




Nasal swab as an alternative specimen for the detection of severe acute respiratory syndrome coronavirus 2

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Abstract

Background and Aims: The coronavirus disease 2019 (COVID-19) has brought serious threats to public health worldwide. Nasopharyngeal, nasal swabs, and saliva specimens are used to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, limited data are available on the performance of less invasive nasal swab for testing COVID-19. This study aimed to compare the diagnostic performance of nasal swabs with nasopharyngeal swabs using real-time reverse transcription polymerase chain reaction (RT-PCR) considering viral load, onset of symptoms, and disease severity.

Methods: A total of 449 suspected COVID-19 individuals were recruited. Both nasopharyngeal and nasal swabs were collected from the same individual. Viral RNA was extracted and tested by real-time RT-PCR. Metadata were collected using structured questionnaire and analyzed by SPSS and MedCalc software.

Results: The overall sensitivity of the nasopharyngeal swab was 96.6%, and the nasal swab was 83.4%. The sensitivity of nasal swabs was more than 97.7% for low and moderate C_t values. Moreover, the performance of nasal swab was very high (>87%) for hospitalized patients and at the later stage >7 days of onset of symptoms.

Conclusion: Less invasive nasal swab sampling with adequate sensitivity can be used as an alternative to nasopharyngeal swabs for the detection of SARS-CoV-2 by real-time RT-PCR.

KEYWORDS

COVID-19, nasal swab, nasopharyngeal swab, real time RT-PCR, SARS-CoV-2, sensitivity

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly worldwide, causing more than 649 million confirmed cases and 6.6 million death till November 2022 (Worldometer,

November 2022). Considering the high transmissibility of SARS-CoV-2 and the financial toll on healthcare systems, early and precise detection is critical to its control.¹ Many asymptomatic SARS-CoV-2 patients, before being identified as symptomatic, have interacted with healthy people due to the lack of appropriate detection assays.²

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Several sampling methods and commercially available kits are used to detect SARS-CoV-2. Nasopharyngeal swab is used as the gold standard recommended by World Health Organization (WHO).^{3,4} However, specimen collection from nasopharynx can be uncomfortable and relatively invasive procedure and may cause sneezing, coughing, and even bleeding. This may also produce aerosols which pose a risk of infection to healthcare personnel. In contrast, nasal swab, saliva, and oropharyngeal (throat) swabs are used as alternatives.⁵⁻⁸ Especially nasal swab has been used as a preferred specimen for detecting many respiratory viral RNA, as it can avoid the uncomfortable sampling procedure.⁹⁻¹¹ Additionally, it can be self-collected which is quicker, more bearable, and reduce the use of stringent personal protective equipment (PPE). As a result, self-collected nasal swab may encourage patients to provide specimen and can help in early detection and prevent transmission.^{12,13} Thus, nasal swab is more suitable than nasopharyngeal sampling for mass screening.^{14,15}

The literature review and meta-analyses currently showed that nasal swab had parallel or acceptable sensitivities compared with nasopharyngeal swab⁶ with some inconsistent results.^{12,13,16-19} Most of the studies were based on the limited number of specimens¹⁹⁻²¹ focused on particular groups, especially adults (above 18 years),^{13,22} used nasal swabs in combination with other types,^{13,17} or symptomatic cases only.²³ Moreover, the performance of nasal swabs in these studies was dependent on collection media and procedure.⁶ In this study, we report a comparative analysis of the overall performance of nasal swab and nasopharyngeal swab based on viral load, diseases severity, hospitalization, and incubation periods of SARS-CoV-2.

2 | MATERIALS AND METHODS

2.1 | Ethical clearance

This study was approved by the icddr,b Institutional Review Board (PR-21065). We obtained informed consent from all the participants before collecting specimen and data. Proper biosafety and biosecurity protocols were maintained during specimen collection and transportation.

2.2 | Participant enrollment and specimen collection

A total of 449 COVID-19 suspected individuals were recruited from February 6 to March 7, 2022. The following enrollment criteria were set for the study participants: (i) all age groups, and either sex; (ii) acute onset of fever or cough or any three or more of the presented signs and symptoms recommended by WHO for COVID-19 testing; (iii) both hospitalized and community people; (iv) both symptomatic and asymptomatic cases who visited the health facility. Even though no infants or babies attended our clinic for COVID-19 testing in the present study. The patients were enrolled at different time points

after infections and we categorized them into three arms based on the onset of illness at sample collection: 0-3 days, 4-7 days, or > 7 days of onset of symptoms.

