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Review

Clinical and Laboratory Diagnosis of SARS-CoV-2, the Virus Causing COVID-19

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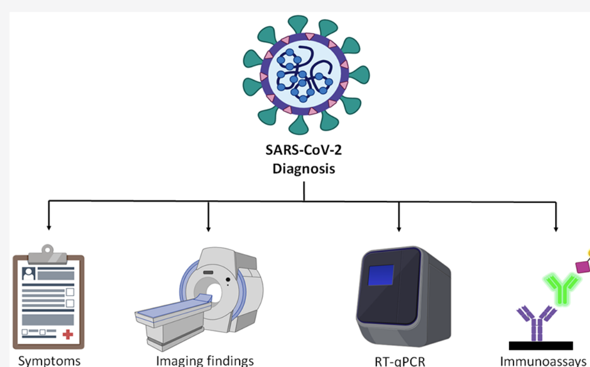
ABSTRACT: In December 2019, a novel beta (β) coronavirus eventually named SARS-CoV-2 emerged in Wuhan, Hubei province, China, causing an outbreak of severe and even fatal pneumonia in humans. The virus has spread very rapidly to many countries across the world, resulting in the World Health Organization (WHO) to declare a pandemic on March 11, 2020. Clinically, the diagnosis of this unprecedented illness, called coronavirus disease-2019 (COVID-19), becomes difficult because it shares many symptoms with other respiratory pathogens, including influenza and parainfluenza viruses. Therefore, laboratory diagnosis is crucial for the clinical management of patients and the implementation of disease control strategies to contain SARS-CoV-2 at clinical and population level. Here, we summarize the main clinical and imaging findings of COVID-19 patients and discuss the advances, features, advantages, and limitations of different laboratory methods used for SARS-CoV-2 diagnosis.



KEYWORDS: *coronavirus, coronavirus disease 2019, COVID-19, diagnostics, imaging findings, laboratory, respiratory pathogens, SARS-CoV-2, pandemic*

The recent emergence of a novel coronavirus in the human population has caused dramatic and unprecedented impact of the economy and prompted mobilization of public health authorities around the world to counter the rapid spread of the virus. Coronaviruses (CoVs) are members of the *Coronaviridae* family and are an important group of viruses that infect a large number of animals including mammalian and avian species.[1] The *Coronavirinae* subfamily is divided into four genera based on genetic features: *Alphacoronavirus* (α-CoVs), *Betacoronavirus* (β-CoVs), *Gammacoronavirus* (γ-CoVs), and *Deltacoronavirus* (δ-CoVs). The α-CoVs (HCoV-229E and HCoV-NL63) and β-CoVs (HCoV-OC43 and HCoV-HKU1) cause human infection and have been associated with mild respiratory diseases.[2] In the 21st century, however, three β-CoVs have emerged from animal reservoirs to cause severe disease in humans: severe acute respiratory syndrome coronavirus (SARS-CoV),[3] the Middle East respiratory syndrome coronavirus (MERS-CoV),[4] and the pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[5,6] The genome of CoVs consists of a single-stranded positive sense (+ssRNA) of around 30 kb in size. The genomic RNA is capped at the 5′ end and has a poly(A) tail at the 3′ end, allowing it to act as an mRNA for translation of the replicase polyproteins.[1,7] The 5′ terminal region of the genome encodes a polyprotein that is cleaved into 16 nonstructural proteins involved in the transcription and replication process, and the 3′ terminal region encodes viral structural proteins.[8]

In December 2019, the world was on alert due to a cluster of severe pneumonia cases of unknown origin in Wuhan, Hubei province, China. This outbreak was epidemiologically linked to a wholesale animal and seafood market where live and freshly slaughtered animals were kept and sold.[9] Of the initial 41 patients hospitalized with pneumonia, two-thirds had a history of direct exposure to this market.[10] On the basis of the clinical presentation and the link with the animal market, similar to SARS epidemiology, a CoV was suspected as the causative agent and therefore pan-CoV PCR primers were used to test the samples followed by sequencing.[11] The causative agent was identified as a novel CoV, eventually named SARS-CoV-2, and the respiratory syndrome associated with the infection was designated as coronavirus disease-2019 (COVID-19) by the

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Table 1. Clinical Characteristics of COVID-19 Patients

	44	40	195	196	197	32	198	199	200	201	202
country	China	China	China	China	China	China	China	China	Netherlands	USA	USA
medical description	191 inpatients	140 inpatients	1099 inpatients and outpatients	99 patients	138 inpatients	171 infected children	51 patients	204 patients	86 health care workers	12 patients	16 inpatients
median age, years	56	57	47	55, 5	56	6, 7	49	52, 9	49	53	67
sex	62% male	50, 7% male	58, 1% male	68% male	54, 3% male	60, 8% male	49% male	52, 4% male	17% male	67% male	75% male
comorbidity	48%	64, 3%	23, 7%	51%	46, 4%	not reported	22%	48, 5%	not reported	42%	not reported
fever	94%	78, 5%	88, 7%	83%	98, 6%	41, 5%	96%	46, 5%	53%	58%	94%
dry cough	79%	64, 2%	67, 8%	82%	59, 4%	48, 5%	47%	not reported	77%	67%	88%
shortness of breath or dyspnea	not reported	31, 4%	18, 7%	31%	31, 2%	28, 7% (tachypnea)	14%	not reported	38%	8%	81%
sore throat	not reported	not reported	13, 9%	5%	not reported	not reported	6%	not reported	40%	8%	12, 5%
sputum	23%	not reported	33, 7%	not reported	26, 8%	not reported	not reported	not reported	not reported	not reported	not reported
fatigue	23%	64, 2%	38, 1%	not reported	69, 6%	7, 6%	31%	26, 4%	not reported	42%	50%
myalgia or arthralgia	15%	not reported	14, 9%	11%	34, 8%	not reported	16%	7, 35%	63%	not reported	25%
diarrhea	5%	12, 8%	3, 8%	2%	10, 1%	8, 8%	10%	17, 1%	19%	8%	6%
nausea or vomiting	4%	17, 1%	5%	1%	10, 1% nausea and 3, 6% vomiting	6, 4%	6%	1, 96%	17%	8%	13%
lack of appetite	not reported	not reported	not reported	not reported	not reported	not reported	18%	39, 7%	17%	not reported	not reported
headache	not reported	not reported	13, 6%	8%	6, 5%	not reported	16%	not reported	57%	25%	25%
chills	not reported	not reported	11, 5%	not reported	not reported	not reported	not reported	not reported	not reported	8%	not reported
pharyngeal erythema	not reported	not reported	not reported	not reported	not reported	46, 2%	not reported	not reported	not reported	not reported	not reported

