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Original Article

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Diagnostic Performance, Stability, and Usability of Self-Collected Combo Swabs and Saliva for Coronavirus Disease 2019 Diagnosis: A Case-Control Study

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ABSTRACT

Background: Self-sampling procedures to detect severe acute respiratory syndrome coronavirus 2 is important for patients who have difficulty visiting the hospital and may decrease the burden for health care workers (HCWs). The objective of this study was to evaluate the diagnostic performance, stability and usability of self-collected nasal and oral combo swabs and saliva specimens.
Materials and Methods: We conducted a case-control study with 50 patients with coronavirus disease 2019 (COVID-19) and 50 healthy volunteers from March, 2021 to June, 2021. We performed real-time reverse-transcription polymerase chain reaction to compare the diagnostic performance of self-collected specimens using positive percent agreements (PPAs).
Results: The PPAs between self-collected and HCW-collected specimens were 77.3 - 81.0% and 80.5 -86.7% for the combo swabs and saliva specimens, respectively. The PPAs increased to 88.9 - 89.2% and 81.2 - 82.1% with a cycle threshold value ≤30.
Conclusion: The diagnostic performance of self sampling was comparable to that of HCW sampling in patients with high viral loads and may thus assist in the early diagnosis of COVID-19.

Keywords: SARS-CoV-2; COVID-19; Self sampling; Self-collected specimens

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Conflict of Interest

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Author Contributions

Conceptualization: SKH, KES, PKU. Data curation: CSJ, JJ, LH, LE, CPG, KJY, and LEJ. Formal analysis: SKH, CSJ. Funding acquisition: SKH. Investigation: CSJ, JJ, HBK, PJS, LH, LE, CPG, KJY, and LEJ. Methodology: SKH. Project administration: SKH. Resources: SKH, JJ, LH, LE, CPG, KJY, and LEJ. Software: CSJ. Supervision: SKH. Validation: CSJ, JJ, LH, LE, CPG, KJY, and LEJ. Visualization: CSJ. Writing - original draft: CSJ. Writing – review & editing: SKH, JJ, KES, KHB, PJS, PKU, LH, LE, CPG, KJY, LEJ.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a global pandemic and after its first appearance in December 2019, it still threatens the lives of over a million people. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative virus of COVID-19, is a positive-sense RNA virus that belongs to the family *Coronaviridae* [1, 2]. The RNA of SARS-CoV-2 encodes four structural proteins, including the spike (S), matrix (M), envelope (E), and nucleocapsid (N), and other non-structural proteins, such as RNA-dependent RNA polymerase (RdRP), that are required for viral replication [3, 4].

Standard confirmatory analyses for COVID-19 are based on the detection of the specific gene sequences of SARS-CoV-2, such as E, N, S, and RdRP, by nucleic acid amplification tests (NAATs). One of the most common NAATs is the real-time reverse-transcription polymerase chain reaction (rRT-PCR) test, which requires respiratory specimens [5]. There are two types of respiratory specimens obtained for swab testing: the upper respiratory, such as nasopharyngeal and oropharyngeal specimens, and the lower respiratory, such as sputum, endotracheal aspirate, and bronchoalveolar lavage. Nasopharyngeal swabs obtained by a trained health care worker (HCW) are widely used for the diagnosis of COVID-19 in screening clinics in the Korea.

However, during the surge of highly transmissible variants of SARS-CoV-2, such as the Omicron variant [6], or in outbreak situations, the collection of nasopharyngeal swabs by the HCWs could be problematic, especially when older people with dementia or young children are involved [7]. As the global COVID-19 pandemic continues and many confirmatory tests are currently being conducted, continuous efforts to simplify and optimize the SARS-CoV-2 specimen collection are much needed. One approach to overcome this problem is to implement self-sampling procedures instead of sampling by trained personnel. Self-collection of specimens has several advantages, such as convenience, improved accessibility for older patients and people with disabilities who have difficulty visiting COVID-19 screening clinics, and a lower transmission risk and burden for the HCWs. Therefore, many studies were conducted on the investigation of the sensitivity of self-sampling specimens compared with specimens collected by a trained HCW [8-14]. Nasal swabs, oral swabs, and saliva collections obtained with or without the supervision of a medical professional were included in the specimen collection guide of the United States Centers for Disease Control and Prevention [7].

In this study, we investigated the diagnostic performance of Allplex™ SARS-CoV-2 Assay and Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene Inc., Seoul, Korea) with self-collected nasal and oral swab (combo swab) specimens and saliva specimens for SARS-CoV-2 detection and compared these samples with nasopharyngeal specimens collected by the HCWs. Allplex™ SARS-CoV-2 Assay is a muliplex real-time polymerase chain reaction (PCR) assay used for detecting the E gene, RdRP/S gene, and N gene of SARS-CoV-2; Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is a multiplex real-time PCR assay used for detecting the N gene, RdRP gene, and S gene for SARS-CoV-2, influenza A, influenza B, and respiratory synctial virus (RSV) A/B. In addition, we evaluated the stability of the test results for the combo swab and saliva specimens in two different storage conditions and determined the usability for self-collection sampling using a questionnaire-based evaluation.

