in an agar gel and filled with either **antigen** or **antibody**.

2.Electric current is applied, causing the antigen and antibody to move toward each other.

3.A **precipitin line** (visible band) forms when the antigen and antibody meet and bind, indicating a positive reaction.

Qualitative

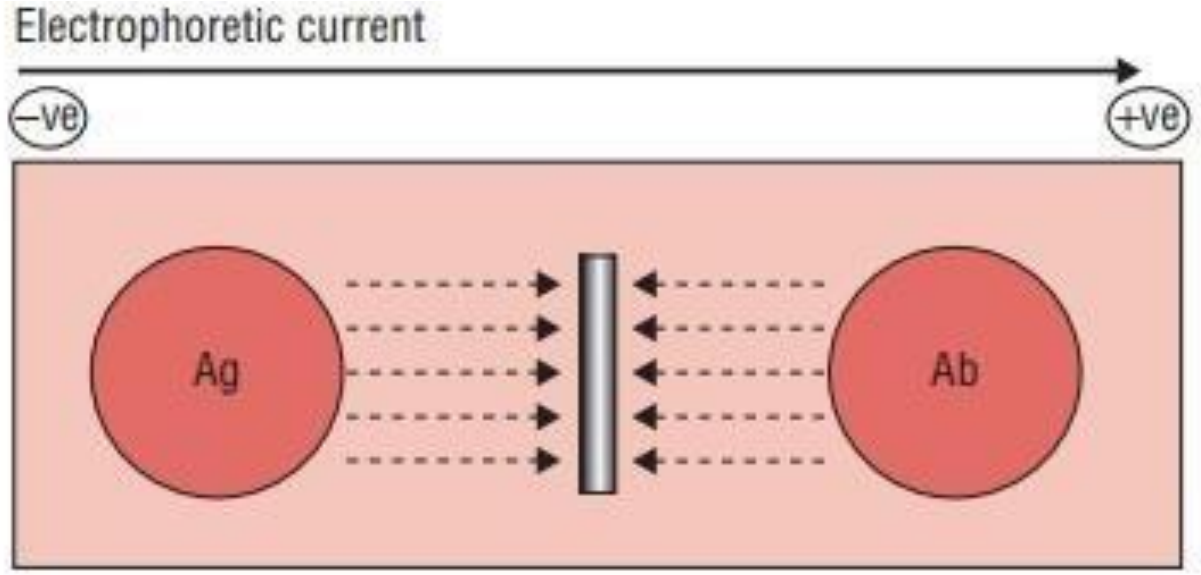


FIG. 14-6. Counter-current immunoelectrophoresis.

Immunoelectrophoresis

Procedure:

1.The agar gel is prepared with **antibodies** uniformly distributed throughout.

2.A sample containing an **antigen** is placed in a well, and an electric current is applied.

3.The antigen moves towards the positive electrode, and a **precipitin curve (rocket shape)** forms.

4.The **height** or **length** of the precipitin "rocket" is directly proportional to the **concentration** of the antigen in the sample.

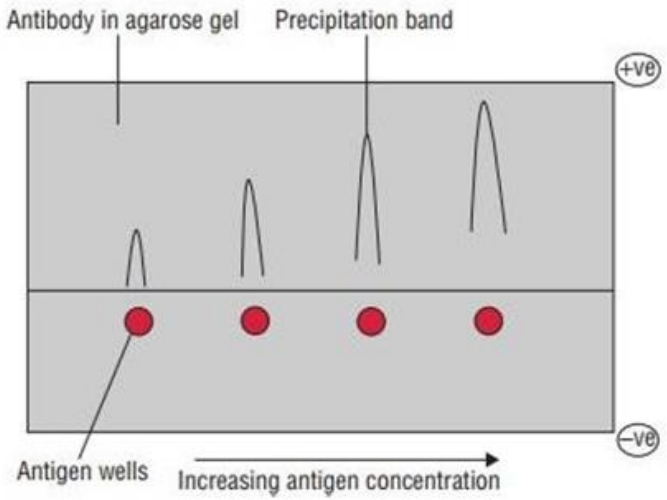
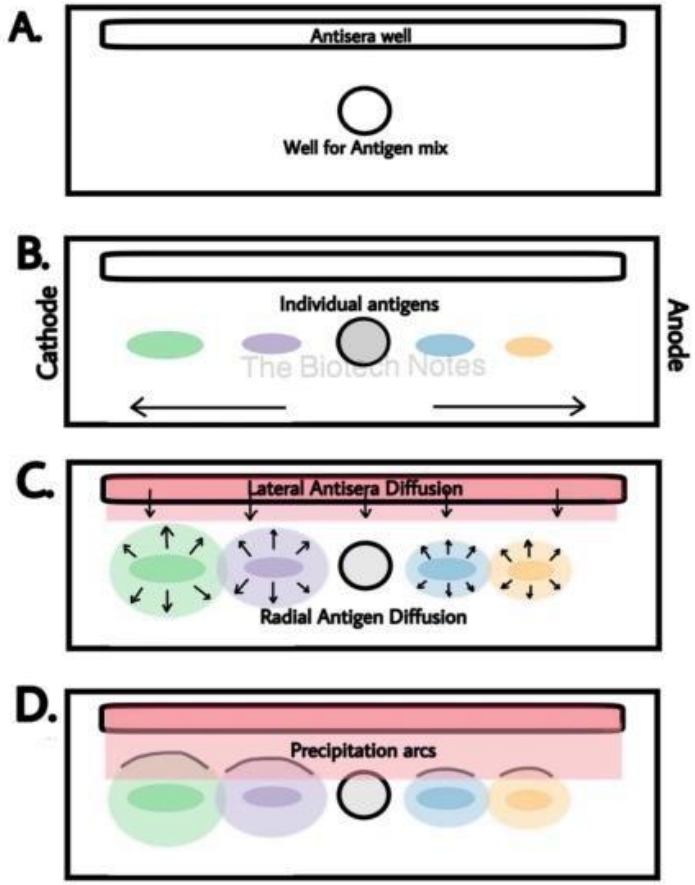


FIG. 14-7. Rocket electrophoresis.



MCQ

Which of the following is used to separate proteins in a patient's blood?

a) Enzyme-linked immunosorbent assay
b) Fluorescent antibody (fluorochromes)
c) FACS (fluorescence-activated cell sorting)
d) Flow cytometer
e) Electrophoresis

Answer:
e) Electrophoresis

Neutralization Tests

Neutralization Tests

1.Purpose:

1. Used to assess the effectiveness of **neutralizing antibodies** against specific **pathogens** or **toxins**.
2. These tests have applications in **virology**, **immunology**, and **vaccine development**.
3. The primary purpose is to determine if a given **antibody** or **serum** can neutralize the **infectivity** or **toxicity** of a particular **pathogen** or **toxin**.

2.Types of Neutralization Tests:

1. **Virus Neutralization Tests**
2. **Toxin Neutralization Tests**

Virus Neutralization Tests

1.Definition:

- The neutralization of **viruses** by their specific **antibodies** is referred to as **virus neutralization tests**.

2.Mechanism:

- Inoculation of **viruses** in **cell cultures**, **eggs**, or **animals** results in their replication and growth.
- When **virus-specific neutralizing antibodies** are injected into these systems, the **replication** and **growth** of viruses are inhibited.

3.Example:

- The **Viral Hemagglutination Inhibition Test** is a frequently used **virus neutralization test** in the diagnosis of viral infections like **influenza**, **mumps**, and **measles**.

1. The test involves:
    1. Mixing the **virus** with **serum** or **antibodies**.
    2. Observing whether the **virus** can infect **host cells**.
    3. If the **antibodies** neutralize the virus, there will be no **infection**, and the test result is **positive for neutralization**

Toxin Neutralization Tests

1.Definition:

- **Toxin neutralization tests** are laboratory assays used to assess the ability of **antibodies** or other agents to neutralize the **toxic effects** of specific toxins, often produced by **bacteria** or other microorganisms.

2.Importance:

- These tests are critical for diagnosing and treating **diseases** caused by toxins.

- **Examples of Neutralization Tests**:
- **In Vivo Tests**:
  1. **Schick Test**: Used to demonstrate **immunity** against **diphtheria**.
  2. **Clostridium perfringens (formerly Clostridium welchii) Toxin Neutralization Test**: Conducted in **guinea pigs** or **mice**.
  3. **Clostridium botulinum Toxin**: Associated with **botulism**.
- **In Vitro Tests**:
  1. (a) **Anti-Streptolysin O (ASO) Test**: A blood test used to measure the level of **anti-streptolysin O antibodies** in the bloodstream. This toxin is produced by **Streptococcus pyogenes**.
  2. (b) **Nagler Reaction**: Used for the rapid detection of **Clostridium perfringens**. This test identifies the presence of **lecithinase**, which hydrolyzes **lecithin**, a component of cell membranes.

