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Short Communication

Current approaches in laboratory testing for SARS-CoV-2

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ABSTRACT

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which began in Wuhan, Hubei Province, China, has rapidly spread to produce a global pandemic. It is now clear that person-to-person transmission of SARS-CoV-2 has been occurring and that the virus has been dramatically growing in recent months. Early, rapid and accurate diagnosis is of great significance for curtailing the spread of SARS-CoV-2. There are currently several diagnostic techniques (e.g. viral culture and nucleic acid amplification test) being used to detect the virus. However, the sensitivity and specificity of these methods are quite different, with the sample source and detection limit varying greatly. This study reviewed all types and characteristics of the currently available laboratory diagnostic assays for detecting SARS-CoV-2 infection and summarized the selection strategies of testing and sampling sites at different disease stages to improve the diagnostic accuracy of Coronavirus Disease 2019 (COVID-19).

An outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was discovered in Wuhan, China, in December 2019. It then rapidly developed into a global pandemic. As of 29 May 2020 a total of 5,701,337 laboratory-confirmed COVID-19 cases had been reported worldwide, with 357,688 deaths confirmed to date. Among the effective control measures to reduce transmission in the community, early and reliable laboratory confirmation of SARS-CoV-2 infection is of crucial importance. This review summarized advances made in technologies for rapid diagnosis and confirmation of respiratory infections caused by SARS-CoV-2, as well as the selection strategies of testing and sampling sites in SARS-CoV-2 detection.

Since the initial cases of pneumonia of unknown cause were first reported, viral culture and genetic sequencing of isolates obtained from these patients identified a novel coronavirus as the etiology within 10 days in January 2020. This benefitted understanding of the disease occurrence and transmission, as well as diagnostic test development (Zhu et al., 2020). Although viral culture is relatively time-consuming and labor-intensive, it is much more useful in the initial phase of emerging epidemics before other diagnostic assays are clinically available. Besides, unbiased, high-throughput sequencing has been proven as a powerful tool for discovering pathogens (Table 1). A detection assay (BGI, Shenzhen, China), based on next-generation sequencing, was approved for emergency use authorization (EUA) by the National Medical Products Administration (NMPA) in China (Table S1). However, whole genome sequencing is time-consuming and requires specialized instruments with high technical thresholds, and thus is not recommended for widespread clinical use.

Real-time reverse transcription polymerase chain reaction (RT-PCR) is routinely used in acute respiratory infection to detect causative viruses from respiratory specimens. The World Health Organization (WHO) recommends that patients who meet the case definition for suspected SARS-CoV-2 should be screened for the virus nucleic acid amplification test (Table 1). Various real-time RT-PCR assays for detecting SARS-CoV-2 RNA have been developed worldwide, with different targeted viral genes or regions (Table S1). To date, 13 and 52 commercial SARS-CoV-2 real-time RT-PCR diagnostic panels have been issued for EUA by China and the US, respectively, with the limit of detection varying from 100 to 1000 copies/mL (Table S1). Although RT-PCR has relatively high sensitivity, there have been reports of multiple false negative tests for the same patients infected with SARS-CoV-2 in China (Xie et al.,

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Table 1
Laboratory testing for detection of SARS-CoV-2.

Testing type	Specimen type	Characteristics	Testing time	Limitation
Viral culture	Respiratory sample	Gold standard for virus diagnosis and useful in the initial phase of emerging epidemics	3–7 days	Time- and labor-consuming, biosafety level 3 laboratory needed, cannot be widely used in clinical settings
NAAT, whole genome sequencing	Respiratory sample and blood	Detects all pathogens in a given specimen, including SARS-CoV-2, as well as viral genome mutations	20 h	Time-consuming, specialized instruments with high technical thresholds, and high cost
NAAT, real-time RT-PCR	Respiratory sample, stool and blood	Most widely used for laboratory confirmation of SARS-CoV-2 infection	1.5–3 h	Time-consuming procedure, requires biosafety conditions, expensive equipment, skilled personnel, and can have false negative results
NAAT, isothermal amplification	Respiratory sample, stool and blood	Requires only a single temperature for amplification, takes less time, but comparable performance with real-time RT-PCR, and does not require specialized laboratory equipment	0.5–2 h	False negative results, as real-time RT-PCR
Serological testing	Serum, plasma and blood	Less time required, simple to operate, useful in disease surveillance and epidemiologic research	15–45 min	Cross-reaction with other subtypes of coronaviruses
Point-of-care test	Respiratory sample	Provides rapid actionable information with good sensitivity and specificity for patient care outside of the clinical diagnostic laboratory	5–30 min	Risk of quality loss and lack of cost-effectiveness

NAAT, nucleic acid amplification test; RT-PCR, reverse transcription polymerase chain reaction.

2020; Xiao et al., 2020), suggesting that negative results do not preclude the presence of SARS-CoV-2 in a clinical specimen. In addition, fluctuating RT-PCR results have been observed in several patients who first tested positive for SARS-CoV-2, then tested negative in the following test, and returned to be positive in a final test (Li et al., 2020a). False negative results may be due to the selection of sampling locations, poor sample quality, low viral load of the specimen, incorrect storage and transportation, as well as laboratory testing conditions and personnel operations. If a highly suspected patient is negative for the virus, repeat the nucleic acid amplification test or consider collecting a more suitable sample.

