


REVIEW



Diagnosis for COVID-19: current status and future prospects

MD Alamgir Kabir ^{a,b}, Rajib Ahmed^c, Sheikh Muhammad Asher Iqbal^{a,b}, Rasheduzzaman Chowdhury^d, Ramasamy Paulmurugan^c, Utkan Demirci^c and Waseem Asghar^{a,b,e}

^aFlorida Atlantic University, Boca Raton, FL, USA; ^bCollege of Engineering and Computer Science, Boca Raton, FL, USA; ^cCanary Center at Stanford for Cancer Early Detection, Department of Radiology, Stanford School of Medicine, Palo Alto, CA, USA; ^dUniversity of California, San Francisco, CA, USA; ^eDepartment of Biological Sciences (Courtesy Appointment, Florida Atlantic University, Boca Raton, FL, USA

ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19), a respiratory illness caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), had its first detection in December 2019 in Wuhan (China) and spread across the world. In March 2020, the World Health Organization (WHO) declared COVID-19 a pandemic disease. The utilization of prompt and accurate molecular diagnosis of SARS-CoV-2 virus, isolating the infected patients, and treating them are the keys to managing this unprecedented pandemic. International travel acted as a catalyst for the widespread transmission of the virus.

Areas covered: This review discusses phenotype, structural, and molecular evolution of recognition elements and primers, its detection in the laboratory, and at point of care. Further, market analysis of commercial products and their performance are also evaluated, providing new ways to confront the ongoing global public health emergency.

Expert commentary: The outbreak for COVID-19 created mammoth chaos in the healthcare sector, and still, day by day, new epicenters for the outbreak are being reported. Emphasis should be placed on developing more effective, rapid, and early diagnostic devices. The testing laboratories should invest more in clinically relevant multiplexed and scalable detection tools to fight against a pandemic like this where massive demand for testing exists.

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1. Introduction

Coronaviruses (CoVs) belonged to the *Coronaviridae* family and had already caused two severe and large-scale outbreaks in humans in the last twenty years [1–3]. The severe acute respiratory syndrome (SARS) first emerged in 2003, while the Middle East respiratory syndrome (MERS) emerged in 2012 as the contributing representative of acute respiratory distress with a high fatality rate [2,4,5]. In December 2019, a new coronavirus emerged in Wuhan Province, China, which caused in patients: respiratory distress, fever, cough, shortness of breath, ‘atypical pneumonia’ [6] and was initially termed as 2019-novel coronavirus (2019-nCoV) [6–8]. 2019-nCoV is now a major public health concern globally [9]. The World Health Organization (WHO) officially named the disease as COVID-19 (**CO**rona **V**irus **D**isease – 2019), and the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses named the virus as SARS-CoV-2 [10].

As of 13 February 2021, SARS-CoV-2 has infected more than 107 million people and claimed the lives of over 2,373,000 people globally [9,11]. Today’s globalization of economic activity and frequent traveling across the borders are likely the main reasons for the worldwide spread of this respiratory virus [12]. In response to the outbreak, initially on 30 January 2020, WHO declared the disease as a Public

Health Emergency, and later on 11 March 2020, as a ‘pandemic’. The asymptomatic behavior and ease of transmission (Figure 1) *via* respiratory droplets, feces, and close contacts made it more crucial to control its widespread transmission [13–15]. Although the disease is transmitting at an alarming rate, the disease symptoms [16], evolution [17,18], transmission dynamics [18], molecular testing [19,20], and genome sequence of the virus [21] were evaluated within weeks after the initial reports.

Accurate and fast diagnosis of the disease plays the most crucial role in mitigating the virus spread (Figure 1). Currently, reverse transcriptase polymerase chain reaction (RT-PCR) based molecular diagnostic method and computed tomography (CT) based medical imaging technologies are being used for diagnosing the COVID-19 infection and pathological status, respectively, in clinical settings [16,22]. However, RT-PCR is time-consuming and can often result in false negatives in lower viral load and limit monitoring the disease progress [22]. Moreover, higher logistical periods and supports related to sample collection and sending it to test facilities potentially raise the testing turnaround time to a few days even though only RT-PCR testing requires 3–6 hours. CT Imaging can be used as an auxiliary method to initially diagnose COVID-19

Article highlights

- (1) The etiology of SARS-CoV-2 and clinical manifestations of COVID-19 are highlighted.
- (2) The conventional and state of the art biosensing approaches for diagnosing COVID-19 are emphasized.
- (3) FDA-approved diagnostics techniques with their sensing mechanism and performance are compared.
- (4) Diagnostic management and selection of detection tools to face the future emergence of the virus are also discussed.

infection and monitor disease progression [22]. Besides, antigen antibody-based serological tests play a promising role for rapid detection, especially under resource constraint settings as well as point of care. Yet, they lack early disease detection capabilities as well as sensitivity [23].

This article aims to review primarily SARS-CoV-2 virus etiology with its detection strategy for COVID-19. This review discusses phenotypic features, routes of transmission, existing laboratory-based technologies in the market, recent development in state of the art diagnostic technologies applicable for point of care, FDA-approved diagnostic techniques with their sensing mechanism, performance as well as updating the topics previously covered in other reviews [24–29]. This review will be concluded by discussing the gaps within the diagnostics mechanism and disease management and how to overcome those limitations to do proper management and prevention of the disease. This review will not discuss Computed Tomography (CT), Next-generation sequencing technologies due to unconventional use in laboratories. The review also discusses articles focusing on development in detection tools submitted to preprint repositories to be compatible with the timely and most recent updates.

2. Phenotype, structure, and evolution

Previously, six coronavirus species from the *Coronaviridae* family have shown the potential to cause mild (*HKU1*, *NL63*, *OC43*, and *229E*) to acute (SARS-CoV and MERS-CoV) respiratory diseases in human; SARS-CoV-2 is the seventh CoV to infect humans responsible for acute respiratory disorder [30]. CoVs can be divided into four genus (α - β - γ - δ -CoV), and SARS-CoV-2 belongs to β genus due to its potential to infect mammals [31,32]. The clinical manifestations of COVID-19 are similar to the common cold with symptoms like fever, dry cough, myalgia, and fatigue. Occasionally, there are enteric symptoms, including acute dyspnea and anorexia [33]. However, atypical symptoms such as diarrhoea, nausea, and even abdominal pain can also occur [34].

The initial patients from the Wuhan outbreak epicenter are elderly males and mostly connected to the seafood market or closely related to the infected patients; CT imaging showed ground glass opaque like shadows in their lungs even though this was also evident with postmortem CT examination for dead patients [35,36]. Respiratory distress syndrome occurs due to the impairment of respiratory cilia by the SARS-CoV-2 on human bronchial epithelial cells [37]. The histological examination of COVID-19 infected dead patients exhibited extensive microvascular damage, with diffuse alveolar damage, including vascular congestion [33]. However, on the other hand, in Germany, asymptomatic cases were also reported, increasing the infection risk to another person without knowing [38–40]. In Iceland, a large number of volunteered testing revealed 43% of the asymptomatic cases [41]. Thus, the infected individuals' asymptomatic appearance endangers the screening process and attests that rigorous testing is required to understand the infection's actual spreading. The viral genome of SARS-CoV-2 has been sequenced to have 29,891 nucleotides, including 9860 amino acids with 79%

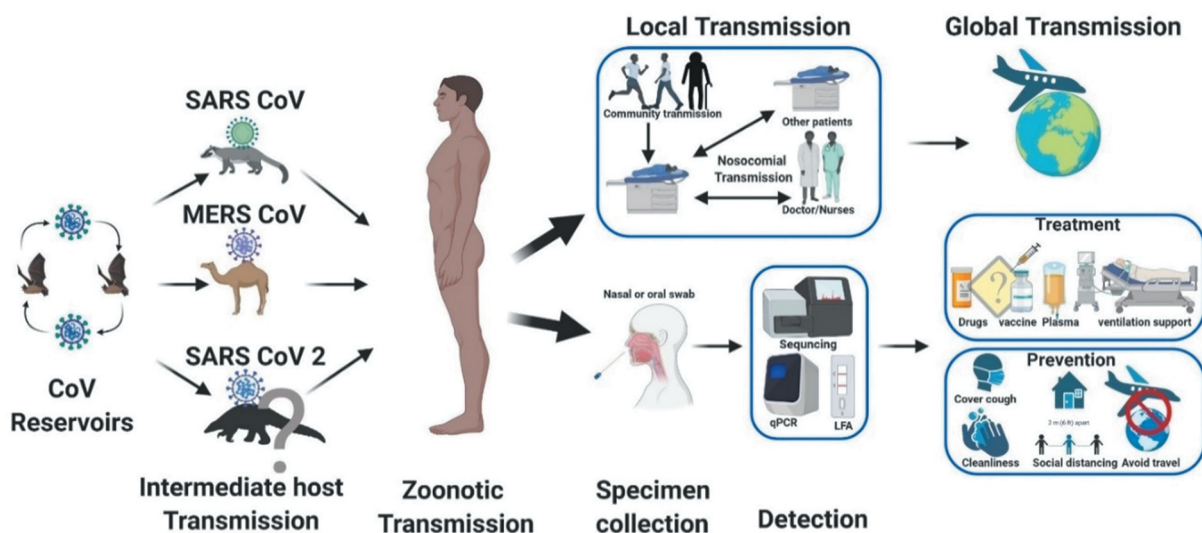


Figure 1. The schematic illustration of emergence of SARS-CoV, MERS-CoV, and SARS-CoV-2 and their diagnosis, treatment, and prevention mechanism. The deadliest human coronaviruses are SARS-CoV, MERS-CoV and SARS-CoV-2, all of which likely have evolved from a common origin in bats. Interestingly, all three of them were likely introduced into human populations through different intermediate zoonotic hosts, for example, SARS CoV via palm civets, MERS CoV via camel, and SARS-CoV-2 via pangolins. The spreading of the virus is related to close contacts with infected patients, nosocomial transmission in healthcare providers as well as fast global spread by intercontinental travel.

pairwise genomic level identity to human SARS-CoV [42] and 50% identity to human MERS-CoV [43]. Due to the higher sequence identity with SARS-CoV, this new virus was named SARS-CoV-2. The whole-genome sequence has 96% similarity with bat coronavirus; thus, it was considered a zoonotic transformation from one of the evolved strains from bat [44,45]. Even though bats are likely reservoir hosts, another study suggests that Pangolins may also act as an intermediate reservoir of CoVs before transmitting to humans [46,47]. CoVs are positive RNA viruses that have the most extensive known non-segmented RNA genomes of ~30 kb length. RNA viruses are more prone to mutation with a mutation rate ($\approx 10^{-4}$) during replication, which substantially makes them more adaptable for survival [48,49].