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MATERIALS AND METHODS

1. Patients

Samples from patients with COVID-19 (n = 50) and healthy volunteers without COVID-19 (n = 50) were obtained from the Seoul National University Bundang Hospital, Seoul National University Hospital, Seoul Metropolitan Government Boramae Medical Center, and Seongnam Citizens Medical Center in Korea from March to June 2021. Patients younger than 13 years and older than 80 years were excluded from the study because of perceived difficulties in performing self-collection of specimens and conducting the survey. Only patients who were admitted to the hospital within 7 days after a diagnosis of COVID-19 were included.

2. Ethics statement

This study was approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (IRB no. B-2101-663-003) and also the IRBs of the other participating hospitals and all participants provided informed consent prior to enrollment.

3. Sample collection

We obtained self-collected nasal and oral swab (combo swab) specimens, self-collected saliva specimens, and HCW-collected nasopharyngeal specimens. For the self-collected specimens, we instructed the patients not to eat, drink (including water), smoke, or brush their teeth within 30 minutes before the sample collection. For the oral swab, participants were instructed to cough deeply three to five times and rub the swab on both cheeks and gums, above and below the tongue, and on the hard palate more than five times to ensure that the swab was saturated with oral fluid. For the nasal swab, participants were instructed to insert the swab into both nostrils to a depth of 2 - 3 cm and then rotate the swab over 10 times. Next, the participants were instructed to place both the nasal and oral swabs into the tube and to close the lid properly. For the saliva sample, participants were instructed to collect the saliva up to the marked line on the sterile container, taking care not to splash droplets or aerosols around it. All instructions were provided through an instruction booklet.

4. Transport and analysis

Collected specimens were transported to the laboratory of Seegene Inc. and kept between a temperature of 2°C to 8°C for testing on the day of collection. We extracted RNA from the samples with MaqNA 96 DNA and Viral NA Small Volume Kit (Roche Diagnostics, Rotkreuz, Swiss) and performed rRT-PCR with Allplex™ SARS-CoV-2 Assay and Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene Inc., Korea) using the CFX96™ Dx system (Bio-Rad, Hercules, CA, USA). To measure the stability of the samples, remnant specimens from eight patients with COVID-19 and two healthy volunteers were stored at room temperature (20°C) for 5 days and tested on days 1, 2, 3, and 5, and stored in the refrigerator (4°C) for 9 days and tested on Days 1, 3, 5, and 9.

5. Usability evaluation

We conducted a questionnaire-based survey to evaluate the usability of the self-collection process. The questions were selected to identify the physical and psychological comfortability of self collection of specimens or collection by the HCWs, preference about the type of specimen collection, and feasibility of the self-collection process with written instructions.

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6. Statistical analyses

To compare the diagnostic performance between the self-collected specimens and HCW-collected specimens, we calculated the positive percent agreement (PPA), negative percent agreement (NPA), Cohen's kappa coefficient (κ), and inter-class correlation (ICC) [15]. We used Fisher's exact test to find the difference in age distribution in the matching and mismatching results between the self-collected samples and samples collected by the HCWs. We used Spearman rank correlation tests to investigate the correlation between viral loads and days after the onset of symptoms. Statistical significance was set at P <0.05. All analyses were performed using R Version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

1. Diagnostic performance of the self-sampling protocol

Using the Allplex™ SARS-CoV-2 Assay (Seegene Inc., Korea), 46 of the 50 patients confirmed with COVID-19 tested positive through the nasopharyngeal specimens collected by the HCWs (**Supplementary Table 1**). As for the self-collected combo swab and saliva samples, 29 and 31 patients, respectively, tested positive (**Table 1**). Meanwhile, for the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene, Korea), 47 of the 50 patients with COVID-19 tested positive through the nasopharyngeal specimens collected by the HCWs (**Supplementary Table 1**). For the self-collected combo swab and saliva samples, 32 and 36 patients tested positive, respectively (**Table 1**).

The false negative results in the self-collected samples usually occurred in patients with a cycle threshold (Ct) value >30 from the HCW-collected samples (**Fig. 1**). Upon using the Allplex SARS-CoV-2 Assay, the proportion of patients with Ct value >30 among those with false-negative results were 92.9 – 100.0% and 64.7 – 100.0% for the self-collected combo swab and saliva samples, respectively. For the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene Inc., Korea), the proportions were 66.6 - 82.4% and 41.7 - 69.2% for the self-collected combo swab and saliva samples, respectively.

Thus, we performed a subgroup analysis with a Ct value of 30 as the cut-off for positivity for the samples collected by the HCWs, along with the calculated PPA and κ (**Table 2**). Using the Allplex™ SARS-CoV-2 Assay (Seegene Inc., Korea), 24 and 23 of the 25 patients who had Ct

Table 1. SARS-CoV-2 nucleic acid detection results for paired nasopharyngeal swabs collected by the HCWs and self-collected combo swabs or saliva samples

PCR Kit	Sample category	Result	HCWs NP swab			% positive agreement (95% CI)	% negative agreement (95% CI)	Kappa statistics
			Positive	Negative	Total			
Allplex™ SARS-CoV-2 Assay	Combo swab	Positive	29	0	29	77.3 (66.8 - 87.8)	85.5 (78.6 - 92.3)	0.640 (0.489 - 0.767)
		Negative	17	50	67			
		Total	46	50	96			
	Saliva	Positive	31	0	31	80.5 (70.9 - 90.2)	87.0 (80.4 - 93.5)	0.683 (0.532 - 0.803)
		Negative	15	50	65			
		Total	46	50	96			
Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay	Combo swab	Positive	32	0	32	81.0 (71.6 - 90.4)	87.0 (80.4 - 93.5)	0.687 (0.538 - 0.806)
		Negative	15	50	65			
		Total	47	50	97			
	Saliva	Positive	36	0	36	86.7 (79.0 - 94.5)	90.1 (84.3 - 95.9)	0.771 (0.625 - 0.872)
		Negative	11	50	61			
		Total	47	50	97			

SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; HCW, health care worker; PCR, polymerase chain reaction; CI, confidence interval; RSV, respiratory synctial virus.

