R E V I E W

# Current Status of Laboratory Diagnosis for COVID-19: A Narrative Review

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**Abstract:** The outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the related disease (COVID-19) has spread rapidly to pandemic proportions, increasing the demands on healthcare systems for the containment and management of COVID-19. One of the critical issues to be addressed is the improvement in laboratory diagnosis and screening of large portions of the population to stop the virus spreading. Currently, the laboratory diagnosis of SARS-CoV-2 infection and the related disease is based on the research of viral RNA with rt-PCR methods in upper and lower respiratory airways. Serological tests to detect SARS-CoV-2 antibodies could help physicians and healthcare workers to support COVID-19 diagnosis and follow-up and perform population screening. Our review, using MEDLINE and EMBASE, summarizes the current knowledge of direct and serological tests performed to research RNA, antigens, or antibodies for SARS-CoV-2, evaluating the advantages and drawbacks for specific tests.
**Keywords:** COVID-19, SARS-CoV-2, laboratory diagnosis, serological test, PCR

## Introduction

In late December 2019, an outbreak of a respiratory disease of uncertain etiology occurred in Wuhan, the capital of Hubei region, China.[1,2] On January 10, 2020, the first genome of the new virus was deposited by Zhang et al,[3] on GenBank (MN908947) and other platforms. The Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV) renamed the virus, previously 2019-nCoV, as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).[4] Despite increasingly rigorous restrictions, China and most of the primarily involved countries failed to contain COVID-19's global spread, as of May 10, 2020, the WHO Situation Report-111 declared 3,917,366 confirmed cases worldwide (1,707,946 in Europe, 218,268 in Italy), with 274,61 deaths (155,552 in Europe, 30,395 in Italy).[5]

COVID-19 infection has a broad spectrum of severity ranging from an asymptomatic form to a severe acute respiratory syndrome that requires mechanical ventilation; typical symptoms of infected patients by SARS-CoV-2 are fever, fatigue, and dry cough.[6]

To date there is uncertainty about the mode of transmission of SARS-CoV-2, but it is likely similar to SARS, which is spread by contact, droplets, aerosol, and contaminated environments;[6,7] studies have highlighted the higher risk of transmission from patients with respiratory symptoms,[8,39] but it is also possible from asymptomatic patients.[9] Nowadays, to identify the asymptomatic carriers of SARS-

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CoV-2 and patients with COVID-19 is the diagnostic gold standard and seems to be important point in the strategies to fight the spread of the epidemic; on the other hand to identify the subjects with previous contact with the virus seems to also be important in the strategies of epidemiological knowledge.

## Virology

SARS-CoV-2 (Order *Nidovirales*, Family *Coronaviridae*, Sub-family *Orthocoronavirinae*, Genus *Betacoronavirus*) is an enveloped positive-sense single-stranded RNA (+ssRNA) virus.[4] The *Orthocoronavirinae* sub-family comprises four distinct clades: alpha- (α-CoV), beta- (β-CoV), gamma- (γ-CoV), and delta-coronavirus (δ-CoV),[10] among which only the first two can also infect mammals and encompass human pathogens. In contrast with the endemic relatively mild α-CoVs, β-CoVs include highly virulent zoonotic epidemic viruses, already known for the massive outbreaks of SARS (2002) and Middle East respiratory syndrome (2012): SARS-CoV and MERS-CoV, respectively.[11,12] According to genomic and phylogenetic analyses, SARS-CoV-2 is included in the *Sarbecovirus* subgenus (B-lineage of β-CoV genus) comprising SARS-CoV and several bat viruses.[13,14] Surprisingly, SARS-CoV-2 genome is closer to the RaTG13 bat CoV (96.2% identity)[15] than to SARS-CoV (79%) and MERS-CoV (50%).[14]