SARS-CoV-2 has a diameter of 100 to 160 nm in size [50–52]. The SARS-CoV-2 genome (Figure 2(A)) has two flanking untranslated regions (UTR): the 5′-cap structure and 3′-poly-A tail. The full genome encodes 27 proteins containing eleven open reading frames (ORFs) including a single long open reading frame encoding a polyprotein (ORF) 1a/b which is nonstructural, and four structural proteins including spike glycoprotein (S), envelope (E), membrane (M), and nucleocapsid (N) [53–56]. Wang et al. analyzed 95 full-length genomic sequences of SARS-CoV-2 isolates from different databases and found very high (99.99%) homology between all the viral isolates with the highest homology in the ORF region [57]. The ORF region includes RNA-dependent RNA polymerase (RdRp) enzyme, an essential enzyme for viral replication [58]. The four structural proteins are very significant for assembly and infection

of the host cell. The S protein that expresses on the surface of the SARS-CoV-2 has a receptor binding domain (RBD) similar to SARS-CoV. S protein has S1 and S2 subunits, which are crucial for binding to cell receptors (Figure 2(B)) during viral entry (S1) and membrane fusion (S2) [59]. S1 unit of the S glycoprotein appears to interact with the peptidase domain of angiotensin-converting enzyme 2 (ACE2) of host cells, regulating cross-species or human to human transmission [60,61]. Although the S1 subunit of SARS-CoV-2 has 70% homology to SARS-CoV, SARS-CoV-2 exhibits substantially higher binding affinity for its interaction with ACE2 than SARS-CoV [62,63]. The N protein consisting of two domains binds with the RNA genome to make up nucleocapsid. The M protein, which contains three transmembrane domains, acts as a central organizer, determines the shapes of the virions, and binds with the nucleocapsid. Finally, the E protein plays a role in creating the virus's envelope membrane by interacting with M protein [48].

3. Transmission, incubation, and stability

Understanding the SARS-CoV-2 transmission dynamics and media by which it transmits are the most critical factors to develop preventive measures and ensure healthcare providers' safety and implement proper public healthcare actions. Typically, SARS-CoV-2 is spread over by respiratory droplets, direct contact, aspirates, and aerosols transmission from the infected person [35,64]. Even asymptomatic or infected individuals, before exhibiting symptoms, can also transmit the virus [65]. Fecal swabs testing from an acute pneumonia patient

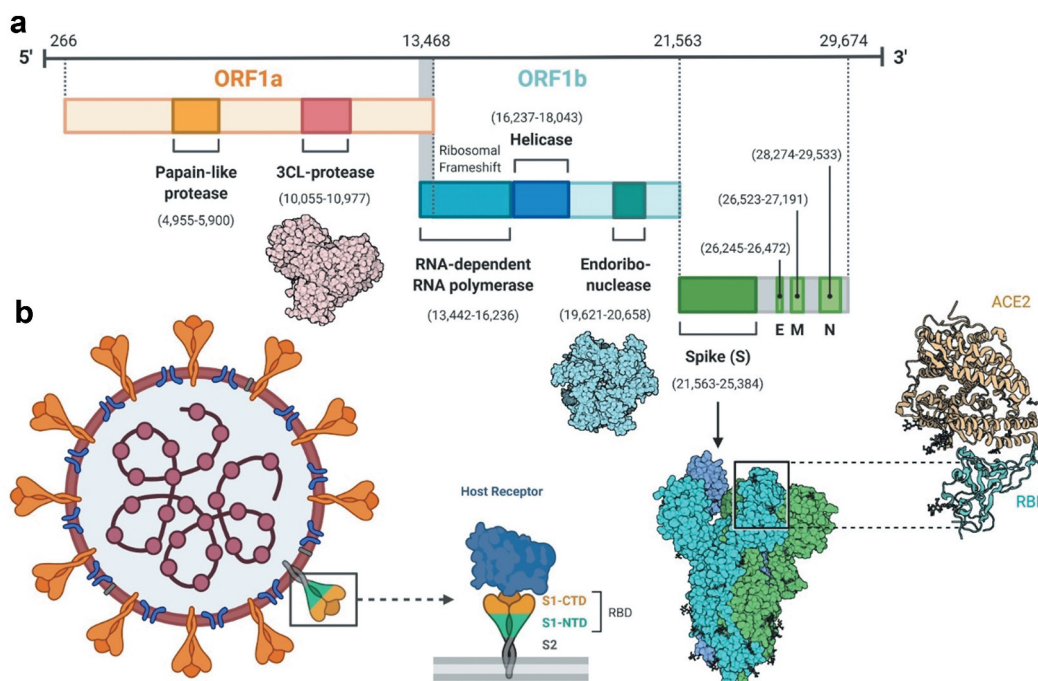


Figure 2. Genome and structure of SARS-CoV-2 revealing mechanism of SARS-CoV-2 infection via its spike protein (S). **A**) The SARS-CoV-2 genome is a non-segmented, positive single-stranded RNA genome, with an approximate size of 30 kb. The full genome arranged in an order of 5′ UTR, replicase genes, four structural proteins including spike glycoprotein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N) with a 3′ poly-A tail. **B**) The spike protein of the SARS-CoV-2 consisting of two subunits S1 and S2 mediates membrane fusion by binding primarily to angiotensin-converting enzyme 2 (ACE2) receptor of the host cells. (Figure 2 (A,B)) are created with Biorender.com).

prove that SARS-CoV-2 has fecal-oral transmission potentiality [6]. The human transmission was reported among the family members or nearby contact persons with infected individuals [66]. A study inside a hospital recently revealed that SARS-CoV-2 could become airborne inside a confined medical facility, especially where infected patients are treated [67]. At the same time, SARS-CoV-2 RNA traces were found on the floor, trash bins in addition to commonly touched areas inside the medical care facility. Droplets containing viruses expelled from both symptomatic and asymptomatic individuals can be transported by aerosol in the environment for up to 4 m and increases infection risk to close contacts [67,68]. Moreover, SARS-CoV-2 can also sustain 48 hours on stainless steel and 72 hours on plastic [69]. Coming into contact with the infected surface and then touching the contaminated hand to mouth, nose, or eyes can potentially deposit the virus to upper respiratory tract, which can penetrate to the depths of the lungs. SARS-CoV-2 is highly stable in lower temperatures (4°C) for 14 days, whereas it could survive 24 hours at 37°C [70]. However, traces of SARS-CoV-2 RNA were found after 17 days of evacuation on the Diamond Princess cruise ship in cabin surfaces contaminated by both symptomatic and asymptomatic carriers, although their viability was undefined [71]. The mean incubation period for SARS-CoV-2 is around 5.1 days (95% CI, 4.5 to 5.8 days), and typically, the infected person starts showing symptoms within 8.2 to 15.6 days with an average of 11.2 days [72]. At the earlier stage of the outbreak, the basic reproductive number or rate of infection of an epidemic (R_0) was considered to be 2.2 (95% CI: 1.4 to 3.9) which means one infected person can infect another 2.2 persons based on the epidemiological data analyzed from 425 confirmed cases [18]. Effective rapid diagnosis of the symptomatic and asymptomatic patients, as well as isolating the infected persons from the non-infected person, can potentially reduce the R_0 , but accurate determination of R_0 is still not possible yet due to inaccurate detection of asymptomatic

patients [73]. An effective way for pandemic mitigation efforts is to introduce travel controlling measures, increase the broader screening for travelers, implementing social distancing, and intervening nosocomial infections by maintaining proper safety protocols.

4. Advances in SARS-CoV-2 diagnosis

Just after the initial report of a cluster of Pneumonia in Wuhan province, China, a group of scientists reported this Pneumonia had emerged due to a new type of virus. They sequenced the genome sequence of the virus using the metagenomic RNA sequencing method from a patient's bronchoalveolar lavage fluid, which was deposited to the GenBank sequence repository (accession number MN908947) [53]. Two other groups were also deposited SARS-CoV-2 genome sequences within a short time after the beginning of the first outbreak [21,42]. Researchers worldwide started analyzing and designing primers for detecting the viral genome effectively and rapidly with the publicly available sequences. For clinical detection of the virus, typically, nasopharyngeal swabs are being tested by nucleic acid amplification method at an early stage of disease onset to measure the presence of viremia (Figure 3) [35]. Other specimens from the patients can also be used for detection of the SARS-CoV-2 RNA including bronchoalveolar lavage fluid (BLF), blood, sputum, feces, urine, oropharyngeal swabs, and even fibro bronchoscope brush biopsies [74]. Besides RT-PCR testing, SARS-CoV-2 specific antibodies can also be an excellent alternative to test people with previous infection or asymptomatic carriers in different rapid and cheaper methods. Usually, Immunoglobulin M (IgM) works as a first defender for any invader into a human body, and usually starts to populate in blood after 3–5 days of infection [75]. Moreover, the Immunoglobulin G (IgG) antibody starts to generate after a week of disease onset, showing very high affinity and adaptive response. As a result, it also represents a detection marker for COVID-19 diagnostics. The IgG response can persist in the

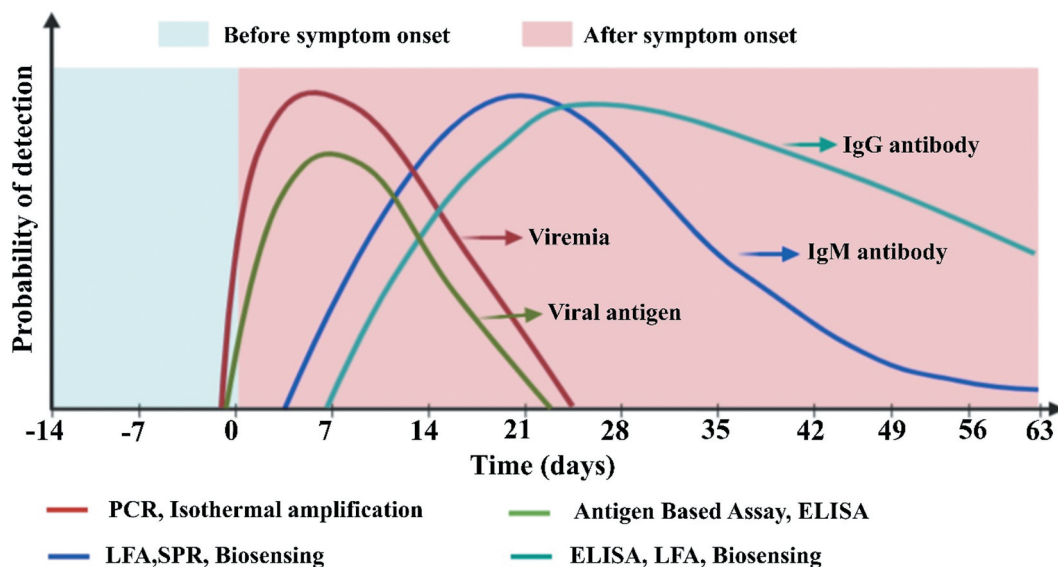


Figure 3. Summary of immune response timeline to the infection of the SARS-CoV-2 virus.

