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Original Article

# Evaluation of the QIAstat-Dx Respiratory SARS-CoV-2 panel, a rapid multiplex PCR method for the diagnosis of COVID-19

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ABSTRACT

*Introduction:* Rapid, simple, and accurate methods are required to diagnose coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This study aimed to evaluate the performance of the QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAstat-SARS-CoV-2), a rapid multiplex PCR assay for SARS-CoV-2 detection.

*Methods:* Nasopharyngeal swabs (NPS) that were obtained from patients with COVID-19 who were diagnosed at the National Center for Global Health and Medicine were used in this study. When the NPS samples were found to be negative for SARS-CoV-2 after treatment, they were used as negative samples. We evaluated the performance of the QIAstat-SARS-CoV-2 comparing SARS-CoV-2 detection with the National Institute of Infectious Diseases in Japan-recommended real-time polymerase chain reaction (RT-PCR) method (NIID-RT-PCR).

*Results:* In total, 45 NPS samples were analyzed. The proportion of overall agreement between QIAstat-SARS-CoV-2 and NIID-RT-PCR on 45 samples was 91.0% with a sensitivity of 84.0% (21/25), specificity at 100% (20/20), negative predictive value at 83.3% (20/24), and positive predictive value at 100% (21/21). There were no patients with co-infections with pathogens other than SARS-CoV-2.

*Conclusions:* QIAstat-SARS-CoV-2 showed a high agreement in comparison with the NIID-RT-PCR for the detection of SARS-CoV-2. The QIAstat-SARS-CoV-2 also provided a rapid and accurate diagnosis for COVID-19, even when the concurrent detection of other respiratory pathogens was desired, and therefore, has the potential to direct appropriate therapy and infection control precautions.

## 1. Introduction

Coronavirus disease (COVID-19), which was caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, was first reported in China at the end of 2019, and the World Health Organization declared it a Public Health Emergency of International Concern (PHEIC) on January 31, 2020. This pandemic has expanded, even after the PHEIC declaration, and 240 million cases including 4.9 million deaths have been reported worldwide through to October 20, 2021 [1].

In Japan, there has been a continuous and acute increase in COVID-19 cases, starting with the Japanese returnees from Wuhan, the Diamond Princess cruise, and community-acquired infections [2–4]. The COVID-19 pandemic is a major problem in terms of public health and socioeconomic activities. Infection prevention and the control of the spread of COVID-19 is an urgent issue; therefore, soon after the initial outbreak, a real-time polymerase chain reaction (RT-PCR) method for the detection of SARS-CoV-2 was developed by the National Institute of Infectious Diseases (NIID) in Japan and distributed to municipal and prefectural institutes, health centers, and quarantine stations for national surveillance [5,6]. However, a variety of respiratory pathogens,

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including viruses, bacteria, and fungi, can also cause respiratory tract infections, resulting in very similar clinical symptoms. Thus, the ability to diagnose respiratory tract infections rapidly and accurately, is important to ensure the administration of appropriate antimicrobial therapy and for the effective implementation of infection prevention and control measures. In fact, the United States reported an increase in the use of macrolides during the first wave of COVID-19 [7].

The QIAstat-Dx Analyzer (QIAGEN) and QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAGEN) are diagnostic methods that were authorized by the Food and Drug Administration (FDA) under an Emergency Use Authorization as of October 2021, although they are not authorized for use in Japan [8]. The QIAstat-Dx Analyzer is a fully automatic diagnostic device that uses a multiplexed RT-quantitative PCR test for the detection of the 21 respiratory viruses and bacteria including SARS-CoV-2 (*Mycoplasma pneumoniae, Clamydophila pneumoniae, Bordetella pertussis,* Influenza A, Influenza A subtype H1N1/2009, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Coronavirus 229 E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Adenovirus, Respiratory Syncytial Virus A/B, Human Metapneumovirus A/B, Rhinovirus/Enterovirus, and SARS-CoV-2 [9]. As for a few other rapid PCR assays such as FilmArray RP2.1 (bioMérieux, BioFire) and Allplex SARS-CoV-2/FluA/FluB/RSV Assay (Seegene), the results are provided in approximately 70 min, compared to the labor-intensive three to 4 h of the NIID-recommended real-time RT-PCR method (NIID-RT-PCR).

