ORIGINAL ARTICLE

DETERMINATION OF INFECTIOUS DISEASES BY POLYMERASE CHAIN REACTION

Zuneera Akram[1*], Rehana Perveen[1], Muzammil Hussain[1],
Aisha Noreen[2], Anum Akram [3], Maryam Inayat [4]
[1] Department of Pharmacology, Baqai Institute of Pharmaceutical Sciences,
Baqai Medical University, Karachi, Pakistan
[2] Department of Pharmaceutical Chemistry, Baqai Institute of Pharmaceutical Sciences,
Baqai Medical University, Karachi, Pakistan
[3] Indus Hospital,Karachi, Pakistan.
[4] Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan
Indus Hospital,Karachi, Pakistan

ABSTRACT
Infectious diseases, which introduced huge public health and financial problems, have been a concern in medical societies since the 1950s. Routine studies are currently carried out based on cell culture, serology and biochemical techniques for laboratory diagnostics of infectious agents. Cell culture-based approaches are mostly applied in laboratories and are less sensitive than serological and molecular methods. The development of molecular techniques, especially in the field of polymerase chain reaction, revolutionized the diagnosis of infectious diseases (PCR). Extensive application of science has provided high sensitivity and ease, with which PCR can identify known genetic sequences. A variety of molecular experiments are focused mainly on what has been described, including in real-time PCRs, multi-plex PCRs, LAMP-PCRs and digital PCRs. This research studies will identify active infections such as arboviruses, sexually transmitted diseases and bacterial infections through the identification of infectious agents and nucleic acids via PCRs methods. There must be stronger molecular science to enhance infectious agent detection capabilities to track the spread of infectious diseases and take appropriate measures to support patients and the health workers themselves.

Key Words: Infectious disease, Diagnosis, Polymerase Chain Reaction, Molecular techniques, Sexually transmitted infections

1. INTRODUCTION
Pathogenic microorganisms such as microbes, viruses, parasites or fungi cause infectious diseases (IDs), and these diseases may spread from human to human, directly or indirectly.Since the first half of the last century, technological development in the biomedical field, including the manufacture of medication, vaccines and state-of-the-art sanitation technology has been taken as part of infectious disease control or prevention. Infectious diseases are among the world's most prominent public health issues.The significance is seen by the number of individuals involving these pathologies due to lack of information on their infectious substances and their socio-economic effects. Adetailed molecular diagnosis analysis and public health research on thestrategies of diagnosis are also observedfor early identification of both the symptomatic carriers and the advancement of diagnostic technologies.

Culture developments, technologies and the micro-organisms itself have helped to evolve new pathogens, to reappear previously regulated diseases and to develop antimicrobial resistance. The World Health Office (WHO) estimates that IDs make up a large proportion of all identified human diseases and an estimated 25% or more of the world's nearly 60 million deaths per year were attributed to

infectious diseases[1,2].

Scientific experiments offer insight into the development of detection tests for certain illnesses and infectious agents, cure medications and preventive vaccines. Previous, specific and efficient diagnoses are one of the better means of treating them. According to Yang & Rothman in 2004, about 5 million infectious cases are registered per year in American hospitals. Besides, most of the cases have not been registered, leading to severe morbidity and death[3]. For the past twenty years, 16S rDNA computing methods have been commonly used rather than traditional cultivation methods.

Unfortunately, considering the reality that these studies suffer from slower processing period from time to time in the clinical laboratories, diagnose infectious diseases are closely related to the time of pathogens detection by traditional methods of culture. Drawbacks of traditional diagnosis include extra research criteria and waiting periods for diagnosed pathogens (i.e., Species discernment, virulence factors, and antimicrobial resistance)[3,4].

Amplification of nucleic acid is one of the most useful methods for diagnosing infectious diseases in nearly every field of life science, including applications, such as clinical medicine. The serological approaches are constrained by the fact that the cross-reactions between different agent forms are not simple enough, and certain infectious agents need to be specifically clinically diagnosed with epitopes. Moreover, IgM-specific antibodies can only be observed at an acute infection level and serological tests are inaccurate, labour intensive and inefficient to diagnose infections. In the last twenty years, nucleic acid-amplified diagnostic techniques have been used as a new tool for diagnosing human diseases such as viruses, bacteria and parasites throughout the life sciences.

