

The yield and consistency of the detection of SARS-CoV-2 in multiple respiratory specimens

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Abstract

Background Few studies compared the yield of the RT-PCR assay in nasopharyngeal swabs, oropharyngeal swabs, and sputum for SARS-CoV-2 detection.

Method We conducted an observational study in Beijing Ditan Hospital, China. Specimens including nasopharyngeal swabs, oropharyngeal swabs and sputum from confirmed COVID-19 patients were collected for RT-PCR testing. Disease duration was calculated from the date of symptom onset to the date of specimens' collection and divided into three groups: ≤ 14 days, 14-21 days, and > 21 days. We compared positive rates across three specimens collected in different disease duration. The kappa coefficient (KC) was used to evaluate consistency of RT-PCR results between different specimens.

Results A total of 291 specimens were collected and tested from 43 confirmed patients. Among specimens collected in disease duration of ≤ 14 days, the positive rate was highest in sputum (79.2%), and significantly higher than that in nasopharyngeal swabs (37.5%, $p=0.003$) and oropharyngeal swabs (20.8%, $p<0.001$). Similar findings were observed in disease duration of 14-21 days and > 21 days. The consistency of testing results between nasopharyngeal swabs and oropharyngeal swabs was low in disease duration of ≤ 14 days and > 21 days. The consistency between the sputum and oropharyngeal swabs, and between the sputum and

nasopharyngeal swabs were very low across all three disease durations with statistical significance.

Conclusion Compared to nasopharyngeal swabs and oropharyngeal swabs, sputum had the highest yield of SARS-CoV-2 detection.

Nasopharyngeal swabs and oropharyngeal swabs had a similar yield. If sputum was not feasible, a nasopharyngeal swab could be recommended for the detection of SARS-CoV-2 and early testing is needed.

Key Words: SARS-CoV-2, sputum, nasopharyngeal swabs, oropharyngeal swabs, positive rate

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Introduction

Since the Novel Coronavirus Disease 2019 (COVID-19) outbreak in Wuhan, China in December 2019, it has caused accumulative more than six million infections across the world by the end of May¹. The World Health Organization (WHO) has declared COVID-19 outbreak a global pandemic². The real-time fluorescence polymerase chain reaction (RT-PCR) testing of respiratory specimens for SARS-CoV-2 RNA is currently widely used for case diagnosis and to guide the duration of patient isolation from hospitalization³. However, it has been reported that there are discrepancies in detection results using different respiratory specimens⁴⁻⁶. The differences in viral presentation among different disease stage of patients with COVID-19 could also play a role⁷. By far, oropharyngeal and nasopharyngeal swabs, as well as sputum, are the recommended respiratory specimens for the detection of SARS-CoV-2. A recent study in Wuhan showed that in suspected individuals the positive rate of RT-PCR test in sputum (76.9%) is significantly higher than that in oropharyngeal swabs (44.2%), but the nasopharyngeal swab was not included in their study⁸. Wang K et al. reported that in 68 patients with COVID-19, SARS-CoV-2 RNA was detected from nasopharyngeal swabs in 48 patients (70.6%), and from sputum specimens in 30 patients (44.1%)⁹. According to the study of Wang X et al., the positive rate was quite different between

nasopharyngeal (32.9%) and oropharyngeal swabs (9.3%) among the inpatients with COVID-19⁶. Notably, dry cough was reported to be common in patients with COVID-19 making sputum collection difficult in many clinical settings^{10,12}, and coughing up sputum can increase the chance of health care workers' exposure. To seek a more applicable specimen for the detection of SARS-CoV-2 to efficiently use the limited public health resources under the current pandemic situation, we would like to propose a study to compare the positive rates of RT-PCR test in nasopharyngeal swabs, oropharyngeal swabs, and sputum for detection of SARS-CoV-2.

