Performance Evaluation of Different RT-PCR Kits for the Direct Detection of SARS-CoV-2 in Preheated Specimens

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Abstract

Background Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has created high demand for molecular kits and consumables for mass screening of suspected individuals. Direct real-time polymerase chain reaction (RT-PCR) assay without nucleic acid extraction has several advantages in saving testing time and cost and helps in the rapid reporting of SARS-CoV-2. The present study evaluated the analytical performance of four SARS-CoV-2 RT-PCR for direct RT-PCR testing using preheated specimens.

Methods A total of 100 clinical specimens were selected and divided into three different groups: (1) group I: 20 SARS-CoV-2 positive specimens with high viral load, viz., low Ct values (< 30 Ct), (2) group II: 50 SARS-CoV-2 positive specimens with low viral load, viz., high Ct values (> 30 Ct), and (3) group III: 30 SARS-CoV-2 negative specimens. Specimens were heat-inactivated at 70°C for 10 minutes and cooled down at 4°C and were evaluated for standard and direct RT-PCR method by using ViralDtect-II Multiplex Real-Time PCR kit, TaqPath COVID-19 Combo kit, COVIDsure Pro Multiplex RT-PCR kit, and Hi-PCR Coronavirus (COVID-19) Multiplex Probe PCR kit.

Results Results showed that except ViralDtect-II kit, the other three TaqPath COVID-19 Combo kit, COVIDsure Pro kit, and Hi-PCR Coronavirus (COVID-19) RT-PCR kit were able to amplify all the SARS-CoV-2 genes in the direct RT-PCR method using preheated specimens. In group I specimens, 100% sensitivity was observed in all three RT-PCR kits. In group II specimens, COVIDsure Pro kit was found to be superior among other kits.

Conclusion Direct RT-PCR method during pandemic situation is valuable and cost effective for the detection of SARS-CoV-2. All three TaqPath COVID-19 Combo kit, COVIDsure Pro kit, and Hi-PCR Coronavirus (COVID-19) RT-PCR kit can be used for direct RT-PCR method and COVIDsure Pro kit performance was found to be superior among all.

Keywords
- COVID-19 disease
- direct RT-PCR
- preheated
- RNA extraction
- SARS CoV-2

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Introduction

The current pandemic of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected approximately 600 million people worldwide with more than 6.5 million death till date.[1,2] Early detection, isolation, and contact tracing are important measures which play a crucial role in controlling the disease spread. During this pandemic, the incidences of cases are rapidly increasing due to the high infectivity and transmissibility rates of new SARS-CoV-2 variants.[3–5] The rise of COVID-19 cases, resulted in the mass screening and testing of suspected individuals. Massive sampling for the SARS-CoV-2 detection leads to increased sample load for the testing laboratory and resulted in high demand for molecular testing kits and consumables.[6–8]

Different approaches like pooling of the specimens,[9] direct real-time polymerase chain reaction (RT-PCR) without nucleic acid extraction,[10] and SARS-CoV-2 detection in saliva[11] have been evaluated and being practiced for the COVID-19 molecular diagnosis to save time and cost involved in it. Detection of viral pathogens without nucleic acid extraction has been previously described for norovirus,[12] human papillomavirus,[13] and Zika virus[14] and most recently for the SARS-CoV-2.[15–19] This method has several advantages in saving testing time and cost and helps in the timely reporting of SARS-CoV-2 diagnosis.

In the present study, the performance of four commercially available SARS-CoV-2 RT-PCR kits were evaluated by using preheated COVID-19 specimens for the direct RT-PCR testing without performing the ribonucleic acid (RNA) extraction processes. The direct RT-PCR method significantly reduced the total testing time involved in specimen preparation, RNA extraction, purification, RT-PCR test, and minimizing total cost and the risk of errors involved in these steps.

Materials and Methods

COVID-19 RT-PCR Kits

To check the compatibility and the performance of RT-PCR assay by using preheated COVID-19 specimens, the following four different, single-tube, multiplex SARS-CoV-2 RT-PCR kits were used (►**Table 1**): (1) VIRALDTECT-II Multiplex Real-Time PCR kit for COVID-19 (Genes2me Pvt Ltd, India), (2) TAQPATH COVID-19 Combo kit (Applied Bio-Systems, United States), (3) COVIDSURE PRO Multiplex RT-PCR kit (Labsystems Diagnostics, India), and (4) HI-PCR Coronavirus (COVID-19) Multiplex Probe PCR kit (HIMEDIA, India). The abovementioned RT-PCR kits were being used for routine diagnosis during the study period. Hence, they have been used in this study for evaluation. None of the kit manufacturers were involved in the conception, assessment, and interpretation of the study results.

