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RESEARCH ARTICLE

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Comparison of extraction-based and elution-based polymerase chain reaction testing, and automated and rapid antigen testing for the diagnosis of severe acute respiratory syndrome coronavirus 2

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Abstract

We aimed to compare the differences in testing performance of extraction-based polymerase chain reaction (PCR) assays, elution-based direct PCR assay, and rapid antigen detection tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We used nasopharyngeal swab samples of patients with coronavirus disease 2019 (COVID-19). We used the MagNA Pure 24 System (Roche Diagnostics K.K.) or magLEAD 12gC (Precision System Science Co., Ltd.) for RNA extraction, mixed the concentrates with either the LightMix Modular SARS-CoV PCR mixture (Roche Diagnostics K.K.) or Takara SARS-CoV-2 direct PCR detection kit (Takara Bio Inc.), and amplified it using COBAS® z480 (Roche Diagnostics K.K.). For elution-based PCR, we directly applied clinical samples to the Takara SARS-CoV-2 direct PCR detection kit before the same amplification step. Additionally, we performed Espline SARS-CoV-2 (Fuji Rebio Co., Ltd.) for rapid diagnostic test (RDT), and used Lumipulse SARS-CoV-2 antigen (Fuji Rebio Co., Ltd.) and Elecsys SARS-CoV-2 antigen (Roche Diagnostics K.K.) for automated antigen tests (ATs). Extraction-based and elution-based PCR tests detected the virus up to 214–216 and 210 times dilution, respectively. ATs remained positive up to 24–26 times dilution, while RDT became negative after 22 dilutions. For 153 positive samples, positivity rates of the extraction-based PCR assay were 85.6% to 98.0%, while that of the elution-based PCR assay was 73.2%. Based on the RNA concentration process, extraction-based PCR assays were superior to elution-based direct PCR assays for detecting SARS-CoV-2.

KEYWORDS

novel coronavirus disease 2019 (COVID-19), polymerase chain reaction, rapid diagnostic test, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

1 | INTRODUCTION

Although more than 2 years have passed since its emergence, the coronavirus disease 2019 (COVID-19) global pandemic continues. The upsurge of the Omicron variant worsens the situation, and many countries are facing unprecedented severe conditions.[1] According to the World Health Organization, more than 300 million confirmed cases and 5.5 million deaths have been reported globally.[2] Early and accurate diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is essential for infection prevention and control, as well as appropriate treatment for the patients.

Polymerase chain reaction (PCR) has a high testing sensitivity for detecting the virus in clinical samples, which is widely used for the diagnosis of COVID-19.[3] PCR test is generally divided into the following two methods: extraction-based PCR and elution-based PCR. In extraction-based PCR, the samples undergo purification and extraction processes, resulting in genome concentration. Although this method is laborious, time-consuming, and expensive, it can achieve a high concentration of nucleic acids; thus, it can yield high testing sensitivity. In elution-based PCR, which is also known as direct PCR, the sample is lysed in a buffer solution without a purification step. This approach is simple, rapid, and economical but it has low sensitivity compared with the extraction-based methods.

Clinicians should be aware of the difference in testing performance of these methods, which is not well recognized.[4] Even a recent review of literature on the diagnosis of COVID-19 does not refer to this essential point.[5] An appropriate interpretation of PCR results is crucial for people in various situations such as patients with clinical symptoms and individuals with close contacts. In particular, a false negative result requires much attention because it may cause the spread of infection and underestimation of the disease prevalence.[6] Although elution-based PCR has less sensitivity, it is widely used in hospital laboratories in Japan owing to its convenience. In this study, we aimed to reveal the differences in testing sensitivity of these methods, along with rapid antigen detection tests.

2 | MATERIALS AND METHODS

This study was performed at Osaka University Hospital, a tertiary medical facility in Japan, between March 2020 and May 2021. We used nasopharyngeal swab samples (FLOQSwab, COPAN Co., Ltd.) of patients diagnosed with COVID-19. The study protocol was approved by the ethics committee of Osaka University (No. 20063-3). The need to obtain informed consent was waived because we retrospectively collected anonymized data.

