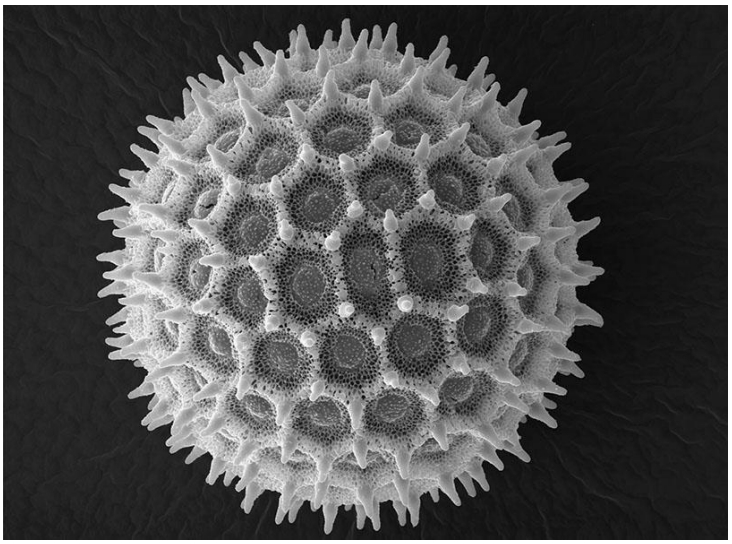

VIOLOGY

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LAB 1: Laboratory safety guidelines and aseptic techniques

Laboratory safety guidelines

The Laboratory Safety Guide is designed to meet the requirements of the Laboratory Safety Standard. It describes policies, procedures, equipment, personal protective equipment, and work practices that can protect employees from health hazards in laboratories. All laboratory personnel must be aware of this plan. Everyone who works in the laboratory is responsible for their own safety and the safety of others.

Personal Practices

1. prevent skin contact with corrosive, toxic, or hot liquids, laboratory protective clothing should cover your arms, main torso, legs, and feet.
2. Do not allow children or pets in laboratories.
3. Never mouth pipette anything.
4. Be aware of dangling jewelry, loose clothing, or long hair that might get caught in equipment.
5. Designate and use non-lab areas for eating and drinking.
6. Chemical/biological refrigerators and storage areas are not to be used to hold or consume food and drinks.
7. Use lab coats, gloves, and other personal protective clothing safely in the lab.
8. Never work alone in the lab if avoidable. If you must, make someone aware of your location so they can check on you periodically.
9. Wash your hands frequently for at least 15-30 seconds throughout the day and before leaving the lab.

10. Caution is always advised when wearing contact lenses in a lab. Chemical liquids and vapors can get behind or penetrate the plastic lens where water cannot wash the eye, causing severe damage.

Biosafety

Biosafety is the safe working practices associated with the handling of biological materials, particularly infectious agents. Level for biosafty (BSL) is a set of biocontainment precautions required to isolate dangerous biological agents in an enclosed laboratory facility. The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4).

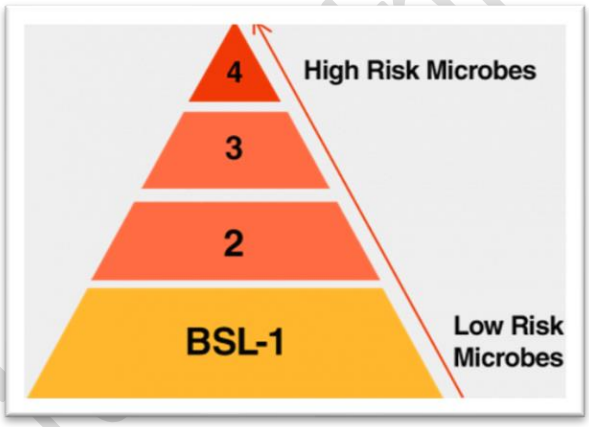


Figure1: Biosafety Levels

*BSL-3 and BSL-4 laboratories are used to deal with viruses such as yellow fever, West Nile virus, or viruses more dangerous include Ebola and Marburg viruses.

The aseptic technique

The aseptic technique is a set of practices that protect patients from healthcare-associated infections and protect healthcare workers (HCWs) from contact with blood, body fluids, and body tissue.

1. The aseptic technique, when performed correctly will:

2. Minimise contamination of key sites.
3. Protect patients from pathogenic microorganisms that may cause infection.
4. Reduce the transmission of microorganisms.
5. Maintain the sterility of equipment and key parts used for aseptic procedures.

LAB 2: Specimen Collection, Transport and Storage

A virus is a microorganism that is smaller than a bacterium and cannot grow or reproduce apart from a living cell. Viruses may contain either DNA or RNA as their genetic material, and they invade living cells and use their chemical machinery to keep themselves alive and replicate themselves.

Viral Specimens Collection

It is important to establish which specimens to send to the laboratory. Viral specimen collection plays a vital role in laboratory diagnosis. Proper sample collection leads to the proper diagnosis of disease. The sample should be collected aseptically. Appropriate samples from the appropriate site and an adequate amount should be collected. Once collected, samples are recommended to be emulsified or mixed properly with a viral transport medium (VTM).

Transporting specimens to the Virology laboratory

1. Most viruses are unable to survive temperatures over 50°C, freezing or fluctuating in temperature.
2. Viruses can also be damaged by light, drying, changes in pH, and bacterial enzymes.

3. The usage of VTM will prevent specimens from drying out and help to preserve viral activity.
4. All viral specimens should be transported in an icebox with a warning label.

Storage and Transportation of Viral Specimens

1. Short-term storage: +4°C
2. Long-term storage: -70°C/-190°C(liquid nitrogen)

Function of VTM

1. Preserves viral infectivity within the specimen
2. Prevents specimen from drying
3. Prevents growth of bacteria and fungi

Criteria for rejecting Vial specimens

1. Mismatch of information on the label and the request
2. Inappropriate transport temperature
3. Excessive delay in transportation
4. Inappropriate transport medium (specimen received in a fixative, dry specimen, Insufficient quantity, or Leakage)

LAB 3: Direct viral examination (Light Microscopy , Electron Microscopy Examination)

Viral examination

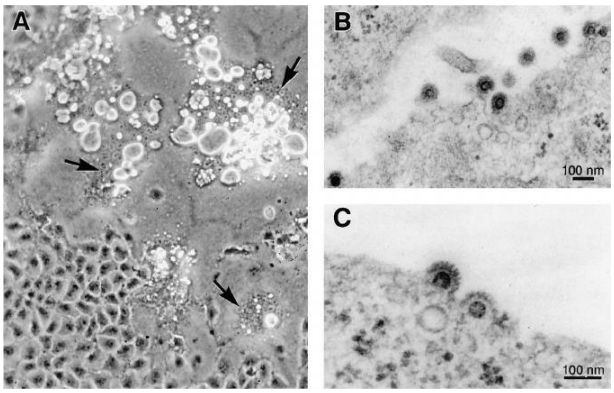
In general, diagnostic tests for viral infection can be grouped into 3 categories:

1. **Direct examination**
    A. Antigen detection: serology (immunofluorescence, ELISA, etc.)
    B. Electron microscopy: (morphology of virus particles, and immune electron microscopy)
    C. Light microscopy: (histological appearance inclusion bodies)
    D. Viral genome detection: hybridization with specific nucleic acid probes polymerase chain reaction (PCR)
2. **Indirect Examination**
    A. Cell Culture: cytopathic effect (CPE), haemabsorption, immunofluorescence
    B. Eggs: pocks on CAM, haemagglutination, inclusion bodies
    C. Animals: disease or death serology
    D. Antibody detection: Serology (Complement fixation tests (CFT), Haemagglutination inhibition tests, Radioimmunoassay (RIA), Immunofluorescence techniques (IF), Particle agglutination, Neutralization tests and Western Blot (WB))

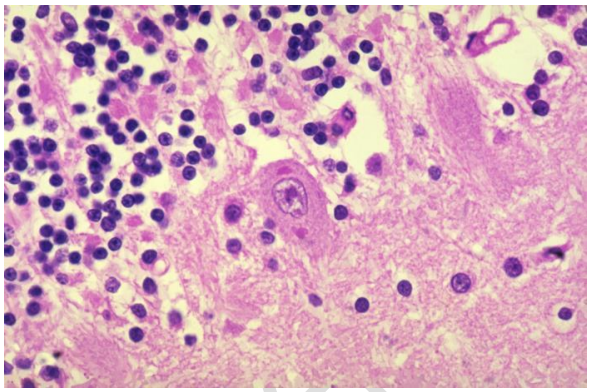
Light microscopy - Replicating viruses often produces histological changes in infected cells. These changes may be characteristic or non-specific. Viral inclusion bodies are basically collections of replicating virus particles either in the nucleus or cytoplasm.

