

Application of serology Vs PCR in infectious disease diagnosis ,eg COVID 19

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ABSTRACT:

The word infection stands for invasion and multiplication of one or more pathogens. They cause infectious diseases in humans after establishing an entry into the human body through routes such as skin, mouth, nose, and genitourinary tract. These infections can escalate into a level of a pandemic such as the current COVID-19 pandemic resulting in severe implications on health, economy, and social life worldwide. Therefore, early diagnosis has become vital to identifying, control, and eradicate these infections. Serological and molecular assays such as Polymerase chain reaction (PCR) have an advantage over conventional diagnostic methods such as culturing, microscopy due to higher specificity and sensitivity exhibited over detecting a large number of infectious diseases. PCR is classified as a direct diagnostic assay. It is a popular diagnostic assay in infectious disease diagnosis due to its multiple advantages such as high sensitivity, specificity, and rapid results. The arrival of real-time PCR is a great achievement in PCR technology and it increased the scope of infectious disease diagnosis. In COVID – 19 caused by SARS-COV 2 virus, diagnosis is carried out mainly by Reverse transcriptase PCR coupled with real-time quantification. Serology is the protein (Antibody) identification and is an indirect diagnosis method of infectious diseases that consists of conventional serological tests such as complement fixation, enzyme immunoassay tests, latex agglutination tests which are widely used and rapid tests include point of care tests. In this review, more emphasis will be made on the applications of serological assays and PCR in infectious disease diagnosis.

Keywords: COVID-19, RT-PCR, infectious diseases , Serology

INTRODUCTION:

The Infectious diseases have become a major cause of morbidity and mortality worldwide. Appropriate and timely diagnosis is the basis for treatments, disease control and prevention. [1]. Availability of rapid, reliable diagnostic assays are important in early and accurate identification of infected individuals and informed early therapeutic interventions [2]. The evolution of infectious disease diagnostics are possible due to advances in chemistry, molecular biology, immunology, automation, and nucleic acid amplification majorly. New technologies are important in bringing advancements in infectious diseases diagnostics [3]. The two tiers of infectious disease diagnosis are direct pathogen detection and specific antibody detection where serological tests involved in the detection of specific antibodies provide a longer diagnostic window than direct methods [4]. Serology is the study of proteins mainly antibodies in body fluids majorly in blood following cerebrospinal fluid and/or saliva and classified as direct or indirect [5]. Classical serological assays widely applicable in total antibody tests are ELISA, agglutination, precipitation, complement fixation, fluorescent antibodies test, and

Chemiluminescence tests which do not provide a rapid diagnosis, where as rapid diagnosis can be achieved via indirect assay that binds the antibodies to the immobilized antigens [6]. Examples of rapid tests done for antigen detection with a specific antibody are detection of the antigens of *Streptococcus pneumoniae*, *Legionella pneumophila* serogroup 1, and *Histoplasma capsulatum* in urine and cryptococcal antigen in blood and CSF [3]. Polymerase chain reaction (PCR) comes under direct diagnostic assays and the application of PCR in infectious disease diagnosis was witnessed for the last two decades [3,4]. This facilitates the rapid diagnosis of microorganisms, which was difficult or not possible with traditional microbiological techniques [7].

The principle of PCR is based on in vitro generation of a large number of copies of the particular DNA fragment, available in small quantities in a DNA extract [8]. This DNA extract can be genomic DNA or complementary DNA obtained by RT-PCR from a messenger RNA extract [9]. This is an enzyme driven process, the oligonucleotide primers that specifically bound to targets are designed from target DNA at the beginning. The targets are then amplified in a

thermocycler by undergoing target DNA denaturation, primer hybridization, and primer extension, leading to exponential amplification using a DNA polymerase, nucleotides, and designed primers.^[2]

Application of Serological assays and PCR for infectious diseases diagnosis:

Serological tests are used in detecting a vast number of infectious diseases and if the viral diagnosis is considered, it is based on either the demonstration of viral-specific IgM antibodies or detection of a significant increase in specific IgG antibodies. The method used for demonstrating specific antibody response is the evaluation of antibody response via serological methods and most commonly experienced serological assays are immunoassays and point of care (POC) tests done for antigens and antibodies^[10].

Applications of serology in the identification of global viral diseases, for example, Human immunodeficiency virus (HIV) which has been infected more than 40 million people and it is of two types as HIV 1/HIV 2^[6]. Serological tests such as enzyme immunoassays (EIA) are sensitive after antibodies formed in patients and this takes around one to two weeks the period after contacting the infection^[11]. The EIA can detect IgG or IgG/IgM Combination of specimens^[12]. The positive results of EIA tests should be confirmed by a Western blot test to detect antibodies for viral antigens^[11]. In certain instances, such as diagnosing HIV positive donors during organ or blood transfusion, rapid serological diagnosis is done by detecting HIV p 24 antigen whereas this antigen is detectable before antibodies in early diagnosis of the infection^[12].

