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Review Article

# In vitro diagnostics of coronavirus disease 2019: Technologies and application

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**Abstract** Laboratory-based diagnostic measures including virological and serological tests are essential for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Real-time reverse transcription-polymerase chain reactions (rRT-PCR) can detect SARS-COV-2 by targeting open reading frame-1 antibodies (ORF1ab), envelope protein, nucleocapsid protein, RNA-dependent RNA polymerase genes, and the N1, N2, and N3 (3N) target genes. Therefore, rRT-PCR remains the primary method of diagnosing SARS-CoV-2 despite being limited by false-negative results, long turnaround, complex protocols, and a need for skilled personnel. Serological diagnosis of coronavirus disease 2019 (COVID-19) is simple and does not require complex techniques and equipment, rendering it suitable for rapid detection and massive screening. However, serological tests cannot confirm SARS-CoV-2, and results will be false-negative when antibody concentrations fall below detection limits. Balancing the increased use of laboratory tests, risk of testing errors, need for tests, burden on healthcare systems, benefits of early diagnosis, and risk of unnecessary exposure is a significant and persistent challenge in diagnosing COVID-19.


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## Introduction

As of May 19, 2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously known as the 2019 novel coronavirus, 2019-nCoV) has caused coronavirus disease 2019 (COVID-19) in 4,731,458 patients, leading to 316,169 deaths worldwide.[1] The World Health Organization (WHO) declared COVID-19 as a pandemic on March 11, 2020.[1] The early diagnosis of COVID-19 is essential for containing and mitigating SARS-CoV-2 infections. However, clinical diagnosis is difficult because of the various clinical manifestations of SARS-CoV-2 infection, which can range from no or mild acute respiratory disease to severe pneumonia and acute respiratory distress syndrome.[2,3] Additionally, travel, occupation, close contact, clustering, and exposure history were relatively less important issues during the early stages of the COVID-19 outbreak. Although most patients with SARS-CoV-2 pneumonia can present with bilateral ground-glass opacity in computed tomography (CT) images,[2,4] specific viruses cannot be identified or distinguished by CT.[5] Therefore, an accurate laboratory method is essential for confirming a diagnosis of COVID-19.[6]

Several diagnostic tools for SARS-CoV-2 infections have been developed since January 2020. Although these tools can help to detect SARS-CoV-2, various limitations have been identified during their clinical application. This article provides an updated and comprehensive review of the current status and pitfalls of laboratory diagnostic tests for SARS-CoV-2 infection.

## Diagnostic methods

Local Centers for Disease Control and Prevention collected and shipped clinical specimens to designated authorized laboratories for pathogen detection (NHC Key Laboratory of Systems Biology of Pathogens and Christophe Mérieux Laboratory, Beijing, China during the earliest stage of the COVID-19 outbreak in Wuhan. A novel coronavirus (initially named 2019-nCoV) was isolated from a lower respiratory tract specimen, and a diagnostic test was developed soon thereafter.[7] In addition to viral cultures, real-time reverse transcription polymerase chain reactions (rRT-PCR) of respiratory specimens have also become a standard diagnostic test for COVID-19 since its introduction in the first three large Chinese studies.[8–10] In addition to the development of many commercial rRT-PCR tests, serological tests using enzyme immunoassays or point-of-care lateral flow immunoassays have been developed to simultaneously or separately detect anti-SARS-CoV-2 IgA, IgM, or IgG antibodies and SARS-CoV-2 antigen; some of these tests have been validated for clinical application in China.[11]

## Real-time RT-PCR

rRT-PCR can identify viral genetic material by targeting SARS-COV-2 open reading frame-1 antibodies (ORF1ab), envelop protein (E), nucleocapsid protein (N), RNA-dependent RNA polymerase (RdRp) genes, and the N1, N2, and N3 (3N) target genes (Table 1), and thus remains a standard method for confirming a diagnosis of SARS-CoV-2.[6,7,12] Real-time RT-PCR using the protocol recommended by the World Health Organization (WHO) and target genes has been applied to detect SARS-CoV-2 in nasopharyngeal and oropharyngeal swabs, sputum, bronchoalveolar lavage (BAL), bronchoscope brush samples, saliva, feces, blood, and urine.[13–16]

**Table 1** Targeted genes for diagnosing SARS-CoV-2 infection using rRT-PCR.

| Source | Gene target |
|---|---|
| Chinese National Institute for Viral Disease Control and Prevention, China CDC (China) | ORF1ab, N |
| WHO, Charité (Germany) | RdRp, E, N |
| University of Hong Kong (Hong Kong) | ORF1ab, N |
| National Institute of Health (Thailand) | N |
| US CDC | 3N primers |
| National Institute of Infectious Disease (JAPAN) | Pancorona and multiple targets, spike protein |
| Pasteur Institute (France) | Two targets in RdRp |

