Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 1993-2002

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 02 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.254

Comparison of the Diagnostic Efficacy among Three Commercially Available RT-PCR Kits for Novel SARS COV-2 at a Tertiary Care Centre Testing Facility

T. Pooja Preethi, P. Shanmugapriya* and Eunice Swarna Jacob

Department of Microbiology Thanjavur Medical College, Tamil Nadu, India

**Corresponding author*

Keywords

Virus-infected individuals, SARS-CoV-2 pandemic, COVID-19 RT-PCR kits

Article Info

Accepted:
18 February 2021
Available Online:
10 March 2021

A B S T R A C T

NOVEL SARS COV 2 is a major pandemic that is threatening the world with its varying clinical presentation. Detection of the SARS COV 2 earlier plays a major role in treating & preventing the spread of infection. The aim of this study is to compare the diagnostic efficacy between three different Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test kits in detecting NOVEL SARS COV -2 Infection and analyzing the efficiency of each kit in diagnosing the infection. Study period: January 2021 – February 2021Type of study: Prospective study. Place of study: Covid Care Centre, Tertiary Care Testing Facility Specimens collected: Nasopharngeal swabs from 20 RT PCR confirmed positive patients admitted in COVID isolation ward and nasopharyngeal swabs from 100 Hospitalized NON COVID patients at Tertiary Care Testing Facility were collected. Three different RT-PCR diagnostic kits (REAL STAR®SARS-CoV-2 RTPCR KIT from ALTONA DIAGNOSTICS, LABGUNTM COVID 19 EXOFASTRTPCR KIT from LABGENOMICS, STANDARD M nCoV Real time detection kit from SD BIOSENSOR) were used for detection of SARS COV 2 infection. Results from three kits were compared. A total of 120 Nasopharyngeal swab samples were collected from patients and enrolled in this study. 20 Nasopharyngeal swabs from RTPCR confirmed positive patients and 100 nasopharyngeal swabs from hospitalised NON COVID patients were taken as standard reference. All samples were tested using three RTPCR diagnostic test kits. Results from each kit were compared by Sensitivity, Specificity, PPV, NPV and kappa value. The Sensitivity, Specificity, PPV, NPV, kappa values for LABGUNTM COVID 19 EXOFAST RTPCR KIT were 90%,100%, 100%, 98%, 0.937 respectively, Sensitivity, Specificity, PPV, NPV, kappa values for STANDARD M nCoV Real time detection kit were 85%,100%, 100%, 97%, 90% respectively, and the Sensitivity, Specificity, PPV, NPV, kappa values for REAL STAR® SARS-CoV-2 RTPCR KIT were 95%, 100%, 100%, 99%, 0.969 respectively. Ct values of all positive cases detected were with in the limits as per each kit protocol. All the three RT-PCR test kits used have shown to provide valid results in detecting the NOVEL SARS COV 2 infection. The diagnostic efficacy of all three kits is almost equal in detecting the NOVEL SARS COV 2. All three kits were highly specific and almost nearly sensitive when compared among them in detecting the virus.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus emerged in the human population in the final months of 2019 and has spread across the globe.

The SARS-CoV-2 pandemic possesses 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. Whereas many COVID-19 RT-PCR kits are currently commercially available, implementation of accurate diagnostic kits is needed for identifying the infection.

Coronaviruses are positive-stranded RNA viruses that express their replication and transcription complex, including their RNA-dependent RNA polymerase (RdRp), from a single, large open reading frame referred to as ORF1ab.

The coronavirus structural proteins, including the envelope (E), nucleocapsid (N), and spike (S) proteins, are expressed via the production of subgenomic messenger RNAs, which during certain stages of the replication cycle far outnumber (anti)genomic RNAs.

The ORF1ab/RdRp, E, N, and S genes are the targets most frequently used for SARS-CoV-2 detection by RT-PCR.

In this study three different RT-PCR diagnostic kits are used to identify these genes and to compare and analyse the efficacy of each kit in detecting the infection.

Materials and Methods

Participants

A cross sectional study was conducted among 20 Covid 19 positive patients and 100 NON COVID patients admitted at Tertiary Care Testing Facility during the period of January 2021- February 2021 for the comparison of the diagnostic efficacy among three different RT PCR test kits. The inclusion criteria included Nasopharyngeal swab positive Covid 19 patients admitted in Covid Isolation ward within one day of admission. For the control group samples were collected from 100 hospitalized Non Covid patients. Patients those who already recovered from SARS COV 2 infection and those who were only radiologically i.e., CT positive (CORADS) patients were excluded from the study.

