

Assessment of 12 Quantitative RT-PCR Commercial Kits for the Detection of SARS-CoV-2

Asmaa M. Altamimi (✉ Asmaamt@moh.gov.sa)

Centers for Disease Control and Prevention SCDC <https://orcid.org/0000-0003-0381-757X>

Dalia A. Obeid

Centers for Disease Control and Prevention SCDC <https://orcid.org/0000-0003-0251-1894>

Taghreed A. Alaifan

CDC Climat

Moroje T. Taha

Saudi CDC

Marwa A. Alhothali

Saudi CDC

Fahad A. Alzahrani

Saudi CDC

Ahmad M. Albarrag

King Khalid University College of Medicine

Research

Keywords: Coronavirus, In vitro diagnostics, nCoV-2019, COVID-19, SARS-CoV-2, RT-PCR

Posted Date: November 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-100605/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background

The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) in the final months of 2019 had the health officials declare a public emergency raising a global response. In response to the burden of the current pandemic, strict measurements were globally implemented to stop further transmission of the virus. These Measurements rely on an accurate diagnosis of individuals infected with the virus by using real-time reverse transcriptase PCR (RT-PCR). The goal of our study is to relate the fundamental clinical and analytical performance of chosen kits of RT-PCR from distinct manufacturers.

Methods

A total of 94 nasopharyngeal and oropharyngeal clinical samples were selected randomly, these samples were previously confirmed as 64 positives, and 30 negatives for SARS-COV-2. Generally, 400 μ l of each respiratory specimen was subjected to extraction using ExiPrep 96 Viral RNA Kit with the ExiPrep 96 Lite Automated NA Purification System. All kits master mix preparation, cycling protocol, and results interpretation were carried out according to the manufactures' instructions of use and recommendations.

Results

In our study, we were able to evaluate the performance of 12 commercial kits in detecting SARS-COV-2 using 5 different targets. The performance of the kits was comparable except for LYRA kit as it was less sensitive ($F=67$, $P < 0.001$). Particularly, the performance of RealStar, BGI, DiaPlexQ, Genesig, LightMix, TaqPath, and RAD1 kits were the most sensitive. Moreover, the performance of these commercial kits by gene target showed no significant change in Ct values which indicates that kits disparities are mainly linked to the choice of the gene target ($F=0.49$, $P=0.73$).

Conclusion

We believe that most of the commercially available RT-PCR kits included in this study can be used for routine diagnosis of SARS-COV-2 patients. Moreover, we recommend that regardless of the laboratory choice of diagnostic commercial kit for the clinical detection of COVID-19 patients the need a for good plan for validation and collaboration with exterior laboratories is essential in order to monitor the virus changes overtime, procedures, technicians, and the different kits performances.

1. Introduction

The emergence of a novel coronavirus, the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) in the final months of 2019 had the health officials declare a public health emergency raising a global response [1]. The new virus (SARS-COV-2) has been identified as a new strain of *Betacoronavirus* from group 2B with approximately 70% genetic similarity to the severe acute respiratory syndrome coronavirus (SARS-COV) [2]. Coronaviruses are positive-sense RNA viruses that express their replication, transcription, and their RNA-dependent RNA polymerase (RdRp) through single, large open reading frame (ORF1ab) [3]. Several structural proteins are found within the virus such as envelope (E), nucleocapsid (N), and spike (S) proteins. In response to the burden of the current pandemic, strict measurements were globally implemented to stop further transmission of the virus. These Measurements rely on an accurate diagnosis of individuals infected with the virus by using real-time reverse transcriptase PCR (RT-PCR) [4]. The most common targets for detecting SARS-COV-2 by (RT-PCR) assays in diagnostic laboratories are the ORF1ab gene, the RdRp gene, the E gene, the N gene, or the S gene [5].

The SARS-CoV-2 pandemic caused a huge burden on the societal, financial, and healthcare systems in the sphere, and various measures are developed to control its spread. Most of the control measures mainly depend on the precise testing of the individuals infected by the virus. Real-time reverse transcription-polymerase chain (RT-PCR) method of detection is the most common and reliable test in detecting viral genome, therefore the world health organization (WHO) has recommended the use of this method as the gold standard during the current time [6]. Many SARS-CoV-2 RT-PCR diagnostic kits are commonly available in the market, however, independent examination of these products is not available in the public sector and is required to give directions of implementation to accurately test [7].

In the Saudi Center for Disease Prevention and Control (SCDC) Laboratories, we have performed an evaluation of twelve RT-PCR SARS-CoV-2 commercial kits from various manufacturers. Most of our chosen kits were CE-IVD certified and can be produced in large amounts. A concise panel of ninety-four clinical samples was used to evaluate the performance of these commercial kits. The goal of our study was to relate the fundamental clinical and analytical performance of chosen kits of RT-PCR from distinct manufacturers. The manufacturers enrolled in our assessment were TIB MOLBIOL, Altona Diagnostics, Thermo Fisher Scientific, Solgent, Quidel, BGI, OPTOLANE Technology, Kogene Biotech, Sansure Biotech, Novacyt/Primer design, GeneReach Biotechnology, and KH Medical. The 12 commercial kits amplified 5 unique targets in SARS-CoV-2 genome, including: N, E, S, RdRp, and ORF1ab/ PP 1ab genes. This study will provide an abundant assessment on the detection of SARS-COV-2 by using different kits and targets to show the importance of accurate testing during these difficult times.