Trained nurses and medical technologists collected nasopharyngeal and nasal swabs from same individuals following the standard sample collection protocol and placed in a separate tube of 1 mL viral transport media for real-time reverse transcription polymerase chain reaction (RT-PCR). Then the specimens were stored at the refrigerator for 2-8°C until tested.

2.3 | Real-time RT-PCR

Viral RNA was extracted from nasopharyngeal swabs and nasal swab using Qiagen miniElute Viral RNA extraction kit. COVID-19 detection was performed by using a WHO-recommended, semi-quantitative, probe-based real-time RT-PCR assay. In brief, we prepared the real-time RT-PCR reaction mixtures using iTaq™ Universal Probes and One-Step Reaction Mix (Bio-Rad Laboratories, Inc.) in CFX96 Touch™ Real-time PCR Detection System (Bio-Rad Laboratories, Inc.). In this PCR system, two genes (RdRp and N genes) of SARS-CoV-2 were targeted and tested according to the protocol suggested by the Chinese Center for Disease Control and Prevention (China CDC) and WHO.^{24,25}

2.4 | Data analysis

If either nasopharyngeal or nasal swab was positive by real-time RT-PCR ($C_t < 37$), the specimen was regarded as true positive. Data were analyzed using SPSS version 22.0. In addition, we calculated the sensitivity, specificity, and accuracy with a 95% confidence interval (95% CI) through online based-software MedCalc (https://www.medcalc.org/calc/diagnostic_test.php). The sensitivity of nasopharyngeal swab or nasal swab is the ability of detecting true positive individuals who had confirmed SARS-CoV-2 using real-time PCR (positive either in nasopharyngeal swab or nasal swab).

The specificity of nasopharyngeal swab or nasal swab is the ability of detecting the negative individuals who were confirmed negative for SARS-CoV-2 using real-time PCR (negative for both nasopharyngeal swab and nasal swab).

The formula was: Diagnostic sensitivity = $a/(a+c) \times 100$ (%) (95% CI), specificity = $d/(b+d) \times 100$ (%) (95% CI) and accuracy = $a+d/(a+b+c+d) \times 100$ (%) (95% CI); where a , true positive (TP); b , false positive (FP); c , false-negative (FN); and d , true negative (TN).

3 | RESULTS

3.1 | Metadata analysis of the study participants

Among 449 suspected cases, (230 hospitalized, 219 nonhospitalized), 279 were male and 170 female and their average age was

41.19 ± 18.25 years. The highest number of the participants were within 18–30 years ($n = 125$) followed by 31–40 years ($n = 100$). Regarding the onset of symptoms, 83, 180, and 168 individuals submitted their specimens within 0–3 days, 4–7 days, and more than 7 days, respectively. Considering different clinical features of the suspected cases, cough (66%) was manifested as the highest followed by runny nose (62.8%), headache (39.4%), and fever (38.5%). Moreover, 124 individuals had comorbid conditions and 18 were asymptomatic (Table 1).

TABLE 1 Demographic and clinical data.

Metadata	Participants, $n = 449$
Sex ($n, \%$)	
Male	279 (62)
Female	170 (38)
Age group (years) ($n, \%$)	
<5	1 (0.2)
5–17	26 (5.8)
18–30	125 (27.8)
31–40	100 (22.3)
41–50	62 (13.8)
51–60	56 (12.5)
>60	79 (17.6)
Age ^a	41.19 ± 18.25
Clinical features	
Fever ($n, \%$)	173 (38.5)
Cough ($n, \%$)	310 (66)
Runny nose ($n, \%$)	282 (62.8)
Sore throat ($n, \%$)	73 (16.3)
Shortness of breath ($n, \%$)	75 (16.7)
Chills ($n, \%$)	16 (3.6)
Vomiting ($n, \%$)	35 (7.8)
Nausea ($n, \%$)	37 (8.2)
Diarrhea ($n, \%$)	20 (4.5)
Altered smell ($n, \%$)	27 (6)
Headache ($n, \%$)	177 (39.42)
Conjunctivitis ($n, \%$)	4 (0.9)
Muscle aches ($n, \%$)	105 (23.4)
Joint aches ($n, \%$)	58 (12.9)
Loss of appetite ($n, \%$)	96 (21.4)
Altered consciousness ($n, \%$)	3 (0.7)
Onset of symptoms	
0–3 days	83 (18.5)
4–7 days	180 (40.1)