The virion presents an almost spherical pleomorphic structure (60–140 nm in diameter) characterized by a peculiar external "crown" of S protein spikes (8–12 nm in length), under transmission electron microscopy.[1] The SARS-CoV-2 genome (30 Kb) encodes 16 non-structural proteins (nsp 1–16),[16] including the RNA-dependent RNA-polymerase (RdRp, nsp12)[17] and the helicase (nsp13), and four structural proteins: the spike (S), the membrane (M), and the nucleocapsid (N) glycoproteins, and the envelope (E) protein.[13,17–19] The viral envelope comprises the S, E, and M proteins, enclosing the N protein and the RNA genome.[19,21] The S glycoprotein, a class I fusion protein,[22,23] is pivotal for the endocytosis-mediated viral entry[22,24] and consists of two subunits (S1, S2);[25] the S1 harbors the receptor-binding domain (RBD),[14,26] which directly binds human angiotensin-converting enzyme 2 (hACE2).[26,28] Crucially, while the M glycoprotein is the most abundant SARS-CoV-2 protein, the S glycoprotein is the main inducer of neutralizing antibodies[29,30] and the most diverging protein, with a high mutation rate,[17,32,34] possibly modifying glycosylation sites and consequently altering hACE2 binding, CTL epitopes,[32,35] and accessibility to proteases and neutralizing antibodies.[22]

## Aim of the Narrative Review

The aim of this narrative review was to evaluate the tools for the etiological diagnosis of SARS-CoV-2 infection and their use in different clinical settings. The article is addressed particularly to physicians providing care to COVID-19 patients and to Healthcare authorities designing screening programs for the general population.

## Methods

We conducted a comprehensive computerized literature research to identify studies analyzing diagnostic tests for COVID-19 using MEDLINE and EMBASE from January 2020 up to April 2020, involving both medical subject heading (MeSH) terminology and relevant keywords for search strings to locate articles that analyzed the diagnostic test for COVID-19. The following items were used to search for the studies: "diagnosis", "laboratory test", "COVID-19," and "SARS-CoV-2". We performed this research to summarize the latest and future perspectives on the laboratory diagnosis for SARS-CoV-2 infection and the related disease.

## Etiological Diagnosis

As for all viral infections, the diagnosis of SARS-CoV-2 infection is based on the direct identification of viral RNA or antigens or the indirect identification of specific antibody responses. A direct diagnosis is the gold standard for an active infection, while the detection of specific anti-SARS-CoV-2 antibodies is the cornerstone for the identification of previous contact with the virus, both for diagnostic and epidemiological aims.[36]

## Direct Diagnosis of SARS-CoV-2 Infection

The direct diagnosis of SARS-CoV-2 infection is based on the detection of SARS-CoV-2 RNA on nasopharyngeal swabs or on lower respiratory tract specimens.[36]

In clinical practice, the most widely used is the former, while tests on lower respiratory tract specimens are performed in some defined cases.[36] In patients with a good outcome, viral RNA is detected for 20 days or longer after the onset of symptoms, and a rebound of the viral load, after undetectable with PCR, is possible.[31]

In addition, rt-PCR positivity for SARS-CoV-2 RNA peaked in upper respiratory tract specimens at 7–10 days after the onset of symptoms and then steadily declined; conversely, rt-PCR RNA detection in lower respiratory tract specimens remained stable for 3 weeks after symptom onset/clinical presentation.[31]

The characteristics for optimal testing for a direct diagnosis of SARS-CoV-2 infection include a short turnaround time, high throughput, minimum batching, low infrastructural requirements, elevated accuracy, low cost to allow access to testing, also considering testing priorities to diagnose vulnerable populations, and to reduce viral spread, especially in nosocomial, family, and closed community settings.[40,47] Nucleic acid testing (real-time rt-qPCR) on respiratory tract specimens have several of these characteristics, thus representing the current gold standard in the diagnosis of SARS-CoV-2 infection.[41] However, various factors, either procedural or virus-related, may impair its reliability,[42] for example a single-time point,[38] and an unmet need for procedural standardization[43] (from sample collection, including swab types, to processing) remains complicated by shortages of personnel and materials.[38,44] For these reasons, new rapid tests (either based on antigens or antibodies),[38,45] point-of-care (POC) assays,[38,46] and digital technologies[47] would support infection detection and containment, also in low- and middle-income settings, but require careful evaluation.