Here, we report an evaluation of the performance of the QIAstat-Dx Respiratory SARS-CoV-2 Panel (QIAstat-SARS-CoV-2) for SARS-CoV-2 detection using clinical samples that had been submitted for the diagnosis of COVID-19. The performance was compared to that of the NIID-RT-PCR that is used as a routine diagnostic tool in Japan [6].

## 2. Materials and methods

### 2.1. Ethics

In this study, we used residual specimens that were collected in clinical settings. Although written consent was not obtained for this study, information about this study was made available on the National Center for Global Health and Medicine website. Patients could, therefore, have declined to participate in the study. Opt-out consent was approved for this study by the Ethics Committee of the National Center for Global Health and Medicine (Approval No.: NCGM-G0003527-00).

### 2.2. Study patients and samples

This study was a single-center, retrospective observational study of patients diagnosed with COVID-19 who were admitted to the National Center for Global Health and Medicine (Tokyo, Japan) between January and May 30, 2020. Patients who were aged ≤18 years were excluded. Nasopharyngeal swabs (NPS) samples that were obtained from patients with or suspected of having COVID-19 were placed in Universal Transport Medium (UTM) (COPAN Diagnostic Inc., USA). SARS-CoV-2 infection was diagnosed using the NIID-RT-PCR according to the "Manual for the Detection of Pathogen 2019-nCoV" issued by the NIID in Japan [6,10]. The NPS samples were collected and stored at −80 °C at the same time from the patients over a total of 5–6 times at prescribed time intervals. When the NPS samples were tested as negative for the SARS-CoV-2 after treatment, the residual samples were used as negative samples. All of samples were taken by trained physicians based on the manual of sample collection in the institution.

### 2.3. Patient demographics and initial symptoms

The patients' medical records were reviewed to collect the following information: basic information of the individuals (sex, age, and underlying diseases), habitus (smoking and drinking), the severity of COVID-19, days from onset to diagnosis, and days from sample collection. The illness severity of patients with COVID-19 at the time of hospitalization was stratified into the following four categories: mild (Sp $O_2$ > 96% and no pneumoniae), moderate I (Sp $O_2$ 93–96% with pneumoniae), moderate II (Sp $O_2$ <93% with pneumoniae), and severe (required intensive care at ICU) as specified in the manual for the clinical guideline for COVID-19 issued by the Japanese Ministry of Health, Labor, and Welfare [11].

### 2.4. NIID-RT-PCR using clinical samples as reference method

A NIID-RT-PCR was performed using NPS samples for the detection and quantitation of SARS-CoV-2. Viral RNA was extracted from 140 μL of the residual NPS samples using QIAamp Viral RNA kits (QIAGEN). For each sample, assays targeting the N gene (N2 set) were carried out as described previously [6,12]. By using a quantitative synthetic SARS-CoV-2 RNA control (AcroMetrix Coronavirus 2019 [COVID-19] RNA Control: Thermo Fisher Scientific), the copy numbers of SARS-CoV-2 RNA in each sample were determined if the SARS-CoV-2 RNA was detected. All the assay were performed in duplicate or triplicate.

### 2.5. QIAstat-SARS-CoV-2 using clinical samples

QIAstat-SARS-CoV-2 was performed according to the manufacturer's instructions [8,9]. Briefly, 300 μL of NPS sample was loaded manually into the single-use QIAstat-Dx Respiratory SARS-CoV-2 Panel cartridge (QIAGEN) and set on the QIAstat-Dx Analyzer (QIAGEN). The presence of SARS-CoV-2 and 21 other respiratory pathogens were determined, and cycle threshold (Ct) values were obtained if the SARS-CoV-2 and/or other respiratory pathogens were detected. The QIAstat-SARS-CoV-2 results were compared against those of the NIID-RT-PCR.

### 2.6. Data analyses

For the identification of the SARS-CoV-2 infection, the sensitivity, specificity, positive predictive value, and negative predictive value of the QIAstat-SARS-CoV-2 were evaluated and compared to the NIID-RT-PCR. The presence of co-infections with pathogens other than SARS-CoV-2 that could be assessed by the QIAstat-SARS-CoV-2 was also assessed.

