



Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19

Yafang Li¹ | Lin Yao² | Jiawei Li³ | Lei Chen¹ | Yiyang Song⁴ | Zhifang Cai⁵ | Chunhua Yang¹

¹Department of Intensive Care Unit, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

²Department of Operation and Management, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

³Natural Language Processing Group, iFLYTEK Research South China, Guangzhou, China

⁴Department of Clinical Medicine, Zhongshan School of Medicine, Guangzhou, China

⁵Department of Respiratory, Hankou Hospital of Wuhan, Wuhan, China

Correspondence

Chunhua Yang, MD, Department of Intensive Care Unit, The Sixth Affiliated Hospital Sun Yat-Sen University, 26th Yuancun Road II, 510655 Guangzhou, China.
Email: yangchunhua_gd@126.com

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Abstract

In this study, we collected a total of 610 hospitalized patients from Wuhan between February 2, 2020, and February 17, 2020. We reported a potentially high false negative rate of real-time reverse-transcriptase polymerase chain reaction (RT-PCR) testing for SARS-CoV-2 in the 610 hospitalized patients clinically diagnosed with COVID-19 during the 2019 outbreak. We also found that the RT-PCR results from several tests at different points were variable from the same patients during the course of diagnosis and treatment of these patients. Our results indicate that in addition to the emphasis on RT-PCR testing, clinical indicators such as computed tomography images should also be used not only for diagnosis and treatment but also for isolation, recovery/discharge, and transferring for hospitalized patients clinically diagnosed with COVID-19 during the current epidemic. These results suggested the urgent needs for the standard of procedures of sampling from different anatomic sites, sample transportation, optimization of RT-PCR, serology diagnosis/screening for SARS-CoV-2 infection, and distinct diagnosis from other respiratory diseases such as influenza infections as well.

KEYWORDS

COVID-19, RT-PCR

1 | INTRODUCTION

As of March 8, 2020, statistical data showed that the outbreak of COVID-19 constitutes an epidemic threat worldwide and 105 631 people have been infected.¹ In China, 80 904 people were diagnosed with COVID-19 and 3123 patients have died, the mortality was 3.9% (3123 patients divided by 80 904 patients).¹ Although the cure rate is rising, the transmission of SARS-CoV-2 remains unoptimistic. Therefore, enhancing the management of hospitalized patients is important for preventing and minimizing the further spread of

COVID-19 during this epidemic. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assay has been widely used to detect SARS-CoV-2.² The Chinese government has also considered RT-PCR result as an indicator of isolation, discharge, or transferring for patients diagnosed with COVID-19.³ Notably, the isolation for patients can be revoked, and the patients will be discharged after two consecutive negative RT-PCR tests.³ However, it was reported on February 12, 2020 that five infected patients had initial negative or weakly positive RT-PCR results.⁴ In another reported case, the third time RT-PCR test result for pharyngeal swab specimen from an