An increasing number of companies have developed rRT-PCR tests to specifically detect SARS-COV-2 genes or syndromic multiplex PCR panels for detecting bacterial and viral respiratory pathogens, including SARS-CoV-2 (Table 2).[17] The Allplex™ 2019-nCoV assay (Seegene Inc., Seoul, Korea) targets the SARS-COV-2 genes, E, N, and RdRp in nasopharyngeal and throat swabs, sputum, and in BAL fluid. The reported limit of detection (LOD) of the Allplex™ assay for detecting SARS-CoV-2 is 100 copies/mL.[18] This assay kit was designated as a Conformité Européenne *in vitro* diagnostic device (CE-IVD) on February 10, 2020 and gained Emergency Use Authorization (EUA) from the Korea Centers for Disease Control and Prevention on February 12, 2020. The LightMix® Modular Wuhan CoV RdRp-gene test, which targets only the RdRp gene (Tib Molbiol GmbH, Berlin, Germany), was developed to detect 2019-nCOV in tracheal aspirates and BAL, but it is now restricted to research applications.[19]

An automated qualitative nucleic acid multiplex test for SARS-CoV-2, *The True Sample-To-Answer Solution*™ ePlexSARS-CoV-2, developed for use with nasopharyngeal swabs, was submitted to the U.S. Food and Drug Administration (FDA) for EUA on March 11, 2020.[20] The Cobas® SARS-CoV-2 test (F. Hoffmann-La *Roche* AG, Basel, Switzerland) received EUA from the United States Food and Drug Administration (USFDA) on March 13, 2020 and is now commercially available. This qualitative method for detecting SARS-CoV-2 in nasopharyngeal and oropharyngeal swab samples can be run on fully automated Cobas® 6800 and Cobas® 8800 systems (F. Hoffmann-La *Roche* AG). This kit enables high-volume testing and facilitates effective responses to the pandemic. The LOD (50% tissue culture infective dose ($TCID_{50}$)/mL) in this kit for SARS-CoV-2 is 0.007 according to the manufacturer. The Cobas® SARS-CoV-2 test is also available as a CE-IVD test in countries that recognize the CE mark.[21]

**Table 2** Commercial rRT-PCR test kits for diagnosing SARS-CoV-2 infection.

| Assay name | Company (country) | Targeted genes | Specimen types | TAT/test | Approval | Reference |
|---|---|---|---|---|---|---|
| **Tests only for SARS-CoV-2** | | | | | | |
| Allplex™ 2019-nCoV Assay | Seegene (Korea) | RdRp, N, E | NPS, NPA, OPS, sputum, BAL | 3—4 h | Korea (Korea CDC)<br>US FDA- EUA<br>CE-IVD | 18 |
| LightMix® Modular Wuhan CoV RdRP-gene | Roche/Tib Molbiol (Switzerland/ Germany) | RdRp | NPS, NPA TA, BAL | 3—4 h | No (RUO) | 19 |
| Cobas® SARS-CoV-2 Test | Roche (Switzerland) | ORF-1a, E | NPS, OPS | 3—4 h | US FDA- EUA<br>CE-IVD | 21 |
| ePlex® SARS-CoV-2 Test | GenMark Diagnostics, (USA) | N, E | NPS | 3—4 h | USA (EUA) | 20 |
| TaqPath COVID-19 Combo Kit | Thermo Fisher Scientific (USA) | ORF-1ab, N, S | NPS, NPA, BAL | 3—4 h | USA (EUA) | 22 |
| Real-Time Fluorescent RT-PCR kit for detecting 2019-nCoV | BGI Biotechnology (China) | Highly conserved region of 2019-nCoV genome | NPS, serum, plasma | 3—4 h | China (NMPA)<br>CE-IVD | 23 |
| SARS-CoV-2 Nucleic Acid Detection Kit (Fluorescence RT-PCR) | Hangzhou Bigfish Bio-tech, Co. Ltd. (China) | ORF-1ab, N | NPS, sputum, BAL | 3—4 h | CE-IVD | 25 |
| Novel Coronavirus (2019 nCoV) RT PCR | Dynamiker Biotechnology (Tianjin) Co., Ltd. (China) | ORF-1ab, N, actin | NPS, OPS, sputum, BAL, conjunctival swabs, serum, plasma, feces | 3—4 h | CE-IVD | 27 |
| ARGENE® SARS-COV-2 R-GENE® | bioMérieux (France) | RdRp, N, E | NPS | 3—4 h | No (RUO) | 26 |
| Xpert® Xpress SARS-CoV-2 | Cepheid (USA) | N2, E | NPS, nasal aspirate, nasal wash | 45 min | US FDA- EUA | 28 |
| BioFire® COVID-19 Test | BioFire Defense, LLC | ORF1ab, ORF8 | NPS | 45 min | US FDA- EUA | 29 |
| CRISPR—Cas12-based assay | Cepheid (USA) | N, E | NA | NA | NA | 31 |
| ID NOW COVID-19 assay | Abbott (USA) | RdRp | NPS, OPS, nasal wash directly or eluted in viral transport media | ≤13 min | US FDA- EUA | 32 |
| **Included in syndromic diagnosis multiplex PCR panel** | | | | | | |
| QIAstat-Dx® Respiratory 2019-nCoV Panel (22 targets) | QIAGEN (Netherlands) | RdRp, E | NPS | <70 min | US FDA- EUA<br>CE-IVD | 40 |
| Biofire Filmarray RP-2.1 (22 targets) | bioMérieux (France) | RdRp, N, E | NPS | 60 min | No | 41 |

BAL, bronchoalveolar lavage; CE-IVD, Conformité Européenne *in vitro* diagnostic device; EUA, Emergency Use Authorization; KCDC, Korea Centers for Disease Control and Prevention; NA, not available; NMPA, National Medical Products Administration; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; OPS, oropharyngeal swab; RUO, research use only; TA, tracheal aspirates, US FDA, Food and Drug Administration of the United States.