2. Methodology

2.1 Samples selection

This study encompasses 12 commercial RT-PCR COVID-19 detection kits available in the market, sent by the manufacturers free of charge to the Saudi Center for Disease Control and Prevention (SCDC) for evaluation. None of the manufacturers were involved in the assessment or data analysis. A total of 94 nasopharyngeal and oropharyngeal clinical samples were selected randomly, these samples included 64 previously confirmed samples and 30 samples confirmed as negatives for SARS-COV-2. The ethical approval was obtained from our institution the Saudi CDC Number: (SCDC-IRB-A012-2020).

2.2 Samples preparation and RT-PCR procedure

Generally, 400 µl of each respiratory specimen was subjected to extraction using ExiPrep 96 Viral RNA Kit, and ExiPrep 96 Lite Automated NA Purification System (Bioneer, Korea). The nucleic acid (RNA) extraction process has been performed twice (with 100 µl elution volume for each) then the extracted RNA was pooled and stored at -80 C until use. This extraction instrument was chosen due to its ability to provide sufficient elution volume to process 10 different assays and its availability in all Saudi's regional laboratories. No exogenous internal controls were added to the extraction or the master mix. All kits master mix preparation, cycling protocol and results interpretation were carried out according to the manufactures' instructions of use and recommendations (Details of the compared kits and instruments used are summarized in Table 1). When the result was inconclusive or invalid, as per the manufactures' results interpretation, retesting was performed, and no PCR inhibition noted spotted.

2.3 Data analysis

For Statistical analysis, Data was collected and analyzed using GraphPad Prism (San Diageo California, USA), version 8.4. Descriptive analysis was done on the reported Cycling Threshold (Ct) and results were compared by the commercial RT-PCR kits and targets. ANOVA test was used to detect the significance of the Ct values reported by the commercial RT-PCR kits and targets. Boxplots and bar graphs were used to show the distribution of CT values and detection results by the different commercial kits. All P values reported are 2-sided and were considered to be statistically significant at alpha < 0.05.

3. Results

3.1 The summary of the SARS-COV-2 Detection by multiple kits

The 94 samples were all processed, and the results were interpreted as recommended by each of the kits' manufacture recommendations. Kits with single targets were reported positive if their cycling threshold (CT) were below the cutoff value. All samples were tested by all kits except for the RADI, we only had enough for 55 samples, therefore we selected the enrolled samples randomly. The summary of the detection results is shown in Fig. 1. Multiple diagnostic kits show that almost 60% of the samples were positive by most of the commercial kits except for LYRA as it shows a lower number.

3.2 Quantitative analysis of the positive samples of CT values

The quantitative analysis of the positive samples' CT values shows a significant difference across the used kits (ANOVA, $F = 67$, $P\text{-Value} < 0.001$). Figure 2 shows the distribution of the CT values reported by the commercial kits and their targets. The lowest CT values were mostly reported with KAIRA (E gene), Sansure (N gene), and TaqPath (N gene). The highest positive CT values were mostly reported with PowerCheck (RdRp gene), LightMix (E gene), and Genesig (RdRp).

3.3 Quantitative analysis of the positive samples by Targets

By target, commercial kits showed similar performance and reported comparable CT values. The commercial kits had 5 different target genes which were N, E, S, RdRp, and ORF1ab/ PP1ab. The results of the ANOVA test showed that by target the CT values of the commercial kits were similar ($F = 1.1$, $P < .05$). Figure 3 shows the distribution of CT values by the target. Overall, the lowest CT values were mostly reported with kits targeting the N gene. The highest CT values were mostly reported with kits targeting the RdRp gene.

Table 2 shows the overall CT summary reported by kits targeting the *ORF1ab* gene which were BGI, Sansure, IQ REAL, DiaPlexQ, LYRA, and TaqPath. For the *ORF1ab* gene, the quantitative analysis showed a similar result across the different kits (ANOVA, $F = 1$, $P \text{ value} = 0.39$).

Table 3 shows the overall CT summary reported by kits targeting the *RdRp* gene which were KAIRA, LightMix, Genesig, RADI, and PowerCheck. For the *RdRp* gene, the kits showed a significant difference in CT values reported by the kits (ANOVA $F = 2$, $P \text{ value} = 0.03$). The change was detected with the Genesig kit which reported distinctive CT values compared to KAIRA, LightMix, and PowerCheck.

Table 4 shows the overall CT summary reported by kits targeting the *E* gene which were reported with KAIRA, LightMix, RealStar, and PowerCheck. For the *E* gene results, the kits showed a significant difference in CT values reported by the kits (ANOVA, $F = 20$, $P \text{ value} < 0.001$). The change was

detected with KAIRA and RealStar CT values which were different compared to LightMix, PowerCheck, and each other.

