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Original Article

Microbiology Section

Investigation of Different Commercially Available Real Time-Polymerase Chain Reaction Kits for SARS-CoV-2 Diagnosis

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ABSTRACT

Introduction: The whole world is facing an ongoing global health emergency of COVID-19 disease caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is a gold standard in the detection of SARS-CoV-2 infection. Presently, many single tube multiple gene target RT-PCR kits have been developed and are commercially available for Coronavirus Disease 2019 (COVID-19) diagnosis.

Aim: To evaluate the performance of seven COVID-19 RT-PCR kits (DiagSure, Meril, VIRALDTECT II, TruPCR, Q-line, Allplex and TaqPath) which are commercially available for COVID-19 RT-PCR diagnosis.

Materials and Methods: This observational study was conducted at the State Virology Laboratory (SVL), Gandhi Medical College, Bhopal, Madhya Pradesh, India. Seven commercially available kits have been evaluated on the basis of: (i) number of SARS-CoV-2 specific gene target; (ii) human housekeeping genes as internal control; (iii) RT-PCR run time; and (iv) kit performances to correctly detect SARS-CoV-2 positive and negative RNA samples. A total of 50 RNA samples (left over RNA) were included, master mix preparation, template addition and RT-PCR test has been performed according to kits literature. At the end of PCR run, mean and standard deviation of obtained cut-off of all kits were calculated using Microsoft Excel.

Results: All seven RT-PCR kits performed satisfactory regarding the reproducibility and they could correctly identify 30 positive and 20 negative RNA samples. RNA samples (group C) having low viral loads with a high Cycle threshold (Ct) value (>30) were also detected by all these seven kits. Obtained Ct values of each group was in parallel range in comparison with the initial testing Ct values. Kits were found to be superior which contains primers and probes for three SARS-CoV-2 specific gene targets, have human housekeeping gene as internal control and taking less time to complete RT-PCR.

Conclusion: All seven COVID-19 RT-PCR kits included in this study demonstrated satisfactory performance and can be used for the routine molecular diagnosis of COVID-19 disease.

Keywords: Coronavirus disease 2019, Genes, Ribonucleic acid, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

The whole world is facing an ongoing global health emergency of COVID-19 caused by the SARS-CoV-2 [1]. Till now no specific antiviral drug for the treatment of COVID-19 is available. However, recently Covishield and Covaxin vaccines have been approved for the emergency use to prevent SARS-CoV-2 infection in India [2]. During this pandemic, the incidences of cases have been increasing rapidly and therefore timely and accurate diagnosis of COVID-19 disease has become indispensable to stop the spread of SARS-CoV-2. This has resulted in an increased need for accurate diagnostic testing [3].

RT-PCR is considered as a gold standard in the detection of SARS-CoV-2 infection. SARS-CoV-2 expresses set of genes including the *ORF1ab gene* (open reading frame 1a and b 226), *N-gene* (the nucleocapsid protein), *E-gene* (envelope protein), *S-gene* (spike protein) and *RdRp gene* (RNA-dependent RNA polymerase), and these are the most common targets for RT-PCR assays [4].

The outcome of RT-PCR depends upon the performance of the RT-PCR kit being used. In the beginning of COVID-19 pandemic, RT-PCR testing was done in two steps: (i) screening test for *E* gene; and (ii) confirmatory test for either of *RdRp gene, N gene* and *ORF gene* [5]. Presently, many single tube multiple gene target RT-PCR kits have been developed and commercially available due to expanding pandemic and demand [6]. Indian Council of Medical Research (ICMR) has approved numerous multiplex RT-PCR kits for commercial use of SARS-CoV-2 testing in India [7]. Different testing laboratories are using different kits and the kits are being changed very frequently especially during the current COVID-19 crisis.

In the present scenario, the testing laboratories are facing main challenges in choosing the appropriate RT-PCR kits on the basis of performance which includes accuracy, duration of the run and compatibility with the RT-PCR machine available in the laboratory. It is a need of the hour to evaluate the performance of various RT-PCR kits and in this paper, we authors have assessed the performance of various COVID-19 RT-PCR kits and this would be helpful for COVID-19 testing laboratories to choose the appropriate kit.

