

Detection of SARS-CoV-2 in Clinical Samples: Target-specific Analysis of Qualitative Reverse Transcription–Polymerase Chain Reaction(RT-PCR) Diagnostic Kits ☆

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ABSTRACT

INTRODUCTION: The Coronavirus disease 2019 pandemic caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to the rise of many available modalities for diagnosis. One such modality is the Reverse Transcription–Polymerase Chain Reaction (RT-PCR) kits which require evaluation amongst the many available commercial kits in the market.

METHODS: We conducted a performance evaluation of twelve RT-PCR SARS-CoV-2 commercial kits. A total of 75 nasopharyngeal and oropharyngeal clinical samples were selected with their cycling threshold (Ct) values. Inclusion of 5 gene targets: E gene, N gene, S gene, RdRp and ORF1ab were assessed. Data was analyzed using R software version 4.1.1 and Microsoft Excel

RESULTS: We observe that, the positive sample's Ct values differs significantly across the 12 diagnostic kits. However, for gene-specific analysis, we observe that, positive sample's Ct values does not differ significantly across gene targets. There is significant difference in Ct values in Commercial kits targeting all genes except S-gene. All the commercial kits Altona (E and S genes), Thermo (ORF1ab and N genes), Multiplex (E, ORF1ab, RdRp genes), Meril (N and ORF1ab genes), S D Biosensor (E and ORF1ab genes), Lab Gun (RdRp and N genes) and Lab systems (ORF1ab and E genes) scored a sensitivity of 100%. All other kits scored sensitivity above 95% and lowest sensitivity with the Genes2me (E gene) and Genes2me (RdRp) at 95.08% each. All kits were 100% specific.

CONCLUSION: This study provides an accurate comprehensive assessment of the different kits in the detection of SARS-CoV-2 which may promote standardization of testing across laboratories.

Introduction

The Coronavirus disease 2019, caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which originated from Wuhan, China became a global pandemic which enforced the World Health Organisation(WHO) to label it as a global health concern. It has led to an estimated 179 million confirmed infections and 3 million deaths worldwide and in India an estimated 30 million cases and 3 hundred thousand plus deaths as of July 2021 (Zhou et. al, 2020; Babu et. al, 2021). As the pandemic progressed, the diagnosis of this disease continuously evolved creating the need of many currently available modalities for di-

agnosis . There are a number of laboratory tests like antigen detection kits, Reverse Transcriptase Polymerase Chain Reactions (RT-PCR), cartridge based nuclear amplification tests etc., however the most widely used and current gold standard for the diagnosis of COVID-19 is Reverse Transcriptase – Polymerase Chain Reaction.

SARS-CoV-2 virus belong to the family *Coronaviridae* and is a positive sense RNA virus which has 14 open reading frames(ORF) that code for the numerous structural and non-structural proteins. The RNA dependent RNA polymerase (RdRp) which is essential for the replication and transcription is encoded by an open reading frame known as ORF1ab. The ORF1ab as well as the genes which encode for the structural pro-

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome CoronaVirus-2; WHO, World Health Organisation; VRDL, State-Level Virus Research and Diagnostics Laboratory; ICMR, Indian Council of Medical Research; RT-PCR, Reverse Transcriptase Polymerase Chain Reactions.

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teins like the spike protein(S), envelope protein(E) and nucleocapsid(N) proteins are the major target sites used for the detection of covid19 by RT-PCR (Kirtipal et. al, 2020; Lu et. al, 2020; Barreto et. al, 2020)

The RT-PCR tests offer several benefits than its other diagnostic counterparts in being very useful for early-stage diagnosis of the infection when antibody titers aren't significantly high. Its added advantages being that it can also be performed on a wide range of specimens including nasal swabs, pharyngeal swabs, sputum, bronchoalveolar lavage fluid, fiber bronchoscope brush biopsies, blood and faces. Furthermore, the RT-PCR has high sensitivity and specificity providing the test results within a few hours making it the most widely used test. Due to the escalating pandemic and the increased demand of easier, more accurate and rapid diagnostic methods, the development of commercial RT-PCR kits has increased, creating different manufactured kits in the market. However there is still insufficient literature on the independent performance assessment among these kits. Increased accuracy of these kits and identifying them would help us to reduce the burden on the health care system and aid in accurately identifying the required population suffering in this pandemic (Li et. al, 2020).