Table 5 shows the overall CT value summary by kits targeting the *N* gene which were reported with TaqPath, DiaPlexQ, and Sansure. For the *N* gene CT values' quantitative analysis, the kits showed no significant difference in CT values reported by the kits (ANOVA $F = 1.27$, P value = 0.27). Similar results were reported with the *S* gene as shown in Table 6, no significance was detected (ANOVA, $F = 1.75$, P value = 0.18).

4. Discussion

During the current stage of the COVID-19 pandemic, many testing kits were developed and available commercially, in our study we were able to evaluate the performance of 12 commercial kits in detecting the SARS-COV-2 virus. The performance of the kits was comparable except for LYRA kit as it was less sensitive. Particularly, the performance of RealStar, BGI, DiaPlexQ, Genesis, LightMix, TaqPath, and RADi kits were the most sensitive. These kits had more than one target except for Genesis and BGI. This finding indicates the importance of having a confirmatory gene to ensure the specificity of diagnostic testing. Moreover, the performance of these commercial kits by gene target showed no significant change in Ct values which indicates that kits disparities are mainly linked to the choice of the gene target. From the 5 targets, ORF1ab/PP1ab, S, and N genes reported similar results by the different kits, however, RdRp and E genes showed significant differences by the reported Ct values. In consideration of time, the shortest tests were KAIRA, LightMix, and RADi with 60 minutes, however, these kits did miss few positive samples.

Indeed, other studies evaluating the performance of RT-PCR kits are in concordance with our results [8]. Comparing commercial kits showed similar results in detection, however, different targets did show variation in CT values. Moreover, kits with multiple targets such as Realstar, Taqpath, LightMix, and Sansure in another evaluation study did show higher sensitivity and specify than with other kits detecting a single target [9]. In our study, we didn't evaluate the limit of detection (LOD) which can play a big rule, as with several of the commercial kits approved for the pandemic situation many were approved without evaluation with appropriate numbers of samples [10]. Another important variation source in many PCR kits results that we didn't manage to evaluate is variation in instrument and technicians, in a multiple center euro surveillance study, the same commercial kits showed variation in results between different centers, many of these centers had different instruments and technicians [11].

One of the biggest challenges in the diagnosis of SARS-COV-2 in clinical settings is the high rate of false-negative cases. As known with RNA viruses the mutation rate is high compared with other pathogens. Moreover, the nucleotide mutation rate reported for SARS-COV-2 was $8E-04$ substitution per site per year [12, 13]. Many studies have already shown the high evolution rate of SAR-COV-2 in many cases and linked its evolution with its origin [5]. Detection methods using RT-PCR are based on a fixed target, however, as the pandemic proceed the virus is changing and many cases are missed. In one study, evaluating multiple RT-PCR targets has shown a high loss of sensitivity, with a total of 11,627 cases missed due to variations in genetic code [14]. In one of the biggest studies worldwide tracking the mutations of SARS-COV-2, one mutation D614 was decreasing and another mutation G614 mutation was growing, the new mutation has lower Ct values which indicate a change in virus virulence [15]. In a genomic surveillance study, a rise in mutation located in the ORF gene was linked to the Middle East SARS-COV-2 cases [16]. Overall these studies indicate the rise of genetic mutations in SARS-COV-2 globally.

This rise will cause many positive cases to be missed in the RT-PCR, which will cause a challenge with using these fixed commercial RT-PCR kits. In one case for a systematic SARS-COV-2 case, the patient was negative in many RT-PCR tests, however he was only positive by the antibody test, moreover, the case was later investigated with sequencing and the virus infecting the patient had two major mutations one located at the NP genes and another located at the ORF gene [17]. The rise of these different mutations in SARS-COV-2 at multiple geographical locations indicates the need for genetic screening periodically in each country to count for these changes as they may significantly play a role in choosing the commercial diagnostic kit used at the testing centers.

We are aware that our study may have several limitations. The first one is that we were not able to study the cross-reactivity of these commercial kits with other viruses which can significantly alter the results of the detection. Even though our conducted study implemented a comparative evaluation it is still not a comprehensive study as we could not include all RT-PCR diagnostic kits available at the market. In our future work we hope to evaluate genetic changes in the virus and its effect on detecting the virus by the available commercial kits.

5. Conclusion

In conclusion, we believe that most of the commercially available RT-PCR kits included in this study can be used for routine diagnosis of SARS-COV-2 patients. Most of the kits succeeded in detecting the virus, however few distinctions were found with specific kits and targets. Kits with multiple targets have shown to be the more sensitive and specific as they counted for the target variations. Moreover, we recommend that regardless of the laboratory choice of diagnostic commercial kit for the clinical detection of COVID-19 patients the need a for good plan for validation and collaboration with exterior laboratories is essential in order to monitor the changes in the virus, procedures, technicians, and the different kits performances.

6. List Of Abbreviation

SARS-COV-2: severe acute respiratory syndrome coronavirus 2

RT PCR: real-time reverse transcriptase Polymerase

LOD: the limit of detection

CT: cycling threshold

CE-IVD: European Conformity-In Vitro Diagnostic; FDA

EUA: U.S Food and drug Administration (Emergency Use Authorization).

RUO: Research Use Only.

