Chapter

In Vitro Diagnostics for COVID-19: State-of-the-Art, Future Directions and Role in Pandemic Response

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Abstract

There have been tremendous advances in in vitro diagnostics (IVD) for coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although the confirmatory clinical diagnosis is made by real-time reverse transcriptase polymerase chain reaction (RT-PCR), lateral flow immunoassay (LFIA) based viral antigen (Ag) detection is used for mass population screening at point-of-care (POC) settings. The rapid RT-PCR tests (such as from Cepheid and Bosch) have an assay duration of less than 40 min, while most rapid Ag tests (such as Abbott’s BinaxNOW™ COVID-19 Ag card) have an assay duration of about 15 min. Of interest is the POC molecular test (ID NOW™) from Abbott that takes less than 13 min. Similarly, many immunoassays (IAs), i.e., automated chemiluminescent IA (CLIA), manual ELISA, and LFIA, have been developed to detect immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) produced in subjects after SARS-CoV-2 infection. Many IVD tests have been approved by the United States Food and Drug Administration (FDA) under emergency use authorization (EUA), and almost all IVD tests are Conformité Européenne (CE) certified.

Keywords: COVID-19, pandemic, in vitro diagnostics, mobile healthcare, antigen, antibodies, molecular assays, CT scans

1. Introduction

SARS-CoV-2 is a positive-sense single-stranded ribonucleic acid (RNA) virus with characteristic spikes on its surface that provide its crown-like appearance. It comprises different structural proteins, namely nucleocapsid protein (NP), spike protein (SP), membrane protein (MP), and envelope protein (EP), which play an important role in the manifestation of SARS-CoV-2 infection (Figure 1). The World Health Organisation (WHO), on February 11, 2020, coined the term COVID-19 for the lung disease that SARS-CoV-2 causes. The WHO declared COVID-19 as a public health emergency of international concern (PHEIC) and a pandemic on January 30, 2020, and March 11, 2020, respectively. The pandemic’s epicenter shifted from
China to Europe and then to the United States of America (USA) and many countries during 2020.

SARS-CoV-2, discovered in the Hubei province of Wuhan city in China in December 2019, has spread to 219 countries and territories. The confirmed COVID-19 cases exceed 129 million [2], while global deaths exceed 2.82 million.

USA accounts for the most COVID-19 cases, i.e., over 31 million, representing about 24% of global COVID-19 cases. With 12.6 and 12.2 million cases, Brazil and India have the subsequent highest incidence of COVID-19. Some nations, such as Singapore, New Zealand, and China, have effectively controlled the COVID-19 incidence by acting proactively and taking the desired measures at the right time.

The origin of SARS-CoV-2 is still unclear and contradictory, but the early reports mention its transmission to humans at the Wuhan’s live animals market in December 2019 [3]. Some researchers claim its origin in bats, although the intermediate hosts are still not identified [4]. The genomic sequence of SARS-CoV-2 has ~82% homology with human SARS-CoV and ~89% homology with bat SARS-like CoVZXC21 [5]. The most vulnerable groups at higher risk of developing severe COVID-19 are persons over 65 years of age; persons with chronic diseases, such as diabetes mellitus, hypertension, cardiovascular diseases, lung disease; and decreased immunity. The intensive large-scale testing of the population to identify and quarantine COVID-19 infected persons is essential to avoid the spread of infection. Rapid LFIAs have played a phenomenal role here, and many of such tests have also been approved for self-use [6]. The use of face masks and social distancing measures has played a significant role in preventing COVID-19 infection [7–9]. There has been considerable improvement in the RT-PCR tests, which are the accepted gold standard for confirmatory COVID-19 clinical diagnosis. However, several deficiencies have also been reported where they were unable to detect COVID-19 during the early stages of infection and provided false negatives results to subjects [10, 11]. The false negative results could be due to the improper extraction of nucleic acid from clinical materials or insufficient cellular material for detection. The chest computerized tomography (CT) scan has further helped physicians detect COVID-19 infection in such RT-PCR false-negative subjects [12, 13]. The last year has also seen the emergence of many mutations in SARS-CoV-2 [14–17], which can only be diagnosed by next-generation sequencing (NGS). There are continuous efforts to develop POC biosensor devices for rapid diagnosis of COVID-19. Further,
many IVD companies and researchers are working on new IVD approaches, such as detecting specific biomarkers that will enable the detection of COVID-19 infection at a very early stage. There is a need for continuous improvement in IVD assays to ensure reliable detection of SARS-CoV-2 infection without being impacted by the SP mutations.

2. Structure of SARS-CoV-2

SARS-CoV-2 has a characteristic crown-like appearance due to the spikes formed by a major glycoprotein (Mol. Wt. ~180 kDa), i.e., SP, which has two subunits, S1 and S2 [1] (Figure 1). S1 has the receptor binding domain (RBD) that recognizes and binds to angiotensin converting enzyme 2 receptor (ACE2) in the lower respiratory tract of SARS-CoV-2 infected subjects [18]. In contrast, S2 has other basic elements needed for membrane fusion. The amino-terminal region of S1 subunit is the most variable immunogenic antigen. SP is the most widely studied viral protein as it is responsible for the SARS-CoV-2 infection. It is the target of all SARS-CoV-2 neutralizing antibodies and COVID-19 vaccines. On the contrary, NP (Mol. Wt. ~40 kDa) is another viral structural protein, which is the most abundant viral phosphoprotein produced and shed during the first two weeks of SARS-CoV-2 infection, with peak shedding around 10 days after infection. It exhibits high immunogenicity and can be detected in either nasal/nasopharyngeal swabs, saliva, stool, serum or urine samples [19]. The sandwich ELISA is used for the detection of NP as it is a large protein with multiple epitopes. The other structural proteins of SARS-CoV-2 are the MP and EP. MP is the most abundant protein on SARS-CoV-2, while EP is the smallest structural protein of SARS-CoV-2 that plays a role in viral assembly, release of virions, and pathogenesis [1].

