Int.J.Curr.Microbiol.App.Sci (2022) *11*(01): 15-19

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 11 Number 01 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1101.003

Evaluate the Four Various Commercial RT-PCR Kits for COVID-19

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Keywords

Coronavirus Disease-2019, Nucleic acid, Polymerase chain reaction

Article Info

Received:
08 December 2021
Accepted:
31 December 2021
Available Online:
10 January 2022

A B S T R A C T

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is a emerged human disease during last month of 2019 in china. The ongoing Coronavirus disease-2019, many diagnostic test available (i.e., TrueNat, Rapid, CBNAAT, etc), amongst them Real Time Polymerase Chain Reaction (RT-PCR) a gold standard and most use technique for the diagnosis of COVID-19. Aim: To evaluate the four various commercial COVID-19 RT-PCR diagnostic kits. The prospective observational study conducted in the Department of Microbiology, Autonomous State Medical College, Firozabad, India, from march to August 2021. A total of 85 sample included in this study. Data should be analysis and maintain in Microsoft Excel version 2007. All 85 respiratory sample tested to four RT-PCR kits. Out of 85, 9 (10.6%) and 73 (85.9%) were negative with all kits. 3 were inconclusive with all four kits. A sensitivity of all four kits at least 100% and a specificity of 99%. Conclusion: The assay exhibited that all four kits high specificity and sensitivity for SARS-CoV-2 and good analytical performance using genes and/or virus.

Introduction

Human Coronaviruses (HCoV's) are members of the subfamily Coronavirinae in the family of Coronaviridae in the order of Nidovirales (Chen, *et al.*, 2020). In December 2019, a novel Human Coronavirus (HCoV) associated to Severe Acute Respiratory Syndrome (SARS) was discovered in the city of Wuhan, Hubei province, China and was later named SARS-CoV-2 (Lu, *et al.*, 2020; Gorbalenya, *et al.*, 20020). In March 11, 2020, World Health Organization (WHO) labeled it pandemic and urge that the most effective way to prevent infection and save lives is breaking the chains of transmission, and do that escalation of covid-19 testing urgently required (4).

Exemplifying the critical need for accurate and rapid diagnostic assays to prompt clinical and public health interventions. In response, several molecular assays (that is, quantitative reverse transcription-PCR (RT-qPCR)) were developed to detect COVID-

19 cases (5). The SARS-CoV-2 pandemic poses an enormous burden on society, economic and healthcare systems worldwide, and various measures are being taken to control its spread. Many of these measures critically depend on the timely and accurate diagnosis of virus-infected individuals. Real-time reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive and specific assay and therefore preferred (6,7).

Along with the advancement in medical diagnosis, nucleic acid detection-based approaches have become a rapid and reliable technology for viral detection. Among nucleic acid tests, the polymerase chain reaction (PCR) method is considered as the 'gold standard' for the detection of some viruses and is characterized by rapid detection, high sensitivity, and specificity. As such, real-time reverse transcriptase-PCR (RTPCR) is of great interest today for the detection of SARS-CoV-2 due to its benefits as a specific and simple qualitative assay (8).

Several COVID-19 RT-PCR diagnostic kits are commercially available, The Centers for Disease Control and Prevention (CDC) has designed a SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel to minimize the chance of false-positive results (9).

In accordance, the negative template control (NTC) sample should be negative, showing no fluorescence growth curves that cross the threshold line. The occurrence of false positive with one or more of the primer and probe NTC reactions is indicative of sample contamination. Importantly, the internal control should be included to help identify the specimens containing substances that may interfere with the extraction of nucleic acid and PCR amplification. Because of the several risks to patients in the event of a false-positive result, all clinical laboratories using this test must follow the standard confirmatory testing and reporting guidelines based on their proper public health authorities (8). The aim of the present to evaluate the four various commercial RT-PCR kits for COVID-19.

Materials and Methods

The prospective observational study conducted in the Department of Microbiology, Autonomous State Medical College, Firozabad, India, from July to September 2021. A total 85 nasopharyngeal and oropharyngeal sample considered. A four COVID-19 commercial RT-PCR kits use for the test the nasopharyngeal and oropharyngeal samples.

Inclusion Criteria

Nasopharyngeal and oropharyngeal swab samples with proper temperature and identification were included in this study.

Exclusion Criteria

Those sample with not proper identification mark and not transport with favorable temperature excluded from this study.

Study Procedure

RNA Extraction

RNA was extracted from clinical samples (oropharyngeal and *nasopharyngeal*) collected in the Viral transport Media Vials (VTM). The oropharyngeal and *naso*pharyngeal VTM vials were opened in biosafety cabinet class-II and 200 µl of the VTM was mix with 1 mL lysis buffer further processed for viral nucleic acid extraction by a GeneMag viral DNA/RNA Kit (Genetix Biotech Asia). To avoid RNA degradation, the study was planned in such a manner that the entire experiment was completed in 24 hours.

Real-time reverse-transcription PCR

All of the four included kits are provided by the state government depot to the lab for COVID-19 testing. The all kits transport and storage at -20°C according to company storage and transport protocol. The target genes for each RT-PCR kit were available in the assay documentation (Table/Fig-1).

