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*Coronavirus Pandemic*

# Review of current in vitro COVID-19 diagnostics methods

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## Abstract

The diagnosis of COVID-19 is considered a significant step in the management of the disease that is causing a major worldwide public health challenge from the time of its emergence in December 2019. Since it has been established that SARS-CoV-2 spreads rapidly, timely detection of the positive cases and isolation of such individuals and their contacts helps in containing viral transmission. In this paper, we review the in vitro technology platforms for testing and diagnosing COVID-19 patients: molecular tests, rapid antigen tests, and serology tests. As part of our review of each category of tests, we discuss the commercialized testing platforms, their analyzing systems, specimen collection protocols, and testing methodologies. Moreover, the efficacy and limitations of each technique are also discussed. The key structural components of the virus are presented to provide an understanding of the scientific principles behind the testing tools.

**Key words:** COVID-19; molecular assay; antigen; diagnostic tests.

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## Introduction

In March 2020, the World Health Organization (WHO) declared COVID-19 a "pandemic". An outbreak of flu-like symptoms that in some cases developed into a deadly respiratory distress was first reported in December 2019 in Wuhan, China. Since then, the number of patients increased rapidly to spread all over the world through international travel [1]. The origin of the causative agent is suspected to be a seafood market where wild animals were sold [2].

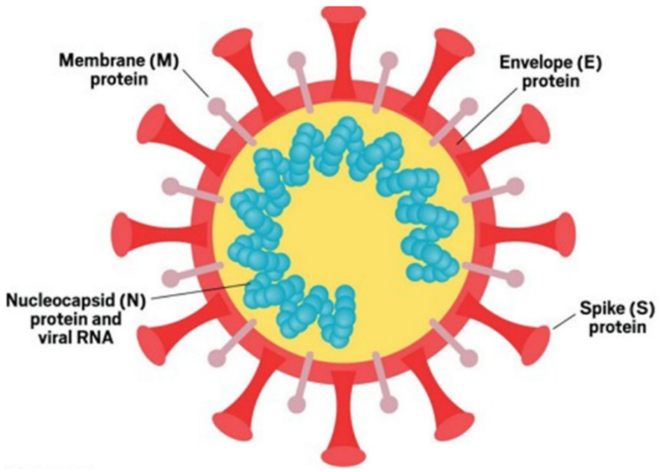
Following the outbreak of COVID-19, severe acute respiratory syndrome coronavirus 2 was found to belong to the same beta-coronavirus family as the Middle East Respiratory Syndrome (MERS-CoV), the virus responsible for the 2012 outbreak of MERS [3], and severe acute respiratory syndrome (SARS) coronavirus [3], the virus responsible for the 2002-2003 outbreak of SARS [4]. Sequence analysis studies reported 50% homology of SARS-CoV-2 with MERS-CoV, 80% homology with SARS-CoV and a significant 96.3% homology with bat coronaviruses (Table 1) [5]. Phylogenetic analysis shows that all the CoVs originated in animals, but SARS-CoV-2 presumably crossed the species barrier quite recently and is distinct from both SARS-CoV and MERS because it has adapted to easily spread from human-to-human [6]. This human-to-human spread among an immunologically naïve population rapidly escalated to a threatening pandemic that affected the entire world. As of April 2021, SARS CoV-2 has spread in over 220 countries resulting in over 130 million reported cases and approximately 3 million deaths with a fatality rate of ~3% [1]. COVID-19 related death is primarily associated with the patient developing acute respiratory distress syndrome (ARDS) [7]. In most cases, an uncontrolled immune activation known as a "cytokine storm" leads to the aggravation of ARDS, intensified tissue damage, multi-organ failure and eventually death [8]. So far, age-dependent host features are believed to be important contributors to the pathophysiology of the

disease [6]. Regardless of geographical location, children and youth have milder disease and less progress to ARDS compared to adults. In addition to age, hypertension, diabetes and obesity among others form major risk factors associated with mortality [9].

Despite the current availability of various vaccines and ongoing vaccination programs, COVID-19 is still regarded as a threat to the public all over the world. Disease transmission and death rates are still high. Vaccination efforts, along with natural infection rates, are still far from reaching the near 60-70% protection rate typical of herd immunity in a population. In the meantime, unequal access to vaccines, vaccine hesitation and the continuous emergence of new variants of the virus are concerns that have an impact on the transition of the epidemiological curve to flattening and eventually, normalcy [10].