For extraction-based PCR, we concentrated the clinical samples using either the MagNA Pure 24 System (MP24; Roche Diagnostics K.K.) or magLEAD 12gC (magLEAD; Precision System Science Co., Ltd.). The extracted samples were mixed with either the LightMix Modular SARS-CoV PCR mixture (Roche Diagnostics K.K.) or Takara SARS-CoV-2 direct PCR detection kit (Takara Bio Inc.), and detected after amplification in COBAS® z480 (Roche Diagnostics K.K.). We performed extraction-based PCR using combinations of MP24 – LightMix (PCR condition 1), magLEAD – LightMix (PCR condition 2), and magLEAD – Takara (PCR condition 3). For elution-based PCR, a clinical sample was directly mixed with sample processing reagents from Takara SARS-CoV-2 direct PCR detection kit (Takara Bio Inc.) and heated at 95°C for 5 min. After adding the PCR mixture to the test kit, we amplified it using COBAS® z480 (PCR condition 4). We also performed rapid antigen detection tests for comparison with each of the PCR conditions. Automated antigen tests (ATs) based on chemiluminescence enzyme immunoassay were performed using Lumipulse SARS-CoV-2 antigen (Fuji Rebio Co., Ltd.) and Elecsys SARS-CoV-2 antigen (Roche Diagnostics K.K.). Rapid diagnostic test (RDT) based on immunochromatographic assay was performed using Espline SARS-CoV-2 (Fuji Rebio Co., Ltd.). In the present study, we applied Dulbecco's phosphate-buffered saline (PBS; Yamagata Plant Co., Ltd.) for bufferization of the clinical samples shortly after the sample arrival. We did not use neither transportation media nor preservation media to avoid an abnormal cross-reaction.

Details of the testing protocols and volumes of clinical samples and reagents are provided in Figure 1. Through these assays, we compared differences in testing performance between (A) sample extraction methods (PCR condition 1 vs. 2), (B) PCR reagents (PCR condition 2 vs. 3), (C) extraction-based PCR and elution-based PCR (PCR condition 3 vs. 4), and (D) AT and RDT.

We first examined a pooled sample of nasopharyngeal specimens to compare testing sensitivity among each condition, using four SARS-CoV-2-positive samples. After adding 2 ml of PBS to each specimen, the pooled sample was diluted with 4^n dilution. The tests were performed in a quintuplicate manner to determine the testing sensitivity. The mean threshold cycles for PCR testing and cutoff index (COI) for rapid antigen detection testing were also calculated when the testing sensitivity was 100%. We then tested 211 nasopharyngeal samples to compare the positivity rates of each testing condition. Cutoff value of the cycle threshold in PCR testing was set at 38.0, and COI of the AT was ≥1.34 for Lumipulse and ≥1.00 for Elecsys.

3 | RESULTS

Comparison of testing sensitivity according to five experiments for each condition of PCR testing and rapid antigen detection tests using a single nasopharyngeal sample is summarized in Table 1. PCR conditions 1, 2, and 3 detected viral genes up to 2^{14}, 2^{16}, and 2^{16} times dilution with 100% positivity, respectively. Thus, differences in (A) sample extraction methods and (B) PCR reagents did not markedly affect testing sensitivity. In contrast, PCR condition 4 identified the viral genes only up to 2^{10} times dilution, indicating that the extraction-based PCR conditions were 16–64 (2^4–2^6) times more sensitive than the elution-based PCR. AT remained positive up to 2^4–2^6 dilutions; thus, the differences in testing sensitivity compared with extraction-based PCR were 1024–4096 (2^{10}–2^{12}) times. RDT results were negative after 2^2 dilutions, which suggested that the difference in testing sensitivity between the extraction-based PCR, and this method was up to 65 536 (2^{16}) times.

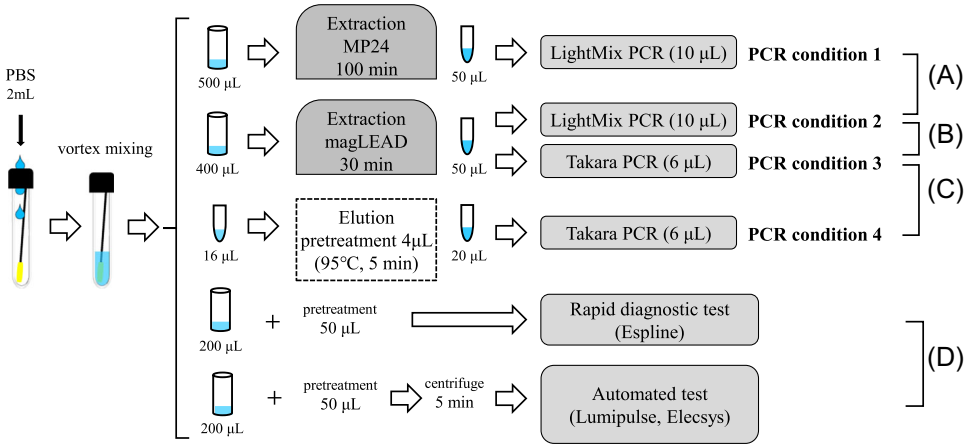

FIGURE 1 Testing protocol. (A) Comparison of sample extraction methods (PCR condition 1 vs. 2); (B) Comparison of PCR reagents (PCR condition 2 vs. 3); (C) Comparison of extraction-based PCR and elution-based PCR (PCR condition 3 vs. 4), and (D) Comparison of automated antigen test and rapid diagnostic test. PBS, phosphate-buffered saline; PCR, polymerase chain reaction