In the State-Level Virus Research and Diagnostics Laboratory (VRDL), Bangalore Medical College and Research Institute, Bangalore, we performed an evaluation of twelve RT-PCR SARS-CoV-2 commercial kits to evaluate the performance characteristics and assess their accuracy. This study targets to provide a comprehensive assessment of the different kits used for the detection of SARS-CoV-2 via RT-PCR which may promote standardization of testing across laboratories, leading to the development of even better diagnostic kits in the future in India.

Methods

1. Sample Selection

A total of 75 nasopharyngeal and oropharyngeal clinical samples were selected in continuum along with their cycling threshold (Ct) values. The ethical approval was obtained from the *Institution Ethics Committee* (IEC) as per Certificate Number: BMCRI/PS/298/2020-21 Dt.06.02.2021/IEC.

2. Selection of Kits

This study encompasses 12 commercial RT-PCR SARS-CoV-2 detection kits available in the Indian market and approved by the Indian Council of Medical Research (ICMR). Kits were obtained from the *Karnataka State Mineral Corporation Limited* for Covid-19 for analysis as a part of the *Karnataka State Government Initiative*. None of the manufacturers were involved in the assessment or data analysis. The ICMR listed diagnostic kits were reviewed and analyzed based on availability and need for study. The details of regulation status, country of manufacturer, reaction volume, cycling time, thermocycler used, RNA template volume(microliter), storage conditions, internal control and target genes for all 12 commercial kits have been elaborated in [Table1](#). Inclusion of 5 gene targets over these 12 kits are as follows: E gene, N gene, S gene, RdRp and ORF1ab. [Figure 1](#). Distribution of COVID detection results by the 12 commercial diagnostic kits and their gene targets.

3. Sample preparation and RT-PCR procedure

All the clinical samples used for kit validations were collected from patients attending the COVID-19 Screening Room at the Out Patient Department along with informed consent. 75 samples were included for the study. Clinical samples collected in Viral Transport Media(VTM) were subjected to thorough vortexing, and 150 µl of the specimen

was further subjected To QIAamp Viral RNA Mini Kit in accordance to the QIAGEN manual RNA extraction protocol as per manufacturer's instruction. Realtime RT-PCR for all the kits were carried out in a span of less than 3 days. The real-time RT-PCR assays were performed on Thermofisher Quant Studio 5 thermal cycler. The assay procedures and interpretation of the results were done as per the manufacturer's instructions.

4. Data analysis

Data was analysed using R software version 4.1.1 and Microsoft Excel. Continuous variables have been mentioned in Mean $\pm$ SD/ Median (Min, Max) format. Kruskal Wallis test was used to compare the Ct values across the 12 diagnostic kits (gene targets) and across the 5 gene targets while Dunn's test was used as post hoc analysis. Box plots and bar graphs show the distribution of Ct values. One Way Analysis of variance (ANOVA) was used to compare the Ct values in Commercial kits by targeting N and ORF1ab genes for which Tukey's HSD was used as post hoc analysis here. Welch's ANOVA was used to compare the Ct values in Commercial kits by targeting E and RdRp genes while Games Howell test was used as post hoc analysis. Two sample t test was used to compare the Ct values in Commercial kits targeting S gene. Diagnostic parameters (Sensitivity, Specificity, negative predictive value and positive values were calculated for all target genes of different commercial RT-PCR kits with the gold standard. In gold standard, samples are considered to be positive if majority kits (>7 kits) agreed on the result as per ([Trevethan et. al, 2017](#)) reference study and ([van Kasteren et. al, 2020](#)) reference study. Kappa agreement was checked for all targets of different kits with gold standard. P-value less than or equal to 0.05 indicates statistical significance

Results

1. Overall summary of SARS-CoV2 Detection through commercial kits

Data contained measurements on 75 samples using 12 commercial kits with different gene targets as mentioned in methodology. The Supplementary Table 1.0 gives the distribution of COVID detection results by the 12 commercial diagnostic kits and their gene targets. Majority kits (7 kits) showed 61 (81.33%) of the samples as positive, while 3 kits showed 60 (80%) of samples to be positive, 1 kit showed 59 (78.67%) of samples to be positive where as Genes2me kit showed that 58 (77.33%) of the samples to be positive. The majority of the kits (7 kits) agreed on 61 (81.33%) positive and 14 (18.67%) negative samples. Hence, the same is considered as gold standard. One positive sample was taken as a positive control.