TABLE 1 (Continued)

Metadata	Participants, $n = 449$
>7 days	168 (37.4)
No symptoms	18 (4.0)
Hospitalization	
Yes	230 (70)
No	219 (30)
Comorbidities ($n, \%$)	
Yes	124 (27.6)
No	325 (62.4)
Body temperature (°C) ^a	36.39 ± 0.58
Respiratory rate (/minutes) ^a	19.49 ± 1.76
Pulse rate (/minutes) ^a	82.25 ± 9.88
Systolic blood pressure (mm/Hg) ^a	115.45 ± 11.11
Diastolic blood pressure (mm/Hg) ^a	75.88 ± 8.10
Oxygen saturation (%) ^a	98.10 ± 1.23

Note: Comorbidity includes obesity, cancer, diabetes, asthma, heart diseases, lung diseases (nonasthma), liver diseases, kidney diseases.

Abbreviation: n , study participant.

^aResults presented as mean ± SD.

3.2 | Diagnostic performance between nasopharyngeal and nasal swab

The diagnostic performance between nasopharyngeal and nasal swab is shown in Figure 1 and Supporting Information: Table S1. We compared the sensitivity, specificity, and accuracy of nasopharyngeal and nasal swabs by testing through the gold standard method real-time RT-PCR. The overall sensitivity of nasopharyngeal and nasal swabs was 96.6% and 83.4%, respectively (Figure 1). The specificity for both nasopharyngeal and nasal swabs was 100% and the accuracy was 98.4% and 92.4%, respectively (Supporting Information: Table S1).

The performance of nasopharyngeal and nasal swabs was analyzed based on real-time PCR-generated C_t values. The sensitivity of nasopharyngeal swabs, in case of low $C_t < 24$, was 100% while nasal swabs showed 97.7%. Interestingly, the sensitivity was 100% for both of nasopharyngeal and nasal swabs with moderate C_t values, 24–31. Although sensitivity for both specimens was declined with high C_t values, >31, the nasopharyngeal swabs showed significantly higher (95.4%) performance compared with nasal swabs (74%) (Figure 1).

We also evaluated the sensitivity based on the onset of symptoms. Participants with 0–3 days of symptoms showed 95.2% sensitivity for nasopharyngeal swabs and 67% for nasal swabs. In case of 4–7 days of symptoms, nasopharyngeal and nasal swabs showed the sensitivity 94.1% and 83.8%, respectively. We observed

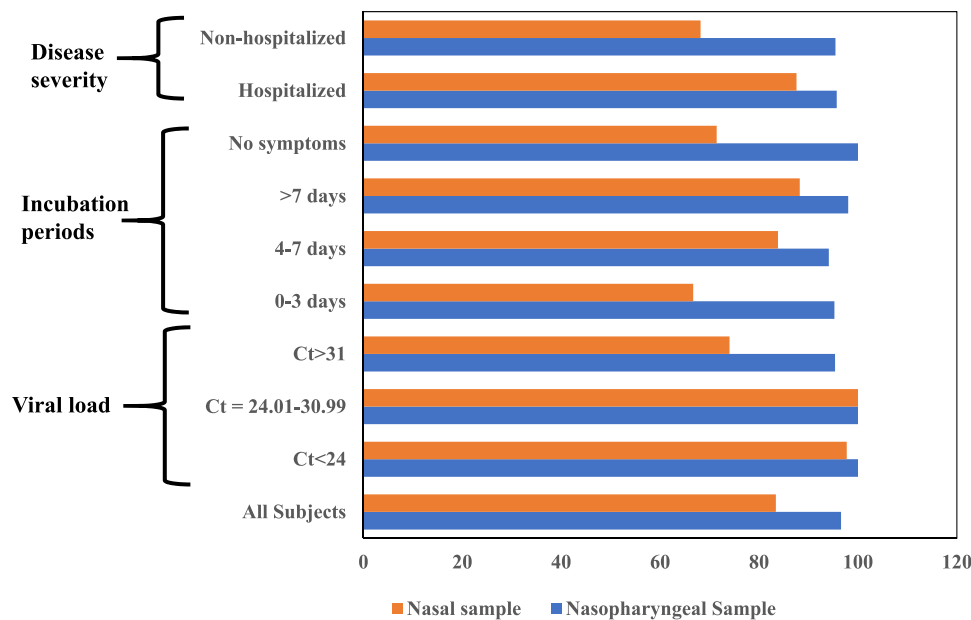


FIGURE 1 Comparison of sensitivity of nasopharyngeal and nasal swabs. Sensitivity was calculated by, True positive/(True positive+ False negative) through MedCalc Software.

the highest sensitivity among the group with >7 days of symptoms; 98% for nasopharyngeal swab, and 88.2% for nasal swab. In case of asymptomatic individuals, the sensitivity of nasopharyngeal swabs was 100% while only 71.4% for nasal swabs (Figure 1).