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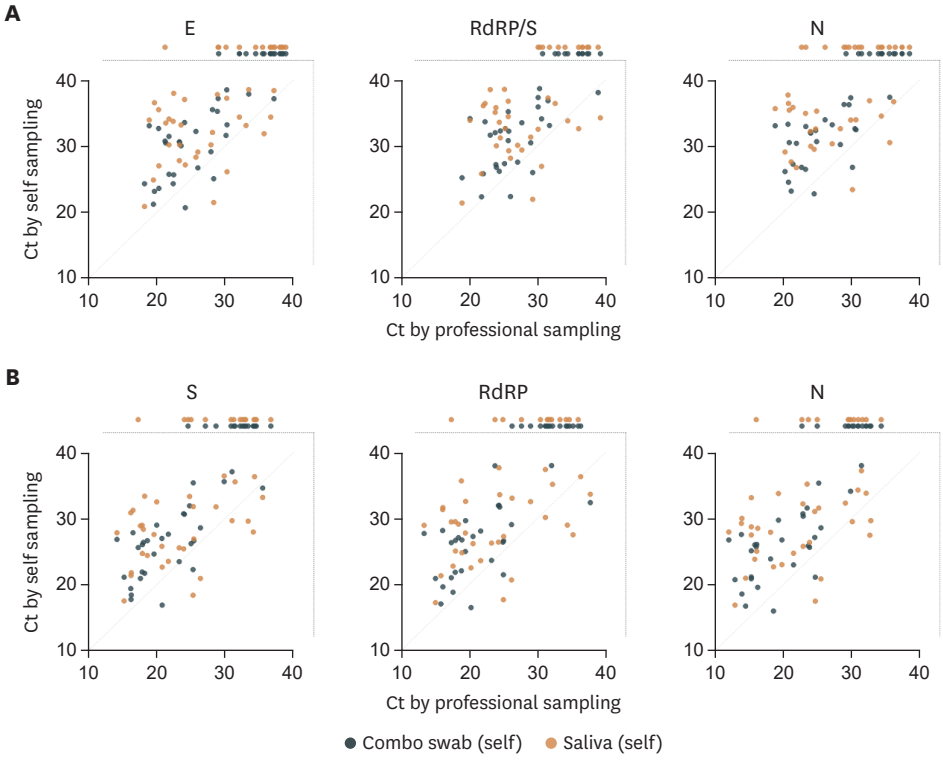

Figure 1. The Ct values of HCW-collected nasopharyngeal swab specimens, self-collected combo swab specimens, and self-collected saliva specimens.
(A) The Ct values of the E, RdRP/S, and N genes of 46 patients who tested positive for the nasopharyngeal specimen collected by the HCWs using the Allplex™ SARS-CoV-2 Assay (Seegene Inc., Seoul, Korea). (B) The Ct values of the S, RdRP, and N genes of 47 patients who tested positive for the nasopharyngeal specimen collected by the HCWs using the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene Inc., Korea). The relationship between the HCW-collected nasopharyngeal swab specimens and self-collected combo swab specimens (gray dots) and the relationship between the HCW-collected nasopharyngeal swab specimens and self-collected saliva specimens (yellow dots).
HCW, health care worker.

Table 2. SARS-CoV-2 nucleic acid detection results for paired nasopharyngeal swabs collected by the HCWs and self-collected combo swabs or saliva samples with a Ct value of 30 as the positive cut-off

PCR Kit	Sample category	Result	HCWs NP swab			% positive agreement (95% CI)	% negative agreement (95% CI)	Kappa statistics
			Positive	Negative	Total			
Allplex™ SARS-CoV-2 Assay	Combo swab	Positive	24	5	29	88.9 (80.1 - 97.7)	95.7 (92.2 - 99.1)	0.856 (0.696 - 0.929)
		Negative	1	66	67			
		Total	25	71	96			
	Saliva	Positive	23	8	31	82.1 (71.3 - 93.0)	92.6 (88.1 - 97.2)	0.749 (0.590 - 0.861)
		Negative	2	63	65			
		Total	25	71	96			
Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay	Combo swab	Positive	29	3	32	89.2 (81.3 - 97.2)	94.6 (90.6 - 98.6)	0.838 (0.689 - 0.924)
		Negative	4	61	65			
		Total	33	64	97			
	Saliva	Positive	28	8	36	81.2 (71.1 - 91.2)	89.6 (84.0 - 95.2)	0.708 (0.546 - 0.830)
		Negative	5	56	61			
		Total	33	64	97			

SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; HCW, health care worker; PCR, polymerase chain reaction; NP, nasopharyngeal; CI, confidence interval; RSV, respiratory synctial virus.

values <30 for the HCW-collected samples also tested positive for the self-collected combo swab and saliva samples, respectively. Meanwhile, for the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene Inc., Korea), 29 and 28 of the 33 patients who had Ct values <30 for the HCW-collected samples tested positive for the self-collected combo swab and saliva samples, respectively (**Table 2**).

