Letter to the Editor

Fecal Nucleic Acid Test as a Complementary Standard for Cured COVID-19 Patients*

HAN Mei[1,&], ZOU Jing Bo[2,&], LI Huan[1,&], WEI Xiao Yu[3], YANG Song[1], ZHANG Hui Zheng[1], WANG Peng Sen[1], QIU Qian[1], WANG Le Le[1], CHEN Yao Kai[1,#], and PAN Pin Liang[4,#]

To date, no vaccine or specific antiviral treatment is yet available for COVID-19. The most effective control measures for the disease mainly depend on early diagnosis, strict patient quarantine, and close contact monitoring[1]. Thus, reliable and accurate diagnostic methods play a critical role in global disease control and prevention. Reverse transcription-polymerase chain reaction (RT-PCR) assays are widely used for the laboratory diagnosis of virus-borne diseases. As a molecular Nucleic Acid Test (NAT), RT-PCR has been approved by the FDA for diagnostic use, such as SARS-CoV-2, with higher sensitivities and specificities compared to other biochemical and immunological based assays. According to the SARS-CoV-2 technical guidance implemented by the Chinese National Health Committee, the open reading frame 1ab and nucleocapsid protein encoding genes are selected as targets for RT-PCR assay[2,3]. A positive result is reported if either of these genes is detected with a cycle threshold (*Ct*) value of less than 37.0. Negative results from nasal/pharyngeal swabs tested for SARS-CoV-2 are an important criterion for the assessment of medical care and discharge of hospitalized patients. However, from January 2020 to June 2020, we noted 22 atypical cases out of 314 cases in our clinical practice; these atypical cases showed positive NAT results in their fecal samples.

Case 1 is a 48-year-old male patient who was infected by his wife, a pneumonia patient who had been previously diagnosed with SARS-CoV-2 infection. The patient had a positive viral NAT result in his respiratory (nasal/pharyngeal swabs) specimens and was admitted to the hospital on January 22, 2020. The NAT result of the patient's respiratory secretions (nasal/pharyngeal swabs) became negative after 12 days of medical treatment, but the results of his stool samples remained positive. The fecal excretion persisted after sputum excretion in for 3 days.

Case 2 is a 9-year-old boy who was diagnosed with COVID-19 and admitted to the hospital on January 24, 2020. He demonstrated mild pneumonia symptoms with a positive test result for the presence of novel coronavirus nucleic acids in his respiratory secretions. The viral NAT results of the patient's respiratory secretions were found to be negative in three consecutive RT-PCR assays over a 24-hour interval after 10 days of hospitalization for medical treatment. Positive RT-PCR results, however, were obtained from his stool specimens, which were collected at the same time as the respiratory specimens. Viral nucleic acids in the patient's stool samples were still detectable on day 14 of hospitalization, although with a relatively high Ct value.

Case 3 is a 48-year-old male patient who was diagnosed with COVID-19 with mild pneumonia and admitted to our hospital. On days 10 and 11 after medical treatment, the patient's samples were collected for viral NATs. Positive results were obtained from fecal samples, but negative results were obtained from respiratory specimens.

Case 4 is a 67-year-old female patient who was admitted to our hospital with a positive NAT result in respiratory secretions on January 30, 2020. After 7 days of treatment, marginal levels of viral nucleic acids remained detectable in the patient's fecal specimens, but respiratory samples were negative for the nucleic acids.

doi: 10.3967/bes2020.128

*This work was supported by the Project on Important Infectious Diseases Prevention and Control [2018ZX10734404] and the National Science and Technology Major Project of China during the 13th Five-year Plan Period [2018ZX10302104].

1. Chongqing Public Health Medical Center, Chongqing 400036, China; 2. Yongchuan district Centre for Disease Control and Prevention, Chongqing 402160, China; 3. Yongchuan Hospital of Chongqing Medical University, Chongqing 402160, China; 4. National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102200, China

Case 5 is a 47-year-old female patient who was confirmed with COVID-19 and admitted to the hospital on January 24, 2020. Viral nucleic acids persisted for 2 weeks in the patient's respiratory swabs and fecal samples. The fecal samples reported positive after the respiratory samples converted negative.