Tables 1 and 2 show the characteristics and the advantages and disadvantages of different direct tests for SARS-CoV-2, respectively.

## Real-Time PCR Tests

Real-time rt-PCR technology is the most used SARS-CoV-2 RNA detection.[36] In several real-time PCR tests, the primers were designed against the envelope (E) and RNA-dependent RNA polymerase (RdRp) regions:[48] the E-region was used for first-line screening, while the RdRp region was employed for confirmatory testing. Other researchers developed one-step rt-PCR assays to detect open reading frame (ORF) 1b and N regions of SARS-CoV-2:[49] the N region assay was used for screening, while ORF1b testing served as a confirmatory test. However, since ORF1b and N regions are highly conserved in Sarbecoviruses, the specific primers could also bind other coronaviruses and closely related viruses. As regards the rt-PCR performances, a recent meta-analysis performed by Kim et al[50] considering 19 studies identified a pooled sensitivity of 89% (95% CI=81–94%; I2=90%), while the positive predictive value (PPV) ranged from 47.3–98.3% and the negative predictive value (NPV) ranged from 93.4–99.9%. In fact, false negative results may not only derive from poor sensitivity, but they can also be due to low amounts of sample, inadequate conservation, or the presence of amplification inhibitors or of mutations in the target regions of the specific primer used.[51,52] For example, Li et al[53] observed that, of the 610 patients enrolled, 384 (63.0%) had a negative nasopharyngeal specimen at baseline: of these 384, 48 patients were positive at the second swab test and 12 at the third. Comparably, Xiao et al,[51] considering 70 patients with COVID-19, in 15 (21.4%) observed a positive rt-PCR result after two

**Table 1** Characteristics of Different Direct Tests for SARS-CoV-2

| SARS-CoV-2 Test | Identification | Specimen | Specific Infrastructural Requirement | Optimal Timing for Testing (Days) | Turnaround Time (minutes) | Se (%) | Sp (%) |
|---|---|---|---|---|---|---|---|
| rt-PCR | RNA | nasopharyngeal and/or oropharyngeal swabs and/or lower respiratory specimen | Yes | At least 2 days after infection until negativization | 190 | ≈89* | 99** |
| RT-LAMP | RNA | nasopharyngeal and/or oropharyngeal swabs and/or lower respiratory specimen | Yes | At least 2 days after infection until negativization | 45–60 | comparable to rt-PCR | comparable to rt-PCR |
| NP antigen detection test | Antigen (Ag) of SARS-CoV-2 | nasopharyngeal and/or oropharyngeal swabs and/or lower respiratory specimen | Yes | At least 2 days after infection until negativization | 240 | 70–86** | 95–97** |

**Abbreviations:** rt-PCR, real time-protein chain reaction; RT-LAMP, reverse-transcription-loop-mediated isothermal amplification; NP, nucleoprotein; Se, sensitivity; Sp, specificity. * Data from meta-analysis; ** Data declared by producer.

**Table 2** Advantages, Disadvantages, and Possible Indications of Different Direct Tests for SARS-CoV-2

| SARS-CoV-2 Test C | Advantages | Disadvantages | Indications |
|---|---|---|---|
| rt-PCR | • Widely used<br>• High sensitivity and specificity | • Needs for infrastructure<br>• Expensive<br>• Medium turnaround time<br>• Qualified personnel<br>• Incorrect sampling<br>• Restrictions on sample transportation | • Currently the gold standard in symptomatic and asymptomatic patients |
| RT-LAMP | • Lowest turnaround time considering direct methods<br>• High sensitivity<br>• Less bias in the analytical phase | • Needs for infrastructure<br>• Expensive<br>• Qualified personnel<br>• Incorrect sampling<br>• Restrictions on sample transportation | • Substitute for rt-PCR where possible in order to reduce the turnaround time |
| NP antigen detection test | • Easier analytical procedures<br>• Possible even in less equipped labs | • No real-life studies<br>• Low sensibility<br>• Qualified personnel<br>• Incorrect sampling<br>• Restrictions on sample transportation | • It could be used in facilities with no equipment for rt-PCR, waiting for the rt-PCR test |

