(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 5, Issue 1, Page No: 613-621
January-February 2022

IJMSCR

Performance Analysis Of 4 Commercially Available Real-Time RT-PCR Kits for SARS-CoV-2 Detection in India

[1]Kala Yadhav M L, [2]Ajay Philips, [1]Aditya Chandrashekar1, [2]Chetana G S
Department of Microbiology,
[1]Bangalore Medical College and Research Institute, Bengaluru, India
[2]Shri Atal Bihari Vajpayee Medical College and Research Institute, Bengaluru, India

***Corresponding Author:**
Chetana G S
Department of Microbiology, Shri Atal Bihari Vajpayee Medical College and Research Institute, Bengaluru, India

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Background: COVID-19 pandemic has led to an unprecedented global health crisis. Diagnostic strategies that are low cost, rapid, and reliable are critical to control the pandemic. RT-PCR based assays remain the standard for the detection of SARS-CoV-2.

Aim: To determine the diagnostic accuracy of 4 commercially available Real-Time RT-PCR Kits for SARS-CoV-2 in both individual as well as pooled samples.

Methods: Four different Real-Time RT-PCR Kits for SARS-CoV-2 were selected. RT-PCR testing was performed on 54 individual and 20 pooled of both previously confirmed positive and negative samples. Limit of detection test was performed using 10 fold dilutions of confirmed positive samples.

Results: All the four kits scored sensitivity, specificity, NPV and PPV of 100% for both individual and pooled samples. We observed that the CT values of positive samples differ significantly across the COVID19 kits. Efficiency in Limit of detection ≥96 % was seen in Qline (E gene), Allplex (E gene), Allplex (N gene) and NIV Inhouse kit (E gene)

Conclusion: The performance parameters of the tested kits were comparable to NIV in house kit. We conclude that all RT-PCR kits assessed in this study may be used for routine diagnostics of COVID-19.

Keywords: Real-Time RT-PCR Kits, SARS-CoV-2, COVID-19

Introduction

The coronavirus disease 2019 (COVID-19) pandemic which initially originated from Wuhan China, still continues to be an obstacle for the healthcare systems as well as on the economy and livelihood of people worldwide. With an estimated 250 million confirmed cases and over 5 million fatalities worldwide, the numbers are still increasing. It is arguably one of the deadliest pandemics in modern time.[1] It is caused by a novel enveloped RNA virus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which belongs to the family Coronaviridae.

The SARS-CoV-2 contains a positive stranded RNA genome with a length of approximately 29.9kb which encodes numerous structural, non-structural as well as accessory proteins [2]. There are 14 open reading frames(ORF) in the genomes with ORF1a and ORF1b which cover more than two thirds of the genome and which encodes the polyprotein pp1ab which is then cleaved into numerous non-structural proteins (nsp) including the RNA-dependent RNA polymerase (RdRp) which is responsible for the replication of the virus. The other third of the genome contains genes for the main structural proteins, primarily S (spike surface glycoprotein), E (envelope

protein), M (membrane glycoprotein), and N (nucleocapsid phosphorylated protein)[3,4].

Early and accurate diagnosis of SARS-CoV-2 infection is the cornerstone for the containment and effective prevention of this pandemic. Infected patients experience a wide range of symptoms, from mild fever and cough to severe respiratory distress leading to fatal consequences. Thus increasing the need for the highest quality diagnostic tests. The current modalities available for the laboratory diagnosis of COVID-19 includes both point of care tests and laboratory tests, with conventional real-time polymerase chain reaction being considered the gold standard among them[5].

Since the first full-length SARSCoV2 genome sequence was published, a variety of RT-PCR assays and kits have been developed and marketed, with over 400 commercially available kits available globally. The most commonly used gene targets for the detection of SARS CoV2 are the ORF1ab, RdRp, E, N, and S genes.

However there is still a lack of data on the relative efficiency of these kits which are available in India. Hence in our study we have made a direct evaluation of the performance characteristics (sensitivity, specificity and LoD) of the 4 kits - Qline, Labsystems, Allpex and Huwel kits which are commercially available in the Indian market on both individual and pooled samples.