Immunofluorescence Tests

1.Definition of Fluorescence:

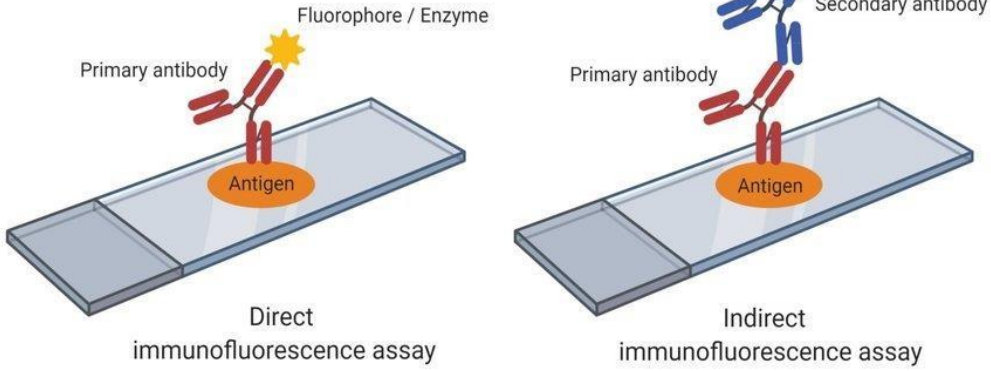
- The property of certain **dyes** absorbing **light rays** at one particular wavelength (**ultraviolet light**) and emitting them at a different wavelength (**visible light**) is known as **fluorescence**.

2.Fluorescent Dyes:

- **Fluorescein isothiocyanate**: Emits **blue-green fluorescence** under **ultraviolet (UV)** rays.
- **Lissamine rhodamine**: Emits **orange-red fluorescence** under **UV rays**.
- These dyes can be **tagged** with **antibody molecules**.

3.Immunofluorescence Tests:

- This forms the basis of an **immunological test**.
- These tests have **wide applications** in **research** and **diagnostics**.
- They are broadly classified into two types:
  1. **Direct Immunofluorescence Test**
  2. **Indirect Immunofluorescence Test**



Direct Immunofluorescence Test

1.Applications:

1. Widely used for the detection of **bacteria**, **parasites**, **viruses**, **fungi**, or other **antigens** in various specimens, including:
    1. **Cerebrospinal Fluid (CSF)**
    2. **Blood**
    3. **Stool**
    4. **Urine**
    5. **Tissues**
    6. Other **clinical specimens**

2.Diagnostic Use:

1. Useful in the diagnosis of:
    1. Suspected **autoimmune diseases**
    2. **Connective tissue diseases**
    3. **Vasculitis**

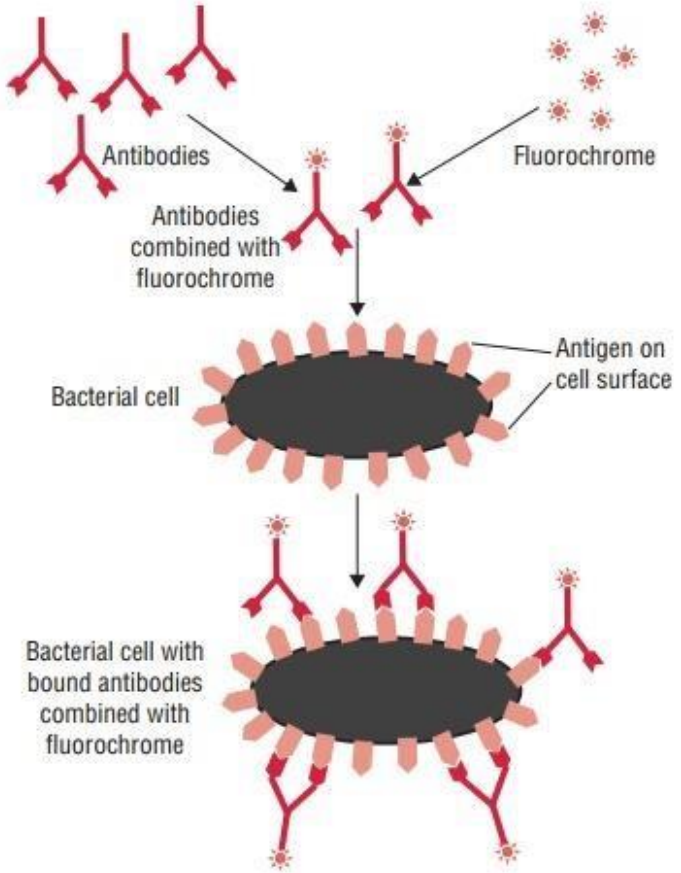
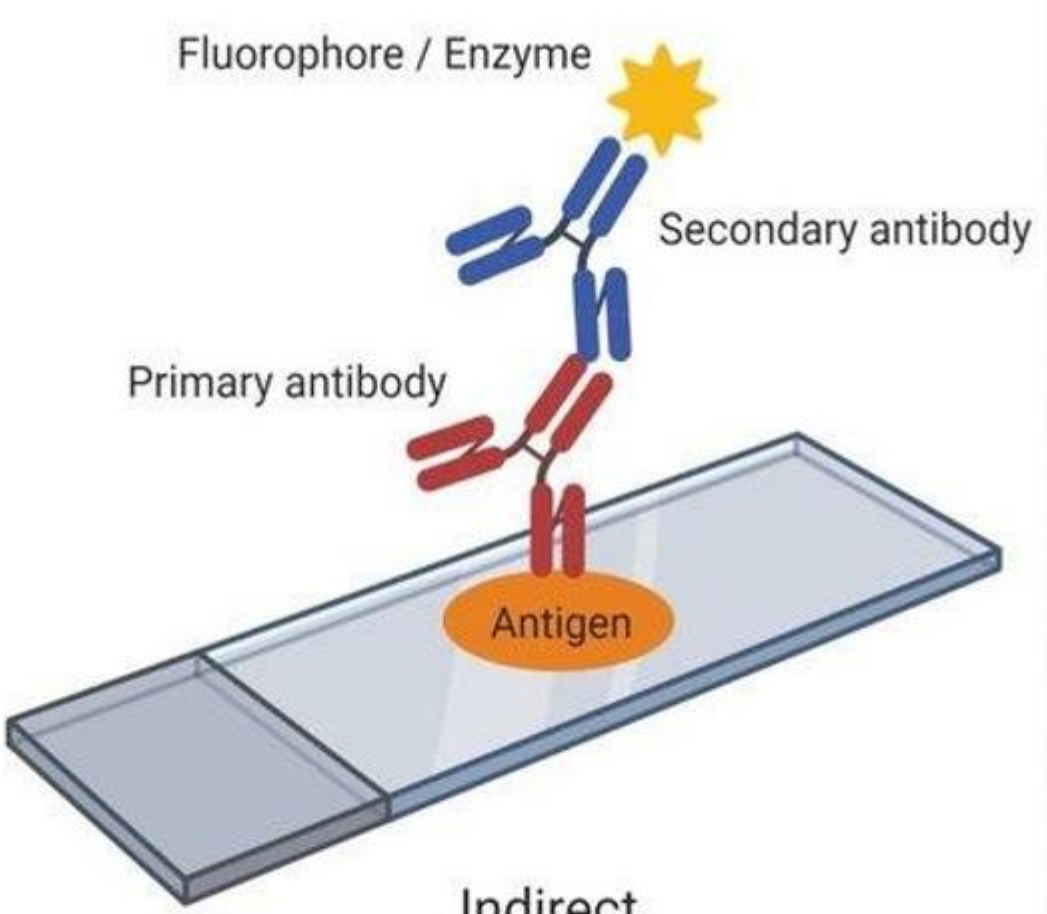


FIG. 14-13. Direct fluorescent antibody test.

Indirect Immunofluorescence

- **Process**:
  - It is a **two-stage process**:
  - **First Stage**:
    1. A known **antigen** is fixed on a **slide**.
    2. The **patient's serum** to be tested is applied to the slide, followed by careful **washing**.
    3. If the patient's serum contains **antibody** against the antigen, it will **combine** with the antigen on the slide.
  - **Second Stage**:
    1. The combination of **antibody** with **antigen** is detected by adding a **fluorescent dye-labeled antibody** (**Secondary Ab**) to human **IgG**.
    2. This is examined using a **fluorescence microscope**



Indirect immunofluorescence assay

Radioimmunoassay (RIA) and Enzyme-Linked Immunosorbent Assay (ELISA)

When an antigen or antibody is labeled with a radioisotope, it can be quantified using instruments that detect radioactive decay events. This method is referred to as a **radioimmunoassay (RIA)**.

