A Case of COVID-19 Patient with False-negative for SARS-CoV-2 of Pharyngeal Swab,
from a Chinese traveller Returning from Wuhan, Hubei Province, China, January 2020

Masahiro Ishikane, Yusuke Miyazato, Satoshi Kustuna, Tetsuya Suzuki, Satoshi Ide, Keiji
Nakamura, Shinichiro Morioka, Harutaka Katano, Tadaki Suzuki, and Norio Ohmagari

Received: April 12, 2020. Accepted: April 23, 2020.
Published online: May 29, 2020.
DOI: 10.7883/yoken.JJID.2020.240

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Short communication

A Case of COVID-19 Patient with False-negative for SARS-CoV-2 of Pharyngeal Swab, from a Chinese traveller Returning from Wuhan, Hubei Province, China, January 2020

Masahiro Ishikane1,2, Yusuke Miyazato1, Satoshi Kustuna1, Tetsuya Suzuki1, Satoshi Ide1, Keiji Nakamura1, Shinichiro Morioka1, Harutaka Katano3, Tadaki Suzuki3, Norio Ohmagari1,2

1Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan
2AMR Clinical Reference Center, Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan
3Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

Corresponding Author:

Masahiro Ishikane, M.D., Ph.D.
Disease Control and Prevention Center, National Center for Global Health and Medicine
1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan

Tel: +81-3-3202-7181
Fax: +81-3-3207-1038
E-mail: ishikanemashiro@gmail.com

**Key Words:** COVID-19; SARS-CoV-2; False-negative of pharyngeal swab

**Running Title:** False-negative for SARS-CoV-2 of pharyngeal swab
著者: 石金正裕 1,2、宮里悠佑 1、忽那賢志 1、鈴木哲也 1、井手聡 1、中村啓二 1、森岡慎一郎 1、片野晴隆 3、鈴木忠樹 3、大曲貴夫 1,2

所属: 1 国立国際医療研究センター病院 国際感染症センター
2 国立国際医療研究センター病院 国際感染症センター AMR 臨床リファレンスセンター
3 国立感染症研究所 感染病理部
Summary

We report a case of patient in Japan with Coronavirus disease 2019 (COVID-19) with false-negative of reverse transcription polymerase chain reaction for Severe Acute Respiratory Syndrome Coronavirus 2 of pharyngeal swab, from a Chinese traveller returning from Wuhan, Hubei Province, China. If a patient is clinically or epidemiologically suspected of COVID-19, appropriate infection and prevention control measures such as standard, contact, and droplet precaution are needed until the patient is proven to be true-negative.
Here, we report a case of Coronavirus disease 2019 (COVID-19) pneumonia patient in Japan with false-negative for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) of pharyngeal swab, from a Chinese traveller returning from Wuhan, Hubei Province, China. Although the possibility of false-negative of reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2 is indicated, the case reports of false-negative are limited (1). Moreover, there are limited scientific case reports of COVID-19 pneumonia patients in Japan (2). SARS-CoV-2 is the third coronavirus to emerge in the human population in the previous two decades that has put the public health institutions worldwide on high alert (3,4). The conditions of COVID-19 patients range from asymptomatic to severe including those leading to death (4-7). Since the initial report of cases on 31 December 2019, as of 11 April 2020, there are 1,610,909 laboratory-confirmed cases of SARS-CoV-2 infection including 99,690 deaths globally reported by WHO (8). The Ministry of Health, Labour, and Welfare, Japan reported 6,005 cases including 94 deaths including Diamond Princess (Cruise) of laboratory-confirmed patients with COVID-19 patient as of 11 April 2020 (9).

On 26 January 2020, a man in his early 50s sought care at the National Center for Global Health and Medicine, Tokyo, Japan, for fever, sore throat, and mild dry cough. He
was from Wuhan, Hubei Province, China, and came to Japan with his family (wife and daughter) on 22 January 2020. After arrival, he traveled from Osaka to Tokyo on a group sightseeing bus with 27 travellers from Wuhan, Hubei Province, China. His medical history included cholecystectomy due to cholecystitis in 2016. On 26 January 2020, he noticed a high fever in the daytime and sought care the same evening. He had not visited the Huanan Seafood Wholesale Market or any other live animal markets in Wuhan. He had no close contact with any person with pneumonia in China.

A physical examination revealed the following findings: systolic blood pressure, 120 mmHg; pulse, 110 beats/min; respiratory rate, 24 breaths/min; body temperature, 38.0°C; and saturation O₂, 97% (room air). His bilateral lower lung fields showed decreased respiratory sounds. His blood biochemistry profile showed 5,340 × 10⁹ leukocytes/l, hemoglobin level 13.0 g/dl, 18.7 × 10⁹ thrombocytes/l, and C-reactive protein 0.50 mg/dl. Liver and kidney function test results were within the normal range. His chest X-ray showed infiltration in the bilateral lower lung fields (Figure 1A). Computed tomography of the chest showed pan lobular centrality ground-glass opacity with bronchial wall thickening on the bilateral lower lobes (Figure 1B). On the first day, a pharyngeal swab was taken 12 hours after onset was examined for reverse transcription polymerase chain reaction (RT-PCR), but negative for SARS-CoV-2 (spike and N proteins) (10, 11). Real-
time RT-PCR for SARS-CoV-2 was performed using Quantitect probe one-step RT-PCR kit (Qiagen) (11). The results of other microbial tests using sputum culture and BioFire Film Array Respiratory Panel (BioFire Diagnostics, Inc.) were negative. Upon admission, we administered symptomatic treatment without antibiotics and antiviral drugs. His symptoms resolved after 3 days of admission, and he was discharged from the hospital on the same day without any complications on 28 January. Although he was discharged, we conducted a re-test for SARS-CoV-2 using reserved specimens from the patient since we had clinical and epidemiological reasons to suspect the patient had COVID-19 pneumonia. Reserved specimens with sputum, serum, and urine on admission day, and sputum, serum, and urine on third admission day were tested for real-time RT-PCR for SARS-CoV-2. We found that the sputum on first and 3rd admission day became positive of SARS-CoV-2 RNA on 4 February (Table 1, Figure 2).