2. Cycle Threshold Values and analysis of Positive samples

We observe that, the positive sample's Ct values differs significantly across the 12 diagnostic kits (gene targets). The lowest Ct values are reported with Lab Gun (N gene target), Lab Gun (RdRp gene target), and Qline (E gene target). The highest positive Ct values are reported with S D Biosensor (E gene target), Allplex (N gene target), Lab Systems (ORF1ab gene target) and S D Biosensor (ORF1ab gene target). The graph in [Figure 2](#) depicts the distribution of the Ct values by the commercial kits and their targets. The Supplementary Table 2.0 gives Comparison of positive sample's Ct values across the 12 diagnostic kits (gene targets).

3. Cycle Threshold Values across the 5 gene targets

The commercial kits had 5 different gene targets (E, N, S, RdRP and ORF1ab). We observe that, positive sample's Ct values does not differ

Table 1
Details of 12 Commercial RT-PCR Kits used for the study.

CODE FOR RT-PCR KIT -Number	MANUFACTURER Name	Country of Manufacturer	Regulatory status	Reaction volume in μ L	Cycling time	Thermocycler	RNA template volume in μ L	Storage Condition	Internal Control	Target Genes	Positive cut off (CUT off) ^K
KIT-1 LAB Systems	Trivitron Health Care Private Limtd./ Channai India	INDIA	ICMR	20	1:22 min	Thermo QS5	5	20°C	Non Human	RdRp, N gene	<36
KIT-2 Tru PCR	Kilpest India Ltd. Bhopal India	INDIA	ICMR	25	1:42 min	Thermo QS5	10	20°C	Human (RNase P)	E gene RdRp+N gene	<35
KIT-3 SARA GENE	COSARA DIAGNOSTICS PVT LTD	INDIA	ICMR	10	1:37 min	Thermo QS5	5	20°C	Non Human	RdRp, E gene	Target not mentioned
KIT-4 Standard M nCoV	SD Biosensor, Germany	South Korea	ICMR	31	1:34 min	Thermo QS5	10	20°C	Non Human	E gene; ORF1 ab (RdRp) gene	<36
KIT-5 Meril	Meril Diagnostics Pvt Ltd India	INDIA	ICMR	20	1:46 min	Thermo QS5	5	20°C	Non Human	ORF1 ab,N gene	<40
KIT-6 Allplex 2019-nCoV assay	Seegene	South Korea	ICMR	25	1:52 min	Thermo QS5	10	20°C	Non Human	E gene, RdRp and N gene	<40
KIT-7 Thermo Taq Path	Life Technologies Corporation, CA	USA	ICMR, US FDA	25	1: 04 min	Thermo QS5	10	20°C	Non Human	ORF1 ab, N gene, S gene	<35
KIT-8 GENES 2 ME	Advanced molecular diagnostic solutions	INDIA	ICMR	20	1:41 min	Thermo QS5	9	20°C	Human (RNase P)	E gene, RdRp/N gene	<37
KIT-9 NIV Multiplex	NIV PUNE,ICMR	INDIA	ICMR	20	1:42 min	Thermo QS5	7	20°C	B actin	E gene,ORF	<35
KIT-10 Lab Gun	Lab genomics Co.,Ltd	INDIA	ICMR	20	00:35 min	Thermo QS5	5	20°C	Human (RNase P)	RdRp,N gene	<30
KIT-11 Q Line	POCT service pvt ltd	INDIA	ICMR	20	01:30 min	Thermo QS5	9	20°C	Human (RNase P)	E gene RdRp	<38
KIT-12 ALTONA	Altona diagnostics	Germany	ICMR, US FDA	30	01:58 min	Thermo QS5	10	20°C	Non Human	E gene, S gene	<35

^K – As per manufacturer provided value. ICMR: Indian Council of Medical Research, US-FDA: United States Food and Drug Administration.

