RSC Advances

REVIEW

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Cite this: *RSC Adv.*, 2022, **12**, 8059

Insight into prognostics, diagnostics, and management strategies for SARS CoV-2

Umay Amara,[ab] Sidra Rashid,[b] Khalid Mahmood,[*a] Mian Hasnain Nawaz,[b] Akhtar Hayat[b] and Maria Hassan[a]

The foremost challenge in countering infectious diseases is the shortage of effective therapeutics. The emergence of coronavirus disease (COVID-19) outbreak has posed a great menace to the public health system globally, prompting unprecedented endeavors to contain the virus. Many countries have organized research programs for therapeutics and management development. However, the longstanding process has forced authorities to implement widespread infrastructures for detailed prognostic and diagnostics study of severe acute respiratory syndrome (SARS CoV-2). This review discussed nearly all the globally developed diagnostic methodologies reported for SARS CoV-2 detection. We have highlighted in detail the approaches for evaluating COVID-19 biomarkers along with the most employed nucleic acid- and protein-based detection methodologies and the causes of their severe downfall and rejection. As the variable variants of SARS CoV-2 came into the picture, we captured the breadth of newly integrated digital sensing prototypes comprised of plasmonic and field-effect transistor-based sensors along with commercially available food and drug administration (FDA) approved detection kits. However, more efforts are required to exploit the available resources to manufacture cheap and robust diagnostic methodologies. Likewise, the visualization and characterization tools along with the current challenges associated with waste-water surveillance, food security, contact tracing, and their role during this intense period of the pandemic have also been discussed. We expect that the integrated data will be supportive and aid in the evaluation of sensing technologies not only in current but also future pandemics.

Received 31st October 2021
Accepted 4th February 2022

DOI: 10.1039/d1ra07988c

rsc.li/rsc-advances

1 Introduction

An initial insight into the current novel infectious ailment, namely, COVID-19, appeared on 31st December 2019 in Wuhan, China.[1,2] A large number of individuals suffering from cough,

[a]*Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 608000, Pakistan. E-mail: khalidmahmood@bzu.edu.pk*

[b]*Interdisciplinary Research Centre in Biomedical Materials (IRCBM), COMSATS University Islamabad, Lahore Campus 54000, Pakistan*

Ms Amara did her Bachelors and Masters from Institute of Chemical Sciences, Bahauddin Zakariya University, Multan. She got enrolled in PhD under supervision of Dr Khalid in the same institute and started working as Research Assistant at IRCBM COMSATS University Islamabad, Lahore Campus. Currently, she is working as a research visitor at Drexel Nanomaterial Institute, USA. Her research interests include the synthesis and characterization of novel organic/inorganic nanohybrids and their biosensing applications.

Sidra received her Bachelor's degree in Chemistry from Mirpur University of Science and Technology (MUST), AJK in 2014. She continued to study Chemistry and received her Master's degree from the University of Kotli (UOK) in 2017. After that, she started working as a research assistant at the Sensors & Biosensors Lab at Interdisciplinary Research Center of Biomedical Materials, COMSATS University Islamabad, Lahore. Her interests are mainly directed toward the use of nanomaterials that can be incorporated for biosensing applications.

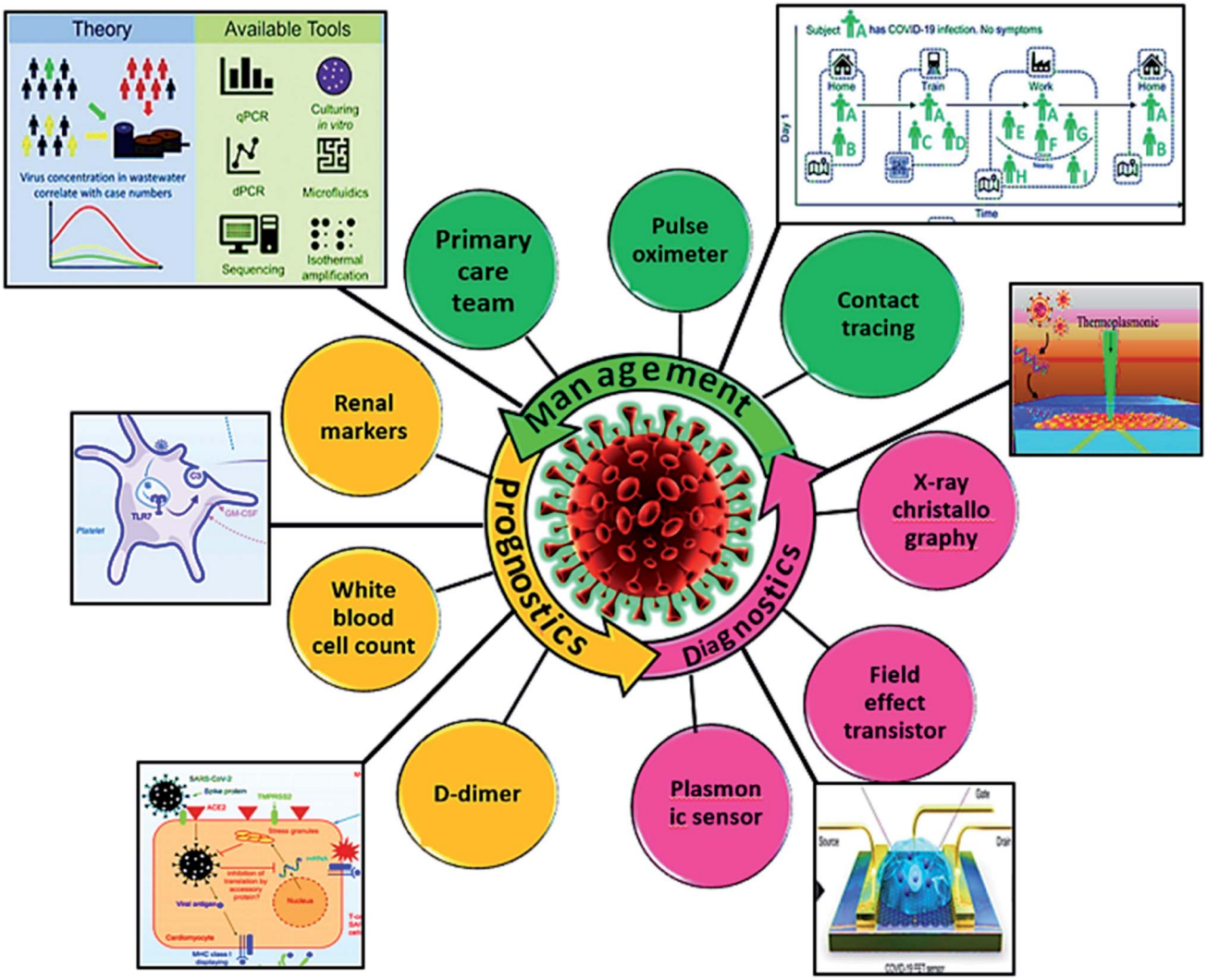
shortness of breath, and fever were hospitalized at the start. A primary evaluation by compound tomography indicated divers as compared to healthy lungs.[3] This finding directed to an early diagnosis of pneumonia. Further, nucleic acid investigations *via* multiplex real-time polymerase chain reaction (PCR) of already known pathogens led to negative outcomes, indicating that the actual cause of pneumonia was unfamiliar (1). A genetic sequence parallel to that of the beta coronavirus B was discovered from a sample of patients suffering from Bronchoalveolar Lavage (BAL) by 10th January 2020. This study also revealed a genomic similarity of 50%, 80%, and 96% to the Middle East Respiratory Syndrome virus (MERS-CoV), Severe Acute Respiratory Syndrome virus (SARS-CoV), and bat coronavirus (RaTG13), respectively.[4,5]Therefore, this unknown pathogen was named as SARS-CoV-2, which is responsible for COVID-19. By April 2, 2020, this infection had blown out to almost 202 countries, infecting approximately 1 million people with 45 526 deaths worldwide. Consequently, on March 11, 2020, this novel COVID-19 outbreak was declared as a pandemic by the World Health Organization (WHO).[6,7] However, the total number of coronavirus cases till December 29, 2021 were 283 374 722 with 5 434 143 deaths by the Worldometer website (https://www.worldometers.info) (Scheme 1).

The percentage of individuals affected by SARS CoV-2 infection that remained asymptomatic has not yet been fully assessed. On the other hand, in symptomatic individuals, clinical manifestations of this infection start usually within a week including cough, fatigue, fever, and upper respiratory tract infection.[8] It can also progress to several other diseases such as dyspnoea and other chest infections in almost 75% of the affected persons. It was also reported by one of the studies that the interaction with the laryngopharynx, nasal mucosa, and trachea of patients can develop innate-immune response for instance tissue damage in different parts of the body. It was also found that this infection may affect the brain *via* different passages also including peripheral nerves.[9] More recently, the investigation of 425 confirmed infected cases demonstrate that the current pandemic may double the number of affected individuals every seven days and that each patient spreads the infection to 2.2 to 3.58 other individuals on an average.[10] Standing against these current circumstances, scientists are actively probing for the important attributes accountable for this pandemic. However, examining the target sequences carried by exogenous nucleic acids along with CT scans[11] suffers from serious limitations produced by nonspecific hybridization or amplification, as well as overpriced infrastructure and

Dr Khalid got his PhD degree from College of chemistry Beijing Normal University, Beijing China (2014) in the field of organic solar cells synthesis. After that he got 2-years post-doctoral fellowship from South China University of Technology, Guangzhou China. Currently, he is working as an assistant professor at the Institute of Chemical Sciences, Bahauddin Zakariya University, Multan. The main area of his research is in the synthesis and characterization of novel organic materials, nanocomposites and pharmaceutical compounds for their applications in energy conversion, sensors and drug delivery.

Dr Hasnain earned his PhD in Materials Science and Engineering from East China University of Science and Technology, Shanghai (2013). During his doctoral dissertation, he mainly focused on polymer chemistry and porphyrin–fullerene nanocomposites. Later, he joined IRCBM as Assistant Professor to focus on the sensing and biosensing aspects of different carbon-based nanocomposites. He earned CAS Presidential Postdoctoral Fellowship to work on the synthesis and characterization of metal nanoparticle-decorated carbon interfaces and their biofunctionalization towards sensing and energy applications. He has published several articles in internationally recognized journals. Currently, he has multidisciplinary research interests in supramolecular chemistry, advance materials, metal nanoparticles, and biomaterials including their potential applications as sensors and biosensors, bioresorbable medical devices, and bio-fuel cells.

Dr Akhtar Hayat is currently working as Assistant Professor at IRCBM, COMSATS Lahore, Pakistan. He received MS/Ph.D. Degree in biosensors from Universite de Perpignan, France and post-doc from Clarkson University, USA in nanobiosensors. He is the founder of the sensors/biosensors research group at IRCBM, COMSATS, Lahore, and is acting as the head of this group. Dr Akhtar Hayat is playing a key role towards the development of this group based on his international collaborations and national research funding.



Scheme 1 Prognostic, diagnostic, and management strategies for SARS CoV-2.

difficult work.[12,13] To mitigate such challenges, protein-based sensing assays that principally depend on identifying antibodies released by individuals on contact with SARS CoV-2 have been introduced.[2,14–16] Certain groups have employed plasmonic sensors as robust and economically viable substitute with much lower detection limits compared to the abovementioned techniques.[17,18] In parallel, nanoscale visualization procedures such as electron microscopy (EM), atomic force microscopy (AFM), and X-ray diffraction (XRD) have also been exploited for timely analysis and enlightening prognostic topographies of a specific virion.[19,20]

Despite huge successes in vaccine development, the COVID-19 variants, especially the most recent one called Omicron, have raised severe concerns as it is significantly limiting antibody-based neutralization and elevated the chances of re-infection.[21] Also, an increased number of cases reported have put the efficacy of functional vaccinations in question for emerging alternates.[13,22] To solve such challenges, there is a need to improve the management strategies by moving toward tele-health care and tele-medicine[23] to provide care from a distance.[24] It would help to decrease the burden on the healthcare system and preventing exposure to highly vulnerable patients. Numerous clinics have implemented this practice globally. The current review aims to report the importance of the state-of-art instruments and technology employed so far to lessen the current and additional waves of COVID-19. The study also underlines the positive impact of contact tracing and quarantine in this critical pandemic situation.[25–28]

2 Sequential and molecular data analysis

Researchers have targeted the analysis of beta-coronavirus including SARS-CoV-1 as well as MERS to elaborate the real nature of the COVID-19 virus.[29] This research shows that ab1 encodes the protein involved in the formation of envelope €, spike protein (S), as well as nucleocapsid (N) (Fig. 1).[30] The sequential data of the viral genome was evaluated from Korean

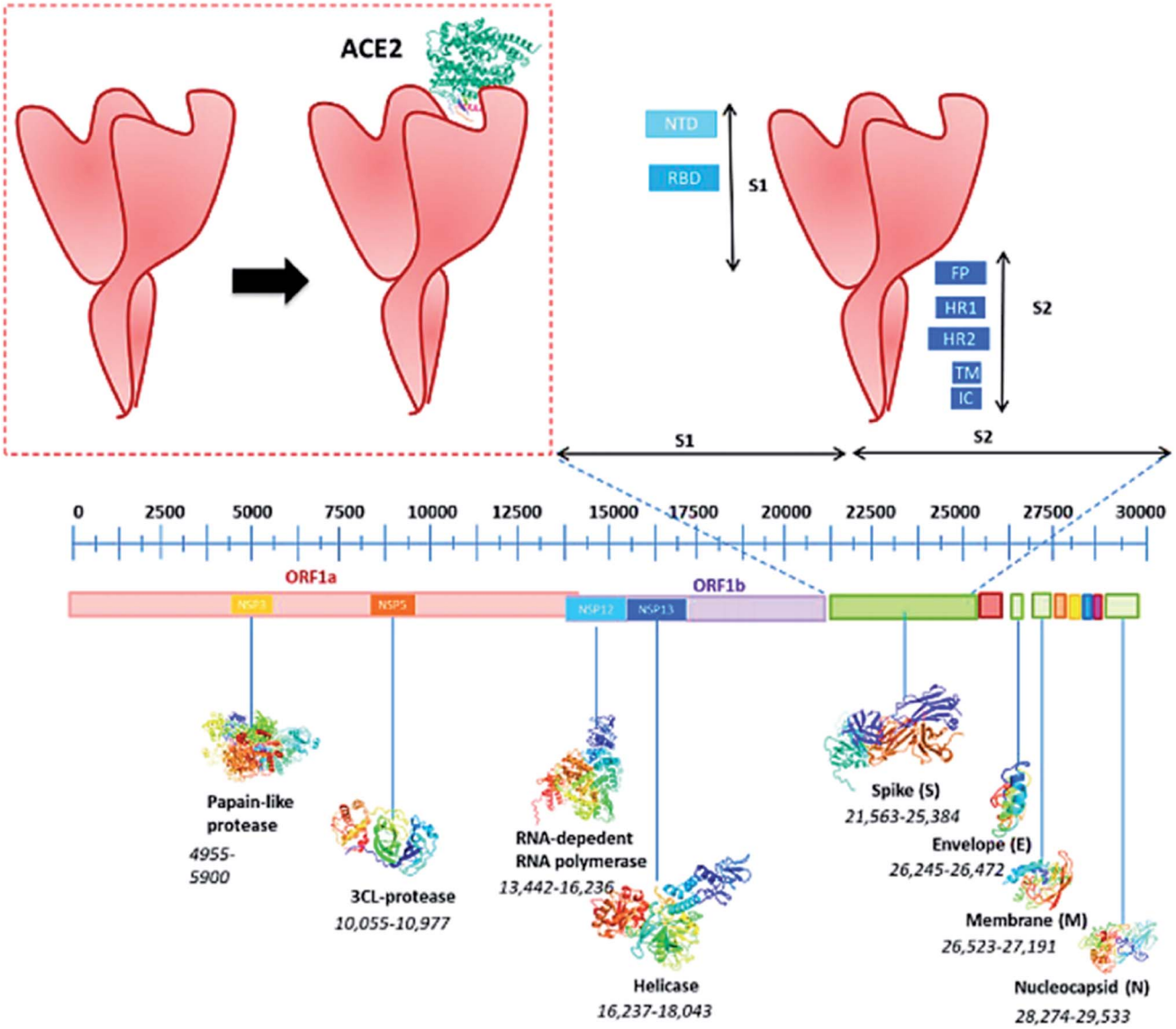


Fig. 1 Structure of SARS-COV-2; prime structure of spike glycoprotein (S1) of SARS-COV-2; lateral and upper views of S1; ACE-2 and RDB of S1; binding sites of spike S1 protein. The corresponding amino acids marked as red for the positive charge, purple for the hydrophilic, and blue for the hydrophobic residues. The figure has been reproduced from ref. 30 with the permission from WILEY, copyright 2020.

patients by Han *et al.*[31] Almost >99.9% of the sequential homology was found for CoV-2 from various other corners of the world. Further, a similar kind of resemblance was also observed by Sah *et al.* in a 32 years old Nepalese patients[32] that is similar to the previously reported viral genome obtained from Wuhan. However, seven new sequences were observed, which vary from MERS and SARS-CoV-1 on the basis of structural evaluation based on biochemical and structural experiments. Meanwhile, a comparative study between α and β coronaviruses revealed that SARS-CoV-2 may have the ability to bind effectively with the human-receptor gene called Angiotensin-Converting-Enzyme-2 (ACE-2).[33] However, computational studies did not prove any linkage between the spike protein and ACE-2.[34] It led the scientists to believe that a greater attraction between ACE-2 and the CoV-2 gene may be the result of mutation to ACE-2.[35] It was also found that this mutation can easily alter the phenotype of SARS-CoV-2, which in turn can alter the diagnosis of the virus.[36] In addition, the mutated portion of the respective genome can forecast the linkage between probe binding and the primer.[37]

