CHAPTER 13

Public health management during COVID-19 and applications of point-of-care based biomolecular detection approaches

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13.1 Overview of human coronavirus infections

Coronaviruses are phylogenetically classified under the family of corona-viridae (a subfamily of orthocoronavirinae) in order Nidovirales, which carries positive-sense single-stranded RNA (ss+RNA) as genetic material. In a study, it has been reported to have the biggest genome among all other types of RNA viruses.[1,2] Naturally, these viruses can be found in a wide range of species including animals as well as humans. The human coronaviruses (HCoVs) include alphacoronavirus (HCoV-229E and HCoV-NL63) and betacoronavirus (HCov-OC43 and HCoV-HKU1).[3] The first HCoVs were discovered in the 1960s from the upper respiratory infections of a patient.[4−6] In 2002, a strain of betacoronavirus (Subgenus Sarbecovirus) originated from bats and later spread to humans via civets in the province of southern China, which caused a severe acute respiratory syndrome (SARS) affecting the lungs, and thus the virus was named as the SARS-CoV.[7] After 10 years, in 2012 a betacoronavirus strain (Subgenus Merbecovirus) has been reported to be transmitted in humans via camels in the Middle East Asian region and was found to have similar symptoms as SARS and thus named as Middle East respiratory syndrome–related coronavirus (MERS-CoV).[8,9] The current outbreak of coronavirus in humans originated from Hubei province of China in December 2019. Studies have shown that it has also been originated sharing similar characteristics as the previous outbreak, thus later named SARS-CoV-2.[10] The genomic

sequencing of this virus revealed it is a group of betacoronavirus 79% distinctive from SARS-CoV.[11,12] Not only this, but it also shares structurally similar features such as the viral capsids proteins, containing S (Spike), E (Envelope), M (Membrane), and N (Nucleocapsid).[13]

13.1.1 Symptomatic characterization of SARS-CoV-2 patients

Infection of the SARS-CoV-2 causes a range of symptoms similar to the previous outbreaks, where apart from abnormal respiratory functioning, mild fever, cough, abnormal gastrointestinal behavior are reported as the major symptoms. In addition, there are asymptomatic cases have also been reported in various studies. A study directed toward asymptomatic infections was conducted by Kim et al., which shows that one-fifth of the patients develop either very mild or no symptoms, which were later considered as asymptomatic cases.[14,15] The most common symptoms of SARS-CoV-2 infection were slight headache and occurrence of cough with a sore throat.[16] The schematic characterization of symptoms in different cases has been represented in Fig. 13.1. Clinical symptoms are generally present with body fever ($\sim 102°$F), fatigue, diarrhea, nausea, continuous dry cough, complete loss of smell and taste, severe respiratory problems, thoracic pain, and breathing apnea.

In addition, a study showed that patients with a premedical conditions such as hypertension, heart illness, chronic bronchitis, diabetes, and cerebral injuries were getting more severe upon contraction of the virus.[17] In severe cases, this virus caused organ damage along with other complications in the lungs, and sometimes death.[18–21]
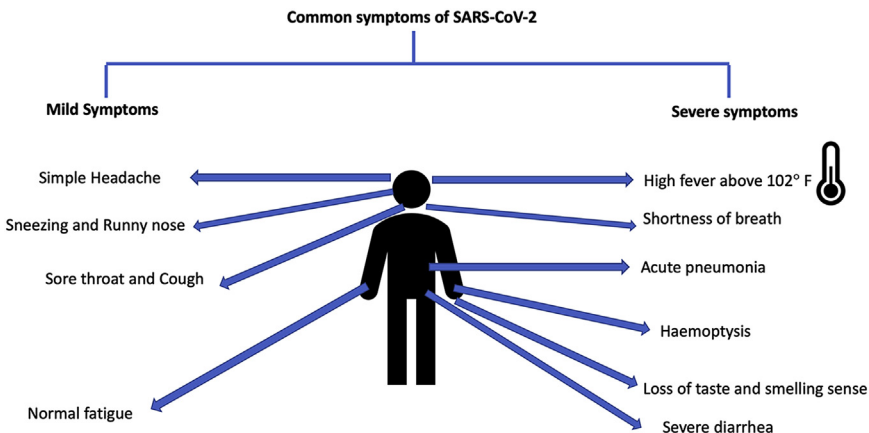

Figure 13.1 Representation of symptoms by COVID-19 patients. Two types of symptoms were observed: (1) mild, and (2) severe symptoms.

13.1.2 Transmission of SARS-CoV-2 agents and pathogenesis

The first epicenter of SARS-CoV-2 was in Wuhan, China. To reduce the rapid spreading of this virus, the Chinese administration had imposed a lockdown in Wuhan and nearby cities to break the transmission from one individual to another.[22,23] The transmission is reportedly air and surface borne, which resulted in rapid spread of the virus. Within four months of its origin, the virus had spread more than 100 countries across the world and thus, the World Health Organization (WHO) declared a pandemic on March 11, 2020.[24] Initially, the major outbreaks were observed in Europe, Iran, South Korea, and the United States. Few reports stated that the initial transmission occurred due to in-person contacts with close ones and overseas traveling, which created the patient zero for the region.[25] Virologists and epidemiologists have suggested that the transmission can be controlled by quick identifications of the carrier and the isolation of infected patients.[26] Another major cause of massive spread was attributed to the latency period of the virus which resulted in delayed symptoms even through the infection was quickly transmitting from one individual to another. The latency period of SARS-CoV-2 was reported for three days, which has greatly impacted the domestic transmission even after the complete lockdown and social distancing measures. In addition, "asymptomatic" cases have played a major role in connecting these domestic spreads. Studies reported that the transmission of this virus is not only human-to-human close contacts, but it is also spreading through human to air in close proximity areas[27] and fomite transmission[28] such as solid objects like plastics, copper, steels, used masks, and gloves. In these cases, when water droplets from infected individuals are expelled onto these solid surfaces,[29] the virus stays viable for a few hours. Another mode of transmission is the contamination of wastewater by fecal and excretory wastes.[30]

