Comparative Analysis of Four Commercial RT-PCR Diagnostic Assay for Detection of Covid-19

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Abstract

SARS-CoV-2 real-time reverse-transcription PCR (rRT-PCR) is currently the only available yet effective testing system to fight COVID-19 epidemics as far as there's not any treatment and vaccine. Moreover, many SARS-CoV-2 rRT-PCR kits are approved by the emergency-use-authorization (EUA) altogether over the world. In this article we've provided a comparison of important performance features of four commercial RT-PCR assays. A total of consecutive nasopharyngeal (NPS) samples and oropharyngeal (OP) swabs were collected from 50 COVID-19 patients for sensitivity and specificity analysis. Specificity of these assays were examined by using extractions of RNA from common human coronavirus cultures. All RT-PCR kits including in this study exhibited acceptable specificity over 90%, except for the Sansure and PowerCheck (88%). Pishtaz teb assays demonstrated a PPA of 95.24% (40/42), while the DaAn Gene, Sansure, Power check of SARS-CoV-2 panel showed the PPA of 85.7% (36/42), 66.66% (28/42), and 64.3% (27/42), respectively. An NPA of 100% (8/8) was observed for four molecular assays. This study gives a technical baseline of four distinct commercial PCR assays for detection of SARS-CoV-2, that can be practical and useful for laboratories interested in buying any of them for more clinical validation.

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Abstract

SARS-CoV-2 real-time reverse-transcription PCR (rRT-PCR) is currently the only available yet effective testing system to fight COVID-19 epidemics as far as there's not any treatment and vaccine. Moreover, many SARS-CoV-2 rRT-PCR kits are approved by the emergency-use-authorization (EUA) altogether over the world. In this article we've provided a comparison of important performance features of four commercial RT-PCR assays. A total of consecutive nasopharyngeal (NPS) samples and oropharyngeal (OP) swabs were collected from 50 COVID-19 patients for sensitivity and specificity analysis. Specificity of these assays were examined by using extractions of RNA from common human coronavirus cultures. All RT-PCR kits including in this study exhibited acceptable specificity over 90%, except for the Sansure and PowerCheck (88%). Pishtaz teb assays demonstrated a PPA of 95.24% (40/42), while the DaAn Gene, Sansure, Power check of SARS-CoV-2 panel showed the PPA of 85.7% (36/42), 66.66% (28/42), and 64.3% (27/42), respectively. An NPA of 100% (8/8) was observed for four molecular assays. This study gives a technical baseline of four distinct commercial PCR assays for detection of SARS-CoV-2, that can be practical and useful for laboratories interested in buying any of them for more clinical validation.

Heighlights

Compared four COVID-19 RT-PCR kits which are approved and available by the Ministry of Health in Iran.

The Pishtaz teb's kit identified the highest number of positive clinical samples.

Keywords: Severe acute respiratory syndrome coronavirus, In vitro diagnostics, Coronavirus, SARS-CoV-2, Real Time RT-PCR

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an infectious disease that emerged in the Middle East (1). It is a concerning public health issue due to its worldwide spread and unexpected high mortality rate (2). SARS-CoV-2 is constantly scattered around the world, and as of October 21st, 2020, over 41 million cases have been confirmed globally, causing over 1,130,496 deaths (3). At the time of writing this paper, the Centers for Disease Control and Prevention (CDC) reported more than 539,670 confirmed COVID-19 cases and 31,034 deaths in Iran (3, 4).

Recently, this epidemic has a heavy burden on society, the global economy, and health care systems

All around the world several measures are being taken to control its prevalence and incidence (5). Many of these measures depend on the accurate diagnosis of infected people. Reverse transcription-polymerase chain reaction (RT-PCR) based on detecting and quantifying a fluorescent signal is the most sensitive and specific method; therefore, it is the most preferred test (6). Although different SARS-CoV-2 RT-PCR kits are commercially accessible, an independent assessment and comparison of these products are not done yet; thus, a study is desperately needed to guide the accuracy of tests in a diagnostic market filled with new tests (7). Although the RT-qPCR assay was attended as the gold-standard method for identification of respiratory viruses such as SARS-CoV and middle eastern respiratory syndrome coronavirus (MERS-CoV), current RT-PCR assays targeting SARS-CoV-2 have some flaws and limitations (8). Initially, due to the high similarity between SARS-CoV-2 and SARS-CoV, the prime probe cross-reacts. Second, these assays are not sensitive enough to confirm suspicious patients at the early stages after admission. In fact, numerous cases have been reported with positive CT scan results showing the onset of infection, but the results of RT-PCR assays were negative at the fist presentation (9).

