Diagnosis of COVID-19: facts and challenges

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Abstract

At the end of 2019, the novel coronavirus disease 2019 (COVID-19) emerged in Wuhan, China, then spread rapidly across the country and throughout the world. The causative agent is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); according to the International Committee on Taxonomy of Viruses, this virus has a nucleic acid sequence that is different from other known coronaviruses but has some similarity to the beta coronavirus identified in bats. Coronaviruses are a large virus group of enveloped positive-sense single-stranded RNA. They are divided into four genera—alpha, beta, delta and gamma—and alpha and beta coronaviruses are known to infect humans. Rapid and early diagnosis of COVID-19 is a challenging issue for physicians and other healthcare personnel. The sensitivity and specificity of the clinical, radiologic and laboratory tests used to diagnose COVID-19 are variable and largely differ in efficacy depending on the disease's stage of presentation.

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Introduction

The novel coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is a member of the *Coronaviridae* family, which comprises large RNA viruses. There are four genera of coronavirus: alpha, beta, gamma and delta [1]. Human infections are mainly caused by seven species. Human coronavirus NL63 (HCoV-NL63) and human coronavirus 229E (HCoV-229E) belong to the alpha genus; human coronavirus OC43 (HCoV-OC-43), human coronavirus HKU1 (HCoV-HKU1), severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2 belong to the beta genus [2]. Most human coronavirus infections involve respiratory tract infections that are mild or moderate, such as common colds, or severe, such as severe pneumonia, which is associated with SARS-CoV, MERS-CoV and SARS-CoV-2. Most people will be infected with one or more of these viruses in their lifetime [3].

SARS-CoV-2, like other coronaviruses, is an enveloped positive-sense single-stranded RNA virus. The genome size ranges from 27 to 32 kb [4]. Its sequence homology with bat SARS-like CoVZXC21 and SARS-CoV is about 89% and 82% respectively [5]. Like SARS-CoV, SARS-CoV-2 uses the angiotensin converting enzyme 2 receptor for cell entry [6]. The important structural proteins are the envelope (E) protein, the protein membrane (M), the spike (S) protein and the nucleoprotein (N) [7]. The N protein is involved in virus genome process and host cellular response. The E protein is important in virus production and maturation, as it is abundantly expressed in infected cells and helps in virus assembly and budding. The S protein assists in the attachment of the virus to the human cell [8]. It is composed of intracellular, transmembrane and extracellular parts; the extracellular part is composed the S1 receptor binding subunit and the S2 membrane fusion subunit. These subunits are arranged in a crown-like structure, which is where the coronavirus got its name.

The increasing demands for sensitive and specific COVID-19 diagnostic methods are important, given the rapid increase in the number of infected people. The US Centers for Disease Control and Prevention (CDC) recommends using an upper nasopharyngeal swab for diagnostic tests, mainly molecular diagnostic tests. There are several reverse transcriptase PCR (RT-PCR) protocols for the detection of SARS-CoV-2 RNA. The CDC developed an RT-PCR diagnostic panel for the universal detection of SARS-like beta coronaviruses and the specific detection of SARS-CoV-2. This protocol, which targets the N region, is composed of three separate reactions. One set of primers/probes is for the detection of all coronaviruses; the other two sets are for the detection of SARS-CoV-2 [9]. The Charité algorithm (Berlin, Germany) begins with two RT-PCR assays that detect E and RNA-dependent RNA polymerase (RdRp) genes of the Sarbecovirus subgenus (SARS-CoV, SARS-CoV-2 and bat-associated beta coronaviruses). Both assays must be positive to advance to the next step in the testing algorithm. The second step consists of a SARS-CoV-2−specific RT-PCR assay that targets RdRp [10]. The University of Hong Kong Li Ka Shing Faculty of Medicine protocol uses two assays (an N gene screening assay followed by an open reading frame 1b (Orf1b) assay for confirmation) to detect the *Sarbecovirus* subgenus [5,11].

These methods can be affected by many factors, such as inadequate sample volume, inappropriate sample collection, inaccurate techniques, inappropriate window for collecting samples and contamination. In addition, these tests may be expensive. There is a need for cheap, rapid alternatives. Imaging techniques such as chest X-ray (CXR) and computed tomographic (CT) scans may be used for COVID-19 diagnosis [12].