The TaqPath™ COVID-19 Combo Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) was developed to qualitatively detect the target genes, ORF-1ab, N, and S, of SARS-CoV-2 RNA in nasopharyngeal swabs, nasopharyngeal aspirates, and BAL fluids from individuals with suspected COVID-19. The LOD for nasopharyngeal swab and BAL samples were both 10 genomic copy equivalents/reaction. The TaqPath™ COVID-19 Combo Kit is available for use only under USFDA EUA.[22]

A fluorescent rRT-PCR kit for detecting SARS-CoV-2 in serum, plasma, or nasopharyngeal swab specimens has been developed (BGI Biotechnology, Shenzhen, China).[23] Primers and sequence-specific fluorescence probes were designed against a highly conserved region of the SARS-CoV-2 genome. The reported limit of SARS-CoV-2 detection for this kit is 100 copies/mL. This kit received emergency approval from Chinese National Medical Products Administration on January 26, 2020, followed by CE-IVD registration on March 2, 2020 and it is now commercially available for clinical use in the USA.[24]

A SARS-CoV-2 Nucleic Acid Detection Kit (Fluorescence RT-PCR) (Hangzhou Bigfish Bio-tech Co., Ltd., Zhejiang, China) was recently registered as a CE-IVD for detecting SARS-CoV-2 ORF-1ab and N genes in nasopharyngeal swabs, sputum, and BAL fluids. This kit has a reported LOD of $<2 \times 10^2$ copies/mL.[25]

ARGENE® SARS-COV-2 R-GENE® (bioMérieux SA., Marcy-l'Étoile, France) was developed to detect SARS-CoV-2 RdRp, N, and E genes in nasopharyngeal swabs. The LOD was determined from an inactivated viral strain spiked in a nasopharyngeal swab sample at 0.43 $TCID_{50}$/mL. This test kit is presently available only for research purposes (Table 2).[26]

The Novel Coronavirus (2019 nCoV) RT-PCR assay (Dynamiker Biotechnology (Tianjin) Co., Ltd., Tianjin, China) can detect the target genes, ORF-1ab, N, and actin, within 1.5 h from oropharyngeal and nasopharyngeal swabs, sputum, BAL fluid, serum, plasma, conjunctival swabs, and feces. The reported detection range is $2 \times 10^2$ copies/mL (LOD) to $2 \times 10^8$ copies/mL.[27] The performance of this assay is presently under evaluation.

The product, Xpert® Xpress SARS-CoV-2 (Cepheid Inc.) detects SARS-CoV-2 RNA (N2 and E genes) in nasopharyngeal swabs, aspirates or wash specimens within 45 min, and received EUA from the USFDA during March, 2020.[28] The claimed LOD for the assay is 250 copies/mL. The performance of the Xpress SARS-CoV-2 test was clinically evaluated in patients with respiratory illnesses from whom contrived nasopharyngeal swab samples were collected into viral transport media. The samples were then spiked with AccuPlex SARS-CoV-2 (a recombinant Sindbis virus particle containing the target sequences of the SARS-CoV-2 genome) at ~2-, 3-, and 5-fold LOD concentrations. The results were in 100% agreement with the predicted results of the AccuPlex SARS-CoV-2 spiked, and negative samples.

The BioFire® COVID-19 test includes assays for SARS-CoV-2a, SARS-CoV-2d, and SARS-CoV-2e to detect SARS-CoV-2 ORF1ab and OR8 sequences in nasopharyngeal swabs, and is a qualitative test on FilmArray® 2.0 or FilmArray® Torch systems. The LOD was 3.3 E+02 GC/mL. This product has received USFDA EUA to detect SARS-CoV-2 RNA.[29]

The LabCorp 2019 Novel Coronavirus (COVID-19) qualitative RT-PCR test for SARS-CoV-2 (LabCorp, Burlington, NC, USA) was launched in the USA on March 5, 2020. LabCorp is rapidly expanding its COVID-19 testing capacity, and it should have the capacity to run 20,000 tests per day by the end of March 2020. This test has been validated for use with nasopharyngeal or oropharyngeal aspirates, washes or swabs, and BAL and is available to ordering physicians and other authorized healthcare providers anywhere in the US. An independent review of the USFDA validation of this test is underway, and LabCorp is applying for EUA.[30]