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We also investigated whether the symptoms of patients affected the concordance between the self-collected and HCW-collected samples. About 87.0% and 89.4% of the patients confirmed as having COVID-19 using the Allplex™ SARS-CoV-2 Assay and the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene Inc., Korea), respectively, through samples collected by the HCWs, had COVID-19 symptoms. First, we found that the Ct values from samples collected by the HCWs tended to increase after the onset of symptoms (**Fig. 2A**). Mismatching results between self-collected samples and samples collected by the HCWs were observed every day after the onset of symptoms (range: 2 to 15 days). Our findings also showed that within 10 days after the onset of symptoms, the patients showed relatively high matching rates, whereas SARS-CoV-2 virus from self-collected samples was usually not detected in patients 10 days after the onset of symptoms (**Fig. 2B**). The PPA of symptomatic patients was higher than that of all patients. Furthermore, in patients within 10 days after symptom onset, the PPAs of combo swab and saliva were over 85.0% (**Supplementary Table 2**). Older age did not affect the concordance between results from self-collected samples and samples collected by the HCWs (**Supplementary Table 3**).

2. Stability of the samples under room temperature or refrigerated conditions

Next, we investigated the changes in Ct values for each sample under room temperature (20°C) and in refrigerated (4°C) conditions. Ten samples (eight from patients who were positive for COVID-19 and two from patients who were negative for COVID-19) were stored at room temperature for 5 days and in refrigerated conditions for 9 days (**Fig. 3**). There were no significant changes in the Ct values (positive to negative result) in all eight patients who were positive for COVID-19.

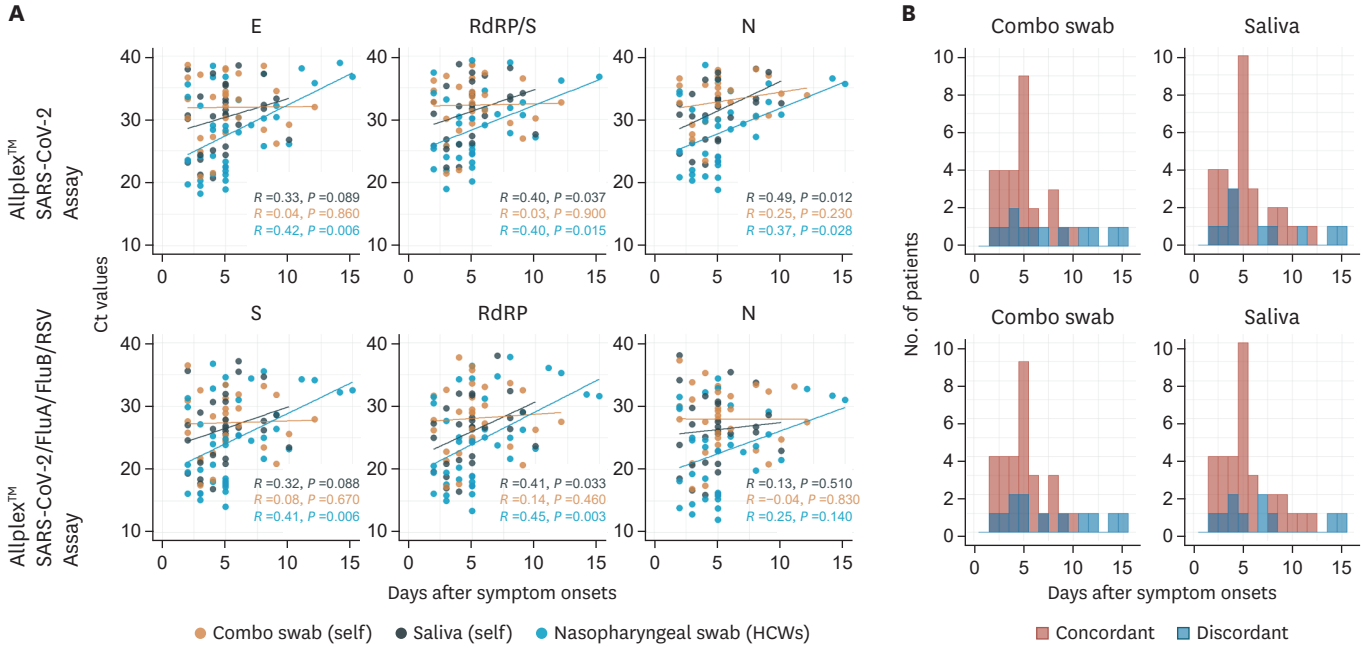

Figure 2. The Ct values of HCW-collected nasopharyngeal swab specimens, self-collected combo swab specimens, and self-collected saliva specimens according to the days after the onset of symptoms.
(A) Relationship between the Ct values of HCW-collected nasopharyngeal specimens (blue dots), self-collected combo swab specimens (gray dots), and self-collected saliva specimens (yellow dots) and the days after the onset of symtoms in 46 patients who tested positive for the nasopharyngeal specimens collected by the HCWs using the Allplex™ SARS-CoV-2 Assay (Seegene Inc., Seoul, Korea) and for the 47 patients who tested positive through the nasopharyngeal specimens collected by the HCWs using the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene Inc., Korea). (B) The relationship between the number of patients with concordant (red) and discordant (blue) relationships between the results of the HCW-collected samples and self-collected samples and the days after the onset of symptoms.
HCW, health care worker.

