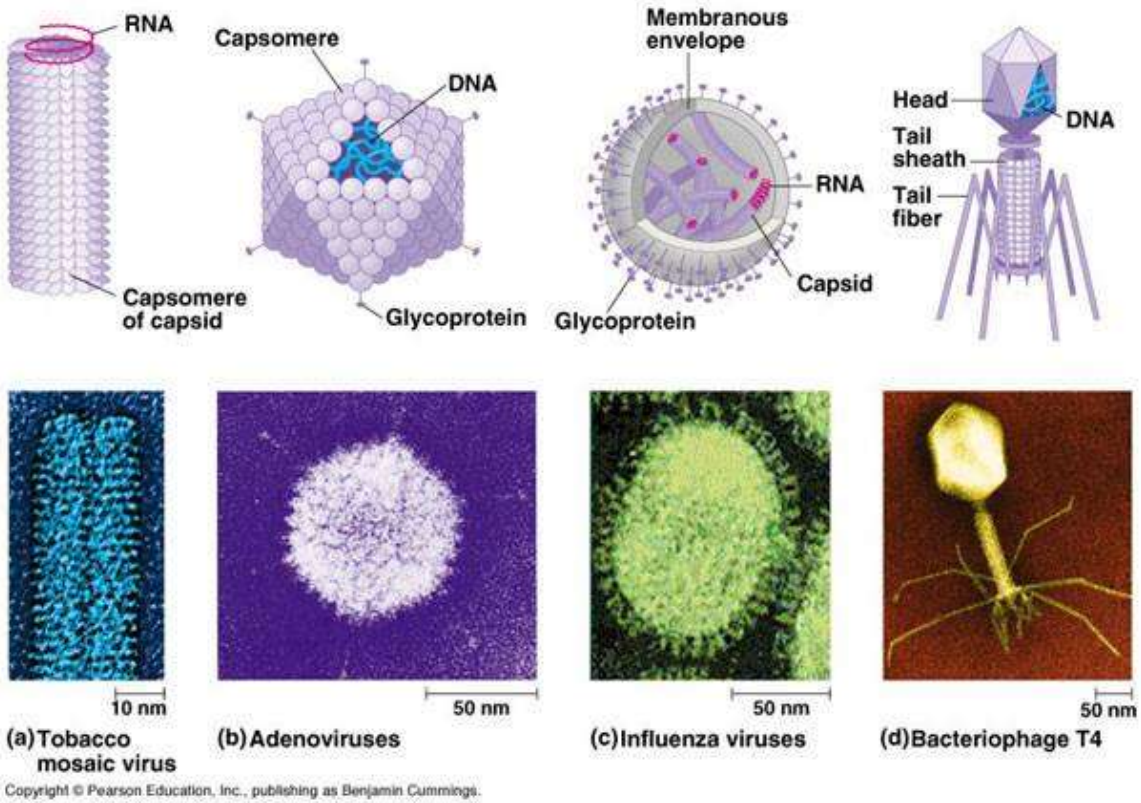
What is a virus ?

Viruses are complexes consisting of protein and an RNA or DNA genome. They lack both cellular structure and independent metabolic processes. They replicate solely by exploiting living cells based on the information in the viral genome.

- Viruses are obligate intracellular parasites.
- Biological structure containing DNA or RNA genome .
- Double stranded (ds) or single stranded (ss).
- Surrounded by a capsid (protein coat).
- The nucleic acid and capsid are termed nucleocapsid.
- Some viruses have an envelope.
- The envelope is a phospholipid bilayer membrane that was obtained from the cell in which the virus arose.



Laboratory Safety

Laboratory safety rules are a major aspect of every clinical lab.

-Each student in clinical laboratory must follow specific safety rules and procedures.

Laboratory Safety Rules

1 - Wear protective clothing e.g. goggles, gloves, lab coat and shoes.

2 - Laboratory personnel should not wear sandals , Jewelry , and Loose or Baggy clothing

3 - Avoid touching objects (e.g. , pencils , cell phones , door handles) while wearing gloves.

4 - Pencils or any other materials should never be placed in your mouth.

5 - Caution must be taken when using gas burners. Be sure gas burners are turned off when finished.

6 - Long hair must be tied back or covered to minimize fire hazard or contamination of experiments.

7 - Do not eat food or drink water in the lab. Do not use lab glassware as food or water containers.

8- Protect your hands safety : wash hands after every lab Handle glassware , sharp tools and heated containers carefully.

9 - Electrical safety : unplug electrical equipment after use .

10 - Chemical safety : never touch , taste or smell a chemical..

11 - Do not engage in practical jokes in the lab.

12 - Keep nonessential books and clothing far away from your work area .

13 - Dispose of waste products according to instructions .

14 - Report all accidents no matter how minor to your supervisor.

15- Lab Safety Equipment : Fire Blanket & Fire Extinguisher .

Lab Safety Symbols (Signs)

Equipment needed for virology lab

The specific materials and equipment needs of virology lab can be divided into three categories:

Essential equipment: you cannot perform job without them that includes:

Laminar flow: include two types of laminar flow hoods:

A. vertical hood (biological safety cabinet) used for hazardous organisms.

B. Horizontal hood used for media preparation.

Both of types are provides with high efficiency filter to continuous filtrated of air from particles, and UV light, which is germicidal lamp to give complete sterility.

Incubators: It should be large enough, safe, fitted with thermostat:

A. Co2 incubator: used for propagation of tissue culture.

B. Egg incubator: used for incubation of inoculated egg

C. Traditional incubator: used for other traditional incubations.

Sterilizer: include humid/pressure sterilizer (e.g. autoclave), and dry sterilizer (e.g. oven).

Refrigerator and freezer: either deep freezer or liquid N2 for storage continuous cell line and kept various reagents.

Microscope: Compound, inverted, and electron microscope used to visualize of cells or viruses practical.

Centrifuges: includes cooling, ultracentrifuge, microfuge, and traditional centrifuge.

Identification of virus

- ❖ All organisms are affected by viruses because viruses are capable of infecting and causing disease in all living species. Viruses affect plants, humans, and animals as well as bacteria.
- ❖ Viruses are considered obligate intracellular parasites since they require living host cells to replicate.
- ❖ Identifying the virus associated with clinical signs and symptoms.

Procedures most commonly used to identify virus include:

1. Detection of a meaningful immune response to the virus (antibody or cell-mediated) by immunologic assays.

• Hemagglutination inhibition (HI)

• Enzyme linked immunosorbent assay (ELISA)

• Indirect fluorescent antibody (IFA)

• Complement fixation (CF)

• Latex agglutination (LA).

2. Identification of the virus by staining of specimens or sections of tissue (light and electron microscopy).

3. Isolation and identification of the agent (cell culture or fertile eggs).

4. Detection of viral nucleic acid (PCR technique using specific primers and probes).

Collection of viral specimens

1. All clinical specimens must be collected in clean sterile containers, with it closed well & avoid contamination of the outside of the container.
2. Put labeling of the all specimens include name, age, sex of the patients, type of specimens and identify their source (nose swab, throat swab…. etc.), date and time of collection.
3. In general, specimens for virus isolation should be collected as soon as possible after the appearance of the symptoms that is when the concentration of the virus is at its highest (within 4 days after onset of illness).
4. Collect an adequate amount of specimen, inadequate amounts of specimen may yield false-negative results.
5. Specimens may be rejected for many reasons including the following:
   - **a)** Hemolysis
   - **b)** Insufficient quantity
   - **c)** Prolonged transport
   - **d)** Unlabeled specimen.

Types of specimens that used for virus infections:

- **Blood:** 5-10 ml of blood is sufficient and put in n an anticoagulant tube, it used to detect Cytomegalovirus, enterovirus and adenoviruses.

  Heparinized, citrated, or ethylenediaminetetraacetic acid (EDTA) anticoagulated blood is acceptable for CMV detection.

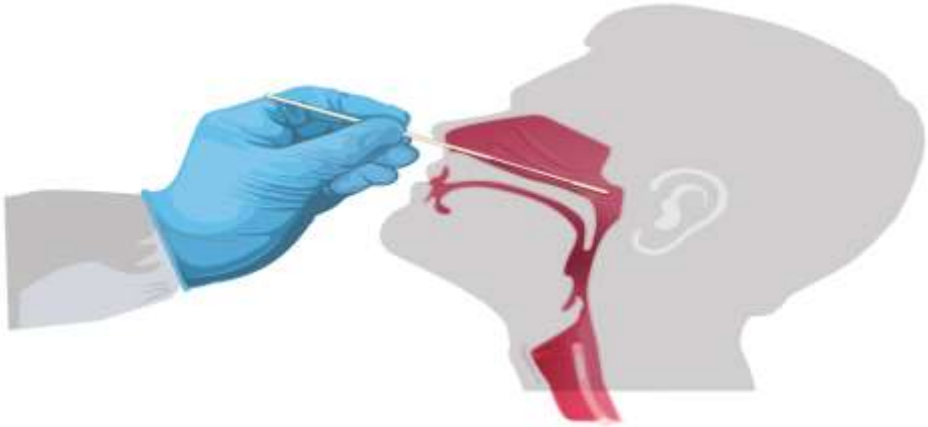
  EDTA should be used for samples collected for nucleic acid testing, because other anticoagulants may interfere with the enzyme functions required for

PCR amplification. Serum may be used for serologic tests and nucleic acid assays.

- **Nasopharyngeal swabs & Throat swabs :** are used for the detection of respiratory viruses infection such as influenza virus (A & B) or parainfluenza virus& coronavirus.

  Throat specimens are collected with a dry, sterile swab by passing the swab over the inflamed, vesiculated, or purulent areas on the posterior pharynx.

  Nasopharyngeal secretion specimens are collected by inserting a swab with a flexible shaft through the nostril into the nasopharynx or by washing and collecting the secretions by rinsing with a bulb syringe and 3-7 mL of buffered saline.



- **Urine:** in the morning, samples are collected in an amount of 5 ml and placed in a clean tube, it used to detect Adenoviruses, CMV, mumps, rubella & measles.

  The urine pH and contaminating bacteria may interfere with viral replication. Virus recovery is improved by centrifugation or filtering to remove contaminants and neutralizing the pH with a 7.5% solution of sodium bicarbonate.

- **Stool specimens:** Used to detect entero viruses, enteric adenoviruses and rotavirus.

  Stool and rectal swabs of fecal specimens are used to detect rotavirus, enteric adenoviruses (serotypes 40 and 41), to investigate poliomyelitis and other diseases caused by enteroviruses.