3. In vitro diagnostics for COVID-19

Various IVD assays have been developed for the detection of SARS-CoV-2 viral RNA, antibodies, and antigens, which encompass assays performed in certified COVID-19 diagnostic laboratories and rapid tests employed at POC settings. The various IVD assays, together with their characteristic features and bioanalytical performances, are specified in this section. Almost all the IVD assays for COVID-19 are CE IVD certified, while several are also approved by the FDA under the EUA. As shown in Figure 2, the RT-PCR is positive for about 3 weeks after the onset of symptoms in COVID-19 patients, while the rapid tests for viral antigen work best during the first week after the onset of symptoms [20]. On the contrary, the serology tests for detecting antibodies work best after seroconversion at the end of 3rd-week post onset of symptoms, when the COVID-19 patients enter convalescence [20, 21].

3.1 Molecular diagnostics

RT-PCR is the gold standard for the confirmatory clinical diagnosis of COVID-19 and the most used IVD assay globally. The first real-time RT-PCR assay, highly specific for SARS-CoV-2 RNA and no cross-reactivity to other coronaviruses, was developed by Tib-Molbiol, Germany, in January 2020 [22]. The assay involved the detection of SARS-CoV-2 RNA by employing envelope (E) and RNA-dependent RNA polymerase (RdRp) gene assays, where E-gene assay enabled the first-line screening and RdRp gene assay did the confirmatory
testing. An alternative format, one-step RT-PCR assay, was developed to detect ORF1b and N regions of SARS-CoV-2 in less than 1.5 h [23]. It employed the N gene assay for screening and Orf1b gene assay for confirmatory analysis. But the assay could also detect SARS-CoV and other closely-related viruses as ORF1b and N regions are highly conserved among sarbecoviruses. The authors distinguished SARS-CoV-2 from SARS-CoV via sequence analysis of positive amplicons if the RT-PCR results are positive. A prospective development was the real-time RT-PCR assay to detect RdRp/helicase (H) genes of SARS-CoV-2 [24]. The assay has high sensitivity and detects COVID-19 in low viral load samples, saliva, plasma, and upper respiratory tract samples. Moreover, it showed no cross-reactivity with other human coronaviruses and respiratory viruses. Subsequently, many innovative RT-PCR assays were developed by many IVD companies and research groups.

A prominent test is the CE certified and FDA EUA approved rapid, real-time RT-PCR test, i.e., the Xpert® Xpress SARS-CoV-2 test, by Cepheid, USA [25]. The assay, requiring GeneXpert Dx or GeneXpert Infinity Systems, enables the qualitative detection of SARS-CoV-2 ribonucleic acid (RNA) in specimens collected from the upper respiratory tract [25]. These include nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab or nasal wash/aspirate specimens. The rapid RT-PCR test is run on GeneXpert Instrument Systems that have automated and fully integrated process steps, i.e., sample preparation, extraction of RNA, amplification of RNA, and detection of the target sequences. The systems employ single-use disposable cartridges, which have all the RT-PCR reagents together with a sample processing control (SPC) and a probe check control (PCC). SPC controls the sample processing and monitors the presence of potential inhibitors in the RT-PCR reaction. It ensures the presence of adequate RT-PCR reaction conditions for the amplification reaction and the proper working of RT-PCR reagents. On the other hand, PCC ensures reagent rehydration, filling of PCR tube, and the presence of all reaction components in the cartridge. It also monitors the integrity of the probe and the stability of the dye. The procedure involves the sample collection and its placement into a viral transport tube that contains 3 mL transport medium or 3 mL of saline. The specimen in the tube is mixed by rapidly inverting it 5 times, followed by transferring the sample to the sample chamber of the Xpert Xpress SARS-CoV-2
cartridge. The cartridge is then loaded onto the GeneXpert Instrument System for the automated sample processing and real-time RT-PCR. The kit comprises freeze-dried beads, lysis reagent, binding reagent, elution reagent, and disposable transfer pipettes, which are sufficient to process 10 specimens or quality control samples. The positive predictive agreement (PPA) and negative predictive agreement (NPA) of the test relative to the expected results were 97.8% and 95.6%, respectively.

Cepheid has developed the same test on GeneXpert Xpress System, a POC system, with similar bioanalytical performance. The SARS-CoV-2 diagnosis is positive if the signal for the N2 nucleic acid target or signals from both nucleic acid targets (N2 and E) have a cycle threshold (Ct) within the valid range. But the diagnosis is presumptive positive if the signal for only the E nucleic acid target has a Ct within the valid range. It may require additional confirmatory testing. The overall assay duration is less than 45 min.

Cepheid has further developed another FDA EUA approved real-time multiplex RT-PCR test, Xpert® Xpress SARS-CoV-2/Flu/RSV, which is performed on GeneXpert Dx or GeneXpert Infinity Systems. It qualitatively detects and differentiates SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA in nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimens. The principle of the assay is very similar to that of Cepheid’s SARS-CoV-2 test. Cepheid recommends the use of external controls in the form of inactivated viruses that are provided by ZeptoMetrix, USA. They should be used to perform external quality control with each new lot and shipment of reagents. The PPA and NPA of the test relative to the predicate RT-PCR tests (FDA EUA approved) for SARS-CoV-2, Flu A, Flu B, and RSV were 97.9% and 100%; 100% and 100%; 100% and 99%; and, 100% and 100%, respectively. The company has developed the same test on GeneXpert Xpress System (a POC system) with similar bioanalytical performance.