MATERIALS AND METHODS

a. Selection of RT-PCR kits

This observational study was conducted at the State Virology Laboratory (SVL), Gandhi Medical College, Bhopal, Madhya Pradesh, India and the laboratory has been recognised as a COVID-19 RT-PCR testing centre by ICMR, India. Till date, the laboratory has used more than 15 RT-PCR kits for the testing and those were supplied through government agencies i.e., National Institute of Virology (NIV), ICMR, Pune and Madhya Pradesh Public Health Services Cooperation Limited (MPPHSCL), Bhopal, Madhya Pradesh, India. For the present study, seven different COVID-19 RT-PCR kits were selected on the basis of multiple SARS-CoV-2 specific gene targets in a single tube with simultaneous detection of each target on different detection channel. Present study was carried in a period of a month time (October 2020) and none of the manufacturers were involved in the assessment and interpretation of the study results.

The following seven kits were included for the study [Table/Fig-1]: (1) DiagSure nCOV-19 Detection assay (GCC Biotech Pvt., Ltd., India); (2) Meril COVID-19 One-step RT-PCR Kit (Meril Diagnostics Pvt.,

Kit name/Manufacturer/Country	Assay format	SARS-CoV-2 genes	SARS-CoV-2 specific genes	Fluorescence probe	Control gene if any	Fluorescence probe
DiagSure nCOV-19 Detection assay/GCC Biotech Pvt. Ltd, 24 Parganas, West Bengal, India	Multiplex (Single Tube)	02	Orf 1ab	FAM	IC	Cy5
			E	HEX/VIC		
Meril COVID-19 One-step RT-PCR Kit/Meril Diagnostics Pvt Ltd. India	Multiplex (Single Tube)	02	Orf 1ab	FAM	IC	ROX
			N	HEX		
TruPCR SARS-CoV-2 RT-qPCR Kit/Kilpest India Ltd. India	Multiplex (Single Tube)	02	N	FAM	RNase P	HEX/VIC
			E	ROX		
Q-line Molecular Coronavirus (COVID19) RT-PCR kit/POCT Services Pvt. Limited, Lucknow, India	Multiplex (Single Tube)	02	Orf 1ab	FAM	IC	ROX
			N	HEX/VIC		
VIRALDTECT II Multiplex real time RTPCR for COVID-19/Genes2me Pvt Ltd, Gurugram, Haryana, India	Multiplex (Single Tube)	03	N	Cy5	RNase P	HEX/VIC
			RdRp	ROX		
			E	FAM		
Allplex 2019-nCoV assay/Seegene, (South Korea)	Multiplex (Single Tube)	03	N	Quasar 670	IC	HEX
			RdRp	Cal Red 610		
			E	FAM		
TaqPath COVID-19 Combo Kit/Applied Bio-Systems (USA)	Multiplex (Single Tube)	03	N	VIC	MS2	JUN
			Orf 1ab	FAM		
			S	ABY		

[Table/Fig-1]: Summary of various RT-PCR kits evaluated in the study.
Fam: 6-carboxyfluorescein; Hex: Hexachloro-fluoresceine; Vlc: 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein; Rox: Carboxy-X-Rhodamine; Cy5: Cyanine5; Ic: Internal control; Ms2: Bacteriophage-*emesvirus zinderi*

Ltd., India); (3) TruPCR SARS-CoV-2 RT-qPCR Kit (Kilpest India Ltd., India); (4) Q-line Molecular Coronavirus (COVID-19) RT-PCR kit (POCT Services Pvt., Ltd., India); (5) VIRALDTECT II Multiplex real time RT-PCR for COVID-19 (Genes2me Pvt., Ltd., India); (6) Allplex 2019-nCoV assay (Seegene, South Korea); and (7) TaqPath COVID-19 Combo Kit (Applied Bio-Systems, USA).

