Rapid antigen test with phlegm is more sensitive and detects SARS-CoV-2 earlier than with nasal swab

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Abstract

Omicron is the current variant of SARS-CoV-2. It has high transmissibility and evades human immunity. Early and accurate diagnosis is essential for therapy, prognosis, and curbing the spread of the virus. Here we reported a new sampling method for the rapid antigen test, with specimens from phlegm, instead of from nasal swabs. Sensitivities in detection of SARS-CoV-2 antigen were compared within the two specimens, and between rapid antigen tests with phlegm specimens and PCR tests. Of 41 volunteers, thirty-one with positive phlegm specimens of SARS-CoV-2 eventually had typical COVID-19 symptoms, which suggested rapid antigen tests with phlegm specimens had 100 % accuracy. Fourteen of these had antigen tests with nasal swab specimens: 13 negative and 1 positive. Combined with that the antigen levels of the 6561-fold diluted phlegm specimen were comparable to those of the original nasal swab specimen, antigen tests with phlegm specimens are more sensitive and earlier detect SARS-CoV-2 than with nasal swab specimens. Interestingly, case studies indicated antigen tests with phlegm specimens earlier notified patients of positive infection than PCR tests. Phlegm specimens enhanced sensitivity in detection of SARS-CoV-2 in antigen tests, resulting in earlier diagnosis (12 to 42 hours, n=6) than nasal swab specimens. The sensitivity of antigen tests with phlegm specimens is comparable to that of PCR tests, but the former earlier outputs test results. Rapid antigen tests with phlegm specimens facilitate monitoring the health of COVID-19 patients and direct recovery.

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ABSTRACT

Omicron is the current variant of SARS-CoV-2. It has high transmissibility and evades human immunity. Early and accurate diagnosis is essential for therapy, prognosis, and curbing the spread of the virus. Here we reported a new sampling method for the rapid antigen test, with specimens from phlegm, instead of from nasal swabs. Sensitivities in detection of SARS-CoV-2 antigen were compared within the two specimens, and between rapid antigen tests with phlegm specimens and PCR tests. Of 41 volunteers, thirty-one with positive phlegm specimens of SARS-CoV-2 eventually had typical COVID-19 symptoms, which suggested rapid antigen tests with phlegm specimens had 100 % accuracy. Fourteen of these had antigen tests with nasal

swab specimens: 13 negative and 1 positive. Combined with that the antigen levels of the 6561-fold diluted phlegm specimen were comparable to those of the original nasal swab specimen, antigen tests with phlegm specimens are more sensitive and earlier detect SARS-CoV-2 than with nasal swab specimens. Interestingly, case studies indicated antigen tests with phlegm specimens earlier notified patients of positive infection than PCR tests. Phlegm specimens enhanced sensitivity in detection of SARS-CoV-2 in antigen tests, resulting in earlier diagnosis (12 to 42 hours, n=6) than nasal swab specimens. The sensitivity of antigen tests with phlegm specimens is comparable to that of PCR tests, but the former earlier outputs test results. Rapid antigen tests with phlegm specimens facilitate monitoring the health of COVID-19 patients and direct recovery.

KEYWORDS

COVID-19, SARS-CoV-2, Antigen Test, PCR Test, Phlegm, Nasal Swab

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic continues worldwide, which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 2]. The current variant of SARS-CoV-2 is omicron (B.1.1.529), having high transmissibility and evading human immunity [3]. SARS-CoV-2 is a positive-sense single-stranded RNA virus [4]. The gold-standard method to detect SARS-CoV-2 is nucleic acid amplification tests (NAAT, here named PCR test) for the reverse transcribed cDNA from viral RNA [5]. Considering the increased number of SARS-CoV-2 infected people, the need for testing for potential infection increased as well. In addition, it is possible to be infected when a nucleic acid specimen is collected by other COVID-19 patients, hence most people choose to use a rapid SARS-CoV-2 antigen self-test for early diagnosis. Omicron appears to be more like a cold for some people, with commonly reported symptoms including a sore throat, runny nose, and headache [6]. The symptoms in each individual are different due to various immunity backgrounds, hence there is a war to fight against SARS-CoV-2 around the world. Early diagnosis, early quarantine, and early therapy are gold rules to conquer SARS-CoV-2 and COVID-19. Among them, early diagnosis is most important, since it helps patients to adjust their lifestyle, protect some special family members with basic diseases, and determine whether they need therapies.

