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Point-of-Care Biosensor-Based Diagnosis of COVID-19 Holds Promise to Combat Current and Future Pandemics

Arpana Parihar, Pushpesh Ranjan, Sunil K. Sanghi, Avanish K. Srivastava,* and Raju Khan*

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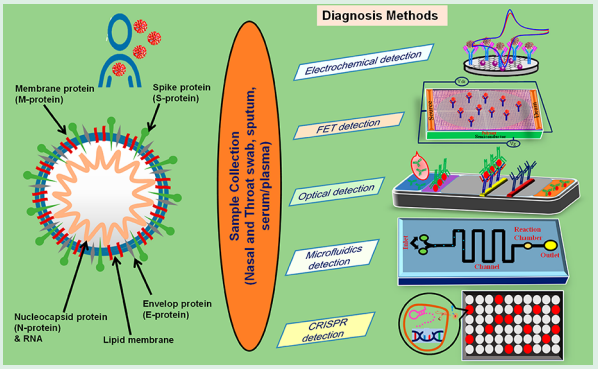
ABSTRACT: Efficient and rapid detection of viruses plays an extremely important role in disease prevention, diagnosis, and environmental monitoring. Early screening of viral infection among the population has the potential to combat the spread of infection. However, the traditional methods of virus detection being used currently, such as plate culturing and quantitative RT-PCR, give promising results, but they are time-consuming and require expert analysis and costly equipment and reagents; therefore, they are not affordable by people in low socio-economic groups in developing countries. Further, mass or bulk testing chosen by many governments to tackle the pandemic situation has led to severe shortages of testing kits and reagents and hence are affecting the demand and supply chain drastically. We tried to include all the reported current scenario-based biosensors such as electrochemical, optical, and microfluidics, which have the potential to replace mainstream diagnostic methods and therefore could pave the way to combat COVID-19. Apart from this, we have also provided information on commercially available biosensors for detection of SARS-CoV-2 along with the challenges in development of better diagnostic approaches. It is therefore expected that the content of this review will help researchers to design and develop more sensitive advanced commercial biosensor devices for early diagnosis of viral infection, which can open up avenues for better and more specific therapeutic outcomes.



KEYWORDS: *Electrochemical biosensor, microfluidics, SARS-CoV-2, optical, FET, COVID-19*

INTRODUCTION

The current coronavirus disease 2019 (COVID-19) pandemic, which originated from Wuhan, China, in December, caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2), has affected approximately 13 million individuals among ∼206 countries and led to the death of ∼0.58 million of people all over the world.[1,2] The WHO declared the SARS-CoV-2 outbreak a pandemic on March 11 and released interim guidelines for its management.[2] Earlier, similar outbreak of SARS (Severe Acute Respiratory Syndrome) in 2002 and MERS (Middle East respiratory syndrome) virus in 2012 caused deaths of several thousand people and posed a severe economic burden to the affected countries. Coronavirus can commonly infect both animals and human beings and can cause a range of respiratory illnesses, but the SARS-CoV-2 infection remains asymptomatic in ∼50% cases; thus, infected people remain indistinguishable from healthy ones but can become a serious threat as they can spread infection unknowingly among large chunks of the population. The main symptoms of SARS-CoV-2 are fatigue and fever, followed by cough, difficulties in breathing, dyspnea, and myalgia; however, more severe respiratory pathologies are observed in infants, immune-compromised patients, and elderly people.[3,4] Genetically novel SARS-CoV-2 belongs to the genus β-coronavirus, which is closely related to SARS-CoV that led to the global outbreak in 2002−2003 and caused severe respiratory pathologies with a rate of fatal conditions of 10%.[4] Another member of the β-coronaviruses is Middle East Respiratory Syndrome (MERS) (outbreak emerged in 2012), which affected millions of people with severe pneumonia.[5,6] Coronaviruses with a diameter of 100−160 nm are spherical enveloped viruses which contain a positive-sense single-stranded (+) RNA genome of size 27−32 kb. The main proteins involved in SARS-CoV-2 infection are spike (S), nucleoprotein (N), membrane (M), and envelope (E). The detailed structural morphology of SARS-CoV-2 and SARS-CoV has been studied using imaging techniques such as