Until now, the pathogenesis of SARS-CoV-2 in humans is not fully known. On the basis of various studies and reports of genetic behavior of SARS-CoV-2 virus, to date, researchers are able to understand the mechanisms about the pathophysiology of this virus (Fig. 13.2).[31] It is also very essential to screen the viral loads, which is different from children to adults to elder individuals. Reports suggested that the amount of viral loads helps to understand whether a patient is having pre- or mild symptoms.[32] Further, in the critical case of a critical patient, the reliability of the epithelial–endothelial wall was interrupted rigorously.[33] Additionally, upon
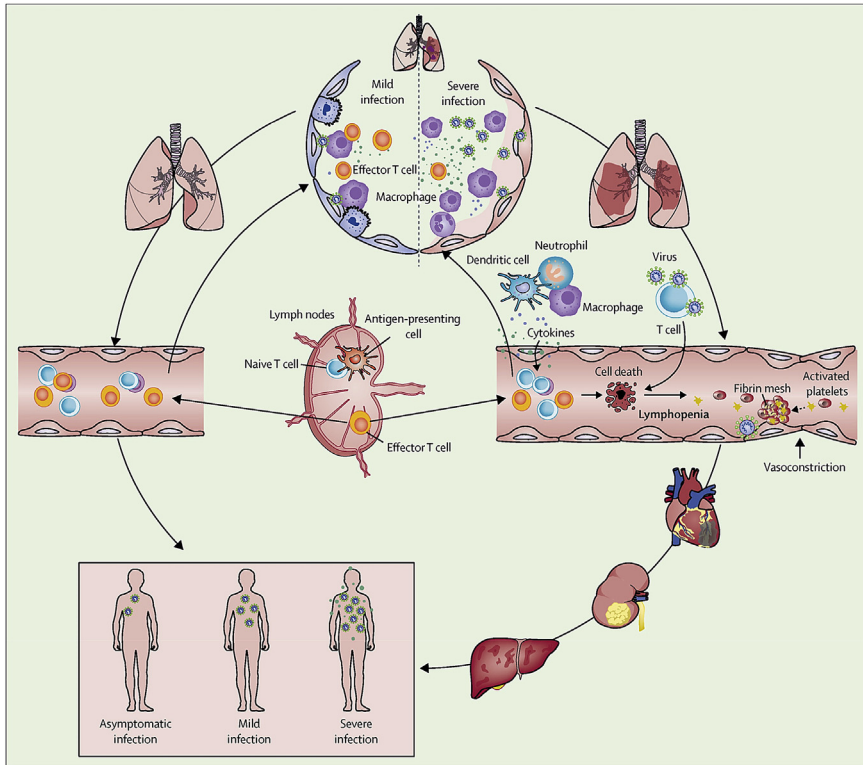
Figure 13.2 Pathophysiology of SARS-CoV-2 in humans. *(This figure is reproduced with permission from Li H, Liu L, Zhang D, et al. SARS-CoV-2 and viral sepsis: observations and hypotheses.* Lancet *2020;**395**(10235):1517—20.)*

the rupture of epithelial cells, SARS-CoV–2 can have an immense effect on the capillary system of the lung endothelial cells, which will cause a diffusion of a large amount of plasma components in the alveolar cavity.[34] On the other hand, the unrestrained virus infection caused by macrophage penetration which will lead to more injuries in the lungs. Simultaneously, the direct infection on other organs by this virus can cause systemic cytokine disturbance in the flow of immune pathogenesis. Hence, the effectiveness of antiviral therapies, immune responses, and study of the new adaptive immune responses are very much important for breaking the vicious cycles and improving the outcome of patients.[31]

Based on several studies and reports, investigators have hypothetically drawn an idea about the pathogenesis of SARS-CoV-2 infections.[35] According to several reports, the virus passes through nasal and larynx mucosa,

after which it enters the lungs via the respiratory tract. During that time patients get a fever, dry cough, and shortness of breath.[36] Investigators explained that once the virus enters the body, it travels through the blood from the lungs to the intestines.[31,37,38] Upon infection, the count of white blood cells (WBC) get reduced to less than the marginal requirements in the body.[5] Hence, lymphopenia has been diagnosed in patients. Researchers have also predicted that B lymphocyte may reduce during this infection, which causes declined antibody production inside the body.[38] However, to confirm this theory more investigations are needed. Immune-related studies are necessary to comprehend and to know the insights of pathogenesis, and treatment of COVID-19 disease.[35]

13.2 Challenges for public health management during the COVID-19 pandemic

The health management of public health is very crucial during the time of a pandemic. Many restrictions are imposed for controlling the transmission within the population. However, there are several challenges associated with following those restrictions which makes it difficult to control the spread of SARS-CoV-2 virus. These challenges will not be mitigated completely even after arrival of vaccines, because postvaccination surveillance is also important, due to which the health workers need to go through numerous undefined challenges.[39] The major challenge to this situation is the asymptomatic cases, in which an individual or a group of individuals spread the virus without even knowing that they have contracted close with the virus. By the time these individuals realize their condition and start to experience the symptoms, they have already become superspreaders. For controlling this situation, a quick plan of action and proper guidelines have been delivered by WHO and the Centers for Disease Control and Prevention (CDC), where the front liners, essential professionals, and investigators have been instructed to use personal protective equipment (PPE) to manage the spread of COVID-19 in the public domain.[40] These organizations have also mentioned the risks factors, symptoms, and preventive measures as well as have suggested when to travel, quarantine, and get tested for COVID-19.

The management of a COVID-19 patient is extremely difficult in terms of contagiousness such as carrying patient's samples regularly for laboratory tests, regular investigations of various diagnostic detection processes,

cleaning the bathrooms and surfaces used by COVID-19 patients, and so forth.[41] Since the majority of transmission of the virus comes from water droplets released by the patient during coughing, sneezing, and talking, therefore, covering the mouth and nose using a surgical mask or N95 mask are extremely necessary.[42] Essential workers need to wear PPE kits all the time when they are in contact with the patients or any suspected individual who came in close contact with the patients. Although wearing PPE is a vital safety measurement for avoiding droplets and airborne transmissions in close vicinity of an infected individual, it is difficult to wear the same for a long period of time due to excessive perspiration. Another important key factor is multidisciplinary management between intensive care units (ICUs), infectious disease (ID) departments, and infection control specialists.[43] After exploring the characteristics of this virus, investigators have drawn few management procedures to implement in the field.