RdRp: RNA-dependent RNA polymerase

ORF1ab: large open reading frame

E gene: envelope gene

N gene: nucleocapsid

S gene: Spike gene

7. Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Saudi Center for Diseases control and prevention ethical committee under the department of public health research and statistics division, (IRB number: SCDC-IRB-A012-2020). Participant consent was waived.

Consent to publication

All authors have reviewed the final version of the manuscript and approve it for publication.

Availability of data and materials

Data available on request from the authors.

Declaration of Competing Interest

Not Applicable.

Funding statement

This work was funded by Saudi CDC. The RT-PCR kits included in this study were provided free of charge.

Acknowledgements

Not Applicable.

Authors' contributions

All Authors contributed to the manuscript equally. AA conducted and planned the experiments. DO ran the analysis and wrote and reviewed the manuscript. TA wrote the methodology section, and MT wrote the introduction section. FA, MA helped with carrying the experiments. AA, reviewed the manuscript.

References

1. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579:270–3. <https://doi.org/10.1038/s41586-020-2012-7>.
2. Zhang Y-Z, Holmes EC. A Genomic Perspective on the Origin and Emergence of SARS-CoV-2. *Cell* 2020;181:223–7. <https://doi.org/https://doi.org/10.1016/j.cell.2020.03.035>.
3. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. *Science* (80-) 2020:eabb2762. <https://doi.org/10.1126/science.abb2762>.

4. Lu X, Wang L, Sakthivel SK, Whitaker B, Murray J, Kamili S, et al. US CDC real-time reverse transcription PCR panel for detection of severe acute respiratory syndrome coronavirus 2. *Emerg Infect Dis* 2020;26:1654.
5. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. *Nat Med* 2020. <https://doi.org/10.1038/s41591-020-0820-9>.
6. Li T. Diagnosis and clinical management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: an operational recommendation of Peking Union Medical College Hospital (V2. 0) working group of 2019 novel coronavirus, Peking union medical college hospital. *Emerg Microbes Infect* 2020;9:582–5.
7. Mattheussen V, Loens K, Lammens C, Vilken T, Koopmans M, Goossens H, et al. Preparedness of European diagnostic microbiology labs for detection of SARS-CoV-2, March 2020. *J Clin Virol* 2020;128:104432. <https://doi.org/https://doi.org/10.1016/j.jcv.2020.104432>.
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;128:104412. <https://doi.org/https://doi.org/10.1016/j.jcv.2020.104412>.
9. Iglói Z, Ieven M, Abdel-Karem Abou-Nouar Z, Weller B, Mattheussen V, Coppens J, et al. Comparison of commercial realtime reverse transcription PCR assays for the detection of SARS-CoV-2. *J Clin Virol* 2020;129:104510. <https://doi.org/https://doi.org/10.1016/j.jcv.2020.104510>.
10. Wang X, Yao H, Xu X, Zhang P, Zhang M, Shao J, et al. Limits of Detection of 6 Approved RT-PCR Kits for the Novel SARS-Coronavirus-2 (SARS-CoV-2). *Clin Chem* 2020;66:977–9. <https://doi.org/10.1093/clinchem/hvaa099>.
11. Muenchhoff M, Mairhofer H, Nitschko H, Grzimek-Koschewa N, Hoffmann D, Berger A, et al. Multicentre comparison of quantitative PCR-based assays to detect SARS-CoV-2, Germany, March 2020. *Euro Surveill* 2020;25:2001057. <https://doi.org/10.2807/1560-7917.ES.2020.25.24.2001057>.
12. Lauring AS, Andino R. Quasispecies theory ,and the behavior of RNA viruses. *PLoS Pathog* 2010;6:e1001005.
13. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* 2018;34:4121–3.
14. Peñarrubia L, Ruiz M, Porco R, Rao SN, Juanola-Falgarona M, Manissero D, et al. Multiple assays in a real-time RT-PCR SARS-CoV-2 panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak. *Int J Infect Dis* 2020;97:225–9. <https://doi.org/https://doi.org/10.1016/j.ijid.2020.06.027>.
15. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell* 2020. <https://doi.org/https://doi.org/10.1016/j.cell.2020.06.043>.
16. Gong Y-N, Tsao K-C, Hsiao M-J, Huang C-G, Huang P-N, Huang P-W, et al. SARS-CoV-2 genomic surveillance in Taiwan revealed novel ORF8-deletion mutant and clade possibly associated with infections in Middle East. *Emerg Microbes Infect* 2020;9:1457–66. <https://doi.org/10.1080/22221751.2020.1782271>.
17. Li Z, Li Y, Chen L, Li S, Yu L, Zhu A, et al. A Confirmed Case of SARS-CoV-2 Pneumonia with Routine RT-PCR Negative and Virus Variation in Guangzhou, China. *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa941>.

Tables

Table 1
Overview of the SARS-COV-2 detection kits encompasses in this study.

