Relative Sensitivity of Common Target Genes For The Detection of SARS-Cov-2 In Real Time-PCR

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Research Article

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Abstract

SARS-CoV-2(COVID-19) currently is the main cause of the severe acute respiratory disease and fatal outcomes in human beings worldwide. Several genes are used as targets for the detection of SARS-CoV-2, including the RDRP, N, and E genes. The present study aimed to determine the RDRP, N, and E genes expressions of SARS-CoV- 2 in clinical samples. For this purpose, 100 SARS-CoV-2 positive samples were collected from diagnostic laboratories of Mazandaran province, Iran. After RNA extraction, the real time RT-PCR assay was performed for differential gene expressions' analysis of N, E, and RDRP. The CT values for N, RDRP, and E targets of 100 clinical samples for identifying SARS-CoV-2 were then evaluated using qRT-PCR. This result suggests N gene as a potential target for the detection of the SARS-CoV-2, since it was observed to be highly expressed in the nasopharyngeal or oropharynges of COVID-19 patients ($P < 0.0001$). Herein, we showed that SARS-CoV- 2 genes were differentially expressed in the host cells. Therefore, to reduce obtaining false negative results and to increase the sensitivity of the available diagnostic tests, the target genes should be carefully selected based on the most expressed genes in the cells.

Introduction

SARS-CoV-2(COVID-19), which is currently known as the global pandemic of Coronavirus, is responsible for the severe acute respiratory disease and fatal outcomes in human beings worldwide (Korber, Fischer et al. 2020). Coronaviruses as a group of enveloped viruses with positive-sense single-stranded RNA belong to the family Coronaviridae, which are able to spread between humans and animals (Holshue, DeBolt et al. 2020).

Unlike three previous epidemics of β-coronaviruses such as Middle East respiratory syndrome-related Coronavirus (MERS-CoV), severe acute respiratory syndrome Coronavirus (SARS-CoV), and severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), which have been potentially associated with acute respiratory distress syndrome (ARDS), most Human Coronaviruses (HCoV) such as OC43, HKU1, 229E, and NL63 cause a moderate upper respiratory infection in human (Hu, Lu et al. 2014). It has been shown that the mortality rate in more than 10,000 cases with both SARS-CoV and MERS-CoV was 10% and 37%, respectively (Prete, Favoino et al. 2020). Some previous studies have suggested that the epidemic potential of the COVID-19 outbreak with the fatality rate of 3.42% is higher than that of both SARS and MERS (Lai, Wang et al. 2020) (Del Rio and Malani 2020).

Coronaviruses ORFs encode four structural proteins, including S-spike, M-membrane, E-envelope, and N-nucleocapsid. Of note, several genes are used as targets identification such as the (RDRP and S), (N and S), and E genes. In this regard, studies have previously shown that SARS-COV-2 N protein is produced in large quantities in infected cells, which is related to the processes of replication, translation, and transcription. Moreover, it causes cell cycle deregulation, consequently inhibiting interferon production and inducing apoptosis (Astuti 2020). In order to have the best RT-PCR performance, the components of these targets should be optimized (Tombuloglu, Sabit et al. 2021). Accordingly, reverse transcription

polymerase chain reaction (RT-PCR) using fluorescent dyes is considered as a gold standard method for detecting bacterial and viral nucleic acid (DNA / RNA). RT-q PCR can also be used as a rapid and accurate assay for screening SARS-CoV-2 in throat samples, nasopharyngeal swabs, and feces (Chaimayo, Kaewnaphan et al. 2020). A cohort study has shown that RT-PCR with sensitivity and specificity values of 70% and 95% could detect viruses in patients, even in those showing no symptoms (Arevalo-Rodriguez, Buitrago-Garcia et al. 2020, Patankar and Zambare 2021). However, a successful detection of this virus depends on some factors such as test time, early or late detection time, viral load, and sample collection procedure (Vickers 2017).

There are some commercial RT-PCR kits such as Primer Design (Chandler's Ford, UK), Seegene (Seoul, South Korea), and CerTest Biotec (Zaragoza, Spain), with different qualities, which are available to be used for the diagnosis of SARS-CoV-2 (van Kasteren, van Der Veer et al. 2020). According to this point that molecular diagnostic method for the diagnosis of SARS-COV-2 must have good sensitivity and specificity, so the present study attempted to analyze the RDRP, N, and E genes expressions of SARS-COV-2 using qRT-PCR through specific primer pairs in the obtained clinical samples.