## New technology

The first clustered, regularly interspaced, short palindromic repeats (CRISPR)-based SARS-CoV-2 test (Cepheid Inc., Sunnyvale, CA, USA and Sherlock Biosciences Inc., Cambridge, MA, USA) is a rapid (~30 min) and inexpensive assay that targets the E and N genes. Its performance is comparable to that of the United States Centers for Disease Control (USCDC) SARS-CoV-2 rRT-PCR assay, with 95% positive predictive agreement and 100 negative predictive agreement.[31] Thus, it could be a visual and faster alternative diagnostic tool for SARS-CoV-2. The automated ID NOW COVID-19 assay[32] (Abbott Laboratories, Lake Bluff, IL, USA) applies isothermal nucleic acid amplification technology to qualitatively detect SARS-CoV-2 RNA by amplifying a unique region of the RdRp segment in direct nasal, nasopharyngeal or throat swabs and in nasal, nasopharyngeal or throat swabs eluted into viral transport media.[33] The claimed LOD of the natural nasopharyngeal swab matrix is 125 genome equivalents/mL. The performance of this assay was evaluated using contrived clinical NPS specimens obtained from individuals with signs and symptoms of respiratory illness.[34] The samples were prepared by spiking a clinical NP swab matrix with purified viral RNA containing target sequences from the SARS-CoV-2 genome at concentrations of ~2 × and 5 × LOD. The test was in 100% (20/20) agreement for 2 × LOD (20/20) and 5 × LOD (10/10). This assay has no significant homologies with other human coronaviruses such as SARS-CoV, MERS-CoV, and coronaviruses 229E, OC43, NKU1, NL63, or human microflora. The ID NOW COVID-19 assay is the most recent breakthrough in terms of an assay *in vitro* that can detect SARS-CoV-2 in <13 min (5 min for positive results). It is authorized for distribution in any patient care setting outside clinical laboratories, such as hospitals, clinics, physicians' offices, and outbreak hotspots.[32] It has received USFDA EUA and is being heralded as a remarkable achievement worldwide. However, investigators in Cleveland recently tested 239 specimens known to contain SARS-CoV-2 using the most prevalent tests developed by the USCDC, Cepheid, Roche, and Abbott. They found a false-negative rate of 14.8% for Abbott ID NOW compared with 0%, 1.8%, 3.5%, and 14.8%, for the USCDC, Cepheid, and Roche assays, respectively.[35]

## Syndromic multiplex panels

In addition to SARS-CoV-2, many viruses including seasonal influenza, adenovirus, coronavirus 229E/NL63/OC43, human bocavirus, human metapneumovirus, parainfluenza

virus 1/2/3, rhinovirus, and respiratory syncytial virus A/B, can cause community-associated pneumonia.[36–39] Although co-infection in patients with COVID-19 is rare, it has been reported.[8,9,40] Therefore, tests that can screen for multiple pathogens, including SARS-CoV-2, provide the additional benefit of detecting possible co-infections, leading to the administration of appropriate antimicrobial agents.

SARS-CoV-2 detection has also been incorporated into extant syndromic multiplex panels, such as the 22-target QIAstat-Dx® Respiratory SARS-CoV-2 Panel (Qiagen GmbH, Hilden, Germany)[41] and the Biofire Filmarray RP-2.1 assay (Biofire FilmArray Respiratory Panel-2 plus SARS-CoV-2) (bioMérieux SA.),[42] to expand the testing capacity for the rapid increase in the number of patients infected by SARS-CoV-2. Both multiplex panels can provide results within 60–75 min and can simultaneously detect many other common bacterial and viral respiratory pathogens. The QIAstat-Dx® Respiratory SARS-CoV-2 Panel became the first syndromic test to become commercially available in the EU and other territories accepting the CE mark on March 18,[41] and received USFDA EUA on March 31.

## Disadvantage of rRT-PCR

Although no cross-reactivity of the above commercial test kits has been reported for respiratory coronaviruses other than SARS-CoV-2 and viral, bacterial, and fungal pathogens associated with respiratory tract infection, rRT-PCR tests have several downsides (Table 2). False-negative rRT-PCR results can arise because of insufficient viral material in specimens, laboratory errors related to the quality of the kit, sample collection, or test performance.[43–45] In addition, rRT-PCR results can be positive in patients who have recovered from SARS-CoV-2, indicating their potential as virus carriers.[45] Lan et al. showed that four patients with COVID-19 who met the criteria for hospital discharge or discontinuation of quarantine in China (absence of clinical symptoms and radiological abnormalities and 2 negative rRT-PCR test results) had positive rRT-PCR test results 5–13 days later.[29] Moreover, many rRT-PCR test kits have a long turnaround time, complex protocols, and require a biological safety level 2 laboratory with expert personnel. Thus, this type of rRT-PCR is unsuitable for rapid and simple diagnosis and screening patients in the field. Accurate and rapid tests that can quickly identify large numbers of infected patients and asymptomatic carriers are urgently needed to prevent viral transmission and assure timely treatment of disease.

## Serology

Numerous diagnostic companies have recently developed serological assays to detect anti-SARS-CoV-2 antibodies, including IgA, IgM, and IgG.[17] Serological tests that can detect SARS-CoV-2 IgG-IgM antibodies are simpler than rRT-PCR, and do not require complicated equipment and protocols (Table 3). Thus, these tests can be used for rapid detection and massive screening, particularly for asymptomatic carriers. During the previous SARS epidemic, the IgM antibody was the first line of defense during viral infections and was detectable in blood samples from patients after 3–6 days. The IgG antibody is responsible for long-term immunity and immunological memory, and was detectable after 8 days.[11] Zhang et al. found relatively low or undetectable IgM and IgG titers against COVID-19 during the early stage of infection (day 0 or the day of first sampling) but serum titers of viral antibodies had increased in nearly all patients by day 5.[15] Overall, IgM-positive rates increased from 50% (8/16) to 81% (13/16), whereas IgG-positive rates increased from 81% (13/16) to 100% (16/16).[15] Lee et al. similarly detected anti-SARS-COV-2 IgG at 11 days following the onset of COVID-19 and on post-exposure days 18–21.[46] Therefore, IgM can be used for early diagnosis and IgG can help to monitor COVID-19 status. A new test kit for detecting IgG and IgM within $<$15 min has recently been validated in a cohort of 397 patients with PCR-confirmed COVID-19 and 128 patients who were SARS-CoV-2 negative at eight clinical sites in China.[11] Overall, this test showed sensitivity and specificity values of 88.7% and 90.6%, respectively.[11]