Specimens

Clinical nasopharyngeal specimens from symptomatic individuals were collected in a viral transport medium (VTM) and sent to the laboratory for the routine diagnosis of COVID-19 disease. Archived VTM samples were retrieved from a –80°C deep freezer and they were selected on the basis of different threshold cycle (Ct) value ranges and used for this comparative evaluation study. A total of 100 clinical specimens were selected and divided into three different groups: (1) group I: 20 SARS-CoV-2 positive specimens with high viral load, viz., low Ct values (< 30 Ct), (2) group II: 50 SARS-CoV-2 positive specimens with low viral load, viz., high Ct values (> 30 Ct), and (3) group III: 30 SARS-CoV-2 negative specimens.

Table 1 Summary of various RT-PCR kits evaluated in the study

Kit name / Batch no/ Expiry date / Manufacturer	SARS-CoV-2 specific genes		Fluorescence probe	Control gene if any	Fluorescence probe
VIRALDTECT II Multiplex Real-Time PCR for COVID-19 / G2M020220/07–2021 Genes2me Pvt Ltd, Gurugram, Haryana, India	03	*N gene*	CY5	*RNase P gene*	HEX/VIC
		RdRp gene	TEXAS RED		
		E gene	FAM		
TAQPATH COVID-19 Combo Kit /SKU#A47814/08–2021/Applied Bio-Systems / USA	03	*N gene*	VIC	MS2	JUN
		Orf 1ab gene	FAM		
		S gene	ABY		
COVIDSURE PRO Multiplex RT-PCR Kit /COVDP500–6/11–2021/ Labsystems Diagnostics / India	03	*E gene*	FAM	IC (Human gene)	CY5
		N gene	TEXAS RED		
		Orf 1ab gene	HEX		
HI-PCR Coronavirus (COVID-19) Multiplex Probe PCR Kit / MBPCR243/04–2022/HIMEDIA / India	03	*N gene*	FAM	*RPPH1 gene*	JOE/HEX
		RdRp gene	CY5		
		E gene	TEXAS RED		

Abbreviations: RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 2 Reaction mix preparation and RT-PCR instrument set-up of kits

			VIRALDTECT II kit		Taqpath kit		Covidsure kit		Hi-PCR kit	
PCR reaction mix			11 µL		20 µL		17 µL		20 µL	
Template (RNA) / inactivated specimen			9 µL		5 µL		8 µL		5 µL	
Total reaction volume			20 µL		25 µL		25 µL		25 µL	
Real-time PCR program setup										
UNG incubation		Temp (°C)	NA		25	1 cycle	NA		NA	
		Time (min)			02					
Reverse transcription		Temp (°C)	55	1 cycle	53	1 cycle	45	1 Cycle	50	1 cycle
		Time (min)	10		10		20		15	
Initial denaturation		Temp (°C)	95	1 cycle	95	1 cycle	95	1 Cycle	95	1 cycle
		Time (min)	03		02		03		03	
PCR	Amplification	Temp (°C)	95	40 cycles	95	40 cycles	95	45 Cycle	95	40 cycles
		Time (s)	15		03		15		15	
	Data collection (fluorescence detection)	Temp (°C)	60		60		63		58	
		Time (s)	60		30		30		30	
Approximate RT-PCR run time			~93 min		~67 min		~89 min		~ 80 min	
Threshold cutoff cycle (Ct)			≤ 37		≤ 37		≤ 40		≤ 38	

Abbreviations: NA, not available; RNA, ribonucleic acid; RT-PCR, real-time polymerase chain reaction; UNG, uracil-DNA glycosylase.