In addition to vaccines, scientists and pharmaceutical/biotechnology companies all around the world are competing to develop anti-viral drugs, immunotherapeutic antibodies and, better diagnostic methods for SARS-CoV-2 infection. However, as with all infectious diseases, a deep understanding of the pathogen's structure, the pathogen's life cycle, host-pathogen interaction and immune response by the host is needed in order to develop diagnostic methods and therapeutic solutions. Therefore, virologists and immunologists worked to discover the fundamental information about the biology of the virus and the pathogenesis of the infection including the mechanisms of infectivity, risk factors, pathogenesis, disease manifestations and immune protection. In fact, it was difficult to keep pace with the large numbers of reports that were published daily. To the best of our knowledge, no successful anti-viral drug against SARS-CoV-2 has been developed. Instead, drug repurposing has been used as means for identifying the potential treatment and management of COVID-19 [11]. On the other hand, various diagnostic kits are available in the market [12]. This review article is an in-depth technical analysis of the current Food and Drug Administration (FDA)-approved diagnostic tests for COVID-19 that were authorized for emergency use. This work also analyzes each testing technique to determine its advantages and disadvantages.

**Figure 1.** Schematic representation of the SARS-CoV-2 structure.




The virions are spherical, with an envelope containing a prominent crown ('corona') of peplomers of S glycoprotein, E (small envelope protein), and M (membrane glycoprotein). The genome is a positive-stranded RNA associated with the N (nucleocapsid phosphoprotein), composing the helical RNP (Ribonucleoprotein).

## Methods

We summarized the current knowledge about the pathogen causing COVID-19 and the various diagnostic methods used to detect this virus, in order to better understand the limitations and nuances of COVID-19 testing. A manual search was conducted in PubMed, Web of Science and Scopus databases. Search strategy used the following terms: ("diagnostic" or "test") and ("COVID-19" or "SARS-CoV-2").

## Coronavirus shape and proteins

The 30 Kb positive-strand RNA genome of SARS-CoV-2 encodes four essential structural proteins and several smaller "accessory" proteins. The spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and envelope (E) protein, are required to produce a structurally complete viral particle. Individually, each protein plays a role in forming and maintaining the structure of the virus particle, and is involved in different aspects of the replication cycle (Figure 1) [13].

SARS CoV-2 S is a 180–200 kDa protein with a total length of 1273 amino acids. The extracellular N-

**Table 1.** Comparison between SARS-CoV, MERS-CoV, and the SARS-CoV-2.

| Virus name | Receptor | Host | Number of cases | Number of deaths | Fatality rate | Countries affected |
|---|---|---|---|---|---|---|
| SARS-COV | ACE-2 | Bat | 8098 | 774 | 10 % | 29 |
| MERS-COV | DPP4 (CD26) | Camels | 2506 | 862 | 34 % | 26 |
| SARS-COV-2 | ACE-2 | Bat | More than 240 million until October, 2021 | More than 4 million until October, 2021 | 3.4% | 223 |

Receptor usage, intermediate hosts, number of cases and deaths, and countries affected (Modified from Ashour et al. [5]).

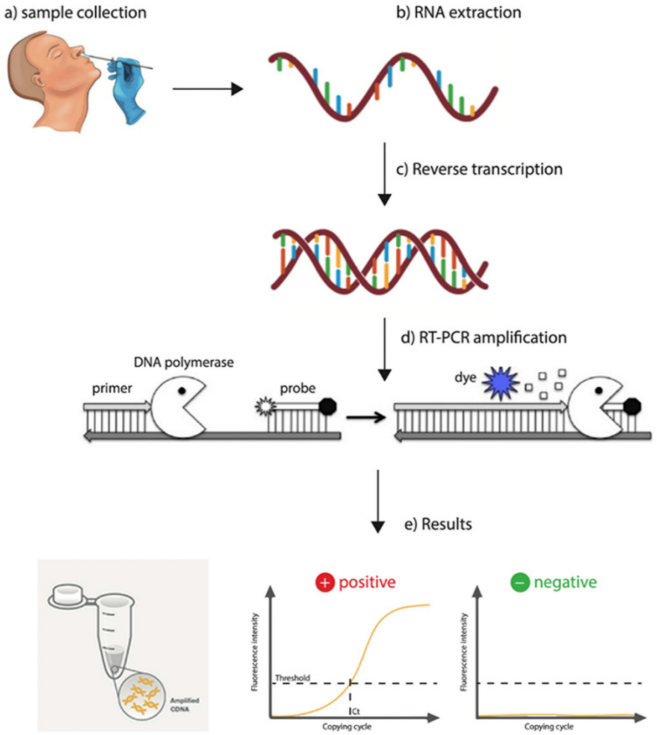
terminus consists of a signal peptide (amino acids 1–13), the S1 subunit (amino acids 14-685), and the S2 subunit (amino acids 686–1273). The S protein is of special importance since it mediates attachment of the virus to the host cell surface receptor(s) and subsequent viral entry into the host cell [14]. Hence, S1, specifically receptor-binding domain (RBD), is the most targeted region for the development of COVID-19 therapeutic antibodies and vaccines [15,16].