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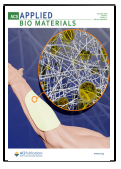

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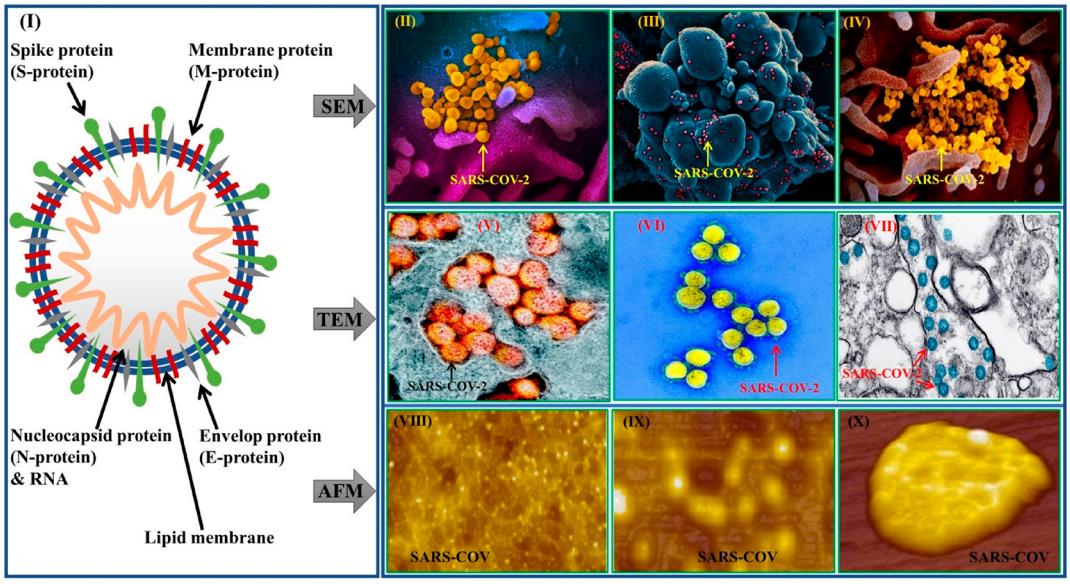

Figure 1. (I) Schematic diagram of SARS-CoV-2. (II, III, IV) Scanning electron microscopy images of SARS-CoV-2. Reproduced from ref 7. Source: NIAID-RML. (V, VI) TEM images of SARS-CoV-2. Reproduced from ref 7. Available free high resolution images. Source: NIAID Flickr. (VII) TEM images of SARS-CoV-2, ref 8. Source: PHIL. (VIII, IX, X) AFM images of SARS-CoV. Reproduced with permission from ref 9. Copyright 2005 WILEY-VCH Verlag GmbH & Co. KGaA.

scanning electron microscope (SEM), transmission electron microscope (TEM), and atomic force microscope (AFM).[7−9] Figure 1 represents the schematic structure of SARS-CoV-2 along with SEM and TEM images and AFM image of SARS-CoV.

