



ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2021; 7(4): 156-161
www.allresearchjournal.com
Received: 07-02-2021
Accepted: 09-03-2021

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Adaptation of molecular and immunological diagnostic techniques of viruses

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Abstract

Viral infections are causing serious problems in human population worldwide. The recent outbreak of coronavirus disease 2019 caused by SARS-CoV-2 is a perfect example how viral infection could pose a great threat to global public health and economic sectors. Therefore, the first step in combating viral pathogens is to get a timely and accurate diagnosis. Early and accurate detection of the viral presence in patient sample is crucial for appropriate treatment, control, and prevention of epidemics. Here, we summarize some of the molecular and immunological diagnostic approaches available for the detection of viral infections of humans. Molecular diagnostic techniques provide rapid viral detection in patient sample. They are also relatively inexpensive and highly sensitive and specific diagnostic methods. Immunological-based techniques have been extensively utilized for the detection and epidemiological studies of human viral infections. They can detect antiviral antibodies or viral antigens in clinical samples. There are several commercially available molecular and immunological diagnostic kits that facilitate the use of these methods in the majority of clinical laboratories worldwide. In developing countries including Ethiopia where most of viral infections are endemic, exposure to improved or new methods is highly limited as these methods are very costly to use and also require technical skills. Since researchers and clinicians in all corners of the globe are working hard, it is hoped that in the near future, they will develop good quality tests that can be accessible in low-income countries.

Keywords: molecular, immunology, diagnostic techniques

Introduction

Viruses are small segments of nucleic acid, deoxyribonucleic acid (DNA), or ribonucleic acid (RNA) within a protein coat or lipoprotein coat (envelope). Viruses require host resources for their replication because they are obligate intracellular parasites. Once viruses enter the host cells, they take over or hijack the cells' biosynthetic machineries for the replication of their genomes and other components. Viral infections are the most common cause of human diseases ^[1, 2, 3]. Millions of people are still dying because of human immunodeficiency virus (HIV) and hepatitis viruses worldwide. (The emerging viruses are also causing serious problems in human population. For example, avian influenza A (H5N1) in 1997, the severe acute respiratory syndrome-coronavirus (SARS-CoV) in 2002–2003, pandemic swine influenza A (H1N1) virus in 2009, Ebola virus in 2014, Zika virus (ZIKV) in 2015, and pandemic recently, among others, have caused several outbreaks in different countries ^[3-9]. (The morbidity and mortality rates of human viral infections are significantly high ^[14, 10]. For example, the pandemic swine influenza A (H1N1) infection in 2009 occurred in 214 countries with more than 18,036 deaths. In 2010 alone, the number of human deaths due to rabies globally was estimated to be 61,000, with 84% of the deaths occurred in rural areas ^[11]. In 2013, approximately, 35,000,000 people were infected with HIV worldwide ^[10]. (The World Health Organization (WHO) reported 1.34 million deaths of viral hepatitis in 2015 ^[12]. As on 6th January 2015, H5N1 viruses have killed 402 out of 694 laboratory-confirmed human infections in 16 countries ^[13], with a mortality rate of around 58%. Recently, the world is challenged by the novel coronavirus disease 2019 (COVID-19). (e disease is caused by the novel coronavirus (SARS-CoV-2). (e pathogen first emerged in Wuhan city, Hubei province, China, which has now quickly gained worldwide spread ^[9, 14, 60]. On 11th March 2020, the WHO declared the COVID-19 outbreak a global pandemic. According to the WHO, 9, 129, 146 confirmed cases of COVID-19 have been reported worldwide, including 473, 797 ^[15-65].