## 3. Results

### 3.1. Detection and quantitation of SARS-CoV-2 using clinical samples by reference method

In total, 45 NPS samples were used for the detection and quantitation of SARS-CoV-2 using the NIID-RT-PCR. Among 30 residual NPS samples from SARS-CoV-2 positive clinical patients, only 23 samples were confirmed to be positive for SARS-CoV-2; 10 samples had over 2500 genome copy equivalent (GCE) per reaction, 12 samples had within 5–2500 GCE per reaction, and one sample has less than 5 GCE per reaction. However, in this study seven samples (sample IDs 4, 14, 15, 18, 19, 24 and 27) were negative (Table 1). In addition, the 15 residual NPS samples that were tested as negative for SARS-CoV-2 in the clinical setting were assayed. Fourteen samples among them were confirmed as negative, however, one sample (sample ID 40) was positive. As the copy numbers of SARS-CoV-2 in the sample IDs 28 and 40 were low, we further performed the NIID-RT-PCR twice using the residual samples, and confirmed that the copy numbers of SARS-CoV-2 in these two samples were below the detection limit (<5 GCE per reaction, Tables 1 and 2). There seems to be a negative correlation between the copy numbers of SARS-CoV-2 and the days from onset to sample collection although it does not reach to the statistical significance. SARS-CoV-2

**Table 1**
Results of NIID in Japan-recommended real-time PCR method (NIID-RT-PCR) and QIAstat-Dx Respiratory SARS-CoV-2 Panel method (QIAstat-SARS-CoV-2) using the samples that were positive for SARS-CoV-2 in the clinical setting, $n = 30$.

| Serial no. | Age | Sex | Underlying diseases | Habitus | Severity[a] | Days from onset to diagnosis | Days from onset to sample collection | Co-infection | NIID-RT-PCR | | | QIAstat-SARS-CoV-2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Viral load, N2 set (/5 μL) | Ct value | Judgement | |
| 1 | 42 | Male | HTN, Hepatitis B, Syphilis | Drinking, Smorking | Severe | 4 | 9 | None | >2500 | 24.7 | Positive | Positive |
| 2 | 78 | Male | HTN, DM, HL, Asthma, HU | Drinking, Smorking | Severe | 11 | 31 | None | 212 | 32.1 | Positive | Positive |
| 3 | 36 | Male | Depression | None | Severe | 6 | 7 | None | >2500 | 24.9 | Positive | Positive |
| 4 | 53 | Male | HTN, DM, Hepatitis C | Smorking | Severe | 10 | 19 | None | UND | UND | Negative | Negative |
| 5 | 50 | Male | HU, Hepatitis C | Drinking, Smorking | Severe | 10 | 20 | None | 1456 | 29.4 | Positive | Positive |
| 6 | 79 | Male | HTN, HL | None | Severe | 9 | 10 | None | >2500 | 23.0 | Positive | Positive |
| 7 | 68 | Female | SAH, DM, HTN, CKD | Drinking, Smorking | Severe | 3 | 14 | None | 946 | 30.0 | Positive | Positive |
| 8 | 36 | Female | None | Drinking, Smorking | Moderate II | 6 | 6 | None | 1832 | 29.2 | Positive | Positive |
| 9 | 51 | Male | HTN | Drinking, Smorking | Moderate II | 4 | 8 | None | 1316 | 29.6 | Positive | Positive |
| 10 | 71 | Male | DM, Asthma, HTN, HL | Drinking, Smorking | Moderate II | 8 | 8 | None | >2500 | 27.0 | Positive | Positive |
| 11 | 79 | Female | HTN, DM, RA | Smorking | Moderate II | 4 | 6 | None | >2500 | 28.5 | Positive | Positive |
| 12 | 68 | Male | None | Drinking, Smorking | Moderate II | 9 | 9 | None | 8 | 37.3 | Positive | Negative |
| 13 | 55 | Male | Depression, HTN, Fatty Liver | Drinking, Smorking | Moderate II | 11 | 12 | None | 137 | 32.4 | Positive | Positive |
| 14 | 74 | Male | Hepatitis A | Drinking, Smorking | Moderate II | 13 | 16 | None | UND | UND | Negative | Negative |
| 15 | 70 | Male | HTN, HU | Drinking | Moderate II | 12 | 17 | None | i) UND<br>ii) 146 | i) UND<br>ii) 34.2 | Positive | Positive |
| 16 | 61 | Male | HL | Drinking, Smorking | Moderate I | 4 | 6 | None | >2500 | 18.6 | Positive | Positive |
| 17 | 38 | Male | None | Drinking, Smorking | Moderate I | 6 | 9 | None | >2500 | 27.3 | Positive | Positive |
| 18 | 79 | Male | HD due to IgA nephropathy, Stroke | Drinking, Smorking | Moderate I | 5 | 13 | None | UND | UND | Negative | Negative |
| 19 | 62 | Male | HTN, HL | Drinking, Smorking | Moderate I | 11 | 18 | None | UND | UND | Negative | Negative |
| 20 | 46 | Female | Breast cancer | Drinking | Moderate I | 6 | 6 | None | >2500 | 24.3 | Positive | Positive |
| 21 | 47 | Female | None | Drinking, Smorking | Moderate I | 4 | 8 | None | 23 | 35.2 | Positive | Negative |
| 22 | 50 | Female | RA, Asthma, Depression | None | Moderate I | 5 | 8 | None | 5 | 36.7 | Positive | Negative |
| 23 | 53 | Male | HTN, HL | Drinking | Moderate I | 1 | 7 | None | 1825 | 28.8 | Positive | Positive |
| 24 | 43 | Male | Epilepsy | None | Moderate I | 6 | 9 | None | UND | UND | Negative | Negative |
| 25 | 50 | Male | None | None | Moderate I | 0 | 4 | None | >2500 | 19.7 | Positive | Positive |
| 26 | 26 | Male | None | Drinking, Smorking | Mild | 5 | 5 | None | >2500 | 26.9 | Positive | Positive |
| 27 | 28 | Female | None | Drinking, Smorking | Mild | 14 | 20 | None | UND | UND | Negative | Negative |
| 28 | 23 | Female | Pregnancy | Smorking | Mild | 6 | 16 | None | i) < 5<br>ii) < 5<br>iii) < 5 | i) 37.6<br>ii) 37.6<br>iii) 37.8 | Probably positive | Negative |
| 29 | 46 | Male | HTN, HL | Drinking | Mild | 2 | 5 | None | 31 | 34.3 | Positive | Positive |
| 30 | 73 | Female | DM | None | Mild | 9 | 11 | None | 340 | 31.0 | Positive | Positive |