In vitro, early diagnosis depends on molecular tests, which consists of PCR test, antibody assays, and antigen testing [7, 8]. Here we focus on PCR tests and antigen testing. PCR test in principle is reverse transcription quantitative PCR (RT-qPCR), which is the most sensitive method to detect SARS-CoV-2. Regarding conventional PCR tests, RNA including viral RNA and host RNA is prepared from respiratory specimens, and then reverse transcribed to cDNA. qPCR is performed to determine the Ct value of the viral RNA in specimens. Generally, the whole procedure takes 3.5-4.0 hours not considering other affected factors, and required professional technicians and instruments. For large-scale PCR tests, normally specimen is collected on the first day, and the output returns on the second day. Nasal and oral swabs are commonly used for PCR tests. Due to discomfort associated with nasal swab collection and the shortage of professional healthcare personnel, an alternative specimen is from saliva (referred to as spit) which is an extracellular fluid produced and secreted by salivary glands in the mouth. Wyllie *et al.* reported that PCR tests with saliva specimens are comparable to those with nasal swab specimens [8], and it is reported that the SARS-CoV-2 virus was detected in the first week of symptom onset.

Antigen test detects viral proteins, rather than viral nucleic acid [8, 9]. Viral proteins from swabs are extracted with sample extraction buffer and applied for nitrocellulose membrane to flow. The viral proteins will be captured by antigen-specific antibodies coupled with colloidal gold in the filter and detected by antigen-specific antibodies at the "T" line. As a technique control, antibodies by flowing will be detected in the "C" line suggesting the quality control of a correct flow. Rapid antigen tests with colloidal gold can detect active infections within 15 min, quite faster than PCR tests. Another advantage of rapid antigen test is that it does not require professional personnel. Currently, a nasal swab is recommended for rapid SARS-CoV-2 antigen tests. Collection of nasal specimens using swabs has discomfort and cannot be applied for all age patients. Alternate specimens for rapid antigen tests are saliva and phlegm. It was reported that saliva specimens were used for rapid SARS-CoV-2 antigen test, but the sensitivity was not concluded against

nasal swab specimens. Different from saliva, phlegm is mucus produced by the respiratory system searched to Wikipedia. It often refers to respiratory mucus expelled by coughing. No conclusive study reported the sensitivity of phlegm specimens against nasal swab specimens in rapid antigen tests for SARS-CoV-2.

This study aims to compare the sensitivities of the methods: rapid SARS-CoV-2 antigen test with nasal swab specimens and with phlegm specimens. We demonstrate rapid antigen tests with phlegm specimens have much higher sensitivity than with nasal swab specimens. The detection time of active SARS-CoV-2 with phlegm specimens is advanced 12-42 hours than with nasal swab specimens. Considering processing time in PCR tests, phlegm antigen tests notify patients of the output report 10 hours earlier than PCR tests.

MATERIALS AND METHODS

Participants

This study was reviewed and approved by the Ethics Committee of Anhui Medical University. Forty-one patients with written informed consent volunteered to have SARS-CoV-2 tests and provide data for analysis.

Specimen with Nasal Swab

A nasal swab is collected as described in the manufacturer's instructions. Carefully insert the swab tip into one nostril about 1-2 cm deep and rub the insides of each nostril in a complete circle at least 5 times.

Specimen with Phlegm Swab

Specimen of phlegm is collected by holding the breath for 2-3 seconds. Use stomach muscles to forcefully expel the air, avoiding a hacking cough or merely clearing the throat. Expel mucus out of the lungs by a deep cough to clean plastic support. Dip the swab into the liquid of phlegm.

Rapid SARS-CoV-2 Antigen Test

Rapid antigen test is carried out as described in the manufacturer's instructions (WANITA, Biohit Healthcare, and Biouhan). Insert the swab tip into the liquid of the specimen collection tube containing the sample extraction buffer. Mix vigorously by rolling the swab tip at least 6 times on the bottom and sides of the tube. Hold the swab tip for 1 minute in the collection tube. Squeeze the swab tip several times from the tube wall releasing as much liquid from the swab as possible. The liquid in the collection tube now is an antigen sample. Add 3 drops of the above-collected antigen into the sample well and read the result after 15 minutes.

