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**“RECENT LABORATORY DIAGNOSTICS OF VIRAL DISEASES” – A  
REVIEW**

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## ABSTRACT

### INTRODUCTION

Viruses infect humans, lower animals, insect, plants, bacteria and fungi. Classification and nomenclature are standardized by the International committee on taxonomy of viruses.<sup>[1]</sup> Viruses are divided into genera and species, as are bacteria; e.g. *simplexvirus* and *pneumovirus*. Viruses of medical importance to humans comprise 7 families of DNA viruses and 14 families of RNA viruses.<sup>[2]</sup>

### Classification of viruses<sup>[1,2]</sup>

- Viruses are classified according to morphology, type of genome.
- Morphological type includes icosahedral and Helical types.
- While genomic type includes DNA or RNA and whether it is single or double stranded.

**DNA VIRUSES.**

<b>FAMILY</b>	<b>VIRAL MEMBERS</b>
1) Adenovirus.	Human Adenovirus.
2) Hepadenavirus.	Hepatitis B virus.
3) Herpesvirus.	Herpes simplex I & II, V-Z Virus, Cytomegalovirus, Epsteins bar virus.
4) Papillomavirus.	Human Papilloma virus.
5) Parvovirus.	Parvovirus B-19
6) Polymavirus.	BK and JC Polymavirus.
7) Poxvirus.	Molluscum Contagiosum.

**RNA VIRUSES**

1) Arenavirus	Lassa fever virus.
2) Astrovirus.	Gastroenteritis causing astrovirus.
3) Bunyavirus.	Encephalitis.
4) Calcivirus.	Hepatitis E virus.
5) Coronavirus.	SARS Coronavirus.
6) Filovirus	Hemorrhagic fever virus.
7) Flavivirus.	Yellow fever, Dengue.
8) Orthomyxomavirus.	Influenza A,B and C.
9) Parvomyxomavirus.	Mumps, Measles and Parainfluenzavirus.
10) Picornavirus.	Poliovirus, Coxsackie A and B virus.
11) Reovirus.	Tick fever virus.
12) Retrovirus.	HIV I and II, HTLV I and II.
13) Togavirus.	Rubellavirus.

- General Approaches for Lab Diagnosis of Viral Infections:
  - Direct detection:
    - Microscopy or staining. (i.e. Cytology and Histology)
    - Electron Microscopy.
    - Immunodiagnosis. (Antigen Detection)
    - ELISA.
  - Virus Isolation:
    - PCR Assays.
  - Viral Serology:
    - Antibodies.
  - Viral Culture:
    - Conventional culture and Shell Vial Cell Culture.

## I) Cytology and Histology

Commonly used techniques for detection of viral inclusions, deals with the study of morphologic study of cells or tissue. Viral inclusions are intracellular structures formed by aggregates of virus or viral components within an infected cell OR abnormal accumulations of cellular materials resulting from viral-induced metabolic disruption. These inclusions occur in single or syncytial cell.<sup>[2,3]</sup>

PAP or Giemsa-stained cytologic smears are examined for inclusions or syncytia.

Cytology is mainly diagnostic for Pemphigus and HSV while, inclusions resulting from infection with CMV, Adenovirus, Parvovirus, Papillomavirus and Molluscum Contagiosum virus are detected by H & E staining.

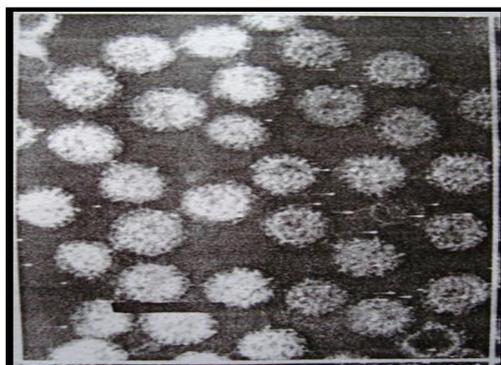
Inclusions of measles and rabies are less commonly detected on routine staining.

