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Comparative Evaluation of RT-PCR Kits Available in Sri Lanka for Diagnosis of COVID-19

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Ethics: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Ethics Review Committee of the Teaching Hospital Jaffna, Sri Lanka (Reg No: S04/07/2022), which was approved on September 01, 2022. Patient's written informed consent was not required for the study.

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Abstract

Identification of gene targets by real-time reverse transcriptase PCR (rRT-PCR) is considered as the gold standard for diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. Although many commercial rRT-PCR kits are currently used in Sri Lanka, analytical performance of these kits have not been investigated adequately. Therefore, the objective of the present study was to evaluate the analytical performance of rRT-PCR kits used in the laboratory of the Faculty of Medicine, University of Jaffna (five kits). Performance of the five rRT-PCR kits selected for this study was compared with the CDC 2019-Novel Coronavirus (2019-nCoV) RT-PCR Diagnostic Panel as reference standard. The sensitivity, specificity, positive predictive value, negative predictive value and Cohen's kappa coefficient of the five different commercial kits were analyzed. SARS-CoV-2 positive (62) and negative (32) respiratory samples collected respectively from symptomatic individuals and asymptomatic healthy individuals were used in this study. Comparison of the cycle threshold (Ct) values of the five commercial kits revealed heterogeneity. Among them, the TaqPath^{TM} kit showed the highest sensitivity (98.4%), and inter-rater reliability (0.976). The HBRT-COVID-19 kit showed the lowest sensitivity (91.9%), specificity (93.7%) and inter-rater reliability (0.838). Although the five RT-PCR kits exhibited varying sensitivity, specificity and Ct values, all of them are suitable for the routine diagnosis of SARS-CoV-2 infections as all values were above 90%.

Keywords: COVID-19; diagnosis; RT-PCR; Ct value; sensitivity; specificity

Introduction

Coronaviruses (CoVs) are a large group of enveloped, single-stranded, positive-sense RNA viruses that have acquired the ability to cause respiratory diseases among a wide variety of animals including humans.[1,2] Although this group of viruses has been known for many decades, they received global attention in December 2019, when a primary epidemic cluster of cases with respiratory tract infections was reported in Wuhan, China.[2] China declared that the severe respiratory tract infections were caused by a novel CoV named as 2019-nCoV. On account of rapid spreading of the virus, renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19), WHO declared a global emergency.[3]

The first case of COVID-19 in Sri Lanka was reported on 27 January, 2020.[4,5] Since then COVID-19 cases have been diagnosed with the aid of SARS-CoV-2 real-time reverse transcriptase PCR (rRT-PCR) kits.[4] According to the data presented in the *National Epidemiological Report* released by the Epidemiology Unit, Ministry of Health, Sri Lanka, a total 422,145 cases were confirmed nationwide by the end of the third wave on 30 September, 2021 and the SARS-CoV-2 rRT-PCR method was used to diagnose the majority of cases.[6] In addition to direct health consequences, the COVID-19 pandemic had a negative impact on the national economy, employment, tourism, poverty, exchange rate, social activity and welfare.[7]

Although promising treatments like antigen neutralizing antibody treatments, which are able to provide long-term protection against COVID-19,[8,9] have been developed, their strict storage conditions, distribution requirements, and high production costs limit accessibility of these

treatment options for patients living in developing countries like Sri Lanka.[9] On the other hand, complete vaccination of the public will take time and investments and vaccines are not effective against emerging variants.[10] Therefore, early diagnosis and adequate preventive strategies are important to combat disease transmission.[11,12] The most commonly used laboratory diagnosis method is the detection of gene targets from SARS-CoV-2 by rRT-PCR, which is considered as the gold standard for diagnosis.[13] According to the principle, the specific genome sequence(s) of SARS-CoV-2 is transcribed and amplified with the aid of the rRT-PCR method. As an initial step, viral RNA is extracted from the biological specimen and subsequently converted into complementary DNA by reverse transcriptase, which is used as the template for the subsequent PCR cycles. A sequence-specific dual-labeled fluorogenic probe and forward and reverse primers are utilized depending on the gene target.[14] Commercially available SARS-CoV-2 rRT-PCR kits target different conserved regions of the viral genome, including the RNA-dependent RNA polymerase gene (*RdRP*) present in the open reading frame (*ORF1ab*) region, the envelope protein gene (*E*), thenucleocapsid protein gene (*N*), and thespike protein gene (*S*).[15,16]

Although there are many commercial rRT-PCR kits available, their comparative sensitivity and specificity have not been reported.[13] Therefore, evaluation of their performance is essential in the context of maintaining SARS-CoV-2 rRT-PCR as a gold standard diagnostic tool. The comparison of different commercially available SARS-CoV-2 rRT-PCR kits has been executed by many research groups from different localities around the world. Although many studies revealed that the majority of the compared kits are reliable for routine diagnosis of the disease, varying cycle threshold (Ct) values for the same samples and varying diagnostic accuracies have been reported.[17-26]

To date there has been only one Sri Lankan study published to evaluate the diagnostic accuracy of different commercially available SARS-CoV-2 rRT-PCR kits.[26] Therefore, the foremost purpose of the current study was to evaluate the analytical performance of five different commercial rRT-PCR kits used in the laboratory of Faculty of Medicine, University of Jaffna.

Results

Five COVID-19 RT-PCR kits were compared to evaluate their efficiency for diagnosis of COVID-19: TaqPath™ COVID-19 CE-IVD RT-PCR (TaqPath™ kit), Real Star® SARS-CoV-2 RT-PCR (Real Star® kit), STANDARD M nCoV Real-Time PCR (STANDARD-M kit), COVID-19 Real-Time PCR (HBRT-COVID-19 kit), and AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR (AccuPower® kit). The comparison was made using the CDC 2019-Novel Coronavirus (2019-nCoV) RT-PCR Diagnostic Panel (CDC RT-PCR kit) as the gold standard reference kit. The descriptive statistical analysis of the results obtained from the five rRT-PCR kits exhibited a diverse range of Ct values, even for the same gene target (Table 1). Distribution patterns of Ct values of the individual gene targets and the respective rRT-PCR kits are illustrated in Figure 1 and Figure 2. Accordingly, the ascending order of lowest Ct values was the *E* gene target (5.56) and *N* gene target (5.36) of the AccuPower® kit, the *ORF1ab* gene target (7.07) and *E* gene target (7.19) of the STANDARD-M kit, and the *N* gene target (7.51) of the TaqPath™ kit. We also observed that there were variations among the mean Ct values across the same gene targets of different rRT-PCR kits. Absolute Ct values for individual samples obtained from the five rRT-PCR kits for the *E* gene, the *ORF1b* gene, the *N* gene and the *S* gene are presented in Table S1.

For the *E* gene target site, significant differences were observed for the pairs of Ct values obtained by the Real Star® kit and STANDARD-M kit ($P < 0.05$) and for the STANDARD-M kit and AccuPower® kit ($P < 0.001$). However, the Real Star® kit and AccuPower® kit were not significantly different. For the *N* gene target site, the pair of Ct values obtained by the TaqPath™ kit and HBRT-COVID-19 kit was significantly different ($P < 0.05$). Furthermore, both pairs of the TaqPath™ kit and AccuPower® kit and the HBRT-COVID-19 kit and AccuPower® kit showed significant differences ($P < 0.001$). For the *ORF1b* gene target site, the pairs of Ct values obtained by the TaqPath™ kit and STANDARD-M kit and the STANDARD-M kit and HBRT-COVID-19 kit showed significant differences ($P < 0.05$). However, the Ct values of the TaqPath™ kit and HBRT-COVID-19 kit were not significantly different ($P > 0.05$). For *S* gene target site, the pairs of Ct values from the TaqPath™ kit and Real Star® kit were not significantly different ($P > 0.05$).