Material And Methods

Multiplex primer and probe design

The specific RT-qPCR primers and probe for the diagnosis of the target regions of the SARS-CoV-2 were designed using the following programs: PrimerPooler, PrimerPlex, and Primer3 (Tombuloglu, Sabit et al. 2021). Moreover, 5' Fluorescein amidites (FAM)-labeled probe was designed for the SARS-CoV-2 RdRp/ N/RP, as well as Hypoxanthine Phosphoribosyltransferase (HPRT) and Yakkima yellow-labeled probe for the viral E gene, which were then synthesized (Fig.1). The sequence of each primer or probe is shown in Table 1.

RNA Extraction from the Clinical Samples

The study was approved by the Mazandaran University of Medical Sciences, Iran, with the number IR.MAZUMS.REC.1399.8671. For the purpose of this study, Nasopharyngeal and oropharyngeal swabs were collected from symptomatic patients, immediately diluted with viral transfer medium (VTM), and finally transferred to the COVID-19 laboratory at Mazandaran University of Medical Sciences for the detection of SARS-CoV-2. RNA extraction was performed in 100 positive samples using the RNJia virus kit (Jivan, Iran) in terms of the manufacturer's instructions. Subsequently, the differential gene expressions of N, E, and RdRp were performed using qRT-PCR.

Real-time RT-PCR assay

In this study, 20-µL reaction containing 4 µL of RNA, 10 µL of one step RT-PCR kit(add bio, korea), 2 µL of enzyme mixture, 0.5 µL of forward and reverse primers, 0.5 µL of each probe, and RNase/DNase-free

ddH2O up to 20 µL, was setup. Final primers and probes concentrations in the reaction were adjusted using the following steps:

1.0.25 pM for RdRP-F, and 0.25 pM for RdRP-R

2. 0.25 pM for E-F, and 0.25 pM for E-R

3. 0.25 pM for N-F, and 0.25 pM for N-R

4. 0.25 pM for HPRT-F, and 0.25 pM for HPRT-R

The reaction was dispensed in 96-well microplates (MicroAmp™ Fast Optical 96-well reaction Plate 0.1 mL, Applied Biosystems) and then sealed with optical film (MicroAmp™ Optical Adhesive Film, Applied Biosystems). Of note, a negative control reaction (RNase/DNasefree ddH2O) was used to check the presence of any contamination. In addition, HPRT and RP genes were used as internal controls (Valadan, Hedayatizadeh-Omran et al. 2015).

Thereafter, Quantitation experiments were conducted using RT-PCR instrument (StepOne^{TM} Real-Time PCR System).

As well, qPCR was performed as follows:

1. Reverse transcription was performed for 20 minutes at 50°C,
2. Inactivation of the reverse transcriptase was done for 10 minutes at 95°C.
3. PCR amplification was performed with 40 cycles for 15 seconds at 95°C and for 30 second at 58°C using StepOne^{TM} Real-Time PCR

Statistical analysis

The obtained results were examined by determining the amplification curve of the target gene and the housekeeping gene. Continuous variables are indicated as means (standard deviation, SD). All the statistical analyses were performed using GraphPad Prism 8 software and p-values less than 0.001 were considered as statistically significant.

Results

In the present study, 100 respiratory samples were collected from nasopharyngeal (NP) and throat swabs in health-care centers of Mazandarn, Iran, from December 2020 to September 2021. Thereafter, Real-time RT-PCR, using E, RDRP, and N targets, was performed for RNA detection of SARS-CoV-2. Firstly, all the primers and probes were analyzed by simplex qRT-PCR. Prior to preparing the reactions, the qRT-PCR instrument was properly calibrated in order to achieve the best fluorescent signal. The simplex reactions were then performed in triplicate for three viral E, N, and RDRP genes as well as internal control genes

(HPRT and RP). The criteria for the diagnosis of positive, negative, and suspicious COVID-19 samples were as follows: (0 < CT < 37.00), (NO CT or CT ≥ 40.00), and (37.00 ≤ CT < 40.00), respectively.

The average cycle threshold (Ct) and ΔCt value with standard deviations (SD) are shown in tables 2 and 3, and the comparative Ct performances of each assay are shown in Fig 2 and 3. In this research, HPRT and RP genes were used as internal controls. Indeed HPRT and RP had significantly increased expression level compared to other targets (including N, E, and RDRP) ($P < 0.0001$). Our findings showed that no detectable difference exists between HPRT and RP internal controls. According to the comparison of ΔCt values among N, E, and RDRP targets, the N gene expression level was found to be higher than that of E and RDRP genes. ($P < 0.0001$). As shown in Fig 4, there is no significant difference between E and N targets (0.611). The result of our study suggest N gene as the most sensitive target compared to E and RDRP for SARS-CoV-2 detection using RT-PCR.

Discussion

In this study, CT values for the N, RDRP, and E targets were evaluated using qRT-PCR in order to detect SARS-CoV-2 in 100 clinical samples. It was observed that N gene has less Ct values (23.73±6.99) than those of E and RDRP. Moreover, our results show a significant difference among the E, N, and RDRP groups.