TABLE 1 Comparison of testing sensitivity according to five experiments of each 4^n dilutions for each condition of PCR testing and antigen testing using single nasopharyngeal sample

	PCR tests								Antigen tests				
	Extraction-based PCR						Elution-based PCR		Automated test				RDT
	PCR condition 1 MP24 – LightMix		PCR condition 2 magLEAD – LightMix		PCR condition 3 magLEAD – Takara		PCR condition 4 Direct – Takara		Lumipulse		Elecsys		Espline
Dilution (times)	Sensitivity (%)	Ct (mean)	Sensitivity (%)	Ct (mean)	Sensitivity (%)	Ct (mean)	Sensitivity (%)	Ct (mean)		COI		COI	Results
1	**100**	21.72	**100**	20.58	**100**	21.34	**100**	26.01	+	285.77	+	8.52	+
2^2	**100**	23.98	**100**	22.60	**100**	23.28	**100**	28.17	+	69.98	+	2.03	–
2^4	**100**	25.84	**100**	24.65	**100**	25.44	**100**	31.70	+	15.9	+	1.02	–
2^6	**100**	27.84	**100**	26.69	**100**	27.24	**100**	33.36	+	3.71	–	0.46	–
2^8	**100**	29.70	**100**	28.67	**100**	29.17	**100**	35.62	–	0.9	–	0.42	–
2^{10}	**100**	31.56	**100**	30.66	**100**	31.70	**100**	37.26	–	0.23	–	0.42	–
2^{12}	**100**	33.90	**100**	32.59	**100**	33.36	0	ND	–	0.07	–	0.48	–
2^{14}	**100**	35.24	**100**	34.66	**100**	35.62	0	ND	–	0.04	–	0.42	–
2^{16}	60	NC	**100**	36.81	**100**	37.78	0	ND	–	0.04	–	0.45	–
2^{18}	0	ND	60	NC	20	NC	0	ND	–	0.04	–	0.48	–
2^{20}	0	ND	0	ND	0	ND	0	ND	–	0.04	–	0.46	–

Note: The positivity rates of the RT-PCR tests were calculated based on five experiments.

Abbreviations: COI, cutoff index; Ct, cycle threshold; NC, not calculated; ND, not detected; RDT, rapid diagnostic test; RT-PCR, reverse transcriptase-polymerase chain reaction.

Subsequently, we examined the positivity rates of these PCR tests and rapid antigen detection tests using 211 nasopharyngeal samples (Table 2). The positivity rates of PCR conditions 1, 2, 3, and 4 were 62.1%, 71.1%, 69.7%, and 53.1%, respectively. In addition, positivity rates of rapid antigen detection tests by Lumipulse, Elecsys, and Espline were 40.8%, 28.9%, and 15.2%, respectively.

Of the 211 samples, 153 samples were positive in both PCR testing methods, which were regarded as positive samples. We then calculated the positivity rates of each method for the positive samples (Figure 2). Consequently, the positivity rates of PCR conditions 1, 2, 3, and 4 were 85.6%, 98.0%, 96.1%, and 73.2%, respectively. Meanwhile, those of Lumipulse, Elecsys, and Espline were 56.2%, 39.9%, and 24.8%, respectively.

TABLE 2 Positivity rates of PCR and antigen tests for 211 nasopharyngeal samples

	PCR tests				Antigen tests		
	Extraction-based PCR			Elution-based PCR	Automated test		RDT
	PCR condition 1 MP24 – LightMix	PCR condition 2 magLEAD – LightMix	PCR condition 3 magLEAD – Takara	PCR condition 4 Direct – Takara	Lumipulse	Elecsys	Espline
Positive	131	150	147	112	86	61	32
Negative	80	61	64	99	125	150	179
Positivity rates	62.1%	71.1%	69.7%	53.1%	40.8%	28.9%	15.2%

Abbreviations: PCR, polymerase chain reaction; RDT, rapid diagnostic test.