NIID, National Institute of Infectious Diseases; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ct, cycle threshold; UND, undetectable; HTN, hypertension; DM, diabetes mellitus; HL, hyperlipidemia; HU, hyperuricemia; CKD, chronic kidney disease; SAH, Subarachnoid hemorrhage; RA, rheumatoid arthritis; HD hemodialysis.

[a] The illness severity of patients with COVID-19 at the time of hospitalization was stratified into the following four categories: mild (Sp O2 > 96% and no pneumoniae), moderate I (Sp O2 93–96% with pneumoniae), moderate II (Sp O2 <93% with pneumoniae), and severe (required intensive care at ICU).

**Table 2**
Results of NIID in Japan-recommended real-time PCR method (NIID-RT-PCR) and QIAstat-Dx Respiratory SARS-CoV-2 Panel method (QIAstat-SARS-CoV-2) using the samples that were negative for SARS-CoV-2 in the clinical setting, $n = 15$.

| Serial no. | Age | Sex | Underlying diseases | Habitus | Days from onset to diagnosis | Days from onset to sample collection | Co-infection | NIID-RT-PCR | | | QIAstat-SARS-CoV-2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Viral load, N2 set (/5 μL) | Ct value | Judgement | |
| 31 | 42 | Male | HTN, Hepatitis B, Syphilis | Drinking, Smorking | 8 | 26 | None | UND | UND | Negative | Negative |
| 32 | 78 | Male | HTN, DM, HL, Asthma, HU | Drinking, Smorking | 11 | 34 | None | UND | UND | Negative | Negative |
| 33 | 53 | Male | HTN, DM, Hepatitis C | Smorking | 10 | 27 | None | UND | UND | Negative | Negative |
| 34 | 79 | Male | HTN, HL | None | 9 | 21 | None | UND | UND | Negative | Negative |
| 35 | 68 | Female | SAH, DM, HTN, CKD | Drinking, Smorking | 3 | 22 | None | UND | UND | Negative | Negative |
| 36 | 71 | Male | DM, Asthma, HTN, HL | Drinking, Smorking | 8 | 16 | None | UND | UND | Negative | Negative |
| 37 | 79 | Female | HTN, DM, RA | Smorking | 0 | 0 | None | UND | UND | Negative | Negative |
| 38 | 55 | Male | Depression, HTN, Fatty Liver | Drinking, Smorking | 4 | 11 | None | UND | UND | Negative | Negative |
| 39 | 74 | Male | Hepatitis A | Drinking, Smorking | 11 | 15 | None | UND | UND | Negative | Negative |
| 40 | 79 | Male | HD due to IgA nephropathy, Stroke | Drinking, Smorking | 13 | 10 | None | i) < 5<br>ii) < 5<br>iii) < 5 | i) 37.3<br>ii) 39.0<br>iii) 37.5 | Probably positive | Positive |
| 41 | 47 | Female | None | Drinking, Smorking | 5 | 15 | None | UND | UND | Negative | Negative |
| 42 | 50 | Female | RA, Asthma, Depression | None | 4 | 11 | None | UND | UND | Negative | Negative |
| 43 | 53 | Male | HTN, HL | Drinking | 5 | 12 | None | UND | UND | Negative | Negative |
| 44 | 28 | Female | None | Drinking, Smorking | 1 | 15 | None | UND | UND | Negative | Negative |
| 45 | 73 | Female | DM | None | 14 | 22 | None | UND | UND | Negative | Negative |