SARS-CoV-2 Nucleic Acid Amplification Test (PCR Test)

PCR test was performed by the third-party Hefei Hehe Medical Laboratory. The patient went to the specimen collection site and a mouth or nasal swab was collected by the professional personnel for a single nucleic acid PCR test. The test result was uploaded to the Alipay app.

RESULTS

Rapid antigen test with phlegm specimens detects SARS-CoV-2 more sensitively than with nasal swab specimens

To know the performance of the rapid antigen test using phlegm specimens in SARS-CoV-2 detection, we compared its sensitivity with that using popular nasal swab specimens. In this test, the "T" band from the phlegm specimen was very strong, but invisible from the nasal swab specimen (Figure 1A, test 1 *vs* test 2). This result indicated that the patient was infected by SARS-CoV-2, which was detected by the rapid antigen test with the phlegm specimen but not with the nasal swab. The patient continued the antigen test with nasal swab specimens (Figure 1A, test 3), and interestingly found 12 hours later the test with a nasal swab specimen was positive. This result reminded us that the sensitivity in detection of SARS-CoV-2 using rapid antigen tests with phlegm specimens is higher than with nasal swab specimens. Hence, we serially diluted the phlegm specimen 3-fold for more tests (Figure 1B). The 2187-fold dilution showed a weak "T"

band (Figure 1B, test 9) compared to the fewer dilutions (Figure 1B, test 2-8), and the 6561-fold dilution had similar levels of SARS-CoV-2 antigen to those of the original nasal swab specimen (Figure 1B, test 10 vs test 1), suggesting the sensitivity in detection of SARS-CoV-2 using an antigen test with phlegm specimens was about 6000-fold higher than that with nasal swab specimens in this case. These results confirmed that rapid SARS-CoV-2 antigen tests with phlegm specimens have higher sensitivity in detection of SARS-CoV-2 than with nasal swab specimens and could be an alternative evaluation technique for COVID-19 diagnosis.

Rapid antigen test with phlegm specimens accurately detects SARS-CoV-2

Among the total of 41 volunteers who volunteered for SARS-CoV-2 tests, 13 patients had a scratchy sensation in the throat when specimens were collected, and their phlegm specimens were positive, but the matched nasal swab specimens were negative; One patient with slight fever had both phlegm and nasal swab specimens tested positive; Seventeen patients with early symptoms of COVID-19 had phlegm specimens tested positive with lack of nasal swab specimens; the rest 10 volunteers without any COVID-19 symptom showed negative phlegm specimens in antigen tests. All 31 patients with detected positive phlegm specimens had typical COVID-19 symptoms in a later stage indicating the accuracy of rapid antigen tests using phlegm specimens was 100%. Considering the collection of phlegm specimens is much milder and easier than that of nasal swab specimens, these results suggested that rapid antigen tests for SARS-CoV-2 with phlegm specimens had high accuracy and can be a good substitute for those with nasal swab specimens.

Rapid antigen test with phlegm specimens earlier detects SARS-CoV-2 infections than with nasal swab specimens

We have demonstrated that the rapid SARS-CoV-2 antigen test with phlegm specimens is more sensitive than that with nasal swab specimens. Hence it is possible that patients can use this test to determine whether they are infected by SARS-CoV-2 earlier than with nasal swab specimens. To test this hypothesis, we chose 3 patients who had positive phlegm in the SARS-CoV-2 antigen test and negative nasal swab to continue antigen tests with nasal swab specimens (Figure 2). Patient 1 had a positive nasal swab specimen 13 hours after SARS-CoV-2 was first detected in the phlegm specimen (Figure 2A, test 4 vs test 2), suggesting the SARS-CoV-2 antigen test with the phlegm specimen detected viral antigen earlier than with the nasal swab specimen for 13 hours. Similarly, Patient 2 and Patient 3 were determined as SARS-CoV-2 infected cases by phlegm specimens earlier than by nasal swab specimens for 24 hours (Figure 2B test 5 vs test 1) and 42 hours (Figure 2C test 5 *vs* test 1) respectively. Taken together, rapid antigen tests with phlegm specimens detect SARS-CoV-2 12-42 hours earlier than those with nasal swab specimens. Considering the rule of early detection, the better specimen for rapid SARS-CoV-2 antigen tests is from phlegm, but not from nasal swabs.

