Letter to the Editor

Qiongying Hu^a, Xue Yang^a, Peiyang Gao, Jianyuan Tang, Qingxiao Liu, Qiurong Du, Fujie Yang, Cheng Wu, Li Liu, Dongming Bi and DaQian Xiong*

Variability between testing methods for SARS-CoV-2 nucleic acid detection 16 days post-discharge: a case report

<https://doi.org/10.1515/cclm-2020-0328>

Received March 16, 2020; accepted May 7, 2020; previously published online June 9, 2020

Keywords: coronavirus disease 2019; laboratory examinations; nucleic acid detection; severe acute respiratory syndrome coronavirus 2.

To the Editor,

By March 17, 2020, there were 80,881 cumulative confirmed cases, 128 suspected cases and 3226 deaths reported by the National Health Commission of the People's Republic of China [1]. Early diagnosis and isolated treatment have been effective strategies to control the spread of disease because of the lack of effective treatment. According to the diagnosis and treatment protocols from the National Health Commission of the People's Republic of China, the diagnostic criteria for COVID-19 are as follows: (1) epidemiological history – travel/history of residence in Wuhan or history of exposure to febrile patients with respiratory symptoms from Wuhan within 14 days before the onset of illness; (2) clinical manifestations – fever, imaging

characteristics of pneumonia and/or normal or decreased white blood cell count or decreased lymphocyte count; and (3) laboratory diagnosis – qualitative reverse transcription polymerase chain reaction (qRT-PCR) revealing positive detection of SARS-CoV-2 in throat swabs or the lower respiratory tract [2, 3]. From trial version 3 to trial version 6, nucleic acid detection by qRT-PCR has always played a definite role in the diagnosis of COVID-19. Based on this background knowledge, many enterprises and scientific research institutions have urgently developed different brands of nucleic acid testing kits, which provide additional choices during clinical investigations.

A 36-year-old woman presented with fever and cough for 7 days and was admitted to the fever clinic at the Hospital of Chengdu University of Traditional Chinese Medicine, complaining of chest congestion, fatigue and headache. A week before admission, the patient had a fever, with the highest body temperature recorded as 38.5 °C after contact with staff returning from Wuhan. The routine blood examination showed only an increased monocyte count (13.20%) at that time. She then took oseltamivir by herself but had no significant improvement in symptoms. On the day of admission, she denied any history of smoking or drinking and had no major illness. She had no respiratory symptoms, a blood pressure of 130/80 mmHg, a pulse rate of 76 beats per minute, a respiratory rate of 18 breaths per minute and a body temperature of 36.9 °C upon complete check-up. CT showed patchy infection and ground-glass opacities with indistinct borders in the upper lobe of the right lung, the middle lobe of the left lung and the lower lobes of both lungs. The laboratory examination results were as shown in Table 1 on the day of admission (23 Jan 2020). Finally, she was diagnosed with COVID-19 via qRT-PCR amplification of SARS-CoV-2 nucleic acid from a throat swab. On January 24, the patient was transferred to a designated hospital for treatment. After treatment, the patient's temperature returned to normal, lasting for more than 3 days, and her respiratory symptoms improved as well. During this time, the results of the SARS-CoV-2

^aQiongying Hu and Xue Yang contributed equally to this work.

*Corresponding author: DaQian Xiong, MD, Department of Laboratory Medicine, Hospital of Chengdu University of Traditional Chinese Medicine, No. 39 Shi-er-qiao Road, Chengdu 610072, Sichuan Province, P.R. China, E-mail: 705006714@qq.com

Qiongying Hu, Xue Yang, Qiurong Du and Fujie Yang: Department of Laboratory Medicine, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, P.R. China

Peiyang Gao: Intensive Care Unit, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, P.R. China

Jianyuan Tang: Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, P.R. China

Qingxiao Liu: Neurology Department, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, P.R. China

Cheng Wu and Li Liu: Pathology Department, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, P.R. China

Dongming Bi: Nuclear Medicine Department, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, P.R. China

Table 1: Serial laboratory results of the patient.

