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Molecular techniques of viral diagnosis

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Abstract

Viral infections are the cause of very serious problems in humans all over the globe. The recent outbreak of coronavirus disease 2019 caused by SARS-CoV-2 is a perfect example of how viral infection could pose a great threat to global public health and economics. Therefore, to defeat the viral pathogens, we need to get a reliable diagnosis. Detection of the viral presence fast and accurate in the patient is important for appropriate treatment, control, and prevention of epidemics. Diagnosis of infectious microbes became easier by the development of molecular techniques, especially with the emerging of the polymerase chain reaction (PCR). The easiness and high sensitivity of the technique made this method reliable enough to be used to detect any known genetic sequence has led to its wide application in the life sciences. Currently, real-time PCR has made remarkable contributions, with an additional fluorescent probe detection system that has increased the sensitivity of this assay over conventional PCR, with the ability to verify the amount of amplification product and to quantitate the target concentration. Further, nucleotide sequence analysis of the amplification products has encouraged epidemiological studies of contagious disease in outbreaks, and the follow up of treatment for infections, particularly the viruses with a high tendency to mutations.

Keywords Virus, Molecular diagnosis, Molecular microbiology, Nucleic acid, PCR techniques, Viral laboratory diagnosis

Introduction

Viruses are tiny microscopic parasites with the genetic material of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) surrounded by protein coat called (envelope). Viruses only replicate in a living cell because they are known as obligate intracellular parasites. Once viruses enter the host cells, they take over and emerge in the cells' biosynthetic types of machinery for the replication of their genomes and other elements (Coboet et al., 2006; Owen et al., 2013). Viruses are relatively the most common causative agent of human diseases. Millions are still dying from AIDS (HIV) virus and hepatitis viruses worldwide. Initiating viruses are also causing serious issues in the human population. Human viral infections have significantly high morbidity and mortality rates (Souf, 2016). Therefore, efficient diagnostic strategies are required to detect these viral infections as early and accurately as possible. Early and accurate detection of viral presence in patients plays an important role in choosing appropriate therapy on time, 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 the development of antiviral vaccines and new therapeutic agents (Shen et al, 2020; Cella et al., 2013; Landry et al., 2016).

Traditionally, laboratory diagnoses of medical viruses are carried out by isolating viruses in embryonated chicken eggs, in tissue culture, or laboratory animals and visual examination of viral particles in a sample using electron microscopy among others (Cella et al., 2013). Many conventional diagnostic tools tend to be indolent, time-consuming, expensive, and poorly performed (Acharya et al., 2013; Kumar, 2013). In contrast, molecular techniques have facilitated diagnostic virology by detecting the existence of viral nucleic acids in the viral sample (Acharya *et al.*, 2013). Here, we describe some of the molecular diagnostic techniques for the detection of viruses.

Molecular techniques for identification and characterization

Reliable detection of causative agents like viruses in viral diseases, distinctive genetic sequences in viral diseases, and protein entity are very objectively required for the management of these diseases with taking into accounts the specificity and sensitivity as important tools in diagnosis. Regular molecular techniques like classical PCR and blotting technique albeit assumed good in diagnosis. Anyway, nowadays molecular techniques like gene and peptide sequencer, real-time PCR may identify in even more accurate and explicit ways without consuming much time.

Molecular diagnostic techniques of viruses

Nucleic acid-based molecular detection techniques have developed diagnostic virology with their faster, highly sensitive, and a highly specific diagnosis (Stone et al., 2014; Canberk et al., 2016). Since these techniques detect specific nucleic acid sequences, nucleic acid-based diagnostic tests can be applied for the detection of any infection that affects humans (Cobo et al., 2006).

Polymerase Chain Reaction (PCR)

PCR is a model example of genetic amplification assay. It has developed the level of molecular diagnosis since developed by Mullis and Faloona (1987). The PCR main principle depends on the extraction and purification of DNA molecule and evidential amplification of the targeted sequence, using a thermostable DNA polymerase and two specific oligonucleotide primers. After the PCR reaction, the amplified product can be detected by several techniques, including gel electrophoresis, colorimetric methods, and sequencing (Cobo, 2012; Levine et al., 2011). Since its introduction, PCR started to be used for the detection of human viral infections with total clinical sensitivity ranging from 77.8% to 100% and clinical specificity ranging from 89% to 100% (Hassan et al., 2006; Demmler et al. 1988; Myerson et al., 1993; Sundaramurthy et al., 2018). These reports recommend that PCR can also be used for the detection of viruses in many types of specimens. PCR is an exceptionally multilateral technique. Several variants of the conventional PCR have been developed, however; the main variants are reverse transcription-PCR and real-time PCR (Cobo et al., 2006; Souf, 2016). The first method was invented to amplify ribonucleic acid (RNA) targets (Cobo et al., 2006); the second technique was introduced to quantify deoxyribonucleic acid (DNA) in real-time throughout the PCR reactions (Gruber et al., 2001).