3 Prognostic strategies using biomarkers for COVID-19

The exploration of various risk factors caused by COVID-19 at different intervals is essential on an urgent basis due to its severity. It would be of great help to take timely actions and reasonable intervention to enhance the cure rate and the prognosis quality.[38] Various biomolecules have been utilized for

the effective prognosis evaluation of COVID-19 in laboratory tests. In the following section, we carefully evaluated the utilization of different prognostic strategies including D-dimer, IL-6, and some other biomarkers particularly during the early stages of infection.[39]

3.1 C-Reactive protein (CRP) for COVID-19

This protein is developed by liver and various inflammatory mediators such as IL-6. Its higher level is parallel to the disease intensity.[40,41] CRP, as a biomarker in disease diagnostics, has been highlighted by a retrospective center in Wuhan. An increased level of CRP (57.9 mg L^{-1}) was reported in the patients in Wuhan.[42] Another study reported CRP levels >41.8 mg L^{-1} and found the likelihood of COVID-19 progression.[43,44] Pathologically, CT scans have been employed to identify lung lesions during COVID-19 progression but are unable to differentiate between mild and severe cases, as we reported previously.[45] The outstanding activity of CRP as a biomarker was revealed in the 'area under the curve' in the receiver operating examination of 0.87 (95% CI, 0.10–1.00), where 83% sensitivity and 91% specificity were reported. Henceforth, CT scans alone along with CRP values have been proved to be authentic for the earlier detection of cases. Many other studies also corroborated the abovementioned findings.[46,47]

3.2 Interleukin-6 (IL-6) for COVID-19 diagnostics

IL-6 is a primary trigger for cytokine storms. Wan *et al.*[48] found that cytokine storm is crucial to the progression of COVID-19 and can lead to severe complications even death. The 5th edition of "Diagnosis and Treatment of COVID-19"[49] suggests that observation of the cytokine level helps to improvise the treatment efficacy with reduced mortality. According to Yang *et al.*, this inflammatory factor plays a crucial role in the progression of the disease from a mild to a severe level.[50,51] Cox-proportional-hazard-model-analysis (CPHMA) showed that IL-6 could be utilized as an independent factor to predict the severity of SARS-COVID-19 hematological diseases.[38] Thus, we can conclude that an increased level IL-6 can be a sign of a higher inflammatory cytokine storm. Thus, the role of IL-6 in this disease deserves special attention.[52,53]

One meta-analysis recounted that the mean IL-6 concentrations were 2.9 times greater in complicated COVID-19 patients in comparison to non-complicated disease, in which ($n = 1302$; 95% CI 1.17–7.19).[54] The findings of the study include ICU admittance, onset of acute respiratory distress syndrome (ARDS), and deaths. Groundbreaking conclusions suggested that the proportional upsurge of IL-6 is directly allied with disease severity. Another group proposed that SARS CoV-2 affects both the upper and lower respiratory tract and causes a high or mild acute respiratory syndrome with the consequent release of IL-6.[55] This enhanced appearance of IL-6 in the serum possibly indicates the severity and prognosis of the disease in SARS COVID-19 patients.[56] In line with the findings of many researchers, the dynamic changes in the level of IL-6 have proven to be an important biomarker for disease diagnostics in severe COVID-19 patients.[57,58]

3.3 White blood cell count

Leucocytes, also known as white blood cells (WBCs), are constituents of blood generated from lymphoid tissue and bone marrow. They are divided into two main categories, *i.e.*, granulocytes and agranulocytes. Eosinophils, neutrophils, and (NC) basophils come under the granulocytes group, while monocytes and lymphocytes (LC) are agranulocytes. Their inconsistent number may cause infection and can be analyzed by blood tests, producing a white blood cell count. A survey was made and numerous differences in the WBC of severe and non-severe COVID-19 patients were noted.[42] Patients of both cases have been reported to have increased leucocytes numbers, while the severe group experienced a comparatively large rise (5.6 *vs.* 4.9 $\times 10^9$ L^{-1}; $P < 0.001$). NCs were found to be primarily driving this increase. More interestingly, the level of monocytes, lymphocytes, eosinophils, and basophils were less, causing a larger neutrophil-to-lymphocyte ratio (NLR; 5.5 *vs.* 3.2; $P < 0.001$). Also, this NLR has been reported as a biomarker that reflects the disease severity.

Another study conducted in China also corroborated the findings that higher NC and low LC count is associated with the severity of affected patients, signifying WBCs as a potent biomarker for the timely recognition of severe COVID-19.[59] Nevertheless, glucocorticoid and other viral and bacterial infections disturb the accuracy of WBCs findings.[60] A specific study made in China reported the depleted LC level in most of the COVID-19 affected patients.[53] Another research group reported low blood lymphocyte percentage (LBL%) in critical cases, proposing that a lower LC count is indicative of a poor prognosis. Many other groups justified the same findings.[61,62] However, the virus can target the mechanisms of IL-6 and lymphoid tissue. Meanwhile, additional reasons for low LC count need to be examined to determine the effect of WBC on COVID-19 severity.[18]

3.4 Lactate dehydrogenase (LDH)

In the glucose metabolic pathway, the enzyme LDH catalyzes the conversion of pyruvate to lactate.

LDH is produced by cell membrane necrosis owing to lung damage or viral infection such as pneumonia caused by SARS CoV-2. Many evidences suggest the strong relationship between the LDH levels and the growth of COVID-19 disease.[63] One study proposed enhanced serum LDH values as one of the unusual diagnostic parameters in SARS CoV-2 patients with severe or fatal development of the disease. Pulmonary injury along with pervasive organ damage are of potential biological and clinical significance at elevated LDH levels.[64] One meta-study reported significantly greater levels of LDH in ICU patients, *i.e.*, 248 U L^{-1} than in non-ICU patients 151 U L^{-1} ($p = 0.002$). Higher LDH level is sustained in ICU patients even after the several post-admission days (160 U L^{-1} *vs.* 218 U L^{-1}, $p = 0.002$). Therefore, LDH may be a prognostic biomarker of severe disease. Multi-center research involving 1099 COVID-19 patients supported the evidence associating inflammation and tissue damage with enhanced LDH levels.[6] Moreover, when LDH was at higher levels, as seen in the associated CT scans, the severity of pneumonia was revealed.[65]

Another study was carried out with 87 confirmed COVID-19 patients, assessed by the LDH level at diagnosis and follow-ups.[66] The enhancement or reduction in the LDH level was noted to be representative of radiographic progress. A positive correlation was observed between the time to LDH normalization (5.67 ± 0.55, days) and the time to radiographic absorption (5.57 ± 0.65 days). The study validates the potential utilization of serum LDH as a biomarker for assessing the severity and observing treatment feedback in COVID-19 pneumonia.[67] Many other studies by different research groups also supported the above findings.[68,69]

3.5 D-Dimer

D-Dimer released from the degradation of the cross-linked fibrin signifies the activation of fibrinolysis and coagulation.[70] Early research has linked elevated levels of D-dimer and hemostatic abnormalities in non-survivors of COVID-19 compared to survivors.[71] A survey performed by a group of researchers on 191 cases reported that a D-dimer level greater than 1.0 μg mL^{-1} (p = 0.0033) resulted in greater mortality among COVID-19 cases. Moreover, they optimized 2.0 μg mL^{-1} or more as a cut-off to predict the mortality in SARS CoV-2 patients in hospital.[72] Another group reported a marked rise in the D-dimer level and enhanced coagulation in almost 90% of patients with pneumonia.[73] The researchers also reported the elevated D-dimer level of 2.4 mg L^{-1} in ICU patients in contrast to non-ICU specimens 0.5 mg L^{-1} ($p = 0.0042$). Another study by Sakkat *et al.* with 1355 patients also justifies the previous findings.[74] Yumeng *et al.* reported a D-dimer elevation of 74.6% (185/248) in patients.[75] Furthermore, another research group suggested that the enhanced level of the D-dimer on admittance can be used as a marker to refer patients to critical care. This, along with the many reported studies, proposed that D-dimer levels should be employed as a prognostic marker and aid doctors to monitor and risk-stratify those who are probable to expire earlier.[76–78]

3.6 Platelet count

As we have discussed in the previous section, the COVID-19 virus leads to severe hematological variation, resulting in thrombocytopenia (Fig. 2). A survey of 1799 CoV-2 victims has revealed a fewer platelet count (WMD -31×10^9 L^{-1}; 95% CI, -35 to -29×10^9 L^{-1}) in patients with severe infection.[79] Likewise, the mortality rate is higher in those with fewer platelets count (WMD -31×10^9 L^{-1}; 95% CI, -35 to -29×10^9 L^{-1}). Considering thrombocytopenia as an endpoint, a fivefold higher possibility of SARS CoV-2 (OR, 5.13; 95% CI, 1.81–14.58) has also been observed. Reconsidering research that castoff Cox proportional hazard regression analysis revealed the platelet count as an independent risk factor for deaths amongst CoV-2 patients, where a 50×10^9 L^{-1} rise is related to 40% mortality.[80] Another study reports the 5.45% death rate (29 subjects died) and decreased platelet count among non-survivors compared to survivors after one week of admission. The difference was enhanced by 50×10^9 L^{-1}, as reported by previous research.[81] Another group endorsed the previously

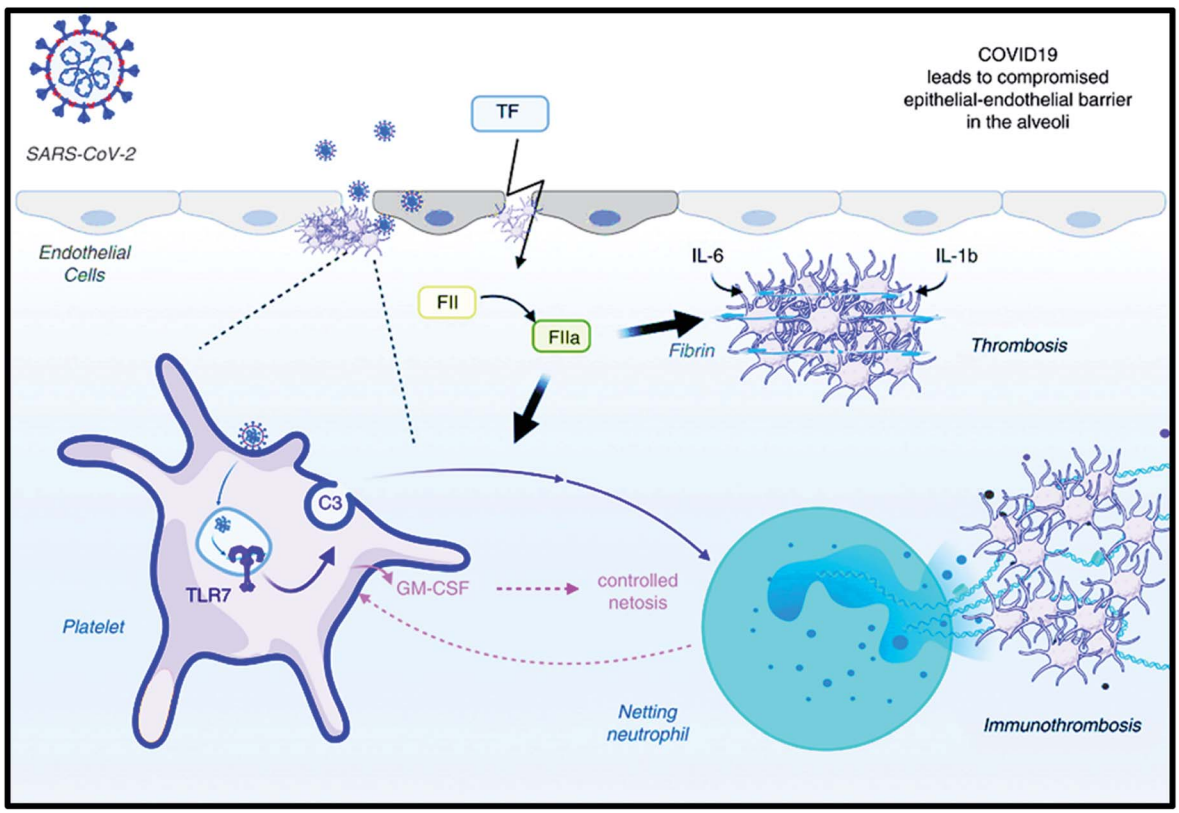


Fig. 2 Potential role of platelets in SARS CoV-2: Implications for thrombosis. The figure has been reproduced from ref. 86 with the permission from WILEY, copyright 2020.

discussed work by suggesting that the lowest platelet count was directly related to death and lower the count, more the association.[82] Hence, the literature and many findings by researchers suggested that the platelet count could be exploited feasibly to clinically analyze the severity of infection.[83–85]

3.7 Cardiac troponin

There are increasing shreds of evidence of higher mortality rates in COVID-19 infected patients with underlying cardiovascular disease.[87,88] Few research groups have examined the utilization of high-sensitivity cardiac troponin I (hs-TnI) as a biomarker of infection development and transience.[89–93] A study conducted on COVID-19 patients exploiting SARS CoV-2 RNA detection depicted an invariable odd ratio for death at 80.1 (95% CI 10.3–620.4, $p < 0.0001$) for hs-TnI.[94] Another study on 416 hospitalized patients with SARS CoV-2 conveyed that hs-TnI was higher in 1 among 5 patients.[95] These patients were more likely to develop infection (59% *vs.* 15%, $p < 0.001$) along with acute kidney injury (9% *vs.* 0%, $p < 0.001$) and needed non-invasive (46% *vs.* 4%, $p < 0.001$) or invasive (22% *vs.* 4%, $p < 0.001$) ventilation. Prompt analysis of myocardial damage identified by raised hs-TnI helps in suitable triage to enlighten the use of vasopressors and inotropes. In addition, the enhanced procoagulant, prothrombotic and inflammatory responses following COVID-19 infection enhanced the likelihood of acute myocardial infection and acute ischemic myocardial injury owing to respiratory failure with hemodynamic instability and hypoxia in severely sick patients. The use of hs-Tn, as discussed above, has the ability to aid in risk stratification and disease phenotyping in hospitalized SARS CoV-2 patients.[96] The probable pathophysiology and anticipated guideline for identification and management has been reported by Bhurint *et al.*, as shown in (Fig. 3).

3.8 Renal markers

Several pieces of evidence have also been reported on the association between the severity of COVID-19 infection and chronic kidney diseases.[98] A study on 28 patients has confirmed suggestively that greater levels of renal biomarkers, for example, creatinine, serum urea, and markers of glomerular filtration have been associated with severe cases.[99] A retrospective study on 701 patients exposed raised serum creatinine levels on admittance, associated with severity, owing to substantial anomalies in the coagulation pathway.[100] The group reported that these cases require intensive care and mechanical ventilation. Univariate Cox regression investigation observing higher creatinine was also related to the in-hospital death rate (HR 2.99, 95% CI: 2.00, 4.47). Likewise, haematuria, proteinuria, and raised urea levels had parallel, if not superior, threat effects. Remarkably, another group of researchers reported a superior role for urinalysis over serum markers for kidney function and suggested the disease severity in case of any abnormalities in the urine test at the time of admission.[101] Another meta-analysis by Michael *et al.* on 3615 patients reported that acute kidney injure is related to poor outcomes in patients with SARS CoV-2.[102] Hence, renal abnormalities in COVID-19 patients at the time of admission might specify greater risks of deterioration, confirming suitable triaging.[103]

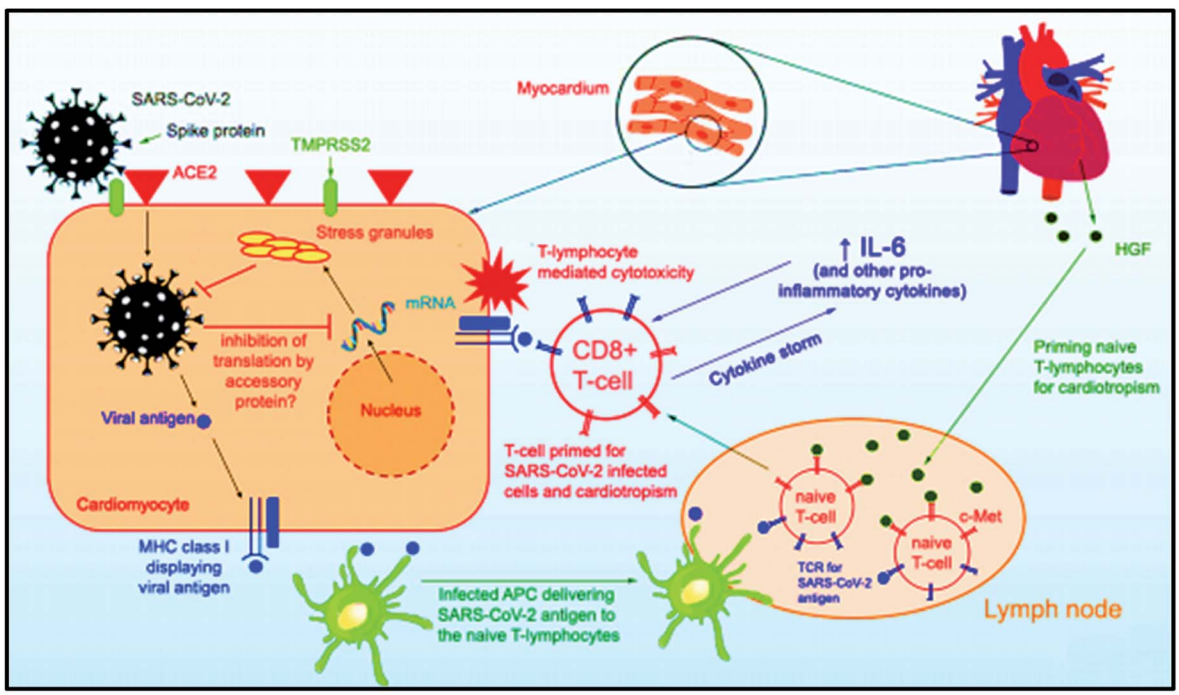


Fig. 3 Proposed pathophysiology of COVID-19 myocarditis. The figure has been reproduced from ref. 97 with permission from Heart Rhythm Society, copyright 2020.

