Molecular Science

AIMS Molecular Science, 13(2): 180–204.
DOI: 10.3934/molsci.2026010
Received: 14 January 2026
Revised: 18 March 2026
Accepted: 26 March 2026
Published: 20 April 2026

https://www.aimspress.com/journal/Molecular

Review

Application of qPCR testing in clinical diagnostics: A brief review of its history, challenges and perspectives

Tuany Rodrigues[1], Bruna Gabriele de Moura[1], Giovanna Tatarem Coelho de Miranda[1], Camila Leitzke Toledo[1], Luma Fernandes Bravo[1] and Alexandre Dias Tavares Costa[1,2,*]

[1] Laboratory of Translational Research in Health, Carlos Chagas Institute (ICC/Fiocruz Paraná), Curitiba, Brazil
[2] National Institute of Science and Technology in Bioanalytics Lauro Kubota (INCTBio-LK), Institute of Chemistry, UNICAMP, Campinas, Brazil

* **Correspondence:** Email: alexandre.costa@fiocruz.br; Tel: +55-41-3316-3230.

Abstract: Real-time polymerase chain reaction (qPCR) is a molecular technique that has transformed clinical diagnostics by enabling the amplification and quantification of specific nucleic acid sequences with high sensitivity, specificity, and speed. This approach supports the detection of pathogens, genetic mutations, and biomarkers across clinical samples. qPCR is widely employed in fields, such as infectious diseases, oncology, parasitology, and medical genetics, playing a pivotal role in the rapid identification of infectious agents, including those responsible for COVID-19, tuberculosis, leprosy, leishmaniasis, and arboviral infections. Compared with conventional diagnostic methods, qPCR offers substantial advantages, including reduced turnaround times, increased analytical accuracy, and compatibility with automated workflows. Recent technological innovations, including portable thermocyclers, simplified workflows, and ready-to-use commercial kits, along with integration into digital platforms, have expanded the applicability of qPCR to low-infrastructure environments and point-of-care settings. Despite these advances, the technique faces challenges, such as high costs of equipment and reagents, reliance on trained personnel, and susceptibility to inhibition by sample-derived compounds. Nevertheless, the adoption of standardized guidelines, such as Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) and Standards for Reporting Diagnostic Accuracy (STARD), has strengthened the reproducibility and reliability of qPCR in clinical research. Future perspectives suggest an increasing role for qPCR in personalized medicine, epidemiological surveillance, and decentralized diagnostic strategies, further consolidating its importance in clinical practice and global public health. Its combined sensitivity, specificity, and adaptability will continue to drive innovation in molecular diagnostics.

Keywords: diagnostic; PCR; qPCR; ddPCR; point of care

1. Introduction

Molecular techniques have been gaining prominence in medicine as important diagnostic tools, enabling accurate detection and identification of pathogens, genetic mutations, and changes in gene expression. Their relevance lies in their ability to provide fast, sensitive, and specific results, which are fundamental for early diagnosis, screening, disease monitoring, and personalized medicine [1].

Among the available techniques, Polymerase Chain Reaction (PCR) is widely used in molecular diagnostics due to its versatility and efficiency [2]. The technique was first described in the late 1980s by Kary Mullis, who received the Nobel Prize in Chemistry in 1993 [2].

Since then, PCR has undergone numerous advances, evolving from its conventional form to newer technologies, such as quantitative real-time PCR (qPCR), digital PCR (ddPCR), and other variations. Initially, PCR was employed to amplify genomic regions associated with diseases such as sickle cell anemia and cystic fibrosis, but its applications quickly expanded to include the detection of polymorphisms and complex diseases, such as cardiovascular disorders [3,4]. qPCR has stood out as an important technique for clinical diagnosis, enabling the quantification of DNA or RNA in real time with high sensitivity and specificity, as well as high adaptability [5].

Our aim of this article is to explore qPCR as a clinical diagnostic tool, examining its advances, applications, advantages, and limitations. We also discuss prospects for the use of qPCR in diagnostic medicine, highlighting its importance as a public health tool.

2. Quantitative real-time Polymerase Chain Reaction (qPCR)

qPCR is a molecular technique that enables simultaneous amplification and quantification of specific nucleic acid targets during the PCR reaction. Unlike conventional PCR, which relies on endpoint detection, qPCR monitors the accumulation of amplified DNA in real time using thermocyclers equipped with optical detection systems that measure fluorescence emission at each amplification cycle [6]. Integrated software records fluorescence signals throughout the reaction and generates amplification curves, from which the Cycle Threshold (Ct) is determined. The Ct corresponds to the cycle at which fluorescence exceeds a predefined threshold above background levels and is inversely proportional to the initial amount of target nucleic acid present in the sample [7].

Quantification in qPCR can be performed using two major approaches. Moreover, absolute quantification is based on a standard curve generated from serial dilutions of known concentrations of target DNA, enabling the number of copies in unknown samples to be determined [8]. The slope of the standard curve is used to estimate PCR efficiency, which typically ranges between 90% and 110%. Alternatively, relative quantification compares the expression of a target gene with that of a stable internal reference gene, enabling the evaluation of changes between experimental conditions [8,9].