Specifically, S protein of SARS-CoV-2 binds to the ACE2 receptor present on the cell surface of the respiratory tract and other parts of the body that include the gastrointestinal tract. After binding to the ACE2 receptor, the virus particle gets internalized into the cell and starts replication. The antiviral drugs such as ritonavir, ribavirin, lopinavir, remdesivir, and favipiravir either alone or in combination with chloroquine, hydroxychloroquine, and interferon-alpha have shown potential to cure the SARS-CoV-2 infection and hence are currently under clinical trial.[10,11] However, the complete remedy of virus infection is vaccination, and several attempts have been made in this regard.[12] Although due to technology advancement the process of design and development of vaccines has become fast and less laborious, it would still take a minimum of ∼18 months or more to clear clinical trial stages 1, 2, and 3 for final approval of a SARS-CoV-2 vaccine. Until that time, early diagnosis of viral infection could have the potential to save lives and prevent the spread of infection. Most of the recent diagnostic kits available for SARS-CoV-2 are polymerase chain reaction (PCR) based such as qRT-PCR.[13,14] Although this technique is sensitive and offers much specificity for a particular infection, it is time-consuming (4−5 h) and requires expensive instruments, reagents, and skilled personnel. Due to its high cost, individuals belonging to low socioeconomic income groups in developing countries cannot afford it. Therefore, there is a need for diagnostic technology which may provide faster, high-throughput results with low cost and simultaneously offer comparable selectivity and sensitivity. Biosensors are attractive tools for detection methods for molecules and small particles, such as virions, as they can produce rapid, sensitive, and specific signals.[15,16] Recently, a large number of studies revealed that biosensor-based diagnosis of viral infection holds promise to provide a low cost, high-throughput, faster way to detect the infection.[16−18] Biosensors are devices which are used to detect biomolecules called biomarkers in patient samples and thus give an indication toward a particular diseased condition. Biosensors mainly comprise a biomarker, a biorecognition element, a transducer, and a signal amplifier digital device. Considering several advantages of electrochemical biosensors such as high sensitivity, ease of operation, cost-effectiveness, and ability to be miniaturized, they are the most appropriate tools among other available diagnostic approaches.[18−20] In this regard, reduced graphene oxide integrated with a microfluidic chip-based electrochemical immunosensor for label-free influenza virus H1N1 detection with a high limit of detection has been reported.[18] Further, Layqah et al. developed a highly sensitive electrochemical biosensor by immobilization of cysteamine on a gold nanoparticle modified carbon electrode for the detection of MERS-CoV and human coronavirus (HCoV), and the detection was done by square wave voltammetry having a low limit of detection of 1 and 0.4 pg mL^{-1} for MERS-CoV and HCoV, respectively.[19] In another report, Kogaki et al. reported an immunosensor for the diagnosis of the N-antigen of SARS-CoV. This immunosensor showed detection limit ranges in picogram concentration of SARS-CoV antigen within 15 min in serum and nasopharyngeal swab samples.[20] However, Kim et al. developed thiol-modified gold nanoparticle-based colorimetric biosensors for rapid diagnosis of MERS-CoV. The biosensor had an ultralow limit of detection and detected up to a picomolar concentration of MERS-CoV.[21] Although biosensors have gained considerable attention as high throughput devices which have potential for cost-effective mass screening, their clinical use in mass testing and commercial availability is still lacking. The roles of government policies, funding agencies, and industries have been critical for biosensor-based diagnostic approaches. Various biosensor-based potential diagnostic approaches which have shown promise for high throughput, cost-effective, early detection of SARS-CoV-2 have been discussed.

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Table 1. Various Biological Parameters and Respective Diagnostic Approaches for Detection of SARS-CoV-2 Infection

S.No.	biological parameters	diagnostic approach	mechanism of detection	time	cost	advantage	limitations	ref
1.	physiological symptoms	thermal scanner/sensors	body temperature measurement	few seconds	low cost	primary indication of infection	nonspecific, cannot distinguish between different types of infection	27
2.	biochemical parameters	complete blood count, C-reactive protein, Cytokines [IL6, IL8, IL10, IL33]	white blood count elevated, platelet count decreased, level of c-reactive proteins and cytokines elevated	3−4 h	moderate	indicate infection and inflammation	nonspecific and unable to differentiate between infections	28−33
3.	anatomical paramaters	X-ray/CT Scan	chest images	∼1 h	moderate	helps in identification of organ damage, virus infilteration, provide higher sensitivity when combined with RT-PCR	hysteresis of abnormal CT, and Incapability of distinguishing between different viral mediated pneumonia	37−41
4.	serological parameters	rapid antibody test [IgM, IgG]	measures level of IgM, IgG in serum	20−30 min	low	rapid, cost-effective; indicate specific viral infection	low sensitivity and specificity as compared to RT-PCR, false positive	52
5.	viral genome and proteins	virus RNA	RNA amplification (RT-PCR), RNA sequencing (NGS)	RT-PCR (5−6 h), NGS (∼2 days)	high	gold standard, provide high specificity, selectivity, and accuracy	costly, time consuming, need skilled person and sample processing, work only in early stage of infection	50,51
		virus RNA	RT-LAMP, CRISPER-Cas9/Cas13	1−2 h	moderate	offer good sensitivity and specificity	gives false positive results due to contamination	50,51
		viral proteins (S, N, E, M)	lateral Immunoflow assays and biosensors	20−30 min	low	rapid, cost-effective, need no sample pretreatment moderate specificity, easy to perform, indicate immunity against infection	fails to indicate early infectious stage (4−5 days) and may give false negative results	22−26
		viral RNA and viral proteins (S, N, E, M)	electrochemical, optical, microfluidic sensors	2−20 min	low	rapid, low cost, capacity of multiplexing, no sample pretreatment, can be integrated with any platform, offer high specificity, sensitivity, and selectivity	promising but needs further attention to be miniaturized, scaled up, and commercialized	58−65 84−93

