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

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Abstract:

Introduction: - At present the whole world is facing pandemic of the Corona virus disease (COVID-19) caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). The knowledge of available laboratory methods is essential for early and correct diagnosis of COVID-19 to identify new cases as well as monitoring treatment of confirmed cases.

Aim: - To compare basic analytical and clinical performance of selected RT-PCR kits from Three different manufacturers (LaboGun, Gene Store, TruPCR).

Materials and Methods: Three 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.

Result: In this study all three RT-PCR kits performed satisfactory regarding the reproducibility and they could correctly identify RNA samples. RNA samples having low viral loads with a high Cycle threshold (Ct) value (>30) were also detected by all these three 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 who 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 three 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: Corona virus disease 2019, Genes, Ribonucleic acid, severe acute respiratory syndrome coronavirus-2, cyclic threshold.

Introduction

Corona virus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2).[1]. In December 2019 a major outbreak from Wuhan, was reported in the World Health Organization (WHO) country office of China. The COVID-19 is a highly infectious disease and after reporting the first case in china it has rapidly covered the entire world and affected the life of millions people. [2] Corona viruses 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 corona virus structural proteins, including the envelope (E), nucleocapsid (N), and spike (S) proteins, are expressed via the production of sub genomic 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. [3]. the COVID-19 Infected patients have clinical manifestation which includes the fever and cough as primary clinical presentations and others are shortness of breath and myalgia etc. Some patients may have serious complications such as acute respiratory distress syndrome (ARDS) and cytokine storm, which may leads to death [4]. 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 [5]. 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 [6]. 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, an independent assessment of these products is not yet publicly available and direly needed to guide implementation of accurate tests in a diagnostic market that is flooded with new tests.[3,7] Commercially available COVID-19 RT-PCR kits were identified via the Find Dx website (www.finddx.org/covid-19/pipeline, March 2020) and requests for information and sample kits were sent via e-mail to approximately 20 manufacturers and/or

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distributors, focusing on those kits that had already obtained CE-IVD certification. Promising commercial kits were selected based on: 1) listing on the Find Dx website; 2) responsiveness to requests; 3) accessible information (in English); 4) compatibility with different PCR platforms; [5} Considerable production capacity. Notably, all of the PCR kits that we had selected for our analysis have in the meantime also been selected for the first round of independent evaluation by Foundation for Innovative New Diagnostics (FIND). [8] This study aimed was, to compare basic analytical and clinical performance of selected RT-PCR kits from three different manufacturers (Labo Gun, Gene Store, Tru PCR) which are commercially available for COVID-19 RT-PCR diagnosis.

Material and Methods

Study Setting: This study was be conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur.
Samples from already known patient related to covid-19 disease were being collected from Rama Medical College Hospital and Research Centre as the source of the sample for the study.
Study Design: Prospective study.
Type Of Study: Observational study.
Study Period: This study will be conducted from 2020 to 2021.
Size of Sample: Total 50 known samples out from 30 positive patient and 20 negative patients already tested by RT- PCR kit for Covid -19 tests.
Inclusion Criteria: All the patients who were having symptoms related to covid-19 disease and confirm by RTPCR either or positive or negative.
Excision Criteria: A symptomatic patient was excluded from the study.
Ethical Consideration: Ethical clearance will be taken from the institutional ethical committee.

Selection of RT-PCR Kits

For the present study, three 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. The following three kits were included for the study [Table/Fig-1]: (1) LaboGun, (2) GeneStore, (3) TruPCR SARS-CoV-2 RT-qPCR Kit (Kilpest India Ltd., India)

Sources Of Sample: This study included left over RNA (RNA samples were stored at -80o C in deep freezer) from the clinical specimens, which were tested earlier in the laboratory as part of routine diagnostics using 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: A total of 50 RNA samples included: 30 different cut-off range SARS-CoV-2 positive RNA samples and 20 negative RNA samples were included.

1) Group A: 10 positive RNA samples with high viral loads with a low Ct- value. (<20)
2) Group B: 10 positive RNA samples with a medium Ct- value. (21-30)
3) Group C: 10 positive RNA samples with low viral loads with a high Ct- value (31- 35)
4) Group D: 20 negative RNA samples were included.

These selected groups were tested for SARS CoV-2 by using six different RT-PCR kits.

Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR)

Earlier the RNA was extracted using Hi PurA Viral RNA purification kit (Hi Media, Mumbai, India). All RT-PCR tests were performed on CFX96 Real Time PCR & CFX Maestro software (BIO-RAD, CA, USA) and thermo cycling settings and result interpretation was performed as per manufacturer's instructions. Positive and negative controls of each kit were included for PCR run validation.

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 three 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 three 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 labeled with basic flurophores that can be detected in the most of the RT-PCR platforms. Those kits contain human housekeeping gene as internal control having advantage over other kits contains normal internal control.

[Table/Fig-1]: Summary of various RT-PCR kits evaluated in the study.

Kit name/manufacturer /country	Assay format	SARS-CoV-2 genes	SARS-CoV-2 specific genes	Fluorescence probe	Control gene if any	Fluorescence probe
LaboGun COVID- 19 One-step RT- PCR Kit/Meril Diagnostics Pvt Ltd. India	Multiplex (Single Tube)	2	RdRp	FAM	IC	HEX/ VIC
			E	Cy5		
TruPCR SARS- CoV-2 RT-qPCR Kit/Kilpest India Ltd. India	Multiplex (Single Tube)	2	N	FAM	R Nase P	HEX/VIC
			E	ROX		
Gene Store Corona virus (COVID19) RT- PCR kit/POCT Services Pvt. Limited, Lucknow, India	Multiplex (Single Tube)	2	N1	FAM	IC	Cy5
			N2	HEX		

[Table/Fig-2]: Amplification program scheme of different RT-PCR kits.

Steps			Labo Gun kit		Tru PCR kit		Gene Store kit	
UNG incubation		Temp	NA		NA		NA	
		Time						
Reverse transcription		Temp	50	1 cycle	50	1 cycle	42	1 cycle
		Time	30		15		5	
Initial denaturation		Temp (C)	95	1 cycle	95	1 cycle	95	1 cycle
		Time(Min)	15		5		5	
PCR	Amplification	Temp (C)	95	45 cycle	95	38 cycle	95	39 cycle
		Time (Sec)	15		5		15	
	Data collection (Fluoresce NCE detection)	Temp (C)	60		60		58	
		Time (Sec)	60		40		30	
		Temp (C)	NA		72		NA	
		Time (Sec)			15			
	Cooling	Temp (C)	NA		NA		NA	
		Time (Sec)						
Master mix volume (µL)			15		15		16.5	
Template (RNA) volume (µL)			5		10		3.5	
Total reaction volume (µL)			20		25		20	
Approximate RT-PCR (Min.) run time			90		87		80	
Threshold Cut-off cycle (Ct)			40		36		40	

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

Initial Ct (Detected by NIV, Pune Kit)	SARS-CoV-2 genes	Lab Gun kit	Tru PCR kit	Gene Store kit
Cut-off threshold (Ct) Mean				
<20 (18±2)	E gene	16.82	16.38	--
	RdRp gene	18.39	--	--
	N gene	--	18.61	19.12
21-30 (22±2)	E gene	24.32	23.54	--
	RdRp gene	26.76	--	--
	N gene	--	25.1	24.56
>30 (32±2)	E gene	34.78	33.67	--
	RdRp gene	33.67	--	--
	N gene	--	34.96	34.67

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 [9].

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 [10]. 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 [11-12, 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 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.

Conclusion

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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