Tentative time intervals of viral load level as well as the immune response to generate IgG and IgM antibodies to fight against the presence of SARS-CoV-2 virus in the infected patient. It also envisages the potentiality of detection mechanism with various diagnostic methods based on the presence of different biomarkers.

human body for a long time, indicating previous infections (Figure 3) [76].

This section discusses different approaches currently used for diagnosing the COVID-19 viral agent, including different approaches of molecular, serological, and rapid antigen-based tests.

4.1. Reverse transcriptase polymerase chain reaction based assays

Polymerase chain reaction (PCR) is considered to be the gold standard for detecting infectious pathogens, and it has been used successfully to detect CoVs in earlier outbreaks [77–82]. PCR is highly sensitive to DNA/RNA target as the primers specifically bind to the viral genome's specific region. Initially, in response to the outbreak of SARS-CoV-2, reverse transcriptase-polymerase chain reaction (RT-PCR) testing (Figure 4(A)) has been used as a frontline quantitative testing method in laboratories all over the world. There are three steps for performing the PCR experiment: (1) Collection and preprocessing of samples from the infected patients, (2) RNA extraction followed by cDNA synthesis by reverse transcriptase technique and (3) amplification using target-specific primers and detection using real-time quantitative PCR method. Quantitative PCR can quantify the viral RNA based on a known source utilizing a fluorescence signal. Alternatively, qualitative PCR gives a fluorescence signal but does not quantify the SARS-CoV-2 RNA [83]. Although the viral dynamics of the COVID-19 patients are still to be determined, preliminary studies reported that the concentration of viral RNA in sputum is 10^6 – 10^{11} copies/mL, nasopharynx 10^6 – 10^9 copies/swab, Oral 10^4 – 10^8 copies/swab, and stool 10^4 – 10^8 copies/g (Figure 4(B)) [84–86]. The amount of viral RNA remains similar for severe cases for both Bronchoalveolar lavage fluid (BLF) and sputum samples, but the sputum sample showed a peaked amount of viral load after 8 days of symptom onset [87].

Nevertheless, throat swabs showed no viral particle 8 days after symptom onset; alternatively, stool samples were highly detectable after 14 days of disease onset [86–88]. However, multiple

specimen testing from an infected patient is recommended because RNA copies can vary specimen-wise at the same time of infection. Although the detection's timeline varies for each patient, RT-PCR is considered the gold standard detection method for SARS-CoV-2 at an early stage of disease onset (Figure 3).

WHO first developed and shared globally the RT-PCR protocol and primers targeting the E gene for screening and RNA-dependent RNA polymerase (RdRp) gene for confirming the specificity of SARS-CoV-2 [89]. The designed method was successful in distinguishing SARS-CoV-2 from SARS-CoV [90]. China CDC initially designed primers targeting ORF1ab and N genes for detecting viral RNA using the RT-PCR method and made the protocols publicly available for widespread distribution [91]. Another group from the University of Hong Kong developed a detection tool targeting ORF1b-nsp14 and N genes of the SARS-CoV-2 RNA [92]. After a few days, a group of researchers from the National Institute of Infectious Diseases, Japan, developed RT-PCR assays targeting multiple targets, including ORF1ab, N, and Spike protein [93]. All these detection schemes pioneered the further development of commercializing kits all around the world.

Until 10 August 2020, the National Drug Administration of China has given emergency use approval to 11 molecular devices based on fluorescence PCR method, joint probe anchored polymer sequencing method, and isothermal amplification method [94]. Centers for Disease Control and Prevention, USA also developed a protocol for RT-PCR-based laboratory detection targeting three targets of N genes approved by the Food and Drug Administration (FDA), USA, on 4 February 2020 [20,95]. In brief, viral RNA was isolated and purified from the upper and lower respiratory specimens, followed by mixing with a mastermix. The mastermix contains forward and reverse primers that target specifically the N gene, and the probe primer targets the RdRp gene. The mixed reagents are then loaded into the PCR thermocycler, and the stepwise incubation temperatures are set to perform the assay [95].

The FDA has approved more than two hundred molecular-based diagnostics devices (Table 1) till 27 December 2020;

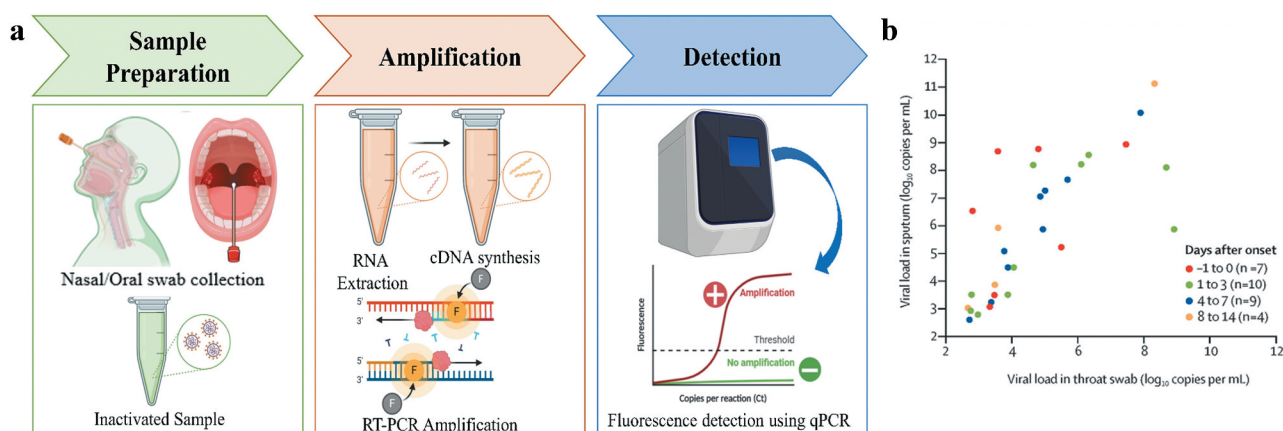


Figure 4. Schematic workflow of COVID-19 Diagnostic Test using RT-PCR. A) Nasal or oral swabs are collected from infected patients containing upper and lower respiratory tract fluids. The collected samples are preserved on specific temperatures and send to labs for testing. Viral samples are deactivated by heat, RNA is isolated using RNA extraction kit and amplified using specific primers targeting segments of different genes (E, N, ORF1ab, and RNase P). Based on the amplified fluorescent signal, test results are interpreted. B) Correlation of a high number of viral loads present in sputum samples than throat swabs on different stages of infection for 30 hospitalized patients (Reprinted from [86]).

most of the molecular test-based assays follow the characteristics of conventional RT-PCR discussed above [20]. The primers included target regions, specially ORF1ab (RdRp), N, E, S, and ORF8 genes. The limit of detections varies from test to test but they showed highly specific detection capability. Some other molecular diagnostic assays are still developing and waiting for FDA approval. All these devices are approved for *in vitro* analysis under emergency usage guidelines and used in designated laboratories to test COVID-19 in the USA. All the RT-PCR-based assays approved by FDA provide qualitative results, including the Accula™ SARS-CoV-2 test that provides visual detection using a lateral flow-based strip within 30 mins [96]. Both FastPlex Triplex SARS-CoV-2 Detection Kit and Bio-Rad SARS-CoV-2 ddPCR test use the droplet-based digital PCR technology to qualitatively detect SARS-CoV-2 combined with Taqman probes [97,98]. Thousands of water-in-oil emulsion nanoliter-sized droplets containing SARS-CoV-2 RNA are randomly partitioned and organized by an automated droplet generator. The emulsions droplets are subjected to amplification using RT-PCR. Interestingly, IntelliPlex™ SARS-CoV-2 Detection Kit uses π Code microdisc-based technology for bio-conjugation, washing, and automated detection combining with RT-PCR [99]. π Code microdisc can generate thousands of discrete image patterns to ensure the specific capture of target analytes from a pool of samples in a single well. However, FDA has approved two multiplexed RT-PCR assays developed by BioFire (BioFire® Respiratory Panel 2.1 (RP2.1)) and QIAGEN (QIAstat-Dx® Respiratory SARS-CoV2 Panel) for the detection of 22 pathogens, including viruses and bacteria [100,101]. BioFire RP2.1 provides real-time detection of pathogens using nested PCR method within 45 mins, and a disposable pouch accommodates all the RNA/DNA extraction to amplification reagents [100]. On the other hand, QIAstat-Dx® also uses a pre-loaded and self-contained cartridge to provide true walk-away operation by automatically transfer samples and fluids *via* the transfer chamber with the help of pneumatic pressure [101]. However, the BioCode® SARS-CoV-2 Assay developed by Applied BioCode uses bar-coded magnetic beads that immobilize viruses by capturing with specifically designed DNA probes that can be amplified using RT-PCR [102]. Moreover, the FDA has approved two other devices that utilize mixed detection and sensing technologies with the combination of RT-PCR method [103,104]. Accula SARS-CoV-2 Test is a rapid (30 mins) which facilitates lysis, reverse transcriptase, amplification, and detection inside a reagent preloaded test cassette [103]. The Accula Dock houses the test cassette as well as controls the temperature, timing, and transfer of fluid. The detection of the SARS-CoV-2 RNA is visualized in a lateral detection strip. The ePlex SARS-CoV-2 testing device, a true Sample-to-Answer solution, provides qualitative detection of the SARS-CoV-2 RNA in a single-use cartridge [104]. Target-specific samples are amplified with the PCR technique. Still, the detection of the amplified target evaluates the voltammetry signal change of a hybridization complex of gold electrode, capture DNA probe, and ferrocene-labeled signal probe.

Canada has authorized almost 100 COVID-19 testing devices (including control, specimen, and assay Kit) based on nucleic acid amplification technology, mostly developed by

US, Germany, South Korea, and Canada-based companies till 27 December 2020 [105]. Higher specificity and significantly lower limit of detection are the main advantages of PCR-based assays. However, the detection limit varies mostly from one testing device to another; thus, high false negative could result. To lower the false negative results and increase sensitivity, multiple types of specimens can be tested as distribution of viral load varies in specimens collected from different sites [74]. Most of PCR assays require a thermocycler, which is costly equipment that makes it unsuitable for POC use. Further, skilled and trained medical personnel are required to collect the specimens and run the assays; hence home/POC testing may not be feasible.