The Simplexa™ COVID-19 real-time RT-PCR assay from DiaSorin Molecular, Italy, is another prospective assay for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal swabs, nasal swabs, nasal wash/aspirate, and bronchoalveolar lavage specimens from COVID-19 suspects. It contains reagents that are sufficient for 24 reactions. The assay targets the ORF1ab and S gene regions of the SARS-CoV-2 genome and is run on the LIAISON® MDX instrument using the Direct Amplification Disc and other accessories. It employs fluorescent probes together with corresponding forward and reverse primers for the amplification of SARS-CoV-2 RNA and internal control RNA. The company provides Simplexa™ COVID-19 Positive Control Pack, which may be used as an external control for quality control testing. The PPA and NPA in various sample matrices were both 100% w.r.t. an established comparator.

The cobas® SARS-CoV-2 is a real-time RT-PCR assay from Roche, which qualitatively detects SARS-CoV-2 RNA in clinically-collected nasal, nasopharyngeal, and oropharyngeal swabs specimens from COVID-19 suspects. The assay is also approved for clinically-instructed self-collected nasal swab specimens. It is performed on cobas® 6800/8800 Systems, which comprise a sample supply module, a transfer module, a processing module, and an analytical module. The cobas® SARS-CoV-2 employs fully automated sample preparation involving RNA extraction and purification, which is followed by PCR amplification and detection. It targets the ORF1a/b and E gene regions of the SARS-CoV-2 genome. The company provides the assay controls, i.e., cobas® SARS-CoV-2 Control Kit and cobas® Buffer Negative Control Kit. The PPA and NPA determined in the clinical evaluation with nasopharyngeal swab samples were both 100%.

The Vivalytic COVID-19 test developed by Bosch, Germany, in collaboration with Randox Laboratories, UK is another prospective assay [26]. The
Biotechnology to Combat COVID-19

A fully-automated POC test can detect SARS-CoV-2 and nine respiratory viruses, including influenza A and B, within 2.5 h. The procedure involves sequentially taking the swab sample from the nose or throat of COVID-19 suspects, placing the swab inside a Vivalytic cartridge containing all the COVID-19 assay reagents, and plugging the cartridge into the Vivalytic analyzer.

The most prominent and rapid POC molecular test is the Abbott ID Now™ COVID-19 test [27], which qualitatively detects the viral RNA from SARS-CoV-2 specimens, i.e., throat, nasal, nasopharyngeal, or oropharyngeal swab samples, in just 5 min [27, 28]. It is a POC molecular test for the RdRp gene, which requires just a portable, touchscreen-operated, lightweight (6.6 pounds) and compact (the size of a small toaster) instrument called ID Now. It enables COVID-19 testing in hospitals, clinics, physicians’ offices, or other POC settings. The test kit comprises 24 tests, positive and negative controls, pipettes, and swabs for sample collection.

3.2 Antigen detection

The LFIA-based rapid Ag tests for SARS-CoV-2 have played a phenomenal role in the COVID-19 pandemic response. They have been extensively used to screen a large population at POC settings as physician office laboratories, offices, schools, businesses, and homes. They have been approved for professional IVD use and self-testing by the FDA and several countries such as Germany. Germany has allowed the use of tens of rapid Ag tests for self-testing via approval provided by the Federal Institute for Drugs and Medical Devices (BfArM) [6]. The US FDA has granted EUA to several rapid Ag tests for SARS-CoV-2, which can be used for professional IVD use, home use, or both. Almost all the approved rapid Ag tests detect the NP of SARS-CoV-2 and have got good analytical performance. Although several reports have shown the contradictory analytical performance of rapid Ag tests, there is no doubt that such tests are of extreme importance as they have extended the outreach of SARS-CoV-2 testing enormously. The most widely used rapid Ag tests are those from Abbott, Becton Dickinson (BD), and Quidel, which have been further approved by FDA recently under EUA for the serial screening of COVID-19 suspects by testing them twice over 3 days with 24–48 h between tests.

The BinaxNOW™ COVID-19 Ag Card from Abbott Diagnostics is an LFIA that enables the qualitative detection of NP antigen from SARS-CoV-2 in direct anterior nasal (nares) swabs without viral transport media [29]. It is an immunochromatography membrane assay that employs highly sensitive and specific antibodies to detect NP from SARS-CoV-2. A test strip is constructed by immobilizing SARS-CoV-2 specific antibodies and a control Ab onto a membrane as two distinct lines and combining it with other reagents/pads. The COVID-19 Ag card is cardboard, book-shaped hinged card that has a well to hold nasal swab and the test strip mounted on opposite sides. The assay procedure involves taking the nasal swab specimen from the COVID-19 suspects and mixing it with the extraction reagent. The extraction agent disrupts the virus particles and exposes internal viral NP. It is followed by closing the card, which brings the extracted sample in contact with the test strip that starts the LFIA assay. The test results are detected visually by naked eyes after 15 min, where the presence of a pink/purple sample line shows the presence of NP in the sample. The test is intended for use in COVID-19 suspects who are within 7 days of symptoms onset. The PPA and NPA of the test were 97.1% and 98.5%, respectively, against the comparator method. FDA also approves it under EUA for the serial screening of COVID-19, where the individuals are tested twice over 3 days with at least 36 h between tests. However, the test does not differentiate between SARS-CoV-2 and SARS-CoV, and the positive results do not rule out bacterial infection or co-infection with other viruses. The negative
test results should be treated as presumptive in patients beyond 7 days post onset of symptoms, where further confirmation with a molecular assay is required. It is essential that the results of the test should be read within 30 minutes, and the nasal swab specimens are used immediately after collection. Apart from the test cards, extraction reagent, and nasal swabs, the BinaxNOW™ COVID-19 Ag Card provides positive and negative control swabs. The positive control swab is a dried swab containing non-infectious recombinant SARS-CoV-2 NP, while the negative control swab has the sample matrix without any NP. It is recommended to test the positive and negative control swabs after each shipment of Ag tests and at least once for each untrained operator.