3.9 Lymphopenia

The control of viral infection necessarily depends on natural killer cells and cytotoxic T lymphocytes. A functional collapse of antiviral lymphocytes has been noted in patients with COVID-19.[104] It has been observed that the degree of pro-inflammatory cytokine storm lymphopenia is greater in severe SARS COV-2 patients than in mild cases, and is related to disease severity.[105,106] The N8R, NLR, and lymphocyte percentage are considered to be useful reliable prognostic factors for the timely diagnosis and progress of severe COVID-19 cases. Literature reports the survey of 12 patients (death case) with an average age of 76 years, in which lymphocyte percentage decreased up to 5% in 2 weeks after disease inception. However, when a survey was performed for 7 patients with severe symptoms within a therapeutic window of 35 days and an average age of 35 years, the lymphocyte percentage was reduced at the start but raised to greater than 10% at the time of discharge. Conversely, another survey with 11 patients having a therapeutic time of 26 days and an average age of 49 years was performed in patients with moderate indications. The lymphocyte percentage was found to be persistent and greater than 20% at the time of discharge. The whole study elucidates the practical applicability of lymphopenia-based prognosis for early SARS COV-19 detection.[94]

4 Detection techniques for SARS-COVID

4.1 Computer tomography (CT)/chest CT scan-based SARS COVID-2 detection

Chest CT scan or computer tomography is an important technique employed for the detection of COVID-19. It is being utilized either alone or in combination with RT-PCR.[32,107,108] The methodology involves a large number of chest X-rays from different dimensions and angles to obtain 3D images, which are later examined by some radiologists to validate the presence of the SARS-CoV2 virus. Consolidation of fluid in lungs along with ground glass opacification are observed in patients of COVID-19 associated with pneumonia. Other abnormalities observed in the CT-scan of COVID-19 patients are bilateral lung, diffused dissimilar airspace, or opaque cavity-like patch, involving lower lobes along with peripheral distribution. For instance, at the initial stages (0 to 2 days) of the infection, the CT scan bears a resemblance to a typical chest condition. However, as the infection develops more, the impermeability of the scan rises, and after 4 days, ground-glass opacity has been observed, which seems to show irregular patterns in the scan images. Fig. 4 illustrates the CT scan image of altered sufferers on different days. Likewise, oddity can be noticeably witnessed.[109,110]

Lately, a 3D framework built on chest CT scan images was constructed for CoV-2 analysis.[112] The described model accomplished a high sensitivity and specificity of 90% and 96%, respectively. Therefore, CT scan images have been employed as a prognostic tool for SARS CoV-2 detection. However, high prices, the requirement of large medical instrumentation, and presence in very few hospitals have limited the use of CT scan technology. Meanwhile, this technology is also unable to distinguish different viruses and unable to distinguish between pre-symptomatic, asymptomatic, and some milder symptomatic patients without pneumonia.

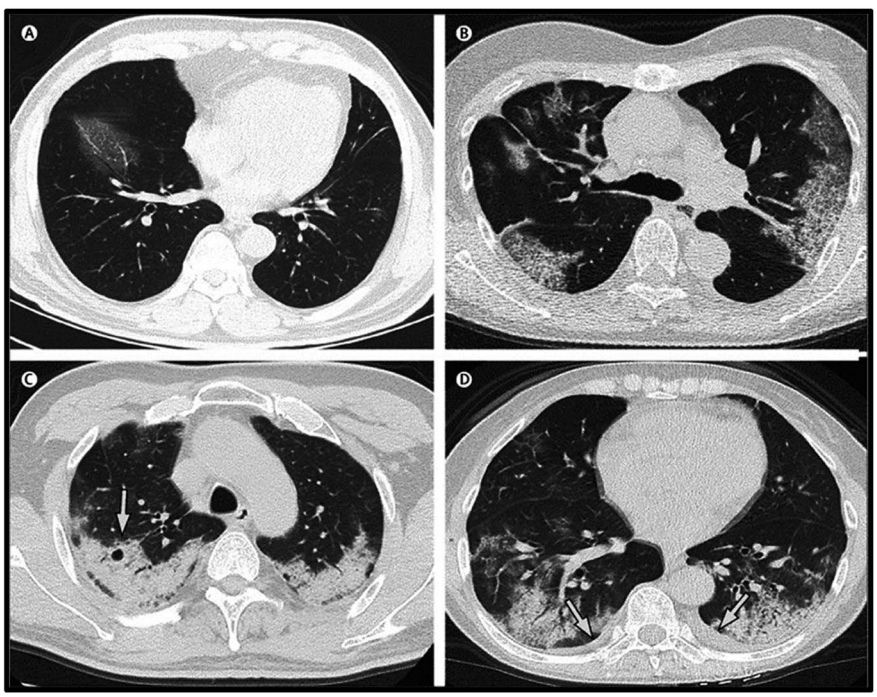


Fig. 4 CT scans (transverse thin-section) in SARS COV-19 patients: (A) a 56 year-old man at 3rd day after symptom onset; (B) a 74 year-old woman at 10th day after symptom onset; (C) a 61 year-old woman at 20th day after symptom onset; (D) 63-year-old woman at 17th day after symptom onset. The figure has been reproduced from ref. 111 with permission from Elsevier, copyright 2020.

4.2 Nucleic acid-based detection

After the recognition of SARS CoV-2 as the main agent for the pandemic, the genome of the virus was sequenced rapidly and distinctive sequences have been recognized for analysis, and different strategies have been proposed for CoVID-19 detection based on the nucleic acid.

4.2.1 Reverse transcription-polymerase chain reaction (RT-PCR)-based SARS COVID-2 detection. Quantitative RT-qPCR has been extensively exploited by Disease Control and Prevention Centre along with the other divisions universally to analyze viruses. This detection strategy has been designed for COVID-19 detection based on the close genetic proximity of 2019-nCoV with other SARS coronavirus, thus exploiting the nucleic acid technology. For detection, first, biological fluids with virus strains are collected from oropharyngeal and nasopharyngeal swabs.[58] Then, viral RNA are isolated through filtration and separation steps. Reverse transcriptase enzyme has been utilized to generate complementary viral DNA (cDNA) from the RNA. It amplifies the specified DNA section through a polymerase chain reaction. The conventional method involves radioactive isotopes as markers to analyze particular nucleic acids (certain noncoding RNAs, mRNA, and pre-mRNAs); however, currently, the real-time detection of DNA probes involves fluorophores and a quencher.

For the SARS-CoV-2 detection process, initially, DNA polymerase enzyme screened envelope gene (E gene) nucleotides, followed by the verification of the nucleocapsid gene (N gene) to the particular fragment of the virus cDNA. Polymerase enzyme encounters the double-stranded DNA and through exonuclease activity, it parts the quencher and fluorophore molecules for the real-time recognition of the viral cDNA using the RT-PCR approach. If the system is well-calibrated, a large number of cDNA copies along with fluorescence signals are formed after a few series of polymerase reactions. The fluorescence intensity and virus concentration are directly proportional. If negative results are obtained, further analysis will be performed by sequencing the RNA polymerase (RdRp) gene. After that analysis, if a positive outcome is achieved, it confirms the existence of SARS-CoV-2 in the suspected patients, and is labeled as COVID-19 positive clinically (Fig. 5). Currently, three regions of the cDNA, *i.e.*, RdRP, E, and N genes have been recognized for the SARS-CoV2 virus detection. The high specificity and

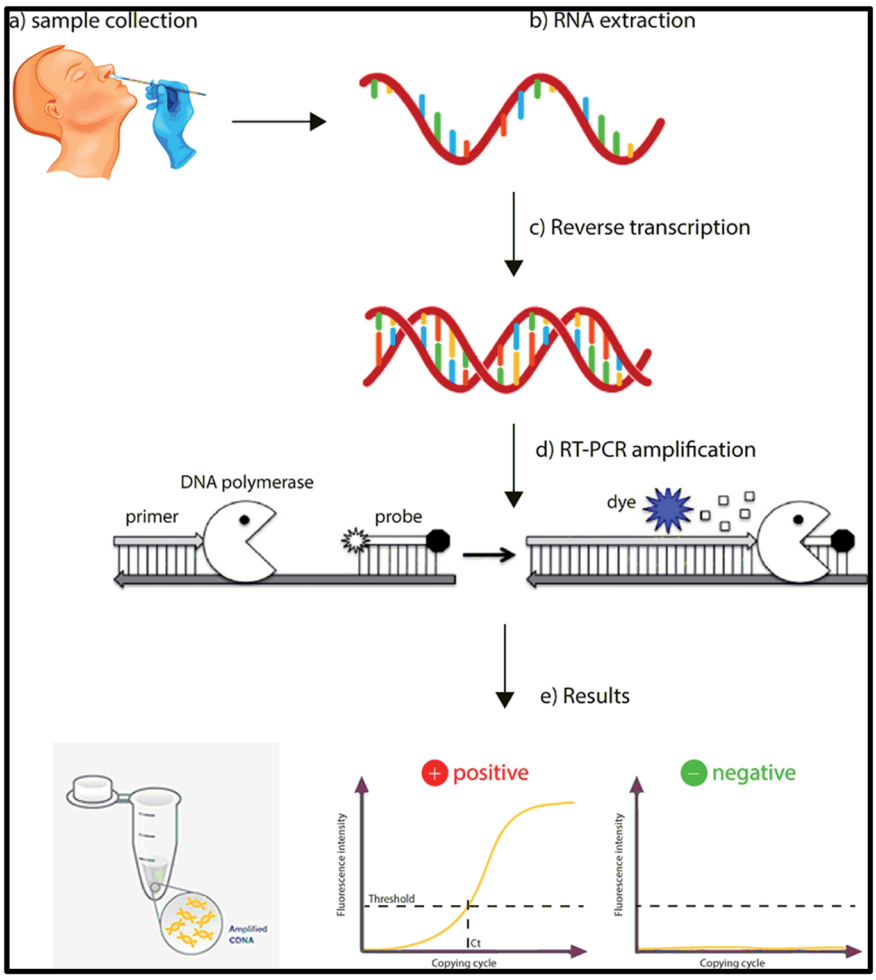


Fig. 5 Steps involved in the qRT-PCR testing methodology. The figure has been reproduced from ref. 114 with permission from WILEY, copyright 2020.

sensitivity of (500 or 1000 copies mL^{-1} of viral RNA) are the main causes of the commercialization of PCR-based diagnostic SARS CoV-2 kits.[113] However, this technique has inherent challenges such as extended turnaround time and a sampling procedure that is affected by the viral load (VL). Meanwhile, SARS-CoV2 destroys the targeted RNA while opening a viral capsid that results in the discharge of small RNA fragments into the bloodstream that challenges detection by RT-PCR. Numerous testing kits have been established worldwide on the principle of RT-PCR.

4.2.2 Digital PCR (dPCR)-based SARS COVID-2 detection. To circumvent the difficult and time-consuming operation of RNA extraction, a new strategy has been developed for viral RNA extraction.[115] This technique employs carboxyl group (PC)-coated magnetic nanoparticles. The conventional column-based extraction of nucleic acid consumes a larger time of 2 h. Meanwhile, pcMNPs-based methodology combines RNA binding and virus lysis into a single step, and these developed pcMNPs-RNA complexes were introduced directly into consequent RT-PCR reactions. This technique requires only 30 min compared to the conventional time consuming RT-PCR. It gives the high sensitivity of 10-copies and wide linear bounds over 5 logs gradient for SARS COV-19 RNA detection. The detection limit of the dPCR technique is approximately 10 folds lower than conventional RT-qPCR.[116] The obtained sensitivity, accuracy, and specificity for RNA extraction utilizing RT-dPCR protocol were 90%, 93%, and 100% respectively.

4.2.3 Loop-mediated isothermal amplification (LAMP)-based SARS COVID-2 detection. The obstacles of great cost and time consumption associated with the previously discussed techniques have been countered by the development of LAMP.[117] The technique can detect nucleic acids. The designed methodology utilizes the chain displacement reaction by DNA polymerase as an alternative to heat to produce the single-stranded template.[118,119] The specifically designed 4 to 6 primers along with DNA polymerase bind to the target genome at different regions, thus amplifying the DNA signal in a short interval of time (Fig. 6). The amplified DNA signal is perceived by variation in turbidity that can be observed by the change in the color owing to pH or fluorescent molecule change, added at the double-stranded DNA. This reaction takes approximately 1 h to complete at 60–65 °C with a sensitivity of ~75 copies along with easy detection.[120] It was potentially implemented for the point-of-care test (POCT).[121] In addition, impure specimens could also be directly sensed by LAMP.[122] Combining impure specimens with non-instrumental calorimetric analysis high-throughput tests is probable.[123] For the robust calorimetric detection of COVID-19, an isothermal LAMP-based method has been developed.[124–126] The reported read-out time was approximately 30 min along with a sensitivity of 97.6% (42/43). The LAMP-based technique is very simple, specific, and easy to visualize owing to the use of a specific number of primers.[127] The technique has the advantage of multiplexing at the amplification or reading step by functionalizing the beads with genes or optical signals to circumvent the likelihood of mutation that leads to false-negative tests. However, the optimization of the primer and other conditions are the challenges associated with LAMP.[118,128] Besides LAMP, many other isothermal amplification approaches for POCT-based nucleic acid detection have been explored at a lower scale, including rolling circle amplification (RCA), recombinase polymerase amplification (RPA), multiple displacement amplification (MDA), and nucleic acid sequence-based amplification (NASBA).

4.2.4 Clustered regularly interspaced short palindromic repeats (CRISPR)-based SARS COVID-2 detection. The turnaround statistics and capacity have limited RT-PCR and many other similar techniques for application in clinical laboratories in this ongoing pandemic, thus forcing scientists to approach new and quick etection strategies. Clustered-Regularly Interspaced Short Palindromic Repeats (CRISPR), along with CRISPR-associated (Cas) protein (CRISPR/Cas) methodologies, were firstly reported in 1987 to protect prokaryotic cells from foreign plasmids and harmful pathogens by identifying and cutting nucleic acid sequences of foreign species that hold short

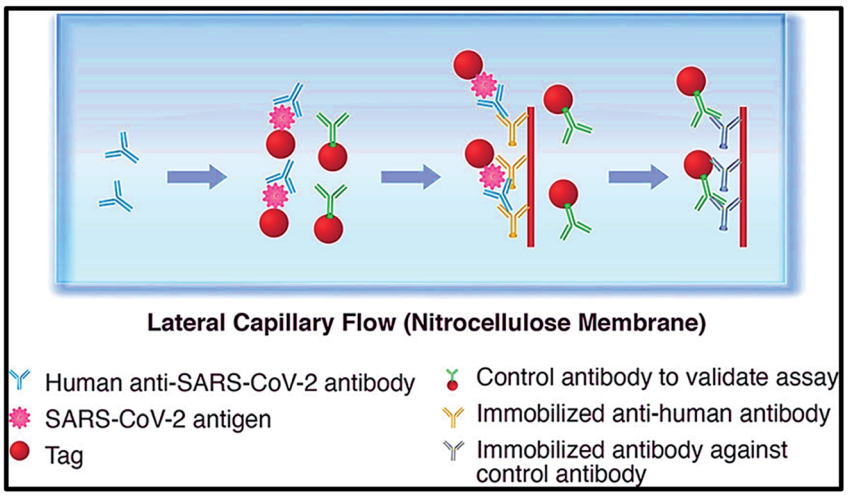


Fig. 6 Lateral flow immunoassay-based sensing of anti-SARS-CoV-2 antibodies. The figure has been reproduced from ref. 129 with permission from ACS, copyright 2020.

palindromic repeating spacer sequences.[130] To date, numerous genome-editing methodologies have been introduced but the latest one is recognized as CRISPR/Cas. Therefore, CRISPR/Cas has enticed much importance in the scientific community for disease diagnosis and management owing to its robust, less expensive, and precise genome editing approaches.[131] CRISP works similar to genomic programming by manipulating isothermal reactions to synthesize and amplify cDNA that again reconvert to RNA. The CRISP editor is programmed to bind to amplified RNA with specified genetic coding to edit at a precise location, thereby cutting off the quencher from fluorescence for signal detection for genotyping, POC virus analysis, and disease monitoring. A number of laboratories have developed CRISP-based SARS COV-2 detection tests with promising LOD, higher sensitivity, and lesser assay time. It includes a lateral flow assay that extracts RNA from respiratory swabs and is labelled as DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) for SARS-CoV-2 analysis.[132] This protocol works by synchronizing reverse transcription and RT-LAMP for NP and OP swab-extracted RNA that was further followed by the detection of Cas12-verified coronavirus sequences. Afterward, corresponding molecule cleavage confirms the occurrence of the virus in the patient (Fig. 7). Furthermore, the reported LOD was 10 copies per μL input with an assay time of 30 to 40 min. Few other diagnostics studies have also been conducted exploiting similar methodology along with relatable findings.[133] Few researchers have freshly reported a FnCas9 Editor Linked Uniform Detection Assay (FELUDA) that utilizes a highly sensitive FnCas9 protein to identify the mismatched region and specific position in the reverse transcribed DNA.[134] Another group reported nucleic acid SARS-CoV-2 detection methodology, involving the PCR amplification of target sequences, employing biotinylated primers that were formerly attached to streptavidin-coated beads. Later on, single guide RNA-containing fluorescently-labeled Cas9 complexes interacted with immobilized target sequences, thereby producing analytical signals. This approach has also been improvised to produce streptavidin-containing lateral flow strips that binds to the biotinylated targets, giving a low detection limit of 110 femtomolar for COVID-19 identification.