The diagnosis of SARS-CoV-2 using molecular tests is known as the gold standard method for the diagnosis of COVID-19 infection. Of note, the RT-PCR is a sensitive assay for the detection of SARS-CoV-2 RNA in clinical specimens (Chaimayo, Kaewnaphan et al. 2020). The study showed that after the onset of the disease's symptoms, the SARS-CoV-2 viral load can be immediately observed in the upper respiratory tract and the antigen can also be detected in the first phase. However, some factors such as clinical manifestations, duration of disease to laboratory test, type of clinical sample, and sample collection procedure (technique process) can be effective on interpreting the results (Zou, Ruan et al. 2020).

In general, many developed laboratory methods use various tools, reagents, and targets in order to identify SRRS-COV-2 (LeBlanc, Gubbay et al. 2020). RDRP, E, and N are three targets proposed by WHO for the SARS-COV-2 identification (Corman[1], Landt et al. 2020). As well, the E gene is the first line screening, the RDRP gene is used as confirmatory test, and the N gene is used for a confirmatory testing, all of which are used in identifying the coronavirus. A previous study has shown that the RdRP_SARSr-P2 target could be specific for the coronavirus, and other probes are suitable for the detection of other types of coronavirus, and if false positive results are obtained regarding the diagnosis of Covid-19, it may possibly indicate that patients with mild symptoms are infected with other types of corona virus (Kakhki, Kakhki et al. 2020). Besides, evidence suggests that other targets such as ORF8 and specific primers / probes, may act as additional confirmatory tests in the diagnosis of SARS-COV-2 (kamali Kakhki, Aryan et al. 2020).

Houda et al. in their study have evaluated three genes of RDRP, N, and E in 187 COVID-19 samples and found gene expression as 22% and 40% in N and N, E genes, respectively. They have also shown that 6% of patients with both E and N genes and 14% of those with N gene still remained positive after a 12-day

treatment period (Benrahma, Diawara et al. 2020). In addition, a study of 114 respiratory specimens has revealed that the N Ct value was more specific for laboratory diagnosis of SARS-CoV-2 (Abbasi, Tabaraei et al. 2021) .

Another study has shown that the one-step real-time RT-PCR can detect SARS-CoV-2 RNA in clinical specimens with a low detection sensitivity (Michel, Neumann et al. 2021). Since January 2020, protocols, tests, and reagents have been developed and introduced for the detection of SARS-COV-2. These laboratory tests that use SARS-CoV-2 RNA for the detection of COVID-19, were compared with commercial kits. A previous study using RT-PCR and two primers (N1 and N2) for SARS-COV-2 identification (Shirato, Nao et al. 2020) has shown that N2 primer has high specificity and sensitivity in this regard. These primers were also assessed using the following commercial kits: LN S & W-E, LN S & W-N, and LMW & RDRP (Hoehl, Rabenau et al. 2020). The results showed that the commercial LN S & W-N kit containing N primer was able to detect the virus better than the LN S & W-E (25 copies detected) and LMW & RDRP kits. It was observed that the LN S & WE targets are strongly conserved in the E gene region on SARS-COV and SARS-COV-2, while the N2 targets are a single region of N gene on SARS-COV-2 virus, so N2 is highly sensitive and specific for the detection of SARS-CoV-2 (Corman, Landt et al. 2020).

This study showed that the differences in gene expressions are associated with the genes of SARS-COV-2. Therefore, to reduce false negative results and to increase the sensitivity, diagnostic tests should be designed based on the targets that have the most differential expression. Correspondingly, RT-PCR method using of N, E, and RDRP targets is known as a reliable and accurate method for SARS-CoV-2 identification that can be used in infection's prevention and control, and in diagnostic laboratories and medical centers.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: This research has been approved with the number of (grant No. IR.MAZUMS.REC.1399.8671) in the ethics committee of Mazandaran University of Medical Sciences, Sari, Iran.

Availability of data and materials: The data and materials are mentioned in the manuscript.

Competing interests: Author declares no conflict of interest.

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Authors' contributions: RV participated in experimental design, SG carried out real time PCR, R A-N performed the statistical analysis, MH participated in data collection, MZ and MGH participated in manuscript preparation, TM contributed to experimental design and manuscript revision.