For RNA targets, such as RNA viruses, it is common to use quantitative reverse transcription PCR (RT-qPCR). In this approach, RNA is first converted to complementary DNA (cDNA) through a reverse transcription step, followed by real-time amplification and detection using qPCR [8,10]. RT-qPCR can be performed using two major strategies: one-step and two-step protocols. In single-step RT-qPCR, reverse transcription and PCR amplification occur sequentially in a single reaction tube,

reducing handling steps and minimizing contamination risks [11]. In contrast, two-step RT-qPCR involves a separate reverse transcription reaction to generate cDNA, which is subsequently used as a template for qPCR amplification [8,10,12].

Fluorescence detection in qPCR is based on two major chemistries: non-specific intercalating dyes and sequence-specific probes. Intercalating dyes, such as SYBR® Green I, bind to double-stranded DNA and emit fluorescence after intercalation during amplification, while hydrolysis probes, such as TaqMan®, increase specificity by hybridizing with a complementary sequence within the target amplicon. Fluorescence is released after cleavage of the probe by the 5′–3′ exonuclease activity of Taq polymerase [8,9,13]. These strategies enable real-time monitoring of amplification without the need for post-PCR analyses such as gel electrophoresis [8,13,14].

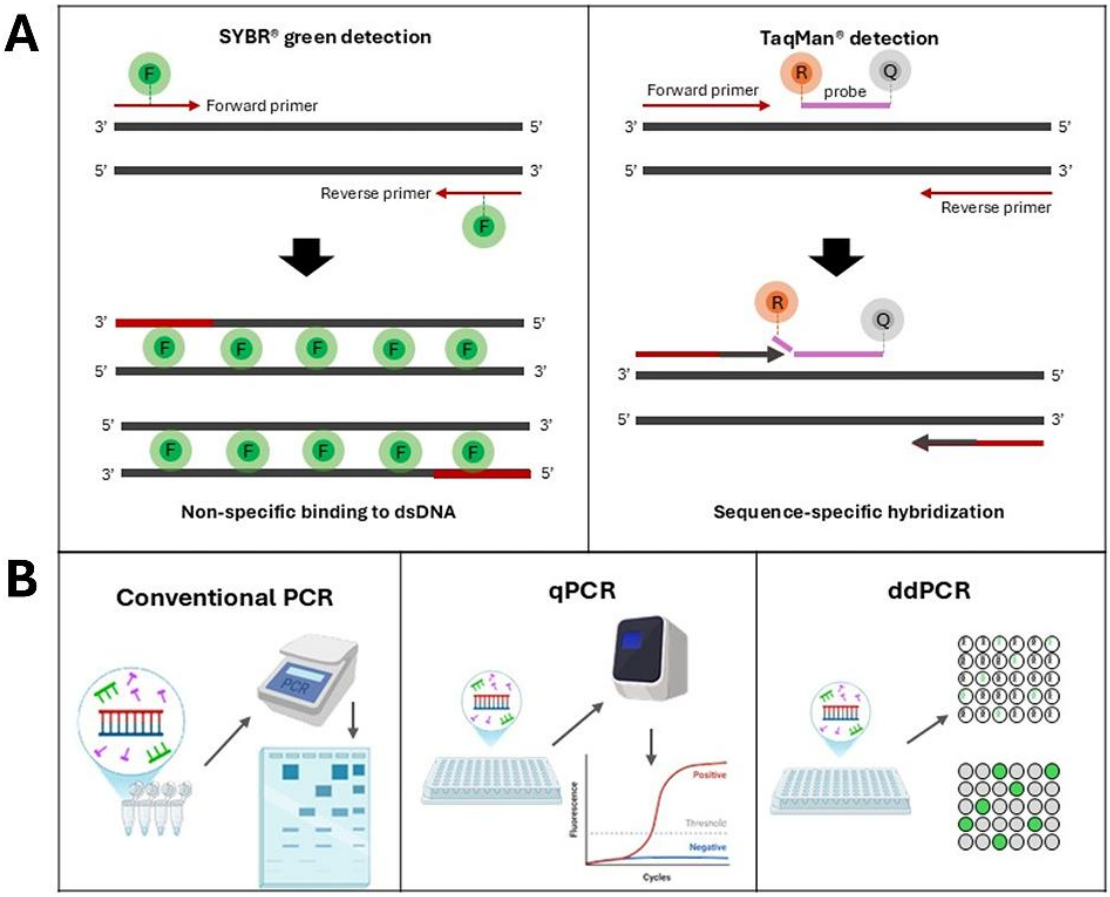

Figure 1. Fundamental principles and technological variations of PCR. (A) Detection chemistries used in qPCR. Comparison between non-specific intercalating dyes (SYBR® Green), which emit fluorescence upon binding to double-stranded DNA, and sequence-specific hydrolysis probes (TaqMan®), in which fluorescence is released after probe cleavage mediated by the 5′–3′ exonuclease activity of Taq polymerase. (B) PCR technological formats. Schematic representation of conventional PCR (endpoint detection), quantitative real-time PCR (qPCR), and droplet digital PCR (ddPCR). In ddPCR, the reaction mixture is partitioned into thousands of droplets, enabling absolute quantification of target molecules using Poisson statistics.