4.3 Protein-based detection

The antibodies and antigens that are released in reaction to SARS CoV-2 have been exploited as a source to assess COVID-19. However, viral load variations during disease progression have limited the long scale application of methodology.

4.3.1 Serological-based tests (SB-T) for SARS COVID-2 detection. SB-T is another currently studied and explored option for COVID-19 detection. It is a blood-based method, which works by measuring protein and antibodies in blood when the body is countering infection (immune response), caused by COVID-19 rather than the virus itself.[136] Presently, serological tests have been employed on asymptomatic patients.[137–139] Nucleocapsid protein is highly abundant in viruses and this immunogenic phosphoprotein is used as a biomarker because of its rare mutation property. A group of scientists has exploited Rp3 nucleocapsid protein to detect IgM and IgG antibodies from SARS CoV-2-suspected patients owing

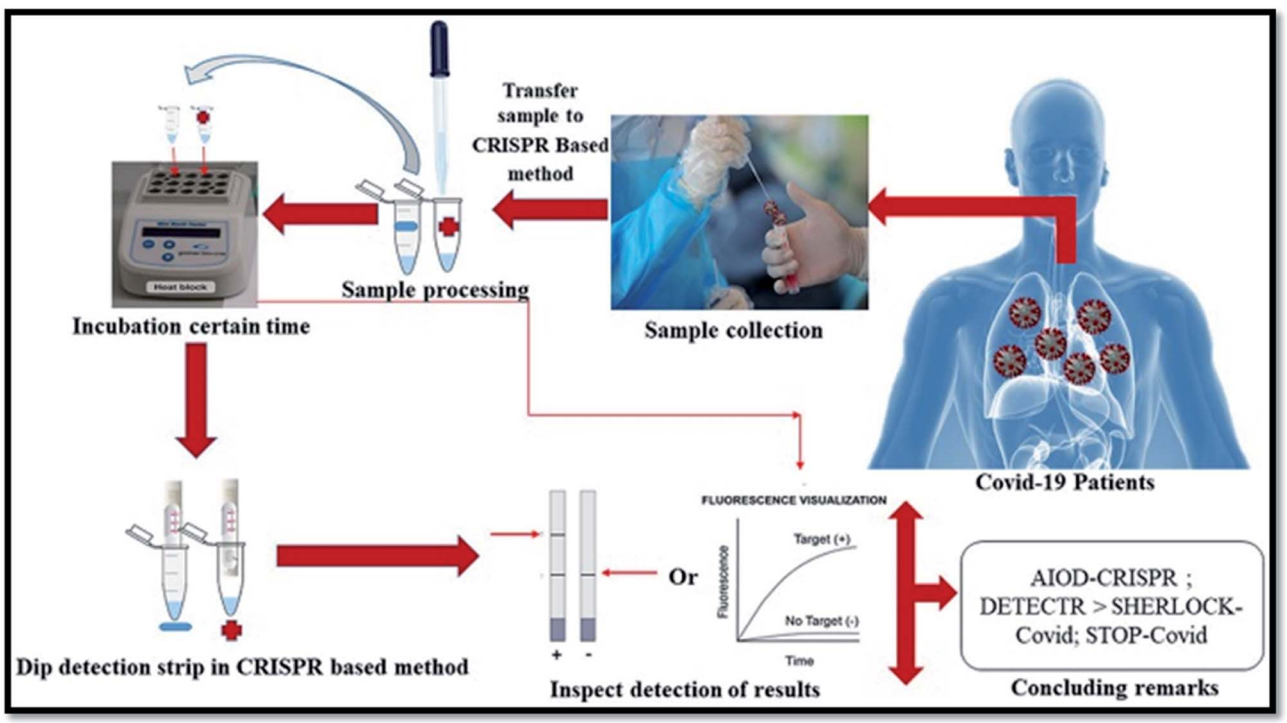


Fig. 7 CRISP-based method for SARS CoV-2 detection. The figure has been reproduced from ref. 135 with permission from Elsevier, copyright 2021.

to the 90% similarity of the mentioned protein with SARS viruses.[80] The SB tests involve the adsorption of recombinant protein to a multi-well plate. Later on, the dilute serum of the suspected patient is mixed to conduct ELISA. The detection signals are obtained by adding horseradish peroxidase-functionalized anti-human IgG antibody. If anti-COVID 19 IgG exists, it will be sandwiched between the adsorbed protein and the antihuman IgG probe. Correspondingly, IgM has also been exploited for sandwiched assay. As the antibody level increases with each passing day in COVID-19 patients, so the reported hike in IgM and IgG was found to be 81% and 100% in COVID-19 positive patients, respectively, after 5 days, compared to 51% and 81% on day 0 of SARS COV-2 contagion. The sensitivity of COVID-19 serological assays is in the linear range of 0–100% for both IgG[140–142] and IgM.[143,144] The determinants for the sensitivity of the SB test include the test and patient factors. Meanwhile, the reported specificity of the COVID-19 serological assays are in the range of 6.9–100% (ref. 145–147) and 0–100% (ref. 140,143) for IgM and IgG, respectively. However, an excellent specificity has been exhibited by several commercial assay kits. Similar to sensitivity, various determinants affect the specificity for serology tests such as test factors and patient factors. This methodology is promising to obtain the prevalence of COVID-19 in the population. Currently, FDA has approved a number of serological tests for early detection and emergency analysis. However, low sensitivity have been reported for the SB-based assay compared to LAMP and rRT-PCR.[128]

4.3.2 Enzyme-linked immunosorbent assay (ELISA)-based SARS COVID-2 detection. Enzyme immunoassays (EIA) along with enzyme-linked immunosorbent assays (ELISA) are widely employed techniques for the quantification and detection of viral antibodies or proteins produced by the body in response to COVID-19 infection for the detection of COVID-19 disease. Both techniques work on the same basic principle and are derived from radioimmunoassay (RIA), which have been employed to measure small quantities of biological molecules such as peptides, protein, and hormones. RIA was modified to current ELISA and EIA owing to safety concerns by replacing the radioisotope with friendly enzymes. Both assays utilize the basic idea of an antigen-binding to its specific targeted antibody for the detection of antibodies and antigens in the fluid samples (Fig. 8). These assays also use enzyme-labeled antibodies and antigens to detect biological molecules.[148] A chromogenic substrate for the enzyme produces a fluorescent, colorimetric, or luminescence detection aided by enzymes used in the reaction. Both qualitative and quantitative measures can be measured based on such detection.[149] However, sometimes the enzyme-mediated color change will keep on reacting indefinitely, thus leading to false-positive results. Likewise, the amount of viral load changes during infection progression, also producing difficulty, thereby leading to the detection of low concentrations of the viral protein.[58]

A study was made on COVID-19 diagnosed cases, in which a group collected ELISA IgM and IgG antibodies from 63 samples. Meanwhile, certain researchers collected 91 plasma specimens for the colloidal gold-immuno-chromatographic assay (GICA). Meanwhile, the sensitivity of ELISA (IgM and IgG) was found to be 55/63 (87.3%). However, the sensitivities of the GICA IgM and IgG were found to be 75/91 (82.4%). Both methodologies showed negative results for healthy controls with 100% specificity.[150] Another study was made for antibody screening to identify SARS-CoV-2 in which IgG and IgA ELISAs were evaluated in unification with the EURO-Lab workstation. The premediated specificities were 91.9% for IgG and 73.0% for IgA.[151]

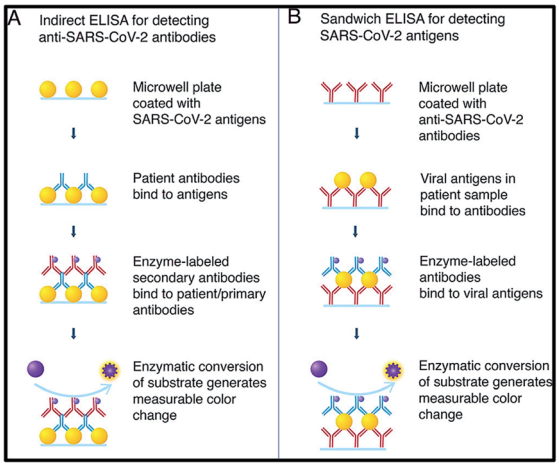


Fig. 8 ELISA assays identify antibodies (A) or antigens (B). The figure has been reproduced from ref. 129 with permission from European Centre for Disease Prevention and Control, Copyright 2020.

4.3.3 Lateral flow immune-chromato-graphic strip (LFICS)-based SARS COVID-2 detection. FICS works on the principle of pregnancy tests and is built up of conjugate pad (CP), sample pad (SP), nitrocellulose membrane (NC), and absorbent pad. An immunofluorescent assay-based methodology was also proposed for the rapid diagnosis and sensitive sensing of IgM and IgG of SARS COVID-2 in human serum specimens within the duration of 10 min. The COVID-19 recombinant nucleocapsid protein was utilized to detect the antigen. Meanwhile, lanthanide-fluorescent microspheres were utilized to evaluate solid-phase immuno-chromatographic assay qualitatively or semi-quantitatively. The specificity and sensitivity of immuno-chromatographic assays were found to be 98.68% and 93.10% for IgM and 98.72% and 100% for IgG in 28 positive and 77 negative clinical serum samples, respectively.[152]

Another (LFICS) has been sanctioned in China for point-of-care disease COVID-19 diagnosis.[153] The colloidal gold immunochromatographic assay (CGICA) based on gold nanoparticles (Au NPs) can identify IgM and IgG antibodies concurrently within 15 min, against the SARS COVID-2 virus in the blood. The gross sensitivity of the LFICS assay was found to be 88.66% with 90.63% specificity.[99] The results demonstrate that immunoassay-based COVID-19 detection can also be used as a tool owing to its portable and economically viable infrastructure, along with good sensitivity and specificity. The inclusive process and data analysis of the abovementioned immunodiagnostic assay are revealed in Fig. 9. The immunoassay involves a few drops of blood to detect a virus qualitatively in a miniaturized set-up. Nonetheless, the analysis of the exact titer is not probable using this methodology.

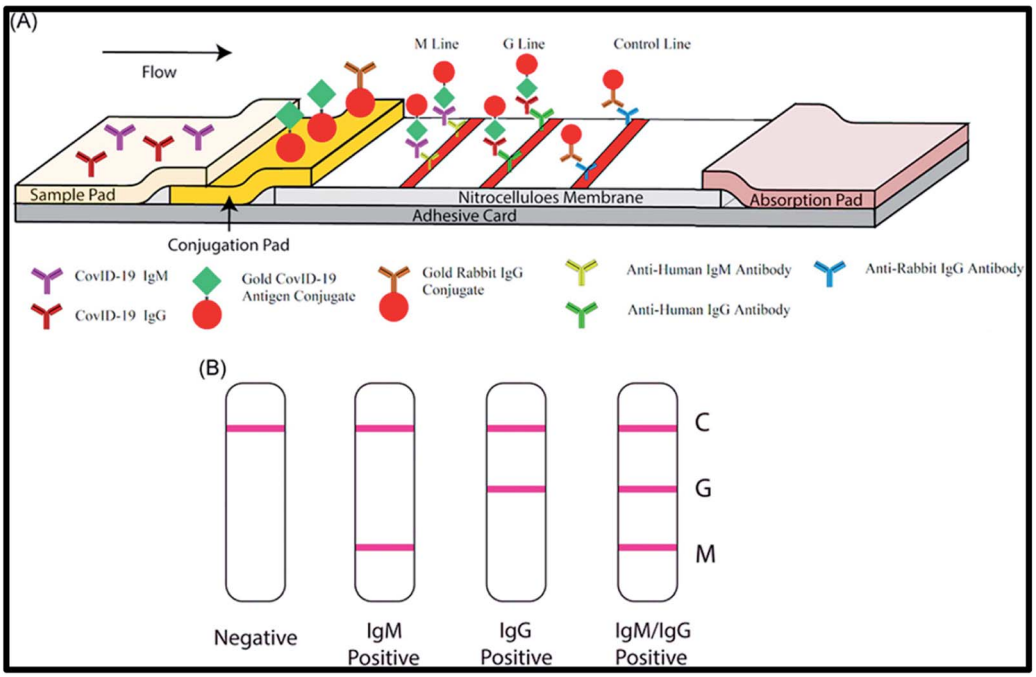


Fig. 9 A systematic diagram of the detection device based on IgM-IgG combined antibody test. The figure has been reproduced from ref. 154 with permission from WILEY, copyright 2020.

4.4 Plasmonic sensors

The ongoing SARS-COV-2 pandemic situation highlights the need for reliable and fast sensing strategies. Deep down, this pandemic is improving virus sensing to some extent, particularly plasmonic techniques, as portable, cheap, reliable, and fast sensors are in demand now. PCR is considered as the most effective and real time detection methodology for SARS-CoV-2 with almost 100% selectivity.[155] However, it still has several drawbacks including cost and long response time along with a complex system.[156] Another major drawback of RT-PCR-based detection has been associated with a large amount of false-positive and negative cases, thus limiting its practical applicability. In this regard, plasmonic detection techniques integrated with lateral flow processes have tremendously increased the detection of viruses in a limited amount of time.[157] Apart from the fastest way with higher sensitivity and selectivity for different applications,[158] plasmonic sensors in point-of-care (POC) devices for the early detection of viral diseases remain nascent.[159] Efforts are being made for the improved functionality of such sensors by implementing various techniques, namely, interference lithography, nanoimprinting microsphere lithography, roll-to-roll pattering, and oblique angle deposition. These methods not only advance the accuracy of the sensors but also bringing the cost down.[157,160,161] Besides, such modifications also simplify the detection of coronavirus and thus may help in disease control.[162]

Recently, a plasmonic sensor with dual functionality combining the plasmonic photothermal (PPT) as well as surfaced plasmonic resonance effects has been employed as a robust and cost-effective substitute to RT-PCR. A selective sequence of CoV-2 has been analyzed *via* nucleic acid hybridization by exploiting complementary DNA-functionalized 2-D gold nanoislands (AuNIs). The two dissimilar incident angles were employed for the excitation of LSPR and plasmonic resonance of PPT, which desirably improved the sensing stability, reliability, and sensitivity, as shown in Fig. 10. A label-free and real-time recognition of viral sequences comprising ORF1ab, RdRp, and the E genes from the COVID-19 has been made owing to the attained ideal configuration. In addition, the enhanced specificity along with the hybridization kinetics about nucleic acid-based recognition have also been achieved by the utilization of PPT enhancement on AuNPs-chip. Such a biosensor exhibited a low detection limit of 0.22 pM along with multigene combination.[163]

Another group developed a selective methodology for the N-gene detection of SARS CoV-2 by the naked eye. They reported a colorimetric assay using AuNPs integrated with thiolated antisense oligonucleotides (ASOs). This protocol was employed for confirming positive COVID-19 cases in a short period of 10 min. The capped AuNPs showed aggregation, while its targeted RNA sequence came into the picture. Furthermore, the existence of RNaseH (enzyme) enhances the splits of the RNA, particularly in RNA-DNA fusion, directing the additional accumulation of AuNPs, as shown in (Fig. 11). The assay worked selectively for MERS-CoV viral RNA having an LOD of 0.18 ng μL^{-1}. Consequently, the current work reported an effective reproducible and reliable methodology for the naked eye detection of CoV-2 without the need for any advanced instrumental techniques.[164]

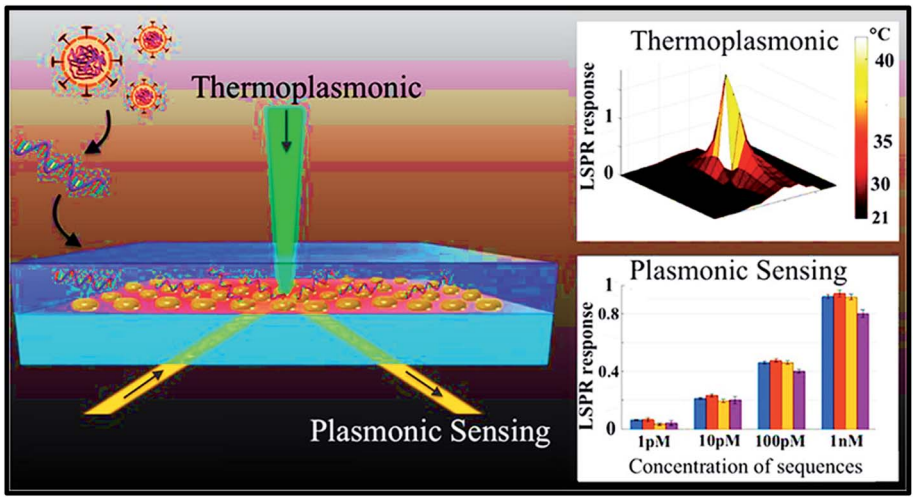


Fig. 10 Graphical presentation of plasmonic sensing of SARS CoV-2 at lower concentrations. The figure has been reproduced from ref. 58 with permission from European Centre for Disease Prevention and Control, Copyright 2020.