Sample collection

The samples were collected under strict aseptic precautions in accordance with the current Institutional Standard Operating Procedures (SOP).Using Sterile Flexible Nasopharyngeal Swab, Trained Health Care Professional after wearing personal protective equipment passed the Swab through the patient's nostril until the posterior nasopharynx was reached. It was left for 10 seconds in place to absorb secretions and removed slowly while rotating. The Swab was put in 3 ml sterile Viral Transport Medium (VTM) and sealed securely and taken to lab for processing.

Test kits used

Three different RT-PCR diagnostic test kits were used to detect NOVEL SARS COV-2 infection from the Nasopharyngeal swabs. Three kits used were Real Star®SARS-CoV-2 RTPCR Kit from Altona Diagnostics, Labgun™ Covid 19 Exofastrtpcr Kit from Labgenomics, Standard M nCoV Real time

detection kit from SD Biosensor. Basic information of all three kits is described below (Table 1–3).

Table.1 Labgun™ Covid-19 ExoFast RT-PCR Kit (Labgenomics)

Genes detected		Master mix volume	Nucleic acid volume	Flurophores used	Amplification Cycles & Temperature	Total Cycles	Result Analysis	
RdRp	N gene	15µl	5µl	RdRp-FAM N gene-Cy5 IC-HEX	Cycles- 32 Temp-60^0 C	44	Positive Ct value ≤ 30	Negative Ct value ≥ 30 or NA

Table.2 Standard M nCoV Real-time Detection kit (SD Biosensor)

Genes detected		Master mix volume	Nucleic acid volume	Flurophores used	Amplification Cycles & Temperature	Total Cycles	Result Analysis	
ORF1 ab (RdRp)	E gene	20.5µl	10µl	RdRp-FAM E gene- HEX IC-Cy5	CYCLES- 40 TEMP 95^0 C – 5 SECS 60^0 C – 40 SECS	47	POSITIVE Ct value ≤ 36	NEGATIVE Ct value ≥ 36 or NA

Table.3 RealStar® SARS-COV-2 RT-PCR Kit (Altona Diagnostics)

Genes detected		Master mix volume	Nucleic acid volume	Flurophores used	Amplification Cycles & Temperature	Total Cycles	Result Analysis	
S gene	E gene	20µl	10µl	Egene -FAM IC- HEX S gene-Cy5	Cycles- 45 Temp 95^0 C – 15 Secs 55^0 C – 45 Secs 72^0C- 15 Secs	47	Positive Ct value ≤ 35	Negative Ct value ≥ 35 or NA

Sample processing and nucleic acid extraction

Nasopharyngeal swabs collected from the patients were taken to the laboratory under sterile conditions. Both positive and negative samples were labeled with specific laboratory numbers. Details about the patients like the name & the contact details were not known to the laboratory personnel who performed the processing and nucleic acid extraction.

Nasopharyngeal swabs from both set of samples were opened in a biosafety cabinet and were treated with lysis buffer to inactivate the SARS-COV 2 Virus. Using Helini Automated Nucleic Acid Extraction system Viral RNA was extracted from 200ml of the samples strictly following manufacturer's instructions. Internal control was added only during master mix preparation and not during nucleic acid extraction.

RTPCR workflow using each kit

For detection of SARS-CoV 2 gene fragments from the samples three SARS-COV 2 detection kits approved by Indian Medical Council were used. Programming and master mix preparation for RT-PCR analysis by CFX96 ™ detection instrument (BIORAD) were done for all three kits based on the manufacturers protocol as follows.

Table.4 Labgun™ Covid-19 ExoFast RT-PCR Kit (Labgenomics) Programming is done as follows

Reaction	Temperature	Time	cycles
Reverse transcription	50° C	5 min	1
Denaturation	95° C	1 min	1
Pre amplification	95 ° C	1 sec	10
	60 ° C	1 sec	
Amplification	95 ° C	1 sec	32
	60 °C*	1 sec	

*indicates dye acquisition/fluorescence detection

Table.5 Fluorescence detectors

Target	Fluorophore
RdRp gene	FAM
N gene	Cy5
Internal Control	HEX

Table.6 Master mix preparation for 1 reaction

Components	Volume (µl)
5x ExoFast 1step Buffer	4
ExoFast 1step Enzyme	2
Assay	4
RNase free water	5
Template RNA/Control	5
Total volume	**20**

Positive control: instead of template RNA PC in the kit is used
Negative control: instead of template RNA RNase free water is used

Basis of result analysis

The test results were analysed based on the cycle threshold (Ct) values and the amplification curves separately for each kit as per the manufacturers protocol

Labgun Exofast

Cycle threshold values between 10 – 30 with a S- shaped amplification curve is considered positive. Cycle threshold values <10 &> 30 with no amplification curve is considered negative.

SDBIO Sensor

Cycle threshold values ≤36 with a S- shaped amplification curve is considered positive. Cycle threshold values ≥36 with no amplification curve is considered negative.

