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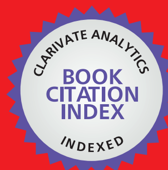

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Chapter

PCR and Infectious Diseases

Danielle Alves Gomes Zauli

Abstract

Since the 1950s, the medical community has been faced with infectious diseases, which have brought significant public health and financial challenges. Currently, routine testing for the laboratory diagnosis for infectious agents is based on cell culture, serological, and molecular methods. However, cell culture-based methods are used mainly in research laboratories and are less sensitive methods when compared with serological and molecular methods. The diagnosis of infectious diseases has been revolutionized by the development of molecular techniques, mainly with the applications of polymerase chain reaction (PCR). The high sensitivity, specificity, and ease with which the PCR can be used to detect genetic sequences known have led to your wide application in science. A great number of qualitative and quantitative molecular assays are mostly based on what have been described such as real-time PCR, multiplex PCR, LAMP-PCR, and digital PCR. These assays could identify active infection by detecting infectious agents and nucleic acid in various clinical conditions including arboviruses, sexually transmitted infections, and bacterial infections. Further advancement of molecular technology is needed to improve the capacity to detect infectious agents in order to control the spread of infectious diseases and lead to appropriate actions which help to benefit patients and health-care workers themselves.

Keywords: infectious disease, PCR, molecular techniques, diagnosis

1. Introduction

Infectious diseases (ID) are caused by pathogenic microorganisms, such as bacteria, viruses, parasites, or fungi, and the diseases can be spread, directly or indirectly, from one person to another. Scientific advances in the biomedical area since the first half of last century, represented by the development of therapeutic drugs, vaccines, and advanced sanitation technologies, were carried as result of the control or prevention of infectious diseases. These diseases are considered, at the global level, as some of the most common public health problems. The relevance of these pathologies is evidenced by the number of individuals reached, the lack of knowledge about the infectious agents, their socioeconomic impact, the deepening of the molecular studies involving a precise and fast diagnosis, and appeals public health agency studies aimed at the development of diagnostic techniques for the early detection of symptomatic carriers as well as the asymptomatic carriers of these infections.

Changes in society, technology, and the microorganisms themselves are contributing to the emergence of new diseases, the reemergence of diseases once controlled, and to the development of antimicrobial resistance. According to the World Health Organization (WHO), the IDs constitute a significant proportion of all human diseases known, and at least 25% of about 60 million deaths that occur worldwide each year are estimated to be due to infectious diseases [1, 2].

Scientific studies of infectious agents and diseases provide a knowledge for the development of diagnostic tests for such diseases, drugs to treat, and vaccines for prevention. Earlier, specific and effective diagnosis is one of the most appropriate forms and strategies for managing. According to [3], in American hospitals, about 5 million cases of infectious disease are reported annually. Besides that, most of the cases are unreported, resulting in substantial morbidity and mortality [3]. In the last 20 years, analysis methods based on the detection and sequencing of 16S rDNA have been widely used in place of conventional culture methods.

Unfortunately, despite in clinical laboratories, the diagnosis of infectious diseases is directly associated with time of pathogen identification by conventional culture methods as these tests suffer from long turnaround times, from hours to days. A technical difficulty encountered in these methods performed before the advent of the molecular techniques was that not all pathogens are cultivable, and culture conditions ordinarily are not known. Other limitations of the traditional diagnostic include requirements for additional testing and wait times for characterizing detected pathogens (i.e., discernment of species, virulence factors, and antimicrobial resistance) [3, 4].

Nucleic acid amplification is one of the most valuable tools in virtually all life science fields, including application-oriented fields such as clinical medicine, for diagnosis of infectious diseases.

The serological methods are limited by the cross-reactions between the types of agents and by the fact that some infectious agents have no clearly identified epitopes that are sufficiently specific, requiring them as a stringent clinic diagnostic. In addition, the specific IgM antibodies are detected only in acute phase of infection, and for detecting infections, the serological tests are inaccurate, labor-intensive, and unreliable. In the last 20 years, analysis methods based on the nucleic acid amplification have been widely used in all life science fields as a new way for the diagnosis of human pathogens like virus, bacteria, and parasites.

The use of amplification techniques such as Polymerase Chain Reaction (PCR) has long been used to detection, genotyping, and quantification of virus and bacteria in various clinical specimens, such as serum, plasma, urine, semen, and liquid cerebrospinal fluid (CSF).

PCR-based diagnostics have been effectively developed for a wide range of microorganisms. Due to its incredible sensitivity, specificity, reproducibility, broad dynamic range, and speed of amplification, PCR has been championed by infectious disease experts for identifying organisms that cannot be grown in vitro, or in instances where existing culture techniques are insensitive and/or need prolonged incubation times [5].