The implemented strategy was to explore the relevant publications indexed by the Google Scholar, PubMed and/or Science Direct databases. Keywords such as SARS-CoV-2, COVID-19, lateral flow immunoassay, enzyme-linked immunosorbent assay (ELISA), PCR, loop-mediated isothermal amplification (LAMP) and CRISPR were used to search for publications which appeared between December 2019 and July 2020. The initial search found approximately 230 articles related to the selected keywords. However, only ten articles were relevant after searching within the scope of the current review and excluding books, duplicate entries, abstracts, conference proceedings and case reports.

Laboratory tests

The decision to test should be based on clinical and epidemiologic factors and should be linked to an assessment of the likelihood of infection [13].

Antibody-based detection tests

It is well known that both innate and adaptive immunity play roles in controlling SARS-CoV-2 infection. In addition, adaptive immunity creates a memory immunity that helps prevent reinfection. One of the components of adaptive immunity is humoral (B cell or antibody)-mediated immunity, which is important in the clearance of the virus and the prevention of reinfection through the memory immune response. The B-cell immune response elicits a virus-specific antibody response, including IgM, IgG, IgA and neutralizing IgG antibodies, in the days after SARS-CoV-2 infection [14]. In most COVID-19 patients, antibodies appear 7 to 14 days after infection and persist for weeks after virus clearance [15−17]. The most commonly detected antibodies are against the internal N protein and the external S protein, such as a neutralizing antibody targeted against the receptor binding domain of the S protein, which is highly immunogenic [18,19].

Antibody detection by rapid point-of-care testing (lateral flow immunoassay). The difficulty in controlling the spread of COVID-19 stems from the fact that some patients, especially those with high virus loads, can transmit the virus to others despite being asymptomatic [20]. Therefore, urgent and rapid tests are needed to solve this problem. Li et al. [21] studied the development of a rapid and simple point-of-care lateral flow immunoassay that can simultaneously detect IgM and IgG antibodies against SARS-CoV-2 in human blood within 15 minutes in patients at different infection stages. Samples were collected from 397 PCR-confirmed cases and 128 negative patients at eight different clinical sites; overall testing sensitivity was 88.66% and specificity was 90.63% regardless of the type of blood sample [21]. Imai et al. [22] showed that immunochromatography (IC) sensitivity was 34.2% and specificity was 98%. The IgM antibody was detected in 27.8% of specimens collected within 1 week of onset, 48.0% collected within 1 to 2 weeks and 95.8% collected more than 2 weeks after onset. The corresponding IgG antibody detection rates were 3.3%, 8.0% and 62.5%. The study also evaluated the diagnostic sensitivity of CT scans alone and in combination with the IC assay for asymptomatic patients (57.9% (22/38) and 68.4% (26/38) respectively) and symptomatic patients (74.3% (55/74) and 82.4% (61/74) respectively) [22]. A German study conducted on 49 samples from a screening centre in a high-prevalence area showed low sensitivity (36.4%) for the rapid test, while specificity was 88.9% with no statistically significant correlation between the rapid test results and the time from potential exposure. Another study showed that the median seroconversion times for neutralizing antibodies (NAb), IgM and NAb, IgM and IgG were at days 11, 12 and 14 respectively [15]. The presence of antibodies was <40% among patients within 1 week of onset and

rapidly increased to 100.0% (NAb), 94.3% (IgM) and 79.8% (IgG) within 15 days of onset [15]. A different study compared the sensitivity and specificity of four point-of-care tests (using N and S antigens); the specificity of the kits was nearly identical at different times during the course of infection, but the sensitivity was variable based on the infection time; it was 41% to 52% within 14 days after symptom onset and increased over time, reaching 100% after 21 days [23]. As of July 2020, the tests of only four companies have been evaluated by the Foundation for Innovative New Diagnostics (FIND), which found that detection sensitivity for SARS-CoV-2 antibodies (IgM, IgG or both) increased as the days after symptom onset increased [24]. It costs about US$20 per test.

An important question has arisen about the duration of protective immunity for SARS-CoV-2 and acquired immunity to reinfection. Given the short time since the start of the COVID-19 outbreak, observations from studies on other human coronaviruses, such as SARS-CoV-1 and MERS-CoV, may provide answers. Immunity against HCoV-229E wanes 1 year after infection [25]; immunity against SARS-CoV-1 wanes after 2 or 3 years, although some authors have detected IgG levels after 12 years [26,27]; and MERS-CoV antibody response can be detected 3 years after infection [28].