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Cohen's kappa coefficient for each rRT-PCR kit are summarized in Table 2. Inter-test agreement was evaluated between the CDC RT-PCR kit and each of the five commercial rRT-PCR diagnostic kits. The TaqPath™ Kit showed the highest sensitivity at 98.4%, followed by the Real Star® kit and AccuPower® kit with a sensitivity of 95.2%. The lowest sensitivity was observed for the HBRT-COVID-19 kit (91.9%). The TaqPath™ kit, Real Star® kit and AccuPower® kit exhibited 100% specificity. The lowest specificity (93.8%) was reported for the STANDARD-M kit and HBRT-COVID-19 kit (Table 2).

The kappa coefficient for all five commercial kits was higher than 0.80. The highest kappa coefficient of 0.98 was obtained with the TaqPath™ kit. In addition, the total PCR running-time of each kit was also compared. The longest run time was reported for the Real Star® kit (1h 55min) and the minimum run time was reported for the TaqPath™ kit (1h 5min).

Three randomly selected positive samples among the categories of strong positive (S1), moderate positive (S2) and low positive (S3) were used to evaluate both repeatability and imprecision. The values obtained for repeatability and imprecision were tabulated in Table 3. Repeatability and imprecision of the rRT-PCR kits for the *E, N, ORF1ab* and *S* gene targets were analyzed. The repeatability ranged from 0.34 (S3) to 4.32 (S1) for the *E* gene target, from 0.42 (S3) to 4.33 (S1) for the *N* gene target, from 0.79 (S3) to 3.59 (S1) for the *ORF1ab* gene target and from 0.96 (S2) to 8.81 (S1) for the *S* gene target. The total imprecision ranged from 0.32 (S3) to 4.35 (S1) for the *E* gene target, from 0.41 (S3) to 4.33 (S1) for the *N* gene target, from 0.79 (S3) to 3.60 (S1) for the *ORF1ab* gene target and from 0.94 (S2) to 8.92 (S1) for the *S* gene target.

AccuPower® kit had the best repeatability and imprecision for both the *E* gene and *N* gene targets for all three categories. The best repeatability and imprecision for the *ORF1ab* gene target were reported for the STANDARD-M kit and the Real Star® kit showed the best repeatability and imprecision for the *S* gene target. Poorest repeatability for *E* gene target was reported for the STANDARD-M kit. HBRT-COVID-19 kit showed the poorest repeatability and imprecision for both the *N* gene and *ORF1ab* gene targets. Comparatively, the repeatability and imprecision of all gene targets in S3 (low positive) were lower than for S1 (strong positive) and S2 (moderate positive) samples. Overall, the repeatability was lower than 5% for all the kits, except for the *S*

gene target of the TaqPath™ kit, and imprecision was lower than 10% for all the five kits.

Discussion

In the context of confirming the diagnosis and promptly isolation of patients from the community to prevent disease transmission, a reliable test is important. Furthermore, it should help to monitor the patients and to provide appropriate disease management at the right time. SARS-CoV-2 rRT-PCR is considered as a reliable laboratory test. The current study focused on comparative evaluation of five commercially available rRT-PCR kits that were used in the laboratory of the Faculty of Medicine, University of Jaffna, for the diagnosis of SARS-CoV-2 infections. The sensitivity, specificity, PPV, NPV and the Ct values obtained by each of the rRT-PCR kits were compared for different SARS-CoV-2 gene targets. Inter-test agreement was evaluated between the reference CDC RT-PCR kit and the five commercial rRT-PCR diagnostics kits. The five rRT-PCR kits showed varied sensitivity, specificity and Ct values. The sensitivity of a diagnostic kit is the ability to detect true positives. A 100% sensitivity reflects a test's ability to correctly identify all samples and thereby people who have the disease condition.[27] None of the five rRT-PCR kits compared in the present study displayed 100% sensitivity. Sensitivity among the five rRT-PCR kits ranged from 93.5% to 98.4%. Although the TaqPath™ kit had the highest sensitivity (98.4%) and inter-rater agreement (0.98), relatively low values for both sensitivity (88%) and inter-rater agreement (0.85) have been reported for this kit by a previously conducted Indian study in 2021.[18] On the other hand, another study carried out in India in 2021 reported 100% sensitivity of the TaqPath™ kit.[23] A study conducted in Ecuador in 2021 reported a lower sensitivity (75%) for the AccuPower® kit than the sensitivity of 93.5% reported in the present study.[19] The differences in sample number and reference assay between our study and the

aforementioned studies might be the possible reason for the discrepancy in results (the sample number comparison of the current study with previously conducted studies is presented in Table S2.

Clinical specificity is defined as the probability of correctly differentiating the healthy individuals from individuals who have the diseased condition.[27] Indeed, the specificity is an indicator to validate the ability of a diagnostic kit to screen the true negatives. When a diagnostic kit has a 100% specificity, it means that particular kit does not give any false positive results.[27] Except the STANDARD-M kit and HBRT-COVID-19 kit, the other three kits showed specificities of 100%. Although the two kits with lower specificity (STANDARD- kit and HBRT-COVID-19 kit) showed two false positive results, the Ct values were marginal to the cutoff Ct values, which may be associated with the limit of detection of low viral loads or cross contaminations between adjacent samples. The studies conducted in India in 2021[18,23] and Ecuador in 2021[19] have recorded 100% specificity for the TaqPath™ kit and AccuPower® kit. The same results (100% specificity) were reported in the current study for both kits.

Overall, all five kits have a high efficiency as they have almost perfect (strong) inter-rater agreements (> 0.80) with the reference assay (Table 2), suggesting that all five evaluated kits are reliable for the diagnosis of COVID-19.[28] Among them, the highest diagnostic accuracy was achieved by the TaqPath™ kit and the lowest by the HBRT-COVID-19 kit with regards to sensitivity and specificity.

Ct values reported by rRT-PCR assays are not only used to determine the existence of viruses in clinical samples, but also as an indirect surrogate marker to determine the viral loads in the patients.[29] It has been reported in previous studies that patients infected with SARS-CoV-2 are assumed to be noninfectious if the Ct value exceeds 32.[29-31] However, it was observed in the present study that there is a possibility to have different Ct values for the same sample depending on the type of commercial kits used. This is in agreement with similar type of comparative studies conducted in the Netherlands in 2020,[17] India in 2021,[18,23] Ecuador in 2021,[19] South Korea in 2020,[20] Serbia 2021,[21] Japan in 2021,[22] Colombia in 2021,[24] and China in 2021.[25]

All commercial rRT-PCR kits have incorporated an internal control, either an exogenous control or an endogenous control, or both. The main purpose to add exogenous controls is to quantify the number of copies per inspected sample and to monitor PCR inhibition. Unlike the exogenous control, endogenous controls target human genes within the inspected sample in addition to the viral gene targets, with the aim of determining the quality of the collected sample.[32] MS2 phage control is used in the TaqPath™ kit as an exogenous control, while B2M RNA is used in the HBRT-COVID-19 kit as an endogenous control. In contrast to the tested commercial kits, the CDC RT-PCR kit has a separate human specimen extraction control (HSC) as an exogenous control to evaluate the quality of the extraction process and the human RNAase P gene target as an endogenous control to validate the quality of the sample, reagent integrity and occurrence of PCR inhibition.