Use the light microscopical examination for viral investigation by detecting infected tissue and detecting the cytopathic effect caused by a viral infection. With light microscope use, proper technique, and adequate control, enzymes, for example, alkaline phosphatase, can also serve as indicator systems.

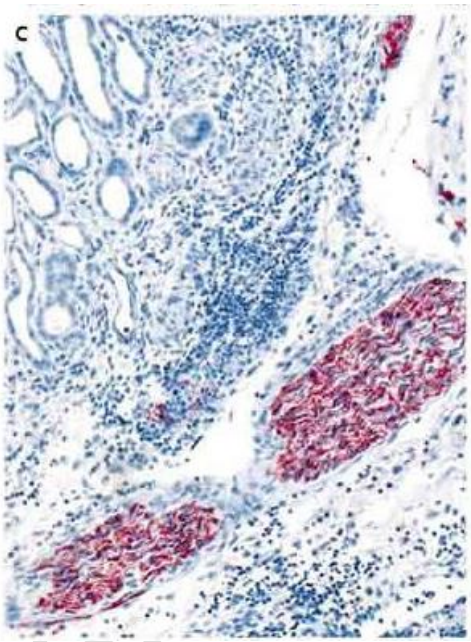
*Light microscopy histological appearance - e.g. inclusion bodies Antigen detection immunofluorescence, etc.



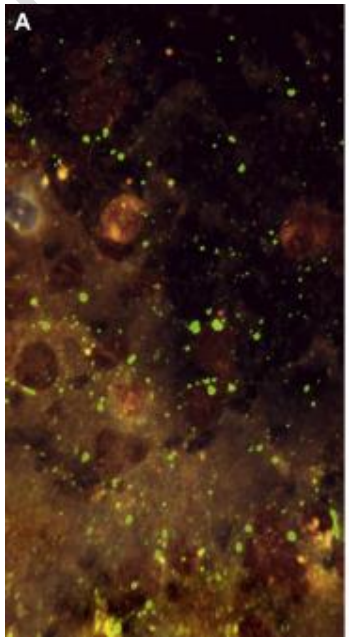
Cytopathic effect in vitro on human cells



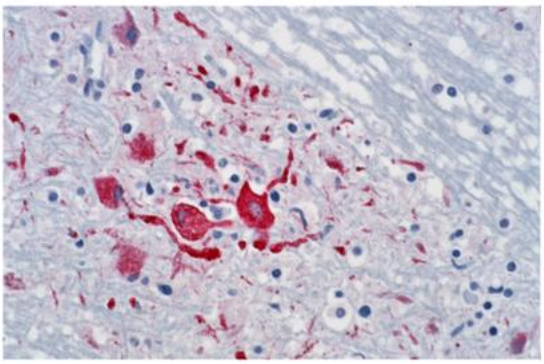
Histopathology on human postmortem brain section. Finding Negri bodies (shown here in the cytoplasm of a Purkinje cell in the cerebellum.



Immunohistochemistry used in rabies patients infected via organ transplants. Here viral antigen fills the axoplasm of peripheral nerves. Human rabies, biotin-conjugated anti-rabies globulin, avidin-alkaline phosphatase, and naphthol fast-red method



Direct immunofluorescence on impression smear of fox brain (possible human contact), showing prominent brilliant apple-green masses of viral antigen (Negri bodies of light microscopy) and punctate "dust," against a dark background. FITC-labeled anti-rabies globulin



Immunohistochemistry performed on human central nervous tissue showing WNV antigen (red) in the cytoplasm of neurons and cell processes.

Electron Microscopy (EM)

Electron microscopy is a method that can take a picture of a whole virus and reveal its shape and structure.

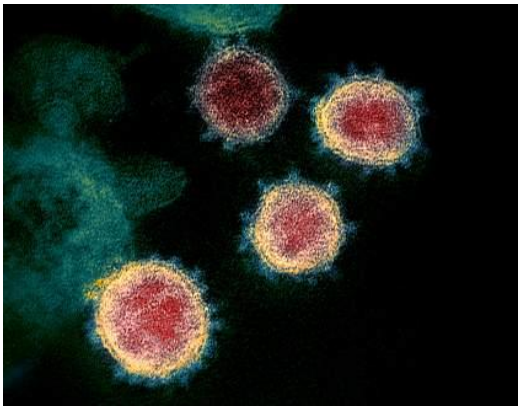
Virus particles are detected and identified based on morphology. A magnification of around 50,000 is normally used.

EM is now mainly used for the diagnosis of viral gastroenteritis by detecting viruses in feces e.g. rotavirus, and adenovirus.

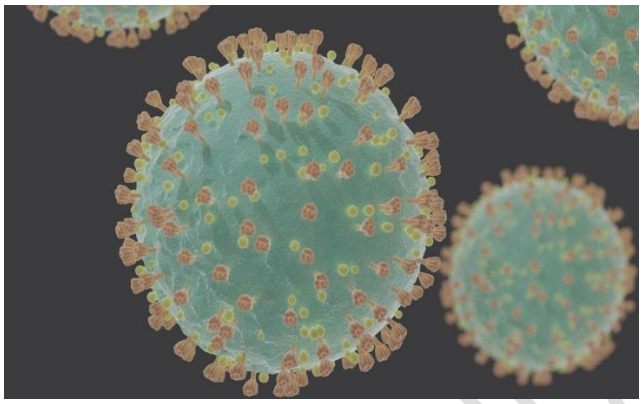
The sensitivity and specificity of EM may be enhanced by immune electron microscopy, whereby virus-specific antibody is used to agglutinate virus particles together thus making them easier to recognize or to capture virus particles onto the EM grid.

*The main problem with EM is the expense, as well as It is not typically used as a routine diagnostic test as it requires a highly specialized type of sample preparation, microscope, and technical expertise.

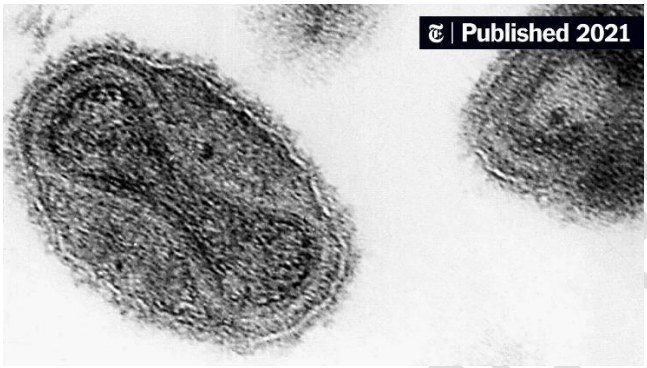
*some viruses dignosis by EM: HSV-1,



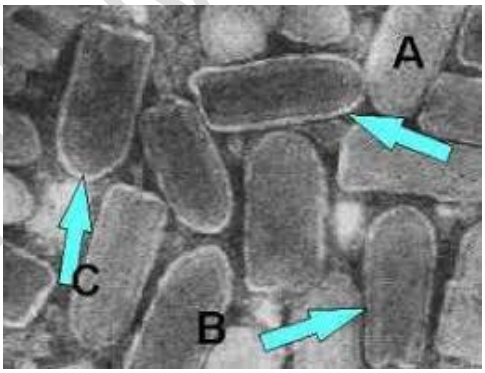
Transmission electron microscope image shows SARS-CoV-2



Electron microscope SARS-CoV-2



Smallpox virus under electron microscope



Rabies virus under electron microscope

LAB 4: Isolation and cultivation of viruses

Virus Isolation is a highly relevant and important testing method used to diagnose viral infections, including emerging, re-emerging, and novel viral pathogens. Isolation and identification of viruses using cell culture methods or embryonated chicken eggs are performed at the AHDC on a variety of species including but not limited to bovine, equine, canine, feline, camelids, avian, reptiles,

and various wildlife and exotic species. Turnaround time for the testing varies and is largely dependent on the rate of growth/replication of the virus and the method ultimately used to identify the virus.

