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RESEARCH ARTICLE

Diagnostic evaluation of qRT-PCR-based kit and dPCR-based kit for COVID-19

Cherl-Joon Lee[1] · Wonseok Shin[2] · Seyoung Mun[3,4,5] · Minjae Yu[5,6] · Young-Bong Choi[7] · Dong Hee Kim[8] · Kyudong Han[1,2,4,5,6]

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Abstract

Background Coronavirus disease of 2019 (COVID-19) is well known as a fatal disease, first discovered at Wuhan in China, ranging from mild to death, such as shortness of breath and fever. Early diagnosis of COVID-19 is a crucial point in preventing global prevalence.

Objective We aimed to evaluate the diagnostic competency and efficiency with the Allplex™ 2019-nCoV Assay kit and the Dr. PCR 20 K COVID-19 Detection kit, designed based on the qRT-PCR and dPCR technologies, respectively.

Methods A total of 30 negative and 20 COVID-19 positive specimens were assigned to the diagnostic test by using different COVID-19 diagnosis kits. Diagnostic accuracy was measured by statistical testing with sensitivity, specificity, and co-efficiency calculations.

Results Comparing both diagnostic kits, we confirmed that the diagnostic results of 30 negative and 20 positive cases were the same pre-diagnostic results. The diagnostic statistics test results were perfectly matched with value (1). Cohen's Kappa coefficient was demonstrated that the given kits in two different ways were "almost perfect" with value (1). In evaluating the detection capability, the dilutional linearity experiments substantiate that the Dr. PCR 20 K COVID-19 Detection kit could detect SARS-CoV-2 viral load at a concentration ten times lower than that of the Allplex™ 2019-nCoV Assay kit.

Conclusions In this study, we propose that the dPCR diagnosis using LOAA dPCR could be a powerful method for COVID-19 point-of-care tests requiring immediate diagnosis in a limited time and space through the advantages of relatively low sample concentration and small equipment size compared to conventional qRT-PCR.

Keywords Coronavirus disease of 2019 · Severe Acute Respiratory Syndrome Coronavirus 2 · quantitative real-time PCR · Digital PCR

Cherl-Joon Lee and Wonseok Shin contributed equally to this work.

✉ Dong Hee Kim
anedhkim@hanmail.net

✉ Kyudong Han
kyudong.han@gmail.com

[1] Department of Bio-Convergence Engineering, Dankook University, Jukjeon 16890, Republic of Korea

[2] NGS Clinical Laboratory, Dankook University Hospital, Cheonan 31116, Republic of Korea

[3] Department of Nanobiomedical Science, Dankook University, Cheonan 31116, Republic of Korea

[4] Center for Bio-Medical Engineering Core Facility, Dankook University, Cheonan 31116, Republic of Korea

[5] DKU-Theragen institute for NGS analysis (DTiNa), Cheonan 31116, Republic of Korea

[6] Department of Microbiology, College of Science and Technology, Dankook University, Cheonan 31116, Republic of Korea

[7] Department of Chemistry, College of Science and Technology, Dankook University, Cheonan 31116, Republic of Korea

[8] Department of Anesthesiology and Pain Management, Dankook University College of Medicine, Cheonan 31116, Republic of Korea

Introduction

Coronavirus disease of 2019 (COVID-19) is a human respiratory infectious disease caused by a novel type of coronavirus (Severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) that was first reported in Wuhan, China, in December 2019 and has become a worldwide pandemic (Kumar et al. 2021; Zhang et al. 2020). As far as is known, SARS-CoV-2 spreads from an infected person to others through respiratory fluids and aerosols carrying the infectious virus. The rapid spread of the COVID-19 pandemic worldwide has been caused by misdiagnosis and asymptomatic transmission (Syal 2021). COVID-19 can cause mild symptoms ranging from loss of smell and taste, fever, muscle pain, and acute shortness of breath (Cascella et al. 2021; Garg et al. 2020). However, since senior citizens, immunocompromised patients, and patients with underlying diseases cause death in severe cases, accurate early diagnosis and comprehensive infectious disease prevention and control are required worldwide before developing of a COVID-19 therapeutic agent. Early diagnosis of COVID-19 is important because it helps determine the direction of treatment and can prevent several fatal complications at an early stage (Peck 2020).

There are several methods for COVID-19 diagnostics and detection, including cell culture and microscopy, radiology-based detection, nucleic acid amplification tests, quantitative real-time PCR (qRT-PCR), and immunoassay-based detection (Russo et al. 2020; Udugama et al. 2020). Among the methods mentioned above, the qRT-PCR and the immunoassay-based detection methods are predominantly used for COVID-19 diagnosis. First, the immunoassay-based detection method uses the point that when the SARS-CoV-2 enters the human body, it stimulates the adaptive immune system to generate antibodies. This method uses IgM, which is produced 7 days and peaks at 28 days after SARS-CoV-2 viral infection, and IgG, which is generated 10 days and peaks at 49 days after SARS-CoV-2 viral infection (Padoan et al. 2020; Xu et al. 2020). The advantage of this method is that the result can be checked after approximately 10 min, it is not expensive, and it can be checked quickly and easily confirmed with a single drop of blood. However, the limitations of the immunoassay-based detection method are: First, in the primary antibody test, it may be challenging to determine whether a SARS-CoV-2—positive individual is infected due to the lack of antibodies in the body. Second, even in SARS-CoV-2—positive individuals, there are cases in which no antibody response or antibody levels cannot be maintained. Third, the low accuracy of this test (50–70%) has been reported (Dhamad and Rhida 2020; Watson et al. 2020). On the other hand, the qRT-PCR is a method that can confirm in real time using specific primers and probes for a specific viral gene sequence capable of diagnosing COVID-19. Currently, qRT-PCR is used as a gold standard in the COVID-19 diagnosis (Goudouris 2021; Huergo and Thanh 2021; Murphy and Bustin 2009). The reason is that SARS-CoV-2 detection shows very high sensitivity (98.2%) and specificity (100%), and quantitative analysis is straightforward (Chung et al. 2021). However, the qRT-PCR method can only be used under laboratory condition because it requires the medium-sized equipment and various laboratory tools for pre-sample preparation operation (Gupta et al. 2021).

