BHARATHIDASAN UNIVERSITY

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Programme: M.Sc., Biomedical Science

Course Title : Medical Virology

Course Code : BM59C19MV

Unit-V

Commonly Used Diagnostic Method for Individual Methods

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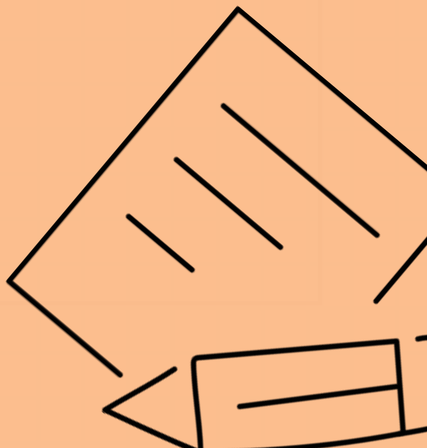
COMMONLY USED DIAGNOSTIC METHOD FOR INDIVIDUAL METHODS

COLLECTION OF SPECIMENS FOR VIROLOGIC EXAMINATION

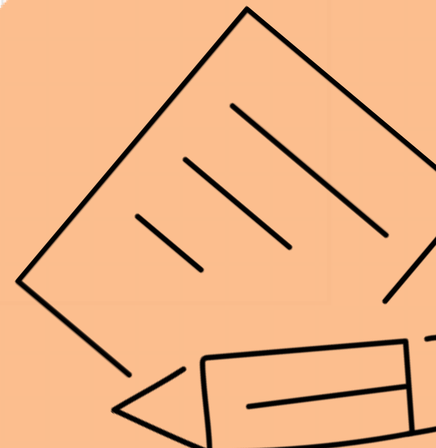
- *Swabs*
- *urine, stool, anticoagulated blood, tissue samples (biopsy)*
- *Bone marrow*
- *Eyes, conjunctival swab*
- *Nasal wash (optimal for RSV)*
- *Nasopharyngeal swab*
- *Cerebrospinal Fluids (CSF)*
- *Throat swab*



DIAGNOSTIC METHODS IN VIROLOGY

1. Direct Examination

2. Indirect Examination Serology



DIRECT DIAGNOSTIC METHODS:

Virus Isolation and Culture

Virus isolation and culture is a classical method in virology for detecting, studying, and identifying viruses.

It involves growing viruses from a patient’s sample by infecting living cells (cultures) under controlled laboratory conditions.

The process can provide a definitive diagnosis and allows further study of the virus, such as its genetics and structure, but it is often more time-consuming and technically challenging than modern molecular methods.

DIRECT DIAGNOSTIC METHODS:

Applications of Virus Isolation and Culture:

1. Clinical Diagnostics: Used to diagnose viral infections, especially when a live virus is needed for further testing, such as in influenza and herpesvirus infections.

2. Vaccine Development: Viruses must be cultured and studied to develop vaccines, particularly when live or attenuated vaccines are being developed.