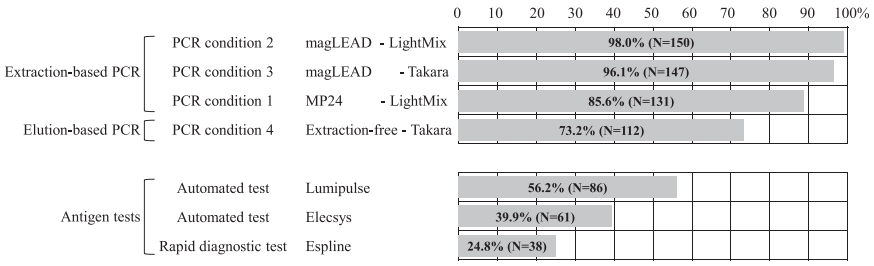

FIGURE 2 Comparison of positivity rates of each PCR and antigen detection test for 153 positive samples. We used 153 nasopharyngeal samples that were positive in either PCR testing method. PCR, polymerase chain reaction

4 | DISCUSSION

We demonstrated the superiority of extraction-based PCR in testing performance compared with elution-based PCR. Based on the evaluation of PCR conditions 1–3 and 4, the extraction-based PCR was 16–64 times more sensitive than the direct elution-based PCR. Recent literature reports an improvement of the extraction-free, direct PCR assays.[7,8] However, the present results endorsed the advantage of extraction-based PCR in detecting viral genes with higher sensitivity. In addition, the comparison between PCR conditions 1 and 2 suggested that the extraction efficiency for SARS-CoV-2 might be different between the equipment, even though their extraction methods are based on the magnetic particle method. However, the influence of PCR reagents on testing sensitivity would be negligible, according to the comparison between PCR conditions 2 and 3.

The RNA extraction process constitutes a major impediment to rapid testing; however, it is an important step to increase the testing sensitivity. Recent studies have proposed several methods to circumvent RNA extraction while maintaining testing sensitivity.[9,10] However, a previous study suggested that direct PCR is acceptable only when samples are collected in universal transport medium or molecular water, whereas extraction is necessary if samples are collected in saline water or Hank's medium.[11] Practically, because of a high RNA concentration, pooling tests for the diagnosis of COVID-19 are allowed only for extraction-based PCR.[12,13] Thus, there is a difference between extraction-based and elution-based PCRs.

A highly sensitive diagnostic assay is indispensable in both clinical and nonclinical settings during the pandemic. However, PCR assays without the RNA extraction process have been widely used in many laboratories in Japan owing to their speed and affordability; for example, Ampdirect™ 2019-nCoV detection kit (Shimadzu Corporation; calculated sample volume, 5 µl), SARS-CoV-2 direct detection RT-qPCR kit (Takara Bio Inc.; calculated sample volume, 4.8 µl), GENECUBE® HQ SARS-CoV-2 (TOYOBO Co., Ltd.; calculated sample volume, 8 µl), and Loopamp™ SARS-CoV-2 detection kit (EIKEN CHEMICAL Co., Ltd.). The calculated sample volumes of these direct assays are much lower (4.8–8 µl per test) than that of the extraction-based PCR (200 µl per test), which is the main reason for the difference in testing performance.

Compared with PCR testing, antigen testing can offer multiple advantages, such as rapidity, low cost, easy availability, and convenience of performance.[14] Although it can detect individuals with replication-competent viruses with high sensitivity and specificity as PCR assays, the rapid AT generally has a lower testing sensitivity, which means that larger amounts of viral genes are required to show a positive result.[15] Thus, clinical application of the AT should be carefully determined. The testing sensitivity of AT is reportedly higher than that of RDT. In the present study, the positivity rates of RDT for positive samples were nearly half of that of AT. Thus, an indication for RDT should be limited to a certain at-risk situation.

Our study had certain limitations. First, we examined four PCR conditions for the purpose of the present study. However, there are many more combinations of extraction equipment and PCR reagents. In addition, as mentioned above, various methods for direct PCR assays are available for the diagnosis of COVID-19. Thus, our data may not be sufficient to conclude the difference in testing performance of extraction-based and elution-based PCRs. Further investigations using other PCR equipment are required. Second, we tested only symptomatic patients with COVID-19, and the samples tested were from patients in whom the disease had progressed. Samples obtained from asymptomatic cases should be included in further studies. Despite these limitations, we believe our study

corroborated that the RNA extraction step is vital to reduce false-negative results in patients with COVID-19.

In summary, extraction-based PCR appears to be superior to elution-based PCR for detecting SARS-CoV-2. We should recognize the advantages and disadvantages of each assay and choose accordingly.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors meet the ICMJE authorship criteria: Kazunori Tomono, Yoh Hidaka, and Ikuhiro Maeda were in charge of the organization and coordination of the trial; Matsuo Deguchi designed the study; Nori Yoshioka, Masanori Kagita, Hiroko Tsukamoto, and Miyuki Takao carried out the measurements; Nori Yoshioka, Matsuo Deguchi, Hideharu Hagiya, Hisao Yoshida, and Shigeto Hamaguchi were in charge of data analysis. Hideharu Hagiya drafted the manuscript, and all authors contributed to writing the final manuscript. All authors contributed to the revision of final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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