Recently, in the diagnosis of COVID-19, a dPCR analysis method that compensates for the weakness of qRT-PCR is emerging and difficulty in field diagnosis (Dong et al. 2021; Verhaegen et al. 2016). The dPCR method for diagnosing COVID-19 is similar to qRT-PCR by hybridizing specific primers and probes to specific viral gene sequences (Dang et al. 2020). Among the currently commercialized dPCR systems, Optolane's LOAA digital real-time PCR analyzer (LOAA dPCR, Optolane, Seongnam, Republic of Korea) released in 2020 integrates light source control and thermal control sensors to miniaturize the equipment, so that it takes less space than the qRT-PCR system. In addition, LOAA dPCR can monitor real-time amplification, and the target gene is placed in each well. Amplification and fluorescence analysis are performed simultaneously in each well, so the probability of false negatives by the experiment is low. Therefore, LOAA dPCR with a small size and high accuracy is expected to play a role as a point of care testing (POCT) device in the future.

Currently, reagent products approved for emergency use for COVID-19 diagnosis in Korea have been developed by Kogen Biotech, Seegene, Solgent, SD Biosensor, Biosewoom, and etc. (Garg et al. 2021; Park and Chung 2021; Sung et al. 2020). Among them, Allplex™ 2019-nCoV Assay kit (Seegene, Seoul, Republic of Korea) is a qRT-PCR-based COVID-19 diagnostic kit and has received the US Food and Drug Administration (FDA) Emergency Use Authorization (EUA) and the Korean Ministry of Food and Drug Safety (KMFDS) EUA approval (Lai et al. 2021). In the previous study, the Allplex™ 2019-nCoV Assay kit (Seegene, Seoul, Republic of Korea) showed high performance in sensitivity as a result of confirming clinical performance by applying KCDC qRT-PCR protocol as a gold standard (Freire-Paspuel and Garcia-Bereguiain 2021). Therefore, currently in Korea, the Allplex™ 2019-nCoV Assay kit (Seegene, Seoul, Republic of Korea) is typically used for the COVID-19 diagnosis. However, since this assay is a qRT-PCR-based method, it has disadvantages such as space limitation, relatively high qPCR equipment cost, and relatively long experimental time. In order to overcome these shortcomings, Optolane Co., Ltd. launched the LOAA dPCR assay (Optolane, Seongnam, Republic of Korea) in 2020,

a compact and relatively short test time. In particular, the semiconductor chip-based Dr. PCR 20 K COVID-19 detection kit (Optolane, Seongnam, Republic of Korea) is the first dPCR-based COVID-19 diagnostic kit developed in Korea. In addition, this kit was approved for COVID-19 diagnostic reagent from the Korea Ministry of Food and Drug Safety (MFDS) on May 18, 2020.

Here, we performed qRT-PCR with the Allplex™ Assay kit currently used as a gold standard and dPCR with the Dr. PCR 20 K COVID-19 Detection kit using actual COVID-19 patient samples, respectively. In addition, based on the results derived from each method, the effectiveness of COVDI-19 diagnosis was evaluated and comparatively analyzed.

Materials and methods

Collection clinical specimens

The Korean government has sufficiently secured and supported various types of COVID-19 samples, including respiratory and blood, from a total of six medical institutions with proven clinical evaluation capabilities. Here, we obtained a total of 50 specimens, of which were derived from 38 pharyngeal swabs (upper respiratory samples) and 12 sputa (lower respiratory samples) from Seoul Clinical Laboratories (SCL, Yongin, Republic of Korea) among those institutions. The specimens obtained are the residual samples from 20 positive and 30 negative specimens diagnosed with COVID-19 at the SCL medical institution. All specimens were stored in 3 mL of viral transport media (Cat. No UTNFS-3B-1, Noble Biosciences, Inc., Hwaseong, Republic of Korea). This study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines and approved by relevant regulatory from SCL Institutional Review Board (IRB-20-008). All specimen donors submitted written consent before they participated in the study.

Viral RNA isolation

In a total of 50 specimens, viral RNAs were isolated from 38 pharyngeal swabs using each 200 µL of a viral transport media with the KingFisher Flex system (ThermoFisher Scientific Inc., Worcester, MA, USA), an automated nucleic acid extraction system, following the manufacturer's guidelines protocol (Lin et al. 2021). In addition, we isolated viral RNAs using the Advansure™ E3 system (LG Chem, Ltd., Seoul, Korea), an automated nucleic acid extraction system, and 200 µL each of 12 viral transport media obtained from sputum specimens according to the manufacturer's guideline (Lee et al. 2021). The viral RNAs isolated by each system were dissolved in 50 µL of RNase-free water. Then, the eluted samples were stored at − 80 ℃ in a deep freezer.

qRT-PCR assay with Allplex™ 2019-nCoV Assay kit

We carried out qRT-PCR using the stored RNA samples from the 50 specimens to evaluate the COVID-19 detection capability of Allplex™ 2019-nCoV Assay kit (Cat. No RP10250X, Seegene, Seoul, Republic of Korea). The qRT-PCR assay was performed according to the protocol of the manufacturer (Fig. 1). The Allplex™ 2019-nCoV Assay kit includes primer sets and probes targeting the RdRp, E and N genes of SARS-CoV-2 (Ambrosi et al. 2021). The target viral genes amplification was performed by following process: cDNA synthesis step of 20 min at 50 ℃, pre-denaturation step of 15 min at 95℃, followed by 45 cycles of denaturation step 15 s at 94 ℃, 30 s at 58 ℃ for annealing and extension steps. CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) was used for qRT-PCR assay.

dPCR assay with Dr. PCR 20 K COVID-19 detection kit

dPCR systems is divided into droplet type and separation type according to the method of dispensing the samples (Fig. 2) (Cao et al. 2017). Among them, the LOAA dPCR system (Optolane, Seongnam, Republic of Korea) released in 2020 is a separation type dPCR equipment. To evaluate the COVID-19 detection capability of Dr. PCR 20 K COVID-19 Detection kit (Cat no. DCM402-X, Optolane, Seongnam, Republic of Korea), we performed LOAA dPCR assay using the same 50 viral RNAs that we carried out qRT-PCR analysis on. The dPCR assay with Dr. PCR 20 K COVID-19 Detection kit was performed following to the manufacture's protocol (Fig. 1). Dr. PCR 20 K COVID-19 Detection kit includes primer sets and probes that is targeting the RdRp and E genes of SARS-CoV-2. The target viral genes amplification was conducted by following process: cDNA synthesis step of 10 min at 50 ℃, pre-denaturation step of 10 min at 95 ℃, followed by 45 cycles of denaturation step 10 s at 95 ℃, 60 s at 60 ℃ for annealing & extension step.