Unlike the other major structural proteins, N is the only protein that functions primarily to bind to the SARS-CoV-2 RNA genome, making up the nucleocapsid. Although N is largely involved in processes relating to the viral genome, it is also involved in other aspects of the SARS-CoV-2 replication cycle, namely viral assembly, budding, and the host cellular response to viral infection [17].

The M protein is the most abundant structural protein and defines the shape of the viral envelope. It stretches the membrane bilayer three times and plays a predominant role in the intracellular formation of virus particles. It is also regarded as the central organizer of SARS-CoV-2 assembly, interacting with all other major coronaviral structural proteins. In addition, it mediates nutrient transport across the transmembrane, bud release and envelope formation [13].

The E protein is the smallest of the major structural proteins, but also the most mysterious. During the replication cycle, E is abundantly expressed inside the infected cell, but only a small portion is incorporated into the virion envelope. Most of the protein is localized at the site of intracellular trafficking machinery where it participates in SARS-CoV-2 assembly and budding, demonstrating the importance of E in virus production and maturation [18]. The S/N proteins are targeted as implied antigens for serodiagnosis of COVID-19, similar to other diagnostic methods that were implemented for diagnosing the SARS disease [19].

**Figure 2.** RT-PCR procedure.




(**A**) Specimen is taken from the nose or throat of an individual. (**B**) RNA is extracted. (**C**) RNA is transcribed into complementary DNA. (**D**) primers bind to the DNA and provide a starting point for the DNA polymerase to help copy it. DNA polymerase then degrades the bound probe which results in an increased fluorescence signal. (**E**) the fluorescence increases as copies of the virus DNA are made. If the level crosses a certain threshold the test is positive.

## SARS-CoV-2 diagnostic methods

*Molecular assays*

The diagnosis of COVID-19 is considered a crucial step in the management of the disease. The containment of the rapidly spreading virus requires a rapid, reliable, and easy-to-implement protocol to detect and isolate positive cases and their contacts. Additionally, diagnostic tools must be able to test a large number of samples within a short time [20]. The Center for Disease Control and Prevention (CDC) and WHO recommended that SARS CoV-2 infection be diagnosed through the use of quantitative real-time reverse-transcription PCR (qRT-PCR) (Figure 2). RT-qPCR tests are currently available globally to detect ORF1ab, E, N, or S gene sequences in various combinations. The sensitivity, stability, and examination time of these tests vary. The test regimen is complicated and expensive, and it is best suited for large, centralized diagnostic labs. Tests typically take 4–6 hours, but logistical requirements for shipping clinical specimens limit execution time to 24 hours. According to recommendations by the WHO, RT-qPCR tests must be able to detect three genes (E gene, N gene, and RdRP gene) in a single reaction. This helps to recognize SARS-CoV-2 virus as well as viruses from the beta-coronavirus group. The design ensures two-fold confirmation in situations of infection and reduces the danger of false negative results if only one target for SARS-CoV-2 is detected [21].

The qRT-PCR protocol requires a well-equipped molecular diagnostic lab with trained staff and expensive equipment. Additionally, the test also has a long turnaround time, thereby limiting the scaling up of the testing capability [16]. There are more than 350

conventional RT-PCR COVID-19 testing kits available commercially, of which 29 kits have been approved by the US-FDA [22] (Table 2).

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems have also been used as it enables the detection of femtomolar level of viral particles. CRISPR associated enzyme 13 (CRISPR-Cas 13) [23] was recently considered as a powerful technique for SARS-CoV-2 detection (Figure 3). CRISPR-Cas13 has a unique feature in comparison with the other types of CRISPR system that is referred to as 'collateral cleavage' in which the Cas13 enzyme binds the targeted sequence on one site and starts cutting other non-target nucleic acid sequences that exist in the surroundings from the other site of the enzyme [24]. This feature has been harnessed by researchers to design approaches based on the CRISPR system and is referred to as Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) and relies on CRISPR-Cas13 [25,26] which has attomolar sensitivity [27]. Once the specimen is taken and the RNA is extracted and added into the SHERLOCK reaction, the pre-amplification step starts by recombinase polymerase amplification (RPA). Next, the T7 RNA polymerase is introduced allowing for RNA transcription and then detection by Cas13. After binding between crRNA, the complementary sequence for the viral genome combines with Cas13 to enable the detection. Although this technique is rapid, more sensitive, and less expensive in comparison with RT-PCR, it requires technical expertise for reaction optimization.

