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Case Report

Three SARS-CoV-2 PCR-negative cases of COVID-19 diagnosed using isothermal amplification methods

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ABSTRACT

Coronavirus disease (COVID-19) is a viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 can be detected by polymerase chain reaction (PCR) and isothermal nucleic acid amplification tests, including loop-mediated isothermal amplification (LAMP) and nicking endonuclease amplification reaction (NEAR) tests. Although PCR is the most sensitive and specific method and is generally considered to be the gold standard, it is time-consuming and costly. Isothermal nucleic acid amplification tests have lower sensitivity and specificity than PCR, but are less time-consuming and costly. We encountered three cases of SARS-CoV-2 infection in which the isothermal amplification test was positive but the PCR test was negative on the day of admission; however, the PCR test was positive the next day. These cases showed that some COVID-19 patients can test negative by PCR but positive using isothermal nucleic acid amplification methods. As PCR tests have the possibility of false-negative results, tests that use isothermal amplification methods which can be performed in a shorter time and at a lower cost than PCR tests, may be able to diagnose patients who have false negative PCR results.

1. Introduction

Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a pandemic by the World Health Organization on March 11, 2020 [1]. Various methods are available for detecting SARS-CoV-2. Among them, polymerase chain reaction (PCR) tests are considered to be the most reliable and accurate, and are the standard for assessing the accuracy of loop-mediated isothermal amplification (LAMP), nicking endonuclease amplification reaction (NEAR), and other tests.

Although PCR is regarded as the gold standard test for diagnosing COVID-19, false-negative PCR test results have been reported [2]. Isothermal amplification methods are less expensive and can be tested more rapidly, but are considered to be less sensitive than PCR [3]. Tests that are deemed to be inaccurate based on studies using PCR as the gold standard, such as NEAR and LAMP tests, may be able to detect SARS-CoV-2 infection, even if the PCR test is negative. In the summer of 2021, all patients admitted to our hospital which was located in Tokyo were screened for SARS-CoV-2 using isothermal nucleic acid amplification tests because of a large increase in the number of COVID-19 patients in Tokyo [4]. Herein, we describe three cases of SARS-CoV-2 infection in which tests for SARS-CoV-2 were positive using isothermal amplification methods, but the PCR results were negative.

2. Case reports

2.1. Case 1

A 53-year-old man, who had not been vaccinated against COVID-19, was referred to our hospital in July 2021 because of a disturbance of consciousness. His body temperature was 37.0 °C. A nasopharyngeal swab sample was tested for SARS-CoV-2 using ID-Now® (Abbott Diagnostics, Lake Forest, IL, USA), as a screening test, which was positive. Because he had only slight fever and no other typical symptoms of COVID-19, we considered the screening test to be false positive, and he was also tested on the same day using a PCR test (GeneXpert® , Cepheid, Sunnyvale, CA, USA), which was negative. Chest computed tomography (CT) showed lung consolidation. The patient was provisionally

Abbreviations: COVID-19, coronavirus disease; Ct, cycle threshold; CT, computed tomography; E, envelope; LAMP, loop-mediated isothermal amplification; N2, nucleocapsid; NEAR, nicking endonuclease amplification reaction; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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diagnosed with COVID-19. The next day, the PCR test was repeated, and it was positive (cycle threshold [Ct]: envelope [E], 22.0; nucleocapsid [N2], 24.2), confirming the diagnosis of COVID-19. Both times, the test samples were collected by a nurse of the emergency department. The patient required 1L/min oxygen therapy, therefore, he was classified as a moderate case. He was treated with remdesivir for 5 days, dexamethasone 6mg once a day for 4 days and heparin (10,000 units a day) for 4 days. His condition improved and he was discharged after 10 days.

2.2. Case 2

A 79-year-old woman presented to our hospital in August 2021 for an endoscopic submucosal dissection of the stomach. She had a history of chronic obstructive pulmonary disease, but had only a slight cough and her body temperature was 36.4 °C. She had been fully vaccinated against COVID-19. On admission, her nasopharyngeal swab was positive for SARS-CoV-2 using Loopamp® 2019-nCoV detection kit (Eiken Chemical Co., Ltd, Tokyo, Japan), a LAMP test. We considered the result as false positive because she had no typical symptoms of COVID-19 excepting slight cough, and she was subjected to a PCR test. The PCR test (GeneXpert) was negative. Chest CT showed lung consolidation. The patient was provisionally diagnosed with COVID-19. The next day, the PCR test was repeated and was positive (Ct: E, 40.4; N2, 38.7), confirming the diagnosis of COVID-19. The tests were collected by clinical technologist. The patient required 2L/min oxygen therapy, therefore, she was classified as a moderate case. She was treated with remdesivir for 5 days. Her condition improved and she was discharged after 10 days.

