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POLYMERASE CHAIN REACTION (PCR)–BASED DIAGNOSTIC APPROACHES IN INFECTIOUS DISEASES WITH SPECIAL EMPHASIS ON COVID-19

Juamamurodov Sobirjon Tursunboy ogli [1,2]
Izbasarov Islomjon Ilkham ogli [3]
[1]Assistant Professor, Department of Microbiology, Virology and Immunology, Tashkent State Medical University
[2]Virologist, Virology Laboratory, Chilonzor District Department, Tashkent City Department of the Committee for Sanitary and Epidemiological Wellbeing and Public Health
[3]Student of Group 202, Faculty of General Medicine No. 2, Tashkent State Medical University, Tashkent, Uzbekistan.
E-mail: izbosarovislomjon@gmail.com Phone: +998900160699

Abstract

Polymerase Chain Reaction (PCR) is currently considered the gold-standard molecular method for detecting pathogenic nucleic acids in clinical specimens. Its exceptional sensitivity and specificity have enabled rapid diagnosis of a wide range of infectious diseases, ranging from classical bacterial infections to emerging viral pathogens. The recent COVID-19 pandemic demonstrated the enormous clinical and epidemiological significance of PCR-based diagnostics, as these methods allowed early detection, patient isolation, monitoring of viral load, and assessment of treatment efficacy. This article reviews the cellular and molecular principles of PCR, the varieties of PCR assays used in modern clinical microbiology, and the diagnostic importance of PCR for SARS-CoV-2—the causative agent of COVID-19. Additionally, the paper discusses limitations,

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interpretation challenges, and the future direction of PCR technology in infectious disease management.

Keywords: Polymerase chain reaction (PCR), reverse transcription, SARS-CoV-2, COVID-19 diagnosis, viral load quantification, molecular diagnostics, infectious disease detection, qPCR (quantitative PCR)

Introduction

Infectious diseases continue to represent a major global health challenge, causing millions of deaths annually, particularly in developing countries with limited diagnostic capacities. Classical diagnostic tools such as culture, microscopy, and serological assays have substantially contributed to infectious disease detection, yet these methods have important limitations. They often require large pathogen quantities, can be time-consuming, and may generate false-negative results in early stages of infection. The advent of genetic amplification techniques dramatically transformed clinical microbiology, enabling detection of minute amounts of pathogen nucleic acids even in presymptomatic individuals.

PCR was introduced by Kary Mullis in 1983 and has since become a cornerstone of molecular biology. In clinical diagnostics, PCR enables exponential amplification of pathogen DNA or RNA, allowing the detection of microorganisms that are difficult or impossible to culture, including many viruses. During the COVID-19 pandemic, qRT-PCR rapidly emerged as the standard method for detecting SARS-CoV-2 in respiratory specimens. The global reliance on PCR assays revealed both the power and the limitations of molecular diagnostics, producing unprecedented data that reshaped public-health strategies, quarantine policies, and global surveillance systems.

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Principles and mechanisms of PCR amplification

PCR relies on repetitive cycles of denaturation, annealing, and extension, mediated by a thermostable DNA polymerase. In every cycle, the amount of specific nucleic acid doubles, leading to exponential amplification. The reaction requires a set of primers complementary to the target sequence, a DNA polymerase enzyme, nucleotides, and thermal cycling conditions. When pathogen detection is required, primers are designed to recognize unique regions of the microbial genome, enabling highly specific molecular identification.

COVID-19 diagnostic PCR is usually performed as reverse-transcription PCR (RT-PCR), because SARS-CoV-2 is an RNA virus. In this approach, viral RNA is reverse-transcribed into complementary DNA (cDNA), which is subsequently amplified. The amplification process is monitored in real-time using fluorescent probes, allowing quantitative evaluation of viral RNA levels. The number of amplification cycles needed to reach fluorescence threshold is defined as Ct value, which correlates with viral load and infectious potential.

Types of PCR used in infectious disease diagnostics

Multiple PCR variants are applied clinically. Conventional PCR identifies pathogen DNA following amplification using gel electrophoresis. Although useful, this method is slower and less sensitive than modern approaches.