  In general, stool specimens are preferable to rectal swabs and should be required for rotavirus and enteric adenovirus testing.

  Rectal swabs are acceptable for detecting enteroviruses in patients suspected of having an enteroviral disease, such as aseptic meningitis.

  A stool sample is preferred over a rectal swab because of the potential for decreased viral recovery from a small sample size.

  Five to 10 mL of freshly passed diarrheal stool or stool collected in a diaper from young infants is sufficient and preferred for rotavirus and enteric adenovirus detection.
- **Genital specimens:** Often are required for detection of HSV and human papillomavirus (HPV).

  Genital swabs should be used for ulcerations.

  Cervical specimens may be collected using a swab or brush.
- **Tissue:** Useful for detecting viruses that commonly infect the lungs (CMV, influenza virus, adenovirus), brain (HSV), and gastrointestinal tract (CMV).

  Specimens are collected during surgical procedures.

  Fresh tissue is preferred for nucleic acid assays, but formalin-fixed and paraffin embedded tissues may be used after removal of the paraffin (deparaffinization) and extraction.
- **Cerebrospinal fluid**: put 1 ml in sterile screw-cap tube**,** it used to diagnosis the coxsackievirus & rabies virus.

Viral Specimen transport and storage:

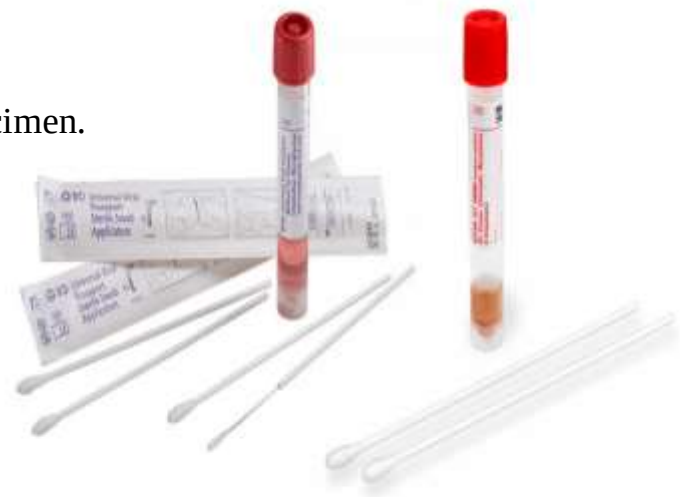
Collection the specimens for viruses detection and transported immediately to the laboratory by using viral transport media (VTM) and should be placed in ice, specimens for viral isolation should not be kept at room or higher temperature. For storage up to 4 days, hold specimen at 4°C. Storage for 6 or more days should be at – 20°C or preferably at – 70°C.

Some antibiotics (e.g penicillin, streptomycin & nystatin) should be added to viral transport media, especially when contamination with microbial flora is expected, Examples of successful transport media include: Stuart's medium & Amie's medium.

Viral Transport Media (VTM)

VTM used to:

1. preserve viral infectivity within the specimen.
2. prevent specimen from drying.
3. stop the growth of bacteria and fungi.



Viral Transport Media (VTM)

VTM contains:

- ✓ **Saline** (adequate ion concentration).
- ✓ **Proteins** (albumine or gelatine).
- ✓ **Buffer** (adequate pH).
- ✓ **Antibiotics and fungicides.**

Specimen Storage:

1. All specimens collected for detection of viruses should be processed by the laboratory immediately.

2. Specimens for viral isolation should not be allowed to sit at room or higher temperature.

3. Specimens should be kept cool (4°C) and immediately transported to the laboratory.

4. If a delay in transport is unavoidable, the specimen should be refrigerated, not frozen, until processed.

5. Every attempt should be made to process the specimen within 12 to 24 hours of collection.

6. Under unusual circumstances, specimens may need to be held for several days before processing.

For storage up to 5 days, specimens are held at 4°C. Storage for 6 days or longer should be at –20° or preferably–70°C.

7. Specimens for freezing should first be diluted or emulsified in viral transport medium.

Direct Examination: Electron microscopy for virus identification

What is an electron microscope?

Electron Microscopes are scientific instruments that use a beam of highly energetic electrons to examine objects on a very fine scale. In electron microscopy, high resolution images are the result of using electrons as the source of illumination. The resolution is about 0.01 nanometers (magnification up to 300,000X).

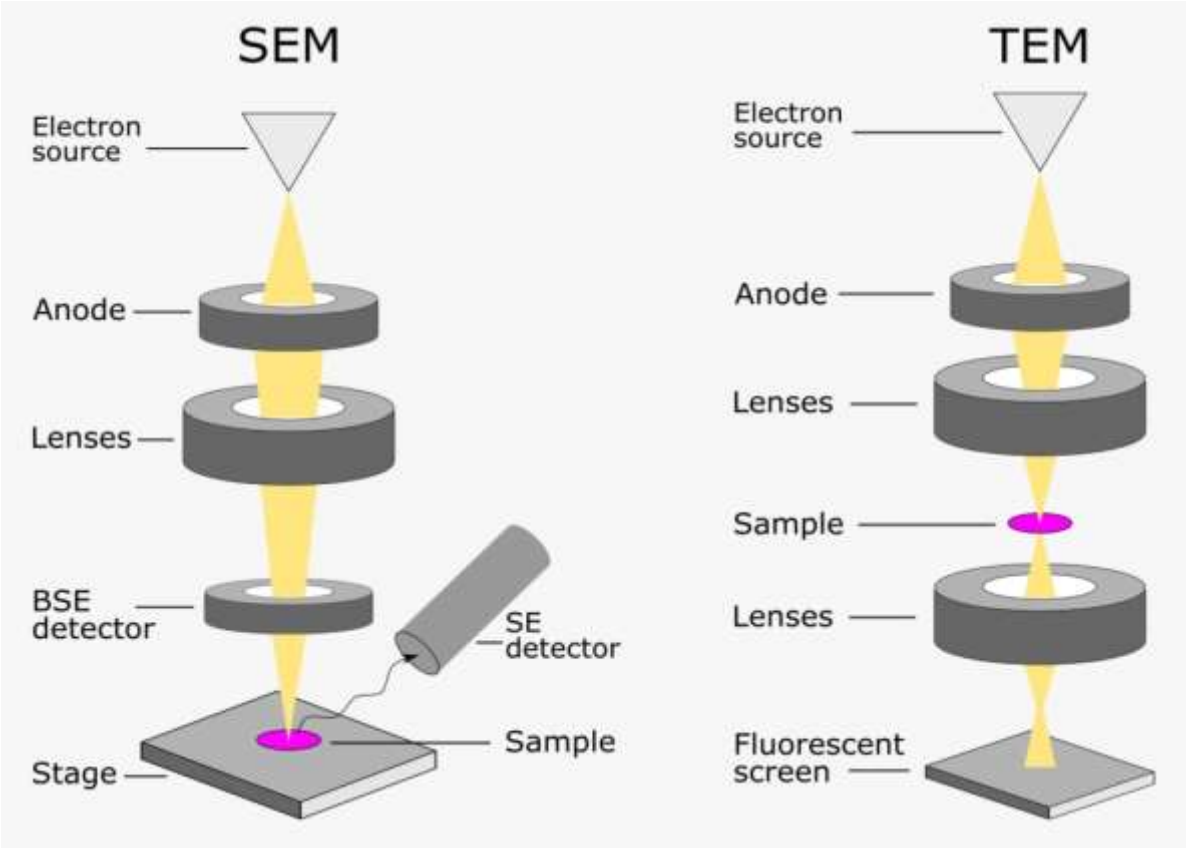
Most microscopes you may have seen are light microscopes, they use a lightbulb to illuminate the specimen.

There are two main types of electron microscopes (EM)

- Transmission electron microscope (TEM)
- Scanning electron microscope (SEM).

TEM

SEM



Transmission Electron Microscope (TEM)

The TEM is a little different than the SEM. A tungsten filament is used to make an electron beam in a vacuum chamber, then, instead of bouncing off the surface of the sample, the beam passes through it. The sample is a very thin slice of material, less than 100nm. When the electrons pass through, they hit a phosphor screen, CCD or film and an image is made.

Scanning Electron Microscope (SEM)

The main parts to an SEM are: source of electrons, a column for them to travel with electromagnetic lenses, an electron detector, sample chamber, and a computer and display to view the images.

A high energy beam of electrons is aimed at the sample. As they interact with the sample, secondary electrons and X-rays are produced. Those signals are collected by the detectors and an image is formed on the computer screen.

Virus diagnosis by electron microscopy: relies on the detection and identification of viruses on the basis of their characteristic morphology.

Advantage of virus diagnosis

The ability to visualize the virus. Speed is another advantage of (EM) as the specimen can be processed within minutes of receipt.

Disadvantage of virus diagnosis

Its inability to examine multiple specimens coincidentally. Secondly, there must be a minimum number of virus particles present (around 106 virus particles per ml for detection). Some viruses may give a non distinct morphological appearance which may make detection very difficult.

- Many investigations of the structure of virions or of virus-infected cells involve electron microscopy.
- Large magnifications are achievable with a transmission electron microscope but the specimen, whether it is a suspension of virions or an ultrathin section of a virus infected cell, must be treated so that details can be visualized.

Negative staining techniques generate contrast by

- Using heavy-metal-containing compounds, such as potassium phosphotungstate and ammonium molybdate.
- In electron micrographs of virions the stains appear as dark areas around the virions, allowing the overall virion shape and size to be determined.

- Further structural detail may be apparent if the stains penetrate any crevices on the virion surface or any hollows within the virion.
- Negative staining techniques have generated many high quality electron micrographs, but the techniques have limitations, including structural distortions resulting from drying.

There are two types of (EM) methods:

1. Direct methods: negative staining is normally used which requires little special equipment, in contrast to thin sectioning techniques.

2. Immune electron microscopy (IEM): is a means of increasing the sensitivity and specificity of (EM) and is particularly useful in the following situations:

- The number of virus particles present in small.
- Many different viruses have different morphology.

In an outbreak situation where the pathogens responsible has been identified, so that it may be useful to go back to look at the negative specimens again with IEM.

There are two types of IEM:

1. Simple IEM: where the specimen is inoculated with specific antibody before staining in the hope that the antibody will agglutinate the specimen.

2. Solid phase IEM (SPIEM): where the copy grid is coated with specific antibody which is used to capture virus particles from the specimen.