2.3. Case 3

A 39-year-old man presented with vertigo in August 2021. He had undergone surgery for a cerebellar hemangioblastoma in 2008. He had been fully vaccinated against COVID-19. His body temperature was at 37.0 °C. He was diagnosed with a cerebellar cyst and was admitted to hospital. As with Case 2, the patient was screened for SARS-CoV-2 using ID-Now, which was positive. We considered the result to be false positive because he had no typical symptoms of COVID-19, and he was again tested by PCR. The PCR test (GeneXpert) result was indeterminate (Ct: E, 41.5; N2, 0) on the same day. The patient was provisonally diagnosed with COVID-19 although he had no manifestations other than vertigo and no specific findings on blood testing and chest CT. The next day, the PCR test was repeated and was indeterminate again (Ct: E, 0; N2, 44.2). Both the tests were collected by clinical technologist. However, because the PCR tests detected both E and N2, the patient was diagnosed as COVID-19. As he was on betamethasone treatment for cerebral edema, he was treated with remdesivir for 5 days, although he had mild disease. He did not experience any side effects. His surgery was postponed and he was quarantined for 14 days.

The details of these three cases, including each patient's condition and the results of each test, are summarized in Table 1.

2.4. Methods of collecting specimens and testing for COVID-19

Flocked swabs NP were used to collect specimens from the patient's nasopharyngeal mucosa, according to the hospital protocol which is based on the US Centers for Disease Control and Prevention recommendations [5]. We checked all patients on admission by isothermal amplification methods. Other than case 1, who had a slight fever, all three patients had no typical symptoms of COVID-19, and therefore the initial positive results were treated as false positive and the patients were subjected to PCR tests.

The LAMP test was performed using Loopamp 2019-nCoV detection kit on samples that had been pre-treated with saline. The ID-Now and GeneXpert assays were performed according to each manufacturer's instructions [6,7].

We detect Ct value witch is less than 40 as positive and witch is 40–45 as indeterminate according to the instruction of GeneXpert [7].

Table 1
Characteristics of the case patients and their SARS-CoV-2 test results.

	Case 1	Case 2	Case 3
Background			
Age (years)	53	79	39
Sex	Male	Female	Male
COVID-19 vaccination	No	Fully vaccinated	Fully vaccinated
Comorbidities	Cerebral hemorrhage	COPD	Cerebellar hemangioblastoma
Clinical manifestation on admission			
Fever	37.0 °C	36.4 °C	37.0 °C
Dyspnea	No	No	No
Cough	No	Yes	No
Other	Disturbance of consciousness	None	Vertigo
Pneumonia on CT scan	Yes	Yes	No
SARS-CoV-2 test results			
LAMP[a] test on admission	…	Positive (Tt 17.24; Positive control Tt 11.54)	…
NEAR[b] test on admission	Positive	…	Positive
PCR[c] test on admission	Negative	Negative	Indeterminate (E, 41.5; N2, 0)
PCR test on the day after admission	Positive (Ct: E, 22.0; N2, 24.2)	Positive (Ct: E, 40.4; N2, 38.7)	Indeterminate (Ct: E, 0; N2, 44.2)

COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease; Ct, cycle threshold; CT, computed tomography; E, envelope; LAMP, loop-mediated isothermal amplification; N, nucleocapsid; NEAR, nicking endonuclease amplification reaction; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2, Tt, threshold time.

[a] The LAMP test was performed using Loopamp 2019-nCoV detection kit (Eiken Chemical Co., Ltd, Tokyo, Japan).

[b] The NEAR test was performed using ID-NOW (Abbott Diagnostics, Lake Forest, IL, USA).

[c] The PCR test was performed using GeneXpert (Cepheid, Sunnyvale, CA, USA).

3. Discussion

We encountered three cases in which tests using isothermal amplification methods were positive for SARS-CoV-2 but the PCR test was negative on the same day, and in all three cases, the PCR test was positive the next day.