On the other hand, when an antigen or antibody is covalently coupled to an enzyme, the rate at which the enzyme converts a clear substrate into a colored product can be quantified using a spectrophotometer. The absorbance at specific wavelengths is then plotted against a corresponding concentration of the target antigen on a slope (with a distinct pattern for each antigen). This method is known as an **enzyme-linked immunosorbent assay (ELISA)**

These techniques are used to measure the quantities of antigens or antibodies (proteins), including (applications):

- Hormones
- Drugs
- Tumor markers
- Antibodies (Abs)
- Viral and bacterial antigens

Enzyme immunoassays (EIAs)

•**Enzyme immunoassays (EIAs)** can be used for detection of either **antigens** or **antibodies** in **serum** and other **body fluids** of the patient.

•In **EIA techniques**, **antigen** or **antibody** labeled with **enzymes** are used:

- **Alkaline phosphatase**
- **Horseradish peroxidase**
- **Galactosidase**

•Following the **antigen–antibody reaction**, a **chromogenic substrate** specific to the enzyme (**peroxidase**, **alkaline phosphatase**, etc.) is added.

•The reaction is detected by reading the **optical density**.

•Usually, a **standard curve** based on known concentrations of **antigen** or **antibody** is prepared, from which the **unknown quantities** are calculated

There are four main general steps to completing an **ELISA immunoassay**:

1.Coating: With either **antigen** or **antibody**.

2.Blocking: Typically with the addition of **bovine serum albumin (BSA)**.

3.Detection.

4.Final read

Types of ELISA

There are four major types of **ELISA**:

1.Direct ELISA:

- Antigen-coated plate.
- Used for screening **antibody**.

2.Indirect ELISA:

- Antigen-coated plate.
- Used for screening **antigen/antibody**.

3.Sandwich ELISA:

- Antibody-coated plate.
- Used for screening **antigen**.

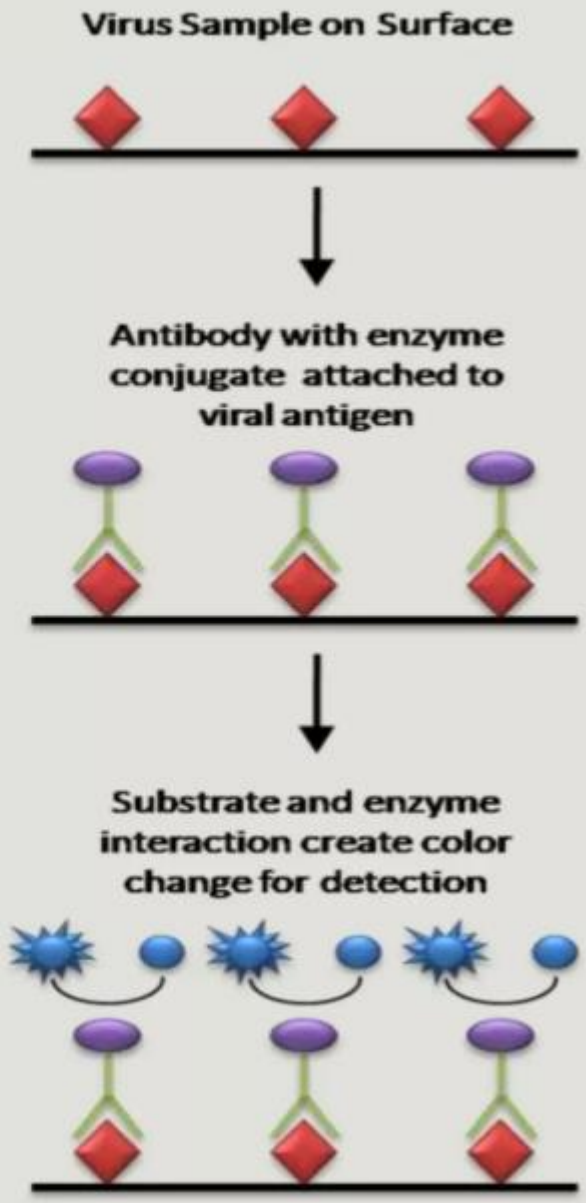
4.Competitive ELISA:

- Used for screening **antibody**

Direct ELISA

DIRECT ELISA

- Apply a sample of known antigen to a surface.
- Enzyme linked primary antibody is applied to the plate.
- Washed, After this wash, only the antibody-antigen complexes remain attached.
- Apply a substrate which is converted by the enzyme to elicit a chromogenic signal.



Indirect ELISA

Antibody detection

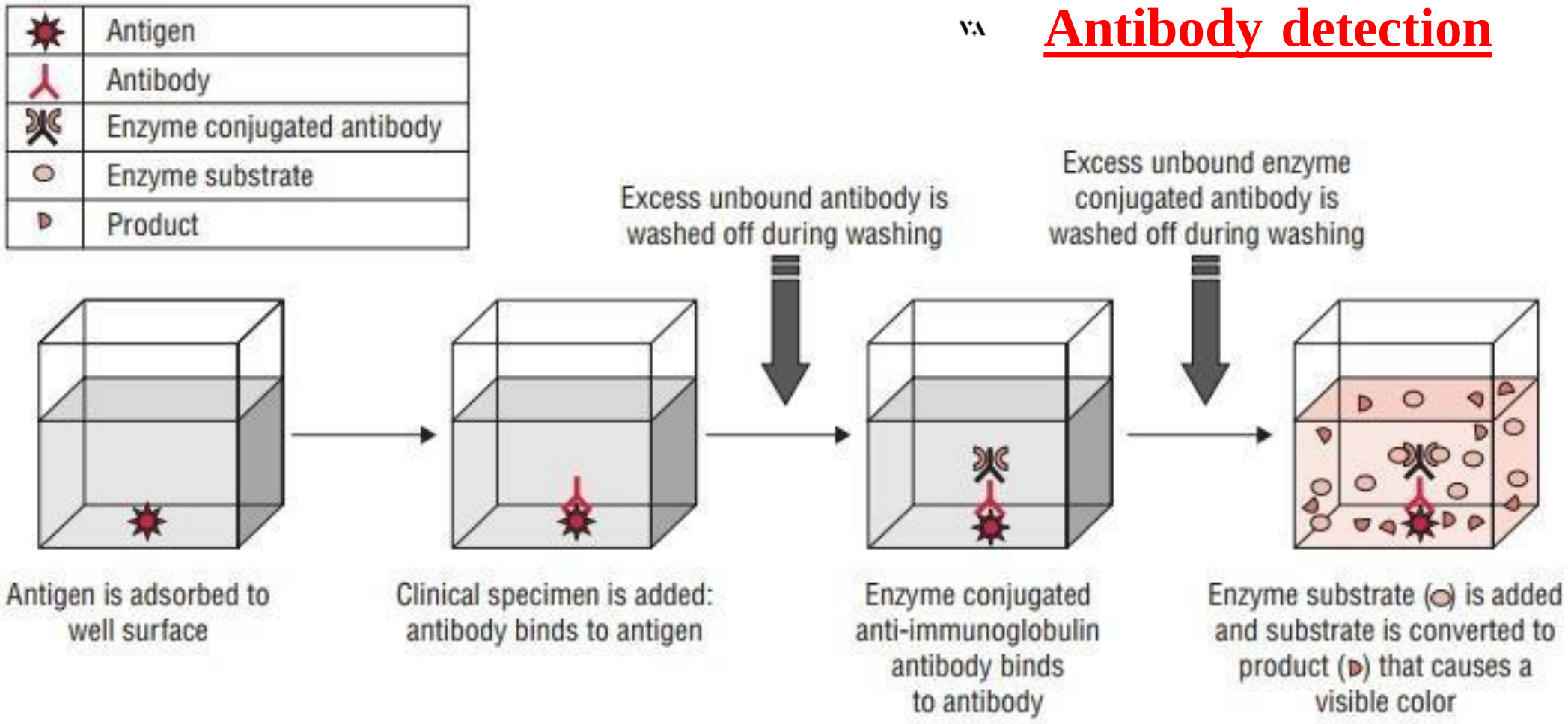


FIG. 14-14. Indirect ELISA test.

