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RESEARCH ARTICLE

Rapid identification of SARS-CoV-2 in the point-of-care using digital PCR-based Dr. PCR™ Di20K COVID-19 Detection Kit without viral RNA extraction

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Abstract

Background Since COVID-19 was declared the pandemic by the WHO, it has continued to spread. There is a need for rapid, efficient, and accurate diagnostic kits and techniques to control its spread.

Objective The diagnostic capability of the qRT-PCR-based Real-Q 2019-nCoV Detection Kit and dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit was compared and evaluated.

Methods Diagnostic tests for COVID-19 were performed using two different COVID-19 kits and 301 individual specimens with confirmed COVID-19 positive/negative at the government-accredited medical institution. Assessment of diagnostic capability was measured through diagnostic sensitivity, specificity, Cohen's Kappa coefficient, and dilutional linearity tests.

Results The COVID-19 diagnostic test results using two kits and 301 individual specimens perfectly matched the pre-diagnosis results of the medical institution. In addition, the measurement results of diagnostic sensitivity and specificity were "1", indicating high diagnostic capability. Cohen's Kappa coefficient value is "1", which means that the diagnosis concordance between the two kits is "Almost Perfect". As a result of dilutional linearity tests to evaluate their detection capability, both kits were measured with very high detection reliability.

Conclusion Here, we propose that the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit has the advantages of the dPCR method reported in the previous study and is suitable for point-of-care testing (POCT) by overcoming the limitations of space, test time, cross-over contamination, and biosafety due to omitting RNA extraction process.

Keywords COVID-19 · qRT-PCR · dPCR · Point-of-care testing · POCT

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Introduction

Currently, coronavirus disease of 2019 (COVID-19), which has continued to spread throughout the world, was officially declared a "pandemic" in March 2020 by the World Health Organization (WHO) (Balkhair 2020; Lone and Ahmad 2020; Mo et al. 2021). The causative virus of COVID-19 is Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a new type of coronavirus. This virus belongs to the subgenus Sarbecovirus of the genus Betacoronavirus and has been reported to cause fatal respiratory disease in humans (Utku et al. 2020; Wang et al. 2020). The main route of transmission COVID-19 is from an infected person to uninfected people through respiratory aerosols and droplets conveying the SARS-CoV-2 (Harrison et al. 2020; Lotfi et al. 2020; Syal 2021). To date, vaccines against SARS-CoV-2 have been developed by various global pharmaceutical companies such as Pfizer Inc., AstraZeneca PLC, Moderna Inc., and Janssen Inc. to prevent the spread of COVID-19 (Kaur and Gupta 2020). Nevertheless, new SARS-CoV-2 mutations (Alpha, Beta, Gamma, Delta, Omicron, etc.) with 1.5–2 times higher infectivity have appeared, and the disease continues to spread due to the absence of an effective therapeutic agent (Khan et al. 2022). In addition, poor diagnosis and asymptomatic transmission have accelerated the rapid spread of COVID-19. Therefore, it is crucial to control the further spread of COVID-19 through prompt and accurate diagnosis in SARS-CoV-2 infected patients along with the development of therapeutic agents and vaccines.

Globally, quantitative Real-time reverse transcription polymerase chain reaction (qRT-PCR), Digital PCR (dPCR), Whole-genome sequencing, Nanopore target sequencing (NTS), Antibody detection, Antigen detection, and CRISPR-Cas system-based method (e.g., SHERLOCK) are being applied as detection methods for SARS-CoV-2 (Eftekhari et al. 2021; Giri et al. 2021; Liu et al. 2020). Among various detection methods, the qRT-PCR is a method based on viral RNA amplification (Falzone et al. 2021). It detects using primers and probes sets composed of specific viral sequences of SARS-CoV-2 such as E gene, RdRp gene, and N gene. Most antibody detection methods determine whether the virus is detected by measuring the levels of IgM and IgG in the immune system. Typically, lateral flow type assays (LFA) and enzyme-linked immunosorbent type assays (ELISA) are mainly used (Mahmoudinobar et al. 2021). However, antibody detection methods can lead to false-positive results due to cross-reactivity in which an antibody binds to an antigen with high molecular similarity other than the target antigen (Liu and Rusling 2021). A person infected with SARS-CoV-2 usually develops antibodies after about 14 days (Cevik et al. 2020; Wolfel et al. 2020). According to the results of a recent study, it has been reported that IgG and IgM can be detected 5 days after infection (Loeffelholz and Tang 2020). Thus, antibody detection methods have a disadvantage that can lead to false-negative results if sufficient antibodies for diagnosing COVID-19 are not formed (Chang et al. 2020). Antigen detection methods are intuitive and straightforward diagnostic methods for COVID-19 (Yuce et al. 2021). Like antibody detection methods, these methods can be performed by LFA or ELISA. However, these methods have low sensitivity compared to nucleic acid testing and do not satisfy the clinical sensitivity criteria (96%) of the Ministry of Food and Drug Safety of the Republic of Korea (MFDS) guideline (Department 2020). In particular, the qRT-PCR method based on viral RNA amplification is considered a gold standard because it has almost 100% specificity and high accuracy (> 97%), unlike other molecular tests that do not have perfect diagnostic specificity (Garg et al. 2021; Teymouri et al. 2021). Although most laboratories diagnose COVID-19 with the qRT-PCR method, there are some weaknesses. It is still difficult to accurately diagnose low viral load in the early stages of SARS-CoV-2 infection (Gupta et al. 2021). In general, this method requires the RNA extraction step and thus has several problems such as cross-contamination, additional experiment cost, and time (Ref 24, 25). In addition, the cost of the qRT-PCR equipment is relatively high, and diagnostic space is limited due to its size.

Nowadays, the dPCR method, which complements the relative disadvantages of the qRT-PCR method, is drawing attention in COVID-19 diagnostics. In a previous study, LOAA dPCR equipment (Optolane, Seongnam, Republic of Korea) with dPCR-based Dr. PCR 20 K COVID-19 Detection Kit (Cat no. DCM402-X, Optolane, Seongnam, Republic of Korea) system has been reported that it is less affected by PCR inhibitors than the typical qRT-PCR assay and can be detected even at low viral loads (Lee et al. 2021a; Sidstedt et al. 2020). In addition, this system has high diagnostic sensitivity and specificity. However, this system requires the RNA extraction step and various resources such as consumables costs and experimental time, as well as carry-over contamination and biosafety risks when handling clinical samples (Wee et al. 2020). Approved by MFDS in March 2021, Dr. PCR™ Di20K COVID-19 Detection Kit (Optolane, Seongnam, Republic of Korea) was newly released. It can be applied to the LOAA dPCR system. This system is designed to overcome the weak points of the existing qRT-PCR and dPCR methods. In addition,

since it is possible to diagnose COVID-19 without a viral RNA extraction step, rapid test results and efficient point-of-care testing (POCT) can be predicted. Through this system, it is expected to quickly and accurately screen patients suspected of having COVID-19 to inhibit the spread of the virus.

