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DIAGNOSTIC AIDS IN VIRAL DETECTION: A REVIEW

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ABSTRACT

The practice of pathology is currently undergoing significant change due to advances in the analysis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins in tissues. These advances have permitted improved biologic insights into many infectious diseases. It is likely that in the near future these methods will increasingly enter into the day-to-day diagnosis and management of patients.

KEYWORDS: Viruses, Diagnosis, Deoxyribonucleic acid, Ribonucleic acid.

INTRODUCTION

The study of viruses has historically provided and continues to provide the basis for much of our fundamental standings of modern biology, genetic and medicines. Thus, the replication and propagation of the virus are frequently manifests with the occurrence of an infectious disease that spreads between the individuals. Therefore, early diagnosis will not only serve to facilitate the early detection of new viral variant but will provide information to problem.[1]

Collection of Specimen: Specimens for the detection of viruses should be collected as early as possible following the onset of disease. Virus may no longer be present as early as two days after the appearance of symptoms. Various specimens for the detection of viruses can be-

Throat or Nasopharyngeal swab or aspirate: Nasopharyngeal aspirates are superior to swabs for recovering viruses. Throat swabs are acceptable for recovering Entero-viruses,

Adenoviruses and Herpes Simplex virus (HSV) whereas nasopharyngeal swab or aspirate specimens are preferred for the detection of respiratory syncytial, influenza, and Para influenza viruses.[2]

Bronchial and Broncho alveolar washes: Wash and lavage fluid collected during bronchoscopy are excellent specimens for the detection of viruses that infect the lower respiratory tract, especially influenza and Adenoviruses.[2]

Skin and mucous membrane lesions: Collection of specimen from cutaneous vesicles for detection of HSV or Vericella zoster virus (VZV) requires a Tzanck smear which are prepared by carefully un-roofing the vesicle. With the roof of the vesicle folded back, carefully remove the excess fluid by dabbing with sterile gauze. Press a clean glass microscope slide against the base of the ulcer. Lift, move slide slightly, and press again. Cells from the base of the ulcer will stick to the slide making an "impression smear" of infected and non infected cells.[3]

Serum for antibody testing: Acute and convalescent serum specimens may be needed to detect antibody to specific viruses. Acute specimens should be collected as soon as possible after the appearance of symptoms. The convalescent specimen is collected a minimum of 2 to 3 weeks after the acute phase by venipuncture.[4]

Specimen Transport and Storage: Ideally Specimens should be placed in ice and transported to the laboratory at once. Every attempt should be made to process the specimen within 12 to 24 hours of collection. Under unusual circumstances specimens may need to be held for days before processing. For storage up to 5 days, hold specimen at 4° C. Transport media contain proteins such as serum, albumin, or gelatin to stabilize virus and antimicrobials to prevent overgrowth of bacteria and fungi. Examples of successful transport media include: Stuart's medium, Amie's medium, Leibovitz-Emory medium, Hanks balanced salt solution and Eagle’s tissue culture medium.[5]

Specimen Processing: Specimens for viral culture should be processed immediately upon receipt in the laboratory. If delays occur, specimens should be stored in viral transport medium at 4°C as described earlier. Each specimen for virus isolation should be accompanied by a requisition that provides the following information in addition to patient identification and demographics: source of specimen, clinical history or viruses suspected and date and

time of specimen collection. Processing viral specimens should occur in a biological safety cabinet (BSC) whenever possible. If not then, it should occur behind a protective plexi glass shield.[4]

DISCUSSION

Various techiniques available to detect the viruses are as follows-

Cytology and histology

Electron microscopy

Immuno diagnosis

Immunofluorescence

Direct Immunofluorescence

Indirect Immunofluorescence

Radioimmunoassay

Enzyme-linked immuno-sorbent assay (ELISA)

Latex agglutination

Enzyme-linked virus-inducible system (ELVIS)