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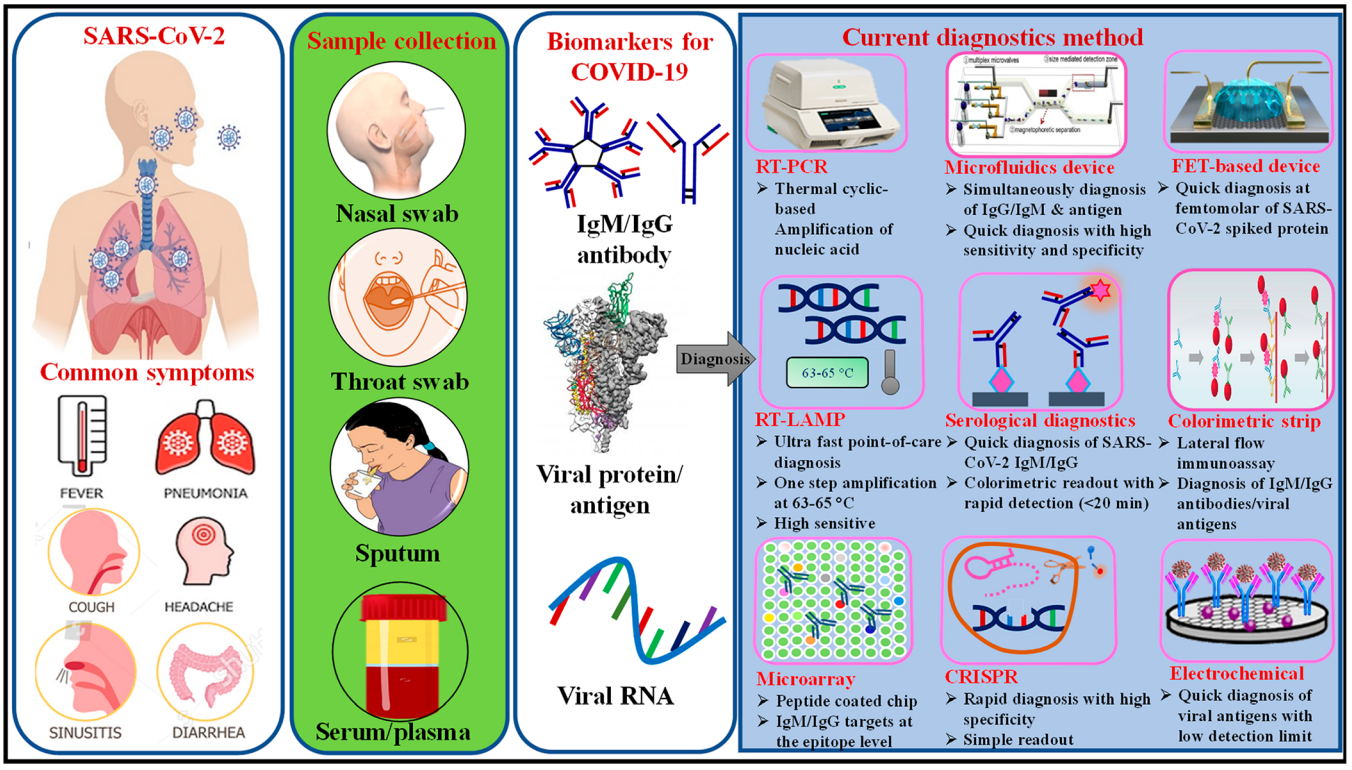