Direct RT-PCR Assay

For the detection of SARS-CoV-2 by direct RT-PCR method, 200 µL aliquots from the specimens were heat-inactivated at 70°C for 10 minutes and cooled down at 4°C. These preheated specimens were directly used as RNA templates. All the preheated specimens were directly used for the amplification and the detection of SARS-CoV-2 by the abovementioned four different RT-PCR kits in triplicates. Respective controls were included in all the assay procedures and result analysis was done as per kit instructions. The RT-PCR instrument setup of each kit is summarized in ►**Table 2**. For the comparative analysis, the standard RNA extraction was performed using an automated RNA extractor Genolution Nextractor NX-48S instrument and NX-48S Viral NA kit according to the manufacturer's instructions and a standard RT-PCR assay was performed using ViralDtect-II Multiplex Real-Time PCR kit for COVID-19 (Genes2me Pvt. Ltd, India).

Statistical Analysis

Ct values of all SARS-CoV-2 genes of all four RT-PCR kits were recorded and analyzed. Percentage of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy with their 95% confidence intervals (CIs) were calculated by using online statistical software Med Calc Version 20.011.

Results

A total of 100 clinical specimens (group I: 20 specimens [< 30 Ct], group II: 50 specimens [> 30 Ct], and group III: 30 negative specimens) were evaluated for standard and direct RT-PCR method. Results showed that except ViralDtect-II Multiplex Real-Time PCR kit, all other three RT-PCR kits used in the study (TaqPath COVID-19 Combo kit, COVIDsure Pro Multiplex RT-PCR kit, and Hi-PCR Coronavirus (COVID-19)) Multiplex Probe PCR kit) were able to amplify all the SARS-CoV-2 genes as well as respective kit internal controls using preheated specimens in the direct RT-PCR method.

No concordances were observed among group I (< 30 Ct) specimens in both standard and direct RT-PCR methods (►**Table 3**). However, a slight variation of the Ct values was observed in the direct RT-PCR method as compared with that of the standard method. Results of group I established the performance of direct RT-PCR tests in low Ct and high viral load clinical specimens. Note that 100% sensitivity was observed in all three RT-PCR kits with no false-negative results recorded.

Further, the performance of RT-PCR kits was evaluated for the direct RT-PCR method in 50 borderline SARS-CoV-2 positive specimens (group II Ct > 30 and low viral load) and 30 SARS-CoV-2 negative specimens (group III) and the results were compared with that of the standard method.

Out of 50 specimens of group II (> 30 Ct), 14 specimens showed complete amplification of SARS-CoV-2 genes in all three RT-PCR kits in the direct RT-PCR method. In group II specimens, COVIDsure Pro Multiplex RT-PCR kit was found to be superior among other kits and was able to detect the highest number ($n = 44$) of borderline positive specimens in the direct PCR method as compared with the TaqPath COVID-19 Combo kit and Hi-PCR Coronavirus (COVID-19) kits which were able to detect only 26 and 24 SARS-CoV-2 borderline positive specimens, respectively (►**Table 4**).

Table 3 Validation of the direct RT-PCR test with low Ct value SARS-CoV-2 positive samples ($n = 20$)