Enzyme-linked immunosorbent assay. ELISA is a commonly used technique for the detection of an antibody against an antigen in serum, and it is low cost (US$5 to $8). There are four ELISA tests with US Food and Drug Administration (FDA) emergency use authorization approval: VITROS Immunodiagnostic Products Anti−SARS-CoV-2 Total and IgG Reagent Packs (Ortho Clinical Diagnostics, Rochester, NY, USA); the COVID-19 ELISA IgG Antibody Test (Mount Sinai Laboratory, New York, NY, USA), which is only approved for use at Mount Sinai Laboratory; and LIAISON SARS-CoV-2 S1/S2 IgG (DiaSorin, Stillwater, MN, USA) [29]. Regarding ELISA specificity, a European study showed that IgG and IgA specificity levels against recombinant structural protein (S1) from SARS-CoV-2 were 91.9% and 73% respectively [30].

Microsphere immunoassay. Microsphere immunoassay (MIA), a unique technology which is a variant of ELISA, allows for the multiplexing of several antigens. It is based on magnetic carboxylated microspheres, to which virus antigens attach, and antibodies against the antigen, if present in the patient's serum, can be detected by adding a fluorescently labeled secondary antibody. Both ELISA and MIA are more sensitive and specific than lateral flow immunoassays, but they require a longer time for results to become available (5 hours for ELISA and 3 to 8 hours for MIA) [29].

Antigen detection

Attempts to diagnose COVID-19 using antigen detection have been made. Lambert-Niclot et al. [31] evaluated a rapid diagnostic test, the COVID-19 Ag Respi-Strip CORIS (BioConcept, Gembloux, Belgium), for SARS-CoV-2 antigen detection in 138 samples. The assay is ready to use and is based on a nitrocellulose membrane technology with colloidal gold nanoparticles sensitized with monoclonal antibodies directed against highly conserved SARS-CoV-2 nucleoprotein antigens. The researchers observed that only 47 samples were positive for SARS-CoV-2 antigen from the 98 samples found to be positive by RT-PCR, with a sensitivity of 50%. Compared to RT-PCR, sensitivity was 82.2% for cycle threshold (C_t) values under 25, while the manufacturer states that sensitivity is 76.7% at C_t values of 25. The authors concluded that the assay can be used to diagnose the disease within a few days after symptom onset, when the virus load in the upper respiratory tract is at its peak.

Table 1 summarizes the original articles reporting on SARS-CoV-2 antigen testing. Most available commercial kits are under investigation by FIND on the basis of their test kit criteria [32,33]. However, the FDA approved the emergency use of two kits: the BD Veritor System for Rapid Detection of SARS-CoV-2 (Becton Dickinson (BD), San Diego, CA, USA) and Sofia 2 SARS Antigen FIA (Quidel, San Diego, CA, USA) [34,35].

Molecular method

Polymerase chain reaction. Since the publication of the genetic sequences comprising SARS-CoV-2, molecular diagnosis by nucleic acid amplification using real-time RT-PCR (rRT-PCR) has been the method of choice [36,37]. The E, RdRp and N genes are available for the molecular detection of SARS-CoV-2 [13,38]. More recently, rRT-PCR method targets have included Orf1a/b and the gene encoding the S protein. PCR is variable depending on the reagents and instruments used [39,40]. In addition, some variables, such as low virus loads (which occur in asymptomatic individuals during the early or late stages of COVID-19 disease) and improper specimen collection [41,42], could greatly affect PCR performance.

Chan et al. [43] developed and compared rRT-PCR assay targets (RdRp/Hel, S and N genes) for SARS-CoV-2 with those of the reported RdRp/P2 assay. They concluded that RdRp/Hel has a lower limit of detection *in vitro*. They studied 273 samples from 15 confirmed COVID-19 patients; 77 were positive for both RdRp/P2 and RdRp/Hel, and 42 (29 respiratory and 13 nonrespiratory samples) were positive for RdRp/Hel only. The COVID-19 RdRp/Hel assay did not cross-react with other human pathogenic coronaviruses and respiratory pathogens in cell culture and clinical specimens. Meanwhile, a Canadian study compared the analytical