Droplet digital PCR (ddPCR) has emerged as an advancement in quantitative PCR technologies. In this approach, the PCR reaction mixture is divided into thousands of nanoliter-sized droplets, each acting as an individual amplification microreactor [15]. After the final PCR, the droplets are classified as positive or negative based on fluorescence detection. Absolute quantification is then calculated using Poisson statistics to estimate the number of target molecules in the original sample, eliminating the need for external calibration curves and improving analytical accuracy, particularly in samples with low target abundance [16–19].

Due to its sensitivity, specificity, and quantitative capability, qPCR has become widely used in molecular diagnostics, pathogen detection, gene expression studies, and nucleic acid quantification. Advances in portable qPCR platforms have also enabled their integration into point-of-care (POC) diagnostic systems, facilitating rapid molecular detection in decentralized clinical settings [20–22].

3. Advantages and limitations

Although qPCR is widely recognized for its high sensitivity and specificity, a balanced evaluation of the technique requires consideration of its strengths and its practical limitations (Table 1). qPCR is characterized by excellent analytical sensitivity and specificity, enabling the reliable detection and quantification of low-abundance nucleic acid targets in complex biological samples [23,24]. In addition, it provides a quick return and enables real-time monitoring of amplification, reducing the risk of post-amplification contamination when compared with conventional PCR [14]. Its broad dynamic range and capacity to generate quantitative data make qPCR particularly valuable for applications such as pathogen detection, gene expression analysis, and viral load monitoring. Furthermore, the availability of standardized protocols, commercial reagents, and validated platforms contributes to its reproducibility and facilitates implementation in routine diagnostic laboratories.

Table 1. Major advantages and limitations of qPCR in molecular diagnostics.

Aspect	Advantages	Limitations
Analytical performance	High sensitivity and specificity for detecting low-abundance nucleic acid targets.	Susceptible to inhibitors present in biological matrices.
Quantification	Enables accurate quantification through Ct values and standard curves.	Quantification may vary depending on reaction efficiency and calibration.
Dynamic range	Broad dynamic range allowing detection across several orders of magnitude.	Requires careful optimization to maintain accuracy across range.
Contamination risk	Closed-tube system reduces post-PCR contamination compared to conventional PCR.	Contamination during sample preparation can still affect results.
Speed	Rapid detection with real-time monitoring of amplification.	Requires specialized instrumentation.
Reproducibility	Standardized reagents and validated protocols improve reproducibility.	Inter-laboratory variability may occur without proper calibration.
Infrastructure	Widely established in diagnostic laboratories	High equipment and reagent costs
Operational requirements	Automated data acquisition through thermocycler software.	Requires trained personnel and controlled laboratory conditions.

Despite these advantages, the implementation of qPCR in routine clinical settings faces practical and technical challenges. One of the major constraints is the high cost of equipment and reagents, as well as the need for specialized laboratory infrastructure and trained personnel. These factors often restrict the use of qPCR to centralized laboratories, limiting its applicability in resource-limited settings or in scenarios that require rapid, decentralized diagnostic testing [25].

In this context, point-of-care (POC) testing has emerged as an important strategy for expanding access to molecular diagnostics, especially in settings with limited laboratory infrastructure. POC tests are designed to provide rapid and reliable results close to the patient, often using compact and portable analytical platforms that integrate sample processing, amplification, and detection into a simplified workflow. These systems reduce the need for centralized laboratory facilities and highly specialized personnel, enabling molecular diagnostics to be performed in decentralized settings, such as field laboratories [26]. Their relevance is especially evident in developing regions, where the high prevalence of infectious diseases coincides with financial and logistical barriers to centralized testing [25,27,28].

In addition, the development of gel-based PCR reagents represents an important technological strategy to facilitate the implementation of molecular assays in decentralized settings and point-of-care platforms [29]. In these systems, the major components of PCR, such as primers, nucleotides, buffer components, and DNA polymerase, are immobilized within a gel matrix, forming a preloaded and stabilized reaction environment. This configuration simplifies assay preparation, reduces pipetting steps, and minimizes the risk of handling errors or contamination. In addition, gel-based reagents generally exhibit better storage stability under refrigerated conditions and less degradation over time. These characteristics reduce reliance on the strict cold chain logistics required for many temperature-sensitive molecular reagents, which represent a significant cost in the transport and storage of diagnostic kits. As a result, gel-based formulations are particularly well suited for portable diagnostic platforms and field applications, where laboratory infrastructure and temperature-controlled distribution systems may be limited [30–33].

Despite these advances, qPCR has inherent technical limitations that must be considered. The technique is highly susceptible to inhibition by components present in biological matrices, such as blood and feces, as well as by factors intrinsic to the reaction itself, including primer design and fluorescent dye chemistry [34]. These inhibitory effects vary depending on the sample matrix. For example, in blood samples, substances such as heme and immunoglobulins can reduce Taq polymerase efficiency, while in stool samples, matrix complexity and differences in extraction methods can affect assay sensitivity [35,36]. Strategies such as sample dilution, the use of modified polymerases, and the inclusion of appropriate internal controls are effective in minimizing these effects and improving overall assay performance [34].

Therefore, although qPCR has practical and technical limitations, ongoing advances and methodological optimizations improve its applicability and reinforce its role as a valuable tool in clinical molecular diagnostics.