In multiple clinical samples, such as plasma, serum, semen, vomit, and liquid CSF, use of amplification methods, such as PCR, has been commonly used to detect, genotyped, measure viruses and bacteria (CSF).

PCR-based diagnostics for a wide variety of microorganisms have been effectively established. PCR is advocated by infectious disease specialists for detection of species not cultivable in vitro or in instances where there is a lack of sensitive and/or a need for prolonged incubating techniquesbecause of its unbelievable sensitivity, accuracy, reproducibility, large dynamic ranges and speed of amputation.[5]

Advancement of molecular technology and diagnostics parameters have improved the knowledge of aetiology, pathogenesis and molecular epidemiology of the IDs that provide a framework for sufficient diagnosis, assessments of prevention, regulation and rational design of vaccinations to eradicate those diseases effectively.

After 1985, several PCR amplification-based techniques, such as PCR (LAMP-PCR), PCR (M-PCR), real-time PCR and,digital PCR (dPCR) were built for detection and identification.

2. CLINICAL AND PCR APPROACHES IN INFECTIOUS DISEASE DIAGNOSTICS.

2.1. PCR Multiplex

The use of PCR is often restricted in diagnostic laboratories by their expense and also by the provision of appropriate sample volume. A type of multiplex PCR is available to address these problems and to improve PCR's diagnostic capabilities (mPCR). The mPCR refers to the use, by way of gel electrophoresis, of various couples of primers to stimulate simultaneously many areas of the nucleic acid of the sample. The use of multiple pairs in mPCRs provides valuable cost, time and exact diagnostic advantages. This technology provides the principal advantage of reducing the number of different responses, for example in a single specimen, to detect multiple pathogens simultaneously, such as sexually transmitted pathogens.[6-8]With this treatment, a variety of diseases can be diagnosed with a single diagnostic examination, with sensitivity, speed and accuracy, important values for diagnostic testing.

This approach has been a staple in therapeutic and scientific applications including diseases acquired sexually (STIs). Provided a big problem with public health, STIs are widespread in developed and emerging countries everywhere. About 340 million new bacterial STI cases are reported to occur per year, including gonorrhoea, chlamydia, and syphilis, and in adults aged 15 to 49 years, the prevalence is growing worldwide.[9]The vulnerability to sexually transmitted infections, including HIV, and the high cost of care has contributed to the need for fast and effective laboratory pathogens detection techniques. Nucleic acid amplification procedures were designed for the identification of sexually transmitted pathogens, in particular mPCR methods[10].

In single reactions, the Multiplex PCR can examine several samples and is helpful in multipathogenic infection diagnosis. However, it has a range of drawbacks, including non-specific materials, which can compete with amplifying targets, limiting sensitivity and selectivity of reactions by primary interaction.

2.2. Isothermic loop-mediated enhancement
In the field of life science analysis, nucleic acid amplification is also used. Many new molecular diagnostic methods were developed subsequently with the advancement of molecular biology[11].

The LAMP-PCR was developed over 15 years earlier and is an effective way to simultaneously detect several pathogens[12-13]. The latter has also been an important tool. The procedure uses a DNA polymerase with strand displacement operation and a series of four internal and external primers that identify six distinct sequences of the target DNA in total. Besides, the process contains two consecutive amplification steps with the first step containing the LAMP and the mPCR. The first stage amplicons act in the second phase as models. The amplification procedure needs only a single reaction temperature, which is diagnosed using in-situ detection of colorimetric dye or a fluorescent dye without the need for electromagnetic techniques. In less than one hour, 10^9 copies of the target DNA are accumulated as final products. LAMP-PCR was considered to be revolutionary development and was developed in a single-phase, substantiated increase in detection limits, effectiveness and selectivity as an alternate to PCR-based methodologies in the clinical laboratory.