Isolation and Cultivation of Viruses

Viruses are obligate intracellular parasites so they depend on the host for their survival. They cannot be grown in non-living culture media or on agar plates alone, they must require living cells to support their replication. Viruses can be grown in vivo (within a whole living organism, plant, or animal) or in vitro (outside a living organism in cells in an artificial environment).

The primary purpose of virus cultivation is:

a. To isolate and identify viruses in clinical samples.
b. To prepare vaccine and antigen
c. To study viral expression and replication (viral structure, genetics).
d. To study the pathogenesis of viral diseases and of viral oncogenesis (effects on a host cell).

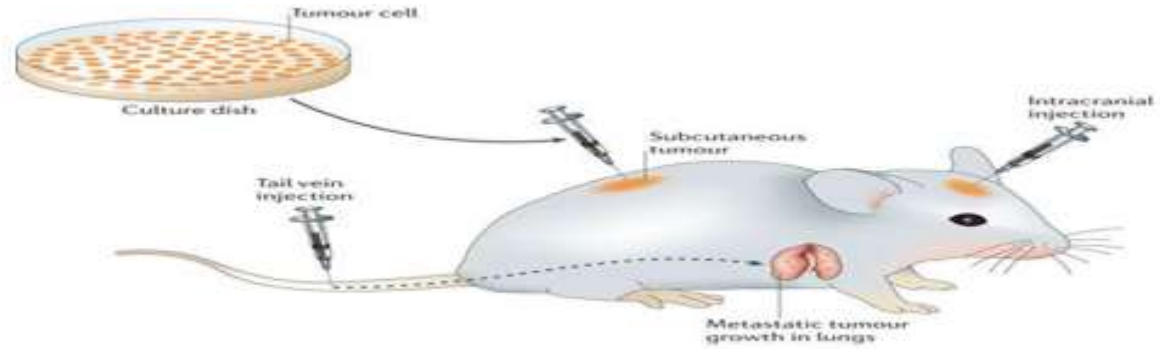
There are three methods of Viruses Cultivation:

- Inoculation of virus into animals.
- Inoculation of virus into embryonated eggs.
- Tissue culture (Cell Culture).

Animal Inoculation

Viruses which are not cultivated in embryonated egg and tissue culture are cultivated in laboratory animals such as mice, guinea pig, hamster, rabbits, and primates are used.

- The selected animals should be healthy and free from any communicable diseases.
- Laboratory animals are widely used for routine cultivation of virus; they play an essential role in studies of viral pathogenesis.
- Live animals such as monkeys, mice, rabbits, guinea pigs, ferrets are widely used for cultivating virus.
- Monkeys were used for the isolation of Poliovirus. But due to their risk to handlers, monkeys find only limited applications in Virology.
- Mice are the most widely employed animals in virology. The different routes of inoculation in mice are intracerebral, subcutaneous, intraperitoneal or intranasal.
- After the animal is inoculated with the virus suspension, the animal is observed for signs of disease, visible lesions or is killed so that infected tissues can be examined for virus.



Advantages of Animal Inoculation

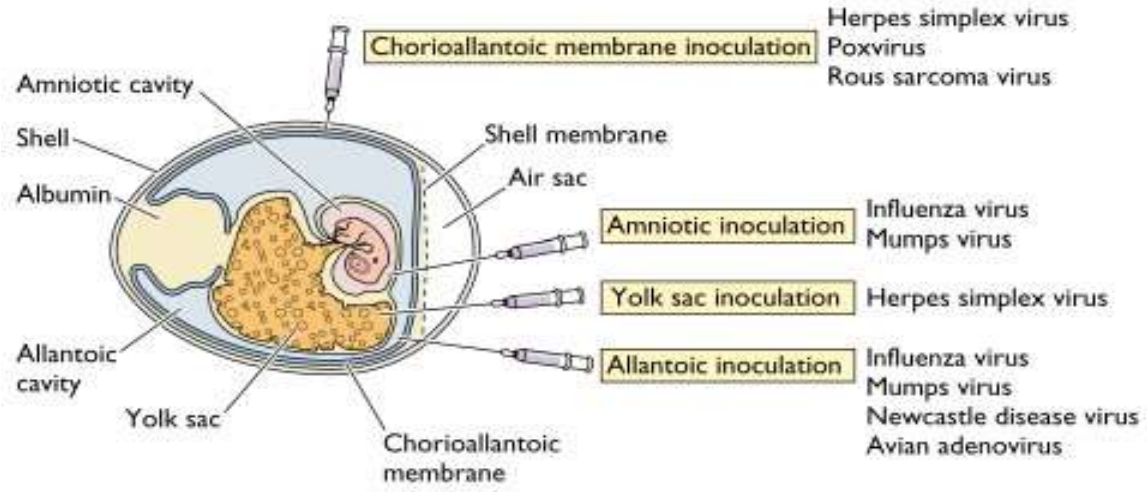
1. Animal inoculation may be used as diagnostic procedure for identifying and isolating a virus from a clinical specimen.
2. Mice provide a reliable model for studying viral replication.
3. Primary isolation of certain viruses.
4. Gives unique insight into viral pathogenesis and host virus relation.
5. Used for the study of immune responses, epidemiology and oncogenesis.
6. Production of antibodies can be identified.

Disadvantages of Animal Inoculation

1. Expensive and difficulties in the maintenance of animals.
2. Difficulty in choosing animals for a particular virus
3. Some human viruses cannot be grown in animals or can be grown but do not cause disease.
4. Mice do not provide models for vaccine development.
5. It will lead to the generation of escape mutants
6. Issues related to animal welfare systems.

Inoculation Into Embryonated Egg

- Good pasture in 1931 first used the embryonated hen's egg for the cultivation of virus.
- The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used.
- Viruses are inoculated into chick embryo of 7-12 days old.
- For inoculation, eggs are first prepared for cultivation, the shell surface is first disinfected with iodine and penetrated with a small sterile drill.
- After inoculation, the opening is sealed with gelatin or paraffin and incubated at 37°c for 2-4 days.
- After incubation, the egg is broken and the virus is isolated from tissue of egg.
- Viral growth and multiplication in the egg embryo are indicated by the death of the embryo, by embryo cell damage, or by the formation of typical pocks or lesions on the egg membranes.
- Viruses can be cultivated in various parts of egg-like:
    1. Chorioallantoic membrane.
    2. Allantoic cavity.
    3. Amniotic cavity.
    4. Yolk sac.

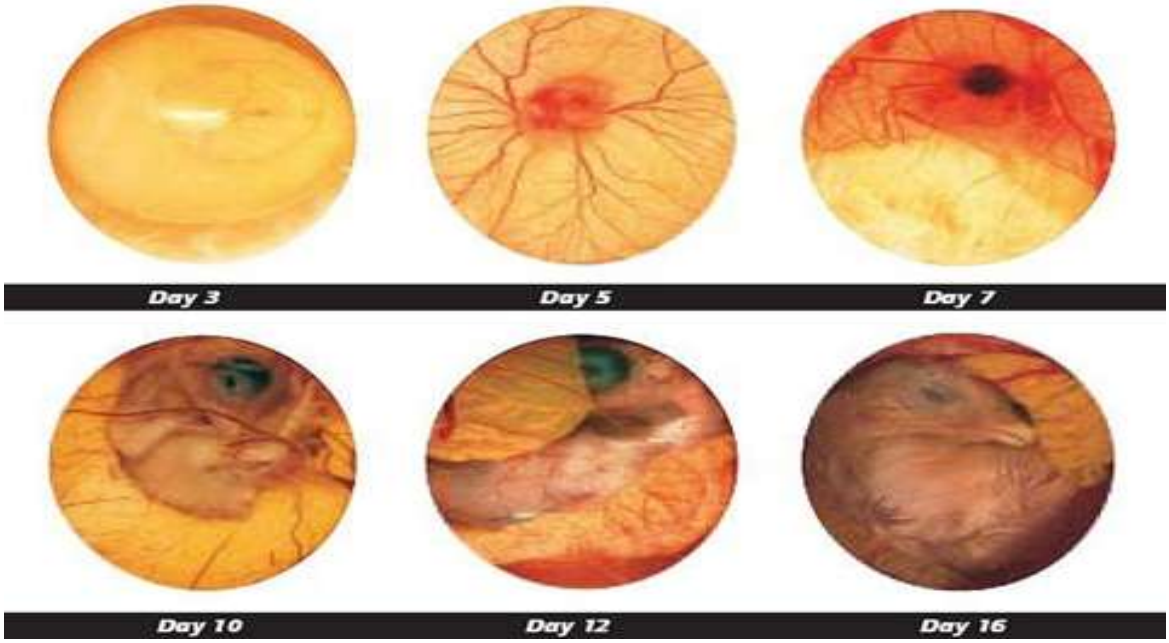


Advantages of Inoculation into embryonated egg

1. Widely used method for the isolation of virus, growth and vaccine production.
2. Ideal substrate for the viral growth and replication.
3. Isolation and cultivation of many avian and few mammalian viruses.
4. Cost effective and maintenance is much easier.
5. Less labor is needed.
6. The embryonated eggs are readily available.
7. Sterile environment (free from contaminating bacteria and many latent viruses).
8. Inability to antibody produce.

Disadvantages of Inoculation into embryonated egg

The site of inoculation for varies with different virus. That is, each virus has different sites for their growth and replication.



Cell Culture (Tissue Culture)

There are three types of tissue culture; organ culture, explant culture and cell culture. Explant culture is rarely done.

- Cell culture is mostly used for identification and cultivation of viruses.
- Cell culture is the process by which cells are grown under controlled conditions.
- Cells are grown in vitro on glass or a treated plastic surface in a suitable growth medium.
- At first growth medium, usually balanced salt solution containing 13 amino acids, sugar, proteins, salts, calf serum, buffer, antibiotics and phenol red are taken and the host tissue or cell is inoculated.
- On incubation the cell divides and spread out on the glass surface to form a confluent monolayer.

Types of cell culture

There are three basic types of cell cultures:

A. Primary cell cultures:

- ✓ These are normal cells derived from animal or human cells (Prepared from cells obtained directly from the tissues).
- ✓ Cut into single cells (by enzymatic digestion or mechanical dispersion)
- ✓ They are able to grow only for limited time and cannot be maintained in serial culture (5-10 passage in culture).
- ✓ They are used for the primary isolation of viruses and production of the vaccine.
- ✓ **Examples:** Monkey kidney cell culture, Human amnion cell culture, Heterogeneous population of cells.