4. Clinical applications of qPCR in infectious diseases

The first clinical applications of qPCR focused on prenatal and oncological diagnostics, including the detection of trisomy 21 during pregnancy and the monitoring of treatment response in follicular lymphoma [37–39]. Since then, the technology has expanded into multiple areas of clinical medicine, including cancer diagnostics, hematological diseases, and metabolic disorders, where qPCR is used

for the detection and quantification of genetic and circulating molecular biomarkers. Comprehensive reviews discussing these applications are available elsewhere [40–43].

Soon after its introduction, qPCR was rapidly adopted for the detection and monitoring of infectious diseases. In this review, we focus on these applications, where the high analytical sensitivity and quantitative capability of qPCR have proven particularly valuable for pathogen detection and surveillance.

qPCR has been widely applied for the detection and quantification of protozoan pathogens. Notable examples include *Trypanosoma cruzi*, the causative agent of Chagas disease, and *Plasmodium* spp., responsible for malaria [44–49]. In these infections, qPCR enables the detection of parasite DNA, enabling the identification of low levels of parasitemia, often undetectable by conventional microscopy or serological methods. Furthermore, quantitative PCR has been successfully implemented for the detection and quantification of *Toxoplasma gondii*, enabling sensitive and specific diagnosis of toxoplasmosis through the identification of multicopy elements, such as the B1 gene or repetitive elements [50,51]. qPCR assays have also been developed for *Leishmania* spp., demonstrating high sensitivity and precise quantification of parasite load in clinical samples from cases of cutaneous, mucosal, and visceral leishmaniasis [52–54]. In addition to diagnosis, these assays enable early detection, monitoring of parasite load during treatment, and evaluation of therapeutic efficacy, especially in endemic regions where rapid and sensitive diagnostic tools are essential.

qPCR has also been instrumental in the diagnosis of bacterial infections, including leprosy and tuberculosis. Detection of *Mycobacterium leprae* DNA using qPCR has been validated across studies employing different sample matrices and genetic targets [55–59]. Similarly, numerous researchers have applied qPCR for *Mycobacterium tuberculosis* detection, exploiting its high analytical sensitivity to differentiate *M. tuberculosis* from nontuberculous mycobacteria and to identify genetic markers associated with drug resistance [60–65].

The application of qPCR in virology has advanced the detection of viral pathogens, providing rapid, sensitive, and quantitative analyses essential for clinical management and outbreak control. It is routinely used for the detection and monitoring of Herpes simplex virus type 1 (HSV-1), enabling accurate quantification of viral load and assessment of disease progression [65]. qPCR assays have also been critical in the surveillance and control of emerging and reemerging viral infections. For instance, multiplex RT-qPCR platforms have been developed for the detection of arboviruses such as dengue, Zika, chikungunya, and yellow fever viruses, demonstrating high analytical sensitivity, minimal cross-reactivity, and robust internal controls [66–69]. The yellow fever assay was tested in three national reference centers and approved by ANVISA for clinical use in Brazil, exemplifying rapid development of molecular tools in response to public health emergencies [69]. In the same year, ANVISA approved the first nationally developed qPCR Nucleic Acid Testing kit for leprosy diagnostics (NAT Hans Kit, IBMP, Brazil) [58]. Similar qPCR kits are also vital for rapid screening during outbreaks of highly virulent pathogens such as Ebola and MERS-CoV, enabling swift clinical isolation and infection control measures [70–73].

qPCR-based assays are widely used in blood banks to screen for infectious diseases such as hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV [74]. Detection of hepatitis A and E viruses by qPCR enhances outbreak investigations, providing 10–100-fold higher sensitivity compared with conventional RT-PCR methods [75]. Moreover, qPCR can detect infections up to 14 days earlier than serological assays, an advantage particularly evident during the first week of illness when antibody-

based methods often yield false-negative results. This early detection enables more timely case identification and supports effective public health interventions [76].

qPCR is also applied for detecting Epstein–Barr virus (EBV) and cytomegalovirus (CMV), which are critical in immunocompromised patients and transplant recipients, enabling early identification of viral reactivation and effective therapeutic management [77,78]. These applications collectively underscore qPCR importance in managing chronic, latent, and acute viral infections across clinical contexts.

The COVID-19 pandemic further highlighted the relevance of qPCR in public health, as RT-qPCR rapidly became the global gold standard for SARS-CoV-2 detection [12,79,80]. Its scalability, sensitivity, and quantitative precision enabled large-scale testing, genomic surveillance, and monitoring of viral variants, demonstrating how molecular diagnostics can be mobilized quickly during global health crises.

Beyond individual diagnostics, qPCR plays a pivotal role in population-level surveillance. Wastewater-based epidemiology (WBE) has emerged as a non-invasive method for tracking viral circulation within communities, as shown during SARS-CoV-2 monitoring. Additionally, qPCR-based WBE supports the One Health framework by detecting zoonotic viruses such as human and rat hepatitis E virus (HEV) in environmental samples, linking human, animal, and environmental health. This expansion from clinical to environmental monitoring illustrates qPCR evolution into an integrated tool for early outbreak detection and comprehensive public health management [81,82].