NIID, National Institute of Infectious Disease; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ct, cycle threshold; UND, undetectable; HTN, hypertension; DM, diabetes mellitus; HL, hyperlipidemia; HU, hyperuricemia; CKD, chronic kidney disease; SAH, Subarachnoid hemorrhage; RA, rheumatoid arthritis; HD hemodialysis.

were not detected almost in the samples collected more than 11 days post symptom onset (Table 1).

### 3.2. Performance of the QIAstat-SARS-CoV-2, compared to the reference method

The presence of SARS-CoV-2 in the 45 NPS samples were determined using the QIAstat-SARS-CoV-2. There were no samples that were positive for multiple pathogens including SARS-CoV-2. Among the 30 redNPS samples that were positive for SARS-CoV-2 in the clinical setting, 20 samples were found to be positive for SARS-CoV-2, and 10 samples negative, using the QIAstat-SARS-CoV-2 (Table 1). Among the 23 NPS samples that were found to be positive for SARS-CoV-2 using the NIID-RT-PCR, 19 samples were identified as positive using the QIAstat-SARS-CoV-2. However, four samples (sample IDs 12, 21, 22 and 28) were identified as negative using the QIAstat-SARS-CoV-2, in which the copy number of SARS-CoV-2 per reaction was ranged around 10–20 copies. In addition, among the six NPS samples that were found to be negative for SARS-CoV-2 using the NIID-RT-PCR, all were found to be negative using the QIAstat-SARS-CoV-2 (Table 1). There was one sample (ID 15) that showed conflicting results, i.e. the sample is found to be negative using the NIID-RT-PCR, positive using the QIAstat-SARS-CoV-2. We analyzed this sample again using the residual sample, and was found to be positive using both the NIID-RT-PCR (146 GCE per reaction) and the QIAstat-SARS-CoV-2 (Ct value: 33.4) (Table 1).

Next, we assayed 15 NPS samples that were negative for SARS-CoV-2 in the clinical setting using the QIAstat-SARS-CoV-2. As shown in Table 2, 14 samples among them were negative, and one sample positive for SARS-CoV-2. Among the 14 NPS samples that were found to be negative for SARS-CoV-2 using the NIID-RT-PCR, all the samples were found to be negative using the QIAstat-SARS-CoV-2. In the remaining sample (ID 40), both NIID-RT-PCR and QIAstat-SARS-CoV-2 produced consistent results (<5 GCE per reaction and Ct value: 33.3, respectively).

Finally, we compared the performance of the QIAstat-SARS-CoV-2 with the NIID-RT-PCR. When plotting individual Ct-values obtained with the QIAstat-SARS-CoV-2 against those for each copy number of SARS-CoV-2 as determined using the NIID-RT-PCR, a proportional relationship was observed over the whole range of experimental Ct-values (Fig. 1). Table 3 shows the performance of the QIAstat-Dx Respiratory SARS-CoV-2 Panel, compared to NIID-RT-PCR in all 45 samples. It showed that the sensitivity, specificity, positive predictive value, and negative predictive value of the QIAstat-SARS-CoV-2 were 84.0%, 100.0%, 100.0%, and 83.3%, respectively. There were four samples (IDs 12, 21, 22, and 28) with conflicting results that were obtained using the NIID-RT-PCR and the QIAstat-SARS-CoV-2 (i.e. the NIID-RT-PCR results were positive, but the QIAstat-SARS-CoV-2 results were negative for SARS-CoV-2); all with low copy numbers of SARS-CoV-2 (from <5 to 23 GCE per reaction).