b. Sources of specimen

This study included left over RNA (RNA samples were stored at -80° C in deep freezer) from the clinical specimens, which were tested earlier in the laboratory as part of routine diagnostics using (NIV Pune supplied) two step PCR kit; (i) screening test for *E gene and RNP gene;* and (ii) confirmatory test for *RdRp gene* and *ORF-1ab gene.*

Sample size for this study was selected by analysing previous related research work done by other scientific groups [8-10]. In this study, 30 different cut-off range SARS-CoV-2 positive RNA samples and 20 negative RNA samples were included. RNA samples with inconclusive test results were excluded in this study. A total of 50 RNA samples (left over RNA) included: (i) Group A: 10 positive RNA samples with high viral loads with a low cut-off threshold (<20); (ii) Group B: 10 positive RNA samples with a medium cut-off threshold (21-30); (iii) Group C: 10 positive RNA samples with low viral loads with a high Ct value (>30); and (iv) Group D: 20 negative RNA samples were included. These selected groups were tested for SARS CoV-2 by using seven different RT-PCR kits.

c. Real Time-PCR Assay

Earlier the RNA was extracted using HiPurA Viral RNA purification kit (HiMedia, Mumbai, India). All RT-PCR tests were performed on QuantStudio 7 Flex (Applied Biosystems, CA, USA) or CFX96 Touch (BIO-RAD, CA, USA) and thermocycling settings and result interpretation was performed as per manufacturer's instructions. Positive and negative controls of each kit were included for PCR run validation. RT-PCR instrument set up of each kit is summarised in [Table/Fig-2].

STATISTICAL ANALYSIS

Ct values of all the genes of the RT-PCR kits were recorded. Mean and Standard Deviation (SD) of the tested genes were analysed using Microsoft Excel 2010.

RESULTS

All seven RT-PCR kits were evaluated with four different groups of known RNA samples. Obtained cut-off threshold values of each group were recorded. Mean and SD of the Ct value of the individual tested genes were calculated for the analysis of the results [Table/Fig-3]. All RT-PCR kits performed satisfactorily regarding the reproducibility and they could correctly identify 30 positive and 20 negative RNA samples. RNA samples (group C) having low viral loads with a high Ct value (>30) were also detected by all these seven kits. Obtained Ct values of each group was in parallel range in comparison with the initial testing Ct values.

PCR run time and other kit parameters were determined for each RT-PCR kits [Table/Fig-2]. As compared to previously used two step PCR kit (screening and confirmatory tests), all these single tube multiplex kits were found to be user friendly, time and resource saving. Among all the RT-PCR kits evaluated, TaqPath COVID-19 combo kit was able to complete the PCR run in the least time (~67 minute) and this is the only kit targeting the *S gene* of SARS-CoV-2 besides *ORF-1ab and N gene.* Most of the genes included in the kits are labelled with basic flurophores that can be detected in the most of the RT-PCR platforms, while Allplex 2019-nCoV assay kit and TaqPath COVID-19 combo kit need higher end RT-PCR instruments having wider fluorescence detection range. Those kits contain human housekeeping gene as internal control having advantage over other kits contains normal internal control.

DISCUSSION

The present SARS-CoV-2 pandemic resulted in the quick setup of laboratories for the COVID-19 molecular diagnosis. The correct diagnosis is more important to identify, control and break the chain of SARS-CoV-2 transmission. Poor diagnosis may lead to false negative test which increases the spread of infection and false positive result may lead to the unnecessary treatment and mental trauma to the patients and their families [11].

RT-PCR is a gold standard test for COVID-19 diagnosis and the result outcome depends upon the performance of the RT-PCR kit being used. Till date, ICMR, New Delhi has evaluated the performance of 321 COVID-19 RT PCR kits and 147 kits performance were found satisfactory [12]. In-house performance assessment of RT-PCR kits in COVID-19 testing laboratory is still limited. However, some studies evaluated performance of COVID-19 RT-PCR kits in single and pooled clinical specimens [8-10,13].