Here, we measured the diagnostic capability of the LOAA dPCR assay with dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit. In this study, the Real-Q 2019-nCoV Detection Kit (Biosewoom, Seoul, Republic of Korea), one of the qRT-PCR-based method kits considered as the gold standard in diagnosing of COVID-19, was selected as a comparison kit for the assessment of COVID-19 diagnostic capability. This kit was reported to have high sensitivity (98.2%) and specificity (100%) in previous studies (Chung et al. 2021). We compared and analyzed the diagnostic capability between the two kits using residual samples from the upper respiratory of 101 positive and 200 negative samples tested with COVID-19 diagnosis a government-certified medical institution.

Materials and methods

Clinical sample collection

The government of the Republic of Korea has selected medical institutions with excellent clinical evaluation capabilities and supported these institutions to sufficiently secure various COVID-19 specimens such as blood and respiratory-derived samples. In this study, a total of 301 upper respiratory samples were collected from nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) in Samkwang Medical Laboratories (SML, Seoul, Republic of Korea) among these institutions. The collected samples are 101 positive and 200 negative specimens tested with COVID-19 diagnosis at the SML institution, which are residual samples extracted from adult males and females over twenty. All swabs were each stored in 3 mL of viral transport media (VTM, Cat. No UTNFS-3B-1, Noble Biosciences, Inc., Hwaseong, Republic of Korea). This study was conducted considering the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and approved through SML Institutional Review Board (S-IRB-2020–032-10–22). Donors for this study submitted written consent prior to participation.

Viral RNA extraction

Viral RNAs were extracted from 301 upper respiratory samples using each 250 µL of the VTM with the MagNa pure 96 system (Roche, Basel, Switzerland), an automated nucleic acid extractor, following the manufacturer's instructions (Edelmann et al. 2013; Hindiyeh et al. 2019). The extracted viral RNAs were each dissolved in 50 µL of RNase-free water. The eluted RNA samples were stored at − 80 °C in a deep freezer.

qRT-PCR assay with Real-Q 2019-nCOV Detection Kit

To evaluate the COVID-19 detection capability of the Real-Q 2019-nCOV Detection Kit (BioSewoom, Seoul, Republic of Korea), the qRT-PCR assay was performed using the stored RNA samples from the 301 specimens. It was carried out following the protocol of the manufacturer. The Real-Q 2019-nCOV Detection Kit includes primer sets and probes targeting the E and RdRp genes of SARS-CoV-2 (Fig. 1). The qRT-PCR was performed by the following process: cDNA synthesis of 30 min at 50 °C, pre-denaturation of 15 min at 95 °C, followed by 40 cycles of denaturation 15 s at 95 °C, 45 s at 62 °C for annealing and extension. CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) was applied to qRT-PCR assay (Fig. 2) (Cho et al. 2020).

dPCR assay with Dr. PCR™ Di20K SARS-CoV-2 Detection Kit

The LOAA dPCR System (Optolane, Seongnam, Republic of Korea) is a compact separation type dPCR instrument released in 2020 (Lee et al. 2021b). This system was applied to the dPCR assay. To evaluate the SARS-CoV-2 detection capability of Dr. PCR™ Di20K COVID-19 Detection Kit (Cat no. COM401-Z, Optolane, Seongnam, Republic of Korea), we carried out LOAA dPCR assay using the 301 VTMs. This assay with Dr. PCR™ Di20K COVID-19 Detection Kit was conducted according to the user guideline of the manufacturer (Fig. 3). Since the experimental procedure of this kit omits the viral RNA extraction step, VTMs were directly used for dPCR assay. Dr. PCR™ Di20K COVID-19 Detection Kit contains the sets of primers and probes that target the E and RdRp genes of SARS-CoV-2. The dPCR was performed by the following process: cDNA synthesis of 10 min at 50 °C, pre-denaturation of 30 min at 95 °C, followed by 40 cycles of denaturation 5 s at 95 °C, 10 s at 57 °C for annealing and extension.

Data analysis

The results of the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit are obtained as C/µL values based on the positive wells of the semiconductor chip and the laboratory diagnosis of COVID-19 is automatically determined

