

# Conjunctival polymerase chain reaction-tests of 2019 novel coronavirus in patients in Shenyang,China

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## Research article

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## Abstract

**Background** We report the laboratory results of conjunctival PCR-tests and some clinical features of these patients infected with COVID-19 in shenyang China.

**Methods** We collected conjunctival samples of the patients to do the laboratory tests by PCR. Medical observed patients were enrolled if they had clinical symptoms. Then we analysed the PCR results and clinical features in order to find some relationships.

**Results** The study include 14 confirmly diagnosed cases ,16 suspected cases and some medical observed patients. 1 of the identified case covered from suspected case. 22 medical observed cases were removed because none of them converted to identified cases. 1 of the suspected converted to identified case recently. All the conjunctival results of PCR-test were negative.

**Discussion** The reasons of the negative detection results of PCR in conjunctival swabs were discussed.

**Conclusion** Conjunctiva may be a transmission way of COVID-19. And ocular conjunctival swabs could be a diagnostic method for identifying the infection of COVID-19. Emphasis on the false-negative results is vital.

## Background

Recently, a new strain of coronavirus, designated as COVID-19 by WHO, firstly emerged in Wuhan, Hubei, China, and spreaded to multiple cities rapidly. Coronaviruses are enveloped non-segmented positive-sense RNA viruses belonging to the family Coronaviridae and the order Nidovirales, which are widely detected in humans and other mammals, and commonly denoted in etiologies of respiratory tract infections in humans [1–3]. With similarity to SARS and MERS, they are all dangerous zoonotic coronaviruses which may have the potential to cause a threatening pandemic among human beings [4–5].

Based on clinical experience, COVID-19 is mainly disseminated by person-to-person through inhalation of respiratory droplets. However, some researchers reported that the viruses could also be transmitted by direct contact [6] and digestive tract [7], and conjunctiva may also be possible transmission route without validation [8]. As previously reported, two clinical doctors were also infected with COVID-19 who wore N95 masks during his work, but they did not wear goggles at that time leaving their ocular surface directly to the room air.

In this study, we make an effort to testify whether conjunctiva is a possible way for the transmission of COVID-19 and whether it could be an effective diagnostic approach to detecting the infection of COVID-19.

## Methods

### Data Collection

In this cross-sectional non-randomized study, 14 identified cases, 16 suspected cases and 22 medical observed cases that infected with from January, 2020 to February, 2020 were included. The study was approved by local ethics committee, sponsored by Ministry of Shenyang science and technology and in accordance with Declaration of Helsinki. At the time of collecting conjunctival samples, every patient was informed of purposes and methods, and informed consent was obtained from all patients. Fundamental information on age, gender, underlying systemic disease and severity of diseases. Ophthalmic information on ocular signs and symptoms, history of ocular surgery and the results of conjunctival PCR-tests were recorded. Detailed research methods were listed as follows.

### Nucleic acid extraction and real time RT-PCR

After obtaining conjunctival samples by sterile swabs, the samples were placed in EP tubes filled with virus sampling fluid. Then the samples were transported immediately to the  $-80^{\circ}\text{C}$  refrigerator in order to preserve and isolate COVID-19 subsequently.

### Nucleic acid extraction and PCR analysis

Specimens were extracted using a viral nucleic acid detection kit (QIAGEN). Briefly, 200  $\mu\text{L}$  sample was used to prepare reaction mixture. After 30 minutes' standing, the reaction mixture was centrifuged for 10 seconds. The reaction system consisted of 13  $\mu\text{L}$  Cov test buffer and 5  $\mu\text{L}$  reaction fluid. After extracting the viral DNA, then the samples were used for COVID-19 PCR analysis. The assay was performed in the laboratory with a real-time LightCycler (ABI7500). Reactions were set up performed according to the manufacturer's instructions. Real-time PCR was performed using the LightCycler and targeted agents (made by Jienuo Biotechnology Limited Company in Shanghai) to amplify the COVID-19 at  $42^{\circ}\text{C}$  for 10 s, followed by  $95^{\circ}\text{C}$  for 10 s, and finally 40 cycles for 10 s at  $95^{\circ}\text{C}$  and for 45 s at  $60^{\circ}\text{C}$ .

The results mainly depended on the CT values of two targets, the ORF (Open reading frame) and core-shell protein gene.