The sensitivity of nasopharyngeal swabs was almost similar for both of the hospitalized and nonhospitalized patients (96%). Whereas, the sensitivity of nasal swabs was found higher in severe cases for example, hospitalized patients (87.6%) compared with the nonhospitalized patients (68.2%) (Figure 1).

4 | DISCUSSION

In this study, we compared the diagnostic performance of nasopharyngeal and nasal swabs for the COVID-19 detection using real-time PCR results. Although, nasopharyngeal swab was found to be more sensitive, nasal swabs could also be used as an alternative specimen type for symptomatic and severe cases with sensitivity and specificity >80%.

The performance of nasal swabs was analyzed considering different factors such as C_t value, the onset of symptoms, and disease severity. We observed that the sensitivity of the nasopharyngeal and nasal swabs was almost similar in lower to moderate C_t values due to high to medium viral load while the sensitivity of nasal swabs was declined to a greater extent with higher C_t value than nasopharyngeal swab. Different studies showed similar performance (75%–100%) of nasal swab.^{20,22,26–28} It is likely that the nasopharynx consumes the highest SARS-CoV-2 viral load. Several studies explained that the patients tested with high C_t >31 value had lower viral load and noninfectious.^{29,30} So, the low sensitivity in high C_t value will not affect the transmissibility of live SARS-CoV-2 which is observed in our study.

Based on the onset of symptoms, nasal swabs showed lower performance at the earlier stage (less than 3 days of onset of symptoms) while nasopharyngeal swabs showed higher performance all through the disease progression. Similarly, the performance of nasal swab was very low in an asymptomatic patient (73.4%).

We also observed that sensitivity and specificity of nasal swabs were higher among hospitalized patient rather than nonhospitalized patient that was similar with other studies.^{21,31} Most of the hospitalized patients had a severe illness and more chance of spreading the virus into different parts of the body. Therefore, more viruses were detected in the nasal swabs of hospitalized patients with higher sensitivity. In contrast, nonhospitalized patients had mild symptoms and the virus might be localized in the respiratory tract only. Therefore, the sensitivity of nasal swab in nonhospitalized patients was lower than in hospitalized ones. As it is difficult to collect nasopharyngeal swabs from hospitalized, especially intensive care unit (ICU)-admitted patients with auxiliary oxygen supply, nasal swab could be a right choice. Another important point from our finding is that, if the participants with low viral load are excluded from the study, the sensitivity of the nasopharyngeal and nasal swabs are almost similar in all conditions (data not shown).

Nasal swab has definite advantages over nasopharyngeal swab, such as more comfortable, less invasive, and easy for self-collection. In addition, nasal swab collection does not require trained medical staff and minimize the risk of transmission.^{32–37} Thus, nasal swab sampling may encourage more people for COVID-19 testing and reduce the transmissibility of the diseases.

The study had one limitation. We could not enroll an equal number of patients in different groups such as very few participants were enrolled in 0–3 days of symptoms onset. It might have some effects on the sensitivity of nasal swab.

In conclusion, nasal swabs can be an easy and potential alternative sample source for SARS-CoV-2 detection which is less invasive and have adequate sensitivity.

AUTHOR CONTRIBUTIONS

Kamrun Nahar: Data curation; investigation; writing—original draft. **Mst Noorjahan Begum:** Investigation; validation; writing—review & editing. **Selim Reza Tony:** Formal analysis; writing—review & editing. **Mohammad Jubair:** Data curation. **Md. Abir Hossain:** Data curation. **Yeasir Karim:** Investigation. **Abdullah Al. Faisal:** Investigation. **Mohammad Enayet Hossain:** Writing—review & editing. **Mohammed Ziaur Rahman:** Writing—review & editing. **Mustafizur Rahman:** Conceptualization; funding acquisition; methodology; project administration; supervision; writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

TRANSPARENCY STATEMENT

The lead author Mustafizur Rahman affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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