The spectrum of its use is now no longer confined to bacteria detection and identification [14], with increasing scientific researchers concentrating their attention on the application of LAMP technology. The LAMP-PCR has been developed and used to diagnose chorioamnionitis and premature labour, ureaplasma parvum, and urealyticurea[15].

It was also used for the treatment of parasites and viruses [16-18]. A LAMP-PCR detection test for the plasma, serum and urine Zika virus samples obtained from 120 suspect cases of arboviral infection in Brazil was recently developed by Kurosaki et al. in their 2017 report[19].

2.3. Real-time PCR
Clinical diagnostic approaches rely on quantitative PCR as a medium for the detection and quantification of infectious agents (qPCR). Fluorescence chemistry methods have revolutionized molecular diagnostics and have become the standard for calculating viral loads and detecting viral and bacterial pathogens. Amplification of nucleic acid in the QPCR before a signal level is generated, given by the color of the intercalation of DNA or a fluorescent sequence-specific test. In the measurement of the number of target molecules originally present on standard curves, the cycles threshold (Cq), defined as the number of amplification cycles required to attain this signal level.A cycle threshold (Cq) defined as the number of amplification cycles necessary to reach a signal level will be used to calculate the number of target molecules originally found on a standard curve [20]. In qPCR, the targets are sensed on the sealed PCR platform in real-time and no processing after PCR is required; the probability of false-positive results due to the transportation of amplicons is thus dramatically reduced compared with the norm [21].

During this past decade, advantages in the detection of infectious disorders, in particular viral diseases such as arboviruses, have been the Gold Standard treatment for PCR. Arboviruses in particular in Latin American countries are a worldwide unparalleled cause of health catastrophe with growing everyday statistics. Only clinical manifestations make it impossible to detect these diseases. A significant proportion of such infections are asymptomatic, although some patients may have clinical symptoms similar to arboviral, such as the Chikungunya, Dengue, and Zika viruses. The qPCR is sensitive and more precise than serological checks. Furthermore, in the serum samples, the acute phase of the condition can only be diagnosed for up to 4–7 days after the start of symptoms. For instance, recent literature findings suggest that viral RNA was detected more in human urine than serum up to twenty days after the initial effects of Zika virus have begun (ZIKV). Semen containing the genetic material of ZIKV was found for weeks and months after infection [22]. The viral load using qPCR methods is, however, extremely difficult to determine. The precise results of absolute qPCR concentration are dependent on test performance, measurements for instrument calibration as well as a comparison with an established benchmark to convert measurements of Cq into an unknown sample.

2.4. Digital PCR

The Digital PCR (dPCR) employs an additional process, unlike qPCR, that does not rely on an amplification period determination to transcend the threshold of the reporter dye signal. The dPCR samples are separated into many thousand individual PCR reactions before the amplification, which are either positive or negative to amplify the interest of viral sequence. The positive wells are counted in the original sample and translated into a target concentration. This binary distribution of each reaction substantially minimizes the calculation dependency on factors such as the evaluation quality and instrument calibration. dPCR, too, is the absolute form of quantification with the highest quantification potential of low-load viral nucleic acids.

The positive outcomes of qPCR obtained by the detection limit for this technique are usually accomplished in the diagnostic routine, which can cause concerns about the outcome. dPCR is an additional approach which focused on the Poisson distribution and functions outside the limits of detection of qPCR. Consequently, both analysis and medical implementations have an important effect on this technique[23-25].

There are some benefits tothe virology of modern PCR[25].

- Quantification of viral genomes in experiments without using a natural curve in samples;
- Detection of viruses of very low viral load;
- The poor concentration of the sample used;
- Reduced effect on complex samples of inhibitors.

Table 1: The two methodologies: qPCR and dPCR are compared

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

Table 1: The analysis of RT-QPCR and dPCR is a comparative analysis.

A wide variety of experiments have used dPCR to detect infectious viruses, such as hepatitis B viruses, human flu, cytomegalovirus, and human immunodeficiency virus[26-28], bacterial infection (Chlamydia trachomatis and mycobacterium tuberculosis) [29-31], as well as parasite infections like Plasmodium, vivax and Plasmodium falciparum[32].