Variables	18 Jan 2020	23 Jan 2020	31 Jan 2020	16 Feb 2020
Leukocyte count, $\times 10^9/L$	3.64	2.37↓	3.95	4.19
Neutrophils, %	40.20	38.50↓	57.80	60.70
Lymphocytes, %	44.50	49.8	29.20	27.90
Monocytes, %	13.20↑	10.10↑	9.30	8.80
Eosinophils, %	1.60	0.80	3.10	1.90
Haemoglobin, g/L	154.00↑	148.00	135.00	137.00
Platelets, $\times 10^9/L$	177.00	154.00	254.00	207.00
CRP, mg/L	<0.5	<0.5	1.53	<0.5
Glucose, mmol/L	Not detected	4.94	5.21	5.61
Urea, mmol/L	Not detected	1.94	3.00	2.29↓
Creatinine, mmol/L	Not detected	51.20	46.00	45.20
Uric acid, mmol/L	Not detected	194.00	188.00	220.00
Total bilirubin, $\mu\text{mol/L}$	Not detected	13.20	6.90	13.50
ALT, U/L	Not detected	22.00	32.00	15.00
AST, U/L	Not detected	22.00	19.00	17.00
CK, U/L	Not detected	54.00	Not detected	130.00
ALP, U/L	Not detected	48.00	46.00	42.00
LDH, U/L	Not detected	191.00	Not detected	137.00
Sodium, mmol/L	Not detected	137.60	141.90	137.60
Potassium, mmol/L	Not detected	3.98	4.81	4.10
Calcium, mmol/L	Not detected	2.11	2.26	2.14
Chloride, mmol/L	Not detected	103.40	105.2	102.40
Total protein, g/L	Not detected	69.90	59.50↓	69.70
Albumin, g/L	Not detected	41.00	38.40	44.40
PT, s	Not detected	13.10	12.90	13.60
PT INR	Not detected	1.0	1.07	1.0
APTT, s	Not detected	40.90	27.50	39.30
D-dimer, $\mu\text{g/mL}$	Not detected	Not detected	0.61	0.31

ALP, alkaline phosphatase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CK, creatine kinase; CRP, C-reactive protein; INR, international normalised ratio; LDH, lactate dehydrogenase; PT, prothrombin time.

nucleic acid detection test from her throat swab were negative on the fourth day, the sixth day and the eighth day in the designated hospital. In addition, re-examination of chest CT showed that the lesions demonstrating bilateral lung infection were improved compared with the results of the CT scan on January 23. Her laboratory results on January 31 were as shown in Table 1.

All of the aforementioned results met the discharge criteria of the ‘Diagnosis and treatment protocols for pneumonia caused by SARS-CoV-2 (trial version 4)’ [4]. Thus, the patient was discharged after hospitalisation and isolated treatment for 8 days. Subsequently, the patient was isolated at home alone for 16 days. On February 16, alarmingly, her re-examination for SARS-CoV-2 nucleic acid showed detectable nucleocapsid protein (N) gene from a throat swab using a different testing kit Daan (DAANGENE, Guangzhou, China, Lot: 20200809, genes: N and Open reading frame 1 ab: ORF1ab) in the Department of Laboratory Medicine, the Hospital of Chengdu University of Traditional Chinese Medicine.

On February 17, throat and sputum samples were processed and tested using two different kits Daan (N and ORF1ab) and Genuo (GENEODX, Shanghai, China, Lot: GZTRM21202002, genes: N, ORF1ab and Envelop E gene: E). The results were positive for both throat swab and sputum by Daan (N and ORF1ab), while they were positive for only sputum by Genuo (N, ORF1ab and E) (Table 2). Because the Daan (N and ORF1ab) detected a single viral nucleic acid in the throat swab (Sample number: 2020021707) as shown in Table 2, the interpretation was considered not indicative of COVID-19 according to the ‘Laboratory guidelines for novel coronavirus pneumonia (version 4)’ [5]. Moreover, amplification efficiency and system problems led to a low-detection fluorescence signal and atypical amplification curve, which were not conducive to assessing the results by Daan (N and ORF1ab) on February 17 (Figure 1). For example, as shown in Figure 1A, the amplification curve of Daan (N) was atypical, with a detection value of 38.00 (cut-off value: 40), which was determined as positivity. Similarly, the amplification curve of Daan

Table 2: Partial qRT-PCR test results of the patient.