Company	Kit	Country	Regulatory status	Target gene	RNA template Vol.	Reaction Vol.	Thermocycler	Cycling Time	Positivity Cut off (CT value)
TIB MOLBIOL	LightMix Modular Wuhan CoV E gene & RdRP gene + Multiplex RNA Master (Roche Diagnostics)	Germany	RUO ¹	E/ RdRP	5 µl	10 µl	LightCycler 480 II Instrument (Roche)	1 h	< 39
Altona Diagnostics	RealStar SARS-CoV2	Germany	FDA (EUA) CE-IVD ²	E / S	10 µl	20 µl	LightCycler 480 II Instrument (Roche)	2h: 20 min	ND
Thermo Fisher Scientific	TaqPath COVID19 RT-PCR Kit + TaqPath 1-Step Multiplex Master Mix (Applied Biosystems)	U.S. A	CE-IVD	N/ Orf-1b/ S	5 µl	20 µl	ABI 7500 Fast (Applied Biosystems)	1h:10 min	≤ 37
Solgent	DiaPlexQ Novel Coronavirus (2019-nCoV) Detection Kit	Korea	FDA (EUA) CE-IVD	N/ Orf1a	5 µl	20 µl	ABI 7500 Fast (Applied Biosystems)	2 h	≤ 40
Quidel	Lyra SARS-CoV-2 assay	U.S. A	CE-IVD	Orf1ab (PP1ab)	5 µl	15 µl	LightCycler 480 II Instrument (Roche)	1h: 20 min	≤ 40
BGi	BGI Real-Time Fluorescent RT-PCR kit for SARS-COV-2	China	FDA (EUA) CE-IVD	Orf1ab	10 µl	20 µl	LineGene 9600 Plus (BIOER)	1h: 45 min	≤ 38
OPTOLANE Technology	Kaira 2019-nCoV Detection Kit	Korea	CE-IVD	E/ RdRP	5 µl	15 µl	ABI 7500 Fast (Applied Biosystems)	1h: 10 min	≤ 36 / ≤37.5
Kogene Biotech	PowerChek™ 2019-nCoV Real-time PCR Kit	Korea	FDA (EUA) CE-IVD	E/ RdRP	5 µl	15 µl	ABI 7500 Fast (Applied Biosystems)	2 h	≤ 37
Sansure Biotech	Sansure COVID-19 Nucleic Acid Test Kit	China	FDA (EUA)	N/ Orf1ab	20 µl	30 µl	ABI 7500 Fast (Applied Biosystems)	2 h	≤ 40
Novacyt/Primerdesign	Genesig coronavirus COVID-19 Real-Time PCR Assay	UK	FDA (EUA) CE-IVD	RdRP	8 µl	12 µl	LightCycler 480 II Instrument (Roche)	1h: 20 min	ND

(1) CE-IVD: European Conformity-In Vitro Diagnostic; FDA (EUA): U.S Food and drug Administration (Emergency Use Authorization).

(2) RUO: Research Use Only. E: envelope gene; ORF: an open reading frame; N: nucleocapsid gene; RdRp RNA dependent RNA polymerase gene; S: spike gene.

Company	Kit	Country	Regulatory status	Target gene	RNA template Vol.	Reaction Vol.	Thermocycler	Cycling Time	Positivity Cut off (CT value)
GeneReach Biotechnology	IQ REAL SARS-COV-2 Qualitative System	Taiwan	NA	Orf1ab	2 µl	23 µl	ABI 7500 Fast (Applied Biosystems)	1h: 40 min	ND
KH Medical	RADI COVID-19 Detection Kit	Korea	CE-IVD	S /RdRP	15 µl	15 µl	ABI 7500 Fast (Applied Biosystems)	1 h	≤ 40
(1) CE-IVD: European Conformity-In Vitro Diagnostic; FDA (EUA): U.S Food and drug Administration (Emergency Use Authorization).									
(2) RUO: Research Use Only. E: envelope gene; ORF: an open reading frame; N: nucleocapsid gene; RdRp RNA dependent RNA polymerase gene; S: spike gene.									

Table 2
Commercial Kits Targeting *ORF1ab/PP1ab* gene *Ct* values summary.

	BGI	Sansure	IQ REAL	DiaPlexQ	TaqPath	LYRA
No. Samples	64	62	63	64	60	42
Minimum	13.00	11.00	13.00	12.00	10.00	14.00
25% Percentile	18.25	19.00	22.00	19.00	17.25	25.00
Median	26.00	28.50	29.00	26.50	24.50	31.00
75% Percentile	32.00	32.00	33.00	32.00	29.00	35.50
Maximum	38.00	38.00	37.00	38.00	38.00	40.00
Mean	25.39	24.7	27.40	25.50	24.07	29.74
Std. Deviation	7.39	6.12	6.36	6.99	7.32	5.96
Global ANOVA Test	F = 0.45, P-value = 0.76					

Table 3
Commercial Kits Targeting *RdRP* gene *Ct* values summary.