3. Infectious Disease Detection PCR applications

3.1 Tuberculosis: HIV and multi-drug-resistant strains of mycobacteriumtuberculosis with an elevated prevalence. Early detection of tuberculosis is important for diagnosis and care. Classical infection detection methods are disadvantageous since the organism is tedious and slowly growing. A variety of molecular techniques have been developed to identify mycobacteria. Relevant ones are PCR, amplification mediated by transcription, amplification dependent on nucleic acid sequence and ligase chain reaction[33]. Multiplex PCR is comparatively and readily distinguishable by mycobacterial speciation that is time-consuming and taxing by traditional methods[34]. The fact that the first line of INH and rifampicin was mutated in the cat gene and the rpoB gene is based on molecular susceptibility research. The gene fragment can be identified by a PCR amplification accompanied by a basic electrophoresis for single streaming conformational Polymorphism (SSCP) analysis in denaturated gels. The above treatment enables early identification of infection by mycobacterium tuberculosis and initiation of suitable therapy. This approach is sensitive and also detected in point mutations[35-36].

3.2 Streptococcal pharyngitis: is becoming increasingly relevant in developing countries since PCR diagnosis of Gp A haemolytic streptococci by PCR (Gp A direct Streptococcus test or GASD) could restrict empirical therapy of pharyngitis, cut beta-lactam antibiotics and lower overall therapeutic cost[36].

3.3 Atypical pneumonia: A PCR-based multiplexing technique that may help reshape the treatment of pneumonia atypical infections through a quick diagnosis of Chlamydia pneumonia, Mycoplasma pneumonia and legionellae[37].

3.4 Persistent disease: Persistent HSV, CMV, EBV, VZV, HHV, JC, measles, hepatitis infections are hard to diagnose and cure and seldom respect organ borders. These diseases typically develop in the immune-competent host as an acute illness, and a high defensive immunity accompanies a recovery. These infections can however be reactivated in the immunocompromised host. Not only can they cause a diagnostic problem, but their nucleic acid may also be not clinically important by amplification procedures. Quantitative tests are important and have been established to help their presence[37].

3.5 The use of PCR has been reported for acute fever causing disease, such as falciparum malaria, salmonellosis, babesiosis. The use of a single PCR reaction and hybridization testing with different samples is especially used in falciparum infections in the identification of species[38].

3.6 Ulcerative Urogenital Infections:Multiplex PCR use for distinguished aetiology causes of ulcerative urogenital infections caused by Haemophilusducreyi, Treponema pallidum, and HSV was reported[39].

4. CONCLUSION

To reliably diagnose infectious diseases as well as recognise coinfections, the capacity to simultaneously recognise several pathogens that invade a host is necessary for efficient disease handling. PCR has commonly been used to identify and measure pathogens that cause multiple infectious diseases, including some arboviruses, STIs and bacterial infections. The high precision of molecular diagnostics and the accuracy for identifying infectious agents revolutionise this approach. Besides, the length and expense of given pathogens in a medicinal specimen were reduced under conventional cultivating approaches. The key benefits of PCR are their greater sensitivity and accuracy in contrast with other diagnostics approaches, such as serological

tests and culturing methods.

While traditional PCR is the most used molecules, other processes such as PCRs, Multiplex PCRs, LAMP-PCRs and digitalised PCRs have been developed. The biochemical pathways for such techniques are focused on processes of enzyme mediation, signal or probe, amplification target, and isothermal conditions. Given the therapeutic importance of diseases, there is a great deal of concern in laboratories for the reliable, quick and sensitive diagnosis of these illnesses in the number of infectious people in the world and for the severe health impacts on the population.However, improvement is also important to provide the basis for precise diagnostic and molecular infectious disease epidemiologies and to provide prevention and vaccine formulation regulation, as well as personal surveillance of pathogens that are used for clinical procedures, in fundamental scientific research and advancement of molecular technologies.

