Air and Water Borne Diseases

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Real-time PCR: Transforming Modern Diagnostics

Dr. Ivan Dimitrov*

Department of Molecular Biology, Sofia University, Sofia, Bulgaria

***Corresponding Author:** Dr. Ivan Dimitrov, Department of Molecular Biology, Sofia University, Sofia, Bulgaria, E-mail: idimitrov.molbio@su.bg

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Abstract

Real-time PCR technology is rapidly advancing diagnostics across diverse fields. New multiplex assays detect multiple diarrheal pathogens, human adenovirus, and fungal infections, offering superior sensitivity and specificity for timely clinical decisions. The technology also tackles antibiotic resistance by identifying resistance genes and aids malaria diagnosis with low parasite loads. Beyond clinical settings, it enhances water quality monitoring for Legionella, ensures food safety by detecting major foodborne pathogens, and enables early diagnosis of plant diseases. Real-time PCR proves crucial for complex challenges like tuberculosis detection and rifampicin resistance, and for rapid identification of zoonotic agents such as Brucella and Coxiella burnetii, thus providing comprehensive and efficient solutions for public and environmental health.

Keywords

Real-time PCR; qPCR; Multiplex assay; Pathogen detection; Antibiotic resistance; Fungal infections; Malaria; Legionella; Foodborne pathogens; Plant diseases; Tuberculosis; Zoonotic diseases

Introduction

Real-time Polymerase Chain Reaction, or PCR, stands as a cornerstone technology in modern molecular diagnostics, offering rapid, sensitive, and specific detection and quantification of nucleic acids. Its versatility makes it an indispensable tool across diverse fields, from clinical diagnostics and public health surveillance to food safety and agricultural management. This collection of studies highlights the broad impact and continuous innovation within real-time PCR applications, showcasing its power in tackling complex biological challenges and improving diagnostic capabilities.

One significant advancement involves designing a multiplex quantitative PCR assay capable of simultaneously detecting three prevalent bacterial pathogens responsible for diarrhea: E. coli, Shigella, and Salmonella. This innovation signifies a major step towards providing a faster, more efficient diagnostic method for these common infections, which could profoundly impact swift clinical decision-making due to its high accuracy [1].

Detecting human adenovirus with high accuracy and sensitivity remains critical for both effective clinical treatment and robust public health monitoring. Research in this area describes a novel real-time PCR assay specifically designed for this purpose, demonstrating superior sensitivity and specificity. This represents a crucial development, enabling the detection of the virus even when present in extremely low concentrations, which is vital for early intervention and control efforts [2].

The escalating global challenge of antibiotic resistance is being directly addressed through advanced PCR techniques. One paper delves into how real-time PCR can rapidly identify antimicrobial resistance genes along with the mobile genetic elements that facilitate

their transfer within Enterobacterales bacteria, commonly isolated in clinical environments. What this really means is a faster route to identifying drug-resistant strains, leading to more informed and targeted infection control strategies and better treatment choices [3].

Prompt and accurate diagnosis of fungal infections, particularly in vulnerable immunocompromised patients, is a major clinical priority. This research introduces an innovative multiplex real-time PCR assay capable of the simultaneous detection of three significant opportunistic fungal pathogens: Aspergillus fumigatus, Candida albicans, and Pneumocystis jirovecii. This integrated approach substantially streamlines diagnostic processes and ensures more timely therapeutic interventions [4].

Let's talk about malaria, a parasitic disease demanding precise and sensitive diagnostic tools. This article focuses on the validation of a real-time PCR method tailored for detecting and quantifying Plasmodium vivax and Plasmodium falciparum directly in clinical samples. Its high sensitivity and specificity are particularly advantageous for diagnosing malaria in cases with very low parasite loads, conditions where conventional methods frequently fail [5].

Effective public health hinges on diligent monitoring of water quality for dangerous bacteria such as Legionella. A recent study details the development of a novel duplex quantitative PCR assay, specifically designed to simultaneously detect and quantify both Legionella pneumophila and other non-pneumophila Legionella species present in environmental water samples. This marked improvement in detection capacity directly translates to enhanced surveillance and swifter responses to potential outbreaks, safeguarding public health [6].

Food safety remains an enduring global concern, necessitating rapid and comprehensive testing methods. This research describes a multiplex real-time PCR assay tailored for the simultaneous detection of five primary foodborne bacterial pathogens, including Salmonella, Escherichia coli O157:H7, Staphylococcus aureus, Listeria monocytogenes, and Vibrio parahaemolyticus. This advanced and rapid testing tool proves invaluable for guaranteeing food product safety, accelerating the identification of contamination, and supporting efficient outbreak investigations [7].