Rapid antigen test with phlegm specimens earlier notifies patients of SARS-CoV-2 infections than PCR test

Since PCR test is a gold standard in SARS-CoV-2 detection and phlegm specimens in rapid antigen test enhance sensitivity in detection, next we would like to know which of the two methods is better for detection of SARS-CoV-2. We picked a volunteer who was exposed to a positive case in the early stages of COVID-19 for further study. On Day 0, the volunteer had a negative phlegm specimen by the rapid SARS-CoV-2 antigen test (Figure 3, test 1), and the PCR test was notified of a negative result on the next day (Figure 3, 0 h). On Day 1, the volunteer collected phlegm specimens for antigen tests and determined that the sample was positive (Figure 3, 0 h). The SARS-CoV-2 antigen levels increased in a later stage shown by the stronger "T" bands with time increment (Figure 3, test 2, 4, 5, 7). On Day 2, 35 hours later, his antigen test with a nasal swab showed a positive result (Figure 3, test 12), as confirmed our conclusion that phlegm specimens in rapid antigen tests determine SARS-CoV-2 infection earlier than nasal swab specimens for about 12-42 hours. More interestingly, the specimen was collected from the patient for a PCR test on Day 1 when the phlegm specimen was first positive (Figure 3, 0 h), and was notified of a positive result 11 hours (Figure 3, 11 h). Hence, technically rapid antigen tests with phlegm specimens earlier notify patients of a positive result than PCR tests, though the two tests have a similar sensitivity in detection of SARS-CoV-2.

Rapid antigen test with phlegm specimens facilitates monitoring health of COVID-19 patients

Considering rapid antigen tests with phlegm specimens can identify the early SARS-CoV2 infection, it is possible to monitor the health conditions of COVID-19 patients using this test. On Day 1, one volunteer had a positive phlegm specimen in the rapid antigen test (Figure 4, test 1). Due to the weak "T" band in the antigen test with the phlegm specimen and negative nucleic acid sample two days ago, we deduced Day 1 was the earliest time in detection of SARS-CoV-2 in the patient. Simultaneously, the patient had a PCR test. Since the report of PCR tests took time, but the patient was notified of a positive nucleic acid RNA of SARS-CoV-2 on Day 2, 10 hours after the first phlegm specimen was positive (Figure 4, 10 h *vs* 0 h), as is consistent with the previous result (Figure 3, 11 h *vs* 0 h). On Day 2, the phlegm specimens remained positive, but the nasal swab specimen tested positive 21 hours after the first phlegm specimen was positive (Figure 4, test 9*vs* test 1). The patient was then thirsty and had a slight cough and an increased body temperature (37.9 degC) 29 hours after the first phlegm specimen was positive (Figure 4, 21 h and 29 h). Hence, rapid antigen test with phlegm specimens facilitates monitoring health of COVID-19 patients.

Rapid antigen test with phlegm specimens better determines patient recovery from SARS-CoV-2 infection

The sensitivity in detection of SARS-CoV-2 using phlegm specimens in rapid antigen tests is higher than using nasal swab specimens, and comparable to that using oropharyngeal swab specimens in PCR tests. Hence, it is possible that a rapid antigen test with a phlegm specimen could be used to determine whether a patient is recovered from SARS-CoV-2 infection. To this end, we investigated the virus antigen levels using rapid antigen tests with phlegm specimens in 3 volunteers, and the tests with nasal swab specimens were used as a contrast (Figure 5). These volunteers were all infected by SARS-CoV-2 and had common symptoms of COVID-19 by omicron, such as a sore throat, runny nose, and headache. Till Day 9 – Day 11, SARS-CoV-2 was still detectable, but the titer was very low, as shown by the weak "T" band in the rapid antigen test with phlegm specimens (Figure 5A test 1 & 3, 5B test 1, 5C test 3), but not with nasal swab specimens (Figure 5A test 2 & 4, 5B test 2, 5C test 4). Depending on the variation of immunity background, each patient may take a different time to recover. As expected, we found while the phlegm specimen was negative in the rapid antigen test, the nuclei acid sample was simultaneously negative in the PCR test on the same day for both patients (Figure 5B, test 3 and greenV; 5C test 5 and greenV). Taken together, rapid SARS-CoV-2 antigen tests with phlegm specimens better direct patients' recovery from COVID-19 infection.