Data analysis

The qRT-PCR-based Allplex™ 2019-nCoV Assay kit results were measured as Ct values and dPCR-based Dr. PCR 20 K COVID-19 Detection kit results were measured as C/µL values. The Ct values of the Allplex™ 2019-nCoV Assay kit results were automatically determined for a diagnosis of COVID-19 using the Seegene Viewer

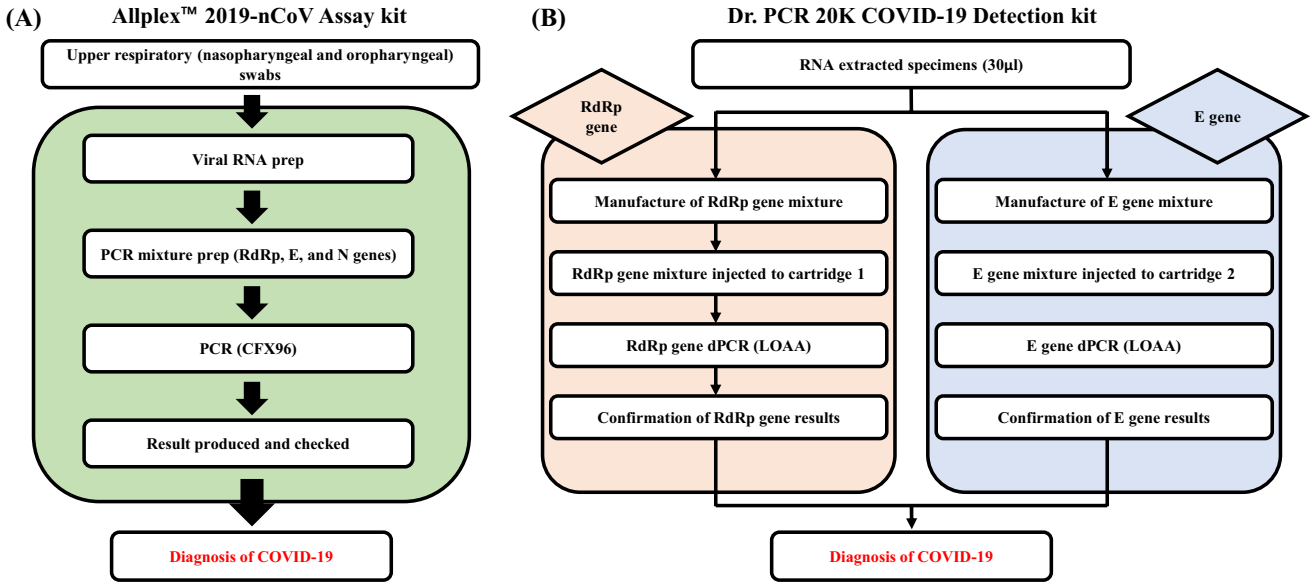

Fig. 1 The workflow of COVID-19 diagnosis using Allplex™ 2019-nCoV Assay and Dr. PCR 20 K COVID-19 Detection platform. **A** The Allplex™ 2019-nCoV Assay kit is a qRT-PCR-based method for COVID-19 detection that is diagnosed using the RdRp gene, E gene and N gene of SARS-CoV-2. **B** The Dr. PCR 20 K COVID-19 Detection kit is a dPCR-based product that diagnoses COVID-19 using the RdRp and E genes of the SARS-CoV-2

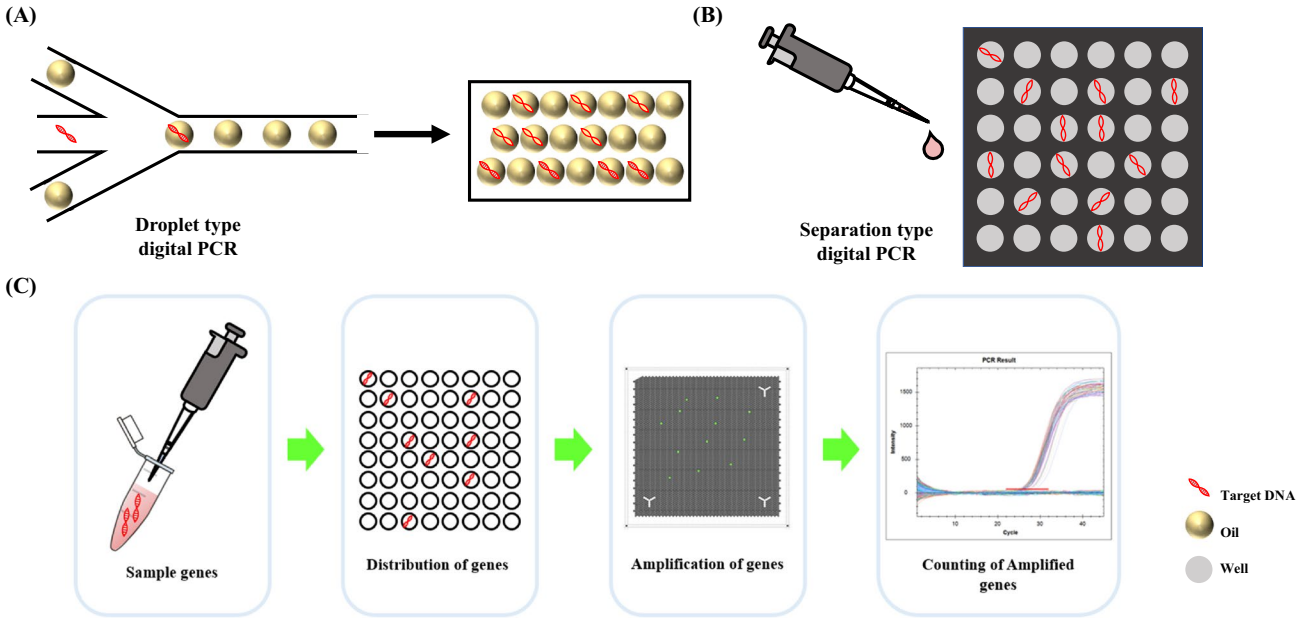

Fig. 2 Schematic diagram of the digital PCR assay. **A** In the droplet type dPCR method, the red and yellow molecules represent target DNA and droplet oil, respectively. **B** In the separation type digital PCR method, the gray circles represent the wells already divided in a semiconductive chip. **C** The workflow of the LOAA digital PCR assay

