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Progress in research on the detection of the novel coronavirus in human samples of different groups

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Abstract. – OBJECTIVE: Among the illnesses that may develop from COVID-19, the disease caused by the novel coronavirus (SARS-CoV-2), is pneumonia, a severe acute respiratory infectious disease. SARS-CoV-2 continues to spread worldwide and has caused hundreds of thousands of deaths thus far and has disrupted the world economy.

PATIENTS AND METHODS: This review summarized the reported distributions of SARS-CoV-2 in 13 biological samples of the human body, including nose, feces, sperm, tears, breast milk, cerebrospinal fluid, urine, organs, sputum, cell lines, bronchial brush, blood, throat, and bronchoalveolar lavage fluid. Moreover, this review briefly describes the detection of SARS-CoV-2 in human body samples of five other coronaviruses.

CONCLUSIONS: This review offers several recommendations for controlling the spread of SARS-CoV-2 control, specifically, sample collection from suspected cases from foreign countries and risk assessment of imported special goods (biological materials).

Key Words:
SARS-CoV-2, Human samples, Novel Coronavirus

Introduction

Among the illnesses that may develop from COVID-19, the disease caused by the novel coronavirus (SARS-CoV-2), is pneumonia, a severe acute respiratory infectious disease. COVID-19 pneumonia is mainly characterized by fever, weakness, and dry coughing. In December 2019, SARS-CoV-2 began to spread and became an epidemic within a short time. It is classified as a Class B infectious disease by the Law of the People's Republic of China on the Prevention and Treatment of Infectious Diseases. Moreover, it is categorized as a Class A infectious disease that requires quarantine measures by the Frontier Health and Quarantine Law of the People's Republic of China. In March 2020, the World Health Organization announced that the COVID-19 epidemic had become a pandemic. By May 24, 2020, the number of cumulative confirmed cases and deaths worldwide has exceeded 5.2 million and 330,000, respectively[1].

Since the occurrence of the COVID-19 epidemic, scientists all over the world have taken the initiative to study the various aspects of SARS-CoV-2, and numerous biopharmaceutical companies have begun conducting urgent research and development of potential drugs and vaccines. Accordingly, international trade of special products, including human tissues, human blood and blood products, and biological products, has been increasing. As COVID-19 continues to spread all around the globe, more stringent requirements for risk assessment and approval of special goods in exports and imports have been proposed. SARS-CoV-2 was first detected in bronchoalveolar lavage fluid (BALF), and nose swab testing is typically done for viral detection[2]. As scientists learn more about this virus, it has been found in numerous human samples, such as urine, feces, cerebrospinal fluid (CSF), tears, and sperm[3-20]. This review summarizes the various detection methods for SARS-CoV-2 in different human body samples that are implemented in China to ensure the safety of its citizens during the pandemic. This review offers several recommendations for controlling the spread of COVID-19, including clinical detection, sample collection from suspected cases from foreign countries, and risk assessment of imported special products (biological materials).

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Detection of SARS-CoV-2 in Different Human Body Samples

SARS-CoV-2 has been discovered in various human body samples, including nose, feces, sperm, tears, CSF, urine, organs, sputum, human cell lines, bronchial brush, blood, throat, and BALF, via nucleic acid tests and virus separation technologies (Table I).

Respiratory Tract, Blood, Urine, and Fecal Samples

The Chinese Center for Disease Control and Prevention has reported the positivity rate of SARS-CoV-2 in different human body samples[3]. The Center examined 205 hospitalized patients and collected a total of 1070 samples. Throat swab was collected from most patients 1-3d after hospitalization. During the entire course of the disease, the Center collected blood, sputum, fecal, urine, and nose swab samples. They also collected biopsy samples of BALF and bronchial brushing cytology from severe cases or patients who required mechanical ventilation. They tested the nucleic acids of SARS-CoV-2 by real-time fluorescence quantitative PCR. The positivity rate in the samples of BALF, sputum, nose swab, biopsy, bronchial brush, throat swab, feces, blood, and urine is 93%, 72%, 63%, 46%, 32%, 29%, 1%, and 0%, respectively. Among respiratory tract samples, the positive rate of throat swab is the lowest (Table II). In Japan, one case reportedly had a negative result in nucleic acid test of throat swab but had positive result in induced sputum test[19]. In the said study of the Chinese Center for Disease Control and Prevention, SARS-CoV-2 was not detected in 72 urine samples. However, the team led by academician Zhong Nanshan detected SARS-CoV-2 from the urine sample of one patient on February 19, 2020[15].