*Antigen diagnostic tests*

At the beginning of the COVID-19 pandemic, techniques previously developed to detect viral infection during the emergence of the earlier SARS-CoV and MERS were employed to detect SARS CoV-2. However, unlike SARS-CoV and MERS, SARS-CoV-2 succeeded in spreading worldwide at an exceptional rate. Hence, in addition to sensitivity and specificity, the development of rapid and high throughput screening testing became a priority. Rapid antigen diagnostic tests (ADTs) were developed for the point-of-care (POC) centers implemented by the frontline healthcare providers to manage and contain COVID-19 (Figure 4) [28,29].

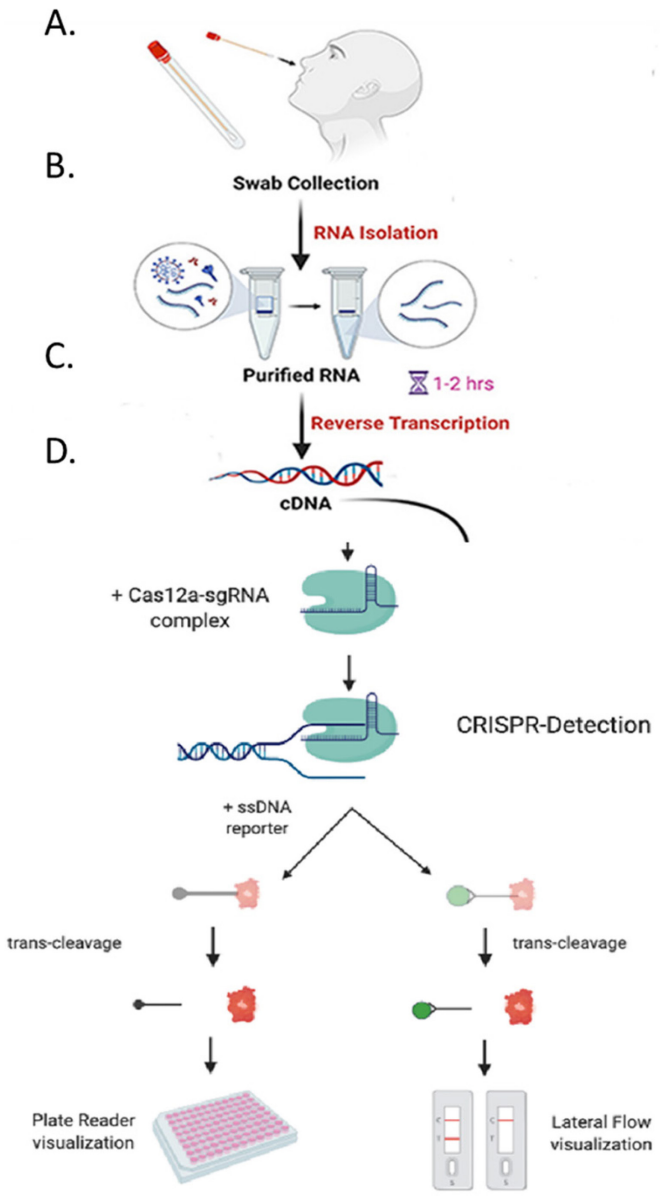
**Table 2.** Overview of SARS-CoV-2 PCR and CRISPR detection reagent kits available in the market.