The Wondfo SARS-CoV-2 Antibody Test (Lateral Flow Method) (Guangzhou Wondfo Biotech Co., Ltd., Guangzhou, China) is a capture-based immunochromatographic assay for the rapid and qualitative detection of SARS-CoV-2 IgG/IgM antibodies in human whole blood, serum, or plasma samples, with a reported sensitivity and specificity of 86.43% (95% CI, 82.4–89.9%) and 99.75% (95% CI, 97.6–99.9%), respectively. Wondfo also completed applications for CE marking the Finecare™ SARS-CoV-2 IgM and Finecare™ SARS-CoV-2 antibody tests on March 5, 2020, giving the Wondfo the most CE marks for novel coronavirus antibody tests in the point-of-care industry.[47]

The ALLTEST 2019-nCoV IgG/IgM Rapid Test Cassette (Hangzhou ALLTEST Biotech Co., Ltd., Hangzhou, China) is a rapid lateral flow immunoassay that uses a recombinant SARS-CoV-2 N protein to detect both IgM and IgG antibodies. This *in vitro* device was registered in Germany in March 2020 (DE/CA22/419-822.3-IVD; catalogue number INCP-402).[48] Compared with a conventional rRT-PCR test (22 positive and 100 negative serum samples), the claimed relative sensitivity, specificity, and accuracy of the ALLTEST 2019-nCoV IgG/IgM Rapid Test Cassette to detect SARS-CoV-2 IgG were $>$99.9% (95% confidence interval [CI], 82.5%–100%), 98.0% (95% CI, 92.6%–99.9%), and 98.4% (95% CI, 93.9%–99.9%), respectively. However, the relative sensitivity and accuracy for detecting SARS-CoV-2 IgM antibodies were only 90.9% (95% CI, 71.0%–98.7%) and 95.9% (95% CI, 90.5%–98.5%), respectively. A recent Taiwanese study of the ability of this rapid test kit to determine anti-SARS-Cov-2 IgM and IgG antibody dynamics in 14 patients with COVID-19, detected SARS-CoV-2 IgM at the earliest on day 5 after initial infection and found that it persisted until day 42. SARS-CoV-2 IgG became detectable also on day 5, and most patients remained persistently SARS-CoV-2 IgG-positive after positive conversion. The overall sensitivity and specificity based on the rRT-PCR results was 78.6% and 100%, respectively.[49]

ASK COVID-19 IgG/IgM Rapid Test (TONYAR Biotech Inc., Taiwan) is another rapid test using lateral flow immunoassay to detect anti-SARS-CoV-2 IgG and IgM. Its sensitivity and specificity varied according to the timing of COVID-19, such as 47.8% and 100% within day 1–14 after symptom onset, 87.0%/100.0% between day 15–21 and 100%/100% after day 21.[50]

**Table 3** Commercial serological diagnosis of SARS-CoV-2 infection.

| Brand (company) | Company (Country) | Methods | Antibodies detected | Specimens | Sensitivity/specificity | TAT/per test | Approval | Reference |
|---|---|---|---|---|---|---|---|---|
| 2019-nCoV IgG/IgM Rapid Test Cassette (ALLTEST) | Hangzhou ALLTEST Biotech Co., Ltd. (China) | LFIA | IgM and IgG | Whole blood, serum, plasma | IgM: 85%/96% IgG: 100%/98% | 10—20 min | CE-IVD | 48 |
| Wondfo SARS-CoV-2 Antibody Test | Guangzhou Wondfo Biotech Co., Ltd, (China) | LFIA | IgM/IgG | Whole blood, serum, plasma | IgM/IgG: 86.43%/99.57% | 15 min | China FDA-EUA | 47 |
| ASK COVID-19 IgG/IgM Rapid Test | TONYAR Biotech Inc. (Taiwan) | LFIA | IgM and IgG | Whole blood, serum or plasma | 1—14 days after symptom onset: 47.8%/100% 15—21 days: 87.0%/100.0% >day 21 days: 100%/100% | 10 min | No | 50 |
| COVID-19 IgG/IgM Rapid Test Cassette | Zhejiang Oriental Gene Biotech Co. Ltd. (China) | LFIA | IgM and IgG | Whole blood, serum, plasma | IgM: 87.9%/100% IgG: 97.2%/100% | 10 min | CE-IVD | 51 |
| 2019-nCoV Ab Test Cassette (Colloidal Gold) | INNOVITA (Tangshan) Biological Technology Co., Ltd. (China) | LFIA | IgM/IgG | Whole blood, serum, plasma | NA | 15 min | China FDA-EUA CE-IVD | 53 |
| 2019 nCOV IgG/IgM Rapid Test | Dynamiker Biotechnology (Tianjin) Co., Ltd. (China) | LFIA | IgM and IgG | Whole blood, serum, plasma | Mixed (IgM and/or IgG): 93.2%/95.3% | 10 min | CE-IVD | 54 |
| qSARS-CoV-2 IgG/IgM Rapid Test | Cellex Inc. (NC, USA) | LFIA | IgM and IgG | Whole blood, serum, plasma | Mixed (IgM and/or IgG): 93.8%/96.4% | 15—20 min | US FDA- EUA CE-IVD | 55 |
| Anti-SARS-CoV-2 ELISA | EUROIMMUN AG (Lübeck, Germany) | EIA | IgA and IgG | Serum | IgG Sensitivity: <10/>10 days after symptom onset: 33%/100% Specificity: 98.5% IgA Sensitivity: <10/>10 days after symptom onset: 50%/100% Specificity: 92.5% IgG and IgA combined Sensitivity: <10/>10 days after symptom onset: 66.7%/100% | 2—3 h/96 samples | CE-IVD | 56 |