Sample ID (Ct < 30)	Cycle threshold (Ct) of SARS-CoV-2 genes												Result	
	Standard PCR method using VIRALDTECT II kit			Direct RT-PCR method										
				Taqpath kit			Covidsure kit			Hi-PCR kit				
	N	RdRp	E	N	ORF 1ab	S	N	ORF 1ab	E	N	RdRp	E	Standard method	Direct method
1	16.54	19.72	21.14	18.52	18.61	19.53	22.65	22.38	22.08	18.91	19.01	19.94	+	+
2	16.85	16.77	18.79	17.31	17.4	18.25	22.43	20.39	20.02	17.67	17.76	18.63	+	+
3	16.87	20.11	21.56	18.89	18.98	19.92	23.1	22.82	22.52	19.28	19.38	20.33	+	+
4	17.43	16.41	18.4	18.78	18.87	19.8	22.91	21.61	20.13	19.17	19.26	20.21	+	+
5	17.72	16.58	18.6	19.73	19.83	20.8	21.51	20.91	23.15	20.14	20.24	21.23	+	+
6	17.77	16.73	18.76	19.15	19.24	20.19	23.36	22.04	20.53	19.55	19.64	20.61	+	+
7	17.9	16.52	19.01	18.51	18.6	19.52	20.99	20.61	20.54	18.9	18.99	19.93	+	+
8	18.07	16.91	18.97	20.12	20.22	21.21	21.94	21.32	23.61	20.54	20.64	21.65	+	+
9	18.35	18.3	20.33	18.61	18.7	19.62	19.97	18.03	17.59	19.02	19.09	20.33	+	+
10	18.44	16.43	18.04	21.11	21.22	22.26	23.05	22.37	21.79	21.55	21.66	22.72	+	+
11	18.77	18.01	19.61	20.66	20.76	21.79	21.22	20.89	19.05	21.09	21.19	22.24	+	+
12	19.14	18.37	20	21.07	21.17	22.22	21.64	21.3	19.43	21.51	21.61	22.68	+	+
13	22.96	21.66	23.49	24.27	25.89	25.34	28.21	28.01	30.09	24.29	25.92	25.37	+	+
14	23.22	21.05	22.89	24.34	24.59	25.44	24.05	23.86	23.71	24.36	24.62	25.47	+	+
15	23.51	23.21	16.05	23.83	24.38	25.14	24.53	24.65	24.02	23.85	24.4	25.17	+	+
16	24.29	23.72	25.22	27.65	27.24	28.12	28.41	23.19	23.2	27.68	27.27	28.15	+	+
17	25.17	22.91	25.12	27.11	27.69	28.14	26.42	27.18	26.32	27.14	27.72	28.17	+	+
18	25.43	23.67	27.05	25.85	25.34	26.87	26.39	26.31	26.24	25.88	25.37	26.9	+	+
19	25.45	23.72	26.86	24.92	24.91	25.61	24.49	24.61	23.95	24.95	24.94	25.64	+	+
20	25.52	23.55	26.31	27.58	26.62	27.29	27.74	26.81	26.47	27.61	26.65	27.32	+	+

Abbreviations: RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 4 Concordance analysis of high Ct value SARS-CoV-2 positive samples ($n = 50$) using the direct RT-PCR test