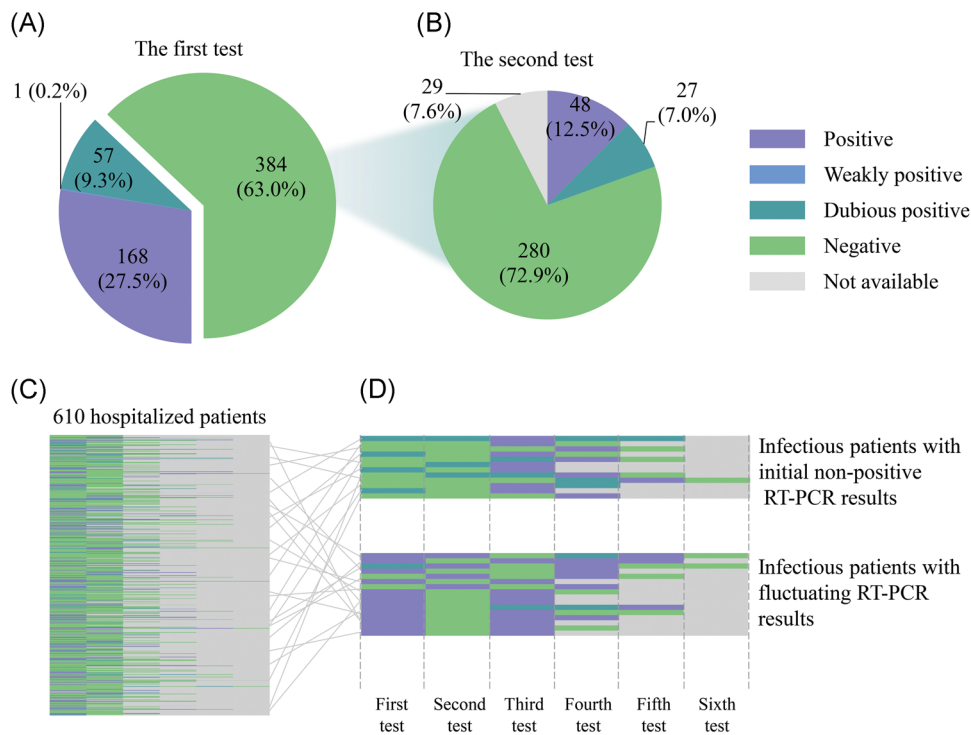


FIGURE 1 Distribution of real-time reverse-transcriptase polymerase chain reaction (RT-PCR) test results of SARS-CoV-2. Distribution of the RT-PCR results of the initial test for all patients (A). Distribution of the RT-PCR results of the second test for patients with initial negative results (B). RT-PCR test results of SARS-CoV-2 of 12 patients with initial non-positive RT-PCR result (C). RT-PCR test results of SARS-CoV-2 of 17 patients with unstable RT-PCR test result among different time points (D)

infected patient turned to be positive after two previous negative results of the PCR test.⁵ Here, we investigated 610 patients in one hospital clinically diagnosed with COVID-19 according to the chest computed tomography (CT) image during the 2019 novel coronavirus outbreak in Wuhan, China and described the stability issues of RT-PCR testing of SARS-CoV-2 for them.

2 | METHODS

We retrospectively identified 610 hospitalized patients clinically diagnosed with COVID-19 between February 2, 2020, and February 17, 2020, in Hankou Hospital of Wuhan, a hospital designated for the treatment of patients with COVID-19. There are 18 doctors and nurses from the Sixth Affiliated Hospital of Sun Yat-sen University supporting the hospital.

According to the recommended protocol,³ patients in Wuhan with a chest CT image demonstrates viral pneumonia were

clinically diagnosed with COVID-19. Confirmed COVID-19 was defined according to the positive (do not contain suspicious positive) RT-PCR test result for pharyngeal swab specimens. Patients with COVID-19 were defined as critically ill when meeting one of the following criteria: (a) respiratory failure and mechanical ventilation are required, (b) shock, (c) combined with other organ failure and need to transfer to the intensive care unit. RT-PCR test of pharyngeal swab specimen was performed in all patients before admission. For patients with an initial negative, dubious positive or weakly positive RT-PCR result, their pharyngeal swab specimens would be retest after 1 or 2 days. For patients with an initial positive RT-PCR result, their pharyngeal swab specimens would be retest after the patients' clinical symptoms improved. Both the RT-PCR results and the test dates were recorded and analyzed. This study was approved by the Ethics Committee of Wuhan Hankou Hospital. The requirement for informed consent was waived because the patients were part of a public health outbreak investigation.

TABLE 1 Distribution of the first real-time reverse-transcriptase polymerase chain reaction test results of SARS-CoV-2 in all patients

	All	Positive	Weakly positive	Dubious positive	Negative	not available
Cases	610	168	1	57	384	0
Rate (%)	100.0	27.5	0.2	9.3	63.0	0

TABLE 2 Distribution of the second real-time reverse-transcriptase polymerase chain reaction test results of SARS-CoV-2 in patients with initial negative results

	All	Positive	Weakly positive	Dubious positive	Negative	not available
Cases	384	48	0	27	280	29
Rate (%)	100.0	12.5	0	7.0	72.9	7.6

TABLE 3 Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) results of SARS-CoV-2 in 12 infectious patients with initial consecutive non-positive^a RT-PCR results

Patient	First test	Second test	Third test	Fourth test	Fifth test	Sixth test
1	D	D	P	D	D	NA
2	N	N	P	N	NA	NA
3	N	N	N	P	N	NA
4	D	N	P	N	NA	NA
5	N	N	D	P	N	NA
6	N	D	P	NA	NA	NA
7	D	N	P	NA	NA	NA
8	N	D	D	P	N	NA
9	N	N	N	D	P	N
10	N	N	P	D	NA	NA
11	D	N	P	NA	NA	NA
12	N	N	N	P	NA	NA

Abbreviations: D, dubious positive; N, negative; NA, not available; P, positive.

^aNon-positive results including dubious positive and negative results.