(*continued on next page*)

**Table 3** (*continued*)

| Brand (company) | Company (Country) | Methods | Antibodies detected | Specimens | Sensitivity/specificity | TAT/per test | Approval | Reference |
|---|---|---|---|---|---|---|---|---|
| SARS-CoV-2 IgG | Abbott Laboratories, IL, USA | CMIA | IgG | Serum or plasma | Sensitivity:<br>3−7 days after symptom onset: 25.0%<br>8−13 days: 86.1%<br>≥14 days: 100%<br>Specificity: 99.6%<br>−100% | NA | CE-IVD, USFDA | 58 |
| Elecsys Anti-SARS-CoV-2 | Roche Diagnostics Basel, Switzerland | ECLIA | Total antibody (including IgG) | Serum or plasma | Sensitivity:<br>0−6 days after symptom onset: 65.5%<br>7−13 days: 88.1%<br>≥14 days: 100%/<br>Specificity: 99.8% | 18 min | CE-IVD, USFDA | 59 |

CE-IVD, Conformité Européenne *in vitro* diagnostic device; CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminescence immunoassay; EIA, enzyme immunoassay; EUA, Emergency Use Authorization; LFIA, lateral flow immunoassay; NA, not available; RUO, research use only; TAT, turnaround time; US FDA, Food and Drug Administration of the United States.

The COVID-19 IgG/IgM Rapid Test (Zhejiang Oriental Gene Biotech Co. Ltd., Zhejiang, China) is a solid phase immunochromatographic assay for detecting IgM and IgG in whole blood, serum, and plasma.[51] The sensitivity and specificity of this test were 87.9% (87/99) and 100% (14/14), respectively, for IgM, and 97.2% (35/36) and 100% (14/14), respectively, for IgG during the convalescence period. Recently, Hoffman et al. evaluated the performance of this assay in 29 patients with rRT-PCR-confirmed COVID-19 and 124 negative control individuals based solely on rRT-PCR results due to the absence of a serological gold standard. The sensitivity was 69% and 93.1%, respectively, and the specificity was 100% and 99.2% for IgM and IgG respectively.[52]

An immunocapture method (2019-nCoV Ab Test Cassette [Colloidal Gold]) (Innovita (Tangshan) Biological Technology Co., Ltd., Tanshan, China) has also been developed to detect SARS-CoV-2 IgM and IgG antibodies in whole blood, serum, and plasma.[53] A clinical study of 447 patients, included 126 clinically confirmed patients and 62 clinically excluded patients from five institutions. The detection sensitivity and specificity were 87.3% (110/126; 95% CI, 80.40%−92.0%) and 100% (95% CI, 94.20−100%), respectively.[53]

The 2019 nCOV IgG/IgM Rapid Test (Dynamiker Biotechnology (Tianjin) Co., Ltd.) uses a capture colloidal gold immunochromatography assay to detect anti-SARS-CoV-2 IgG and IgM antibodies in whole blood, serum, and plasma.[54] The performance of this assay was evaluated in a multicenter clinical study in China that included 162 positive serum samples from patients with confirmed COVID-19 and 300 negative samples. The sensitivity and specificity of this assay were 93.2% (151/162) and 95.5% (286/300), respectively.

The Cellex qSARS-CoV-2 IgG/IgM Rapid Test (Cellex Inc., Durham, NC, USA) is also an LFIA for detecting anti-SARS-CoV-2 IgM and IgG. The performance of this test was assessed in 128 serum or plasma samples from 98 patients with COVID-19 confirmed by rRT-PCR and 70 negative serum or plasma samples collected before September 2019. The overall positive and negative rates (%) of agreement (sensitivity and specificity, respectively, were 93.75% (95% CI, 88.1−97.3%) and 96.40% (95% CI, 92.3−97.8%), respectively.[55]

Several commercial enzyme-linked immunosorbent assay (ELISA) kits for detecting anti-SARS-CoV-2 IgA, IgM, or IgG antibodies are presently in clinical trials. The Anti-SARS-CoV-2 IgG and IgA ELISA (Euroimmun Medizinische Labordiagnostika AG., Lübeck, Germany) detects either IgA or IgG antibodies against SARS-CoV-2 spike protein subunit 1 (S1) and was 100% sensitive for IgA, IgG, and IgA combined with IgG, and 92.5% and 98.5% specificity for IgA and IgG, respectively, at $>$ 10 days after symptom onset.[56] The Wantai SARS-CoV-2 Ab ELISA (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China) is based on a double-antigen sandwich principle that detects total antibody binding to the SARS-CoV-2 spike protein receptor binding domain (RBD) in human serum or plasma.[57]