Figure 2. Representation of SARS-CoV-2 infection, symptoms [Reproduced from ref 41. Copyright 2020 Source: shutterstock.com] along with clinical sampling method [Reproduced with permission from ref 42. Copyright 2020 Nature publishing group. Reproduced from ref 59. Copyright 2020 American Chemical Society], biomarkers for diagnostic approach and point-of-care biosensor [Reproduced from ref 25. Copyright 2020 American Chemical Society. Reproduced with permission from ref 43. Copyright 2020 Elsevier. Reproduced from ref 59. Copyright 2020 American Chemical Society].

In a recent review article, Zhu et al. have gathered information on microfluidic-based lab-on-a-chip devices for detection of viral infection; however, information specific for SARS-CoV-2 and currently available commercial sensors for its detection is lacking in this article.[22] However, Bhalla et al. have compiled thorough information on diagnostic approaches for COVID-19 including available sensors, but it lacks specific and commercial information about sensors developed.[23] Other recent review papers have provided very good information about currently available diagnostic approaches for detection of SARS-CoV-2, but most of these methods are based on RT-PCR and immunoassay.[24−26]

In this review, we basically describe and discuss various techniques such as electrochemical, optical, and microfluidics-based biosensor platforms for viral infection diagnosis. Apart from this, we will also discuss some high-throughput diagnostic approaches specifically designed for SARS-CoV-2 detection and also shed light on their commercial availability. At the end, current challenges faced during development and commercialization of diagnostic approaches are summarized. It is expected that this review will help researchers to design and develop diagnostic kits which can not only be available at low cost but also offer high sensitivity and selectivity.

BIOLOGICAL SAMPLE COLLECTION, BIOMARKERS, AND PRESENT DIAGNOSTIC METHODS FOR DETECTION OF SARS-COV-2

For monitoring status and root cause of any disease condition, the first step involves biological sample collection and the second step is to analyze the presence of a specific biomarker associated with the specific disease condition with the help of an appropriate diagnostic method. Various biological parameters and respective diagnostic approaches along with their pros and cons for detection of SARS-CoV-2 infection are described in Table 1. The preliminary identification of COVID-19 disease has been done via the measurement of physiological symptoms, specifically temperature using a thermal scanner. This is a very nonspecific parameter, as we cannot distinguish between various diseases in which fever (high body temperature) is the primary sign of infection,[27] yet governments around the globe have been using temperature monitoring as the first criterian at various public places such as airports, railway stations, etc., to identify and isolate the suspected COVID-19 individuals from the rest of the population. However, to get better insight, the anatomical and biochemical tests such as X-ray, CT-scan, complete blood count, C-reactive protein, and cytokines [IL6, IL8, and IL10] can provide valuable information about the pathophysiology of a particular disease condition in an individual. So far, however, these are considered nonspecific because X-ray and CT scan indicate about lung infections and the rest of the biochemical test results such as elevated white blood cell count, C-reactive proteins, and cytokine levels have been observed in many inflammatory diseases. Elevated levels of white blood cells, C-reactive proteins, and IL6, IL8, and IL10 have been observed in COVID-19 infection.[28−33] Recent studies have shown that high inflammation led to cytokine storm, which causes more damage to internal organs than the infectious agent in the case of COVID-19.[31,34,35] Therefore, one cannot deny the importance of anatomical and biochemical parameters for

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Table 2. Some Selected Commercial Kits for SARS-CoV-2 Detection