**Abbreviations:** rt-PCR, real-time protein chain reaction; RT-LAMP, reverse-transcription-loop-mediated isothermal amplification; NP, nucleoprotein.

negative specimens. Thus, a negative result does not exclude SARS-CoV-2 infection and should not be used alone for clinical management.

As for the identification of an optimal specimen type, the nasopharyngeal swab remains the gold standard for the detection of SARS-CoV-2 RNA. Azzi et al[54] highlighted the possibility of performing rt-PCR also on saliva samples; all 25 patients with a positive rt-PCR on nasopharyngeal swabs were positive also on saliva samples, more easily collected. Of relevance, the collection of sequential upper respiratory specimens or a single lower respiratory specimen may be necessary to detect the virus in symptomatic subjects with initial negative rt-PCR testing. In fact, Guo et al[55] Pshowed that rt-PCR performed on throat washing samples was more accurate for COVID-19 diagnosis than nasopharyngeal swab samples (*P*<0.031).

## Reverse-Transcription-Loop-Mediated Isothermal Amplification (RT-LAMP)

In recent years, the loop-mediated isothermal amplification (LAMP) method, which includes an exponential amplification of specific nucleic acid sequences at a constant temperature, has been widely utilized for the rapid detection of virus-specific genes, with a specificity and sensitivity comparable to that reported for rt-PCR assays. The LAMP technique eludes some PCR limitations, such as the need for high-purity samples, trained personnel, and expensive laboratory instruments.[56]

A combination of LAMP with reverse transcription (rt-LAMP) allows the direct detection of RNA, including SARS-CoV-2 RNA. In a small study, Wui et al[57] validated the RT-LAMP method employing 16 clinical samples, eight positive and eight negative for SARS-CoV-2 by rt-PCR: the testing results were consistent with the conventional rt-PCR.

## Antigen-Based Tests

Direct methods for COVID-19 diagnosis also encompass a double antibody sandwich enzyme-linked immunoassay identifying the SARS-CoV-2 nucleoprotein (NP) by a microplate pre-coated with specific antibodies against SARS-CoV-2 NP and the use of a horseradish peroxidase (HRP)-labeled secondary antibody against the same protein. This direct method is simple, rapid, and does not require trained personnel and expensive laboratory instruments. However, in a meta-analysis by Castro et al[58] the sensitivity of this test ranged from 70–86%, while the specificity ranged from 95–97%, and thus a single negative test result cannot rule out SARS-CoV-2 infection.[59]

## Indirect Diagnosis of SARS-CoV-2 Infection

The indirect diagnosis of SARS-CoV-2 infection is based on the detection of specific IgG and/or IgM antibodies. Importantly, the WHO underlines a strong need for serological IgM and IgG testing and a rapid design of easy and inexpensive point-of-care (POC) tests.[38,46,60]

The dynamics of antibody responses were studied by several authors. To et al[31] evaluated the viral load and antibody kinetics on deep-throat saliva specimens (posterior nasopharyngeal samples), suggesting that serological testing may be complementary with rt-PCR testing; they observed that IgG and IgM responses against the internal viral NP and the S protein receptor-binding domain (RBD) occurred mostly within 10 days after symptom onset and positively correlated with neutralizing antibody titres; antibodies against RBD showed an earlier rise, when compared to those against NP, and a rise of IgG prior to IgM was noticed. In addition, they showed a more prolonged rt-PCR positivity in patients who formed early antibodies and in those with a poor prognosis.[61,62] Analysing 173 patients with SARS-CoV-2 infection, Zhao et al[63] observed seroconversion in less than 40% of the patients within 1-week from the onset of symptoms and in all patients (94.3% for IgM and 79.8% for IgG) within day 15 after clinical presentation.