B. Diploid cell lines (Semi-continuous cell lines):

- ✓ These are normal cells derived from animal or human cells.
- ✓ They can be sub-cultured up to 50-100 passages by serial transfer following senescence and the cell strain is lost.
- ✓ They are used for the isolation of some fastidious viruses and the production of viral vaccines.

- ✓ Examples: Human embryonic lung strain, Rhesus embryo cell strain, Homogeneous population of cells.

C. Continuous cell lines (Heteroploid cultures):

- ✓ They are derived from cancer cells.
- ✓ They can be serially cultured indefinitely so named as continuous cell lines
- ✓ They can be maintained either by serial subculture or by storing in deep freeze at -70°c.
- ✓ Due to derivation from cancer cells, they are not useful for vaccine production, while its importance lies in the identification and cultivation of the virus.
- ✓ Examples: The established cell lines most frequently employed are HeLa (Human Carcinoma of cervix cell line), HEP-2 (Human Epithelioma of larynx cell line), Vero (Vervet monkey) kidney cell lines, BHK-21 (Baby Hamster Kidney cell line).

Notes: Primary cell culture are widely acknowledged as the best cell culture systems available since they support the widest range of viruses and usually retain many different characteristics of the cell in vivo Disadvantages. However, they are very expensive, the preparation of primary cultures is labor intensive and can be maintained in vitro only for a limited period of time. Continuous cells are the most easy to handle but the range of viruses supported is often limited.

Advantages of cell culture

Relative ease, broad spectrum, cheaper and sensitivity

Disadvantage of cell culture

1. The process requires trained technicians with experience in working on a full-time basis.
2. State health laboratories and hospital laboratories do not isolate and identify viruses in clinical work.
3. Tissue or serum for analysis is sent to central laboratories to identify virus.

Titration of Viruses

Virus Titer – The concentration of viruses ina sample which is a numerical expression of the quantity of virus in a sample. Viral titer is an essential assay for researchers studying infectious disease, pathogenesis, vaccine development, even cell and genetherapy.

Enzyme linked immunosorbent assay (ELISA) for viral identification

- ✓ Is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.
- ✓ The ELISA assay uses the coupling of antigens and antibodies and relies on the specificity and affinity of antibodies for antigens.
- ✓ Specificity is the ability to discriminate among diverse proteins.
- ✓ Affinity is the ability to tightly bind to molecules.
- ✓ In 1971, ELISA was introduced by Peter Perlmann and Eva Engvall at Stockholm University in Sweden. It is a common laboratory technique which is usually used to measure the concentration of antibodies or antigens in blood.
- ✓ ELISA is used in both experimental and diagnostic virology. It's a highly sensitive assay that can detect proteins at the Pico molar and Nano molar range (10^{-12} to 10^{-9} moles per liter). It's the mainstay for the diagnosis of infections by many different viruses.

Principle:

- ✓ ELISAs are typically performed in 96-well polystyrene plates.
- ✓ The serum is incubated in a well, and each well contains a different serum.
- ✓ A positive control serum and a negative control serum would be included among the 96 samples being tested.
- ✓ Antibodies or antigens present in serum are captured by corresponding antigen or antibody coated on to the solid surface.
- ✓ After some time, the plate is washed to remove serum and unbound antibodies or antigens with a series of wash buffer.
- ✓ To detect the bound antibodies or antigens, a secondary antibodies that are attached to an enzyme such as peroxidase or alkaline phosphatase are added to each well.
- ✓ After an incubation period, the unbound secondary antibodies are washed off.
- ✓ When a suitable substrate is added, the enzyme reacts with it to produce a color.

- ✓ This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample. Any, the color (usually blue) appears in the wells. Then add a Stop solution that leads to color change of the blue to yellow.
- ✓ The intensity of color/ optical density is measured at 450nm.
- ✓ The intensity of the color gives an indication of the amount of antigen or antibody.
- ✓ The antibody and antigen concentration to be measured with color intensity is Directly or Inversely proportional.

The required materials:

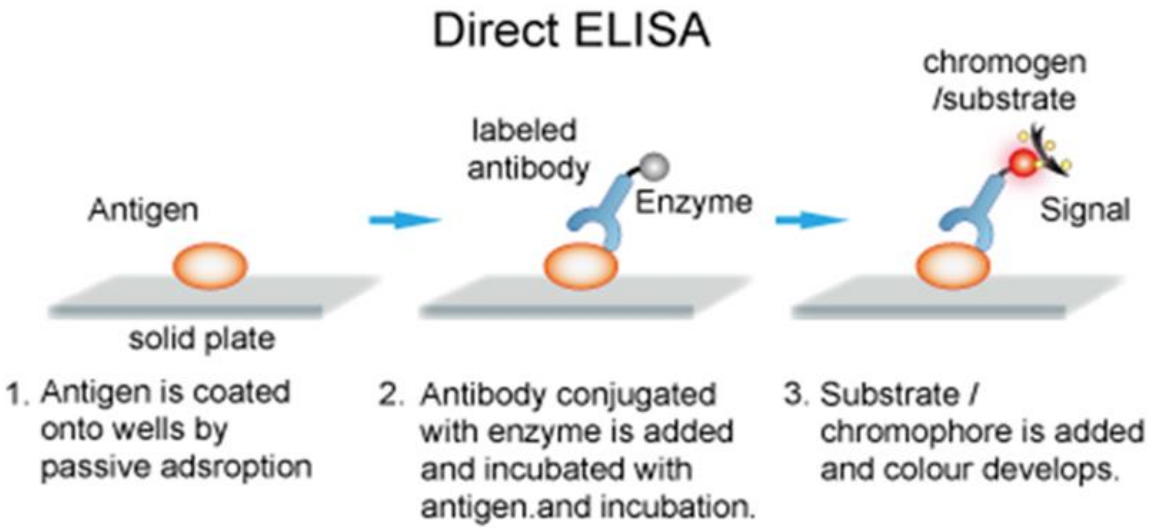
1. Microtiter strips: contain antibody or antibody installed in the wells.
2. Standards: Include:
• Negative control.
• Cut-off standard.
• Weakly positive control.
• Positive control.
3. Enzyme conjugate: Contains antibodies associated with Peroxidase enzyme.
4. TMB substrate solution: contains TMB (Tetramethylbenzidine).
5. TMB stop solution: Contains sulfuric acid (H2SO4).
6. Sample diluents: Contains Phosphate buffer saline + potassium tetraiodomercurate (0.01%).
7. Wash buffer: Contains Phosphate buffer saline.
8. Automatic pipette.
9. Distilled water.
10. Microtiter spectrophotometer or (ELISA reader).
11. Microtiter ELISA washer.

The technique is divided into:

1- Direct ELISA
2- Indirect ELISA
3- Sandwich ELISA
4- Competitive ELISA

1. Direct ELISA

• It uses a primary labeled anti-body that react directly with the antigen.
• It can be performed with the antigen that is directly immobilized on assay plate.



2. Indirect ELISA

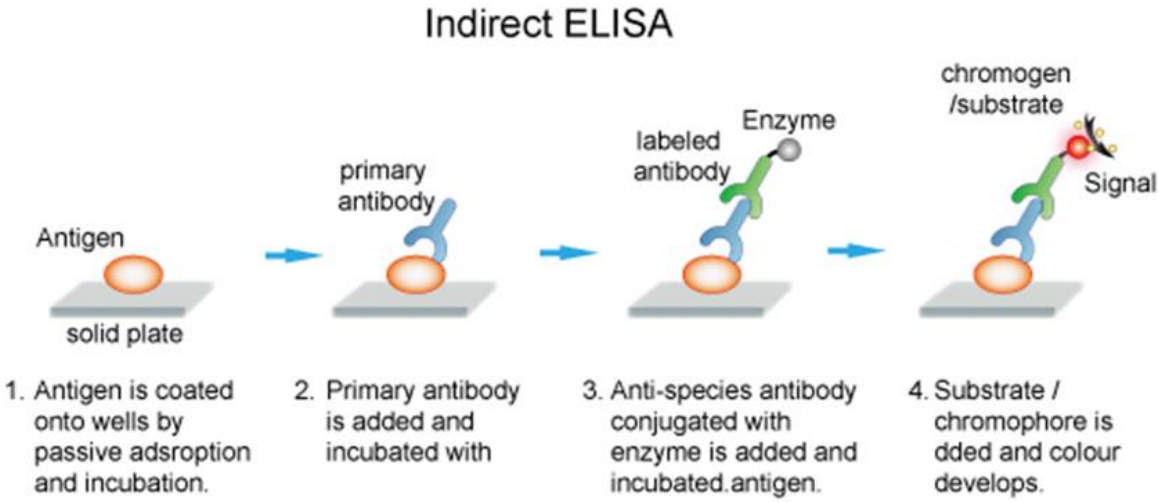
• Antibody can be detected or quantitatively determined with an indirect ELISA.

• Serum or some other sample containing primary antibody (Ab1) is added to an antigen-coated microtiter well and allowed to react with the antigen attached to the well.

• After any free antibody (Ab1) is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody.

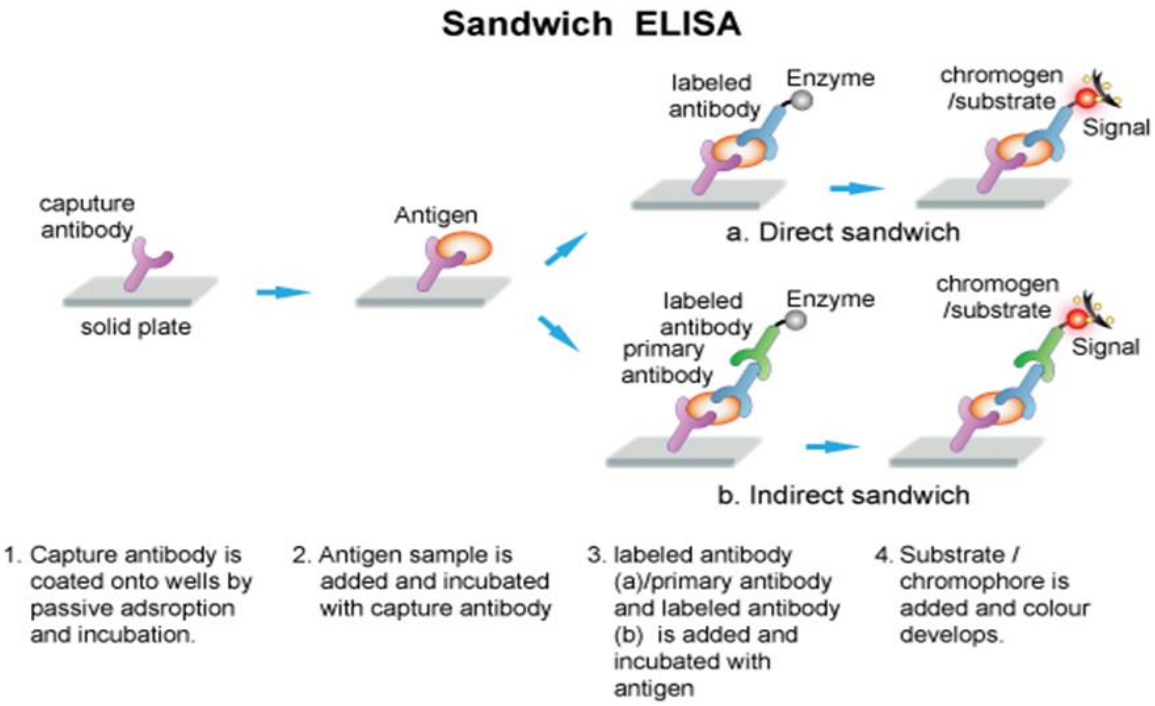
• Any free Ab_2 then is washed away and a substrate for the enzyme is added.

