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Note

Viral load may impact the diagnostic performance of nasal swabs in nucleic acid amplification test and quantitative antigen test for SARS-CoV-2 detection

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ABSTRACT

Introduction: Compared to nasopharyngeal swabs (NPS), there has been insufficient evaluation of the diagnostic performance of nasal swabs (NS) for the detection of severe acute respiratory coronavirus 2 (SARS-CoV-2) in the nucleic acid amplification test (NAAT) and quantitative SARS-CoV-2 antigen test (QAT).

Methods: We prospectively compared healthcare worker-collected and flocked NS within nine days after symptom onset to paired NPS to detect SARS-CoV-2 in NAAT and QAT on the fully automated Lumipulse system. The agreement between sample types was evaluated, and cycle threshold (Ct) values and antigen levels were used as surrogate viral load measures.

Results: Sixty sets of NPS and NS samples were collected from 40 patients with COVID-19. The overall agreements between NAAT and QAT samples were 76.7% and 65.0%, respectively. In NAAT, the Ct value of NS was significantly higher, 5.9, than that of NPS. Thirty-nine (95.1%) NS tested positive in 41 positive-paired NPS with Ct $\leq$ 30. The negative correlation was observed between antigen levels of NS in QAT and Ct values of NS in NAAT ($r = -0.88$). In QAT, the antigen level of NS was significantly lower than that of NPS. Thirty-six (90.0%) NS tested positive in 40 positive-paired NPS with antigen levels >100 pg/mL, which were collected significantly earlier than those with antigen levels ≤100 pg/mL.

Conclusions: In NAAT and QAT, NS had limited performance in detecting SARS-CoV-2 compared to NPS. However, NS may be helpful for patients with COVID-19 with high viral loads or those in the early stages of the illness.

Diagnostic testing is essential in controlling the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. The World Health Organization recommends nasopharyngeal swabs (NPS) as reliable samples to detect the virus [1]. Meanwhile, nasal swabs (NS) and saliva, which are comfortable and effortless to collect and can be self-collected, have been used as alternative sample types to reduce healthcare workers' exposure to the virus. Many researchers have evaluated NS performance for nucleic acid amplification test (NAAT). Some studies showed acceptable results [2,3], while others found them inferior to NPS [4,5]. The criteria for study subjects and sample collection methods differed from study to study, and there has not been sufficient evidence even at this time. In addition, a new quantification reagent for detecting SARS-CoV-2 antigens has been developed. The quantitative SARS-CoV-2 antigen test (QAT) has a high diagnostic performance comparable to that of NAAT and has been used in large hospitals and airport quarantine in Japan because of its rapidity, low cost, and simplicity [6]. However, NS performance in QAT is currently unclear. Thus, in this study, we aimed to evaluate the diagnostic

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Table 1
Diagnostic performance of nasal swabs and nasopharyngeal swabs in nucleic acid amplification test and quantitative SARS-CoV-2 antigen test (n = 60).

a. NAAT between NPS and NS

		NAAT with NS		concordance rate (%) (95% CI)	kappa coefficient (95% CI)
		+	−		
NAAT with NPS	+	45	14	76.7 (66.0–87.3)	0.10 (−0.08–0.27)
	−	0	1		

b. QAT between NPS and NS

		QAT with NS		concordance rate (%) (95% CI)	kappa coefficient (95% CI)
		+	−		
QAT with NPS	+	39	21	65.0 (52.9–77.0)	NA
	−	0	0		

c. NAAT and QAT with NPS

		QAT with NPS		concordance rate (%) (95% CI)	kappa coefficient (95% CI)
		+	−		
NAAT with NPS	+	59	0	98.3 (95.0–100.0)	NA
	−	1	0		

d. NAAT and QAT with NS

		QAT with NS		concordance rate (%) (95% CI)	kappa coefficient (95% CI)
		+	−		
NAAT with NS	+	39	6	90.0 (82.4–97.5)	0.76 (0.59–0.94)
	−	0	15		

NAAT, nucleic acid amplification test; QAT, quantitative SARS-CoV-2 antigen test; NPS, nasopharyngeal swab; NS, nasal swab; CI, confidence interval; NA, not applicable.

performance of NS for NAAT and QAT compared to that of NPS.