## Results

We retrospectively analyze 30 patients who were diagnosed as infection of COVID-19 including 14 identified cases and 16 suspected cases. One suspected case has converted to identified case recently. What's more, 22 medical observed cases who had had intimate contact with identified or suspected cases were included. However, because none of medical observed cases have converted to identified cases and related detection of samples were negative, so they are removed from this study. As displayed in the table 1, the mean age of the identified group is  $48 \pm 13.4$  years old and that of the suspected group is  $40 \pm 16.2$  years old. The numbers of male and female are nearly equal. As for systematic diseases, 3 of them have diabetic mellitus, 4 of them have hypertension and 1 of them has hepatitis B. None of them have the history of general or ocular surgery. Just 1 identified case is complicated with macular degeneration and another 1 identified case felt eye itching before the onset.

With regard to the severity of disease, common pneumoniae accounts for most of identified cases and mild cases are the most common type of the suspected cases. In the identified group, there is just one severe pneumonia case, whereas another one converted to severe pneumonia from a suspected case.

The test results and related time show as below in table 2.

## Discussion

Generally speaking, deduced from the fundamental condition of our cases, we found that the severity of disease in our patients is relatively moderate. In addition, the affected age group is younger without so many underlying diseases. There are only 2 cases manifested as severe pneumonia, and one of them converted from suspected case. By investigating the possible reasons, we speculated this consequence may be caused by the delayed confirmation of infection. However, the condition seems to contradict the epidemiological study done by a cluster of Chinese researchers, in which they found that COVID-19 infection could cause severe respiratory illness similar to severe acute respiratory syndrome and was associated with ICU admission and high mortality [9]. It may be due to the fact that the included are all imported cases who recently have gone to Wuhan or contacted with the identified cases, compared with those who were invaded directly by viruses.

PCR test has been widely used to confirm the viral infection targeted to the virus-specific nucleic acid sequences, but in our study, we found that after isolation for presumed infection of COVID-19, the 2 cases were finally diagnosed by repeated pharynx swabs tests. We postulated that maybe it caused by false sampling position, which means that the viruses are likely to exist in lower respiratory tract other than the upper. So the false-negative rate of PCR may mislead the clinical doctors and even miss the optimal opportunity for treatment. Some Chinese clinicians also found similar phenomenon and reported 5 cases. By evaluating radiographic characteristics of 5 patients with confirmed 2019-nCoV infection and initial negative or weakly positive RT-PCR, they found that the patients presented characteristic radiographic features of COVID-19 pneumonia from the first scan and then were confirmed by positive repeat swabs test during the isolated observation or treatment. They ascribed the possible reasons to laboratory error or insufficient viral material in the specimen [10].

In our cases, all of the conjunctival results of PCR were negative no matter in identified or suspected cases. We analyze and summarize the reasons as follows: (1) The shedding loads of the COVID-19 was below the sensitivity of the test or some individuals were actually not shedding viral DNA at that time. This opinion correlates with Sarah E. Burr's study, where she obtained 28 conjunctival swabs during the outbreak of acute haemorrhagic conjunctivitis in the Gambia, West Africa, caused by the epidemic of coxsackievirus, and 25% of the PCR-test showed negative results. [11] (2) In our study, the conjunctival sampling time of the cases was after identification. The administration of drugs, such as corticosteroid and antiviral drugs, and the mutation of viruses could all affect the results. (3) The sensitivity of the viral nucleic acid kits is low. (4) The conjunctiva lacks of related receptors. A pivotal factor for efficient person-to-person transmission is the ability of the virus to attach to human cells. Because coronaviruses use a spike protein for attachment to host cells [12]. As previously reported, both COVID-19 and SARS-nCov use the same receptor called ACE2, which has been verified to locate mainly on lung alveolar epithelial cells and enterocytes of the small intestine [13]. However, whether ACE2 receptors exist on the conjunctival surface and the level of conjunctival ACE2 expression still need further investigation. Given that ACE2 expression is extremely rare on the ocular surface, the viruses could not attach to the conjunctiva and they may transfer to any organs in our bodies through lacrimal ductule. In our study, a 29-year-old female without any systematic disease converted to identified case from suspected case. She was a moderate suspected case, whose result of pharynx swab was positive at first, and after treatment, the result changed negative. However, the result represented as positive after 3 days. So we could speculate present therapies may only resist the viruses temporarily, and the COVID-19 may seek any opportunity to represent when the human immunity weakens. What's worse, they may transfer to anywhere to find suitable hosts. In addition, we found that the sampling time contributed to obtain a reliable result, pharynx and conjunctiva swab should be collected meantime. In our study, the two swabs were obtained meantime in 3 identified cases, but the results were inconsistent. The coexistence of positive pharynx specimen and negative result of conjunctiva specimen reminded that the viruses may be more likely to attach to respiratory tract other than conjunctiva.