Table.7 Standard™ M nCoV Real-time Detection kit(SD Biosensor) Programming is done as follows

Reaction	Temperature	Time	Cycles
Reverse transcription	50° C	15 mins	1
Initial Denaturation	95° C	3 mins	1
Pre amplification	95° C	5 secs	5
	60° C	40 secs	
Amplification	95° C	5 secs	40
	60° C*	40 secs	

*indicates dye acquisition/fluorescence detection

Table.8 Fluorescence detectors

Target	Fluorophore
ORF1ab(RdRp) gene	FAM
E gene	HEX
Internal Control	Cy5

Table.9 Master mix preparation for 1 reaction

Components	Volume(µl)
2019-nCoV Reaction Solution	14
RTase mix	6
Internal control A	0.5
Sample/Control	10
Total volume	**30.5**

Positive control: instead of template RNA PC in the kit is used
Negative control: instead of template RNA NC in the kit is used

Altona Realstar

Cycle threshold values ≤35with a S- shaped amplification curve is considered positive. Cycle threshold values ≥35 with no amplification curve is considered negative.

Table.10 RealStar® SARS-COV-2 RT-PCR KIT (Altona Diagnostics) Programming is done as follows

Reaction	Temperature	Time	Cycles
Reverse transcription	55° C	20mins	1
Denaturation	95° C	2mins	1
Amplification	95 ° C	15 secs	45
	55 ° C*	45 secs	
	72 ° C	15 secs	

*indicates dye acquisition/fluorescence detection

Table.11 Fluorescence Detectors

Target	Fluorophore
E gene	FAM
S gene	Cy5
Internal Control	HEX

Table.12 Master Mix Preparation for 1 reaction

Components	Volume(µl)
Master A	5
Master B	15
Sample/Control	10
Total volume	**30**

Positive control: instead of template RNA PC in the kit is used
Negative control: instead of template RNA NC in the kit is used

Statistical analysis

The RT-PCR data for both SARS-CoV 2 Positive and Negative patients run by all three kits are presented qualitatively. Cycle threshold values were analyzed separately for each test kit. To detect the diagnostic efficacy of all three kits Sensitivity, Specificity, PPV, NPV, kappa value were calculated separately for all three kits and the results were analyzed.

Results and Discussion

Nasopharyngeal swab from 20 COVID positive patients and 100 NON COVID patients which was taken as standard reference, were analyzed using all three diagnostic kits. All the patients were in the age group of 18-60 years. Out of 20 positive patients 11 were men (55%), 9 were female (45%). Mean age of positive male patients was 35 and positive female patients was 42.Among the control group 66 were male (66%), 44 were female (44%). Mean age of male patients was 26 and female patients was 36. Out of 20 Positive cases, 2 were detected to be false negative by Labgun Exofast kit, 3 were detected to be false negative by SD Biosensor kit, and 1 was false negative by ALTONA REALSTAR kit. There were no false positives detected by any of the kits. All the samples run were found to be valid according to each manufacturer's protocol.

The Sensitivity, Specificity, PPV, NPV, kappa values for LABGUN EXOFAST PCR kit were 0.90,1.000, 1.000, 0.981, 0.937 respectively, Sensitivity, Specificity, PPV, NPV, kappa values for SD BIOSENSOR PCR kit were 0.851,1.000, 1.000, 0.972, 0.900 respectively, and the Sensitivity, Specificity, PPV, NPV, kappa values for ALTONA REALSTAR PCR kit were 0.953, 1.000, 1.000, 0.991, 0.969 respectively. Ct values for positive samples in LABGUN EXOFAST PCR kit was in the range of 10-27 were the median (IQR) Ct values of the RDRP and N genes were 20 (15e27) and 22 (14e28), SD BIOSENSOR it was detected within 22-32 were the median (IQR) Ct values of the RDRP and E genes were 24 (14e32) and 25 (15e30), and for ALTONA REAL STAR it was within 24- 30 were the median (IQR) Ct values of the E and S genes were22 (14e30) and 24 (16e30).The results are shown in the following tables 16-21.

Diagnosis of SARS CoV 2 infection during this pandemic serves to be of prime importance in treating and preventing the spread of infection. There are various RT PCR diagnostic kits manufactured by various manufacturers for the rapid detection of SARS COV2 infection. Because of rapid development of such Nucleic acid detection kits in a very short period of time the diagnostic efficacy may vary among different KITS that are developed.