Coronaviruses are enveloped positive-sense RNA viruses that express their replication and transcription complex, such as open-reading frame (ORF1a and ORF1b), rRNA-dependent RNA polymerase (RdRp). Moreover, they express the coronavirus structural proteins, including the envelope (E), nucleocapsid (N), and spike (S) proteins, by the transcription of subgenomic messenger RNAs, during replication cycle stages of the far outnumber (anti) genomic RNAs. The ORF1ab/RdRp, E, N, and S genes were consequently developed and targeted regions in the SARS-CoV-2 genome (5). To identify coronavirus disease of 2019 (Covid-19), world Health Organization (WHO) suggests the E gene as the first-line screening, the RdRp gene as confirmatory assay, and the N gene as additional confirmatory assay (10). For example, European Virus Archive GLOBAL (EVAg) primer-probe set targets the E and RdRp regions, the DAAN kit targets the ORF1ab, and N coding regions and the BGI kit targets the ORF1ab region (11-13).

In the early stages of the outbreak, both international and national agencies rushed to begin mass production of test reactants and issued an Emergency Use Authorization (EUA) for the CDC COVID-19 RT-PCR method. Despite these efforts, laboratories still face several problems such as shortage of reagents, lack of tools access, inability to perform high-complexity tests, and increase in staffing needs. These issues leave a gap in health care providers' ability to quickly diagnose and manage patients. The urge to find a sensitive, availabe and rapid diagnostic test for the detection of COVID-19 is obvious. Here, we have compared four COVID-19 RT-PCR kits that have been approved and available by the Ministry of Health in Iran to evaluate their sensitivity and specificity.

Material and methods

The study was conducted at the Imam Reza University Hospital in Mashhad (Iran) from March to October 2020. Different RT-PCR commercial assays were selected (Table 1) based on the following criteria: i) The assay can be performed on standard real-time PCR thermocyclers available worldwide, ii) The assay must be available in the market or the test can be made available by the manufacturer in the pre-release version, iii) The assay must be approved by the Ministry of Health of Iran. Our focus was on evaluating the rate of false negatives (FN) reports and assay's sensitivity using 50 COVID-19 patient samples.

Both nasopharyngeal (NP) and oropharyngeal (OP) swabs were collected from 50 hospitalized patients in Imam Reza hospital with suspected COVID-19 symptoms. Their NP/OP swabs were put into a tube containing 3ml of Universal Transport Media. RNA was extracted from clinical samples with the RNJia Virus Kit. we first ran a duplicate 10-fold dilution series of viral RNA for each assay. We determined the slope by linear regression in GraphPad Prism and defined the required levels for PCR efficiency (E) and R2 as > 95 % and > 0.95, respectively. After extraction, target genes were available in the assay documentation or upon request (for an overview, see Table 1). All PCRs were run on a Rotorgene II (Qiagen) and performed according to the manufacturer's instructions. However, the commercial kits included positive and negative controls, which we only used eight negative RNA samples. Each sample was run in duplicate and repeated three times in three consecutive days. The results were analyzed and interpreted as positive (Ct<40) or negative (Ct>40) and the Ct value of each target gene. To establish PCR efficiency Comparison of Ct values and statistical analyses were performed using the SPSS software program, version 15. A *P-value* of <0.05 was considered statistically significant.

Result

In the first step, all samples were analyzed using Covitech diagnostic kits, then the results were compared by the other three kits. Finally, the sensitivity and specificity of each assay were calculated (Table 2).

We first assessed PCR efficiency for each target gene assay by running a duplicate 10-fold dilution series of SARS-CoV-2 viral RNA (Figure 1). All assays showed an efficiency [?]97 % and R squares were <0.97, which are both well above the pre-defined required level. Following testing of 50 clinical specimens, Pishtaz teb assays demonstrated a PPA of 95.24% (40/42), while the DaAn Gene, Sansure, Power check SARS-CoV-2 panel showed the PPA of 85.7% (36/42), 66.66% (28/42), and 64.3% (27/42), respectively. An NPA of 100% (8/8) was observed for all four molecular assays. Therefore, all kits were compared by pishtaz teb. The results from Power check and Pishtaz teb showed that 53.1% of samples were detected by both molecular

assy, 14 samples were detected only by the Pishtaz teb, and only one sample was detected by the Power check. The results of Sansur and Pishtaz teb demonstrated that only 57% of the samples were identified by both assays, 13 tests were detected just by pishtaz teb and only one sample was detected by Sansure. 60.7% of Pishtaz teb and DaAn Gene results were complemented and close to each other. All relations showed a significant difference between the results of these kits (Table 3).