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References

1. Abbasi, H., A. Tabaraei, S. M. Hosseini, A. Khosravi and H. R. Nikoo (2021). "Real-time PCR Ct value in SARS-CoV-2 detection: RdRp or N gene?" Infection: 1-4.
2. Arevalo-Rodriguez, I., D. Buitrago-Garcia, D. Simancas-Racines, P. Zambrano-Achig, R. Del Campo, A. Ciapponi, O. Sued, L. Martinez-Garcia, A. W. Rutjes and N. Low (2020). "False-negative results of initial RT-PCR assays for COVID-19: a systematic review." PloS one **15**(12): e0242958.
3. Astuti, I. (2020). "Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response." Diabetes & Metabolic Syndrome: Clinical Research & Reviews **14**(4): 407-412.
4. Benrahma, H., I. Diawara, I. Smyej, J. Rahoui, N. Meskaouni, R. Benmessaoud, K. Arouro, K. Jaras, Z. Adam and S. Nahir (2020). "Epidemiological description and analysis of RdRp, E and N genes dynamic by RT-PCR of SARS-CoV-2 in Moroccan population: Experience of the National Reference Laboratory (LNR)-UM6SS." medRxiv.
5. Bruce, E. A., S. Tighe, J. J. Hoffman, P. Laaguiby, D. L. Gerrard, S. A. Diehl, D. G. Leonard, C. D. Huston, B. D. Kirkpatrick and J. W. Crothers (2020). "RT-qPCR detection of SARS-CoV-2 RNA from patient nasopharyngeal swab using Qiagen RNeasy kits or directly via omission of an RNA extraction step." BioRxiv.
6. Chaimayo, C., B. Kaewnaphan, N. Tanlieng, N. Athipanyasilp, R. Sirijatuphat, M. Chayakulkeeree, N. Angkasekwinai, R. Sutthent, N. Puangpunngam and T. Tharmviboonsri (2020). "Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of COVID-19 in Thailand." Virology Journal **17**(1): 1-7.
7. Corman[1], V. M., O. Landt, M. Kaiser, R. Molenkamp[4], A. Meijer, D. K. Chu, T. Bleicker[1], S. Brünink[1], J. Schneider[1] and M. L. Schmidt[1] (2020). "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR."
8. Corman, V., T. Bleicker, S. Brünink, C. Drosten and M. Zambon (2020). "Diagnostic detection of 2019-nCoV by real-time RT-PCR." World Health Organization **17**: 1-13.
9. Corman, V. M., O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D. K. Chu, T. Bleicker, S. Brünink, J. Schneider and M. L. Schmidt (2020). "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR." Eurosurveillance **25**(3): 2000045.
10. Del Rio, C. and P. N. Malani (2020). "COVID-19—new insights on a rapidly changing epidemic." Jama **323**(14): 1339-1340.
11. Gregianini, T. S., I. R. S. Varella, P. Fisch, L. G. Martins and A. B. Veiga (2019). "Dual and triple infections with influenza A and B viruses: a case-control study in Southern Brazil." The Journal of infectious diseases **220**(6): 961-968.

12. Hoehl, S., H. Rabenau, A. Berger, M. Kortenbusch, J. Cinatl, D. Bojkova, P. Behrens, B. Böddinghaus, U. Götsch and F. Naujoks (2020). "Evidence of SARS-CoV-2 infection in returning travelers from Wuhan, China." New England Journal of Medicine **382**(13): 1278-1280.
13. Holshue, M. L., C. DeBolt, S. Lindquist, K. H. Lofy, J. Wiesman, H. Bruce, C. Spitters, K. Ericson, S. Wilkerson and A. Tural (2020). "First case of 2019 novel coronavirus in the United States." New England Journal of Medicine.
14. Hu, Q., R. Lu, K. Peng, X. Duan, Y. Wang, Y. Zhao, W. Wang, Y. Lou and W. Tan (2014). "Prevalence and genetic diversity analysis of human coronavirus OC43 among adult patients with acute respiratory infections in Beijing, 2012." PLoS One **9**(7): e100781.
15. Jung, Y. J., G.-S. Park, J. H. Moon, K. Ku, S.-H. Beak, S. Kim, E. C. Park, D. Park, J.-H. Lee and C. W. Byeon (2020). "Comparative analysis of primer-probe sets for the laboratory confirmation of SARS-CoV-2." BioRxiv.
16. Kakhki, R. K., M. K. Kakhki and A. Neshani (2020). "COVID-19 target: A specific target for novel coronavirus detection." Gene Reports **20**: 100740.
17. kamali Kakhki, R., E. Aryan, Z. Meshkat and M. Sankian (2020). "Development of a cost-effective line probe assay for rapid detection and differentiation of mycobacterium species: a pilot study." Reports of Biochemistry & Molecular Biology **8**(4): 383.
18. Korber, B., W. M. Fischer, S. Gnanakaran, H. Yoon, J. Theiler, W. Abfalterer, N. Hengartner, E. E. Giorgi, T. Bhattacharya and B. Foley (2020). "Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus." Cell.
19. Lai, C.-C., C.-Y. Wang, Y.-H. Wang, S.-C. Hsueh, W.-C. Ko and P.-R. Hsueh (2020). "Global epidemiology of coronavirus disease 2019: disease incidence, daily cumulative index, mortality, and their association with country healthcare resources and economic status." International journal of antimicrobial agents: 105946.
20. LeBlanc, J. J., J. B. Gubbay, Y. Li, R. Needle, S. R. Arneson, D. Marcino, H. Charest, G. Desnoyers, K. Dust and R. Fattouh (2020). "Real-time PCR-based SARS-CoV-2 detection in Canadian laboratories." Journal of Clinical Virology **128**: 104433.
21. Michel, J., M. Neumann, E. Krause, T. Rinner, T. Muzeniek, M. Grossegesse, G. Hille, F. Schwarz, A. Puyskens and S. Förster (2021). "Resource-efficient internally controlled in-house real-time PCR detection of SARS-CoV-2." Virology Journal **18**(1): 1-11.
22. Patankar, R. S. and V. P. Zambare (2021). Development of RT-PCR Based Diagnosis of SARS-CoV-2. Biotechnology to Combat COVID-19, IntechOpen.
23. Prete, M., E. Favoino, G. Catacchio, V. Racanelli and F. Perosa (2020). "SARS-CoV-2 inflammatory syndrome. Clinical features and rationale for immunological treatment." International Journal of Molecular Sciences **21**(9): 3377.
24. Shirato, K., N. Nao, H. Katano, I. Takayama, S. Saito, F. Kato, H. Katoh, M. Sakata, Y. Nakatsu and Y. Mori (2020). "Development of genetic diagnostic methods for novel coronavirus 2019 (nCoV-2019) in Japan." Japanese journal of infectious diseases: JJID. 2020.2061.

