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Original Article

# Indications for SARS-CoV-2 nucleic acid amplification test for areas with low endemicity

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ABSTRACT

*Introduction:* The optimal indication for the nucleic acid amplification test (NAAT) in areas with low endemicity for coronavirus disease 2019 (COVID-19) is unclear. This study aimed to identify patients who should undergo the NAAT for COVID-19 diagnosis.

*Methods:* We retrospectively analyzed the clinical data of patients with suspected COVID-19 who underwent NAAT between October 5, 2020, and May 31, 2021 in our institution.

*Results:* A total of 1238 patients were enrolled and NAAT positive results were observed in 40 patients (3.2%). The NAAT positivity rate was 34.3% (23/67) in patients with a history of close contact and 1.5% (17/1171) in patients without a history of close contact. Olfactory/gustatory dysfunction and a history of stay in other prefectures were independent risk factors of COVID-19 in patients without a history of close contact. On the other hand, the NAAT positivity rate was only 0.7% (8/1073) in patients without olfactory/gustatory dysfunction and a history of stay in other prefectures. Among them, the group without respiratory symptoms/sign had only one NAAT-positive case (0.1%: 1/1073).

*Conclusions:* This study revealed that a history of close contact, olfactory/gustatory dysfunction, and a history of stay in other prefectures are key eligibility criteria for NAAT in areas with relatively few patients with COVID-19. On the other hand, NAAT may not be necessary in cases without all of these factors and respiratory symptoms/sign.

## 1. Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in December 2019 in China and rapidly spread as a global pandemic [1,2]. As of June 22, 2021, 178,360,849 cases were diagnosed worldwide [3]. Fever and cough are the most common symptoms of COVID-19 [4,5]. Some patients have gastrointestinal symptoms (e.g., nausea and diarrhea), headache, chest pain, and conjunctivitis [4,6]. In addition, one-third of SARS-CoV-2-infected patients are asymptomatic [7]. Since there are various symptoms of COVID-19, it is sometimes difficult to determine whether COVID-19 should be suspected in a symptomatic patient. In Kagawa prefecture, Japan, the average number of new infections per 100,000 people per week was 5.60 (range: 0.00–34.41) during this research period (from October 5, 2020 to May 31, 2021). In Japan, 15 or more new infections per 100,000 people per week is defined a high incidence rate, so our prefecture is considered to have relatively few cases of COVID-19. Especially in areas with relatively few patients such as our prefecture, the optimal indication for the nucleic acid amplification test (NAAT) is unclear. Identifying the ideal time to administer diagnostic testing is crucial because the pre-test probability is low and excessive testing wastes specimen collection, as well as resources including personal protective equipment, time to release results, and overall cost.

The aim of this study was to identify the group of patients who should undergo the NAAT for COVID-19 diagnosis and the group of patients who may forego the NAAT.

## 2. Patients and methods

This was a retrospective, single-center, observational study. The study period was from October 5, 2020, when NAAT became available in our facility, to May 31, 2021. Patients suspected of having COVID-19 and who underwent NAAT in Kagawa Rosai Hospital were enrolled. We collected the clinical data of these patients from medical records. Nasopharyngeal swab samples were analyzed using the Smart Gene®

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SARS-CoV-2 (Mizuho Medy, Co., Ltd., Saga, Japan) or Xpert® Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA) to diagnose COVID-19. Close contact was defined as living with patients with COVID-19 or contact with patients for 15 min or more. In addition, those who worked in the same workplace as patients diagnosed with COVID-19 or who attended the same school as COVID-19 diagnosed patients, were also considered to have a history of close contact in this study. This study was approved by the institutional review board of Kagawa Rosai Hospital (R3-15). Statistical analyses were performed using the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [8]. Patient characteristics were compared using Fisher's exact test for binary variables and the Mann–Whitney *U* test for continuous variables. Univariate and multivariate analyses with logistic regression models were performed to identify the risk factor(s) of COVID-19. P values $< 0.05$ were considered to indicate statistical significance.