The primary purpose of virus cultivation is:

To isolate and identify viruses in clinical samples. 2. To research viral structure, replication, genetics, and effects on host cells. 3. To prepare viruses for vaccine production.

Testing Strategies

1. **Respiratory Disease**
    A. Nasal Swabs, Pharyngeal Swabs, oral swabs
    B. Tracheal wash, broncho-alveolar lavage
    C. Upper airway tissues, lung
    D. Neurological Disease
    E. Brain, brain stem, spinal cord
    F. CSF (Cerebral Spinal Fluid)
    G. EDTA Whole Blood
2. **Enteric Disease**
    A. Feces
    B. Jejunum, ileum, cecum, colon
3. **Reproductive Disease/Abortion**
    A. Placenta
    B. Fetal Tissues: lung, liver, kidney, spleen, adrenal
    C. Vaginal or preputial swabs

Viruses Cultivation

There are three methods of Viruses Cultivation:

1. Animal Inoculation 2. Inoculation into embryonated egg 3. Cell Culture

1. Animal Inoculation

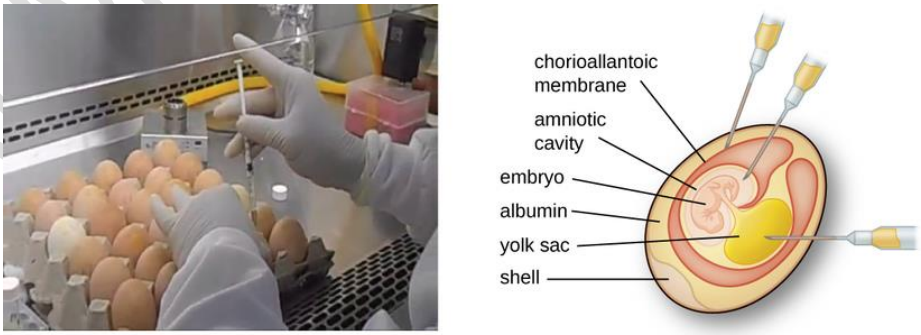
Animal viruses require cells within a host animal or tissue-culture cells derived from an animal. Animal virus cultivation is important for:

A. the identification and diagnosis of pathogenic viruses in clinical specimens,
B. the production of vaccines, and
C. basic research studies.

Selected animals should be healthy and free from any communicable diseases. Viruses are cultivated in laboratory animals such as mice, guinea pigs, hamsters, and rabbits.

2. Inoculation into embryonated egg

In vivo, host sources can be a developing embryo in an embryonated bird's egg (e.g., chicken, turkey) or a whole animal. For example, most of the influenza vaccine manufactured for annual flu vaccination programs is cultured in hens' eggs.



(a) The cells within chicken eggs are used to culture different types of viruses. (b) Viruses can be replicated in various locations within the

3. Cell Culture (Tissue Culture)

There are three types of tissue culture; **organ culture, explant culture, and cell culture.** Organ cultures are mainly done for highly specialized parasites of certain organs e.g., tracheal ring culture is done for isolation of coronavirus. Explant culture is rarely done.

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

Types of cell culture

1. **Primary cell culture**: Examples: Monkey kidney cell culture, Human amnion cell culture
2. **Diploid cell culture (Semi-continuous cell lines)**: Examples: Human embryonic lung strain, Rhesus embryo cell strain

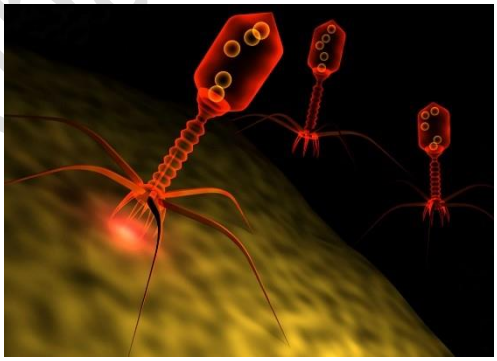
3. **Heteroploid cultures (Continuous cell lines)**: Examples: HeLa (Human Carcinoma of cervix cell line), HEP-2 (Human Epithelioma of larynx cell line), Vero (Vervet monkey) kidney cell lines, BHK-21 (Baby Hamster Kidney cell line).

Cultivation of plant viruses

There are some methods of Cultivation of plant viruses such as plant tissue cultures, cultures of separated cells, or cultures of protoplasts, etc. viruses can be grown in whole plants.

Cultivation of bacteriophages

Bacteriophages are cultivated in either broth or agar cultures of young, actively growing bacterial cells. Bacteriophages are detected by the presence of clear plaques on bacterial lawns.



LAB 5: The Complement Fixation Test

Complement is heat-labeled proteins produced by the liver and circulate in blood. The complement fixation test is an immunological test that can be used to detect the presence of either a specific antibody or specific antigen in a patient's

serum. It was widely used to diagnose infections, particularly with microbes that are not easily detected by culture methods.

The complement fixation test consists of: -

1. **Test system:** patient serum, knowing antigen and complement protein.
2. **Indicator system:** involving red blood cells pre-coated with anti-red blood cell antibody)

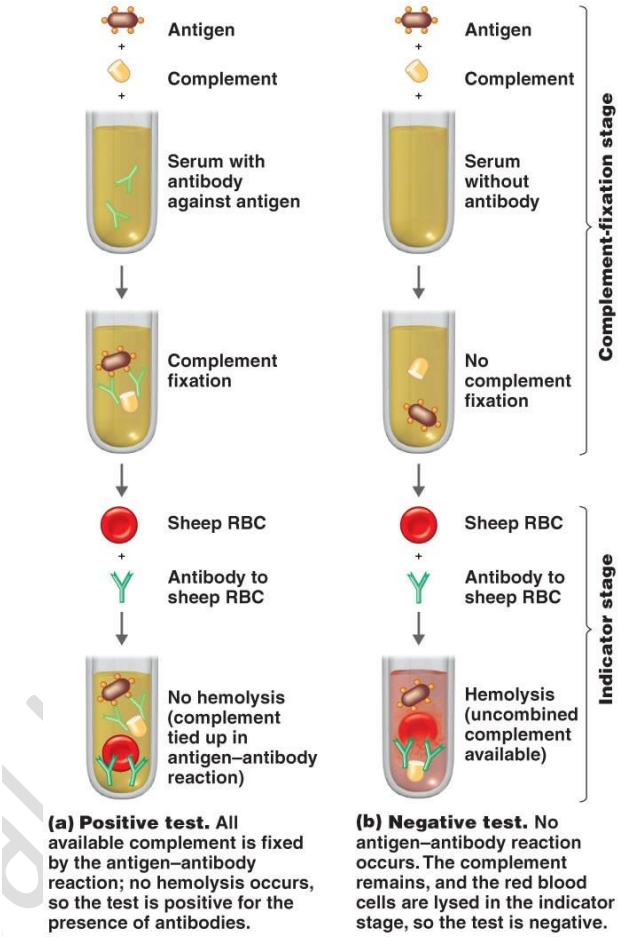
*Sheep Red Blood Cells (sRBCs) + anti-sRBC antibodies

Principle of complement fixation test

- The complement can be activated only when there is a formation of an antigen-antibody complex.
- The first step is to heat the serum at 56°C to destroy the patient's complement.
- A measured amount of complement and antigen are then added to the serum.
- If there is a presence of antibodies in the serum, the complement is fixed due to the formation of the Ag-Ab complex.
- If no antibody is present in the patient's serum, then the complement remains free.
- To determine whether the complement has been fixed, sheep RBCs and antibodies against sheep RBCs are added.
- **In the positive test:** The available complement is fixed by the Ag-Ab complex and no hemolysis of sheep RBCs occurs. So, the test is positive for the presence of antibodies.
- **In the negative test:** No Ag-Ab reaction occurs and the complement is free. This free complement binds to the complex of sheep RBC and its antibody to cause hemolysis, causing the development of a pink color.