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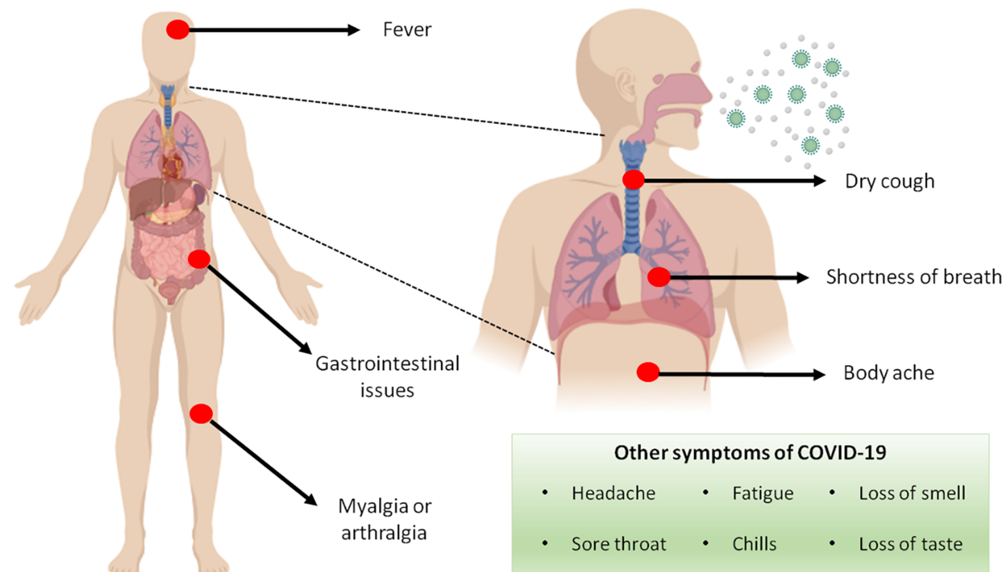

Figure 1. COVID-19 symptoms.

World Health Organization (WHO). The SARS-CoV-2 genome has about 80% sequence identity to SARS-CoV (with whom it is classified into the species *severe acute respiratory syndrome-related coronavirus*)[12] and 50% to MERS-CoV. The most closely related virus to SARS-CoV-2 found so far is a CoV isolated from bats, named RaGT13 CoV, whose nucleotide identity is 96%, suggesting that SARS-CoV-2 is also of bat origin. However, it is not clear whether SARS-CoV-2 jumped to humans directly from bats or through an intermediate host.[13] The rapidly increasing numbers of COVID-19 prompted WHO to declare first a Public Health Emergency of International Concern (PHEIC) on January 30, 2020 and then a pandemic on March 11, 2020.[14] As of July 31, 2020, more than 17 million cases of COVID-19 and 677 549 deaths have been reported in 213 countries and territories around the world. Most of the cases have been reported by the USA, followed by Brazil, India, Russia, South Africa, Mexico, and Peru.[15]

Different from the other highly pathogenic CoVs, SARS-CoV-2 has acquired the ability to establish sustained human-to-human transmission. Its basic reproductive number (R0), i.e., the number of secondary infections generated from one infected individual, is estimated to be between 1.4 and 6.49, with a mean of 3.28.[16] Ultimately, this metric will require further investigations and may vary across settings and locations. On the basis of the travel history and symptom onset of patients in China, the mean incubation period of COVID-19 has been calculated to be 6.4 days, ranging from 2 to up to 14 days.[17] Clinically, the spectrum of COVID-19 manifestations ranges from asymptomatic and mild to severe infections requiring oxygen therapy and ventilation support.[9,18,19]

Since its emergence, a wide variety of methods have been developed for the purpose of the rapid and accurate diagnosis of COVID-19. On the basis of clinical criteria alone, SARS-CoV-2 cannot be reliably distinguished from infections with other pathogens that cause similar symptoms, including influenza, seasonal CoV, adenovirus, bocavirus, human metapneumovirus, parainfluenza, respiratory syncytial virus rhinovirus, *Bordetella pertussis*, *Legionella pneumophila*, *Mycoplasma pneumonia*,[20,21] and even the mosquito borne dengue virus.[22] In this context, the laboratory-based diagnosis assumes a role for the clinical management of patients and the implementation of disease control measures. Here, we review the clinical features, laboratory methods, and imaging findings that are used for COVID-19 diagnosis. In addition, we explore the next steps of the methods under development for COVID-19 diagnosis.

CLINICAL DIAGNOSIS

A rapid presumptive diagnosis based on clinical assessment and epidemiological characteristics is critical to ensuring appropriate patient care and controlling viral transmission, thus contributing to disease control. As mentioned and as with other respiratory viral infections, the signs and symptoms of COVID-19 are nonspecific and the clinical spectrum of disease can range from no symptoms to severe pneumonia and death.[23] Asymptomatic infection has been reported in many settings, but some patients develop clinical disease at a later stage of infection. The proportion of truly asymptomatic infections is unclear, but some estimates indicated that up to 80% of infections do not result in overt clinical signs of disease.[24] A recent mathematical model suggested that undocumented infections might be major drivers of SARS-CoV-2 spread in the world.[25]

According to WHO criteria, a person is suspected of being infected with COVID-19 in three scenarios: (i) a patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath) and a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset; (ii) a patient with any acute respiratory illness who has been in contact with a confirmed or probable COVID-19 case in the last 14 days prior to symptom onset; and (iii) a patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath, and requiring hospitalization) and in the absence of an alternative diagnosis that fully explains the clinical presentation. If a patient then tests positive with a laboratory diagnostic, then the infection becomes a confirmed

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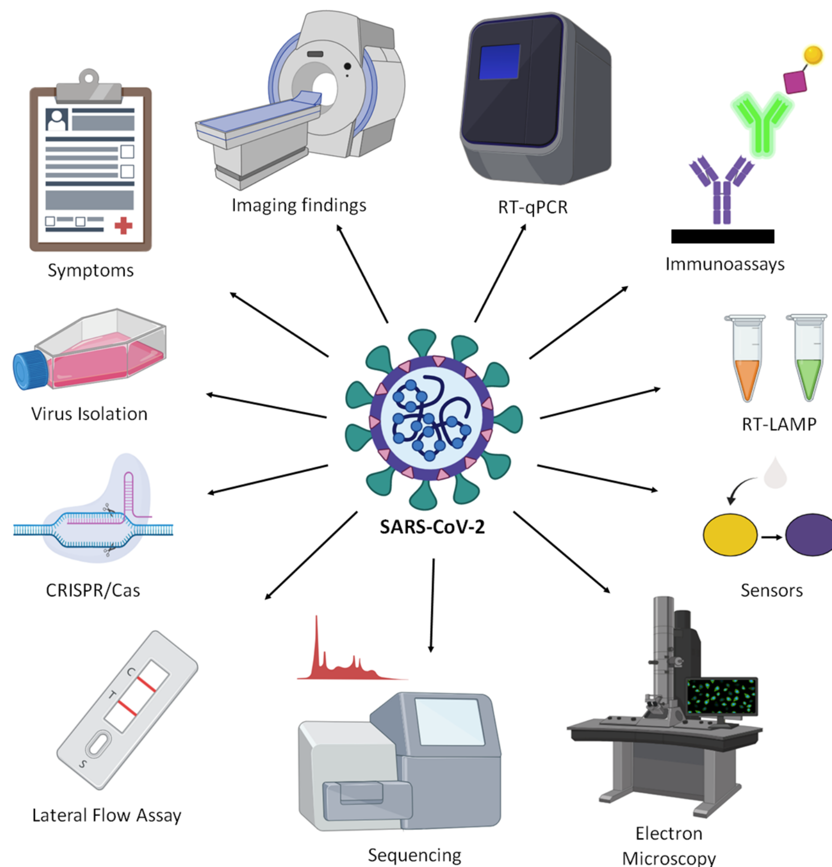

Figure 2. COVID-19 diagnostic approaches.

case of COVID-19, irrespective of clinical signs and symptoms.[26]