Reverse Transcription-PCR (RT-PCR)

RT-PCR was devised to amplify RNA targets. In this technique, reverse transcriptase (RT) is used to convert viral RNA targets into complementary DNA (cDNA), and then amplify the resulting cDNA by conventional PCR. Since its development, RT-PCR has been employed for the diagnosis of human infection by RNA viruses. Conventional RT-PCR demonstrated overall sensitivity ranging from 73% to 100% and specificity ranging from 99% to 100% in the detection of viral infection (Maignan et al., 2019; Falsey et al., 2002;

Formentry et al., 2006). These data indicate that RT-PCR is an exceptional technique for the diagnosis of human infection by RNA viruses. Currently, however, the method is not used commonly in clinical specimens because of its high cost and time-consuming process (Shen et al., 2020).

Multiplex PCR

In a similar response combination, at least two or more primer sets prepared for amplification of a variety of targets are utilized (Chamberlain et al., 1988). In a clinical sample, more than one target sequence can be co-amplified in a single tube. However, the primers used must be carefully selected for them to have similar annealing temperatures and less complementarily. This sort of PCR is less sensitive than PCR with a single primer set. Multiplex PCR assays have been developed and commercialized for viral respiratory pathogens and detection of viral infections of the central nervous system (Boriskin et al., 2004; Templeton et al., 2004).

Real-Time PCR

Real-time PCR is a simple, quantitative assay for amplification of DNA sequence. It was clarified firstly by, Higuchi et al. (1993). It depends on using fluorescent-labeled probes to verify and quantify the PCR products as they are being generated in real-time. The real-time PCR, which has three novel features as temperature cycling occurs considerably faster in cooperation to ordinary PCR assays, hybridization of specific DNA probes are added continuously during the amplification process and a fluorescent dye is cogitated to the probe and fluoresces only when hybridization takes place. Lack of post PCR processing of amplified products would make this technique convenient. The production of amplified products is automatically observed by real-time monitoring of fluorescence. A small-signal can be produced within 30–45 min depending on the amount of target gene. Since the tubes do not have to be opened at the time of reaction, the risk of contamination decreased considerably. In recent years, some automated real-time PCR systems have been available commercially. (Light Cycler & TaqMan). Using these systems, such as the Light Cycler TM and the Smart Cycler, they perform the real-time fluorescence monitoring by using fluorescent dyes such as SYBR-Green I, which binds non-specifically to double-stranded DNA that was previously generated during the PCR amplification (Fakruddin et al., 2012; Holland et al., 1991).

Nested-PCR

This type of PCR possesses relatively high sensitivity and specificity (Haqqi et al., 1988). This technique uses two pairs of amplification primers and two rounds of PCR. In the first round, it uses one primer pair for 15 to 30 cycles. The product's first-round amplification is submitted to the second round of amplification coupled with the second pair of primers. The major setback of nested PCR is the high rates of contamination.

Transcription-based amplification methods

Transcription-based amplification method based on two procedures, nucleic acid sequence-based amplification (NASBA) and transcription-mediated amplification (TMA). NASBA and TMA are not different in terms of principle. They are isothermal amplification methods. The entire amplification process is performed at a temperature of 41°C. In both methods, the viral RNA target is first converted into cDNA with RT and then multiple copies of viral RNA products are synthesized by RNA polymerase. The sole difference between TMA and NASBA in the amplification process is two enzymes (RT and RNA polymerase). They are used in the case of TMA while NASBA utilizes three enzymes (avian myeloblastosis virus reverse transcriptase (AMV-RT), RNase H, and T7 RNA polymerase) (Fakruddin et al., 2012; Cobo, 2012). Transcription-based amplification methods have several advantages, for example, they do not require a thermal cycler, so developing countries and budget-restricted laboratories can afford to perform the assays, they are rapid (requires fewer cycles), and they produce a single-stranded RNA product that is suitable for detection by various techniques (Mohammadi-Yeganeh et al., 2012; Yu et al., 2012). Transcription-based amplification assays are convenient for the diagnosis of human infections caused by RNA viruses because they are capable of amplifying viral genomic RNA, messenger RNA, or ribosomal RNA (Cobo, 2012; Mohammadi-Yeganeh et al., 2012). Ayelet et al. (2004) developed a NASBA assay that uses gag-based molecular beacons to differentiate between HIV-1 subtype C (C and C') occurring in Ethiopia. The assay has high levels of sensitivity and specificity (90.5% sensitivity, 100% specificity for the C beacon, and 100% sensitivity, 95.2% specificity for the C' beacon) by considering sequencing as the gold standard for genotyping.

Conclusion

The introduction of nucleic acid-based diagnostic tests into diagnostic virology has made a remarkable improvement in the detection of human viral infections. Since nucleic acid-based diagnostic tests are highly sensitive and specific, they play a distinctive role in the diagnosis and control of viral infection. Molecular diagnostic methods diagnose viral infections by detecting viral RNA or DNA. Therefore, these techniques can detect infected individuals before the antibody response is raised against the particular virus. This is especially important in young, elderly, and immune-suppressed patients. However, they are unreachable for resource-limited nations due to their high cost, instrumentation complexity, and the requirement for technical expertise.

Authors' contributions

All authors have contributed significantly to the conception and design of the study, the interpretation of data, and the drafting and revision of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors hereby declare no conflict of interest.

Consent for publication

The authors declare that the work has consent for publication

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