Restricted by reality, there are some limitations in our study. Firstly, no positive result was obtained from identified and suspected cases. Secondly, we lack of swabs collected from individuals with normal eyes during the sampling process.

## Conclusions

Undoubtedly, the regular samples for PCR tests, such as pharynx and sputum swabs, have its advantage in some facets, but considering their errors and the viral transmission characteristics, conjunctival swabs for PCR may become a convenient, noninvasive and simple diagnostic methods. And whether conjunctiva is a possible transmission route of COVID-19 still needs further research. Sampling time is also a key factor as well. A more uniformed scheme should be established to guide the clinical practitioners make correct judgements avoiding the cross infection.

## Abbreviations

RT-PCR: reverse transcription polymerase chain reaction; COVID-19: coronavirus disease 2019

## Declarations

### Ethics approval and consent to participate

The need for ethics approval is deemed unnecessary because we obtained samples according to the third version of monitoring scheme for 2019 novel coronavirus infected cases, which is published nationally. Only verbal consent were obtained because the patients with infection of COVID-19 should be isolated at once and accept treatments. The ethics committee in Shenyang Center of Disease control and prevention approved this.

### Consent to publish

The patients gave written consent for the patients' personal or clinical details along with any identifying images to be published in this study.

### Availability of data and materials

All data generated and analyzed during the study are included in this article.

### Competing Interests

The authors declare that they have no competing interests.

### Funding

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### Author's Contributions

LX, WS and BJS collected the data, XYZ and LX drafted the article, JPM, BW, ZQW, XD and YHC analyzed the data, LX carried out critical revision and correction of the manuscript. All authors read and approved the final manuscript.

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Not Applicable

## References

1. Zhu N, Zhang D, Wang W, Li X et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*, 2020 Jan 24, DOI: <https://doi.org/10.1056/NEJMoa2001017>
2. Li Q, Guan X, Wu P, Wang X et al. Early transmission dynamics in Wuhan, China, of novel coronavirus infected pneumonia. *N Engl J Med*, 2020, Jan 29, DOI: <https://doi.org/10.1056/NEJMoa2001316>
3. Richman DD, Whitley RJ, Hayden FG, eds. *Clinical Virology*, 4th edn. Washington: ASM Press, 2016
4. De Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol*, 2016, 14: 523-534. Yin Y, Wonderlink RG. MERS, SARS and other coronaviruses as causes of pneumonia. *Respirology*, 2018; 23: 130-137

5. Yin Y,Wonderlink RG.MERS,SARS and other coronaviruses as causes of pneumonia.Respirology,2018;23:130-137
6. Ping-Ing Lee,Po-Ren Hsueh.Emerging threats from zoonotic coronaviruses-from SARS and MERS to 2019-nCov.Journal of Microbiology,Immunology and Infection,https://doi.org/10.1016/j.jmii.2020.02.001.
7. Michelle L.Holshue,Chas DeBolt,Scott Lindquist et al.First case of 2019 novel coronavirus in the United States,The New England Journal of Medicine,2020,DOI:10.1056/NEJMoa2001191
8. Cheng-wei Lu,Xiu-fen,Liu,Zhi-fang Jia.2019-nCov Transmission through the ocular surface must not be ignored.2020,Feb 6,www.Lancet.com.https://doi.org/10.1016/S0140-6736(20)30313-5
9. Chaolin Huang,Yeming Wang,Xingwang Li et al.Clinical features of patients infected with 2019 novel coronavirus in Wuhan,China.2020,Jan 24,http://doi.org/10.1016/S0140-6736(20)30183-5
10. Xingzhi Xie, Zheng Zhong ,Wei Zhao , Chao Zheng et al.Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing.2020,Radiology,200343.doi:10.1148/radiol.2020200343
11. Sarrah E.Burr,Ansumana Sillah,Hassan Joof et al.An outbreak of acute haemorrhagic conjunctivitis associated with coxsackievirus A24 variant in the Gambia,West Africa.2017,BMC Res Notes,10:692
12. Yushun Wan,Jian Shang,Rachel Graham et al.Receptor recognition by novel coronavirus from Wuhan:An analysis based on decade-long structural studies of SARS.2020,J. Virol. doi:10.1128/JVI.00127-20
13. I Hamming,W Timens,AT Lely et al.Tissue distribution of ACE2 protein,the functional receptor for SARS coronavirus.A first step in understanding SARS pathogenesis.2004,Journal of Pathology,203(2):631-637