The BD Veritor™ System [30] for Rapid Detection of SARS-CoV-2 is another prospective rapid LFIA-based test that detects qualitatively the presence of SARS-CoV-2 NP in direct anterior nasal swabs from COVID-19 suspects within the first five days of the onset of symptoms. FDA also authorizes it under EUA for the serial screening of COVID-19 where the subjects are tested twice over 2 or 3 days with 24–48 h between tests. The swab specimens are placed in the extraction reagent tube for sample processing, and the processed sample is then added to the BD Veritor System test device. The SARS-CoV-2 NP in the sample form Ag-conjugate complexes by binding to antibodies conjugated to detector particles in the test strip. The complexes are then captured by the specific antibodies bound to the membrane at the test line. The BD Veritor™ System test device's test results are read after the completion of test in 15 min using the BD Veritor™ Plus Analyzer Instrument. The SARS-CoV-2 test kit includes BD Veritor™ System test devices, extraction reagent, nasal swabs, SARS-CoV-2 (+) Control Swab, and SARS-CoV-2 (−) Control Swab. Most of the assay characteristics in terms of interferences, specificity, and analysis are similar to that of Abbott's BinaxNOW™ COVID-19 Ag Card test. The specimens should be tested immediately after collection. The PPA and NPA of the test were 84% and 100%, respectively, against the RT-PCR method.

The QuickVue At-Home OTC COVID-19 Test from Quidel Corporation is another rapid test to detect SARS-CoV-2 NP qualitatively in direct anterior nasal swabs from COVID-19 suspects within the first six days of the onset of symptoms. FDA also authorizes it under EUA for the serial screening of COVID-19 suspects where they are tested twice over 2 or 3 days with 24–36 h between tests. The test procedure is very similar to that of the BD Veritor™ System for Rapid Detection of SARS-CoV-2 test except that the test strip is read visually by naked eyes after 10 min. The presence of a pink/purple-colored test line indicates the presence of SARS-CoV-2 NP in the specimen. Most assay characteristics are like that of Abbott’s BinaxNOW™ COVID-19 Ag Card test. The PPA and NPA of the test were 83.5% and 99.2%, respectively, against the EUA molecular comparator assay.

All other SARS-CoV-2 rapid Ag tests detect the NP antigen qualitatively and have demonstrated good analytical performance. However, some SARS-CoV-2 rapid Ag tests, such as that from Sensing Self, Singapore, have also shown good analytical performance for the qualitative detection of SP antigens. But as most of these tests targeting the SP were developed last year, there is a need to demonstrate that they can work in subjects affected by various spike mutations. The preference for clinical decision-making is certainly the NP detection-based rapid Ag tests. Apart from nasal swabs, several companies have demonstrated the use of saliva, sputum, and stool samples to detect SARS-CoV-2 viral Ag.

3.3 Antibodies detection

Various IVD companies have developed serological IAs to detect anti-SARS-CoV-2 Ab in the serum, plasma, or whole blood samples. They enable identifying
individuals with an adaptive immune response to SARS-CoV-2 either due to prior or recent infection [31]. Despite several reports stating the persistence of immunity after SARS-CoV-2 infection for several months [32, 33], it is still unclear how long the anti-SARS-CoV-2 Ab persists and whether they confer protective immunity. Therefore, serology tests are not used to diagnose acute SARS-CoV-2 infection. In the case of SARS-CoV-2, IgM, IgG, and IgA antibodies appear in the subjects at nearly the same time and have the seroconversion between 14 and 23 days post onset of symptoms. SARS-CoV-2 IgG and IgM antibodies may be below the detectable levels in COVID-19 patients who are within 14 days after the onset of symptoms. However, the COVID-19 samples should be handled with care as there is a possibility of detectable SARS-CoV-2 in samples even after seroconversion. Almost all assays have a poor PPA with RT-PCR in patient samples taken from subjects within 14 days from the onset of symptoms. But they have good PPA for samples taken from subjects more than 14 days post onset of symptoms. However, there is always a risk of false-positive results due to the presence of pre-existing antibodies or other possible causes. The most widely used IAs to detect anti-SARS-CoV-2 Ab are automated CLIA, manual ELISA, and rapid LFIA tests that are specified in more detail below.

3.3.1 Automated CLIA

The magnetic particles-based CLIAs are the gold standard in clinical diagnostics for the automated high-throughput analysis of many disease biomarkers on a random-access CLIA analyzer. The desired throughput can be customized from low- to high-throughput by selecting an appropriate CLIA analyzer. Many IVD companies have developed several SARS-CoV-2 serology tests for the detection of IgG and IgM. Most of these assays have an overall assay duration of less than 30 min and employ SP, NP, or both for the detection of anti-SARS-CoV-2 Ab.

The DiaSorin LIAISON® SARS-CoV-2 S1/S2 IgG assay [34, 35], used on the LIAISON® XL Analyzer, detects the anti-SARS-CoV-2 IgG qualitatively in human serum or plasma (sodium heparin, lithium heparin, and potassium EDTA). The assay employs the specific recombinant S1 and S2 antigens coated magnetic particles, which detect the anti-SARS-CoV-2 IgG in the patient sample. This is followed by subsequent binding to mouse monoclonal Ab to human IgG linked to an isoluminol derivative (isoluminol-Ab); adding the starter reagents to induce flash chemiluminescence (CL) reaction; and, measuring the CL signal of isoluminol-Ab conjugate via a photomultiplier. The assay employs two calibrators, one containing low and another having high anti-SARS-CoV-2 IgG levels. The assay has a PPA of 97.6% in samples taken from COVID-19 subjects ≥15 days after diagnosis by RT-PCR. DiaSorin also developed LIAISON® SARS-CoV-2 S1/S2 IgM assay for the qualitative detection of anti-SARS-CoV-2 IgM in human serum or plasma (sodium heparin, lithium heparin, and dipotassium EDTA). The assay principle is similar to that of IgG assay except that magnetic particles were coated with spike receptor-binding domain (RBD) antigen and mouse monoclonal IgG antibodies were linked to an isoluminol derivative, N-(4-Amino-Butyl)-N-Ethyl-Isoluminol (ABEI isoluminol-Ab conjugate). It employs two calibrators, one containing anti-SARS-CoV-2 human IgM monoclonal Ab while the other has no IgM. The PPA to PCR was 92.6% in samples taken from COVID-19 subjects between 15 and 30 days after PCR diagnosis.