Based on the difference in detection rates for each gene, Pishtaz teb showed 11/42 positive samples in both channels, while 29 samples were identified only in the yellow channel (N gene) and samples were considered positive if repeated. Compared to Pishtaz teb, DaAn Gene diagnostic kit was able to detect seven specimens in the green channel (N gene), which were detected in Pishtaz teb only in the yellow channel. In contrast, the Pishtaz teb detected seven specimens in the yellow channel that DaAn Gene could not detect. Sensure kit detected four samples in the green channel (ORF1ab gene), which Pishtaz teb identified only in yellow. Sansure was detected in one sample in the yellow channel (N gene), which Pishtaz cannot identify. In contrast, eleven samples were identified by the Pishtaz teb, which the Sensure has not detected. The PowerCheck showed a good level of identification in the green channel (RdRp gene), so it has identified sixteen samples that Pishtaz found only in the yellow channel, and it identified one sample in the yellow channel that Pishtaz has not identified. In contrast, eight samples were identified by Pishtaz Kit, which PowerCheck did not identify. This difference in detection rate was significant between the Pishtaz teb and other kits (P-value$<$0.05). Except for the yellow channel's results of the Sensure, which did not show a significant P-value.

When CT values were considered in both channels, Pishtaz teb exhibited the lowest mean CT (Figure 2) with a significant difference from the mean CT of other assays ($P < 0.001$). The remaining three kits had statistically higher CT values compare to the Pishtaz teb assay.

Discussion

Here we have provided a comparison of four commercially available RT-PCR kits for the detection of SARS-CoV-2 in clinical samples (n = 50). All RT-PCR kits performed satisfactorily regarding PCR efficiency ([?]96 %).

The Pishtaz teb's kit diagnosed 48 positive results out of 50 samples, which is the highest number of positive clinical samples. Dangen kits were able to positively identify 44 out of 50 samples. Sansure and PowerCheck exhibited fourteen negative results, which are the most; therefore, needs a further survey. Particularly, we performed our analysis with a small number of clinical specimens; thus, we advise that diagnostic laboratories conduct additional clinical examinations upon the application of novel RT-PCR kits. One of the advantages of all these diagnostic kits based on multiple target genes is that the results can be interpreted as those of a combination of target genes, which complements both sensitivity and specificity.

A major challenge encountered in standard real-time PCR analysis is false negative reports, caused by inhibitors or inefficient PCR conditions (14, 15). Internal controls are used to address reliability, by adding extra primer-probe sets to target other endogenous DNA sequences or exogenous targets (16).

Regarding our conclusion, we believe that all of the commercially available RT-PCR kits included in this study can be used for the diagnosis of patients with symptoms of COVID-19. When performing virus diagnostics tests in populations that low viral loads expected to display, such as health-care workers with mild or no symptoms or patients during the early stages of the infection (17), it might be advisable to use those kit with the best performance regarding the identification of clinical samples, i.e., RT-PCR kits from Pishtaz teb and Dangene Which showed high identification rate among other kits.

Abbreviations

Covid-19: coronavirus disease of 2019; CDC: Centers for Disease Control and Prevention; E: Envelope; EVAg: European Virus Archive GLOBAL; EUA: Emergency Use Authorization; FN: False negative; MERS-CoV: Middle Eastern Respiratory Syndrome Coronavirus; N: Nucleocapsid; NP: Nasopharyngeal; ORF: Open-Reading Frame; OP: Oropharyngeal; RT-PCR: Reverse transcription-polymerase; chain reaction; RdRp:

rRNA-dependent RNA polymerase; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; S: Spike; WHO: World Health Organization.

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Figure 1 PCR efficiency for four commercially available RT-PCR kits for the detection of SARS-CoV-2 RNA**.** 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 Graphpad Prism to obtain the slope and R^2. The percentage efficiency was calculated from the slope using the formula $E = 100*(-1 + 10^{-1/\text{slope}})$.

Figure 2 Different RT-PCR kits showed variations in Ct values.

Table 1. Overview of kits for RT-PCR-based detection of SARS-COV-2 included in the study.

Manufacturer	Country	Storage condition	Target gene(s)	Instruments Channels	Ct value
Sansure	China	-20 ±5	ORF1ab, N	FAM, ROX	[?] 40
DaAn Gene	China	-20±5	ORF1ab, N	VIC, FAM	[?] 40
Power check	Korea	-25–15	RdRP, E	FAM, JOE	[?] 37
Pishtaz teb	Iran	-20±5	RdRp, N	FAM, HEX	[?] 40