Beyond viral surveillance, qPCR has also been extensively applied in fungal diagnostics. For instance, qPCR protocols targeting the CYP51C gene of *Fusarium tricinctum* provide a robust framework for early detection, complementing other molecular techniques such as nested PCR and LAMP [83]. Standardized assays targeting IGS2 and ITS1 regions of rDNA enable accurate differentiation among clinically relevant *Candida* species, supporting rapid species-level identification for appropriate antifungal therapy [84]. For *Cryptococcus* spp., optimized DNA extraction and amplification protocols have improved diagnostic precision in cerebrospinal fluid and clinical management of neurocryptococcosis [85]. Similarly, validated qPCR assays integrated into clinical protocols for invasive aspergillosis, particularly alongside biomarkers such as galactomannan, have improved diagnostic consistency [86].

qPCR is also widely employed for the diagnosis of intestinal and zoonotic parasites, reinforcing its versatility across human, animal, and environmental health. Multiplex qPCR significantly improves diagnostic sensitivity and specificity compared to conventional microscopy or antigen-based methods for detecting protozoa and helminths [89–91]. A prospective study of 3,495 stool samples over three years demonstrated that multiplex qPCR significantly outperformed microscopy for pathogens such as *Giardia intestinalis*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Dientamoeba fragilis*, and *Blastocystis* spp. [92]. The major clinical applications of qPCR in infectious diseases are summarized in Table 2.

Table 2. Overview of the major clinical applications of qPCR in infectious diseases.

Application area	Target organisms	Clinical relevance
Protozoal infections	*Plasmodium* spp., *T. cruzi, Toxoplasma* spp., *Leishmania* spp.	Early diagnosis, parasite load
Bacterial infections	*M. tuberculosis*, *M. leprae*	Drug resistance, differentiation
Viral infections	SARS-CoV-2, arboviruses, HBV/HCV	Surveillance, outbreak control
Fungal infections	*Candida spp.*, *Cryptococcus*	Species-level diagnosis

Despite its wide applications, clinical implementation of qPCR requires rigorous validation and adherence to regulatory standards. Diagnostic assays must undergo comprehensive analytical and clinical evaluation, including sensitivity, specificity, reproducibility, and limits of detection, before approval by agencies such as the FDA [93]. qPCR experiments are expected to follow guidelines such as Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE), which standardize parameters including primer design, amplification efficiency, and internal controls [8]. Additionally, the Standards for Reporting Diagnostic Accuracy Studies (STARD) framework is recommended for reporting diagnostic performance, particularly in clinical validation of novel technologies or point-of-care platforms. These frameworks, available through the EQUATOR Network (https://www.equator-network.org/), provide a foundation for transparent, high-quality reporting across clinical applications.

Industry surveys conducted by the Global CRO Council (GCC) highlighted the growing reliance on qPCR and ddPCR in regulated bioanalysis, including gene therapy, cell therapy, and vaccine development, and the ongoing lack of comprehensive regulatory guidance, particularly for ddPCR. Many laboratories rely on MIQE guidelines or consensus recommendations from FDA, EMA, and industry white papers to define validation parameters and acceptance criteria, underscoring the need for harmonization in molecular assay validation [94].

In summary, qPCR plays a central role in the detection, monitoring, and surveillance of infectious diseases, supporting applications ranging from clinical diagnostics to population-level epidemiological surveillance and One Health initiatives. Continuous improvements in assay design, rigorous validation, and regulatory oversight are essential to fully realize qPCR's potential as a global diagnostic tool.

5. Technological advances and perspectives in molecular diagnostics

Despite its numerous advantages, the translational implementation of PCR in clinical diagnostics faces significant challenges [95]. These include the high cost of specialized reagents and instruments, the need for trained personnel, and performance variability across platforms and laboratories [5,96–98]. In resource-limited settings, constraints related to cost and infrastructure remain particularly critical, underscoring the demand for simplified and portable PCR platforms that preserve analytical sensitivity and specificity while reducing operational complexity [95,99].

To mitigate these limitations, technological innovations have prioritized workflow simplification, reagent stabilization, and the development of portable and automated platforms capable of extending molecular diagnostics beyond centralized laboratories.

Reagent-stabilization strategies have notably improved the feasibility of performing PCR outside controlled laboratory environments. Carbohydrate-stabilized, vacuum-dried qPCR mixes have been shown to retain accuracy, sensitivity, and specificity for extended periods under elevated temperatures [29,32,33,100]. Gelification provides an alternative, milder preparation method, enabling complete reaction mixes to be stored for up to 90 days at 2–8°C without performance loss [30,31,101]. Similarly, lyophilized bead-based PCR mixes have achieved prolonged room-temperature stability, maintaining analytical performance for over one year [102,103]. Moreover, glycerol-free lyophilized formulations supplemented with trehalose or dextran have demonstrated robust stability for several months at room temperature, enabling reliable viral detection across a wide range of clinical matrices [104–107].

In parallel, innovative extraction methods have been developed to simplify sample preparation. The SmartLid system enables rapid, electricity-free nucleic-acid purification using pre-aliquoted

magnetic beads and low-cost, 3D-printed magnetic lids [108–110]. "Dipstick" methods using cellulose strips enable nucleic-acid adsorption, washing, and elution within minutes, requiring no specialized equipment [111]. Ultra-simplified field protocols employing chemical or thermal lysis paired with portable qPCR devices have also demonstrated adequate sensitivity for pathogen surveillance in low-resource settings [112–115]. Collectively, these strategies demonstrate that optimized stabilization and simplified extraction can generate ready-to-use PCR reagents suitable for decentralized diagnostic workflows. A notable example is the development of a simplified molecular assay for tuberculosis that integrates sample preparation and bacillus DNA detection in infrastructure-limited regions [116,117].