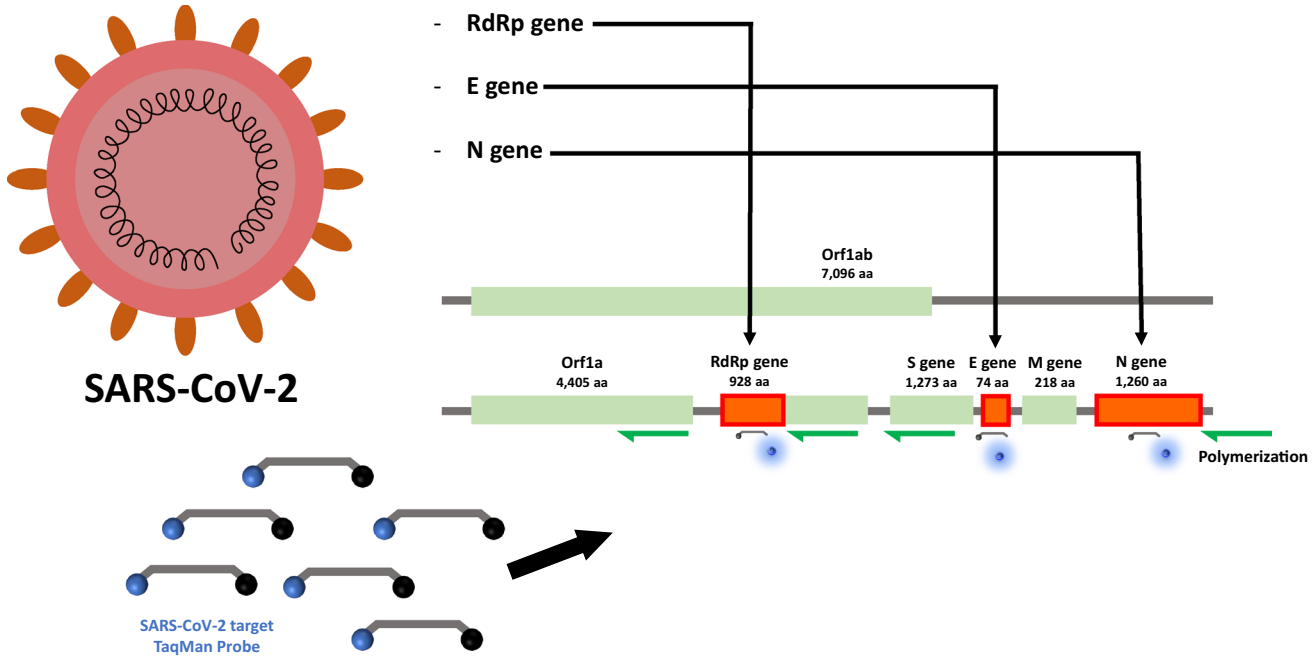

Fig. 1 SARS-CoV-2 detection of qRT-PCR and dPCR method. The World Health Organization (WHO) has recommended targeting the E, RdRp, and N genes for SARS-CoV-2 detection. The specific sets of primers and probes targeting these genes can be used to diagnose COVID-19

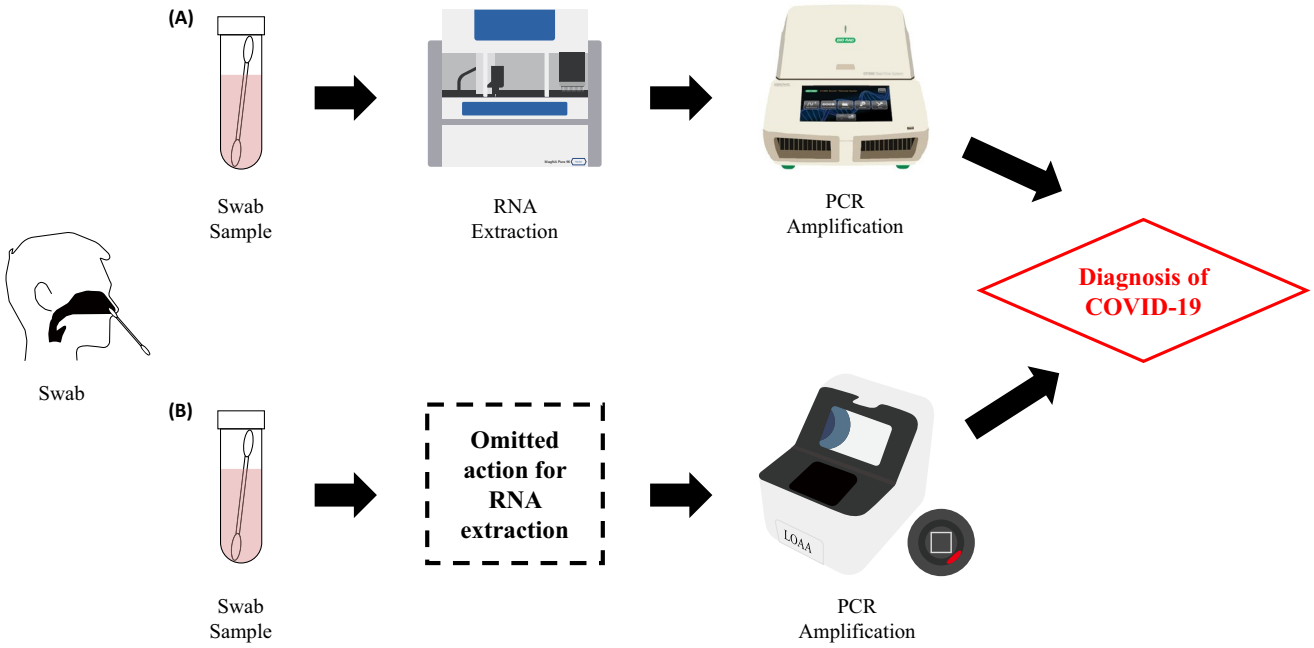

Fig. 2 Schematic diagram of qRT-PCR-based Real-Q 2019-nCoV Detection Kit and dPCR-based Dr. PCRTM Di20K COVID-10 Detection Kit. **A** The COVID-19 diagnosis process of the qRT-PCR-based Real-Q 2019-nCoV Detection Kit. **B** The COVID-19 diagnostic method using dPCR-based Dr. PCRTM Di20K COVID-19 Detection Kit can save the test time of about 1–2 h due to omitting the RNA extraction step

using the LOAA Dr. PCR software 3.0.0. The results of the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit are obtained as C/μL values based on the positive wells of the semiconductor chip and the laboratory diagnosis of COVID-19 is automatically determined using the LOAA Dr. PCR software 3.0.0. Clinical sensitivity and specificity were