Despite successful detection of the *ORF1ab* and *N* gene targets, *S* gene dropout/*S* gene target failure was observed for the TaqPath™ kit in the present study. The phenomenon of *S* gene

dropout is considered as one of the proxy indicators to determine the presence of variants of concern with alterations in the targeted region of the *S* gene. A recent study has suggested that the TaqPath™ kit is a useful tool in this context as it is cost-effective and it enables a rapid large scale screening of SARS-CoV-2 variants with the ΔH69/ΔV70 polymorphism.[33] However, the latest hotspots of new coronavirus transmission suggest that SARS-CoV-2 continues to evolve and the global authorities are expected to prioritize equity in vaccine access and genomic surveillance in order to fully unveil the biological properties of Omicron.[34] Therefore, the capability to detect new variants should also be considered when selecting appropriate diagnostic methods in the future.

RT-PCR is globally still the main detection method for the genome of SARS-CoV-2.[10,35,36] Despite its long sample processing time and instrument running time, the average accuracy of RT-PCR for diagnosis of COVID-19 has been reported as 97.7%.[10] Indeed, RT-PCR remains the gold standard method for diagnosing COVID-19.[10,35,36] Although there are many other advanced and improved methods available, such as biosensors, droplet digital PCR, nano PCR, and the clustered regularly interspaced short palindromic repeats (CRISPR) method, with promising accuracy and rapid detection time, healthcare authorities from low income countries like Sri Lanka cannot afford those diagnostic tools on a large scale for community screening.[36] Other serological and rapid antigen detection kits are not well-recommended methods due to their lack of sensitivity, specificity, and overall accuracy, which limits their use as screening tests.[10,35,36] With the dissemination of vaccines and decreasing number of deaths every day, the end of the threat from COVID-19 might be in sight.[36] However, this does not mean that the threat of COVID-19 will disappear. Evolution and emerging new variants may evade the protection

acquired from vaccines and previous infections.[36,37] Therefore, accurate diagnosis and effective management will remain the most effective option for combating the pandemic. According to recent literature, some rRT-PCR kits have been found to exhibit lowest accuracy levels for diagnosis of COVID-19, despite having received the US FDA approval.[35] Therefore, in house validation of diagnostic kits is essential for effective diagnostic practices.

The current study has some limitations, eg, reference RNA samples with known initial nucleic acid concentrations were not used in our study, and only a limited number of samples and rRT-PCR kits were investigated. Another limitation was that repeated freezing and thawing of extracted RNA while testing the different PCR kits may have had some influence on the results obtained. Finally, differences in PCR thermal conditions, gene targets, fluorescence detection channels and the usage of passive reference dyes might have affected the outcome and comparison between rRT-PCR kits.

Although in accordance with overall analyses and results, we observed varying sensitivity, specificity, and Ct values among all five RT-PCR kits investigated in this study. We believe that all the tested rRT-PCR kits can be used for routine diagnosis of COVID-19. However, we observed that there are variations in Ct values while analyzing the same samples using different rRT-PCR kits. Therefore, the efficiency of the PCR kit used should be considered as an important factor while using the Ct value as a surrogate marker to identify the infection stage of a COVID-19 patient.

Material and Methods

Selection of rRT-PCR kits

When COVID-19 became prevalent in Jaffna district, the PCR-Diagnostic Laboratory, Faculty of Medicine, University of Jaffna was transformed to diagnose COVID-19. It was accredited by WHO and was validated twice in 2020 and 2021 by external assessors. The following kits were used: TaqPath™ kit (Life Technologies Corporation, CA, USA), Real Star® kit (Altona Diagnostics, Germany), STANDARD-M kit (SD Biosensor Inc. Korea), HBRT-COVID-19 kit (Chaozhou Hybribio-Biochemistry Ltd., China), and AccuPower® kit (Bioneer Corporation, Korea. These five commercially available SARS-CoV-2 rRT-PCR kits were certified by the National Medicines Regulatory Authority in Sri Lanka. Specifications were detailed in Table S3, according to the manufacturers' instructions.

Selection of samples from stored specimens

Considering the possible deterioration due to prolonged storage of the samples, all the samples included in this study were collected within a period of one month (from 1 September, 2022 to 30 September, 2022) from the samples received for routine diagnosis at the COVID-19 PCR-Diagnostics Laboratory, Faculty of Medicine, University of Jaffna-Sri Lanka (WHO/50231).

A total of 94 nasopharyngeal swab specimens among the leftover samples received for diagnosis, 62 SARS-CoV-2-positive samples and 32 SARS-CoV-2-negative samples, were selected, anonymized and used for the study. All the positive samples were collected from patients meeting the selected criteria of having significant clinical features and a clear contact history with confirmed SARS-CoV-2-positive patients. Similarly, the negative samples were collected

from asymptomatic healthy individuals without any contact history. The positivity and negativity of all the included samples were confirmed by using the CDC RT-PCR kit with replicates. All selected samples were stored at -80 °C, following interim guidance of WHO, 2021, for laboratory testing of COVID-19 in suspected human cases.[38]

Nucleic acid extraction

Viral RNA was extracted manually from all the clinical samples using the Biospin Virus DNA/RNA extraction kit (Hangzhou Bioer Technology Co., Ltd., China) as per the manufacturer's instructions. Briefly, a mixture of 200 µL of patients' sample was added to 200 µL of lysis buffer consisting of 10 µL of proteinase K and subjected to lysis at 56 ℃ for 15 minutes. Total viral RNA was cleaned by the centrifugation steps with 250 µL of absolute ethanol, 500 µL of wash buffer-1 and 500 µL of wash buffer-2, respectively. Viral RNA was eluted with 60 µL of elution buffer and immediately stored at -80 ℃ until the subsequent PCR steps were executed.

Molecular assay: rRT-PCR

In order to maintain homogeneity, RNA was extracted from each sample only once, and the same RNA sample was used to compare the efficiency of all five commercial rRT-PCR kits selected for this study. It was done using the 96 well-plate, QuantStudio™ -5 Real-Time PCR system thermal cycler (Life Technologies Holdings Pte Ltd., Singapore). Instructions provided by manufacturers were strictly followed in the preparation of reaction-mixtures and setting up the thermal cycler conditions (Table S4 and S5). For quality assessment, positive, negative and internal controls were incorporated in each and every individual PCR run. Positive and negative

results were interpreted according to the instructions given by respective rRT-PCR kit manufacturer, based on the performance of the individual gene targets and the controls.

Repeatability and imprecision test

Three randomly selected positive samples among the categories of strong positive (S1), moderate positive (S2) and low positive (S3) were used to evaluate both repeatability and imprecision. The modified EP15-A protocol was followed, each of three positive samples was tested five times per day over a period of five adjacent days. Mean, SD, and coefficient of variation (CV) were calculated for each kit used in the current study.

Statistical analysis

Numerical data (Ct values) obtained were compiled in MicroSoft Excel for descriptive statistical summarization of variables and analysis of specificity, sensitivity, PPV and NPV determination.[27] Cohen's kappa coefficient was determined with the aid of the quantify inter-rater agreement with the kappa-Graphpad online calculator to check the proportion of agreement. Differences in the Ct values of different gene targets of rRT-PCR kits were compared by pairwise *t*-tests. The minimum statistically significant level was considered as $P < 0.05$.