• The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds.



3. Sandwich ELISA

• Antigen can be detected or measured by a 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 colored reaction product is measured.



4. Competitive ELISA

• Another variation for measuring amounts of antigen is 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.

• Addition of an enzyme-conjugated secondary antibody (Ab_2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA.

Quantitative Evaluation:

In order to quantify the specific antibodies or antigens required for the virus:

1. A standard curve is made between optical density (O.D.) and concentrations of standard control solutions,
2. In this curve, the standard curve equation is extracted.
3. Thus, the concentration of the antibody or antigen can be measured based on the optical density measured by the spectra.

Qualitative evaluation

In order to perform a qualitative assessment of viruses is measured by the optical density of the patients serum and compares with optical density for Cut-off standard. Where:

1. If the value of the sample optical density is higher than cut-off: positive sample.
2. If the value of the sample optical density is less than cut-off: negative sample.

Radioimmunoassay (RIA)

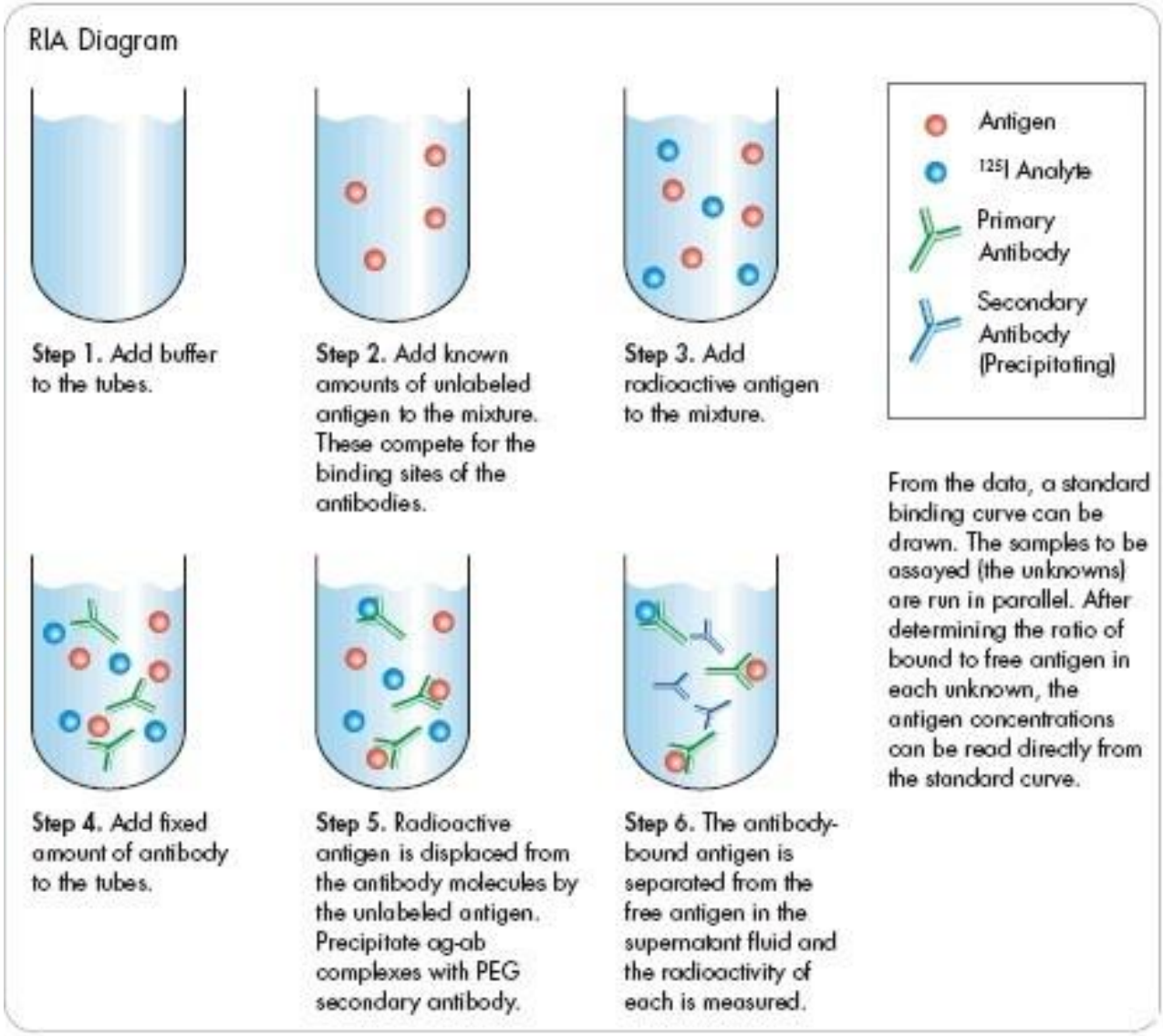
Radioimmunoassay (RIA) techniques use radioactive isotopes as a label (usually I_{125}, H_3 or C_{14}), which emits radiation that can be measured with a beta or gamma counter. It is a very sensitive in vitro assay technique used to measure concentrations of antigens or antibodies, RIA technique is extremely sensitive and extremely specific, requiring specialized equipment.

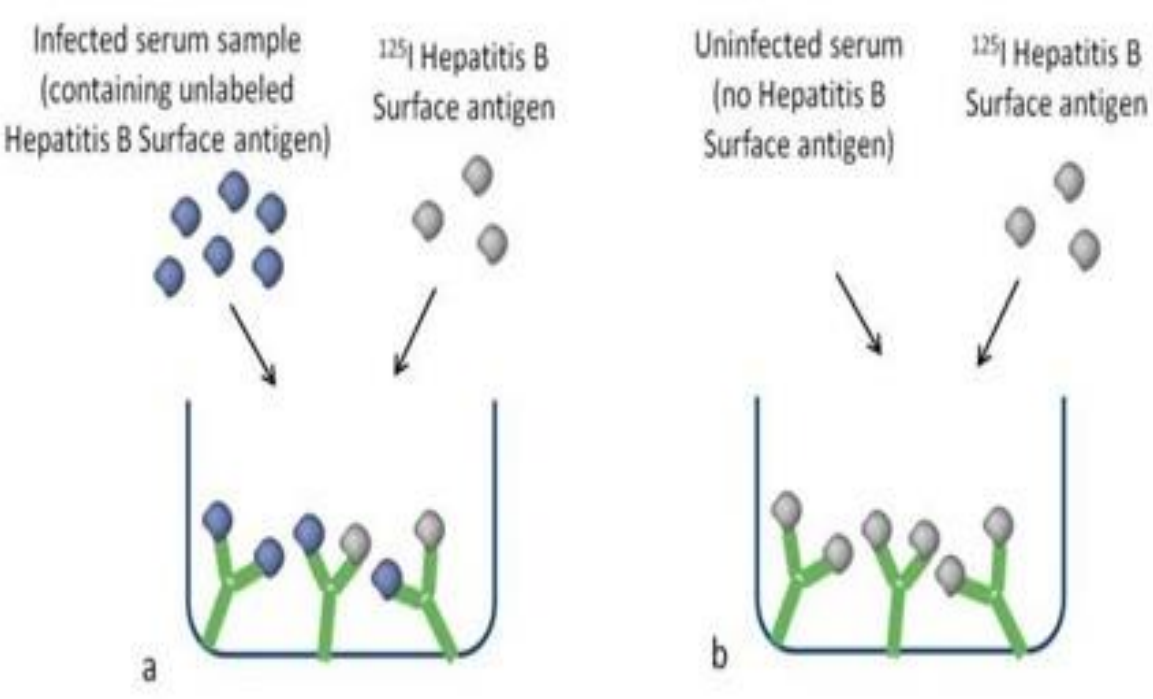
PRINCIPLE AND PROCEDURE OF RIA

Radioimmunoassay combines the principles of radioactivity of isotopes and immunological reactions of antigen and antibody, hence the name. The principle of RIA is primarily based on the competition between the labelled and unlabeled antigens to bind with antibody to form antigen-antibody complexes (either labelled or unlabelled). The unlabeled antigen is the substance (say some hormone or virus) to be determined. The antibody to it is produced by injecting the antigen to a goat or a rabbit. The specific antibody (Ab) is then subjected to react with unlabeled antigen in the presence of excess amounts of isotopically labelled (125I) antigen (Ag+) with known radioactivity. There occurs a competition between the antigens (Ag+ and Ag) to bind the antibody. Certainly, the labeled Ag+ will have an upper hand due to its excess presence.

As the concentration of unlabeled antigen (Ag) increases the amount of labelled antigen-antibody complex (Ag+-Ab) decreases. Thus, the concentration of Ag+- Ab is inversely related to the concentration of unlabeled Ag i.e., the substance to be determined. The labelled antigen-antibody (Ag+-Ab) complex is separated by precipitation. The radioactivity of 125I present in Ag+-Ab is determined. To determine

the amount of labeled antigen bound, the Ag-Ab complex is precipitated to separate it from free antigen (antigen not bound to Ab), and the radioactivity in the precipitate is measured.





Advantages:

- It is structurally specific as antigen antibody reaction are highly specific.
- It is indirect method of analysis.
- Highly specific and sensitive.
- Measure small amount.