In addition, chemiluminescence immunoassay including Abbott SARS-CoV-2 IgG assay and Elecsys Anti-SARS-CoV-2 were developed to detect the presence of anti-SARS-CoV-2 antibodies. For Abbott SARS-CoV-2 IgG assay, it used

**Table 4** Summary of reality and pitfalls of virological and serological methods for the diagnosis of COVID-19.

| Modality | Benefits | Pitfalls | False negative | Uncertain |
|---|---|---|---|---|
| rRT-PCR (SARS-CoV-2 RNA) | 1 Confirmatory test | 1 Long turnaround (3—4 h)<br>2 Cannot confirm viral viability<br>3 Technique-dependent<br>4 Specimen source-dependent<br>5 Equipment-dependent<br>6 Expensive | 1 Low viral load<br>2 Improper sampling procedure<br>3 Inappropriate sampling site<br>4 Unqualified reagent<br>5 Errors in storage and processing specimens and interpretation | 1 Number of negative results required to confirm absence of COVID-19.<br>2 Whether sputum is more appropriate than other upper respiratory tract specimens at any degree of severity or disease stage.<br>3 Useful for mass screening |
| Serology (anti-SARS-CoV-2 IgA, IgM, or IgG antibodies) | 1 Rapid<br>2 User friendly<br>3 Inexpensive | 1 Unknown clinical significance<br>2 Repeat tests needed<br>3 Time lags between viral infection, illness onset, and IgM, IgG, IgA antibody development. | 1 Low antibody concentration<br>2 Early stage of disease<br>3 Immunocompromised patients | 1 Unknown performance in immunocompromised patients<br>2 Appropriate timing of tests<br>3 Cross-reactivity<br>4 Correlation between severity of diseases and serological response |

chemiluminescent microparticle immunoassay for the qualitative detection of IgG antibodies to SARS-CoV-2 N protein in human serum and plasma on the ARCHITECT i System (Abbott Laboratories, IL, USA), which is presently undergoing clinical evaluation and preliminary findings have shown sensitivity and specificity of 100% and 99.5%, respectively.[58] For Elecsys Anti-SARS-CoV-2, it used an electrochemiluminescence immunoassay for the determination of antibodies (including IgG) against SARS-CoV-2 N protein in Cobas e immunoassay analyzers (Roche Diagnostics Basel, Switzerland). Its overall sensitivity was 65.5%, 88.8% and 100.0% when tested within 0—6 days, 7—13 days and ≥14 days after the onset of symptoms but the specificity was greater than 99.8%.[59]

Lassaunière et al.[57] recently investigated the sensitivity and specificity of nine commercially available serological tests (Wantai SARS-CoV-2 Total Antibody ELISA, Euroimmun Anti-SARS-CoV-2 IgG and IgA ELISA and six LFIA kits- 2019-nCOV IgG/IgM Rapid Test (Dynamiker Biotechnology), OnSite™ COVID-19 IgG/IgM Rapid Test (CTK Biotech Inc., Poway, CA, USA), Anti-SARS-CoV-2 Rapid Test (AutoBio Diagnostics CO., Ltd., Zhengzhou, China), Coronavirus Diseases 2019 (COVID-19) IgM/IgG Antibody Test (Artron Laboratories, Burnaby, Canada), 2019-nCoV IgG/IgM Rapid Test Cassette (Acro Biotech Inc., Rancho Cucamonga, CA, USA), and 2019-nCoV IgG/IgM Rapid Test Cassette (Hangzhou ALLTEST Biotech Co., Ltd.). The rates of sensitivity (specificity) of the three Wantai SARS-CoV-2 Total Antibody, Euroimmun IgA, and Euroimmun IgG ELISA kits in 30 and 82 patients with and without COVID-19, respectively, were 93% (100%), 93% (93%), and 67% (96%), respectively. The sensitivity rates of the LFIA kits (AutoBio Diagnostics, Dynamiker Biotechnology, CTK Biotech, and Artron Laboratories) determined in 30 patients with and 32 without COVID-19, were 93%, 90%, 90% and 83%, respectively, and all specificity rates were 100%. The performance of the Actro Biotech and ALLTEST Biotech assays were comparatively poorer in the initial round of evaluation, having false positive results due to IgM antibodies cross-reacting with other viruses. These findings suggest that further studies are needed to develop serological assays that can detect specific antibodies against SARS-CoV-2- to confirm a diagnosis of COVID-19.

Although serology tests presently cannot confirm SARS-CoV-2, positive findings can at least indicate a recent SARS-CoV-2 infection and provide epidemiological information about the time course of COVID-19. However, it is possible that no antibody response can be detected in the case of transient colonization of SARS-CoV-2 in the upper respiratory tract.[60] In addition, false-negative results might arise when antibody concentrations fall below the detection limit (Table 4). Overall, anti-SARS-CoV-2 IgG/IgM tests might comprise an ideal adjuvant for directing SARS-CoV-2 detection, supporting a diagnosis of COVID-19, and clarifying patients with suspected COVID-19 who are asymptomatic or characterized by negative rRT-PCR results.[15]