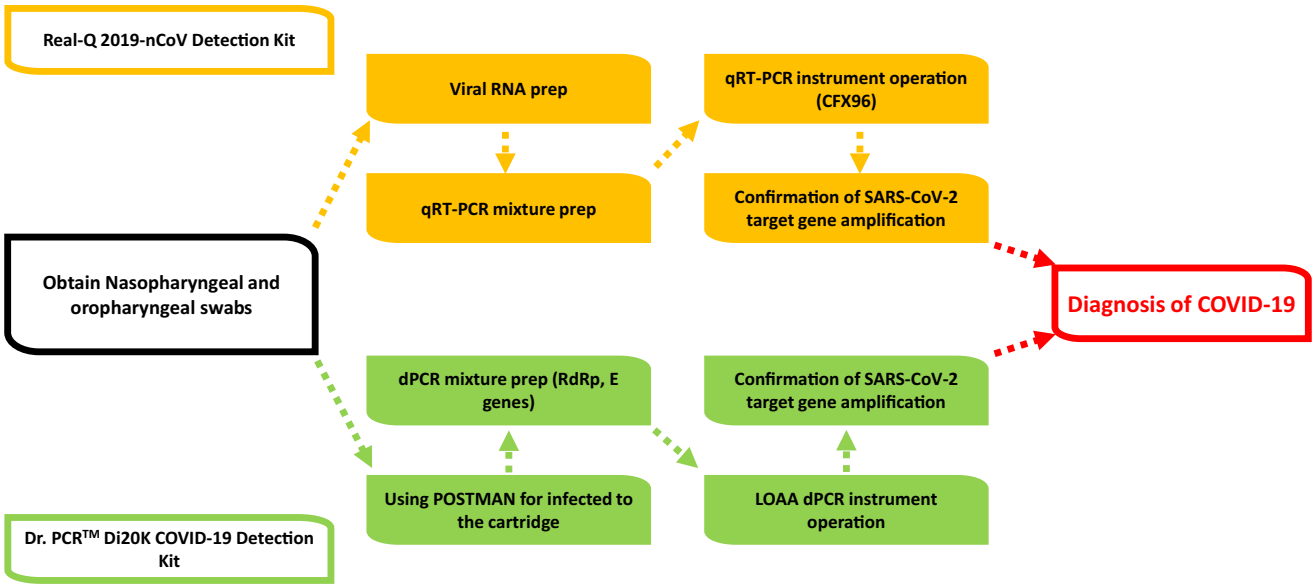

Fig. 3 Workflow of qRT-PCR-based Real-Q 2019-nCoV Detection Kit and dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit. The two Kits based on viral RNA amplification target the E and RdRp genes of SARS-CoV-2. Real-Q 2019-nCoV Detection Kit is a method based on qRT-PCR, and Dr. PCRTM Di20K COVID-19 Detection Kit is based on the dPCR method

calculated on the assay results of each kit to investigate the accuracy of the laboratory diagnosis of COVID-19. In addition, we measured the COVID-19 diagnosis concordance of two different kits using Cohen's Kappa analysis (Daly et al. 2019; Inaba et al. 2021). The interpretation of the Kappa (K) value is as follows: < 0, No agreement; 0.21–0.4, Slight; 0.41–0.6, Moderate; 0.61–0.8, Substantial; 0.81–1, Perfect. To compare the SARS-CoV-2 detection capabilities of each kit following the dilution factor (10^{-1} to 10^{-7}), we carried out a dilution linearity analysis by the spike-in high concentration of SARS-CoV-2 positive sample (almost Ct value > 20) to the negative sample.

Results and discussion

Evaluation of the Real-Q 2019-nCoV Detection Kit for COVID-19 diagnosis

The Real-Q 2019-nCoV Detection Kit with high clinical specificity and sensitivity is commonly used for COVID-19 diagnosis in South Korea. To evaluate the diagnostic capability of the qRT-PCR-based Real-Q 2019-nCoV Detection Kit, we carried out a qRT-PCR assay with 301 residual samples derived upper respiratory tested for COVID-19 at the Samkwang Medical Laboratories (SML, Seoul, Republic of Korea. The SARS-CoV-2 detection results of the qRT-PCR-based Real-Q 2019-nCoV Detection Kit were measured as Ct values. The PCR amplification targets were the E gene, RdRp gene of SARS-CoV-2, and internal control (IC, Human RNase P gene). As shown in Table 1, the classification of results using this assay is reported as a total of five CASE types (CASE 1 to 5). When the Ct values of each E gene (HEX/VIC dye) ≤ 38, RdRp gene (FAM dye) ≤ 38, and IC (Cy5 dye) ≤ 35, it is described as "Detection" (+), and exceeding each cut-off is indicated as "Not detection" (–). According to the (+) or (–) result of each gene, CASE 1 and 2 are determined as "2019-nCoV positive". In the case of CASE 3, IC is (±), E gene is (+), and RdRp gene is (–), which is determined as "Presumptive positive for 2019-nCoV" and retesting is recommended (BioSewoom 2020). If the IC is (+) and the E and RdRp genes are (–) in the sample, it is classified as CASE 4 and finally determined as "Negative" for COVID-19. A sample with results obtained as (–) for all target genes is "invalid" and corresponds to CASE 5.

As a result of carrying out a COVID-19 diagnosis using the qRT-PCR-based Real-Q 2019-nCoV Detection Kit with a total of 301 individual samples, 101 specimens tested positive for COVID-19, and 200 specimens determined negative for COVID-19 (Supplementary Table S1). The positive samples were OP001 to OP020, OP061 to OP080, OP121 to OP140, OP181 to OP200, OP241 to OP261, all of which were individual samples of patients diagnosed with COVID-19 at the medical institution. In addition, the negative samples were OP021 to OP060, OP081 to OP120, OP141 to OP180, OP201 to OP240, OP262 to OP301, and were all individual samples derived from normal individuals in which SARS-CoV-2 was not detected at the same medical

Table 1 Result interpretation of qRT-PCR assay with Real-Q 2019-nCoV Detection Kit

Potential result type	E gene (HEX/VIC dye)	RdRp gene (FAM dye)	Internal control (IC) (Cy5 dye)	Auto-interpetation	Comment
CASE 1	+	+	±	2019-nCoV positive	
CASE 2	–	+	±	2019-nCoV positive[a]	
CASE 3	+	–	±	Presumptive positive for 2019-nCoV[b]	Retest in recommended. If the repeated result remains presumptive positive, contact your local public health laboratory or CDC for further guidance
CASE 4	–	–	+	Negative	
CASE 5	–	–	–	Invalid	Repeat the test

Retest is recommended

[a]E gene (–) and/RdRP gene (+) result could be casued by (1) a specimen at concentrations near or below the limit of detection of the test, (2) a mutation in the E gene target region in the oligonucleotide binding sites, (3) other factors

[b]E gene (+) and/RdRP gene (–) result could be caused by (1) a specimen at concentrations near or below the limit of detection of the test, (2) a mutation in the RdRP target region in the oligonucleotide binding sites, (3) infection with some other human coronavirus, (4) other factors

institution. The qRT-PCR assay results using the Real-Q 2019-nCoV Detection Kit exactly matched results of the COVID-19 diagnostic test at the medical institution in all individual samples. Thus, for COVID-19 diagnostic evaluation using this kit, the false-positive and false-negative ratio values were "0". To evaluate clinical COVID-19 diagnostic accuracy of the Real-Q 2019-nCoV Detection Kit, the diagnostic sensitivity (eps 1) and specificity (eps 2) were calculated by comparing the qRT-PCR assay results of individual samples with the diagnostic results obtained at the medical institution (Feuerman and Miller 2008; Trevethan 2017).

$$Sensitivity(\%) = \frac{Ture\ positive}{True\ posivite + False\ negativie} \quad (1)$$

$$Specificity(\%) = \frac{Ture\ negative}{True\ negative + False\ positive} \quad (2)$$

Although the sample size was relatively small, it was measured with very high diagnostic sensitivity (100%) and specificity (100%) (Supplementary Table S2). Thus, we confirmed that the qRT-PCR-based Real-Q 2019-nCoV Detection Kit has high diagnostic accuracy in the COVID-19 diagnosis.

