2nd Edition

Concise Workbook in Practical Microbiology

As per the Revised Competency-based Medical Education Curriculum (NMC)

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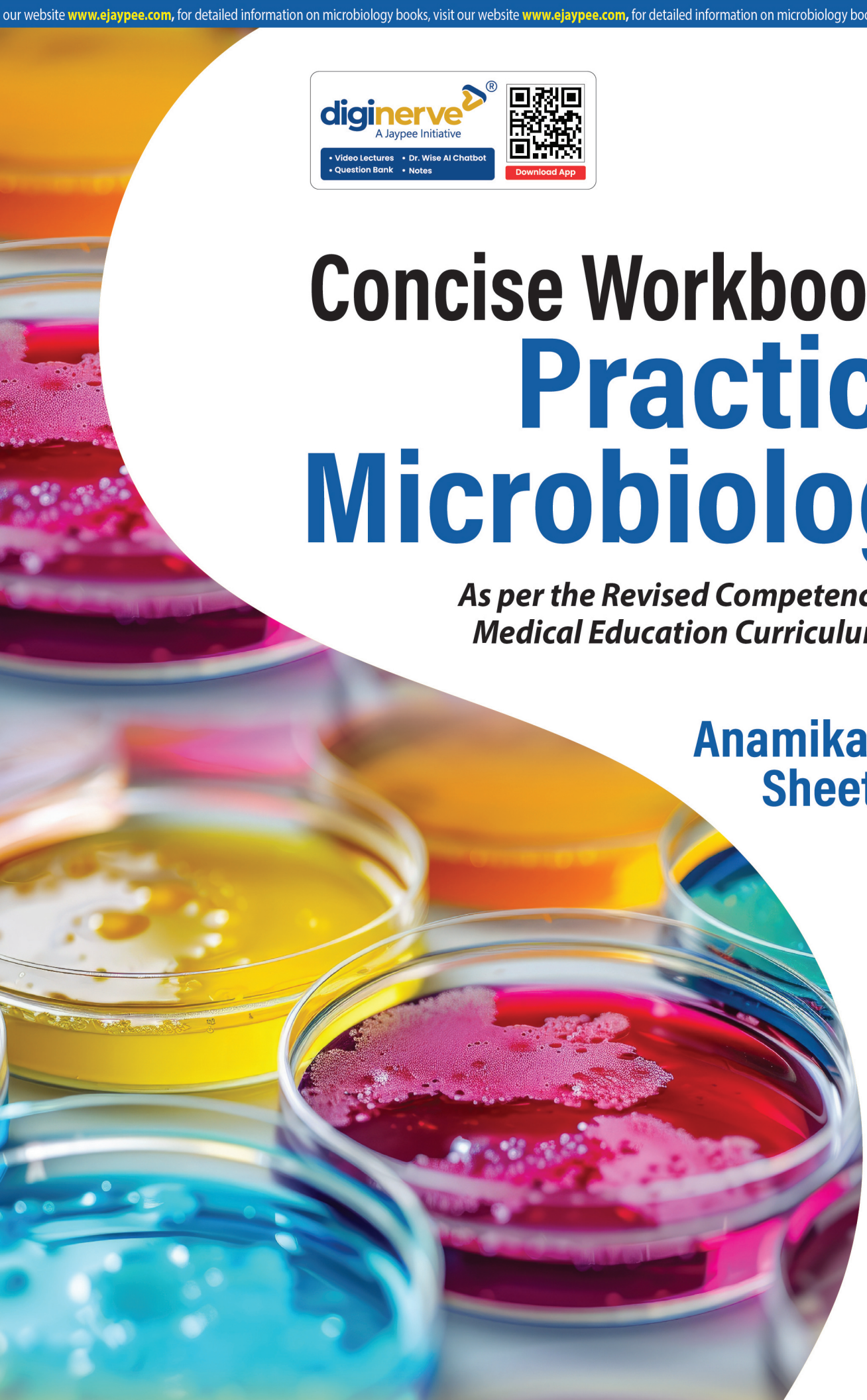
Sheethal S



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CHAPTER

15 Serological Tests in Microbiology

Date:

MI2.5: Discuss the principles and applications of laboratory tests used in diagnostic microbiology based on host's immune response.
MI9.3: Choose the most suitable microbiological investigation in a given clinical situation and interpret the results of the laboratory tests for the diagnosis of infectious diseases.

SPECIFIC LEARNING OBJECTIVES

- Enumerate the various serological tests used for the diagnosis of infectious diseases.
- Describe the principle, types and applications with appropriate examples of the various serological tests performed in the laboratory.
- Choose the correct test and interpret it for the diagnosis of a particular infection.

INTRODUCTION

The diagnostic tests based on antigen-antibody (Ag-Ab) reactions are called immunoassays. Serological tests are immunoassays that are used to identify antigens and antibodies in a patient's serum sample. However, other immunoassay methods use other samples such as cerebrospinal fluid (CSF), urine, etc.

Immunoassay can be:

- **Qualitative:** Reported as only positive and negative.
- **Quantitative:** Reported in exact values or titre.

The test performance is evaluated in two parameters.

1. **Sensitivity:** It is the ability of a particular test to detect even very minute quantities of Ag or Ab, i.e., to correctly identify all those who have the disease (true positives). A highly sensitive test is one where false negative results are absent or minimal.
2. **Specificity:** It is the ability of a particular test to detect reactions between homologous antigens and antibodies only and with no other, i.e., to correctly identify all those who do not have the disease (true negatives). A highly specific test is one where false positive result, are absent or minimal.

TYPES OF ANTIGEN–ANTIBODY REACTIONS

1. **Conventional techniques:**
   a. Precipitation reaction
   b. Agglutination reaction
   c. Neutralization reaction
   d. Complement fixation test (CFT)
2. **Newer techniques:**
   a. Immunofluorescence (IF)
   b. Flow cytometry (FC)
   c. Enzyme-linked immunosorbent assay (ELISA)
   d. Enzyme-linked fluorescent assay (ELFA)
   e. Immunohistochemistry (IHC)
   f. Radioimmunoassay (RIA)

g. Chemiluminescence immunoassay (CLIA)
h. Blotting techniques
i. Rapid tests:
   - ♦ Lateral flow assay (immunochromatographic test or ICT)
   - ♦ Flow through assay

PRECIPITATION REACTIONS

When soluble antigen reacts with its specific antibody in the presence of electrolytes at optimum temperature and pH, the antigen-antibody forms an insoluble complex called as a precipitate (in gel, e.g., agar, agarose, polyacrylamide) and the reaction is called as precipitation. In a liquid medium, precipitates are called floccules which floats on the surface of the liquid and the reaction is termed as flocculation.

Clinical Applications

- ❖ May be used either as qualitative or quantitative tests. Precipitation tests are more sensitive to detect antigens than to detect antibodies.
- ❖ Detection of antibody for diagnostic purposes, e.g., the VDRL test for syphilis.
- ❖ Identification of bacteria, e.g., Lancefield's grouping of *Streptococcus*.
- ❖ In standardizing toxins and toxoids.
- ❖ To detect toxigenicity of diphtheria toxin.