2019-nCoV software v1 (Freppel et al. 2020). The C/μL values of Dr. PCR 20 K COVID-19 detection kit results were automatically determined by LOAA Dr. PCR software 3.0.0 as a diagnosis of COVID-19. We calculated the COVID-19 diagnosis agreement of both kits using Cohen's Kappa analysis (Daly et al. 2019; Inaba et al. 2021). Kappa (K) value is interpreted as poor ($K < 0.00$), slight ($0.00 < K < 0.20$), fair ($0.20 < K < 0.40$), moderate ($0.41 < K < 0.60$), substantial ($0.61 < K < 0.80$), and almost perfect ($0.81 < K < 1.00$). In addition, we performed the dilutional linearity experiments by spike-in the high concentration of COVID-19 positive sample (Ct value near 10) to the negative sample to compare the detection capability according to the dilution factor (10^{-1} to 10^{-10}) of two kits (Kim et al. 2021).

Results and discussion

Evaluating COVID-19 diagnosis with the Allplex™ 2019-nCoV Assay kit

We selected the most popular qRT-PCR-based Allplex™ 2019-nCoV Assay kit in Korea to evaluate the efficiency of COVID-19 diagnosis. In addition, pharyngeal swab or sputum samples from 20 COVID-19 positive patients and 30 normal individuals (i.e., COVID-19 negative) were obtained from Seoul Clinical Laboratories (Gyeonggi, Republic of Korea) (Supplementary Table S1). The COVID-19 diagnosis result using the Allplex™ 2019-nCoV Assay kit is evaluated by the Ct value of qRT-PCR. As shown in Table 1, the potential outcome types of this kit are reported for a total of 9 CASE types (CASE-1 to -9) (Seegene 2021). When the Ct value of each internal control (IC), E gene, RdRp gene, or N gene is ≤ 40, it is indicated as "Detected" (+), and when it is > 40 or not applicable (N/A), it is indicated as "Not detected" (−). Depending on the (+) or (−) result of each gene, CASE-1 to -6 are determined to be "2019-nCoV positive" (Table 1). In the case of CASE-7, if IC is (+/−), E gene is (+), and RdRp gene and N gene are (−) determined as "Presumptive positive for 2019-nCoV", and re-experiment is required. If the IC is (+) and all viral genes are (−) in the sample, it is determined as "Negative" (CASE-8). Samples with all (−) results for IC (HEX) and all viral genes are determined as "Invalid" (CASE-9) (Supplementary information).

As a result of performing a COVID-19 diagnostic test using the qRT-PCR-based Allplex™ 2019-nCoV Assay kit with a total of 50 individual specimens, all ODP1 to ODP30 specimens extracted from normal individuals not infected with COVID-19 were "negative". In addition, all ODP31 to ODP50 specimens extracted from patients diagnosed with COVID-19 obtained "2019-nCoV positive" results (Table 2). Therefore, in the diagnostic evaluation of COVID-19 using the Allplex™ 2019-nCoV Assay kit, the false-negative and false-positive rates were 0. In addition, the diagnostic sensitivity and specificity were 1 (van Stralen et al. 2009). Although we obtained results with a relatively small number of specimens, we confirmed that the qRT-PCR assay showed very high sensitivity and specificity using the Allplex™ 2019-nCoV Assay kit (Supplementary Table S2).

Table 1 Result interpretation of qRT-PCR with Allplex™ 2019-nCoV Assay kit

Potential result type	Internal control[†] (HEX dye)	E gene (FAM dye)	RdRp gene (CalRed 610 dye)	N gene (Quasar 670 dye)	Auto-interpretation
CASE 1	+/−	+	+	+	2019-nCoV positive
CASE 2	+/−	+	−	+	2019-nCoV positive
CASE 3	+/−	+	+	−	
CASE 4	+/−	−	+	+	
CASE 5	+/−	−	−	+	
CASE 6	+/−	−	+	−	
CASE 7	+/−	+	−	−	Presumptive positive for 2019-nCoV
CASE 8	+	−	−	−	Negative
CASE 9	−	−	−	−	Invalid

[†]This internal control (IC) material verifies all steps of the analysis process, including sample extraction, reverse transcription and PCR to demonstrate proper specimen processing and test validity of each specimen