Cytology and histology are less sensitive than culture but are especially helpful to detect those viruses which are difficult to culture e.g. Parvo and rabiesvirus.<sup>[4,5]</sup>

## II) Electron Microscopy

EM is very helpful for the detection of viruses that do not grow in cell culture. Immune EM allows visualization of virus particles present in numbers too small for easy direct detection.

In this specific anti-serum is added which allow virus particle to form antibody-bound aggregates, which are more easily detected than are single virus particles.<sup>[2]</sup>



**Fig: Gastroenteritis Virus.**

- EM can detect Gastroenteritis viruses that cannot be detected by other methods (Noroviruses, coronaviruses and Astroviruses) and viruses causing Encephalitis that are not detected with cell culture (HSV, Measles virus and Polyomavirus).

- In addition, the etiology of newly recognized viral syndromes can be recognized rapidly by identifying characteristic viral morphology by EM in infected tissue.

### III) Immunodiagnosis (Antigen Detection)

High quality viral antibody reagents have led to the development of Fluorescent Antibody, Enzyme Immunoassay, Radioimmunoassay, Latex Agglutination, and Immunoperoxidase Tests that detect viral antigen in patient's specimens.<sup>[6]</sup>

A technique that uses a Fluorochrome to indicate the occurrence of a specific antigen-antibody reaction. The Fluorochrome labels either an antigen or an antibody. The labeled reactant is then used to detect the presence of the unlabeled reactant.

- The use of a labeled reactant (such as an antibody which both detects and indicates the antigen) to reveal the presence of an unlabeled one is termed as Direct Immunofluorescence.<sup>[1,2]</sup>
- The use of a labeled indicator antibody, which reacts with an unlabeled detector antibody that has previously reacted with an antigen, i.e. termed as Indirect immunofluorescence.<sup>[3]</sup>
- Direct immunofluorescent testing involves use of a labeled antiviral antibody; i.e. Fluorescein isothiocyanate (FITC), which is layered over specimen suspected of containing homologous viruses.<sup>[2]</sup>
- The indirect immunofluorescent procedure is a two-step test in which unlabeled antiviral antibody is added to the slide first, followed by a labeled (FITC) antiglobulin that binds to the first step antibody bound to virus in the specimen.
- Direct immunofluorescence is more rapid and specific than indirect immunofluorescence, but less sensitive.<sup>[7]</sup>
- Increased sensitivity of the indirect test, results from signal amplification that occurs with the addition of the second antibody.
- Signal amplification decreases specificity by increasing nonspecific back-ground fluorescence.<sup>[8]</sup>
- DIF is best suited to situations in which large quantities of virus are suspected or when high-quality, concentrated monoclonal antibodies are used.
- IDIF should be used when lower quantities of virus are suspected, such as detection of respiratory virus.
- High quality monoclonal antibodies improve the sensitivity and specificity of IF testing.<sup>[3]</sup>
- When possible direct test should be used because it is faster.

- Strict criteria is used for the interpretation of fluorescent patterns must be used. This includes standard interpretation of fluorescent intensity and recognition of viral inclusion morphology.

<b>INTENSITY</b>	<b>INTERPRETATION</b>
NEGATIVE	No Apple-green fluorescence.
1+	Faint Equivocal Apple-green fluorescence.
2+	Apple-green fluorescence.
3+	Bright apple-green fluorescence.
4+	Brilliant apple-green fluorescence.

**Fig: Interpretation of fluorescence intensity**

- Nuclear and cytoplasmic staining patterns are typical for influenza virus, adenovirus, and the herpes viruses; cytoplasmic staining only is typical for respiratory syncytial, parainfluenza, and mumps viruses; and staining within multinucleated giant cells is typical of measles virus.
- False-positive staining can occur with specimens containing yeasts, certain bacteria, mucus or leukocytes.
- IF stains are useful to detect RSV, Influenza and Parainfluenza viruses, Adenovirus, HSV, VZV, and CMV.<sup>[1,2]</sup>

**IV) ELISA**

Enzyme-Linked Immunosorbent Assays.<sup>[1,2]</sup> Enzyme immunoassay methods used most in clinical virology are the solid-phase enzyme-linked immunosorbent assay (solid-phase ELISA) and the membrane bound enzyme-linked immunosorbent assay (membrane ELISA).