Evaluation of the Dr. PCR ™ Di20K COVID-19 Detection Kit for COVID-19 diagnosis

To evaluate the diagnostic accuracy of the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit, a COVID-19 diagnostic test was performed with the same 301 individual samples evaluated above. The results of the dPCR assay with Dr. PCR™ Di20K COVID-19 Detection Kit were analyzed based on positive wells. This dPCR assay amplifies the E gene, RdRP gene of SARS-CoV-2, and PCR Control (PCRC) to determine the diagnosis of COVID-19. The dPCR assay results are valid when the number of positive wells is 10,000 or more. In addition, positive control material is RdRp (FAM dye) ≥ 2, E gene (FAM dye) ≥ 2, PCRC (Cy5 dye) ≥ 10, and NTC is RdRp (FAM dye) < 2, E gene (FAM dye) < 2, PCRC (Cy5 dye) ≥ 10, it is determined to be valid. The COVID-19 diagnostic test for individual samples is automatically determined as CASE 1–9 based on positive wells (Table 2).

A total of 301 identical individual samples evaluated through the above qRT-PCR assay was analyzed for COVID-19 diagnostic capability using the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit. As a result of the dPCR assay with Dr. PCR™ Di20K COVID-19 Detection Kit, 101 specimens were positive for COVID-19, and 200 specimens were negative. The 101 positive individual specimens were OP001 to OP020, OP061 to OP080, OP121 to OP140, OP181 to OP200, OP241 to OP261, all of which were extracted from patients diagnosed with COVID-19 at the medical institution. The 200 negative individual specimens were OP021 to OP060, OP081 to OP120, OP141 to OP180, OP201 to OP240, OP262 to OP301, all of which were tested negative for COVID-19 at the same medical institution. The results of the dPCR assay with Dr. PCR™ Di20K COVID-19 Detection Kit are 100% consistent with the results of the COVID-19 diagnostic test at the medical institution in all individual samples. Therefore, in the case of COVID-19 diagnostic evaluation using this kit, the false-positive and false-negative ratio values were confirmed as "0". In addition, to evaluate diagnostic accuracy, we calculated the diagnostic sensitivity and specificity of the Dr. PCR™ Di20K COVID-19 Detection Kit by comparing the results of the

Table 2 Result interpretation of dPCR assay with Dr. PCR™ Di20K COVID-19 Detection Kit

Potential result type	Valid well (In number of positive wells)	Green channel result		Red channel result	Auto-interpetation
		SARS-CoV-2			
		E gene (FAM dye) (In number of positive wells)	RdRp gene (FAM dye) (In number of positive wells)	PCR control (PCRC) (Cy5 dye) (In number of positive wells)	
Positive control	≥ 10,000	≥ 2	≥ 2	≥ 10	Valid
Negative control		< 2	< 2	≥ 10	Valid
CASE 1		≥ 2	≥ 2	≥ 10	COVID-19 positive
CASE 2		≥ 2	≥ 2	< 10	COVID-19 positive[a]
CASE 3		< 2	≥ 2	≥ 10	Undetermined[b]
CASE 4		≥ 2	< 2	≥ 10	SARS-CoV positive[c]
CASE 5		≥ 2	< 2	< 10	SARS-CoV positive[a,c]
CASE 6		< 2	≥ 2	< 10	Undetermined[a,b]
CASE 7		< 2	< 2	≥ 10	COVID-19 negative
CASE 8		< 2	< 2	< 10	Invalid[d]
CASE 9	< 10,000	–	–	–	Invalid[e]

[a]In the case of a high viral load, the PCRC FRET signal (ch2, red channel) may be reduced

[b]If the E gene is negative but the RdRP gene is positive, it is determined as "Undetermined", and if the same result is obtained after retesting, it is determined as positive

[c]If the E gene is positive but the RdRP gene is negative, it is determined as "SARS-CoV"

[d]If the 'Valid' condition is met in NTC, PC, or PCRC, use DEPC treated water to dilute 1/5 and then proceed with re-reaction

[e]If the number of valid wells is less than 10,000, it is determined as invalid, and the cartridge is discarded and retested with a new cartridge

dPCR assay with diagnostic results of the medical institutions in the individual specimens. Although the individual specimen size was relatively small, we confirmed that it was measured with very high diagnostic sensitivity (100%) and specificity (100%). As with the qRT-PCR assay, the sample size was relatively small. However, we confirmed that the dPCR assay with Dr. PCR™ Di20K COVID-19 Detection Kit has the same high diagnostic accuracy as the qRT-PCR assay used as a gold standard in diagnosing COVID-19 (Supplementary Table S3). This dPCR is expected to be easy to save experimental time and resources, which is important for the diagnosis of COVID-19 by omitting the RNA extraction step in the diagnostic test process.

Table 3 The dilution factors for detection capability evaluation

	Negative matrix	Positive specimen	Note
Dilution factor I	720µL	80µL	10^{-1} diluent
Dilution factor II	720µL	Dilution factor I 80µL	10^{-2} diluent
Dilution factor III	720µL	Dilution factor II 80µL	10^{-3} diluent
Dilution factor IV	720µL	Dilution factor III 80µL	10^{-4} diluent
Dilution factor V	720µL	Dilution factor IV 80µL	10^{-5} diluent
Dilution factor VI	720µL	Dilution factor V 80µL	10^{-6} diluent
Dilution factor VII	720µL	Dilution factor VI 80µL	10^{-7} diluent

Comparison of diagnostic capability evaluation for COVID-19 between qRT-PCR-based Real-Q 2019-nCoV Detection Kit and dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit

Through the COVID-19 diagnostic test of the qRT-PCR-based Real-Q 2019-nCoV Detection Kit and dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit, we confirmed that the diagnostic results using the two different kits were completely consistent. We calculated using Cohen's Kappa coefficient to measure the agreement and reliability between the two diagnostic results. The Kappa coefficient is a probability value, and the closer to 1, the higher the agreement between two different data (Czodrowski 2014). When the COVID-19 diagnostic test results using the two kits were calculated by Cohen's Kappa coefficient, the value was "1" meaning "Almost Perfect".