Method

Study site and study population

An observational study was conducted at Beijing Ditan Hospital, which is one of the three designated medical centers to treat and manage suspected and confirmed COVID-19 patients in Beijing. We collected nasopharyngeal swabs, oropharyngeal swabs, and sputum from February 2nd to 19th from 43 confirmed COVID-19 patients, who administrated in our hospital from January 21th to February 19th, 2020. All 43 patients were diagnosed according to Chinese management guidelines for COVID-19³ (meet the criteria for suspected cases and have at least two positive results by the RT-PCR assay for SARS-CoV-2 or a genetic

sequence that matches SARS-CoV-2). Each patient was set up an electronic case file to collect basic demographics, medical history, as well as biochemical and radiological tests results during hospitalization.

Sample collection

We collected nasopharyngeal swab, oropharyngeal swab and sputum at the same sampling time and each patient might be sampled more than once during their disease course. Nasopharyngeal swab and oropharyngeal swab were both collected using Applied Cell® Nasopharyngeal Swab with a flocking head. A nasopharyngeal swab was collected from single nostril and an oropharyngeal swab was collected from both sides of throat according to detailed sampling videos respectively¹³(<http://www.cslm.org.cn/cn/news.asp?id=74.html>). When collected sputum specimens, patients were instructed to deep breath in and cough hard, then produce sputum from deep inside the chest. To control the quality, all specimens were collected at 8 am by trained nurses. Specimens were stored in a collection tube with 5 mL virus preservation solution and transferred to laboratory within 2-3 hours by a biosafety box keeping temperature of 0~4 °C.

Laboratory testing

RT-PCT test was conducted in the P2+ laboratory of Beijing Ditan Hospital, which is the officially recognized laboratory to confirm the SARS-CoV-2 infection. Viral RNA was extracted within two hours using

the QIAamp® Viral RNA Mini Kit with primers and probes targeting the open reading frame 1ab (ORF1ab) and nucleocapsid protein (N). ORF1ab: forward primer CCCTGTGGGTTTTACACTTAA; reverse primer ACGATTGTGCATCAGCTGA; and the probe 5' -VIC-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3' . N gene: forward primer GGGGAAGTTCTCCTGCTAGAAT; reverse primer CAGACATTTTGCTCTCAAGCTG; and the probe 5' -FAM- TTGCTGCTGCTTGACAGATT-TAMRA-3' . Reaction system and amplification conditions were performed according to the manufacturer's specifications (Shanghai BioGerm Medical Technology Co. LTD, China). The result was considered valid when the cycle threshold (Ct) value of the reference gene was ≤ 38 . The result was considered positive when the Ct values of both target genes were ≤ 38 . If one of the target genes had a Ct value ≤ 38 and the other >38 , it was interpreted as a single-gene positive. A single-gene positive was also considered as positive in our study.

Definitions

The positive rate of the RT-PCR testing was calculated as the number of specimens tested for positive divided by the total number of specimens being tested. The positive rate was compared among three specimens collected in three disease duration.

The disease duration was calculated from the date of symptom onset to

the date of sample collection and was divided into three groups: ≤ 14 days, 14-21 days, and > 21 days.

Statistical Analysis

Continuous variables were described as the means (Standard Deviation, SD) or medians (interquartile ranges, IQR). Categorical variables were described as counts and percentages. Chi-square test and Fisher's exact test were used for comparison and Bonferroni adjustment was used to account for multiple comparison between different sample types ($P < 0.016$ was considered statistically significant). A kappa coefficient (KC) was used to evaluate testing consistency across three different specimens (highly consistent if $KC \geq 0.75$, consistent if $KC \geq 0.4$, and low consistent if $KC < 0.4$, $P < 0.05$ was considered statistically significant). All analyses were conducted with SAS statistical software (Version 9.2).

Patient Consent Statement

Each patient included in this study signed an informed consent form when they admitted to hospital and any information of patients were anonymised throughout the study and writing process. The design of our study has been reviewed and approved by the Ethics Review Board of Beijing Ditan Hospital (IRB 2020-011-02).