| Kit name | Company | Target gene | Sample source | Time | Detection limit |
|---|---|---|---|---|---|
| SARS-CoV-2 Nucleic Acid Detection Kit | Zybio Inc. | N gene | NS, TS, BAL stool | | 500 copies/mL |
| LiliF™ COVID-19 Real-time RT-PCR Kit | Lilif Diagnostics | RdRP genes, E and N genes | BAL, NS, OS | | N/A |
| ViroQ® & ViroQ® Rapid SARS-CoV-2 | BAG Diagnostics | E and RdRP | NP, OP, NS | 90 minutes | 5 copies/20 µL RdRP and 10 copies /20 µL target E gene |
| PowerChek™ 2019-nCoV Real-time PCR Kit | Kogenebiotech | RdRP gene<br>E gene | NP, OP, NS | | 4 copies/µL |
| ePlex® SARS-CoV-2 Test | GenMark Diagnostics | | NPS | | $1\times10^3$ copies/mL with WA1 viral RNA |
| Easy SARS-CoV- 2 WE kit | Diatech Pharmacogenetics | N and RdRp, and S gene (HV69-70 del and N501Y) and the E gene | NPS, OPS, BAL | less than 2 hours | 10 copies/reaction |
| COVID-19 Real Time Multiplex RT-PCR Kit | Labsystems Diagnostics | ORF1ab, N & E besides internal control. | entire respiratory tract can be used | approximat ely 1 hour | < 5 copies/µL |
| TaqPath™ COVID-19 Combo Kit | Thermo Fisher Scientific | ORF1ab, N gene, S gene, MS2) | NP, OP NS, BAL | | 10 GCE/reaction |
| SNP COVID-19 REAL TIME PCR KIT | SND Biotechnology | RdRp, N gene | NP, OP, ANS. | 83 minutes | between 1-10 Copies/Rxn. |
| Clarigene® SARS-CoV-2 CE-IVD | Yourgene Health plc | N and E gene | NPS | 1 hr 20 min | 5 copies per reaction |
| GeneFinder™ COVID-19 PLUS RealAmp Kit | GeneFinder | RdRp gene, the E gene, the N gene, and the RNAse P gene. | NPS, NS, BAL | | N/A |
| The SARS-CoV-2 Fluorescent PCR Kit | Maccura Biotechnology | ORF1ab, N and E genes | OPS, NPS, NS | | $1.0\times10^3$ copies/mL. |
| VIASURE SARS-CoV-2 & UK Variant Real Time PCR Detection Kit | CerTest BIOTEC | S UK, ORF1ab and N genes | NS, NPS, OPS Saliva | | 40 copies/rxn for S gene (HV 69/70 deletion), and ORF1ab gene 80 copies/rxn for N gene |
| Sherlock™ CRISPR SARS-CoV-2 | Sherlock Biosciences | ORF1ab and N genes | NS, NPS, OPS | 1 hr | 6.75 cp/µL VTM |
| TATA MD CHECK CRISPR SARS-COV-2 KIT | TATA MD | S gene | NS, OPS | 90 min | N/A |

NS: Nasal Swab; TS: Throat Swab; BAL: bronchoalveolar lavage; OPS: oropharyngeal swab; NP: nasopharyngeal; RdRp: RNA-dependent RNA polymerase gene; N: Nucleocapsid; VTM: Viral Transport Medium. (Online sources according to manufacturers' specification).

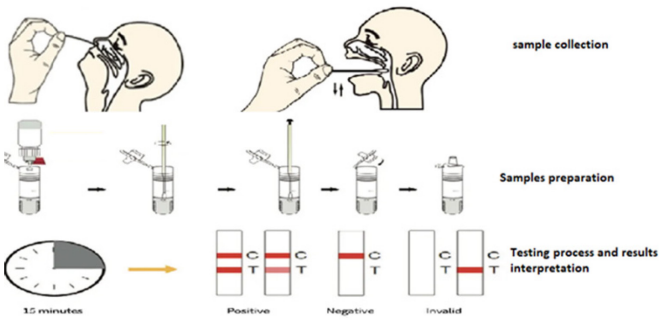
ADTs are used to detect SARS-CoV-2 specific antigen(s) in the respiratory specimens collected from individuals suspected to have COVID-19 and from individuals with epidemiological risk factors (such as travelers coming from epidemic regions and patient contacts) [1,30]. Numerous ADTs are now commercially available and used for rapid detection of COVID-19. Table 3 lists some of diagnostic rapid antigen tests approved for clinical use by the US-FDA and/or by the European Community (CE) [31]. These tests are meant to be employed for detecting active COVID-19 early in infection when timely access to molecular testing is not available. However, the results should be confirmed by qRT-PCR [32].

**Figure 3.** Nucleic acid detection of SARS-CoV-2 using CRISPR assays.




(**A**) Patient swab can be collected from different types of clinical samples. (**B**) RNA is extracted from the specimen. (**C**) DNA must be amplified from the nucleic acid extraction. (**D**) The nucleic acid of SARS-CoV-2 can now be detected. If a person has COVID-19 then the CRISPR/Cas complex will bind to the target region of the amplified nucleic acid and collateral cleavage activity can occur by cleaving the nearby fluorescence reporter nucleic acids. This can be detected by the naked eye under specific light, a fluorescence plate reader, or a lateral flow assay that can indicate the presence of the virus's nucleic acid.