## 4. Discussion

For the first time in Japan, the performance of the QIAstat-SARS-CoV-2 was evaluated. The QIAstat-SARS-CoV-2 workflow is very simple. Compared to other rapid PCR assay, QIAstat-SARS-CoV-2 involves only one step to load the NPS resuspended in transport medium through the liquid port or to insert the NPS directly into the cartridge without additional manipulation. This lessens manipulation and may help to reduce contamination. Compared to the NIID-RT-PCR, the sensitivity,

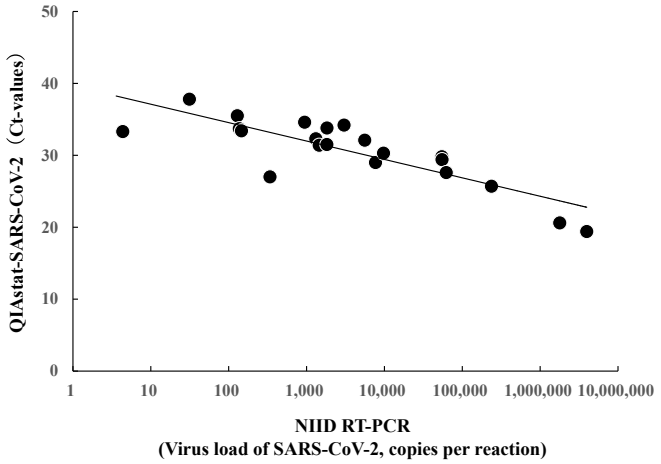


**Fig. 1.** The relationship between the cycle threshold (Ct)-values obtained with the QIAstat-Dx Respiratory SARS-CoV-2 Panel method (QIAstat-SARS-CoV-2) and those for each copy number of SARS-CoV-2 determined using the National Institute of Infectious Diseases in Japan-recommended real-time RT-PCR method (NIID-RT-PCR)|
Individual Ct-values obtained with QIAstat-SARS-CoV-2 (vertical axis) and those for each copy number of SARS-CoV-2 in the N2-gene by the NIID-RT-PCR were plotted in vertical and horizontal axes, respectively. A proportional relationship was observed over the whole range of experimental Ct-values.

**Table 3**
Performance of the QIAstat-Dx Respiratory SARS-CoV-2 Panel method (QIAstat-SARS-CoV-2), compared to NIID in Japan-recommended real-time PCR method (NIID-RT-PCR), $n = 45$.

| | | NIID-RT-PCR | | | Predictive values |
|---|---|---|---|---|---|
| | | Positive | Negative | Total | |
| QIAstat-SARS-CoV-2 | Positive | 21 | 0 | 21 | 100.0% |
| | Negative | 4 | 20 | 24 | 83.3% |
| | Total | 25 | 20 | 45 | |
| Sensitivities/specificities | | 84.0% Sensitivity | 100.0% Specificity | | |

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NIID, National Institute of Infectious Diseases; PCR, polymerase chain reaction.

specificity, positive predictive value, and negative predictive value of the QIAstat-SARS-CoV-2 were high (84.0%, 100.0%, 100.0%, and 83.3%, respectively). There were no patients with co-infections with pathogens other than SARS-CoV-2. The advantages of the QIAstat-SARS-CoV-2 were considered to be its relatively high sensitivity and specificity. A previous report from France that used 69 clinical samples showed a high sensitivity (100% [40/40]) and specificity (93% [27/29]). No cross-reactions were encountered for any other respiratory viruses or bacteria in that report [12]. The sensitivity and specificity of the QIAstat-Dx Respiratory SARS-CoV-2 Panel were higher than the sensitivity (70.7%) and specificity (96.0%) of the rapid antigen test (Roche, Switzerland), one of the most commonly used methods to diagnose COVID-19 in Japan [13]. A significant advantage of the system is that it allows the user to obtain a Ct-value for each detected pathogens and the internal control. These values, while not truly quantitative, do allow semiquantitative assessment of target amounts as shown in Fig. 1, which can be useful in troubleshooting or other quality control measures. Another advantage was that it was more suitable for measuring heterogeneous NPS specimens because of the larger volume of samples (300 μL) used compared to the NIID-RT-PCR (140 μL) [9,10]. Of all 45 samples, one sample (ID 15) with the positive result of the QIAstat-SARS-CoV-2 had no virus detected in the first evaluation using the NIID-RT-PCR; however, the virus was detected in the re-evaluation. The amount of SARS-CoV-2 RNA extracted from the NPS samples may not have been consistent as some parts of the NPS samples had high viscosities, although attention was paid to the preparation of the SARS-CoV-2 RNA solution, such as using parts with low viscosity. It is expected that if samples with non-uniform viscosities such as the NPS are used for measurement, false-positive or false-negative results may be reduced. In addition, the simple operation, short measurement time (approximately 70 min) compared to NIID-RT-PCR (3–4 h), and the ability to differentiate 21 similar respiratory diseases simultaneously, which were not detected in this study, were also considered as advantages [9].