3. Research: Virus isolation allows scientists to study the life cycle, pathogenesis, and mutation rates of viruses.

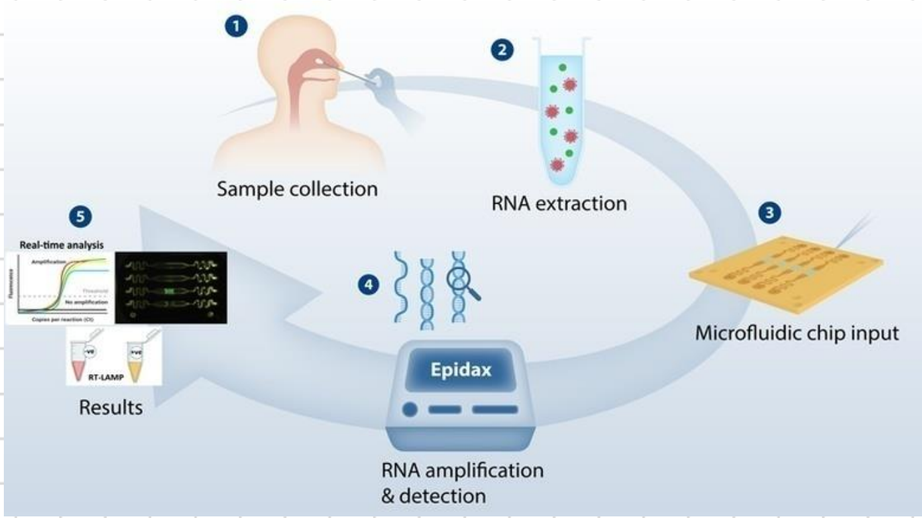
4. Antiviral Drug Testing: Cultured viruses are used to test the efficacy of antiviral drugs in vitro.

DIRECT DIAGNOSTIC METHODS:

Polymerase Chain Reaction (PCR)

PCR is a molecular biology technique used to amplify and detect the genetic material (DNA or RNA) of viruses.

It is one of the most sensitive and widely used diagnostic methods in virology, capable of detecting minute quantities of viral DNA or RNA in clinical samples.



PCR has revolutionized viral diagnostics by providing rapid, specific, and sensitive results.

Applications of PCR

PCR is used to detect and diagnose a variety of viral infections

In chronic infections like HIV or hepatitis C

. ***Detecting Emerging Viruses:***
PCR was crucial during the COVID-19 pandemic for the detection of SARS-CoV-2. PCR-based tests were rapidly developed to identify the virus in clinical samples.

. ***Genotyping and Resistance Testing:***
PCR is used to study viral genomes and mutations that confer drug resistance, such as in HIV or hepatitis B.

. ***Viral Surveillance and Epidemiology:***
PCR is used to track virus spread, detect outbreaks, and monitor changes in viral genomes for epidemiological studies.

DIRECT DIAGNOSTIC METHODS:

Electron Microscope (EM)

Electron microscopy (EM) is a powerful technique used for the direct visualization of viral particles in clinical specimens, such as feces, vesicular fluid, or tissue samples. It allows for the detection and identification of viruses based on their size, shape, and structural features. EM is particularly useful for identifying large viruses, including:

Rotaviruses

Poxviruses

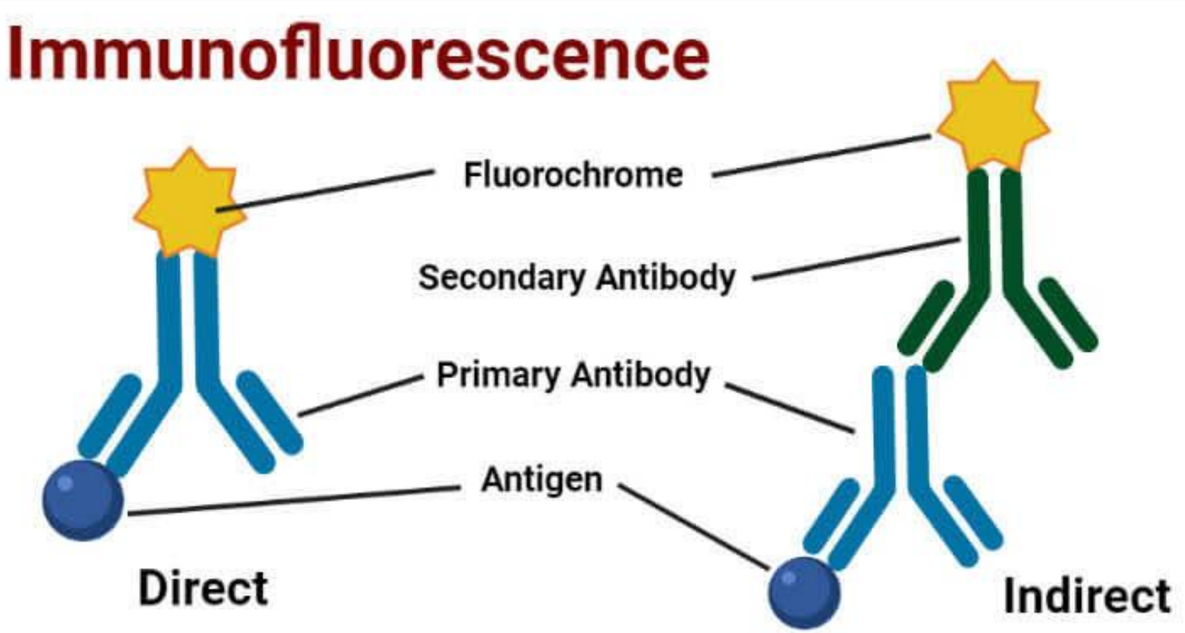
Herpesviruses

Although EM is not a first-line diagnostic tool due to its lower sensitivity compared to molecular methods like PCR, it remains valuable in certain cases, particularly for rapid identification during outbreaks or when specific viral infections are suspected.

DIRECT DIAGNOSTIC METHODS:

Immunofluorescence Assay (IFA)

Immunofluorescence Assay (IFA) is a technique that uses fluorescently-labeled antibodies to detect the presence of specific viral antigens in infected cells or tissue samples. This method provides a way to visualize the interaction between antibodies and their corresponding viral antigens under a fluorescence microscope.



DIRECT DIAGNOSTIC METHODS:

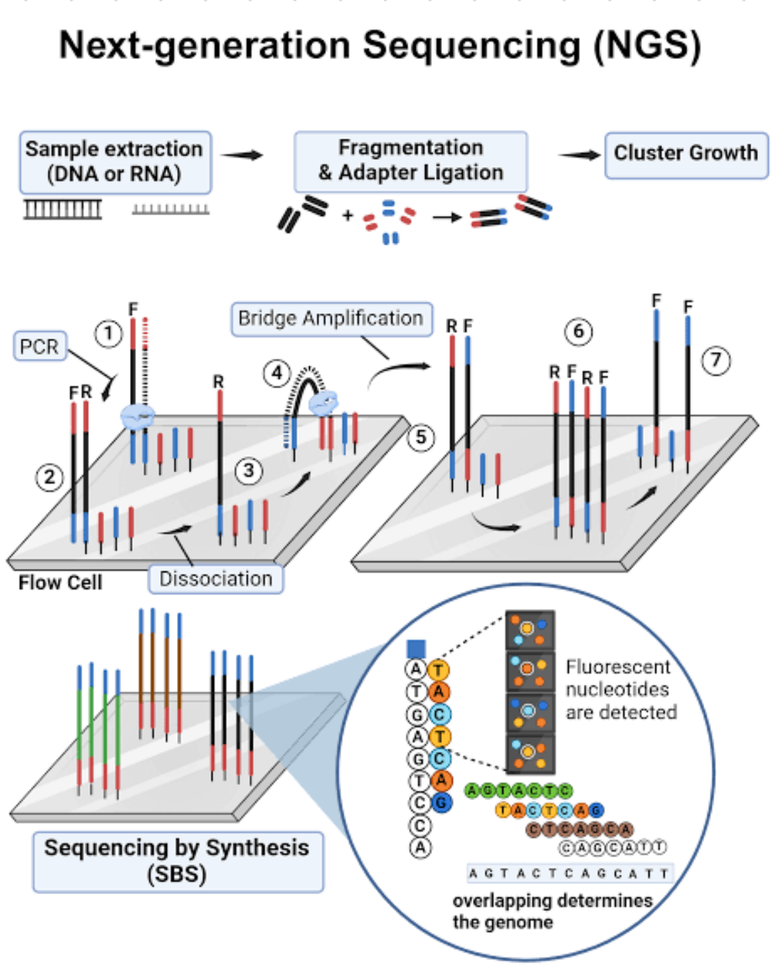
Rapid antigen tests

Rapid Antigen Tests (RATs) are diagnostic tools designed to detect specific viral proteins (antigens) in clinical samples, often providing results within minutes. These tests are widely used for detecting respiratory viruses, such as SARS-CoV-2 (the virus responsible for COVID-19) and influenza, especially in point-of-care settings.