Table 2 Result interpretation of dPCR with Dr. PCR 20 K COVID-19 detection kit

Sample no.	Sample type	E gene	C(t)	RdRp gene	C(t)	N gene	C(t)	Internal control (IC)	C(t)	Automatic analysis
ODP01	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	23.54	Negative
ODP02	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.55	Negative
ODP03	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.57	Negative
ODP04	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.2	Negative
ODP05	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	23.65	Negative
ODP06	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.45	Negative
ODP07	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.47	Negative
ODP08	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.2	Negative
ODP09	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	23.6	Negative
ODP10	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.41	Negative
ODP11	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.28	Negative
ODP12	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.03	Negative
ODP13	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.32	Negative
ODP14	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	23.54	Negative
ODP15	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	23.98	Negative
ODP16	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	23.16	Negative
ODP17	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.2	Negative
ODP18	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.11	Negative
ODP19	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.74	Negative
ODP20	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.84	Negative
ODP21	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.16	Negative
ODP22	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.23	Negative
ODP23	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.27	Negative
ODP24	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.87	Negative
ODP25	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.89	Negative
ODP26	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.04	Negative
ODP27	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.72	Negative
ODP28	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	23.95	Negative
ODP29	Pharyngeal Swab	–	N/A	–	N/A	–	N/A	+	22.96	Negative
ODP30	Sputum	–	N/A	–	N/A	–	N/A	+	21.96	Negative
ODP31	Pharyngeal Swab	+	27.9	+	29.11	+	28.74	+	27.26	2019-nCoV detected
ODP32	Pharyngeal Swab	+	20.87	+	22.42	+	23.29	+	39.93	2019-nCoV detected
ODP33	Pharyngeal Swab	+	22.2	+	23.93	+	24.63	+	27.96	2019-nCoV detected
ODP34	Pharyngeal Swab	+	25.62	+	27.54	+	27.85	+	26.78	2019-nCoV detected
ODP35	Pharyngeal Swab	+	25.36	+	27.18	+	26.65	+	32.34	2019-nCoV detected
ODP36	Pharyngeal Swab	+	27.14	+	28.21	+	28.86	+	26.87	2019-nCoV detected
ODP37	Pharyngeal Swab	+	29.32	+	30.16	+	30.69	+	26.82	2019-nCoV detected
ODP38	Pharyngeal Swab	+	24.76	+	26.27	+	26.71	+	27.34	2019-nCoV detected
ODP39	Pharyngeal Swab	+	23.84	+	25.08	+	25.35	+	26.19	2019-nCoV detected
ODP40	Sputum	+	27.3	+	28.71	+	28.25	+	29.1	2019-nCoV detected
ODP41	Sputum	+	29.99	+	30.06	+	30.12	+	29.16	2019-nCoV detected
ODP42	Sputum	+	25.68	+	26.91	+	27.25	+	27.55	2019-nCoV detected
ODP43	Sputum	+	27.42	+	29.36	+	28.91	+	28.34	2019-nCoV detected
ODP44	Sputum	+	28.64	+	28.98	+	29.51	+	28.23	2019-nCoV detected
ODP45	Sputum	+	23.39	+	24.33	+	25.57	+	29.17	2019-nCoV detected
ODP46	Sputum	+	23.06	+	24.29	+	24.55	+	28.2	2019-nCoV detected
ODP47	Sputum	+	24.82	+	27.48	+	26.57	-	N/A	2019-nCoV detected
ODP48	Sputum	+	27.78	+	29.97	+	29	+	27.91	2019-nCoV detected
ODP49	Sputum	+	25.64	+	28	+	27.66	+	30.04	2019-nCoV detected

Table 2 (continued)

Sample no.	Sample type	E gene	C(t)	RdRp gene	C(t)	N gene	C(t)	Internal control (IC)	C(t)	Automatic analysis
ODP50	Sputum	+	26.46	+	29.37	+	28.06	-	N/A	2019-nCoV detected

Table 3 Result interpretation of dPCR with Dr. PCR 20 K COVID-19 detection kit

Type	Valid well	Green channel result		Red channel result	Result
		SARS-CoV-2			
		RdRp gene (FAM dye)	E gene (FAM dye)	PCR control (PCRC)[§] (Cy5 dye)	
Positive control	≥ 10,000	≥ 0.257	≥ 0.24	≥ 1	Valid
Negative control		< 0.257	< 0.24	≥ 1	Valid
CASE 1		≥ 0.257	≥ 0.24	≥ 1	COVID-19 positive
CASE 2		≥ 0.257	< 0.24	≥ 1	Undetermined[†]
CASE 3		< 0.257	< 0.24	≥ 1	COVID-19 negative
CASE 4		< 0.257	< 0.24	< 1	Invalid[‡]
CASE 5	< 10,000	–	–	–	Invalid[*]

*The change of the pattern of the whole well is confirmed and if the normal well is less than full, the experiment result for Digital PCR is not effective in the whole, so it is retested with a new cartridge after discarding the cartridge.

[§]Determination of nucleic acid amplification of each tube according to PCR reaction and determination of inhibition of PCR reaction by specimen.

[†]If E gene (beta coronavirus identification) is negative or RdRp gene (for new corona virus identification) is positive, it should be determined as undetermined.

[‡]The validity of the NTC and PC is determined based on the range defined by the manufacturer based on each C/uL number when the PCRC 'Valid' condition is satisfied.

Evaluating COVID-19 diagnosis with the Dr. PCR 20 K COVID-19 detection kit

We performed a COVID-19 diagnostic test using the LOAA dPCR (Optolane, Seongnam, Republic of Korea) equipment and the Dr. PCR 20 K COVD-19 Detection kit (Optolane, Seongnam, Republic of Korea) with the same samples evaluated above. As shown in Table 3, LOAA dPCR assay results are reported as CASE-1 to -5. To determine the diagnostic test efficacy, dPCR assay was performed using non-template control (NTC), positive control (PC), and PCR control (PCRC), respectively. The PC is composed of when RdRp gene and E gene. A valid decision can only be made when the number of valid wells is greater than 10,000. The experiment is valid when RdRp gene (FAM dye) has a value of ≥ 0.257 C/μL, E gene (FAM dye) ≥ 0.24 C/μL, and PCRC has a value of ≥ 1 C/μL (Table 3).

As a result of the dPCR assay using the Dr. PCR 20 K COVD-19 Detection kit, 30 out of a total of 50 specimens obtained SARS-CoV-2 negative results, and 20 specimens obtained SARS-CoV-2 positive results (Table 4). Similar to the Allplex™ 2019-nCoV Assay kit, the ODP1 to ODP30 specimens were extracted from normal individuals not infected with COVID-19, and all of the SARS-CoV-2 negative results were obtained. In addition, the ODP31 to ODP50 specimens were samples extracted from patients diagnosed with COVID-19, and all of them obtained SARS-CoV-2 positive results. Therefore, in the diagnostic evaluation of COVID-19 using the dPCR assay with Dr. PCR 20 K COVD-19 Detection kit, the false-negative and false-positive rates were 0. In addition, the diagnostic sensitivity and specificity were 1 (Supplementary Table S2).