S.No.	test name	test type	sample source	detection gene	manufacture company/institute/research lab	sensitivity/LOD	approval
1.	BioMerieux nucliSENS Easy MGA	real time RT-PCR	nasopharyngeal swab, nasal swab, mid-terbinate nasal swab, oropharyngeal swab, bronchoalveolar lavage, lower respiratory tract aspirates, sputam	nucleic acid	Nationwide Children's Hospital	-	Review by FDA under this EUA
2.	ThermiFisher Scientific TaqPath COVID-19 combo kit	RT-PCR	nasopharyngeal, nasal swab, midterbinate nasal swab, oropharyngeal swab, bronchoalveolar lavage	Orf1ab, N gene, and S gene	Biocerna	250 GCE/mL for the Genotek or 100 collection device and 375 GCE/mL for the copan eSwab	EUA
3.	ThermiFisher TaqMan 2019-nCOV assay kit v1 (singleplex) combo kit	RT-PCR	nasopharyngeal, nasal swab, mid-terbinate nasal swab, oropharyngeal swab	Orf1ab, N gene, and S gene	Altru Diagnostics, Inc.	0.625 copies/μL	Review by FDA under this EUA
4.	UTHSC/UCH SARS-CoV-2 RT-PCR assay	RT-PCR	nasal swabs	nucleic acid	UTMG Pathology Laboratories	100 PFUs	Review by FDA under this EUA
5.	SARS-CoV-2 assay	real time RT-PCR	nasopharyngeal, nasal swab, mid-terbinate nasal, oropharyngeal swab, bronchoalveolar lavage	nucleic acid	Biocollections Worldwide, Inc.	1 copy/μL	Review by FDA under this EUA
6.	OZO gold SARS-CoV-2 assay IgM/IgG rapid test kit	lateral flow immunoassay	serum/plasma/blood	IgM/IgG antibody, N protein	JETTA lab LLP (India)	-	Review by FDA under this EUA
7.	OZO diamond SARS-CoV-2 assay IgM/IgG rapid test kit	lateral flow immunoassay	serum/plasma/blood	IgM/IgG antibody, N protein and S protein	JETTA lab LLP	-	Review by FDA under this EUA
8.	ErbaLisa COVID-19 IgG	ELISA	serum	IgG antibodies	Calbiotech Inc.	98.3%	Review by FDA under this EUA
9.	Platelia SARS-CoV-2	EIA	serum/plasma	Antibodies IgM, IgG, and IgA	Bio-Rad laboratories Inc.	-	Review by FDA under this EUA
10.	VITROS Total and IgG COVID-19 antibody test		-	-	Ortho clinical diagnostics	-	Review by FDA under this EUA
11.	Patho detect	RT-PCR	-	-	MY LAB	-	ICMR India

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Table 3. Currently Available Biosensor Based Diagnostic Approaches for Detection of SARS-CoV-2 Infection