Reagents	Date	Hospital	Sample number	Specimen type	Genes		
					N	ORF1ab	E
Genuo	Jan 23, 2020	DH	N/A	Throat swab	N/A	N/A	N/A
	Jan 27, 2020	DH	N/A	Throat swab	N/A	N/A	N/A
	Jan 29, 2020	DH	N/A	Throat swab	N/A	N/A	N/A
	Jan 31, 2020	DH	N/A	Throat swab	N/A	N/A	N/A
	Feb 17, 2020	HCUTCM	2020021707	Throat swab	Not detected	Not detected	Not detected
	Feb 17, 2020	HCUTCM	2020021708	Sputum	34.80	32.10	31.59
	Feb 17, 2020	HCUTCM	2020021609	Throat swab	Not detected	Not detected	Not detected
Daan	Feb 16, 2020	HCUTCM	2020021608	Throat swab	37.76	Not detected	N/A
	Feb 16, 2020	HCUTCM	2020021609	Throat swab	39.05	Not detected	N/A
	Feb 17, 2020	HCUTCM	2020021707	Throat swab	38.00	41.23	N/A
	Feb 17, 2020	HCUTCM	2020021708	Sputum	31.38	33.32	N/A
	Feb 17, 2020	HCUTCM	2020021609	Throat swab	36.26	40.43	N/A
	Feb 18, 2020	HCUTCM	2020021810	Serum	Not detected	Not detected	N/A

DH, designated hospital; HCUTCM, Hospital of Chengdu University of Traditional Chinese Medicine; N/A, not applicable, the sample number and Ct values by Genuo (N, ORF1ab and E) in the designated hospital were not available for privacy. The results of the SARS-CoV-2 nucleic acid detection were negative on Jan 27–31, 2020 in the designated hospital.

Figure 1: The amplification curve of hallmarker genes from different samples.

A comparison of the atypical amplification curve (A) and normal amplification curve (B) for SARS-CoV-2 nucleic acid detection by qRT-PCR.

(ORF1ab) was atypical, and the detection value of 41.23 (cut-off value: 40) was negative. In Figure 1B, the curves were all normal amplification curves. The detection value of Daan (N) and Daan (ORF1ab) were 31.38 and 33.32, respectively, which were both determined as positivity, according to the cut-off value of 40. The general characteristics of SARS-CoV-2 detection reagents and the partial test results are shown in Supplementary Table 1, Table 2 and Figure 1 for qRT-PCR. The same sample number meant that the sample was tested twice at different dates using the same reagent or parallelly tested at the same time using different reagents. As a supplement, we also measured the anal swab by Daan (N and ORF1ab) and Genuo (N, ORF1ab and E), and both results were negative on February 17. On February 21, we obtained positive IgM results from serum with the SARS-CoV-2 IgM antibody detection kit (colloidal gold immune chromatography) (Beijing Hot

King Biotechnology Co., LTD, China, Lot: 20200205). The CT scan showed that some lesions had obvious absorption and tended to show local fibrosis. In addition, physical and other examinations of the patient basically appeared normal. On February 18, the patient was isolated again, and her complete clinical course is shown in Figure 2.

According to the disease course, the patient history included exposure to an infected person followed by symptoms consistent with COVID-19, specifically weakness and cough. Consequently, her CT scan and initial positive SARS-CoV-2 nucleic acid test both confirmed this disease.

Undeniably, early diagnosis becomes the key to controlling the epidemic, and qRT-PCR technology is the preferred screening method. Nucleic acid detection has the advantages of early diagnosis, high sensitivity and specificity, and easy operation, but the accuracy of nucleic

Figure 2: Diagram showing the clinical course of the patient.

acid detection results needs to be comprehensively analysed according to the sample types, quality, experimental factors, kit performance and patient infection cycles [6]. Whether assessing this case or other cases, most nucleic acid test results have been unsatisfactory (low detection rate). Faced with the problems of nucleic acid testing and substantial social pressure, the National Health Commission of the People's Republic of China promptly updated the 'Laboratory guidelines for novel coronavirus pneumonia (version 4)' to final version 5 [7]. The new guidelines (version 5) make it clear that positive results can be determined by the simultaneously positive results of a single target (N or ORF1ab) from two samples with qRT-PCR or by the positive results of a single target from two samples of the same type [7].

The quality of nucleic acid detection kits is the key to determining the detection rate of the same sample in the same laboratory except for the error of the clinical laboratory practitioner's inter-batch operation. Although both SARS-CoV-2 nucleic acid detection kits Daan (N and ORF1ab) and Genuo (N, ORF1ab and E) detect genes at least including N and ORF1ab, the primers were designed at different sites, which directly determined the amplification efficiency [8]. In addition, the selection of reverse transcriptase enzymes and reverse transcriptase primers can also affect reverse transcriptase efficiency because RT enzymes do not have a proofreading function to check for errors in newly synthesised DNA [9]. Both SARS-CoV-2 nucleic acid detection kits Daan (N and ORF1ab) and Genuo (N, ORF1ab and E) use one-step RT-PCR, and in this system, researchers should take the activity of reverse transcriptase and DNA amplification enzymes into account, which would affect the overall detection rate [10].

