Journal of Virology & Antiviral Research

Opinion

A SCITECHNOL JOURNAL

Evaluation of N Gene, S Gene and ORF Based Real-Time RT-PCR Assays by Taqpathcovid 19 Combo Kit for Confirmation of SARS-Cov-2 Infection: An Observational Study

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Introduction

Coronaviruses (CoVs) represent a major group of viruses mostly affecting human beings through zoonotic transmission. In the past two decades, this is the third instance of the emergence of a novel coronavirus, after severe acute respiratory syndrome (SARS) in 2003 andMiddle East respiratory syndrome coronavirus (MERSCoV) in 2012 [1,2].

COVID-19 caused by SARS-CoV-2 has spread to most countries across the globe including India [3]. Laboratory diagnosis depends on the detection of viral RNA in nasopharyngeal and/or oropharyngeal swabs using real-time reverse transcription polymerase chain reaction (qRT-PCR) [4]. In India, based on guidelines of the Indian Council of Medical Research-National Institute of Virology (ICMR-NIV) at Pune, it is a one-step strategy for the diagnosis of COVID-19 using qRT-PCR. Primers and probes by TaqPath COVID-19 Combo Kit. TaqPath COVID-19 Combo Kit contains the assays and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory and bronchoalveolar lavage (BAL) specimens during the acute phase of infection. Taq Path COVID-19 Control Kit contains an external positive RNA control that is run on each plate. The RNA control contains in vitro transcribed (IVT) RNA, which is specific to N, S, and ORF1ab regions of SARS-CoV-2. Complete workflow enabling clinical laboratories to evaluate 94 specimens in under 3 hours, or 382 specimens in under 6.5 hours, with a single King Fisher purification system and Applied Biosystems real-time PCR system combination (when additional approved instruments are utilized, throughput can increase significantly) Targeted specificity to 100% of currently available complete genomes for SARS-CoV-2Assay targets spike (S) protein and nucleocapsid (N) protein regions having higher specificity and exhibiting lower risk for mutation. COVID-19 Interpretive Software, which automatically converts genetic analysis data into diagnosis, helping reduce risk of user interpretation error. Recently, the Rutgers Clinical Genomics Laboratory developed an RT-PCR assay (TaqPath COVID-19 Combo kit) that uses self-collected saliva samples, which is quicker and less painful than other sample collection [5,6].

Coronaviruses possess the largest genomes (26.4–31.7 kb) among all known RNA viruses, with G + C contents varying from 32% to 43%. Variable numbers of small ORFs are present between the various conserved genes (ORF1ab, spike, envelope, membrane and nucleocapsid) and, downstream to the nucleocapsid gene in different coronavirus lineages. The viral genome contains distinctive features, including a unique N-terminal fragment within the spike protein. Genes for the major structural proteins in all coronaviruses occur in the 5′–3′ order as S, E, M, and N [7].

In an April, 2020 bioRxiv paper, Brown et al., from the Great Ormond Street Hospital (NHS, London, UK), compared One Step Prime Script III RT-qPCR Mix to Quanti Fast Multiplex PCR +R master mix (Qiagen), TaqMan Fast Virus 1-Step Master Mix (Thermo), and TaqPath One Step RT-qPCR Master Mix (Thermo) in order to test compatibility and sensitivity for SARS-CoV-2 detection. In the initial experiment to test the limit of detection, One Step Prime Script III RT-qPCR Mix showed superior sensitivity by consistently detecting 1 viral copy/µl with all three primer/probe assays. The authors showed that the lower limit of detection for this kit is 10 to 100 times greater than the competitors' solutions.

The Prime Script III one-step RT-qPCR experiment can be run in less than 1 hour (33% faster than Quantifast Multiplex PCR +R master mix), meaning even faster detection of SARS-CoV-2 infection. Faster reaction speeds will help increase the throughput for COVID-19 diagnostic labs. Brown et al. conclusively showed that the PrimeScript III One Step RT-qPCR Mix outperforms other one-step RT-qPCR solutions in both sensitivity and speed. In a time where screening for SARS-CoV-2 infection is of utmost importance, the efficiency and rapidity of One Step PrimeScript III RT-qPCR Mix plays a critical role in the optimal management and control of this pandemic [8].

In this context, we analyzed the qRT-PCR data of 752 SARS-CoV-2- cases tested at VRDL MGMMC INDORE to find out the sensitivity of N gene, S gene and ORF-based assays to confirm SARS-CoV-2infection. In the 752 cases 94 were positive by N gene, S gene and ORF [cycle threshold (Ct) values forall three geneswere ≤ 35]. The modified algorithm involving three-stage assay strategy islead to a reduction in the number of reactions required for a positive sample. Instead of the four reactions required for a positive sample (screening, internal control and 2 confirmatory assays).

Conflicts of Interest

None

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Received: October 18, 2021 **Accepted:** November 16, 2021 **Published:** November 30, 2021

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