Materials and Methods

Selection of samples

A total of 54 individual nasopharyngeal and oropharyngeal clinical samples were selected out of which 44 were confirmed positive and 10 were confirmed negative samples. Samples with inconclusive RT PCR and extreme CT values (<15,>33) were not included.

Selection of kits

Commercially available ICMR approved COVID-19 RT-PCR kits were identified and were selected based on availability, lower limit of detection and compatibility with different PCR platforms. The kits included for the study are

1-QLIne molecular ER nCOV19 qRT-PCR kit. 2-Quantiplus CoV detection KIT 3- Allplex 2019 -ncov assay. 4 COVIDsure Multiplex qRT-PCR kit. 5- NIV In-house covid kit.

All of the kits included in our analysis were provided free of charge and none of the manufacturers were involved in the assessment and interpretation of the results.

Sample preparation and RT PCR procedure

All the samples used for kit validation were received at Bowring and Lady Curzon Hospital , RT-PCR lab. 54 samples were included in the study. Clinical samples were collected in Viral transport media (VTM) and were subjected to through vortexing. RNA extraction was performed by MagMAX Viral/Pathogen Nucleic Acid Kit as per manufacturer's instruction using Thermo Scientific King Fisher Flex Purification system and run on Bio-Rad CFX 96 according to the manufacturer's instruction. All samples were tested in triplicate before they were included in the study.

To establish PCR efficiency a duplicate 10-fold serial dilution of viral RNA for each assay was prepared. Viral RNA was isolated from a previously confirmed COVID-19 positive sample. The slope was determined by linear regression and defined the required levels for PCR efficiency (E) and R2 as > 95 % and > 0.95, respectively.

Pooling of samples

Positive pools will be created using 200µl VTM from an RT-PCR confirmed COVID-19 positive patient specimen added to 200µl VTM from each of four negative patient samples to prepare a final volume of 1 ml. From that 1 ml, 200µl will be used as the starting material for RNA extraction. Similarly, negative sample pools will also be created. Nucleic acid extraction will be performed on each pool using viral nucleic acid extraction by MagMAX Viral/Pathogen Nucleic Acid Kit on Thermo Scientific King Fisher Flex Purification System

Data analysis

Data is analyzed using R software version 4.1.1 and Excel. Categorical variables are given in the form of frequency table. Continuous variables are given in Mean ± SD/ Median (Min, Max) form. Kruskal Wallis test is used to compare the CT values across the diagnostic kits (gene targets) and across gene targets. Dunn's test is used as post hoc analysis. Box