Plant diseases represent a significant threat to agricultural productivity, underscoring the importance of early and accurate detection. This paper introduces a highly sensitive real-time PCR assay specifically for detecting and quantifying Phytophthora ramorum, a serious plant pathogen. Such a tool is critical for early disease diagnosis within plant tissues, thereby enabling effective management strategies to curb its spread and protect vital agricultural resources [8].

The diagnosis of tuberculosis demands both speed and a comprehensive understanding of resistance profiles. This study presents an evaluation of a new multiplex real-time PCR assay uniquely designed to simultaneously detect the Mycobacterium tuberculosis complex and concurrently identify rifampicin resistance. This dual diagnostic capability is critically important for guiding effective treatment regimens and for implementing robust control measures against the dissemination of drug-resistant tuberculosis [9].

Zoonotic diseases, which can spread between animal populations and humans, continue to pose substantial public health threats. This research details the successful development of a duplex real-time PCR assay aimed at the rapid, simultaneous detection of Brucella species and Coxiella burnetii in clinical samples. What this means is a more efficient and streamlined diagnostic utility for two important zoonotic pathogens, significantly enhancing our capacity to respond to and effectively manage these complex infections [10].

Description

Real-time PCR assays are revolutionizing the speed and accuracy of diagnosing bacterial and viral infections. For instance, new multiplex quantitative PCR assays provide a faster, more efficient way to diagnose diarrheal infections caused by common pathogens like E. coli, Shigella, and Salmonella simultaneously. This capability is a game-changer for quick clinical decisions due to its high accuracy [1]. Similarly, detecting human adenovirus accurately and sensitively is crucial for both clinical treatment and public health monitoring, and a new real-time PCR assay boasts superior sensitivity and specificity, allowing detection even when present in very small amounts [2].

The critical issue of antibiotic resistance is being directly addressed through real-time PCR, which can rapidly identify antimicrobial resistance genes and the mobile genetic elements that carry them in Enterobacterales bacteria found in clinical settings. This means faster identification of drug-resistant strains, informing better infection control and treatment choices [3]. Beyond bacteria, diagnosing fungal infections quickly, especially in immunocompromised patients, is a significant challenge. Multiplex real-time PCR assays now simultaneously detect three important opportunistic fungal pathogens: Aspergillus fumigatus, Candida albicans, and Pneumocystis jirovecii. This comprehensive approach streamlines diagnostics and helps ensure timely intervention [4].

Real-time PCR methods are also crucial in diagnosing parasitic diseases like malaria. Validation of a real-time PCR method for detecting and quantifying Plasmodium vivax and Plasmodium falciparum in clinical samples offers high sensitivity and specificity, making it particularly useful for diagnosing malaria in cases with very low parasite loads that traditional methods often miss [5]. Furthermore, monitoring water quality for harmful bacteria like Legionella is vital for public health. A novel duplex quantitative PCR assay can simultaneously detect and quantify both Legionella pneumophila and other non-pneumophila Legionella species in environmental water samples. This improvement in detection capability means better surveillance and quicker responses to potential outbreaks [6].

Food safety remains a constant concern, demanding rapid and reliable detection of pathogens. Multiplex real-time PCR assays are now designed for the simultaneous detection of five major foodborne bacterial pathogens, including Salmonella, Escherichia coli O157:H7, Staphylococcus aureus, Listeria monocytogenes, and Vibrio parahaemolyticus. This comprehensive and rapid testing tool is invaluable for ensuring food safety, speeding up contamination detection, and aiding in outbreak investigations [7]. In agriculture, plant diseases can devastate crops, making early detection key. A highly sensitive real-time PCR assay for detecting and quantifying Phytophthora ramorum, a serious plant pathogen, helps diagnose the disease early in plant tissues, which is crucial for managing its spread and protecting agricultural resources [8].

Tuberculosis diagnosis requires both speed and comprehensive insights into drug resistance. A new multiplex real-time PCR assay simultaneously detects the Mycobacterium tuberculosis complex and identifies rifampicin resistance. This dual capability is incredibly important for guiding effective treatment and controlling the spread of drug-resistant tuberculosis [9]. Zoonotic diseases, those that spread between animals and humans, also pose significant health threats. The development of a duplex real-time PCR assay for rapidly detecting Brucella species and Coxiella burnetii simultaneously in clinical samples provides a more efficient diagnostic tool for two important zoonotic pathogens, improving our ability to respond to and manage these infections [10].

Conclusion

Recent advances in real-time PCR technology are transforming diagnostics across various fields, from clinical medicine to environmental monitoring and agriculture. For instance, new multiplex quantitative PCR assays are making it possible to detect multiple diarrheal bacterial pathogens like E. coli, Shigella, and Salmonella simultaneously, leading to faster and more efficient diagnoses for quick clinical decisions. The superior sensitivity and specificity of these real-time PCR assays are also vital for accurately detecting and quantifying viruses such as human adenovirus, even in minute amounts, which is crucial for public health.