DISCUSSION

Early and accurate diagnostic tests are essential to curb the spread of SARS-CoV-2 and control the ongoing COVID-19 pandemic. Since the current variant of the SARS-CoV-2 virus has high transmissibility and evades human immunity, it is urgent to determine whether one is infected by SARS-CoV-2 who has some early common COVID-19 symptoms such as cough, low fever, and headache. Early and accurate detection helps patients to adjust their lifestyle by increasing sleep, stopping alcohol and smoking, separating them from young or old family members or members having basic diseases, and storing some required foods and medicines for recovery time. Considering the reduction of available nuclei acid test sites and cross infections by other positive patients, people tend to have a self-test with rapid SARS-CoV-2 antigen test with nasal swab specimens at home to know whether they are infected.

PCR test has been a gold standard to diagnose SARS-CoV-2 infection because of its sensitivity. However, it has shortcomings such as false negative results, crossing infections, long procedure time, and professional personnel and instrument involvement. As a substitutive test, rapid SARS-CoV-2 antigen tests are carried out using a specimen of nasal swabs. The rapid antigen test has several advantages such as high accuracy, portable use, and no requirement for professional background. However, the essential shortcoming is the low sensitivity in detection of SARS-CoV-2 compared to the PCR test. Currently, our research updated the collection method of specimens and used phlegm samples for rapid SARS-CoV-2 antigen tests. Using this new sampling method, we demonstrated the sensitivity in detection of SARS-CoV-2 in rapid antigen tests is about 6000-fold higher than that using nasal swab specimens. Importantly this sensitivity enhancement

of rapid antigen tests with phlegm specimens made it comparable to PCR tests for diagnosing SARS-CoV-2 infection. Two reasons possibly contribute to the elevated sensitivity. First, the common symptom caused by the omicron variant of SARS-CoV-2 is sore throat suggesting virus titer is high in throat secretion; Second, more viruses are picked in the tests by dipping phlegm.

Using the rapid antigen test with phlegm specimens, from 41 volunteers we diagnosed 31 positive cases of SARS-CoV-2 infection, and these cases all developed as typical COVID-19 suggesting the method has 100% accuracy. Thirteen cases showed negative rapid antigen tests with nasal swab specimens but positive phlegm specimens, indicating rapid antigen tests with phlegm specimens can earlier detect SARS-CoV-2 infection cases than with nasal swab specimens. From 6 cases, we found the advanced time by rapid antigen test with phlegm specimens is about 12-42 hours compared to the tests with nasal swab specimens (Figure 1-4). The advanced time is a big window for patients to arrange the following plan and prevent their family members from further infection, and for physicians to start specific therapy against severe symptoms at the early stage of COVID-19 development. Even though the antigen test with phlegm specimens and the PCR test detected SARS-CoV-2 at the same time, technically the former test notified of the output result ˜10 hours earlier than the latter one (Figure 3, 4). During recovery from COVID-19, antigen tests with phlegm specimens better determine a negative time, which is comparable to PCR tests (Figure 5). It helps patients to wisely make decisions for recovery avoiding the side effects of improper treatments. Taken together, antigen tests with phlegm specimens accurately and early detect SARS-CoV2, and better determine the recovery time suggesting a good aid to monitor the health conditions of COVID-19 patients.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

XS, BC, and SZ designed the study. XS, CH, NH, BC, and SZ performed the experiments. XS, BC, and SZ analyzed the data, and XS wrote the paper with help from all authors.

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FIGURE LEGENDS

Figure 1. Rapid antigen test with phlegm specimens is more sensitive than that with nasal swab specimens. (A) A patient with mild symptoms of COVID-19 volunteered simultaneously for rapid antigen tests with both phlegm and nasal swab specimens. Viral antigens of phlegm liquids from the deep throat or specimens from the nostril were extracted with antigen extraction buffer from a rapid antigen test kit. Three drops of extracted antigen were applied for tests. Test time 0 h indicated when the phlegm specimen was initially positive, and 12 h was the passed time after the phlegm specimen was positive; **(B)** The phlegm sample was consecutively diluted at 3-fold for a test of sensitivity in detection of SARS-CoV-2. Sixty microliters of the original or diluted sample were applied for rapid antigen tests. SARS-CoV-2 antigen was still detectable in the 2187-fold diluted phlegm. The antigen levels in the 6561-fold diluted phlegm sample are comparable to that in the nasal swab sample indicated in rapid SARS-CoV-2 antigen tests. Control is the antigen extraction buffer only.