In April 2020, Long et al[64] evaluated the antibody dynamics in a Chinese cohort of 285 COVID-19 patients, using a magnetic chemiluminescence immunoassay (MCLIA). All patients had virus-specific IgG approximately at day 17–19 after the onset of symptoms, while 94.1% had virus-specific IgM at day 20–22. Moreover, higher IgG titers were observed in patients with a severe course of illness compared to those without (*P*=0.001).[64] Of the 285 patients enrolled, a serological course was observed in 26 who were initially seronegative with three different dynamics: synchronous IgG/IgM seroconversion (9 patients), IgM seroconversion earlier than that of IgG (7 patients), and IgM seroconversion later than that of IgG (10 patients).[64]

Several serological immunoassays have been developed for the detection of SARS-CoV-2 viral proteins and antibodies in serum or plasma samples. The most widely used commercial tests are based on rapid lateral flow immunoassay (LFIA), automated chemiluminescence immunoassay (CLIA), and enzyme-linked immune assay (ELISA).[65]

Tables 3 and 4 show the characteristics and the advantages and disadvantages of different indirect tests for SARS-CoV-2, respectively.

## Enzyme-Linked Immune Assay (ELISA)

Enzyme-linked immune assay (ELISA) is a method to detect anti-SARS-CoV-2 IgG and IgM responses by identifying antibodies against the NP and spike proteins, but the absence of defined standards represents a limitation. In a recent meta-analysis whose aim was to analyze the accuracy of available tests to detect SARS-CoV-2 infection in Brazil, the pooled diagnostic measures of ELISA tests were: for IgM antibodies, sensitivity of 82% [95% CI=76–87], specificity of 97% [95% CI=96–98]; for IgG antibodies, sensitivity of 97% [95% CI=90–99], specificity of 98% [95% CI=97–99].[58]

## Chemiluminescent Immunoassay (CLIA)

CLIA offers significant advantages over traditional assay detection methods, especially in the quantification of antibodies. Light-producing chemical reactions (a substrate reacts with the Avidin-Horseradish Peroxidase resulting in light development) allowed estimating the titers of IgG and IgM by the amount of the emitted luminous

**Table 3** Characteristics of Different Indirect Test for SARS-CoV-2

| SARS-CoV-2 Test | Type | Specimen | Specific Infrastructural Requirement | Optimal Timing for Testing (days) | Turnaround Time (minutes) | Se (%) | Sp (%) |
|---|---|---|---|---|---|---|---|
| ELISA | IgG and IgM | human serum, plasma, whole blood | Yes | After 17–19 days from infection | 240 | ≈82* | ≈97* |
| CLIA | IgM and IgG | human serum, plasma, whole blood | Yes | After 17–19 days from infection | 30 | ≈77.9§ | ≈100§ |
| Rapid detection test | IgG and IgM | fingerprick samples | No | After 17–19 days from infection | 10–30 | ≈88.6§ | ≈90.63§ |

**Abbreviations:** ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence immunoassay; Se, sensitivity; Sp, specificity. * Data from meta-analysis; § Data from small cohort.