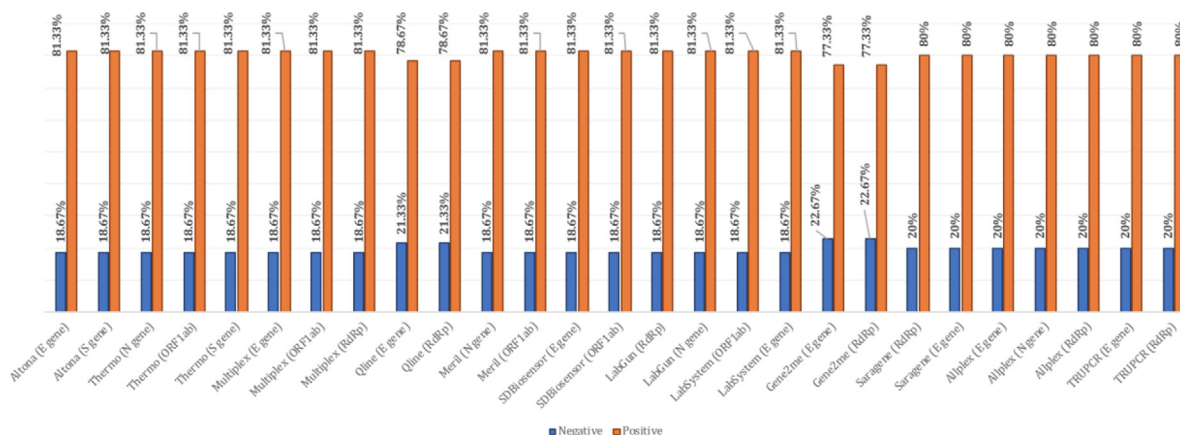


Figure 1. Distribution of COVID detection results by the 12 commercial diagnostic kits and their gene targets.

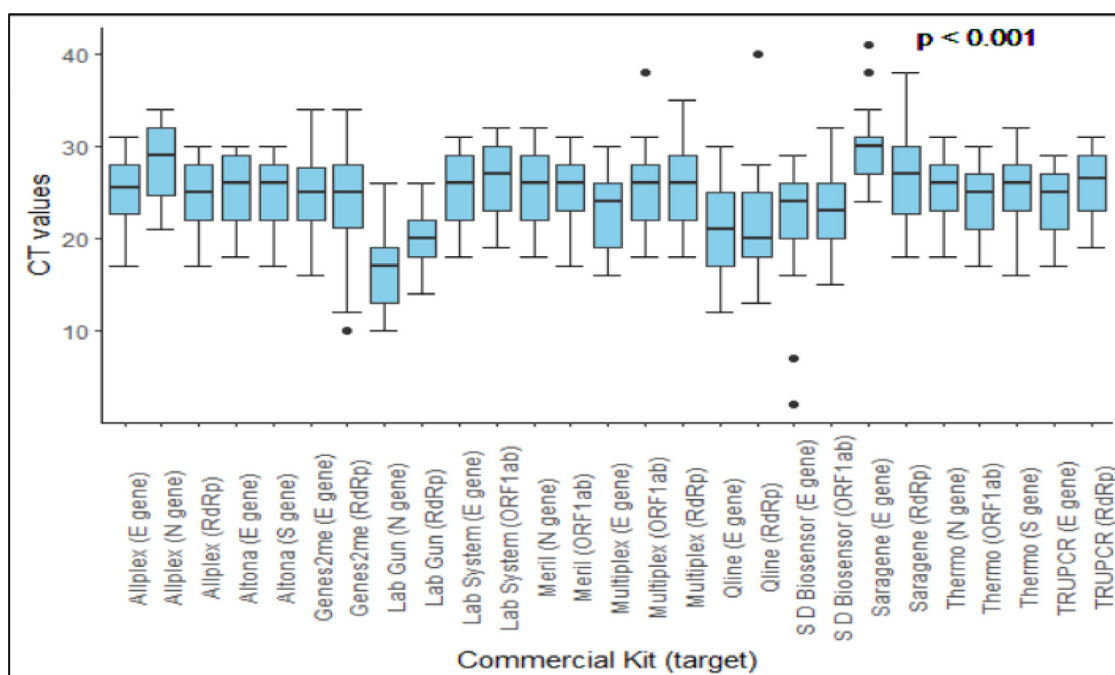


Figure 2. Positive samples' cycling threshold (CT) values across the 12 diagnostic kits (gene targets).

significantly across gene targets. The graph in Figure 3 depicts the distribution of the Ct values by the gene target while the Supplementary Table 3.0 gives the data for the same.