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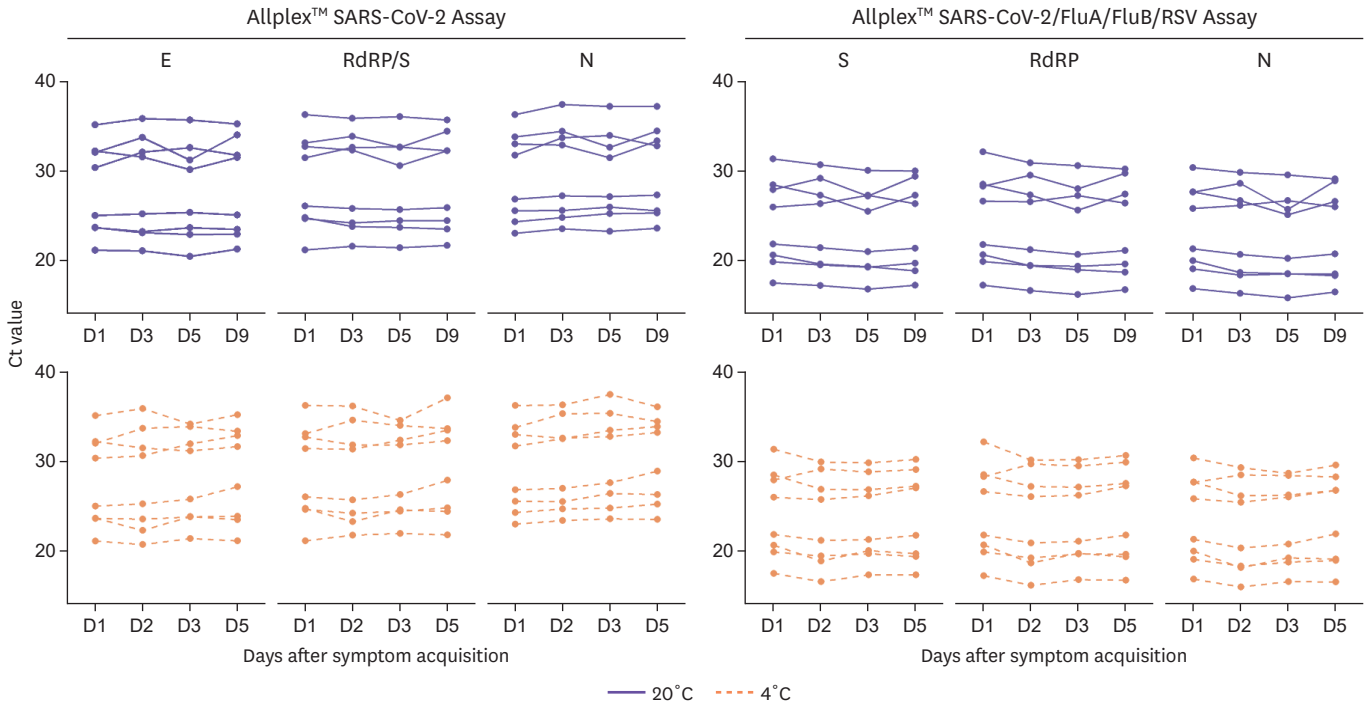

Figure 3. Stability of the SARS-CoV-2 RNA at room temperature and in the refrigerator.
Changes in the Ct values of the samples from eight patients with COVID-19 and two healthy donors at room temperature and in refrigeration.
SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; COVID-19, coronavirus disease 2019; RSV, respiratory synctial virus.

Table 3. Investigation of the degree of comfort, preference, and feasibility of self-sampling

Number	Question	1[a]	2[b]	3[c]	4[d]	5[e]
1	It was physically comfortable when the nasopharyngeal swab samples were collected by the HCWs (n = 98)	10	28	17	26	17
2	It was psychologically comfortable when the nasopharyngeal swab samples were collected by the HCWs (n = 98)	6	13	23	34	22
3	It was physically comfortable to self-collect nasal swab samples by self (n = 98)	0	0	9	48	41
4	It was psychologically comfortable to self-collect nasal swab samples by self (n = 98)	0	2	9	45	42
5	It was physically comfortable to self-collect oral swab samples by self (n = 98)	0	0	3	44	51
6	It was psychologically comfortable to self-collect oral swab samples by self (n = 98)	0	2	6	44	46
7	It was physically comfortable to self-collect saliva samples by self (n = 98)	1	0	8	43	46
8	It was psychologically comfortable to self-collect saliva samples by self (n = 97)	0	2	9	40	46
9	It is preferable to self-collect nasal swab samples rather than have nasopharyngeal swab samples collected by HCWs (n = 98)	2	8	11	48	29
10	It is preferred to self-collect oral swab samples rather than have nasopharyngeal swab samples collected by HCWs (n = 98)	2	9	11	41	35
11	It is preferred to self-collect saliva samples rather than have the nasopharyngeal swab samples collected by the HCWs (n = 98)	2	6	11	42	37
12	It was easy to self-collect nasal swab samples by self (n = 98)	0	0	4	39	55
13	It was easy to self-collect oral swab samples by self (n = 98)	0	0	2	39	57
14	It was easy to self-collect saliva samples by self (n = 98)	0	1	6	33	58
15	The quick guide for instruction of the nasal swab self-collection method was easy to understand (n = 98)	1	1	7	40	49
16	The quick guide for instruction of the oral swab self-collection method was easy to understand (n = 98)	1	0	7	37	53
17	The quick guide for instruction of the saliva self-collection method was easy to understand (n = 98)	1	0	6	38	53

(a = strongly disagree, b = disagree, c = neutral, d = agree, e = strongly agree).