All PCRs were run on a CFX96 Real Time System (BIO RAD) and performed according to the manufacturer's instructions for use.

Data analysis

The collected data were entered into Microsoft Excel 2007 and analyzed using Microsoft Excel. Test positivity was represented as a proportion with a 95% confidence interval.

Results and Discussion

All samples that were initially screened for the internal control gene and positive samples were confirmed by detection of specific RdRP or ORF1ab gene. The cut-off threshold (Ct value) of each sample was recorded. All the samples were tested with all four kits. The positive and negative samples were identified by kits instructions (Table-2). Out of 85 samples, 9 (10.6%) were positive, 73 (85.9%) negative and 3 (3.5%) inconclusive from all four commercial kits selected for study.

All four of the nucleic acid detection kits evaluated in this study could detect SARS-CoV-2 in respiratory samples (known to be positive or negative for SARS-CoV-2) at a sensitivity of at least 98.9% and a specificity of 100%, when compared with the results of the RT-PCR assay of this study (Table-3).

In this study we compare four commercial RT-PCR kits. According to manufacturer interaction, all RT-PCR kits performed satisfactorily regarding PCR efficiency.

In present study included all commercially RT-PCR kits can be used for routine diagnostics of symptomatic or asymptomatic COVID-19 diagnosis. Molecular methods are more rapid, accurate, and sensitive for virus detection than culture methods. In this study, we established a consensus method using molecular tools for detecting SARS-CoV-2 that did not require the use of EUA-approved reagents or kits. Early diagnosis of SARS-CoV-2-infected patients is essential for controlling the dynamics of the COVID-19 pandemic.

Amidst the ongoing COVID-19 pandemic, the World Health Organization has globally emphasized the importance of the molecular diagnosis of SARS CoV-2 to limit the spread as well as to appropriately treat those patients who have a serious infection (10).

The recommended test for diagnosis of COVID-19 is RT-PCR, and to conduct this test, a fully functional molecular laboratory is required equipped with specialized equipment like biosafety cabinets, automated RNA extractors, a Real-time PCR machine, and trained manpower to process the samples while ensuring biosafety and biosecurity and give the accurate and timely results (11).

In present study, 100% sensitivity found in all four kits, it's also found in another study Kasteren P B *et al.,* (12). The RT-PCR assay showed excellent specificity and sensitivity.

Present study showed that the all four kits are highly specific for both of COVID-19 target genes. Similar result were seen by Chung S Y *et al.,* (13).

In present study, out of 85 samples, 3 (3.5%) were inconclusive. Similar results were also seen in Chung S Y *et al.,* (13). The sensitivity of COVID-19 RT-PCR diagnostic kits is not only related to the types, sampling, transportation, and preservation of the viral specimens but also to the quality of the kits, which is considered the most important factor (14).

All the four commercial RT-PCR COVID-19 kits gives satisfactory result, we don't found any difference between positive and negative sample.

Table.1 Kits for RT-PCR based detection of SARS-COV-2.

Manufacturer	Storage condition	Regulatory status	Target gene(s)	Catalog number	Country
Genetix Biotech Asia Pvt. Ltd.	-20℃	CE-IVD	RdRp	CovizONE-100	India
General Biologicals Corporation	≤-15℃	CE-IVD	ORF1ab (RdRp)	4PCO052E	Taiwan
POCT Services Pvt. Ltd.	-20℃	CE-IVD	ORF1ab	COVIDM96PS	India
GCC Biotech Pvt Ltd.	-20℃	CE-IVD	ORF1ab	DG6001-OSTM-200R	India

Table.2 Overview of RT-PCR Kits.

Kits	Total Reaction volume (µL)	Extracted RNA volume (µL)	Master Mix volume (µL)	Reaction Time (hours)	Target gene CT value
COVISure	20	5	15	1:30	≤36
GB SARS	25	10	15	1:40	<37
Q-line Molecular	20	5	15	1:35	<40
DiAGSure	25.5	10	15.5	1:00	≤36

Table.3 Comparison of sensitivity and specificity of SARS-CoV-2 detection in respiratory samples.

Variables		COVISure			GB SARS			Q-line Molecular			DiAGSure		
		Pos	Neg	Inc	Pos	Neg	Inc	Pos	Neg	Inc	Pos	Neg	Inc
Positive	9	9			9			9			9		
Negative	73		73			73			73			73	
Inc	3			3			3			3			3
Sensitivity (%)		100%			100%			100%			100%		
Specificity (%)		98.9%			98.9%			98.9%			98.9%		

We evaluated the four commercial COVID-19 RT-PCR kits. The assay exhibited that all four kits high specificity and sensitivity for SARS-CoV-2 and good analytical performance using genes and/or virus. The all four RT-PCR kits are daily used in laboratories for diagnosis of COVID-19.

Conflict of Interests

The authors declare that there is no conflict of interests.

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How to cite this article:

Bitesh Kumar and Sugandh Rathore. 2022. Evaluate the Four Various Commercial RT-PCR Kits for COVID-19. *Int.J.Curr.Microbiol.App.Sci.* 11(01): 15-19. doi: https://doi.org/10.20546/ijcmas.2022.1101.003