13.2.1 Patient management

Health workers at hospitals need to take care of a COVID-19 patient starting from their admission until their recovery or ICU admissions or in a later-stage ventilation process. During this whole process, the hospital's administrative workers need to collaborate with ID physicians. COVID-19 suspected patients need to be kept in an isolated hygienic single room.[44] All CDC and the infection prevention control (IPC) guidelines should be followed by each personnel.[40] Later, on a regular basis those patients need to be tested by RT-PCR and CT scanning to confirm the case and to know the viral loads inside the patient's body.[45] In that case, samples are taken from upper and lower respiratory tracts (mostly from nasal and oropharyngeal swabs specimens). Additionally, a few days later blood, urine, and fecal samples should be collected for further analysis.[43,46,47] Thereafter, if the patient is recovering well, then they need to have regular checkups for testing the viral loads and when it will be confirmed by RT-PCR testing that the patient does not have any trace of SARS-CoV-2 elements then they will be discharged with proper hospital guidelines. On the other hand, if the patient's condition worsens, they need to be shifted to ventilators in ICUs with various life supporting systems. Thus, the overall process of an infected patient from SARS-CoV-2 is maintained through good health management decided by the health sector's expert professionals.[48]

13.2.2 Precision medicine management (PM)

Precision medicine (PM) management is a budding area in pathogen disease management, which develops with various advances in molecular biology, immunology sciences, bioinformatics, etc. for the treatment of health diseases.[49] Four major approaches that mainly occur under this management consist of prediction, prevention, personalization, and participation.[50] The first step is "prediction," which mainly focuses on the expectation of the manifestations of diseases by analyzing the risk factors, symptoms, and lifestyle, spreading social awareness with proper information to the public to prepare themselves to face the pandemic situation.[51] The next step is "prevention," where this management is applied for offering proper programs that will direct the progression of the pathology before the appearances occur. This process will also convey secondary prevention when the disease will be stable. The third step is "personalization," where various kinds of biological testing will occur, such as molecular, serological, and genetic analysis to know the behaviors of SARS–CoV-2 virus within infected patients. The last step of this management is called "participation," which includes a detailed treatment process and the involvement of academic institutes, health workers, biomedical professionals, and the patients themselves.[52] Fundamentally, PM involves the integration of a broad array of community and distinctive data that embraces clinical, molecular, genetic, and more improved biomarkers that can be used for the detection. This study mainly emphasizes how and why it is necessary to detect COVID-19 at an early stage, with a major focus on the strategies adopted for screening and detection of SARS–CoV-2. Hence, depending on the knowledge of detection and management of the virus, we aim toward contributing in controlling the COVID-19 pandemic.

13.3 Importance of early diagnosis and treatment of SARS-CoV-2

Because scientific studies have reported that SARS-CoV-2 is spreading rapidly, quick detection of this virus is a very crucial step to stop the spread. The rapid replication of this virus increase the viral loads. The very first step is to ban all kind of travel process and local transportations except essentials belongings The very first step is to restrict all kinds of traveling processes and local transportations except essentials belongings.[53] The next step is to isolate all the immediate travelers and test them by quick diagnostic

procedures with 14 days of the quarantine course. Already, many countries have implemented these steps to evaluate COVID-19 patients.[54,55] Hence, early diagnosis is important to isolate patients quickly from healthy people.[56] Researchers are focusing on more POC-based diagnostic processes to detect the virus at earliest. The gold standards for detection SARS–CoV–2 is reverse transcriptase polymerase chain reaction (RT–PCR).[57] Many other crucial POC strategies with advanced biomolecular bioanalysis processes have been implemented for early diagnostic purposes. Numerous rapid detection kits are available in the market to control the spread.[58] Presently, nucleic acid–based and genetic sequencing are widely being used to detect this contagious infection.[59] Nucleic acid amplification tests like isothermal amplification diagnostic processes are rapid and straightforward as compared to the conventional processes such as cell culture based, ELISA–based techniques.[60] Thus, these techniques are playing a major role in initial diagnosis of SARS–CoV–2. These test specimens are collected from upper and lower respiratory tract.[61,62] Serological testing is also important. In this process lateral flow assays (LFA), ELISA techniques have been used for population screening, identification of plasma donors, and evaluation of immunity power.[63,64] The third essential part is biomedical monitoring by advanced POC devices, and the major role of these strategies are to check the severity of the virus, evaluation, and therapeutic monitoring of patients. Many biomarkers like cardiac,[65] renal,[66] inflammatory,[67] and hepatic[68] markers have been implicated in the COVID-19 pandemic.

Additionally, early-stage sample collection is a vital factor in terms of reducing the viral loads for patients. To know the acute phase of this infection time of repeated testing is needed. In serological testing, monitoring the IgA response is also important. By this response, it will be easy to know when patients are exposed to the virus. Generally, the peak time is for IgM antibody is around 18−21 days from contracting the virus to get a positive response.[69] Although detailed study of antibody testing is not yet confirmed and many factors are unknown. In addition the serological processes have poor sensitivity issues. Henceforward, the biosensors approaches come into the picture where diagnosis process is very fast and affordable to the general health public.

On the other hand, medical diagnosis of SARS–CoV–2 at an early stage is also very much essential, so that the increase of viral loads can be reduced. Following all the preventive and control measures can reduce the risk of exposure with an infected person but at early stage of infection proper medication is a critical issue. According to studies, COVID-19 patients with

pneumonia, high fever, and chest congestion require oxygen therapy with a flow rate of 5 L/min. The concentration of oxygen obtained for adults is >90% SpO_2, for children and pregnant women is 95% SpO_2. Patients with acute respiratory problems and hypoxemic respiratory failure require invasive mechanical ventilation.[68,70] For the mild symptoms cases noninvasive and high-flow nasal oxygen therapy can be implemented at an early stage. Further, if the condition of patients get worse within 1 h then endotracheal intubation and mechanical lung ventilations must be applied.[71] However, specific pharmacological treatment is not yet confirmed in the case of COVID-19. In many places, doctors have used empirical antiviral with lopinavir, ribavirin, nelfinavir, or other same derivatives.[72] In Japan, doctors have used inhibitor enzyme transmembrane protease, serine 2 (TMPRSS2).[73,74] Antimalaria drugs such as chloroquine and its derivatives hydroxychloroquine are used in many countries.[75] In a few cases, therapeutics strategies are also used like steroids, immunoglobulin, Janus kinase inhibition, and cytokine blockade.[76] These strategies are not only used for quick relief for patients, but these treatments are curable for COVID-19. More accurate medicines and vaccines are yet to come. Thus, in the current situation, development of technologies for early detection is crucial. Later in this chapter the various advances in sensor technology to improve COVID-19 situations are discussed.