4.5 Surface-enhanced raman spectroscopy (SERS)

For the evaluation of organic and inorganic compounds, Raman spectroscopy (RS) is a well-known technique.[165] It has been employed for the recognition of bacteria in food 22 as well as for HIV-1 detection.[166–168] Recently, RS was applied for SARS-COVID-19 detection using Au nanoparticles.[169] However, signal amplification was required due to the very low signal intensity, for which the plasmonic effects of different metallic nanoparticles can be applied. This plasmonic field is able to enhance the signal by $\sim 10^8$ to 10^9 times, based on the chemical composition or the aspect ratio (shape).[170] Further, various 2-D materials can easily provide an additional signal amplification of approximately 10^2 because of the effective transfer of electrons.[171] This signal enhancement is known as Surface-Enhanced Raman Spectroscopy (SERS) for the detection of viruses as well as other pathogens[172] with improved specificity and sensitivity.[173]

According to Sanchez et al.,[175] Raman-SERS spectral analysis of any viral particle is complex and can give broad peaks due to multiple chemical species (Fig. 12). Lee *et al.*[176] reported a similar trend in the spectra of HIV-1 viral infection. An interesting fact regarding virion and S-protein spectra is that they lack the peaks characteristic (1640–1678 cm^{-1}) of amide-I bonds. Kuroski *et al.* explained this with reference to the SERS studies of various homopeptides composed of Tyr-, Ala-, Trp-, and Gly-chains with a variety of investigational circumstances.[177] In this study, it was found that the lack of some bands is due to amino side chains. This side chain increased

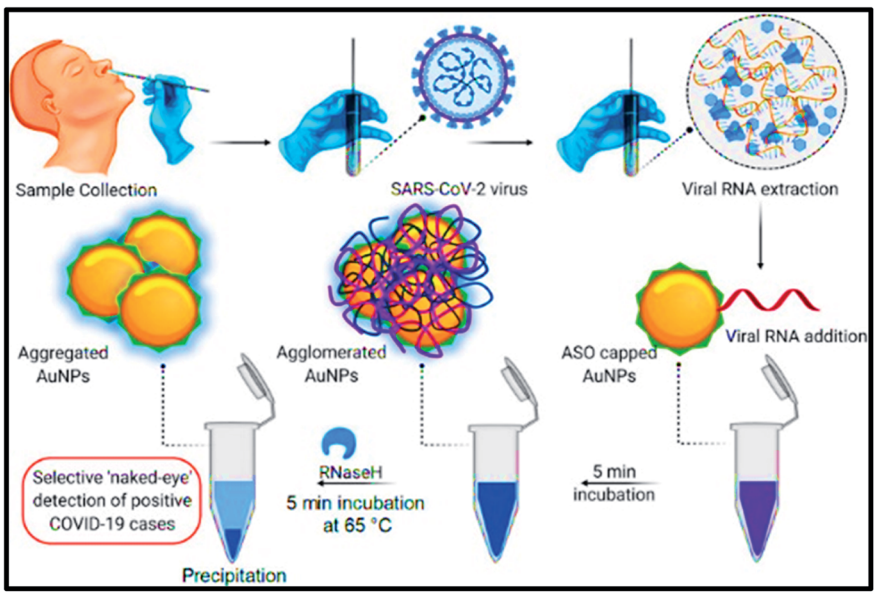


Fig. 11 Schematic illustration of naked eye sensing of COVD-19 by AuNPs capped by ASO. The figure has been reproduced from ref. 164 with permission from ACS, copyright 2020.

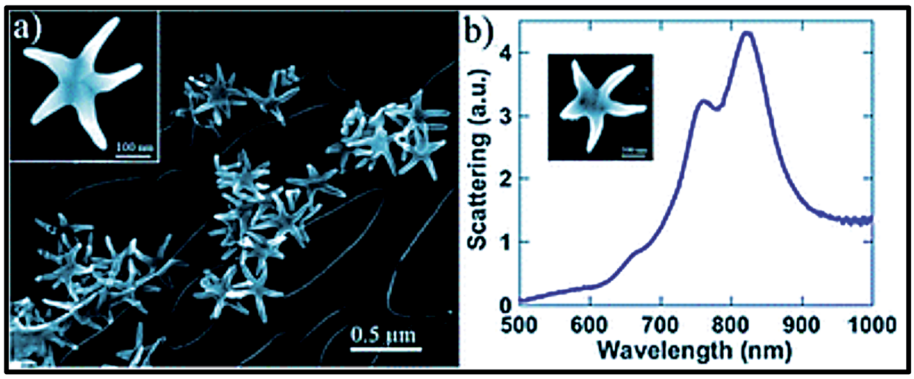


Fig. 12 Application of SERS indicated by (a) the SEM image of Au–Cu nanostars, (b) optical absorption spectra (OAS) of these nanostars. The figure has been reproduced from ref. 174 with permission from ACS, copyright 2020.

the distance among the particles and the peptide bond. However, another possibility might be the deactivation that can disrupt amino acids. In another study, Zhang *et al.* employed SERS based on silver nanorods, which were functionalized by binding protein and cellular-receptor angiotensin converting enzyme 2 (ACE2) (Fig. 13).[174] A very weak peak for amide I was found in this case, which was closer to the noise. They used the 1189 cm^{-1} peak in ACE-2 with a peak shift of 1182 cm^{-1} as the

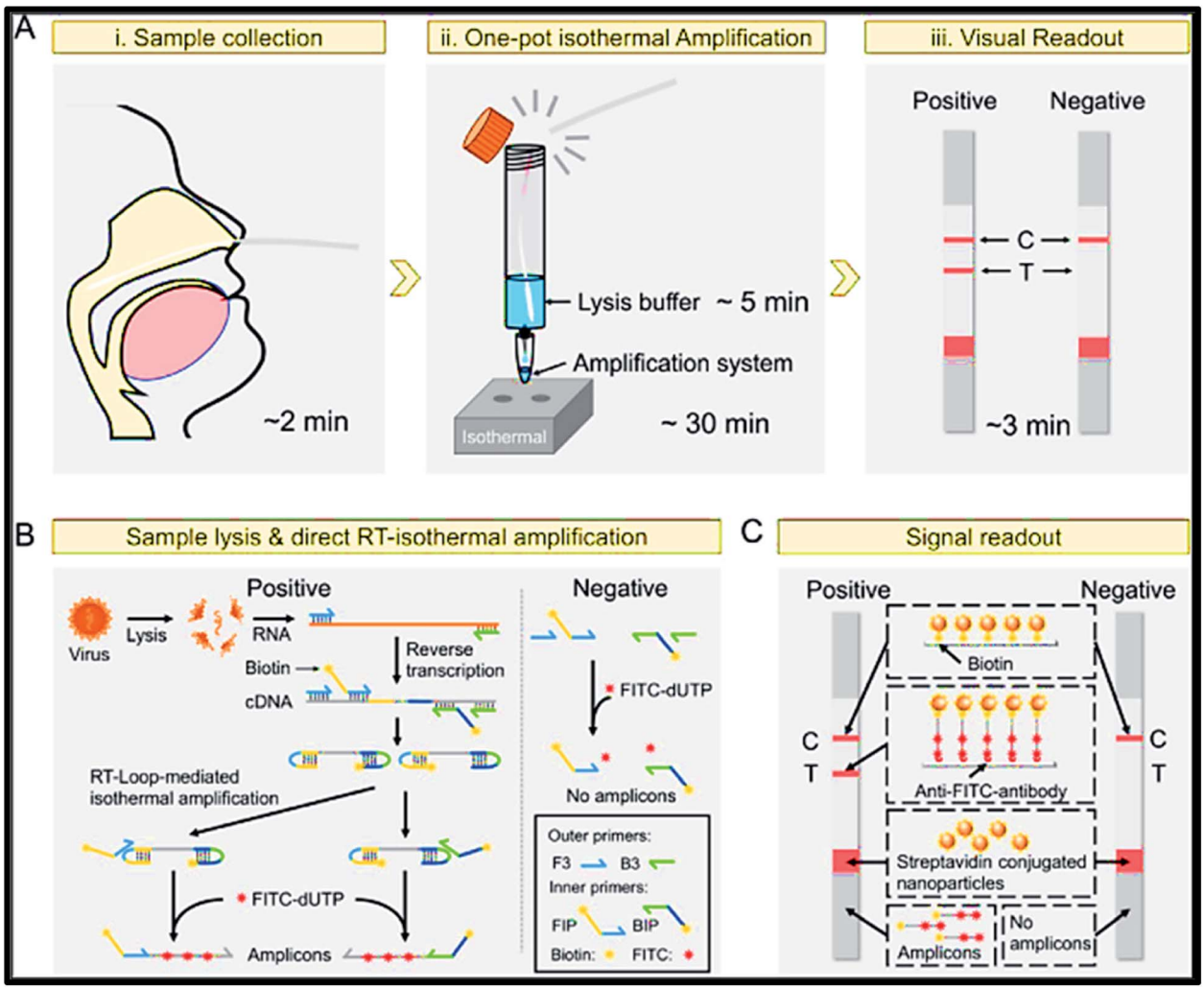


Fig. 13 Diagram illustration of the RT-LAMP-LFA scheme. (A) Workflow of the developed scheme for the detection of COVD-19, (B) principle involved in RT-LAMP amplification. (C) Principle of visual detection with LFA. The figure has been reproduced from ref. 174 with permission from ACS, copyright 2020.

Table 1 Pros and cons of the diagnosis techniques for SARS-CoV 2

Diagnostic techniques	Pros	Cons
Computer topography/Chest CT scan	1. Provides high quality, detailed image of patient organs	1. Higher price
	2. Images are problem-solving for cases that do not show up in initial PCR tests	2. Requirement of large medical instrumentation
		3. Presence in very few hospitals
		4. Can result in a fast negative result, especially at early infection
		5. Unable to distinguish different viruses and unable to detect pre-symptomatic, asymptomatic, and some milder symptomatic patients without pneumonia
Nucleic acid-based detection	1. High specificity	1. Extended turnaround time
	2. High sensitivity	2. The sampling procedure is affected by the viral load (VL)
	3. Promising LOD	3. SARS-CoV2 destroys the targeted RNA while opening viral capsid that results in the discharge of small RNA fragments into the bloodstream that challenges detection by RT-PCR.
	4. Less assay time	4. A large amount of false negative and positive cases
Protein-based detection	1. Good specificity	1. Viral load variations during disease progression
	2. Promising method to obtain the pervasiveness of COVID-19 in a larger population	2. Sometimes false-positive results
	3. Economic and portable infrastructure along with good sensitivity	3. Detection of low concentrations of viral protein
Plasmonic sensor based detection	1. Take a few to tens of minutes or even less	1. Early diagnostics of viral diseases remains nascent
	2. Rapid sampling	
	3. Broad linear range	
	4. Lower LOD.	
	5. High sensitivity	
	6. Higher selectivity	
Signal enhanced Raman spectroscopy (SERS)	1. High selectivity	1. Low signal amplification
	2. High specificity	
A field-effect transistor (FET)	1. Miniaturization	1. Less sensitive
	2. Robust	
	3. Real-time	
	4. Selective	
	4. Label-free probing	
	5. Use of ultralow concentration	

spike protein was attached. Then, they used a ratio of these two peaks in order to predict viral presence. Their SARS-CoV-2 spectra showed very few weak peaks.

Besides, SERS amplification requires a molecule, which should be in contact with any suitable metallic nanoparticle. This method was used by Jhon *et al.* by combining SERS with a particular substrate, which is able to combine plasmonic amplification with excitation amplification and provides us with a greater number of peaks. A consistent spectrum with almost 5 peaks was obtained by this methodology. These peaks were connected to S and N proteins. However, fewer peaks were assigned to the particles deactivated due to the destruction of some viral parts. The range of 744–7 nM was obtained in the SERS-spectra of InBios-Spike-Protein. Afterward, for the calibration curve, PLSR (Partial Least Squares Regression) was employed with the variance of 99.9%. Finally, a limit of detection (LOD) of 8.89×10^{-9} M with a Root Mean Square Error (RMSE) of $\approx 2.27 \times 10^{-9}$ was obtained, which is a good approximation for concentrations in the nM range. Meanwhile, we have summarized the pros and cons of all the discussed techniques as shown in (Table 1).

4.6 Field-effect transistor (FET)

The FETs and amperometric systems are important electronic and digital devices that are broadly employed for the sensing of biomolecules and pathogens. The remunerations of low cost, mass manufacturing, and miniaturization make these systems most desirable and applicable. FET biosensors have also been applied for the clinical diagnosis of COVD-19. The sensor was established by integrating the COVD-19 spike antibody with the graphene sheets of FET. The developed transducing interface detected the COVID-19 protein in phosphate buffer saline (PBS)

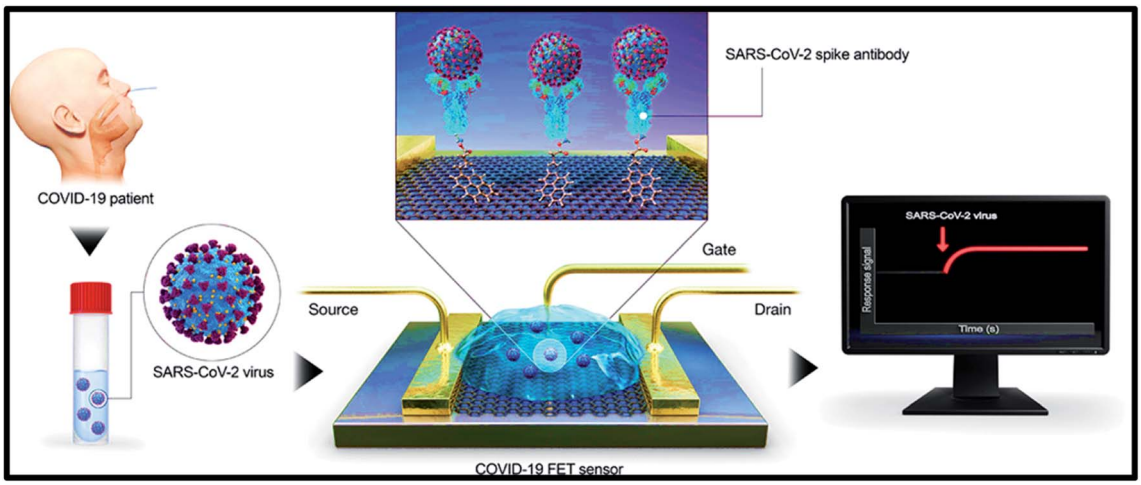


Fig. 14 Representation of the operational setup of FET-based COVID-19 sensor. The figure has been reproduced from ref. 179 with permission from ACS, copyright 2020.

in the concentration range of 1–100 fg mL^{-1}. Meanwhile, the OFET was also apt to analyze COVID-19 in clinical trials with an LOD of 2.42×10^2 copies mL^{-1} and a detection bound (DB) of 1.6×10^1 pfu mL^{-1}.[178] Thus, the developed FET-based interfaces can be employed for the production of compact POC devices and provide the advantages of robust, real-time, selective, and label-free probing of SARS Cov-2 at an ultralow concentration. The working methodology of FET-based COVD-19 detection has been shown in Fig. 14.

4.7 Visualization and characterization tools for COVID-19 detection

These basic techniques are usually employed separately or with the combination of other detection techniques for the viral structure. The outstanding proficiencies of these techniques provide a deep examination of the viral structure and function along with a detailed examination of viral influence on the extracellular environment and host cells, which are beneficial in drug discovery applications.[180–190]

4.7.1 Electron microscopy (EM). An EM utilizes an electronic beam as an illumination source. The resolving power of the EM is much greater compared to optical microscopes owing to a small wavelength of electrons than visible light photons. Therefore, EM has the ability to visualize the particle structure at the nanoscale, thus making it a potent apparatus for viral diagnosis. The most employed molecular and serological approaches need a specified probe to identify the virus; the EM approach does not require organism-specific reagents to detect pathogenic agents. As for anonymous disease analysis, molecular tests require details about potential agents, whereas EM gives an open outlook of the unknown given sample under analysis.[183] Besides the direct analysis of the virus by visualization, it also gives directions regarding the ultrastructure and structural dynamics of the virus associated to attachment and replication. These traits make EM a worthwhile technique for the detection and design of antiviral agents and vaccines.[186,188,191,192] To examine the viral structure, three main techniques are mostly employed. In the negative staining method, the sample remains untouched with a stained background, making the sample visible. Cryo-EM is an image-based technique that is utilized to obtain a high resolution three dimensional image of cells and other biological constituents.[193,194] SEM has been extensively employed for virus quantification. SEM, when combined with TEM, is also be able to provide a high resolution image of the viral structure.[183] Recently, an image of SARS CoV-2 was taken *via* TEM,[183,195] as shown in Fig. 15.

Recently, cryo-EM was utilized for the identification of multiple mono-clonal antibodies, aiming at the coronavirus, particularly using S-protein from memory B-cells of the infected

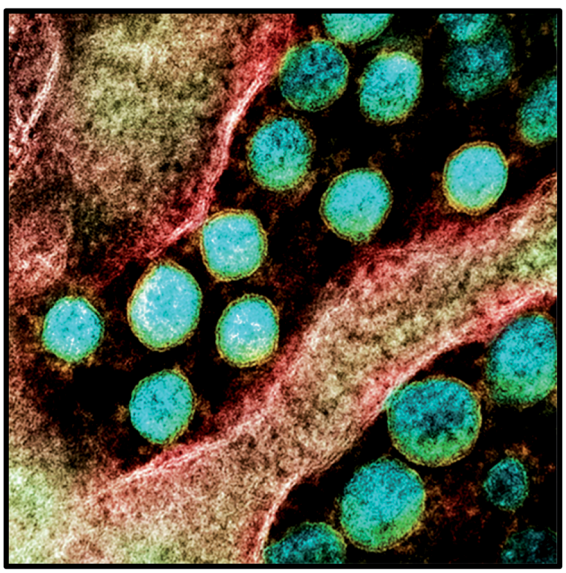


Fig. 15 SARS CoV-2 virus particles envisioned by TEM. Viral particles are revealed in blue-green with yellow viral envelopes. This TEM was captured and color-magnified. The figure has been reproduced from ref. 199 under Creative Commons license agreement, copyright 2020.

person. It was found that an antibody called S309 has the potential to neutralize CoV-2 and SARS-CoV.[196]

The methodology has been utilized for the investigation of RNA-dependent RNA polymerase nsp12 of the COVID-19 structure, which catalyzes the synthesis of viral RNA in complexation with 2 cofactors, namely, nsp7 and nsp8.[197] In ref. 198, detailed information about the 3D structure and the morphological surface of the causative agent in SARS-CoV was achieved. These results suggest that such techniques can easily be exploited for the detailed analysis of SARS COVID-19.