25. Tombuloglu, H., H. Sabit, E. Al-Suhaimi, R. Al Jindan and K. R. Alkharsah (2021). "Development of multiplex real-time RT-PCR assay for the detection of SARS-CoV-2." Plos one **16**(4): e0250942.
26. Valadan, R., O. Amjadi, M. Tehrani, A. Rafiei, A. Hedayatizadeh-Omran and R. Alizadeh-Navaei (2015). "Pseudogene-free amplification of HPRT1 in quantitative reverse transcriptase polymerase chain reaction." Analytical biochemistry **485**: 46-48.
27. Valadan, R., A. Hedayatizadeh-Omran, M. N. Alhosseini-Abyazani, O. Amjadi, A. Rafiei, M. Tehrani and R. Alizadeh-Navaei (2015). "Data supporting the design and evaluation of a universal primer pair for pseudogene-free amplification of HPRT1 in real-time PCR." Data in brief **4**: 384-389.
28. van Kasteren, P. B., B. van Der Veer, S. van den Brink, L. Wijsman, J. de Jonge, A. van den Brandt, R. Molenkamp, C. B. Reusken and A. Meijer (2020). "Comparison of seven commercial RT-PCR diagnostic kits for COVID-19." Journal of Clinical Virology **128**: 104412.
29. Vickers, N. J. (2017). "Animal communication: when i'm calling you, will you answer too?" Current biology **27**(14): R713-R715.
30. Zou, L., F. Ruan, M. Huang, L. Liang, H. Huang, Z. Hong, J. Yu, M. Kang, Y. Song and J. Xia (2020). "SARS-CoV-2 viral load in upper respiratory specimens of infected patients." New England Journal of Medicine **382**(12): 1177-1179.

Tables

Table 1. The sequences and concentrations of primer and probe sets used in the PCR reactions.

Target	Sequence(5'-3')	Label	Reference
2019-nCoV_N1-F	GAC CCC AAA ATC AGC GAA AT	None	(Jung, Park et al. 2020)
2019-nCoV_N1-R	CT GGT TAC TGC CAG TTG AAT CTG	None	(Jung, Park et al. 2020)
2019-nCoV_N1-P	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1	FAM, BHQ1	(Bruce, Tighe et al. 2020)
E_Sarbeco_F1	ACAGGTACGTTAATAGCGT	None	(Corman, Bleicker et al. 2020)
E_Sarbeco_R1	ATATTGCAGCAGTACGCACACA	None	(Corman, Bleicker et al. 2020)
E_Sarbeco_F1	Yakkima yellow-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1	Yakkima yellow, BHQ1	(Corman, Bleicker et al. 2020)
2019-nCoV_RDRP-F1	GTGARATGGTCATGTGTGGCGG	None	(Corman, Bleicker et al. 2020)
2019-nCoV_RDRP-R	CARATGTTAAASACACTATTAGCATA	None	(Corman, Bleicker et al. 2020)
2019-nCoV_RDRP-P	FAM-CAGGTGGAACCTCATCAGGAGATGC-BHQ1	FAM, BHQ1	(Corman, Bleicker et al. 2020)
HPRT-F	GGACTAATTATGGACAGGACTG	None	(Valadan, Amjadi et al. 2015)
HPRT-R	GCTCTTCAGTCTGATAAAATCTAC	None	(Valadan, Amjadi et al. 2015)
HPRT-P	FAM-CCTCCCATCTCCTTCATCACATCTC–BHQ1	FAM, BHQ1	(Valadan, Amjadi et al. 2015)
RP-F	AGA TTT GGA CCT GCG AGC G	None	(Gregianini, Varella et al. 2019)
RP-R	GAG CGG CTG TCT CCA CAA GT	None	(Gregianini, Varella et al. 2019)
RP-P	FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1	FAM, BHQ1	(Gregianini, Varella et al. 2019)