- Solid-phase ELISA is performed in a small test tube or microtiter tray. Breakaway strips of microtiter wells are available for low-volume test runs.
- The remaining, unused wells can be saved for future testing.
- A solid-phase ELISA used for the detection of Rotavirus in stool specimens.
- Membrane ELISA tests have been developed for low-volume testing and where rapid results are needed. They can be performed by those with minimum training and usually require less than 30 mins to complete. The membrane method uses a handheld reaction

chamber with a cellulose-like membrane. Specimen and reagents are applied to the membrane.

- Following a short incubation time, a chromogenic (color) reaction occurs on the surface of the membrane and is read visually.
- The most used ELISA for antigen detection is those for RSV, HIV, Rotavirus and Influenza Viruses.

#### • Applications

- 1) The presence of antigen or the presence of antibody in a sample, it is a useful tool for determining serum antibody concentrations (such as with the HIV test or West Nile Virus).
- 2) It has also found applications in the food industry in detecting potential food allergens such as milk, peanuts, walnuts, almonds, and eggs.
- 3) ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs.

#### • Limitations

False negative results may occur, although on rare occasions, in the advent of very early or acute HIV infection or if the specimen volume is inadequate or the specimen quality is inappropriate.

False-positive results do occur, although the laboratory attempts to minimize this event from being reported. These false-positive results can be caused by a number of factors, namely:

- Interfering substances within the specimen;
- Severely hemolysed specimens;
- Cross reaction within the test method matrix;
- Cross-reaction events due to other underlying diseases such as;
- Autoimmune diseases.

Radio Immune assay, Immunoperoxidase staining and latex agglutination are additional techniques used to detect viral antigens.

- RIA has been largely replaced by ELISA because of the expensive materials and disposal procedures needed for radioactive materials.

- Immunoperoxidase staining is commonly used to stain histologic section for virus but is less popular than IF staining.
- Latex agglutination is an easy and inexpensive method but lacks sensitivity compared with ELISA and fluorescent immunoassays.

#### V) Nucleic Acid Probes and Polymerase Chain Reaction Assays<sup>[9,10,11]</sup>

Introduction of nucleic acid detection techniques into the clinical virology has resulted in a major shift in testing strategy.

- With the use of both nucleic acid detection and amplification based system, in conjunction with automated nucleic acid isolation techniques for sample preparation, nearly all virology labs have access to commercial or in house molecular assays, which leads generate results within 2-3 hrs.
- Nucleic acid detection done using nucleic acid probes, which are short segments of DNA that hybridize with complementary viral DNA or RNA segments.
- These probes are labeled with a fluorescent, chromogenic, or radioactive tag that allows detection if hybridization occurs.
- It is most useful when the amount of virus is relatively abundant, viral culture is slow or not possible, and when immunoassays lack sensitivity or specificity.
- When DNA target fragments that are too few in number in the original specimen, then it is detected by probes which amplified by using molecular techniques such as PCR.
- PCR is the method which can duplicate short DNA targets thousands to a million-fold. This provides enough target, referred to as Amplicons, to readily identify the presence of a specific virus.
- The PCR reaction with ensuing amplicon identification has been automated and made very rapid, and this rapid testing is known as Real-time PCR. It Can be used to detect viruses that are non cultivatable. Rapid identification (e.g. RT-PCR—4 Corners outbreak of hantavirus or FRET in the field) Can be used to manage patients (e.g. HIV viral load).

- The PCR test also can be used to amplify and detect RNA viruses by using the enzyme RT.
- The first step in RT-PCR includes making complementary DNA strands of the RNA segment in question.
- The usual PCR steps used to multiply the DNA target are then performed, leading to DNA amplicons whose identification signifies the presence of original RNA sequence.
- The rapid occurrence and broad application of molecular diagnostics will require the introduction and use of standardized materials and quality control programs.