4.7.2 X-ray crystallography. To achieve highest the resolution image for the viral structure along with macro-molecular associations at the atomic level, single crystal XRD is the most promising methodology. Five steps are involved in identifying the viral structure *via* XRD, including virus particles' preparation and purification, crystallization, diffracted data analysis, phase calculation through isomorphous replacement or molecular replacement (MR),[198,200] and, lastly, building the model.[200–202] Kits are freely available commercially for the preparation and purification of viruses from the extracellular matrix.[203] Handling should be performed as gently as possible to uphold the icosahedral symmetry of the virus. Consequently, crystallization is mandatory to achieve the supersaturation of the aqueous protein solution. Crystallization involves nucleation by developing a crystal nucleus, followed by a growth process. Batch crystallization, dialysis, liquid–liquid diffusion, and vapor diffusion are the major four steps involved in growing the crystals of virus samples.[203] Furthermore, the crystallographic data is collected at 100 K, *i.e.*, cryogenic temperature, to prevent secondary radiation damage to the virus specimen and to improve the resolution. However, growing high-quality virus crystal *via* X-ray crystallography is quite challenging and the currently used methods are slow and centered on trial and errors.

Nonetheless, this cryo technique could be an analogous process for viral protein crystallography.[204] The details provided by 3D cryo-EM could be assimilated with the obtained X-ray data to ameliorate the constructed model of the virus particles.[205] Serial femtosecond X-ray crystallography (SFX) also uses X-ray free-electron lasers (XFELs). It has been exploited to scrutinize the viruses as single particles and crystals owing to its unmatched brilliance of XFEL beams and the pulse duration in a femtosecond. The variations that occurs in the life cycles of viruses, such as response to the changes in pH and interaction of the viral protein with the receptors, could be sensed using time-resolved SFX.[206–208] Recently, a 3D crystal structure of unliganded CoV-2 was determined by a resolution power of 1.75 Å, as shown in Fig. 16 (ref. 209) and was utilized to determine the optimization of α-ketoamide inhibitor series.

However, EM and XRD methodologies are established on the average of countless particles that exist in the crystal or electron micrographs. Consequently, it generates inadequacy in the data acquired from the structural differences between the discrete particles present in a large population. Moreover, the above-mentioned methodologies require an atmosphere that is far from the physiological conditions of viral functioning and prevent the characterization of vibrant properties in real-time. Thus, it obscures the study of the viruses that lack distinct structural symmetry, such as SARS CoV-2, which is an enveloped virus. Lastly, AFM is also a direct imaging technology that offers noteworthy impression of the virus study. It offers prospects for studying the COV-2 virus particles.

4.7.3 Atomic force microscopy. AFM has developed into a significant instrument for the characterization and visualization of nanoscale images of specimens in air as well as liquid.[210] Normally, the deflection of the microcantilever is utilized in AFM measurement to examine the contact between the nanometric tip located at the end of the microcantilever and the surface of the sample. A broad information pool including mechanical, thermal, chemical, physical, atomistic details of the surface, and viscoelastic characteristics of nanobiomaterials entities could be achieved based on time scales, range, and types of interactions.[211–223] The very first invented mode of AFM is "contact mode", in which the tip is raster scanned on the specimen interface by sustaining a constant force generated on the sample through deflection recognition by regulating the height of the tip. However, the foremost challenge associated with contact mode utilization while imaging soft entities such

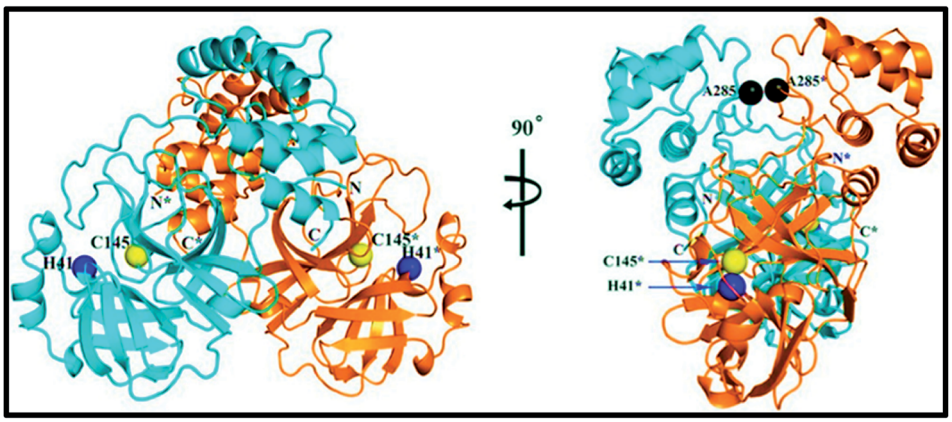


Fig. 16 The structure of SARS CoV-2 Mpro from two altered 3D viewpoints. The figure has been reproduced from ref. 209 with permission from AAAS, copyright 2020.

as biological specimens and viruses is the force (applied) to circumvent undesirable friction, impairment to the sample, and reversible or irreversible deformation.[224] Dynamic AFM was invented to minimize the applied force and friction and to enhance image resolution.[225] In dynamic SFM, while scanning the sample surface, the microcantilever oscillates near its resonance frequency. The two main dynamic AFM modes are tapping mode, also known as amplitude modulation atomic force microscopy (AM-AFM), and the second one is frequency modulation atomic force microscopy (FM-AFM).[225] The friction is reduced and the risk of the damage to the specimen is significantly lessened in the dynamic mode owing to the reduction of the contact time amid the tip and the specimen to the small fraction of the oscillation time. Recently, some radical techniques have been established to enhance the resolution and find more detailed information on these materials. These dynamic advanced methodologies can be categorized as multifrequency methods[226] as the cantilever is excited at many different frequencies and altered signals are utilized as feedback parameters. AFM can also quantify and measure the structural and mechanical properties of the viruses, in addition to visualizing and imaging the virus particles.[227] In addition, it can also be exploited to operate and dissect biological entities along with viruses.[228–231]

Furthermore, to examine the chemical, physical, and viscoelastic traits of the viruses, the force–distance curve-based AFM (FD-based AFM) and currently, multifrequency methods, are the frequently employed approaches. In the latest FD curve-based AFM, while imaging the biological sample, several FD curves are recorded.[232] Meanwhile, chemical and biological properties mapping methodology has been developed on the idea of FD-based imaging.[233,234] In the above methodology, the tips are modified by particular ligands. Furthermore, based on the mechanical strength and adhesion of bonds developed among the modified tips and the receptors of the specimen, the biological traits can be investigated during imaging.[235] The time of data acquisition and high volume are the major challenges associated with FD-based AFM. Numerous multifrequency AFM approaches have been projected to decrease the amount of data and increase the imaging speed.[236,237] However, their complicated physical principle, mechanism of data interpretation, and theoretical development require more research.

The former research on coronaviruses exploiting AFM techniques[238–241] indicates the substantial potential of AFM to visualize, image, and investigate the morphological topographies of the SARS-CoV virus, as shown in Fig. 17. Generally, characterization/visualization tools are valuable as they deliver surface and subsurface information of COVID-19 affected cells,

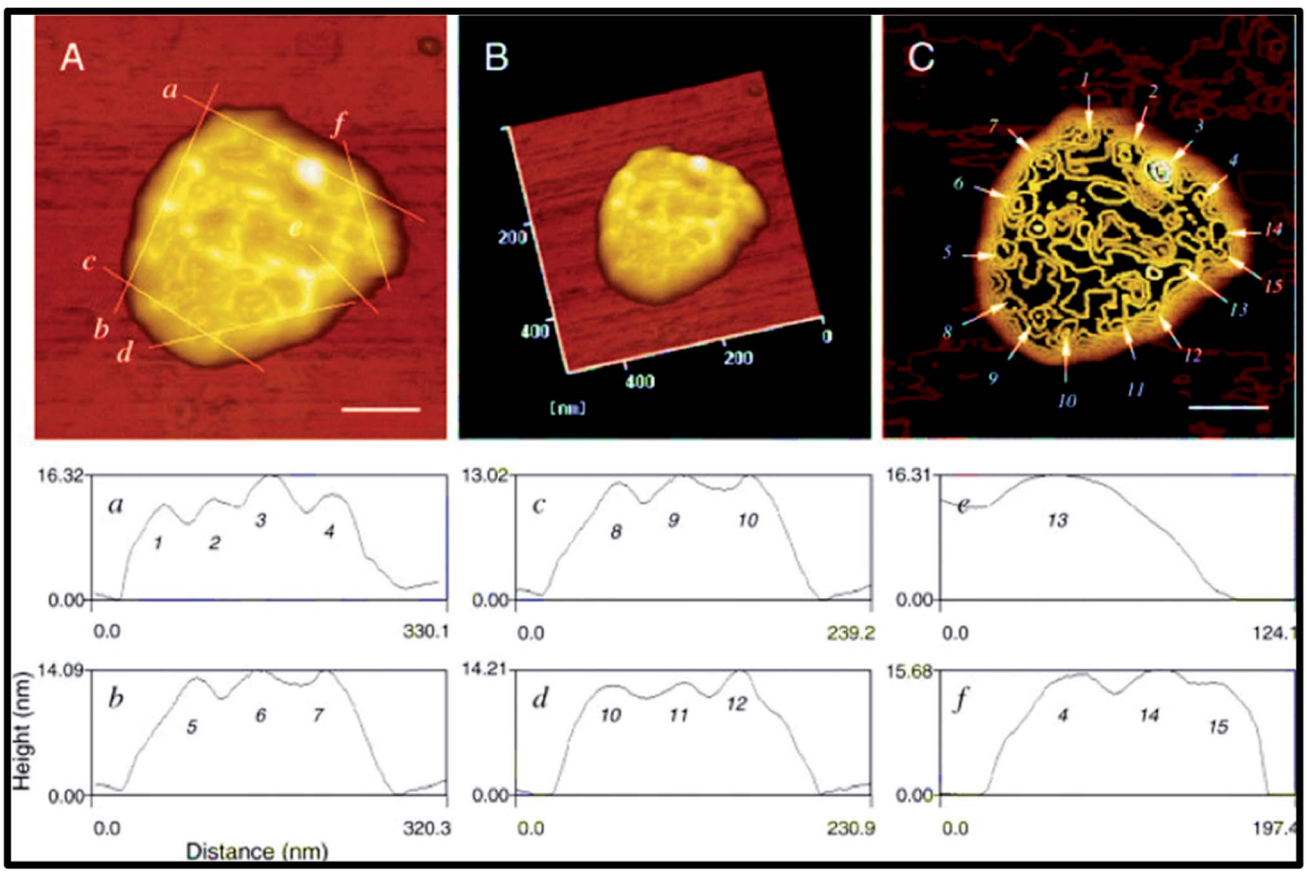


Fig. 17 (A) 2D, (B) 3D AFM images, and contour map (C) of a single SARS-CoV virion. The scale bar = 100 nm in (A) and (C). The resultant cursor profiles (middle and bottom row) give quantitative measurements of the dimensions for the spike proteins (1–15) revealed in (C). The figure has been reproduced from ref. 238 with permission from WILEY, copyright 2005.

perceive multiple versatile interactions amid host cell and SARS CoV-2 virus, explore the mechanism of the COVID-19 virus to pass the cellular membrane and transport its genome into the host cell, study its replication into the cell along with SARS-CoV2 virus nucleic acids characterization, and recognize the way it is packaged and condensed inside capsids. Recently, anticipated techniques at the nanoscale map that the directional flow patterns can also be castoff to examine the effects of ion-specific sieving traits of the host cell and different pH on the binding of the SARS CoV-2 S protein with the receptor at the interface of the cell.[242] Currently, Yang *et al.* characterized molecular binding and studied the inhibition relation of SARS CoV-2 with ACE2 receptor by exploiting AFM.[243]

4.8 Detection of COVID-19 from common sources

4.8.1 Saliva-based detection. Salivary glands release an exocrine secretion named saliva that has multiple functions such as protection and cleansing of oral cavity, aid in the digestion, and most importantly, the antimicrobial activity. Speedy progress in the field of saliva omics resulted in the acceptance of saliva as a biological biomarker that fluctuates from changes in the nucleic acid, proteins, and microflora. Saliva has an edge over other diagnostic fluids owing to its economic perspective, reliable collection methodology, and non-invasive procedure for monitoring systematic health. Formerly, it has been previously manifested that saliva has a higher consistency rate (90%) with nasopharyngeal specimens for the sensing of coronaviruses.[244]

The systematic study publicized 96 records after the removal of duplicates and among those, 5 records were included for quantitative and 26 records for qualitative synthesis. The findings proposed 91% (CI 80–99%) sensitivity of saliva-based diagnostics and 98% (CI 89–100%) sensitivity for nasopharyngeal-based diagnostics for CoV-2 patients with moderate heterogeneity in the studies. The same group also recognized 18 registered patients with ongoing clinical trials of saliva-centered prognosis for COVID-19 diagnosis.[245] In Hong Kong, a study was made, in which patients were considered to be diseased if SARS CoV-2 was identified in their sputum or nasopharyngeal specimen. Saliva of a total of 12 patients was obtained and subjected to nucleic acid extraction and RT-PCR for COVID-19 prognosis. Saliva samples were collected at an average of almost 2 days after hospitalization (range, 0–7 days) and initially, SARS CoV-2 was found to be present in saliva samples of 110 patients (91.7%). Meanwhile, there were 33 cases whose nasopharyngeal samples were found to be negative for COVID-19. Their saliva specimen also showed the same negative response. Recently, a group of scientists has effectually endorsed saliva as a viable source compared to oropharyngeal swabs for COVID-19 detection. They reported the utilization of saliva as a robust source to extract viral RNA for COVID-19 detection. Saliva-based tests will help with the global scarcity of swab-based sampling for COVID-19 analysis.[246] Likewise, many other groups reported the early detection of COVID-19 based on saliva specimen findings.[247,248] However, the low concentration of the analyte in the saliva compared to blood limits its practical application, which is circumvented by the development of precise molecular methodologies and nanotechnology.

4.8.2 Wastewater-based detection. Sewage or waste water-based epidemiology (WBE) has been employed to track and give timely forewarnings of outbreaks of pathogenic diseases such as poliovirus, norvirus, hepatitis, salivirus, enterovirus, rotavirus, and astravirus, and for the regular monitoring of the diversity and concentration of viruses in wastewater.[249] Commonly, WBE evaluates the spatial and temporal trend of virus in wastewater inside the catchment of sewage treatment plants. As we all know, the outburst of COVID-19 has brought a substantial threat to human health. Recently, SARS CoV-2 has been identified in fecal samples of COVID-19 patients from various regions such as China, Korea,[250] United States,[251] Singapore,[252] and Germany.[253] Another research including 10 pediatric COVID-19 patients provided proof for the occurrence of SARS CoV-2 in feces.[254] Even in most negatively reported nasopharyngeally tested cases, rectal testing was found to give positive results for COVID-19, demonstrating the shedding of the gastrointestinal and fecal-oral pathway. Current evidence suggest that analysis of SARS CoV-2 in wastewater is useful to scrutinize the viral transfer in humans as SARS CoV-2 can be released *via* urine or feces.[255] Medema *et al.* revealed the detection of SARS CoV-2 from sewage in the Netherlands.[256] Likewise, many other attempts have been conducted to analyze SARS CoV-2 through wastewater in countries such as the United States,[257] Australia,[258] France,[259] and Sweden.

RT-qPCR has been utilized to test N(N1–N3) and gene E, and they all were identified at different sites and validated the presence of SARS CoV-2. In the United States, the SARS-CoV2 at high titers was also detected in wastewater specimens by RT-PCR.[260] Their work further revealed that the longitudinal examination of wastewater can give an approximation of the population level without onsite analysis. The existence of SARS CoV-2 RNA was also confirmed in 6 WWTP wastewater specimens from a lower frequency area in Spain.[261] Environmental surveillance outcomes elucidated that SARS CoV-2 had been transmitted among the population before initial reporting by the municipality. Therefore, WBE can be employed as a surveillance tool for the detection and pervasiveness of COVID-19 and gives a detailed insight into the development magnitude of COVID-19 than clinical analysis. RT-PCR assays have been executed for SARS CoV-2 assessment in many disease control and research centers, as we have discussed previously.[58,262] However, the requirement of skilled technicians, longstanding data processing, and examination limits its practical application globally. Digital PCR has also been utilized and its sustainability for certain samples has been evaluated.[263]

Therefore, it is of paramount importance to manufacture efficient robust and transportable analytical tools to analyze low-level SARS CoV-2 specimens through WBE to validate doubtful cases and monitor asymptomatic patients without the need for centralized laboratories. Thus, exploiting the WBE approach for timely warning and intervention needs a prompt biosensing approach for the on-site recognition of all viruses. The latest progress in sensing devices makes field analysis

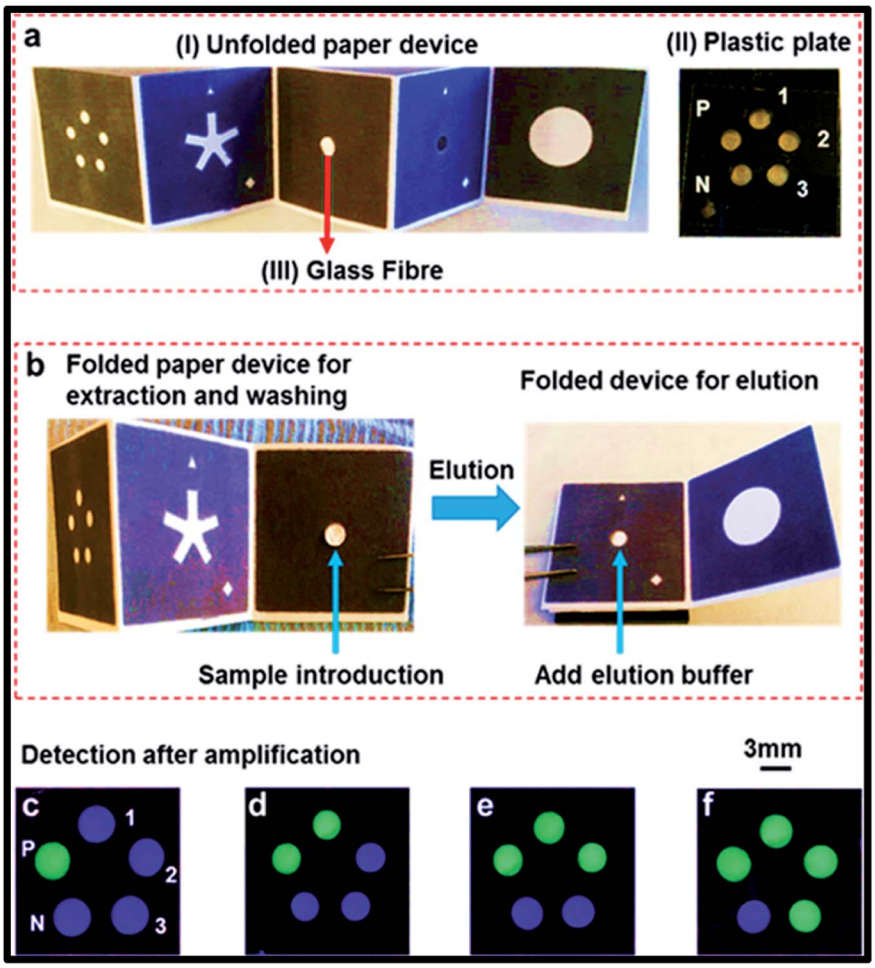


Fig. 18 Design of the established sensor for the recognition of multiplex infectious disease pathogens. (a) Components of the sensor, (b) sketch of the whole sample processing methodology ranging from sample addition to pathogen detection. After amplification, the signal is read out with a UV flashlight (365 nm). (c–f) The figure has been reproduced from ref. 266 with permission from ACS, copyright 2018.