Table 2: The Ct value emerged in RT-PCR assay for the SARS-COV-2

Characteristics	Result
Ct-value of E Mean±SD (min, max)	26±6.53 (14.24, 39.24)
Ct-value of N Mean±SD (min, max)	20.19±5.93 (9.72, 33.82)
Ct-value of RDRP Mean±SD (min, max)	26.92±7.04 (13.85, 40)
Ct-value of HPRT Mean±SD (min, max)	31.34±2.75 (26.94, 40)
Ct-value of RP Mean±SD (min, max)	24.43±1.63 (20.56, 27.94)

Table 3: The ΔCt value emerged in RT-PCR assay for the SARS-COV-2

Characteristics	ΔCt value (HPRT control)	ΔCt value (RP control)
E gene Mean±SD (min, max)	-5.34 ±6.76 (-15.15, 0)	1.57±6.74 (-8.71, 19.07)
N gene Mean±SD (min, max)	-11.15±6.28 (-22.45, 0.43)	-4.23±6.13 (-13.23, 13.65)
RDRP gene Mean±SD (min, max)	-4.42±7.27 (-16.91, 11.42)	2.49±7.32 (-6.84, 17.59)

Figures

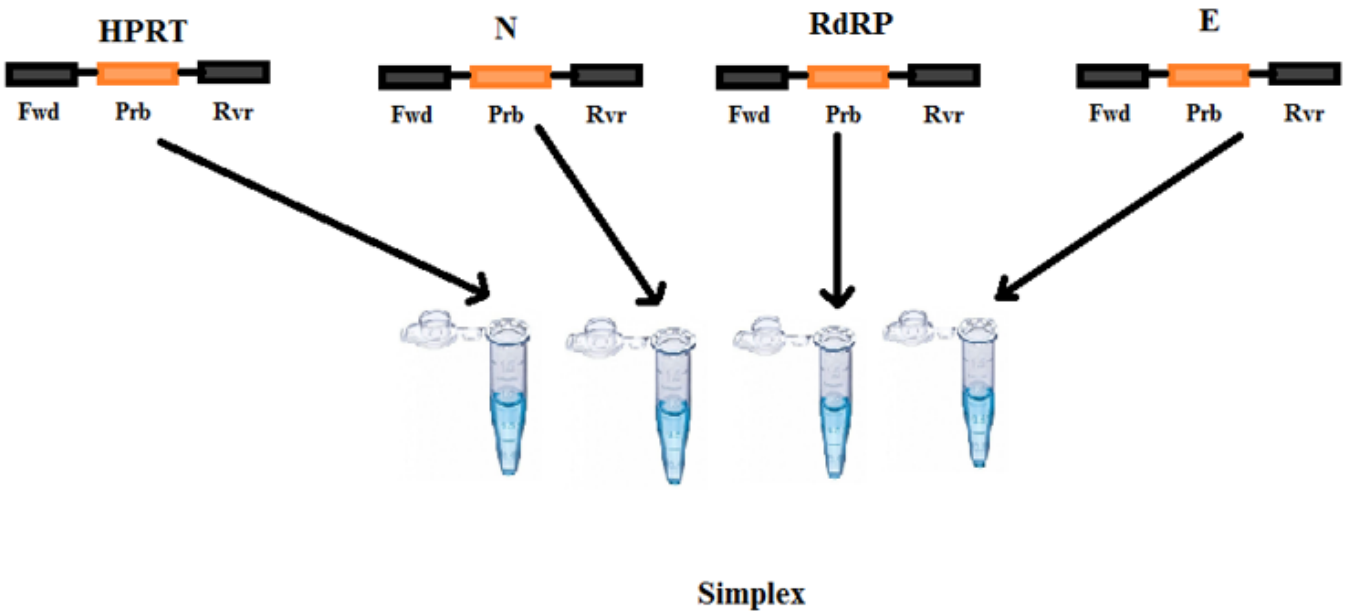

Figure 1

Experimental design of qRT-PCR.