Advances in portable PCR platforms have further expanded the feasibility of decentralized molecular diagnostics. A comparative overview of representative portable and automated qPCR platforms, including their design, analytical performance, turnaround time, and infrastructure requirements, is presented in Table 3.

The Truenat™ system exemplifies how portable PCR devices can achieve sensitivity and specificity comparable to laboratory-based systems, reaching 100% sensitivity and specificity for HBV while operating with sealed cartridges and automated analysis [118]. Its versatility includes validated applications in the detection of *M. tuberculosis* (94.7% sensitivity), malaria (up to 99.3%), high-risk HPV (97.5%), and SARS-CoV-2 (100%) [119–122].

The diaxxoPCR system integrates lyophilized cartridges and streamlined workflows to enable portable qPCR without cold-chain requirements, supporting pathogen identification, quantification, and genotyping with minimal operator input. It has been successfully applied to detection of SARS-CoV-2 variants, *Plasmodium* spp., and soil-transmitted helminths, achieving sensitivities above 97% and average turnaround times of approximately 34 minutes [123–125]. The MEDIC-PCR platform, a palm-sized real-time RT-qPCR device, uses a photothermal thermal cycler and disposable PCR chips heated by carbon-black thin films [126]. The Q3-Plus system is another compact platform performing real-time PCR on microchips with integrated heating and fluorescence detection, achieving performance comparable to standard qPCR. Its applications include tuberculosis screening, detection of *Trypanosoma cruzi* and *Plasmodium* spp., and simplified molecular diagnosis of leprosy and tuberculosis [48,54,114,115,127]. More broadly, this platform has also been successfully applied in healthcare settings for viral, bacterial, parasitic, and host genetic analyses, demonstrating high analytical sensitivity, reduced turnaround times, and suitability for decentralized testing in clinical, veterinary, food safety, and pharmacogenomic contexts [54,128,129].

Additional portable PCR systems include ultrafast devices, such as GeneSoC, capable of performing 50 amplification cycles in approximately 15 minutes, showing full concordance with conventional qPCR in the detection of Treponema pallidum from ulcer and lesion swab samples, although sensitivity decreases in more complex matrices such as saliva [130]. Approaches combining mobile qPCR platforms with multiplex detection have also expanded diagnostic coverage for respiratory and arboviral infections, as well as improved applications in environmental and wastewater surveillance [131].

However, beyond developed handheld and chip-based systems, cartridge-based automated PCR platforms have long represented a reference standard for decentralized molecular diagnostics. Among these, the GeneXpert system stands out as a fully automated, closed-cartridge nucleic acid amplification platform that integrates sample processing, nucleic acid extraction, amplification, and real-time detection into a single workflow. Extensive clinical validation has demonstrated its high diagnostic accuracy; for instance, the Xpert MTB/RIF assay achieved sensitivity and specificity of

100% and 97%, respectively, when compared with culture in large clinical cohorts [132]. In populations co-infected with HIV, where conventional smear microscopy often performs sub optimally, an integrative review reported sensitivities for tuberculosis detection range from approximately 68% to 100%, with specificities between 91.7% and 100% [133]. Importantly, the platform has also shown reliable performance in extrapulmonary specimens, supporting its application in more complex clinical presentations [134].

Similarly, the BIOFIRE® FILMARRAY® TORCH System exemplifies the utility of multiplex, sample-to-answer PCR platforms in acute care settings. This system employs self-contained pouches that require minimal hands-on time while enabling the simultaneous detection of multiple pathogens and resistance markers within approximately one hour. A prominent example is the FilmArray Meningitis/Encephalitis (ME) Panel, which processes a single cerebrospinal fluid sample through integrated nucleic acid extraction, reverse transcription for RNA viruses, and real-time PCR within a sealed workflow. Meta-analytical evidence from 13 studies reports a pooled sensitivity of approximately 90% (95% CI: 86–93%) and a specificity of 97% (95% CI: 94–99%) for the detection of common central nervous system pathogens, including viruses, bacteria, and *Cryptococcus* spp. [135]. Although reagent storage typically requires refrigeration (2–8°C), the system provides rapid, clinically actionable results that can substantially improve early therapeutic decision-making in suspected meningitis and encephalitis.

While these cartridge-based systems are less portable than handheld or lab-on-chip devices, they illustrate how automation, sealed consumables, and reduced operator dependence have substantially lowered technical barriers to molecular testing outside centralized laboratories. Together with newer portable qPCR instruments and microfluidic platforms, these technologies reflect the ongoing transition toward more compact, rapid, and decentralized molecular diagnostics.

In parallel, advances in microfluidics and PCR miniaturization continue to drive the development of highly integrated diagnostic devices with reduced reagent consumption and shorter assay times. Microfluidic chips enable PCR and isothermal amplification at nanoliter volumes, enhancing speed and multiplexing capacity while maintaining analytical sensitivity [25,26]. Reported applications include autonomous microfluidic systems for influenza A/B subtyping suitable for field deployment, self-heating platforms capable of detecting respiratory pathogens directly from whole blood, and ultrafast photothermal PCR devices achieving dozens of amplification cycles within minutes [136–138]. DNA-hydrogel microfluidic platforms have also been developed, enabling SARS-CoV-2 detection within 5–15 minutes [139]. Additional examples include fully automated systems for rapid protein quantification via qPCR and high-speed RT-qPCR devices for SARS-CoV-2 detection [140]. These innovations demonstrate the potential of microfluidics to support sensitive, rapid, and portable diagnostics suitable for field surveillance and primary care.