4.2. Isothermal amplification based assays

The PCR based assays require multiple temperature steps to amplify the nucleic acids. As an alternative to PCR, isothermal amplification-based assays have been developed to detect the viral RNAs. Isothermal-based assay does not require an expensive thermocycler for amplification and demonstrates high sensitivity and specificity with the potential to be used in resource-limited settings. Conventional isothermal-based assays include loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), Transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), and rolling circle amplification (RCA).

4.2.1. Loop-mediated isothermal amplification (LAMP) based assay

Loop-mediated isothermal amplification (LAMP) is a highly specific, sensitive, and rapid amplification method that amplifies the DNA/RNA with the help of four to six primers, which bind distinctively six regions of the target genome under a constant temperature of 60–65°C [106–108]. The RT-LAMP method includes step-by-step process for amplifying the viral RNA and starts with collecting the SARS-CoV-2 patient's sample. The samples are stored in virus transport media, extracted RNA is mixed with primers and mastermix, and then heated up to 60–65°C to amplify the RNA (Figure 5 (A)). Forward, reverse, inner and outer primers are the four mandatory primers required in a LAMP reaction, but a higher number of primers (e.g. loop primers) can be used to increase sensitivity and specificity to target and reduce the amplification time [107]. The LAMP-based assays can be performed in a small PCR tube, producing qualitative results visible to the naked eye by measuring byproducts of the amplification (turbidity transformation), and color change by adding pH-sensitive dye or fluorescence change by adding intercalating dyes [109]. Lu *et al.* recently developed an RT-LAMP assay targeting N (four primer sets), S (one primer set), and RdRp (one primer set) genes, among them primer set targeting RdRp gene showed highest specific detection of SARS-CoV-2 RNA with a sensitivity of three copies per reaction (Figure 5(B)) [110]. The amplification occurs at 63°C within less than 40 mins. The positive samples change pH-sensitive cresol red color from burgundy to orange or yellow and visible in naked eyes [110]. Another group of researchers developed an RT-LAMP assay targeting the ORF1a gene and S gene with

Table 1. Partial list of molecular testing devices approved by FDA for *in vitro* diagnostics of SARS-CoV-2 under emergency use authorizations [20].

| Product Name | Producer | Technology | Sample specimen | Target Gene | Readout time | Limit of Detection |
|---|--|------------------------------|--|--|---------------------------------------|--|
| CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel | Centers for Disease Control and Prevention | Real-Time RT-PCR | Nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate | N gene and RNase P gene | N/A | 10 ⁶ RNA copies/ μ L (QIAGEN DSP Viral RNA Mini Kit) 10 ⁶⁻⁵ RNA copies/ μ L (QIAGEN EZ1 Advanced XL) 25 genome copies/reaction (0.007 TCID50/mL) |
| New York SARS-CoV-2 Reverse Transcriptase (RT)-PCR Diagnostic Panel | Wadsworth Center NYSDOH | Real-Time RT-PCR | Nasopharyngeal/oropharyngeal swabs and sputa | N gene and RNase P gene | N/A | 25 genome copies/reaction (0.007 TCID50/mL) |
| cobas® SARS-CoV-2 | Roche Molecular Systems, Inc | Real-Time RT-PCR | Nasopharyngeal and oropharyngeal swab | ORF1 a/b and E gene | About three hours | 0.007 TCID50/mL |
| TaqPath COVID-19 Combo Kit | Thermo Fisher Scientific, Inc. | Real-Time RT-PCR | Nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar Lavage | ORF1ab N Protein and S Protein | Under three hours. | 10 Genomic Copy Equivalents/reaction (1x10 ⁻² TCID50/mL) |
| Panther Fusion® SARS-CoV-2 Assay | Hologic, Inc | Real-Time PCR | Nasopharyngeal and oropharyngeal swab | Two conserved regions of the ORF1ab gene | Three hours | 6.25 genome copies/ μ L |
| COVID-19 RT-PCR Test | Laboratory Corporation of America | Real-Time RT-PCR | Nasopharyngeal or oropharyngeal Swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and Nasopharyngeal wash/aspirate or nasal aspirate | Three conserved regions of the N gene and RP | N/A | 968 copies/mL |
| Quest SARS-CoV-2 rRT-PCR | Quest Diagnostics Infectious Disease, Inc | Real-Time RT-PCR | Nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and Nasopharyngeal wash/aspirate or nasal aspirate | N gene (N1 & N3) | N/A | 8 x 10 ⁻¹ genomic RNA copies/ μ L |
| Lyra SARS-CoV-2 Assay | Quidel Corporation | Real-time RT-PCR | Nasopharyngeal or oropharyngeal swab | Non-structural polyprotein (pp1ab) | Less than 75 minutes after extraction | 100 virus copies/mL |
| Abbott RealTime SARS-CoV-2 assay | Abbott Molecular, Inc | Real-Time RT-PCR | Nasopharyngeal and oropharyngeal swab | RdRp and N genes | Under two hours | 1 x 10 ⁵ copies/mL |
| ePlex SARS-CoV-2 Test | GenMark Diagnostics Inc | Nucleic acid multiplex assay | Nasopharyngeal swab | - | Under two hours | 500 copies/mL |
| Simplexa COVID-19 Direct assay | DiaSorin Molecular LLC | Real-Time RT-PCR | Nasopharyngeal swabs | ORF1ab and S gene | Little more than an hour | 250 copies/mL |
| Xpert Xpress SARS-CoV-2 | Cepheid | Real-Time RT-PCR | Nasopharyngeal swab and/or nasal wash/Aspirate | N2 and E | 45 minutes | 0.33 copies/ μ L |
| Primerdesign Ltd COVID-19 genesig Real-Time PCR assay | Primerdesign Ltd | Real-Time RT-PCR | Oropharyngeal swab | ORF1ab | Under two hours. | 200 copies/reaction (Clinical) |
| Accula SARS-CoV-2 Test | Mesa Biotech Inc | PCR and lateral flow | Throat swab and nasal swab combined | N gene | 30 minutes | 3.3 x 10 ² Genomic copies/mL |
| BioFire COVID-19 Test | BioFire Defense, LLC | Real-Time RT-PCR | Nasopharyngeal swabs | ORF1ab and ORF8 | 45 minutes | 9,307 copies/mL (ORF1ab) 24,884 copies/mL (N) |
| PerkinElmer New Coronavirus Nucleic Acid Detection Kit | Perkin Elmer, Inc. | real-time RT-PCR | Oropharyngeal swab and Nasopharyngeal swab | N gene and ORF1ab | N/A | 55 copies/ μ L |
| Avellino CoVz test | Avellino Lab USA, Inc. | real-time RT-PCR | Oropharyngeal swab and Nasopharyngeal swab | N gene and RNase P | Three hours | |

(Continued)

Table 1. (Continued).

| Product Name | Producer | Technology | Sample specimen | Target Gene | Readout time | Limit of Detection |
|---|--------------------------------------|---|--|---|--|--|
| Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV | BGI Genomics Co. Ltd | real-time RT-PCR | Throat swabs and bronchoalveolar lavage fluid | ORF1ab | Three hours | 150 copies/mL |
| NXTAG CoV Extended Panel Assay | Luminex Molecular Diagnostics, Inc. | RT-PCR | Nasopharyngeal swab | ORF1ab, N, and E | Three hours | 5x10 ³ Genomic Copy Equivalents/mL |
| ID NOW COVID-19 | Abbott Diagnostics Scarborough, Inc. | isothermal nucleic acid amplification | Direct nasal, Nasopharyngeal or throat swabs and nasal, nasopharyngeal or throat swabs | RdRp segment | Positive results in five minutes and negative results in 13 minutes. | 125 Genome Equivalents/mL |
| NeuMoDx SARS-CoV-2 Assay | NeuMoDx Molecular, Inc. | real-time RT-PCR | Nasal, nasopharyngeal and oropharyngeal specimens | (Nsp2) gene and N gene | Just over an hour | 150 copies/mL |
| QIAstat-Dx Respiratory SARS-CoV-2 Panel | QIAGEN GmbH | real-time PCR | Nasopharyngeal swabs | Orf1b poly gene (RdRp gene) and E genes | About an hour | 500 copies/mL |
| qSARS-CoV-2 IgG/IgM Rapid Test | Cellex Inc | Lateral flow assay | Serum, plasma or venipuncture whole blood | SARS-CoV-2 recombinant antigens conjugated with colloidal gold N gene and RNase P | 15–20 minutes | - |
| COV-19 IDx assay | Ipsium Diagnostics, LLC | Real-time RT-PCR | Nasopharyngeal and oropharyngeal swab | N1, N2 genes and RNase P | 24 hours | 8.5 Genome copies/ μ L |
| BioGX SARS-CoV-2 Reagents for BD MAX System | Becton, Dickinson & Company (BD) | Real-time RT-PCR | Nasopharyngeal and oropharyngeal swab | N1, N2 genes and RNase P | Under three hours | 40 genome equivalents/mL |
| ARIES SARS-CoV-2 Assay | Luminex Corporation | Real-time RT-PCR | Nasopharyngeal swabs | ORF1ab, N and RNase P | Two hours. | 7.5 x10 ⁴ Genomic Copy Equivalents/mL |
| ScienCell SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit | ScienCell Research Laboratories | RT-qPCR | nasal, nasopharyngeal, oropharyngeal swab specimens, and bronchoalveolar lavage f | N1, N2 genes and RNase P | Under three hours | 10 ⁶⁻⁵ copies/ μ L. |
| Logix Smart Coronavirus Disease 2019 (COVID-19) Kit | Co-Diagnostics, Inc. | Real-time RT-PCR | Bronchoalveolar lavage, sputum, tracheal aspirate and nasopharyngeal and oropharyngeal swabs | RdRp gene | Less than two hours. | 4.29 copies/ μ L |
| Gnomegen COVID-19 RT-Digital PCR Detection Kit | Gnomegen LLC | Real-time digital PCR | Nasal, nasopharyngeal, and oropharyngeal swab | N1, N2 genes and RNase P | More than three hours | 8 Genomic Copies/reaction |
| Smart Detect SARS-CoV-2 rRT-PCR Kit | InBios International, Inc | Real-time digital PCR | nasopharyngeal swab, anterior nasal swab and mid turbinate nasal swab | E gene, N gene and ORF1b | about four hours. | 1.1 x 10 ³ genome equivalents/mL |
| BD SARS-CoV-2 Reagents for BD MAX System | Becton, Dickinson & Company | real-time RT-PCR | Nasal, nasopharyngeal and oropharyngeal swab | N1, N2 genes and RNase P | - | 40 genome equivalents/mL |
| Quantivirus SARS-CoV-2 Test kit | DiaCarta, Inc | real-time RT-PCR | Nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, and sputum | N, ORF1ab and E gene | within 2 hours after extraction | 100 copies/mL |
| iAMP COVID-19 Detection Kit | Atlia BioSystems, Inc. | real-time fluorescent OMEGA amplification | Nasopharyngeal and oropharyngeal swab | N and ORF1ab genes | less than 1.5 hours | 4 copies/ μ L |