In order to evaluate the detection capability between the two different kits depending on the virus concentration, a high concentration of SARS-CoV-2 positive specimens (OP003, OP009, OP010, OP013, OP015, OP017, OP137,

Table 4 Evaluation of SARS-CoV-2 detection capability according to dilution factors using qRT-PCR-based Real-Q 2019-nCoV Detection Kit and dPCR-based Dr. PCR™ Di20K

Sample No	Dilution	Real-Q 2019-nCoV Detection Kit				Dr. PCR™ Di20K COVID-19 Detection Kit										
		E gene	RdRp gene	Internal Control (IC)	Auto-interpetation	Valid well	E gene		PCRC		Valid well	RdRp gene		PCRC		Auto-interpetation
							Positive well	Conc. (C/µL)	Positive well	Conc. (C/µL)		Positive well	Conc. (C/µL)	Positivie well	Conc. (C/µL)	
OP003	Di_01	16.95	17.11	32.54	Positive	15,006	14,964	41,013.04	1347	656.17	15,371	15,358	49,362.48	2158	1055.45	Positive
	Di_02	20	20.37	29.68	Positive	15,739	15,521	29,856.29	4000	2045.76	17,544	16,086	17,355.67	2797	1211.67	Positive
	Di_03	23.6	24.03	28.42	Positive	14,802	3892	2128.48	3546	1910.66	16,169	2886	1371.70	1891	867.74	Positive
	Di_04	26.45	27.06	26.64	Positive	12,387	393	224.94	2350	1467.69	13,314	236	124.78	2162	1236.26	Positive
	Di_05	31.46	32.58	27.68	Positive	10,833	20	12.89	133	86.19	16,020	35	15.26	2353	1108.28	Positive
	Di_06	34.35	35.8	27.55	Positive	14,838	6	2.82	3578	1925.13	15,286	8	3.65	2437	1211.66	Positive
	Di_07	N/A	N/A	27.61	Negative	14,933	0	0	2641	1357.85	14,937	0	0	3257	1716.02	Negative
OP009	Di_01	18.43	18.82	32.91	Positive	14,454	14,427	43,834.11	3399	1870.38	16,258	16,194	38,633.42	2870	1355.07	Positive
	Di_02	22.17	22.58	29.84	Positive	13,707	12,780	18,793.31	3573	2107.05	15,928	9038	5846.56	2171	1022.31	Positive
	Di_03	25.3	26.09	28.02	Positive	17,937	1863	765.09	3457	1493.70	16,763	2658	1204.50	3763	1773.66	Positive
	Di_04	28.52	29.29	27.61	Positive	16,166	163	70.7	2414	1128.32	15,886	110	48.48	3562	1771.32	Positive
	Di_05	30.08	30.45	27.01	Positive	16,506	12	5.07	2575	1183.31	15,234	11	5.04	3640	1904.94	Positive
	Di_06	38.43	38.17	26.63	Negative	14,495	1	0.48	3080	1666.57	14,099	0	0	2848	1574.28	Negative
OP010	Di_01	22.1	22.54	29.6	Positive	14,119	11,962	13,107.93	1283	664.66	17,380	10,013	5988.21	111	44.7	Positive
	Di_02	25.37	26.2	28.56	Positive	15,558	2999	1493.98	3601	1836.68	16,931	2072	910.75	2076	912.63	Positive
	Di_03	28.48	29.31	27.85	Positive	15,630	374	168.97	3387	1703.98	15,770	282	125.89	2612	1263.35	Positive
	Di_04	32.22	33.04	27.38	Positive	16,667	31	12.99	3116	1443.98	16,521	22	9.3	4337	2124.48	Positive
	Di_05	36.15	36	27.2	Positive	17,042	3	1.23	2489	1101.51	16,403	3	1.28	1839	829.62	Positive
	Di_06	N/A	39.04	27.67	Negative	15,922	1	0.44	2988	1450.06	13,415	0	0	554	294.24	Negative
OP013	Di_01	23.47	23.84	30.21	Positive	15,635	15,367	28,369.40	796	364.56	12,718	12,470	27,469.85	1428	830.94	Positive
	Di_02	24.46	25.22	28.81	Positive	14,428	5375	3251.68	615	303.91	16,290	3488	1681.05	1188	528.31	Positive
	Di_03	26.85	27.53	27.48	Positive	13,792	650	336.81	736	382.61	14,090	1595	838.16	1407	733.97	Positive
	Di_04	30.44	31.27	26.57	Positive	13,277	134	70.77	1788	1009.14	16,921	119	49.24	2862	1292.74	Positive
	Di_05	33.17	34.39	27.17	Positive	14,807	5	2.36	509	244.05	17,727	3	1.18	3012	1299.22	Positive
	Di_06	38.41	38.5	27.36	Negative	15,071	0	0	1718	844.4	15,319	1	0.46	996	469.03	Negative
OP015	Di_01	20.42	20.47	29.65	Positive	16,182	14,772	17,025.42	362	157.85	16,885	13,223	10,663.37	1154	493.9	Positive
	Di_02	23.54	23.74	28.63	Positive	16,450	3997	1942.07	579	249.99	14,208	3716	2115.29	1439	745.01	Positive
	Di_03	27.4	28.05	26.9	Positive	16,750	730	310.89	1748	768.94	18,125	363	141.15	1632	658.3	Positive
	Di_04	30.6	31.57	26.88	Positive	15,536	33	14.84	768	353.7	16,186	52	22.45	2576	1209.36	Positive
	Di_05	39.13	35.37	26.71	Positive	12,841	4	2.17	791	443.57	15,290	4	1.83	1384	661.94	Positive
	Di_06	38.01	38.14	27.72	Negative	15,795	0	0	611	275.24	18,259	0	0	3308	1394.51	Negative

Table 4 (continued)