Case 6 is a 28-year-old male patient who was confirmed with COVID-19 with symptoms of fever lasting for 5 days and admitted to the hospital on February 3, 2020. The presence of viral nucleic acids persisted for 28 days. On day 18 after medical treatment, positive NAT results were obtained from fecal samples but negative results were obtained from respiratory specimens. However, 2 days later, the NAT results of both sample types became positive.

Case 7 is a 67-year-old male patient who was diagnosed with COVID-19 with mild pneumonia on February 1, 2020. The viral nucleic acid could be detected via fecal sample 14 days later while respiratory sample converted to negative.

Case 8 is a 44-year-old male patient who was determined to be NAT-positive on June 12, 2020 with no novel coronavirus pneumonia (NCP)-related symptoms. Computed tomography showed some cloudiness in the patient's right lung after traveling to India. Positive NAT results were obtained from the patient's fecal and respiratory samples 3 days after the admission.

Case 9 is a 27-year-old male who was diagnosed asymptomatic with no NCP-related symptoms but with positive NAT results in both fecal and respiratory samples on June 12, 2020. The patient's fecal sample became negative without any antiviral treatment 5 days later, but his respiratory sample remained positive for the nucleic acids for 15 days.

Cases 10–22 are patients with positive NAT results in their fecal and respiratory samples and diagnosed as mild NCP. The ages of these cases ranged from 18 years to 73 years. Five cases were female and eight were male.

In all of the cases presented above, specimens for COVID-19 diagnostic testing were obtained according to the guidelines issued by the Chinese Health Committee[2,4]. The necessary procedures were conducted by experienced clinical providers, and the negative results obtained were unlikely to have originated from improper or poor clinical specimen collection or poor specimen handling after collection and prior to testing. Laboratory confirmation of SARS-CoV-2 nucleic acids was completed and verified in two independent institutions. All patients who presented symptoms received treatment of lopinavir, ritonavir, thymalfasin, oxygen uptake, and traditional Chinese medicine.

In the above cases, all patients with NCP remained medically stable after 10+ days of hospitalization for medical antiviral treatment. Patients who recovered well and with vital signs (e.g., oxygen saturation, multiple cytokines, body temperature) in the normal range were categorized as cured or convalescent according to the current clinical guidelines. However, viral NAT was detected in the fecal specimens of cases 1–7 despite repeated negative results observed in specimens of respiratory secretions. We noted this gender-independent discrepancy in 22 cases with diverse clinical backgrounds and age ranges. Two patients in our study demonstrated gastrointestinal symptoms with diarrhea. It is worth noting that for case 2, three consecutive negative results in nasopharyngeal swabs were observed with 24-hour intervals, which satisfied the discharge standards stipulated by the Novel Coronavirus Pneumonia Prevention and Control guideline issued by the Chinese National Health Committee[2]. However, the strong positive result (low *Ct* values) of viral RNA in fecal specimens suggests the existence of viable virions in the patients, which implies highly infectious and transmissible capabilities.

Cases 1–7 are in our clinical practices, since January, 2020, got our attention, which have controversial viral NAT results in respiratory and fecal specimens after treatments for 10–15 days. The viral receptor angiotensin converting enzyme 2 (ACE2) could be detected in gastrointestinal epithelial cells, and infectious viral particles have been isolated from feces[5]. These findings may explain why positive NAT results were consistently obtained in fecal samples. In fact, fecal specimens are as accurate as respiratory specimens in PCR testing with a 2–5 day lag. It is reported approximately 23%–82% COVID-19 patients' fecal excretion persisted positive after SARS-CoV-2 banding gastrointestinal epithelial cells, and these feces-positive COVID-19 patients are potentially infectious[5].