## 3. Results

We enrolled 1238 patients who were suspected to have COVID-19 and underwent NAAT. The clinical characteristics of the patients are presented in Table 1. The median age was 69 years (interquartile range [IQR], 42–81 years), and 663 patients (53.6%) were male. The median body temperature was 37.2 °C (IQR, 36.6–38.0 °C) and oxygen desaturation was observed in 280 patients (22.6%). Oxygen desaturation was defined as an oxygen saturation ($SpO_2$) of ≤94% at room air. If oxygen was administered, this was also defined as decreased oxygenation regardless of $SpO_2$ value. The major symptoms were fever, cough, sore throat, and dyspnea. Sixty-seven patients (5.4%) had a history of close contact with patients with COVID-19, and 89 patients (7.2%) had a history of stay in other prefectures within the last 2 weeks. NAAT-positive results were observed in 3.2% of patients (40/1238). The patients in the NAAT-positive group were younger than those in the NAAT-negative group (48 vs. 70 years). Cough, sore throat, olfactory dysfunction, and gustatory dysfunction were more frequently observed in the NAAT-positive group. The percentage of patients with a history of close contact was higher in the NAAT-positive group than in the NAAT-negative group (57.5% vs. 3.7%). History of stay in other prefectures within the last 2 weeks was also more frequently observed in the NAAT-positive group (25.0% vs. 6.6%).

The NAAT positivity rate was 34.3% (23/67) in patients with a history of close contact compared to 1.5% (17/1171) in patients without a history of close contact. It is important to determine the characteristics of NAAT-positive cases among those without a history of close contact, since most patients with a history of close contact will be tested in clinical practice. We compared the characteristics of NAAT-positive cases and NAAT-negative cases in patients without a history of close contact (Table 2). The patients in the NAAT-positive group were younger than those in the NAAT-negative group (43 vs. 71 years). Cough, olfactory dysfunction, and gustatory dysfunction were more frequently observed in the NAAT-positive group. The percentage of patients with a history of stay in other prefectures within the last 2 weeks was higher in the NAAT-positive group than in the NAAT-negative group (29.4% vs. 6.5%).

In patient without a history of close contact, to identify patient conditions for which NAAT should be performed, univariate analysis was performed on the following five factors: fever, respiratory symptoms/sign, gastrointestinal symptoms, olfactory/gustatory dysfunction, and a history of stay in other prefectures (Table 3). Respiratory symptoms/sign included cough, nasal discharge, sore throat, dyspnea, and

**Table 2**
Comparison between NAAT-positive and -negative cases in patients without a history of close contact.

| Characteristics | positive (n = 17) | negative (n = 1154) | P value |
|---|---|---|---|
| Age, median (IQR) – year | 43 (28–59) | 71 (45–81) | <0.01 |
| Male sex – no. (%) | 10 (58.8) | 615 (53.3) | 0.81 |
| Body temperature, median (IQR) –°C | 37.1 (36.5–37.6) | 37.3 (36.7–38.0) | 0.32 |
| Oxygen desaturation[a] – no. (%) | 4 (23.5) | 276 (23.9) | 1.00 |
| Symptoms – no. (%) | | | |
| Fever | 13 (76.5) | 1000 (86.7) | 0.27 |
| Cough | 9 (52.9) | 246 (21.3) | <0.01 |
| Nasal discharge | 1 (5.9) | 86 (7.5) | 1.00 |
| Sore throat | 4 (23.5) | 160 (13.9) | 0.28 |
| Dyspnea | 4 (23.5) | 162 (14.0) | 0.29 |
| Olfactory dysfunction | 5 (29.4) | 2 (0.2) | <0.01 |
| Gustatory dysfunction | 5 (29.4) | 14 (1.2) | <0.01 |
| Abdominal pain | 0 (0) | 120 (10.4) | 0.24 |
| Diarrhea | 2 (11.8) | 51 (4.4) | 0.18 |
| Nausea/Vomiting | 0 (0) | 117 (10.1) | 0.40 |
| Headache | 3 (17.6) | 76 (6.6) | 0.10 |
| Myalgia/Arthralgia | 2 (11.8) | 65 (5.6) | 0.25 |
| History of stay in other prefectures – no. (%) | 5 (29.4) | 75 (6.5) | <0.01 |

IQR, interquartile range.
[a] Oxygen desaturation was defined as $SpO_2$ of ≤94% in room air or cases with oxygen supplementation.