Statistical analysis was conducted and 95% confidential intervals were fixed in R (version-3.6.3) and Python (version 3.9). Scatter plots and box plots were plotted in Python (version 3.9).

Patient and public involvement

Patients or the public were not directly involved in the design, conduct, or the reporting or dissemination plans of this study.

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Figure legends

Figure 1. Boxplot for gene targets of various rRT-PCR kits. $**P < 0.001$, $*P < 0.05$. Ct, cycle threshold; rRT-PCR, real-time reverse transcriptase PCR; TaqPath_*ORF1ab* Gene, *ORF1ab* gene target of TaqPath™ COVID-19 CE-IVD RT-PCR Kit; TaqPath_*N* Gene, *N* gene target of TaqPath™ COVID-19 CE-IVD RT-PCR Kit; TaqPath_*S* Gene, *S* gene target of TaqPath™ COVID-19 CE-IVD RT-PCR Kit; Real Star_*E* Gene, *E* gene target of Real Star® SARS-CoV-2 RT-PCR Kit; Real Star_*S* Gene, *S* gene target of Real Star® SARS-CoV-2 RT-PCR Kit; STANDARD M _*E* Gene, *E* gene target of STANDARD M nCoV Realtime Detection Kit; STANDARD M_*ORF1ab* Gene, *ORF1ab* gene target of STANDARD M nCoV Realtime Detection Kit; HBRT_O*RF1ab* Gene, *ORF1ab* gene target of Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19); HBRT_*N* Gene, *N* gene target of Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19); AccuPower_*E* Gene, *E* gene target of AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit; AccuPower_*N* Gene, *N* gene target of AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit.

Figure 2. Scatter plot to illustrate the distribution of Ct values for gene targets of the rRT-PCR kits used in this study. A, Ct value comparison of *E* gene; B, Ct value comparison of *N* gene; C, Ct value comparison of *ORF1ab* gene; D, Ct value comparison of *S* gene. Ct, cycle threshold; rRT-PCR, real-time reverse transcriptase PCR; Real Star_*E* Gene, *E* gene target of Real Star® SARS-CoV-2 RT-PCR Kit; STANDARD M _E Gene, E gene target of STANDARD M nCoV Realtime Detection Kit; AccuPower_*E* Gene, *E* gene target of AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit; TaqPath_*N* Gene, *N* gene target of TaqPath™

COVID-19 CE-IVD RT-PCR Kit; HBRT_*N* Gene, *N* gene target of Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19); AccuPower_N Gene, *N* gene target of AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit; TaqPath_*ORF1ab* Gene, *ORF1ab* gene target of TaqPath™ COVID-19 CE-IVD RT-PCR Kit; STANDARD M_*ORF1ab* Gene, *ORF1ab* gene target of STANDARD M nCoV Realtime Detection Kit; HBRT_*ORF1ab* Gene, *ORF1ab* gene target of Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19); TaqPath_*S* Gene, *S* gene target of TaqPath™ COVID-19 CE-IVD RT-PCR Kit; Real Star_*S* Gene, *S* gene target of Real Star® SARS-CoV-2 RT-PCR Kit.