In this study, 7% of the patients (22/314) with NAT-positive fecal specimens were admitted in two designated hospitals in Chongqing. Several independent clinical reports have demonstrated the detection of viral RNA by RT-PCR in rectal swabs after obtaining negative nasopharyngeal test results[6,7]. Analysis of a meta-analysis[8] revealed that

17.6% of patients with COVID-19 have gastrointestinal symptoms and that viral RNA could be detected in the stool samples of 48.1% patients even after negative test results are obtained from respiratory samples. In our study, viral RNA was detected in the stool specimens of a patient 20 days after hospitalization for medical treatment and over 8 days after respiratory specimens tested negative for viral nucleic acids (Table 1, case 1). Another study reported a duration of 7 days of viral shedding from

Table 1. Case information and clinical features of the patients

Case No	Gender	Age	Data of illness	Hospital days when sample was collected	Sample type*	RT-PCR results (*Ct* value)		Clinical characteristics	
						ORF1ab gene	*N* gene	Diarrhea	Other symptoms
1	M	48	1/21/20	12	R	0	0	Yes	No symptom of pneumonia; cough; fever; streaky opacities in both lungs
				12	F	33.04	34.46		
				13	R	0	0		
				13	F	31.59	37.14		
				15	R	0	0		
				15	F	34.41	33.62		
2	M	9	1/24/20	20	R	0	0	No	Mild pneumonia; cough
				20	F	34.53	32.76		
				10	R	0	0		
				11	R	0	0		
				12	R	0	0		
				12	F	32.32	31.7		
				14	R	0	0		
				14	F	33.56	32.02		
3	M	48	1/18/20	7	R	36.46	35.05	No	Mild pneumonia; cough; streaky opacities in both lungs
				7	F	39.1	36.88		
				10	R	0	0		
				10	F	28.07	27.07		
				11	R	0	0		
				11	F	36.90	0		
				23	R	32	32.27		
				23	F	33.36	32.59		
4	F	67	2/2/20	2	R	30.81	28.95	No	Mild pneumonia
				2	F	37.24	36.58		
				7	R	0	0		
				7	F	39.5	38.76		
5	F	47	1/24/20	10	R	30.43	29.96	No	Mild pneumonia; opacities in both lungs; cough; fever
				10	F	35.07	34.41		
				11	R	0	0		
				12	R	39.35	0		
				12	F	33.94	34.70		
				14	R	0	38.82		
				14	F	0	41.59		
				16	R	0	0		
				16	F	0	40.45		
6	M	22	2/3/20	18	R	0	0	No	Mild pneumonia; fever
				18	F	39.37	0		
				20	R	32.31	34.21		
				20	F	32.40	34.51		
7	M	67	2/1/20	14	R	0	0	No	Mild pneumonia
				14	F	0	38.55		

Continued

Case No	Gender	Age	Data of illness	Hospital days when sample was collected	Sample type*	RT-PCR results (*Ct* value)		Clinical characteristics	
						ORF1ab gene	*N* gene	Diarrhea	Other symptoms
8	M	44	6/13/20	2	R	33.91	30.87	No	Mild pneumonia
				2	F	38.17	35.80		
9	M	27	6/13/20	2	R	39.89	36.66	No	Asymptomatic
				2	F	38.38	36.59		
				5	R	39.64	0		
				5	F	0	0		
10	F	47	2/3/20	0	F	Positive		Yes	Mild pneumonia; fever; cough
				10	R	32.81	29.40		
				10	F	31.24	28.85		
				12	R	39.15	0		
				12	F	33.94	34.70		
				14	R	0	38.82		
				14	F	0	41.59		
				16	R	33.40	36.78		
				17	F	Positive			
11	M	41	1/30/20	7	R	39.51	38.26	No	Mild pneumonia
				7	F	39.67	38.26		
12	M	37	2/17/20	21	R	35.51	34.63	No	Mildpneumonia
				24	F	33.51	21.03		
13	F	44	2/28/20	17	F	30.76	0	No	Mild pneumonia
				17	R	34.02	34.63		
14	M	32	1/18/20	29	R	34.19	34.46	No	Mildpneumonia
				29	F	35.68	36.12		
15	M	61	1/21/20	21	R	0	41.49	No	Mildpneumonia
				19	F	40.01	34.52		
				31	R	37.95	0		
				30	F	39.73	40.04		
16	M	64	2/7/20	2	R	41	0	No	Mildpneumonia
				2	F	34.38	41.37		
17	M	73	1/24/20	15	R	0	38.65		Sever pneumonia
				15	F	34.64	36.97		
18	F	46	2/13/20	8	R	0	40.234	No	Mildpneumonia
				8	F	42.31	35.61		
19	M	18	1/31/20	8	R			No	Mildpneumonia
				8	F	35.79	37.89		
				10	R	31.94	33.49		
				10	F	37.47	36.96		
				19	R	37.47	36.96		
				19	F	38.66	39.92		
20	F	45	1/23/20	16	R	0	36.19	No	Mildpneumonia
				16	F	32.05	33.86		
21	M	23	2/5/20	15	R	38.07	39.59	No	Mildpneumonia
				15	F	36.32	37.01		
22	F	47	2/9/20	9	R	30.05	29.36	No	Mildpneumonia
				9	F	29.07	30.15		