We conducted a prospective observational study of adult patients with COVID-19 admitted to Sapporo Medical University Hospital between October 2020 and September 2021. The researchers collected NPS and NS at admission and on multiple occasions up to nine days after the onset of symptoms if consent was obtained and tested them using the SARS-CoV-2 NAAT and QAT. Both samples were collected using Copan FLOQSwab and a sterile tube containing 3 mL of Universal Transport Medium (UTM) (Copan Italia s.p.a, Brescia, Italy). Firstly, for NS sampling, one researcher inserted a swab 2–3 cm into the anterior nostril and rotated it along the nasal mucosa for 10 s. The other anterior nostril was sampled in the same way with the same swab. Then, NPS was sampled with a different swab. All sampling was performed by a single researcher, a medical doctor, to prevent sampling technique bias. Just after the samples were collected, we performed the NAAT and QAT simultaneously using the fresh samples. The NAAT was performed on a LightCycler480 System (Roche Diagnostics K.K., Basel, Switzerland) using the Ampdirect™ 2019-nCoV Detection Kit (Shimadzu Corporation, Kyoto, Japan) [7]. The QAT was performed on the fully automated Lumipulse L2400 (Fujirebio Inc., Tokyo, Japan) using Lumipulse Presto SARS-CoV-2 Ag (Fujirebio Inc., Tokyo, Japan) [8]. According to the manufacturer's protocol, samples with ≥1.0 pg/mL and <10.0 pg/mL were re-tested and judged as positive if >1.34 pg/mL. Statistical analyses were performed using JMP Pro15 statistical software (SAS Institute, Cary, NC, USA). Compared to NPS tests, the NS tests' diagnostic accuracy was evaluated with the agreement and kappa coefficient. As appropriate, continuous data were analyzed using a *t*-test, paired *t*-test, or the Mann–Whitney *U* test. A two-tailed *p*-value < 0.05 was considered statistically significant. The Clinical Research Review Committee of Sapporo Medical University Hospital approved this research (approval number: 322-167). We obtained written informed consent from all patients included in this study.

Sixty paired samples (NPS and NS) were collected from 40 patients with COVID-19 at a median of six days (interquartile range [IQR], 4–7 days) after symptom onset. The median age was 62 years (IQR, 46–69 years), and 27 of the patients were men. We evaluated the agreement between the NPS and NS tests. The concordance rate in NAAT was 76.7% (95% confidence interval [CI]: 66.0–87.3) with a kappa coefficient of 0.10 (95% CI: −0.08–0.27) (Table 1a). In the QAT, the concordance rate was 65.0% (95% CI: 52.9–77.0) (Table 1b).

We compared the characteristics of positive and negative NS samples. There was no significant difference in the distribution of the day of sample collection after onset (positive NS samples: mean 6.0 ± 2.0 days, negative NS samples: mean 6.5 ± 2.0 days; $p = 0.43$). However, negative NS samples in NAAT had significantly higher cycle threshold (Ct) values in paired NPS samples than positive NS samples ($p < 0.01$, Fig. 1a). Additionally, among 44 positive paired samples in NAAT, Ct values of NS were significantly higher than those of NPS, with a difference of 5.9 (95% CI: 3.7–8.1, $p < 0.01$) (Fig. 1b). Of the 41 samples with a Ct value of NPS ≤30, 39 tested positive for NS, with a positive agreement of 95.1% (95% CI; 90.1–99.3), while 6 (33.3%) of 18 samples with a Ct value of NPS >30 tested positive for NS. There was no significant difference in the distribution of the day of sample collection between the samples with Ct values of NPS ≤30 and >30 (5.8 ± 0.3 days vs. 6.6 ± 0.4 days after onset, $p = 0.17$). Moreover, the negative correlation was observed between antigen levels of NS in QAT and Ct values of NS in NAAT ($r = -0.88$, Fig. 2a). Antigen levels in QAT seemed to reflect Ct values and viral loads. Negative NS samples in QAT had significantly lower antigen levels in NPS samples than positive NS samples ($p < 0.01$, Fig. 2b). Among the 39 positive paired samples in QAT, antigen levels of NS were significantly lower than those of NPS ($p < 0.01$, Fig. 2c). Of the 40 samples with antigen levels of NPS >100 pg/mL, 36 tested positive for NS, with a positive agreement of 90.0% (95% CI: 84.1–95.8), while 3 (15.0%) of 20 samples with antigen levels of NPS ≤100 pg/mL tested positive for NS. There was a significant difference in the distribution of the day of sample collection between the samples with antigen levels of