Molecular detection using Nucleic acid probes

Molecular detection using Probe identification following amplification by PCR

Conventional cell culture

Shell vial culture

Hem adsorption

Viral Serology

Cytology and histology: The most readily available technique for the detection of virus is cytologic or histological examination for the presence of characteristic viral inclusions. 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[6] Papanicolaou (PAP) or Giemsa-stained cytologic smears are examined for inclusions. Cytology is most frequently used to detect infections with Varicella-zoster and Herpes simplex viruses.[7]

Electron microscopy: EM is most helpful for the detection of viruses that do not grow readily in cell culture and works best if the titer of virus is at least 106 to 107 particles per ml. EM allows visualization of virus particles that are present in too small numbers for easy direct detection. The addition of specific antiserum to the test suspension causes the virus

particles to form antibody-bound aggregates, which are more easily detected. Advantage is that it is labor intensive, relatively insensitive and is used for Rotavirus, Enteric adenovirus, Herpes simplex virus and measles virus.[3]

Immunodiagnosis: Immunodiagnostic techniques include - Immuno fluorescence, Radioimmunoassay, Enzyme-linked immunosorbent assay (ELISA), Latex agglutination, Immunoperoxidase.[4]

Immunofluorescence: It was discovered by Coons which involves direct and indirect immunofluorescent antibody method.

- **Direct immuno fluorescent technique**

Antibody testing involves use of a labeled antiviral antibody. The label is usually fluorescein isothiocyanate (FITC), which is layered over specimen suspected of containing homologous virus.

- **Indirect immunofluorescence technique**

Antibody procedure is a two-step test in which unlabeled antiviral antibody is added to the slide first followed by a labeled fluorescein isothiocyanate (FITC) antiglobulin that binds to the first-step antibody bound to virus in the specimen. Increased sensitivity of the indirect test results from signal amplification that occurs with the addition of the second antibody.[4]

Radioimmunoassay: This method uses labels called as radioisotopes & enzymes. The method is used to determine the concentration of antigen. The antigen (analyte or ligand) conjugates with antibody (binder). Ag is separated into free and bound state. Radioactive counts are measured.[4]

Enzyme-linked immuno-sorbent assay (ELISA): Enzyme immunoassay methods are used most frequently in clinical virology. Solid phase ELISA is performed in a small test tube or micro titer tray. Breakaway strips of micro titer wells are available for low-volume test runs. Membrane ELISA tests have been developed for low volume testing and situations in which rapid results are needed. The membrane method uses a hand held 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.[4]

Latex agglutination: This method helps in detection of antigen by clumping or agglutination of latex beads with antibody bound to their surfaces. Antigen, if present in the specimen to be tested, binds to the combining sites of antibody exposed on the surfaces of latex beads, forming cross-linked aggregates of latex beads and antigen.[4]

Enzyme-linked virus-inducible system (ELVIS)

The ELVIS system uses a BHK (baby hamster kidney) cell culture system with a cloned (added) beta-galactosidase gene that is expressed only when cells are infected with a virus. The genetically engineered BHK cells are sold in multi welled micro titer plates. Following inoculation of specimens and overnight incubation, growth of virus results in production of the beta-galactosidase enzyme by the BHK cells. Beta-galactosidase serves as the "reporter" molecule. When cells are fixed and stained for galactosidase activity, positive staining indicates the presence of virus. Wells not containing virus show no staining.[4]

Molecular detection using Nucleic acid probes

Nucleic acid probes are short segments of DNA that are designed to hybridize with complementary viral DNA or RNA segments. The probe is labeled with a fluorescent, chromogenic, or radioactive tag that allows detection, if hybridization occurs.[8] Acid probes are most useful in situations in which the amount of virus is relatively abundant, viral culture is slow or not possible and immunoassays lacking sensitivity or specificity.[9]