Overall, 81% of laboratory-confirmed COVID-19 patients develop mild to moderate disease, which includes non-pneumonia and pneumonia cases, 14% have severe disease (dyspnea, tachypnea, blood oxygen saturation ≤93%, and PaO2/FiO2 ratio of 50% of the lung field within 24−48 h), and 5% of cases reach critical state (respiratory failure, septic shock, and/or multiple organ dysfunction/failure).[27] Risk factors for severe COVID-19 include age ≥65 years and people with preexisting concurrent conditions such as cardiovascular disease, hypertension, diabetes, chronic respiratory disease, immunodeficiencies, cancer, and obesity.[28−30] Accurate estimates of the COVID-19 case fatality rate are still lacking, but it is believed to be around 3%[9] and can be as high as 14.8% in patients >80 years of age.[27] However, this number is dependent on the level of testing in countries, testing accuracy, and death-reporting policies. Interestingly, men have a much higher risk of death than women.[27,31]

Table 1 summarizes the clinical signs and symptoms of COVID-19 patients seen in several studies. In general, the most common clinical manifestations are fever, dry cough, fatigue, sputum production, dyspnea, sore throat, headache, myalgia or arthralgia, and chills (Figure 1). Less common symptoms include nausea or vomiting, nasal congestion, diarrhea, hemoptysis, and conjunctival congestion.[23] In children, SARS-CoV-2 infection is generally mild and in some cases asymptomatic; however, when presented, the main symptoms include fever (about 41%), cough (48%), and pharyngeal erythema (46.2%).[32] More recently, several studies have been suggested that COVID-19 infection was associated with cutaneous manifestations in patients.[33−35] Major manifestations observed in COVID-19 patients including different types of lesions such as purpuric, papulovesicular, livedoid, urticarial, maculopapular, and thromboticischemic.[36]

A recent study in European COVID-19 patients reported that 85.6 and 88.0% of patients had olfactory and gustatory dysfunctions, respectively. These disorders persisted after the resolution of other symptoms.[37] Finally, a single retrospective case series in Wuhan, China, reported neurologic manifestations in 78 of 214 hospitalized patients (36.4%) with a laboratory-confirmed diagnosis of COVID-19. These symptoms were more common in severe cases, along with acute cerebrovascular events, headache, dizziness, and impaired consciousness. It is unclear whether the neurologic manifestations were caused by SARS-CoV-2 directly or by pulmonary disease or other organ damage indirectly or by cytokines.[38] Since this study included only hospitalized patients in a single location, the true percentage of neurological manifestations in COVID-19 needs further evaluation.

IMAGING FINDINGS IN COVID-19 PATIENTS

Imaging techniques such as chest X-rays, pulmonary computed tomography (CT) scans, and lung ultrasounds are important tools in the early diagnosis of pneumonia in patients with COVID-19. Although chest X-rays are less expensive and more convenient for follow up in pneumonia cases, the technique has low-resolution and projection overlapping, which could lead to many false-negative COVID-19 cases.[39] Reports indicate that COVID-19 patients submitted to chest CT scans on admission presented abnormal results (about 90%), showing bilateral multiple ground-glass and patchy opacity.[40] A

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study with 101 patients in China showed that COVID-19 pneumonia displayed typical imaging features, such as ground-glass opacities (GGO) (86.1%) or mixed GGO and consolidation (64.4%), vascular enlargement in the lesion (71.3%), and traction bronchiectasis (52.5%).[39] Importantly, a CT scan has a sensitivity that is greater than that of RT-qPCR (97.2 versus 83.3% (discussed below) for the diagnosis of COVID-19 patients.[41] Despite its high sensitivity in diagnosing COVID-19, chest CT findings in COVID 19 could be indistinguishable from other viral pneumonia findings, resulting in false positives.[42] However, taken together, the association of a clinical assessment with imaging findings has a complementary role in the diagnosis of COVID-19.

■ LABORATORY-BASED DIAGNOSIS OF COVID-19

Clinical Laboratory Findings and Biomarkers in COVID-19 Patients. Several studies have reported hematologic and blood chemistry alterations in patients infected by SARS-CoV-2.[43,44] Major laboratory findings in COVID-19 patients identified by meta-analysis include leukopenia, leukocytosis, decreased albumin levels, increased levels of C-reactive protein, lactate dehydrogenase (LDH), creatinine kinase, and biliarubin, and a high erythrocyte sedimentation rate (ESR).[45] Increased levels of creatine kinase and lactate dehydrogenase were associated with myalgia.[38]

A growing body of evidence suggests that SARS-CoV-2 infection can trigger the overproduction of cytokines in some patients, known as a cytokine storm, which is associated with poor outcomes.[10,46−49] As for other severe viral infections, the exacerbated production of proinflammatory cytokines may be involved in some of the pathophysiology of COVID-19, including pulmonary edema and lung failure and damage to the liver, heart, and kidneys. Compared to healthy adults, COVID-19 patients had higher levels of IL-1β, IL-1RA, IL-7, IL-8, IL-9, IL-10, basic FGF, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1A, MIP-1B, PDGF, TNF-α, and VEGF. Serum biomarkers associated with severe disease included IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, and TNF-α.[10] A recent retrospective study of 150 confirmed COVID-19 cases (68 fatal and 82 discharged cases) in Wuhan, China, identified several serological markers that were more elevated in lethal cases than in survivors: elevated ferritin, IL-6, myoglobin, C-reactive protein, and cardiac troponin.[50] Together, these findings suggest that COVID-19 mortality might be due to infection-driven hyperinflammation.

Diagnostic Virology of COVID-19. Laboratory virology tests are essential for a correct diagnosis and the population level prevalence of COVID-19, given the number of asymptomatic cases or nonspecific clinical symptoms. Results from these tests guide clinicians and health officials in the management, control, and prevention of COVID-19. Several analytical parameters are used to determine the performance of COVID-19 assays, including clinical sensitivity, clinical specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy.[51]

The confirmation of a SARS-CoV-2 infection in the laboratory can be achieved by direct and indirect virology methods. While direct detection is more specific, indirect methods allow a greater opportunity for virus detection after the acute phase of the disease. In direct tests, the clinical sample is examined directly for the presence of particles, virus antigens, or viral nucleic acids, whereas indirect methods detect the serological response against the infection (Figure 2).

Biosafety for Handling and Processing COVID-19 Samples. The World Health Organization (WHO) recommends that all procedures performed with COVID-19 be done only by trained professionals and should take place in a laboratory with an appropriate level of biosafety. Non-propagative diagnostic assays, such nucleic acid amplification tests (NAATs), sequencing, and some serological tests (e.g., ELISA type assays) can be performed in biosafety level 2 (BSL-2) laboratories provided that the initial processing (before virus inactivation) of samples takes place in a validated biological safety cabinet. However, procedures that involve propagative virus work, such as virus culture, isolation, or neutralization assays, should be performed in laboratories equivalent to BSL-3 using validated practices. The work team must use appropriate personal protective equipment including eye protection (goggles or a disposable face shield), a respirator or facemask, a long-sleeved gown, and gloves and follow all standard operating procedures regarding sample collection and handling. Respirators that offer a higher level of protection such as N95 respirators or powered air-purifying respirators should be used instead of a face mask when performing an aerosol-generating procedure. Samples from patients with a suspected or confirmed case must be transported to UN3373, a code referring to "biological substance category B"; however, virus-infected cultures or isolates must be transported as category A - UN2814, "an infectious substance that affects humans".[52,53]

Specimens for Diagnosis. Choosing the correct sample for diagnosis tests is an essential step in a reliable diagnosis. SARS-CoV-2 infection can be detected in a variety of clinical specimens, such as nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, sputum, tracheal aspirates, and bronchoalveolar lavage.[54−58] The median duration of SARS-CoV-2 shedding in respiratory samples is 24 (IRQ, 18−31) days in survivors, but shedding can last for up to 42 days.[59]