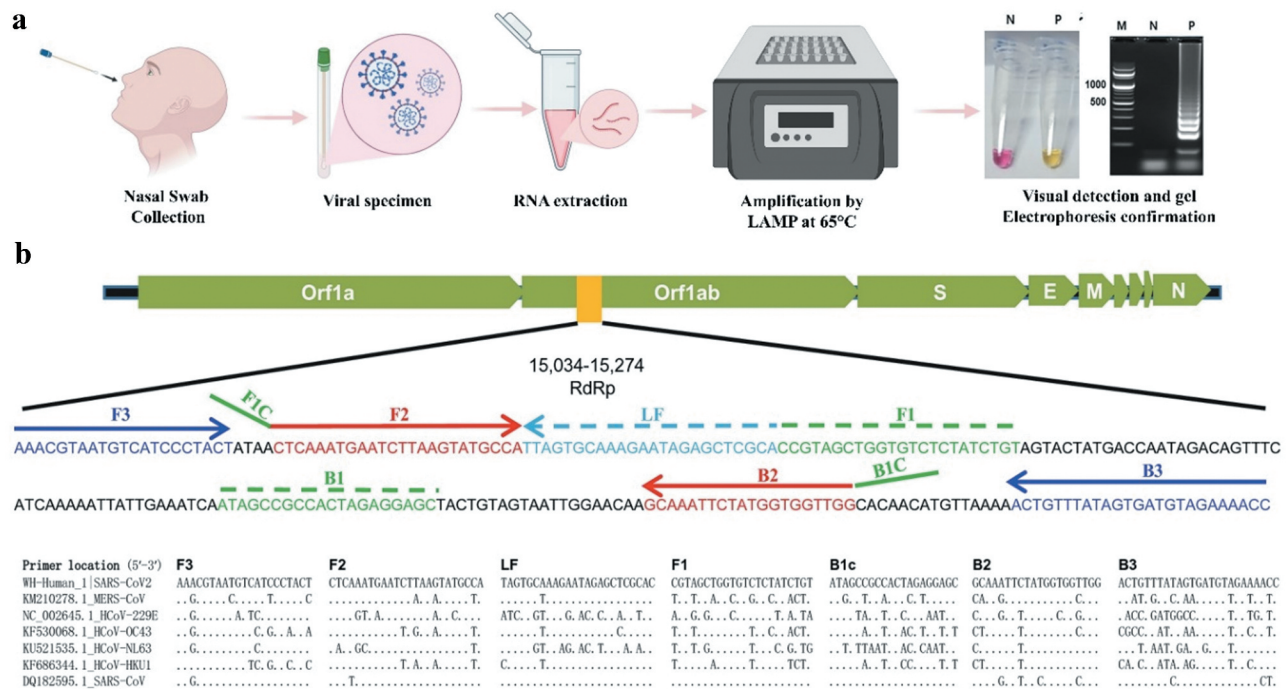


Figure 5. Detection of SARS-CoV-2 using the RT-LAMP method. A) Respiratory fluids are collected by using a nasal or oral swab from the infected patients, and RNA of SARS-CoV-2 are collected from the specimen, and LAMP primer and reaction agents are added, followed by amplification at 60–65°C. The RT-LAMP product's final outcome represents positive reaction changed color from pink to yellow due to the decrement of pH of target DNA polymerase reaction, which is easily confirmed by naked-eye detection. Detection of the amplified samples was visualized using gel electrophoresis (modified from [115]) **B)** Design of a set of RT-LAMP primers targeting the RdRp gene of the SARS-CoV-2. Primers sets include two inner primers containing sequence complementary, two outer primers, and two loop primers. (Reprinted from [110]).

a 100% detection sensitivity and specificity for 130 clinical samples in just about 30 mins [111]. Yang *et al.* developed an RT-LAMP assay with a 99% sensitivity and specificity equal to RT-PCR based on 208 clinical samples [112]. The assay outcome was measured by the solution's turbidity change, which was also visualized by the naked eye. Another approach for detecting SARS-CoV-2 in resource-limited settings was developed using RT-LAMP technology but integrated with nanoparticle-based biosensors to visualize the result in a lateral flow strip [113]. In the first one-step RT-LAMP reaction occurs in a single tube at 63°C targeting ORF1ab and N genes. During the amplification step, ORF1ab was labeled with FITC and biotin. On the other hand, the N gene binds with Dig and biotin. After the amplification, RT-LAMP amplicons reagents were visualized in a lateral flow-based strip. In one line, ORF1ab amplicons bind with anti-FITC antibody and N amplicons captured by an anti-Dig antibody in the second line. This method is highly sensitive (12 copies per reaction) and specific (100%). The whole sample collection to answer takes 60 mins. Separate RNA isolation and several manual steps before running the amplification reaction are the considerable drawbacks for the discussed LAMP method. However, designing LAMP primers are the more crucial compared to PCR which in terms makes it less flexible. The potentiality of primer-primer interaction is higher in LAMP as a large number of primers are used [114]. Additionally, aerosol generation at the time of sample handling also can show false positive results.

4.2.2. Recombinase polymerase amplification (RPA) based assay

RPA is another rapid isothermal amplification method, which requires a reduced number of primers than LAMP. This recombinase polymerase amplification is based on strand displacement using single-strand DNA binding protein and reaction using recombinase and polymerase enzymes [116]. RPA requires a lesser number of primers than LAMP to amplify the target exponentially. RPA method is also highly sensitive and suitable for POC-based testing as no thermocycler is needed [117]. Few approaches from academic institutes for developing diagnostic assays targeting SARS-CoV-2 using the RPA technique are being reported recently [118–121]. Though RPA-based assays allow testing in resource-limited settings, the main constraints for these assays remain the same as the LAMP method, the RNA isolation procedure. This is also limited by using multiple reagents, which need to be mixed at the time of reaction without having a master mix. On the other hand, RPA uses a very low temperature for amplification of the target; thus, primer-primer interaction can happen [122].

4.2.3. Transcription-mediated amplification (TMA) based assay

Transcription-mediated amplification (TMA) is another simple, rapid, single tube-based isothermal amplification-based assay that can create billions of copies of RNA within a concise time by using RNA polymerase and reverse transcriptase [123]. In

this method, both RNA and DNA can be targeted, utilizing two primers, and the amplicons are ssRNA. However, this method uses 37–41°C temperature with no requirement of expensive thermocyclers. This method has been used to detect different viral and bacterial pathogens [124–127]. Aptima SARS-CoV-2 assay developed by Hologic Panther recently got FDA approval for testing COVID-19 patients using TMA based detection method [128]. Magnetic microparticles are used to capture the ORF1ab gene of SARS-CoV-2 RNA from the infected patients' nasopharyngeal or oral swabs. The target is then amplified at room temperature by attaching the deoxyadenosine region on the capture oligomer and the polydeoxythymidine molecules with microparticles. Photon signals generated by the light emitting labeled acridinium ester and amplicons are measured to provide the detection signal [128]. This assay takes around ~4.5 hours to complete and another 2 hours to prepare the samples [129]. One recent study shows promising negative percentage agreement (100%) but reported 94.7% positive percentage agreement [130]. However, the assay was able to detect as low as 5.5×10^3 copies/mL [131].

4.2.4. Nucleic acid sequence-based amplification (NASBA) based assay

Nucleic acid sequence-based amplification is a single-step isothermal amplification assay that has the capability to create a very high number of copies ($\sim 10^9$) within a very short time; as a result, it shows great potentiality to be used at POC in a low resource setting [132,133]. In NASBA, RNA of the virus samples is directly amplified at 41°C within 1.5–2 hrs with the help of reverse transcriptase and T7 RNA polymerase targeted by primers [134]. NASBA was used to detect other coronaviruses (SARS-CoVs) in earlier outbreaks [135]. A manuscript reporting two staged NASBA-based detection assay has been deposited to bioRxiv preprint recently [132]. They used the NASBA-based amplification strategy in the first stage to amplify the SARS-CoV-2 RNA from the saliva sample and then used the next generation of sequencing to improve the test's accuracy.

4.2.5. Rolling circle amplification based assay

Rolling circle amplification (RCA) is another versatile and straightforward isothermal nucleic acid amplification method with ultrahigh sensitivity for detecting different bio targets [136–138]. In the RCA method, padlock probes target the specific genome sequence and hybridize that target's complementary sequence. A simplified cascade-based circle-to-circle amplification RCA has been developed to facilitate amplification and real-time detection in one pot [139]. The method was successful in detecting and differentiating synthetic SARS-CoV-2 RdRp cDNA from synthetic SARS-CoV RdRp cDNA. Another similar approach has also been just reported detecting SARS-CoV-2 synthetic DNA recently [140]. Although none of the approaches have been tested against clinical samples, RCA can be an advantageous candidate for point of care detection. Moreover, RCA does not require a thermocycler as it has room temperature amplification capability and requires a minimal

amount of reagents to generate rapid and visually highly specific results.

However, from the laboratory testing point of view, recently the FDA has approved an isothermal amplification-based testing device named ID NOW COVID-19 by Abbott diagnostics to qualitatively represent the result in less than 13 mins very high detection limit of 125 copies/mL [141]. The device is housed with a sample receiver, test base, and buffers to facilitate the sample to answer detection. The primers target RdRp segment of the SARS-CoV-2 RNA for amplification and the fluorescence-labeled probe to create fluorescence. A recent study shows this device is 94% sensitive, but some other studies have reported lesser sensitivity, including a higher false-negative rate [142–147]. Another FDA approved isothermal-based assay can detect SARS-CoV-2 RNA within 25 mins named as Cue COVID-19 Test. This device targets and amplify N gene of the viral RNA. RNA extraction to amplification occurs inside a single cartridge where a wand having nasal sample is inserted into the cartridge. A nano ampere level current change inside the electrodes determines the positive and negative results based on the amplification inside the cartridge with the help of Cue Health Monitoring System [148]. However, FDA also approved two other isothermal amplification-based devices one of them used RT-LAMP-based technology (AQ-TOP™ COVID-19 Rapid Detection Kit) and another one used their proprietary technology named as OMEGA amplification (iAMP COVID-19 Detection Kit) [149,150]. All the FDA-approved testing devices are suitable for rapid detection and can be used at the point of care. Nevertheless, the device could revolutionize the testing for Covid-19, considering high throughput, cost, and simplifying the testing workflow.