**Figure 4.** COVID-19 antigen rapid test.




The sample is applied to the test strip and if antigen is present, it is bound by antibodies linked to detector molecules, as well as antibodies immobilized in the test line further down the strip.

ADTs are designed as simple to use paper based or lateral flow immunochromatography formats. Rapid ADTs require neither special equipment nor operator skills. They are highly specific and quick with results available in less than 30 minutes. However, a major disadvantage of these tests is low sensitivity. Unfortunately, samples with low viral loads give false negative results in most of rapid ADTs developed so far, necessitating adjunct diagnosis using PCR [33–37]. The majority of rapid ADTs developed until now are designed to detect nucleocapsid antigen in nasopharyngeal samples collected from patients in a qualitative rather than quantitative manner. In brief, paper or gel immobilized anti-SARS-CoV-2 N protein specific antibodies are used to capture the presence of viral particles in tested samples. The resulting antibody–antigen complex can then be detected with an additional tracer antibody to produce a colorimetric, chemiluminescent or fluorescent-based readout [25].

Many "test validation" studies were performed to evaluate the sensitivity and specificity of the various rapid ADT kits available for SARS-CoV-2 detection. All validations were made in comparison with RT-PCR. Generally speaking, sensitivity varied widely between kits, and within kit variability was mainly dependent on the viral load or the timing of sampling post onset of symptoms.

**Table 3.** COVID-19 antigen detection kits.

| Company | Kit name | Time | Antigen type | Overall Sensitivity |
|---|---|---|---|---|
| Abbott Diagnostics[a] | BinaxNOW COVID-19 Ag Card | ~15 min | Nucleocapsid protein antigen | 73.3-76.6% |
| Amedica SA[b] | Amela Covid-19 Antigen test | ~30 min | Nucleocapsid protein antigen | N/A |
| ArcDia International Ltd[b] | mariPOC SARS-CoV-2 | ~20 min | Nucleocapsid protein antigen | 0.92 (95% CI, N/A) |
| Becton Dickinson[a] | BD Veritor System for Rapid Detection of SARS-CoV-2 | ~15 min | Nucleocapsid protein antigen | 0.84 (95% CI, 66–95) |
| Beijing Kewei Clinical Diagnostic Reagent[b] | Kewei COVID-19 Antigen Rapid Test Kit | ~15 min | Nucleocapsid protein antigen | 0.85 (95% CI, N/A) |
| Beijing Savant Biotechnology[b] | SARS-Cov-2 Antigen Fluorescence Rapid Detection Kit | ~15 min | Nucleocapsid protein antigen | N/A |
| Liming Bio-Products [b] | Strongstep COVID-19 Antigen Rapid Test Device | ~15 min | Nucleocapsid protein antigen | 0.73 (95% CI, 0.60–0.83) |
| LumiraDx UK Ltd [a] | LumiraDx SARS-CoV-2 Ag Test | ~12 min | Nucleocapsid protein antigen | 0.98 (95% CI, 0.92–1.00) |
| Quidel Corporation[b] | Sofia SARS Antigen FIA | ~15 min | Nucleocapsid protein antigen | 0.97 (95% CI, 0.83–1.00) |
| RapiGEN Inc[b] | Biocredit COVID-19 Ag | 5–8 min | Recombinant SARS-CoV-2 antigens | 0.90 (95% CI, 0.79–0.97) |
| SD Biosensor Inc | Standard F COVID-19 Ag FIA | ~30 min | Nucleocapsid protein antigen | 70.6% |
| Shenzhen Bioeasy Biotechnology [b] | Bioeasy 2019-nCoV Ag | ~15 min | SARS-CoV-2 antigens | N/A |
| Sugentech, Inc[b] | SGTi-flex COVID-19 Ag | ~20 min | Nucleocapsid protein antigen | 0.80 (95% CI, 0.61–0.9 |
| Roche | SARS-CoV-2 Rapid Antigen Test | 15–30 min | Nucleocapsid protein antigen | 95.5% (Ct value ≤ 30) |
| JOYSBIO Biotechnology's | Coronavirus Antigen Rapid Test Kit | 15 min | Nucleocapsid protein | 98.13% |
| NanoEntek [b] | The FREND™ COVID-19 Ag | 3 minutes | Nucleocapsid protein | 95% CI |
| Genrui Biotech Inc[b] | SARS-CoV-2 Antigen Test Kit (Colloidal Gold) | 10-15 min | N/A | 95% CI |
| Verify Diagnostics [b] | COVID-19 Antigen Rapid Test Device | ~15 min | Nucleocapsid protein antigen | 0.80 (95% CI, 0.74–0.86) |

Antigen test diagnostics approved for clinical use by the US Food and Drug Administration (FDA) and/or by the European Community (CE) (Modified from Mattiuzzi et al. [31]. Ct: cycle threshold; N/A: not available (not found). aUS Food and Drug Administration – Emergency Use Authorizations for Medical Devices (FDA-EUA); bCE.