Note. *Sample type: F: Fecal; R: Respiratory.

feces after the negative conversion of pharyngeal swabs[9]. These findings suggest that clinicians should pay attention to the negative result of viral NATs when evaluating treatment effects and the discharge standard. Parallel tests should be conducted to ensure accurate evaluations or assessments of different types of specimens, such as saliva, sputum, alveolar lavage fluid, and feces. We also propose the inclusion of viral NATs of fecal specimens in the screening of suspected patients and adoption of extra precautionary measures when necessary for medical care providers. Molecular assays such as RT-PCR can detect viral RNA over longer durations and are more sensitive than other biochemical tests. However, our clinical findings highlight the concern that, although molecular assays have high sensitivity, negative molecular assay results may not always exclude a diagnosis of infection. Strict hygiene and sanitation precautions are required during hospitalization or quarantine on account of the extra-pulmonary viral shedding of COVID-19 patients.

We will continue to track the viral NAT results of fecal and other specimens from patients and the hospital environment to reveal associations between the presence of virions and the clinical features of COVID-19. Because live SARS-CoV-2 virus has been detected in stool samples by scanning electron microscopy[10], cell culture of positive stool or respiratory secretions from recurrent patients and PCR samples of asymptomatic patients may be conducted to confirm that the virus is transmissible.

We strongly recommend that the national guidelines for COVID-19 diagnosis, especially the suggestion of 14 days of quarantine management and health monitoring, should be strictly executed. Asymptomatic patients may transmit the virus. Thus, good personal hygiene and environmental monitoring should be emphasized.

Acknowledgment We thank LUO Ming, HE Ying, LI Mei, DENG Reng Li, LI Jun Gang, LI Tong Xing, LUO Fu Long, and ZHOU Feng for their hard work in laboratory. We also thank all of the patients who participated in our study.

Competing interests The authors declare that they have no competing interests.

[&]These authors contributed equally to this work.

[#]Correspondence should be addressed to PAN Pin Liang, E-mail: panpinliang@chinaaids.cn; CHEN Yao Kai, E-mail: yaokaichen@hotmail.com

Biographical notes of the first authors: HAN Mei, female, born in 1976, MD, majoring in microbiology; ZOU Jing Bo, male, born in 1970, Bachelor, majoring in Microbiology; LI Huan, born in 1979, MD, majoring in Medicine.

Received: July 6, 2020;

Accepted: September 21, 2020

REFERENCES

1. Interim guidelines for collecting, handling, and testing clinical specimens from persons under investigation (PUIs) for coronavirus Disease 2019 (COVID-19) [https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html]. [2020-07-08].
2. The Novel coronavirus Pneumonia Prevention and Control guideline (5th Edition) [http://www.nhc.gov.cn/yzygj/s7653p/202002/3b09b894ac9b4204a79db5b8912d4440.shtml]. [2020-02-05].
3. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet, 2020; 395, 565−74.
4. Holshue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States. N Engl J Med, 2020; 382, 929−36.
5. Tian Y, Rong L, Nian W, et al. Review article: gastrointestinal features in COVID-19 and the possibility of faecal transmission. Aliment Pharmacol Ther, 2020; 51, 843−51.
6. Xiao F, Tang M, Zheng X, et al. Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology, 2020; 158, 1831−3.e3.
7. Xu Y, Li X, Zhu B, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat Med, 2020; 26, 502−5.
8. Cheung KS, Hung IFN, Chan PPY, et al. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from a Hong Kong cohort: systematic review and meta-analysis. Gastroenterology, 2020; 159, 81−95.
9. Chen Y, Chen L, Deng Q, et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J Med Virol, 2020; 92, 833−40.
10. Hindson J. COVID-19: faecal-oral transmission? Nat Rev Gastroenterol Hepatol, 2020; 17, 259.