Sandwich ELISA

Antigen Detection

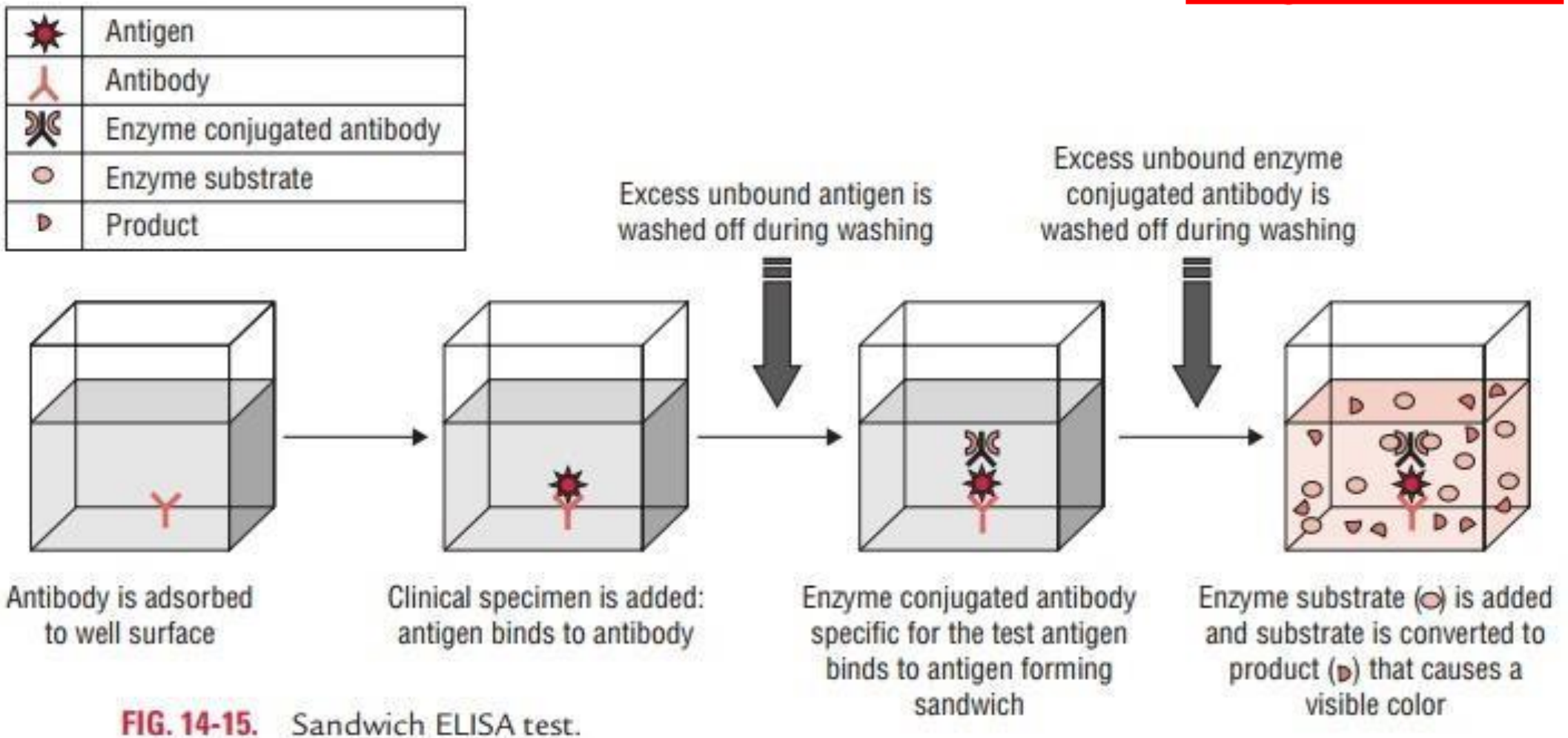
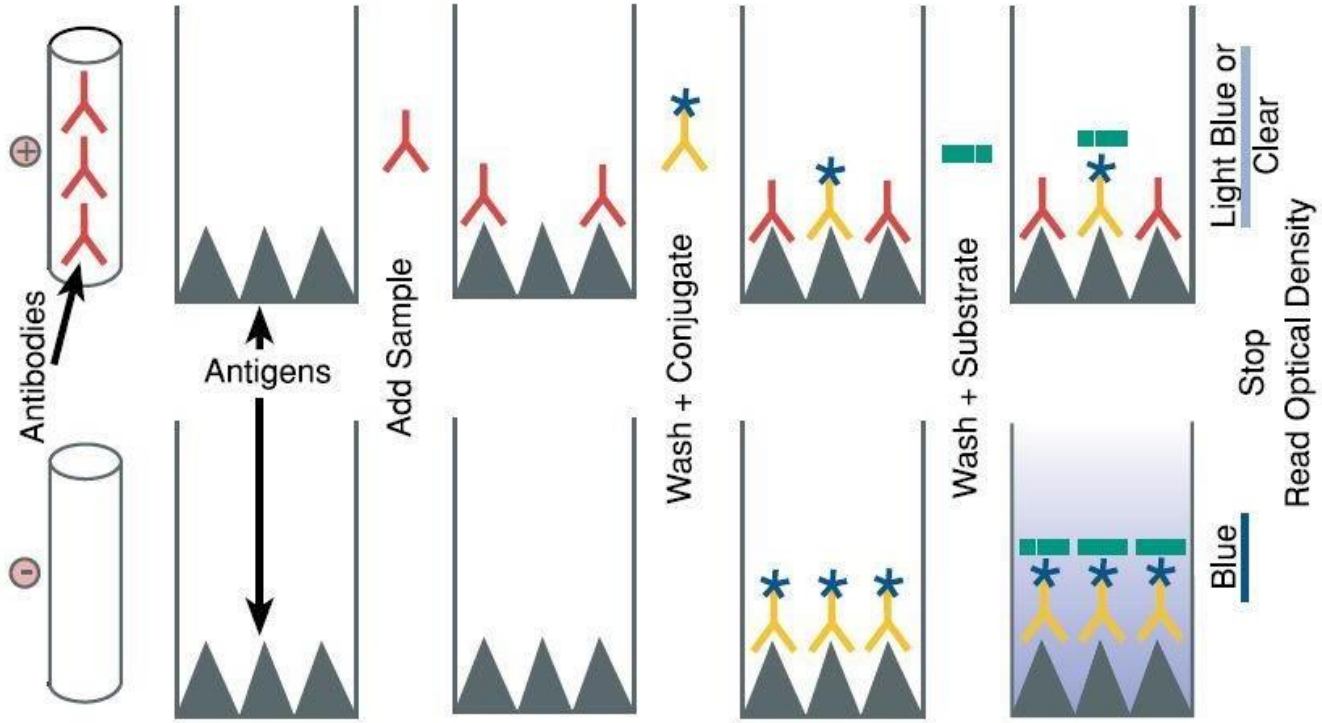


FIG. 14-15. Sandwich ELISA test.

Competitive ELISA

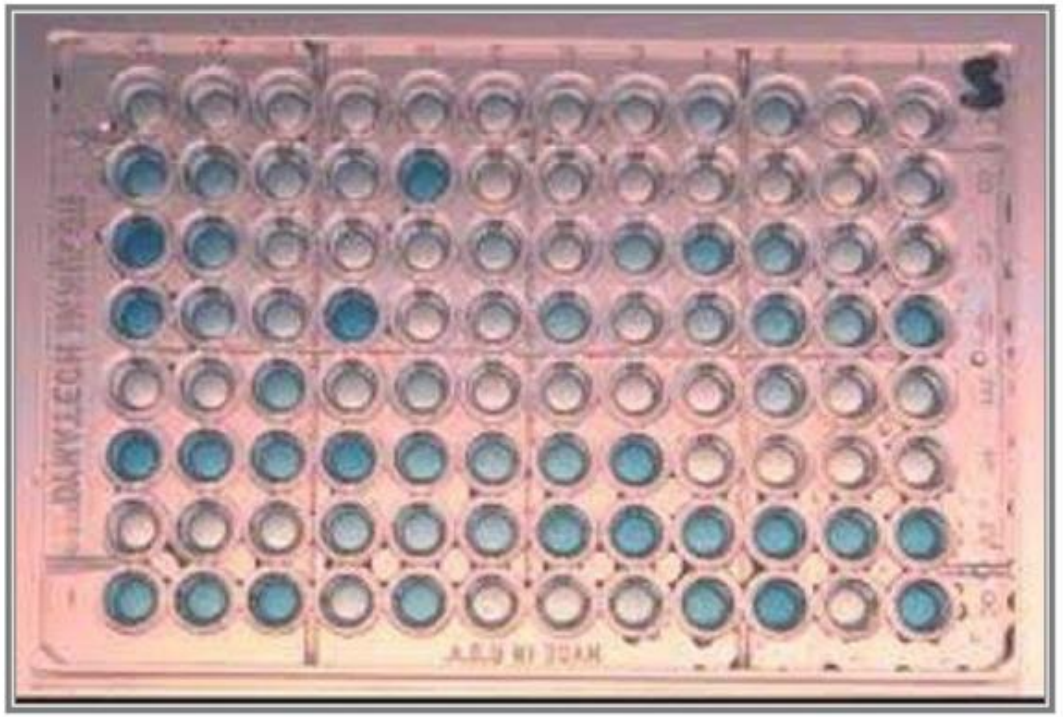
- **Purpose:**
  - Used for the **estimation of antibodies** present in a specimen, such as **serum**.
- **Principle:**
  - Two specific **antibodies** are involved:
    - One **conjugated with an enzyme**.
    - The other **present in the test serum** (if the serum is positive for antibodies).
  - **Competition** occurs between the two antibodies for the **same antigen**.
- **Interpretation of Results:**
  - **Appearance of color**: Indicates a **negative test** (absence of antibodies).
  - **Absence of color**: Indicates a **positive test** (presence of antibodies)