The Elecsys anti-SARS-CoV-2 S cobas® [34, 36] is an electrochemiluminescent IA developed by Roche for the qualitative and semi-quantitative detection of Ab against the SP RBD in human serum and plasma (lithium heparin, dipotassium-EDTA (K₂-EDTA), tripotassium EDTA (K₃-EDTA), and sodium
The IA is performed on cobas® e analyzers and has a total assay duration of just 18 min. It employs double-antigen sandwich principle, where the antigens present in the reagent capture mainly anti-SARS-CoV-2 IgG but also IgM and IgA. The assay procedure comprises of two incubations and a measurement step. The 1st incubation of the sample with biotinylated SARS-CoV-2 SP RBD-specific recombinant antigen and biotinylated SARS-CoV-2 SP RBD-specific recombinant antigen labeled with a ruthenium complex leads to the formation of a sandwich complex. It is followed by the 2nd incubation, during which the addition of streptavidin-coated magnetic particles binds to the sandwich complex via streptavidin-biotin interaction. Subsequently, the reaction mixture is aspirated into the measuring cell where the electrode magnetically captures the magnetic particles and the unbound substances are removed. Finally, a voltage is applied to the electrode, which induces the CL signal that is measured by a photomultiplier. The results are determined using a calibration curve with 2-point calibration and a master curve. The assay demonstrated a PPA to PCR of 96.6% in samples taken from COVID-19 subjects ≥15 days after PCR positive result and a NPA to PCR of 99.98%.

Another prospective assay is the IDS SARS-CoV-2 IgG assay developed by Immunodiagnostic Systems, United Kingdom (UK), which detects the anti-SARS-CoV-2 IgG antibodies qualitatively in human serum and plasma (lithium heparin, K$_3$-EDTA, and sodium citrate). The assay involves the incubation of magnetic particles coated with recombinant SARS-CoV-2 NP and SP antigens with the patient sample (4 μL), which is followed by a wash step and subsequent incubation with anti-SARS-CoV-2 Ab labeled with acridinium. The magnetic particles, having the specific immune complexes, are then captured by a magnet, and a wash step removes the unbound substances. Subsequently, the addition of trigger reagents induces the CL signal that is measured by a photomultiplier. The IA employs two calibrators, one calibrator having low anti-SARS-CoV-2 IgG level while other calibrator has high anti-SARS-CoV-2 IgG level. The assay demonstrated a PPA to PCR of 97.6% in samples taken from COVID-19 patients ≥15 days after symptoms onset and a PPA to PCR of 100% in samples taken from COVID-19 subjects ≥15 days after PCR method. The NPA to PCR was 99.6%.

Of interest is the SNIBE’s MAGLUMI 2019-nCoV IgM/IgG assay [37], which employs the 2019-nCoV recombinant antigen, expressing the full-length SP and NP. The assay employs two separate cassettes; one cassette detects IgM via a capture CLIA while another cassette detects IgG via an indirect CLIA. The CL signal detection mechanism is similar to that of DiaSorin. It employs two calibrators and two controls both for the IgG as well as IgM assays. One calibrator and control have high anti-SARS-CoV-2 IgG/IgM levels, while another calibrator and control have low anti-SARS-CoV-2 IgG/IgM levels. The results are generated by a calibration curve generated by 2-point calibration for a specific SNIBE’s analyzer and a master curve. The IgG and IgM assays demonstrated PPAs to PCR of 100% and 77.46%, respectively, in samples taken from COVID-19 patients ≥15 days post symptoms onset. The combined PPA to PCR was 93.94% in samples taken from COVID-19 patients ≥15 days post symptoms onset.

There are several other CLIA developed by Siemens, Abbott, Beckman Coulter, Ortho Clinical Diagnostics, etc., which employ similar assay formats and have high analytical performance.

### 3.3.2 ELISA

Many IVD manufacturers have developed several CE-certified ELISA kits for the detection of anti-SARS-CoV-2 IgG and IgM antibodies. The most prominent ELISA
kits from Euroimmun, InBios, Wantai, Epitope Diagnostics, and Thermo Fisher Scientific, have been approved by FDA under EUA.

The most widely used ELISA kit is the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) kit [35, 38], which enables the qualitative detection of anti-SARS-CoV-2 IgG antibodies in human serum and plasma (K⁺-EDTA, Li⁺-heparin, and Na⁺-citrate). The test kit contains microplate strips, where each strip has 8 break-off reagent wells that are pre-coated with S1 domain of SP of SARS-CoV-2, which is expressed recombinantly in the human cell line HEK 293. The assay procedure involves incubating reagent wells with diluted patient sample (diluted 1:101 in sample buffer), which leads to the binding of anti-SARS-CoV-2 IgG antibodies (and also IgA and IgM) with the coated viral Ag. It is followed by subsequent incubation with HRP labeled anti-human IgG detection Ab and TMB substrate reaction. The absorbance of the colored solution after stopping the enzyme-substrate reaction with a stop solution is measured at the wavelength of 450 nm with a reference wavelength of 620–650 nm. The provided two controls, i.e., a positive and a negative control, and calibrator must be used with each run. The overall assay duration is about 2 h and 15 min. The test is evaluated by calculating a ratio of the OD of the control/patient sample over the OD of the calibrator. The results are interpreted as negative, borderline, or positive if the ratio is <0.8, 0.8–1.1, or > 1.1, respectively. It is recommended to retest the patient after 1–2 weeks if he has a borderline result. The assay demonstrated a PPA to PCR of 100% in samples taken from COVID-19 patients ≥21 days post onset of symptoms and a PPA to PCR of 81.1% in samples taken from COVID-19 patients ≥11 days post PCR confirmation. The NPA to PCR was 98.6%. The independent validation study by Frederick National Laboratory for Cancer Research (FNLCR) determined the PPA to comparator method of 90% and NPA of 100%.