3. Usability of self-sampling protocol

We also conducted a participant survey for the degree of comfort, preference, and feasibility of the self-sampling protocol (**Table 3**). Most patients felt physically comfortable about performing the specimen collection by themselves compared to it being done by the HCWs. In addition, most patients also felt psychologically comfortable about collecting the specimens on their own than when the collection was performed by the HCWs (**Table 3**). Compared to nasopharyngeal swab collection done by the HCWs, participants preferred to collect the samples by themselves. The instructions provided for the self-collection of

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specimens were easy to understand and most of the participants performed the self-sampling protocol with ease (**Table 3**).

DISCUSSION

We performed a head-to-head comparison of the SARS-CoV-2 test for self-collected combo swab and saliva samples with HCW-collected nasopharyngeal swabs. Compared with previous studies that PPA of nasal swab or self-collected combo swab were 75.0 – 100.0% and PPA of saliva samples were 87.0 – 100.0% [8-12, 16, 17], the PPAs were relatively low in our study (77.3 – 81.0% and 80.5 – 86.7%, respectively). Mismatch of tests between the self-collected and HCW-collected samples was usually seen in patients with low viral loads (Ct values over 30). The differences in the sites from which samples were acquired could explain the variation in the results between the self-collected and HCW-collected samples. In a meta-analysis published in 2021 [18], the diagnostic performance in the nasal sample collected by the HCWs (68% [95% confidence interval (CI): 47.0 – 86.0%]) was lower than that in the nasopharyngeal sample collected by the HCWs (96% [95% CI: 92.0 – 99.0%]). In addition, the difference in the diagnostic performance between the nasal and nasopharyngeal swab was greater in a more sensitive assay with a limit of detection <1,000 cp/mL (61% [95% CI: 40.0 – 79.0%] *vs.* 97% [95% CI: 92.0 – 100.0%], respectively) than in a less sensitive assay with a limit of detection ≥1,000 copies/mL (87% [95% CI: 82.0 –91.0%] *vs.* 99% [99% CI: 93.0 – 100.0%], respectively). Lower viral burden in the anterior nasal area compared to that in the nasopharyx could explain the variations between the nasal and nasopharyngeal samples. Furthermore, the viral load of SARS-CoV-2 declines over time following symptom onset [19]. Therefore, samples obtained from the hospitalized patients with COVID-19 rather than those with the initial diagnosis could have contributed to the relatively low PPA in our study. Thus, we performed a subgroup analysis with a Ct value of 30 as the cut-off for positivity, and there was a comparable positive agreement between the HCW-collected samples and the self-collected combo swab tests for patients with high SARS-CoV-2 viral loads (PPA = 88.9 - 9.2%). However, a positive agreement between the HCW-collected samples and the self-collected saliva specimens in the subgroup analysis (PPA = 81.2 - 82.1%) was still lower than that in the previous report [17]. The self-collected combo swab samples showed excellent agreements when the Ct values of the samples collected by the HCWs were ≤30. The agreements between the self-collected saliva and nasopharyngeal swabs collected by the HCWs in the subgroup analysis were similar to the conventional Ct cut-off value. Good inter-class correlation (ICC) between the Ct values of self-collected combo swabs and HCW-collected nasopharyngeal swabs and poor ICC between the saliva and nasopharyngeal samples collected by the HCWs were also observed. This could explain the excellent agreements of the combo swabs with a Ct value >30 as the cut-off for positivity and the saliva samples with a similar Ct as the conventional Ct cut-off value for positivity (**Supplementary Table 4**). Considering the evidence suggesting the high viral loads during the early stages of infection or prior to the onset of symptoms [20, 21], self-collected specimens (especially combo swabs) can be useful for the early diagnosis of people who were recently exposed to patients with COVID-19.

The advantage of the self-collection of samples is that it can be performed at home or at work. To perform rRT-PCR, the collected samples should be transported to the laboratory within the appropriate time with the adequate storage temperature maintained. In our study, there were no changes in test positivity in 10 patients within 5 days of storage at room

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temperature and within 9 days of storage in the refrigerator. This result suggests that as long as the self-collected samples are stored at the right temperature and analyzed within the appropriate time, there will be no significant change in the interpretation of the test even if the specimen is collected at home or at work by the patient.

Self-collection of specimens also has the advantage of high accessibility. Most participants felt comfortable with and preferred collecting the specimens by themselves. For the participants aged between 13 to 80 years, it was easy to self-collect specimens with the aid of an instruction guide.