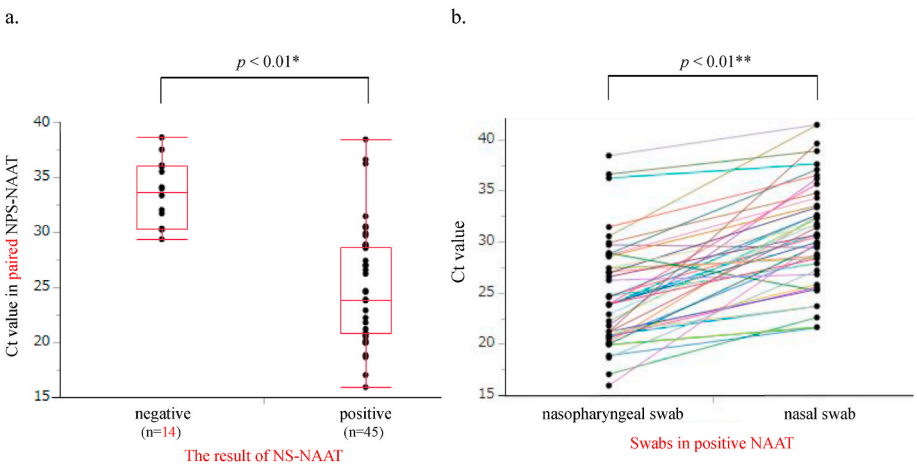

Fig. 1. a. The difference of Ct values in paired NPS-NAAT between positive and negative NS-NAAT
b. The difference of Ct values in positive NAAT between nasopharyngeal swabs and nasal swabs (n = 44)
NAAT, nucleic acid amplification test
NS-NAAT, nucleic acid amplification test using nasal swabs
NPS-NAAT, nucleic acid amplification test using nasopharyngeal swabs
* Mann–Whitney *U* test was performed.
** Paired *t*-test was performed.

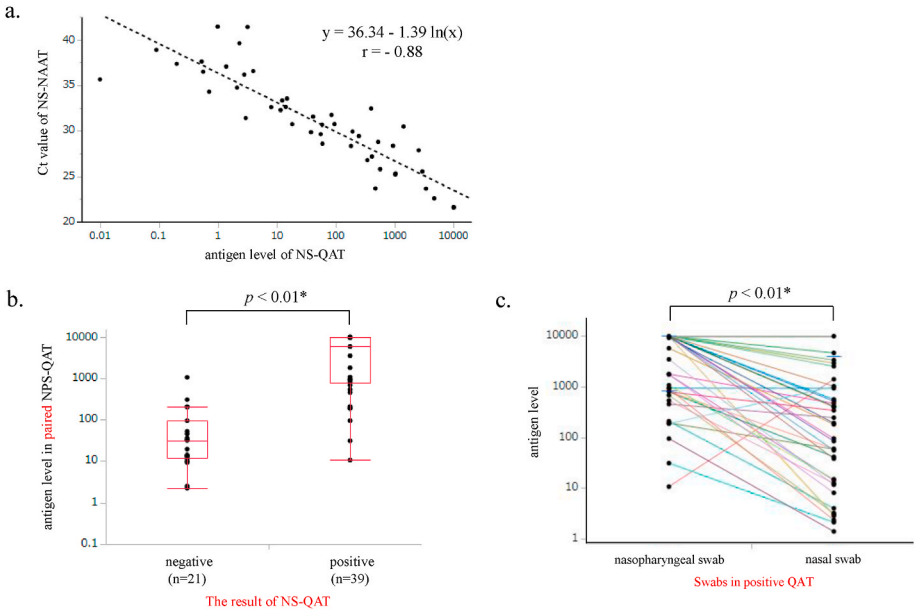

Fig. 2. a. Correlation between antigen levels of NS-QAT and Ct values of NS-NAAT (n = 45)
b. The difference of antigen levels in paired NPS-QAT between positive and negative NS-QAT
c. The difference in antigen levels in positive QAT between nasopharyngeal swabs and nasal swabs (n = 39)
QAT, quantitative antigen test
NS-QAT, quantitative antigen test using nasal swabs
NPS-QAT, quantitative antigen test using nasopharyngeal swabs
NS-NAAT, nucleic acid amplification test using nasal swabs
* Mann–Whitney *U* test was performed.