Comparison of detection evaluation for COVID-19 between Allplex™ 2019-nCoV Assay kit and Dr. PCR 20 K COVID-19 detection kit

Comparison of diagnostic results between qRT-PCR-based Allplex™ 2019-nCoV Assay kit and dPCR-based Dr. PCR 20 K COVD-19 Detection kit showed the same results (Supplementary Table S3). Cohen's Kappa coefficient was tested to measure the reliability and agreement between the two results (Czodrowski 2014). The Kappa Coefficient has a value between 0 and 1, and the closer to 1, the more consistent the data to be compared. Therefore, when the detection results of the Allplex™ 2019-nCoV Assay kit and the Dr. PCR 20 K COVID-19 Detection kit were confirmed with

Table 4 The results of the dPCR method with Dr. PCR 20 K COVID-19 detection kit

Sample no.	Sample type	E gene			PCRC		RdRp gene			PCRC		Result
		Valid well	Positive	Conc.(C/μL)	Positive	Conc.(C/μL)	Valid well	Positive	Conc.(C/μL)	Positive	Conc.(C/μL)	
ODP01	Pharyngeal Swab	18,464	0	0	3001	312.23	18,286	0	0	3133	437.06	SARS-CoV-2 negative
ODP02	Pharyngeal Swab	17,539	0	0	2766	399.13	18,293	0	0	3066	426.62	SARS-CoV-2 negative
ODP03	Pharyngeal Swab	18,079	0	0	2793	390.26	16,879	0	0	3072	467.2	SARS-CoV-2 negative
ODP04	Pharyngeal Swab	17,467	0	0	2854	414.89	18,284	0	0	3075	428.23	SARS-CoV-2 negative
ODP05	Pharyngeal Swab	18,122	0	0	2742	381.99	17,932	0	0	2966	420.48	SARS-CoV-2 negative
ODP06	Pharyngeal Swab	17,091	0	0	2530	372.57	17,802	0	0	3029	433.74	SARS-CoV-2 negative
ODP07	Pharyngeal Swab	18,244	0	0	2916	405.01	16,046	0	0	2837	452.47	SARS-CoV-2 negative
ODP08	Pharyngeal Swab	16,831	0	0	2661	400.22	16,538	0	0	2992	464.12	SARS-CoV-2 negative
ODP09	Pharyngeal Swab	17,991	0	0	2878	405.39	16,523	0	0	2859	441.83	SARS-CoV-2 negative
ODP10	Pharyngeal Swab	18,403	0	0	3256	452.82	17,668	0	0	3145	455.86	SARS-CoV-2 negative
ODP11	Pharyngeal Swab	17,078	0	0	3016	451.9	15,856	0	0	2777	447.77	SARS-CoV-2 negative
ODP12	Pharyngeal Swab	17,463	0	0	5790	936.76	17,120	0	0	2925	435.72	SARS-CoV-2 negative
ODP13	Pharyngeal Swab	17,399	0	0	3022	443.68	17,700	0	0	3062	441.73	SARS-CoV-2 negative
ODP14	Pharyngeal Swab	15,660	0	0	2688	437.95	17,032	0	0	2995	449.76	SARS-CoV-2 negative
ODP15	Pharyngeal Swab	16,598	0	0	2835	435.58	17,357	0	0	3031	446.33	SARS-CoV-2 negative
ODP16	Pharyngeal Swab	18,316	0	0	3022	454.62	14,773	0	0	2615	453.06	SARS-CoV-2 negative
ODP17	Pharyngeal Swab	17,019	0	0	3022	454.62	13,174	0	0	2406	468.99	SARS-CoV-2 negative
ODP18	Pharyngeal Swab	16,320	0	0	2784	434.97	17,342	0	0	3102	458.32	SARS-CoV-2 negative
ODP19	Pharyngeal Swab	18,026	0	0	3200	454.49	16,617	0	0	2984	460.31	SARS-CoV-2 negative
ODP20	Pharyngeal Swab	17,397	0	0	3146	463.89	15,210	0	0	2790	471.27	SARS-CoV-2 negative
ODP21	Pharyngeal Swab	17,173	0	0	3173	475.07	14,643	0	0	2580	450.74	SARS-CoV-2 negative
ODP22	Pharyngeal Swab	17,754	0	0	3190	460.6	16,259	0	0	2703	422.83	SARS-CoV-2 negative
ODP23	Pharyngeal Swab	16,821	0	0	2812	425.41	17,416	0	0	3044	446.76	SARS-CoV-2 negative
ODP24	Pharyngeal Swab	17,641	0	0	3064	443.68	18,570	0	0	3370	465.7	SARS-CoV-2 negative
ODP25	Pharyngeal Swab	16,596	0	0	2839	436.31	17,263	0	0	3154	469.19	SARS-CoV-2 negative
ODP26	Pharyngeal Swab	18,883	0	0	3263	441.19	17,486	0	0	3067	448.5	SARS-CoV-2 negative
ODP27	Pharyngeal Swab	16,013	0	0	2693	428.22	16,521	0	0	3035	472.05	SARS-CoV-2 negative
ODP28	Pharyngeal Swab	16,013	0	0	2693	428.22	17,586	0	0	3378	496.04	SARS-CoV-2 negative
ODP29	Pharyngeal Swab	18,425	0	0	3377	470.84	17,282	0	0	3241	482.99	SARS-CoV-2 negative
ODP30	Sputum	15,900	0	0	969	146.23	17,663	0	0	260	34.49	SARS-CoV-2 negative
ODP31	Pharyngeal Swab	17,510	298	39.92	3065	447.5	16,686	133	18.61	3094	476.95	SARS-CoV-2 positive
ODP32	Pharyngeal Swab	18,630	14,757	3652.89	3235	443.56	18,627	13,423	2965.55	5501	813.99	SARS-CoV-2 positive
ODP33	Pharyngeal Swab	18,423	8967	1551.05	3240	449.82	16,560	6075	1062.89	3795	605.31	SARS-CoV-2 positive
ODP34	Pharyngeal Swab	18,458	1025	132.87	3261	452.09	18,087	746	97.95	3089	435.53	SARS-CoV-2 positive
ODP35	Pharyngeal Swab	18,521	1629	214.1	3142	432.33	16,137	928	137.74	2937	467.2	SARS-CoV-2 positive
ODP36	Pharyngeal Swab	16,780	361	50.58	3036	464.15	17,286	545	74.5	3125	463.73	SARS-CoV-2 positive