Results

Basic characteristics

We conducted 97 times sampling (24 in the disease duration ≤ 14 days, 33 in the disease duration of 14-21 days, and 40 in the disease duration of > 21 days) and collected nasopharyngeal swab, oropharyngeal swab and sputum at each time. A total of 291 specimens from 43 confirmed COVID-19 patients, including 97 in each type of specimens, were collected and tested. Among 43 included patients, 21 (48.8%) patients were male and the average age was 43.8 (SD:17.1) years old. 36 patients (83.7%) were mild illness, 6 patients were severe illness and 1 patient was critical illness. (Supplementary Table)

Comparison of the testing yield

As showed in Figure 1, the positive rate of RT-PCR testing in sputum was the highest, followed by nasopharyngeal swabs and oropharyngeal swabs across three disease durations. In disease duration of ≤ 14 days, the positive rate in sputum (79.2%) was significantly higher than that in nasopharyngeal swabs (37.5%, $p=0.003$) and oropharyngeal swabs (20.8%, $p<0.001$). In disease duration of 14-21 days, the highest positive rate was also found in sputum (63.6%), and was significantly higher than that in nasopharyngeal swabs (18.2%, $p<0.001$) and in oropharyngeal swabs (9.1%, $p<0.001$). A similar finding was observed in the disease duration of > 21 days that the positive rate was the highest in sputum

(70.0%), and significantly higher than that in nasopharyngeal swabs (15.0%, $p<0.001$) and oropharyngeal swabs (10.0%, $p<0.001$).

We also compared the positive rates between nasopharyngeal swabs and oropharyngeal swabs. There was no statistical significance in each disease durations, even though the positive rates seemed generally higher in nasopharyngeal swabs than that in oropharyngeal swabs (Figure 1).

Consistency test

Table 1-3 showed the KC value of the pairwise comparison of three different specimens collected across three disease durations. For nasopharyngeal swabs and oropharyngeal swabs, the testing consistency was low in the disease durations of ≤ 14 days and >21 days with the KC value of 0.22 ($p=0.157$) and 0.09 ($p=0.480$), respectively. However, the differences in positive rates were not statistically significant. In the disease duration 14-21days, the testing consistency between the two specimens was higher but not significantly (KC=0.62, $p=0.083$).

Between sputum and oropharyngeal swabs, the testing consistency was very low across all three disease durations with statistical significance.

The KC value in the disease duration of ≤ 14 days, 14-21days and >21 days were 0.13($p=0.000$), 0.11($p<0.001$) and 0.02($p<0.001$), respectively (Table 2). Similar findings were observed in the consistency test between sputum and nasopharyngeal swabs that there was a low consistency across three disease durations with statistical significance. The KC value

were 0.13($p=0.004$), 0.12($p=0.000$) and -0.09 ($p<0.001$) for the disease duration of ≤ 14 days, 14-21days and >21 days, respectively (Table 3).

Discussion

In our study, we found the positive rate in sputum was significantly higher than that in nasopharyngeal swabs and oropharyngeal swabs in any disease duration we defined. It is similar to other respiratory viruses, such as influenza A and respiratory syncytial virus, of which the detection rate in sputum was significantly higher than that in nasopharyngeal swabs¹¹. Several articles have revealed that the viral load of SARS-CoV-2 in sputum was significantly higher than that in both throat swabs and nasopharyngeal swabs^{23, 14}. While a report of 28 patients from the Korean Cohort Study on COVID-19 showed viral shedding was higher in nasopharyngeal swabs than sputum¹⁵. We found the positive rate was slightly higher in nasopharyngeal swabs than that in oropharyngeal swabs. It was consistent with the research of Wang X et al., in which higher positive rate was observed in nasopharyngeal swabs than oropharyngeal swabs ($P=0.000$)⁶. Another study conducted by China Center for Disease Control and Prevention (CCDC), the detection of SARS-CoV-2 also showed a higher positive rate (5/8,63%) in nasopharyngeal swabs than oropharyngeal swabs (126/398,32%)⁵, but the number of nasopharyngeal swabs was very small making the researcher hard to conclude. In our

result, the difference of positive rate between nasopharyngeal swabs and oropharyngeal swabs did not have statistical significance either, it might relate to our small sample size.