4. Individual analysis of various gene targets across all 12 commercial kits

4.1 Analysis with respect to E-Gene

We observe that, there is significant difference in Ct values in commercial kits targeting E gene. From post hoc analysis (Games-Howell test), we observe that, Ct values from Allplex differs significantly from QLINE (p-value<0.001) and Saragene (p-value<0.001), Altona differs significantly from QLINE (p-value<0.001) and Saragene (p-value<0.001), Genes2me differs significantly from Qline (p-value < 0.001) and Saragene (p-value<0.001), Lab Systems differs significantly from NIV Multiplex V 3.1 (p-value = 0.025), QLINE (p-value < 0.001), S D Biosensor (p-value = 0.02) and Saragene (p-value<0.001), Multiplex varies significantly from Saragene (p-value < 0.001), QLINE differs significantly from Saragene (p-value < 0.001) and TRUPCR (p-value < 0.001), S D Biosensor differs significantly from Saragene (p-value = 0.002) and Saragene differs significantly from TRUPCR (p-value

< 0.001). The Figure 4 depicts the Mean plot of Ct values in Commercial kits targeting E gene.

4.2 Analysis with respect to S-Gene

We observe that, there is no significant difference in Ct values in Commercial kits targeting S gene (Altona and Thermo, p value= 0.6595). Figure 4 depicts Mean plot of Ct values in Commercial kits targeting S gene.

4.3 Analysis with respect to N- Gene

We observe that, there is significant difference in Ct values in Commercial kits targeting N gene. From post hoc analysis (Tukey's HSD), we observe that, Ct values from Allplex differs significantly from Lab Gun (p-value<0.001), Meril (p-value<0.01) and Thermo (p-value = 0.0014), Lab Gun differs significantly from Meril (p-value < 0.001) and Thermo (p-value<0.001). Figure 4 depicts the Mean plot of Ct values in Commercial kits targeting N gene.

4.4 Analysis with respect to RdRp target

We observe that, there is significant difference in Ct values in Commercial kits targeting RdRp gene. From post hoc analysis (Games-

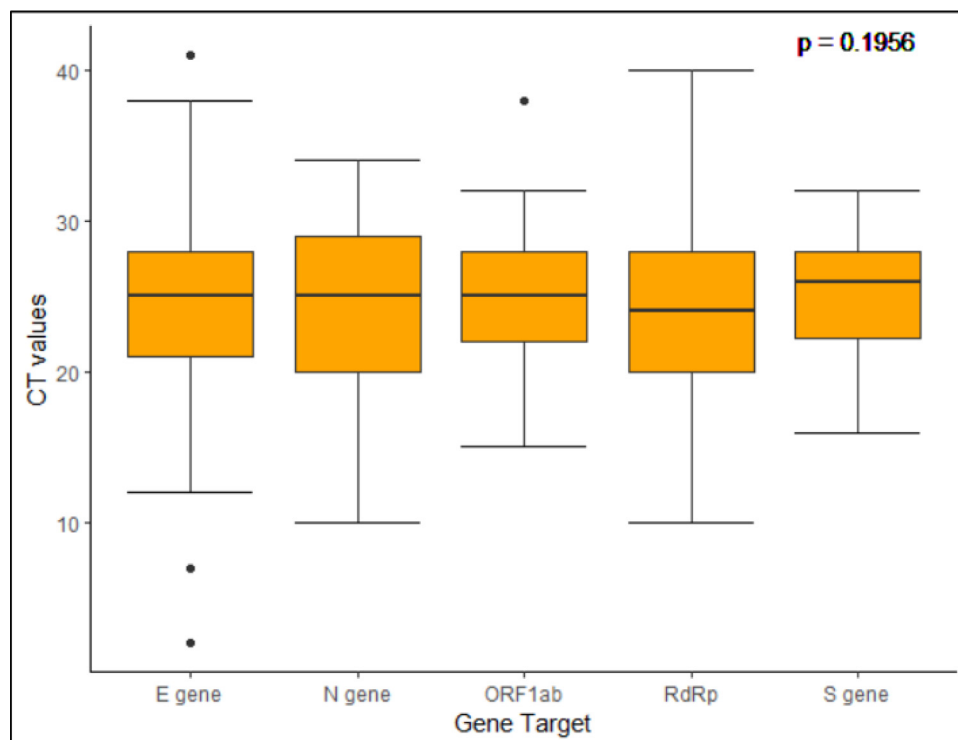


Figure 3. Positive samples cycling threshold (CT) values across the testing kits gene targets.