The CF test is interpreted as follows:

- Antibody present (Positive)= No hemolysis
- Antibody absent (Negative)= Hemolysis



(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.

Advantages and disadvantages of CFT

Advantages

- Ability to screen against a large number of viral and bacterial infections at the same time.
- CFT is used to detect and quantify antibody that does not agglutinate or precipitate with its antigen.
- CFT can detect antibody at level less than 1 microgram per milliliter.
- CFT is also used to detect antigen
- CFT is Economical

Disadvantages

- Not sensitive - cannot be used for immunity screening
- Time consuming and labor intensive
- Often non-specific e.g. cross-reactivity between HSV and VZV

LAB 6: Virus Neutralization test

In a neutralization test, serum and virus are reacted together in equal volumes and inoculated into a susceptible animal host or cell culture. Viral neutralization tests are used to either identify an unknown virus using known reference antisera/monoclonal antibody or measure virus-neutralizing antibody levels in serum samples against a known infectious virus.

The virus neutralization test is sensitive and specific, but also more complex, time-consuming, and expensive than many other assays. Virus neutralization tests are now done using microtiter systems (as for a quantal assay), which are economical, easier to perform, and use smaller amounts of reagents. Plaque reduction tests are performed for a plaque assay following the reaction of the virus with an antibody.

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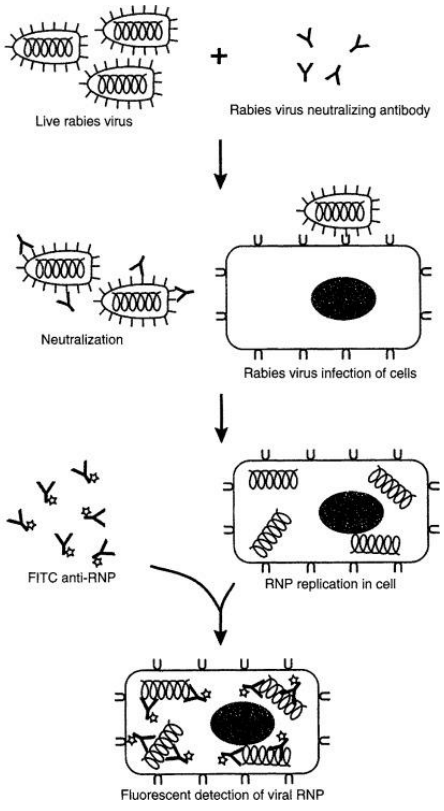


LAB 7 & 8: Hemagglutination test (HA) and Hemagglutinationinhibition test (HAI)

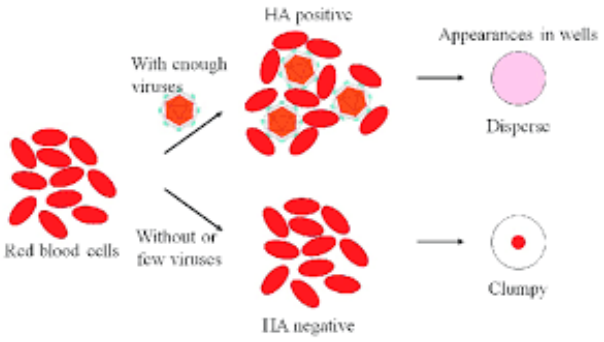
Haemagglutination (HA) and haemagglutination inhibition tests (HAI): These tests detect antibodies to viruses (rubella, influenza) that possess a haemagglutinin antigen. These are also relatively insensitive and can give non-specific reactions, and have mostly been replaced by more sensitive and specific techniques.

A Hemagglutination Assay: is a laboratory technique where red blood cells coated with antigens are used to detect the presence of antibodies in a sample based on the degree of agglutination observed after incubation.

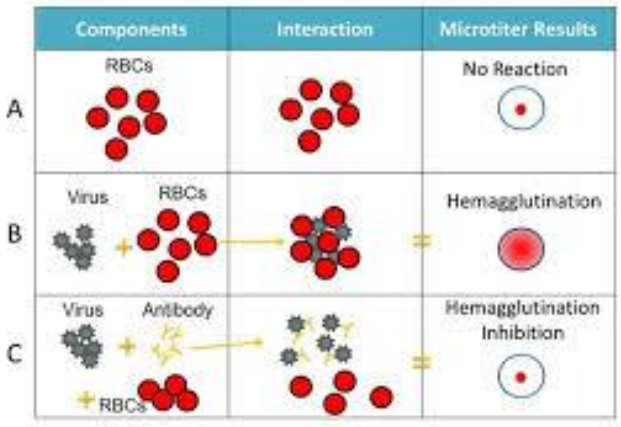
Hemagglutination is used for the diagnosis of some enveloped viruses such as influenza viruses. This method relies on the specific feature of some enveloped viruses that can adsorb to red blood cells (RBCs). Specifically, hemagglutinin5 (HA), an envelope glycoprotein of some enveloped viruses, imparts this property. In the absence of virus particles, RBCs precipitate by gravity to the bottom of the well, giving rise to a distinct red-colored dot in a conical-shaped well.

Negative HA result = a sharp button

Positive HA result = a diffuse film



In the presence of virus particles, RBCs clump together as a result of interaction between HA proteins of virus particles and RBC, leading to a lattice formation. In this case, as RBCs are dispersed as a clump, a red dot is not formed.



Tests	Result	Interpretation
Rapid HA	Positive	Presence of viral particles that may or may not be infectious.
Rapid HA	Negative	Absence of viral particles or presence of viral particles in levels too low to detect

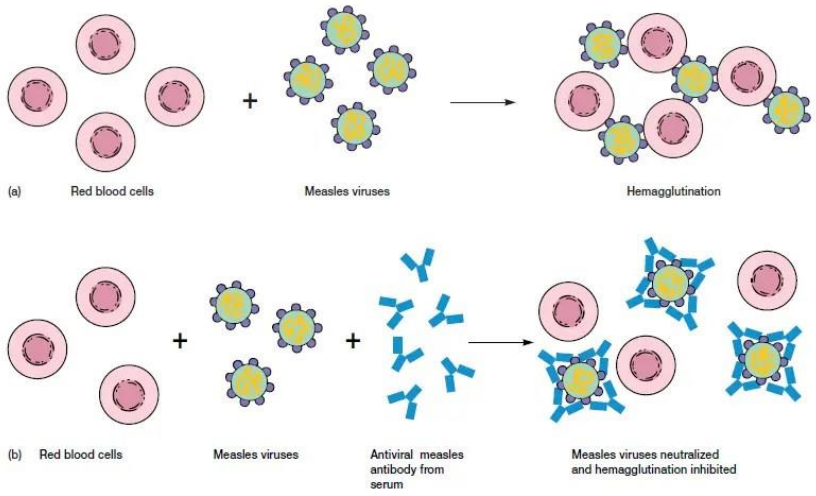
Hemagglutination-inhibition (HI) Test

It is assay is a classical laboratory procedure for the classification or subtyping of hemagglutinating viruses. For the influenza virus, HI assay is used to identify the hemagglutinin (HA) subtype of an unknown isolate or the HA subtype specificity of antibodies to the influenza virus. The assay may be utilized to detect or quantify antibodies to influenza A viruses and can be used to characterize differences in antigenic reactivity between influenza isolates.