Real-time PCR (qPCR) provides high sensitivity, rapid results, and quantitative interpretation. Multiplex PCR can detect several pathogens simultaneously by using multiple primer sets within a single reaction mixture, a feature critically relevant for respiratory infections where co-infection may occur. Digital PCR, an emerging method, offers extreme quantification precision, useful for viral evolution research and monitoring residual viral load after treatment.

Among these methods, real-time RT-PCR remains the most frequently employed tool for COVID-19 diagnosis due to its accuracy, scalability, and compatibility with automated laboratory platforms.

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Clinical application of PCR in COVID-19

Early detection of SARS-CoV-2 infection

PCR became the primary diagnostic test worldwide during the initial spread of COVID-19, allowing identification of infected individuals even before symptoms developed. This capability significantly reduced transmission, especially among asymptomatic carriers who would otherwise remain undetected by serological or antigen-based assays.

Quantification of viral load

RT-PCR enables estimation of viral copy numbers, providing clinically relevant information about infectiousness, disease progression, antiviral treatment response, and risk of complications. Lower Ct values correspond to high viral load, correlating with increased transmission potential.

Identification of viral variants

Genomic sequencing combined with PCR allowed classification of SARS-CoV-2 strains, including Alpha, Delta, and Omicron variants. Certain PCR assays incorporate mutation-specific probes to rapidly screen for variants without the need for full genome sequencing. This accelerated public health decision-making and vaccine strategy adjustment.

PCR Sensitivity and Sample Type

The diagnostic sensitivity of RT-PCR varies depending on the type of specimen collected. Table 1 summarizes sensitivity values reported in the literature for different sample types.

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Table 1: Diagnostic Sensitivity of RT-PCR by Sample Type

Sample Type	Sensitivity (%)	Reference
Nasopharyngeal swab	70–90	Wang et al., 2020
Oropharyngeal swab	60–80	Yang et al., 2020
Sputum	80–95	Wang et al., 2020
Bronchoalveolar lavage fluid	93–100	Yang et al., 2020
Saliva	72–91	Vogels et al., 2020

Notes: Sensitivity varies by stage of infection and sample collection quality.

False-Negative Considerations

The likelihood of false-negative results depends on the timing of testing relative to symptom onset. Table 2 shows false-negative rates across different days post-symptom onset.

Table 2: False-Negative Rate of RT-PCR by Days Since Symptom Onset

Days Since Symptom Onset	False-Negative Rate (%)	Reference
1	100	Kucirka et al., 2020
4	67	Kucirka et al., 2020
8	20	Kucirka et al., 2020
21	66	Kucirka et al., 2020

Notes: Viral RNA levels peak around Day 5–7, then decline; timing is critical for testing accuracy.

Advantages of PCR in COVID-19 diagnosis

PCR offers unmatched sensitivity, capable of detecting very small viral quantities. The specificity of PCR positively reduces false-positive results caused by cross-reactivity with other microorganisms. Rapid turnaround time enables early

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intervention. The automated nature of PCR platforms ensures reproducibility, while quantitative capability provides clinically informative viral-load measurements. Together, these features made PCR indispensable in global pandemic control.

Limitations and challenges

Despite its strengths, PCR is not free of challenges. False-negative results can occur if specimen collection is inadequate or viral load is below detection level, particularly in late infection stages. False-positives may arise from laboratory contamination or detection of non-infectious viral fragments, leading to unnecessary isolation measures. PCR requires laboratory infrastructure and skilled personnel, limiting access in resource-poor settings. Variants of SARS-CoV-2 may escape detection if primer-binding regions mutate, although modern assays are designed to target conserved gene regions to mitigate this problem.

Future perspectives

Future development of PCR technology will enhance diagnostic capabilities through increased portability, speed, and multiplexing. Point-of-care PCR devices are already emerging, allowing molecular testing outside of laboratories. CRISPR-based molecular detection methods may further expand rapid viral screening. Integration of digital PCR with epidemiological surveillance will enable more precise tracking of viral evolution. Broader implementation of PCR in low-resource settings will remain a global priority.

Conclusion

PCR-based diagnostics revolutionized infectious disease detection. The COVID-19 pandemic illustrated the indispensable value of PCR for early diagnosis, patient management, and epidemiological control. As molecular diagnostic technologies continue evolving, PCR will maintain its central role in global

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infectious-disease surveillance. Continued innovation aimed at improved accessibility and interpretation will define the future of PCR-driven medical microbiology.

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