Sample ID	Standard PCR Method using VIRALDTECT II kit				Direct RT-PCR Method											
					Taqpath kit				Covidsure kit				Hi-PCR kit			
	N	*RdRp*	*E*	Result	*N*	*ORF 1ab*	*S*	Result	*N*	*ORF 1ab*	*E*	Result	*N*	*RdRp*	*E*	Result
SCOV-01	35.58	33.26	33.46	+	ND	ND	ND	−	34.28	36.61	35.33	+	ND	ND	ND	−
SCOV-02	32.76	33.43	29.65	+	32.59	33.54	33.80	+	32.01	33.34	31.85	+	ND	ND	ND	−
SCOV-03	33.86	31.54	31.32	+	35.54	35.53	35.55	+	ND	ND	ND	−	ND	ND	ND	−
SCOV-04	32.47	31.2	31.1	+	35.85	35.21	35.70	+	34.99	37.58	34.64	+	ND	ND	ND	−
SCOV-05	31.73	32.84	28.1	+	33.24	32.10	32.31	+	31.12	32.77	31.05	+	36.81	35.27	34.43	+
SCOV-06	31.83	31.96	32.29	+	ND	ND	ND	−	32.81	34.16	32.80	+	34.84	35.38	35.29	+
SCOV-07	32.37	31.57	31.57	+	ND	ND	ND	−	33.30	34.14	32.88	+	36.45	33.48	32.84	+
SCOV-08	31.03	29.25	29.06	+	ND	ND	ND	−	34.97	37.19	34.55	+	35.34	39.1	33.15	+
SCOV-09	32	31.18	31.02	+	33.39	26.76	32.98	+	33.39	37.10	33.79	+	35.28	34.78	31.42	+
SCOV-10	33.93	31.21	30.76	+	35.54	33.91	34.41	+	33.02	35.15	33.39	+	ND	ND	ND	−
SCOV-11	35.19	33.45	32.03	+	35.30	35.74	35.44	+	34.99	36.15	33.81	+	ND	ND	ND	−
SCOV-12	33.9	36.79	31.01	+	ND	ND	ND	−	35.62	36.75	36.43	+	ND	ND	ND	−
SCOV-13	32.15	32.7	27.05	+	34.72	35.16	35.65	+	31.83	33.58	31.75	+	33.8	35.32	32.7	+
SCOV-14	35.51	34.16	32.24	+	30.69	29.58	30.25	+	30.68	32.41	30.55	+	ND	ND	ND	−
SCOV-15	31.09	31.37	27.88	+	35.28	34.01	35.38	+	ND	ND	ND	−	35.53	33.51	33.39	+
SCOV-16	31.34	15.44	28.39	+	31.25	31.36	32.01	+	31.67	32.73	31.31	+	34.51	33.37	28.52	+
SCOV-17	32.52	31.43	28.76	+	ND	ND	ND	−	34.98	36.01	34.33	+	ND	ND	ND	−
SCOV-18	34.66	32.83	32.87	+	ND	ND	ND	−	33.32	35.11	33.76	+	ND	ND	ND	−
SCOV-19	33.45	31.43	31.33	+	ND	ND	ND	−	ND	ND	ND	−	ND	ND	ND	−
SCOV-20	35.00	32.76	32.77	+	ND	ND	ND	−	33.21	34.37	32.66	+	ND	ND	ND	−
SCOV-21	33.4	30.96	30.87	+	34.30	34.62	35.44	+	34.70	35.76	34.39	+	ND	ND	ND	−
SCOV-22	32.65	30.54	29.94	+	ND	ND	ND	−	35.24	37.58	34.96	+	ND	ND	ND	−
SCOV-23	31.35	33.04	29.31	+	35.92	35.57	35.86	+	ND	ND	ND	−	32.96	32.54	31.15	+
SCOV-24	31.98	27.88	28.0	+	ND	ND	ND	−	ND	ND	ND	−	33.74	31.5	31.27	+
SCOV-25	31.08	28.08	27.87	+	33.78	32.48	34.56	+	33.67	35.13	32.96	+	37.62	42.38	36.31	+
SCOV-26	31.66	31.84	27.61	+	32.92	31.18	31.92	+	35.31	37.95	33.52	+	34.75	31.28	31.4	+
SCOV-27	31.7	29.85	29.82	+	ND	ND	ND	−	36.27	37.47	35.89	+	34.09	33.82	35.46	+
SCOV-28	31.76	29.75	29.42	+	35.35	35.14	35.09	+	32.95	33.85	32.64	+	35.17	37.07	32.88	+
SCOV-29	31.76	29.54	29.36	+	ND	ND	ND	−	31.23	32.80	31.08	+	36.43	35	34.89	+
SCOV-30	31.67	29.87	29.76	+	34.91	35.24	35.72	+	34.62	36.03	33.74	+	35.6	36.84	31.52	+

(*Continued*)

Table 4 (*Continued*)

Sample ID	Standard PCR Method using VIRALDTECT II kit				Direct RT-PCR Method											
					Taqpath kit				Covidsure kit				Hi-PCR kit			
	N	*RdRp*	*E*	Result	*N*	*ORF 1ab*	*S*	Result	*N*	*ORF 1ab*	*E*	Result	*N*	*RdRp*	*E*	Result
SCOV-31	33.89	32.28	32.26	+	ND	ND	ND	−	34.08	35.50	33.01	+	ND	ND	ND	−
SCOV-32	33.47	33.61	33.92	+	ND	ND	ND	−	36.60	38.71	35.98	+	ND	ND	ND	−
SCOV-33	34.59	32.64	32.03	+	ND	ND	ND	−	35.54	35.25	36.94	+	ND	ND	ND	−
SCOV-34	32.9	29.77	30.59	+	ND	ND	ND	−	34.75	35.91	34.55	+	ND	ND	ND	−
SCOV-35	31.15	34	26.92	+	33.34	34.92	34.02	+	34.44	34.38	33.02	+	35.57	35.37	35.66	+
SCOV-36	31.96	30.81	31.96	+	32.62	29.85	31.18	+	32.11	34.06	30.78	+	36.48	35.26	32.26	+
SCOV-37	31.02	35.33	29.2	+	ND	ND	ND	−	33.21	35.79	33.27	+	35.16	17.32	31.85	+
SCOV-38	33.35	30.52	30.24	+	34.17	35.85	35.90	+	33.49	35.63	33.76	+	ND	ND	ND	−
SCOV-39	31.11	13.31	28.27	+	35.78	34.02	35.39	+	ND	ND	ND	−	ND	ND	ND	−
SCOV-40	31.45	31.00	28.01	+	31.24	32.62	33.86	+	33.19	35.44	33.18	+	36.07	36.68	30.35	+
SCOV-41	34.07	35.92	31.05	+	ND	ND	ND	−	36.39	35.21	35.34	+	ND	ND	ND	−
SCOV-42	31.5	34.85	29.55	+	35.28	34.25	35.12	+	33.98	37.44	34.05	+	34.81	32.81	32.6	+
SCOV-43	31.73	33.11	28.76	+	ND	ND	ND	−	35.30	35.71	33.27	+	35.9	33.86	33.68	+
SCOV-44	33.95	33.58	34.83	+	35.47	35.84	35.45	+	34.66	38.97	34.83	+	ND	ND	ND	−
SCOV-45	31.2	26.62	26.33	+	35.25	31.94	33.39	+	31.64	32.95	31.73	+	36.31	35.42	35.42	+
SCOV-46	31.94	28.46	28.18	+	35.38	31.81	34.77	+	32.71	33.41	32.24	+	34.59	34.73	36.22	+
SCOV-47	32.49	29.84	29.27	+	ND	ND	ND	−	34.02	35.71	34.66	+	ND	ND	ND	−
SCOV-48	33.47	31.54	31.46	+	ND	ND	ND	−	36.32	36.33	35.03	+	ND	ND	ND	−
SCOV-49	33.34	31.04	30.93	+	ND	ND	ND	−	36.61	35.51	34.67	+	ND	ND	ND	−
SCOV-50	33.3	31.49	31.3	+	ND	ND	ND	−	37.89	35.32	35.4	+	ND	ND	ND	−