## Viral dynamics

Although rRT-PCR is highly sensitive, performance might be affected by viral load, which can vary according to disease stage and type of clinical specimens.[13,14,61] Viral loads are similar between asymptomatic and symptomatic patients,

supporting the notion that SARS-CoV-2 can be transmitted by asymptomatic carriers. In addition, viral loads are higher, as reflected by lower mean cycle threshold values, in patients with severe symptoms than in those with mild-to-moderate symptoms.[14] Furthermore, Zou et al. also showed that viral loads were higher in the nose than in the throat.[14] Similarly, viral loads were generally lower in throat swab, than in sputum samples in a study of two patients who underwent serial daily sampling.[57] A study of SARS-CoV-2 bio-distribution in 1070 clinical specimens collected from 205 patients with laboratory-confirmed COVID-19, found the highest positive rates in BAL specimens (14/15, 93%), followed by sputum (72/104, 72%), nasal swabs (5/8, 63%), bronchoscope brush biopsies (6/13, 46%), pharyngeal swabs (126/398, 32%), feces (44/153, 29%), and blood (3/307, 1%).[13] Similar to clinical samples obtained by invasive means such as bronchoscopy, nasal swab samples have higher viral loads and higher positive detection rates than throat/pharyngeal swabs, suggesting that nasal swabbing is a superior sampling method. However, several issues remain. Like nasal swabs, viral loads and detection rates were also high in sputum samples.[13,61] However, in contrast to nasal swabs, sputum sampling does subject healthcare workers to risk of exposure while collecting clinical specimens. In addition, whether sampling sputum has advantages over nasal swabs for detecting SARS-CoV-2 requires further clarification. Although several studies have shown that SARS-CoV-2 is detectable in stool samples,[13,61,62] the clinical significance of positive rRT-PCR results in stool specimens also remains unclear. However, precautionary measures should be taken when handling fecal samples from patients with COVID-19. The sensitivity of SARS-CoV-2 detection can be enhanced by adding tests of numerous different clinical specimens. However, the appropriate number of samples and negative results required to confidently exclude COVID-19 infection remain unknown (Table 4).

Only 11.8% of convalescent patients tested positive for SARS-CoV IgG antibodies at 7 days after SARS-CoV onset 17 years ago, and positive detection of the IgG antibody peaked four months later. Thereafter, the titers gradually declined, but more than 90% remained detectable.[63–65] These findings might help to explain the immunological responses of patients who recover from SARS-CoV. However, the clinical course of SARS-CoV-2 might not be identical to that of the previous SARS outbreaks, and thus antibody titers should be monitored after SARS-CoV-2 infection to determine the appropriate timing of tests and clarify associated immunological responses.[66]

## Viral culture

Viral culture is a gold standard for diagnosing SAS-CoV-2 infections. Virus isolation in cell cultures (usually Vero cells) is critical for locally and globally characterizing viral strains and for supporting the further development of vaccines and therapeutic agents.[67–69] However, cell culture of SARS-CoV-2 is time-consuming, labor-intensive, expertise-dependent, and biosafety (BSL-3) has to be considered. Therefore, it is not recommended for screening suspected COVID-19 in routine diagnostic laboratories.[64]

## Rapid antigen tests

Rapid antigen tests are theoretically time- and labor-saving, user-friendly, and cost-effective tools for detecting SARS-CoV-2 antigen and diagnosing early COVID-19. However, sensitivity remains uncertain based on experience with such tests of influenza viruses.[69] A multi-center (n = 7) study evaluated a fluorescence immunochromatographic assay to detect SARS-CoV-2 nucleocapsid protein in nasopharyngeal swabs (n = 239) and urine (n = 20) samples within 10 min.[70] The study found that all nucleocapsid protein positive and negative samples were in accordance with the conventional rRT-PCR results for same samples. Notably, infection could be identified in patients using this assay after 3 days of fever. Moreover, in an additional preliminary study, nucleocapsid protein was detected in urine from 73.6% of patients with confirmed COVID-19.[71] The investigators concluded that nucleocapsid protein can be accurately, rapidly, and simply assayed for an early diagnosis of COVID-19. A point-of-care lateral-flow test for rapidly screening patients with suspected COVID-19 is under development (Sona Nanotech Inc., Halifax, NS, Canada) and is expected to produce results in 5–15 min.[71]

## The challenges

The rapidly increasing number of COVID-19 infections and deaths has defined SARS-CoV-2 as a global threat to public health and has imposed numerous burdens on the healthcare system, including the availability of diagnostic tests.[72,73] During the early stage of the outbreak, rRT-PCR was recommended only for patients with suspected infection and a characteristic history and typical manifestations, with limited tests being approved by the USFDA. However, the exponential growth of COVID-19 infections resulted in the abandonment of diagnostic test guidelines, and the USFDA began to permit laboratory-developed SARS-CoV-2 tests without prior agency approval.[74] An increasing number of commercial and in-house diagnostic tests has been developed, although most have not yet obtained FDA approval.[75] However, diagnostic capacity has yet to meet current demands in many areas, and widespread tests or test-seeking is likely to overwhelm healthcare systems. Furthermore, although drive-through tests might largely reduce the risk of exposure to SARS-CoV-2 for patients with mild or no illness by avoiding waiting rooms, the application of this type of test remains limited. Overall, balancing the increasing use of laboratory-developed tests, the risk of test errors, the need for tests, the burden on healthcare systems, the benefits of early diagnosis, and the risk of unnecessary exposure remain significant challenges.

## Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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