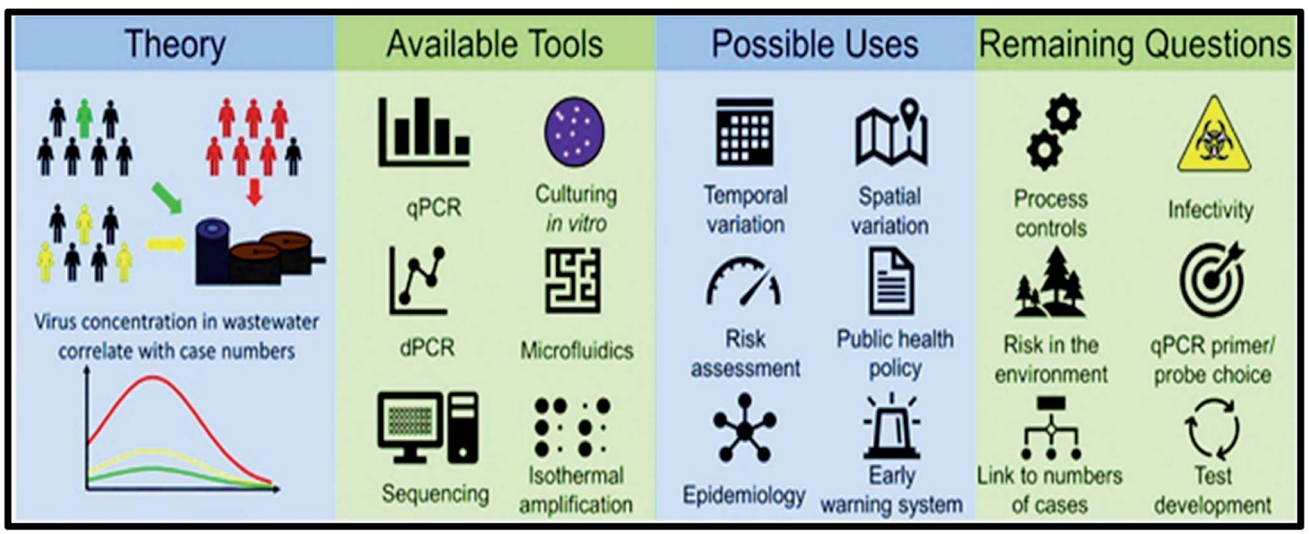


Fig. 19 Wastewater surveillance potential for COVID-19 monitoring. The figure has been reproduced from ref. 268 with permission from Elsevier, copyright 2020.

Table 2 Molecular detection (*in vitro*). Tests that identify constituents and infection produced by the SARS CoV-2 virus

Product	Detail	Sponsor
CDC 2019-nCoV real-time RT-PCR diagnostic panel	Manufactured by CDC and firstly distributed to public health laboratories but testing is limited to complex labs	Center for Disease Control and Prevention
New York SARS-CoV-2 real-time reverse transcriptase (RT)-PCR diagnostic panel	Developed on CDC's principal and works incapable labs. However, testing is restricted to complex laboratories	Wadsworth – New York State Public Health
Panther fusion SARS-CoV-2 assay	Reagents are disseminated as a kit. However, testing is restricted to complex laboratories only	Hologic, Inc.
Lyra® SARS-CoV-2 assay	Kits are commercialized but restricted to complex labs only	Quidel Corporation
Abbott RealTime SARS-CoV-2 assay	Reagents are commercialized as kits but testing is confined to extraordinary complex labs	Abbott Molecular, Inc.
Xpert Xpress SARS-CoV-2 test	Reagents are commercialized and circulated as kits to laboratories. It can run 2000 specimens in a day and tests are confined to moderately to highly complex labs along with Point of Care (POC) settings	Cepheid
Actual SARS-CoV-2 test	Reagents commercialized and disseminated as a kit to laboratories, run only a single sample at a time, and tests are restricted to moderately to highly complex laboratories along with Point of Care (POC) infrastructure working under a CLIA Certificate	Mesa Biotech inc
BioFire COVID-19 test	Reagents distributed commercially to laboratories, test 264 samples a day, and can perform in both moderate or complex labs	BioFire Defense, LLC
AvellinoCoV2 test	Tests are restricted to high complexity Avellino laboratories USA, Inc.	Avellino Labs USA
NxTAG CoV extended panel assay	Reagents circulated to labs and tests are restricted to highly complex laboratories only	Luminex Molecular Diagnostics, Inc.
ID NOW™ COVID-19	Reagents commercialized and disseminated as a kit, need a specific platform, only one sample runs at a time, and every specimen takes greater than 13 min. The test is restricted to moderately as well as highly complex labs along with Point of Care (POC) sets working under a CLIA Certificate of Waiver	Abbott Diagnostics Scarborough, Inc.
NeuMoDx SARS-CoV-2 assay	The reagents are commercialized, 288 or 96 samples run at a single time, and sometimes takes 80 samples depending on the instrument. However, tests are restricted to moderate as well as complex laboratories	NeuMoDx Molecular, Inc.
QIAstat-Dx respiratory SARS-CoV-2 panel	Identifies many other respiratory viruses and bacterial organisms, and reagents are distributed commercially as kits to laboratories. It takes one hour and runs one sample at a time	QIAGEN GmbH
COVID-19 IDx assay	Utilizes already commercialized reagents and the test is restricted to complex laboratories by Ipsum	Ipsum Diagnostics
BioGX SARS-CoV-2 reagents for BD MAX system	Commercialized reagents are distributed as a kit to moderate to high complexity fully automated laboratories that run 8 samples per hour	Becton, Dickinson & Company (BD)
ARIES SARS-CoV-2 assay	Reagents distributed commercially as a kit to labs and tests are conducted at moderate and complex labs	Luminex Corporation
Logix Smart Coronavirus Disease 2019 (COVID-19) kit	Reagents are commercialized and distributed as a kit to laboratories and testing is bound to highly complex labs	Co-Diagnostics, Inc. 4-3-2020
Gnomegen COVID-19 RT-Digital PCR detection kit	Reagents are spread commercially to labs as kits. Tests are restricted to very complex labs	Gnomegen LLC
Smart detect SARS-CoV-2 rRT-PCR kit	Reagents are distributed and commercialized as a kit to laboratories and testing is restricted to complex laboratories	InBios International, Inc

Table 2 (*Contd.*)

Product	Detail	Sponsor
QuantiVirus SARS-CoV-2 test kit	Reagents are commercialized and testing is confined to highly complex labs only	DiaCarta, Inc.
iAMP COVID-19 detection kit	Reagents are circulated commercially as kits and tests are restricted to extraordinary complex laboratories only	Atila BioSystems, Inc.
SARS-CoV-2 fluorescent PCR kit	Reagents are distributed commercially to laboratories as a kit and are restricted to very complex laboratories for testing	Macura Biotechnology (USA) LLC
Curative-Korva SARS-cov-2 assay	Tests are restricted to KorvaLabs as that is developed specifically for laboratories	KorvaLabs inc
GeneFinder COVID-19 plus RealAmp kit	Reagents are commercialized to the lab and restricted to complex labs for testing	OSANG Healthcare
PhoenixDx 2019-CoV	Reagents are distributed to labs and tests are confined to highly complex labs	Trax Management Services Inc.
Allplex 2019-nCoV assay	Reagents are commercialized and distributed to labs and are restricted to highly complex laboratories for tests	Seegene, Inc.
Rheonix COVID-19 MDx assay, COVID-19	Reagents are commercialized and distributed to laboratories as kits and testing is restricted to complex labs	Rheonix, Inc.
LabGunCOVID-19, RT-PCR kit	Reagents are commercially divided into laboratories and testing is restricted to complex laboratories	LabGenomics Co., Ltd.
Bio-Rad, SARS-CoV-2 ddPCR test	Reagents are commercialized and distributed to laboratories as kits; the test is restricted to highly complex laboratories	Bio-Rad Laboratories, Inc.
Sherlock CRISPR SARS-CoV-2 kit	Commercialized reagents are circulated to highly complex laboratories as kits	Sherlock Biosciences, Inc.
Gnomegen COVID-19-RT-qPCR detection kit	Reagents are commercialized and distributed to laboratories and are limited to highly complex labs for testing	Gnomegen LLC
NeoPlex COVID-19 detection kit, COVID-19	Reagents are distributed commercially to labs as kits and testing is restricted to highly complex labs	GeneMatrix, Inc.
Optima SARS-CoV-2 assay	Reagents are commercialized and disseminated to labs; tests are restricted to complex labs	Hologic, Inc.
Lyra direct SARS-CoV-2 assay	Reagents are commercialized and distributed to labs; testing is confined to complex labs	Quidel Corporation
AQ-TOP COVID-19 rapid detection kit	Reagents are commercially disseminated to complex laboratories	Season Biomaterials, Inc.
DiaPlexQ novel coronavirus detection kit	Reagents are circulated commercially as a kit to highly complex laboratories for testing	SolGent Co., Ltd
BioCore 2019-nCoV real time PCR kit	Reagents are commercialized and distributed to highly complex labs only for testing	BioCore Co., Ltd
FRL SARS CoV-2 test	Laboratory-based testing restricted to UAB Fungal Reference Laboratory, a high complexity lab.	The University of Alabama at Birmingham Fungal Reference Lab
LifeHope 2019-nCoV real-	Laboratory manufactured test, Time RT-PCR Diagnostic Panel. Tests are narrowed to complex Life Hope Laboratory in plainview	LifeHope Labs
COVID-19 RT-PCR peptide nucleic acid (PNA) kit	Reagents are distributed commercially in the laboratory as a kit; testing is restricted to highly complex laboratories	TNS Co., Ltd (Bio TNS)
Psoma COVID-19 RT test	Tests are confined to the complex laboratory of Psomagen, Inc. in RockvilleMD.	Psomagen, Inc.
Solaris multiplex SARS-CoV-2 assay	Laboratory manufactured to test and is restricted to Solaris Diagnostics, a highly complex lab situated in NicholasvilleKY.	Solaris diagnostics
SARS CoV-2 RNA STAR	Reagents distributed commercially to labs as kits and limited to highly complex laboratories	LumiraDx UK Ltd.
SARS CoV-2 Real-Time RT-PCR Test	Reagents are commercially distributed to labs as kits and tests are confined to highly complex laboratories	Biomeme, Inc.

Table 2 (*Contd.*)

Product	Detail	Sponsor
Pro-Amp RT SARS-CoV-2 Test	Lab-based test and restricted to Pro-Lab Diagnostics, a highly complex lab in Round Rock, TX	Pro-Lab Diagnostics
SalivaDirect	Recognition of SARS CoV-2 in specimens of saliva obtained without preserving. Commands for utilization is spread to laboratories and all constituents are available commercially	Yale School of Public Health, Department of Epidemiology of Microbial Diseases
Advanta Dx SARS-CoV-2 RT-PCR assay	Sensing of COVID-19 in saliva and reagents are disseminated commercially as kits to labs. Testing is restricted to highly complex laboratories	Fluidigm Corporation

possible so that the system can deliver real-time monitoring and public health information, as shown in Fig. 18. Recently, it has been proposed by a few scientists that paper-based biosensors can be employed for WBE.[262] Owing to the merits such as budget-friendliness, robustness, accuracy, specificity, and sensitivity, these biosensors have been employed previously in the clinical analysis of different analytes,[264] food protection, and environmental monitoring systems. Recently, biosensors have been exploited for electrochemical, acoustic, thermal, electrical, and piezoelectric analysis of contagious diseases. The indicators of these contagious diseases are typically recognized in the nasal mucosa, plasma, sputum, serum, blood, urine, feces, and saliva. Exploiting high-affinity probes for sensing wastewater specimens can also decrease the matrix effects.[265] In addition, biosensors can also be employed for the multiplex analysis of contagious ailments.

Moreover, biosensors as medical tools can be established for the POC recognition of contagious ailments in resource-limited areas. For instance, Yang *et al.* introduced a fast "sample-to-answer" detection process, which can offer quantitative analysis of nucleic acids along with the genetic data by the exploration of sewage waste,[267] and these findings were further validated by agarose gel image assay and robust electrophoresis, displaying good consistency for wastewater examination. The anticipated biosensors will display advantages such as excellent sensitivity, affordability, superior specificity, rapid sensing time, and low sample consumption for the user-friendly recognition of SARS CoV-2 in sewage water despite conventional detection methods. Under this standpoint, the advanced study on the environment for the examination of disease biomarkers is needed to efficiently exploit WBE sensors for the updated status of COVID-19 in a community (Fig. 19).

4.8.3 Food and other surface-based detection. As the human health system is globally affected by the current pandemic, adverse effects on the food system and its associated population are also evident. Therefore, to guarantee food safety and to avert the interruption of food supply chains, the development of SARS CoV-2 recognition tools for food analysis is a mandatory need. However, a reliable detection strategy in food is challenging owing to the heterogeneous distribution of virus particles, non-optimal tedious isolation, and low viral load.[269] Previously RT-qPCR, ELISA,[270] and nanoELISA[271] methodologies have been employed for this purpose, but currently, molecular and serological tests are in practice for SARS CoV-2 identification. Most studies recommend that the immune response to the virus commences after 7 days of the beginning of symptoms.[272,273] As we discussed, considering the recognition of CoV-2 in food, on surfaces and adjacent mediums, or surrounding is a major concern as currently not much research has been performed and no evidence exists that coronavirus spreads through surfaces, surroundings, and food. Nevertheless, there are maximum chances of the virus spreading from diseased workers *via* surfaces and adjacent milieu of the food industries and the food supply chain. Cai *et al.* reported that the transmission of the virus by faucet taps and elevator buttons has also contributed to a number of cases in China.[274] RT-qPCR have been employed to detect COVID-19 from all fomite samples.[275–277] Though these are preliminarily findings, some companies have designed commercially available kits for environmental swabs.[278–280] Meanwhile, sampling kits for surfaces are also offered by few companies.[281] However, their high prices limit their broad application, especially in the food sector.