Abbreviations: ND, not detected; RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 5 Performance characteristics of RT-PCR kits by direct RT-PCR assays

	Taqpath kit	Covidsure kit	Hi-PCR kit
Sensitivity (95% CI)	67.57% (55.68%–78.00%)	89.29% (78.12%–95.97%)	65.79% (54.01%–76.29%)
Specificity (95% CI)	100% (88.43%–100%)	100% (88.43%–100%)	100% (88.43%–100%)
PPV	100%	100%	100%
NPV (95% CI)	55.56% (47.36%–63.46%)	83.33% (70.13%–91.42%)	53.57% (45.79%–61.18%)
Accuracy (95% CI)	76.92% (67.64%–84.62%)	93.02% (85.43%–97.40%)	75.47% (66.16%–83.31%)

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; RT-PCR, real-time polymerase chain reaction.

The overall sensitivity, specificity, and accuracy of the COVIDsure Pro Multiplex RT-PCR kit was 89.29% (95% CI = 78.12–95.97), 100% (95% CI = 88.43–100), and 93.02% (95% CI = 85.43–97.40), as compared with TaqPath COVID-19 Combo kit 67.57% (95% CI = 55.68–78), 100% (95% CI = 88.43–100), and 76.92% (95% CI = 67.64–84.62) and Hi-PCR Coronavirus (COVID-19) kit 67.79% (95% CI = 54.01–76.29), 100% (95% CI = 88.43–100), and 75.47% (95% CI = 66.16–83.31), respectively (►**Table 5**). Results for group III demonstrate that all three RT-PCR kits were able to correctly amplify and detect respective internal control genes of all the SARS-CoV-2 negative specimens by direct RT-PCR method (data not shown).

Discussion

During this ongoing SARS-CoV-2 pandemic, more samples are being collected and sent to the testing laboratories for the molecular diagnosis of the COVID-19 disease. Molecular diagnosis involves critical and multistep time-taking processes, that is, preanalytical (sample sorting and labeling), analytical (RNA extraction and RT-PCR test), and postanalytical (data analysis and reporting). At present, one-step, multiplex SARS-CoV-2 RT-PCR kits are available which give results in one shot and save time.

On the contrary, the RNA extraction step is time-consuming especially, in manual RNA extraction procedures as most testing laboratories are not equipped with automated RNA extraction systems. This leads to delays in testing and an increase in the total turnaround time. Pendency in testing may result in sample deterioration due to the limited sample storage capacity in the laboratories, which eventually affects COVID-19 diagnosis.