Given the invasiveness and requirements for equipment and skilled labor, the collection of specimens other than sputum from the lower respiratory tract should be considered only in special situations. A nasopharyngeal swab collected with a fiberplastic shaft swab is the preferred choice for swab-based SARS-CoV-2 testing because it provides reliable results while not being too invasive. Calcium alginate swabs and wooden shaft swabs are not recommended because they may contain substances that inactivate some viruses and inhibit PCR testing. When collection with a nasopharyngeal swab is not possible, oropharyngeal swabs, nasal midturbinate swabs, or anterior nares (nasal swab) swabs are also acceptable alternatives. Swabs should be placed immediately after collection into sterile tubes containing 2 to 3 mL of a viral transport medium to preserve viral integrity. Upon collection and with the appropriate storage medium, specimens can be stored at 2−8 °C for up to 72 h. In cases of delayed testing or shipping, samples should be stored at −70 °C or below.[57] Inadequate sample collection, handling, and storage are important variables that may result in false-negative test results.

In addition to respiratory tract specimens, SARS-CoV-2 can also be detected in other samples such as stool, anal swabs, and blood but not in urine.[54,60,61] SARS-CoV-2 detection in blood is not frequent and is associated with disease severity.[60,61] The detection rate of SARS-CoV-2 RNA in fecal specimens is similar to that of pharyngeal swabs;[54,62] however, importantly, fecal viral shedding appears to occur for a longer period of

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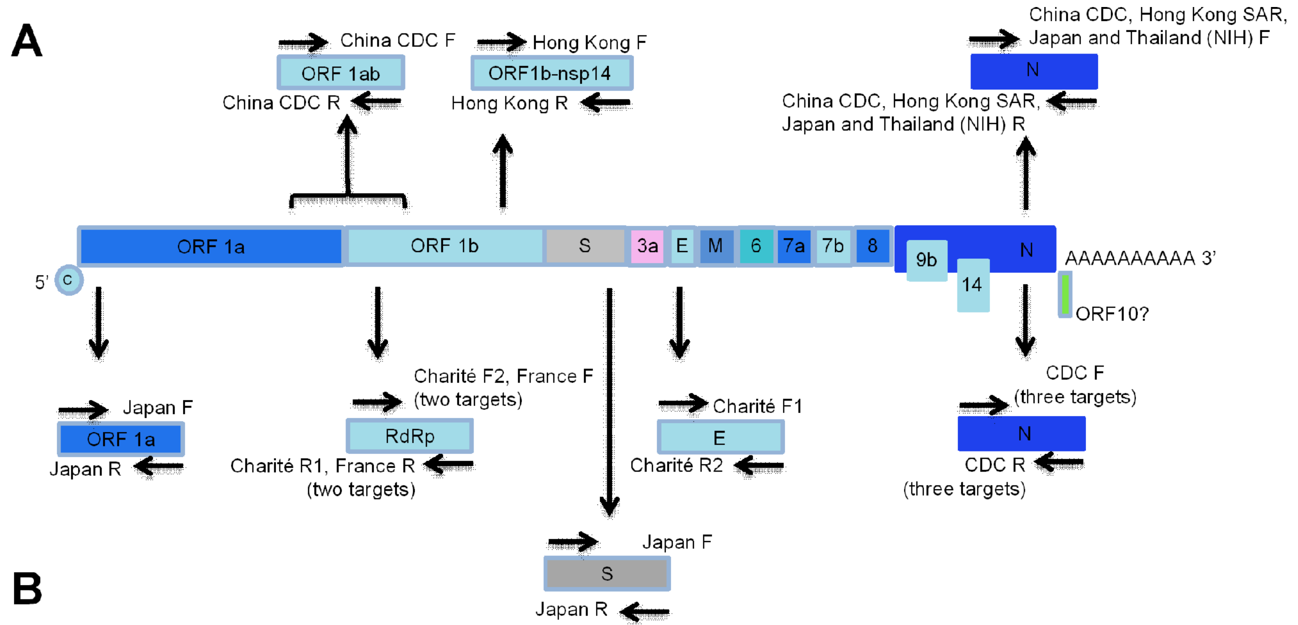

Country	Institute	Gene targets	Primers and probes sequence (5'-3')	Reference
China	China CDC	ORF1ab	F: CCCTGTGGGTTTTACACTTAA R: ACGATTGTGCATCAGCTGA P: FAM-CGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1	203
		N	F: GGGGAACTTCTCCTGCTAGAAT R: CAGACATTTTGCTCTCAAGCTG P: FAM-TTGCTGCTGCTTGACAGATT-TAMRA	
Germany	Charité	RdRP (confirmatory)	SARSr-F2: GTGARATGGTCATGTGTGGCGG RdRP_SARSr-R1: CARATGTTAAASACACTATTAGCATA RdRP_SARSr-P1*: CCAGGTGGWACRTCATCMGGTGATGC-BBQ RdRP_SARSr-P2#: FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	204
		E (first line)	E_Sarbeco_F1: ACAGGTACGTTAATAGTTAATAGCGT E_Sarbeco_R2: ATATTGCAGCAGTACGCACACA E_Sarbeco_P1: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	
Hong Kong SAR	HKU	ORF1b-nsp14	HKU-ORF1b-nsp14F: TGGGGYTTTACRGGTAACCT HKU-ORF1b-nsp14R: AACRCGCTTAACAAAGCACTC HKU-ORF1b-nsp141P: FAM-TAGTTGTGATGCWATCATGACTAG-TAMRA	205
		N	HKU-NF: TAATCAGACAAGGAACTGATTA HKU-NR: CGAAGGTGTGACTTCCATG HKU-NP: FAM-GCAAATTGTGCAATTTGCGG-TAMRA	
Japan	National Institute of Infectious Diseases	Pancorona and multiple targets ORF1a Nested PCR	NIID_WH-1_F501: TTCGGATGCTCGAACTGCACC NIID_WH-1_R913: CTTTACCAGCACGTGCTAGAAGG NIID_WH-1_F509: CTCGAACTGCACCTCATGG NIID_WH-1_R854: CAGAAGTTGTTATCGACATAGC	206
		S Nested PCR	WuhanCoV-spk1-f: TTGGCAAAATTCAAGACTCACTTT WuhanCoV-spk2-r: TGTGGTTCATAAAAATTCCTTTGTG NIID_WH-1_F24381: TCAAGACTCACTTTCTTCCAC NIID_WH-1_R24873: ATTTGAAACAAAGACACCTTCAC	
		N	NIID_2019-nCOV_N_F2: AAATTTTGGGGACCAGGAAC NIID_2019-nCOV_N_R2: TGGCAGCTGTGTAGGTCAAC NIID_2019-nCOV_N_P2: FAM-ATGTCGCGCATTGGCATGGA-BHQ	
Thailand	National Institute of Health	N	WH-NIC N-F: CGTTTGGTGGACCCTCAGAT WH-NIC N-R: CCCCACTGCGTTCTCCATT WH-NIC N-P: FAM-CAACTGGCAGTAACCA-BQH1	207
USA	USA CDC	Three targets in N gene N1	2019-nCoV_N1-F: GACCCCAAAATCAGCGAAAT 2019-nCoV_N1-R: TCTGGTTACTGCCAGTTGAATCTG 2019-nCoV_N1-P: FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1	208
		N2	2019-nCoV_N2-F: TTACAAACATTGGCCGCAAA 2019-nCoV_N2-R: GCGCGACATTCCGAAGAA 2019-nCoV_N2-P: FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1	
		N3	2019-nCoV_N3-F: GGGAGCCTTGAATACACCAAAA 2019-nCoV_N3-R: TGTAGCACGATTGCAGCATTG 2019-nCoV_N3-P: FAM-AYCACATTGGCACCCGCAATCCTG-BHQ1	
France	Institut Pasteur, Paris	Two targets in RdRP gene IP2	nCoV_IP2-12669Fw: ATGAGCTTAGTCCTGTTG nCoV_IP2-12759Rv: CTCCCTTTGTTGTGTTGT nCoV_IP2-12696bProbe: Hex-AGATGTCTTGTGCTGCCGGTA-BHQ-1	209
		IP4	nCoV_IP4-14059Fw: GGTAACTGGTATGATTTCG nCoV_IP4-14146Rv: CTGGTCAAGGTTAATATAGG nCoV_IP4-14084Probe: FAM-TCATACAAACCACGCCAGG-BHQ-1	