Automation further strengthens point-of-care molecular testing. Self-heating, multi-channel cassette systems enable automated processing of multiple samples with minimal user input [141]. Veterinary point-of-care platforms, such as those used for canine distemper virus detection, demonstrate the feasibility of integrated workflows from extraction to analysis with high sensitivity and specificity [142].

Digital PCR (dPCR) technologies represent a major analytical advance, providing absolute quantification without external standards and enabling highly sensitive detection of rare mutations, low viral loads, and minimal residual disease in oncology [15,16,143,144]. The high cost and

operational complexity of commercial dPCR systems have prompted the development of point-of-care adaptations. The lab-on-a-disc dPCR platform integrates centrifugal microfluidics, droplet generation, thermocycling, and fluorescence detection in a single device, providing sample-to-answer dPCR capable of detecting concentrations as low as 20 copies/μL [145]. The SPEED device, a smartphone-integrated handheld dPCR system weighing ~400 g, performs 45 cycles in ~50 minutes using reusable silicon chips with 26,448 partitions. It has demonstrated analytical precision comparable to commercial systems for detecting SARS-CoV-2, cancer-associated genes, and prenatal chromosomal alterations, highlighting its potential for cost-effective point-of-care diagnostics [146].

In summary, advances in molecular diagnostics have substantially expanded the capacity to perform rapid, precise, and portable testing outside centralized laboratories. Progress in reagent stabilization, simplified extraction, portable qPCR and dPCR devices, and integrated microfluidic have improved efficiency, reduced operational costs, and enhanced accessibility. These innovations not only support accurate detection of bacterial and viral pathogens in clinical and field settings but also lay the foundation for next-generation diagnostics in low-resource environments. Continued efforts toward integration, automation, and regulatory harmonization will be important to overcoming challenges and consolidating molecular diagnostics as a central tool for disease surveillance, screening, and clinical decision-making across contexts.

Table 3. Representative qPCR-based platforms for decentralized and point-of-care molecular diagnostics.

Platform	System type	Turnaround time	Sensitivity/Specificity	Cold-chain requirement	Integrated extraction	Main applications	Ref
Truenat™	A portable, battery-operated micro-PCR platform that performs real-time PCR at the point of care	~35–60 min (varies by assay)	HBV: Clinical sensitivity = 100 % (31/31 positives detected) and clinical specificity = 100 % (76/76 negatives correctly identified)	No (sealed, room-stable cartridges)	Automated nucleic-acid extraction is performed inside the TruePrep™ AUTO cartridge, which is part of the Truenat system	Quantitative HBV-DNA testing for early diagnosis and monitoring of antiviral therapy in resource-limited or remote settings; the platform is also suggested for potential quantification of HCV and HEV RNA in similar point-of-care contexts	[120]
diaxxoPCR	Portable qPCR with lyophilized cartridges	~34 min	verall sensitivities ≥97 % and specificities ≥ 94 % for the three targets. Exact values: T. *trichiura* 97.9 % / 94.2 %; *A. lumbricoide*s 100 % / 95.6 %; *S. stercoralis* 100 % / 100 %	No (lyophilized reagents)	Extraction is performed separately (Qiagen QIAamp kit with bead-beating) and is not built into the instrument	Species-specific population surveys for STH control programs; Individual patient diagnosis and case management; Monitoring efficacy in clinical trials (e.g., ALIVE trial); Potential multiplex testing for simultaneous detection of multiple helminths	[125]
PlasmoPod - diaxxoPCR	Cartridge-based RT-qPCR assay run on the portable diaxxoPCR instrument	≤30 min	Sensitivity/Specificity – Overall sensitivity 93.6 % (95 % CI 78.6–99.2) and specificity 100 % (95 % CI 85.2–100) versus laboratory RT-qPCR; in the asymptomatic cohort detection rate 81.4 %	No (lyophilized reagents)	Extraction is performed separately (Rapid Chelex-based nucleic-acid extraction)	Rapid, quantitative malaria diagnosis and high-sensitivity surveillance of asymptomatic carriers; decentralised testing in peripheral health facilities and elimination settings; replacement of antigen RDTs and microscopy for field-based monitoring.	[123]