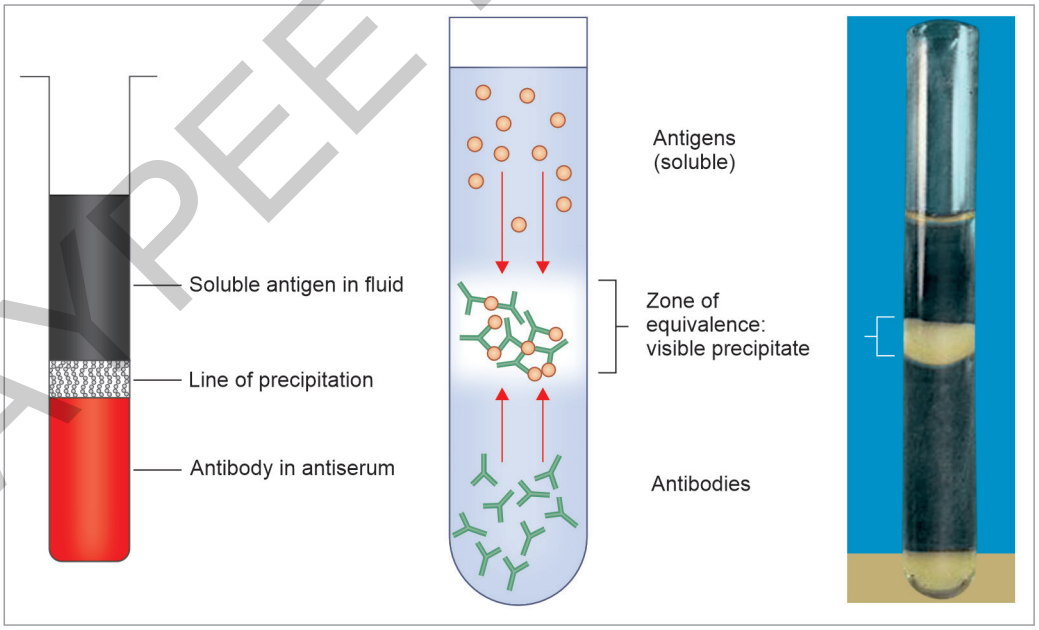
Common Precipitation Reactions

I. Precipitation in Liquid Medium

1. Ring Test

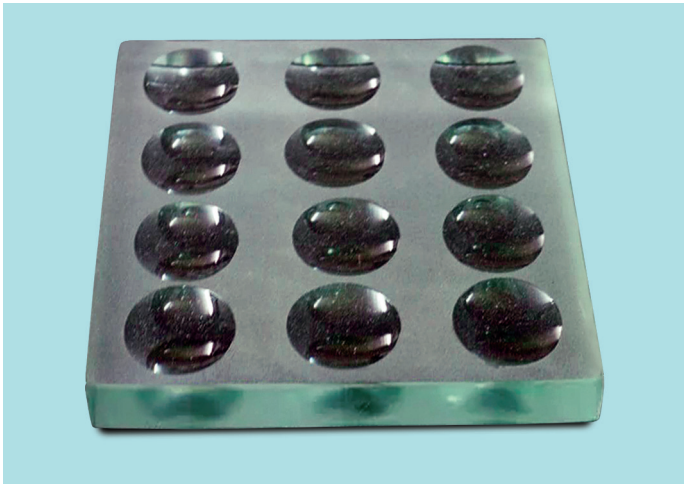
Antigen solution is layered over a column of antiserum in a narrow tube. A precipitate forms at the junction where the two liquids meet.

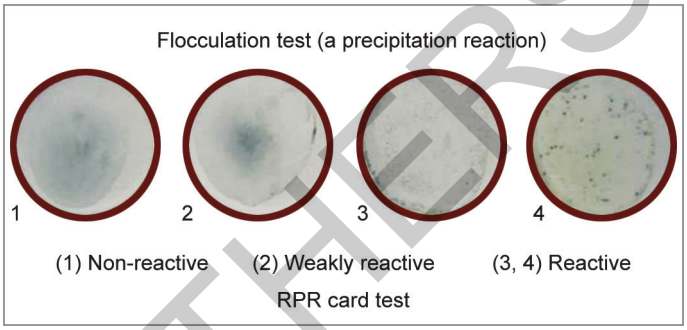
Examples:
- ❖ Ascoli's thermo-precipitation test used for diagnosis of anthrax
- ❖ Streptococcal grouping by Lancefield technique



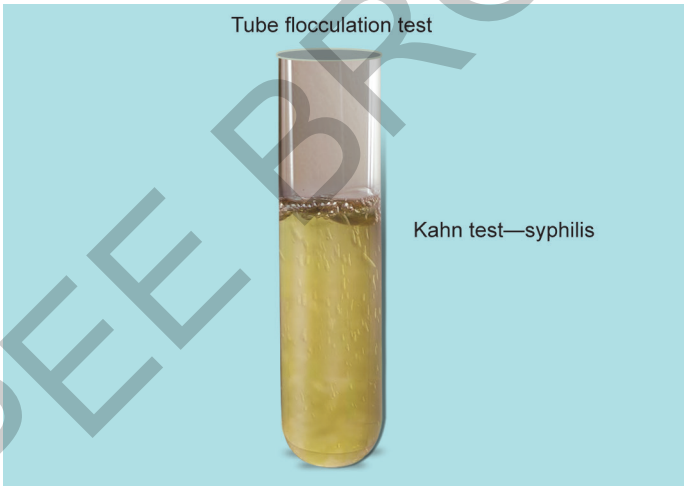
2. Flocculation Test

- **Slide test:** When a drop of each antigen and antibody are placed on a slide and mixed by shaking, floccules appear. *Examples:* VDRL and RPR tests used for diagnosis of syphilis.





- **Tube test:** An antigen and serum are placed in a tube and mixed by shaking—the result is the formation of floccules. *Example:* Kahn test—previously used for diagnosis of syphilis.



II. Precipitation in Gel Medium

Immunodiffusion tests (generally done in 1% agar gel).

Advantages of gel over liquid medium are:

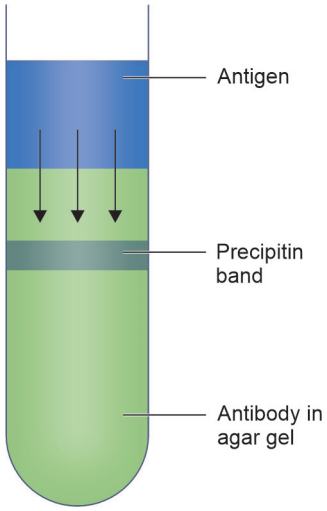
- Reaction visible as a distinct band
- Stable
- Can be stained and preserved
- Number of different antigens can be observed

This test is based on two principles:

1. Only antigen diffuses (single diffusion) or both antigen and antibody diffuse (double diffusion).
2. When immunodiffusion is done in a test tube layered with gel, vertical diffusion occurs (one dimension). When the test is done on a slide or petri dish layered with gel, diffusion occurs in both X- and Y-axes (two dimensions).

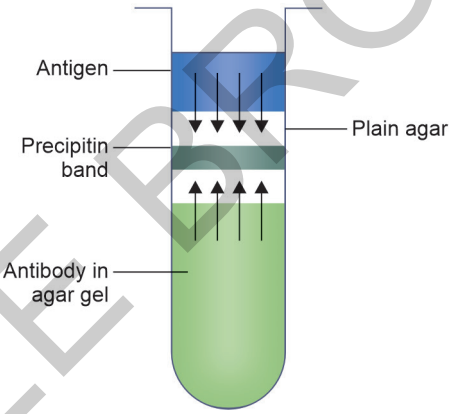
Types of immunodiffusion tests:

1. **Single diffusion in one dimension (Oudin procedure).**



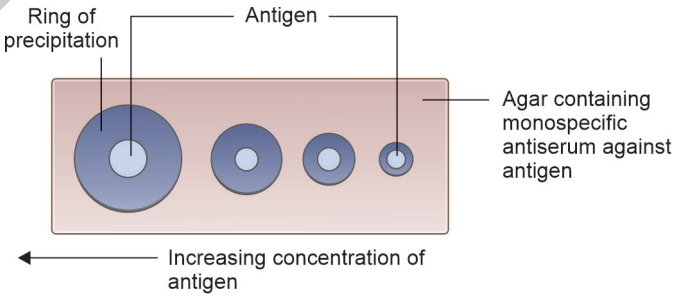
Single diffusion in one dimension (Oudin procedure)

2. **Double diffusion in one dimension (Oakley–Fulthorpe procedure).**



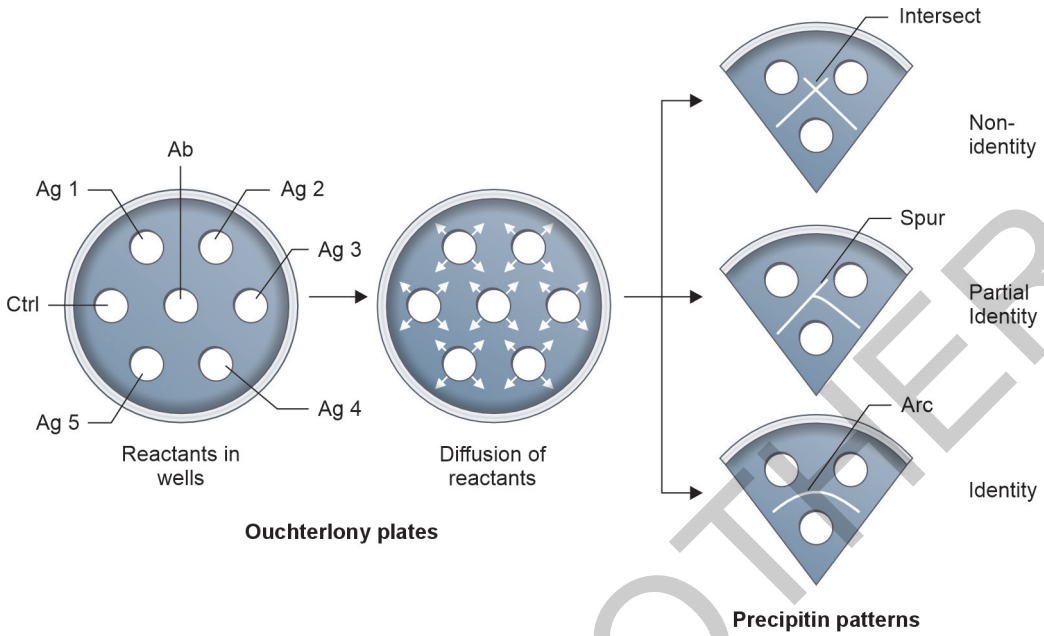
Double diffusion in one dimension (Oakley–Fulthorpe procedure)