Advances in development of molecular technology and diagnostics have enhanced understanding IDs' etiology, pathogenesis, and molecular epidemiology, which provide basis for appropriate detection, quantification, prevention, and control measures as well as rational design of vaccine, by which some diseases have been successfully eliminated.

Since 1985, many PCR amplification-based techniques have been designed for detection and identification, including: multiplex PCR (M-PCR), LAMP-PCR, digital PCR (dPCR), and real-time PCR.

2. PCR techniques and clinical applications in diagnostic of infectious disease

2.1 Multiplex PCR

In diagnostic laboratories, the use of PCR is often limited by its cost and sometimes by the availability of adequate sample volume. To overcome these issues and

also to increase the diagnostic capacity of PCR, there is a type termed multiplex PCR (mPCR). The mPCR refers to the use of different pairs of primers to simultaneously amplify multiple regions of the nucleic acid of the sample with visualization of the amplified products by gel electrophoresis. The use of multiple primer pairs in mPCRs is an innovation that offers significant benefits in cost, time, and exact diagnosis. The main advantage of this technology is to minimize the number of separate reactions, for example, to detect several pathogens at the same time in a single specimen such as sexually transmitted pathogens [6–8]. This technique makes it possible to diagnose several diseases with a single diagnostic test, with sensitivity, specificity, and speed, indispensable values in diagnostic tests.

This technique has become a mainstay of research and clinical diagnostic applications, such as sexually transmitted infections (STIs). Considering a major public health problem, the STIs are common everywhere from developed countries and developing countries. It is estimated that each year more than 340 million new cases of bacterial STI arise, including gonorrhea, chlamydia, and syphilis, and the incidence is increased worldwide in adults of 15–49 years of age [9]. The susceptibility to sexually transmitted infections, including the human immunodeficiency virus (HIV), and the high cost of treatment have led to the need for fast and reliable laboratory techniques for the identification of pathogens. Procedures for nucleic acid amplification to detect sexually transmitted pathogens have been developed, especially mPCR methods [10].

The multiplex PCR has the potential to analyze many samples in a single reaction, and it is useful for diagnostic of multipathogenic infection. However, it has some limitations such as the nonspecific products generated through primer-primer interactions that may interfere with the amplification of targets, decreasing sensitivity, and selectivity of reactions.

2.2 Loop-mediated isothermal amplification

Nucleic acid amplification is commonly used in the field of life science research. With the development of molecular biology, many new molecular diagnostic technologies have been developed subsequently [11].

The loop-mediated isothermal amplification (LAMP-PCR) was first developed over 15 years ago, and it has emerged as powerful method to concurrently detect multiple pathogens [12, 13]. The method employs a DNA polymerase with strand displacement activity and a set of four inner and outer primers that recognize a total of six distinct sequences of the target DNA. Moreover, the method involves two successive steps of amplification, with the first step comprising mPCR and the second step LAMP. Amplicons of the first step serve as templates in the second step. The amplification protocol requires only a single temperature for the reaction, and the amplification is diagnosed without the need for electrophoretic techniques, using in situ detection process with colorimetric dye or with a fluorescent dye. The final products are the accumulation of 10^9 copies of target DNA in less than an hour. The LAMP-PCR has been regarded as an innovative technology and emerged as an alternative to PCR-based methodologies in clinical laboratory with significant increase of detection limits, efficiency, selectivity, and specificity over single-stage.

With more and more scientists focusing their attention on the application of LAMP technology, the range of its use is not limited to the bacteria detection and identification any more [14]. The LAMP-PCR was developed and employed to detect species that cause chorioamnionitis and premature labor, *Ureaplasma parvum*, and *Ureaplasma urealyticum* [15].

It was also applied to the parasite and virus detection [16–18]. Recently, Kurosaki et al. in their study [19] in 2017 developed a LAMP-PCR assay for the detection of

Zika virus plasma, serum, and urine samples collected from 120 suspected cases of arbovirus infection in Brazil.

2.3 Real-time PCR

According to [20], clinical diagnostic approaches rely on quantitative PCR (qPCR) as a method to detect and quantify infectious agents. Fluorescence chemistry-based methods have revolutionized molecular diagnostics and become the gold standard for viral load quantification and detection of bacterial and viral pathogens. During qPCR, the nucleic acid is amplified until it produces a certain level of signal which is supplied through a DNA intercalating dye or sequence-specific fluorescent probe. The cycle threshold (Cq), defined as the number of amplification cycles required to reach that signal level, is used to calculate the number of target molecules originally present based on a standard curve [20]. In qPCR, the targets are detected in real time from the sealed PCR plate, and there is no post-PCR processing required; therefore, the risk of false-positive results due to amplicon carryover is substantially decreased compared to conventional [21].