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Platform	System type	Turnaround time	Sensitivity/Specificity	Cold-chain requirement	Integrated extraction	Main applications	Ref
MEDIC-PCR	Portable RT-qPCR device that utilizes a near-infrared LED-driven photothermal thermocycler and a roll-printed carbon black disposable PCR chip.	~15 min	Sensitivity ≈ 94 %; Specificity ≈ 98 % (diagnostic accuracy 97 %)	Not explicitly stated in the paper	Integrated extraction Direct RT-qPCR eliminates RNA extraction; lysis buffer is added directly to the sample	SARS-CoV-2	[126]
Q3-Plus	Portable, battery-operated qPCR instrument that integrates electrical temperature control, illumination and optical detection on a silicon-chip cartridge.	~40 min–1h	*Plasmodium spp.*: 95 % LOD of 1.7 parasites μL^{-1}; TB: Diagnostic performance (vs. GeneXpert): Sensitivity ≈ 90 %, Specificity ≈ 100 % (vs. culture): Sensitivity ≈ 100 %, Specificity ≈ 80 % (95 % CI 44.4–97.5 %); leprosy: sensitivity of 55 % and specificity of 87 %	Gelified (ready-to-use) reagents eliminate the need for a strict cold chain	No on-board DNA extraction	*Plasmodium spp*.; *Trypanosoma cruzi; Mycobacterium tuberculosis; Mycobacterium leprae*	[48, 54, 114, 115, 127]
GeneSoC	micro-fluidic, rapid quantitative PCR (qPCR) system	~15 min	Ulcer/lesion swabs: sensitivity 100 % and specificity ≈ 100 %; Saliva: Positive agreement 63.6 % with crude DNA and 84.8 % with purified DNA compared with conventional qPCR.	The study does not specify reagent storage conditions	The "direct-rapid" assay can be performed on ulcer/lesion swab suspensions without DNA extraction; for saliva, an extraction step is required.	Point-of-care testing for primary syphilis using ulcer/lesion swabs	[130]

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Platform	System type	Turnaround time	Sensitivity/Specificity	Cold-chain requirement	Integrated extraction	Main applications	Ref
GeneXpert	Automated, cartridge-based real-time PCR (molecular) platform that performs nucleic-acid amplification and detection in a single closed system.	~90–120 min (assay-dependent)	(vs. culture): 100 % sensitivity and 97 % specificity for *Mycobacterium tuberculosis* detection.	Cartridges are stable ambient temperature (2–28°C) and do not require refrigeration	Yes (complete sample-to-answer)	Rapid diagnosis of pulmonary (and extrapulmonary) tuberculosis, simultaneous detection of rifampicin resistance	[132]
BIOFIRE ® FILMARRAY® TORCH	Fully automated, moderately complex multiplex PCR platform	~60 min	FilmArray Panel for Meningitis/Encephalitis (ME): Pooled sensitivity ≈ 90% (95% CI 86-93%) and specificity ≈ 97% (95% CI 94-99%) in 13 studies.	Reagents must be stored refrigerated (2–8°C) according to manufacturer specification	Yes	apid detection of the most common CNS pathogens (7 viruses, 6 bacteria, Cryptococcus). spp.) in patients with suspected meningitis or encephalitis	[135]

6. Conclusions

qPCR has become one of the leading tools in molecular diagnostics, offering high sensitivity, specificity, and quantitative capability for nucleic acid detection. In particular, it plays a critical role in the detection, monitoring, and surveillance of infectious diseases, enabling rapid identification of pathogens and supporting effective clinical management and public health responses.

In recent years, technological advances, including portable platforms, simplified extraction methods, stabilized reagents, and ready-to-use systems, have substantially expanded the accessibility of the technique to low-infrastructure settings. The development of microfluidics, DNA hydrogels, and digital approaches reinforces a trend toward faster, more accessible, and operationally streamlined molecular diagnostics, including at the point of care.

Despite this progress, the clinical implementation of qPCR demands rigorous validation, standardization according to international guidelines (such as MIQE and STARD), and regulatory compliance. In well-equipped diagnostic laboratories, these requirements can generally be met through access to specialized instruments, controlled laboratory environments, and trained personnel. However, smaller laboratories and healthcare facilities, particularly in low-resource settings, often face significant challenges related to infrastructure, reagent storage, equipment costs, and technical expertise.

In this context, the development of simplified and portable molecular diagnostic platforms, including point-of-care technologies, is becoming increasingly important. By reducing operational complexity and minimizing dependence on specialized infrastructure, these systems have the potential to enable rapid molecular testing closer to the patient and to expand access to reliable diagnostics. Continued technological innovation, together with robust validation and regulatory harmonization, will further strengthen the role of qPCR as a key tool for infectious disease diagnostics and global health preparedness.

Author contributions

T.R.: Writing–original draft, Conceptualization, Visualization; B.G.M: Writing–original draft; G.T.C.M: Writing–original; C.L.T: Writing–original; L.F.B: Writing–original; ADTC: Conceptualization, Supervision, Validation, Writing – Review & Editing.

Use of Generative-AI tools declaration

The author declare they have used Artificial Intelligence (AI) tools in the creation of this article.

AI tools used: ChatGPT (OpenAI). The AI tools were used exclusively to support language review, text organization, and improvement of the clarity of manuscript sections. They were not used for generating original scientific content, data analysis, or interpretation of results. The use of AI tools was selectively applied throughout the manuscript, especially during the text review and editing stages.

Acknowledgments

This work was funded by grants from Presidência da Fundação Oswaldo Cruz/ Vice Presidência

de Pesquisa e Coleções Biológicas VPPCB/Fiocruz (VPPIS-004-FIO-22-2-64 and ICC-008-FIO-21-2-25-39) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq process 308384/2025-3). ADTC is a CNPq Productivity Fellow (level C). TR, BGM and CLT are fellowship holders at CAPES (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). GTCM and LFB are fellowship holders at Fiotec (Fundação para o Desenvolvimento Científico e Tecnológico em Saúde). The funders played no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest

The authors have no competing interests to declare.

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