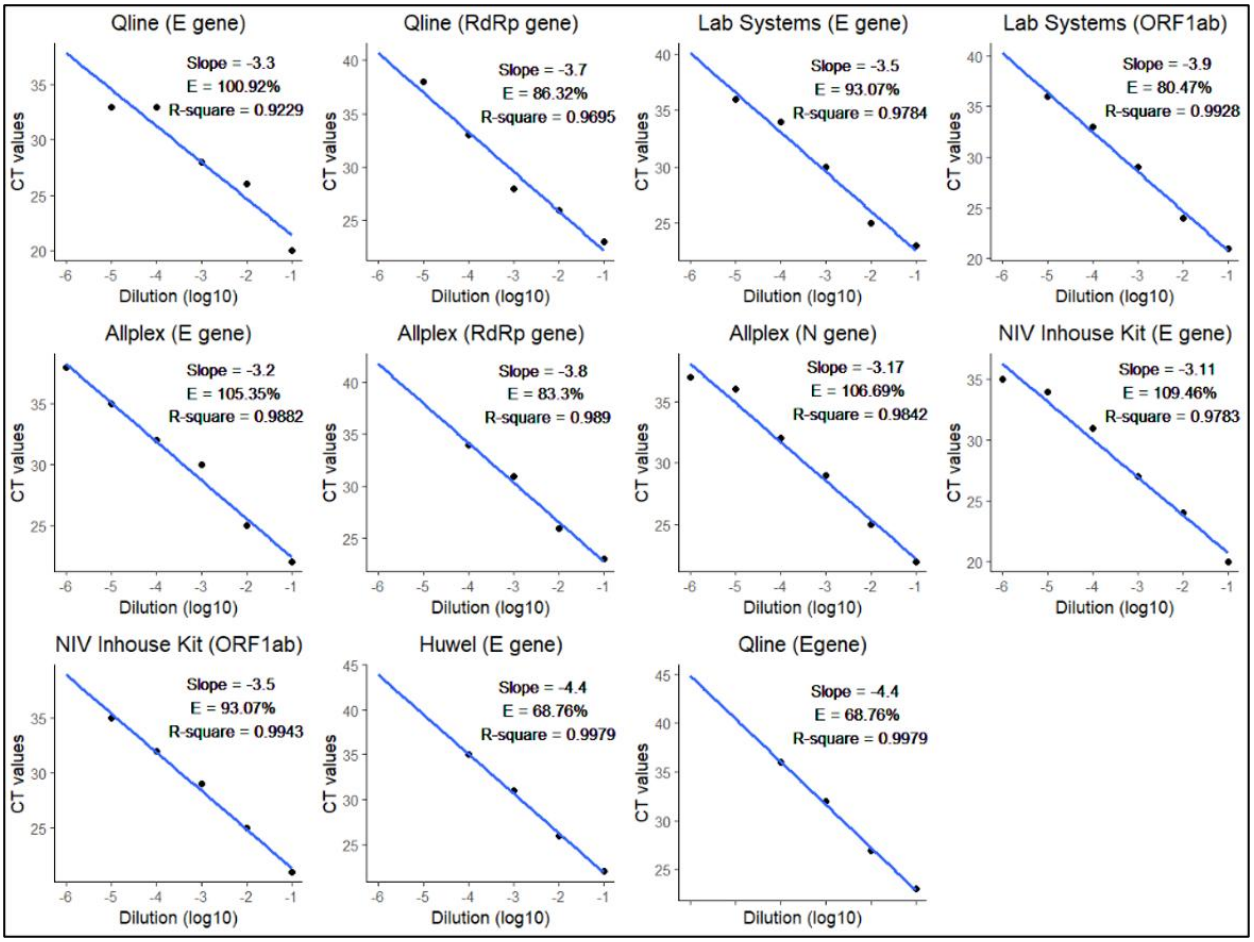
Volume 5, Issue 1; January-February 2022; Page No 613-621

plots and bar graphs are used to show the distribution of CT values. One Way Analysis of variance (ANOVA) is used to compare the CT values in COVID kits by targeting E genes. Tukey's HSD is used as post hoc analysis. Mann Whitney U test is used to compare the CT values in COVID kits targeting RdRp, N and ORF1ab genes. Diagnostic parameters (Sensitivity, Specificity, negative predictive value and positive predictive values) are calculated for different kits with the NIV inhouse kit as gold standard for individual samples and pooled samples. Kappa agreement is checked for different kits with NIV inhouse kit as gold standard for individual samples and pooled samples. Linear regression was performed to obtain the slope, R^2 and PCR efficiency. P-value less than or equal to 0.05 indicates statistical significance.

Results

1-PCR Efficiency

Figure 1: PCR efficiency for COVID-19 RT-PCR kits for the detection of each gene



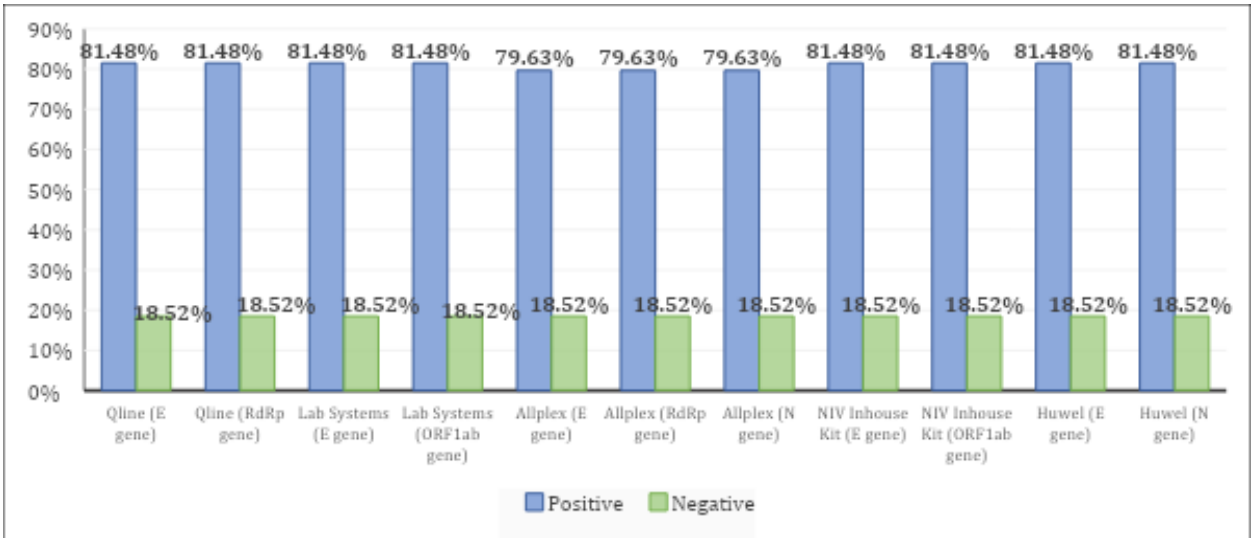
PCR efficiency (E) for each target gene was assessed using a duplicate 10-fold dilution series of SARS-CoV-2 viral RNA. Linear regression was performed in to obtain the slope and R^2. The percentage efficiency was calculated from the slope using the formula $E = 100*(-1 + 10^{-1/slope})$.