TABLE I. Articles reporting on SARS-CoV-2 antigen testing

Study (year)	Study design	N	Country	Antigen used	Methodology	Main findings or conclusions	Sensitivity	Specificity	PPV (%)	NPV (%)
Mak (2020) [75]	Retrospective	368	China	NA	Lateral flow IC	RAD was 10^3 fold less sensitive than virus culture; RAD was 10^5 fold less sensitive than RT-PCR. RAD detected 11.1% to 45.7% of RT-PCR–positive samples from COVID-19 patients.	NA	NA	NA	NA
Mertens (2020) [76]	Retrospective	328 pharyngeal samples	Belgium	Nucleoprotein	IC	COVID-19 Ag Respi-Strip represents a promising rapid SARS-CoV-2 antigen assay for first-line diagnosis of COVID-19 in 15 minutes at pandemic's peak. Its role in proposed diagnostic algorithm complements currently used molecular techniques.	57.6	99.5	98.7	77.7
Scohy (2020) [77]	Retrospective	148 nasopharyngeal samples	Belgium	Nucleoprotein	IC	Higher virus loads are associated with better antigen detection rates. Overall poor sensitivity of COVID-19 Ag Respi-Strip does not allow its use alone for frontline testing for COVID-19 diagnosis.	30	100	NA	NA
Blairon (2020) [78]	Prospective	774	Belgium	Nucleoprotein	IC	Sensitivity of this rapid test is poor and improvements are needed to enhance its performance.	23.9	NA	NA	NA

COVID-19, coronavirus disease 2019; IC, immunochromatography; NA, not available; NPV, negative predictive value; PPV, positive predictive value; RAD, rapid antigen test; RT-PCR, real-time PCR; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

sensitivity of various laboratory-developed tests and some commercially available rRT-PCR assays, showing that laboratory-developed test analytical sensitivity was consistent between the laboratories, while few exceptions were noted in the commercial assays. Most laboratory-developed tests had limits of detection between 3.4 and 4.5 $\log_{10}$ copies/mL [44]. Several manual molecular tests with two near point-of-care automated tests passed evaluations by FIND [45].

Loop-mediated isothermal amplification. LAMP is a single-tube technique for amplification of DNA, and RT-LAMP is used for detection of RNA [46]. In contrast to PCR, the reaction is carried out at a constant temperature. Studies have shown that the technique has good sensitivity and specificity. Kitagawa et al. [47] studied 76 nasopharyngeal swab samples from suspected COVID-19 patients; sensitivity and specificity were 100% and 97.6% respectively. In another study, sensitivity and specificity of the technique were both 100% compared to RT-PCR [48]. Because of its simplicity and low cost (about US$1 per test), LAMP can potentially be used as a simple screening assay in the field or at the point of care, and can be used in low- and middle-income countries for detection of SARS-CoV-2 [49,50] as well as other infectious diseases, such as malaria [51] and sleeping sickness [52]. The iAMP COVID-19 Detection Kit (isothermal detection) (Atila Biosystems, Mountain View, CA, USA) passed the FIND round 1 evaluation, but it is used for research purposes only [45].

CRISPR-Cas–based COVID-19 testing methods. Clusters of regularly interspaced short palindromic repeats (CRISPR) comprise a family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria and archaea. These sequences are derived from DNA fragments of bacteriophages that have previously infected the prokaryote. They are used to detect and destroy DNA from similar bacteriophages during subsequent infections [53]. The CRISPR-Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements, such as those present within plasmids and phages [54]. CRISPR-Cas12 and CRISPR-Cas13 are used in combination with LAMP for the diagnosis of different viral infections, such as Zika and dengue viruses [55]. This rapid, inexpensive (about US$6 per test) and sensitive nucleic acid detection method may aid point-of-care pathogen detection [56]. Broughton et al. [57] developed a CRISPR-Cas12–based assay for detection of SARS-CoV-2 from extracted patient sample RNA called the SARS-CoV-2 DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) [58]. Meanwhile, the FDA approved the emergency use of the CRISPR SARS-CoV-2 Kit (Sherlock BioSciences, Cambridge, MA, USA) [59,60].

Imaging techniques

Chest X-ray

CXR is an important tool in the diagnosis of pneumonia, lung abscess and many other lung diseases. It is readily available in any hospital, and the results can be obtained within minutes [61].