Outcome of this study indicate detection of SARS-CoV-2 was comparable by all RT-PCR kits and kits performed satisfactorily regarding the reproducibility. Authors found that all kits performed similarly in all

Steps			DiAGSure kit		Meril kit		TruPCR kit		Q-line molecular kit		VIRALDTECT II kit		Allplex kit		Taqpath kit	
UNG incubation		Temp	NA		NA		NA		NA		NA		NA		25	1 Cycle
		Time													02	
Reverse transcription		Temp (°C)	50	1 Cycle	50	1 Cycle	50	1 Cycle	50	1 Cycle	55	1 Cycle	50	1 Cycle	53	1 Cycle
		Time (Min.)	15		15		15		15		10		20		10	
Initial denaturation		Temp (°C)	95	1 Cycle	95	1 Cycle	95	1 Cycle	95	1 Cycle	95	1 Cycle	95	1 Cycle	95	1 Cycle
		Time (Min.)	10		03		05		03		03		15		02	
PCR	Amplification	Temp (°C)	95	40 Cycle	95	45 Cycle	95	38 Cycle	95	45 Cycle	95	40 Cycle	94	45 Cycle	95	40 Cycle
		Time (Sec.)	10		15		05		15		15		15		03	
	Data collection (Fluorescence detection)	Temp (°C)	60		55		60		55		60		58		60	
		Time (Sec.)	40		40		40		40		60		30		30	
		Temp	NA		NA		72		NA		NA		NA		NA	
		Time					15									
	Cooling	Temp	NA		25	-	NA		NA		NA		NA		NA	
		Time			10											
Master mix volume (µL)			16		10		15		10		11		17		20	
Template (RNA) volume (µL)			10		10		10		10		9		8		5	
Total reaction volume (µL)			26		20		25		20		20		25		25	
Approximate RT-PCR (Min.) run time			~84		~96		~87		~96		~93		~104		~67	
Threshold Cut-off cycle (Ct)			≤36		≤40		36		≤40		≤37		≤40		≤37	

[Table/Fig-2]: Amplification program scheme of different RT-PCR kits.
UNG: Uracil N-glycosylase; PCR: Polymerase chain reaction; Min: Minute; Sec: Second; NA: Not applicable

Initial Ct of *Orf gene* (Detected by NIV, Pune Kit)	SARS-CoV-2 genes	DiAGSure kit	Meril kit	TruPCR kit	Q-line molecular kit	VIRALDTECT II kit	Allplex kit	Taqpath kit
		Cut-off threshold (Ct) Mean						
<20 (18±2)	E gene	19.05	-	16.98	-	19.20	17.31	-
	RdRp gene	-	-	-	-	18.26	20.14	-
	Orf 1ab gene	16.61	17.07	-	16.48	-	-	16.74
	N gene	-	19.30	17.73	18.99	17.91	19.09	17.56
	S gene	-	-	-	-	-	-	18.29
21-30 (22±2)	E gene	22.26	-	21.10	-	23.86	19.14	-
	RdRp gene	-	-	-	-	22.47	22.87	-
	Orf 1ab gene	23.31	22.55	-	22.04	-	-	22.18
	N gene	-	24.48	22.19	24.76	22.33	21.04	23.38
	S gene	-	-	-	-	-	-	22.96
>30 (32±2)	E gene	31.27	-	31.31	-	31.33	27.73	-
	RdRp gene	-	-	-	-	31.16	32.20	-
	Orf 1ab gene	32.17	32.16	-	31.97	-	-	31.83
	N gene	-	32.92	32.21	33.66	31.08	31.17	32.74
	S gene	-	-	-	-	-	-	31.98

[Table/Fig-3]: Result showing mean Cut-off threshold (Ct value) of positive RNA samples detected by different RT-PCR kits.

low, medium and high Ct group (100% sensitivity and 100% specificity). Authors have not included inconclusive RNA samples as our key purpose of this study was to evaluate kits performance among each other and our analysis indicates that all RT-PCR tests look good to diagnose and differentiate COVID-19 positive and negative samples.