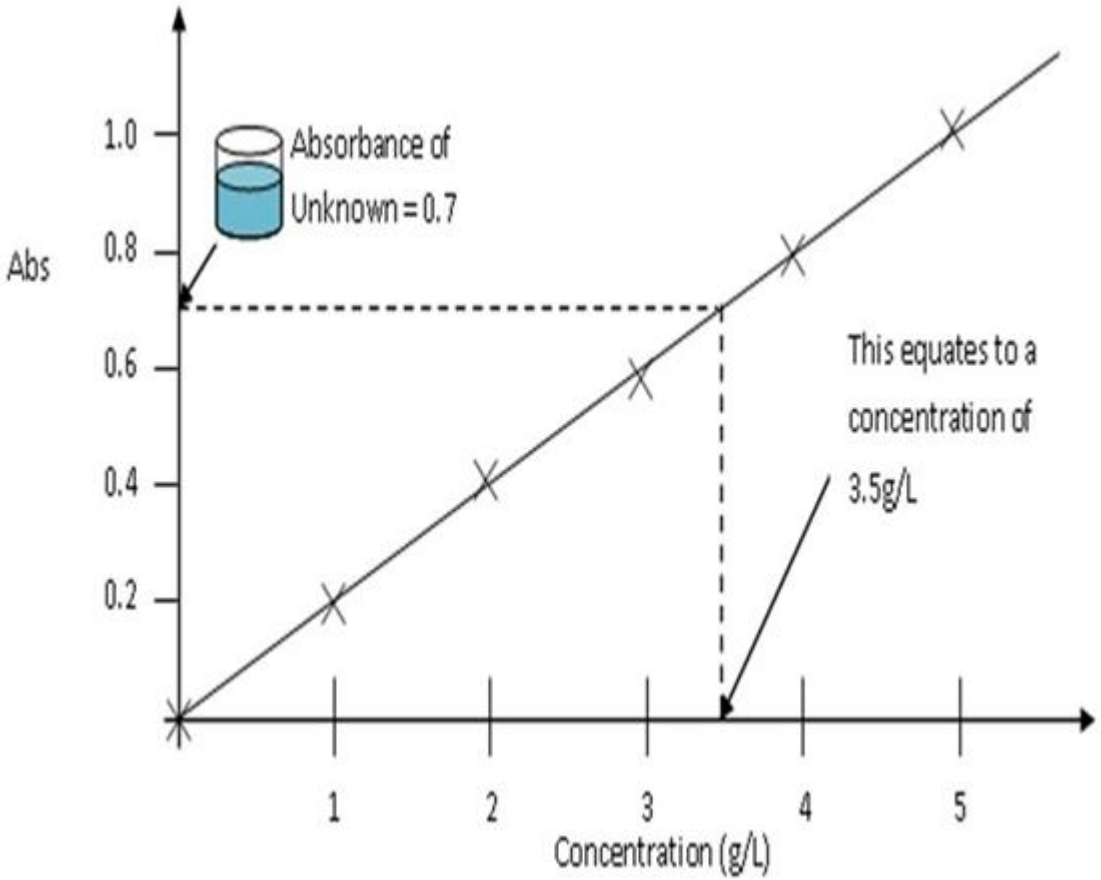
Competitive ELISA

We can use ELISA as well with titration technique

ELISA plate



Or just use Spectrophotometer for reading the color



Spectrophotometry absorbance and antigen concentration slop

What is the basic schematic for an indirect ELISA test?

a) Antigen, Primary antibody, Secondary Antibody, Enzyme
b) Antigen, Primary antibody, Enzyme
c) Antibody, Antigen, Enzyme
d) Antibody, Enzyme
e) Antibody, Antigen, Antibody, Enzyme

Answer:
a) Antigen, Primary antibody, Secondary Antibody, Enzyme

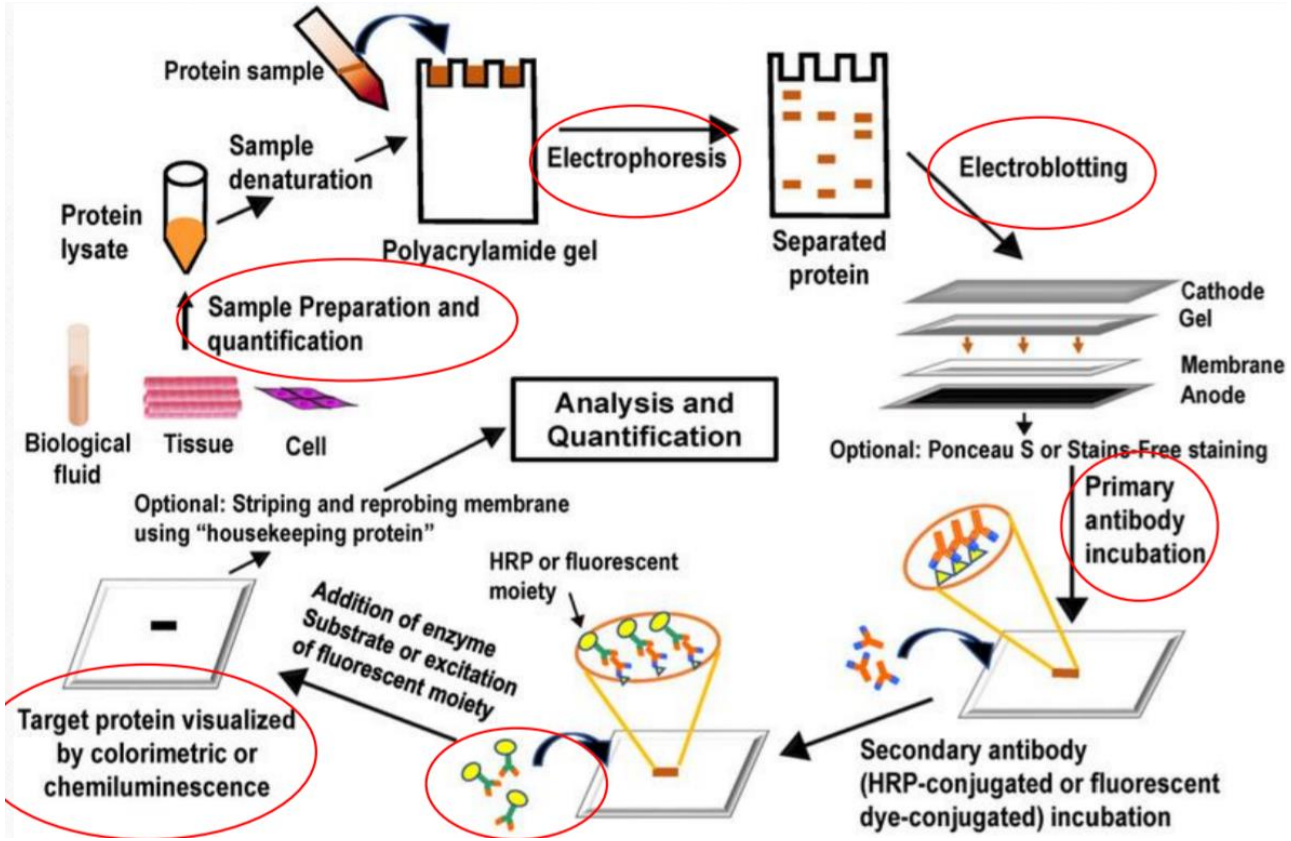
Western Blotting Techniques

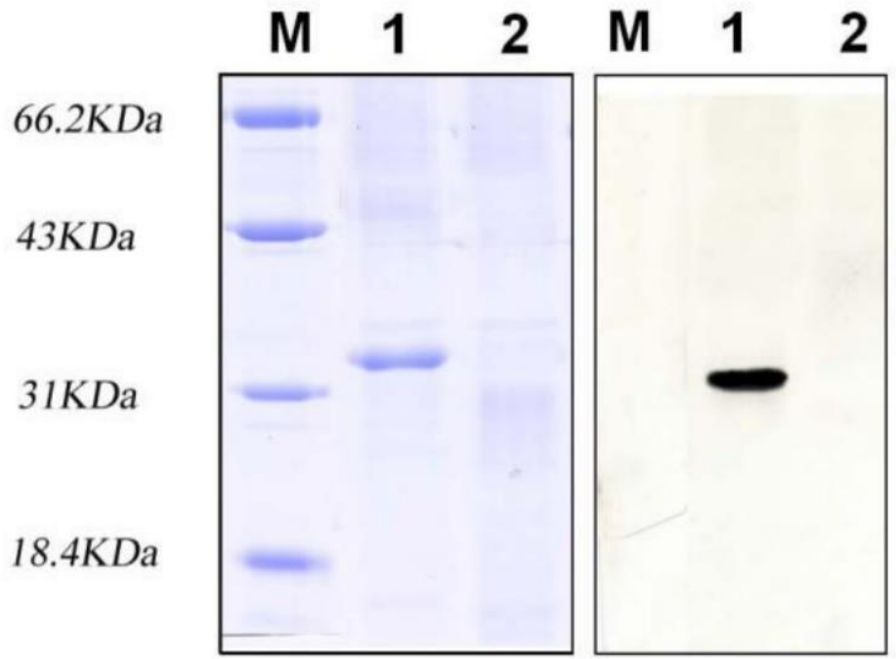
- **Blotting** is a technique by which a **macromolecule** (such as **DNA**, **RNA**, or **protein**) is:
  - Resolved in a **gel matrix**.
  - Transferred to a **solid support**.
  - Detected with a **specific probe**.
- These powerful techniques allow the **identification** and **characterization** of specific molecules in a **complex mixture** of related molecules.
- **Common Blotting Techniques:**
  - **Southern blotting**: For detecting **DNA**.
  - **Northern blotting**: For detecting **RNA**.
  - **Western blotting**: For detecting **protein**