Disadvantages

- Radiation hazards.
- Labs require special
- Radioactive waste disposal.
- Expensive equipment.

LATEX AGGLUTINATION TEST

Agglutination Test

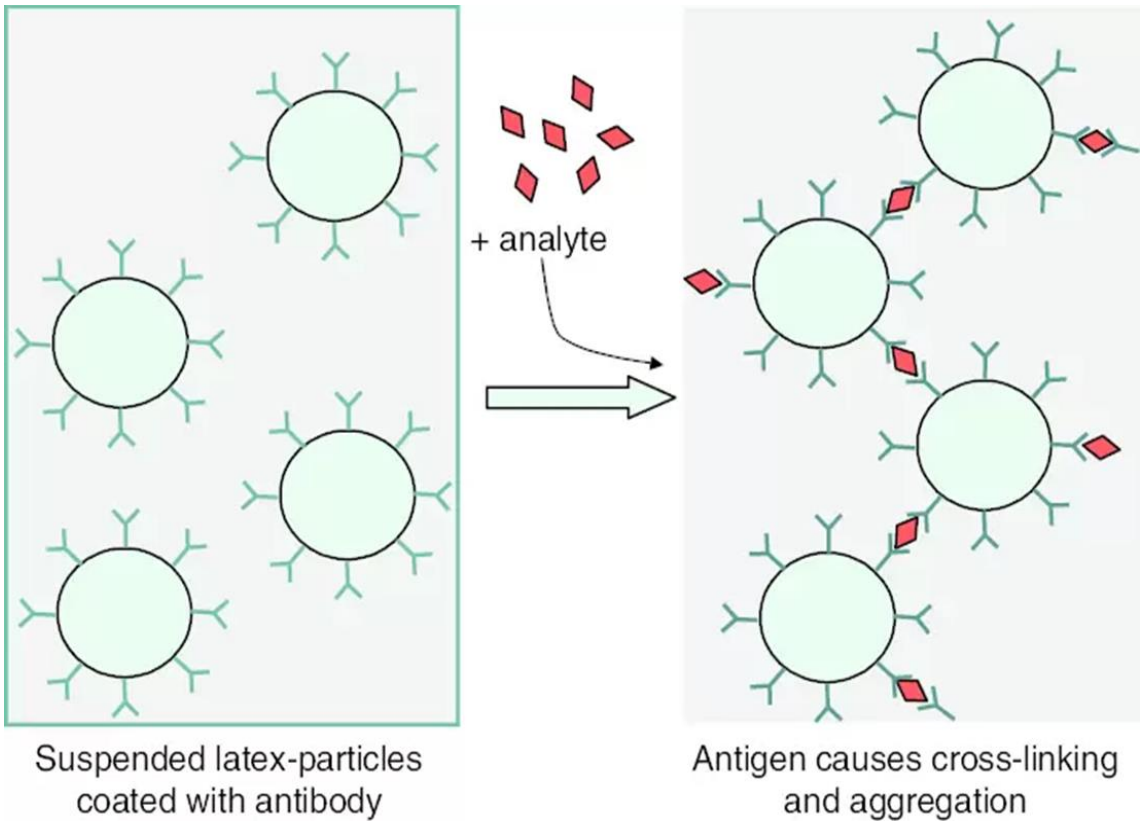
Agglutination Test is a clinical assay used for the detection of Antibody or Antigen in body fluids such as saliva, urine, cerebrospinal fluid, or blood.

In agglutination test antibodies react with antigens on cells and form visible clumps or aggregates which is called agglutinates. We can see these clumping in naked eyes. The antibodies are involved in the formation of clumping in the Agglutination test is known as agglutinins.

This agglutination test is specific, hence the antigen combines homologous antibody and form an antigen-antibody complex or clumps.

Latex Agglutination Test

- This type of agglutination test is performed by coating antibody or antigen on the surface of an artificial carrier particle, which is called latex bead (polystyrene).
- If any antigen present in the test sample it will bind with at the combining site of antibody which is coated on the surface of the latex bead, by forming a cross-linked aggregates of latex beads and antigen. The size of latex beads varies from 0.8µm to 1um.
- Latex agglutination test is also known as the latex fixation test. Clinically this test is used for the identification and typing of many important microorganisms.



Aim of Latex Agglutination Test

The main purpose of the Latex agglutination test is to detect the presence of antibodies produced against a particular antigen or virus or bacteria.

Types of Latex Agglutination

Latex agglutination test is divided into two classes based on the processes of detection;

A. Latex Agglutination Test (LAT) for Antibody Detection

It is a passive agglutination test , in this method antigen is coated on the surface of latex beads to detect the antibody in the test sample.

B- Latex Agglutination Test (LAT) for Antigen Detection

It is a Reverse Passive Agglutination Test. In this method, antibody is coated on the surface of latex beads to detect the antigen in the test sample.

Principle of Latex Agglutination

Antibody or antigen molecules can be bound in random alignment to the surface of latex (polystyrene) beads. The number of antibody or antigen molecules bound to each latex particle is large, resulting in a high number of exposed potential binding sites. Antigen or antibody present in a specimen binds to the combining sites of the corresponding antigen/antibody exposed on the surfaces of the latex beads, forming crosslinked aggregates of latex beads and antigen/antibody. A large particle size of latex facilitates the visualization of the of the antigen - antibody reaction.

Result of Latex Agglutinatio

Positive Agglutination Result: The presence of White clumps indicates a positive result i.e suspected particle is present.

Negative Agglutination Result: The absence of white clumps indicates a negative result i.e Suspected particle is not present.

Use of Latex Agglutination

1- In the clinical laboratory, it is used for the detection of antigen to Cryptococcus neoformans in CSF or serum.

2- Used for the confirmation test of beta-hemolytic streptococcus from the culture plates.

3- It also used for the detection of Streptococcus agalactiae, Clostridium difficile toxins A and B and rotavirus.

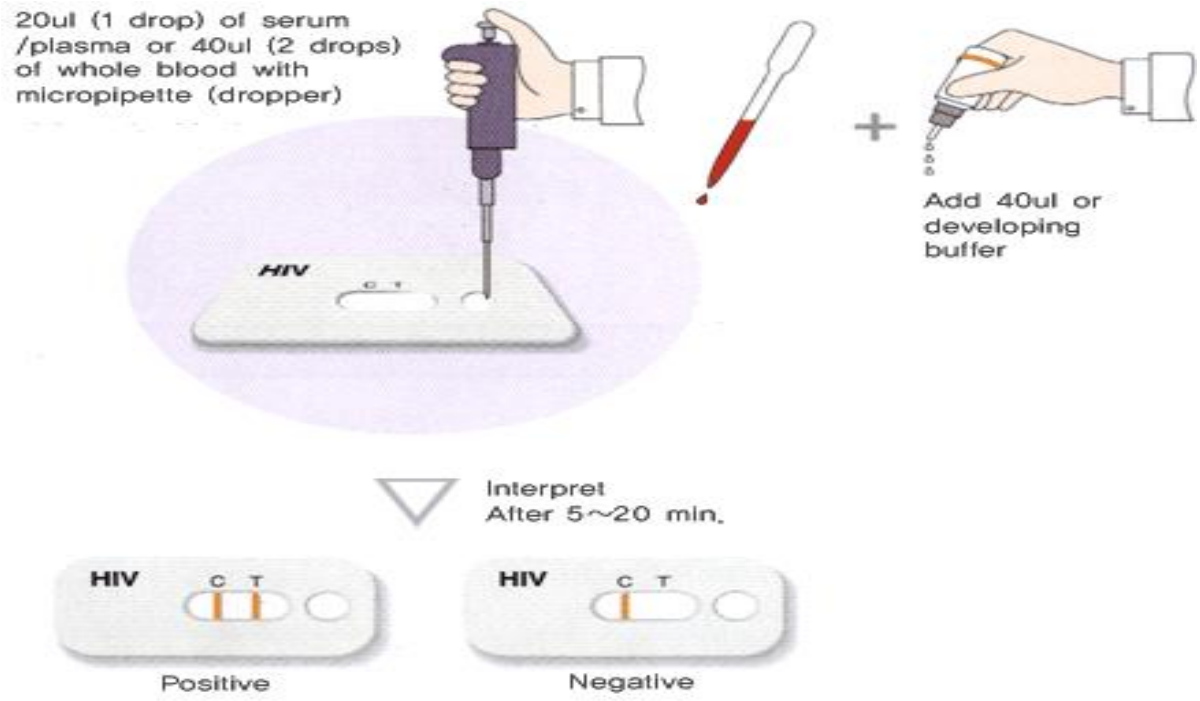
Rapid diagnosis of virus

- Rapid test is a qualitative immunoassay that is quick and easy to perform to aid in the diagnosis of viral infection such as: HIV, influenza virus, adenovirus & COVID-19.
- These tests in comparison to the cell culture (required days), few hours required after receiving the specimens to detect the virus.
- Suitable for preliminary or emergency, POCT and home use.
- Designed to be single use and disposable.
- Membrane coated with antigen or antibody produces color reaction.
- This is used to measure present the IgM &IgG (IgM useful for diagnosis of current infection while presence the IgG indicates past infection).
- Rapid tests generally give a result in 5 to 30 minutes.
- Although these tests have high specificity, false positives do occur. Any positive test result should be confirmed by a lab using the PCR.