Figure 3. RT-qPCR assays for the detection of SARS-CoV-2. (A) SARS-CoV-2 annealing sites for the primers and probe. (B) Commonly used SARS-CoV-2 RT-qPCR assays.

time.[63] SARS-CoV-2 detection in stool is not associated with the presence of gastrointestinal symptoms or the severity of illness.[64−67] Despite high virus RNA levels in feces, successful isolation of SARS-CoV-2 from patient stool has been reported.[68] The isolation of SARS-CoV-2 from urine and ocular secretions from infected patient has been described.[69,70] Viral shedding in human breast milk and semen has been detected,[71,72] while in tears it has either been undetected[73] or

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detected at very low frequency.[74] Serum and plasma samples are used for serological assays, especially 1 week postinfection. The median seroconversion time for total anti-SARS-CoV-2 antibodies, IgM and then IgG, were days 11, 12, and 14, respectively.[75]

Virus Isolation. SARS-CoV-2 was first isolated from bronchoalveolar lavage from a patient with pneumonia in Vero E6 and Huh7 cells. The viral identity was confirmed by immunofluorescence using the now-known cross-reactive anti-SARSr-CoV Rp3 N antibody, RT-qPCR, and metagenomics sequencing. The cellular infectivity of the isolated virus was confirmed by virus neutralization assay using sera from convalescing patients.[11] Culture-based methods for SARS-CoV-2 detection have been used in research and public health laboratories in different parts of the world, but virus isolation is not recommended as a routine diagnostic procedure because it has low sensitivity, it is time-consuming, and it requires BSL-3 containment.[56] SARS-CoV-2 is also culturable in several cell lines, including human airway epithelial,[5,76] Vero E6, Vero CCL-81, and Huh-7 cells.[11,58,77,78] Vero E6 cells express high levels of angiotensin-converting enzyme 2 (ACE2),[79] which has been identified as a key a cell receptor for SARS-CoV-2 infection.[80] Similar to SARS-CoV-1 and MERS-CoV, SARS-CoV-2 isolation is enhanced in an engineered Vero E6 that expresses TMPRSS2 (transmembrane serine protease 2) levels that are 10-fold higher than in human normal lung tissue and other human cell lines, suggesting that TMPRSS2 protease is important for SARS-CoV-2 infection.[81] Recently, Zang and colleagues demonstrated that TMPRSS2 and TMPRSS4 promote SARS-CoV-2 entry into enterocytes.[82]

Harcourt and co-workers studied the susceptibility of several cell lines such as Vero CCL-81, Vero E6, HEK-293T, A549, and Huh-7 as well as the big brown bat kidney cell line (EFK3B) to support the productive replication of SARS-CoV-2. No cytopathic effect (CPE) was observed in any of the cell lines except in Vero E6 and Vero CCL81 cells, in which the virus grew to >10^7 PFU/mL at 24 h postinfection. Huh-7 and HEK-293T cells showed only modest viral replication, whereas no virus replication was detected in either A549 or EFK3B cells. SARS-CoV-2 produced distinct plaques in Vero E6 cells, but plaques in Vero CCL-81 cells were not as clear, suggesting that Vero E6 cells might be the best choice for the propagation, quantification, and study of plaque phenotypes of different SARS-CoV-2 strains.[78] However, should virus isolation be attempted, Vero E6 cells clearly stand out as a cell line that supports infection and virus production.

A recent cross-sectional study used 90 SARS-CoV-2 RT-qPCR confirmed specimens and evaluated their ability to infect Vero cell lines.[83] For the study, 90 patient samples were incubated on Vero cells (CCL-81) during 4 days, and then the cytopathic effect was evaluated. Of these, 26 samples (28.9%) demonstrated viral growth, but there was no growth in samples with a Ct value >24,[83] which suggests using samples with low Ct values for successful viral isolation.

Electron Microscopy. Human CoV was first isolated in 1965 from a patient with a common cold.[84] Later, the virus was named “coronavirus” due to the virus’ appearance under the electron microscope that resembles the solar corona.[85] CoV particles are pleomorphic, and their surfaces are covered with a distinct layer of projections, which corresponds to the long Spike protein.[80] Given the virus’ distinctive morphology, electron microscopy has been used to observe and identify virus particles after isolation in culture systems in the initial outbreak in Wuhan[11] and subsequently by others.[11,86,87] The first attempts to recover SARS-CoV-2 virions with the characteristic fringe of surface spike proteins from the first COVID-19 case in Australia failed, but adding trypsin to the cell culture medium immediately allowed the visualization of the virus with characteristic CoV morphology, with particles of 90−110 nm diameter displaying prominent spikes (9−12 nm) on the surface.[88]

Real-Time RT-qPCR. The gold standard diagnostic test for SARS-CoV-2 infection is viral RNA detection by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) given its sensitivity, specificity, and speed. RT-qPCR shows better performance than serology because it can identify positive cases in the early stage of infection, even during the incubation period of the disease and after symptoms have disappeared.[58] Many laboratory-developed tests based on the RT-qPCR assay have been published or are in development for SARS-CoV-2 detection. Some of these developed assays are specific to the pandemic strain of the virus, and others also recognize genetically similar strains such as SARS-CoV. Molecular targets for SARS-CoV-2 RT-qPCR include the genes that encode the nucleocapsid (N), envelope (E), spike (S), and RNA-dependent RNA polymerase proteins given their high degree of genetic conservation (Figure 3).[56,89] Most assays use two targets in the viral genome, but in the event of reagent shortages, RT-qPCR screening with only one set of primers instead of two may be considered after thorough validation in the individual laboratory.[90] Performing a sample preheating step instead of RNA extraction can also be considered during a shortage of viral nucleic acid extraction kits. In a study with 87 oropharyngeal swab samples (65 positive and 22 negative for SARS-CoV-2) in Denmark, it was found that heating for 5 min at 98 °C resulted in a sensitivity, specificity, and accuracy of 97.4, 100.0, and 98.3%, respectively, as compared with MagNA pure 96 RNA-extracted samples.[91] Although some laboratories have recommended heat inactivation of the virus (56−60 °C for 30−60 min) before RNA extraction to protect laboratory personnel, this procedure adversely affects the efficacy of RT-qPCR for SARS-CoV-2 detection.[92] High rates of false-negative SARS-CoV-2 RT-qPCR results and prolonged nucleic acid conversion have been reported in some studies, which have implications for returning to normal activities and COVID-19 control.[93−95]