Western Blot

- **Principle:**
  - A **mixture of proteins** is separated based on **molecular weight** using **gel electrophoresis**.
  - The separated proteins are transferred to a **membrane**, producing a **band** for each protein.
  - A **specific protein** is identified by binding a **radiolabeled** or **enzyme-linked antibody**.
- **Key Features:**
  - Common application: **Identification of HIV antibodies**.
  - The position of the **protein antigen** on the membrane depends on its **molecular weight**.
  - Detection can be achieved by:
    - An **enzyme-conjugated antibody** (e.g., **horseradish peroxidase**) that generates signals and produces images on **photographic film**.
    - A **radiolabeled antibody**.
- **Steps Involved in Western Blotting:**
  - Sample preparation.
  - Gel electrophoresis.
  - Blotting (or transfer).
  - Blocking.
  - Antibody probing.
  - Detection

Steps Involved in Western Blotting





MCQ

DNA is detected by:

a) Southern blot
b) Western blot
c) Eastern blot
d) Rapid blot
e) Northern blot

Answer:
a) Southern blot

Tests for Cell/Tissue-Associated Antigens

- **Immunocytochemistry/Immunohistochemistry:**
  - Used for the **detection** of **cell surface** or **intracellular antigens**.
- **Immunofluorescence:**
  - A technique where an **antibody** is labeled with a **fluorescent molecule** (e.g., **fluorescein**).
  - Used to determine the **anatomic distribution** of antigens in tissues, based on the fluorescence emitted by the bound antibody.
  - Common applications:
    1. **Tumor antigen detection**.
    2. **ANA in SLE** and **rheumatoid arthritis**.
    3. **Autoimmune diseases**.
- **Types of Immunofluorescence:**
  1. **Direct Immunofluorescence**:
     - **Fluorescein** is attached directly to a specific **mouse antibody** directed against the antigen of interest.
  2. **Indirect Immunofluorescence**:
     - **Fluorescein** is attached to a second **anti-antibody** (e.g., **rabbit anti-mouse Ig antibody**), which binds to the first unlabeled antibody.
     - **Indirect fluorescence** is more **sensitive** than direct immunofluorescence, as it amplifies the signal

Direct Immunofluorescence Test

•Is widely used for detection of **bacteria**, **parasites**, **viruses**, **fungi**, or other **antigens** in **CSF**, **blood**, **stool**, **urine**, **tissue biopsy**, and other **specimens**

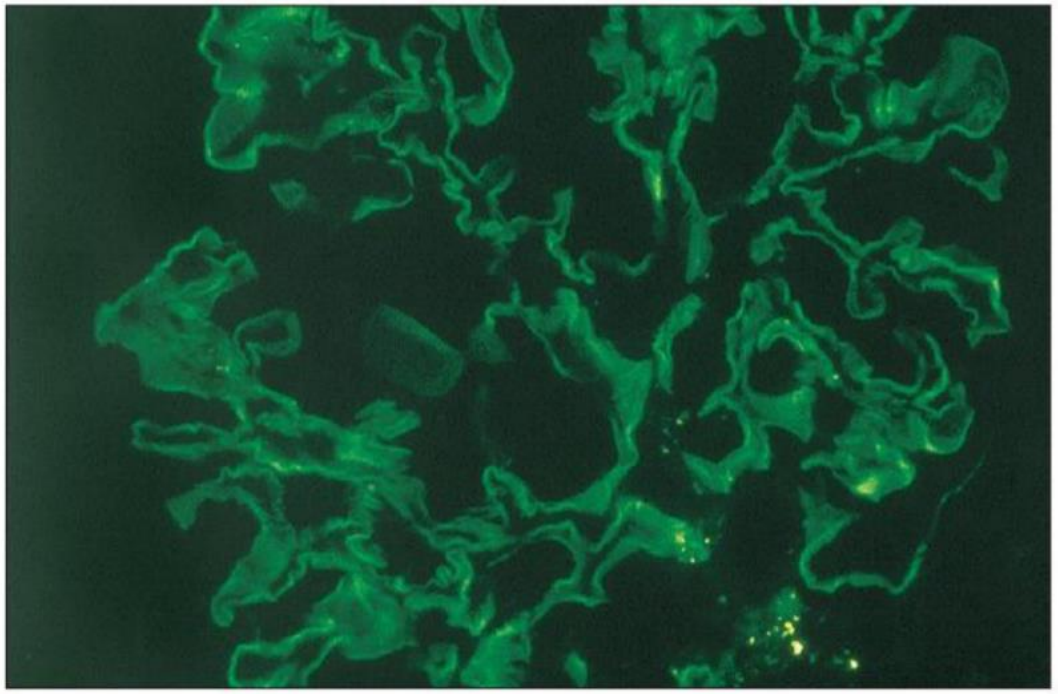


Figure 53-10 Direct immunofluorescence microscopy reveals global and linear glomerular basement membrane immunofluorescence in anti–glomerular basement membrane disease (×400).

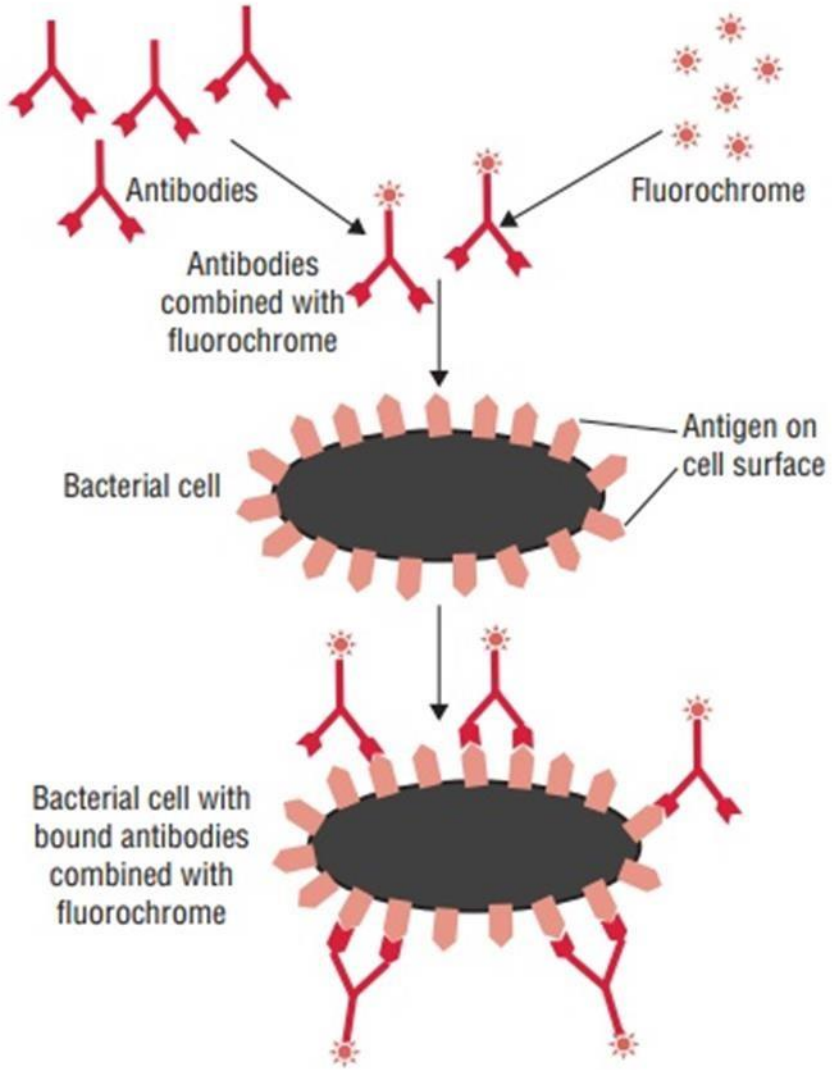
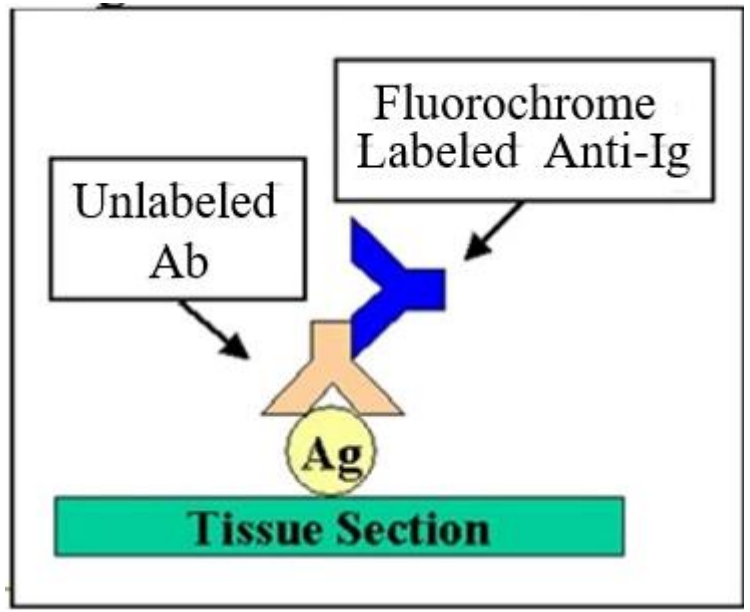