**Table 1**
Patient characteristics.

| Characteristics | NAAT positive (n = 40) | NAAT negative (n = 1198) | All patients (n = 1238) |
|---|---|---|---|
| Age, median (IQR) – years | 48 (28–61) | 70 (43–81) | 69 (42–81) |
| Male sex – no. (%) | 26 (65.0) | 637 (53.2) | 663 (53.6) |
| Body temperature, median (IQR) –°C | 37.0 (36.4–37.6) | 37.3 (36.6–38.0) | 37.2 (36.6–38.0) |
| Oxygen desaturation[a] – no. (%) | 4 (10.0) | 276 (23.0) | 280 (22.6) |
| Symptoms – no. (%) | | | |
| Fever | 35 (87.5) | 1012 (84.5) | 1047 (84.6) |
| Cough | 19 (47.5) | 255 (21.3) | 274 (22.1) |
| Nasal discharge | 3 (7.5) | 91 (7.6) | 94 (7.6) |
| Sore throat | 11 (27.5) | 168 (14.0) | 179 (14.5) |
| Dyspnea | 6 (15.0) | 164 (13.7) | 170 (13.7) |
| Olfactory dysfunction | 5 (12.5) | 4 (0.3) | 9 (0.7) |
| Gustatory dysfunction | 5 (12.5) | 16 (1.3) | 21 (1.7) |
| Abdominal pain | 1 (2.5) | 121 (10.1) | 122 (9.9) |
| Diarrhea | 3 (7.5) | 52 (4.3) | 55 (4.4) |
| Nausea/Vomiting | 1 (2.5) | 118 (9.8) | 119 (9.6) |
| Headache | 6 (15.0) | 79 (6.6) | 85 (6.9) |
| Myalgia/Arthralgia | 6 (15.0) | 67 (5.6) | 73 (5.9) |
| History of close contact with patients with COVID-19 – no. (%) | 23 (57.5) | 44 (3.7) | 67 (5.4) |
| History of stay in other prefectures – no. (%) | 10 (25.0) | 79 (6.6) | 89 (7.2) |

NAAT, nucleic acid amplification test; IQR, interquartile range.
[a] Oxygen desaturation was defined as $SpO_2$ of ≤94% in room air or cases with oxygen supplementation.

**Table 3**
Univariate analysis of the risk of COVID-19 in patients without a history of close contact.

| Characteristics | OR | 95% CI | P value |
|---|---|---|---|
| Fever | 0.51 | 0.16–1.55 | 0.23 |
| Respiratory symptoms/sign | 2.81 | 0.91–8.67 | 0.07 |
| Gastrointestinal symptoms | 0.55 | 0.12–2.44 | 0.43 |
| Olfactory/gustatory dysfunction | 31.60 | 9.91–101.00 | <0.01 |
| History of stay in other prefectures | 5.99 | 2.06–17.50 | <0.01 |

Respiratory symptoms/sign included cough, nasal discharge, sore throat, dyspnea, and oxygen desaturation, whereas gastrointestinal symptoms included abdominal pain, diarrhea, and nausea/vomiting. CI, confidence interval; OR, odds ratio.

**Table 4**
Multivariate analysis of the risk of COVID-19 in patients without a history of close contact.

| Characteristics | OR | 95% CI | P value |
|---|---|---|---|
| Respiratory symptoms/sign | 3.45 | 1.04–11.40 | 0.04 |
| Olfactory/gustatory dysfunction | 38.50 | 10.90–135.00 | <0.01 |
| History of stay in other prefectures | 6.98 | 2.18–22.40 | <0.01 |

Respiratory symptoms/sign included cough, nasal discharge, sore throat, dyspnea, and oxygen desaturation. CI, confidence interval; OR, odds ratio.

oxygen desaturation, whereas gastrointestinal symptoms included abdominal pain, diarrhea, and nausea/vomiting. Olfactory/gustatory dysfunction and a history of stay in other prefectures were significant risk factors of COVID-19. From a multivariate analysis including the following factors: respiratory symptoms/sign, olfactory/gustatory dysfunction and a history of stay in other prefectures, olfactory/gustatory dysfunction and a history of stay in other prefectures were confirmed as significant risk factors of COVID-19 (Table 4). Respiratory symptoms/sign were also significant risk factors in multivariate analysis.

Fig. 1 shows the NAAT positivity rate in patients without a history of close contact based on the three risk factors described above. The positivity rate in patients with olfactory/gustatory dysfunction was high regardless of a history of stay in other prefectures. In addition, a history of stay in the other prefectures alone group had relatively high-positively rate (5.1%). On the other hand, the NAAT positivity rate was only 0.7% (8/1073) in patients without olfactory/gustatory dysfunction and a history of stay in other prefectures. Among them, the group without respiratory symptoms/sign had only one NAAT-positive case (0.1%: 1/1073).

## 4. Discussion

We aimed to identify patients eligible to take the NAAT for COVID-19 diagnosis. In our study, we observed a high positivity rate of NAAT (34.3%) in patients with a history of close contact. In addition, olfactory/gustatory dysfunction and a history of stay in other prefectures were independent risk factors of COVID-19 even in patients without a history of close contact. On the other hand, the positivity rate was only 0.1% in patients without all of the following factors: a history of close contact, olfactory/gustatory dysfunction, a history of stay in other prefectures, and respiratory symptoms/sign.