13.4 Strategies adopted for the screening and detection of SARS-CoV-2

The screening, early detection, and public health management of the SARS-CoV-2 infected individuals have been done in various ways, where the preliminary ways for the screening have been performed by biomolecular technologies. Currently the gold standards technology is RT-PCR based process. Based on RT-PCR tests, many advanced technologies have been developed. Many commercial kits are available based on RT-PCR and nucleic acid testing in the market. Apart from these, recently diagnostics-based screening, biosensor, and nanomaterial-based POC detections are also employed for early diagnosis of SARS-CoV-2 virus. This section describes all such advanced in technologies used for SARS-CoV-2 preliminary screening and monitoring in daily bases.

13.4.1 Technology empowered screening of patients

Infected individuals by SARS–CoV–2 commonly show a mild fever at first and then it develops severity at a later stage. Targeting these mild symptoms, temperature based screening have been initiated in public domains such as at airports, public parks, and various public transportations.[77] Recently in Switzerland, dual functional plasmonic biosensor combined with plasmonic photo thermal effect and localized surface plasmon resonance (LSPR) sensing process had a very advantageous solution for SARS-CoV-2 diagnoses.[78] This study showed high sensitivity and rapid reliable assay for this virus detection. In this study, researchers have used two-dimensional gold nanoislands, which were functionalized with complementary DNA (cDNA) receptors. This principle worked to sense the selected sequences from SARS-CoV-2 strain via nucleic acid hybridization. To improve the sensitivity, researchers employed thermoplasmonic heat on gold nanoislands and illuminated at particular plasmonic resonance frequency (Fig. 13.3). The detection limit (LOD) of this process is 0.22 pM.[78] Previously developed body temperature sensor and thermal imaging based infrared thermography (IRT) technology can be used for controlling recent
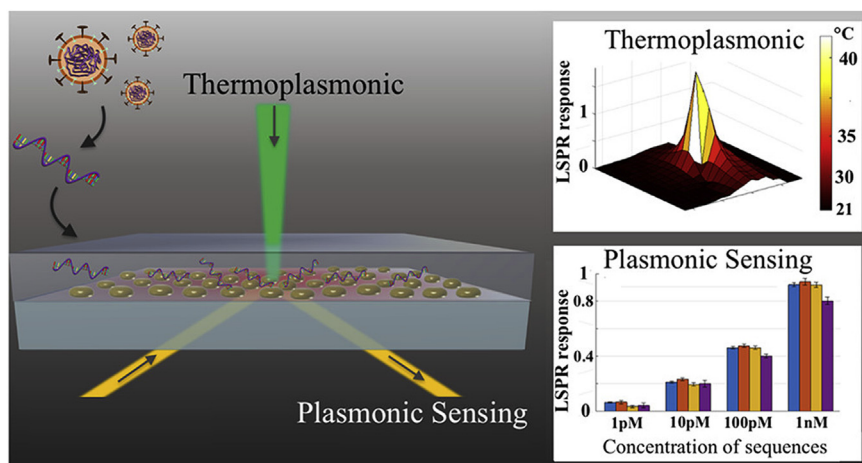
Figure 13.3 Schematic representation of dual-functional plasmonic photothermal biosensors for SARS-CoV-2 detection. Two-dimensional gold nanoislands functionalized with cDNA receptors for sensing the selected sequences from SARS-CoV-2 via nucleic acid hybridization. *(This figure is reproduced with the permission from Qiu G, Gai Z, Tao Y, Schmitt J, Kullak-Ublick GA, Wang J. Dual-functional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection. ACS Nano. 2020;14(5):5268−77.)*

outbreaks by SARS-CoV-2 screening.[79] Apart from these technologies, recent geotagging-based cellphone screening is also helpful for separating the infected people from healthy populations.[80] In this process, when a person is infected by the SARS-CoV-2 virus, then the individual will be detected by geotagging and cellular code. This is linked with the device the person carries. Hence, this process gives alerts and signals to healthy individuals to separate themselves from the infected ones.[81] These important technologies are gaining in demand to help further detect SARS-CoV-2 in early stages.

In several cases, it has been reported that the infected personnel who is asymptomatic is difficult to be diagnosed and isolated those individuals from healthy population. In these cases, thermal sensors fail. For these incidents, screening at a large scale for each individual is necessary by biomolecular, colorimetric sensor, electrochemical sensor, or any clinical assisted diagnostics methods. Therefore, the aforementioned techniques empower the screening and quick detection schemes that can evaluate healthy people from infected ones. In the following section, we have described such frequently used techniques for the screening/detection SARS-CoV-2.

13.4.2 Biomolecular techniques for SARS-CoV-2 detection

In clinics and diagnostic centers, many gold–standard biomolecular techniques have been used, where they examine the samples from the infected symptomatic/asymptomatic individual using thermal cycler by RT-PCR, enzyme-linked immunoassays (ELISA), and many next generation sequencing (NGS) platforms. For this, the sample from an infected patient is collected either in the normal laboratory settings or in the clinical settings. These techniques have mainly been targeted for identifying the specific signature sequence from the viral genome. The most common biomolecular technique is based on the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).[82] Usually, viral RNA is converted into cDNA by RT-PCR/qRT-PCR amplification. Infected samples such as swabs from respiratory tracts and bronchoalveolar lavage fluid were collected and transferred into sterile tubes that contais universal transport medium for RT-PCR reactions (Fig. 13.4.1). Most of the commercial kits target four different viral genes such as RdRp genes for RNA dependent RNA polymerase, ORF1a/b gene for human RNA polymerase protein, N genes for nucleocapsid protein, and E gene for envelope protein[83] (Table 13.1). However, there are limitations to this process, which needs to be addressed
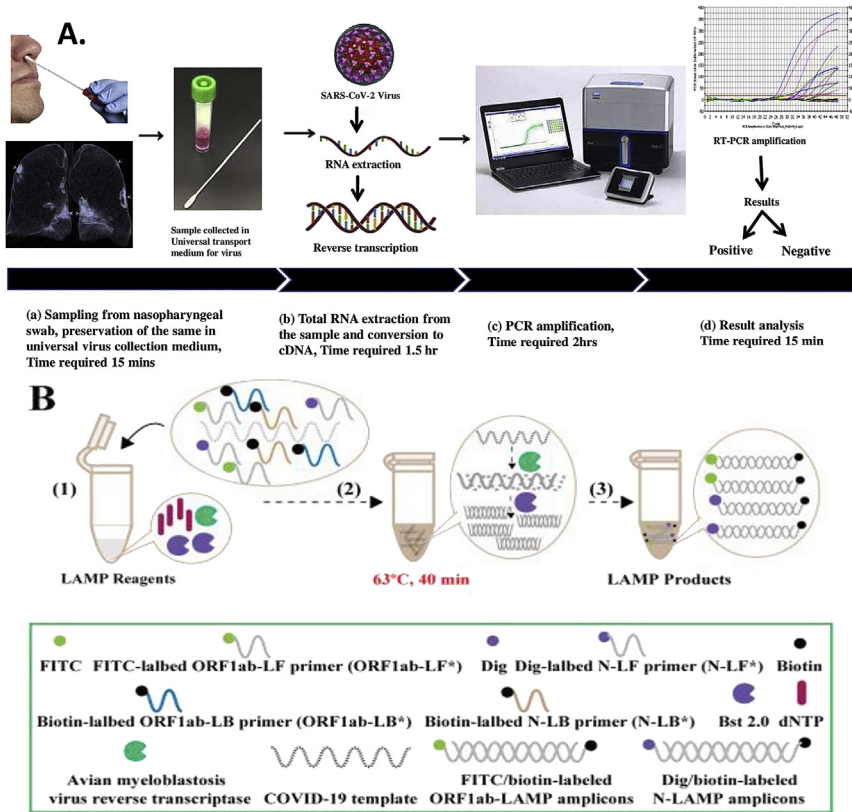
Figure 13.4 Schematic representation of biomolecular detection of SARS-CoV-2: (1) workflow of RT-PCR based detection; (2) mechanisms of RT-LAMP reaction for the detection of SARS-CoV-2. *(This figure is reproduced with permission from Das Mukhopadhyay C, Sharma P, Sinha K, Rajarshi K. Recent trends in analytical and digital techniques for the detection of the SARS-Cov-2.* Biophys Chem. *2021;**270**:106538 and Zhu X, Wang X, Han L, et al. Reverse transcription loop-mediated isothermal amplification combined with nanoparticles-based biosensor for diagnosis of COVID-19 166. medRxiv; 2020. p. 112437. 2020.2003.2017.20037796.)*[85]