3. **Single diffusion in two dimensions (radial immunodiffusion).**



Single diffusion in two dimensions (radial immunodiffusion)

4. **Double diffusion in two dimensions (Ouchterlony procedure),** e.g., Elek's gel precipitation test for toxigenicity testing in *Corynebacterium diphtheriae.*

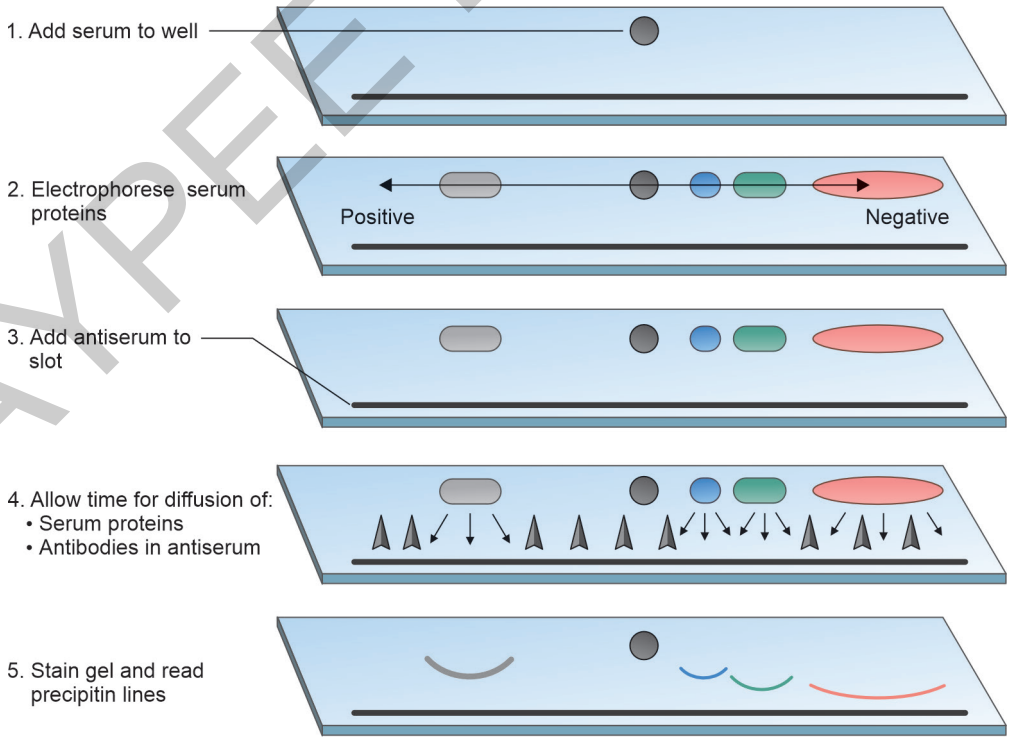


Double diffusion in two dimensions (Ouchterlony procedure)

III. Electroimmunodiffusion Tests (Precipitation in Gel in the Presence of an Electric Current)

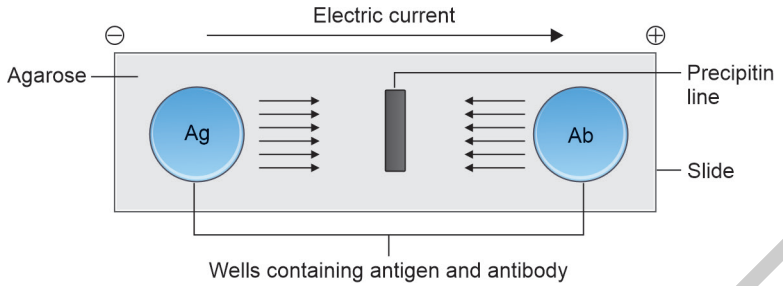
Types of electroimmunodiffusion tests:

1. **Immunoelectrophoresis:**
   - A number of antigens can be identified in serum.
   - Used to detect normal as well as abnormal proteins, such as myeloma proteins in human serum.



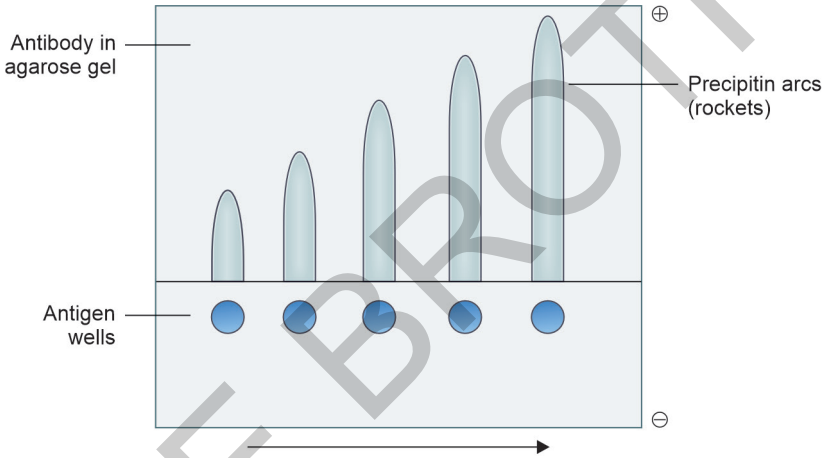
Immunoelectrophoresis (IEP)

2. **Counterimmunoelectrophoresis:**
   - Rapid and highly specific method for detection of both antigen and antibodies in the serum, CSF and other body fluids.
   - Commonly used for hepatitis B surface antigen (HBsAg), α fetoprotein, hydatid and amoebic antigens in the serum and cryptococcal antigen in the CSF.



Counterimmunoelectrophoresis

3. **Rocket electrophoresis:**
   - Appearance of precipitin bands in the shape of cone like structures (rocket appearance) at the end of the reaction.
   - Used for the quantitation of proteins and other antigens in various clinical specimens.



Rocket electrophoresis

4. **Two-dimensional immunoelectrophoresis (Laurell's procedure):**
   - A variant of rocket electrophoresis.
   - Used for qualitative and quantitative analysis of sera for a wide range of antigens.

AGGLUTINATION REACTIONS

Principle

When a particulate or insoluble antigen reacts with its antibody in the presence of electrolytes at optimum pH and temperature, the particles are clumped or agglutinated. The reaction is called agglutination.

Advantages

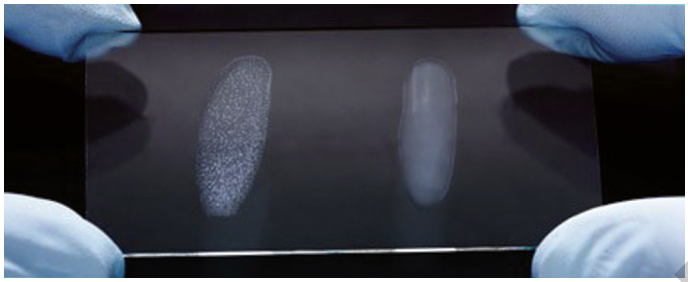
- ❖ More sensitive than precipitation for the detection of antibodies.
- ❖ Clumps are better visualized and interpreted than bands or floccules.
- ❖ The agglutination reaction takes place better with IgM antibody.

Types of Agglutination Reactions

1. **Direct agglutination reactions:**
   - *Slide agglutination test:* Basic type of agglutination reaction that is performed on a slide in which a suspension of bacteria/serum is prepared to which a drop of standardized antiserum/antigenic suspension is added. A positive reaction is indicated by clumping of bacteria and clearing of the background solution.