	KAIRA	PowerCHECK	LightMix	Genesig	RADI
No. Samples	63	62	60	61	31
Minimum	11.00	11.00	13.00	18.00	16.00
25% Percentile	16.00	17.00	19.00	25.00	20.00
Median	25.00	24.00	25.50	31.00	24.00
75% Percentile	30.00	29.00	29.00	35.00	32.00
Maximum	38.00	37.00	37.00	40.00	39.00
Mean	23.35	23.03	24.70	29.74	25.77
Std. Deviation	7.769	6.799	6.212	5.961	6.859
Global ANOVA Test	F = 2.7, P-value = 0.0305*				
Tukey Comparison	<i>Kaira vs Genesig</i>		Mean diff = -6.4	P-value < 0.0001***	
(Paired t-Test) *	<i>PowerCheck vs Genesig</i>		Mean diff = -6.7	P value < 0.0001*	
if Significant	<i>LightMix vs Genesig</i>		Mean diff = -5.04	P value = 0.005**	

Table 4
Commercial Kits Targeting *E* gene *Ct* values summary.

	KAIRA	PowerCHECK	LightMix	RealStar
No. Samples	64	63	68	59
Minimum	10.00	14.00	15.00	13.00
25% Percentile	13.00	21.00	21.00	19.00
Median	18.50	30.00	28.00	25.00
75% Percentile	26.00	34.00	34.00	29.00
Maximum	37.00	39.00	39.00	36.00
Mean	19.67	27.89	27.31	24.20
Std. Deviation	7.224	6.809	7.061	5.542
Global ANOVA Test	F = 20, P value < 0.0001***			
Tukey Comparison (Paired t Test) * if Significant	<i>Kaira vs PowerCHECK</i>	Mean diff = -8.217	P value < 0.0001***	
	<i>Kaira vs LightMix</i>	Mean diff = -7.637	P value < 0.0001***	
	<i>Kaira vs. RealStar</i>	Mean diff = -4.532	P value = 0.0013**	
	<i>PowerCHECK vs RealStar</i>	Mean diff = 3.68	P value = 0.014*	
	<i>LightMix vs RealStar</i>	Mean diff = 3.101	P value = 0.048*	

Table 5
Commercial Kits Targeting *N* gene *Ct* values summary.

	Sansure	DiaPlexQ	TaqPath
No. Samples	66	64	62
Minimum	11.00	11.00	11.00
25% Percentile	17.75	18.25	16.00
Median	26.00	26.00	23.00
75% Percentile	32.00	31.75	29.00
Maximum	40.00	37.00	39.00
Mean	25.1	24.47	23.48
Std. Deviation	7.1	7.517	7.894
Global ANOVA Test	F = 1.28, P-value = 0.27		

Table 6
Commercial Kits Targeting S gene Ct values summary.

	<i>RealStar</i>	TaqPath	RADI
No. Samples	60	60	31
Minimum	14.00	12.00	14.00
25% Percentile	20.00	17.25	20.00
Median	26.50	24.00	27.00
75% Percentile	29.75	29.00	34.00
Maximum	36.00	38.00	39.00
Mean	25.22	23.92	26.65
Std. Deviation	5.41	7.42	7.45
Global ANOVA Test	F = 1.75, P-value = 0.178		

