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Lab Investigation Of Viral Diseases – A Brief Review Literature

[1]Dr. Vitheeyasree S , [2]Dr. Keerthana G, [3]Dr. Mathumala Subramanian , [4]Dr.Pradeep Sankar S , [5]Dr.Sathish Kumar M

[1]Under Graduate, [2,3]Post Graduate, [4]Senior Lecturer, [5]Head of the Department

Department of Oral and Maxillofacial Pathology

Karpaga Vinayaga Institute of Dental Sciences

Chengalpattu, India

ABSTRACT:

Viral diseases pose a significant threat to global health, with a vast array of viruses infecting humans and animals, causing widespread morbidity and mortality. This review aims to provide a comprehensive overview of the laboratory investigation of viral diseases, highlighting the importance of understanding the biology and pathogenesis of viruses. Viruses, consisting of genetic material encased in a proteinaceous capsid, rely on host cells for replication and spread of disease. This review will delve into the diverse range of viral diseases, including arboviruses, bunyaviruses, cytomegalovirus, papillomavirus, and many others, which affect various organ systems and manifest in different clinical presentations. The laboratory investigation of viral diseases is crucial for diagnosis, surveillance, and prevention of outbreaks. This review will summarize the current laboratory methods and techniques used in the detection, identification, and characterization of viruses, as well as discuss the challenges and limitations faced in the laboratory investigation of viral diseases. The review will also highlight the significance of laboratory investigation in informing public health policy and guiding the development of effective vaccines and treatments.

KEYWORDS – Genetic material, Detection, Identification, Public health policy

INTRODUCTION

The minimal proteins and genetic material present in viruses, which are microscopic particles of either DN A or RNA, are encased in a proteinaceous capsid material[1]. Viruses need a host cell and depend on the host's cellular machinery to reproduce and spread disease. Arbovirus and renavirus, bunya viruses, cytomegalovirus, papillomavirus, Epstein-Barr virus, genital herpes, German measles (rubella), hanta viruses, hemorrhagic viruses, herpes simplex, influenza A, Kaposi's sarcoma, measles (rubeola), poliomyelitis, poxvirus infections, rabies, respiratory syncytial virus infection, Varicella-Zoster viral disease, viral encephalitis, viral hepatitis, and West Nile virus are some of the important viruses and viral diseases.

DEFINITION

Viral diseases are caused by organisms that are particles and do not have a self-sustaining or autoreplicatory cellular machinery, and contain nucleic acid as the genetic material.

CLASSIFICATION

Based on their genetic makeup, viruses are divided into two categories:

- RNA viruses and
- DNA viruses.

A minimal set of proteins and genetic material are present in every form of virus[1]. The genetic material can be either double-stranded (hepatitis B virus or herpes simplex virus) or single-stranded (poliovirus or human immunodeficiency virus). The genomes of most RNA viruses are single-stranded. Arena virus, rotavirus, influenza A, bunya viruses, and other reoviruses are exceptions to this rule[3]. Except for the parvoviridae, the majority of DNA viruses have a double-stranded genome (Kok and Pechere, 1999). Other virus categories are based on the form of the viral particle, the type of nucleocapsid, or the polarity of the genomes**.**

ETIOLOGY

Exposure to a specific virus causes individual viral illnesses.

Viral diagnosis has advanced significantly, going from the periphery to the center of clinical care[4]. Numerous factors have contributed to this shift, but most significantly, efficient antiviral treatments. Conventional virus isolation was the gold standard for viral diagnosis for many years because it was "open-minded" and sensitive[5]. Cell culture, however, is a specialized method that calls for highly qualified staff. Furthermore, the clinical significance of conventional cell culture is limited by the intrinsic delay that comes with growth. Rapid culture and viral antigen techniques are less sensitive and detect fewer pathogens than conventional culture, but they also need less skill and produce results much faster[1]. In virology, polymerase chain reaction (PCR) has brought about a new age, particularly in the detection of neurological disorders. Molecular amplification techniques, such as PCR, are quick and extremely sensitive. They can identify viruses that are not susceptible to standard culture, and they can be quantified and automated[6].