(Therefore, good diagnostic techniques are required to detect these viral infections early and accurately. Early and accurate detection of viral diseases plays a significant role in selecting appropriate therapy timely, minimizing therapy costs, minimizing unnecessary loss of human lives, and controlling the disease. It also helps to develop appropriate disease prevention and treatment strategies, like development of antiviral vaccines and new therapeutic agents [14, 16, 17, 47]. Traditionally, laboratory diagnoses of medical viruses are carried out by isolating viruses in embryonated chicken eggs, in tissue culture, or in laboratory animals and visual examination of viral particles in sample using electron microscopy among others [16]. Many conventional diagnostic tools tend to be cumbersome, time-consuming, expensive, and poorly reproducible [18, 19, 55]. Diagnostic virology is rapidly moving into the mainstream of clinical medicine as a result of the convergence of several independent developments. First, dramatic progress in antiviral therapeutics has increased the need for specific viral diagnoses. Second, technological developments, particularly in the area of nucleic acid chemistry, have provided important new tools for viral diagnosis. Third, the number of patients at risk for opportunistic viral infections has expanded greatly as a result of the HIV/AIDS epidemic. Finally, modern management of HIV infection and hepatitis C is providing a new paradigm for the integration of molecular techniques into management of chronic viral infections. These developments are not only increasing the use of diagnostic virology but are reshaping the field. The purpose of this article is to review the field of diagnostic virology at the beginning of the 21st century, to provide guidance about current use of the tools of diagnostic virology, and to provide a glimpse of important future developments.

In contrast, molecular techniques have revolutionized diagnostic virology by detecting the presence or absence of viral nucleic acids in a patient's sample [18]. Immune-based techniques still play a great role for the detection and serosurveillance of human viral infections despite the fact that many of the traditional methods are replaced by nucleic acid-based techniques [20]. Immunological methods detect viral infections by identifying antiviral antibodies or viral antigens in clinical samples. Here, we describe some of the molecular and immunological diagnostic approaches for the detection of medical viruses. Nucleic acid-based molecular detection techniques have revolutionized diagnostic virology with their faster, highly sensitive, and highly specific diagnosis [14, 23, 24, 74, 76]. Since these methods detect specific nucleic acid sequences, nucleic acid-based diagnostic tests can be applied for the detection of virtually any virus that affects humans [1, 3, 5]. Molecular techniques that involve the amplification of viral genomic material are extremely sensitive and specific, provide rapid diagnosis, and allow the detection of several viruses at same time [67, 68, 69, 16]. Nucleic acid amplification techniques are very useful for the detection of viruses that are uncultivable or difficult and harmful to culture, slow growing viruses in culture, and viruses that display antigenic variations.

Problem in hand: The statement of the research problem is reported as under:

“Adaptation of Molecular and Immunological Diagnostic Techniques of Viruses”

Objectives of the study: The purpose of the study was to explore the adaptation of Adaptation of Molecular and Immunological Diagnostic Techniques for determining the Viruses.

Rationale of the study: The humoral branch of the immune system makes antibodies in response to viral infections. This natural response of the human body against viral infection is utilized for the development of immunological diagnostic methods. Several immunological diagnostic techniques are available for the detection human viral infections in clinical samples, including enzyme-linked immunosorbent assay, western blotting, immunofluorescence assay, and hemagglutination inhibition assay. The principles of these assays rely on the formation of antigen-antibody complex and consist of clinical specimens, whole virus or viral antigen, and an indicator.

Enzyme-Linked Immunosorbent Assay (ELISA): In ELISA, enzyme conjugated antibody is utilized to detect the presence of specific antiviral antibody or viral antigen in human specimens. In positive sample, the reaction between an enzyme conjugated with an antibody and colourless chromogenic substrate leads to the formation of a colourful product. In the absence of antigen/antibody in the clinical specimen, no colour is produced. The intensity of the colour is directly proportional to the amount of antigen-antibody complex formed. The colour change can be observed by the naked eye or read by a spectrophotometer, which can measure the absorbance. Several enzymes, including alkaline phosphatase, horseradish peroxidase (HRP), and β -galactosidase, have been used for ELISA. There are several variants of ELISA, but the two main types are antigen-capture ELISA (also called sandwich ELISA) and antibody-capture ELISA (also called indirect ELISA) [19, 15]. As illustrated in the first method detects viral antigen by immobilizing antibody specific for the viral protein of interest on a microliter well [22]; the second technique detects antiviral antibody in a patient sample by coating whole virus or viral protein on a microtiter well