- **Limitations**

- False negative and positive if not performed correctly.
- Tests are used for special purposes and not for routine diagnosis.
- Due to the fact that this assay is highly specific and the technique amplifies mainly DNA, false positive reactions may occur.
- The test is only accurate when requested at least 14 days after exposure or at least 6 weeks after the birth of a baby born to an HIV-positive mother.

- **Advantages**

- Capable of detecting pro viral DNA irrespective of viral expression.
- Highly sensitive and requires less sample.
- Nucleic acid can be detected in fresh/archival samples.
- Results within 24-48 hrs.
- Less expensive than virus culture.

## VI) Cell Culture

1. **Conventional cell culture:** Intracellular parasites requires host cell to grow, cell culture media and techniques in cell culture maintenance are necessary.<sup>[12]</sup>

- Cell cultures are incubated in a roller drum that holds cell cultures test tubes 5 to 7 degrees while they slowly revolve (1/2 to 1 rpm) at 35<sup>0</sup> to 37<sup>0</sup>. Incubation of cell culture tubes in a stationary rack can be used in place of a roller drum.

- Rapidly growing viruses, such as HSV, appear to be detected equivalently by this way.
- Once inoculated with specimen, cell cultures are incubated for 1 to 4 weeks, depending on virus suspected. Cells are inspected microscopically for the presence of virus, indicated by areas of dead or dying cells called cytopathic effects.

- **Cytopathic effects**

It is a visible result occurred in a cell due to viral infections. It produces Cell death by multiplying viruses. It produces Inhibition of DNA, RNA or protein synthesis, which affects on permeability of membrane.

- Cytopathic effects (CPEs) of infected cells can be observed with inverted light microscopes. It gives following interpretation:
  - Rounding/detachment from plastic flask.
  - Syncytia/fusion.
  - Fusion of cells.
  - Shrinkage.
  - Increased refractility.
  - Aggregation.
  - Loss of adherence.
  - Cell lysis/death.

Two kinds of media, growth medium and maintenance medium are used for cell culture.

- Both are prepared with Eagle's Minimum Essential Sodium (EMEM) in Earle's Balanced Salt Solution (EBSS) and includes antimicrobials to prevent bacterial contamination.
- *Growth medium* is a serum-rich (10% fetal, newborn, or a gammaglobulinemic calf serum) nutrient medium designed to support rapid cell growth.
- *Maintenance medium* is similar to growth but contains less serum (0% to 2%) and is used to keep cells in a steady state of metabolism.

2. **Shell vial cell culture**<sup>[2]</sup>: (centrifugation culture) it is a rapid modification of earlier conventional cell culture.

- In this viruses are detected more quickly because the infected cell monolayer is stained for viral antigens produced soon after infection, before the development of CPE.
- Viruses that take days to weeks to produce CPE can be detected within 1 to 2 days by detecting early produced viral antigens.
- A shell vial culture tube a 15 x 45 mm 1-dram vial, is prepared by adding a round cover slip to the bottom, covering this with growth medium, and adding appropriate cells.
- It is commonly used to detect viruses having long incubation before producing CPE i.e. CMV and VZV.
- Advantage is its speed; most viruses are detected within 24 hrs.
- Disadvantage is that only one type of virus can be detected per shell vial.

**TABLE 5-4** Detection of Virus: Traditional CPE Method vs. Shell Vial Technique

Virus	Days to Detect CPE Conventional Cell Culture Method [avg(range)]	Days to Detect CPE Shell Vial Centrifugation Method [avg(range)]
RSV	6(2-14)	1-2
Influenza A	2(1-7)	1-2
Influenza B	2(1-7)	1-2
PIV 1-4	6(1-14)	1-2
Adenovirus	6(1-14)	2-5
HSV	2(1-7)	1-2
VZV	6(3-14)	2-5
CMV	8(1-28)	1-2

QUANTIFICATION	INTERPRETATION
Negative	Uninfected Monolayer.
Equivocal	Atypical alteration of monolayer involving few layer.
1+	1%-25% of monolayer exhibits CPE.
2+	25%-50% of monolayer exhibits CPE.
3+	50%-75% of monolayer exhibits CPE.
4+	75%-100% of monolayer exhibits CPE.