RNA extraction step may be skipped from the molecular diagnosis process without affecting the outcome of the test. This will reduce the total turnaround time and will be more economical in resource-limited settings and the risk of human error can be reduced substantially. In recent times, many studies have shown rapid molecular diagnosis of SARS-CoV-2 by omitting the standard RNA extraction step.[20–24]

To increase the testing capacity and to reduce the total turnaround time and cost, this study aimed to evaluate the preheated clinical specimens in the direct RT-PCR testing by omitting the routinely performed RNA extraction step. For the direct RT-PCR assay, using preheated clinical specimens four different commercially available SARS-CoV-2 RT-PCR kits (ViralDtect-II Multiplex Real-Time PCR kit for COVID-19, TaqPath COVID-19 Combo kit, COVIDsure Pro Multiplex RT-PCR kit, and Hi-PCR Coronavirus (COVID-19) Multiplex Probe PCR kit) were evaluated for the compatibility and analytical performance. None of the kit manufacturers endorses their RT-PCR kits to be used in direct RT-PCR assay using preheated clinical specimens. All the RT-PCR kits used in the study except the ViralDtect-II Multiplex Real Time PCR kit were found to be compatible in the direct RT-PCR testing. The failure of detection of the SARS-CoV-2 genes by the ViralDtect-II kit might be due to the absence of reagents in the kit master mix which protects the PCR reaction from the PCR inhibitors present in the VTM. Three groups of samples (group I [< 30 Ct], group II [> 30 Ct], and group III [negative]) were evaluated for standard and direct RT-PCR method in this study. There were slight differences in Ct values as compared with the standard method and this may be due to the uneven distribution of nucleic acid present in the clinical specimens

Group II samples showed a difference in sensitivity and accuracy among all three RT-PCR kits. However, the performance of the COVIDsure Pro Multiplex RT-PCR kit was superior than other kits and also showed the highest sensitivity and accuracy as compared with the standard RT-PCR method (►**Table 5**). The performance of the TaqPath COVID-19 Combo kit and Hi-PCR Coronavirus (COVID-19) Multiplex Probe PCR kits were comparable with each other; however, sensitivity and accuracy of both the kits were considerably less than the COVIDsure Pro Multiplex RT-PCR kit. The reason for the difference in Ct, sensitivities, and accuracy in direct RT-PCR method and standard method can be explained as this study utilized the archived specimens. In the standard RNA extraction method, the lysis step is more crucial in inactivating the SARS-CoV-2 wherein the direct RT-PCR method there is a chance of infection to the laboratory personnel. Although some studies[25–27] have documented that SARS-CoV-2 loses infectivity at temperature above 56° C. Therefore, in this study clinical specimens were heat-inactivated and cooled down before the direct RT-PCR test.

During pandemic situation, massive sampling and testing are being performed and in this scenario, direct RT-PCR method can be the best alternative for rapid testing besides substantially decreasing the cost and time. Nonetheless, before switching into

the direct RT-PCR method, RT-PCR kits should be evaluated for compatibility with the direct RT-PCR method as well as limit of detection (LOD) in high and low viral load specimens. This alternative and fast method can be employed during the pandemic situation and quality of the direct RT-PCR method should be checked intermittently to avoid any false reporting.

Limitations

In this study, LOD assessment of RT-PCR kit was not done as the main focus was to evaluate the potential of routinely used SARS-CoV-2 RT-PCR kits as RNA extraction-free method and fast alternative for the routinely used standard method. This study also compared and validated the performance of commercially available RT-PCR kits. Further, archived specimens were used for this study which might have an impact on the Ct of different RT-PCR kits and the total number of specimens was less in number.

Conclusion

The present study concludes that nucleic acid extraction-free direct RT-PCR method during pandemic situation is a valuable substitute for routinely performed COVID-19 testing. COVIDsure Pro Multiplex RT-PCR kit performance was superior to other kits used in this study and can be used for direct RT-PCR method for the diagnosis of COVID-19 disease. However, it is important to confirm whether a RT-PCR kit is compatible with this alternative method or not. In the long run, this extraction-free method will facilitate the enlargement of COVID-19 disease testing facility by reducing testing time, reagents, and needed tools. At last, direct RT-PCR method facilitates mass testing and early reporting which eventually helps to control SARS-CoV-2 pandemic.

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Conflict of Interest
None declared.

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