In addition to in-house tests, many molecular assay kits have been approved by the Food and Drug Administration’s (FDA) and have been made commercially available for the detection and amplification of the SARS-CoV-2 RNA genome (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations). However, diagnostic laboratories should rigorously validate these commercial kits before routine use since the analytical sensitivity of some commercial RT-qPCR tests differs substantially, which could lead to false-negative results.[96]

Two of the most widely used assays in the Western world have been developed by Charité, Hospital Germany[97] and the U.S. CDC.[98] Nalla and co-workers compared the performance of the Charité (N, polymerase, and E targets) and the CDC (N1, N2, and N3 targets) primer/probe sets side-by-side using clinical samples. It was found that all assays were highly specific to SARS-CoV-2, with no cross-reactivity with other respiratory viruses. The CDC N2 and the Charité Hospital E-gene primer/probe sets performed equally well and were the most sensitive assays for detecting SARS-CoV-2.[99] We anticipate

that this list will grow as several new RT-qPCR assays are being developed to promote the accurate and rapid detection of SARS-CoV-2.[97,100−102]

Viral shedding patterns in COVID-19 patients have been studied.[55,59,103−110] Xiao and colleagues investigated the viral shedding profile of SARS-CoV-2 in 56 recovered patients with COVID-19 disease.[59] The results demonstrated that the median duration between the onset of symptoms to nucleic acid conversion was 24 days (IQR, 18−31) and that the longest duration was 42 days after the onset of symptoms.[59] It was found that higher viral loads (inversely proportional of the Ct value) can be observed in upper respiratory samples soon after symptom onset[55] and that the peak occurs within the first week of illness onset of COVID-19 confirmed patients.[55,103] In another study, the authors evaluated the viral shedding patterns demonstrated in patients with mild and severe COVID-19 disease using specimens from 76 patients, including 46 individuals classified as mild cases and 30 classified as severe cases.[104] The results showed that the viral load of severe cases was around 60 times higher when compared with that of mild cases, indicating that higher viral loads might be associated with severe clinical findings. In addition, it was revealed that the Ct values of severe cases were significantly lower than those of mild cases in infected patients at the time of admission and that early viral clearance was observed in patients classified as mild cases (10 days after onset).[104] SARS-CoV-2-positive RT-qPCR detection has also been demonstrated even after the resolution of COVID-19 symptoms.[58,111] For this reason, the CDC has recommended obtaining at least two negative upper respiratory tract samples, collected in intervals of 24 h or longer, to document SARS-CoV-2 clearance.[112]

On the other hand, the viral shedding in asymptomatic patients with COVID-19 also has been investigated.[113,114] Zhou and co-workers analyzed the viral shedding pattern in 31 patients that were confirmed to have COVID-19 using RT-qPCR but were asymptomatic during admission to the hospital.[113] Subsequently, the study divided the 31 patients into 2 groups: 9 patients who remained asymptomatic during hospitalization (APs) and 22 patients who presented symptoms after admission to the hospital (APIs). The results demonstrated that the median Ct value of APs (39.0, interquartile range [IQR] 37.5−39.5) was higher than when compared to APIs (34.5, IQR 32.2−37.0), showing a lower viral load in APs. Also, it was found that the duration of viral shedding remained similar in the two patient groups, which reflects the possibility of SARS-CoV-2 transmission in the community during the asymptomatic period. In addition, the study findings revealed that the viral load of APs peaked during the first week after admission to the hospital while that of APIs peaked during the second week of COVID-19 infection. These findings alert professionals and health authorities to the possibility of transmission during the asymptomatic period of patients with COVID-19 disease. However, further scientific studies with more patients are required to elucidate the real role of asymptomatic patients in the transmission chain.

Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP). The recent increase in the number of COVID-19 cases in the world has encouraged a global effort to develop point-of-care platforms for diagnosing SARS-CoV-2. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is perhaps one of the most promising platforms for rapid development and accessible SARS-CoV-2 testing and has many advantages, compared to RT-qPCR, such as high specificity and sensitivity, simple operation, rapid amplification, and low cost,.[115,116] RT-LAMP assays have been developed for other CoVs of the same genus (*Betacoronavirus*), including SARS-CoV[117,118] and MERS-CoV.[119,120] Not surprisingly, several studies have already demonstrated the use of RT-LAMP for SARS-CoV-2 detection.[121−125]

CRISPR/Cas-Based Diagnostic Methods. The clustered regularly interspaced short palindromic repeats (CRISPR)/Cas machinery has recently been adapted as a POC tool for the rapid detection of nucleic acids (DNA or RNA).[126,127] Overall, this CRISPR machinery is programmed to cleave specific sequences in the DNA/RNA target where the results can be easily observed by combination with a lateral-flow strip. Initially, Zhang's team developed a CRISPR/Cas-based platform called specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) that, combined with isothermal preamplification to detect strains of single-strand RNA viruses, identifies mutations and human genotype DNA, and distinguishes pathogenic bacteria.[126,127] More recently, using the same knowledge, they adapted a protocol using the SHERLOCK system for SARS-CoV-2 detection.[128] On the other hand, Mammoth Bioscience Company developed another platform based on the CRISPR/Cas system named the endonuclease-targeted CRISPR trans reporter (DETECTR) to detect any RNA or DNA target, which has now been used to detect the SARS-CoV-2 RNA genome from respiratory swab RNA extracts.[129] The suitability of DETECTR technology for the detection of SARS-CoV-2 was evaluated using 78 patient specimens, including 36 patients with COVID-19 infection and 42 patients with other viral respiratory infections, and then compared with the CDC RT-qPCR as a reference method to confirm COVID-19 infection. The SARS-CoV-2 DETECTR test had 95% positive predictive agreement and 100% negative predictive agreement when compared with RT-qPCR results. Despite these promising results, CRISPR/Cas-based diagnostic methods are not widely used by diagnostic laboratories and need further implementation. Taken together, these results highlight the great potential of CRISPR-based diagnostic methods as a rapid, specific, portable, and accurate detection platform for the detection of the SARS-CoV-2 genome in patient samples.

Sensors. Sensors represent another alternative detection method with rapid and high throughput. Since the emergence of SARS-CoV-2, several research groups from different parts of the world have also focused on alternative sensing modalities based on sensors for the diagnosis of SARS-CoV-2 that reduce the use of expensive laboratory equipment, trained laboratory personnel, and extensive sample preparation and provide fast and accurate results.[130] In this context, Qiu and co-workers developed a dual-functional plasmonic biosensor combining the plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) to detect SARS-CoV-2.[131] In another study, the authors used a field-effect transistor (FET)-based sensor to detect SARS-CoV-2 from patient samples.[132] In general, these recent research findings are based on technologies previously used for the detection of other viral pathogens that now can be adapted for the detection of SARS-CoV-2. For instance, due to its high sensitivity and specificity, simple operation, low cost, and other advantages, the sensors using programmable biomolecular components named the toehold switch developed in response to previous Ebola and

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Zika outbreaks represent another powerful platform for detecting the SARS-CoV-2 genome.[133−135]

Genome Sequencing. Whole genome sequencing was used to identify potential etiological agents involved in the index cases of the COVID-19 pandemic in Wuhan.[11] In addition to unequivocally confirming the diagnosis of a SARS-CoV-2 infection, regular sequencing of a percentage of patient samples from clinical cases can be used to monitor changes in the viral genome over time and trace transmission patterns. For this purpose, several sequencing protocols based on Sanger and next-generation sequencing (NGS) are being applied to rapidly generate the genome sequences.[5,136,137] Lu and colleagues used a combination of Sanger, Illumina, and Oxford nanopore MinION sequencing technologies to obtain the whole-genome sequences of SARS-CoV-2 from six patient specimens from Wuhan, China.[5] Holshue and colleagues reported the first case of the novel coronavirus in the United States and used a combination of the Sanger method, Illumina, and MinION to generate the whole-genome sequences.[136] Whole-genome sequencing using MinION technology coupled with phylogenetic analyses and travel history allowed the identification of SARS-CoV-2 entry routes into Latin America during its emergence in Brazil.[138] As of July 31, 2020, 13 303 genome sequences had been deposited in Genbank (https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/), including complete genome sequences from different countries around the world and partial genome sequences.