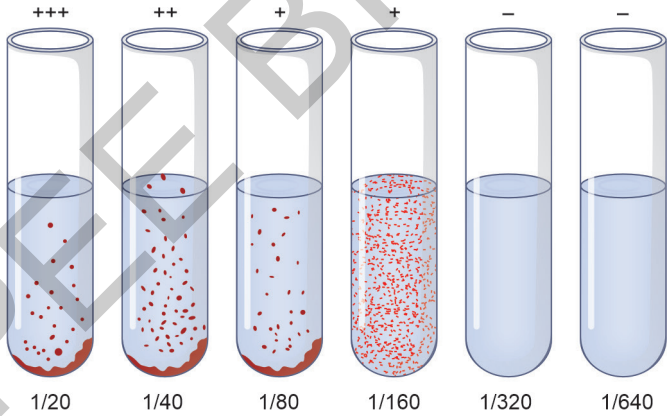
Uses:

- To confirm the identification and serotyping of bacterial colonies grown on agar (especially *Salmonella* spp., *Shigella* spp. and *Vibrio cholerae*).
- Blood grouping and cross matching.
- Slide Widal test for diagnosis of enteric fever.



Serotyping of S. Typhi showing positive (left) and negative (right) tests

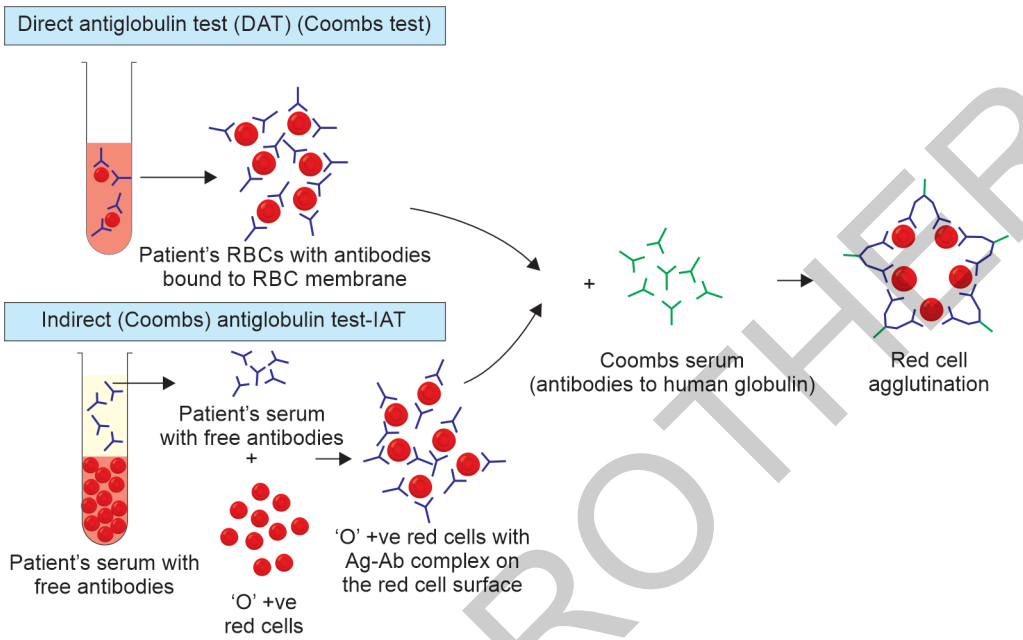
- *Tube agglutination test:* It is carried out to quantitate antibody in serum. This is performed in tubes. Patient's serum is diluted in a series of tubes and a prefixed volume of bacterial antigens specific for the suspected disease is added to it. This is then incubated. The positive reaction is demonstrated by visible clumps of agglutination. The highest dilution of serum that gives a positive agglutination reaction is given as the antibody titre.
  Uses:
  - For serological diagnosis of:
    - Enteric fever (Widal test)
    - Acute brucellosis (standard agglutination test)
    - Heterophile agglutination tests, e.g., Typhus fever (Weil Felix reaction), infectious mononucleosis (Paul Bunnell test), *Mycoplasma pneumoniae* (cold agglutination test)
  - For diagnosis of primary atypical pneumonia (*Streptococcus* MG agglutination test)



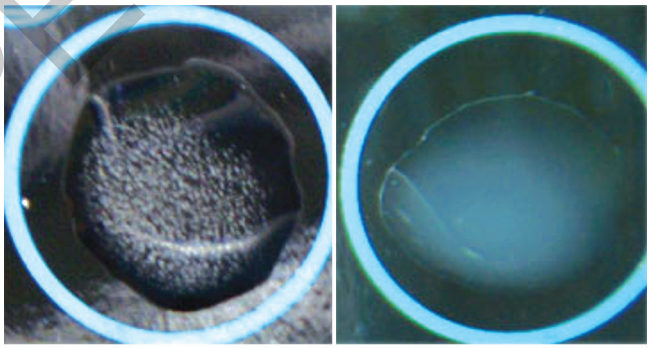
Widal test by tube agglutination method

- *Microscopic agglutination test (MAT):* Test is performed on a microtiter plate and the result is read under a microscope.
  Uses: Demonstration of specific antibodies in leptospirosis.
- *Heterophile agglutination test:* This test depends on demonstration of heterophilic antibodies in serum present in certain bacterial infections:
  - Weil Felix test: This test is used for serodiagnosis of rickettsial infections. In this test, the cross-reacting antibodies produced against rickettsial pathogen are detected by using cross-reacting related antigens of *Proteus* strains OXK, OX19 and OX2.
  - Paul Bunnell test: This test is used to detect antibodies in infectious mononucleosis by using sheep erythrocytes as antigens.
- *Antiglobulin test (Coombs test):* Devised by Coombs *et al.* (1945) to detect incomplete anti-Rh antibodies that do not agglutinate Rh + erythrocytes in saline.
  - Direct Coombs test: It is done to detect monovalent maternal antibodies already present on the surface of fetal RBCs. In this test, sensitization of RBCs with incomplete antibodies takes place in vivo.
    Fetal RBCs with monovalent antibody + antihuman immunoglobulin serum (Coombs reagent) → Agglutination.
    Use: In hemolytic diseases of newborn due to Rh incompatibility.

- Indirect Coombs test: In this test, sensitization of RBCs with incomplete antibodies takes place in vitro.
  Antibodies in mother's serum + Target RBCs → Antibody bound RBCs.
  Antibody bound RBCs + antihuman immunoglobulin (Coombs reagent) → agglutination of RBCs.
  Uses:
  - For detection of anti-Rh antibodies in patient's serum.
  - Used to demonstrate incomplete antibodies, e.g., in brucellosis.



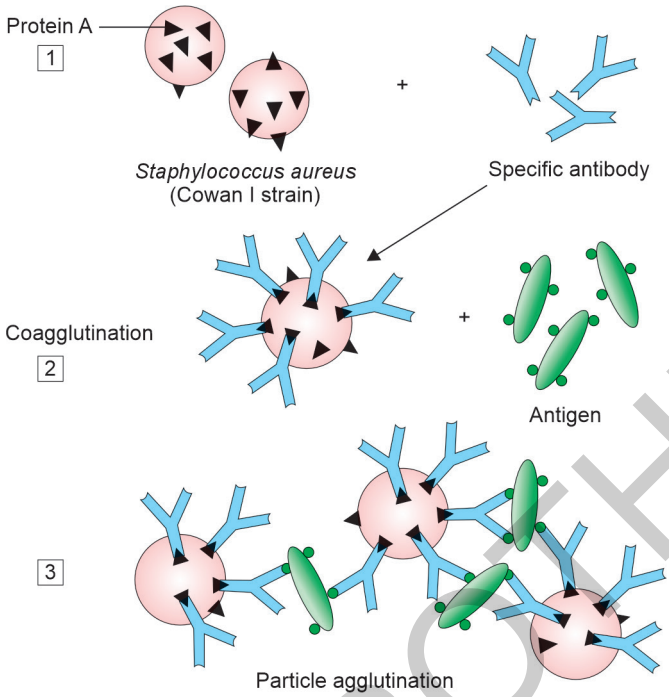
2. **Indirect (passive) agglutination reactions:** For antibody detection.
   - Conversion of precipitation reaction into agglutination reaction by coating the soluble antigen on the surface of a carrier molecule (e.g., RBC, latex, carbon or bentonite), so that antibody binds to the coated antigen and agglutination occurs on the surface of the carrier molecule.
     - Indirect hemagglutination test: For example, Rose-Waaler test for detection of RA factor in rheumatoid arthritis (carrier molecule here is sheep RBC).
     - Latex agglutination test for antibody detection: Used for detection of antistreptolysin O (ASO) antibody (carrier molecule here is latex particle).