further. RT–PCR method uses more time for expertized sample handling, post–PCR analysis. This process also has cross–contaminating issues and the sensitivity is inferior compared to other POC-based detection processes.[84] In a few reports it is stated that the sampling process (RNA samples) is very complicated. Thus, the sample preparation for the RT–qPCR is one of the tedious part especially when it comes to the larger scale of examinations.

To address such limitations, researchers have developed isothermal amplification processes like loop–mediated isothermal amplification (LAMP),[86–88]

Table 13.1 Diverse biomolecular processes used for detection of SARS-CoV-2.

Diagnostic platforms	Sample type	Detection limit	Analytes	Detection time	Advantages	Limitations	References
RT–PCR	Fecal sample	3.9 copies/ reaction	Nucleic acids	Not mentioned	High sensitivity and high specificity	High-end laboratory procedures with trained labors	104
qRT– PCR	Respiratory samples (swabs)	M Gene 10^6 copies/ reaction	RNA	Not mentioned	Good sensitivity	High-end procedures and Carry over issues	105
Real-time RT–PCR	Respiratory swabs	11.2 copies/ reaction	Genomic RNA	Not mentioned	Good sensitivity and specificity	Different COVID– 19 gene assays were performed with different commercial kits which created problem in determination of sensitivity	59
RT–PCR	Nasal swab and sputum	20 folds of diluted samples	Viral RNA	3–4 h	Moderate sensitivity	High-end laboratory process	106
ELISA	Blood samples	92.37% sensitive	Antigen and antibodies (IgG)	1–5 h	Good sensitivity	ELISA is a very conventional laboratory based procedure with high risk of contamination	107

Continued

Table 13.1 Diverse biomolecular processes used for detection of SARS-CoV-2.—cont'd

Diagnostic platforms	Sample type	Detection limit	Analytes	Detection time	Advantages	Limitations	References
ELISA	Plasmid	IgG = 0.95, IgA = 0.975 IgM = 0.916	Antigen and antibody	Limited time frame	Quantitative ELISA method for SARS-CoV-2 detection with good sensitivity	High risk of contamination, need high-end instruments for sample analysis	108
RT-LAMP	Serum, saliva and nasal swab, urine	1.02 fg	Nucleic acids	30 min	Very high sensitivity with high specificity	This study was performed with nucleotide oligos of COVID-19 from GeneBank not directly test related samples	109
RT-LAMP	Clinical samples from direct COVID-19 patients	3 copies/reaction	RNA	30−50 min	Very good specificity and high accuracy with high sensitivity on-spot biomolecular assay	Not mentioned	110

RCA	DNA Oligo nucleotides	~0.96 p.m.	Circular DNA	10 min	Rapid, robust and highly sensitive assay	RCA has limitation for the length of circular template	101
One step RT–LAMP	Clinical samples	500 copies/mL	Nucleocapsid protein	60 min	Good specificity, and straight forward process	This study is not as good as other quantitative process and sensitivity is also poor	111
NASBA	Saliva samples	10 copies/reaction	Viral RNA	60 min	Portable, scalable, high accuracy, rapid and affordable	NASBA has poor RNA integrity issues and NASBA needs to improve the specificity of the reactions	100

rolling circle amplification (RCA),[89] nucleic acid sequence–based amplification (NASBA) (Table 13.1).[90] These isothermal amplifications, especially LAMP reaction, reduces time, multiple steps, costs, and development on-site based on rapid detection (Fig. 13.4.2).[87,91–93] Based on this principle, researchers have developed a LAMP-assisted colorimetric rapid detection method for SARS-CoV-2 in clinical samples. This method demonstrated excellent results and qualitative visual detection of the SARS-CoV-2 virus.[94] Likewise, using the same LAMP principle, one group of researchers have detected the SARS-CoV-2 virus from the unpurified clinical samples. Due to the versatile nature of LAMP assay, it is easy to detect target analytes from unpurified samples. As a result, this process leads to the whole reaction quickly and more cost-effectively using less reagents and high-end instruments.[95] Since LAMP reaction is an isothermal process, which means for the whole reaction, it needs only one single temperature; thus this process required only one heat block to perform the whole experiment.[96] Based on this concept LAMP assay can be used as a noninstrumental and POC system.[97,98] Accordingly, paper-based LAMP reaction, based on a colorimetric process provides better sensitivity and time of response. Researchers developed a LAMP-based method for the rapid colorimetric detection of COVID-19; the sensitivity was ∼97.6% (42/43) and read-out time was ∼30 min[99] based on the isothermal concept. NASBA is also used for SARS-CoV-2 RNA detection. Reported sensitivity by NASBA was 10 copies/reaction within 10 min.[100] RCA isothermal process is also one of the helpful methods for rapid detection of SARS-CoV-2 at this current situation. One of the reports stated that RCA can detect SARS-CoV-2 circular DNA within 10 min and the sensitivity of the assay was ∼0.96 pM.[101] On the other hand, for a detailed study of SARS-CoV-2 behavior and determination of mutations, NGS technology is playing a major role.[102] Additionally, advanced molecular biology techniques based on gene editing have also been used to develop the assay for the detection of the SARS-CoV-2 in clinical samples. In recent evolution, a clustered regularly interspaced short palindromic repeat (CRISPR)-based technique has been employed to achieve higher sensitivity and prompt discovery in molecular assay.[103]