The First Affiliated Hospital of Zhejiang University School of Medicine assessed viral loads in different human samples[4]. The team collected 3497 respiratory, serum, fecal, and urine samples from 96 patients and found substantial differences in their viral loads. The found the highest viral loads in respiratory tract and fecal samples, whereas they recorded the lowest loads in serum samples. The duration of SARS-CoV-2 in fecal samples was considerably longer than that in respiratory tract and serum samples. This finding indicates that the management of fecal samples must be strengthened for the effective prevention and control of COVID-19. Guangzhou Medical University and the Chinese Center for Disease Control and Prevention successfully detected SARS-CoV from fecal samples[5,6].

CSF Samples

Beijing Ditan Hospital and the Institute of Infectious Diseases of the Chinese Center for Disease Control and Prevention collaborated and analyzed SARS-CoV-2 sequences from the CSF sample of one patient with COVID-19 and encephalitis. Computed tomography and magnetic resonance imaging of patients with COVID-19 and acute hemorrhagic necrosis brain diseases revealed that SARS-CoV-2 can invade the brain directly in extremely few cases. Moreover, clinicians and radiologists must consider the neuropathic conditions of patients with COVID-19[12]. Recent studies[13,14] have achieved progress in identifying the clinical manifestations and infection mechanisms of SARS-CoV-2 invasion into the central nervous system.

Table I. SARS-CoV-2 detection in different human body samples.

No.	Sample type	Detection method	Detection results
1	Nose swab	Nucleic acid test	Detected[3]
2	Feces	Nucleic acid test, virus separation and identification	Detected[3-6]
3	Sperm	Nucleic acid test	Detected[7]
4	Tears	Nucleic acid test	Detected[8,9]
5	Breast milk	Nucleic acid test	Not detected[10]
6	CSF	Gene sequencing	Detected[11-14]
7	Urine	Virus separation and identification	Detected[15]
8	Organ	Modeling and autopsy	Detected[16-18]
9	Sputum	Nucleic acid test	Detected[3,19]
10	Bronchial brush	Nucleic acid test	Detected[3]
11	Blood	Nucleic acid test	Detected[3,4]
12	Throat swab	Nucleic acid test	Detected[2,3]
13	BALF	Nucleic acid test	Detected[2,3]

Table II. Positive rates of SARS-CoV-2 in different human body samples reported by the Chinese Center for Disease Control and Prevention.

No.	Sample type	Positive rate (%)	Corresponding proportion of detection
1	BALF	93	14/15
2	Sputum	72	72/104
3	Nose swab	63	5/8
4	Bronchial brush	46	6/13
5	Throat swab	32	126/398
6	Feces	29	44/153
7	Blood	1	3/307
8	Urine	0	0/72

Tear and Conjunctiva Secreta Samples

The First Affiliated Hospital of Zhejiang University School of Medicine tested for SARS-CoV-2 in tear and conjunctiva secreta sample of 30 patients with COVID-19 via nucleic acid test and detected the virus in one patient[8]. This result indicates that SARS-CoV-2 can also exist in the tears and conjunctiva secreta. On the basis of their assessment of keratoplasty risk under the current COVID-19 epidemic, the United States and Global Eye Bank Association have suggested to "completely preventing use of donor tissues which has been infected with SARS-CoV-2 or exposed to SARS-CoV-2 environment recently".