Table 4 (continued)

Sample no.	Sample type	E gene			PCRC		RdRp gene			PCRC		Result
		Valid well	Positive	Conc.(C/μL)	Positive	Conc.(C/μL)	Valid well	Positive	Conc.(C/μL)	Positive	Conc.(C/μL)	
ODP37	Pharyngeal Swab	17,949	103	13.38	3120	444.07	17,557	103	13.68	3113	453.89	SARS-CoV-2 positive
ODP38	Pharyngeal Swab	17,407	1930	273.3	3843	580.12	15,845	1421	218.51	4010	678.59	SARS-CoV-2 positive
ODP39	Pharyngeal Swab	17,506	5912	958.27	3131	458.26	16,528	4175	677.11	4339	708.19	SARS-CoV-2 positive
ODP40	Sputum	16,785	598	84.37	2963	451.68	16,404	389	55.81	2837	441.59	SARS-CoV-2 positive
ODP41	Sputum	15,402	149	22.61	2684	445.3	15,586	103	15.42	2539	413.52	SARS-CoV-2 positive
ODP42	Sputum	17,072	1843	265.67	3136	472.01	15,716	1013	154.95	2634	426.61	SARS-CoV-2 positive
ODP43	Sputum	17,685	671	89.95	2923	420.14	17,437	407	54.93	2838	413.12	SARS-CoV-2 positive
ODP44	Sputum	18,711	350	43.91	3102	421.54	16,892	260	36.07	2777	417.68	SARS-CoV-2 positive
ODP45	Sputum	17,926	8012	1377.45	2974	421.88	16,755	5834	995.37	3376	523.28	SARS-CoV-2 positive
ODP46	Sputum	18,273	9012	1580.49	3237	453.44	18,577	7074	1114.69	3696	515.9	SARS-CoV-2 positive
ODP47	Sputum	17,344	3466	518.47	2660	387.18	17,156	2587	380.12	2692	396.94	SARS-CoV-2 positive
ODP48	Sputum	16,512	390	55.59	2790	430.43	16,069	265	38.67	2625	414.79	SARS-CoV-2 positive
ODP49	Sputum	16,349	1375	204.31	2853	445.98	17,154	1161	162.98	2898	430.36	SARS-CoV-2 positive
ODP50	Sputum	18,158	615	92.46	3533	503.21	17,235	462	63.19	2566	374.9	SARS-CoV-2 positive

Cohen's Kappa Coefficient, the value was "Almost Perfect" with "1".

In order to evaluate the detection capability between the two kits according to the extraction efficiency of viral RNA, a high concentration of COVID-19 positive sample (Ct value near 10) was prepared. The high concentration of COVID-19 positive sample was spike-in treated with the negative patient sample, diluted, and then the dilutional linearity experiments were performed.

The correlation between the detection capability between the two kits was confirmed with the spike-in treatment of the negative patient sample on the high concentration of COVID-19 positive sample. The treated sample was diluted from 10^{-1} to 10^{-10} (Supplementary Table S4), and then the dilutional linearity experiments were performed. As shown in Table 5, the Allplex™ 2019-nCoV Assay kit result can confirm that COVID-19 was detected up to 10^{-6}. On the other hand, the results of the Dr. PCR 20 K COVID-19 Detection kit were capable of detecting COVID-19 up to 10^{-7} (Table 6). This indicates that the Dr. PCR 20 K COVID-19 detection kit was about 10 times higher than the Allplex™ 2019-nCoV Assay kit in the detection capability according to the extraction efficiency of viral RNA. In addition, the regression analysis results of each kit according to the dilution factor showed that the Allplex™ 2019-nCoV Assay kit had an average of $R^2 = 0.9972$, and the Dr. PCR 20 K COVID-19 Detection kit was $R^2 = 0.9948$, confirming very high detection reliability for both kits (Fig. 3). In summary, we represent that the diagnosis of COVID-19 using dPCR is advantageous compared to qRT-PCR in samples infected with infinitesimal amounts of SARS-CoV-2 in the early stages of infection.

Correlation analysis of qRT-PCR and dPCR for RdRp gene and E gene

We performed correlation analysis on the detection levels of RdRp gene and E gene from diluted viral RNA extracted from qRT-PCR-based Allplex™ 2019-nCoV Assay kit and dPCR- based Dr. PCR 20 K COVID-19 Detection kit. As shown in Fig. 4, R^2 was confirmed through regression analysis in which qRT-PCR was the Ct value and dPCR was the C/μL value for each dilution factor extract. As a result, the R^2 value was 0.994 in the RdRp gene and 0.9964 in the E gene, which means that the two experiments have a very high correlation for each gene.

Conclusions

In this study, Optolane's Dr. PCR 20 K COVID-19 Detection kit was compared with Seegene's Allplex™ 2019-nCoV Assay kit, a product that has already received the US FDA

Table 5 qRT-PCR results with Allplex™ 2019-nCoV Assay kit of SARS-CoV-2 genes according to dilution factor

Dilution factor	E gene	RdRp gene	N gene	PCRC
Dilute① (10^{-1})	16.07	17.16	18.76	N/A
Dilute② (10^{-2})	18.83	20.75	22.10	26.22
Dilute③ (10^{-3})	22.67	24.23	25.41	24.31
Dilute④ (10^{-4})	26.02	27.84	28.46	25.54
Dilute⑤ (10^{-5})	28.41	30.64	30.89	24.79
Dilute⑥ (10^{-6})	32.58	35.06	34.63	25.67
Dilute⑦ (10^{-7})	N/A	N/A	37.86	25.08
Dilute⑧ (10^{-8})	N/A	N/A	37.98	24.90
Dilute⑨ (10^{-9})	N/A	N/A	N/A	24.56
Dilute⑩(10^{-10})	N/A	N/A	N/A	24.83