Here's the thing: real-time PCR is proving invaluable in addressing antibiotic resistance by rapidly identifying resistance genes and mobile genetic elements in clinical isolates of Enterobacterales, informing better infection control and treatment. Moreover, diagnostics for fungal infections, especially in immunocompromised patients, are being streamlined with multiplex assays that detect pathogens like Aspergillus fumigatus, Candida albicans, and Pneumocystis jirovecii concurrently. Let's talk about malaria: real-time PCR methods offer high sensitivity for detecting and quantifying Plasmodium vivax and Plasmodium falciparum, crucial for low parasite loads that traditional methods often miss.

Beyond clinical applications, real-time PCR is enhancing public health and safety. Novel duplex quantitative PCR assays are now used to simultaneously detect and quantify Legionella pneumophila and other non-pneumophila species in environmental water samples, allowing for better surveillance and rapid responses to outbreaks. Food safety is another constant concern, with multiplex real-time PCR assays designed for the simultaneous detection of five major foodborne bacterial pathogens, including Salmonella and Listeria monocytogenes, significantly speeding up contamination detection. In agriculture, highly sensitive real-time PCR assays help diagnose plant diseases early, such as those caused by Phytophthora ramorum, protecting vital resources.

The technology also addresses complex diagnostic challenges, such as the simultaneous detection of Mycobacterium tuberculosis complex and rifampicin resistance, guiding effective treatment for drug-resistant tuberculosis. Finally, rapid detection of zoonotic diseases like Brucella species and Coxiella burnetii in clinical samples is now more efficient through duplex real-time PCR assays, improving our ability to respond to and manage these cross-species infections. This collective body of research underscores the versatility and critical importance of real-time PCR in modern diagnostics, offering faster, more accurate, and comprehensive solutions for a wide range of microbial and pathological threats.

References

1. Mahdi A-H, Fatemeh S, Reza R, Seyed MH, Mehdi Z et al.

(2023) Development of a multiplex real-time PCR assay for simultaneous detection of three major diarrheagenic bacterial pathogens. J Microbiol Methods 214:106516

2. Sarah TW, Kevin MD, Mark KA, Lauren KH, Janelle RR et al. (2022) Highly Sensitive and Specific Real-Time PCR for Detection and Quantification of Human Adenovirus. Viruses 14:1703

3. Agata TR, Karolina W, Mateusz G, Anna MK, Joanna KS et al. (2021) Detection of Antimicrobial Resistance Genes and Mobile Genetic Elements in Clinical Isolates of Enterobacterales by Real-Time PCR. Pathogens 10:1609

4. Ana MA-I, Maika B-F, Maria LG-T, Jose PC, Julio G-R et al. (2020) Multiplex Real-Time PCR for the Simultaneous Detection of Aspergillus fumigatus, Candida albicans, and Pneumocystis jirovecii. J Clin Microbiol 58:e01026-20

5. Laura GG, Carlos MS, Ana FR, María MM, Melina DM et al. (2019) Real-Time PCR for the Detection and Quantification of Plasmodium vivax and Plasmodium falciparum in Clinical Samples. Diagnostics 9:105

6. Li W, Xiaodong S, Linlin Y, Lingling Z, Chunxia L et al. (2023) A Novel Duplex Quantitative PCR Assay for Simultaneous Detection and Quantification of Legionella pneumophila and L. non-pneumophila in Environmental Water Samples. Pathogens 12:438

7. Saima K, Muhammad S, Muhammad TJ, Muhammad A, Muhammad W et al. (2022) Multiplex Real-Time PCR for Simultaneous Detection of Five Major Foodborne Pathogens: Salmonella, Escherichia coli O157:H7, Staphylococcus aureus, Listeria monocytogenes, and Vibrio parahaemolyticus. Foods 11:3810

8. María AR-R, Silvia PL-R, Beatriz VG-F, Ana IM, Elena MA-M et al. (2021) Development of a highly sensitive real-time PCR assay for detection and quantification of Phytophthora ramorum in plant tissues. Eur J Plant Pathol 161:863-875

9. Hossein N, Seyed MM, Amir MK, Vahab VM, Mohammad RZ et al. (2020) Evaluation of a novel multiplex real-time PCR assay for simultaneous detection of Mycobacterium tuberculosis complex and rifampicin resistance. J Forensic Leg Med 76:101997

10. Jae-Hoon K, Young-Ran J, Jun-Young K, Young-Jin C, Seung-Won K et al. (2023) Development of a Duplex Real-Time PCR Assay for Rapid Detection of Brucella spp. and Coxiella burnetii in Clinical Samples. Diagnostics 13:965