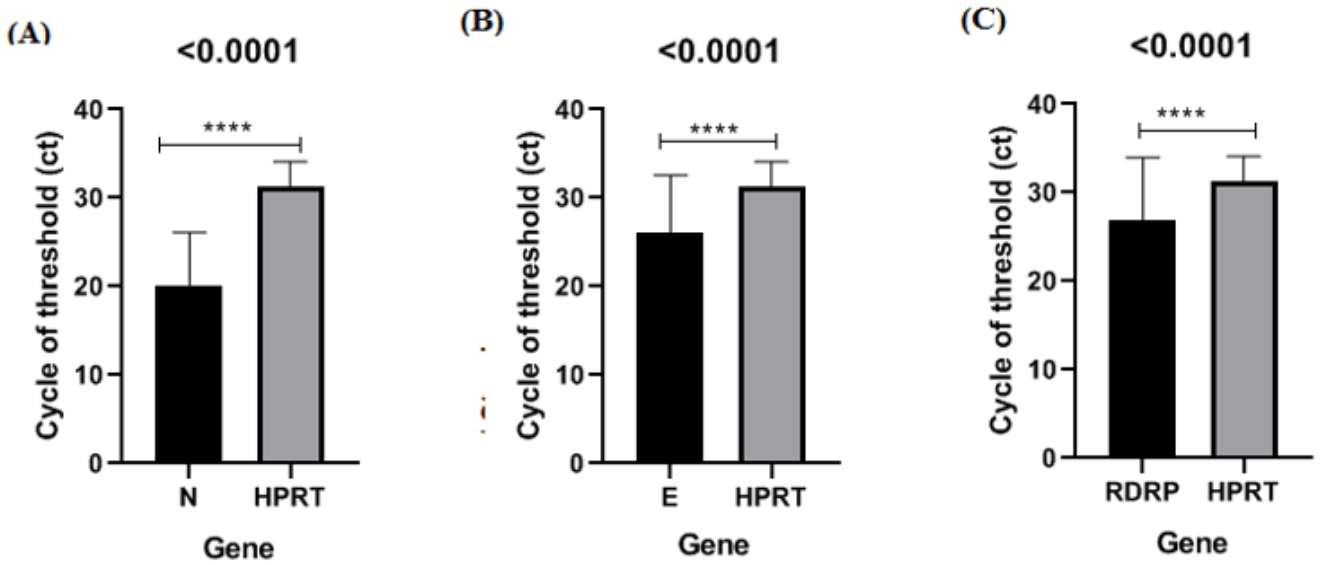

Figure 2

Cycle threshold (Ct) value of qRT-PCR. HPRT gene was used as an internal control. A: Comparison of N target and HPRT, B: Comparison of E target and HPRT, and C: Comparison of RDRP target and HPRT. A

significant difference is indicated by *P<0.05. **** = (<0.0001).