Uses of Hemagglutination-Inhibition Test

The hemagglutination inhibition test is widely used for the diagnosis of infection caused by orthomyxoviruses (influenza), paramyxoviruses (measles, mumps),

mononucleosis, arboviruses-togaviruses (including rubella), flaviviruses, and bunyaviruses.



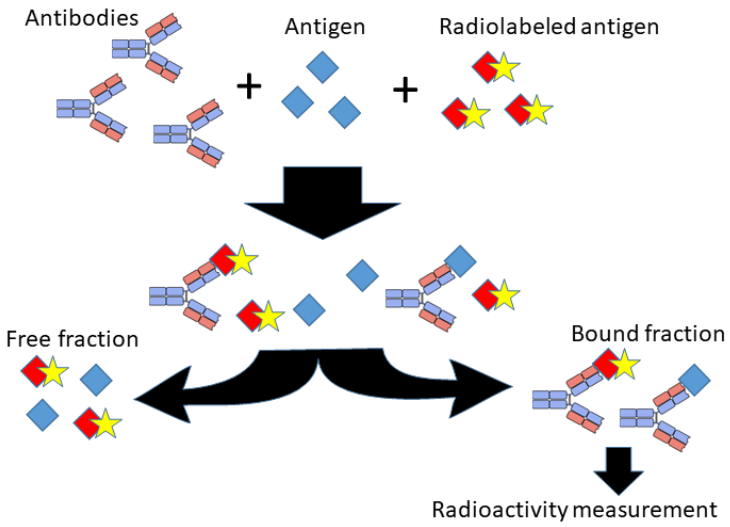
* The basis of the HAI assay is that antibodies to that particular virus (for example; measles virus) will prevent the attachment of the virus to RBC. Therefore hemagglutination is inhibited when antibodies are present.

* If the serum contains no antibodies that react with the measles virus, then hemagglutination will be observed in all wells.

LAB 9: Radioimmunoassay (RIA)

A radioimmunoassay (RIA): is an immunoassay that uses radiolabeled molecules in a stepwise formation of immune complexes. A RIA is a very sensitive in vitro assay technique used to measure concentrations of substances, usually measuring antigen concentrations (for example, hormone levels in blood) by use of antibodies.

The RIA technique is extremely sensitive and extremely specific, and although it requires specialized equipment, it remains among the least expensive methods to perform such measurements. It requires special precautions and licensing since radioactive substances are used.



Antigens and antibodies bind specifically to form the Ag-Ab complex. The antigen can be labeled or conjugated with radioisotopes. The unlabeled antigens from the sample compete with radiolabeled antigens to bind on paratopes of specific antibodies. The unlabeled antigens replace labeled antigens that are already linked with the antibodies.

It involves a combination of three principles.

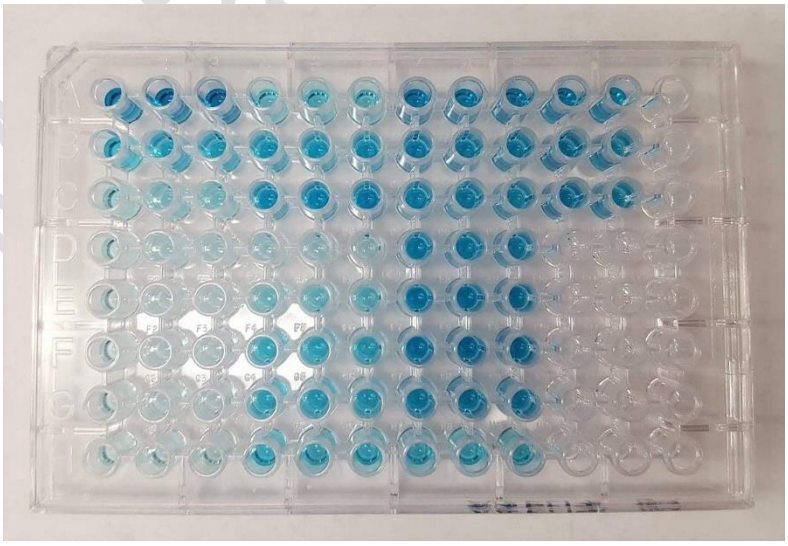
- An immune reaction i.e. antigen, antibody binding.
- A competitive binding or competitive displacement reaction. (It gives specificity)
- Measurement of radio emission. (It gives sensitivity).

*Some viruses diagnosis by radioimmunoassay (RIA): HIV,

LAB 1 0: The enzyme-linked immunosorbent assay ELISA test

The enzyme-linked immunosorbent assay ELISA: is a common laboratory testing technique that detects and counts certain antibodies, antigens, proteins, and hormones in bodily fluid samples. This includes blood, plasma, pee, saliva (spit), and cerebrospinal fluid (CSF). The assay used most widely to detect or diagnose virus infection, especially infection of blood-borne viruses e.g. HBV, HCV, HIV, and HTLV, is the enzyme-linked immunosorbent assay (ELISA), whose sensitivity and practicability have rendered it the most common primary screening assay.

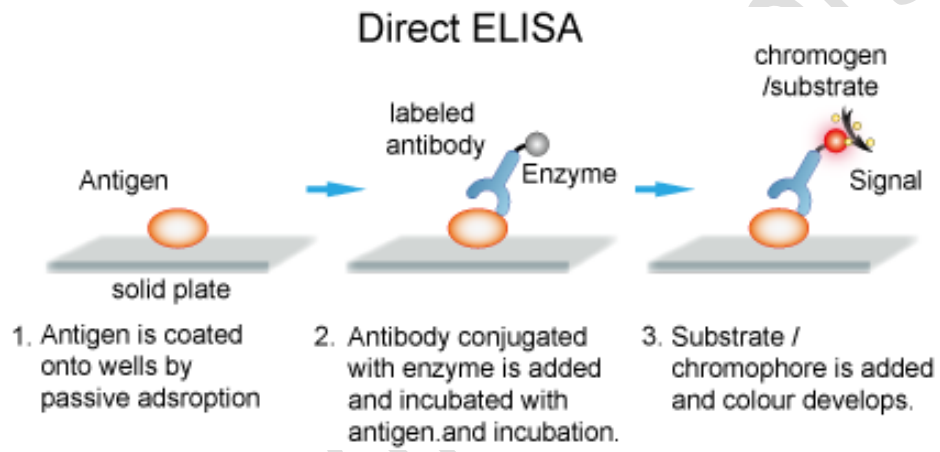
The enzyme-linked immunosorbent assay (ELISA) is one of the most commonly used labeled immunoassay techniques. It is based on an enzyme-labeled antibody capable of detecting an antigen immobilized to a solid surface, 96-well polystyrene plates. A substrate is added to produce either a color change or light signal correlating to the amount of the antigen present in the original sample, it is a simple and rapid technique to detect antibodies or antigens attached to a solid surface.