Supplemental digital content

Supplemental materials are available at

Figure 1

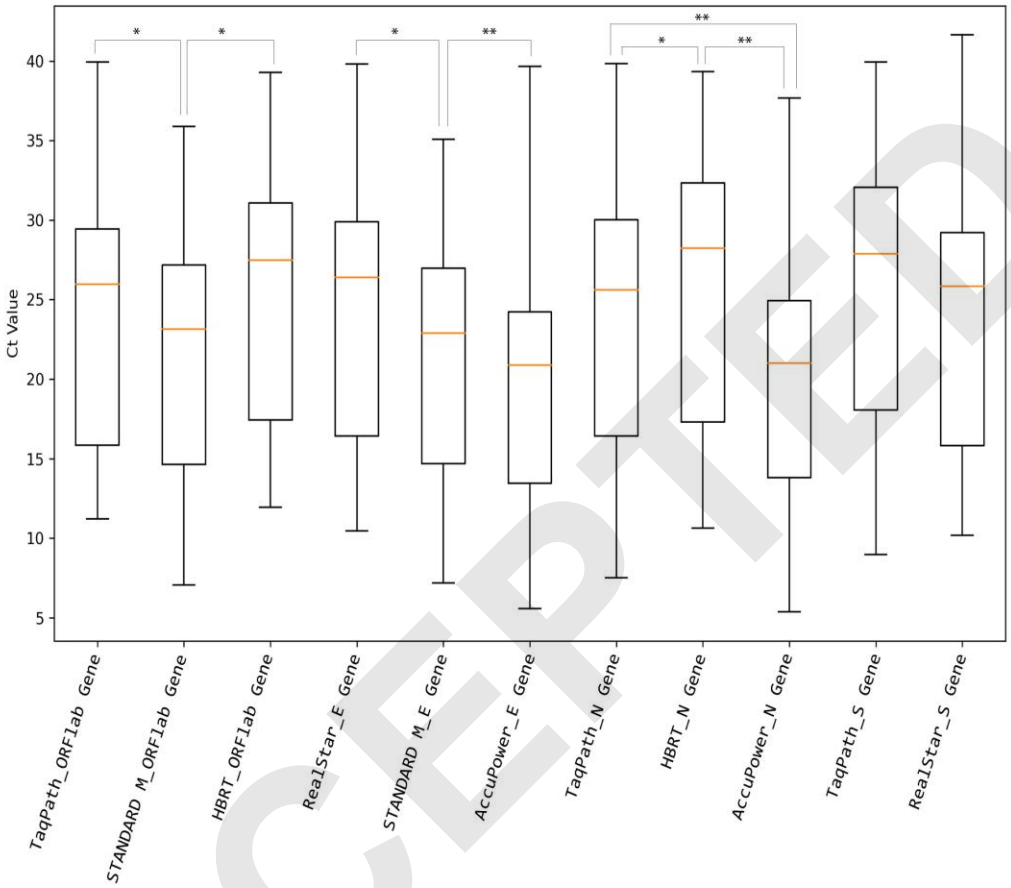

Figure 2

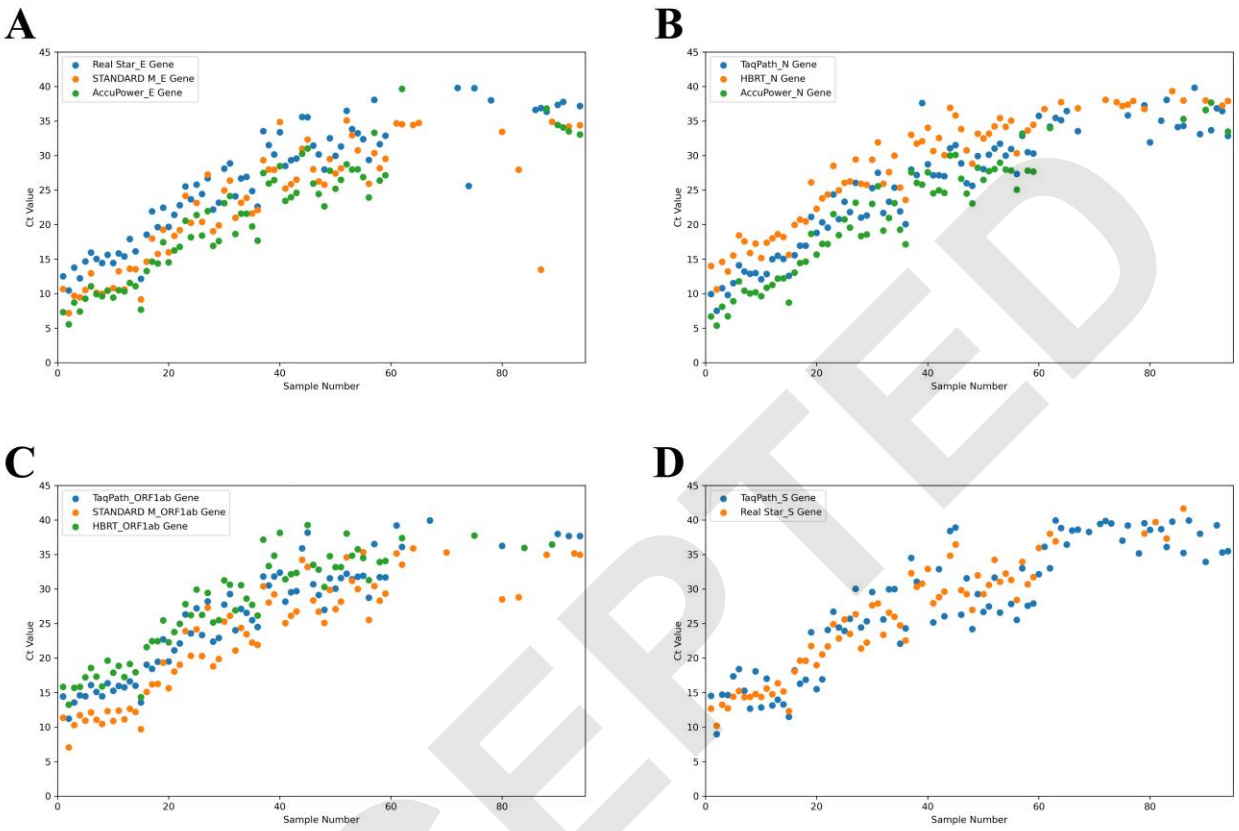

Table 1 Descriptive statistical analysis values for Ct values reported for the amplified gene targets of the rRT-PCR kits

Description	**TaqPath_*ORF1ab* gene**	**TaqPath_*N* gene**	**TaqPath_*S* gene**	**RealStar_*E* gene**	**RealStar_*S* gene**	**STANDARD M_*E* gene**	**STANDARD *M*_*ORF1ab* gene**	**HBRT_*ORF1ab* gene**	**HBRT_*N* gene**	**AccuPower_*E* gene**	**AccuPower_*N* gene**
N	66	79	82	69	66	68	67	61	72	65	64
Mean Ct (SD)	25.96 (±7.85)	25.62 (±8.54)	27.98 (±9.03)	26.38 (±8.20)	25.45 (±8.12)	22.89 (±8.41)	23.14 (±8.37)	27.48 (±7.44)	28.22 (±7.96)	20.87 (±8.45)	21.00 (±8.31)
Median Ct (IQR)	27.17 (12.54)	27.16 (13.05)	27.89 (13.61)	26.91 (13.18)	26.47 (12.13)	24.57 (13.89)	25.07 (13.39)	28.62 (11.93)	30.03 (12.72)	21.60 (12.52)	22.26 (13.53)
Min Ct	11.22	7.51	8.97	10.45	10.18	7.19	7.07	13.25	10.62	5.56	5.36

Max Ct	39.95	39.83	39.94	39.81	41.65	35.09	35.89	39.29	39.33	39.66	37.67

Ct, cycle threshold; N, Number of samples; IQR, Interquartile range; Min, Minimum value; Max, Maximum value; TaqPath_*ORF1ab* gene, *ORF1ab* gene target of TaqPathTM COVID-19 CE-IVD RT-PCR Kit; TaqPath_*N* gene, *N* gene target of TaqPathTM COVID-19 CE-IVD RT-PCR Kit; TaqPath_*S* gene, *S* gene target of TaqPathTM COVID-19 CE-IVD RT-PCR Kit; Real Star_*E* gene, *E* gene target of Real Star® SARS-CoV-2 RT-PCR Kit; Real Star_*S* gene, *S* gene target of Real Star® SARS-CoV-2 RT-PCR Kit; STANDARD M _*E* gene, *E* gene target of STANDARD M nCoV Realtime Detection Kit; STANDARD M_*ORF1ab* gene, *ORF1ab* gene target of STANDARD M nCoV Realtime Detection Kit; HBRT_*ORF1ab* gene, *ORF1ab* gene target of Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19); HBRT_*N* gene, *N* gene target of Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19); AccuPower_*E* gene, *E* gene target of AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit; AccuPower_*N* gene, *N* gene target of AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit.

Table 2 Sensitivity, specificity, PPV, NPV, and kappa coefficient for the rRT-PCR kits used in this study

rt-PCR Kit	% Sensitivity (CI 95%)	% Specificity (CI 95%)	% PPV	% NPV	Kappa coefficient (CI 95%)	True positives	True negatives	False positives	False negatives
TaqPath™ Kit	98.4 (91.3-99.9)	100.0 (89.1-100.0)	100.0	96.9	0.98 (0.93-1.00)	61	32	0	1
Real Star® Kit	95.2 (86.5-98.9)	100.0 (89.1-100.0)	100.0	91.4	0.93 (0.85-1.00)	59	32	0	3
STANDARD-M Kit	95.2 (86.5-98.9)	93.7 (79.2-99.2)	96.7	90.6	0.88 (0.78-0.98)	59	30	2	3
HBRT-COVID-19 Kit	91.9 (82.2-97.3)	93.7 (79.2-99.2)	96.6	85.7	0.84 (0.72-0.95)	57	30	2	5
AccuPower® kit	93.5 (84.3-98.2)	100.0 (89.1-100.0)	100.0	88.9	0.91 (0.82-0.99)	58	32	0	4

PPV, Positive predictive value; NPV, Negative predictive value; TaqPath, TaqPathTM COVID-19 CE-IVD RT-PCR kit; Real Star, Real Star® SARS-CoV-2 RT-PCR kit; HBRT COVID-19, Hybribio COVID-19 Real-Time PCR kit (HBRT-COVID-19); AccuPower, AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit; STANDARD M, STANDARD-M nCoV Realtime Detection kit.