Western Blotting Analysis: Western blotting (also known as immunoblotting) assay detects viral proteins or antiviral antibodies. For detection of viral proteins, denatured whole viral proteins are first separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Viral proteins are then electro transferred onto nitrocellulose membrane. The membrane is then incubated with enzyme conjugated antibodies specific for the viral proteins. If the viral proteins are bound by enzyme labelled antibody, addition of a chromogenic substrate leads to the formation of coloured bands at the sites of the viral antigens [19, 132]. For detection of antiviral antibodies, viral specific denatured proteins are electrophoretic ally blotted onto nitrocellulose membrane after subjected to SDS-PAGE. The membrane is then incubated with patient serum. If the patient serum contains antibodies against the viral proteins, they will bind to their specific viral proteins. The addition of enzyme conjugated secondary anti-human antibody and a chromogenic substrate results in the production of coloured bands at the locations of the viral proteins immunoblotting has been used in clinical diagnosis for serosurveillance and as confirmatory tests for human viral infection. He *et al.* [143] developed western blot assay for detection of antibodies

against SARS-CoV in human serum samples. The assay demonstrated a sensitivity of 98.3% and specificity of 90.9%, compared to IFA. Western blotting assay was also used for the detection of anti-Chikungunya virus antibody in human serum. Sensitivity of 83.3% and specificity of 96.7% were demonstrated by the assay using 30 sera from confirmed Chikungunya virus infected patient and 30 normal sera [144]. In one study, western blotting was a promising method for surveillance of HIV-1 infection in resource-limited regions.

Immunofluorescence Assay: Immunofluorescence assay is commonly conducted for the detection of viral antigens or antiviral antibodies in clinical samples. The assay is conducted in two formats: direct immunofluorescence assay (DFA) that detects viral antigens in patient sample [149] and indirect immunofluorescence assay (IFA) that detects antiviral antibody [150] or viral antigen [151] in clinical specimen. In the DFA, antibody that recognizes viral antigen is directly conjugated to fluorescent dye. In the IFA, viral antigen specific antibody is unlabelled and is detected with a second fluorescently labeled anti-human antibody. IFA is more sensitive than DFA because several fluorescently labeled anti-immunoglobulin antibodies bind to each antiviral antibody, increasing the intensity of fluorescence at the site of each antiviral antibody. The most widely used fluorescent dye in diagnostic virology is fluorescein isothiocyanate (FITC), which emits an intense yellow-green fluorescence, but rhodamine, which emits a deep red fluorescence, is also available. After staining, the specimen is examined under fluorescence microscope with a source of incident UV light

Hemagglutination Inhibition (HI) Assay: Some viruses such as dengue virus, adenovirus, rubella virus, measles virus, and influenza virus have hemagglutinin antigen on their surfaces that binds and agglutinates RBCs termed hemagglutination (HA). The inhibition of the ability of the viruses to agglutinate RBCs is utilized for the development of HI assay. In the HI assay, serial dilutions of serum sample are prepared in a microtiter plate. Then, a specified amount of viral hemagglutinin is added. Finally, appropriate RBCs are added. The absence of HA indicates a positive reaction. This is judged by tilting the microtiter plate, which allows free RBCs to stream. The dilution rate where complete inhibition of agglutination of RBCs occurred is recorded. The HI titer, therefore, is the reciprocal of the last serum dilution which completely inhibits HA [10, 17, 18, 19]. HI was utilized for a number of applications in diagnostic virology. The assay was used for serosurveillance of influenza A (H1N1) pdm09 virus [45, 80, 79] and measles virus [49]. In one study, HI assay was applied to assess the efficacy of pandemic influenza vaccine [34, 78, 74]. In a validation study using sera from 79 RT-qPCR-confirmed cases and 176 sera from a non-exposed population, HI assay showed high sensitivity (92%) and specificity (91%) for the detection of human infection.

Conclusion

The introduction of nucleic acid-based diagnostic tests into diagnostic virology has made tremendous improvement in the detection of human viral infections. Since nucleic acid-based diagnostic tests are highly sensitive and specific, they play a crucial role in the diagnosis and control of medical

viruses. Molecular diagnostic methods diagnose viral infections by detecting viral RNA or DNA. Therefore, these techniques can pick infected individuals before antibody response is mounted against the virus in question. This is especially important in young, elderly, and immunosuppressed patients. However, they are beyond the reach of resource-limited nations due to their high cost, instrumentation complexity, and requirement for technical expertise. Immunoassays also play a significant role in the diagnosis and serosurveillance of viral infections worldwide. Although immune techniques are easy to perform and inexpensive compared to molecular methods, they are not widely available in low-income countries. Consequently, scientists are working hard to develop inexpensive good quality tests in low-income nations. Moreover, most countries in the developing world are training their citizens abroad and inland at postgraduate level by opening relevant departments and institutes.

Computing interest

The authors declare that there are no conflicts of interest.

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