4.3. Clustered regularly interspaced short palindromic repeats (CRISPR) based assays

The CRISPR (clustered regularly interspaced short palindromic repeats) modules are acquired immunity systems encoded by prokaryotic organisms, especially in bacteria, and act against invading genetic elements, including viruses [151]. CRISPR-associated proteins (Cas) such as Cas9, Cas12, and Cas13 cut viral RNA and organize the sequence to be programmed for the adaptation (Figure 6) [152]. Several CRISPR-based assays have already been reported for diagnosing SARS-CoV-2 using different isothermal amplification technique. An RT-LAMP-based DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) assay has been developed to detect SARS-CoV-2 [153]. The RT-LAMP primers were designed to target the E and N gene to perform the amplification using RT-LAMP. Cas12 protein was programmed for target recognition and detect SARS-CoV-2 gRNA. However, the programmed and amplified target is visualized by lateral flow strip, and the total assay requires minimum laboratory equipment [153,154]. This assay can detect as low as 10 copies/ μ l with a total sample assay completion time of around 45 mins. The assay is waiting for FDA EUA approval.

On the other hand, another portable CRISPR-based specific high-sensitivity enzymatic reporter unlocking (SHERLOCK)

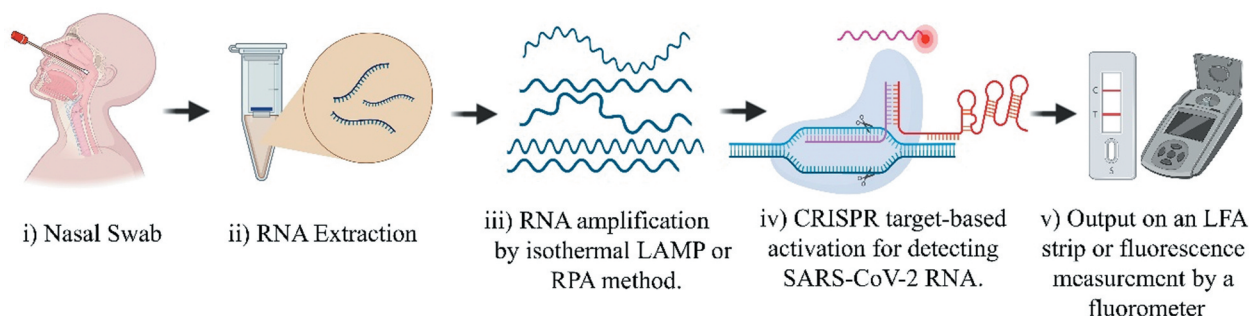


Figure 6. Detection strategy of SARS-CoV-2 amplicons using the CRISPR.

platform with the multiplexing capability has been successfully implemented to detect RNA or DNA using Cas13- or Cas12 [155,156]. By applying the same technology targeting the ORF1ab and N gene, a kit has been designed to target SARS-CoV-2 RNA by SHERLOCK Biosciences (Sherlock™ CRISPR SARS-CoV-2 kit), and it was the first CRISPR-based kit approved by FDA to be used for COVID-19 diagnostics [157]. In this assay, viral RNA was extracted first, followed by isothermal mediated amplification of the RNA. Then the amplified RNA is added with reporter enzyme-specific and sensitive to Cas-13. An engineered or programmed Cas-13 contains a guide RNA that only binds with the target, activating the cleavage mechanism to create a unique labeling signal [158]. A dipstick lateral flow strip then visualizes the signal. It has the capability to detect 1×10^4 – 1×10^5 copies/mL of SARS-CoV-2 RNA and the total assay takes less than an hour. However, a Cas12b-mediated DNA detection known as CDetection has also been developed and tested for SARS-CoV-2 like Pseudo virus without any cross-reactivity with a limit of detectability of 1×10^4 RNA copies/mL [159]. Another, CRISPR Cas-13-based portable and low-cost platform has been developed recently, which combines real-time PCR amplification of the RNA and the Cas-13-based reporter enzyme for visualizing the enzymatic reaction [160]. However, a Cas-12-based CRISPR assay utilizes isotachopheresis on a microfluidic chip to automatize the RNA purification [161]. Moreover, a few other CRISPR Cas-12-based SARS-CoV-2 detection assays are in the pipeline [162–164]. CRISPR-based detection assays are highly sensitive and selective. It also has multiplexing capabilities and can be implemented in point of care settings. In short, CRISPR has the great potentiality to be used as a useful diagnostic tool during disease outbreaks although, sometimes it shows off target editing [165].

4.4. ELISA based assay

Serological diagnostic assays play a crucial role in contact tracing, determining the reservoir host as well as diagnosing asymptomatic patients during pandemics [166,167]. Nucleic acid-based tests are prioritized for the symptomatic patients, but testing IgM and IgG antibodies is also considered indirect detection markers of SARS-CoV-2. Enzyme-Linked Immunosorbent Assay (ELISA) is considered as the ‘gold standard’ for laboratory-based serological specimens to detect SARS-CoV-2 [168]. Indirect ELISA uses immobilized proteins on a solid phase, especially on 96 well plate, to assess the

presence of antibodies from the patient’s serum sample by creating an antigen-antibody complex (Figure 7(A)) [169]. On the other hand, sandwich ELISA utilizes anti-SARS-CoV-2 antibody coated solid surface to create a capture antibody-antigen complex when the different concentration of SARS-CoV-2 viral antigen was added to the plate (Figure 7(B)) [170]. For both cases, in the presence of an enzyme, the samples’ indicative color generation qualitatively represents the result. By measuring the intensity of the color change, the antibody titer or presence of antigen can be quantified [171,172]. To detect the SARS-CoV-2, S protein, including S1, S2 subunit, and RDB and N protein, mainly works as the primary immunogen for detecting the virus neutralization antibody from patients sera [166,173].

A group of scientists developed an indirect ELISA to detect seroconversion using SARS-CoV-2 spike protein generated from the first patient’s viral isolate [169]. They constructed one full and steady S protein and another smaller than RDB to test against the serum/plasma samples collected from the infected patients. Both cases showed strong binding reactivity but strongest in full-length spike due to a higher number of binding epitopes. However, another group developed another ELISA method to detect low-level seroconversion, mostly in asymptomatic or mildly symptomatic patients [174].

Until 25 October 2020, FDA has given EUA to twelve ELISA-based assays [175]. Siemens has developed a streptavidin and biotin-conjugated S protein-based high throughput sandwich ELISA named Vista SARS-CoV-2 total antibody assay to target both IgG and IgM antibodies [176]. Sandwich chemiluminescent immunoassay-based high throughput IgG and IgM detection assay were developed by Siemens Healthcare [177]. Biorad’s one-step antigen capture-based ELISA named as Platelia SARS-CoV-2 total Ab can detect all three (IgM/IgG/IgA) antibodies from human plasma/serum [178]. However, Emory Medical Laboratories, EUROIMMUN, InBios, and Mount Sinai hospital’s developed ELISA can only detect IgG antibody from the human plasma/serum sample [175]. InBios has developed another ELISA kit that can detect only IgM antibody [175]. Thermo Fisher’s OmniPATH tests total antibody, whereas ZEUS Scientific tests the presence of IgG targeting spike protein in both systems [175]. ELISA assay takes around 4–5 hrs to perform in a laboratory setting by skilled personnel. However, it is a noteworthy assay to test seroconversion for all patients, alongside it is also used to detect the presence of antigen and antibody when RT-PCR is negative [179,180].

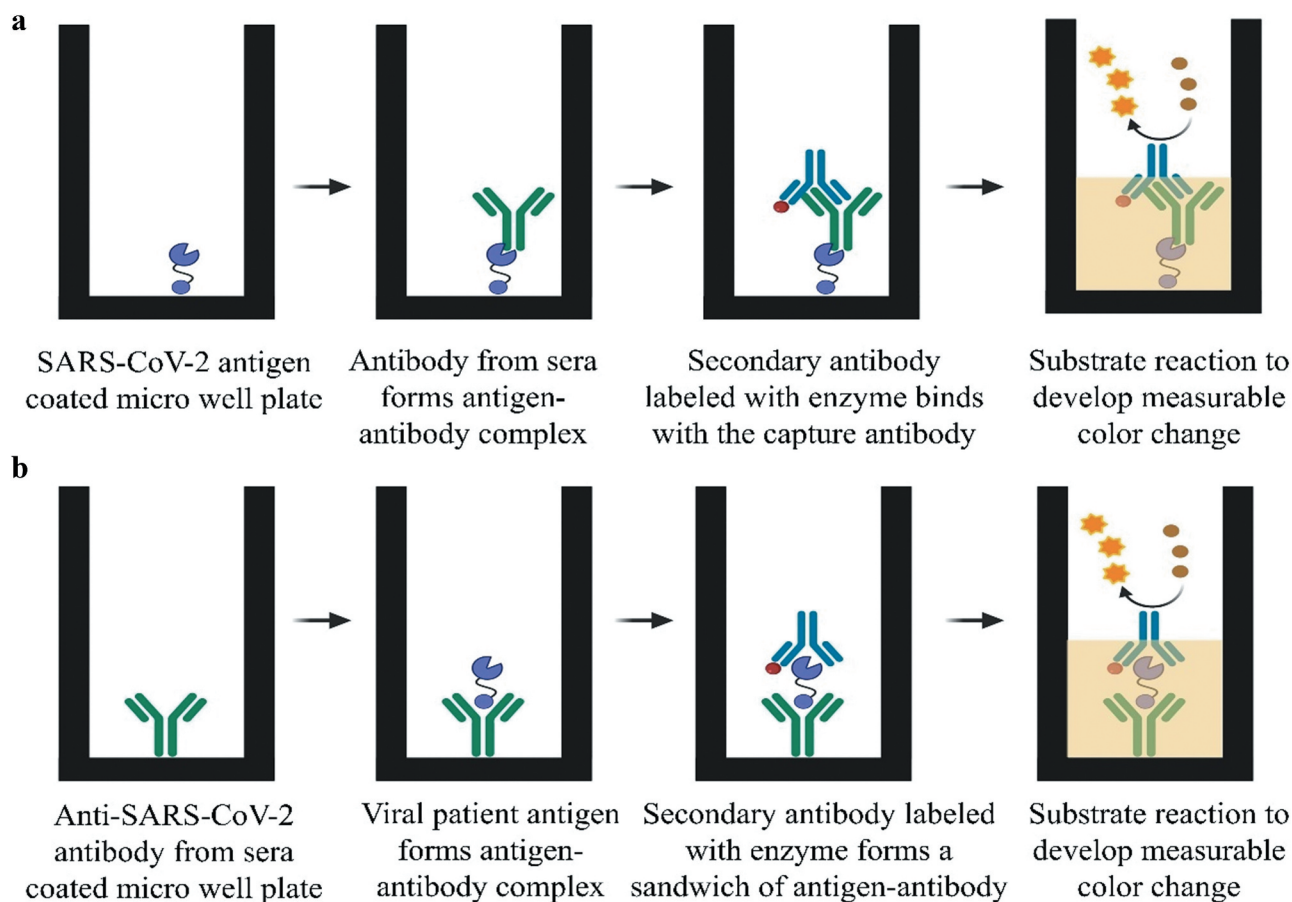


Figure 7. Detection of SARS-CoV-2 antibody by indirect ELISA (A) and antigen by sandwich ELISA (B).