Table 3 Repeatability and imprecision of rRT-PCR kits for the *E*, *N*, *ORF1ab* and *S* gene targets

Kits	Sample	***E* gene target**					***N* gene target**					***ORF1ab* gene target**					***S* gene target**				
			Repeatability		Total imprecision			Repeatability		Total imprecision			Repeatability		Total imprecision			Repeatability		Total imprecision	
		Mean	±SD	CV (%)	±SD	CV (%)	Mean	±SD	CV (%)	±SD	CV (%)	Mean	±SD	CV (%)	±SD	CV (%)	Mean	±SD	CV (%)	±SD	CV (%)
TaqPath	**S 1**						13.02	0.38	2.88	0.38	2.92	14.71	0.52	3.56	0.53	3.60	15.03	1.32	8.81	1.34	8.92
Real Star		14.50	0.33	2.29	0.33	2.28											14.48	0.26	1.81	0.26	1.79
STANDARD M		10.79	0.47	4.32	0.47	4.35						11.37	0.18	1.59	0.18	1.58					
HBRT COVID-19							16.63	0.72	4.33	0.72	4.33	18.36	0.66	3.59	0.66	3.59					
AccuPower		11.04	0.14	1.24	0.14	1.27	8.96	0.10	1.14	0.10	1.12	22.45	0.40	1.79	0.40	1.78	28.97	1.07	3.68	1.07	3.69
TaqPath	**S 2**						20.79	0.40	1.95	0.40	1.92						22.20	0.21	0.96	0.21	0.94
Real Star		22.27	0.23	1.05	0.23	1.03						19.39	0.33	1.71	0.33	1.70					
STANDARD M		18.92	0.64	3.37	0.64	3.38						25.98	0.68	2.63	0.68	2.62					
HBRT							24.20	0.70	2.91	0.70	2.89	31.02	0.54	1.73	0.53	1.71	30.87	1.14	3.68	1.13	3.66
AccuPower		19.18	0.07	0.37	0.07	0.36	17.01	0.11	0.65	0.11	0.65						30.34	0.34	1.13	0.34	1.12
TaqPath	**S 3**						30.01	0.54	1.81	0.54	1.80	27.96	0.22	0.79	0.22	0.79					
Real Star		30.41	0.12	0.38	0.40	1.31						34.56	0.67	1.94	0.67	1.94					

STANDARD M		27.30	0.42	1.55	0.42	1.54														
HBRT COVID-19							33.83	0.49	1.45	0.49	1.44									
AccuPower		28.18	0.09	0.34	0.09	0.32	26.52	0.11	0.42	0.11	0.41									

CV, coefficient of variation; TaqPath, TaqPath™ COVID-19 CE-IVD RT-PCR kit; Real Star, Real Star® SARS-CoV-2 RT-PCR kit; HBRT COVID-19, Hybribio COVID-19 Real-Time PCR kit (HBRT-COVID-19); AccuPower, AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit; STANDARD M, STANDARD-M nCoV Realtime Detection kit.

Comparative Evaluation of RT-PCR Kits Available in Sri Lanka for Diagnosis of COVID-19

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Supplemental digital content: Supplemental Tables

Table S1 Cycle threshold (Ct) values reported by rRT-PCR kits

Sample No:	CDC_N1	CDC_N2	TaqPath_ORF1ab Gene	TaqPath_N Gene	TaqPath_S Gene	Real Star_E Gene	Real Star_S Gene	STANDARD M_E Gene	STANDARD M_ORF 1ab Gene	HBRT_ ORF1ab Gene	HBRT_ N Gene	AccuPower_E Gene	AccuPower_N Gene
S01	13.302	13.522	14.439	9.926	14.534	12.533	12.681	10.661	11.364	15.812	13.993	7.306	6.7
S02	11.231	11.421	11.223	7.507	8.972	10.455	10.183	7.19	7.066	13.255	10.624	5.564	5.359
S03	14.143	14.334	13.588	10.783	14.706	13.784	13.237	9.708	10.311	15.696	14.594	8.711	8.127
S04	12.967	12.990	14.597	9.801	14.633	12.235	12.724	9.48	11.706	15.813	13.21	7.423	6.725
S05	15.543	15.601	14.481	11.529	17.342	14.682	14.415	10.544	10.929	17.208	15.521	9.27	8.905
S06	16.913	16.984	16.086	14.09	18.419	15.948	15.229	12.946	12.12	18.579	18.445	11.053	11.786
S07	15.827	15.904	15.1	13.206	15.28	15.049	14.339	10.143	11.067	17.305	17.58	9.933	10.434
S08	15.217	15.483	14.455	12.888	12.666	14.445	14.375	9.989	10.474	15.882	15.89	9.632	10.035
S09	16.602	16.730	16.345	12.988	18.065	15.633	14.813	10.479	12.311	19.621	17.258	10.428	10.215
S10	15.232	15.374	15.255	12.075	12.845	14.445	14.375	10.783	10.905	17.886	15.161	9.434	9.627
S11	16.732	16.861	15.964	12.857	17.012	15.814	15.584	13.253	12.389	18.865	17.382	10.497	10.846
S12	16.495	16.321	15.756	15.015	13.134	15.386	14.781	10.629	11.141	17.246	17.997	10.325	11.242
S13	18.986	18.972	16.623	15.504	13.961	17.919	16.374	13.621	12.655	19.123	18.615	11.558	12.169
S14	17.063	17.241	15.991	15.022	13.264	16.125	15.168	13.544	12.2	17.931	18.227	11.091	12.208
S15	12.938	12.966	13.573	12.592	11.484	12.165	12.314	9.179	9.708	14.324	15.623	7.693	8.715

Sample No:	CDC_ N1	CDC_ N2	TaqPath _ORF1ab Gene	TaqPath _N Gene	TaqPath _S Gene	Real Star_E Gene	Real Star_S Gene	STANDARD M_E Gene	STANDARD M_ORF 1ab Gene	HBRT_ ORF1ab Gene	HBRT_ N Gene	AccuPower_E Gene	AccuPower_N Gene
S16	19.863	19.907	19.056	15.565	18.2	18.557	18.064	14.632	15.107	21.583	19.973	13.277	13.035
S17	23.204	23.362	18.454	16.952	16.276	21.905	19.641	17.968	16.18	22.458	20.73	14.621	14.434
S18	20.770	20.871	19.477	16.965	16.853	19.643	19.606	15.773	16.265	22.44	20.464	14.358	14.63
S19	23.845	23.851	22.707	21.113	23.753	22.458	21.762	19.278	19.335	25.479	26.141	17.438	18.668
S20	20.881	20.900	19.506	18.824	15.492	19.683	18.97	15.988	15.621	22.278	22.29	14.538	15.657
S21	22.632	22.681	21.12	20.321	16.925	21.432	20.528	18.385	18.033	23.769	23.805	16.262	17.206
S22	23.985	24.024	22.128	19.568	24.086	22.803	21.674	19.198	19.033	24.965	24.362	16.778	17.188
S23	26.702	26.913	26.317	24.357	26.712	25.535	24.896	24.179	23.922	27.806	28.513	20.578	21.507
S24	24.763	24.609	23.582	20.803	24.441	23.663	22.84	20.257	20.344	26.235	24.968	18.178	18.479
S25	26.880	26.901	27.208	23.315	23.942	25.795	25.598	23.152	24.158	29.927	26.073	21.395	20.748
S26	25.740	25.789	23.356	21.832	25.694	24.422	23.517	20.384	20.299	26.265	26.245	18.403	19.564
S27	28.003	28.109	28.226	26.076	30.021	26.73	26.345	27.238	27.302	29.425	29.425	21.945	23.171
S28	22.973	22.990	22.376	21.043	24.436	22.174	21.4	19.05	18.815	25.168	25.92	16.924	18.34
S29	24.102	24.243	22.902	21.326	25.321	23.182	22.242	19.911	19.852	25.508	25.763	17.601	18.578
S30	29.948	30.104	27.751	25.304	29.58	28.112	27.634	24.954	25.303	31.252	29.402	23.098	23.024
S31	30.341	30.448	29.273	27.509	Not Detected	28.862	27.929	26.402	26.14	30.645	31.924	24.135	25.573