Four of the selected kits (DiagSure, Meril, TruPCR and Q line) contain two sets of SARS-CoV-2 specific gene primers and probes while VIRALDTECT, Allplex and Taqpath kits are containing three sets of SARS-CoV-2 specific gene primers and probes including internal control gene. Kits having three SARS-CoV-2 specific gene targets are superior than two gene targets as some studies indicate mutation of SARS-CoV-2 genome [14,15] as RNA viruses have high mutation rates [16] which leads to false-negative results and that can be reduced by targeting more sequences within the viral genome [17]. Most recently, a new SARS-CoV-2 variant (SARS-CoV-2 VOC 202012/01) has been identified in United Kingdom which is believed to be more infectious [18]. However, at present only sequencing method is available for the detection of the same [19-21].

The final outcome of the RT-PCR results also depends upon the proper sample collection of nasopharyngeal and throat swab. Inappropriate sample collection and improper sample transportation temperature will eventually influence the entire process and leads to false results [22-24]. These false results can be avoided by using human housekeeping gene as an internal control in RT-PCR kits. Meril, Q line, VIRALDTECT II and TruPCR kits contains primers and probes for endogenous internal control and have an additional advantage to find out proper sample collection and RNA extraction process.

During this outbreak situation, thousands of samples are collected and being sent to the laboratories for the diagnosis of COVID-19 and most labs do not have sufficient space to store thousands of samples at a time and improper storage leads to sample degradation which increases the chance of false results. Hence, prompt processing of this sample is recommended. Turnaround time is purely depending

upon the sample processing and the RT-PCR run time. RT-PCR kits having shorter run time will reduce the turnaround time. In this study, authors have observed that RT-PCR run time of kits ranged between approx one to two hours. TaqPath COVID-19 combo kit able to complete the PCR run in the least time compared with other kits.

Quant Studio 7 Pro and BIO-RAD CFX96 Touch Real-Time PCR instruments were used for this study and both were calibrated with the most of the fluorescent dyes generally used in PCR application like FAM, HEX/VIC, ROX, Cy5, Texas Red etc., [25,26]. However, normally many labs do not have such high-end calibrated PCR instruments and they further need to compensate the same with additional cost burden. This study can help to the laboratories engaged in COVID-19 testing for selecting appropriate kits which best suit the machine available in their laboratory.

Limitation(s)

This study has some limitations. First, authors have received kits free of cost for SARS-CoV-2 diagnosis from NIV, Pune via NIREH, Bhopal and later on from MPPHSCL, Bhopal, Madhya Pradesh consequently were not able to compare the cost effectiveness of RT-PCR kits on the basis of cost per test. Second, this evaluation used small sample size and total 50 RNA sample (30 SARS-CoV-2 positive RNA sample with three different Ct range and 20 negative RNA sample) were chosen in this study and aimed to check whether all kits were able to detect positive and negative sample equally and correctly.

CONCLUSION(S)

It was concluded that all commercially available RT-PCR kits included in this study can be used for the routine molecular diagnosis of COVID-19. Considering high sample load per day, it might be advisable to use those kits having less RT-PCR run time for timely diagnosis of symptomatic COVID-19 patients.