In this study we compared three RT-PCR diagnostic kits approved for testing on the nasopharyngeal samples collected from the patients. All three kits used for detection had same Specificity (100%) and Positive Predictive Value (100%). There were slight variations in the Sensitivity, Negative Predictive Value and kappa values among all three kits. Labgun™ Covid-19 ExoFast RT-PCR Kit (Labgenomics) had a Sensitivity of 90%, Negative Predictive Value of 98%,and kappa value 0.939 whereas Real Star® SARS-COV-2 RT-PCR Kit (Altona Diagnostics) showed a Sensitivity of 95%, Negative Predictive Value of 99%, and kappa value 0.969. Standard™ M nCoV Real-time Detection kit (SD Biosensor) showed a Sensitivity of 85%, Negative Predictive Value of 97% and kappa 0.904. All the three kits gave valid result. There was no sample for which the result was found to be inconclusive or invalid. The minor differences in Sensitivity may be due to difference among the target gene detection between three kits used. The clinical samples with low viral load i.e., Ct value > 30 (SD Biosensor, ALTONA) and> 25 (Labgun Exofast) may also account for their low detectability rate. If the minimum detection limit cannot reach the detection concentration, weakly positive samples might show a false-negative result.

The analysis was done using a small number of clinical samples, and only one kit represented each manufacturer hence All three kits used (Real Star® SARS-CoV-2 RTPCR KIT from Altona Diagnostics, LABGUN™ Covid 19 Exofast RTPCR KIT from Labgenomics, Standard M nCoV Real time detection kit from SD BIOSENSOR)do not represent the overall performance of the manufacturer. In summary all the three RT-PCR kits used has shown to provide valid results in detecting the SARS COV 2 infection. The diagnostic efficacy of all three kits are almost equal in detecting the SARS COV 2.

Table.13 Labgun™ Covid-19 ExoFast RT-PCR Kit (Labgenomics) Result Interpretation

PC	NC	RdRPFAM	N Gene Cy5	IC HEX	Interpretation
+	-	+	+	+/-	SARS-CoV-2 Positive
+	-	+	-	+/-	
+	-	-	+	+/-	
+	-	-	-	+	Negative
+	-	-	-	-	Invalid Result/Retest
+	+	+/-	+/-	+/-	
-	+	+/-	+/-	+/-	
-	-	+/-	+/-	+/-	

Table.14 Standard™ M nCoV Real-time Detection kit (SD Biosensor)

ORF1ab(RdRp) Gene FAM	E gene HEX	IC Cy5	INTERPRETATION
+	+	+	SARS-COV2 POSITIVE
+	-	+	INCONCLUSIVE
-	+	+	NEAR-SOURCE POSITIVE
-	-	+	NEGATIVE
-	-	-	INVALID

Table.15 RealStar® SARS-COV-2 RT-PCR KIT (Altona Diagnostics)

E gene FAM	S gene Cy5	IC HEX	Interpretation
+	+	+	SARS-COV2 Positive
-	+	+	
+	-	+	Negative
-	-	+	
-	-	-	Invalid

Table.16 Labgun™ Covid-19 ExoFast RT-PCR Kit (Labgenomics)

NPS RT-PCR			
	Disease positive	Disease negative	Total
Test positive	18	-	18
Test negative	2	100	102
Total	20	100	120

Table.17 Standard™ M nCoV Real-time Detection kit (SD Biosensor)

NPS RT-PCR			
	Disease positive	Disease negative	Total
Test positive	17	-	17
Test negative	3	100	103
Total	20	100	120

Table.18 RealStar® SARS-COV-2 RT-PCR KIT (Altona Diagnostics)

NPS RT-PCR			
	Disease positive	Disease negative	Total
Test positive	19	-	19
Test negative	1	100	101
Total	20	100	120

Table.19 Median Ct value Labgun Exofast

Type of gene	Ct value NPS by RT-PCR
	Median10-27
N gene	22 (14e28),
RDRP gene	20 (15e27)

Table.20 Median Ct value SD Biosensor

Type of gene	Ct value NPS by RT-PCR
	Median22-32
E gene	25 (15e30)
RDRP gene	24 (14e32)

Table.21 Median Ct value Altona Realstar

Type of gene	Ct value NPS by RT-PCR
	Median24-30
E gene	22 (14e30)
S gene	24 (16e30)

This study helps to find the detection performance and diagnostic efficacy of all three SARS COV 2 Nucleic acid and Gene Detection RTPCR kits. All three kits were highly specific and almost nearly Sensitive when compared among them in detecting the virus. We assert that all three kits can be used as Diagnostic tests for detection of NOVEL SARS COV 2.

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How to cite this article:

Pooja Preethi, T., P. Shanmugapriya and Eunice Swarna Jacob. 2021. Comparison of the Diagnostic Efficacy among Three Commercially Available RT-PCR Kits for Novel Sars Cov-2 at a Tertiary Care Centre Testing Facility. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 1993-2002.
doi: https://doi.org/10.20546/ijcmas.2021.1003.254