During the past decade, advantages on PCR have become gold standard procedure in the diagnosis of infectious diseases, particularly the diagnosis of viral diseases, such as arboviruses. Arboviruses are causing an unprecedented health calamity in world, especially in Latin American countries, with rising statistics on a daily basis. The diagnosis of these diseases is difficult to establish only by clinical features. A substantial proportion of these infections are asymptomatic, but some patients may also present clinical symptoms similar to the arboviruses such as those caused by the Dengue, Zika, and Chikungunya virus. The qPCR technique is more sensitive and specific than serological tests. Besides that, in serum samples, it is only possible to perform the diagnosis in the acute phase of the disease that lasts up to 4–7 days after the onset of symptoms. In relation to the detection of Zika virus (ZIKV), for example, recent data from the literature indicate that in human urine, viral RNA was found longer compared to serum, up to 20 days after the onset of the first symptoms. In semen, studies show that the genetic material of ZIKV was found for weeks and even months after infection [22]. However, the determination of viral load presents some particular challenges when using qPCR methodology. This is because reliable absolute concentration results of qPCR are dependent on assay efficiency, instrument calibration metrics, and comparison with a known reference sample to convert the Cq measurements to a sample unknown.

2.4 Digital PCR

In contrast to qPCR, the digital PCR (dPCR) uses an alternative method that is not dependent upon the determination of the amplification cycle that the reporter dye signal exceeds a threshold. Instead, prior to amplification, the samples to be subjected to dPCR are divided into thousands of independent PCR reactions and are scored as either positive or negative for amplification of the viral sequence of interest. The positive wells are counted and converted to a target concentration in the original sample. This binary assignment of each reaction greatly minimizes the dependence of measurement on parameters such as the efficiency of the assay and the calibration of the instrument. Therefore, dPCR is the absolute quantification methodology with the greatest potential for quantification of low-load viral nucleic acids.

In the diagnostic routine, it is very common to obtain positive qPCR results obtained at the detection limit of this methodology, which may generate uncertainty of the result. dPCR is a complementary methodology that works beyond

	qPCR	dPCR
Results	Cq, ΔCq, or ΔΔCq	Copies/mL
Quantification	Relative quantification	Absolut quantification, without standard curve
Factors affecting the signal	Standard curve	Results are not affected by any parameter
	Instrument	
	Primers and probes	

Table 1.
Comparative analysis between RT-qPCR and dPCR.

the limit of detection of qPCR, since it is based on the Poisson distribution. Consequently, this methodology has a significant impact on research as well as on diagnostic applications [23–25].

Some benefits of digital PCR in virology can be cited [25]:

- Quantification of viral genomes in samples without the need to use a standard curve;
- Detection of viruses that have a very low viral load;
- Use of low concentration of samples;
- Reduction of the impact of inhibitors present on complex samples.

Table 1 shows a comparison between the two methodologies: qPCR and dPCR.

A great number of studies use dPCR to diagnose infectious disease-related viruses, including hepatitis B virus (HBV), cytomegalovirus, human influenza, and HIV [26–28]; bacterial infections (*Mycobacterium tuberculosis*, *Chlamydia trachomatis*, and *Staphylococcus aureus*) [29–31]; and parasitic infectious such as detection of *Plasmodium falciparum* and *Plasmodium vivax* [32].

3. Conclusions

The ability to concurrently detect multiple pathogens infecting a host is crucial for accurate diagnosis of infectious diseases, identification of coinfections, and assessment of disease state for an effective patient management. PCR technology has been widely used to detect and quantify pathogenic microorganisms that cause various infectious diseases including some arboviruses, STIs, and bacterial infection. This methodology is revolutionizing the area of molecular diagnostics because of its high sensitivity of detection and specificity for the determination of infectious agents. In addition, there is a reduction in run time and cost over traditional cultivation methods, for example, to determine the amount of a particular pathogen in a clinical specimen. The main advantages of PCR are its higher sensitivity and specificity compared with other diagnostic methods such as serological assays and culture methods, as well as its rapidity, utility, and versatility in clinical laboratory.

Although the conventional PCR is the most widely used molecular technique, other methodologies have been developed including real-time PCR, multiplex PCR, LAMP-PCR, and digital PCR. The biochemical mechanisms of these techniques are based on enzyme-mediated processes, target, signal or probe amplification, and isothermal conditions.

Considering the clinical importance of these diseases, the number of infected individuals worldwide and the serious health consequences for population is extremely relevant to the precise, rapid, and sensitive diagnosis of these diseases in laboratories. Regardless, there is still the need for advances in basic science research and development of molecular technologies, which provide basis for the precise diagnostic and molecular epidemiology of infectious diseases as well as control measures, prevention, and design of vaccines and monitoring of infections face-to-face the treatment applied in clinical practice.

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Conflict of interest

The author declares that he has no conflict of interest.

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