4.5. Paper-based lateral flow assay

The paper-based devices are simple and easy to integrate or apply materials such as sample/absorbent pads, fluids, and bioreagents [181,182]. It also facilitates functionalized bioreagents (gold particle conjugated antibody, antigens, proteins) to develop paper-based lateral flow viral load assay having cost-effective and extensive volume manufacturing capability [183,184]. The paper-based lateral flow assays (LFAs) are a small analytical tool that printed different sections (such as sample extraction, amplification, and detection labels) with a wax printer. The working principle of paper-based LFAs is robust and straightforward, samples (fluids, buffer, and functionalized gold conjugate antibody, antigens, proteins) loaded on sample pad flow toward absorbent pad through capillary force. Manufacturing of LFAs is based on simple steps such as membrane/pad coating, drying, cutting, and membrane/pad assembly (Figure 8(A,B)) [185]. It has already been demonstrated as a powerful tool for pathogens and viral diagnosis [186–188]. The PCR-based diagnostic is based on viral RNA detection, mainly in respiratory samples for COVID-19 patients. The PCR-based tests able to detect viral cargos in early days (say 1–2 days) of infections, however immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies which can be detected approximately 4 days and 8 days respectively after the infection (Figure 3) [75,168]. The LFA-based assays provide qualitative

and rapid detection (10–30 min) of IgG and/or IgM antibodies, especially during this detection window. Subsequent stepwise progression in a paper-based LFA to detect SARS-CoV2 antibody and antigen are shown in (Figure 8(C)). The patient's sample is loaded to the sample inlet, followed by buffer solutions. Samples flow through capillary action into the sample pad, conjugate pad, incubation and detection pad, and finally collected in the absorbent pad. The detection principle is based on colorimetric binding between gold functionalized COVID-19 antigen and IgG/IgM antibody at incubation and detection pad. The patient's sample's antibody-antigen complex binds to the anti-human IgG/IgM antibody functionalized at the test line. Similarly, Rabbit-gold conjugate binds with an anti-human IgG/IgM antibody at the control line. Finally, based on the test/control line's color, positive, negative, and invalid (false positive or false negative) results will occur. Therefore, the captured antibody-antigen complexes are visualized through color bands [189]. These LFAs are suitable for point-of-care qualitative detection (within 15 min.) of SARS CoV-2 infections from a drop of human serum, plasma or even whole blood [190]. A group of Chinese researchers developed a paper-based LFAs to detect both IgM and IgG antibodies from COVID-19 patients' blood [75]. This diagnostic tool is simple, easy to use, and rapid, requiring less than 15 minutes of assay time. This

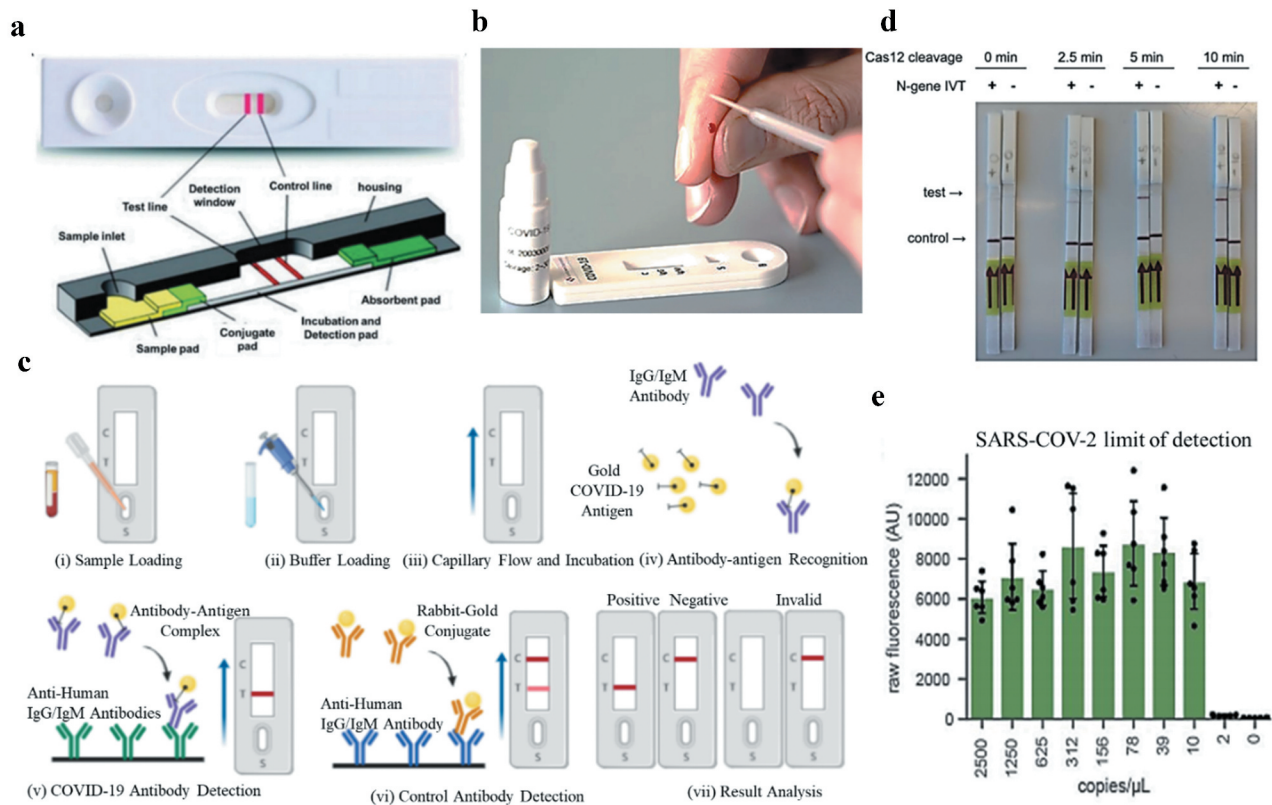


Figure 8. Paper-based LFAs for COVID-19 detection caused by SARS-CoV-2 coronavirus. **A)** Paper-based LFAs are composed of an in-line sample pad, conjugate pad, incubation and detection pad (test and control line), and absorbent pad. **B)** A picture of IgM/IgG COVID-19 detection kit based on paper-based LFA [193]. **C)** Sample loading, processing, and colorimetric detection of IgG/IgM antibody from paper-based LFAs. **D, E)** HybriDetect Strip based COVID-19 detection and further quantification of the limit of detection [158].

product is validated by china CDC and used in eight Chinese hospitals in the earlier stage of the outbreak. Another immunochromatographic strip-based LFA has also been utilized to detect COVID-19. Determination of IgG concentration changes at different stages of infection can be useful for COVID-19 treatment and projection as well as indicative of previous infection [191]. However, a polystyrene nanoparticle doped with lanthanide and recombinant nucleocapsid phosphoprotein based LFIA has been developed recently to detect IgG antibody against SARS-CoV-2 in patients serum [192]. The assay was able to detect the presence of IgG within just 10 mins and has 89% agreement of samples tested with RT-PCR. The FDA, USA, has given 'Emergency usage authorization' to eight LFA devices for detecting IgM and/or IgG till 17 July 2020 [175]. Autbio Anti-SARS-CoV-2 Rapid Test, Beijing Wantai Biological Pharmacy Enterprise, RightSign COVID-19 IgG/IgM Rapid Test Cassette, LYHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit, and Healgen COVID-19 IgG/IgM Rapid Test Cassette uses Spike protein as target whereas, Biohit SARS-CoV-2 IgM/IgG Antibody Test Kit targets nucleocapsid protein for the development of the assay. But, Assure COVID-19 IgG/IgM Rapid Test Device and Cellex qSARS-CoV-2 IgG/IgM Rapid Test targets both Spike and nucleocapsid protein [175].

Moreover, the HybriDetect technique is used to detect COVID-19 by using lateral flow dipstick (LFD), capable of detecting different molecules, including gene amplification,

antibodies, and proteins (Figure 8(D, E)) [194]. In this technique, the gene expression of virions can be expressed regardless of any organism that needs to be detected. Finally, detected primers are introduced during the amplification step; fragments are labeled and detected. To accelerate the rapid diagnostic test of COVID-19, paper-based LFAs with isothermal amplification combined with CRISPR are also being reported (SHERLOCK, DETECTOR techniques) [153,158].

In summary, paper-based LFAs are a potentially small, portable diagnostic tool to detect SARS-CoV-2 and track virus carriers or infected patients in our community. Paper-based serological LFAs suffer from a significant problem related to antibody cross-reactions and may provide false-positive results with other types of coronavirus or influenza viruses [195]. However, this paper LFAs provide rapid qualitative detection (positive or negative) and suitable for point-of-care diagnosis at resources limited settings [189]. These paper-based LFAs have already been developed, approved, and demonstrated worldwide, including Australia, China, Germany, Singapore, South Korea, and the USA [190]. These LFAs are a cost-effective and fast-response approach that can help provide a warning to our local government and agencies to isolate infected patients to avoid further spreading of epidemics. For example, Germany provides 'immunity certificates' based on rapid antibody tests and stops their lock-down [27]. The USA also started providing free antibody tests to their community and reopen social activities at small scale.

4.6. Surface plasmon resonance based assay

Surface plasmon resonances (SPR) based biosensors provide sensitive and real-time diagnosis of biomolecules, real-time interaction, and binding affinity at the atomic scale [196]. In the 1980s, SPR-based biosensor was first introduced to detect chemicals and biomolecules [197]. SPR-based biosensors are based on plasmonic metal layers that produce a large number of free electrons. These free electrons move or oscillate with light illumination, producing negative permittivity required for SPR generation [198,199]. The conventional prism-based SPR sensing technique is also known as the Kretschmann configuration. The prism is coated with plasmonic materials and covered with bioanalytes. Light is illuminated from one side of the prism and then reflected light was measured through a spectrometer or photodetector on the other side. As we change the refractive index of bioanalytes, the propagation constant of SPR mode is altered to vary coupling conditions or light properties. Besides prism-based SPR biosensing techniques, other optical sensing techniques are also available such as microring resonators [200], waveguides [201], photonic crystal fiber [202], and gold nanoparticles [203].