NPS >100 pg/mL and ≤100 pg/mL (5.6 ± 1.8 days vs. 7.0 ± 1.9 days after onset, $p < 0.01$).

Next, we evaluated the agreement between the NAAT and QAT using the same sample types. The concordance rates in NPS and NS were 98.3% (95% CI: 95.0–100.0) and 90.0% (95% CI: 82.4–97.5), respectively (Table 1c and d). The kappa coefficient in NS was 0.76 (95% CI: 0.59–0.94] (Table 1d). The NAAT and QAT results indicated high agreement when the same sample type was used.

In our study, we evaluated the diagnostic performance of NS for NAAT and QAT for SARS-CoV-2 compared to that of NPS. Both NAAT and QAT indicated limited agreement between NPS and NS. NS had a worse performance in detecting SARS-CoV-2 than NPS among patients with low viral loads. However, NS may be a potential sample type in the early stages of illness.

Lee et al. performed a pooled meta-analysis of 11 studies using NS and found a lower positive rate (82%) for NS than for NPS (98%) [9]. The positive rate of NS was also lower, but not significantly, for collection using unflocked swabs, from both nares, by healthcare workers, and more than seven days after the onset. Although the conditions of sample collection varied by study, they suggested that the viral loads in the samples had a significant impact on NS performance. A similar finding was observed in our study, with NS having a significantly higher Ct value (5.9) in NAAT than NPS among paired samples taken simultaneously from the same patients. In addition, there have been no reports of NS studies on QAT, which has a diagnostic performance comparable to that of NAAT [6]. Our study indicated that NS's positivity rate and antigen levels were also significantly lower in QAT than NPS. These findings suggest a lower viral burden in the nasal region than in the nasopharynx. NS provides the advantages of comfort, effortless technique, and safety in sample collection, particularly for children, as well as possible self-collection [10]. Therefore, the NS is an alternative sample type. However, false-negative results may miss true-positive patients due to limited sensitivity, which would have a significant clinical impact, especially in hospital cases. Given the available medical resources, NPS is preferable whenever possible.

However, NS may have sufficient diagnostic performance in patients with COVID-19 with high viral loads in the upper respiratory tract. Pinninti et al. indicated that 94% of the corresponding NS were positive for NAAT among patients with positive NPS with Ct ≤30 and 41% among those with Ct >30 [11]. Due to the high viral loads in the early stage of the illness [12], some studies have suggested that NS might be an alternative to NPS in NAAT at this time [11,13]. Similar results were obtained in our study; however, there were no significant differences in the distribution of the day of sample collection in NAAT, suggesting that

other factors may have influenced viral loads. Furthermore, we also compared the NS performance in QAT using a cutoff value of 100 pg/mL of antigen levels for NPS, which would be the antigen level a week after disease onset in a previous study [14]. In our study, samples with NPS >100 pg/mL antigen levels were collected significantly earlier, and NS had sufficient diagnostic performance. Therefore, if we choose the appropriate situation in a community setting or the early stages of the illness, NS may be a potentially helpful sample type.

Our study has several limitations. This study had a small sample size and was conducted at a single institution. Only symptomatic adult patients were enrolled, and the NS performance in asymptomatic patients and children was not evaluated. In addition, only unvaccinated patients were included in this study, and NS performance in patients who were fully vaccinated or infected with variants was unclear. However, several researchers have reported that the viral load does not differ according to vaccination status or variant type [15]. Further research in various situations is required.

In conclusion, NS would be less reliable than NPS for detecting SARS-CoV-2; however, NS may provide a diagnostic performance comparable to NPS in patients with high viral loads. Sample types should be selected based on the advantages and timing of sample collection and diagnostic accuracy, and the results should be appropriately interpreted according to the situation.

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Authorship statement

All authors met the ICMJE authorship criteria. YF, SN, KA, KK, and ST contributed to the organization and coordination of the trial. YF was the chief investigator and was responsible for data analysis. All authors developed the trial design, conducted the investigation, and contributed to writing the final manuscript.

Declaration of competing interest

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