Cieszanowski et al. [62] recommended CXR with a portable X-ray machine as the first and primary imaging study for COVID-19. There is no need for imaging in patients with mild symptoms because CXR may give false-negative results if the lung lesions are mild. Follow-up by CXR is not needed unless the disease progresses and CXR would affect patient management. In patients with acute respiratory distress syndrome, especially those who are ventilated, portable CXR is indicated.

Ippolito et al. [63] demonstrated that the most common X-ray patterns in COVID-19 patients are multifocal and peripheral, and are associated with interstitial and alveolar opacities. Lung lesions primarily manifested as interstitial opacities (71.7%) or alveolar opacities (60.5%) and were frequently bilateral (64.5%) or peripheral (62.5%). Patients admitted to the emergency radiology department more than 5 days after symptom onset more frequently had interstitial and alveolar opacities than those admitted within 5 days, and lung lesions were more frequently bilateral and peripheral. Older patients more frequently had interstitial and alveolar opacities than younger ones, as well as a higher rate of pleural effusion (77.1% vs. 22.9%) [63].

Computed tomography

Although CXR is a simple and rapid radiologic test for diagnosis in suspected COVID-19 cases, certain findings, such as infiltrate, patchy or hazy opacity pneumonia, remain difficult to interpret [64,65]. The predominant CT scan findings in COVID-19 patients are bilateral, peripheral and basal predominant ground-glass opacity; consolidation; or both [66,67]. Although CT scan sensitivity is high (98%) with low specificity, it may aid but not replace molecular methods for the diagnosis [68] and is considered an important tool to diagnose complications and to assess the severity of the disease in order to improve the quality of care [69]. Another important aspect of CT scanning is that it is useful in diagnosing asymptomatic patients with COVID-19–related pneumonia and in the early stage of the disease [70]. However, CT scans are not effective in diagnosing patients who are asymptomatic or presymptomatic, or who have mild symptoms without pneumonia [71]. The limitation of CT scanning is that it only available in large hospitals; in addition, images need to be checked by two radiologists. The risk of CT scanning in COVID-19 patients, especially in severely symptomatic patients, is the cumulative radiation dose that these patients will receive as a result of repeated examinations; in addition, there are certain patient groups, like children and pregnant patients, who are at risk from repeated exposure to radiation [72]. To overcome this risk, conventional CT scanning with a radiation dose of 7 mSv can be replaced with low-dose CT with a radiation dose of 1 to 1.5 mSv, or ultra-low-dose CT with a radiation dose of 0.3 mSv [73]. Most of the studies regarding the use of low-dose CT and ultra-low-dose CT in COVID-19 patients were performed on intermediate to severely ill patients; the results of studies assessing this modality in patients with very early-stage disease have not yet been published. However, low-dose and ultra-low-dose CT have the ability to detect ground-glass appearance and consolidation in COVID-19 patients with pneumonia but lack the ability to detect pulmonary findings [74].

Conclusion

Molecular tests for nucleic acid detection are considered to be the reference standard for laboratory diagnosis of COVID-19 disease. They are highly sensitive and specific techniques that can be used as the first line in the diagnosis of acutely infected COVID-19 patients. RT-PCR is the mostly commonly used technique; however, it may be not available in low-income countries because of its high costs as well as its need for sophisticated instruments and qualified technicians. Alternatives such as LAMP and CRISPR-Cas may be considered in those countries because they are low-cost, simple procedures, and because manufacturers have received authorization from the FDA. Antigen detection techniques have lower sensitivity than molecular detection techniques, but the former are easier and require less time for the results to become available.

Antibody detection methods such as lateral flow IC, ELISA and MIA can be used for the supplementary detection of SARS-CoV-2 in patients who test negative for nucleic acids, especially 7 days after appearance of symptoms. These methods can be used to track disease progression, but they have limitations because they cannot detect whether the patient is still infectious. Although lateral flow IC is rapid and requires no technical skills, it has low sensitivity; ELISA and MIA have high sensitivity but take a longer time to perform and require qualified technicians.

CXR is simple and accessible; it can be used as the first and primary imaging technique for COVID-19 patients even though it may give false-positive results in mild lung lesions. A CT scan has a sensitivity of 98% and can help diagnose patients with asymptomatic pneumonia; however, it is of no benefit in diagnosing patients who are asymptomatic or presymptomatic, or who have mild symptoms without pneumonia.

Conflict of interest

None declared.

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