GENERAL PRINCIPLES OF LABORATORY VIRAL DIAGNOSIS

Viral diagnostic testing is done to help diagnose an acute sickness or to record a previous infection. We will concentrate on diagnosing diseases. The development of immunoglobulin M (IgM) and IgG antibodies, viral replication, clinical symptoms, virus elimination, and disease resolution all follow a predictable course in an idealized viral infection[2]. Samples should be taken as early in illness as feasible, when virus titers are at their highest, and diagnostic efforts should be concentrated on virus identification when disease results from viral replication and host cell lysis. On the other hand, antibody detection is crucial in cases when the host's reaction to viral infections mediates some disease processes. Hepatitis B virus (HBV), parvovirus B19, and Epstein-Barr virus (EBV) are viruses linked to immune-mediated illnesses[7]. Viral persistence, latency, reactivation, and late illness are all significant departures from the model of acute infection leading to viral clearance. Therefore, while ordering tests and interpreting the findings, it is important to take into account the pattern for a certain virus as well as the host's immunological capability.

1.VIRAL ISOLATION METHODS

CONVENTIONAL CELL CULTURE

Living cells are necessary for virus replication. Suckling mice, primates, embryonated eggs, and other vulnerable animal hosts were used in early virology research. Primary, diploid, and continuous or heteroploid cell lines are the three fundamental types utilized in clinical laboratories [2]. According to Land and Ginocchio (2007), the majority of labs that carry out standard viral culture will inoculate a clinical specimen into two or three distinct cell lines. After one to three weeks of incubation, cultures are checked for the emergence of typical viral cytopathic effects (CPE). The virus inoculum, the particular virus's growth pattern, the cell line or lines inoculated, whether the culture is rotated, the incubation temperature, and the frequency of examinations all affect how long it takes to discover CPE in a conventional culture[9]. Immunofluorescence (IF) staining of the grown cells is the most common method of identifying the virus isolate if CPE is observed. When CPE is not available, hemadsorption—which involves adding a mild guinea red cell solution to the cell monolayer—can be used to check for the expression of hemagglutinin proteins on the surface of influenza or parainfluenza virus-infected cells. Enteroviruses (acid-stable) and rhinoviruses (acid-labile) can be distinguished using an acid lability test.

ASSAY TIME - 1-21 DAYS

ADVANTAGES

More sensitive than antigen detection, it enables the isolation of many viruses and can identify unexpected or novel viruses.

LIMITATION

Requires skill to maintain cell cultures and interpret CPE; a full culture requires more than 1 mL of CSF; some viruses do not thrive in standard cultures; Biosafety issues with emerging and zoonotic viruses

RAPID CULTURE

A more focused strategy, like quick culture utilizing shell vials, can be employed when a small number of particular viruses are being taken into consideration in the differential diagnosis. Shell vials, which get their name from their resemblance to shotgun shells, are tiny vials with a coverslip coated in cell monolayer at the base. The vials are centrifuged to boost sensitivity following sample inoculation, resulting in the nickname quick centrifugation cultures The cover slips are taken out of the shell vials at prearranged intervals following inoculation, and the cell monolayers are then preserved with acetone and exposed to IF staining using monoclonal antibodies (MAbs) for certain viruses [10]. The most frequent examination of shell vial cultures occurs about 1-2 days after incubation and prior to the emergence of CPE (Gleaves et al., 1984). Shell vials might occasionally undergo a prolonged incubation period and be evaluated for CPE.

ASSAY TIME – 1-5 DAYS

ADVANTAGES

Use of "mixed cell" cultures enables the identification of numerous viruses in a single vial; most findings occur in 1-2 days; and IF staining takes less training to interpret than CPE.