S.No.	biomarker	biological samples	biorecognition element	method	LOD/sensitivity	ref
1.	SARS-CoV-2 protein	gargle solution	-	mass spectroscopy	10^5–10^6 genome equivalents μL^{-1}	49
2.	nCovid-19 antigen	saliva	FTO/AuNPs/nCOVID-19 Ab	DPV	10 fM	58
3.	SARS-CoV-2 antigen protein	nasopharyngeal swab	graphene sheets with SARS-CoV-2 Ab	FET	2.42×10^2 copies mL^{-1}	59
4.	Viral sequences including RdRp COVID, ORF1ab-COVID, and E genes	respiratory sample	gold nanoislands functionalized with complementary DNA	PPT-LSPR	0.22 pM	62
5.	N-gene	-	thiolated modified ASO AuNPs	colorimetric	0.18 ng μL^{-1}	64
6.	Nucleocapsid Ab	serum	peptide monolayer functionalized with SARS-CoV-2 nucleocapsid recombinant protein	SPR	~1 μg mL^{-1}	65
7.	IgM/IgG Ab	blood/serum/plasma sample	gold nanoparticle conjugated with COVID-19 antigen and rabbit IgG	colorimetric	88.66%	69
8.	Anti-SARS-CoV-2 IgG	serum	mouse anti-human IgG antibody labeled lanthanide-doped polystyrene NPs	colorimetric	-	70
9.	IgM/IgG	serum	lanthanide Eu(III) fluorescent microsphere	colorimetric	98.72%	71
10.	IgM Ab	serum	anti-human IgM conjugated colloidal gold nanoparticles	colorimetric	-	72
11.	IgG Ab	serum	NC protein and anti-human IgG modified AuNPs	colorimetric	69.1%	73
12.	IgM/IgG Ab	serum	SARS-CoV-2 antigen and rabbit IgG antibodies labeled colloidal AuNPs	colorimetric	85.29%	74
13.	SARS-CoV-2 spike protein	-	monoclonal antibodies labeled AuNPs	plasmonic metasensor	~4.2 fM	75
14.	IgM, IgG Ab	serum	-	chemiluminescence	≥10 AU mL^{-1}	76
15.	SARS-CoV-2 Ab	blood	bioconjugate labeled RRBC	gel card agglutination	-	77
16.	S1 Ab	-	cell-based detection	membrane engineering	1 fg mL^{-1}	78
17.	N-gene	serum	ssDNA aptamers	aptamer	-	79
18.	IgG/IgM/Antigen	serum/pharyngeal swab	fluorescent microsphere coated IgG/IgM Ab and SARS-CoV-2 capture Ab	microfluidic	-	83
19.	E and N gene	respiratory swab	CRISPR-Cas12 based DETECTR	CRISPR	10 copies μL^{-1}	84
20.	orf1ab and N genes	nasal swab	CRISPR Cas12a/gRNA with fluorescent probe	CRISPR	2 copies mL^{-1}	89
21.	orf1ab, S and N gene	throat swab	primer and DNA fragment	RT-LAMP	80 copies viral RNA mL^{-1}	91
22.	Nucleocapsid gene	nasal swab	RT-LAMP primer	RT-LAMP	10^2 RNA copies	92
23.	orf1ab and S genes	swab/bronchoalveolar lavage fluid	viral RNA extracted using QIAamp Viral RNA Mini Kit	RT-LAMP	2×10^1 copies and 2×10^2 copies/reaction with primer sets orf1ab and S gene	93
24.	orf1ab and N genes	saliva	LAMP primers	RT-LAMP	~10^2 viral genome/reaction	94

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better disease prognosis and therapeutic management. In the case of COVID-19, digitalized chest imaging revealed the typical ground glass opacity (GGO) in the lungs.[36] Other studies revealed that, apart from the GGO pattern, the abnormal testimony with bilateral lung involvement, in a few cases opacities with rounded morphology and peripheral distribution of disease have also been observed;[36,37] in addition, CT images help as a prognostic tool for COVID-19 infection. As the disease progresses, the opacity in images increases.[36] Furthermore, consolidation of fluid in the lung CT-scan has been observed in some cases.[38] It is pertinent to note that the COVID-19 CT-scan finding is similar to the reported CT imaging for MERS and SARS-CoV, and hence, one cannot distinguish among these infections on the basis of a CT-scan.[39] However, clinical CT-scan findings have shown promise for initial screening of SARS-CoV-2 infection[40] and can be used for followup for the individual recovery process. To identify the root cause of disease and the specific infectious agent, serological and nucleic acid amplification methods are generally recommended. While blood serum is the preferred biological sample for estimation of serological markers, samples such as oropharyngeal swab, nasopharyngeal swab, sputum, and bronchoalveolar lavage have been considered ideal for nucleic acid amplification/detection in COVID-19 patients.

Biomarkers, comprising biomolecules specifically associated with a particular disease, are the most important component of any diagnostic method; therefore, detection of a specific biomarker in a patient's sample can be exploited to design a diagnostic method for early detection of infection or other disease conditions. Figure 2 depicts common symptoms (fever, cough, headache, pneumonia, sinusitis, and diarrhea), types of biological samples (nasal/throat swab, sputum, and serum/plasma), biomarkers, and respective diagnostic methods for COVID-19. Basically, three types of biomarkers have been widely considered for detection of SARS-CoV-2 infection: viral proteins, viral genome, and antibodies specific to viral components generated by host immune system.