13.5 Nanobiosensing techniques for SARS-CoV-2 detection

Even though biomolecular technologies are considered as gold-standard procedures due to their constant, efficient, and reliable outcomes, but these processes have many drawbacks, such as requiring high-end instruments, taking too much time, and consuming the use of many reagents and skilled personnel to perform the experiments.[112,113] Furthermore, their requirement for these reliable instruments has many restrictions, which are very challenging in rural areas with less-developed facilities. To conquer such drawbacks and to expand the prospect for the prompt detection of the SARS-CoV-2 at on-site for rapid assessment, nanomaterial-based biosensing methods have been employed to improve the unsophisticated devices that can be handled by anyone as a POC system.[114] These devices are established using nanomaterial-based processes, which help to detect pathogens and are robust, rapid, and with very good sensitivity.[115] Nanomaterials provide outstanding transducing capabilities to the device due to their astonishing optical and electronic properties.[114] In recent years, these nanomaterial-based applications in biological sciences are becoming an emerging technology due to their outstanding analytical performance, lower LOD determination, wide detection range, and less involvement of high-end instruments.[116] Apart from these advantages, the main benefits of nanomaterial-based technologies are reduction of reagent volumes, detection and response time, user-friendly applications, and high stability. The major application of nanomaterials is based on different kinds of biosensor devices, which include, for example, colorimetric, fluorescence, plasmonic, and electrochemical devices.[117,118]

Biosensor contains a bioreceptor as a sensing component and a signal transducer to transform any kind of biological signal into a physical, electrochemical, optical, or any other quantifiable signal. Transformation of a biological signal into a measurable signal, biosensor is performed by a physicochemical transducer, such as an optical, thermal, and mass process.[119] To amplify the signal, a signal processor is necessary, including a bioreceptor such as DNA, RNA, antibodies, and enzymes, which is linked to the target analytes (Fig. 13.5).

Based on these simple principles, researchers are applying this concept for early diagnosis of SARS-CoV-2 screening. Many nanobiosensing-based detection methods have been developed for on-site monitoring of SARS-CoV-2.[121] However, these techniques are also having some limitations such
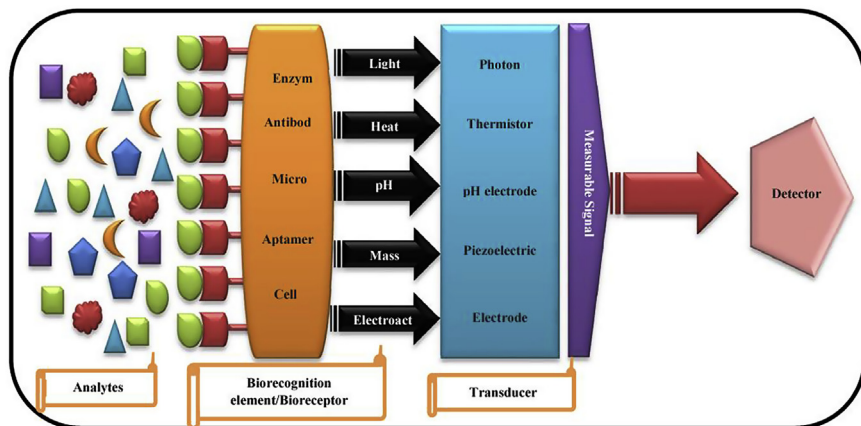
Figure 13.5 Schematic representation of a typical nanobiosensor principle. *(This figure is adopted from Girigoswami K, Akhtar N. Nanobiosensors and fluorescence based biosensors: an overview. Int J Nano Dimens 2019;**10**(1):1—17.)*[120]

as the designing of sensor platforms to obtain the lowest LOD and to improve the signal-to-noise ratio. To overcome these problems, researchers are attempting to improvise more by incorporating different unique surface modifications to the biosensor platforms using advanced nanomaterials. Among all the sensor strategies, electrochemical-based biosensing platforms are used widely. In a later section we have summarized studies that have been conducted using electrochemical biosensors for SARS-CoV-2 detection.

13.5.1 Electrochemical-based biosensors for the detection of SARS-CoV-2

Fundamentally, an electrochemical biosensor is a biosensor device with an electrochemical transducer. It consists of a chemically modified electrode with conducting or semiconducting material. These materials are coated with a biochemical film.[122] Based on this concept, several electrochemical based biosensor studies were employed in detection of SARS-CoV-2 (Table 13.2). In general, electrochemical-based colorimetric detection was used in viral detection such as LFA, lateral flow immunochromatographic strips (LFICS), fluorescence resonance energy transfer (FRET), Field effect transistors (FET), etc.[123] These processes are not only used before the clinical/real sample but also allows for the controlled release of samples to the detection zone, which assist a device to obtain a better sensitivity. Employing this strategy, Lin et al.

Table 13.2 Summary table of available electrochemical biosensing strategies for detection SARS-CoV-2.