SARS-CoV-2 has evolved continuously since its emergence. The binding regions of primers and probes should be monitored continuously for matching to the virus genome as more sequence information becomes available. In this scenario, Artesi and colleagues reported that changes within the SARS-CoV-2 primer binding region negatively affected the performance of commercial RT-qPCR assays (unpublished data, https://www.medrxiv.org/content/10.1101/2020.04.28.20083337v1). In another study, Rana and co-workers analyzed the whole genome sequence of 93 patients with COVID-19 and found variations in the primer and probe binding sites that could produce false-negative results.[139] A comprehensive bioinformatics analysis of 17 000 SARS-CoV-2 sequences from around the world identified the presence of mutations/mismatches in the primer/probe binding regions of 7 assays out of 27 RT-qPCR assays studied, including the Charité-ORF1b reverse primer and the US-CDC-N-1 probe.[140] However, it should be noted that except at the 3′ end, RT-qPCR can tolerate mismatches at the 5′ extremity or in the middle of a primer. Thus, oligonucleotide binding regions should be continuously monitored by bioinformatics and also by wet-laboratory experiments in order to identify changes that may influence RT-qPCR performance.[139,141,142]

Taken together, whole genome sequencing and bioinformatics will be important in global efforts to combat the pandemic, with the resulting analyses being used to guide a wide range of studies, including diagnostics,[97,121,122] molecular epidemiology studies,[5] viral evolution,[143−145] elucidatation of possible hosts of the virus,[5,146−148] identification of targets for drugs and vaccines,[149,150] molecular determinants involved in virulence and pathogenicity,[151,152] and factors related to the host's immune response to the virus.[153,154]

Serology. Serological methods are being increasingly used and can be used for diagnosis, contact tracing, and herd immunity assessment, and vaccine efficacy evaluation. Seroepidemiologic studies can assist in the investigation of the ongoing pandemic and the retrospective assessment to determine the attack rate or the progress of the pandemic through antibody detection. These studies can assist health authorities and governments in making sound decisions with respect to the implementation of public health measures during the course of the current pandemic. Population-based serosurveys for SARS-CoV-2 has been applied in several sites to assess the burden of COVID-19 and more accurately determine SARS-CoV-2 prevalence and transmission dynamics.[155−157]

Moreover, serology can also be useful in situations where RT-qPCR is negative and there is a strong epidemiological link to COVID-19 infection.[94] In these cases, paired serum samples collected in the acute and convalescent phase can be of diagnostic value.

The adaptive immune response to SARS-CoV-2 infection has been studied,[75,94,158,159] despite several knowledge gaps in this area. Long and co-workers studied the antibody responses to SARS-CoV-2 in 285 patients with COVID-19 in China. Seroconversion for IgM and IgG occurred simultaneously or sequentially, and the median day of seroconversion for both immunoglobulins was 13 days after COVID-19 onset. The seroconversion rate for IgG reached 100% 19 days after symptom onset.[94] In a related work, Zhao and colleagues showed that the median seroconversion times for total antibodies, IgM, and IgG were 11, 12, and 14 days after SARS-CoV-2 infection.[75] Severe patients tend to have higher antibodies titers than patients with mild COVID-19.[94,160]

To investigate the antibody responses to SARS-CoV-2 in convalescing individuals, Robbiani and co-workers analyzed 149 plasma samples collected an average of 39 days after the onset of symptoms from convalescing individuals. Most convalescent plasmas obtained from recovered patients did not contain high concentrations of neutralizing activity.[161] A related study with convalescing patients detected serum neutralizing antibodies in 13 out of 14 patients, and there was a strong correlation among neutralization antibody titers, the numbers of virus-specific T cells, and anti-S-RBD IgG but not with anti-NP IgG.[159] Notably, the antibody response and viral clearance can be delayed in immune-compromised individuals and people posteriorly infected with SARS-CoV-2.[162]

In the past few months, a variety of serological tests have been designed to detect SARS-CoV-2, mainly enzyme-linked immunosorbent assays (ELISA), immunofluorescence assays (IFA), chemiluminescence enzyme immunoassays (CLIA), and lateral flow assays (LFA).[163] Of these serological methods, ELISA, CLIA, and LFA are used for the first-line screening of patient samples with COVID-19. Most serological assays are based on the SARS-CoV-2 nucleocapsid protein (N), transmembrane spike protein (S), or S receptor-binding domain (RBD) because of their high antigenicity.[164−166] As mentioned before, SARS-CoV-2 enters host cells using its spike protein and represents the main target of neutralizing antibodies produced by the host.[167] In this context, several studies have demonstrated that serological tests using the S antigen are more sensitive than those using N-antigen-based antibody assays.[163] Instead of a single antigen, some companies have combined N- and S-based antigens to develop serological assays for SARS-CoV-2 detection.[163] Cross-reactivity between SARS-CoV-2 and SARS-CoV-1 has been reported against the N protein but not against the S1 subunit of the S protein.[94] Cross-reactive antibodies between SARS-CoV-2 and SARS-

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CoV-1 have been detected in both the RBD region located in the S1 subunit and also in non-RBD regions.[168] Anderson and colleagues analyzed patient samples with SARS-CoV-1 and SARS-CoV-2 infections and showed that there is no detectable cross-neutralization by SARS-CoV-1 patient serum against SARS-CoV-2, despite significant cross-reactivity between the N proteins from the two viruses[169]

Many ELISA-based assays have been developed to evaluate the human antibody response (IgA, IgM, and IgG) against SARS-CoV-2.[11,170−172] Cross reactivity to other coronaviruses has been reported, and therefore test results should be interpreted in light of the local epidemiological scenario.[11] For example, it has been shown that IgM ELISA allows a higher detection efficacy than RT-qPCR after 5.5 days of symptom onset, and the combined use of IgM ELISA and RT-qPCR increases the positive detection rate to 98.6% when compared with a single RT-qPCR test (51.9%).[171] The median time of appearance of IgM and IgA antibodies in plasma was 5 days, while IgG was detected 14 days after symptom onset. In the same study, SARS-CoV-2 N ELISA did not show any cross-reactivity with other CoVs, with the exception of SARS-CoV-1, a virus that has not been in circulation since the 2002 to 2003 outbreak.[171]

Perera and co-workers developed an ELISA based on the receptor-binding domain (RBD) of the SARS-CoV-2 S protein to detect IgG and IgM antibodies in human sera and compared the ELISA results with confirmatory microneutralization and 90% plaque reduction neutralization tests ($PRNT_{90}$). They found that $PRNT_{90}$ were more sensitive than microneutralization to detecting antibodies against SARS-CoV-2, indicating that ELISA should be used for screening and $PRNT_{90}$ should be used for confirmation in large-scale seroepidemiological studies.[173]