Figures

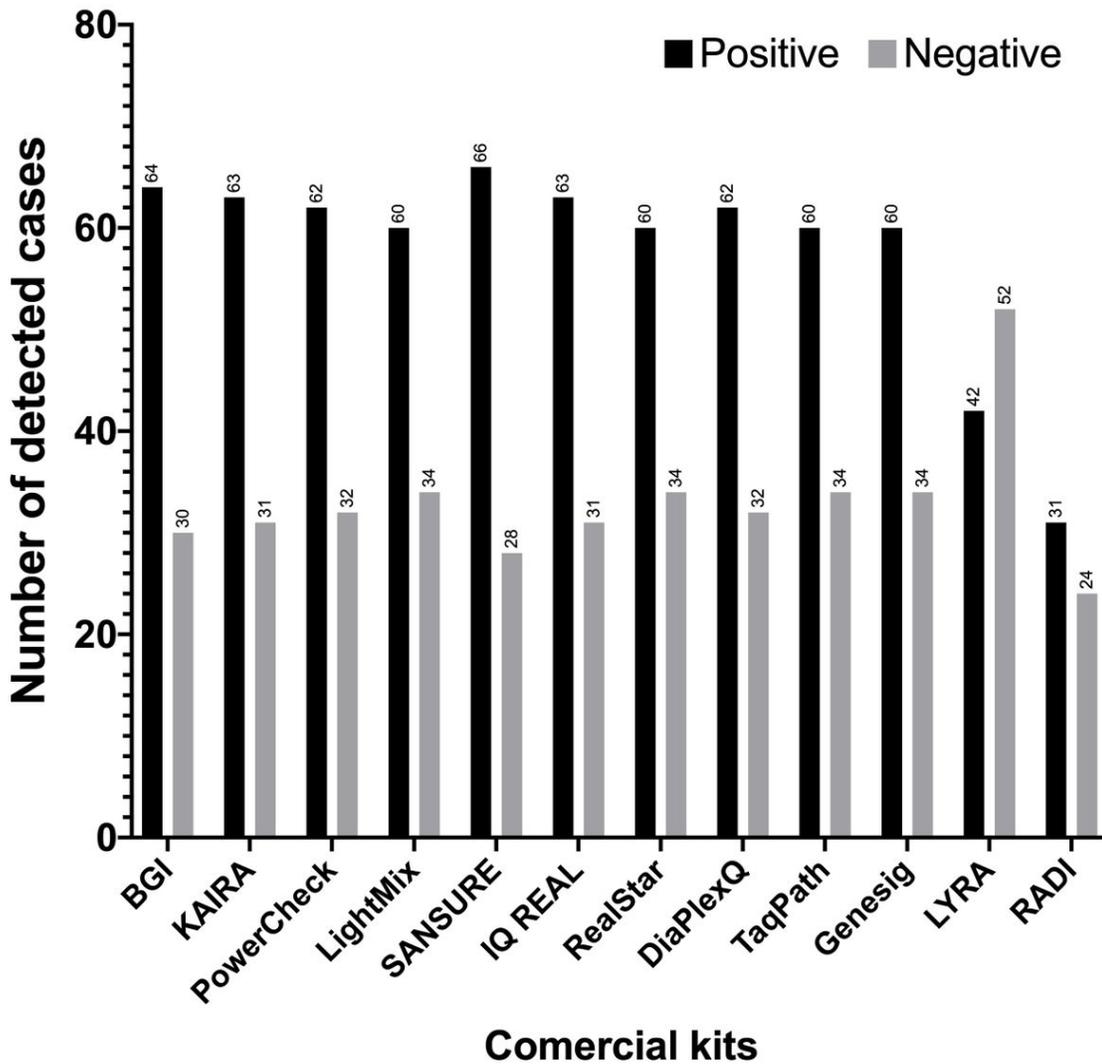


Figure 1

The summary of the SARS-COV-2 detection results by the 12 commercial diagnostic kits. The bar graph shows a similar proportion by the kits, except for the LYRA kit, as it shows more negative results than positive.