Except for the influence of nucleic acid detection kit performance, the dynamic balance between SARS-CoV-2 and the host is also an important factor affecting the

outcomes of the disease. According to the disease course of the patient, when she was released from quarantine, her nucleic acid detection result was negative. Given that low viral loads below the minimum detection limit cannot be measured using the current kits, a small amount of virus might continue to replicate, especially after treatment is ceased; then, the number of viruses may increase to the detection level. For this case, we could not conclude whether she had relapsed or had not been cured. However, she remained in isolation until her nucleic acid detection was positive again. Therefore, there was very little chance for reinfection.

In summary, based on the current situation of novel coronavirus nucleic acid detection, the outcomes of patients with isolation treatment need to be supported by more clinical evidence. In terms of nucleic acid testing, in addition to examining respiratory specimens multiple times, the use of more types of samples is also recommended (such as serum, faeces and even urine). When selecting the types of samples, the samples from the lower respiratory tract (such as deep sputum, lavage fluid, etc.) were preferred, followed by samples from the upper respiratory tract (such as nasopharyngeal swabs, etc.), at last faeces, urine and blood samples were selected [7]. We can even choose other supplementary methods, such as the use of serum-specific antibodies IgM or IgG. Because there are no specific drugs for the treatment of COVID-19, it seems that the infusion of specific antibodies from cured patients may provide an exciting therapeutic strategy. However, we still know little about SARS-CoV-2. The definitions of incubation and recovery are also being revised. We believe that the use of serum-specific antibodies from cured patients for infused treatment requires more careful consideration. Finally, the management and timely review of discharged patients should be considered.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Research funding: This study was supported by the Study on the Prevention and Control Technology of Traditional Chinese Medicine Community in New Coronavirus Epidemic Situation (2020YFS0012).

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: Authors state no conflict of interest.

Informed consent: Written informed consent was obtained from all individuals included in this study.

References

1. National Health Commission of the People's Republic of China. <http://www.nhc.gov.cn/xcs/yqfkdt/202003/28d026a0422844969226913ee3d56d77.shtml>.
2. Pan F, Ye T, Sun P, Gui S, Liang B, Li L, et al. Time course of lung changes on chest CT during recovery from 2019 novel coronavirus (COVID-19) pneumonia. *Radiology* 2020;295:715–21.
3. National Health Commission of the People's Republic of China. Diagnosis and treatment protocols of pneumonia caused by a novel coronavirus (trial version 6). <http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2/files/b218cfeb1bc54639af227f922bf6b817>.
4. National Health Commission of the People's Republic of China. Diagnosis and treatment protocols of pneumonia caused by a novel coronavirus (trial version 4). <http://www.nhc.gov.cn/xcs/zhengcwj/202001/4294563ed35b43209b31739bd0785e67.shtml>.
5. National Health Commission of the People's Republic of China. Laboratory guidelines for novel coronavirus pneumonia (trial version 4). <http://www.nhc.gov.cn/xcs/zhengcwj/202002/573340613ab243b3a7f61df260551dd4.shtml>.
6. Zhu L, Qing Z, Hou L, Yang S, Zou Z, Cao Z, et al. Direct detection of nucleic acid with minimizing background and improving sensitivity based on a conformation-discriminating indicator. *ACS Sens* 2017;2:1198–204.
7. National Health Commission of the People's Republic of China. Laboratory guidelines for novel coronavirus pneumonia (trial version 5). <http://www.nhc.gov.cn/xcs/zhengcwj/202002/a5d6f7b8c48c451c87dba14889b30147.shtml>.
8. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinform* 2012;13:134.
9. Ellefson JW, Gollihar J, Shroff R, Shivram H, Iyer VR, Ellington AD. Synthetic evolutionary origin of a proofreading reverse transcriptase. *Science* 2016;352:1590–3.
10. Okano H, Baba M, Kawato K, Hidese R, Yanagihara I, Kojima K, et al. High sensitive RNA detection by one-step RT-PCR using the genetically engineered variant of DNA polymerase with reverse transcriptase activity from hyperthermophilic. *J Biosci Bioeng* 2018;125:275–81.

Supplementary Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/cclm-2020-0328>).