For all COVID 19 kits (gene targets), R squares were > 0.97. Efficiency ≥96 % for Qline (E gene), Allplex (E gene), Allplex (N gene) and NIV Inhouse kit (E gene). For others efficiency was <96%.

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

Volume 5, Issue 1; January-February 2022; Page No 613-621

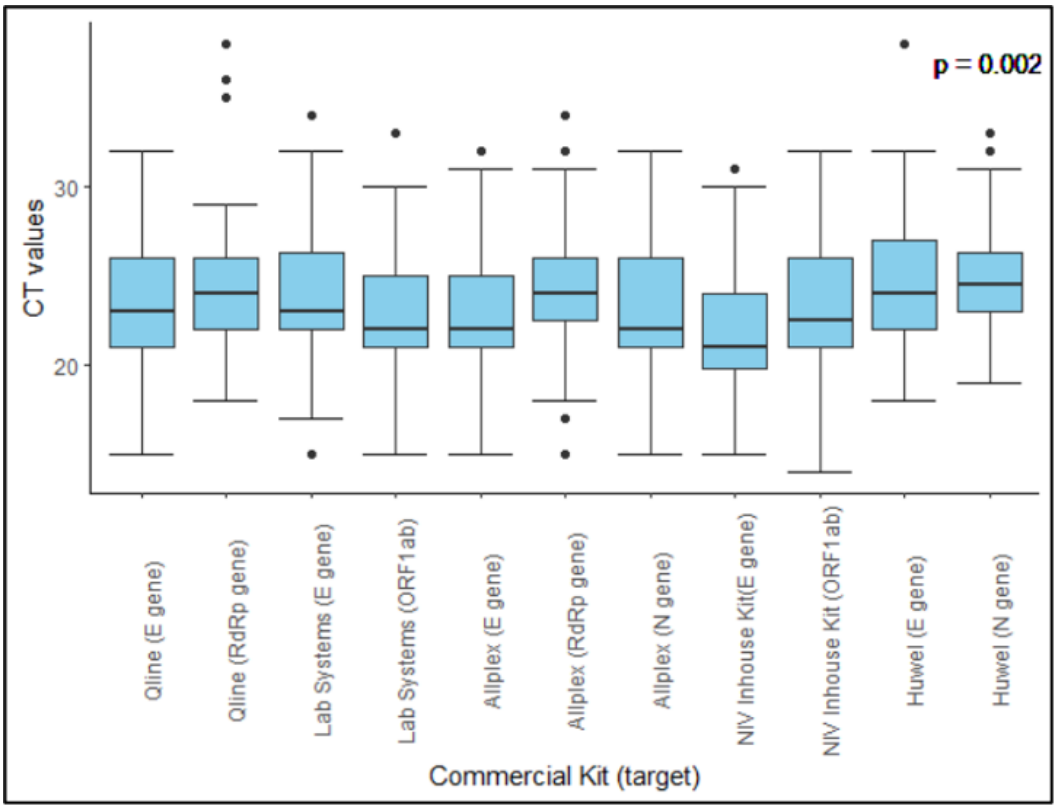
Figure 2- gives the distribution of COVID detection results by different COVID diagnostic kits and their gene targets



Majority kits showed 44 (81.48%) of the samples as positive, while Allplex kit with E gene, RdRp gene and N gene showed 43 (79.63%) of samples to be positive and inconclusive result for one sample.