These case reports illustrate two important clinical findings. First, some COVID-19 patients test negative by PCR but positive by isothermal nucleic acid amplification methods. There are various detection methods for SARS-CoV-2, including antigen testing and gene amplification using PCR and isothermal amplification methods such as LAMP and NEAR. Among them, PCR is considered to be the most reliable and accurate method, and is the reference method for assessing the accuracy of LAMP, NEAR, and other tests [8,9]. Despite the use of PCR as the gold standard test for the diagnosis of COVID-19, it has several limitations, such as the requirement of sophisticated laboratories, need for skilled staff, long waiting times for results, and the high cost per test [9,10].

Isothermal amplification methods are less expensive and can be performed more rapidly than PCR but are considered to be less sensitive [11]. The sensitivity and specificity of LAMP are 17–100% and 73–100%, respectively; and the sensitivity and specificity of ID-Now are 78.7% and 100%, respectively [12,13]. However, according to one review, PCR tests have a false-negative rate of 9.3% (95% confidence interval [CI]: 1.5–17%) [14].

While a PCR test for one patient costs about 5,000 yen and requires 1 hour, ID-Now takes 15 minutes and costs 6,000 yen, and LAMP takes

about 1 hour and costs 2,000 yen. We use ID-Now or LAMP at admission because of the costs of time and money.

Considering that the sensitivity and specificity of PCR tests for detection of SARS-CoV-2 were 99% (95% confidence interval [CI], 97–99%) and 97% (95% CI, 95–98%), PCR tests are the most reliable test, but ID-Now and LAMP are superior in terms of cost and time consumption as many patients are checked at admission [15].

In the diagnosis of COVID-19, PCR is recognized as a very sensitive test, but there is a possibility of false negative, and isothermal amplification tests may compensate for this. PCR and NEAR tests usually have a low sensitivity at a low viral load [16]. We cannot show the reason as to why these three cases were positive at NEAR tests but gave false negative results with PCR. Based on Ct values of Case 2 and 3 checked the next day, there were found to have a low viral load. There was a possibility that PCR tests were false negative because of the very low viral load. Some studies report that NEAR has higher amplification efficiency than RT-PCR, and NEAR may be more effective at very small viral load [17]. We have no clinical evidence to support this hypothesis, but we could prevent misdiagnosis by checking with two other methods at low levels of viral load.

Second, patients with asymptomatic COVID-19 can be identified by testing all patients for SARS-CoV-2 on admission using a screening test in settings with a high prevalence of COVID-19. Cases 2 and 3 were hospitalized in August 2021, when an average of 4200 people per day were diagnosed with COVID-19 in Tokyo [4].

Because a previous study by He et al. [18] had shown that 46% of cases of SARS-CoV-2 infection are asymptomatic, all patients admitted to our hospital were screened for SARS-CoV-2 infection on admission using isothermal nucleic acid amplification methods, NEAR or LAMP, even if they had no symptoms of COVID-19. We tested for SARS-CoV-2 using ID-Now or Loopamp because of their low cost and rapid results.

It is particularly important to screen patients for SARS-CoV-2 on hospital admission in high-prevalence settings. ID-Now and Loopamp are suitable for use as screening tests because of their low cost and short turnaround time.

This study had several limitations. First, it is not possible to draw conclusions about the incidence of false-negative PCR results because there were only three cases. To assess how often and why some patients SARS-CoV-2 were positive by the isothermal amplification method but negative by PCR, further studies with more cases are needed. Second, a second nasopharyngeal specimen was collected for PCR testing after the first nasopharyngeal swab specimen had tested positive using isothermal amplification methods, so it is possible that the viral load might have decreased. To our knowledge, there have been no reports of the SARS-CoV-2 viral load being reduced by collecting multiple nasopharyngeal swabs. Third, it is possible that different collectors could have affected the quality of the sample because of their skills. But, considering our hospital's well-established collecting protocol, there was little difference in the quality of collection by different collectors. However, for accuracy, the results of PCR and isothermal amplification tests should be compared using the same nasopharyngeal swab.

We encountered three cases in which tests for SARS-CoV-2 were positive using isothermal amplification methods, but the PCR results were negative. As PCR tests can have false-negative results, it is important to carry out the COVID-19 testing using various methods. Tests that use isothermal amplification methods can be performed in a shorter time and at a lower cost than PCR tests, and it may be able to diagnose patients who have false negative PCR results.

Authorship statement

SS wrote the manuscript. YM was in charge of the treatment of the patients as the clinical infectious disease physician. YM reviewed the manuscript. Both authors meet the ICMJE authorship criteria.

Consent for publication

The three case patients provided written informed consent for publication of their case details.

Declaration of competing interest

None.

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