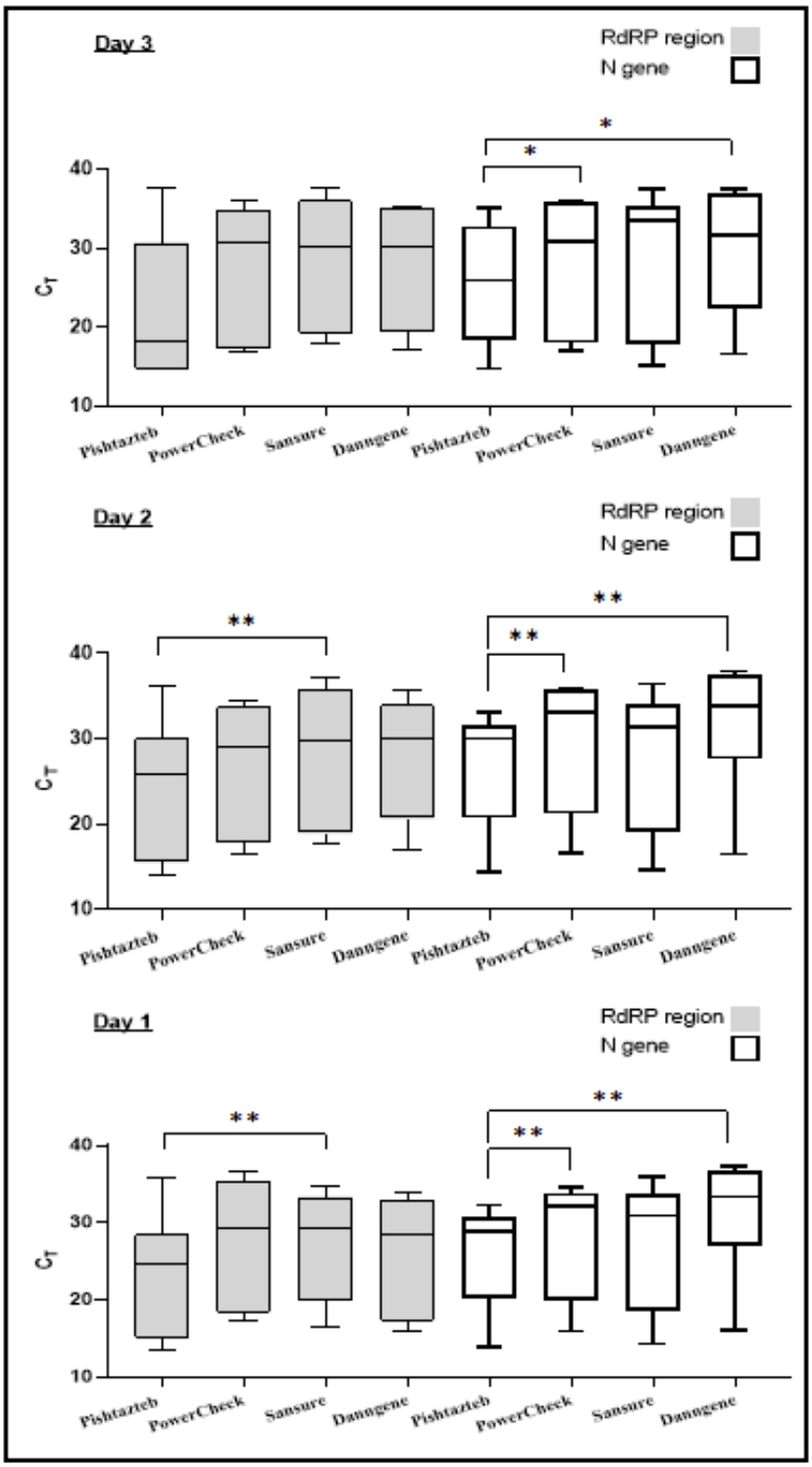
Table 2. Sensitivity and specificity of the PCR kits.

Company	Sensitivity	Specificity	Accuracy
Pishtaz teb	100%	100%	100%
Da An Gene	85%	100%	89.29%
Sansure	67.5%	88.89%	71.43%
Power check	65%	88.89%	69.39%

Table 3 . Clinical performance comparison of four molecular assays for the detection of SARS-CoV-2

Company	No. Of Results	No. Of Results	No. Of Results	No. Detection rate Of Each Gene	No. Detection rate Of Each Gene	No. Detection rate Of Each Gene	No. Detection rate Of Each Gene	P- Val Mc Nem test
	Positive	**Negative**	**ND[1]**	**N**	**RDRP**	**E**	**Orf1ab**	
Pishtaz teb	40	8	2	40	11	-	-	
Da An Gene	35	8	7	36	-	-	32	0.016
Sansure	28	8	14	28	-	-	15	0.002
Power check	27	8	15	-	16	27	-	0.001

ND. Not Detected



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analysis-of-four-commercial-rt-pcr-diagnostic-assay-for-detection-of-covid-19