Detection principles	Samples source	Nanomaterial used in probe	LOD	Linear range	Signal process	Response time	Recognition element	References
Functionalized TiO$_2$ Nanotube-based electrochemical biosensor	Nasal and saliva	Co-TNT [Cobalt functionalized TiO$_2$ nanotubes]	$\sim$0.7 nM	14–1400 nM	Amperometry	$\sim$30s	Spike protein of SARS-CoV-2 (Receptor-binding domain, RBD)	133
Paper-based electrochemical biosensors	Nasopharyngeal swab specimens	ssDNA capped with AuNPs on graphene-coated filter paper	6.9 copies/μL	585.4 -5.854×10^7 copies/μL	CV and EIS	Less than 5 min	Nucleocapsid phosphoprotein (N genes)	134
Ultrasensitive super sandwich electrochemical sensor using smartphone	Sputum, throat swabs, urine, plasma, feces, oral swabs and serum samples	Calixarene functionalized graphene oxide targeting RNA	200 copies/μL	10-fold serial dilution	EIS and DPV	Not reported	ORF1ab gene	135
Paper-based electrochemical biosensor	Human Serum	Redox conversation [Fe(CN)$_6$]$^{3-/4-}$ between captured immunoglobulins	$\sim$1 ng/mL	1–1000 ng/mL	EIS, CV	30 min	Spike protein	136
Impedance-based	Serum samples from positive	RBD-Monoclonal	Not reported	Not reported	EIS	Less than 5 min	RBD	137

Table 13.2 Summary table of available electrochemical biosensing strategies for detection SARS-CoV-2.—cont'd

Detection principles	Samples source	Nanomaterial used in probe	LOD	Linear range	Signal process	Response time	Recognition element	References
electrochemical sensor	COVID-19 patients	antibody CR3022						
Electrochemical immunosensor	Nasal swabs	Carbon nanofiber–modified screen printed	0.8 pg/mL	1–1000 ng/mL	SWV	Not reported	Nucleocapsid N protein	138
Plasmonic electrochemical biosensor detection	Not reported	Functionalized AuNPs conjugated specific monoclonal antibody	$\sim$4.2 fmol	Not reported	Plasmonic metasensors	$\sim$80 min	Spike protein	139
FET-based electrochemical sensor	Nasal swabs	Graphene-SiO$_2$/Si substrate	2.42×10^2 copies/mL	Not reported	FET	Not reported	Spike protein	125
Microfluidic chip based electrochemical sensor	Saliva and nasal swabs	Gold nanospikes in a microfluidic chip	$\sim$0.5 p.m.	Not reported	Surface plasmon resonance	30 min	Spike protein	140

have developed an LFA–based device for rapid and sensitive determination of the anti–SARS-CoV-2 antibody, which is a biomarker that originates in the infected individuals.[124] Simultaneously, Seo et al. developed FET–based detection process of SARS-CoV-2 spike protein detection with high sensitivity and specificity. The sensitivity of this process was discovered as 1 fg/mL in phosphate-buffered saline and 100 fg/mL in clinical medium from nasal swabs (Fig. 13.6A).[125] The detection of the antibody is the indirect format and carries reliability issues; thus to obtain improved detection, the sensing is based on highly precise techniques, where CRISPR-Cas12 based lateral flow system has been adopted to effectively detect the specific RNA from the viral genome.[126] Other than these types of biosensor systems, few biosensors were made using conventional biological processes such as ELISA-LFA techniques for preliminary detection of SARS-CoV-2 virus. This process has also successfully served as an efficient tool for screening a wide range of populations for managing the public health.[127] Besides, LFICS based detection has been developed by Huang et al. for analysis of SARS-CoV-2. This knowledge incorporates gold nanoparticles colloid-based procedures, which can detect IgM and IgG antibodies against a SARS-CoV-2 viral strain in human blood within a very short period of time, $\sim$ 15 min.[128] In addition, another report by Li et al., who discovered that the testing sensitivity of LFICS procedure is 88.66% and the specificity is 90.63%, which is also an outstanding outcome (Fig. 13.6C and D).[129] Further, detecting and destroying this virus at an early stage is extremely important. Hence, targeting this issue, researchers suggested a strategy based on metallic-nanomaterials in disinfectants such as silver, titanium dioxide, and copper nanoparticles by emitting toxic ions, which will destroy the viral particles.[130]

Due to the rapid and sensitive detection capability, the electrochemical-based biosensing platforms have widely been accepted worldwide and were impressively discovered for the detection and screening of various disease biomarkers.[131] In the current circumstances of SARS-CoV-2 detection, these electrochemical procedures have been significantly exploited, which includes voltammetry, amperometry, impedance-based, and FET–assisted detection for screening the infected individuals. For example, Mahari et al. have developed a system to detect the SARS-CoV-2-specific antigen, using the differential potential voltametric technique, where the antibody against SARS-CoV-2 has been immobilized on the sensing probe surface. Here, the analytical signal has been recorded using Zobell's solution and obtained a wide range of the antigen concentration between one fM and 1M with the sensitivity of 90 fM.[132]
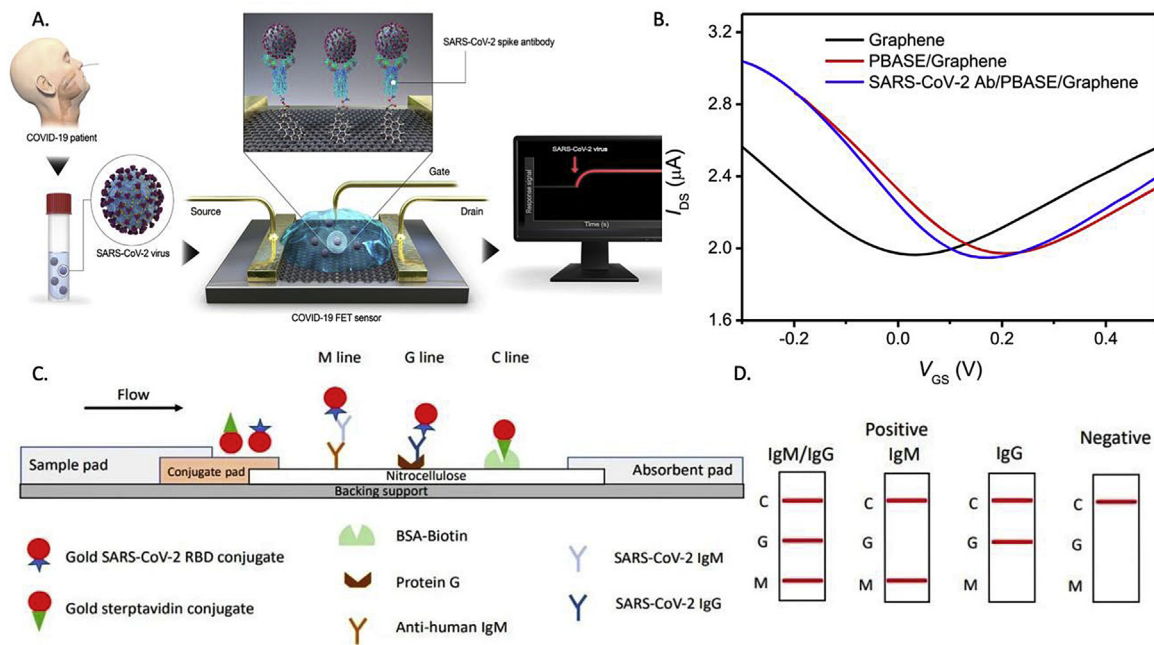
Figure 13.6 A schematic diagram of FET sensor for SARS-CoV-2: (A) working principle; (B) measurement of SARS-CoV-2 signals through FET sensors in steps of the antibody conjugation; (C) schematic representation of SARS-CoV-2 IgM-IgG antibody test diagram of detection device; (D) diagram of different testing outcomes where C indicates control line, G indicates IgG line, M indicates IgM line, and G means IgG immunoglobulin. *(These figures are adopted with the permission from Seo G, Lee G, Kim MJ, et al. Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor.* ACS Nano *2020;**14**(4):5135—42. and Li Z, Yi Y, Luo X, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis.* J Med Virol *2020;**92**(9):1518—24.)*