**Table 4.** Comparison of selected serology tests for SARS-CoV-2 based on sensitivity, sample type and size and test type.

| Company | Kit name | Status | Sample size | Sensitivity | Test Type |
|---|---|---|---|---|---|
| AccuBioTech Co. Ltd. | Accu-Tell COVID-19 IgG/IgM Rapid Test Cassette | CE | 10 µL of whole blood, serum or plasma | IgG 97.4% IgM 86.8% | Immunochromatographic |
| BIOMAXIMA S.A | -2019nCoV IgG/IgM Rapid Test Cassette | CE-IVD | 10-20 µL whole blood, serum or plasma | IgG100% IgM 85% | Immunochromatographic |
| BioMedomic, Inc | COVID-19 IgM-IgG Dual Antibody Rapid Test | CE-IVD | 10-20 µL whole blood, serum or plasma | 89% | Immunochromatographic |
| Cellex Inc. | Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test | CE-IVD USA | 10 µL whole blood, serum or plasma | 93.9%- | Immunochromatographic |
| Innovita Biological Technology | -2019nCoV Ab Test (Colloidal Gold) IgM/IgG | CE-IVD; China, Brazil | 20 µL whole blood, and 10 µl serum or plasma | 93.3% | Immunochromatographic |
| Getein Biotech, Inc | One Step Test for Novel Coronavirus (2019-nCoV) IgM/IgG Antibody | CE | 10 µL of serum or plasma; 20 µL of fingertip blood or whole blood | 94.1% | Immunochromatographic |
| EUROIMMUN AG | Anti-SARS-CoV-2 ELISA (IgG) | CE-IVD; Brazil; USA | Serum or plasma | 94.4% | ELISA |
| Epitope Diagnostics, Inc. | EDI™ Novel Coronavirus COVID-19 IgM ELISA Kit | CE-IVD | 20 µl of serum | ----- | ELISA |
| Mokobio Biotechnology R&D Center | SARS-CoV-2 IgM IgG Quantum Dot Immunoassay | CE-IVD | Serum, plasma, whole blood | %89.52 | Immunofluorescence |
| EUROIMMUN AG | Anti-SARS-CoV-2 ELISA (IgA) | CE-IVD | Serum or plasma | 88.2% < 10 days after symptoms | ELISA |
| Hangzhou Biotest Biotech | COVID-19 IgG/IgM Rapid Test Cassette | US FDA EUA CE-IVD | 10 µL of whole blood, serum or plasma | IgM 91.8% IgG 100% | Immunochromatographic |
| CTK Biotech | OnSite COVID-19 IgG/IgM Rapid Tes | Australia TGA India CDSCO | 10-15 µL of serum or plasma | %96.9 | Immunochromatographic |
| BIOMAXIMA S.A | -2019nCoV IgG/IgM Rapid Test Cassette | India CDSCO - CE-IVD | 10-20 µL whole blood, serum or plasm | IgG100% IgM 85% | Immunochromatographic |
| Hunan Lituo Biotechnology Co., Ltd | COVID-19 IgG/IgM Detection Kit (Colloidal Gold) | India CDSCO - CE-IVD) | 10 µL of serum, plasma and whole blood | 94.4% | Immunochromatographic |

(Online sources according to manufacturers' specification).

As an example, Scohy *et al*. reported a low 30.2% sensitivity of the COVID-19 Ag Respi-Strip (Coris BioConcept, Wallonia, Belgium) [37]. A 45.7% sensitivity for the Biocredit COVID-19 Ag Detection Kit (BioVendor, Brno, Czech Republic) was reported by Mak *et al*. [36]. The STANDARD Q COVID-19 Ag Test (SD Biosensor, Suwon, Republic of Korea) showed a 70.6% sensitivity [34]. Comparably, the Panbio COVID-19 Ag Rapid Test (Abbott, Abbott Park, IL, USA) showed a sensitivity range of 73.3–79.6% by two separate studies (Table 3) [1,35].