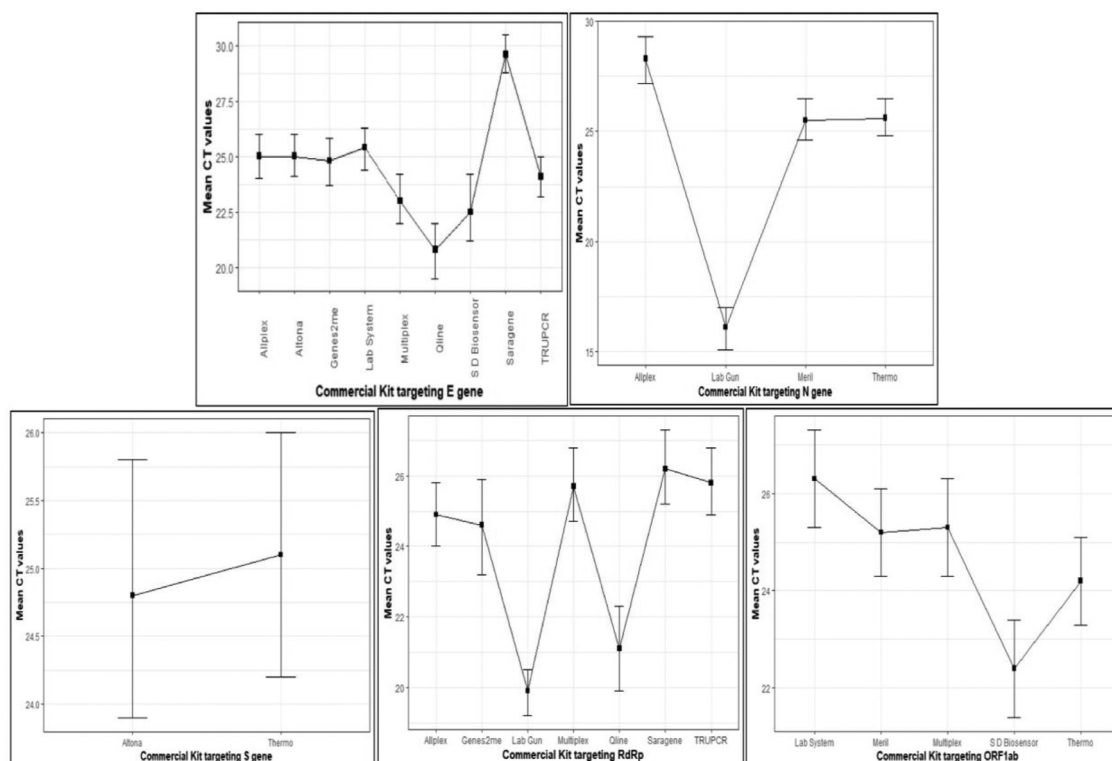


Figure 4. Mean plot of CT values in Commercial kits targeting E Gene, S Gene, N-Gene, RdRp Gene and ORF1ab Gene.

Howell test), we observe that, Ct values from Allplex differs significantly from Lab Gun (p-value<0.001) and QLINE (p-value<0.001), Genes2me differs significantly from Lab Gun (p-value<0.001) and QLINE (p-value = 0.005), Lab Gun differs significantly from NIV Multiplex V 3.1 (p-value < 0.001), Saragene (p-value < 0.001) and

TRUPCR (p-value<0.001), Multiplex varies significantly from QLINE (p-value < 0.001), QLINE differs significantly from Saragene (p-value < 0.001) and TRUPCR (p-value < 0.001). Figure 4 depicts the Mean plot of Ct values in Commercial kits targeting RdRp gene.

Table 2

Statistical summaries for the performance of 12 commercial kits.