- **Identification of Viruses in cell culture**

Viruses are detected in cell culture by the CPE.

Virus infected cells change their morphology and eventually lyse or detach from the glass surface while drying. CPE is quantified as follows:

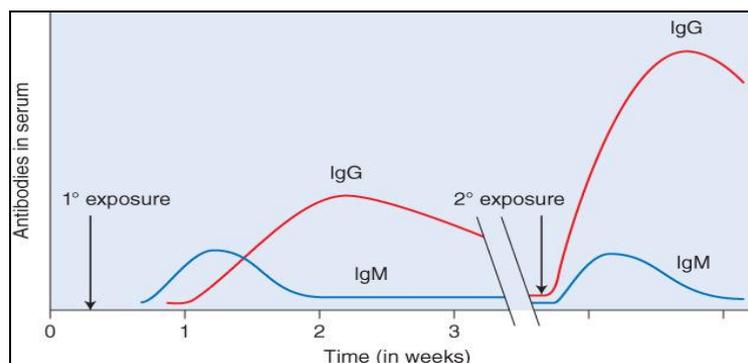
- Identification of viruses can be made based on the cell line that supports viral replication, how quickly the virus produced CPE and a description of a CPE on a particular cell culture.
- Most of the viruses can identify on these criteria but for confirmation, some can use Fluorescent labeled anti-sera.

## VII) Viral Serology<sup>[10,12]</sup>

Viral serology was first used in 1970's. It is used to detect immune status and to make the diagnosis of infections in situations in which the virus cannot be cultivated in cell culture, or detected readily by immunoassay or molecular assays. In most viral infections, IgM is undetectable 1 to 4 months after acute infection resolves but detectable levels of IgG remain for the life of the patient.

If a patient is infected by an antigenically similar virus or the original strain has remained latent and reactivates at a later time, these viral-specific IgG and IgM antibody levels may again rise.

- The secondary IgM response may be difficult to detect; however a significant IgG titer rise is readily apparent in immunocompetant patients.
- Primary and secondary responses to viral infections
  - IgM (1st exposure)
  - IgG (2nd exposure)



**Fig: Primary (1 degree) and secondary (2 degree) antibody responses toward a viral pathogen.**

- Serological methods are used to detect antiviral antibody.
- Prominently in that is Western Blotting.

- **The Western Blot assay<sup>[1]</sup>**

The method originated from the laboratory of George Stark at Stanford. The name western blot was given to the technique by W. Neal Burnette and is a play on the name Southern blot, a technique for DNA detection developed earlier by Edwin Southern.

Detection of RNA is termed northern blotting and the detection of post-translational modification of protein is termed Eastern blotting.

**Principle:** The western blot is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide or by the 3-D structure of the protein. The proteins are then transferred to a membrane (typically nitrocellulose, where they are detected using antibodies specific to the target proteins. The stained bands then indicate the proteins to which the patient's serum contains antibody.

- **Limitations:** Since the Western Blot is an antibody test, like the initial ELISA test, it is not fully independent, and the very common use of this as a confirmatory test for ELISA is thus questionable.

- **Advantages**

1. Rapid and easy to perform
2. Can usually discriminate between HIV-1 & HIV-2
3. Do not require sophisticated equipment
4. The results are read by development of colour
5. Sensitivity and specificity of these tests is comparable to ELISA is high.

- **Disadvantage**

1. Only drawback is the high cost

- **Uses**

single test application i.e. in emergency, autopsy room and peripheral blood banks.

- **Serology Panels & Immune Status tests for Common Viral Syndromes.**

Situation	Viruses under consideration
AIDS	Human immunodeficiency virus.
CNS infections	Arbovirus, Lymphocytic Choriomeningitis virus, Enterovirus, Measles, Mumps, HSV, Rabies.
Exanthems	Measles, Rubella, Parvovirus.
Vesicular	HSV, Varicella-Zoster virus.
Hepatitis A	Hepatitis A virus.
Hepatitis B	Hepatitis B virus.
Infectious Mononucleosis	Cytomegalo virus, Epstein – Barr Virus.

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