The SCoV-2 Detect™ IgG ELISA from InBios International, Inc., USA employs indirect ELISA for the qualitative detection of anti-SARS-CoV-2 IgG antibodies in diluted serum samples (diluted 1:100 in sample dilution buffer). The assay procedure and steps are similar to that of Euroimmun ELISA kit, and the overall assay duration is about 2 h. The kit has a positive and a negative IgG control and a cut-off IgG control. All the controls should be run whenever an assay is performed. The assay achieved a PPA to PCR of 100% in samples taken from COVID-19 subjects ≥22 days post onset of symptoms and a PPA to PCR of 100% in samples taken from COVID-19 subjects 0–28 days post PCR confirmation. The PPA to PCR was 95.45% in samples collected from suspects ≥15 days post onset of symptoms. The NPA to PCR was demonstrated to be 98.95%. The independent validation study by FNLCR showed both PPA and NPA to comparator method to be 100%.

InBios has also developed the SCoV-2 Detect™ IgM ELISA that enables the qualitative detection of anti-SARS-CoV-2 IgM antibodies in human serum and plasma (K₂-EDTA) from individuals that are 7–64 days post symptoms onset. The assay procedure is similar to that of Euroimmun and InBios IgG kits, except that detection is done by HRP labeled anti-human IgM. The IA involves dilution of the patient sample, i.e., 1:100 in sample dilution buffer, and has an assay duration of about 2 h. It employs a positive and a negative IgM control, and a cut-off IgM control, which must be run for every assay. The assay achieved a PPA to PCR of 93.75% in samples taken from COVID-19 subjects ≥15 days post onset of symptoms. The NPA to PCR was demonstrated to be 98.95%. The independent validation study by FNLCR showed PPA and NPA to comparator method of 96.7% and 98.8%, respectively.

Bio-Rad has developed the Platelia SARS-CoV-2 Total Ab ELISA that enables the qualitative detection of total Ab (IgM/IgG/IgA) against SARS-CoV-2 in human serum and plasma (K₂-EDTA, K₃-EDTA, lithium heparin, acid citrate dextrose,
or sodium citrate) [39, 40]. It is a one-step antigen capture format that employs the addition of pre-diluted patient samples to recombinant SARS NP coated wells, and the simultaneous addition of HRP labeled recombinant SARS NP. The mixture is incubated for 1 h at 37°C that leads to the formation of immune complex. Thereafter, the TMB substrate is added, and after 30 min, the colorimetric reaction is stopped by adding a stop solution. The optical density is read by a spectrophotometer at 450 nm with a reference wavelength of 620 nm. The assay employs a positive control, a negative control, and a cut-off control that should be run for each assay. The positive and cut-off controls consist of rabbit polyclonal anti-SARS NP antibodies in human serum and buffer, respectively, with other components. The analysis of results is similar to that of Euroimmun IgG ELISA. The assay has a PPA to PCR of 100% in samples taken from COVID-19 suspects ≥15 days post onset of symptoms while the NPA to PCR was 98.3%. A similar ELISA kit is the WANTAI SARS-CoV-2 Ab ELISA that enables the qualitative detection of total antibodies against SARS-CoV-2 in human serum and acid citrate dextrose plasma. The PPA to PCR was 98.72% in samples taken from COVID-19 suspects ≥15 days post onset of symptoms while the NPA to PCR was 98.3%. The FNLCR's independent validation showed PPA and NPA to comparator method of 96.7% and 97.5%, respectively.

A novel ELISA is the cPassTM SARS-CoV-2 Neutralization Activity Detection Kit from GenScript USA, Inc., which detects qualitatively the total anti-SARS-CoV-2 neutralization Ab in human serum and K$_2$-EDTA plasma (diluted 1:9 in sample dilution buffer) in just less than 1 h 15 min. The test mimics the virus-host interaction in a test tube or microplate by coating the surface of the well with human ACE2 and analyzing its specific binding to the purified recombinant SARS-CoV-2 SP RBD protein conjugated to HRP (HRP-RBD). The specific interaction between human ACE2 and HRP-RBD is blocked when the neutralization Ab against SARS-CoV-2 SP-RBD are present in the patient sample. The patient samples and controls are diluted and incubated with HRP-RBD so that the neutralization Ab could bind to HRP-RBD. It is followed by the addition of mixture to human ACE2 protein coated capture plate, where the unbound HRP-RBD and HRP-RBD bound to non-neutralizing Ab is captured on the plate. The neutralization Ab HRP-RBD immune complexes in the supernatant are removed during the washings. Subsequently, the TMB substrate is added, and the enzymatic reaction is stopped by adding a stop solution. The absorbance of the solution is read at 450 nm in a microplate reader. The assay employs a positive and a negative control that should be run for each assay. It has PPA and NPA of 100% with the comparator Plaque Reduction Neutralization Test at 50% viral neutralization (PRNT$_{50}$) and 90% viral neutralization (PRNT$_{90}$). The PRNTs employ the SARS-CoV-2 virus (WA01/2020 isolate).