LIMITATION

Needs knowledge of IF and cell culture; only finds specific viruses; less sensitive than traditional culture

2.ANTITIBODY DETECTION

When antibodies are present, it can be a sign of previous viral exposure, vaccination, cross-reaction to a closely related virus, or even passive antibody transfer from blood products or immunoglobulins. Evidence testing methods include ELISA, EIA, CLIA, IF, IC and IgG

Solid-phase immunoassays (SPIAs)

The process of immobilizing an antigen or antibody on a solid phase, like a microtiter plate or microparticle, is known as "solid-phase immunoassay." The most popular kind of SPIA is the non-competitive indirect enzyme immunoassay (EIA), while these tests come in a variety of formats[1]. An MAb coupled to an enzyme (such as alkaline phosphatase or horseradish peroxidase) that, in the presence of a suitable substrate, generates a colorimetric or fluorescent signal whose intensity is directly correlated with the amount of antibody present in the original sample is used to detect the presence of bound virus-specific antibodies in indirect EIAs, where patient serum is allowed to react with immobilized viral antigens[1]. Almost all non-competitive EIAs employ an IgG-specific antibody for detection, even though the enzyme-coupled antibody could be specific for any isotype.

ASSAY TIME – 30 MINS – 24 HOURS

ADVANTAGES

Can be automated; certain tests can be performed at the point of care; it can record primary, recent, and previous infections as well as carrier statuses; Fourth-generation HIV tests detect both antigen and antibody in a single reaction.

LIMITATION

IgM assays have a somewhat high probability of false-positive results; immunocompromised hosts may not produce antibodies; diagnosis is sometimes retrospective; and cross-reactivity between comparable viruses is frequent (e.g., arboviruses).

3. ELECTRON MICROSCOPY

Conventional light microscopy cannot view viruses because they are too small, and the only method that can directly visualize viruses is electron microscopy (EM) (Biel and Gelderblom, 1999)[2]. The foundation of viral identification by EM is the recognition of viral morphology and ultrastructural features of virus-infected cells, which calls for a high level of knowledge.

THIN SECTIONING

When histopathologic results point to a viral infection, tissue biopsies or cells undergo thin sectioning. Similar to paraffin-embedded tissues in pathology, samples are sectioned after fixation and embedding in polymer resin; however, the resulting sections are far thinner. After that, staining is done with heavy-metal salts**.**

ASAAY TIME – 3 DAYS

ADVANTAGES

Permits the viewing of virus particles, the identification of novel viruses, and the detection of unexpected infections**.**

LIMITATION

Costly; labor-intensive; necessitates a large viral burden and proficiency in viral recognition

NEGATIVE STAINING.

In negative staining, samples are treated with fine-grained, heavy-metal, electron-dense salts, and the staining material builds up inside the viral envelope or capsid and around the virus[3].Stain-free areas show up as white on a black background, while viral structures that have acquired stain can take on various gray tones.

ASSAY TIME – 1 HOUR

ADVANTAGES

Enables quick visual inspection of virus particles in respiratory secretions, urine, stool, or vesicle fluid; unexpected pathogen detection and viral discovery

LIMITATION

Costly and time-consuming; little experience

4.HISTOPATHOLOGY AND CYTOLOGY

Although light microscopy cannot reveal virus particles, morphologic characteristics and specialized staining techniques can be used to identify viral infections in tissue samples. Viral replication can create cytoplasmic and/or nuclear inclusions, which are a characteristic of a certain viral infection, or it can alter the normal cellular and nuclear architecture, resulting in the appearance of "ground-glass" nuclei and multinucleate cells[4]. To find viruses in tissue, more specialized techniques such in situ hybridization (ISH) and immunohistochemistry may be used.

GIEMSA STAIN

For both thin and thick smears, Giemsa stain is the gold standard staining method used to check for malaria parasites, perform routine blood parasite checks, and morphologically distinguish between the nucleus and cytoplasm of erythrocytes, leucocytes, platelets, and parasites

Giemsa stain is a traditional differential stain used extensively in histology and cytogenetics to diagnose the following conditions[3]. It is composed of a mixture of reagents (eosin dye, methylene blue, and azure).