There are four types of ELISA

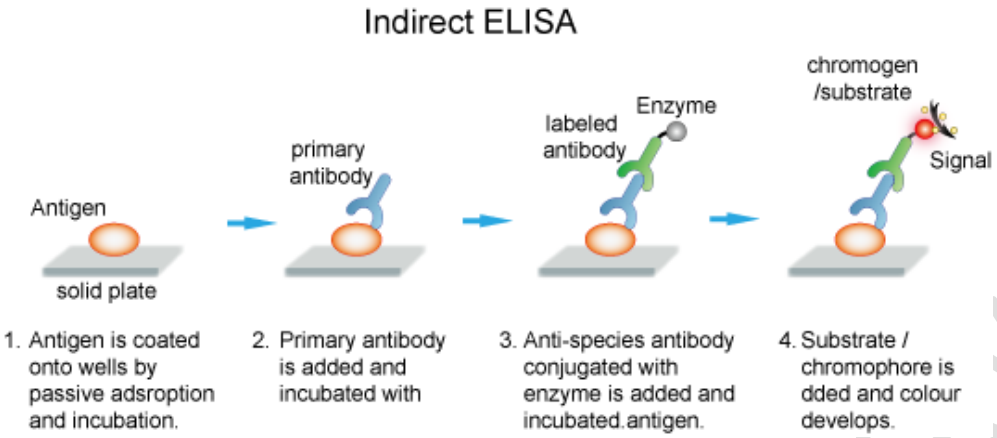
1- Direct ELISA

Direct ELISA, only an enzyme-labeled primary antibody is used. The enzyme-labeled primary antibody "directly" binds to the target (antigen) that is immobilized to the plate (solid surface). Next, the enzyme linked to the primary antibody reacts with its substrate to produce a visible signal that can be measured. In this way, the antigen of interest is detected. Color is read in a spectrophotometer.



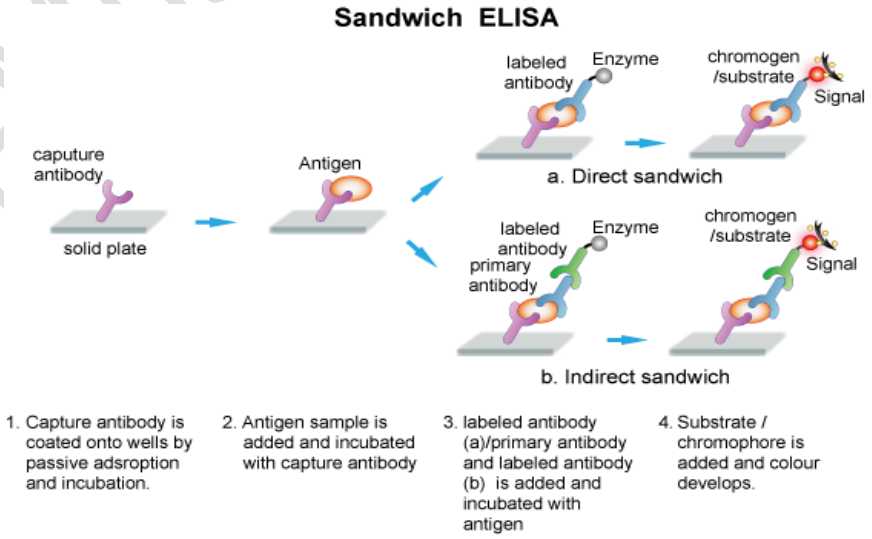
2- Indirect ELISA

Indirect ELISA, both a primary antibody and a secondary antibody are used. But in this case, the primary antibody is not labeled with an enzyme. Instead, the secondary antibody is labeled with an enzyme. The primary antibody binds to the antigen immobilized to the plate, and then the enzyme-labeled secondary antibody binds to the primary antibody. Finally, the enzyme linked to the secondary antibody reacts with its substrate to produce a visible signal that can be measured.



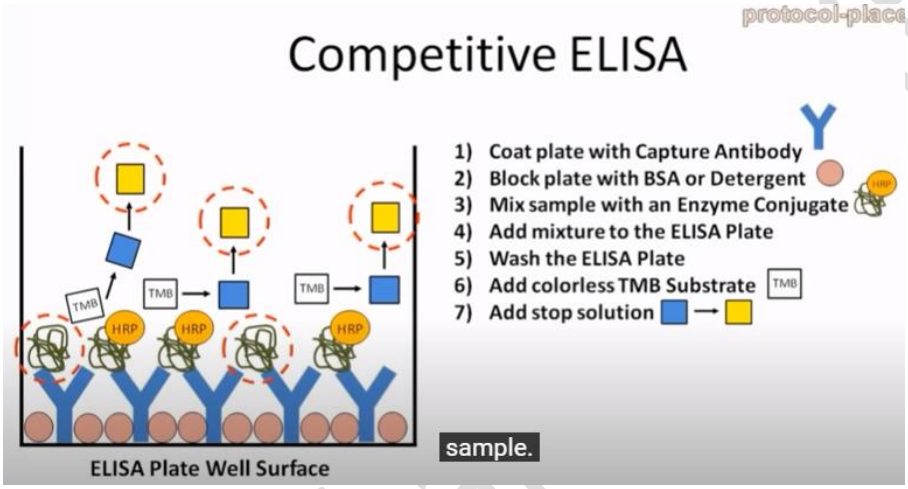
3- Sandwich ELISA (or sandwich immunoassay)

Sandwich ELISAs are the most common type of ELISA. Two specific antibodies are used to sandwich the antigen, commonly referred to as matched antibody pairs. The capture antibody is coated on a microplate, the sample is added, and the protein of interest binds and is immobilized on the plate. A conjugated-detection antibody is then added and binds to an additional epitope on the target protein. Substrate is added and produces a signal that is proportional to the amount of antigen present in the sample. Sandwich ELISAs are highly specific since two antibodies are required to bind to the protein of interest. These systems are useful when antigens are in a crude form (contaminated with other proteins) or at low concentrations.



4- Competitive ELISA

Compared with the three ELISA types above, competitive ELISA is relatively complex because it involves the use of inhibitor antigens, so competitive ELISA is also known as inhibition ELISA. In competitive ELISA, the inhibitor antigen and the antigen of interest compete for binding to the primary antibody.

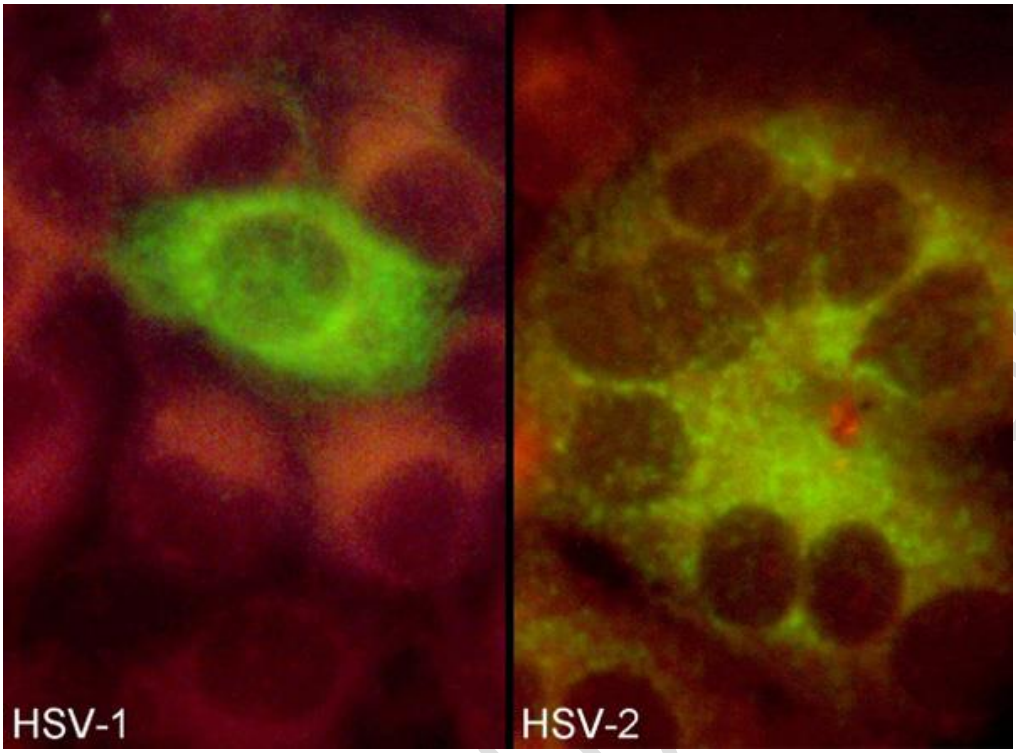


*Some viruses diagnosis by ELISA: variola virus, HIV,

LAB 11: Immunofluorescence test (IF or IFT)

Immunofluorescence tests (IF or IFT)

These assays use the same principle as EIA, and like EIA they can be constructed to detect either viral antibodies or antigens in the patient specimen. However, instead of the enzyme/substrate detector system of EIA, the fluorescein-labeled anti-human antibody is used to detect a positive reaction, which appears as apple-green fluorescence under a light microscope.