ASO antibody test showing positive and negative reactions

3. **Reverse passive agglutination reactions:** For antigen detection.
   - Same as indirect (passive) agglutination reactions except that in this test antibody is coated on carrier molecule which detects antigen in patient's serum.
     - Reverse passive hemagglutination assay (RPHA): RBCs are used as carrier molecules.
       Uses: For detection of hepatitis B surface antigen (HBsAg) which is now obsolete.
     - Latex agglutination test for antigen detection: Latex particles are adsorbed with antibodies.
       Uses: For detection of C reactive protein (CRP), capsular antigen detection in CSF (for *Pneumococcus, Meningococcus* and *Cryptococcus*) and streptococcal grouping.

- Coagglutination test: Protein A in *Staphylococcus aureus* (Cowan 1 strain) acts as a carrier molecule.
  Uses: For detection of bacterial antigen in blood, CSF and urine.



COMPLEMENT FIXATION TEST

Principle

The ability of antigen-antibody complexes to fix complement is made use of in complement fixation test. To detect the fixation of complement, an indicator system is used such as guinea pig RBCs.

Applications

- Wassermann test for syphilis.
- Tests for demonstration of antibodies to *Mycoplasma pneumoniae, Bordetella pertussis*, many different viruses such as arboviral infections and fungi such as *Cryptococcus* species, *Histoplasma* and *Coccidioides immitis.*
- Various serological tests such as *Treponema pallidum* immobilization test for syphilis and Sabin-Feldman dye test for *Toxoplasma gondii.*

OTHER COMPLEMENT DEPENDENT SEROLOGICAL TESTS

- **Immune adherence test:** This facilitates phagocytosis of bacteria, e.g., *Vibrio cholera, Treponema pallidum.*
- **Cytocidal or cytotoxic test:** Forms the basis of test used to measure anticholera antibodies in the serum.
- **Conglutinating complement absorption test:** An alternative method for systems that do not fix guinea pig complement. Sheep erythrocytes, sensitized with bovine serum are used as the indicator system.

NEUTRALIZATION TEST

Principle

It is an antigen-antibody reaction in which the biological effects of viruses and toxins are neutralized by homologous antibodies known as neutralizing antibodies.

Examples:

I. **In vivo tests:**
   - Toxigenicity test, e.g., diphtheria toxin-antitoxin neutralization tests in animals.
   - Schick test: Diphtheria toxin-antitoxin neutralization test in humans.

II. **In vitro tests:**
   - Virus neutralization tests.
   - Nagler reaction: Used for detection of α-toxin of *Clostridium perfringens.*

IMMUNOFLUORESCENCE ASSAY (IFA)

Principle

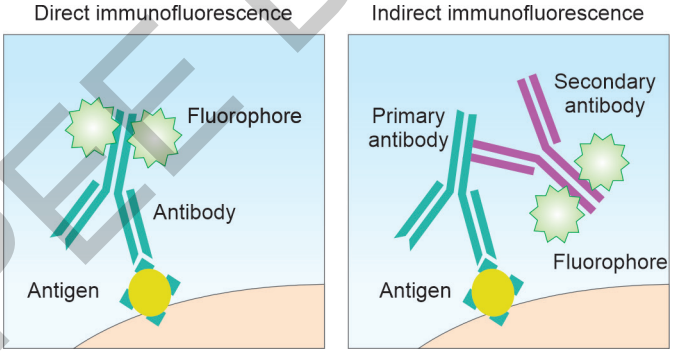
Fluorescence refers to absorbing high energy shorter wavelength ultraviolet light rays by a fluorescent compound and in turn emitting visible light rays with a low energy longer wavelength. A fluorescent dye is used to conjugate an antibody. If antigen is present in the specimen, the same will bind the fluorescein labeled antibodies and the antibody bound fluorescein will emit a fluorescence which is observed with the help of a fluorescent microscope using ultraviolet radiations.

Fluorescent compounds normally used are fluorescein isothiocyanate (FITC) (most common), rhodamine, dansyl and phycoerythrin.

Types

I. **Direct immunofluorescence assay:** Sample containing cells carrying surface antigen is smeared on a slide → primary antibody specific to the antigen, tagged with fluorescent dye is added → slide is washed to remove unbound antibodies and observed under a fluorescent microscope.
Disadvantage: Different antibodies, tagged with fluorescent dye have to be used for different antigens.
Uses:
- Commonly employed for detection of bacteria, viruses or other antigens in blood, CSF, urine, feces, tissues, and other specimens.
- A sensitive method to diagnose rabies virus antigens in brain smears.

II. **Indirect immunofluorescence assay:** Suspected antigen of the organism is smeared on a slide → test serum containing primary antibody is added to the slide → slide is washed to remove the unbound antibodies → a secondary antibody (antihuman antibody conjugated with fluorescent dye) is added → slide is washed and viewed under a fluorescence microscope.
This detects antibodies in sample. Slides smeared with cells carrying known antigens are commercially available.
Advantage: A single antihuman globulin fluorescent conjugate can be employed for detection of antibody to any antigen.
Uses:
- Detection of autoantibodies (e.g., antinuclear antibody) in autoimmune diseases.
- Detection of viral antigens in cell lines inoculated with the specimens.



FLOW CYTOMETRY

It is a laser-based technique that quantitatively analyses and separates the cells as they pass through the laser beam that can analyze various parameters of cells like cell counting, cell sorting by size, shape, granularity, DNA/RNA content of cell, etc.

Uses:
- ❖ CD4 T cell count in HIV infected patients.
- ❖ Detection of leukocytes with specific markers for diagnosis of various lymphoma.

ENZYME-LINKED IMMUNOSORBENT ASSAY

Principle

Enzyme-linked immunosorbent assay (ELISA) is an immunoassay that detects either antigen or antibodies in the specimen by using enzymes-substrate-chromogen system for detection.

ELISA has two components:

1. **Immunosorbent:** As absorbing material (e.g., polystyrene, polyvinyl) is used that specifically absorbs the antigen or antibody present in serum.
2. **Enzyme:** Used to label one of the components (antigen or antibody) of immunoassay, e.g., horseradish peroxidase.

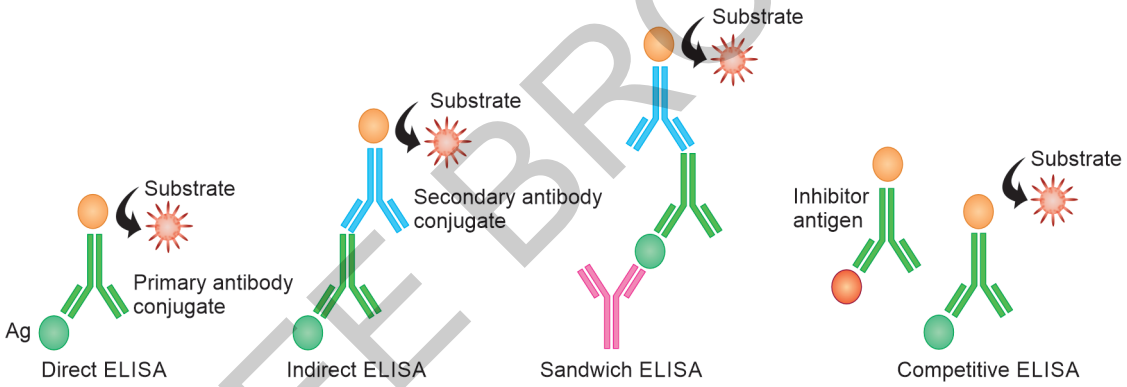
Substrate-chromogen system: It is added at the final step of ELISA, e.g., hydrogen peroxide-tetramethyl benzidine.
The enzyme reacts with the substrate which in turn activates the chromogen to produce a color. The color change is detected by spectrophotometry in an ELISA reader at the wavelength of 492 nm. Intensity of color is directly proportional to the amount of antigen or antibody present in the serum.

Various Generations of ELISA

- **1st generation:** Crude antigen (infected viral cell lysate as antigen) is used for detection of antibodies.
- **2nd generation:** Recombinant antigen is used for detection of antibodies.
- **3rd generation:** Recombinant antigens and/or synthetic peptides are used for the detection of antibodies.
- **4th generation:** Recombinant antigens or synthetic peptides along with monoclonal antibodies are used that detect two components in the blood: antibodies and antigens.

Types of ELISA

1. **Direct ELISA:** Used for detection of antigen in the test serum. Primary antibody that is targeted against the serum antigen is labeled with enzyme. The microtitre plates are not precoated with antigen/antibody.
   Principle: Antigen present in test serum + enzyme labeled primary antibody specific to the antigen present in test serum + substrate-chromogen → color change → positive reaction.