1. Spirochetes, malaria, and other blood parasites
2. Inclusion bodies for Chlamydia trachomatis
3. Borrelia species
4. Yersinia pestis
5. Histoplasma species
6. Cysts of pneumocystis

Staining procedure 1:

Thin Film staining

- Create a thin layer of the specimen (blood) on a dry, clean microscopic glass slide, then let it air dry.
- To fix the smear, dip it twice or three times in pure methanol, then let it air dry for 30 seconds.
- For 20 to 30 minutes, cover the slide with 5% Giemsa stain solution.
- Use tap water to flush, then let it dry**.**

Staining Procedure 2:

Thick Film Staining

1. On a staining rack, apply a thick layer of blood and let it air dry for an hour.

The thick blood smear should be dipped into diluted Giemsa stain, which can be made by mixing 1 milliliter of the stock solution with 49 milliliters of phosphate buffer or distilled water, but the results may differ.

3. Rinse the smear for three to five minutes in distilled water that has been buffered.

4. Let it air dry.

Interpretation

A basic dye, such as methylene blue or azure, attaches itself to the acid nucleus to produce a blue-purple hue. The cytoplasm and its alkaline granules are drawn to the acidic dye eosin, which gives them a reddish-orange hue**.**

Advantages

Easily accessible, simple to maintain, prepare, and operate

Limitations

The working Giemsa stain needs to be ready right before usage.

PAPANICOLAOU STAINING

A pap smear is the process used to apply Papanicolaou stain, which is also referred to as the pap stain[2] .It is a polychromatic stain that differently stains different cell components using a number of dyes.

PROCEDURE

- Use 95% ethanol for 15 minutes to fix the stain.
- Use tap water to rinse.
- For one to three minutes, add the Harris Hematoxylin dye.
- Rinse with Scott's tap water or tap water.
- Make ten dips in 95% ethanol using the preparation.
- For one and a half minutes, add orange G-6 stain.
- Take ten dips in 95% ethanol
- For two and a half minutes, add Eosin dye, EA-50, Modified EA-50, or EA-65 stain.
- Make ten dips and two changes in 95% ethanol.
- For one minute, add 100% ethanol.
- Use two xylene changes for two minutes each to clean.
- Mount using permanent mounting media.

ADVANTAGES

- Used in the Pap test, often known as the Pap smear.
- Cervical cancer screening.
- Analysis of hepatic myeloma cancer cells.
- Thyroid cancer screening.
- Cell carcinoma screening.
- Benign tumors are examined and described.
- The species of Candida was identified.
- Chlamydia trachomatis identification

LIMITATION

It has low sensitivity and limited accuracy; it is merely a screening test that needs to be followed up with more sophisticated diagnostic procedures.

5.VIRAL ANTIGEN DETECTION

Direct proof of viral infection is provided by the identification of viral antigens in clinical samples. Viral antigens can be detected using many of the same assay formats that are used to detect antibodies.

IMUNOFLUORESCENCE

Direct immunofluorescent assays (DFA) for the detection of viral antigens in infected cells are widely used for the diagnosis of respiratory virus infections and herpetic skin lesions. DFA can be performed on many cellular sample types, especially nasopharyngeal washes, aspirates or swabs, bronchoalveloar lavage, skin and mucosal lesions, PBL and tissue biopsies. Cells collected from a patient are pelleted by centrifugation, applied to glass slides, fixed, stained with antibodies coupled to fluorophores, then examined under a fluorescence microscope for virus-specific staining[5] . The interpretation is influenced by the stain's color and intensity, the distribution of viral proteins within the infected cell, the infection of particular cell types, and the quantity of positive cells

ASSAY TIME – 1-2 HOURS

ADVANTAGES

As samples enter the lab, it can be completed "on demand"; reagents for eight respiratory and four herpesviruses are available; sample quality can be evaluated.

LIMITATIONS

Demands a significant amount of experience to get correct results; it is labor-intensive and manual; it needs a sufficient number of target cells to produce reliable results.