TABLE 4 Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) results of SARS-CoV-2 in 17 infectious patients with fluctuating RT-PCR results

Patient	First test	Second test	Third test	Fourth test	Fifth test	Sixth test
1	P	P	N	D	P	N
2	P	N	P	P	P	NA
3	D	P	N	P	N	N
4	P	N	N	P	NA	NA
5	N	P	N	P	N	NA
6	P	N	P	NA	NA	NA
7	N	P	N	P	NA	NA
8	P	N	P	N	NA	NA
9	P	N	P	NA	NA	NA
10	P	N	P	NA	NA	NA
11	P	N	P	NA	NA	NA
12	P	N	D	D	P	NA
13	P	N	P	N	N	NA
14	P	N	P	P	NA	NA
15	P	N	P	NA	NA	NA
16	P	N	P	N	NA	NA
17	P	N	P	NA	NA	NA

Abbreviations: D, dubious positive; N, negative; NA, not available; P, positive.

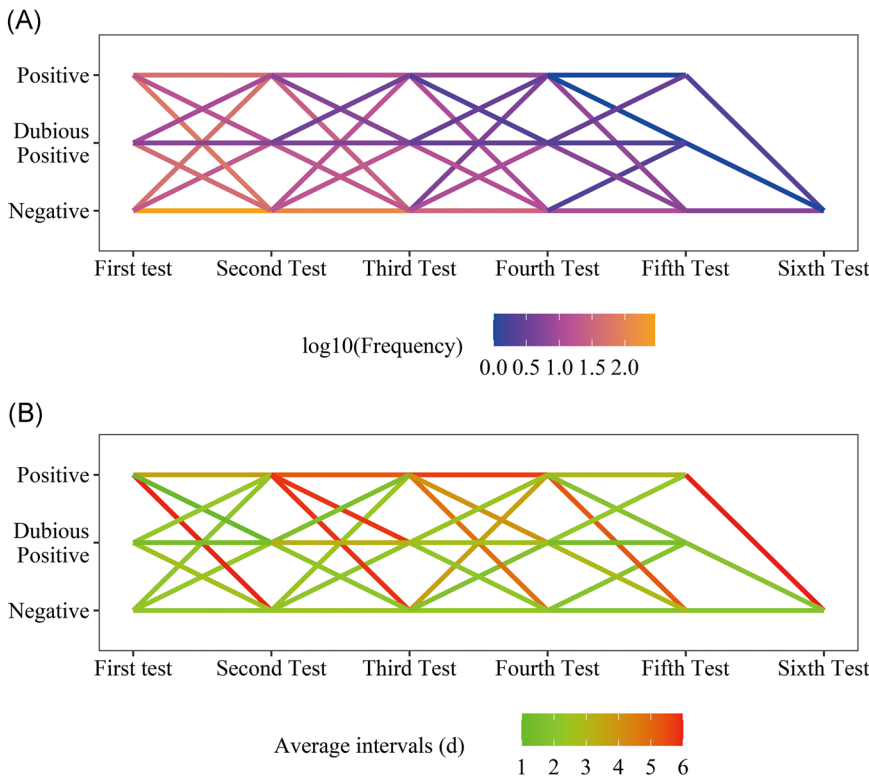


FIGURE 2 The networks of real-time reverse-transcriptase polymerase chain reaction (RT-PCR) result transformations about frequencies and average intervals. Frequencies of RT-PCR results transformations (A). Average intervals of RT-PCR results transformations (B)

3 | RESULTS

A total of 610 hospitalized patients diagnosed with COVID-19 were identified between February 2, 2020, and February 17, 2020. From whom 241 (39.5%) patients were finally confirmed with COVID-19 with at least one positive RT-PCR test result. Of all the patients, the median age was 52.7 years (ranging from 20 to 88 years), 55.8% were male, and 56.1% were critically ill. All the patients resided in Wuhan city.

Observational analysis of RT-PCR results revealed the following findings. In the first test for all patients, 168 cases were positive (27.5%), one was weakly positive (0.2%), 57 were dubious positive (9.3%), and 384 were negative (63.0%) (Figure 1A and Table 1). Among the 384 patients with initial negative results, the second test was performed. For these patients, the test results were positive in 48 cases (12.5%), dubiously positive in 27 patients (7.0%), negative in 280 patients (72.9%), and results were not available for 29 patients (7.6%) (Figure 1B and Table 2). Among the patients with initial non-