Commercial Kits (Gene target)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95%CI)	Kappa (95% CI)	p-value ^{MC}
Altona (E gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Altona (S gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Thermo (N gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Thermo (ORF1ab)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Thermo (S gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Multiplex (E gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Multiplex (ORF1ab)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Multiplex (RdRp)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Qline (E gene)	96.72% (88.65% - 99.6%)	100% (76.84% - 100%)	100% (93.94% - 100%)	87.5% (61.65% - 84.5%)	0.92 (0.8 - 1)	<0.001*
Qline (RdRp)	96.72% (88.65% - 99.6%)	100% (76.84% - 100%)	100% (93.94% - 100%)	87.5% (61.65% - 98.45%)	0.92 (0.8 - 1)	<0.001*
Meril (N gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Meril (ORF1ab)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
S D Biosensor (E gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
S D Biosensor (ORF1ab)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Lab Gun (RdRp)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Lab Gun (N gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Lab System (ORF1ab)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Lab System (E gene)	100% (94.13% - 100%)	100% (76.84% - 100%)	100% (94.13% - 100%)	100% (76.84% - 100%)	1	<0.001*
Genes2me (E gene)	95.08% (86.29%-98.97%)	100% (76.84% - 100%)	100% (93.84% - 100%)	82.35% (56.57% - 96.20%)	0.88 (0.74 - 1)	<0.001*
Genes2me (RdRp)	95.08% (86.29%-98.97%)	100% (76.84% - 100%)	100% (93.84% - 100%)	82.35% (56.57% - 96.20%)	0.88 (0.74 - 1)	<0.001*
Saragene (RdRp)	98.36% (91.2% - 99.96%)	100% (76.84% - 100%)	100% (94.04% - 100%)	93.33% (68.05% - 99.83%)	0.96 (0.87 - 1)	<0.001*
Saragene (E gene)	98.36% (91.2% - 99.96%)	100% (76.84% - 100%)	100% (94.04% - 100%)	93.33% (68.05% - 99.83%)	0.95 (0.87 - 1)	<0.001*
Allplex (E gene)	98.36% (91.2% - 99.96%)	100% (76.84% - 100%)	100% (94.04% - 100%)	93.33% (68.05% - 99.83%)	0.96 (0.87 - 1)	<0.001*
Allplex (N gene)	98.36% (91.2% - 99.96%)	100% (76.84% - 100%)	100% (94.04% - 100%)	93.33% (68.05% - 99.83%)	0.96 (0.87 - 1)	<0.001*
Allplex (RdRp)	98.36% (91.2% - 99.96%)	100% (76.84% - 100%)	100% (94.04% - 100%)	93.33% (68.05% - 99.83%)	0.96 (0.87 - 1)	<0.001*
TRUPCR (E gene)	98.36% (91.2% - 99.96%)	100% (76.84% - 100%)	100% (94.04% - 100%)	93.33% (68.05% - 99.83%)	0.96 (0.87 - 1)	<0.001*
TRUPCR (RdRp)	98.36% (91.2% - 99.96%)	100% (76.84% - 100%)	100% (94.04% - 100%)	93.33% (68.05% - 99.83%)	0.96 (0.87 - 1)	<0.001*

Abbreviation: MC – Chi square test with Monte Carlo simulation, * indicates statistical significance.

4.5 Analysis with respect to ORF1ab target

We observe that, there is significant difference in Ct values in Commercial kits targeting ORF1ab gene. From post hoc analysis (Tukey's HSD), we observe that, Ct values from Lab Systems differs significantly from S D biosensor (p-value<0.001) and Thermo (p-value = 0.0248), S D Biosensor differs significantly from Meril (p-value <0.001) and Multiplex (p-value<0.001). [Figure 4](#) depicts the Mean plot of Ct values in Commercial kits targeting ORF1ab gene target.

5. Analysis of Sensitivity and Specificity of Commercial kits

The commercial kits Altona (E and S genes), Thermo (ORF1ab and N genes), Multiplex (E, ORF1ab, RdRp genes), Meril (N and ORF1ab genes), S D Biosensor (E and ORF1ab genes), Lab Gun (RdRp and N genes) and Lab systems (ORF1ab and E genes) scored a sensitivity of 100%. All other kits scored sensitivity above 95%. The lowest sensitivity was observed with the Genes2me (E gene) and Genes2me (RdRp) with 95.08% each. For specificity, all the tested kits scored 100%. For the Kappa agreement tests, the highest score was 100% and was observed with Altona (E and S genes), Thermo (ORF1ab and N genes), Multiplex (E, ORF1ab, RdRp genes), Meril (N and ORF1ab genes), S D Biosensor (E and ORF1ab genes), Lab Gun (RdRp and N genes) and Lab systems (ORF1ab and E genes) kits, the other kits scored an agreement above 90% except for Genes2me (E gene) and Genes2me (RdRp) which had 88% agreement each. The statistical summaries of all commercial kits on genes, sensitivity, specificity, Positive Predictive Value(PPV), Negative Predictive Value(NPV) and kappa value have been illustrated in [Table 2](#).