Sample No	Dilution	Real-Q 2019-nCoV Detection Kit				Dr. PCR™ Di20K COVID-19 Detection Kit										
		E gene	RdRp gene	Internal Control (IC)	Auto-interpeta-tion	Valid well	E gene		PCRC		Valid well	RdRp gene		PCRC		Auto-inter-petation
							Positive well	Conc. (C/µL)	Positive well	Conc. (C/µL)		Positive well	Conc. (C/µL)	Positivie well	Conc. (C/µL)	
OP017	Di_01	20.55	20.96	29.84	Positive	13,405	3642	2211.82	442	233.92	15,286	1684	814.33	1091	516.61	Positive
	Di_02	27.8	28.56	28.56	Positive	14,353	286	140.42	397	195.69	15,728	186	83	1083	497.75	Positive
	Di_03	30.5	31.85	27.1	Positive	17,817	61	23.93	2337	980.96	15,743	25	11.09	1314	608.06	Positive
	Di_04	34.44	35.66	27.89	Positive	14,651	6	2.86	200	95.9	15,299	2	0.91	1865	906.97	Positive
	Di_05	38.18	39.8	27.17	Negative	13,658	0	0	552	95.94	15,203	0	0	1226	586.6	Negative
OP137	Di_01	20.99	21.22	29.85	Positive	11,942	11,457	22,351.17	1012	617.79	14,122	13,655	23,784.84	2734	1501.21	Positive
	Di_02	24.46	25.01	28.35	Positive	12,061	3867	2697.03	1974	1246.95	14,629	1636	827.41	1947	996.43	Positive
	Di_03	27.99	28.73	27.51	Positive	14,512	374	182.16	1915	987.33	15,632	183	82.16	2037	974.08	Positive
	Di_04	31.08	32.16	26.68	Positive	13,546	52	26.83	3079	1799.05	16,479	20	8.47	2867	1333.50	Positive
	Di_05	35.01	36.53	27.1	Positive	14,571	7	3.35	3907	2177.86	12,832	2	1.09	1994	1178.26	Positive
	Di_06	N/A	N/A	27.62	Negative	14,794	1	0.47	4122	2278.59	15,111	1	0.46	589	277.38	Negative
OP190	Di_01	19.37	20.02	30.22	Positive	16,636	16,548	36,572.01	2768	1269.66	13,892	13,396	23,249.95	2670	1489.10	Positive
	Di_02	22.52	23.15	28.78	Positive	15,291	5643	3212.89	2785	1402.72	15,169	4996	2787.33	3431	1788.99	Positive
	Di_03	25.32	26.06	27.64	Positive	15,103	833	395.82	3107	1606.88	12,505	244	137.48	1667	998.16	Positive
	Di_04	30.11	31.01	27.69	Positive	15,640	67	29.95	2422	1173.85	13,119	29	15.44	2352	1378.43	Positive
	Di_05	33.37	34.28	26.6	Positive	17,147	7	2.85	2790	1238.97	12,482	2	1.12	2739	1728.41	Positive
	Di_06	N/A	38.07	27.67	Negative	15,808	0	0	2939	1435.28	15,798	1	0.44	3757	1894.65	Negative
OP253	Di_01	19.6	19.57	30.45	Positive	17,250	17,198	40,495.28	3447	1555.30	15,787	15,714	37,510.34	3505	1751.53	Positive
	Di_02	22.29	22.34	29.88	Positive	15,735	6311	3576.47	2255	1079.17	15,416	5223	2886.31	3573	1839.55	Positive
	Di_03	26.57	27.11	27.09	Positive	16,244	582	254.55	2927	1386.15	15,575	430	195.33	3359	1694.79	Positive
	Di_04	29.56	30.12	27.25	Positive	14,840	62	29.21	3840	2089.05	17,818	54	21.18	4320	1937.22	Positive
	Di_05	30.89	31.22	27.38	Positive	15,977	4	1.75	3513	1732.37	15,585	3	1.34	3164	1583.17	Positive
	Di_06	N/A	N/A	27.71	Negative	17,503	1	0.4	2956	1290.61	16,214	0	0	3840	1885.66	Negative
OP260	Di_01	23.64	24.15	28.56	Positive	12,338	3629	2430.19	1716	1044.81	16,118	4120	2059.51	3578	1751.25	Positive
	Di_02	27.47	28.15	27.91	Positive	10,924	543	355.71	325	210.72	17,237	382	156.36	3640	1654.95	Positive
	Di_03	30.04	30.46	27.04	Positive	15,886	61	26.84	2013	945.3	15,508	25	11.26	3731	1920.05	Positive
	Di_04	35.14	36.82	27.14	Positive	16,397	4	1.7	3590	1724.00	14,886	2	0.94	3373	1792.63	Positive
	Di_05	38.4	38.4	26.73	Negative	16,610	1	0.42	3149	1466.57	16,763	0	0	3515	1641.82	Negative

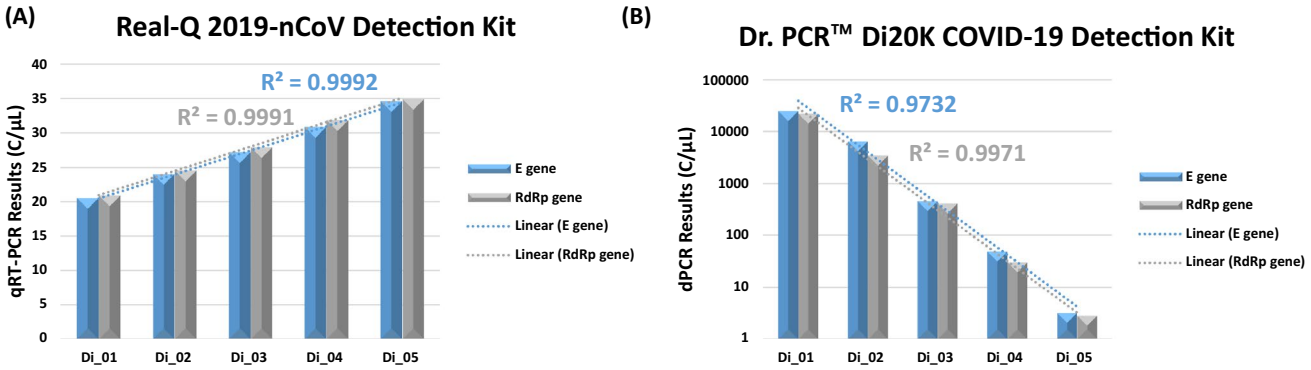

Fig. 4 Dilutional linearity test results depending on the dilution factors of the qRT-PCR based Real-Q 2019-nCoV Detection Kit and dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit. **A** The average R^2 dilutional linearity test depending on the dilution factors using a qRT-PCR assay with Real-Q 2019-nCoV Detection Kit in the 10 individual specimens (OP003of, OP009, OP010, OP013, OP015, OP017, OP137, OP190, OP253, OP260) with a high concentration of SARS-CoV-2. **B** The average R^2 of dilutional linearity test depending on the dilution factors using dPCR assay with Dr. PCRTM Di20K COVID-19 Detection Kit in the 10 individual specimens (OP003, OP009, OP010, OP013, OP015, OP017, OP137, OP190, OP253, OP260) with a high concentration of SARS-CoV-2

OP190, OP253, OP260) was selected. The high concentration SARS-CoV-2 positive specimens were spike-in treated with the negative patient specimen, diluted, and then the dilutional linearity test was carried out based on C/uL.