Sample No:	CDC_ N1	CDC_ N2	TaqPath _ORF1a b Gene	TaqPath _N Gene	TaqPath _S Gene	Real Star_E Gene	Real Star_S Gene	STAND ARD M_E Gene	STAND ARD M_ORF 1ab Gene	HBRT_ ORF1ab Gene	HBRT_ N Gene	AccuPo wer_E Gene	AccuPo wer_N Gene
S32	25.321	25.726	24.034	21.705	25.608	24.105	23.377	20.997	21.09	26.911	25.938	18.639	19.155
S33	28.081	28.142	27.127	23.311	29.956	26.699	26.603	23.165	24.362	30.548	27.617	21.59	20.947
S34	28.342	28.398	26.563	25.365	29.991	26.914	25.993	23.87	23.483	28.619	29.994	21.602	23.118
S35	25.928	26.104	25.501	21.928	22.099	24.878	24.726	21.622	22.264	27.722	25.349	19.723	19.262
S36	23.941	23.980	24.489	20.066	24.291	22.608	22.534	22.135	21.917	26.151	23.576	17.681	17.167
S37	35.438	35.422	31.806	27.996	34.489	33.534	32.298	29.34	30.412	37.162	33.01	27.473	27.571
S38	33.205	33.286	30.526	27.199	31.044	31.517	30.342	27.978	28.041	33.35	31.766	25.947	26.068
S39	32.007	32.214	31.819	37.619	Not Detected	30.15	30.789	27.946	29.24	34.818	32.074	26.468	25.792
S40	35.541	35.682	32.385	28.736	Not Detected	33.362	32.923	34.875	Not Detected	38.134	34.049	28.463	27.596
S41	30.009	30.172	28.185	27.162	25.163	28.524	27.934	25.229	25.068	31.425	30.666	23.44	24.552
S42	31.450	31.623	29.522	27.147	32.884	29.337	28.85	25.848	26.139	32.147	32.537	23.977	24.96
S43	31.541	31.594	29.711	27.01	26.057	29.565	29.62	26.512	26.746	32.349	30.063	24.607	24.6
S44	38.201	38.836	35.886	31.055	38.406	35.601	34.823	30.977	34.251	Not Detected	36.918	30.271	30
S45	38.105	38.694	38.171	31.508	38.899	35.553	36.473	32.321	33.203	39.287	35.826	31.039	30.111
S46	34.556	34.582	30.683	28.882	26.303	31.441	29.841	28.024	28.334	33.514	33.846	25.955	26.672
S47	32.459	32.565	29.153	25.996	31.561	30.166	29.269	26.256	26.714	32.827	30.769	24.453	24.51

Sample No:	CDC_ N1	CDC_ N2	TaqPath _ORF1ab Gene	TaqPath _N Gene	TaqPath _S Gene	Real Star_E Gene	Real Star_S Gene	STANDARD M_E Gene	STANDARD M_ORF 1ab Gene	HBRT_ ORF1ab Gene	HBRT_ N Gene	AccuPower_E Gene	AccuPower_N Gene
S48	29.502	29.783	26.994	25.615	24.2	27.967	26.983	25.793	25.101	30.309	28.845	22.639	23.091
S49	35.834	36.342	31.561	29.929	29.276	32.505	31.983	29.504	29.854	34.771	33.188	27.824	28.232
S50	32.657	33.320	30.081	28.038	26.681	29.97	29.286	27.409	27.074	33.187	32.487	25.184	26.482
S51	34.372	34.834	31.596	30.136	27.494	31.324	30.602	28.15	28.165	33.191	33.193	26.485	27.737
S52	39.872	39.732	32.226	31.041	31.656	36.492	34.248	35.092	34.608	38.058	34.226	28.752	28.062
S53	36.283	36.364	31.433	31.733	26.601	33.849	31.047	32.932	31.178	34.85	35.432	27.985	28.973
S54	36.384	36.843	31.8	29.995	Not Detected	33.261	32.247	30.753	29.995	35.748	34.168	28.024	27.99
S55	35.702	35.947	31.932	30.954	27.859	32.386	31.327	Not Detected	35.321	34.492	35.062	26.883	27.853
S56	33.745	33.986	28.738	27.366	25.545	29.355	28.399	25.943	25.518	31.285	30.324	23.935	25.076
S57	39.623	39.873	36.536	32.889	32.997	38.067	33.943	30.35	30.416	Not Detected	Not Detected	35.317	35.179
S58	34.573	34.875	31.713	30.481	27.578	31.657	30.702	28.194	28.317	33.911	33.643	26.385	27.822
S59	35.876	36.163	31.676	30.31	27.923	32.868	31.73	29.545	29.333	34.065	34.477	27.138	27.689
S60	38.565	38.861	Not Detected	35.749	32.163	Not Detected	35.947	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S61	Not Detected	Not Detected	39.201	Not Detected	36.132	Not Detected	Not Detected	34.658	35.15	Not Detected	36.752	Not Detected	Not Detected
S62	Not Detected	Not Detected	36.08	34.197	33.021	Not Detected	38.025	34.539	33.534	37.37	Not Detected	39.659	34
S63	Not Detected	Not Detected	Not Detected	35.479	39.927	Not Detected	36.921	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Sample No:	CDC_ N1	CDC_ N2	TaqPath _ORF1a b Gene	TaqPath _N Gene	TaqPath _S Gene	Real Star_E Gene	Real Star_S Gene	STAND ARD M_E Gene	STAND ARD M_ORF 1ab Gene	HBRT_ ORF1ab Gene	HBRT_ N Gene	AccuPo wer_E Gene	AccuPo wer_N Gene
S64	Not Detected	Not Detected	Not Detected	35.134	38.854	Not Detected	Not Detected	34.442	35.894	Not Detected	37.748	Not Detected	Not Detected
S65	Not Detected	Not Detected	Not Detected	36.46	36.472	Not Detected	Not Detected	34.742	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S66	Not Detected	Not Detected	Not Detected	Not Detected	38.472	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S67	Not Detected	Not Detected	39.947	33.538	38.6	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	36.861	Not Detected	Not Detected
S68	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S69	Not Detected	Not Detected	Not Detected	Not Detected	38.262	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S70	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	35.3	Not Detected	Not Detected	Not Detected	Not Detected
S71	Not Detected	Not Detected	Not Detected	Not Detected	39.428	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S72	Not Detected	Not Detected	Not Detected	Not Detected	39.853	39.81	Not Detected	Not Detected	Not Detected	Not Detected	38.095	Not Detected	Not Detected
S73	Not Detected	Not Detected	Not Detected	Not Detected	39.489	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S74	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	25.581	Not Detected	Not Detected	Not Detected	Not Detected	37.741	Not Detected	Not Detected
S75	Not Detected	Not Detected	Not Detected	Not Detected	37.024	39.774	Not Detected	Not Detected	Not Detected	37.762	37.196	Not Detected	Not Detected
S76	Not Detected	Not Detected	Not Detected	35.832	39.217	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	37.394	Not Detected	Not Detected
S77	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	37.923	Not Detected	Not Detected
S78	Not Detected	Not Detected	Not Detected	Not Detected	35.179	38.012	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S79	Not Detected	Not Detected	Not Detected	37.274	39.545	Not Detected	38.053	Not Detected	Not Detected	Not Detected	36.758	Not Detected	Not Detected