Discussion

Having passed two major waves of the coronavirus disease 2019 (COVID-19) pandemic, many testing kits were developed and were made available commercially. The ICMR has approved several diagnostic kits for several gene-specific analysis and data validation. In our study we evaluate and analyse the performance of 12 commercial kits in detecting the various genes of the SARS-CoV-2 virus. We performed target gene

specific analysis from which we derived that although the commercial kits had 5 different gene targets (E, N, S, RdRp and ORF1ab), the positive sample's cycling threshold (Ct) values differs significantly across gene targets.

From post hoc analysis, we observe that, Ct value of different gene targets did not differ significantly as elaborated in the results above. Among the kits we also notice that Altona (E and S genes), Thermo (ORF1ab and N genes), Multiplex (E, ORF1ab, RdRp genes), Meril (N and ORF1ab genes), S D Biosensor (E and ORF1ab genes), Lab Gun (RdRp and N genes) and Lab systems (ORF1ab and E genes) scored a sensitivity of 100%. All other kits scored sensitivity above 95%. The lowest sensitivity was observed with the Genes2me (E gene) and Genes2me (RdRp) with 95.08% each.

The study conducted by ([Altamimi et. al, 2021](#)) on the performance of these commercial kits by gene target showed no significant change in Ct values which indicates that kits disparities are mainly linked to the choice of the gene target. Their study showed that RdRp and E gene targets show significant differences by the reported Ct values. However in our study Ct-value for different gene targets did not differ significantly with the others. Comparing commercial kits showed similar results in detection, however, different targets did not show variation in Ct values. Moreover, kits with multiple targets such as in another evaluation study did show higher sensitivity and specificity than with other kits detecting a single target ([Iglói et. al, 2020](#); [Basawarajappa et. al, 2021](#))

The escalation of these various mutations in SARS-CoV-2 in multiple geographic locations indicates the need for regular genetic testing in each country to capture these changes as they are used in the selection of the commercial diagnostic kit used in health centres. An issue regarding the current situation in India is regarding the rise of the delta variant. India has experienced multiple outbreaks of SARS-CoV2 in 2020 and has reached a population sero-positivity of more than 50% by 2021. We hope by the results, new data and analysis can be done for the analysis of RT-PCR versus genomic detection of the new strains and variants ([Dhar et. al, 2021](#)). But recent reports and current evidence from scientific literature tell a different story. There was speculation that SARS-CoV-2 escaped RT-PCR due to a series of mutations received. Host-dependent RNA editing and high human-to-human transmission may have mutated

the virus, thereby allowing the virus to spread faster and even escape RT-PCR (Jindal et al., 2021).

Limitations in our study includes the absence of evaluation of the limit of detection which can play a big role, as with several of the other commercial kits approved for the pandemic situation. Another limitation is that we were unable to examine the cross-reactivity of these commercial kits with other viruses, which can significantly alter the detection results. As we were not able to include all the RT-PCR diagnostic kits available on the market and the performance of some kits may vary depending on the extraction method, there may be a limitation posed as well. Lastly, in our study, our sample size was limited to the number of tests per kit for that particular Lot/Batch number and might vary with manufacturer provisions.

Conclusion

In conclusion, we entrust upon the fact that most of the commercially available RT-PCR kits endorsed in this study can be used for routine diagnosis of clinical samples suspicious of SARS-CoV-2 patients. Most of the kits were validated in detecting the virus, however, few distinctions were found with specific kits and targets. In comparison with other studies our study covers the difference in Ct-values with respect to target specific gene-analysis in a detailed comprehensive manner. Multiple targets have been shown to be more sensitive and specific as they counted for target variations. New methods for diagnosis have been recently mentioned in literature, such as, saliva samples and oral dry swabs amongst many others (Teo et al., 2021). In addition, we recommend that regardless of the choice of commercial diagnostic kit for the clinical detection of COVID-19 patients in the laboratory, a good validation plan and collaboration with external laboratories and government agencies are required to identify virus changes, procedures, technicians and the various kit services.

Data summary

The authors confirm all supporting data and protocols have been provided within the article or through supplementary data files. Additional data available upon request.

Conflicts of interest

The author(s) declare that there are no conflicts of interest

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Ethical approval

The ethical approval was obtained from the *Institution Ethics Committee* (IEC) as per Certificate Number: BMCRI/PS/298/2020-21 Dt.06.02.2021/IEC.

Consent for publication

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ijregi.2021.11.004](https://doi.org/10.1016/j.ijregi.2021.11.004).

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