REFERENCE

1. Nii-Trebi NI. Emerging and neglected infectious diseases: Insights, advances, and challenges. Biomed Res Int. 2017;2017:1-15.
2. Cohen ML. Changing patterns of infectious disease. Nature. 2000;406:762-767
3. Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: Uses, limitations, and future applications in acute-care settings. Lancet Infect Dis. 2004;4:337-348.
4. Vouga M, Greub G. Emerging bacterial pathogens: The past and beyond. Clin Microbiol Infect 2016;22:12-21.
5. Louie M, Louie L, Simor A. The role of DNA amplification technology in the diagnosis of infectious disease. CMAJ. 2000;163:301-309
6. Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE.ÊMultiplex PCR: Optimization and application in diagnostic virology. Clin Microbiol Rev. 2000;13:559-570
7. McIver CJ, Jacques CFH, Chow SSW, Munro SC, Scott GM, Roberts JA, et al. Development of multiplex PCRs for detection of common viral pathogens and agents of congenital infections. J. Clin. Microbiol. 2005;43:5102-5110.
8. Ratcliff RM, Chang G, Kok T, SlootsÊTP. Molecular diagnosis of medical viruses. Curr Issues Mol Biol. 2007;9:87-102
9. Tucker JD, Bien CH, Peeling RW. Point-of-care testing for sexually transmitted infections: Recent advances and implications for disease control. Current Opinion in Infectious Diseases. 2013;26:73-79.
10. Zauli DAG, Lima LM, Fradico JRB, Menezes CLP, Diniz CG, Silva VL, et al. In house real-time PCR assays for detection of sexually transmitted pathogens: Neisseria gonorrhoeae, Chlamydia trachomatis, Ureaplasmaurealyticum, Mycoplasma hominis and Mycoplasma genitalium. IJRAMR. 2017;4:2561-2565
11. Yanmei L, Penghui F, Shishui Z, Li Z. Loop-mediated isothermal amplification (LAMP): A novel rapid detection platform for pathogens. Microb. Pathog. 2017;107:54-61.
12. Iwamoto T, Sonobe T, Hayashi K. Loop-mediated isothermal amplification for direct detection of complex, and in sputum samples. J. Clin. Microbiol. 2003;41:2616-2622
13. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop mediated is other mal amplification of DNA. Nucleic Acids Res. 2000;28:e63
14. Maruyama F, Kenzaka T, Yamaguchi N. Detection of bacteria carrying the stx2 gene by in situ loopmediated isothermal amplification. Appl Environ Microbio. 2003;69:5023-5028.
15. Fuwa K, Seki M, Hirata Y, Yanagihara I, Nakura Y, Takano C, et al. Rapid and simple detection of Ureaplasma species from vaginal swab samples using a loop- mediated isothermal amplification method Am. J. Reprod. Immunol. 2017;79:1-8.
16. Kaneko H, Iida T, Aoki K. Sensitive and rapid detection of herpes simplex virus and varicella-zoster virus DNA by loop-mediated isothermal amplification. J. Clin. Microbiol. 2005;43:3290-3296.
17. Enomoto Y, Yoshikawa T, Ihira M. Rapid diagnosis of herpes simplex virus infection by