**Table 4** Advantages, Disadvantages, and Possible Indications of Different Indirect Tests for SARS-CoV-2

| SARS-CoV-2 Test | Advantages | Disadvantages | Indications |
|---|---|---|---|
| ELISA | <ul><li>Not very expensive</li><li>Medium turnaround time</li><li>Data confirmed by meta-analysis and cohort data</li><li>Easy collection sampling</li><li>Widely used technique</li></ul> | <ul><li>Needs infrastructure</li><li>Qualified personnel</li></ul> | Population screening and/or second level test in order to confirm Rapid detection test results |
| CLIA | <ul><li>High throughput and sensitivity method</li><li>Assist early detection of suspicious cases with nucleic acid false negative</li><li>High production capacity with advanced automatic production line</li></ul> | <ul><li>Needs infrastructure</li><li>Qualified personnel</li><li>Data from small cohort</li></ul> | Population screening and/or second level test in order to confirm Rapid detection test results |
| Rapid detection test | <ul><li>Does not need infrastructure</li><li>Easy collection sampling</li></ul> | <ul><li>Low specificity and sensitivity</li><li>Data from small cohort data</li></ul> | Weekly screening in high risk population, eg, healthcare personnel |

**Abbreviations:** ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence immunoassay.

signal. Padoan et al[66] analyzed the kinetics of anti-SARS-CoV-2 IgG and IgM antibodies by CLIA, showing a rapid increase in IgM and IgG after 6–7 days from symptom onset. In particular from day 1 to day 5 after symptom onset, no patient had detectable IgM and IgG; of the six patients observed between day 6 and 7, three (50%) were positive for IgM and four (66.6%) for IgG; of the nine patients observed between day 12 and 13, seven (77.8%) were positive for IgM and all (100%) for IgG.

## Rapid Serological Tests

Many rapid IgM/IgG tests have been developed by several companies, mainly based on immunoassay technology[67] providing results in 10–15 minutes. These devices contain colloidal gold-labeled SARS-CoV-2 recombinant protein and murine anti-human IgG antibodies immobilized in the G area, murine anti-human IgM antibodies immobilized in the M area, and the corresponding antibody in quality control area C.

However, the clinical accuracy of rapid tests needs to be stringently evaluated before they are authorized for mass screening of COVID-19. The manufacturers declare a relative specificity of 100%, a relative sensitivity of 81% in subjects observed between day 4 and 10 after the onset of symptoms, and a relative sensitivity of 97% between day 11 and 24. Cassaniti et al[68] compared the results of rapid serological tests with those of nasopharyngeal swab in 30 healthy subjects, 30 rt-PCR-SARS-CoV-2-positive subjects, and 50 subjects admitted to the emergency department with fever and respiratory syndrome: all 30 healthy subjects were negative by rapid IgM/IgG testing; of the 30 positive subjects, 19 (63.3%) showed clear positivity for both IgM and IgG antibodies, five (16.7%) weak positivity for both, one (3.3%) an isolated IgM positivity, and five (16.7%) negativity for both IgG and IgM antibodies. Interestingly, among the 50 patients admitted to the emergency department, 38 tested COVID-19-positive by rt-PCR; of these 38, only seven patients showed a positive rapid test with a sensitivity of 18.4% and a specificity of 91.7%, NPV 26.2%, PPV 87.5%. Based on current data, the WHO does not recommend the use of antibody-detecting rapid diagnostic tests for patient care, but encourages the continuation of the present work to establish their usefulness in disease surveillance and epidemiological research.[36]

## Conclusions

The COVID-19 pandemic poses a major global challenge, with a massive yet possibly underestimated burden and several unknowns. With a subtle clinical presentation and asymptomatic carriage, and in the absence of specific treatment and vaccines, it is clear that an early and accurate diagnosis is crucial for the control of the disease.[69,71] Although rt-PCR represents a cornerstone for SARS-CoV-2 laboratory diagnosis, several limitations have been observed. Therefore, a combined approach harmonizing laboratory methods (ie, rt-PCR and serology) with imaging features and clinical findings is essential to guide patient management and infection control.

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## Author Contributions

NC and AR were involved in the review concept and design and critical revision for important intellectual content. MS, CM, RA, FC, and AR performed the literature search and drafting of the manuscript. RA, CM, and MS were involved in the critical revision of the manuscript. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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