Isothermal amplification techniques offer a good alternative to real-time RT-PCR, with comparable performance (Table 1). They take less time and generally do not need specialized laboratory equipment. These techniques include loop-mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), and cross priming amplification (CPA). A recent study suggested that reverse transcription LAMP (RT-LAMP) assay could detect as low as 20 copies of SARS-CoV-2 ORF1ab RNA, with 100% agreement with the commercial real-time RT-PCR in 130 swabs and bronchoalveolar lavage fluid samples (Yan et al., 2020). Another RT-LAMP assay, targeting the N gene of the virus, displayed a detection limit of 100 RNA copies in 30 min combined with colorimetric visualization (Baek et al., 2020). These results suggest that RT-LAMP assays could be used as a sensitive and specific early detection method with which to identify SARS-CoV-2 cases. Currently, several isothermal amplification-based nucleic acid tests for SARS-CoV-2 detection have received EUAs from China's NMPA (Table S1).

Serological assays provide an alternative diagnostic approach for the current rapidly growing demand for rapid diagnosis of suspected patients and asymptomatic infections. The entire test could be completed in a short time, and be independent of specific equipment or places. They are suggested to be used either in combination with molecular testing or for additional testing in suspected cases with negative nucleic acid results to improve detection accuracy of COVID-19. In a study of 397 real-time RT-PCR-confirmed COVID-19 patients and 128 virus-negative patients, IgM/IgG assays showed a sensitivity and specificity of 88.66% and 90.63% in blood samples, respectively (Li et al., 2020b). Combined IgM–IgG tests provided better sensitivity than tests for only IgM or IgG. However, cross-reactivity of the serological assay to other coronaviruses has been observed (Guo et al., 2020). Besides, serological testing is critically useful in disease surveillance and epidemiologic research. A community seroprevalence study of 863 individuals showed that the prevalence of antibodies to SARS-CoV-2 was 4.65% in Los Angeles County (Sood et al., 2020); 367,000 people were estimated to be infected with SARS-CoV-2, which is 43.53 times higher than the cumulative number (8430) of confirmed cases by the time of the survey.

Table 2
Sampling location recommended for patients with COVID-19.

Specimen type	Positive rate[a]	Priority of specimen	Early stage/ initial diagnosis	Advanced stage	Recovery/ follow-up	Remarks
Oropharyngeal swab	32–48%	★	Recommended	Recommended	Recommended	Viral loads in the upper respiratory tract peak soon within one week after symptom onset then steadily decline after that.
Nasopharyngeal swab	63%	☆	Highly recommended	Highly recommended	Highly recommended	Nasopharyngeal swab samples generally show higher viral loads and positive rates than oropharyngeal swab samples.
Bronchoalveolar lavage fluid (BALF)	79–93%	★	Not recommended	Highly recommended	Not recommended	BALF could be collected from patients presenting with more severe disease or undergoing mechanical ventilation.
Sputum	72–76%	★	Highly recommended	Highly recommended	Highly recommended	For patients who develop a productive cough, sputum should be collected and tested for SARS-CoV-2.
Stool/anal swab	29%	★	Not recommended	Not recommended	Highly recommend	Fecal testing for SARS-CoV-2 is highly recommended after viral clearance in the respiratory samples.

[a] All patients were confirmed by SARS-CoV-2 detection (Wang et al., 2020; Yang et al., 2020).

Point-of-care (POC) diagnostic tests provide rapid actionable information for patient care outside of centralized facilities such as airports, local emergency departments and clinics, and other locations. It has been shown to have an immediate impact on patient management and control of infectious disease epidemics (Kozel and Burnham-Marusich, 2017). At the time of writing, three detection assays have been issued EUAs for point of care diagnosis of SARS-CoV-2 in the US (Table S1), including Xpert Xpress SARS-CoV-2 test (Cepheid, USA) (real-time RT-PCR assay), ID NOW COVID-19 test (Abbott, USA) (isothermal nucleic acid amplification), and Sofia 2 SARS Antigen FIA assay (Quidel, USA) (antigen test). These emerging POC assays would be a powerful tool for effective patient care and outbreak containment of SARS-CoV-2 infection.

Lastly, the selection of specimens for molecular assays is crucial in the laboratory diagnosis of SARS-CoV-2 (Table 2). To prevent misdiagnosis caused by insufficient viral load, bronchoalveolar lavage fluid (BALF) is the most preferred specimen, as the viral loads of respiratory tract specimens are highest in BALF, followed by sputum, nasopharyngeal swabs, and oropharyngeal swabs (Wang et al., 2020; Yang et al., 2020). Due to the prolonged presence of SARS-CoV-2 viral RNA in fecal samples and potential fecal-oral transmission, fecal testing for SARS-CoV-2 is highly recommended when there is virus negativity in respiratory tract specimens (Wu et al., 2020). In addition, sampling different sites in suspected people or repeatedly sampling at different infected stages may help to prevent false negative results.

This comprehensive review examined all available diagnostic assays of SARS-CoV-2 infection, including virus culture, whole genome sequencing, real-time RT-PCR, isothermal amplification, antibody test, and POC test. The choice of a diagnostic assay for COVID-19 should take the characteristics and advantages of various technologies, and different clinical scenarios and requirements into full consideration. Moreover, to improve the detection accuracy of infectious diseases with COVID-19, proper collection of specimens is of great importance.

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Ethical approval

This manuscript does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

Conflict of interest

The authors declare that they have no competing financial interests.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2020.08.041.

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