Types of ELISA

2. **Indirect ELISA:** Used for detection of antibody and less commonly for antigen detection.
   Here, secondary antibody, i.e., an antihuman antibody, targeted against Fc region of any human immunoglobulin (antibody) is labeled with enzyme.
   Principle: Wells precoated with antigen + primary antibody present in test serum + secondary antihuman antibody labeled with enzyme + substrate-chromogen → development of color → positive reaction.
3. **Sandwich ELISA:** It detects the antigen in test serum. Antigen gets sandwiched between a capture antibody and a detector antibody.
   Principle: Wells coated with capture monoclonal antibody specific to the antigen to be detected + antigen in test serum + primary detector antibody specific for the antigen labeled with enzyme + substrate-chromogen → development of color → positive reaction.
4. **IgM antibody capture (MAC) ELISA:** It is an enzymatically amplified sandwich type immunoassay widely used for dengue, Japanese encephalitis and West Nile virus, scrub typhus, leptospirosis, toxoplasmosis, etc.
   Principle: Wells coated with capture antihuman IgM antibody + IgM antibody present in test serum + recombinant antigen + enzyme labeled secondary antibody specific for the antigen-biotin-avidin + substrate-chromogen → development of color → positive reaction.
5. **Competitive ELISA:** Most commonly used for antigen detection and less commonly for antibody detection in test serum. In this type, antigen present in the test serum competes with another antigen of the same type coated on well to bind to the primary antibody.

Principle: Primary antibody is incubated in a solution with a test serum containing the test antigen + same type of antigen precoated on the wells → free primary antibodies bind to the antigens coated on wells → after washing, secondary antibody labeled with enzyme + substrate-chromogen → color does not develop → positive reaction.
Here intensity of color is inversely proportional to the amount of antigen present in the test serum.

6. **Cassette or cylinder ELISA:** It is a simple modification of ELISA for testing one or few samples of sera at a time.
Principle: Specific types of antigens are immobilized on nitrocellulose membrane in a cassette + test serum containing primary antibodies specific to the immobilized antigens → after washing to remove unbound primary antibodies enzyme labeled secondary antihuman antibodies (conjugate) are added → washed again to remove unbound conjugate + substrate added → positive result shows a color dot on control spot and test spot.

Advantages of ELISA

- Large volume of samples can be processed at the same time.
- Reports are released within 2–3 hours.
- Highly sensitive and highly specific (with use of more purified recombinant and synthetic antigens and monoclonal antibodies).

Disadvantages of ELISA

- Takes more time (2–3 hours) than rapid tests (10–20 minutes).
- Needs expensive equipment such as ELISA washer and reader.

Applications

- **For antigen detection,** e.g., hepatitis B surface antigen (HBsAg) and pre-core antigen (HBeAg), NS1 antigen for dengue infection, p24 antigen for HIV infection (in window period), rotavirus in feces, etc.
- **For antibody detection** against HIV, HBV, HCV, dengue, EBV, HSV, toxoplasmosis, leishmaniasis, etc.

ENZYME-LINKED FLUORESCENT ASSAY

Enzyme-linked fluorescent assay (ELFA) is a modification of ELISA. It differs from ELISA in the following ways:

- This is an automated system.
- Antibody-enzyme complex is detected by fluorometric method.

Advantages over ELISA:

1. Fully automated system
2. User friendly, easy to perform
3. Less chances of contamination
4. Quantitative results are produced
5. More sensitive and specific

Disadvantages:

1. Expensive
2. Only 12-24 numbers of tests can be run at a time

Uses:

1. To detect a number of parameters.
   a. Markers of hepatitis viruses and HIV (antigen and antibodies).
   b. Antibodies against TORCH, measles, mumps and varicella.
   c. Antigens of *C. difficile* and Rotavirus.
2. Biomarkers of procalcitonin, hormones like thyroid, tumor markers and cardiac markers.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) is the process of detecting surface or intracellular antigens in cells of a tissue section on the basis of the principle of fluorescent dye (e.g., fluorescein isothiocyanate, phycoerythrin, rhodamine) or enzyme (e.g., peroxidase – immunoperoxidase staining) tagged antibodies that bind specifically to antigens in biological tissue. This method helps to visualize antigen- antibody reactions in situ.
Uses: Diagnosis of abnormal cell population in cancerous tumors.

RADIOIMMUNOASSAY

Radioimmunoassay (RIA) is based on competitive binding. A fixed amount of antigen labeled with radioisotopes and unknown amount of antigen unlabeled (in the test specimen) competes with each other to bind with prefixed amounts of antibody when incubated together with corresponding antibody. The amount of radiolabeled antigen precipitated with the antibody is determined by counting radioactivity. A standard curve is constructed by plotting the percentage of antibody bound radiolabeled antigen against concentrations of a standardized unlabeled antigen.

Advantage: Highly sensitive assay.

Disadvantages:
- ❖ High cost of equipment and reagents.
- ❖ Short shelf-life of radiolabeled compounds.
- ❖ Problems associated with the disposal of radioactive waste.

Uses:
- ❖ To determine very small quantities (e.g., nanogram) of antigens and antibodies in the serum.
- ❖ For quantitation of hormones, drugs, HBsAg and other viral antigens.

CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence immunoassay (CLIA) uses chemiluminescent compounds such as luminal and acridinium ester that emit energy in the form of light during the antigen-antibody reactions. The emitted lights are measured and the concentration of the analyte is calculated.

Uses:
- ❖ For detection of biomarkers such as procalcitonin.
- ❖ For detection of antigens and antibodies against infections caused by hepatitis viruses, HIV and TORCH.

BLOTTING TECHNIQUES

The blot analysis technique is a powerful method to detect specific biomolecules in samples of complex composition. Blotting methods are the adjunct to gel electrophoresis, a method for separating DNA, RNA and proteins with exceptional resolving power.

Types of Blotting Techniques

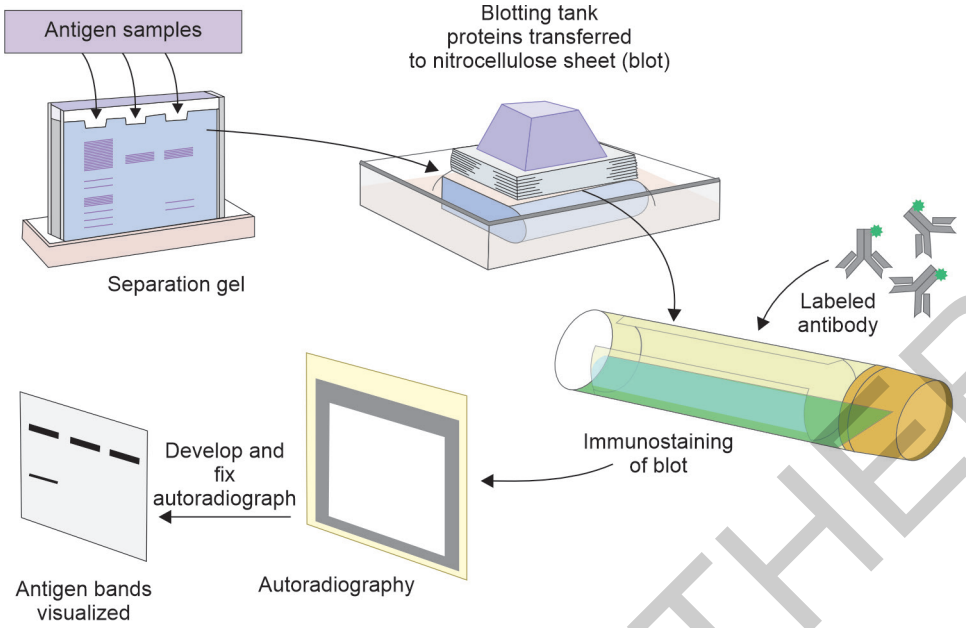
1. **Southern blot**: Detects DNA fragments.
2. **Northern blot**: Detects mRNAs.
3. **Western blot**: Detects specific proteins (antibodies) in a sample containing mixture of antibodies each targeted against different antigens of same microbe.
4. **Eastern blot**: Modification of western blot that detects the carbohydrate epitopes present on proteins or lipids.

Principle

All the blotting methods are fairly simple and usually consist of four separate steps:
1. Electrophoretic separation of protein or of nucleic acid fragments in the sample.
2. Transfer to and immobilization on paper support.
3. Binding of analytical probe to target molecule on paper.
4. Visualization of bound probe.