Figure 2. The time to detect SARS-CoV-2 in antigen tests with phlegm specimens is earlier than with nasal swab specimens. (A) In patient 1, the SARS-CoV-2 antigen in the phlegm specimen was not detectable on Day 0 and was detected on the next day (Day 1, 0 h). SARS-CoV-2 antigen in the nasal swab specimen was not detectable at the beginning of Day 1 (0 h), but detected 13 hours after the phlegm specimen was positive on the same day (13 h); **(B)** In patient 2, on Day 1, SARS-CoV-2 antigen was detected in phlegm specimens (0 h and 13 h). In nasal swab specimens, antigen was not detected (0 h, 13 h) till Day 2 (24 h) and the relative intensity of the "T" band was stronger after 35 hours (35 h); **(C)** In patient 3, SARS-CoV-2 antigen was detected in the phlegm specimen on Day 1 (0 h), but not detected in nasal swab specimens until 42 hours later on Day 3 (42 h). The yellow triangle indicates the time when the SARS-CoV-2 antigen was initially detected in the phlegm specimen; The blue triangle stands for the time point when the antigen was initially detected in the nasal swab specimen.

Figure 3. The rapid antigen test with phlegm specimens notified the patient of SARS-CoV-2 infection earlier than the PCR test.A patient was exposed to a positive case of COVID-19 and collected specimens for viral detection. On **Day 0** , a phlegm specimen was collected for a rapid antigen test, and the nucleic acid specimen was collected for a PCR test (the result was notified on Day 1). Both tests showed negative results. On **Day 1** , both phlegm and nasal swab (N. Swab) specimens were tested for rapid SARS-CoV-2 antigen as indicated. Viral antigens in the phlegm specimen were detected (0 h), and a nucleic acid specimen was collected for a PCR test, with notification of the positive nucleic acid specimen 11 hours after the phlegm sample was positive (11 h). On Day 2, SARS-CoV-2 antigens from the nasal swab specimen were detected, 35.5 hours after positive phlegm was determined (35.5 h). The yellow or blue triangle indicates the time when SARS-CoV-2 antigens were detected in phlegm or nasal swab specimen respectively. The green or red triangle designates the time when the results of a PCR test of SARS-CoV-2 RNA were notified.

Figure 4. Rapid antigen test with phlegm specimen facilitates monitoring the health of COVID-19 patients. On **Day 1** , a tiny amount of SARS-CoV-2 antigen was detected at a weak band in phlegm (0 h), and the nucleic acid specimen was collected for PCR test on the same day. On **Day 2** , the patient was

notified of the positive nucleic acid sample 10 hours later than the first positive phlegm specimen, and the specimen of the nasal swab in antigen test was detected 21 hours after the first positive phlegm specimen (21 h). The patient had no symptoms until the nasal swab specimen was positive 21 hours after the first positive phlegm specimen. The patient had a slight cough, and an increase in body temperature 29 hours post the first positive phlegm specimen. The yellow, blue, or red triangle indicates the time when SARS-CoV-2 was initially detected in the phlegm specimen, the nasal swab specimen, or the nucleic acid sample by a PCR test (time with the notified result) respectively.

Figure 5. Rapid antigen tests with phlegm specimens can be used to direct recovery. Day 9 - 12 is the 9^{th} – 12^{th} day after the first positive phlegm specimen.**(A)** On Days 9 – 11, the nasal swab specimens of Patient 1 were negative but the phlegm specimens were still positive, indicating a rapid antigen test with phlegm specimens more accurately showed the patient's infection condition. On Day 12, both specimens were negative;**(B)** On Day 9, both the phlegm and the nucleic acid samples of Patient 2 were still positive, but the nasal swab specimen by an antigen test was negative. On Day 10, both the PCR test and the phlegm antigen test showed negative results. The red or green triangle designates the time when SARS-CoV-2 nucleic acid specimens were collected and tested as positive or negative in PCR tests; **(C)** On Day 11, the phlegm specimen of Patient 3 was positive but the nasal swab one was negative. On Day 12 both antigen specimens and the nucleic acid sample with PCR test became negative in SARS-CoV-2 detection, suggesting the rapid antigen test with phlegm was comparable to the PCR test directing recovery from COVID-19.

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