## Tables

Table 1:Demographics and baseline characteristics of identified and suspected cases infected with COVID-19

	All patients(N=30)	Identified(n1=14)	suspected(n2=16)
Characteristics			
Age,years	43.7±14.3	48±13.4	40±16.2
Sex			
Men	14(46.7%)	7(50%)	7(43.75%)
Women	16(53.3%)	7(50%)	9(56.25%)
Earliest Contact Time		2020.01.04	2020.01.13
Last Contact Time		2020.01.31	2020.01.31
Onset Time,numbers			
January	16(55.2%)	10	6
February	13(44.8%)	4	9
Identifying Time,numbers			
January	4(18.2%)	4	0
February	18(81.8%)	10	8
Underlying Systematic Disease			
Diabetes	3(37.5%)	3	0
Hypertension	4(50%)	4	0
Hepatitis	1(12.5%)	1	0
Ocular or General Surgery History	None	None	None
Ocular Symptoms			
eye itching	1	1	0
Ocular Comorbidity			
macular degeneration	1	1	0
Severity of Disease (8 cases were removed from suspected group without abnormalities)			
mild	6(27.3%)	2	4
moderate	3(13.6%)	2	1
ordinary	4(18.2%)	3	1
severe	1(4.5%)	1	0
common pneumonia	6(27.3%)	5	1
severe pneumonia	2(9.1%)	1	1
Total	22(100%)	14(63.6%)	8(36.4%)

Table 2: The detection results of identified patients with infection of COVID-19

Number	Onset Time	Identified Time	First sampling	Sampling type			Second sampling	Sampling type			Third sampling	Sampling type			Fourth sampling	Sampling type		
				Pharynx	sputum	blood		Conjunctiva	Pharynx	sputum		blood	Conjunctiva	Pharynx		sputum	blood	Conjunctiva
1	2020/1/26	2020/2/6	2020/2/2	+			2020/2/5				2020/2/13	-						
2	2020/1/29	2020/2/5	2020/2/4	+		-	2020/2/5				2020/2/13	+						
3	2020/1/28	2020/2/6	2020/2/2	+			2020/2/5				2020/2/13	+						
4	2020/1/31	2020/2/5	2020/2/2	-	-	-	2020/2/3	-			2020/2/4	+			2020/2/6			-
5	2020/2/5	2020/2/7	2020/2/6	+		-	2020/2/13	+										
6	2020/1/25	2020/1/28	2020/1/27	+			2020/2/2	+		+	2020/2/9			-	2020/2/13			-
7	2020/2/2	2020/2/5	2020/2/2	+	+		2020/2/3	+	+	+	2020/2/9			-	2020/2/13			-
8	2020/2/2	2020/2/4	2020/2/3	+		+	2020/2/9											-
9	2020/1/21	2020/1/27	2020/1/26	+			2020/2/10	-		-	2020/2/12	-		-	2020/2/13	-		-
10	2020/1/24	2020/2/3	2020/2/2	+	+	+	2020/2/8	-			2020/2/10	+	+	-	2020/2/12	-		-
11	2020/1/23	2020/2/5	2020/2/2	-	-	-	2020/2/3	+	+		2020/2/8	-		-	2020/2/10	-		-
12	2020/1/23	2020/1/31	2020/1/30	+	+	+	2020/2/10	-		-	2020/2/12	-		-				
13	2020/1/23	2020/1/25	2020/1/24	+			2020/2/10	-		-	2020/2/13	-		+				
14	2020/2/6	2020/2/7	2020/2/6	+			2020/2/10				2020/2/13	-						
15	2020/1/31	2020/2/3	2020/2/2	+			2020/2/5				2020/2/6	-			2020/2/8	+		+

The number 15 is an identified case converted from the suspected without typical clinical symptoms. She was identified as suspected on 2020/2/3 and was finally confirmed on 2020/2/8 representing with positive of pharynx swabs at the third sample.