Received: 2 April 2020 | Accepted: 13 April 2020

DOI: 10.1002/jmv.25892

COMMENTARY

JOURNAL OF MEDICAL VIROLOGY WILEY

COVID-19: Recommended sampling sites at different stages of the disease

Fang Song[1] | Xiangyan Zhang[2] | Yan Zha[3] | Weijia Liu[2]

[1]Department of Cardiology, Guizhou Provincial People's Hospital, Guiyang, Guizhou, China

[2]Department of Respiratory and Critical Care Medicine, Guizhou Provincial People's Hospital, Guiyang, Guizhou, China

[3]Department of Nephrology, Guizhou Provincial People's Hospital, Guiyang, Guizhou, China

Correspondence

Dr Weijia Liu, Department of Respiratory and Critical Care Medicine, Guizhou Provincial People's Hospital, Guiyang, Guizhou 550002, China.
Email: weijia902@126.com

Coronavirus Disease 2019 (COVID-19), an acute respiratory infectious disease caused by a novel coronavirus (officially named severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) in December 2019, is currently a worldwide pandemic and mainly causes novel coronavirus pneumonia (NCP). At present, we mainly rely on real-time reverse transcription-polymerase chain reaction (RT-PCR) to detect SARS-CoV-2 virus nucleic acid collected from the clinical specimens of patients as the standard for diagnosis, discontinuation of quarantine, and discharge.[1,2] It is worth noting that not only are the nucleic acid test results of a significant proportion of patients "false negative" in the process of diagnosis[3,4] but also the nucleic acid reexaminations positive again in some discharged patients who have reached the standards of discontinuation of quarantine or discharge.[5,6]

The main reason for the above phenomenon is that the sensitivity and accuracy of real-time RT-PCR itself are affected by some factors, including sampling location and methods, quality of detection reagent and PCR instrument, training of operators, and so forth. Some test results are false negative, meaning the virus is present somewhere in the patient's body, and the difference is whether the virus is detected successfully or not.[3,7] Thus, the suspected cases in Hubei province of China with typical imaging features of viral pneumonia but negative nucleic acid results were once included in the clinical diagnosis of NCP.

At present, in the case of a mature detection reagent, standardized laboratory quality control, and sampling methods, more attention should be paid to the sampling location and timing. COVID-19 patients have different virus loads or positive rates in different stages of the disease and in different parts of the body, such as absence in the nasopharynx or oropharynx, and possible presence in sputum, feces, or blood.[3,7,8,9] A high viral load can be detected in the early stage of the disease by pharyngeal swab,[3] and detection of SARS-CoV-2 viral RNA is better in nasopharynx samples than the oropharynx.[10] In the middle stage, the viral load of the lower respiratory tract will be significantly higher than that of the upper respiratory tract.[11] According to the existing evidence, the positive rate from high to low is bronchoalveolar lavage fluid, sputum, nasal swab, fibrobronchoscope brush biopsy, pharyngeal swab, and feces.[3,10,11] The positive rate of nasal swabs is close to that of sputum, which may be related to detoxification mainly through nostril breathing. It is important to note that with the recovery of the disease, the positive rate of oropharyngeal swabs in mild patients declines the fastest, and in the later course of the disease, positive results of anal swabs are more than that of pharyngeal swabs.[3,7,9,12] The viral nucleic acids in the stool of the recovered patients turn to negative later than the oropharyngeal swabs.[9,12-14] Due to the convenience of operation and the acceptability of patients, the most commonly used specimens at discharge in practice are also oropharyngeal swabs, and sometimes, nasal swabs are collected at the same time. However, in the middle and later stages of the disease, the amount of virus remaining in the pharyngeal cells is small or very low in some patients. If only the pharyngeal specimens are taken, the viral nucleic acid cannot be detected. Although alveolar lavage fluid is easier to detect viruses, due to its inconvenient operation and high risk of exposure, it is mainly used on critically ill patients who have been intubated.[15]

Different from the suspected cases with typical clinical characteristics, the diagnosis rate can be improved by detecting nucleic acid in fecal samples, but the latest discharge standards in China still requires only the collection of respiratory specimens.[1] However, it has been emphasized in the discharge standard to collect "nasal swab, sputum" and other upper and lower respiratory tract specimens at the same time.[1] Wang et al[16] reported that 10.1% of the patients had diarrhea and nausea 1 to 2 days before the onset of fever and respiratory symptoms, and at the late stage of the disease, viable viruses were found in stool samples from patients with high

TABLE 1 Sampling sites recommended for patients with COVID-19 at different disease stages

Sampling sites	Disease stages			
	Early stage (diagnosis)	Intermediate stage (advanced)	Convalescence (discharge)	Isolation period after discharge (follow-up)
Nasal swabs	Highly recommend	Highly recommend	Highly recommend	Highly recommend
Pharyngeal swabs	Highly recommend	Recommend	Recommend	Recommend
Sputum	Highly recommend	Highly recommend	Highly recommend	Highly recommend (severe, critical type)
Bronchoalveolar lavage fluid	Not recommend	Highly recommend (intubated patients)	Not recommend	Not recommend
Feces/anal swabs[a]	Recommend	Recommend	Recommend	Recommend
Blood	Not recommend	Recommend (critical type)	Not recommend	Not recommend
Fibrobronchoscope brush biopsy	Not recommend	Recommend (intubated patients)	Not recommend	Not recommend