Nevertheless, we also identified disadvantages of the QIAstat-SARS-CoV-2. In our study, there were four samples (IDs 12, 21, 22, and 28) in which there were conflicting results between those obtained using the NIID-RT-PCR and the QIAstat-SARS-CoV-2 (the NIID-RT-PCR was positive, but the QIAstat-SARS-CoV-2 was negative). The copy numbers of SARS-CoV-2 in these four samples were low (from <5 to 23 GCE per reaction), so it is possible that these four samples were true positive but resulted in an evaluation as negative by QIAstat-SARS-CoV-2 due to being below the sensitivity level of the assay [8,9]. The reason for the low copy of virus in these false-negative samples was the relatively long days from onset to sample collection, although statistical analysis was not performed due to the small number of these samples. Although the sensitivity of the QIAstat-SARS-CoV-2 was not high, it was considered to be sufficient for actual clinical use [12]. The other disadvantage was that the QIAstat-SARS-CoV-2 could only evaluate one sample at a time; therefore, multiple samples could not be evaluated at the same time [8,9]. However, since each operation takes only approximately 70 min, this disadvantage can be compensated for by repeating the test [8,9].

### 4.1. Limitation

This study had several limitations. First, the number of samples used in this study was small (30 positive samples and 15 negative samples in a clinical setting). However, it met the criteria stated in the “Performance evaluation of a genetic testing method for SARS-CoV-2" issued by the NIID in Japan, which indicates the minimum necessary sample size (10 positive samples and 15 negative samples). Second, there were inconsistent results for the detection of SARS-CoV-2 used as positive and negative samples diagnosed in the clinical settings. However, consistent results for the detection of SARS-CoV-2 in each sample were obtained using the NIID-RT-PCR in this study, and then with a performance comparison with the QIAstat-SARS-CoV-2. Furthermore, while two samples (IDs 28 and 40) were found to be positive for SARSC-CoV-2 in this study, their copy numbers of SARS-CoV-2 were below the detection limit, which gave a negative result for SARS-CoV-2. Therefore, when the accuracy of the QIAstat-SARS-CoV-2 was re-evaluated with 43 samples excluding these two samples, the sensitivity, specificity, positive predictive value, and negative predictive value were 87.0%, 100.0%, 100.0%, and 83.3%, respectively, which were similar to the results when these two samples were found to be positive.

## 5. Conclusion

In conclusion, the sensitivity, specificity, positive predictive value, and negative predictive value of the QIAstat-Dx SARS-CoV-2 were high (84.0%, 100.0%, 100.0%, and 83.3%, respectively), compared to that of NIID-RT-PCR. In response to the outbreak of SARS-CoV-2 infection, the QIAstat-SARS-CoV-2 is expected to contribute to the development of a stable system for genetic testing. Further studies using more samples to assess the usefulness of the QIAstat-SARS-CoV-2 in the clinical setting are recommended.

## Authorship statement

MI designed the research, obtained clinical samples, contributed to data collection and verification, and wrote the manuscript. SK received research grants from QIAGEN K.K., Japan, and reviewed the study design and manuscript. TS conducted the sample collection, and wrote the manuscript. HU, HA, YK, and AM conducted the SARS-CoV-2 detection assays, analyzed the data, and wrote the manuscript. NI, MK, and NO reviewed the study design and the manuscript. All the members contributed to the management or administration of the trial. All authors meet the ICMJE authorship criteria.

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## Declaration of competing interest

S.K. received research grants from QIAGEN K.K., Japan. The other authors declare no conflicts of interest.

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