3-Cycling threshold (CT) values across the diagnostic kits

Figure 3 gives the Comparison of positive sample's CT values across the COVID kits



The lowest CT values are reported with NIV Inhouse Kit (E gene) and Lab Systems (ORF1ab gene). The highest positive CT values are reported with Huwel (N gene).

We observe that, positive sample's CT values differs significantly across the COVID kits (gene targets). From post hoc analysis (Dunn test), we observe that, there is significant difference in distribution of CT value

Volume 5, Issue 1; January-February 2022; Page No 613-621

between Huwel (N gene) & Lab Systems (ORF1ab gene) (p-value = 0.0302), NIV Inhouse Kit (E gene) & Allplex (RdRp gene) (p-value =0.0267), Huwel (E gene) & NIV Inhouse Kit (E gene) (p-value = 0.0385), Huwel (N gene) & NIV Inhouse Kit (E gene) (p-value = 0.0064) and NIV Inhouse Kit (E gene) & Qline (RdRp gene) (p-value = 0.0296).

4-Cycling Threshold Values across gene targets

Table 1: Comparison of positive sample's CT values across the gene targets

Gene targets	Mean ± SD	Median (Min, Max)	p-value
E gene	23.43 ± 3.96	23 (15, 38)	
N gene	24.13 ± 4.09	24 (15, 33)	
ORF1ab gene	23.06 ± 3.93	22 (14, 33)	$0.0187^{K}*$
RdRp gene	24.66 ± 4.17	24 (15, 38)	

The COVID kits had 4 different gene targets (E, N, RdRP and ORF1ab). We observe that, positive sample's CT values differ significantly across gene targets.

From post hoc analysis (Dunn test), we observe that, the distribution of CT value of RdRp gene differs significantly from E gene (p-value = 0.0359) and ORF1ab gene (p-value = 0.0435).

5-Cycling Threshold Values across COVID kits targeting E gene.

Table2: Comparison of CT values in COVID kits targeting E gene

COVID kits (Gene target)	Mean ± SD	Median (Min, Max)	p-value
Qline (E gene)	23.5 ± 3.93	23 (15, 32)	
Lab Systems (E gene)	23.93 ± 4.07	23 (15, 34)	
Allplex (E gene)	23 ± 3.7	22 (15, 32)	$0.0293^{A}*$
NIV Inhouse Kit (E gene)	22.07 ± 3.77	21 (15, 31)	
Huwel (E gene)	24.66 ± 4.03	24 (18, 38)	

We observe that, CT values differs significantly across the COVID kits targeting E gene. From post hoc analysis (Tukey's HSD), we observe that, there is significant difference in mean CT value between Huwel (E gene) & NIV Inhouse Kit (E gene) (p-value = 0.0177).

6-Cycling Threshold Values across COVID kits targeting ORF1ab gene.

Table3: Comparison of CT values in COVID kits targeting ORF1ab gene.

COVID kits (Gene target)	Mean ± SD	Median (Min, Max)	p-value
Lab Systems (ORF1ab gene)	22.77 ± 3.84	22 (15, 33)	0.4305^{MW}
NIV Inhouse Kit (ORF1ab gene)	23.34 ± 4.03	22.5 (14, 32)	

We observe that, there is no significant difference in the distribution of CT values between Qline (RdRp gene) and Allplex (RdRp gene).

Volume 5, Issue 1; January-February 2022; Page No 613-621

7-Cycling Threshold Values across COVID kits targeting RdRp gene.

Table 4: Comparison of CT values in COVID kits targeting RdRp gene.

COVID kits (Gene target)	Mean ± SD	Median (Min, Max)	p-value
Qline (RdRp)	24.77 ± 4.46	24 (18, 38)	0.7007^{MW}
Allplex (RdRp)	24.53 ± 3.91	24 (15, 34)	

We observe that, there is no significant difference in the distribution of Ct values between Qline (RdRp gene) and Allplex (RdRp gene).