Interestingly, a group of researchers studied the performance of a new ADT, namely LUMIPULSE, based on a chemiluminescence enzyme immunoassay. The results showed rapid identification of SARS-CoV-2-infected individuals with moderate to high viral loads, exhibiting 55.2% sensitivity and 99.6% specificity, with a 91.4% overall agreement rate (286/313). In specimens with > 100 viral copies and between 10 and 100 copies, the antigen test showed 100% and 85% concordance with RT-qPCR, respectively, and helped in monitoring viral clearance in hospitalized patients [38]. Another study performed in September 2020 studied the efficacy of (nucleocapsid protein) antigen testing by fluorescence immunochromatographic FIC assay in detecting SARS-CoV-2 infection. Using RT-PCR assay as a reference standard, NP antigen testing showed high specificity and relative high sensitivity in SARS-CoV-2 diagnosis in the early phase of infection. The sensitivity, specificity, and percent agreement of the FIC assay was 75.6% (95% CI 69.0%-81.3%), 100% (95% CI 91.1%-100%), and 80.5% (95% CI 75.1%-84.9%), respectively [39].

Reassuring results were also obtained using Panbio™ COVID-19 ADT Test Device for the detection of SARS-CoV-2 antigen compared to RT-qPCR using nasopharyngeal swabs. This test also showed high sensitivity and specificity in samples obtained during the first week of symptoms and with high viral loads. Patients with less than seven days onset of symptoms showed a higher viral load and sensitivity for rapid antigen test (86.5 %), compared to those with more days (sensitivity of 53.8 %) ($p < 0.004$) [35].

On the other hand, a study performed in Thailand suggested that rapid antigen detection showed comparable sensitivity and specificity with the RT-PCR assay and proved to be a potential screening assay, especially in high prevalence areas. The only disadvantage was its low positive predictive value (PPV) in low prevalence areas [40].

Interestingly, the appearance of false positive results was reported for the COVID-19 rapid ADT Respi-Strip, except in cases of high viral loads [37]. Serious concerns about potential false positive results were raised by Taku Ogawa *et al.* because the false-positive patients may be admitted to the same medical room as patients truly infected with COVID-19, increasing the risk of nosocomial infection [41].

In summary, rapid ATDs seem to be reliable in cases of moderate to high viral loads, typical of the beginning of the infection. Based on this, the WHO recommended that rapid diagnostic tests should be used for symptomatic individuals within the first 5–7 days following symptom onset but should not be used for asymptomatic individuals [1].

*Antibody detection methods*

The immune system produces antibodies in response to infections in order to control the spread of the infectious agent and destroy it by complement activation of antibody dependent cell cytotoxicity. Serological tests are applied to identify the presence of such humoral responses to SARS-CoV-2. Antibody isotypes IgA, IgM, and IgG specific to different virus proteins were detected by the most popular serology technique, ELISA, or the modified highly sensitive method, chemiluminescence immunoassays (CLIA). Sensitivity and specificity of serological tests vary according to the testing technique, specificity of the antibody studied, duration of symptoms at the time of collection, and immunocompetence of the individual [42].

The panic that flooded the globe after the spread of COVID-19 and the need for sensitive diagnostic techniques resulted in lack of scientific evaluation of most of the tests that are in use (Table 4) [43]. However, assessment of specific antibodies to N protein have been found to be sensitive but less specific, whereas antibodies directed to S protein are more specific to SARS-CoV-2 [44]. Other factors that may interfere with results include the duration of symptoms at time of blood collection and severity of the disease. IgM can be identified from the fifth day of symptoms, and more significantly, from the eighth day onwards. Specific IgG values are detectable from the tenth day of symptom onset, and more significantly, from the 14$^{th}$ day onwards [10]. These tests are therefore not appropriate for the early diagnosis of COVID-19, and were not approved by the WHO and CDC for the diagnosis of COVID-19. Many countries approved the use of antibody detection after the vaccination campaigns started for immune status evaluation and vaccine efficacy testing.

## Conclusions

Several techniques for diagnosing COVID-19 infection have been developed within a short period of time. However, many factors have to be considered in order to correctly diagnose COVID-19. In addition to the test itself, the patient's medical history, the time of the suspected SARS-CoV-2 exposure, the type of sample to be collected and analyzed, and how to interpret the results must be considered.

Although RT-PCR tests are considered the gold standard in COVID-19 testing, rapid antigen assays are a viable alternative as a low-cost, at-home, self-testing method. It will remain important to test for antibodies to SARS-CoV-2 as more people are vaccinated or exposed to the virus. The current technologies for in vitro diagnosis (IVD) testing need to be improved continuously if they are to effectively detect emerging and circulating SARS-CoV-2 strains. Consequently, further research and studies are needed to uncover new methods for SARS-CoV-2 detection that are rapid, more sensitive, highly specific, accurate, and capable of detecting infection at an early stage.

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