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REFERENCES

[1] Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature. 2020;583(7816):459-68.
[2] COVID-19 vaccine tracker. https://www.raps.org/news-and-articles/news-articles/2020/3/covid-19-vaccine-tracker.
[3] Premraj A, Aleyas AG, Nautiyal B, Rasool TJ. Nucleic acid and immunological diagnostics for SARS-CoV-2: Processes, platforms and pitfalls. Diagnostics (Basel). 2020;10(11):866.
[4] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045.
[5] Gupta N, Potdar V, Praharaj I, Giri S, Sapkal G, Yadav P, et al. Laboratory preparedness for SARS-CoV-2 testing in India: Harnessing a network of Virus Research & Diagnostic Laboratories. Indian J Med Res. 2020;151(2-3):216-25.
[6] Dharavath B, Yadav N, Desai S, Sunder R, Mishra R, Ketkar M. A one-step, one-tube real-time RT-PCR based assay with an automated analysis for detection of SARS-CoV-2. Heliyon. 2020;6(7).
[7] Kits validation and batch testing. https://www.icmr.gov.in/ckitevaluation.html.
[8] van Kasteren PB, van der Veer B, van den Brink S, Wijsman L, de Jonge J, van den Brandt A, et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J Clin Virol. 2020;128:01-05.
[9] Garg A, Ghoshal U, Patel SS, Singh DV, Arya AK, Vasanth S, et al. Evaluation of seven commercial RT-PCR kits for COVID-19 testing in pooled clinical specimens. J Med Virol. 2020 ;93(4):2281-86. https://doi.org/10.1002/jmv.26691.
[10] Wang M, Chen D, Wu W, Tang H, Kan L, Zong Z, et al. Analytical performance evaluation of five RT-PCR kits for severe acute respiratory syndrome coronavirus 2. J Clin Lab Anal. 2020;35(1):e23643. Doi: 10.1002/jcla.23643.
[11] Mathuria JP, Yadav R, Rajkumar. Laboratory diagnosis of SARS-CoV-2- A review of current methods. J Infect Public Health. 2020;13(7):901-05.
[12] Performance evaluation of commercial kits for real time PCR for COVID-19 by ICMR identified validation centres. https://www.icmr.gov.in/pdf/covid/kits/RT_PCR_Tests_Kits_Evaluation_Summ_28122020.pdf.
[13] 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. 2020;35(1):e23605. https://doi.org/10.1002/jcla.23605.
[14] Sun J, He WT, Wang L, Lai A, Ji X, Zhai X, et al. COVID-19: Epidemiology, evolution, and cross-disciplinary perspectives. Trends Mol Med. 2020;26(5):483-95.
[15] Phan T. Genetic diversity and evolution of SARS-CoV-2. Infect Genet Evol. 2020;81:104260. 10.1016/j.meegid.2020.104260.
[16] Sanjuán R, Domingo-Calap P. Mechanisms of viral mutation. Cell Mol Life Sci. 2016;73(23):4433-48.
[17] Abduljalil JM. Laboratory diagnosis of SARS-CoV-2: Available approaches and limitations. New Microbes New Infect. 2020;36:100713.
[18] SARS-CoV-2 variants. https://www.who.int/csr/don/31-december-2020-sars-cov2-variants/en/.
[19] Bal A, Destras G, Gaymard A, Stefic K, Marlet J, Eymieux S, et al. Two-step strategy for the identification of SARS-CoV-2 variant of concern 202012/01 and other variants with spike deletion H69-V70, France, August to December 2020. Euro Surveill. 2021;26(3):2100008. Doi:10.2807/1560-7917.ES.2021.26.3.2100008.
[20] Sequencing of SARS-CoV-2- first update. https://www.ecdc.europa.eu/en/publications-data/sequencing-sars-cov-2.
[21] Genomic Surveillance for SARS-CoV-2 In India Indian SARS-CoV-2 Genomics Consortium (INSACOG). https://www.mohfw.gov.in/pdf/IndianSARSCoV2PDFGenomicsConsortiumGuidanceDocument.pdf.
[22] Kinloch NN, Ritchie G, Brumme CJ, Dong W, Dong W, Lawson T, et al. Suboptimal biological sampling as a probable cause of false-negative COVID-19 diagnostic test results. J Infect Dis. 2020;222(6):899-902.
[23] Higgins TS, Wu AW, Ting JY. SARS-CoV-2 nasopharyngeal swab testing—false-negative results from a pervasive anatomical misconception. JAMA Otolaryngol Head Neck Surg. 2020;146(11):993-94.
[24] Loeffelholz MJ, Tang YW. Laboratory diagnosis of emerging human coronavirus infections- The state of the art. Emerg Microbes Infect. 2020;9:747-56.
[25] QuantStudio™ 6 and 7 Flex Real-Time PCR Systems. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/4489821.pdf.
[26] CFX96 and CFX384 Real-Time PCR Detection systems. https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10010424.pdf.

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