Application of serological tests in the detection of antibodies of patients having fungal infections is advantageous over non culture-based diagnosis since the middle of the last century. Serology gains importance in diagnosing endemic mycoses where diagnostic methods include immuno diffusion (ID), complement fixation (CF), and an enzyme immunoassay (EIA). Histoplasmosis is a fungal infection that is more commonly diagnosed by CF and immuno diffusion. Immuno diffusion is capable of detecting precipitating antibodies for H and M antigens of *Histoplasma* and also these tests involved in diagnosing coccidioidomycosis, where immuno diffusion can be used to determine coccidioidal IgM and CF detects IgG antibodies^[13].

Serological tests apply in detecting many bacterial infections whereas *Yersinia enterocolitica*, *Campylobacter jejuni*, *Salmonella*, *Shigella*, and *Chlamydia trachomatis* are associated with reactive arthritis and disease diagnosis is performed by indirect hemagglutination test and complement fixation test^[14].

Serological assays are useful in providing information about etiology after the acute stage during primary infection and can result in epidemiological data also involve in determining vaccine induces immunity^[10].

Also advantageous in detecting invasive fungal infections since it reduces the necessity of culturing of hazardous fungi such as *Coccidioides* spp. if serological results are positive^[13]. Also, early diagnosis of acute infections such as Epstein–Barr virus and diagnosis of chronic infections such as HIV (human immunodeficiency virus) or viral hepatitis can be done and further benefits include enhanced performance, traceability, and cost-effectiveness^[6].

Not only advantages but there are disadvantages also since serology depends on the immunological response of the patient that may take weeks to a month to develop antibodies^[15]. False-negative results and diminished specificity may result when already available or chosen serological antigens for assays become too specific or mismatched to the pathogens present. Antibody cross-reactivity where certain viruses in a group share antigenic determinants and serological tests cannot differentiate on past and current infections are limitations^[12]. It encounters limitations as in some infections strong antibody response is not witnessed or the specificity of the antigens become decreased therefore distinct interpretation of the results does not happen and when comes to the serological response of immunocompromised patients it is often too weak to allow the demonstration of specific responses^[10]. Same time certain assays are time-consuming, for example, complement fixation, and also it is difficult to distinguish between current or previous infection^[16].

The role of serology is diminished in the early stage of infection due to the difficulty in obtaining specific antisera required for serological tests and with the arrival of PCR technology, for example, multiplex PCR, real-time PCR with improved efficiency the role of molecular methods in infectious disease diagnosis was further increased^[7].

Antibody detection for certain viruses becomes relatively insensitive but an improved detection of a number of these viruses can be obtained by performing the PCR technique. Diagnosis of Herpes simplex virus (HSV) encephalitis termed as a serious infection was carried out before, requiring a brain biopsy in certain cases where low sensitivity was reported for serology and cerebrospinal fluid culture (CSF) and With PCR brain biopsy was replaced with HSV DNA detection from CSF with high sensitivity. Compared to culturing PCR can relatively detect viral meningitis caused by enteroviruses or HSV. Genital ulceration caused by HSV type 2 infection, can be routinely detected by PCR due to its increased sensitivity over viral culture, and the detection of blood-borne virus infection is improved with PCR. Intrauterine infection of a fetus caused due to cytomegalovirus (CMV), rubella, and varicella zoster virus is detected by carrying out a PCR in amniocentesis fluid^[7].

The infectious disease diagnosis caused by fastidious bacteria has benefited greatly from molecular detection where PCR, which has a higher sensitivity replaced direct fluorescent-antibody and culture methods in the detection of *Bordetella pertussis*. Laboratory detection of *Bordetella pertussis* during an outbreak resulted in a 48% PCR detection and only 5% by culturing. Neuro-Whipple's disease and endocarditis can be diagnosed after carrying out PCR to detecting *Tropheryma whippelii*, the causative agent [16].

PCR is used to detect fungal pathogens causing diseases to humans and offer several features that may aid in overcoming current shortcomings for the diagnosis of fungal infections [17]. *Pneumocystis jirovecii* is fungi involve in causing severe pneumonia in immuno compromised and HIV-infected patients but the pathogen detection is limited to the microscopy of respiratory tract specimens but PCR is useful in detecting *Pneumocystis jirovecii* in HIV-non-infected individuals and this microbe, a ubiquitous commensal can be detected by PCR in the absence of pneumonia [16].

There are many advantages of PCR. It is highly sensitive, specific, and produces rapid results [8]. Advantageous over serological testings in early diagnosis of infection and different PCR primers can be designed to specifically target disease-causing microbes at their species or strain levels [9]. PCR can make many copies, a billion number of the particular genetic sequence for further analysis [8].

Application of PCR in infectious disease diagnosis is advantageous since rapid multiplication of viral sequences can be obtained by using PCR with specific primers and it replaces viral isolation also PCR provides a diagnostic advantage for viruses that cannot be cultivated such as papilloma viruses, parvoviruses, and hepatitis viruses also capable of detecting emerging viruses [10].