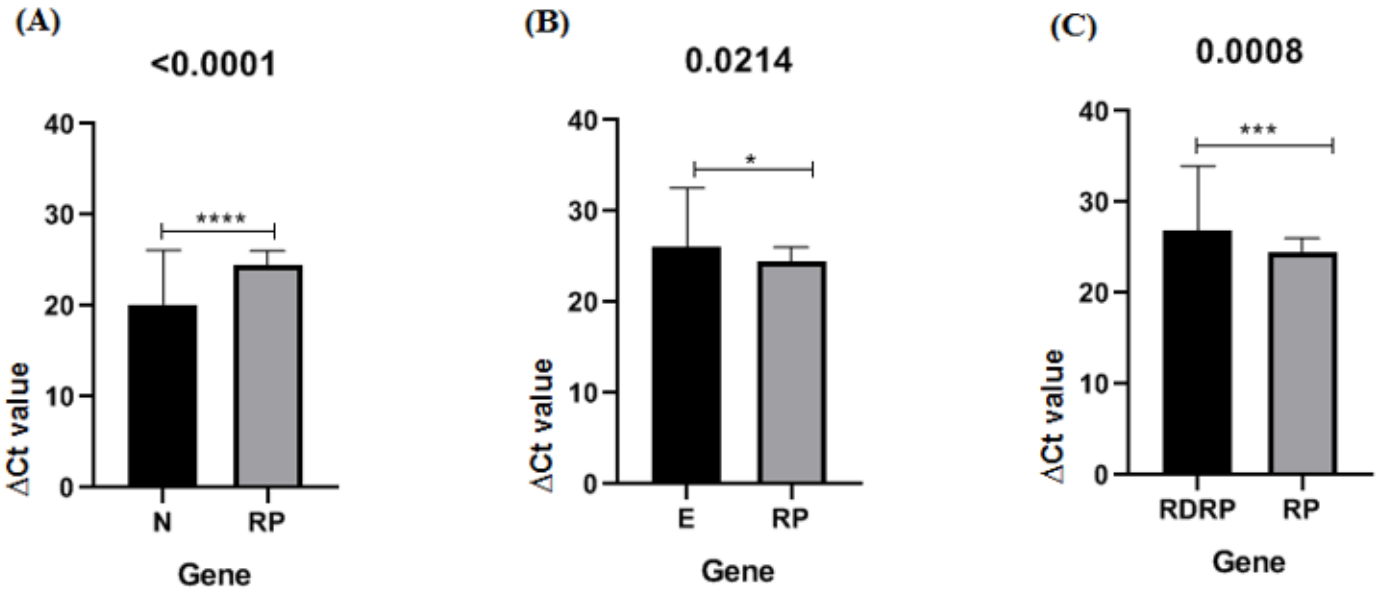

Figure 3

Cycle threshold (Ct) value of qRT-PCR. RP gene was used as an internal control. A: Comparison of N target and RP, B: Comparison of E target and RP, and C: Comparison of RDRP target and RP. A significant difference is indicated by *P<0.05. **** = (<0.0001).

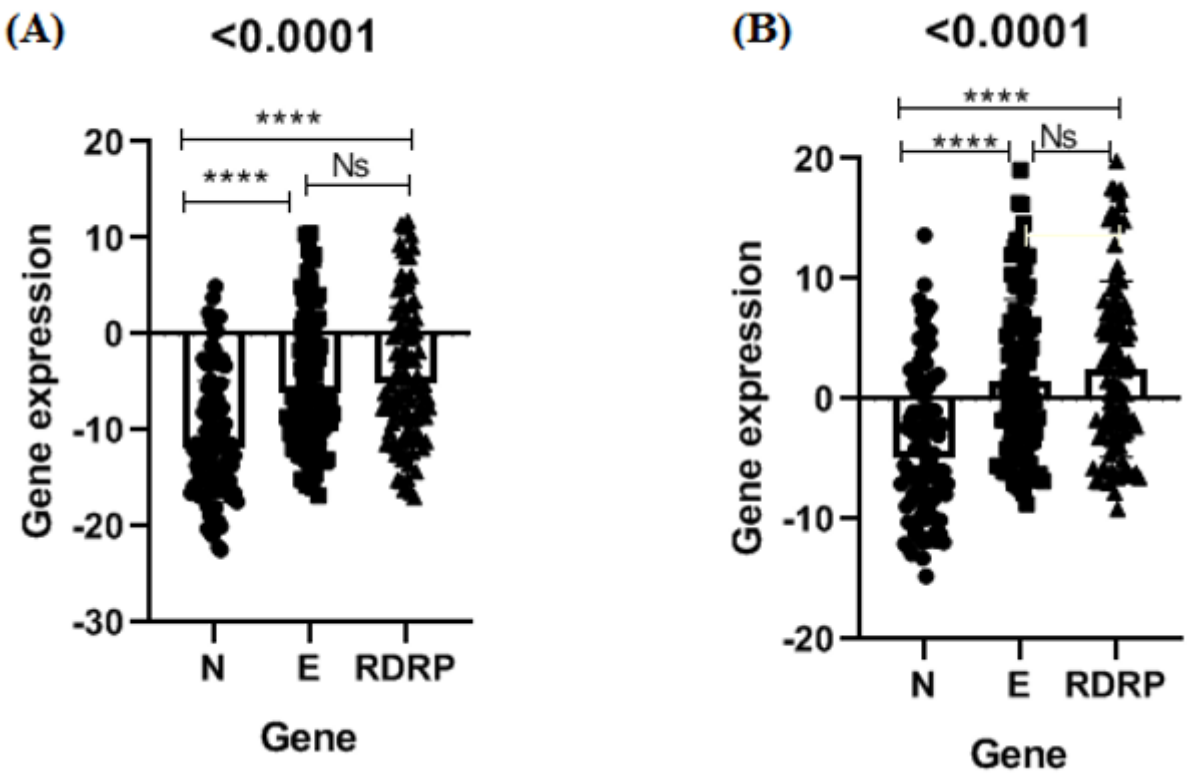

Figure 4

Comparison of the cycle threshold (ΔCt) value of SARS-COV-2 expression. A: HPRT gene was used as an internal control. A significant difference is indicated by *P<0.05. ****, Ns=Not significant (0.611). B: RP gene was used as an internal control. A significant difference is indicated by *P<0.05. ****, Ns=Not significant, (0.608).