The discrepancies of consistency tests between sputum and nasopharyngeal swabs, and between sputum and oropharyngeal swabs indicates sputum could be a more reliable specimen for SARS-CoV-2 detection. However, not all patients with COVID-19 produce sputum¹². In many situations, sputum would be hard to get especially among patients with dry cough. The majority of patients with COVID-19 are mild but the virus has high infectivity²¹ so induced sputum in patients might cause further transmission. Therefore, oropharyngeal swabs and nasopharyngeal swabs are the most commonly used specimens for SARS-CoV-2 detection in China and the US^{16,17}. However, the collection of oropharyngeal swabs or nasopharyngeal swabs requires the patient to open mouth wide enough or might cause discomfort and epistaxis^{13,17}. The close contact between healthcare workers and patients was considered high risk of transmission. As the pandemic is still accelerating nowadays, the global public health system is being challenged. In the case of lacking specific antiviral treatment and vaccine for COVID-19, it is critical to efficiently use limited public health resources to detect SARS-CoV-2. The choice of a specimen is not only based on the efficiency of viral detection, but also the cost and available expertise. Researchers have reported that there is a

high expression of ACE-2 receptor of SARS-CoV-2 on cell surfaces in tongue and salivary tissues¹⁸ and the positive rates for saliva were reported nearly 31%-91% in different studies^{19,20}. Considering that sputum is not a common clinical manifestation of COVID-19 and there is a probability of collecting saliva as sputum based on the method of sputum sampling, researchers have demonstrated the potential for saliva to be an ideal specimen type for the detection of SARS-CoV-2²². In our study, we found that nasopharyngeal swabs had a slightly higher yield of SARS-CoV-2 detection compared to oropharyngeal swabs. The collection of nasopharyngeal swabs is considered to be more convenient and safer than oropharyngeal swabs for health workers which made us would like to choose nasopharyngeal swabs for testing. Currently, in our hospital, we have tried to use nasopharyngeal swabs for SARS-CoV-2 detection instead of oropharyngeal swabs when sputum is not available. The outcomes of the strategy change would be coming shortly.

According to our study, the positive rates in nasopharyngeal swab and oropharyngeal swab were not satisfactory among our confirmed patients. In Wang X et al.'s study, false negative result may occur in the late stage of hospitalization especially in oropharyngeal swabs⁶. Chen C et al. have reported that a certain proportion of COVID-19 patients had positive RT-PCR results for SARS-CoV-2 in the sputum or feces after oropharyngeal swabs became negative²⁴. These findings suggest that we

should be cautious when using the negative result of RT-PCR assay in oropharyngeal swabs as the criterion to rule out the infection or to determine the cure of COVID-19. Besides, we also found that not only sputum but also nasopharyngeal and oropharyngeal swabs had a better yield when they were collected within 14 days of symptom onset. When the duration of the symptom onset is greater than 21 days, the profitability of the harvests is lower in all types of samples collected. There are studies suggested that the viral shedding from the upper respiratory tract specimens may reach its peak during the early days after illness onset^{4, 15}. Early testing might be critical to increase the yield for SARS-CoV-2 detection in nasopharyngeal and oropharyngeal swabs, especially when sputum was not available⁵. It is possible that some of these patients are already in the convalescence phase and that is why the negative tests are actually true negative. Further studies are needed to confirm our hypothesis. Our current study included all three commonly used respiratory specimens, in different disease durations, we compared the positive rates and evaluated testing consistency across these three specimens simultaneously, which enabled us to add more comprehensive information in the literature.

There are several limitations to our study. Firstly, the generalizability of our study results might be restricted by the single-center source and a small number of patients included in our study. Patients with severe

diseases were somewhat under-represented (n=7) and sample size were too small to stratify analysis based on disease severity. Further studies are needed to include multiple centers and large numbers of patients.

Secondly, we did not detect viral load in these specimens which is a more representative indicator of the viral distribution. Ct value of samples were also not available in this study to provide semi-quantified virus level.

Thirdly, the patients we have included and tested are mostly with mild to severe diseases. The results cannot be generalized to asymptomatic patients. Other types and larger size of specimens for SARS-CoV-2 detection need to be further studied.

Conclusion

In conclusion, compared to nasopharyngeal swabs and oropharyngeal swabs, sputum had the highest yield in the RT-PCR testing for SARS-CoV-2 detection. Nasopharyngeal swabs and oropharyngeal swabs had a similar yield of SARS-CoV-2 detection. When sputum is hard to get, nasopharyngeal swabs could be a better option and early testing might be needed.