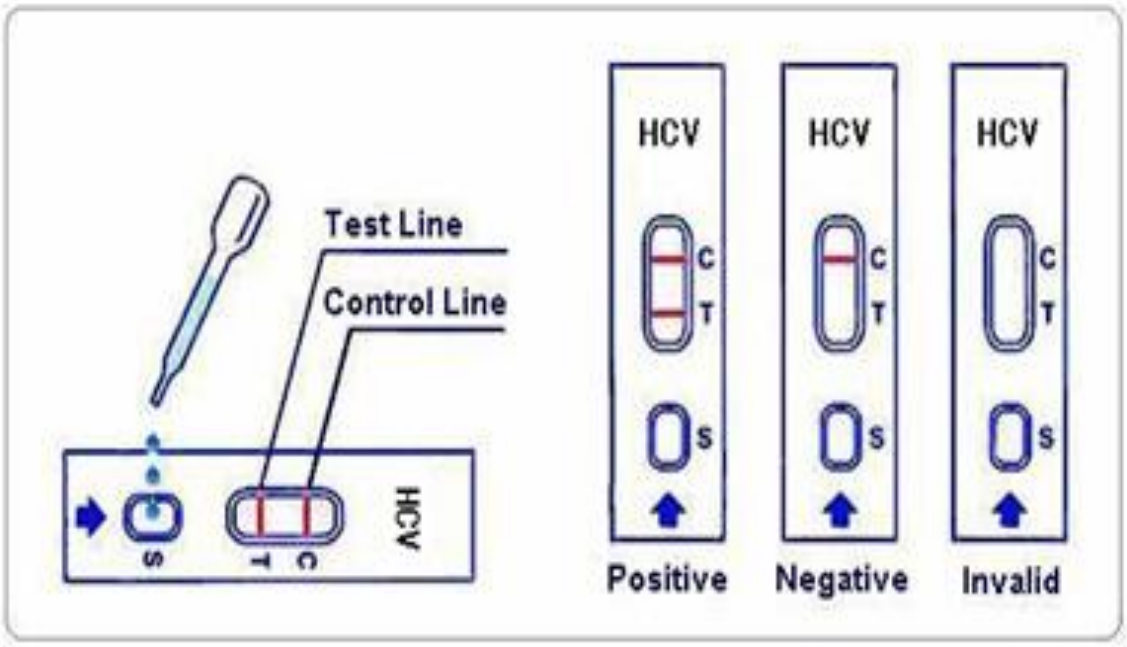
The cassette detects Ag through visual interpretation of color development on the internal strip. Antibodies are immobilized on the test region of the membrane.

During testing, the specimen reacts with antibodies conjugated to colored particles and pre-coated onto the sample pad of the test. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane. If there is enough Ag in the specimen, a colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

Procedure of Rapid Test for HIV:



Procedure of Rapid Test for HCV:



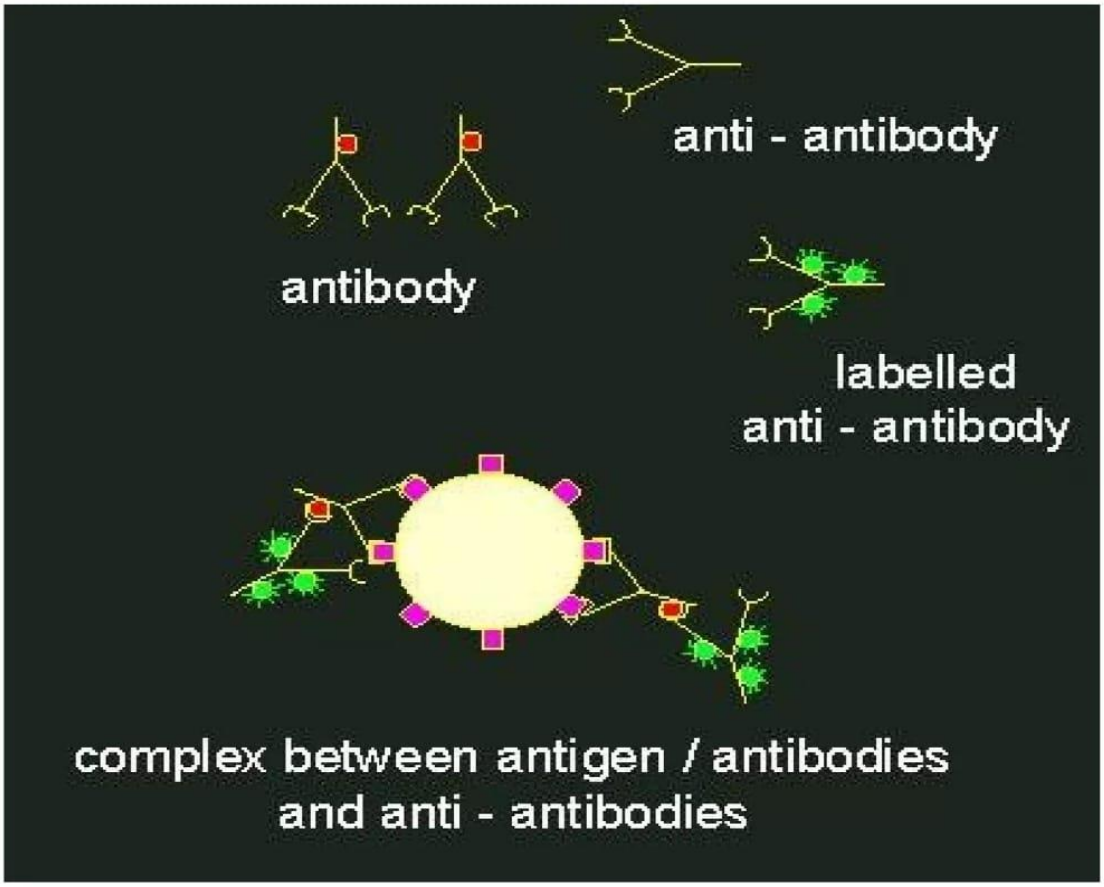
Immunofluorescence (IF)

Immunofluorescence is a powerful technique that utilizes fluorescent-labeled antibodies to detect specific target antigens.

- Fluorescein is a dye which emits greenish fluorescence under UV light. It can be tagged to immunoglobulin molecules.
- This technique is sometimes used to make viral plaques more readily visible to the human eye.

Immunofluorescence assay

- Immunofluorescence is a technique allowing the visualization of a specific protein or antigen in tissue sections by binding a specific antibody chemically conjugated with a fluorescent dye such as fluorescein isothiocyanate (FITC).
- The specific antibodies are labeled with a compound (FITC) that makes them glow an apple-green color when observed microscopically under ultraviolet light.
- Fluorescence is the property of certain molecules or fluorophores to absorb light at one wave length and emit light at longer wave length (emission wavelength) when it is illuminated by light of a different wavelength (excitation wavelength).
- The incident light excites the molecule to a higher level of vibrational energy. As the molecules return to the ground state, the excited fluorophore emits a photon.



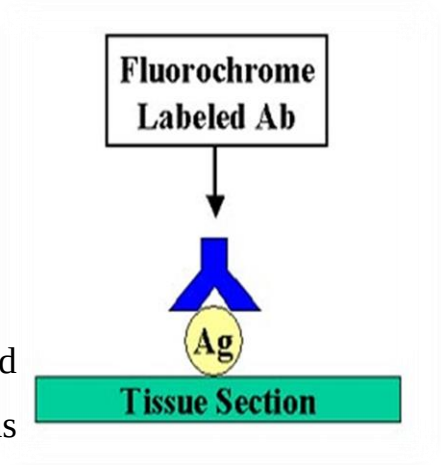
Principle: The sample was smeared on the slide and allowed for incubation, after washed the smear is treated with a fluorescent labeled antibody to detect antigens or antibodies according to test systems. Ag-Ab complex must form for fluorescence to occur. The result should be read immediately under fluorescent microscope. Bright object against dark background is positive result.

There are two ways of doing IF staining

1. Direct immunofluorescence
2. Indirect immunofluorescence

1) Direct immunofluorescence

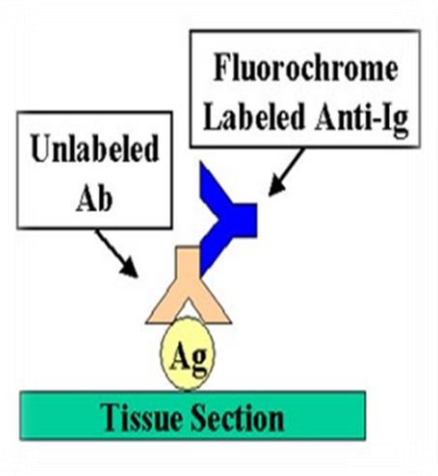
This technique is used to detect viral antigen in clinical specimens using specific fluorochrome labeled antibody against the virus. Sensitive method for identification of rabies viral antigen in brain smears. The steps of direct immunofluorescence are:



1. Fixation of sample (antigen) on the slide.
2. Slide was washed to remove free antigens.
3. Treating with labeled antibody.
4. Incubation.
5. Washing to remove unbound excess labeled antibody
6. visualization under fluorescent microscope. when viewed under fluorescent microscope, the field is dark and areas with bound antibody fluoresce green.

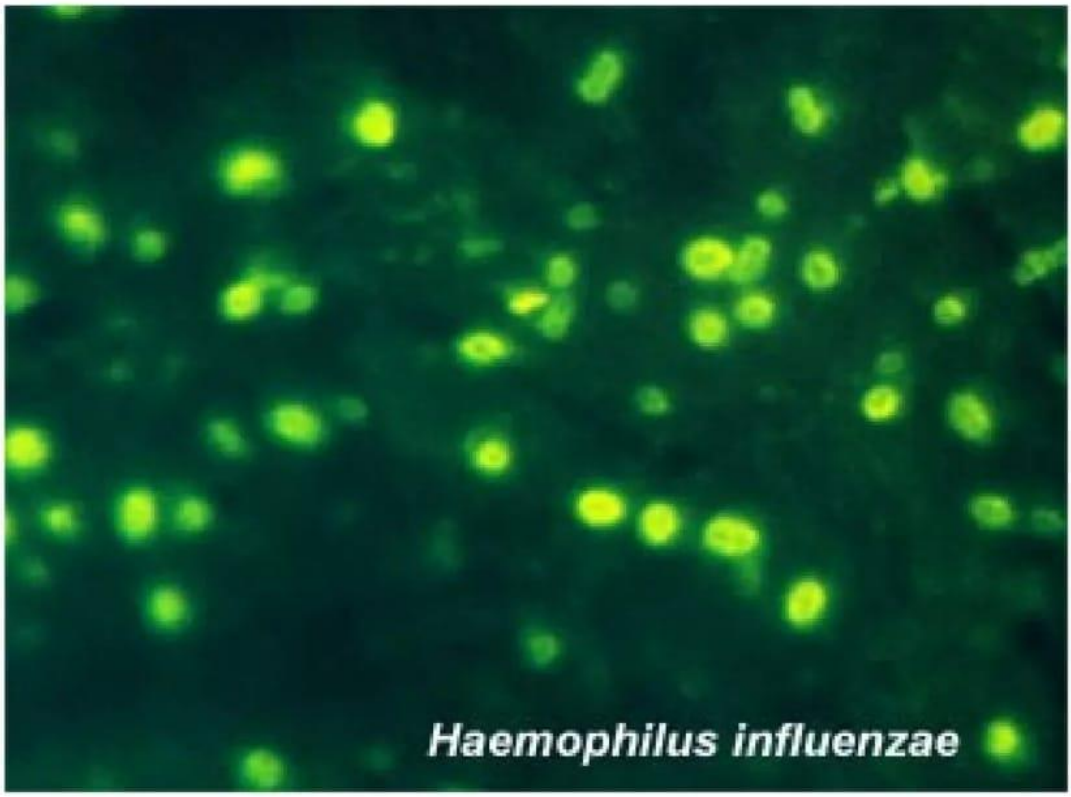
2) Indirect immunofluorescence

Indirect immunofluorescence is employed to detect antibodies in patient serum. The presence of specific antibody in serum bound to the antigen on smear is detected by adding another antibody



1) The antigen on smear are made to react with specific unlabeled antibody (serum).
2) Incubation.
3) Washed for remove the unbound antibody.
4) Add the second antibody (labeled antibody) which it binds to Fc portion of first antibody and persists despite washing.