Molecules in a sample are first separated by electrophoresis and then transferred on to an easily handled support medium or membrane. This immobilizes the protein or DNA fragments, provides a faithful replica of the original separation and facilitates subsequent biochemical analysis. After being transferred to the support medium the immobilized protein or nucleic acid fragment is localized by the use of probes such as antibodies or DNA that specifically bind to the molecule of interest. Finally, the position of the probe that is bound to the immobilized target molecule is visualized usually by autoradiography.

Uses of western blot:
- ❖ Supplementary test to confirm the result of ELISA or other immunoassay having higher sensitivity.
- ❖ To detect antibodies in various diseases such as HIV, Lyme's disease, Herpes simplex virus infection, cysticercosis, hydatid disease, toxoplasmosis, etc.

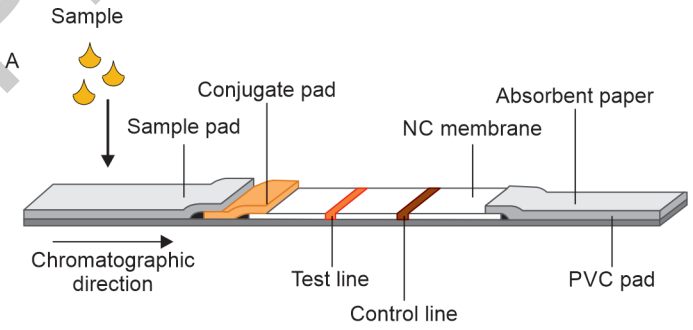


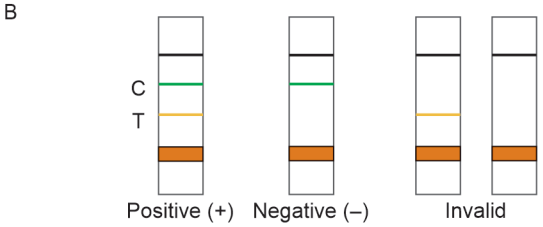
RAPID TESTS

These tests are qualitative, single step test techniques that can be performed without any specialized equipment, laboratory or training within 10–20 minutes. They are also called point of care tests (POCT) as these tests can be performed at the bedside.

Principles

1. **Lateral flow assays,** e.g., immunochromatographic tests: This test system (available in two formats, i.e., cassette or strip) consists of a nitrocellulose membrane and an absorbent pad. The nitrocellulose membrane is coated at two places in the form of lines—a test line, coated with monoclonal antibody targeted against the test antigen and a control line, coated with antihuman immunoglobulin. Specific antibodies (tagged with colloidal gold or silver) against the target antigen labeled with chromogenic marker are infiltrated in the absorbent pad lining the sample window where the test serum is added. Both antigen-antibody-colloidal gold complex as well as free colloidal gold labeled antibodies move laterally along the nitrocellulose membrane.
   Test line: The antigen labeled antibody complex is immobilized by binding to monoclonal antibodies in the test line to form a colored band.
   Control line: Free colloidal gold-labeled antibodies move further and bind to the antihuman immunoglobulin to form a color control band.





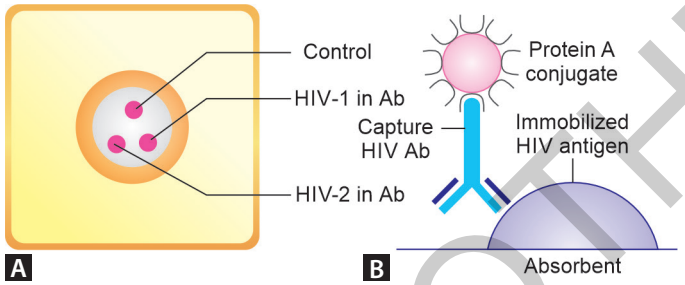
2. **Flow through assays,** e.g., TRI-DOT assay. These assays are different from lateral flow assays in the following aspects:
   a. Protein A is used for labeling antibody instead of gold conjugate.
   b. The sample flows vertically through the nitrocellulose membrane.

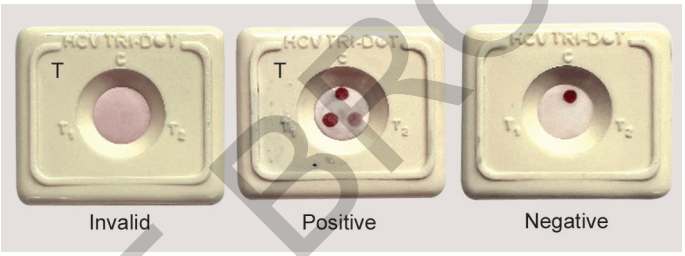
   This assay is available in cassette format only consisting of nitrocellulose membrane and absorbent pad. The nitrocellulose membrane is coated in two or three regions, a control region coated with antihuman immunoglobulin and test regions coated with antigen-specific to the testing antibody. The specimen and buffer are added from the top subsequently which pass through the membrane and if the antibodies are present, they bind to the coated specific antigen and form a color dot at the test region.

   Test dot: Protein A conjugate binds to the Fc portion of the test antibodies, if present to give colored dot.

   Control dot: Protein A binds to any antibody present in the specimen and antibody-protein A complex further binds to the antihuman antibody to give a colored dot. This dot must be present in all the performed tests irrespective of the presence of the test antibody.

   Use: For detection of antibodies, e.g., in infections with HIV and HCV.



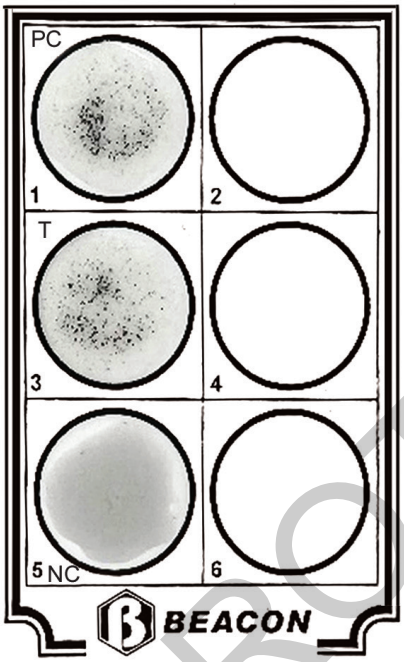


SOME LABELED IMMUNOASSAYS

Immunoassay	*Molecule used for labeling*	*Visible effect*
Immunofluorescence assay (IFA)	Fluorescent dye	Emits light, detected by fluorescent microscope
Enzyme-linked immunoassay (ELISA)	Enzyme	Color change detected by spectrophotometer
Enzyme-linked fluorescent assay (ELFA)	Enzyme	Fluorometric detection
Chemiluminescence immunoassay (CLIA)	Chemiluminescent compounds	Emits light, detected by luminometer
Radioimmunoassay (RIA)	Radioisotopes	A standard curve is plotted
Immunohistochemistry (IHC)	Enzyme or fluorescent dye	Color change (naked eye) or fluorescence (fluorescent microscope)
Western blot	Enzyme	Color band (naked eye)
Immunochromatographic lateral flow assay	Colloidal gold or silver	Color band (naked eye)
Flow through assay	Protein A conjugate	Color band (naked eye)

QUESTIONS

1. **A commercial sex worker with a history of painless, indurated genital lesions a few months back which healed spontaneously now presented to the outpatient department with rashes on the palms and soles. Screening for sexually transmitted diseases was performed as demonstrated below.**



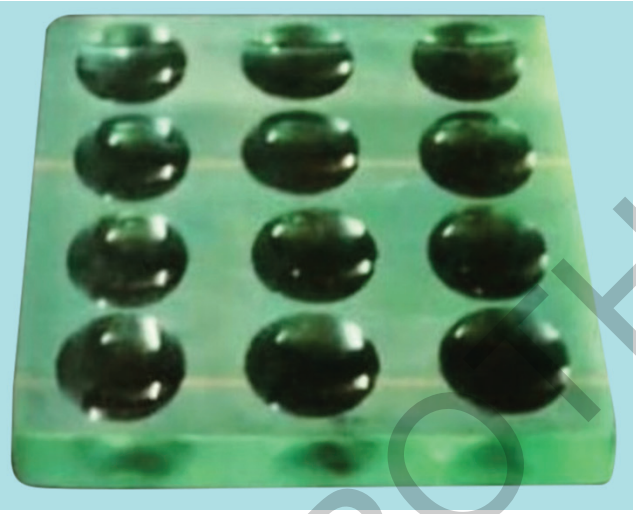
   a. **Identify the test and in which condition it is done.**

   b. **Interpret the test results.**

   c. **Name the antigen used in this test.**

d. Which type of antigen-antibody reaction does this test denote?