Sperm Samples

The Eight Medical Center of Chinese PLA General Hospital detected SARS-CoV-2 from the sperm of patients in the acute and recovery stages[7]. They collected sperm samples from 38 patients, 23 of which were in the recovery stage, whereas 15 were in the acute stage. Nucleic acid test detected SARS-CoV-2 in the sperm samples of six patients, four of which were in acute stage (26.7%) and two in the recovery stage (8.7%). Although this study confirmed that SARS-CoV-2 can exist in sperm, it failed to prove that SARS-CoV-2 can be transmitted through sexual acts or intercourse.

Human Tissues

SARS-CoV-2 infection models of human organs predict that this virus can infect biliary epithelial cells[16]. Autopsy examinations revealed that SARS-CoV-2 can directly infect the kidneys[17,18].

Human Cell Lines

The receptor for SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2), which is the same for SARS-CoV[20]. ACE2 can be expressed in HeLa cells, COS7 cells, and NIH 3T3 cells. ACE2 genes (Hace2) are transferred to these cells through a virus carrier. Several cell lines have been found to be susceptible to SARS-CoV-2 and SARS-CoV.

Breast Milk

A prior study did not detect SARS-CoV-2 in breast milk samples from the first lactation of nine puerperal patients with COVID-19[10].

Detection of Other Coronaviruses in Different Human Body Samples

Before the first recorded case of SARS-CoV-2, six types of coronavirus, namely, human coronavirus 229E (HCoV-229E), human coronavirus NL63 (HCoV-NL63), human coronavirus OC43 (HCoV-OC43), human coronavirus HKU1 (HCoV-HKU1), Middle East respiratory syndrome–coronavirus (MERS-CoV), and severe acute respiratory syndrome–coronavirus (SARS-CoV), have been documented to infect people. The detection results of these coronaviruses in different human body samples are listed in Table III.

Detection Results of HCoV-NL63, HCoV-HKU1, HCoV-OC43, and HCoV229E in Different Human Body Samples

A total of 538 nose swab samples from several children with acute respiratory infection in winter and spring in Fuzhou City were tested for coronaviruses via nucleic acid test. Among them, 41 tested positive: 8 tested positive for HCoV-NL63, 5 for HCoV-229E, 6 for HCoV-HKU1, and 22 for HCoV-OC43[21].

A study in Canada obtained brain tissue samples from 90 donors with nervous system diseas-

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7	Blood	1	3/307
8	Urine	0	0/72

es for coronavirus detection via nucleic acid test. Forty tested positive for HCoV229E and 21 for HCoV- OC43[22].

The Centers for Disease Control of Guizhou Province collected nose swab, thoracocentesis liquid, and bronchoalveolar lavage fluid samples from 118 patients with pneumonia for unknown reasons and tested them for four coronaviruses (i.e., HCoV- NL63, HCoV-HKU1, HCoV-OC43, and HCoV229E) via nucleic acid test. Four tested positive for HCoV- OC43, of which three were detected from nose swab samples and one from thoracocentesis liquid sample[23].

A study in France detected HCoV-HKU1 in the fecal samples of patients who tested positive for HCoV-HKU1 in nose swab samples (2/6 cases)[23].

Detection Rresults of MERS-CoV in Different Human Body Samples

The *Diagnosis and Treatment for MERS Cases* published by the National Health and Family Planning Commission of the People's Republic of China proposed requirements on samples for MERS-CoV detection. It recommends that multiple samples should be collected in time (throat swab, nose swab, extracts from nose, throat or trachea, phlegm or lung tissues, blood, and feces) to allow viral detection via nucleic acid test. The detection rate of patients that tested positive for MERS-CoV was higher in respiratory tract samples than in other samples[24]. In Germany, MERS-CoV was detected in the urine and feces of one patient with MERS-CoV.