Sample No:	CDC_ N1	CDC_ N2	TaqPath _ORF1a b Gene	TaqPath _N Gene	TaqPath _S Gene	Real Star_E Gene	Real Star_S Gene	STAND ARD M_E Gene	STAND ARD M_ORF 1ab Gene	HBRT_ ORF1ab Gene	HBRT_ N Gene	AccuPo wer_E Gene	AccuPo wer_N Gene
S80	Not Detected	Not Detected	36.259	31.911	38.571	Not Detected	Not Detected	33.448	28.526	Not Detected	Not Detected	Not Detected	Not Detected
S81	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	39.695	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S82	Not Detected	Not Detected	Not Detected	35.058	38.662	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S83	Not Detected	Not Detected	Not Detected	38.09	36.091	Not Detected	37.321	27.962	28.793	Not Detected	Not Detected	Not Detected	Not Detected
S84	Not Detected	Not Detected	Not Detected	Not Detected	39.765	Not Detected	Not Detected	Not Detected	Not Detected	35.976	39.328	Not Detected	Not Detected
S85	Not Detected	Not Detected	Not Detected	34.144	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S86	Not Detected	Not Detected	Not Detected	34.298	37.239	36.627	41.653	Not Detected	Not Detected	Not Detected	37.991	Not Detected	35.314
S87	Not Detected	Not Detected	Not Detected	Not Detected	39.939	36.878	Not Detected	13.479	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
S88	Not Detected	Not Detected	Not Detected	39.83	Not Detected	36.344	Not Detected	Not Detected	34.967	Not Detected	Not Detected	36.817	Not Detected
S89	Not Detected	Not Detected	Not Detected	33.112	38.015	Not Detected	Not Detected	34.888	Not Detected	36.448	Not Detected	Not Detected	Not Detected
S90	Not Detected	Not Detected	37.989	36.599	33.947	37.34	Not Detected	Not Detected	Not Detected	Not Detected	37.973	34.444	36.61
S91	Not Detected	Not Detected	Not Detected	33.687	Not Detected	37.783	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	34.083	37.672
S92	Not Detected	Not Detected	37.687	36.858	39.23	Not Detected	Not Detected	34.206	Not Detected	Not Detected	Not Detected	33.502	Not Detected
S93	Not Detected	Not Detected	Not Detected	36.457	35.282	Not Detected	Not Detected	Not Detected	35.178	Not Detected	37.25	Not Detected	Not Detected
S94	38.521	38.959	37.673	32.856	35.485	37.178	Not Detected	34.403	34.973	Not Detected	37.902	35.048	35.484

False positive results were shaded in yellow color; False negative results were shaded in blue color; The cut-off Ct value for CDC N1 and CDC N2 genes is <40 Ct; CDC_N1, N1 gene target of CDC 2019-Novel Coronavirus (2019-nCoV) RT-PCR Diagnostic Panel; CDC_N2, N2 gene target of CDC 2019-Novel Coronavirus (2019-nCoV) RT-PCR Diagnostic Panel; TaqPath_ORF1ab Gene, ORF1ab gene target of TaqPathTM COVID-19 CE-IVD RT-PCR Kit; TaqPath_N Gene, N gene target of TaqPathTM COVID-19 CE-IVD RT-PCR Kit; TaqPath_S Gene, S gene target of TaqPathTM COVID-19 CE-IVD RT-PCR Kit; Real Star_E Gene, E gene target of Real Star® SARS-CoV-2 RT-PCR Kit; Real Star_S Gene, S gene target of Real Star® SARS-CoV-2 RT-PCR Kit; STANDARD M _E Gene, E gene target of STANDARD M nCoV Realtime Detection Kit; STANDARD M _ORF1ab Gene, ORF1ab gene target of STANDARD M nCoV Realtime Detection Kit; HBRT_ORF1ab Gene, ORF1ab gene target of Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19); HBRT_N Gene, N gene target of Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19); AccuPower_E Gene, E gene target of AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit; AccuPower_N Gene, N gene target of AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit.

Table S2 Sample number comparison of current study with previously conducted studies

	Sample number		
Study	Positives	Negatives	Total
Current study	62	32	94
(Singh *et al.*, 2021)	92	60	152
(Freire-paspuel, Garcia-bereguiain and Kit, 2021)	57	32	89
(Garg *et al.*, 2021)	40	10	50

Table S3 Comparative overview of five different SARS-CoV-2 multiplex rRT-PCR kits

No.	Name of The Kit	Manufacturer	Target Regions	Sample Volume (μl)	Reaction Volume (μl)	Number of Amplification Cycles	Running Time of the PCR	Cut-Off Ct values	
								Target Gene	Internal Control
1	TaqPath™ COVID-19 CE-IVD RT-PCR Kit	Life Technologies Corporation, CA, USA	*ORF1ab*, *N* gene, *S* gene	10.0	25.0	40	1h 5min	<37	≤32
2	Real Star® SARS-CoV-2 RT-PCR Kit	Altona Diagnostics , Germany	*E* gene, *S* gene	10.0	30.0	45	1h 55min	<40	<40
3	STANDARD M nCoV Realtime Detection Kit	SD Biosenser Inc., Korea	*ORF1ab*, *E* gene	10.0	30.5	40	1h 24min	≤36	≤36
4	Hybribio COVID-19 Real-Time PCR Kit (HBRT-COVID-19)	Chaozhou Hybribio Biochemistry Ltd., China	*ORF1ab*, *N* gene	5.0	30.0	45	1h 23min	≤40	≤40
5	AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit	Bioneer Corporation, Korea	*RdRP*/ *N* gene, *E* gene	10.0	20.0	40	1h 27min	≤35	<33

Table S4 Overview of the PCR conditions for SARS-CoV-2 multiplex rRT-PCR kits

	Name of the Kit											
Step	TaqPath™ COVID-19 CE-IVD RT-PCR Kit		Real Star® SARS-CoV-2 RT-PCR Kit		STANDARD M nCoV Realtime Detection Kit		COVID-19 Real-Time PCR Kit (HBRT-COVID-19)		AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit	
UNG Incubation	25 ℃, 2 min	1								
Reverse Transcriptase Activation	53 ℃, 10 min	1	55 ℃, 20 min	1	50 ℃, 15 min	1	55 ℃, 15 min	1	50 ℃, 20 min	1
(Initial Denaturation	95 ℃, 2 min	1	95 ℃, 2 min	1	95 ℃, 5 sec	1	95 ℃, 30 sec	1	95 ℃, 5 min	1
					95 ℃, 5 sec				95 ℃, 5 sec	
Pre-Amplification					60 ℃, 40 sec	5			60 ℃, 30 sec	5
Denaturation	95 ℃, 3 sec		95 ℃, 15 sec		95 ℃, 5 sec		95 ℃, 30 sec		95 ℃, 5 sec	
Anneal or Anneal/Extension	60 ℃, 30 sec	40	55 ℃, 45 sec		60 ℃, 40 sec	40	60 ℃, 35 sec	45	58 ℃, 30 sec	40
Extension			72 ℃, 15 sec	45						
Final extension							38 ℃, 30 sec	1		

Table S5 Overview of the fluorescence detector settings of SARS-CoV-2 multiplex rRT-PCR kits

	Name of the Kit				
Target Gene	TaqPath™ COVID-19 CE-IVD RT-PCR Kit	Real Star® SARS-CoV-2 RT-PCR Kit	STANDARD M nCoV Realtime Detection Kit	COVID-19 Real-Time PCR Kit (HBRT-COVID-19)	AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR kit
ORF1ab	FAM		FAM	FAM	
RdRP					TET/JOE
N gene	VIC			HEX/JOE	TET/JOE
E gene		FAM	JOE (VIC/HEX)		FAM
S gene	ABY	CY5			
Internal Control (IC)	JUN	JOE	CY5	CY5	CY5