4.9 Role of nanotechnology for SARS-CoV-2 detection

As mentioned previously, the first technique employed for the detection of SARS-CoV-2 and to gain information regarding primers, genetic biomarkers, molecular probes, and different concentration of antibodies in samples was rRT-PCR. But the complex infrastructure, longstanding time requirements,[282,283] and excessive use of reagents for the diagnostic test are the major drawbacks of the technique. Therefore, simplistic diagnostic tools with robust response are the immediate requirement. In this regard, nanotechnology has played an important role to counter the aforementioned challenges. For instance, nanodiagnostics works on the principle of the binding capacity of the nanomaterials and the biomolecules under consideration to generate a quantifiable signal for pathogen detection.[284] Meanwhile, nanotechnology has also aided in providing effectual results with prompt and timely diagnosis of the disease.[285] In addition, the role of nanomaterials in bioengineering can

Table 3 Molecular diagnostics (Home collection). It can be exploited for specimens that can be self-obtained or collected at homes and then forwarded to laboratories for further analysis

Product	Detail	Sponsor
COVID-19 RT-PCR Test	Manufactured and functions in LabCorp labs; not for larger laboratories	Laboratory Corporation of America
COVID-19 RT-PCR Amendment	Amendment allows utilization of the Pixel *via* LabCorp COVID-19 testing and home collection kit permitting suspected victims to self-collect nasal swab specimens. It also offers the transfer of the collected sample materials safely from homes to an authorized laboratory	Amended
SARS-CoV-2 RNA, Qualitative Real-Time RT-PCR	Manufactured to function in Quest labs only and not for distribution, and tests are restricted to highly complex laboratories	Quest Diagnostics Infectious Disease, Inc.
P23 Labs TaqPath SARS CoV-2 Assay	Laboratory manufactured test, confined to P23 Labs, situated in Little Rock, a high complexity lab and utilized in-home for collecting saliva samples using OMNIgene ORAL Collection Device and then referred to the laboratory for further tests	P23 Labs, LLC.
LetsGetChecked Coronavirus (COVID-19) Test	Laboratory-based test restricted to PrivaPath Labs, high complexity labs and utilized only for nasal swabs comprised of COVID-19 LetsGetChecked Home-based Kit and directed to the laboratory for tests	PrivaPath Diagnostics, Inc.
KPMAS COVID-19 Test	Lab-based testing confined to KPMAS Regional Lab and can be utilized with nasal swab samples collected *via* KPMAS Health Plan members	Kaiser Permanente Mid-Atlantic States
Compass Laboratory Services SARS-CoV2 Assay	Laboratory-based testing restricted to Compass Lab Services, a high complexity laboratory. It can be utilized for nasal swab samples obtained at home also *via* a video witnessed by a healthcare worker	Compass Laboratory Services, LLC
Quest Diagnostics PF SARS-CoV-2 Assay	Laboratory-based test, confined to labs selected through Quest Diagnostics that meet the necessities to conduct complex testing. It could be cast-off for nasal swab samples at home under the supervision of a healthcare worker by a COVID-19 Self-Collection Quest Diagnostics Kit	Quest Diagnostics Infectious Disease, Inc.
CRL Rapid Response	Lab manufactured test, restricted to the Clinical Reference Library laboratory placed in Lenexa, high complexity laboratory. It could be exploited for saliva samples obtained at home or under the supervision of a healthcare provider	Clinical Reference Laboratory, Inc
QDX SARS-CoV-2 Assay	Laboratory-based test, restricted to QDx Pathology Services situated in Cranford a giant complex laboratory. It could be applied for samples that are collected at home by utilizing the Qdetect collection kit or any other approved home-based collection kit	QDx Pathology Services 8-25-2020

also be of significant importance for SARS-CoV-2 detection as this virus itself has a core shell nanostructure with the size ranging from 60 nm to 140 nm.[286,287] Thus, it allows the bio-engineered nanomaterials to specifically bind with the virus,[288] permitting the evaluation, engineering, and development of procedures for diagnosis, treatment, as well as the preventive measures of SARS-COV-2.[289,290] These traits of nanotechnology also lead to the development of handheld devices/tools that can be commercialized with additional benefits of stability, sensitivity, and high accuracy (Tables 2–8).

The below table contains the data regarding the confirmed *in vitro* detection tests, which can be exploited for COVID-19 patients' management.

5 Management of Covid-19

Still various rational and distinguishing management strategies are critical even after the development of intensive detection methodologies to overcome the after effects from this pandemic situation.[291] For intense cases, survivors of SARS COV-19 could

Table 4 Antigen test-based detection of SARS COV-19

Product	Detail	Sponsor
BinaxNOW COVID-19 Ag Card	Moderate to highly complex laboratories are exploited for tests and POC sets working below a CLIA Certificate. It visually recites assays that do not need an instrument	Abbott Diagnostics Scarborough, Inc.
LumiraDx SARS-CoV-2 Ag Test	Tests are confined from moderately to highly complex laboratories and POC settings work under a waiver of CLIA Certificate	LumiraDx UK Ltd.
Sofia SARS Antigen FIA	Tests are confined from moderately to highly complex laboratories and POC settings functioning under a waiver of CLIA Certificate	Quidel Corporation

have a higher risk of long-term lung infection. Moreover, some other serious problems such as serious interstitial, upper, and lower respiratory body infection can be possible.[292]

5.1 Contact tracing-based SARS COV-19 detection

The isolation of symptomatic COVID-19 patients and tracing of contacts along with social distancing have been employed as an early measure to mitigate COVID-19 spread as outbreaks have grown. Reducing the disruption to the population while controlling infection, there is a dire need to recognize what combination of measures are required to decrease the transmission. Thus, in this area, the use of smartphone-based digital technologies have been executed effectively to cope with the spread of COVID-19.[293] Recently, the application of contact tracing has been developed and employed as shown in Fig. 20. The main idea of this application is to substitute manual contact tracing with an immediate broadcast of signal toward central/main server. Principally, the information of virus patients has been transferred to the server that recommends risk-stratified quarantine along with robust social distancing to

Table 5 Serology test-based detection of SARS COV-2

Product	Detail	Sponsor
Serology Test qSARS CoV-2 IgG/IgM Rapid Test	The very first authorized serological test by EUA, spots CoV-2 antibodies in the blood, distinguishes between IgM and IgG antibodies. It is a robust test and gives an outcome in 15–20 min and is restricted to adequate to high complexity labs	Cellex Inc.
VITROS immunodiagnostic products anti-SARS-CoV-2 IgG reagent pack	Identifies IgG antibodies in human serum in moderate to high complexity labs	Ortho-Clinical Diagnostics, Inc.
COVID-19 ELISA IgG Antibody Test	Used to identify IgG antibodies in human plasma and serum. Tests are restricted to the high complexity Mount Sinai Lab	Mount Sinai Laboratory
LIAISON SARS-CoV-2 S1/S2 IgG	Identifies IgG antibodies in serum and human plasma and tests are restricted from moderately to largely complex labs	DiaSorin Inc.
SARS-CoV-2 IgG assay	Perceives IgG antibodies in human plasma, as well as serum and tests, and are restricted from moderate to large complexity laboratories	Abbott Laboratories Inc.
Elecsys Anti-SARS CoV-2	Senses Anti-SARS Cov-2 antibodies in human serum and plasma, and tests are restricted to moderate as well as highly complex laboratories	Wadsworth Center, NY State Department of Health
Vibrant COVID-19 Ab Assay	Detects IgM and IgG in dried blood and human serum, functional in the highly complex Vibrant America Clinical Laboratories	Siemens Healthcare Diagnostics Inc.
Biohit SARS-CoV-2 IgM/IgG Antibody Test Kit	Senses IgM and IgG in human plasma, serum, and venipuncture whole blood; tests are constrained to medium and high complexity laboratories	Biohit Healthcare (Hefei) Co. Ltd.
DZ-Lite SARS-CoV-2 IgM CLIA Kit	Identifies IgM in serum along with plasma	Diazyme Laboratories, Inc.
SARS-CoV-2 IgG and IgM Combo Test	Identifies and distinguishes between IgM and IgG in human serum	BioCheck, Inc.
Tell Me Fast Novel Coronavirus (COVID-19) IgG/IgM Antibody Test	Senses and differentiates between IgM and IgG in venous whole blood, plasma, and serum	Biocan Diagnostics, Inc

Table 6 Individual (*in vitro*) diagnostic tests for COVID-19 patient management

Product	Detail	Sponsor
Elecsys IL-6	To help in recognizing severe inflammatory response in COVID-19 confirmed cases and for analyzing the risk of intubation with mechanical ventilators	Roche Diagnostics

Table 7 Decontamination systems for personal protective equipment

Product	Detail	Sponsor
Battelle Decontamination System	A well-suited respirator was reused and recycled up to twenty times utilizing the Battelle Decontamination System. It has been approved to ameliorate their actions in order to purify approximately 120,000 respirators every day through 12 satellite amenities that transfer data to the FDA	Battelle
Duke Decontamination System	Purifies compatible N95 or analogous respirators	Duke University Health System
STERIS STEAM Decon Cycle in AMSCO Medium Steam Sterilizers	Disinfects N95 respirators for single-user reprocess by HCP to stop acquaintance to pathogenic airborne particulates in shortage of face-filtering respirators (FFRs) owing to COVID-19	STERIS
Stryker Sustainability Solutions VHP Decontamination System	Purifies N95 respirators for numerous user reuses *via* healthcare workers to avoid acquaintance to infectious airborne particles in shortage of face-filtering respirators as consequences of the pandemic	Stryker
Nova2200	Disinfects N95 respirators for one person reuse for healthcare personnel (HCP) to stop contact to airborne pathogenic species in insufficient supply of face-filtering respirators (FFRs) owing to the SARS COV-19	NovaSterilis, Inc.

possible contacts. The tests of symptomatic patients have been requested through developed applications, while maintaining the anonymity of the infected patients. This developed software-based technology can be tuned to more informative infrastructure owing to its accessibility to almost every community member.[294] Nevertheless, to achieve the complete exploitation of this technology, the tests number also needs to be increased. Meanwhile, privacy consideration also limits the application of this software-based technology.[26] However, as the virus responsible for COVID-19 is continuously emerging, thus, such an improved developed system of contact tracing could play an important role in lowering the intensive impact generated by COVID-19.[295]

5.2 Use of pulse oximetry

Intense shallow breathing has been commonly observed after severe SARS-COVID-19 infection. The Pulse Oximeter (PO) can play an important role in monitoring different respiratory symptoms after SARS-COVID-19 without any extreme anxiety or stress.[297] Infected persons should be given an observation diary with a pulse oximeter and proper guideline to self-monitor. Normally, in this practice, a daily reading would be taken by a neat and warm finger after an interval of 20 min. However, the important thing to keep in mind while taking the reading is that the applied device should be allowed to stabilize first and then the highest reading should be considered.[298] Another major

Table 8 Infusion pumps for COVID-19 patients. Infusion pumps are therapeutic machines that distribute fluids and medications into the patient's body in a measured quantity

Product	Detail	Sponsor
Space and Outlook Pumps (Infusion Pump)	A pump (infusion) for the transport of medicines into a nebulizer for the treatment of SARS CoV-2 patients of all ages	B. Braun

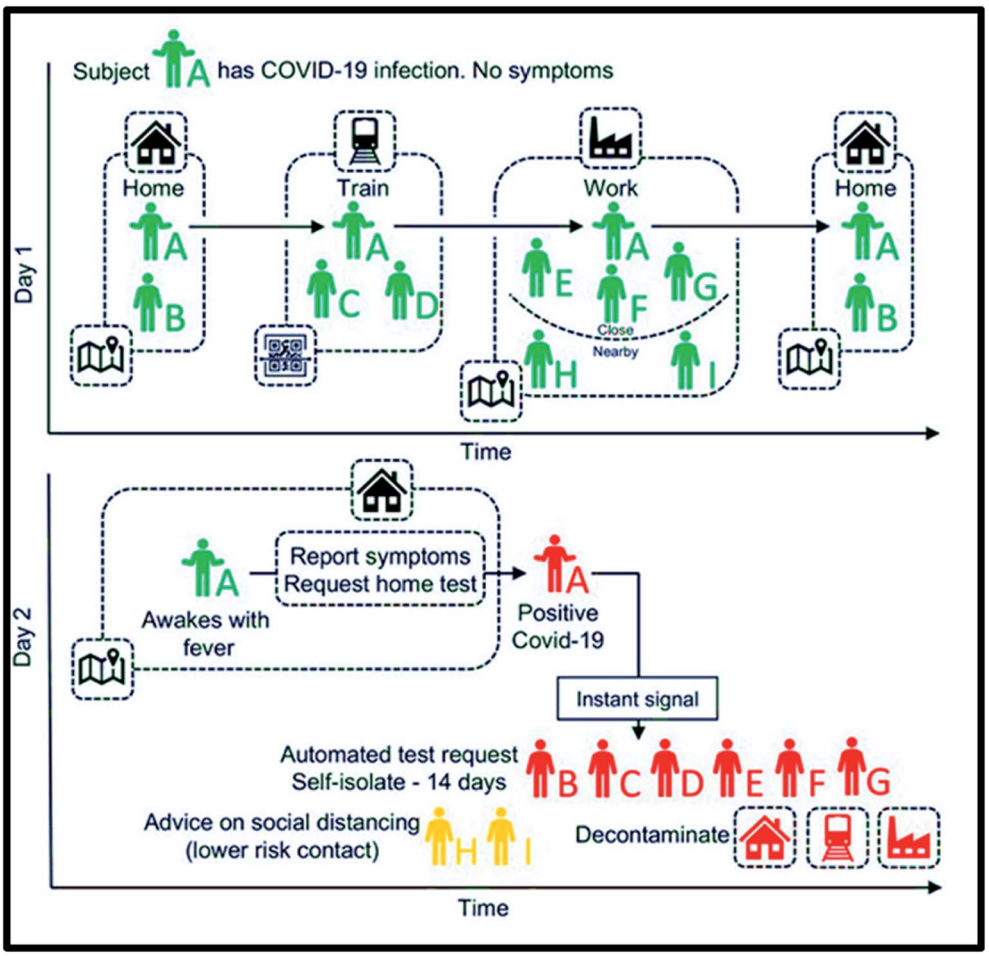


Fig. 20 Schematic representation of contact tracing application. The figure has been reproduced from ref. 296 with permission from AAAS, copyright 2020.

consideration is that commercially available O-saturated probes with a normal range of 92% should be used instead of smartphone apps.[299]

5.3 Repercussions of the primary care Team

Most of the COVID-19 patients suffered from prolonged recovery without any special input through a holistic approach. Much of it could be achieved *via* community rehabilitation strategies and inter-professional services, which improve a person's self-management and harness the possible video and related technologies. Also, an informative as well as rehabilitation platforms should be established at the community level.[300] In this time of uncertainty, the major role of these platforms should be the witness of "honoring the story" of a patient whose recovery was found to be unexpected.[301]

6 Recent trends and future perspective

To date, no accurate treatment/therapy has been proved to circumvent the mortality from this pandemic completely. Researchers are continuously struggling to invent a universal treatment to reduce the severity of disease. Various COVID-19 vaccines across the world have been developed, as listed in Table 9. Experts consider Remdesivir as a significant and clear-cut vaccine for reducing the recovery time by blocking this virus. It could be considered as a magic bullet. Further, Favilavir has been considered as an antiviral drug by China. However, its sample size is still very small. Experts also consider that any new compound could take a decade from its initial discovery to reach the final marketplace; many compounds would never be able to make it that far.

This pandemic situation needs intensive actions in various areas from prevention to detection and then to final treatment. A cheap, simple, and fast testing methodology is required to detect the antibodies able to neutralize COVID-19. In this regard, some of the opportunities have to intervene in long as well as short terms to save lives. But before that, we need a guideline from science. Besides all these scientific efforts, everyone has to contribute to control this uncertain situation to lessen the risk assessment. At this stage, food, shelter, and healthcare for the needy sections of the community are massive

Table 9 Vaccines investigated and implemented against SARS-CoV-2[a]

No	Vaccine	Manufacturer	Brand Name
1	Janssen	Janssen Pharmaceutica	Johnson & Johnson
2	Sinopharm BIBP	China National Pharmaceutical Group	NA
3	Sputnik V	Russian Gamaleya Research Institute of Epidemiology and Microbiology	NA
4	CoronaVac	Sinovac Biotech	NA
5	Novavax	Novavax and the Coalition for Epidemic Preparedness Innovations	Covavax
6	Covaxin	Bharat Biotech in collaboration with the Indian Council of Medical Research-National Institute of Virology	Covaxin
7	Sputnik Light	Russian Gamaleya Research Institute of Epidemiology	NA
8	Convidecia	Chinese company CanSino Biologics and the Beijing Institute of Biotechnology of the Academy of Military Medical Sciences	NA
9	Sinopharm WIBP	China National Pharmaceutical Group	Sinopharm
10	Abdala	Center for Genetic Engineering and Biotechnology in Cuba	NA
11	EpiVacCorona	State Research Center of Virology and Biotechnology	NA
12	Zifivax	Chinese company Anhui Zhifei Longcom Biopharmaceutical	NA
13	Soberana O2	Finlay Institute in Cuba	NA
14	QazCovid-in	Research Institute for Biological Safety Problems in Kazakhstan	QazVac
15	Minhai	Minhai Biotechnology Co. and Shenzhen Kangtai Biological Products Co. Ltd. in China	NA
16	CoviVac	Chumakov Centre at the Russian Academy of Sciences	NA
17	COVIran Barekat	Shifa Pharmed Industrial Co. in Iran	NA
18	Medigen	Taiwan's Medigen Vaccine Biologics and Dynavax Technologies	NA
19	FAKHRAVAC	Organization of Defensive Innovation and Research	MIVAC
20	COVAX-19	Australian-based company Vaxine and Iran-based company CinnaGen	NA
21	Razi Cov Pars	Razi Vaccine and Serum Research Institute	NA
22	Turkovac	Health Institutes of Turkey and Erciyes University	NA
23	Corbevax	Indian biopharmacutical firm Biological E. Limited (BioE), the Baylor College of Medicine in Houston, United States, and American company Dynavax Technologies (DVAX)	NA

[a] NA = not applicable.

challenges. Everyone including migrants and refugees need special attention.

Shaping the future as a global nation is anticipated. The education sector is one of the highly affected areas by SARS-COV-2. All the nations must have strategies to protect children's education. Teenagers are the major group affected by this pandemic-isolation. As the situation prolongs, the need for healthcare is increasing, which also includes the counseling of young generation missing out on school meals amid school closures.

7 Conclusion

The SARS-CoV-2 pandemic offers an incomparable global humanitarian and medical trial. Although this has impelled unprecedented growth in the development of therapeutics and vaccines, it has also highlighted the vulnerability of limited resources. The limited testing capacity along with the infrastructure to produce therapeutic drugs was largely absent to combat COVID-19 in the beginning. Efforts were made to exploit available resources to manufacture cheap and robust diagnostic methodologies. The review discusses in detail the standard diagnostic methods along with their pros and cons for the detection of COVID-19 as well as to avoid consequent secondary blow-out. Nucleic acid plus protein-based diagnosis along with biomarkers to evaluate the disease severity have been employed at the start to sense SARS CoV-2. Furthermore, as research proceeded, non-invasive sensing protocols were explored for wastewater surveillance for early detection. Similarly, nanoscale tools have also been discussed to examine viral morphology. Although various nanobiosystems have clearly revealed the viral inhibitory properties of COVID-19, still, most studies have shown the significance of initial findings of the virus. The perseverance of nanosystems has not fully been evaluated, regardless of their endpoint applications including therapeutics, surface protection, and water disinfection. Further, the administration of investigative nanobiosystems to animal models should be validated along with its development. This validation would lead to subsequent antiviral coatings for medical devices. The review was an effort to provides insights into the diagnostics and prognostic approaches for current and future pandemics.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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