Risk Ranking of Unique Imported and Exported Goods

According to risk factors of unique goods, such as pathogenicity, pathogenic route, mode of use, purpose, and controllability, unique goods are divided into four classes: A, B, C, and D. Unique goods classified under class A and B are relatively of high risk. Class A unique goods include those containing type I and type II pathogenic microorganisms in the *List of Pathogenic Microorganisms of Human Infection*, imported human blood, plasma, tissues, organs, cells and marrows, exported unique goods involving genetic resources of humans, and environmentally friendly microbial agents. Class B unique goods are composed of goods containing type III pathogenic microorganisms in the *List of Pathogenic Microorganisms of Human Infection*; unique goods that might contain type I, type II, or type III pathogenic microorganisms and goods that have unknown infectivity; unique goods containing or might contain parasites; complete or modified genome nucleic acid substances of type I and type II pathogenic microorganisms; and toxins generated by pathogenic microorganisms and proved harmful to human health. Various human biological samples of SARS-CoV-2 that can be detected in unsplit or incompletely purified protein products all belong to unique goods of high risk. All possibilities of the presence of SARS-CoV-2 in different samples should be considered in assessing imported and exported goods to ensure safety and prevent the further spread of this virus in China.

Spreading Pathway of SARS-CoV-2 and Requirements on Laboratory Detection Samples

According to the *Diagnosis and Treatment Scheme for Novel Coronavirus Infected Pneumonia* published by the National Health and Family Planning Commission of the People's Republic of China, respiratory droplets and close contact are two major ways by which SARS-CoV-2 is spread. Spread via aerosols is another major possibility. Thus far, no evidence is available that shows that

SARS-CoV-2 can be spread through blood, breast milk, and sexual acts or intercourse. During laboratory detection of SARS-CoV-2, respiratory tract samples, including the upper and lower respiratory tract, should be collected in patients in the acute stage. However, samples should be collected first from the lower respiratory tract of severe patients. Fecal samples, whole blood, or serum samples can be collected as clinically necessary. The types of samples that should be collected from different body parts are as follows. Upper respiratory samples: nose swab and throat swab; lower respiratory samples: deep coughing sputum, BALF, bronchial lavage fluid, and respiratory aspirate; fecal sample: about 10 g (about the size of a peanut) of feces (anal swab is acceptable if collection of fecal sample is inconvenient; blood sample: 5 mL of anticoagulation sample from patients in the acute stage within 7 d after the onset (blood samples should be collected via vacuum angiography, which contains an EDTA anticoagulant); serum samples: serum samples (5 mL) from patients in both the acute and recovery stages. The first serum sample should be collected as early as possible (preferably 7 d after symptom onset), whereas the second serum sample should be collected 3–4 weeks after symptom onset. The sample should be collected via vacuum angiography without an anticoagulant. Serum is mainly used to test antibodies and thus nucleic acid test is not necessary.

Conclusions

COVID-19 has affected over 210 countries worldwide and currently poses a serious threat to humans. Guterres, the Secretary General of the United Nations, emphasized that the COVID-19 pandemic is the biggest international challenge since WWII. This pandemic cannot be defeated without technological support. Since the onset of the epidemic, China has made science-based decisions and conducted large-scale and immediate laboratory detections of suspected cases to prevent missing cases. Samples from patients must be properly collected and these should be tested via nucleic acid tests so that the COVID-19 pandemic can be effectively controlled. China has received international accolades for its effective prevention and control of COVID-19 and has achieved progress in technological R&D. Quarantine lines must be consolidated and biomedical R&D should be facilitated if we wish to win this fight against this pandemic. This review summarized the distribution of SARS-CoV-2 in different human body samples. Aside from respiratory samples, SARS-CoV-2 can be detected in blood, urine, fecal, CSF, tear, sperm, human tissue, and human cell line samples. The viral load in respiratory samples is relatively higher compared with that in other samples. The positivity rate of urine and blood samples for SARS-CoV-2 is lower than that of other samples, indicating that they carry a lower risk of SARS-CoV-2. This review offers recommendations for clinical detection of SARS-CoV-2. Specifically, samples for viral detection should be collected from suspected cases from foreign countries at customs and imported unique goods should be assessed for risk.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Statement

All patients signed an informed consent approved by the Institutional Ethics Committee before participation in this study.

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