13.5.2 Colorimetric-based biosensors for the detection of SARS-CoV-2

Colorimetric biosensors are one of the best options for SARS-CoV-2 detection and random screening due to its simple, rapid outcome and on field experiments. This kind of setup is much more important at this current scenario in terms of isolating infected individuals from healthy ones. Using the colorimetric principle, several biosensor devices have been proposed for on-site monitoring of SARS-CoV-2 detection (Table 13.3). In contrast, conventional processes and electrochemical-based detection colorimetric detection can be more portable since these processes do not require a laboratory facility for the whole experiment. For example, Moitra et al. developed naked eye detection of SARS-CoV-2 DNA by using colorimetric assay. In this case, the group has used gold nanoparticles capped with thiol-based antisense oligonucleotides for N gene from SARS-CoV-2. This report said that this process can detect positive SARS-CoV-2 strain within 10 min with the sensitivity of 0.18 ng/μL.[141] Another group of researchers have developed colorimetric biosensors based on gold nanoparticles functionalized with antibodies targeting three different proteins of SARS-CoV-2. The remarkable feature of this biosensor is to detect the quantification of viral load which is similar to threshold cycle 36.5 targeting RdRp and N genes. The application of this method is claimed as POC testing.[142] Considering these facts, we can conclude that, in the field of lab-on-chip diagnostic sensors for SARS-CoV-2, colorimetric biosensors play major roles in terms of rapidity and cost.

13.6 Conclusion and future direction

To address COVID-19 encounters, proper health management is much needed in terms of controlling the spread of the virus. The isolation process and the infrastructure of patient units such as ICU rooms, ventilators, operating rooms, and washrooms need to be maintained in a hygienic way using disinfectants, sanitization, and continuous hand washing. The demand for PPE has increased significantly, since more patients are getting admitted to hospitals. So, distributing PPE to health workers at the correct time is very much needed. In addition, to protect patients, medical professions, health care systems, virologists, and other investigators who are associated with COVID-19 from unnecessary infections, health care management is very much essential at this moment. Additionally, it is important to perform

Table 13.3 Various colorimetric biosensors available for SARS-CoV-2 detection.

Testing approaches	Testing type	Sample source	Gene/region detection	LOD	Detection time	Target analyte	References
Colorimetric biosensor based on gold nanoparticle (AuNP)	POC	Nasal swabs,	RdRp and N genes	Threshold cycle (Ct) = 36.5	3 min	Spike protein, membrane protein, envelope proteins	142
Colorimetric biosensor based on botulinum neurotoxin receptor	POC	Blood, urine, Stool and Nasopharyngeal swabs	N genes	1a.m.	1−2 h	Recombinant plasmid	143
RT-PCR colorimetric assay	Laboratory based	Upper respiratory specimens	ORF1ab and N genes	10^5 dilution factors	Total ∼2 −3 h But observable color change found within 10 min	Nucleic acids template	144
Colorimetric detection using CRISPER/ dCas9	Laboratory based	Nasopharyngeal aspirates and sputum specimens	N1, N2, and N3 genes	30 pM	∼2 h	Nucleic acids	145
Colorimetric detection using DNAzyme sensor	Laboratory and POC– based	Swabs from nasopharyngeal and oropharyngeal	N genes	10^1 to 10^7 copies	Total duration ∼1 h and visual observation ∼5 min	RNA	146

Paper-based colorimetric biosensor using electrochemical assay	Laboratory and POC-based	Not mentioned	Immunoglobulins IgG and IgM	∼1 ng/mL	30 min	Spike protein	136
Colorimetric biosensor using LAMP assay	POC	Nasal swabs	N gene	∼62.5 viral copies/LAMP reaction	50−60 min	Plasmids	147
Colorimetric detection using RT–LAMP	POC	Nasal swabs	N gene	10^2 RNA copies	30 min	RNA	148
Colorimetric detection using paper-based ELISA	POC	Not mentioned	SARS-CoV-2 antibody	100 ng/μL	20 min	Recombinant nucleocapsid	149
Colorimetric detection using binding chemistry	POC	Cells infected with SARS-CoV-2	N gene	0.18 ng/μL	10 min	Nucleic acids	141

regular self-monitoring and self-care by using easily accessible diagnostic support, which could improve the outcomes for many individuals and health care professionals without having close contact with infected patients. Due to lack of space in the hospital for 14 days isolation is difficult, rather the isolation process can be possibly done at a patient's home instead if the symptoms of COVID-19 is mild. Instead of admitting into the hospital and occupying the hospital beds that can be used for those severely affected or who are having heavy symptoms, patients could monitor themselves using rapid and on-site diagnostic approaches. Therefore, advancements in biosensor systems are helping patients for quick, accurate, and easy identification of their condition. The biosensor-based quick methodologies have the potential to reduce the transmission of the respiratory virus. The rapid and early detection of SARS-CoV-2 is very much essential in terms of stopping the infections. Biosensor-based POC systems are not only useful for on-the-spot diagnostics, but it is also helpful for environmental monitoring in public areas such as airports, ports, railways stations, and various working institutes. Scaling up the number of preliminary tests across the world, biosensor-based facilities would be the best way to stop the virus. In the near future, advancements in biosensor-based technology integrated with microfluidic sensors and the establishment of nanotechnologies such as nanosized sensors, nanovaccines, and antiviral nanocomposites will help to bring some solution in terms of stopping this virus. Henceforth, the development of biosensor-based technologies and health management will have great potential to combat the present scenario and to overcome any other pandemic situations in the future.

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