Figur: HSV infected epithelial cell (foe skin lesion and genital lesion)

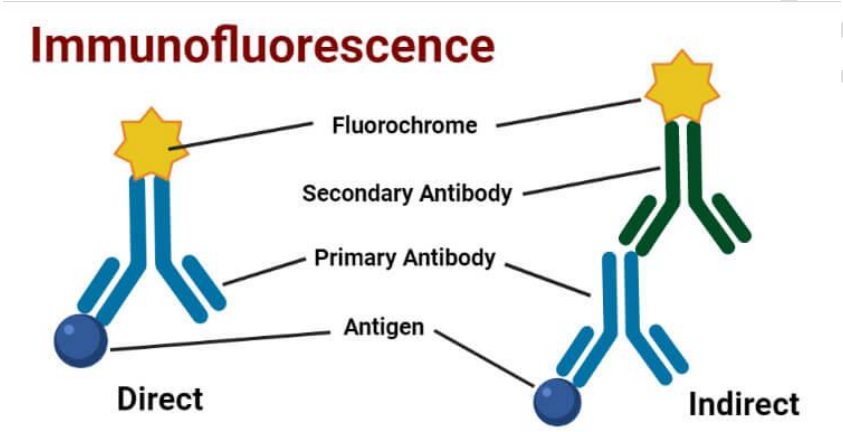
To look for viral antigens, cells from the patient's secretions (e.g. nasopharyngeal aspirate) are fixed to a spot on the glass slide and fluorescein-labeled monoclonal antibody against the virus (RSV, influenza A, etc.) is added. A mixture of these monoclonal antibodies can be added at the same time to detect a panel of viruses (e.g. respiratory viruses, all at one go). Immunofluorescence tests are also rapid serological tests, but the disadvantage is that they require subjective interpretation and are therefore labor-intensive to carry out, and are dependent upon operator expertise.

There are two types of Immunofluorescence

- Direct Immunofluorescence Test
- Indirect Immunofluorescence Test

Direct Immunofluorescence Test

Single antibody i.e. primary antibody is used that is chemically linked to a fluorochrome. If the antigen is present, the primary antibody directly reacts with it and fluorescence can be observed under the fluorescent microscope. This method is used for the detection of rabies virus antigen in the skin smear collected from the nape of the neck in humans and the saliva of dogs.



Indirect Immunofluorescence Test

Double antibodies are used i.e. primary and secondary antibodies. The primary antibody is not labeled and a fluorochrome-labeled secondary antibody is used for detection. The antigen used is known and it binds to the specific primary antibodies of interest in the sample. The secondary antibody then binds to the Fc region of the primary antibody.

Uses of Indirect Immunofluorescence Test

In the detection of specific antibodies for diagnosis of syphilis, amoebiasis, leptospirosis, toxoplasmosis, and other diseases.

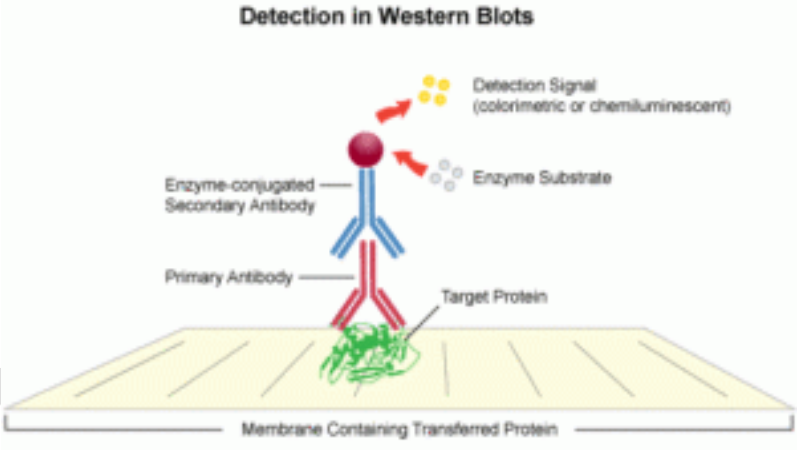
*Also used in the detection of autoantibodies that cause autoimmune disorders.

* Some viruses diagnosis by Immunofluorescence Test: HIV, Respiratory syncytial virus

LAB 12: Western Blot Test (WB)

Western Blot Test (WB)

Specific viral proteins are transferred on blotting paper either from a gel (western blot) or produced by recombination or peptide synthesis (line immunoassays). Further steps are similar to those of EIA (see above). The viral antigen band on the blotting paper develops color if a specific antibody to that particular antigen is present in the serum.



The advantage of these techniques is that the assays can distinguish antibodies directed against specific virus proteins and are therefore very specific.

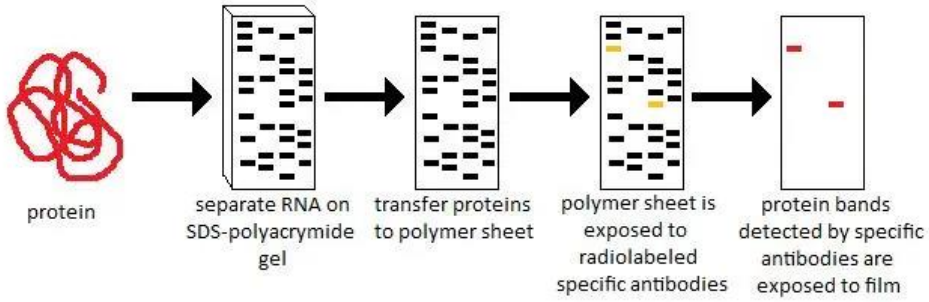
Western blotting is frequently used for the confirmatory medical diagnosis of infectious diseases such as Lyme disease, HIV infection, bovine spongiform encephalopathy (BSE), hepatitis C infection, syphilis, inflammatory muscle conditions such as myositis, and certain autoimmune disorders (e.g., paraneoplastic disease). Lyme disease and HIV infection, are the only two microbial diseases for

which a confirmatory Western blot must follow an initial borderline or positive ELISA.

A Western blot test is typically used to confirm a positive HIV diagnosis. During the test, a small sample of blood is taken and it is used to detect HIV antibodies, not the HIV virus itself. The Western blot test separates the blood proteins and detects the specific proteins (called HIV antibodies) that indicate an HIV infection. The Western blot is used to confirm a positive ELISA, and the combined tests are 99.9% accurate.

Principles of Western Blotting

The key principles of western blotting are equal loading of proteins, separation of proteins by molecular weight, electrophoretic transfer to a suitable membrane, and antibody probing. Western blot samples are first prepared by extracting proteins using specialized cell lysis buffers and protease and phosphatase inhibitors.



LAB 13 & 14: PCR (Conventional PCR) and Real Time- PCR

PCR (Conventional PCR)

PCR or Polymerase Chain Reaction is a technique used in molecular biology to create several copies of a certain DNA segment. This technique was developed in

1983 by Kary Mullis, an American biochemist. PCR has made it possible to generate millions of copies of a small segment of DNA. This tool is commonly used in the molecular biology and biotechnology labs.