### 3.3.3 Rapid LFIA

Numerous rapid LFIA-based POC tests have been developed to detect and differentiate anti-SARS-CoV-2 IgM and IgG antibodies produced in individuals after SARS-COV-2 infection. They provide the test results in less than 20 min using only a few microliters of the sample.

The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) test from Healgen Scientific LLC, USA, is rapid LFIA for the qualitative detection and differentiation of anti-SARS-CoV-2 IgM and IgG antibodies in human venous whole blood, serum, and plasma from anticoagulated blood (Li’ heparin, K$_2$-EDTA, and sodium citrate) [41]. It employs anti-human IgG, anti-human IgM and rabbit IgG coated on a nitrocellulose strip at the test line IgG, test line IgM and control line C,
respectively. The colloidal gold particles conjugated to recombinant SARS-CoV-2 S1 antigen (COVID-19 conjugates) are stored in the burgundy-colored conjugate pad. The assay procedure involves the addition of patient sample and assay buffer to the sample well. The presence of anti-SARS-CoV-2 IgM and/or IgG antibodies in the sample will lead to the formation of immune complex with COVID-19 conjugates, which will migrate through nitrocellulose membrane via capillary action and forms a burgundy-colored band at the test line IgM and/or IgG. The absence of colored test bands indicates a negative result. The control line C serves as a procedure control, which will change from blue to red if the proper volume of sample has been added and membrane wicking has occurred. The manufacturer recommends using positive and negative controls as a good laboratory practice to confirm the test result. These are not provided by the company but need to be identified by the laboratory themselves. The assay takes only 10 min and must be read visually within 15 min. The PPA and NPA for the detection of anti-SARS-CoV-2 IgG and IgM antibodies were 96.7% and 98%; and, 86.7% and 99%, respectively. The independent clinical study determined the overall PPA of 100% and NPA of 97.5%.

Another rapid test, the BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test from Xiamen Biotime Biotechnology Co., Ltd., China, detects and differentiates anti-SARS-CoV-2 IgM and IgG antibodies in human serum, potassium EDTA venous whole blood and plasma (potassium EDTA) [35]. It takes only 20 min and must be read visually within 30 min. The company has SARS-CoV-2 IgG/IgM Control Set, which can be purchased separately by customers. The PPAs to PCR for the detection of anti-SARS-CoV-2 IgG and IgM antibodies in serum and plasma were 100% in samples collected from COVID-19 patients ≥15 days post onset of symptoms. The NPA to PCR for the detection of anti-SARS-CoV-2 IgG and IgM antibodies in serum and plasma were 98.46% and 100%, respectively. The FNLCR validation study demonstrated sensitivity and specificity for anti-SARS-CoV-2 IgM and IgG to be 100% and 98.8%; and, 96.7% and 97.5%, respectively. The combined sensitivity and specificity were 100% and 96.2%, respectively.

Of interest is the SGTi-flex COVID-19 IgG rapid LFIA from Sugentec, Inc., Korea, for the qualitative detection of anti-SARS-CoV-2 IgG antibodies in human serum, plasma (sodium heparin, lithium heparin, sodium citrate, and K$_3$-EDTA), and venous whole blood (sodium heparin, lithium heparin, sodium citrate, and K$_3$-EDTA). The assay principle is very similar to that of Healgen's test except that recombinant SARS-CoV-2 NP and SP RBD protein is used in the conjugate. The assay takes only 10 min, and must be read visually within 30 min. Sugentec also provides a separate SGTi-flex COVID-19 IgG Control, which contains a positive and a negative control that are prepared from processed human plasma or serum. The sensitivity (PPA) and specificity (NPA) of the test to PCR were 92.43% and 99.15%, respectively. The sensitivity (PPA) was 98.6% in samples taken from COVID-19 patients ≥15 days post onset of symptoms.

The INNOVITA 2019-nCoV Ab Test (Colloidal Gold) (IgM/IgG Serum/Plasma/Venous whole blood Combo) from Innovita (Tangshan) Biological Technology Co., Ltd., China detects and differentiates anti-SARS-CoV-2 IgM and IgG antibodies in human serum, plasma (lithium heparin, sodium citrate, and K$_2$-EDTA), and venous whole blood (lithium heparin, sodium citrate, and K$_2$-EDTA) [36]. The assay duration is 10 min, and results must be read visually within 15 min. The test strip has two windows, with their distinct sample wells, to selectively detect anti-SARS-CoV-2 IgM and IgG antibodies. The assay principle is similar to that of Healgen's test except that recombinant SARS-CoV-2 NP and S1 antigen is used in the conjugate. The T lines in the IgM and IgG result windows are coated with mouse anti-human monoclonal IgM (μ chain) antibodies and mouse anti-human monoclonal IgG (γ chain) antibodies, respectively. The control C lines in both IgG and IgM result
windows are coated with goat anti-mouse IgG antibodies. Innovita provides separate 2019-nCoV IgM Positive Control, 2019-nCoV IgG Positive Control, and 2019-nCoV IgM/IgG Negative Control, which can be purchased separately. The positive controls are lyophilized humanized anti-SARS-CoV-2 IgM and IgG antibody in negative control serum matrix while the negative control is lyophilized negative control matrix. The PPAs to PCR for detecting anti-SARS-CoV-2 IgG and IgM antibodies in K$_2$-EDTA plasma samples collected from COVID-19 patients $\geq$15 days post onset of symptoms were 97.78% and 97.67%, respectively. The NPA to PCR for the detection of anti-SARS-CoV-2 IgG and IgM antibodies was 100%. The FNLCR validation study showed sensitivity and specificity for anti-SARS-CoV-2 IgM and IgG w.r.t. the comparator method to be 93.3% and 98.8%; and, 93.3% and 98.8%, respectively. The combined sensitivity and specificity were 100% and 97.5%, respectively.