Molecular detection using Probe identification following amplification by PCR

The polymerase chain reaction (PCR) is a method that duplicates short DNA segments thousands- to a million-fold. DNA fragments that can be identified with a specific probe but are very few in number in the original specimen to be detected and can be duplicated (amplified) using PCR. This provides the probe with enough target to readily identify the presence of a specific virus.[10]

Conventional cell culture

Viruses are strict intracellular parasites, requiring a living cell for multiplication and spread. To detect virus using living cells, suitable host cells and cell culture media the techniques in cell culture maintenance are necessary. Host cells, referred to as *cell cultures* originate as a few cells and grow into a monolayer on the sides of glass or plastic test tubes. Cells are kept moist and supplied with nutrients by keeping them continuously immersed in cell culture medium. Cell cultures are routinely incubated in a roller drum, which holds cell culture test tubes tilted (5 to 7 degrees) while they slowly revolve (upto 1 RPM) at 35° to 37°C.[4]

Metabolism of growing cells in a closed tube results in the production of CO_2 and acidification of the growth liquid. To counteract the pH decrease a bicarbonate buffering system is employed in the culture medium to keep the cells at physiologic pH 7.2. Phenol red, a pH indicator which is red at physiologic, yellow at acidic and purple at alkaline pH is added to monitor adverse pH changes. Once inoculated with specimen, cell cultures are incubated for 1 to 4 weeks depending on the viruses suspected. Periodically, the cells are inspected microscopically for the presence of viruses, indicated by areas of dead or dying cells called as cytopathic effect (CPE). Preliminary identification of a virus can sometimes be made based on the cell culture that supports viral replication, the speed at which the virus produced CPE and a description of the CPE.[11]

Shell vial culture: The shell vial culture is a rapid modification of conventional cell culture. Virus is detected more quickly using the shell vial technique because the infected cell monolayer is stained for viral antigens found soon after infection before the development of CPE. Viruses that normally take days to weeks to produce CPE can be detected within 1 to 2 days.

The shell vial culture technique can be used to detect most viruses that grow in conventional cell culture. It is best used for viruses that require relatively long incubation before producing CPE such as CMV and VZV. The advantage of shell vial is its speed and most viruses are detected within 24 hours. The disadvantage is that only one type of virus can be detected per shell vial.[4]

Hemadsorption: This method is used for detection of viruses that produce little or no CPE. As infected cells contain viral hem adsorbing glycoproteins in their outer membranes, addition of guinea pig red blood cells to the cell culture tube followed by a wash to remove no adsorbed RBCs results in a ring of RBCs around infected cells. This ring can be stained with fluorescent-labeled antisera to identify the specific hem adsorbing virus present. This method is mainly used for used for – Influenza virus, Para influenza virus and Para myxovirus virus.[4]

Viral Serology: It is used primarily to detect immune status and to make the diagnosis of infections caused by virus which cannot be cultivated in cell culture or detected by immunoassay. In most viral infections, IgM is undetectable after 1 to 4 months but detectable levels of IgG remain. If infected by an antigenically similar virus or a latent virus that

reactivates at a later time, virus-specific IgG and IgM antibody levels may again rise. At this stage, secondary IgM response may be difficult to detect. However, a significant (four fold) IgG titer rise can be noted.

Immune status test measures whether a patient previously was infected by a particular virus. A positive result with a sensitive, virus-specific IgG test indicates past infection.

Two approaches to detect current disease i.e. detection of virus-specific IgM in an acute-phase specimen collected at least 10 to 14 days after the onset of infection and detection of a fourfold antibody titer rise between acute and convalescent sera also indicates current or recent disease. Acute-phase serum should be obtained as soon as possible after onset of symptoms while convalescent specimen - 2 to 3 weeks after the acute phase.[12]

CONCLUSION

The analysis of data obtained from diagnostic specimens is revolutionizing the practice of pathology. Nevertheless, the era of the pathologist relying entirely on the examination of tissue stained by histochemical method is being replaced by advanced immunologic and molecular techniques.

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