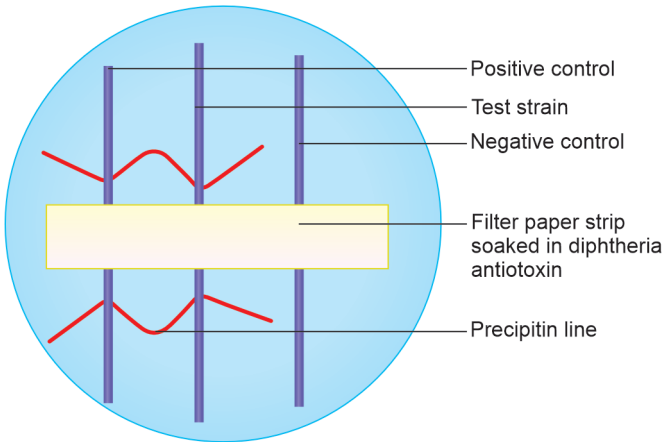
2.



a. Identify the tile in the picture and write down its use.

b. Tabulate the differences between VDRL and RPR tests.

3. A 3-year-old child's throat swab was sent for Albert's staining which showed multiple characteristic purple metachromatic granules on either side of green bacilli. A serological test was performed for confirmation (given below).



a. Identify the test and the clinical condition for which this test can be performed.

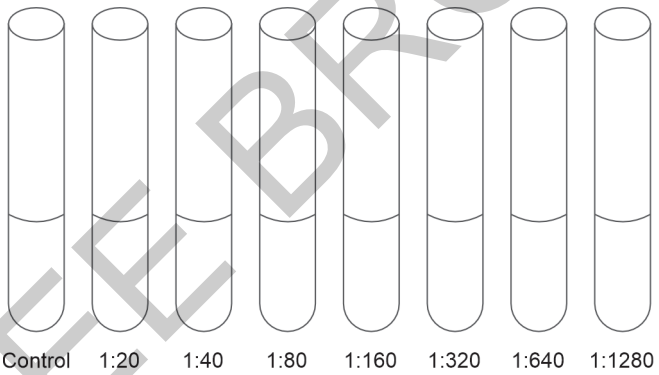
b. Interpret the result of the test strain in the above picture.

c. Which type of antigen-antibody reaction does this test denote?

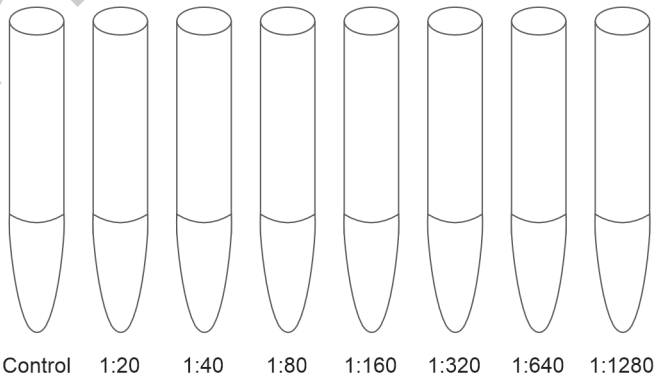
4. A young adult male was admitted to the hospital with step-ladder pattern of fever since 10 days. A provisional clinical diagnosis of enteric fever was made. The following serological examination was performed.

Tube Test

"O" Ag Agglutination



"H" Ag Agglutination



a. Identify the test.

b. Which type of antigen-antibody reaction does this test denote?

c. How to interpret positive and negative results? Demonstrate pictorically in the test tubes above, titres of TO 1:160 and TH 1:320.

d. Name another test based on the same principle.

5. A young child with history of recurrent sore throat was admitted to the hospital with complaints of migratory joint pains. His serum was sent to the laboratory and the below test was performed.

A. Negative control B. Positive control C. Test

a. Identify the test and interpret the result.

b. What is the principle of the test?

c. What is the nature of antigen used in the test?

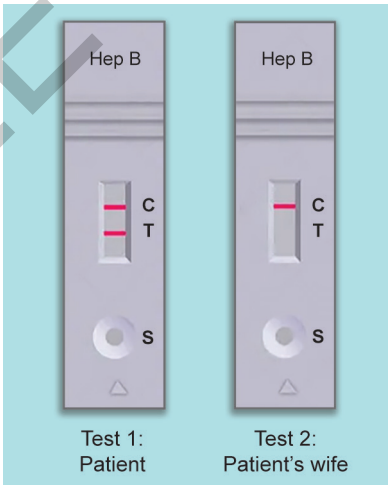
d. Enlist other tests based on the same principle.

6. **A 25-year-old male with history of multiple sex partners is admitted with complaints of unexplained fever, progressive loss of weight, persistent diarrhea and generalized lymphadenopathy for the past 6 months. One of the tests done is demonstrated below.**

a. **Identify the test and write its principle.**

b. **Interpret the test result.**

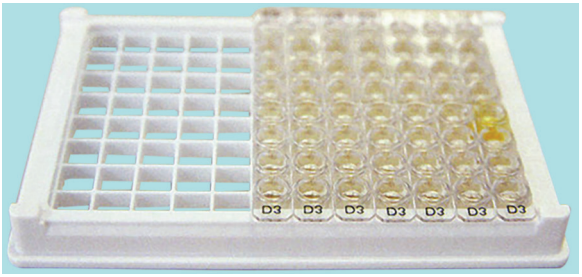
7. **A patient with history of multiple blood transfusions was admitted in the Medicine ward with complaints of jaundice. Examination revealed moderate hepatomegaly. A POCT test was performed at the bedside along with his wife's sample. The test is represented below.**



a. **Identify the test and write its principle.**

b. **Interpret the test results.**

8. Anti-HCV antibodies screening was performed in the Blood Bank. The picture of the test performed is given below.



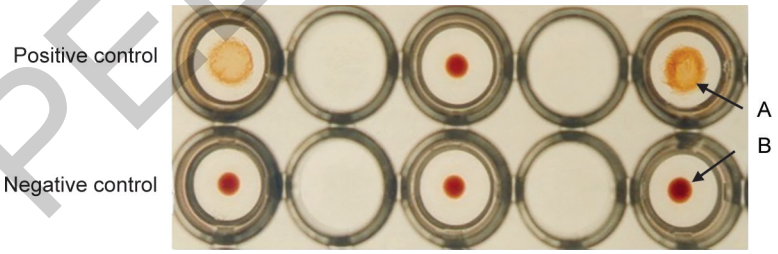
a. Identify the test.

b. What are the various types of this test method?

c. Enlist the advantages and disadvantages of this test method.

d. Enlist the applications of this test format.

9. A 27-year-old female, 16 weeks pregnant visiting the antenatal clinic and a 35-year-old male visiting the Dermatology OPD tested positive for RPR/VDRL test. To confirm the diagnosis, the below test was performed the results of which are seen at A and B respectively.



a. Identify the test.

b. What is the principle of this test?

c. Give your interpretation for both A and B.

Concise Workbook in Practical Microbiology

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Sheethal S is a Clinical Microbiologist and an Infection Preventionist. She completed her MBBS from Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, Karnataka, India, and MD (Microbiology) from Karnataka Institute of Medical Sciences, Hubballi, Karnataka. She is also a recipient of the Post Graduate Diploma in Infection Prevention and Control (PGDIPC), awarded jointly by the University of Hyderabad (UoH) and Infection Control Academy of India (IFCAI). She is a big dreamer and lets nothing stop her in achieving what she sets her mind to. She has pursued a Fellowship in Clinical Microbiology and Infectious Diseases from Deenanath Mangeshkar Hospital, Pune, Maharashtra, India. She has worked as a Clinical Microbiologist and Infection Control Officer in HCG and as an Assistant Professor and Infection Control Officer in the Department of Microbiology in GMCH, Udaipur, Rajasthan. Currently, she holds the post of Consultant Microbiologist and Infection Control Officer at Yashoda Hospital, Hyderabad, Telangana, India. In her short career in the field as an astute teacher, she has mentored MD Microbiology postgraduates, MSc Medical Microbiology students, and MBBS undergraduates. Currently, she has 11 published articles. As a zealous microbiologist, this book is just a small effort from her to help II MBBS students who might find Clinical Microbiology too fleeting to comprehend.

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