Limitations include, more expensive than other detection methods since expensive instruments, chemicals, and trained staff is needed [9]. Multiple pathogens or species detected in multiplex PCR assays but achieving optimal sensitivity for all pathogens is difficult and also co-infecting pathogens may cause competition and cross-reaction in the PCR reaction process [18]. Reduced specificity is observed when primers bind nonspecifically and to design primers, prior knowledge of target sequence is necessary [8].

Applications of PCR and serological assays in the diagnosis of a novel coronavirus, cause for Covid -19:

COVID-19 (coronavirus disease 2019) is caused by a novel enveloped RNA betacoronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and this has been revealed as an international public health concern due to its global spread, molecular methods and serological assays are used for clinical diagnosis of the disease [19].

Better control over Covid -19 can be achieved by rapid and accurate diagnostic methods and this has two categories serological tests for detection of anti-SARS-CoV-2 immunoglobulins and molecular methods for viral RNA detection [20].

Direct Diagnosis of SARS-CoV-2 Infection:

Direct diagnosis of infection of SARS-CoV-2 is by identification of viral RNA in lower respiratory tract specimens and nasopharyngeal swabs, but nasopharyngeal swabs are the gold standard [21]. RT-PCR involves in the detection of SARS-CoV-2 infection [22]. It is reverse transcription of viral RNA into complementary DNA (cDNA) and amplification of a particular region of the cDNA takes place and it is the most widely used method in detection of COVID-19 samples [23]. The genome of this SARS-CoV-2 virus consists of around 30 000 nucleotides and 15 genes and many of these genes are used as primer/probe for diagnosis of infected individuals in reverse-transcription polymerase chain reaction (RT-PCR) assays [21].

Real-time rt-PCR is the most used direct diagnosis method of SARS-CoV-2 viral RNA detection due to the specificity and simplicity as a one-step assay and higher sensitivity obtained in early detection but there is a risk of exhibiting false positive and false negative results yet a combination of Real-time rt-PCR and clinical features has given an improvement in managing SARS-COV -2 pandemic [22].

Indirect Diagnosis of SARS-CoV-2 Infection:

This method of diagnosis of SARS-CoV-2 infection depends on the detection of specific IgG and/or IgM antibodies whereas WHO (World Health Organization) has announced the strong requirement of serological IgM and IgG testing in detecting SARS-CoV-2, therefore, it has been developed many serological assays to diagnose SARS-CoV-2 viral proteins and antibodies in serum or plasma samples. Most widely used are rapid lateral flow immunoassay (LFIA), automated chemiluminescence immunoassay (CLIA), and enzyme-linked immune assay (ELISA) [21].

Enzyme-Linked Immune Assay (ELISA):

Enzyme-linked immune assay (ELISA) was developed to detect anti-SARS-CoV-2 IgG and IgM responses by detecting antibodies produced against the nucleocapsid protein and spike proteins [21]. The sensitivity of ELISA is high in total antibody detection and it is advantageous to measure antibody titers and determining selective isotypes but can be used for point of care testing [24].

Chemiluminescent Immunoassay (CLIA):

Chemiluminescent Immunoassay is advantages in the quantification of antibodies over traditional assay detection methods since the light-producing chemical reactions occur as the substrate reacts with the Avidin-

Horseradish Peroxidase allowed estimating the titers of IgG and IgM considering the emitted luminous signal^[21].

Lateral Flow Immunoassay (LFIA):

Lateral Flow Immunoassay (LFIA) is productive in SARS-CoV-2 testing can be used for point-of-care testing and LFIA provide results for both IgM and IgG where it demonstrated higher sensitivity and specificity in detection of both isotypes, but a higher specificity was observed with the detection of the IgG isotype over the detection of the IgM isotype alone^[24]. Serology is important in detecting people who have been subjected to this disease and recovered but antibody cross-reactivity can be a disadvantage yet can overcome to a greater extent by using RBD-based ELISA and rapid tests done for IgG have given higher sensitivity and specificity as recently performed ELISA, indicating better results with higher specificity and sensitivity in detecting COVID-19 infection^[25].

Summary:

Infectious diseases caused by pathogens can be severe or mild depending on the pathogen virulence, environmental factors, and host immunity. Serological tests and PCR are widely used due to higher specificity and sensitivity than other diagnostic assays. Conventional serological tests applicable to infectious disease diagnosis mainly include complement fixation, hemagglutination, enzyme immunoassays, and immuno diffusion. Rapid diagnostic tests include point of care tests. Serological methods are advantages but disadvantageous to a certain extent whereas most of these disadvantages can overcome by molecular assays such as PCR. PCR assays are more versatile in disease diagnosis and there are many types according to the function required. COVID -19 caused by SARS-COV 2 virus, diagnosis carried out mainly by real-time rt-PCR assay which is sensitive, specific yet results in false positive and negative. The use of real-time-PCR with clinical features aids in managing the COVID-19 pandemic. Serological tests for the identification of specific IgG and/or IgM antibodies are used for the diagnosis of COVID -19. This includes mainly enzyme immune assay, chemiluminescence immunoassay, and lateral flow immune assay.

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