6.NUCLEIC ACID DETECTION

Early molecular techniques were not frequently used in the clinical setting because they were more expensive and esoteric than antigen and culture techniques, but they were also not more sensitive[9]. Since there was no alternative test for HPV, the earliest clinical viral molecular diagnostics detected HPV DNA in cervical samples using non-amplified nucleic acid hybridization with radioisotope-labeled probes[5]. The next development was ISH, which used non-radioactive probes on these samples, like for EBV. For HBV DNA quantification, hybrid capture—in which nucleic acid hybrids in solution were caught by MAbs and identified—was later introduced.

CONVENTIONAL PCR

Three processes make up conventional PCR: nucleic acid extraction and purification, target sequence amplification with certain primers and DNA polymerase, and detection of amplified fragments, or "amplicons.

EXTRACTION-

Other approaches have replaced the conventional phenol/chloroform-based nucleic acid separation procedures. After cells are alkalinely lysed, single-sample spin column kits bond to a resin to separate either DNA or RNA. Nucleic acids are eluted into an aqueous solution following washing[7]. Another popular method involves binding nucleic acids to silica beads in solution, then washing and centrifuging to get rid of contaminants before elution into a buffer. These kits are not suitable for high-throughput testing and necessitate a large amount of hands-on work.

AMPLIFICATION

Small DNA primers (about 20 base pairs (bp) are used in PCR to bind to complementary areas of the target to get the nucleic acid sequence. The length of the primers and the sequence being targeted determine the temperature at which the DNA primers anneal to it; typically, this is between 55 and 60 C. The thermostable DNA polymerase used in PCR was first isolated from the hot spring-dwelling bacteria Thermus aquaticus (Taq), which has an optimum activity temperature of about 72 C[6]. At the higher temperature (about 95 C) needed to "melt" the longer strands of double-stranded DNA (dsDNA) produced by PCR into single strands, Taq polymerase is comparatively stable.

DETECTION -

Products are sorted according to the length of the dsDNA fragment after a tiny volume of the PCR reaction is placed onto an agarose gel soaked with ethidium bromide, a highly carcinogenic fluorescent chemical that firmly binds DNA. Following UV light exposure, amplified PCR products are then visible as discrete bands of the proper size. As an alternative, amplicons can be spotted straight onto nitrocellulose for hybridization with a particular probe or transmitted via Southern blot[4]. Relatively inexpensive thermocyclers and gel visualization equipment are needed for conventional PCR.
But in order to extract an aliquot for detection, vials must be opened, which might aerosolize DNA amplicons and contaminate tools, surfaces, gloves, and clothes.

ADVANTAGES

Employs low-cost conventional thermocyclers; multiplex testing is more feasible than real-time testing, and it is less impacted by genomic variability

LIMITATION

Slower than real-time techniques; ethidium bomide used for amplicon detection is poisonous; prone to carryover contamination from amplified products because the tube is opened after amplification.

REAL TIME PCR

Fluorescent DNA-binding dyes like SYBR green, which is comparable to ethidium bromide in gel electrophoresis, or hybridization with fluorescent DNA probes are frequently used in real-time PCR techniques[8] . Consequently, real-time assays need the utilization of thermocyclers equipped with integrated detectors, filters, and light sources. When DNA binds, SYBR green and related dyes turn fluorescent and have a greater affinity for dsDNA molecules[3]. The fluorescent signal produced by the dyes rises in tandem with the quantity of dsDNA produced by PCR. These assays may be useful for detecting viruses with sequence diversity because they don't require a complementary DNA probe.

ASSAY TIME – 1-5 HOURS

ADVANTAGES

More commercial kits, including walk-away tests, are becoming accessible; lab-developed assays can be easily updated; they are quicker, less susceptible to cross-contamination, and easily measured.

LIMITATION

Lack of standardization; values acquired in various labs can differ by three log10; more likely to provide falsely negative or low values because of genetic variances in virus strains; restricted ability to multiplex

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