We only enrolled participants from March to June 2021. Therefore, it is expected that patients with the Delta variant (B.1.617.2) were rarely included in our study because although this variant was first identified within the local community in April 2021 [22], it was not until July that it became the dominant species in Korea [23]. The Delta variant showed higher viral loads than other variants of concern (VOC) of the SARS-CoV-2 virus [24-26]; thus, the diagnostic performance of the self-collection protocol may be further improved. In addition, the Omicron variant (B.1.1.529), which was designated as a VOC by the World Health Organization in November 2021, has two mutations, R203K and G204R, that are related to the increased sub-genomic RNA and high viral loads. In a retrospective analysis in Italy, the Omicron variant was associated with a higher nasopharyngeal viral load than the alpha variants. In addition, it was confirmed that the Omicron variants could be detected using the Allplex SARS-CoV-2 Assay (Seegene, Korea) [27].

There were several limitations in our study. First, we assigned a selective number of people to the patients with COVID-19 and control patients. Low prevalence of COVID-19 patients in the real world may lower the positive predictive values and Cohen's kappa coefficient (κ) of self-collected specimens [15]. Second, our study included a relatively small sample size without the Delta variant or the Omicron variant and validation with more patients with new variants is needed.

In our study, the overall positive agreement of self-collected specimens was slightly lower than in previous reports of self-sampling [4, 17]. However self-collected nasal and oral swab specimens showed good agreements in the subgroup analysis with Ct values of 30 as the cut-off for positivity; although agreements of saliva specimens were similar after a change in the positivity cut-off. In addition, self-collection was feasible for most of the participants and the specimens were stable for up to 5 days at room temperature and 9 days in refrigeration. That considered, self-collected specimens for the early diagnosis of SARS-CoV-2 infection can increase the testing accessibility and convenience of potentially infected individuals and reduce the exposure of HCWs and other patients to COVID-19. Therefore, it is necessary to encourage the use of self-collection methods for the early diagnosis of COVID-19 during this pandemic.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Pooled results of SARS-CoV-2 detection in 50 COVID-19 patients with HCW-collected nasopharyngeal swab specimens, self-collected combo swab specimens, and self-collected saliva specimens

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Supplementary Table 2

SARS-CoV-2 nucleic acid detection results for paired nasopharyngeal swabs collected by the HCWs and self-collected combo swabs or saliva samples within symptomatic patients

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Supplementary Table 3

Distribution of the age of patients with matching and mismatching results between self-collected samples collected and samples collected by HCWs

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Supplementary Table 4

ICC and 95% CI between the Ct values of the self-collected and HCW-collected samples

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REFERENCES

1. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, Yuan ML, Zhang YL, Dai FH, Liu Y, Wang QM, Zheng JJ, Xu L, Holmes EC, Zhang YZ. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265-9.
PUBMED | CROSSREF
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
PUBMED | CROSSREF
3. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17:181-92.
PUBMED | CROSSREF
4. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395:565-74.
PUBMED | CROSSREF
5. World Health Organization (WHO). Diagnostic testing for SARS-CoV-2: Interim guidance, 11 september 2020. Available at: https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2. Accessed 14 January 2022.
6. Karim SSA, Karim QA. Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. Lancet 2021;398:2126-8.
PUBMED | CROSSREF
7. Centers for Disease Control and Prevention (CDC). COVID-19 interim guidelines for collecting and handling of clinical specimens for COVID-19 testing. Updated October 25 2021. Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html. Accessed 14 January 2022.