FIG. 14-13. Direct fluorescent antibody test.

Indirect Immunofluorescence Test

- **Indirect immunofluorescence** is a **two-stage process**.
- **First stage:**
  - A **known antigen** is fixed on a slide.
  - The **patient's serum** to be tested is applied to the slide, followed by careful washing.
  - If the **patient's serum** contains **antibody** against the antigen, it will combine with the antigen on the slide.
- **Second stage:**
  - The combination of **antibody** with **antigen** can be detected by the addition of a **fluorescent dye-labeled antibody** to **human IgG**.
  - This is examined by a **fluorescence microscope**.
- **Key components:**
  - **Fluorochrome**
  - **Labeled Anti-Ig**
  - **Unlabeled Ab**



What is the main purpose of an indirect immunofluorescence test?

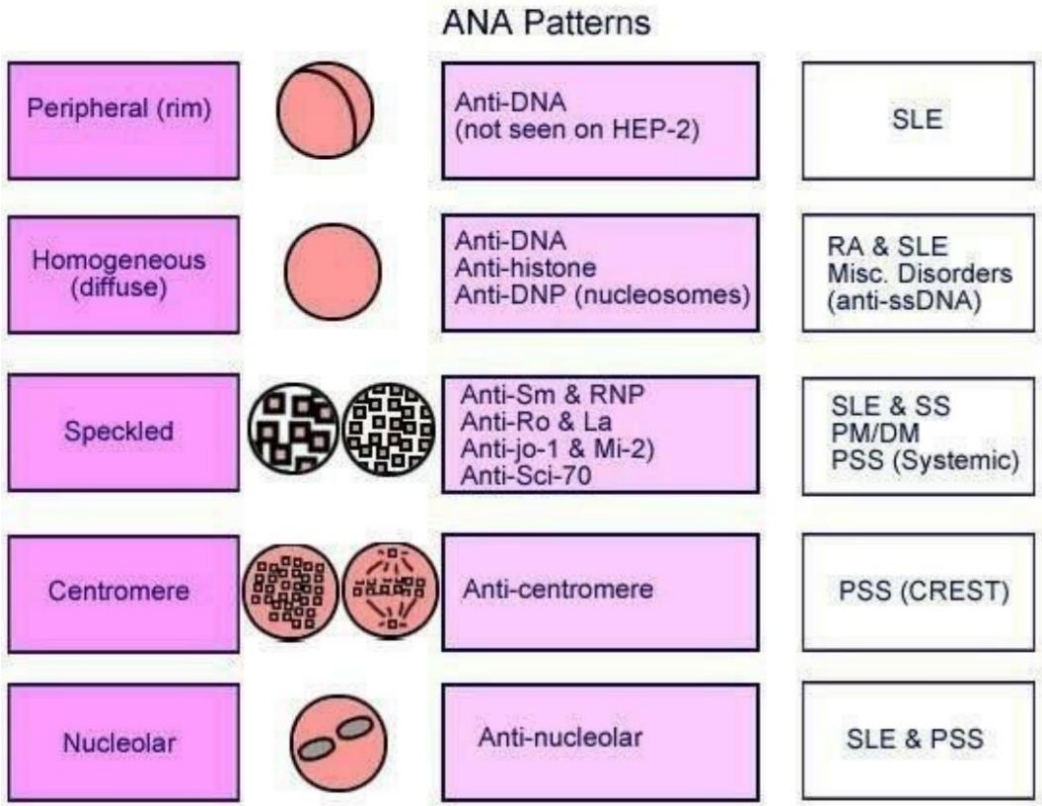
a) Detect ANA
b) Detect viral antigen
c) Detect bacterial antigen
d) Detect level of antibodies
e) Detect level of complements

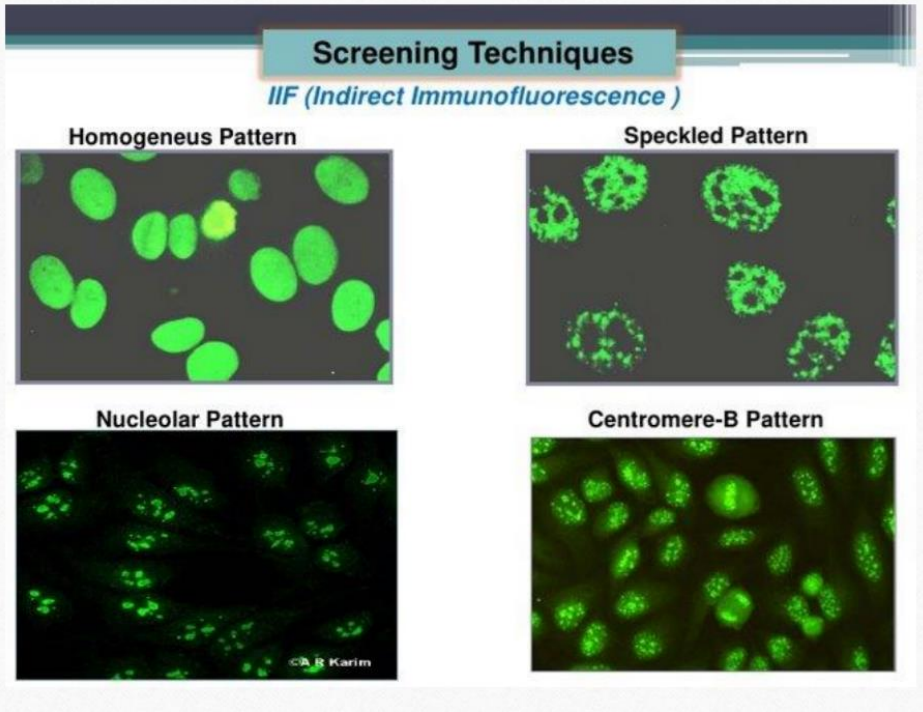
Answer:
d) Detect level of antibodies

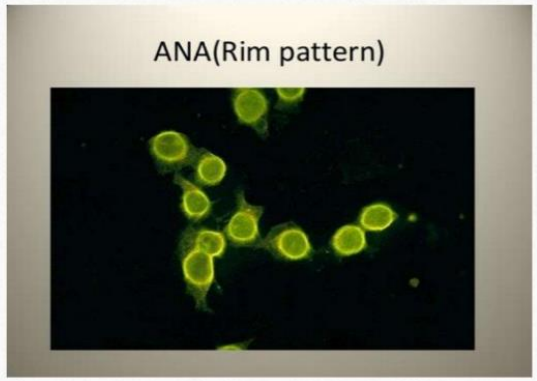
Test for ANA in Autoimmune Diseases

- **Antinuclear antibodies (ANA)** are a category of antibodies that target the **nucleus** of other cells.
- The **ANA blood test** is used to help diagnose certain **autoimmune conditions**, particularly **lupus**.
- **Procedure:**
  - **Patient serum** is added to a slide containing **cells**.
  - If the patient has **autoantibodies** to the nuclei of the cells, they bind to the slide.
  - After washing away any antibodies that don't bind, an **antibody against human antibody** is added.
  - This antibody has **radiolabeled molecules** attached to it, which, when viewed under an **Immunofluorescent microscope**, **light up green**