A magnetic chemiluminescence enzyme immunoassay (MCLIA) has also been developed for virus-specific antibody detection. In a study with 285 COVID-19 patients, sera from these patients did not cross react with the SARS-CoV spike antigen, but some cross-reactivity was found with the SARS-CoV nucleocapsid antigen.[94]

Many lateral flow assays (LFA) have been developed to detect SARS-CoV-2[174−177] and have obtained Emergency Use Authorization from the FDA (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations). A combined IgM/IgG assay seems to be a better choice in terms of performance and sensitivity to either antibody alone (IgM or IgG). Recently, Li and colleagues developed a rapid LFA to simultaneously detect IgM and IgG antibodies against SARS-CoV-2 within 15 min in human blood.[178] The assay uses the receptor binding domain of the SARS-CoV-2 S protein as the antigen. They validated the assay with 525 blood samples previously screened by RT-qPCR, including 397 positive and 128 negative samples. The assay had a sensitivity of 88.66% and a specificity of 90.63% compared to RT-qPCR. In addition, several studies have been used to evaluate the clinical sensitivity of serological tests with COVID-19 patient samples collected on different days after the onset of symptoms.[179−183] Pan and colleagues evaluated a commercial lateral flow immunochromatographic test targeting viral IgM or the IgG antibody and compared it with RT-qPCR. The sensitivity of the assay for SARS-CoV-2 detection was 11.1% in early-stage patients (1−7 days after onset), 92.9% in intermediate-stage patients (8−14 days after onset), and 96.8% in late-stage patients (more than 15 days after onset).[180] In similar study, Tang and co-workers compared the diagnostic performance of two SARS-CoV-2 commercial serologic tests using 103 specimens from PCR-confirmed SARS-CoV-2 patients and 153 control specimens from different days after disease onset (<3, 3−7, 8−13, and ≥14 days). Both assays had poor diagnostic sensitivity during the first 14 days of symptoms, generating a high rate of false-negative results.[179] The time of sampling since the onset of symptoms must be taken into account when performing the serological diagnostic test, and false-negative results can lead to the false assumption that a person is not infected, consequently bringing about serious challenges and implications for the spread or containment of the disease. Most patients will seroconvert only in the second week after infection, and thus negative serological test results obtained during the first 14 days of the disease should be interpreted with caution.[75]

As opposed to classical serum neutralization assays such as the microneutralization and plaque reduction neutralization test (PRNT), pseudovirus neutralization assays for SARS-CoV-2 can be performed in biosafety level 2 facilities. Pseudovirus-based neutralizing assays for SARS-CoV-2 have been developed using vesicular stomatitis virus (VSV) and lentiviral pseudovirus systems.[184,185] Such systems will facilitate the study of COVID-19 humoral immunity and the development and evaluation of vaccines and therapeutics against SARS-CoV-2 once the reliability and cross reactivity have been assessed.

Antigen detection tests represent another alternative to detecting SARS-CoV-2. Antigen detection assays are developed to directly detect viral particles in biological samples such as nasopharyngeal secretions from infected patients. In this context, some rapid antigen assays have been proposed to detect SARS-CoV-2.[172,186−189] A fluorescence LFA antigen test based on the N protein has been developed and validated using nasopharyngeal and oropharyngeal samples from 127 suspected Covid-19 cases (82 positive by RT-qPCR). Compared with RT-qPCR, the test achieved a sensitivity of 93.9% and 100% specificity.[186] However, some commercially available point-of-care tests have very low sensitivity, which hinders their use in COVID-19 diagnosis.[187,190]

For the validation of serological tests, robust test validation with adequate clinical samples representative of a real-world scenario (e.g., timing from disease onset, different disease severities, different geographical locations, and patient age) is of paramount importance. Particular attention should be paid to the low specificity of some assays and the high possibility of false-positive results.[181] A low specificity of serological assays may have important consequences in terms of both diagnosis and population surveillance of COVID-19 patients at the individual level and population level.[163,190] At the individual level, the risks posed by false-positive results can be of great concern. For instance, people who have never been infected may return to work or travel because they are considered to be immune to SARS-CoV-2 infection. At the population level, false-positive results may increase the prevalence of COVID-19 disease and provide a distorted picture of the higher population immunity and lower mortality rate than what is actually happening, which can negatively affect seroepidemiological surveillance studies. Thus, the independent validation of COVID-19 serological tests using samples from different stages of the disease is urgently needed to encourage health care professionals and governments to make sound decisions.

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Differential Diagnosis. Since the clinical manifestations of COVID-19 are similar to those of other respiratory diseases, differential diagnosis is of paramount importance in assisting physicians in the therapeutic management of patients and health officials in establishing disease control policies. In an effort to do so, respiratory pathogens in patients suspected of COVID-19 in Italy have been investigated.[20] Here, researchers tested the nasopharyngeal swabs of 126 suspected causes using SARS-CoV-2 RT-qPCR and a commercial multiplex respiratory cartridge that detects and differentiates viral and bacterial pathogens. Only 3 patients were confirmed to be infected with SARS-CoV-2, and 67 (53.2%) were positive for other respiratory pathogens, including 26 (20.6%) positive for influenza A and 10 (7.9%) positive for influenza B. Other pathogens detected in the patient samples were common cold CoV (H-CoV 229 E, H-CoV NL63, and H-CoV HKU1), rhinovirus, enterovirus, metapneumovirus, adenovirus, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, and *Legionella pneumophila*.[20] These results highlight the diagnostic differential and demonstrate the importance of using a spectrum of molecular kits for the rapid detection of respiratory pathogens in order to improve the clinical management and treatment of patients infected with COVID-19.

In another study, Yan and co-workers reported the cases of two patients in Singapore who initially had moderate symptoms including myalgia, a mild cough, and diarrhea and presented with thrombocytopenia and a normal chest radiograph.[22] Dengue was suspected, and a rapid serological test for dengue produced false-positive results. As patient symptoms worsened, they were later diagnosed with COVID-19 by RT-qPCR. Taken together, these findings suggest that special attention is needed in the differential diagnosis between arboviruses and SARS-CoV-2 infection, especially in countries where is there a circulation of DENV, ZIKV, and CHIKV. Moreover, coinfection with SARS-CoV-2 and other respiratory viruses, such as common cold CoV, influenza, and metapneumovirus, have been reported, highlighting the need for repeat testing based on clinical indications.[191−194]

CONCLUSIONS AND FUTURE DIRECTIONS

The rapid spread of SARS-CoV-2 around the world, with mounting cases of fatal pneumonia and the economic crisis, is a global concern. Diagnostics represent one of the most powerful tools in mitigating these effects until a vaccine can be established. This needed is further highlighted by overlapping symptoms from other pathogens that can confound diagnosis based only on clinical criteria. As we have reviewed, many approaches have been established for the diagnosis of SARS-CoV-2, and we anticipate the coming months to bring about many more innovative strategies. Critically, the development of inexpensive point-of-care diagnostic platforms will accelerate the global response to the current pandemic, especially in countries with health systems devoid of adequate laboratory infrastructure. It will be important that widespread diagnostic development and validation continue in the coming months and that the large-scale implementation of these SARS-CoV-2 diagnostic platforms is successful as this pandemic evolves. Reliable, easy-to-use assays will be absolutely critical, especially as the disease moves through low- and middle-income countries.

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Notes

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