A PCR (polymerase chain reaction) test is a way to look for genetic material (DNA or RNA). After taking a body fluid sample (like blood) or a swab from a body site (like deep in your nose), scientists use PCR to make many copies of DNA (amplification) that was in the original sample. By amplifying with PCR, scientists can better analyze the DNA or RNA that was in the original sample.

*Some PCR tests can determine if a specific virus is present in the sample, which might be making you sick. Other PCR tests can determine if certain genes in your body might have important changes.

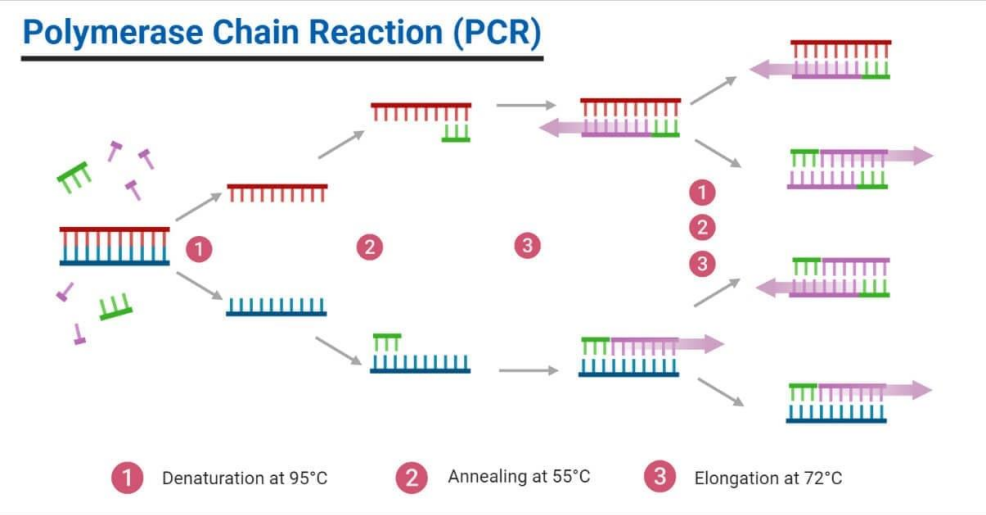
Most of us know the term "PCR test" because of testing for COVID-19. But it's been used in medicine for a long time and has many other uses. Any test that uses a polymerase chain reaction technique is a PCR test. PCR is a type of nucleic acid amplification test (NAAT).

PCR is frequently used to diagnose bacterial pathogens because of its improved sensitivity and high throughput. A range of PCR assay types exist, each with its advantages and limitations. **Conventional PCR** is the most basic type of PCR assay that requires a post-PCR step for detection and visualization of the DNA product.

PCR uses: It is valuable in a number of laboratory and clinical techniques, including DNA fingerprinting, detection of bacteria or viruses (particularly HIV), and diagnosis of genetic disorders.

Principle of PCR

The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer-mediated enzymes.



Components of PCR constitute the following:

- DNA Template– The DNA of interest from the sample.
- DNA Polymerase– Taq Polymerase is used. It is thermostable and does not denature at very high temperatures.
- Oligonucleotide Primers- These are the short stretches of single-stranded DNA complementary to the 3' ends of sense and anti-sense strands.
- Deoxyribonucleotide triphosphate– These provide energy for polymerization and are the building blocks for the synthesis of DNA. These are single units of bases.
- Buffer System– Magnesium and Potassium provide optimum conditions for DNA denaturation and renaturation. It is also important for fidelity, polymerase activity, and stability.

Advantages of PCR: Extremely high sensitivity, may detect down to one viral genome per sample volume– Easy to set up– Fast turnaround time

Disadvantages of PCR– Extremely liable to contamination– High degree of operator skill required– Not easy to set up a quantitative assay.– A positive result may be difficult to interpret, especially with latent viruses such as CMV, where any seropositive person will have a virus present in their blood irrespective of whether they have disease or not.

*some viruses diagnosis by use PCR technique: HSV-1, smallpox virus, HIV

Real-time polymerase chain reaction (real-time PCR)

Real-time polymerase chain reaction (real-time PCR) is commonly used to measure gene expression. It is more sensitive than microarrays in detecting small changes in expression but requires more input RNA and is less adaptable to high-throughput studies. It is best suited for studies of small subsets of genes. Its one major shortcoming is that the sequence of the specific target gene of interest must be known (so you can design the PCR primers), hence real-time PCR can only be used for studying known genes.

*Real-Time RT-PCR (Reverse Transcription Polymerase Chain Reaction) is a sensitive and fast test used for detecting the presence of specific genetic materials within a sample. This genetic material can be specific to humans, bacteria, and viruses like SARS-CoV-2.

LAB 15: In Situ Hybridization Technique

In situ hybridization (ISH): is a type of hybridization that uses a labeled complementary DNA, RNA, or modified nucleic acid strand (i.e., a probe) to localize a specific DNA or RNA sequence in a portion or section of tissue (in situ)

or if the tissue is small enough (e.g., plant seeds, Drosophila embryos), in the entire tissue (whole mount ISH), in cells, and in circulating tumor cells (CTCs). This is distinct from immunohistochemistry, which usually localizes proteins in tissue sections.

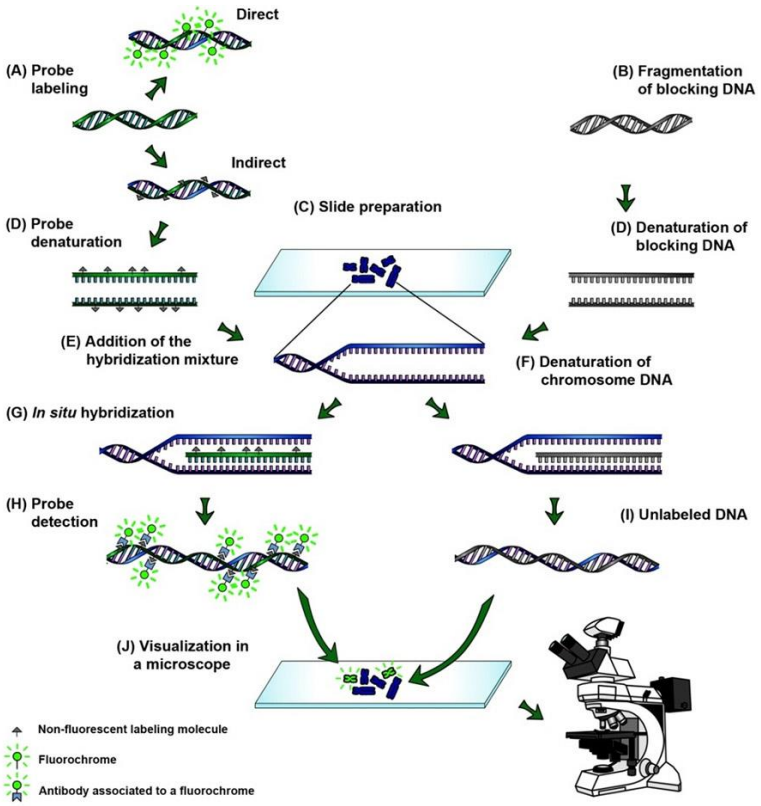


Fig. The process of genomic in situ hybridization

In situ hybridization is used to reveal the location of specific nucleic acid sequences on chromosomes or in tissues, a crucial step for understanding the organization, regulation, and function of genes.

In situ hybridization is a powerful technique for identifying specific mRNA species within individual cells in tissue sections, providing insights into physiological processes and disease pathogenesis.

In situ hybridization for parvovirus DNA can also be used to demonstrate the presence of the genome in acute or chronic infections and hydrops fetalis.