8. Tu YP, Jennings R, Hart B, Cangelosi GA, Wood RC, Wehber K, Verma P, Vojta D, Berke EM. Swabs collected by patients or health care workers for SARS-CoV-2 testing. N Engl J Med 2020;383:494-6.
PUBMED | CROSSREF
9. Altamirano J, Govindarajan P, Blomkalns AL, Kushner LE, Stevens BA, Pinsky BA, Maldonado Y. Assessment of sensitivity and specificity of patient-collected lower nasal specimens for severe acute respiratory syndrome coronavirus 2 testing. JAMA Netw Open 2020;3:e2012005.
PUBMED | CROSSREF
10. McCulloch DJ, Kim AE, Wilcox NC, Logue JK, Greninger AL, Englund JA, Chu HY. Comparison of unsupervised home self-collected midnasal swabs with clinician-collected nasopharyngeal swabs for detection of SARS-CoV-2 infection. JAMA Netw Open 2020;3:e2016382.
PUBMED | CROSSREF
11. Kojima N, Turner F, Slepnev V, Bacelar A, Deming L, Kodeboyina S, Klausner JD. Self-collected oral fluid and nasal swabs demonstrate comparable sensitivity to clinician collected nasopharyngeal swabs for coronavirus disease 2019 detection. Clin Infect Dis 2021;73:e3106-9.
PUBMED | CROSSREF
12. Shakir SM, Barker AP, Hillyard DR, Gilmore N, Barrett JW, Orlandi RR, Hanson KE. Combined self-collected anterior nasal and oropharyngeal specimens versus provider-collected nasopharyngeal swabs for the detection of SARS-CoV-2. J Clin Microbiol 2020;59:e02291-20.
PUBMED | CROSSREF
13. Kagan RM, Rogers AA, Borillo GA, Clarke NJ, Marlowe EM. Performance of unobserved self-collected nasal swabs for detection of SARS-CoV-2 by RT-PCR utilizing a remote specimen collection strategy. Open Forum Infect Dis 2021;8:ofab039.
PUBMED | CROSSREF
14. Hanson KE, Barker AP, Hillyard DR, Gilmore N, Barrett JW, Orlandi RR, Shakir SM. Self-collected anterior nasal and saliva specimens versus health care worker-collected nasopharyngeal swabs for the molecular detection of SARS-CoV-2. J Clin Microbiol 2020;58:e01824-20.
PUBMED | CROSSREF
15. Hripcsak G, Heitjan DF. Measuring agreement in medical informatics reliability studies. J Biomed Inform 2002;35:99-110.
PUBMED | CROSSREF
16. Tsang NNY, So HC, Ng KY, Cowling BJ, Leung GM, Ip DKM. Diagnostic performance of different sampling approaches for SARS-CoV-2 RT-PCR testing: a systematic review and meta-analysis. Lancet Infect Dis 2021;21:1233-45.
PUBMED | CROSSREF
17. Manabe YC, Reuland C, Yu T, Azamfirei R, Hardick JP, Church T, Brown DM, Sewell TT, Antar A, Blair PW, Heaney CD, Pekosz A, Thomas DL; Ambulatory COVID Team. Self-collected oral fluid saliva is insensitive compared with nasal-oropharyngeal swabs in the detection of severe acute respiratory syndrome coronavirus 2 in outpatients. Open Forum Infect Dis 2020;8:ofaa648.
PUBMED | CROSSREF
18. Lee RA, Herigon JC, Benedetti A, Pollock NR, Denkinger CM. Performance of saliva, oropharyngeal swabs, and nasal swabs for SARS-CoV-2 molecular detection: a systematic review and meta-analysis. J Clin Microbiol 2021;59:e02881-20.
PUBMED | CROSSREF
19. Néant N, Lingas G, Le Hingrat Q, Ghosn J, Engelmann I, Lepiller Q, Gaymard A, Ferré V, Hartard C, Plantier JC, Thibault V, Marlet J, Montes B, Bouiller K, Lescure FX, Timsit JF, Faure E, Poissy J, Chidiac C, Raffi F, Kimmoun A, Etienne M, Richard JC, Tattevin P, Garot D, Le Moing V, Bachelet D, Tardivon C, Duval X, Yazdanpanah Y, Mentré F, Laouénan C, Visseaux B, Guedj J; French COVID Cohort Investigators and French Cohort Study groups. Modeling SARS-CoV-2 viral kinetics and association with mortality in hospitalized patients from the French COVID cohort. Proc Natl Acad Sci U S A 2021;118:e2017962118.
PUBMED | CROSSREF
20. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, Lau YC, Wong JY, Guan Y, Tan X, Mo X, Chen Y, Liao B, Chen W, Hu F, Zhang Q, Zhong M, Wu Y, Zhao L, Zhang F, Cowling BJ, Li F, Leung GM. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 2020;26:672-5.
PUBMED | CROSSREF
21. Walsh KA, Jordan K, Clyne B, Rohde D, Drummond L, Byrne P, Ahern S, Carty PG, O'Brien KK, O'Murchu E, O'Neill M, Smith SM, Ryan M, Harrington P. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect 2020;81:357-71.
PUBMED | CROSSREF
22. Hwang H, Lim JS, Song SA, Achangwa C, Sim W, Kim G, Ryu S. Transmission dynamics of the delta variant of SARS-CoV-2 infections in South Korea. J Infect Dis 2022;225:793-9.
PUBMED | CROSSREF

23. Kim IH, Park AK, Lee H, Kim J, Kim DH, Kim JA, No JS, Lee CY, Woo S, Lee J, Rhee JE, Kim EJ. July 2021 status and characteristics of the covid-19 variant virus outbreak in the Republic of Korea. Public Healthy Weekly Report 2021;14:2555-60.

24. Teyssou E, Delagrèverie H, Visseaux B, Lambert-Niclot S, Brichler S, Ferre V, Marot S, Jary A, Todesco E, Schnuriger A, Ghidaoui E, Abdi B, Akhavan S, Houhou-Fidouh N, Charpentier C, Morand-Joubert L, Boutolleau D, Descamps D, Calvez V, Marcelin AG, Soulie C. The Delta SARS-CoV-2 variant has a higher viral load than the Beta and the historical variants in nasopharyngeal samples from newly diagnosed COVID-19 patients. J Infect 2021;83:e1-3.
PUBMED | CROSSREF

25. Singanayagam A, Hakki S, Dunning J, Madon KJ, Crone MA, Koycheva A, Derqui-Fernandez N, Barnett JL, Whitfield MG, Varro R, Charlett A, Kundu R, Fenn J, Cutajar J, Quinn V, Conibear E, Barclay W, Freemont PS, Taylor GP, Ahmad S, Zambon M, Ferguson NM, Lalvani A; ATACCC Study Investigators. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. Lancet Infect Dis 2022;22:183-95.
PUBMED | CROSSREF

26. Kissler SM, Fauver JR, Mack C, Tai CG, Breban MI, Watkins AE, Samant RM, Anderson DJ, Metti J, Khullar G, Baits R, MacKay M, Salgado D, Baker T, Dudley JT, Mason CE, Ho DD, Grubaugh ND, Grad YH. Viral dynamics of SARS-CoV-2 variants in vaccinated and unvaccinated persons. N Engl J Med 2021;385:2489-91.
PUBMED | CROSSREF

27. Salvagno GL, Henry BM, Pighi L, De Nitto S, Montagnana M, Lippi G. SARS-CoV-2 Omicron infection is associated with high nasopharyngeal viral load. J Infect 2022;84:834-72.
PUBMED | CROSSREF