EUA and the Korea MFDS EUA approval for clinical efficacy evaluation. As a result of testing the same COVID-19 diagnostic positive and negative samples, it was confirmed that the results of the dPCR-based Dr. PCR 20 K COVID-19 Detection kit and the results of qRT-PCR-based Allplex™ 2019-nCoV Assay kit were consistent. That is, it was confirmed that these two kits had high sensitivity and specificity in the evaluation of the capability to detect COVID-19 using SARS-CoV-2 viral nucleic acids extracted from sputum and nasopharyngeal smears. However, according to the dilutional linearity experiments results, the Dr. PCR 20 K COVID-19 Detection kit confirmed about ten times higher detection capability at a lower viral load than the Allplex™ 2019-nCoV Assay kit. Taken together, we confirmed that the detection of COVID-19 using LOAA dPCR is advantageous

Table 6 dPCR results with Dr. PCR 20 K COVID 19 Detection kit of SARS-CoV-2 genes according to dilution factor

Dilution factor	RdRp gene	PCRC	E gene	PCRC
Dilute① (10^{-1})	Above linear range	778.15	Above linear range	414.67
Dilute② (10^{-2})	9977.12	905.83	20000.72	455.95
Dilute③ (10^{-3})	933.68	490.65	1644.83	460.05
Dilute④ (10^{-4})	95.13	473.86	163.81	439.83
Dilute⑤ (10^{-5})	9.10	433.92	16.12	433.63
Dilute⑥ (10^{-6})	1.09	454.22	1.09	913.64
Dilute⑦ (10^{-7})	0.26	445.99	0.28	399.05
Dilute⑧ (10^{-8})	0.00	426.27	0.00	455.74
Dilute⑨ (10^{-9})	0.00	421.84	0.00	413.96
Dilute⑩(10^{-10})	0.00	451.08	0.00	460.07

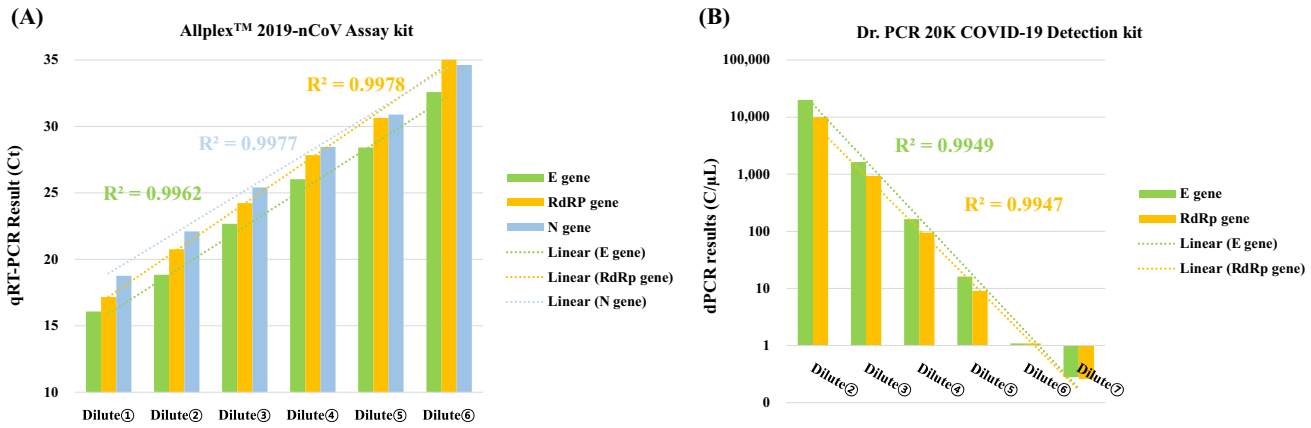

Fig. 3 The dilutional linearity experiments for the detection capability evaluation of each kit according to the dilution factor. **A** The dilutional linearity experiments using a qRT-PCR method with the Allplex™ 2019-nCoV Assay kit according to the dilution factor of SARS-CoV-2. **B** The dilutional linearity experiments using a dPCR method with the Dr. PCR 20 K COVID-19 Detection kit according to the dilution factor of SARS-CoV-2

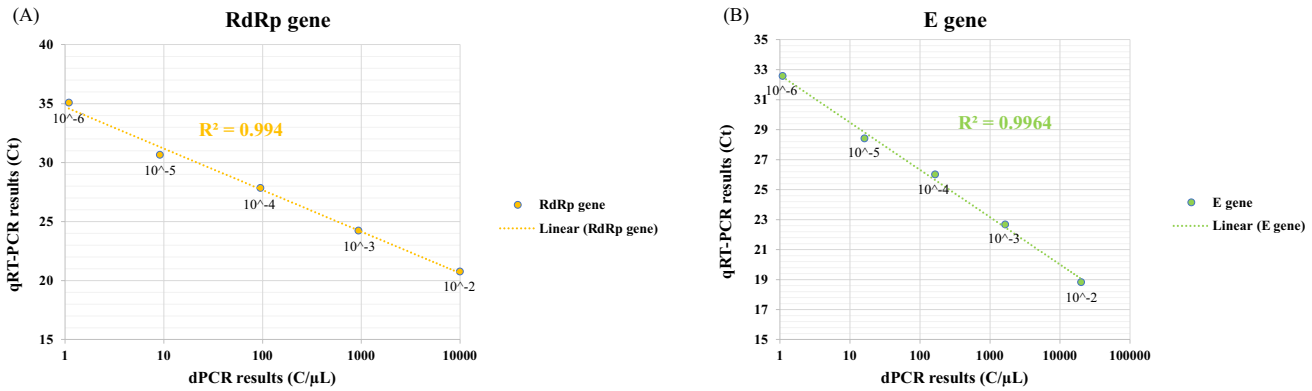

Fig. 4 Correlation analysis between dPCR and qRT-PCR results of SARS-CoV-2 genes according to dilution factors. **A** shows the correlation between dPCR and qRT-PCR results according to the dilution factor of the RdRp gene. **B** Shows the correlation between dPCR and qRT-PCR results according to the dilution factor of the E gene

compared to qRT-PCR in terms of relatively lower sample concentration, the small size of equipment, and low equipment price. Therefore, we propose that the use of LOAA dPCR can be expected for COVID-19 point-of-care testing (POCT) in confined spaces and where the immediate diagnosis is required.

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Declarations

Conflict of interest Cherl-Joon Lee, Wonseok Shin, Seyoung Mun, Minjae Yu, Young-Bong Choi, Dong Hee Kim, 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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