8-Cycling Threshold Values across COVID kits targeting N gene.

Table 5:Comparison of CT values in COVID kits targeting N gene

COVID kits (Gene target)	Mean ± SD	Median (Min, Max)	p-value
Allplex (N gene)	23.09 ± 4.28	22 (15, 32)	0.0197^{MW}*
Huwel (N gene)	25.14 ± 3.66	24.5 (19, 33)	

We observe that, there is significant difference in the distribution of Ct values between Allplex (N gene) and Huwel (N gene).

9-Performance analysis of different kits with NIV inhouse kit as gold standard in individual samples.

Table 6: Statistical summaries for performance of different kits with NIV inhouse kit as gold standard in individual samples

COVID Kits (Gene target)	**Result**	**Positive**	**Negative**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95%CI)**	**Kappa (95% CI)**	**p-value MC**
Qline	Positive	44	0	100% (91.96% - 100%)	100% (69.15% - 100%)	100% (91.96% - 100%)	100% (69.15% - 100%)	1	<0.001 *
	Negative	0	10						
Lab Systems	Positive	44	0	100% (91.96% - 100%)	100% (69.15% - 100%)	100% (91.96% - 100%)	100% (69.15% - 100%)	1	<0.001 *
	Negative	0	10						
Allplex	Positive	43	0	100% (91.78% - 100%)	100% (69.15% - 100%)	100% (91.78% - 100%)	100% (69.15% - 100%)	1	<0.001 *
	Negative	0	10						
Huwel	Positive	44	0	100% (91.96% - 100%)	100% (69.15% - 100%)	100% (91.96% - 100%)	100% (69.15% - 100%)	1	<0.001 *
	Nega	0	10						

Volume 5, Issue 1; January-February 2022; Page No 613-621

	tive								

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

All the four kits (Qline, Lab Systems, Allplex and Huwel) scored sensitivity, specificity, NPV and PPV of 100% for individual samples. For the Kappa agreement tests, the highest score was 100% and was observed with all kits.

10-Performance analysis of different kits with NIV inhouse kit as gold standard in pooled samples.

Table 7: Statistical summaries for performance of different kits with NIV inhouse kit as gold standard in pooled sample.

COVID Kits (Gene target)	Result	Positive	Negative	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95%CI)	Kappa (95% CI)	p-value MC
Qline	Positive	10	0	100% (69.15% - 100%)	100% (69.15% - 100%)	100% (69.15% - 100%)	100% (69.15% - 100%)	1	<0.001 *
	Negative	0	10						
Lab Systems	Positive	10	0	100% (69.15% - 100%)	100% (69.15% - 100%)	100% (69.15% - 100%)	100% (69.15% - 100%)	1	<0.001 *
	Negative	0	10						
Allpex	Positive	10	0	100% (69.15% - 100%)	100% (69.15% - 100%)	100% (69.15% - 100%)	100% (69.15% - 100%)	1	<0.001 *
	Negative	0	10						
Huwel	Positive	10	0	100% (69.15% - 100%)	100% (69.15% - 100%)	100% (69.15% - 100%)	100% (69.15% - 100%)	1	<0.001 *
	Negative	0	10						

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

All the four kits (Qline, Lab Systems, Allplex and Huwel) scored sensitivity, specificity, NPV and PPV of 100% for pooled samples. For the Kappa agreement tests, the highest score was 100% and was observed with all kits.

Discussion

RT-PCR is considered the gold standard for diagnosis of COVID-19. We are now two years into the pandemic and we now have various RT-PCR kits targeting different genes that are commercially available to us today. In this study we report a comparative evaluation of four commercially available RT-PCR kits which have been approved for the diagnosis of COVID-19 in India. The performance of the four kits (Qline, Labsystems, Allpex and Huwel kits) were comparable with all of them showing extremely high sensitivity and specificity(100%) but there was significant difference across the positive samples cycling threshold values (Ct) across the gene targets -E, N, RdRP and ORF1ab where we observe that, the distribution of Ct value of RdRp gene differs significantly from E gene (p-value = 0.0359) and ORF1ab gene (p-value = 0.0435). Among the kits, the lowest Ct values were reported with NIV Inhouse Kit (E gene) and Lab Systems (ORF1ab gene) and the highest positive Ct values

Volume 5, Issue 1; January-February 2022; Page No 613-621

reported with Huwel (N gene). These results were in accordance with study done by Altamimi AM, et al which indicated that the kit disparity was mainly due to the choice of gene target[6]. However Rangaiah A ,et al reported that there were no significant differences in Ct values among different gene targets[7].