WANTAI SARS-CoV-2 Ab Rapid Test is a novel LFIA for the qualitative detection of total anti-SARS-CoV-2 Ab in human serum, venous whole blood, and plasma (K$_2$-EDTA, sodium citrate and lithium heparin). The assay format is similar to that of Healgen test except that SARS-CoV-2 SP RBD antigens are coated at the test (T) and control (C) zones, and colloidal gold particles are conjugated to recombinant SARS-CoV-2 SP RBD antigens. The company provides separate lyophilized positive and negative controls that can be bought separately. The positive control comprises monoclonal mouse anti-SP RBD antibodies in newborn calf serum buffer. The assay duration is 15 min, and results must be read visually within 20 min. The PPA and NPA to PCR were 94.7% and 98.89%, respectively. The PPA to PCR in samples collected from COVID-19 patients $\geq$15 days post onset of symptoms was 91.67%. The FNLCR validation study showed sensitivity (PPA) and specificity (NPA) to be 100% and 98.8%, respectively.

4. Challenges and future directions

Various novel IVD assay formats, based on reverse transcription loop-mediated isothermal amplification (RT-LAMP), lab-on-a-chip, microfluidics, biosensor, multiplex detection, and POC technologies, are being chased by many groups. An automated, fully integrated POC COVID-19 assay, which can perform molecular, Ag, and Ab testing on a single bioanalytical platform, would be a breakthrough. The smartphone-based POC electrochemical test for SARS-CoV-2 biomarkers, similar to the iHealth Align device developed by iHealth, USA, for blood glucose monitoring [42], would be a very useful test for pandemic response. The rapid and accurate early-stage diagnosis of people infected with the SARS-CoV-2 is critical to decreasing the spread of COVID-19.

Apart from the RT-PCR based IVD tests performed in central clinical laboratories, the rapid Ag tests have played a significant role in extending the outreach of COVID-19 diagnostics to remote, decentralized, and POC settings [43]. Many well-performing rapid Ag tests have been approved by FDA under EUA and many countries for the self-testing of COVID-19. There are concerns regarding the performance of rapid Ag tests and it has been shown that they are not as accurate as RT-PCR tests. But there is no doubt that they are still very useful in pandemic response as they have enabled the mass screening of the population and helped in identifying many COVID-19 positive cases. The serology tests to detect anti-SARS-CoV-2 Ab provide the desired information to the healthcare providers about the immune status of the COVID-19 patients and convalescent subjects.

The clinical accuracy of all COVID-19 tests needs to be stringently evaluated and constantly checked. The regulatory bodies and clinical authorities should analyze if the approved IVD tests are still working properly in all the patients or they are impacted by any specific SARS-CoV-2 mutation(s). Several safety alerts have been issued by
the FDA where certain molecular tests impacted by SARS-CoV-2 mutations have been recalled from the market. Additionally, FDA has recalled a large number of COVID-19 tests due to the lacking clinical validation data or performance. The discovery and use of novel biomarkers for the diagnosis of SARS-CoV-2 infection at an early stage could further lead to a novel IVD assay that would be ideal for pandemic response.

There is a need for more extensive research so that novel rapid and highly sensitive diagnostic technologies for the POC detection of SARS-CoV-2 infection with good analytical performance could be developed. The early-stage detection of SARS-CoV-2 infection would enable the healthcare professionals to intervene early and prevent the spread of infection.

The sensitivity of rapid LFIA tests could be further enhanced by employing new nanomaterial labels [44], which could lead to much lower limit of detection for the detection of specific analytes in the patient sample. Colloidal gold nanoparticles (NPs) has been the most used in commercial LFIA rapid tests due to the availability of large number of conjugation and immobilization chemistries, easy synthesis and low cost. However, many prospective nanomaterial labels have been demonstrated to provide much higher sensitivity. These include quantum dots, up-conversion NPs, time-resolved fluorescence NPs, surface enhanced Raman scattering active NPs, magnetic NPs, carbon nanotubes and carbon NPs. In case of COVID-19, there is a need to detect very low concentrations of NP at pg/mL level, which is just at the limit of detection of conventional LFIA formats that are being used commercially in rapid tests. It is possible to increase the accuracy of detection at such low levels by employing such novel nanomaterial labels, which have been demonstrated by several researchers [44]. Several other biomarkers that are being investigated for the early-stage detection of COVID-19, such as cytokines, are also present at very low levels in the patient samples. Therefore, there is a requirement of very high sensitivity for analyte detection. Further, a large number of smartphone-based colorimetry, fluorescent and chemiluminescent readers for rapid LFIA have already been developed by many companies and groups [45–47], which would be ideal for POC readout of rapid tests and rapid transmission of test results to a dedicated healthcare server or Cloud. The current generation of smartphones have advanced imaging, processing, connectivity, and other characteristic features, which would be highly effective in the development of next generation of innovative IVD and healthcare technologies for pandemic response.

5. Conclusions

Several IVD assays have been developed for the diagnosis of COVID-19. The gold standard for the confirmatory clinical diagnosis is the RT-PCR assay, while the mass population screening has only been feasible with rapid Ag tests. The rapid molecular tests have further extended the RT-PCR based COVID-19 diagnosis at POC settings. The POC molecular test (ID NOW™) from Abbott is a breakthrough in IVD testing. A large number of serology IVD tests, such as automated CLIA, manual ELISA, and LFIA, have further enabled the detection of anti-SARS-CoV-2 Ab in COVID-19 patients and suspects. The ongoing research efforts and continuous advances in the field and complementary technologies will lead to improved IVD tests for COVID-19 in the near future.

Conflict of interest

The authors declare no conflict of interest.
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Biotechnology to Combat COVID-19

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