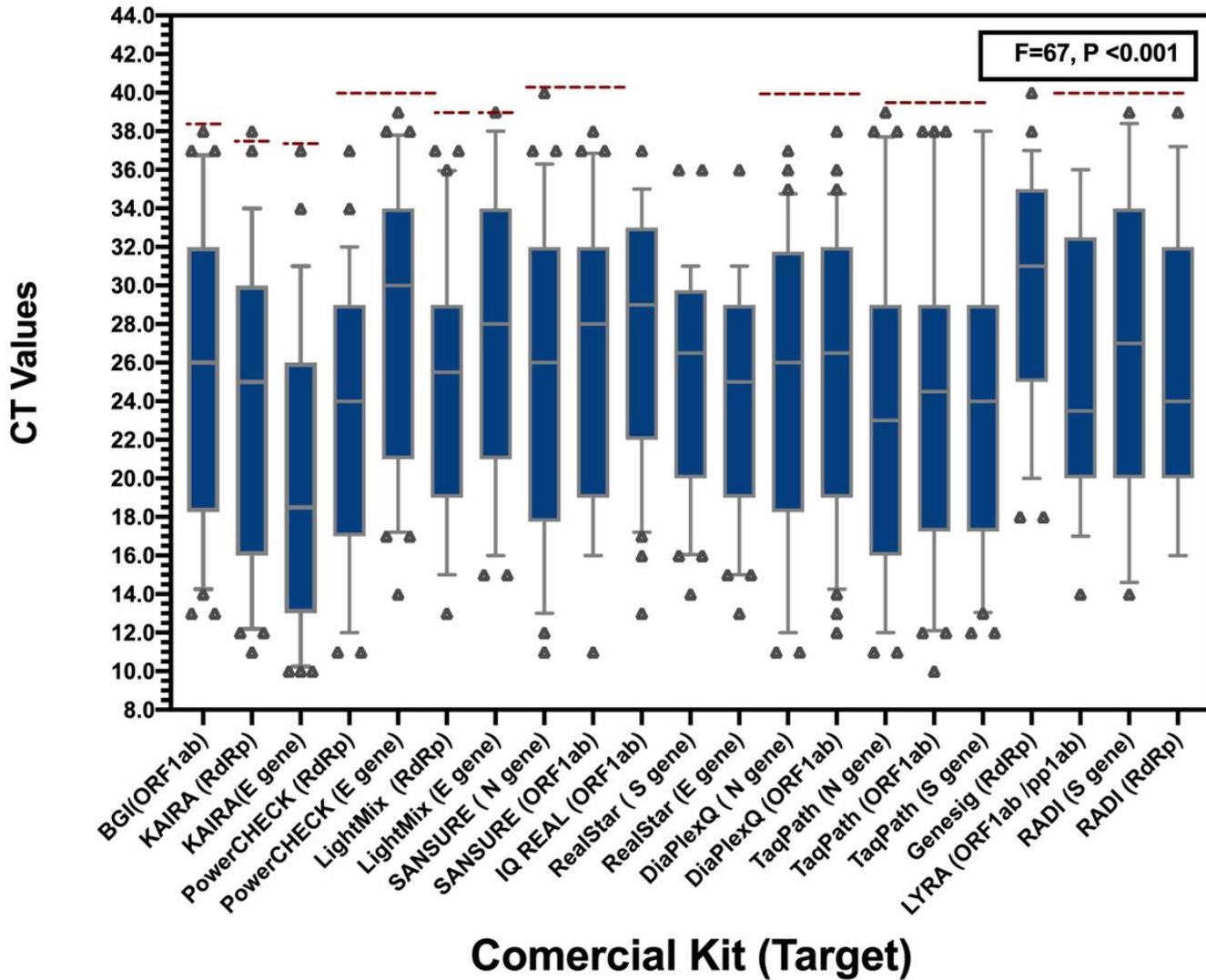


Figure 2

Boxplot of the positive samples' cycling threshold (CT) values across the 12 diagnostic kits (genes). The whisker above the boxplot represents the upper limit of the 95% confidence interval, while the bottom line represents the lower limit of the 95% confidence interval, the red line represents the cutoff value reported in the kit user guide. The lowest CT values were mostly reported with KAIRA (E gene), Sansure (N gene), and TaqPath (N gene). The highest positive CT values were mostly reported with PowerCheck (RdRP gene), LightMix (E gene), and Genesis (RdRp). ANOVA test was significant by the CT value reported by the multiple kits.

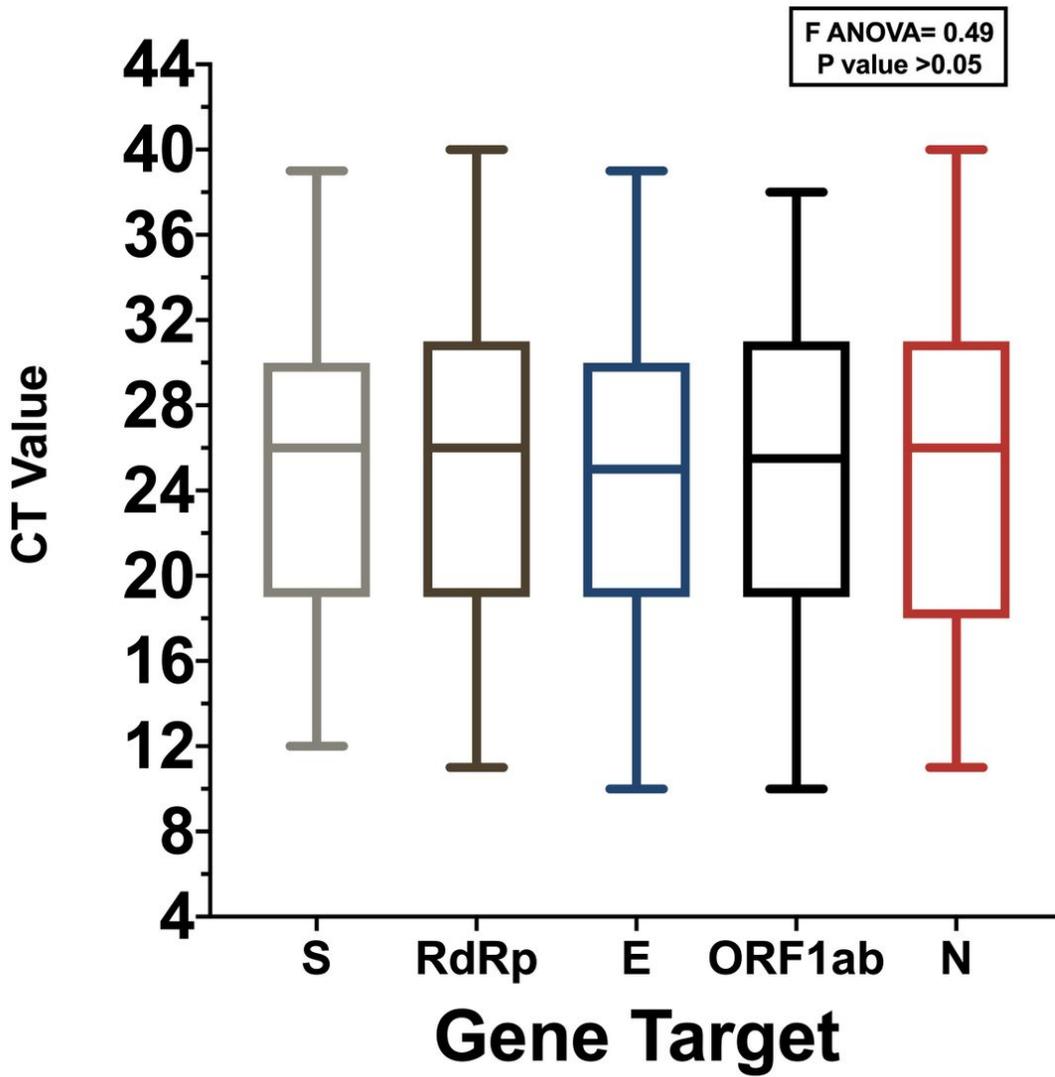


Figure 3

Boxplot of the positive samples cycling threshold (CT) values across the testing kits targets. The whisker above the boxplot represents the 95% upper confident interval, while the bottom line represents the lower 95% confidence interval. The lowest CT value was reported with kits targeting the N gene. The highest CT values were mostly reported with kits targeting the RdRp gene.