[a]Patients with digestive symptoms.

viral load in anal swabs. Although it is still controversial whether positive result in fecal samples is related to the presence of gastrointestinal symptoms and the severity of the disease,[9,17] SARS-CoV-2 was detected in esophageal, gastric, duodenal and rectal specimens of critically ill patients and esophageal mucosal damage was observed.[17] We recommend that nucleic acid testing should be performed on fecal or anal swabs for patients with gastrointestinal symptoms (such as unexplained diarrhea, abdominal pain, and nausea and vomiting) as a measure to reduce the rate of false negatives and improve the discharge accuracy. The recommended sampling sites for patients with COVID-19 at different disease stages are shown in Table 1.

Based on the improvement of clinical symptoms and computed tomography imaging, the guidelines require only two consecutive negative nucleic acid tests (≥24 hours) before discharge can be considered. In clinical practice, there are some severe or critical patients who are discharged from the hospital after 2 days of consecutive negative test of throat swabs after glucocorticoid shock therapy.[18] Due to the long time of virus-carrying and severe pulmonary inflammatory damage, some patients develop an organization of alveolar exudates and interstitial fibrosis,[1] and these lung tissues have incomplete blood circulation perfusion, and the hidden virus may not be completely cleared. The patient's condition may fluctuate after discontinuation of glucocorticoids or antiviral drugs,[19] with intermittent virus shedding.[13] A cohort study from Wuhan, China found that the nucleic acid of SARS-CoV-2 can be shed for up to 37 days in a severely ill patient.[18] Special attention should be paid to the symptoms or nucleic acid retest results during the subsequent rehabilitation or prolonged intensive isolation period for such patients.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

ORCID

Fang Song http://orcid.org/0000-0002-4893-0368

REFERENCES

1. National Health Commission & National Administration of Traditional Chinese Medicine. Diagnosis and treatment protocol for novel coronavirus pneumonia (Trial Version 7). *Chin Med J*. 2020;133(9):1087–1095. https://doi.org/10.3760/cma.j.issn.0366-6999.2020.0027
2. Centers for Disease Control and Prevention. Interim clinical guidance for management of patients with confirmed coronavirus disease (COVID-19). 2020. https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html
3. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 2020;323(18):1843–1844. https://doi.org/10.1001/jama.2020.3786
4. Li YF, Yao L, Li JW, et al. Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19. *J Med Virol*. 2020. https://doi.org/10.1002/jmv.25786
5. Lan L, Xu D, Ye G, et al. Positive RT-PCR test results in patients recovered from COVID-19. *JAMA*. 2020;323(15):1502–1503. https://doi.org/10.1001/jama.2020.2783
6. Zhang J-F, Yan K, Ye H-H, Lin J, Zheng J-J, Cai T. SARS-CoV-2 turned positive in a discharged patient with COVID-19 arouses concern regarding the present standard for discharge. *Int J Infect Dis*. 2020. http://dx.doi.org/10.1016/j.ijid.2020.03.007
7. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect*. 2020;9:386-389.
8. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis*. 2020;20(4):411–412. https://doi.org/10.1016/S1473-3099(20)30113-4
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. http://dx.doi.org/10.1002/jmv.25825
10. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med*. 2020;382:1177-1179. https://doi.org/10.1056/NEJMc2001737
11. Yang Y, Yang M, Shen C, et al. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. *MedRxiv*. 2020. https://doi.org/10.1101/2020.02.11.20021493
12. Wu Y, Guo C, Tang L, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol*. 2020;5(5):434-435. https://doi.org/10.1016/S2468-1253(20)30083-2

13. Ling Y, Xu SB, Yx L, et al. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chin Med J*. 2020; 133(9):1039–1043. https://doi.org/10.1097/CM9.0000000000000774
14. Zhang TQ, Cui XJ, Zhao X, et al. Detectable SARS-CoV-2 viral RNA in feces of three children during recovery period of COVID-19 pneumonia. *J Med Virol*. 2020. https://doi.org/10.1002/jmv.25795
15. Centers for Disease Control and Prevention. Interim guidelines for collecting, handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19). 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
16. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323:1061-1069.
17. Lin L, Jiang X, Zhang Z, et al. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut*. 2020;69(6):997–1001. http://dx.doi.org/10.1136/gutjnl-2020-321013
18. Zhou F, Yu T, Du RH, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395(10229):1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3
19. Shang L, Zhao J, Hu Y, Du R, Cao B. On the use of corticosteroids for 2019-nCoV pneumonia. *Lancet*. 2020;395:683-684.

How to cite this article: Song F, Zhang X, Zha Y, Liu W. COVID-19: Recommended sampling sites at different stages of the disease. *J Med Virol*. 2020;92:1383–1385. https://doi.org/10.1002/jmv.25892