Recently, a prism-based SPR technique has been reported as a photothermal biosensor to detect SARS-CoV-2 coronavirus (Figure 9(A)) [204]. This biosensing technique is a combination of plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) to provide an accurate and promising clinical diagnosis of COVID-19. The sensing principle is based on surface functionalization of gold nanoislands (AuNIs) with complementary DNA receptor and detection of RNA SARS-CoV-2 coronavirus sequence through heat-activated nucleic acid hybridization. This thermoplasmonic effect of AuNIs contributes to the free electron's kinetic acceleration and provides a higher plasmonic response

(Figure 9(B)). Therefore, resonance wavelength shifting was used to detect various viral oligos and nucleic acid concentrations with a minimum detection limit of 0.22 pM (Figure 9(C)). A portable SPR sensor instrument (Affité Instruments, Canada) has also been used for detecting nucleocapsid antibodies of SARS-CoV-2 [205]. Here, the SPR sensor was coated with a peptide monolayer and functionalized with recombinant protein to detected nanomolar anti-SARS-CoV-2 antibodies in undiluted human serum within 15 minutes (Figure 9(D, E)).

Nanotechnology-based plasmonic biosensors are convenient, simple, and easily demonstrable portable platforms to detect layer-by-layer biomolecules, proteins, and virus particles [196,206]. Recently, AuNPs particle-based detection showed impressive attention for colorimetric biosensing applications due to high excitation coefficients, localized surface plasmon resonance, and inherent photostability properties [207–209]. They found a wide variety of applications in a colorimetric-based assay, such as small molecules, proteins, metal ions, and nucleic acids [210]. Further, the 'naked eye' colorimetric detection of COVID-19 caused by SARS CoV-2 was demonstrated (Figure 9(F, G)) [207]. This colorimetric assay was based on thiol-modified functionalized AuNPs to capture thiol-modified antisense oligonucleotides (ASOs) specific for N-gene (nucleocapsid phosphoprotein) of SARS CoV-2. The sensing principle is based on RNA-DNA hybridization and color variation of agglomerated AuNPs that produce a limit of detection of 0.18 ng/ μ L of viral RNA of SARS-CoV-2. This technique has advantages with reliability, reproducibility, and fast-response that takes only 10 minutes to diagnose positive COVID-19 patient samples after RNA isolation. Further, the selectivity of this colorimetric sensor was also tested in the presence of MERS-CoV viral RNA.

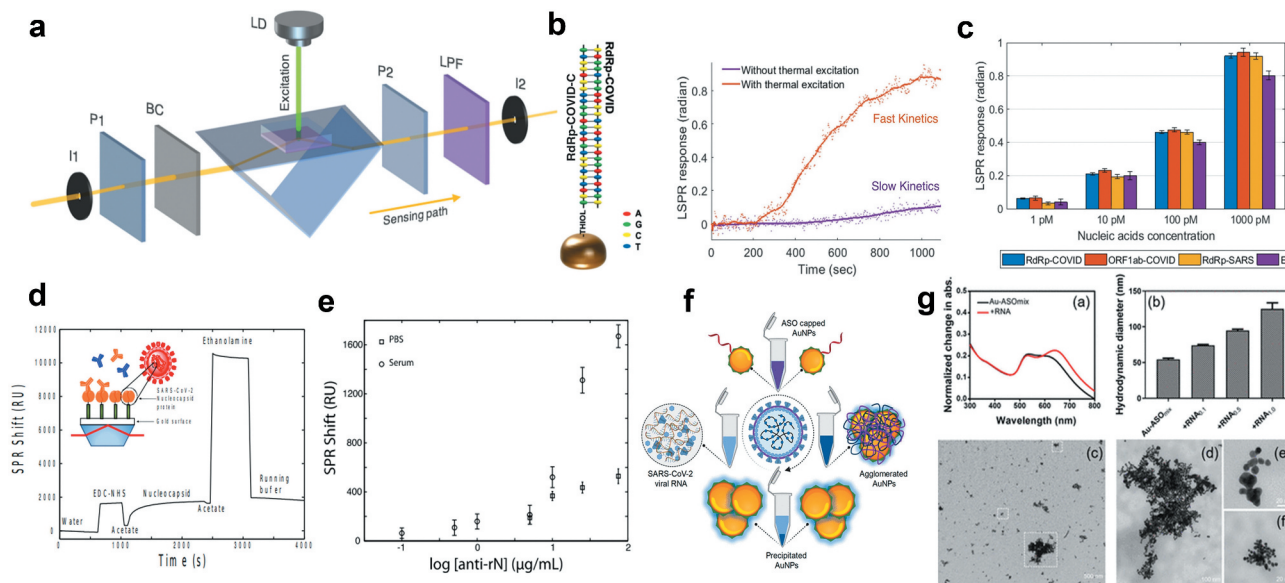


Figure 9. Surface plasmon resonance based COVID-19 detection. A) Experimental setup of the dual-functional PPT enhanced LSPR biosensing system for SARS CoV2 detection [204]. B) Schematic illustration of the hybridization of RdRp-COVID and its cDNA sequence (RdRp-COVID-C) and their plasmonic response with/without thermal activation. C) SARS-CoV-2 viral oligos concentrations variations and their corresponding plasmonic resonance shifting. D, E) SPR sensorgram and resonance wavelength shift of functionalized AffiCoated surface with nucleocapsid protein of SARC-CoV-2 (rN) [205]. F, G) Conceptual block-diagram of AuNP functionalized colorimetric SARS-CoV-2 detection. Normalized optical response of AuNP absorption before and after adding RNA SARS CoV-2 viral load (a), their hydrodynamic diameters variations (b), TEM images of Au-functionalized nanoparticles after addition of SARS-CoV-2 viral RNA [207].

5. Expert opinion

Hospital facilities have been overwhelmed with the skyrocketing increase in COVID-19 patients everywhere from Rome to New York City [211]. Assay selection for diagnosing a highly transmissible virus like SARS-CoV-2 plays an important role for diagnostic management, which in turn helps to implement proper treatment. To ensure correct processing of collected respiratory or other specimens, specimens should be processed inside a biosafety level II cabinet. However, mismanagement of sample collection, storage and transportation to the lab also can provide misleading results. Good laboratory practice should be maintained while extracting and purifying RNA. Importance should be placed on testing of specimens collected from different samples as virus replication site may change at different stages of infection. Nasal/oral swab could be used initially but rectal swab could be used to detect SARS-CoV-2 using RT-PCR in the later part of the infection as it showed an important route of transmission in the later part of the infection [6]. The conventional gold standard RT-PCR method-based diagnostic devices require highly sophisticated and expensive lab facilities, trained personnel, and logistics. COVID-19 suspected individual had to wait a long time from specimen collection to get test results. Moreover, the false-positive result is a significant drawback as the testing device has varying sensitivity to a great extent, which is evident from (Table 1) [212]. Similarly, false negative RT-PCR result can be misleading while considering a person is fully virus free [42]. Additional molecular and serological testing can be considered if the person has a trace of exposure and/or clinical conditions. On the other hand, POC-based devices like Xpert Xpress and BioFire play an essential role in the midst of a pandemic. However, isothermal amplification-based testing devices can be a better alternative to RT-PCR-based devices. It usually provides results within 30 minutes and, most importantly, does not require the expensive thermocycler. ID NOW™ from Abbot which uses isothermal amplification to detect positive samples within 5 mins whereas for negative, it takes 13 mins. Moreover, mix detection and sensing technologies are also being developed where RT-PCR method is used to detect and amplify the viral genome and lateral flow-based techniques (Accula SARS-Cov-2 Test) or gold electrode based electrochemical sensors (ePlex SARS-CoV-2 Test Kit) were used to visualize the results. Paper-based LFA-based antibody biosensing technologies can be a solution for rapid testing as this provides cost effectiveness, less complexity, and flexibility to be used in resource-limited settings. LFA assays require less than 30 mins from the sample to answer. But, the main drawback of antibody-based testing is its limitation to detect early infection. The testing laboratories should invest more in detection tools with high-throughput ability, high sensitivity, and high scalability. Multiplex detection capability can also be an excellent choice to make noteworthy contribution to a pandemic like this where enormous demand for testing exists. Self specimen collection or in home sample collection devices could be a good choice where resources are limited, and then samples can be transported to designated laboratories for further testing. Additionally, more focus should be given for developing point of care platforms that

are suitable for resource-limited settings following the WHO ASSURED criteria [171,180,213,214].

We have seen a worldwide effort for the effective administration of fighting against COVID-19 pandemic. We foresee great advances in the field of developing biosensing diagnostic technology in next five years. These devices or technologies will be rapid, cheaper, user-friendly, field-deployable, and more importantly suitable for resource constraint settings as per guidelines of WHO [213]. However, more sensitive, specific, scalable and multiplexed detection technology will be developed in coming years. Mutations are common phenomena for viruses, and focus should always be given to identify the variant of mutated SARS-CoV-2 [215]. While designing, developing, and testing of new detection tools, mutations or new emergence of strains should be considered. As SARS-CoV-2 sheds through feces, monitoring wastewater could be a good option for predicting future transmission surges inside a community. However, current mitigating measures, especially social distancing and wearing masks, are proven to limit viral spread and be adopted widely in regulation.

The recent pandemic will create a multidimensional adverse effect on the world. Although some regions or countries could mitigate the virus's spread, there is always a fear of the next wave. Every day new results are coming out on different prospects of the virus as well as disease. Changing the mitigating strategy with the evolved results or findings are the keys to fight against this deadly virus. Global leadership, collaboration, accurate surveillance, tracking, and sharing information with the scientific committee require more coordination to overcome the global health emergency.

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Declaration of interest

U. Demirci is a founder of and has an equity interest in: (i) DxNow Inc., a company that is developing microfluidic IVF tools and imaging technologies for point-of-care diagnostic solutions, (ii) Koek Biotech, a company that is developing microfluidic technologies for clinical solutions, (iii) Levitas Inc., a company focusing on developing microfluidic products for sorting rare cells from liquid biopsy in cancer and other diseases, and (iv) Hillel Inc., a company bringing microfluidic cell phone tools to home settings. UD's interests were viewed and managed in accordance with the conflict of interest policies. All other authors declare no financial conflict of interests. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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ORCID

MD Alamgir Kabir  <http://orcid.org/0000-0002-1659-3125>

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