With E gene being the common gene target among the kits, it was compared to the NIV in-house kit and we observe that, there is significant difference in mean Ct value between Huwel (E gene) & NIV Inhouse Kit (E gene) (p-value = 0.0177) while there was no significant difference in the other kits. There was also significant difference in the distribution of CT values between Allplex (N gene) and Huwel (N gene) while no significant difference was noted between RdRP and ORF1ab targets. However Altamimi AM, et al reported that RdRP and E gene targets showed significant differences in the Ct values[6].

This difference between Ct values in the kits targeting the same gene suggests that the primers, probes and master mix components included in the different kits could contribute to this variation[8].

All the four kits (Qline, Lab Systems, Allplex and Huwel) scored sensitivity, specificity, NPV and PPV of 100% for individual samples with other studies also showing comparable results[9,10,18].

The real-time RT-PCR cycle threshold (Ct) represents the number of amplification cycles that is needed for the target gene to cross the threshold level. It is therefore inversely related to viral load and can serve as an indirect marker of viral load[11]. The Ct were also closely related to disease severity, mortality, infectivity, and multiple biomarkers[12]. Although the diagnosis and management of Covid19 is based on positive or negative SARSCoV2 RT-PCR test results, reporting Ct values may be beneficial for clinicians in making clinical decisions and to predict the progression of COVID19 disease. It may also help in guiding decisions about infection control, public health, and occupational health[13]. Hence it is important to develop kits which do not show significant variation in the Ct values. Further development and standardization is required to achieve this goal.

While no Covid-19 RT-PCR kit has been declared as the gold standard in diagnosis of COVID 19, our study shows that all the 4 kits can be used in the diagnosis of COVID 19.

While the diagnosis of COVID-19 has developed rapidly there have also been few limitations, one of which is the cost and availability of the RT-PCR which is an expensive test and which requires skilled personnel and well equipped molecular laboratories. Many countries are facing shortages of diagnostic kits and manufacturers are also struggling to meet the demand [14]. Hence the Indian Council Of Medical Research, New Delhi has recommended pool testing of five-sample pools in an area where COVID-19 prevalence is <5% [15]. Sample pooling conserves PCR Kits and consumables and greatly reduces the manpower required while also significantly increasing the testing capacity [16,17].

In this study we also evaluated the kits for pooled sampling and all the four kits (Qline, Lab Systems, Allplex and Huwel) scored sensitivity, specificity, NPV and PPV of 100% which is in accordance with other studies [14]. Hence all the 4 kits included in this study can be used for individual samples as well as for pooled sample testing.

While we have selected 4 of the commonly used kits for evaluation, this is but a small number of the approved and available kits available in India. The small sample size also serves as a limitation. A comprehensive review of all the available kits is needed for further standardization and approval of COVID 19 RT-PCR kits.

Authors and contributors

Aditya Chandrashekar: data analysis, statistical analysis, literature search, manuscript preparation, manuscript editing and manuscript review.

Ajay Philips: concept, design, definition of intellectual content, experimental studies, data acquisition.

Chetana G S : concept, design, definition of intellectual content, literature search, experimental studies, data acquisition, data analysis, manuscript editing and manuscript review.

Kala Yadhav M L(Guarantor): concept, design, experimental studies, data acquisition, data analysis, manuscript editing and manuscript review.