5) The presence of the second antibody is detecting by observing under fluorescent microscope.



NEUTRALIZATION TESTS

Serological method that detects the presence of viral neutralizing antibodies. The antibodies bind to the viral particles.

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

• These tests are broadly of two types :

• Virus neutralization tests.

• Toxin neutralization tests.

Virus neutralization tests

• Neutralization of viruses by their specific antibodies are called virus neutralization tests. Inoculation of viruses in cell cultures, eggs, and animals results in the replication and growth of viruses.

Virus neutralization tests

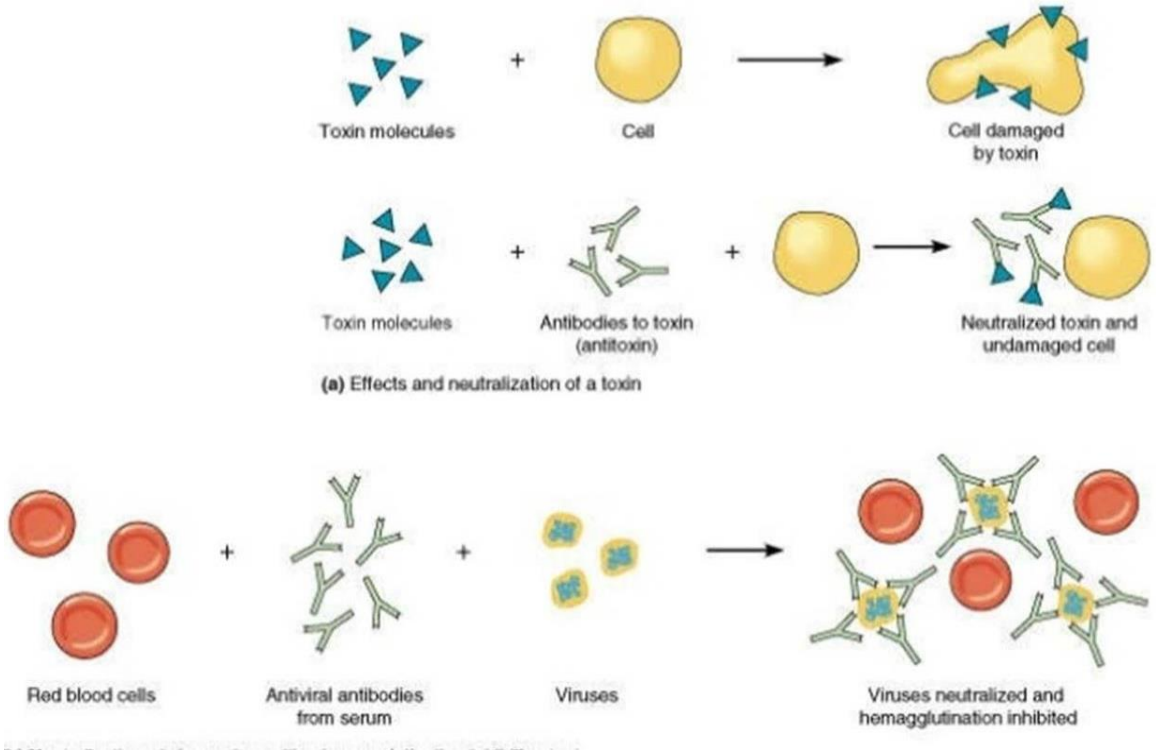
• When virus - specific neutralizing antibodies are injected into these systems replication and growth of viruses is inhibited.

• This forms the basis of virus neutralization test.

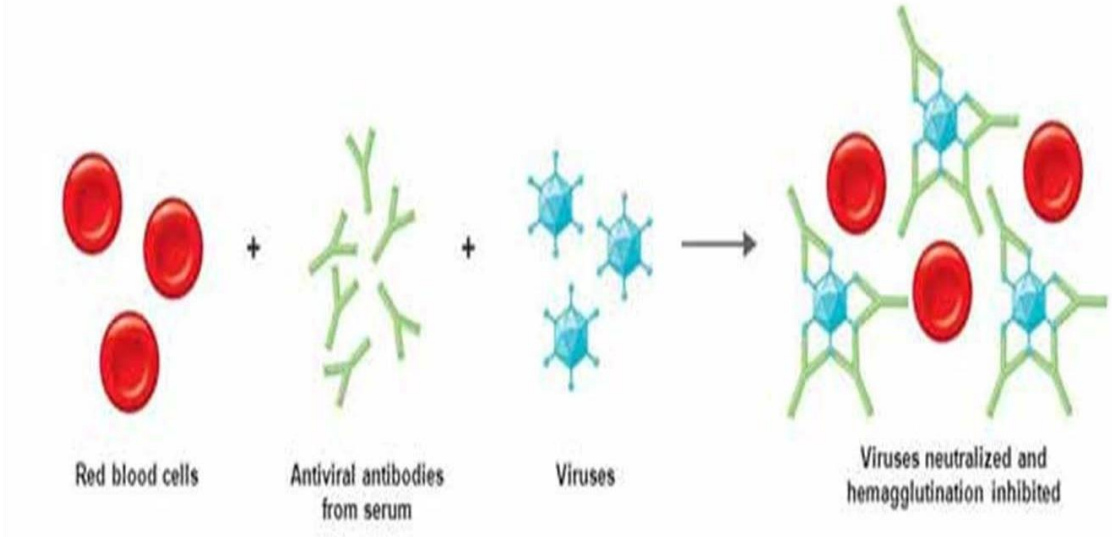
• Viral hemagglutination inhibition test is an example of virus neutralization test frequently used in the diagnosis of viral infections such as influenza, mumps, and measles.

• If patients serum contains antibodies against certain viruses that have the property of agglutinating the red blood cells, these antibodies react with the viruses and inhibit the agglutination of the red blood cells.

NEUTRALIZATION



Neutralization Test



STEPS

• First step:

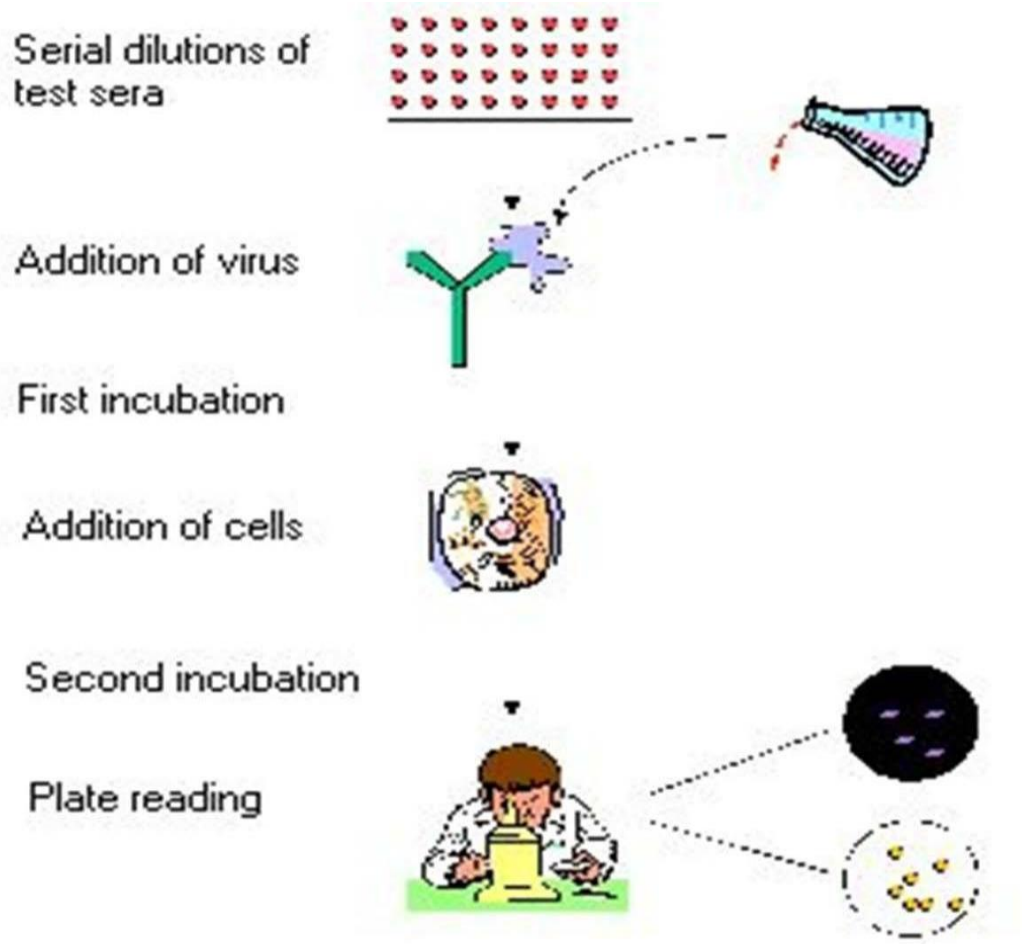
• Serial dilutions of serum(virus-neutralizing Ab?)+ known virus

• Incubated for 1-2hr at 37 degrees Celsius

• Second step;

• Cell culture inoculated with mixture

• Seal the plates & Incubated at 37 degrees Celsius



Uses of the neutralization tests :

• Recognition of the pathogenic causative agents (Toxins / Viruses) and differentiation between their pathogenicity.

• Study the antigenic relation for various toxins and viruses

• Detection the biological protection ability of the neutralizing antitoxins and antiviruses against toxins and Viruses.

• Measure the potency of vaccines and the ability to induce immunity.

Advantages

• Highly specific

• Often used as gold standard

Disadvantages

• Technically demanding

• Time consuming

• Can only be used for viruses that can be grown

• Complexity limits the use beyond gold standard