Contributors

FZ, HZ, GW had the idea for and designed the study and take responsibility for the integrity of the data and the accuracy of the data analysis. YZ, JW, DL, YW and JB contributed to samples acquisition of

patients and data collection. YW, JG, CL, FY and LY contributed to the test of samples. GG, SW and DY contributed to the diagnosis and treatment of patients. GW contributed to the statistical analysis. HZ and MC contributed to data analysis and data interpretation. MC and LMW contributed to writing of the article. All authors reviewed and approved the final version.

Declaration of interests

All authors declare no competing interests.

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Figure 1

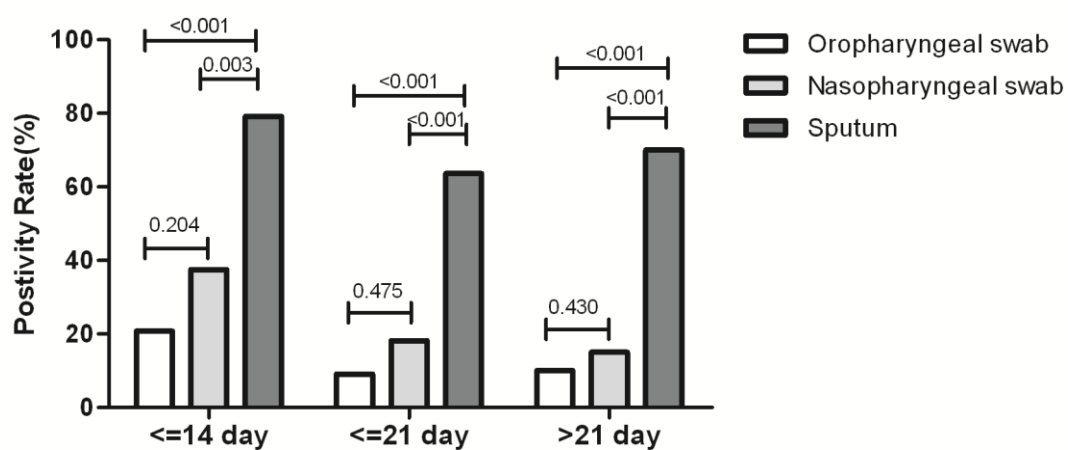


Figure1. The positivity rate of various specimens in different disease duration

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Table 1. The consistency between nasopharyngeal swabs and oropharyngeal swabs

oropharyngeal swabs	nasopharyngeal swabs		kappa	P
	positive	negative		
<=14days			0.22(0.16,0.6)	0.157
positive	3(33.3)	2(13.3)		
negativity	6(66.7)	13(86.7)		
<=21days			0.62(0.24,1.00)	0.083
positive	3(50.0)	0(0.0)		
negativity	3(50.0)	27(100.0)		
>21days			0.09(0.27,0.45)	0.480
positive	1(16.7)	3(8.8)		
negativity	5(83.3)	31(91.2)		

Table 2. The consistency between sputum specimens and oropharyngeal swabs

oropharyngeal swabs	sputum specimens		kappa	P
	positive	negative		
<=14days			0.13(0.02,0.27)	0.000
positive	5(26.3)	0.0(0.0)		
negativity	14(73.7)	5(100.0)		
<=21days			0.11(0.02,0.23)	<0.001
positive	3(14.3)	0(0.0)		
negativity	18(85.7)	12(100.0)		
>21days			0.02(-0.11,0.14)	<0.001
positive	3(10.7)	1(8.3)		
negativity	25(89.3)	11(91.7)		

Table 3. The consistency between sputum specimens and nasopharyngeal swabs

nasopharyngeal swabs	sputum specimens		Kappa	P
	positive	negative		
<=14days			0.13(0.13,0.38)	0.004
positive	8(42.1)	1(20.0)		
negativity	11(57.9)	4(80.0)		
<=21days			0.12(0.08,0.32)	0.000
positive	5(23.8)	1(8.3)		
negativity	16(76.2)	11(91.7)		
>21days			-0.09(-0.28,0.09)	<0.001
positive	3(10.7)	3(25.0)		
negativity	25(89.3)	9(75.0)		