SARS-CoV-2 is primarily transmitted by respiratory droplets [9]. Therefore, patients identified to have a positive history of close contact is recognized candidates eligible for NAAT to identify new infections [10]. A previous study in our country reported that 58.3% of patients confirmed with COVID-19 had a history of close contact [4]. Our study demonstrated a high positively rate of NAAT in patients with a history of close contact (34.3%). Therefore, a history of close contact is the most important factor in the eligibility criteria for NAAT. Olfactory and gustatory dysfunction are common symptoms in patients with COVID-19 [11–13]. A meta-analysis reported that the prevalence of olfactory or gustatory dysfunction was estimated to be 52.73% and 44.93%, respectively [13]. Another study showed that olfactory dysfunction was strongly associated with NAAT positivity in healthcare workers with mild symptoms [14]. In our study, the association between olfactory/gustatory dysfunction and a NAAT-positive result was also strong even in patients without a history of close contact. Therefore, olfactory/gustatory dysfunction is also an important factor in the eligibility criteria for NAAT. In addition, a history of stay in other prefectures is thought to be candidate of the eligibility criteria for NAAT because our study shows that it is an independent risk factor of COVID-19. On the other hand, the positivity rate of NAAT was only 0.7% in patients with no history of close contact, no olfactory/gustatory dysfunction and no history of stay in other prefectures. Whether we should perform NAAT in this condition is thought to be controversial. Furthermore, the negativity rate reached 99.9% in patients without all of the following factors: a history of close contact, olfactory/gustatory dysfunction, a history of stay in other prefectures, and respiratory symptoms/sign. Therefore, it may not be necessary to perform NAAT on these patients in areas with low endemicity, such as our prefecture.

This study had some limitations. First, a NAAT-negative result does not indicate that a patient does not have COVID-19 because this test is not absolute and may have false-negative results [15,16]. Therefore, the NAAT positivity rate may differ somewhat from the true prevalence of COVID-19. Second, the NAAT positivity rate in each subgroup may

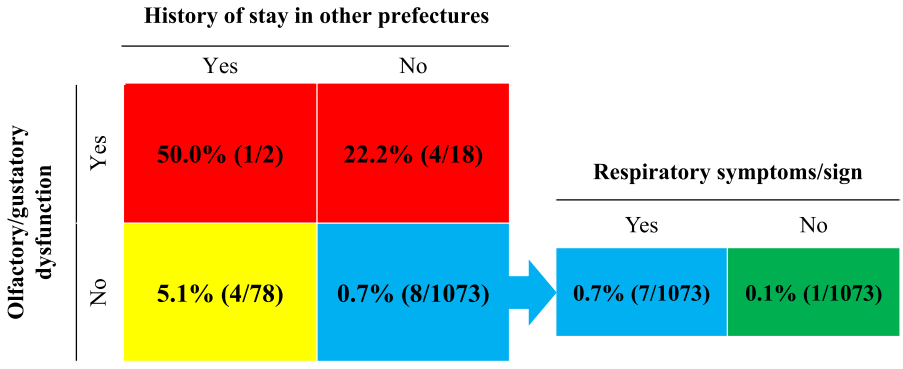


**Fig. 1.** Nucleic acid amplification test positivity rate in patients without a history of close contact based on three risk factors.

change in case of an increase in new infections. For instance, the number of newly infected people in Tokyo in March 2021 was 9,462/month, but the number of infected people increased to reach 125,606/month in August. The proportions of patients with no known route of infection in the respective months were 49.7% and 61.3%, indicating that the NAAT positivity rate of patients without a history of close contact may increase as infection increases. However, even in areas where the number of new infections is currently very high, the incidence rate may be similar to that in our area at some time. Therefore, we believe our findings are generalizable to many different populations.

## 5. Conclusions

This study revealed that a history of close contact, olfactory/gustatory dysfunction, and a history of stay in other prefectures are key eligibility criteria for NAAT in areas with relatively few patients with COVID-19. On the other hand, NAAT may not be necessary in cases without all of these factors and respiratory symptoms/sign.

## Authorship statement

All authors meet the ICMJE authorship criteria. Contributor H.H. drafted the manuscript and performed the statistical analyses. Y.T. collected clinical data. N.S., T.N. and M.M. analyzed the data and revised the manuscript. All authors contributed to the writing of the final manuscript.

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## Declaration of competing interest

I declare on behalf of my co-authors and myself that we do not have any conflict of interest to declare.

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