DIRECT DIAGNOSTIC METHODS:

Next-Generation Sequencing (NGS)

Next-Generation Sequencing (NGS) is an advanced molecular technique that sequences the entire or partial genome of viruses, providing comprehensive data for the detection, characterization, and monitoring of both known and novel viruses. This method has transformed viral diagnostics and epidemiology, especially in tracking viral evolution and outbreaks.



2. INDIRECT EXAMINATION METHODS

Indirect methods detect the host's immune response (antibodies) to the virus rather than detecting the virus itself.

- Serology
- Hemagglutination Inhibition Test
- Complement Fixation Test
- Immunoblotting

SEROLOGY

Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used laboratory technique designed to measure virus-specific antibodies in a patient's blood, helping to diagnose viral infections. It is highly sensitive and commonly used for detecting antibodies like IgM (indicative of recent infection) and IgG (suggestive of past exposure or immunity).

SEROLOGY

Applications in Viral Infections:

HIV: ELISA is routinely used in HIV testing to detect antibodies to the virus, helping diagnose individuals who have been exposed to HIV.

Hepatitis: ELISA can detect antibodies to hepatitis viruses (e.g., hepatitis B or C), helping diagnose both acute and chronic infections.

Dengue: ELISA can detect antibodies to dengue virus, with IgM indicating a recent infection and IgG pointing to past exposure or secondary infection.

HEMAGGLUTINATION INHIBITION TEST (HI)

The Hemagglutination Inhibition Test (HI) is a laboratory technique used to measure the ability of specific antibodies in a patient's serum to inhibit the agglutination of red blood cells (RBCs) by certain viruses. This method is particularly valuable in diagnosing viral infections, such as influenza and dengue.

Influenza: The HI test is widely used to diagnose influenza infections and can help determine the subtype of the virus involved. It is particularly useful during flu outbreaks to monitor immune responses in populations.

COMPLEMENT FIXATION TEST

The Complement Fixation Test (CFT) is an immunological assay that measures the ability of antibodies to bind to specific antigens and activate the complement system, leading to the fixation (binding) of complement proteins to antigen-antibody complexes. Historically, it was a significant method for diagnosing various viral infections but is less commonly used today due to the development of more sensitive and specific techniques.

Historically, the CFT was used for diagnosing a variety of viral infections, including influenza, measles, mumps, and herpes viruses. It was also used for certain bacterial and fungal infections.

IMMUNOBLOTTING

Immunoblotting, also known as Western blotting, is a laboratory technique used to detect specific virus-related antibodies in a patient's serum. This method involves separating viral proteins by size using gel electrophoresis, transferring these proteins onto a membrane, and then probing the membrane with patient serum to identify antibodies specific to the viral proteins.

Detects virus-specific antibodies by transferring viral proteins onto a membrane and probing with patient serum. It is used as a confirmatory test in HIV and other infections.

APPLICATIONS

HIV Diagnosis: Immunoblotting is commonly used as a confirmatory test for HIV after initial screening tests (like ELISA). It can differentiate between HIV-1 and HIV-2 and assess the presence of specific antibodies.

Other Viral Infections: It can also be used for diagnosing infections caused by viruses such as hepatitis B, hepatitis C, and certain arboviruses.

SUMMARY

Direct examination methods aim to detect viral components (antigen, nucleic acid, whole particles) and are useful in early stages of infection.

Indirect examination methods assess the host's immune response (antibodies), which develop later in infection and are helpful for diagnosing past or ongoing infections.

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