a loop-mediated is othermal amplification method. J. Clin. Microbiol. 2005;43:951-955.
18. Varlet-Marie E, Sterkers Y, PerrotteÊM, Bastien P.ÊA new LAMPbased assay for the molecular diagnosis of toxoplasmosis: Comparison with a proficient PCR assay. International Journal for Parasitology. 2018;48: 457-462.
19. Kurosaki Y, Martins DBG, Kimura M, Catena AS, Borba MACSM, Mattos SS, etÊal. Development and evaluation of a rapid molecular diagnostic test for Zika virus infection by reverse transcription loop-mediated isothermal amplification. Nature. 2017;7:13503-13513.
20. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry. 2009;55:611-622.
21. Sibley CD, Peirano G, Church DL. Molecular methods for pathogen and microbial community detection and characterization: Current and potential application in diagnostic microbiology. Infection, Genetics and Evolution. 2012;12:505-521.
22. Wahid B, Ali A, Rafique S, Idrees M. Zika: As an emergent epidemic. Asian Pacific Journal of Tropical Medicine. 2016;9:723-729.
23. Huggett JF, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, et al. The digital MIQE guidelines: Minimum information for publication of quantitative digital PCR experiments. Clinical Chemistry. 2013;59:892-902.
24. Huggett JF, Cowen S, Foy CA. Considerations for digital PCR as an accurate molecular diagnostic tool. Clinical Chemistry. 2015;61:79-88.
25. Sedlak RH, Jerome KR.ÊViral diagnostics in the era of digital polymerase chain reaction. Diagnostic Microbiology and Infectious Disease. 2013;75:1-4.
26. Whale AS, Bushell CA, Grant PR, Cowen S, Gutierrez-Aguirre I, O'Sullivan DM. Detection of rare drug resistance mutations by digital PCR in a human influenza a virus model system and clinical samples. J. Clin. Microbiol. 2016;54:392-400.
27. Huang JT, Liu YJ, Wang J, Xu ZG, Yang Y, Shen F. Next generation digital PCR measurement of hepatitis b virus copy number in formalin-fixed paraffin-embedded hepatocellular carcinoma tissue. Clinical Chemistry. 2015;61:290-296.
28. Sedlak RH, Cook L, Cheng A, Magaret A, Jerome KR. Clinical utility of droplet digital PCR for human cytomegalovirus. J. Clin. Microbiol. 2014;52:2844-2848.
29. Yang J, Han X, Liu A, Bai X, Xu C, Bao F, et al. Use of digital droplet PCR to detect Mycobacterium tuberculosis DNA in whole blood-derived DNA samples from patients with pulmonary and extrapulmonary tuberculosis. Front. Cell. Infect. Microbiol. 2017;11:7-369.
30. Roberts CH, Last A, MolinaGonzalez S, Cassama E, Butcher R, Nabicassa M, et al. Development and evaluation of a next-generation digital PCR diagnostic assay for ocular Chlamydia trachomatis infections. J. Clin. Microbiol. 2013;51:2195-2203.
31. Luo J, Li J, Yang H, Yu J, Wei H. Accurate detection of methicillinresistant Staphylococcus aureus in mixtures by use of single-bacterium duplex droplet digital PCR. J. Clin. Microbiol. 2017;55: 2946-2955.
32. Koepfli C, Nguitragool W, Hofmann NE, Robinson LJ, OmeKaius M, Sattabongkot J. Sensitive and accurate quantification of human malaria parasites using droplet digital PCR (ddPCR). Scientific Reports. 2016;6:39183. DOI: 10.1038/srep39183
33. Shaw RJ. Polymerase chain reaction. In: Davis PDO, editor. Clinical Tuberculosis. 1st edn. Chapman and Hall Medical; London: 1994. 381–391.
34. Cormican M, Glenon M. Ni Riain U, Flynn J. Multiplex PCR for identifying mycobacterial isolates, J Clin Pathol. 1995;48:203–205.
35. Zhang Y, Heym B, Alen B. The catalase peroxidase gene and INH resistance of Mycobacterium tuberculosis Nature. 1992; 358(6387):591-593.
36. Musser JM. Antimicrobial agents resistance to mycobacteria: Molecular genetic insights. Clin Microbial Review. 1995;8:496–514

37. Whelen AC, Persing DH. The role of nucleic acid amplification and detection in the clinical microbiology. Annu Rev Microbiol. 1996;50:349–373.
38. Kimura M, Miyake H, Kim HS, Tanabe M, Arai M. Species specific PCR detection of malaria parasite by microtiter plate hybridisation: clinical study with malaria patients. J Clin Microbiol. 1995;33:2342–2346.
39. Orle KA, Gates CA, Martin DH, Body BA, Weiss JB. Simultaneous PCR Detection of Hducreyi, T. pallidum and Herpes simplex virus from genital ulcers. J Clin Microbiol. 1996;34:49–54.