There were two possible routes of infection: first, primary infection in Wuhan, Hubei Province, China; second, human-to-human transmission during group sightseeing bus tour in Japan. A previous study reported that the incubation period was 2–14 days (12). Our case had a 3-day close contact with the fourth laboratory-confirmed COVID-19 pneumonia patient who was a Chinese traveller returning from Wuhan, Hubei Province in Japan because they participated in the same group sightseeing bus tour from Osaka to
Tokyo (9). Our case was sitting in the seat behind the fourth laboratory-confirmed patient in the sightseeing bus. The fourth laboratory-confirmed patient arrived in Osaka on 22 January 2020 and noticed a high fever on 23 January. This patient sought care and was admitted to a hospital in the Aichi prefecture due to pneumonia. On 26 January 2020, the pharyngeal swab from the patient was shown to be positive for SARS-CoV-2 RNA. Similar to our patient, this patient did not visit the Huanan Seafood Wholesale Market or any other live animal markets in Wuhan and had no close contact with a person with pneumonia (Figure 2) (9). Similar to other reports, this indicates the possibility of human-to-human transmission in Japan (7,13).

Our case showed that a pharyngeal swab which is obtained at the early onset of the disease may be false-negative of RT-PCR for SARS-CoV-2 RNA as well as past report (1). The result on the first day using pharyngeal swab was negative, but sputum from first and third admission day were positive for SARS-CoV-2 RNA. Although the current first specimens of diagnosis for SARS-CoV-2 in Japan are sputum or nasopharyngeal swab, the first choice of that was pharyngeal swab at the time of diagnosis of our case (10). Therefore, we suggest that clinicians should take into consideration two points while making a diagnosis. First, the time period from onset of disease to the testing of the specimen should be considered. In our case, the first specimen taken 12 hours after onset
gave a negative result. A previous report estimated that the period from the onset of illness to the first medical visit ranged from 4.6 to 5.8 days (4). Second, the test result depends on the specimen used for diagnosis. A study from the USA reported that the value of the cycle threshold of a pharyngeal swab was higher than that of a nasopharyngeal swab (14). Therefore, the viral load of a nasopharyngeal swab was higher than that of a pharyngeal swab. On 24 February, the specimen for diagnosis of COVID-19 in Japan was changed from pharyngeal swab to nasopharyngeal swab, based on this report (14).

In conclusion, we report the case of a patient with COVID-19 pneumonia in Japan with false-negative for SARS-CoV-2 of pharyngeal swab, from a Chinese traveller returning from Wuhan, Hubei Province, China. It should be noted that if a patient is clinically or epidemiologically suspected of COVID-19, appropriate infection prevention and control such as standard, contact, and droplet precaution are needed until the patient is proven to be true-negative with testing using appropriate specimen and timing.

Acknowledgments

We thank all the clinical staff at our hospital for their dedication to clinical practice and patient care.
**Funding**

This work was supported by grants from the Japan National Center for Global Health and Medicine (29-1018) and the Japan Agency for Medical Research and Development, AMED (JP19fk0108104).

**Potential conflicts of interest**

All authors declare no conflicts of interest regarding this study.
References


Figure legends

Figure 1. Chest X-ray and computed tomography of the chest on admission

A. Chest x-ray with infiltration in bilateral lower lung fields

B. Computed tomography of the chest with pan lobular centrality ground-glass opacity with bronchial wall thickening on bilateral lower lobes

Figure 2. Timeline for establishing the epidemiological link of close contact with confirmed patients in a group sightseeing bus

Our patient had a 3-day close contact with the fourth laboratory-confirmed COVID-19 pneumonia patient who was a Chinese traveller in Japan returning from Wuhan, Hubei Province.
Table 1. Results of Real-Time Reverse Transcriptase-Polymerase-Chain-Reaction Testing for the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Specimen | illness Day 1 | illness Day 3 |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Real-time RT-PCR (S)</td>
<td>Real-time RT-PCR (N)</td>
</tr>
<tr>
<td>Pharyngeal swab</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Sputum</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Sputum</td>
<td>21.3 (37.8)</td>
<td>4.0 (37.3)</td>
</tr>
<tr>
<td>Serum</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Urine</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Stool</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

Data are presented as virus copies per 1µL (Ct).
Lower cycle threshold (Ct) values indicate higher viral loads.
Real-time RT-PCR (S and N) detected spike and nucleoprotein genes of SARS-CoV-2, respectively.
NT denotes not tested.