TABLE 5 Percentage of different transformations among RT-PCR test results

Transformation	First to second test (n = 540)	Second to third test (n = 225)	Third to fourth test (n = 89)	Forth to fifth test (n = 33)	Fifth to sixth test (n = 7)
N-P	48 (8.9)	15 (6.7)	6 (6.7)	0	0
N-D	27 (5.0)	16 (7.1)	12 (13.5)	2 (6.1)	0
N-N	280 (51.9)	114 (50.7)	35 (39.3)	10 (30.3)	4 (57.1)
D-P	12 (2.2)	3 (1.3)	4 (4.5)	3 (9.1)	0
D-D	6 (1.1)	5 (2.2)	3 (3.4)	2 (6.1)	0
D-N	38 (7.0)	22 (9.8)	12 (13.5)	7 (21.2)	1 (14.3)
P-P	49 (9.1)	15 (6.7)	6 (6.7)	1 (3.0)	0
P-D	11 (2.0)	5 (2.2)	2 (2.2)	1 (3.0)	0
P-N	69 (12.8)	30 (13.3)	9 (10.1)	7 (21.2)	2 (28.6)

Note: Results were presented as n (%).

Abbreviations: D, dubious positive; N, negative; P, positive; RT-PCR, real-time reverse-transcriptase polymerase chain reaction.

TABLE 6 Average days of different transformations among RT-PCR test results

Transformation	First to second test	Second to third test	Third to fourth test	Forth to fifth test	Fifth to sixth test
N-P	2.2	2.5	3.7
N-D	2.3	2.2	1.8	2	...
N-N	2.3	2.5	2.4	2	2
D-P	2.3	1.7	2.5	2.3	...
D-D	1.5	3.2	2.7	1.5	...
D-N	2.6	2.2	2.2	2.9	2
P-P	3.7	5.3	5.7	3	...
P-D	1	5.8	4	2	...
P-N	5.9	5.8	4.8	5.3	6

Note: Results were presented as mean.

Abbreviations: D, dubious positive, N, negative; P, positive; RT-PCR, real-time reverse-transcriptase polymerase chain reaction.

positive results, seven patients were eventually confirmed with COVID-19 by three repeated swab PCR tests, four were confirmed by four repeated tests, and one was confirmed by five repeated tests (Figure 1D and Table 3). In the patients confirmed as COVID-19, 17 patients have positive RT-PCR results for pharyngeal swab specimens at first, and their PCR results turned to be negative after treatment for several days. However, again several days later when the patient's symptoms improved, their PCR results returned to be positive (Figure 1D and Table 4). Among them, one patient's RT-PCR result turned positive after two consecutive negative tests (Figure 1D and Table 4). The sub-networks of frequencies and average intervals of resulting transformations are shown in Figure 2. The sub-networks of result transformations were completed before the fourth test, which means negative results may turn to non-negative (including positive and suspicious positive) results even after three times tests (Figure 2A and Table 5). After the fourth test, no negative case turned to positive, only to suspicious positive. After the fifth test, neither suspicious nor negative results transformed to positive (Figure 2A and Table 5). The RT-PCR tests were implemented in clinic with longer intervals during the positive-suspicious-negative processes than during the negative-suspicious-positive processes (the Wilcoxon rank-sum test: $P = 5.706e-09$) (Figure 2B and Table 6).

4 | DISCUSSION

In our study, we found a potentially high false negative rate of RT-PCR testing for SARS-CoV-2 in hospitalized patients in Wuhan clinically diagnosed with COVID-19. Furthermore, the RT-PCR results showed a fluctuating trend. These may be caused by insufficient viral material in the specimen, laboratory error during sampling, or restrictions on sample transportation.⁶

To increase the survival rate of critically ill patients, when respiratory failure, shock, or multiple organ dysfunction syndromes emerged, timely transfer them to ICU or a designated hospital that has sufficient rescue equipments should be considered even if their results of RT-PCR test for pharyngeal swab specimens are negative.

Eighteen patients were found to have a positive RT-PCR result after two consecutive negative results in this study. If these patients were released from isolation due to the previous negative results, the risk of human-to-human transmission would be inevitably increased. Thus, to reduce the number of new cases, strict adherence to the discharge criteria is needed. In addition, it is recommended that patients should be isolated for several days after discharge, to reduce the risk of transmission of SARS-CoV-2 if the above situation occurs.

This study was limited by the lack of detailed patient information because of the clinical workload of the frontline employees involved in the outbreak in Wuhan. Further research is required to investigate the relationship between the RT-PCR result and the onset time of symptoms such as fever.

Our findings indicate that RT-PCR test results of pharyngeal swab specimens were variable and potentially unstable, and it should not be considered as the only one indicator for diagnosis, treatment, isolation, recovery/discharge and transferring for hospitalized patients clinically diagnosed with COVID-19.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Chunhua Yang  <http://orcid.org/0000-0003-4558-4110>

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