The correlation of the detection capabilities of two different kits was confirmed through the spike-in treatment of the negative patient specimen at the high concentration SARS-CoV-2 positive specimens. The dilutional linearity test was carried out after dilution of the treated individual specimen depending on the dilution factors from 10^{-1} to 10^{-7} (Table 3). As shown in Table 4, we confirmed that SARS-CoV-2 could be detected up to the dilution factor of 10^{-5} in both results of the qRT-PCR-based Real-Q 2019-nCoV Detection Kit and the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit. Thus, this means that the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit has the same detection capability as the qRT-PCR-based Real-Q 2019-nCoV Detection Kit approved by FDA-Emergency Use Authorization (EUA) and MFDS-EUA. In addition, the results of regression analysis measured depending on the dilution factors of 10 randomly selected individual specimens (OP003, OP009, OP010, OP013, OP015, OP017, OP137, OP190, OP253, OP260) were average $R^2 = 0.99915$ in the qRT-PCR assay with Real-Q 2019-nCoV Detection Kit and average $R^2 = 0.98515$ in the dPCR assay with Dr. PCR™ Di20K COVID-19 Detection Kit (Fig. 4; Fig. S1; Fig. S2). This means that both kits have high detection reliability (Gougeon 2005). In summary, dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit has the same high detection reliability as the qRT-PCR method. In addition, this kit has the advantages of the dPCR method as reported in the previous study, and at the same time, omits the RNA extraction step, suggesting that the SARS-CoV-2 can be detected relatively quickly.

Correlation analysis of the qRT-PCR-based Real-Q 2019-nCoV Detection Kit and dPCR-based Dr. PCR™ COVID-19 Detection Kit without RNA extraction step for the SARS-CoV-2 genes

Correlation analysis of the two kits was performed using the detection level results of SARS-CoV-2 E and RdRp genes in the ten individual samples analyzed to the above dilutional linearity test. R^2 values were measured by performing regression analysis based on the Ct value in the qRT-PCR assay and the C/uL value in the dPCR assay. As a result of the measurement, the average $R^2 = 0.9908$ in the E gene and $R^2 = 0.9947$ in the RdRp gene (Fig. 5; Fig. S3; Fig. S4). This means that there is a high correlation between the qRT-PCR assay with Real-Q 2019-nCoV Detection Kit and the dPCR assay with Dr. PCR™ Di20K COVID-19 Detection Kit for these two genes.

Conclusion

As the COVID-19 pandemic continues worldwide, the need for a rapid and accurate detection method of SARS-CoV-2 continues to grow. Thus, we compared the diagnostic capability of the recently released dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit (approved by the Korea MFDS) without the viral RNA extraction step and the qRT-PCR-based Real-Q 2019-nCoV Detection Kit (approved by the US FDA EUA and the Korea MFDS EUA) commonly used in Korea. We obtained the 301 individual specimens with confirmed COVID-19 positive/negative from the government-accredited medical institution and performed the SARS-CoV-2 detection test using these two kits. The results of both kits confirmed that the COVID-19 diagnosis was

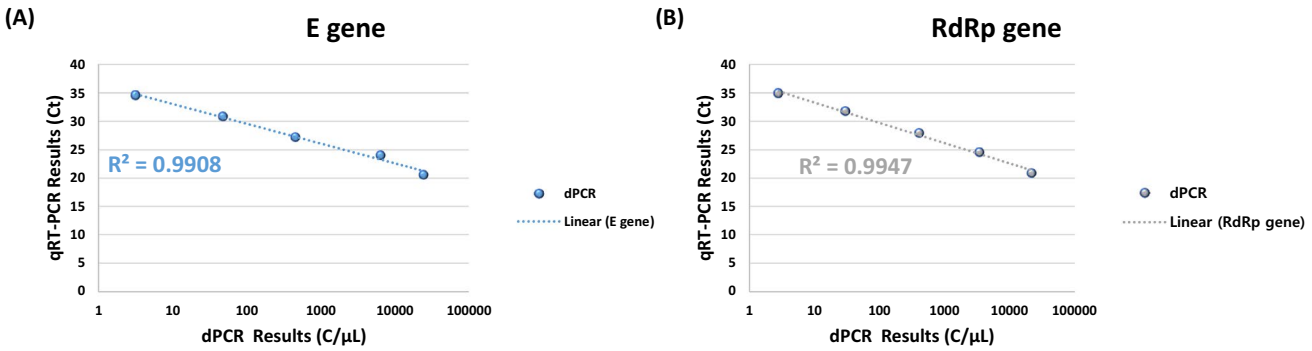

Fig. 5 Correlation analysis of the qRT-PCR and dPCR assay using two different kits for the E and RdRp genes of SARS-CoV-2. **A** Correlation analysis for E gene of SARS-CoV-2 between qRT-PCR and dPCR assay was performed using the detection level results of in the 10 individual specimens with a high concentration of SARS-CoV-2. **B** Correlation analysis for the RdRp gene of SARS-CoV-2 between qRT-PCR and dPCR assay was performed using the detection level results of in the 10 individual specimens with a high concentration of SARS-CoV-2

perfectly matched, and the clinical sensitivity and specificity were 100%. In addition, the dilutional linearity test demonstrated that both of these kits were capable of detection at low viral loads (10^{-5}) with high confidence. However, the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit could cover the weaknesses of the qRT-PCR-based method. In addition, since this kit omits the RNA extraction step, the test time of approximately 1–2 h can be shortened, thereby reducing the risk of carry-over contamination and biosafety. This kit is applied to the LOAA dPCR equipment reported in the previous study, and it can overcome various limitations with this compact size and low equipment price. Thus, we suggest that the dPCR-based Dr. PCR™ Di20K COVID-19 Detection Kit is suitable for the COVID-19 point-of-care testing (POCT), which requires a rapid and efficient method.

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Declarations

Conflict of interest Wonseok Shin, Cherl-Joon Lee, Yong-Moon Lee, Young-Bong Choi, Seyoung Mun, and Kyudong Han declare that we have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee.

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