ANA patterns



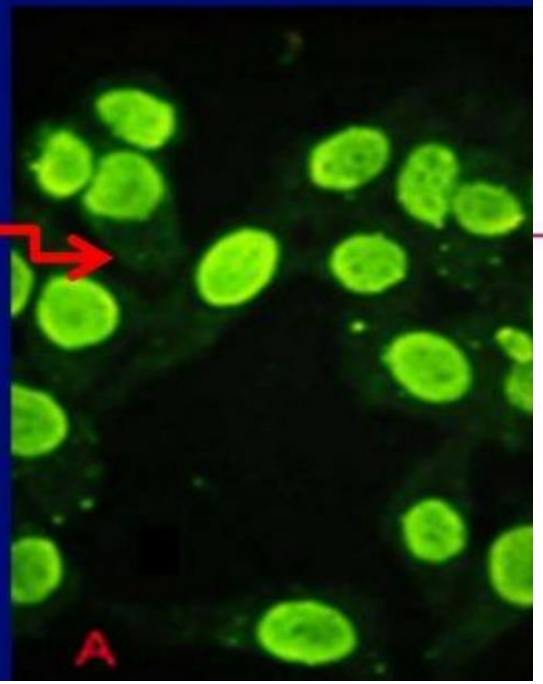




Interpretation

Auto antibodies

- The anti-nuclear antibody (ANA) test is the best screening test for SLE and is determined by **immunofluorescence** or **ELISA** tests
- The ANA is positive in significant titer (usually 1:160 or higher) in virtually all patients with SLE



HOMOGENEOUS PATTERN

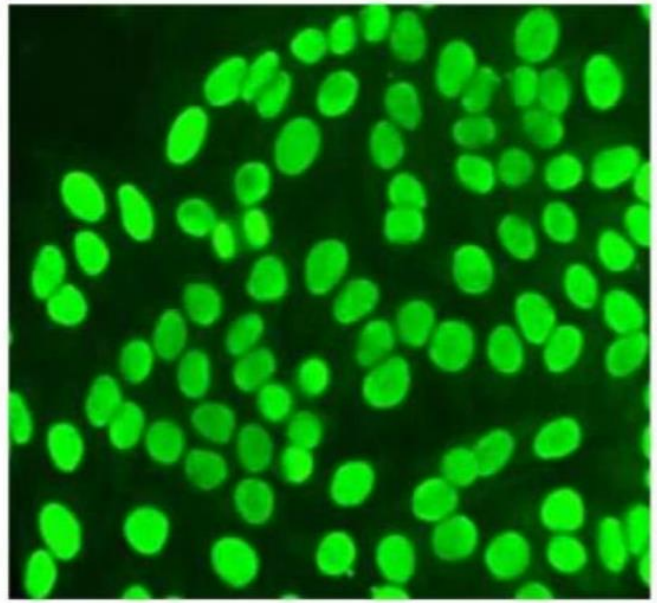
Uniform diffuse fluorescence staining of the entire nucleus in interphase cells.

ANTIGENS:

- DNA, DNA-histone

DISEASES:

- SLE (very specific)
- Drug-induced lupus
- RA (**Rheumatoid Arthritis**)
- Juvenile chronic arthritis
- Systemic sclerosis



Fluorescence Microscope Limitations and Alternatives

- Although **sensitive**, the **fluorescence microscope** is not an ideal tool to identify the **detailed structure** of the cell or tissue because of **low structural details**.
- This problem has been overcome by new technologies, including **confocal microscopy**.
- **Antibody** can be coupled to an **electron-dense probe** such as **colloidal gold**, and the location of the antibody can be determined **subcellularly** by means of an **electron microscope**

MCQ

Which of the following is used to identify autoantibodies such as ANA?

a) ELISA (Enzyme-linked immunosorbent assay)
b) Fluorescent microscope
c) Confocal microscope
d) Western blotting (Immunoblotting)
e) Flow cytometer

Answer:
b) Fluorescent microscope

Which of the following uses ultraviolet light for examining specimens?

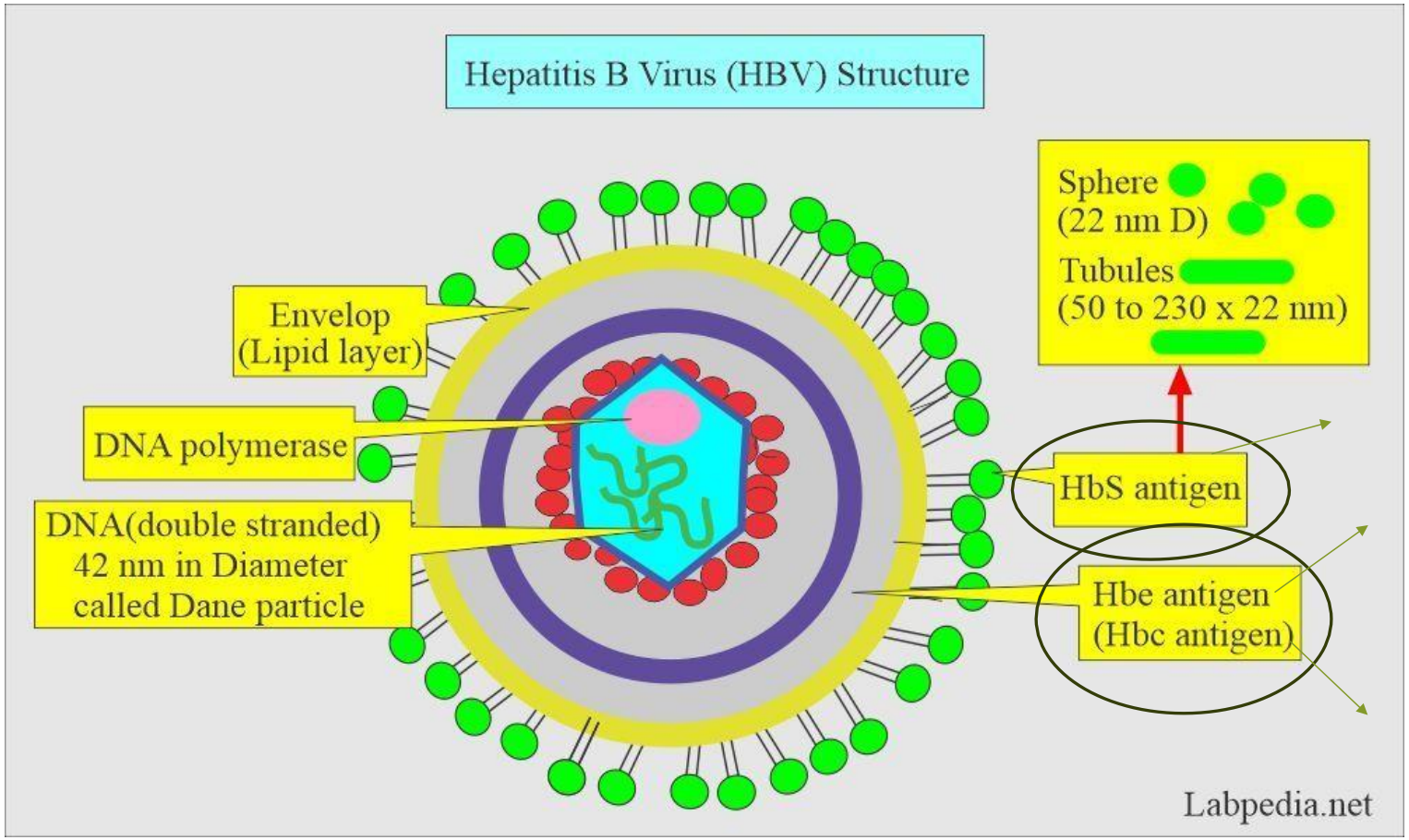
a) ELISA
b) Western blot
c) Fluorescent microscope
d) Western blotting (immunoblotting)
e) Flow cytometer

Answer:
c) Fluorescent microscope

Hepatitis B virus (HBV)

HBsAg (surface antigen); appear during active infection (acute or chronic)

HBeAg (envelope antigen); appear during active replication of virus (acute infection)



MCQ

Extra MCQ;

The affinity of an antibody can be determined by:

a) ELISA (Enzyme-linked immunosorbent assay)
b) Fluorescent antibody (fluorochromes)
c) FACS (fluorescence-activated cell sorting)
d) Western blotting (immunoblotting)
e) Surface plasmon resonance

Answer:
e) Surface plasmon resonance

MCQ

Extra MCQ;

Which of the following is used to detect the production of cytokines inside cells?

a) ELISA (Enzyme-linked immunosorbent assay)
b) Fluorescent antibody (fluorochromes)
c) Flow cytometer
d) Western blotting (immunoblotting)
e) Single radial immunodiffusion

Answer:
c) Flow cytometer

MCQ

Extra MCQ;

The concentration of a solution in a tube is 200 µg/mL. When 9.3 mL of diluent is added, the volume decreases by 0.5 mL after performing 3 dilutions. What is the concentration in Tube 4?

Select one:
a) 2.6 µg/mL
b) 0.025 µg/mL
c) 0.000 µg/mL
d) 0.050 µg/mL
e) 0.1 µg/mL

Answer:
B) 0.025 µg/mL

«Wherever the art of medicine is loved,
there is also a love of humanity.»

- Hippocrates-

Charting New Horizons in Education