Volume 5, Issue 1; January-February 2022; Page No 613-621

References

1- WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/
2. Kim, D.; Lee, J.Y.; Yang, J.S.; Kim, J.W.; Kim, V.N.; Chang, H. The architecture of SARS-CoV-2 transcriptome. Cell-Press 2020, 181, 914–921.e10.
3. Bahrami, A.; Ferns, G.A. Genetic and pathogenic characterization of SARS-CoV-2: A review. Future Virol. 2020, 15.
4. Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.; et al. A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. 2020, 382, 727–733.
5. Buddhisha Udugama, Pranav Kadhiresan, Hannah N. Kozlowski, Ayden Malekjahani, Matthew Osborne, Vanessa Y. C. Li, Hongmin Chen, Samira Mubareka, Jonathan B. Gubbay, and Warren C. W. Chan. ACS Nano 2020 14 (4), 3822-3835 . DOI: 10.1021/acsnano.0c02624
6. Altamimi AM, Obeid DA, Alaifan TA, et al. Assessment of 12 qualitative RT-PCR commercial kits for the detection of SARS-CoV-2. J Med Virol. 2021;93(5):3219-3226. doi:10.1002/jmv.26900
7. Ambica Rangaiah, Sathyanarayan Muthur Shankar, Shantala Gowdara Basawarajappa, Pritik A Shah, Aditya Chandrashekar, Asha Munegowda, Shashiraja Padukone,
8. Detection of SARS-CoV2 in Clinical Samples: Target-specific Analysis of Qualitative Reverse Transcription–Polymerase Chain Reaction(RT-PCR) Diagnostic Kits,IJID Regions, 2021,ISSN 2772-7076, https://doi.org/10.1016/j.ijregi.2021.11.004.
9. Singh, J., Yadav, A.K., Pakhare, A. et al. Comparative analysis of the diagnostic performance of five commercial COVID-19 qRT PCR kits used in India. Sci Rep 11, 22013 (2021). https://doi.org/10.1038/s41598-021-00852-z
10. van Kasteren, P. B. et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J. Clin. Virol. 128, 104412 (2020).
11. Shen, L, Cui, S, Zhang, D, Lin, C, Chen, L, Wang, Q. Comparison of four commercial RT-PCR diagnostic kits for COVID-19 in China. J Clin Lab Anal 2021; 35:e23605. https://doi.org/10.1002/jcla.23605
12. Indian Council of Medical Research: Evidence Based Advisory on Correlation of COVID-19 Disease Severity with Ct Values of the Real Time RT-PCR Test (2020) https://www.icmr.gov.in/pdf/covid/techdoc/Advisory_on_correlation_of_COVID_severity_with_Ct_values.pdf
13. Rabaan, A. A., Tirupathi, R., Sule, A. A.,et al(2021). Viral Dynamics and Real-Time RT-PCR Ct Values Correlation with Disease Severity in COVID-19. Diagnostics, 11(6), 1091. https://doi.org/10.3390/diagnostics11061091
14. Rao SN, Manissero D, Steele VR, Pareja J. A Systematic Review of the Clinical Utility of Cycle Threshold Values in the Context of COVID-19. Infect. Dis. Ther. 2020;9:573–586. doi: 10.1007/s40121-020-00324-3
15. Garg A, Ghoshal U, Patel SS, et al. Evaluation of seven commercial RT-PCR kits for COVID-19 testing in pooled clinical specimens. J Med Virol. 2021;93(4):2281-2286. doi:10.1002/jmv.26691
16. Indian Council of Medical Research:Advisory on feasibility of using pooled samples for molecular testing of COVID-19. https://www.icmr.gov.in/pdf/covid/strategy/Advisory_on_feasibility_of_sample_pooling.pdf
17. -Praharaj I, Jain A, Singh M, et al. Pooled testing for COVID-19 diagnosis by real-time RT-PCR: A multi-site comparative evaluation of 5- & 10-sample pooling. Indian J Med Res. 2020;152(1 & 2):88-94. doi:10.4103/ijmr.IJMR_2304_20
18. Garg, J, Singh, V, Pandey, P, et al. Evaluation of sample pooling for diagnosis of COVID-19 by real time-PCR: A resource-saving combat strategy. J Med Virol. 2021; 93: 1526- 1531. https://doi.org/10.1002/jmv.26475
19. Banko,A.;Petrovic,G.; Miljanovic, D.; Loncar, A.; Vukcevic, M.; Despot, D.; Cirkovic, A. Comparison and Sensitivity Evaluation of Three Different Commercial Real-Time Quantitative PCR Kits for SARS-CoV-2 Detection. Viruses2021,13,1321. https:// doi.org/10.3390/v13071321

Volume 5, Issue 1; January-February 2022; Page No 613-621