Mostly, viruses possess an outer protein capsid and genetic material. Among N, S, M, and E proteins of SARS-CoV-2, the N protein interacts with genetic material, and the other three proteins S, M, and E helped in the spread of infection and pathogenesis. The viral nucleocapsid protein binds with M protein and plays an important role in viral assembly and budding, whereas E protein is involved in viral morphogenesis, its release, and pathogenesis. The S is a homotrimeric protein that forms spikes which recognize and bind with the cellular receptor and facilitate viral entry into target cells.[44−47] During viral infection, virus binds with the cell surface receptor and gets internalized; inside the cell, it starts to replicate its genome and synthesize viral proteins, which upon assembly generate a large number of virus particles. These particles come out from the cell via exocytosis or host cell apoptosis; thereafter, virus particles spread into the body and lead to spread of infection. Several studies exploited the virus surface protein as a potential biomarker for detection of a specific virus in the patient's sample. For instance, Begum et al. detected a spike protein sequence of SARS-CoV-2 isolated from a patient's sample.[48] The spike protein consists of two regions, S1 and S2, where S1 binds with the host cell receptor and S2 is involved in membrane fusion. The spike protein due to its antigenicity and immunogenicity is the main target for neutralizing with antibodies and vaccines. Another SARS-CoV-2 protein is the nucleocapsid protein, which is the most abundant and highly immunogenic phosphoprotein. Therefore, the N protein of SARS-CoV-2 is often used as a marker in several diagnostic assays. Recently, Ihling et al. reported mass spectroscopy-based detection of the SARS-CoV-2 protein. Herein, they extracted protein from a gargle solution with the help of acetone and then digested it before experimental testing. On the basis of fragmentation peaks of protein in mass spectroscopy, they calculated and determined the presence of the SARS-CoV-2 protein. However, this technique has some limitations: it is tedious, requires costly instruments with trained personnel, and takes almost 3 h to complete the detection process.[49]

The nucleic acid or genome of the virus is the most potent biomarker among all the others used to detect the viral infection, with the help of several conventional techniques such as polymerase chain reaction, qRT-PCR, RT-LAMP, CRISPR, and NGS (next generation genome sequencing).[50,51] The viral nucleic acid (RNA or DNA) can also be exploited as a biomarker for designing biosensors. In this regard, several commercial PCR based kits for SARS-CoV-2 detection have been designed to detect target genes, which include mainly the Orf1 gene (human RNA polymerase protein), the N-gene (nucleocapsid protein), and the E-gene (envelope protein). Several studies in which nucleic acid is used as a biomarker for detection of SARS-CoV-2 have been summarized in Table 2.

The immune system of an individual produces a specific antibody against a specific viral infection, and these antibodies can be used as biomarkers for fabrication of lateral immune flow assay and biosensors. In the case of early infection, the PCR test is able to detect viral nucleic acid, but if the patient's immune system eliminates the virus, then the PCR test may provide false negative results. However, specific antibodies generated by the immune system against the virus can stay within the blood for a longer period. Therefore, detection of antibody titer such as IgG and IgM can provide important information in terms of immunity against a specific infection. In this context, several commercial immunoassay tests are available in the market for detection of SARS-CoV-2 antibodies. These kits detect specific IgM and IgG against the virus in the blood of infected patients. Further, an automated fluorescent immunoassay system is available for quantitative or semiquantitative measurement of concentration of the targeted analyte, which could be the viral antigen or IgM/IgG, as summarized in Table 3. Recently, the presence of IgM and IgG antibody in a patient's nasal and pharyngeal swab samples has been used for development of immunoassay for detection of SARS-CoV-2.[52]

Although antibodies have shown broader clinical application in terms of therapeutic and diagnostic intervention, certain advantageous traits of aptamers such as easy chemical modification, thermal stability, and rapid synthesis along with specific target recognition make them suitable molecules for clinical diagnosis of various diseases.[53,54] In this context, Song et al. have identified and prepared two high-binding-affinity aptamers named CoV2-RBD-1C and CoV2-RBD-4C having K_d values of 5.8 nM and 19.9 nM, respectively, against the SARS-CoV-2 receptor binding domain (RBD) of spike protein. They used an ACE2 competition-based selection strategy and machine-learning screening algorithm for selection of RBD specific aptamers. The results of simulated interaction modeling and competitive experiments revealed high binding affinity of aptamers for RBD; hence, they can be exploited for diagnosis and treatment of SARS-CoV-2 infection. However, the diagnostic and therapeutic testing of these aptamers in

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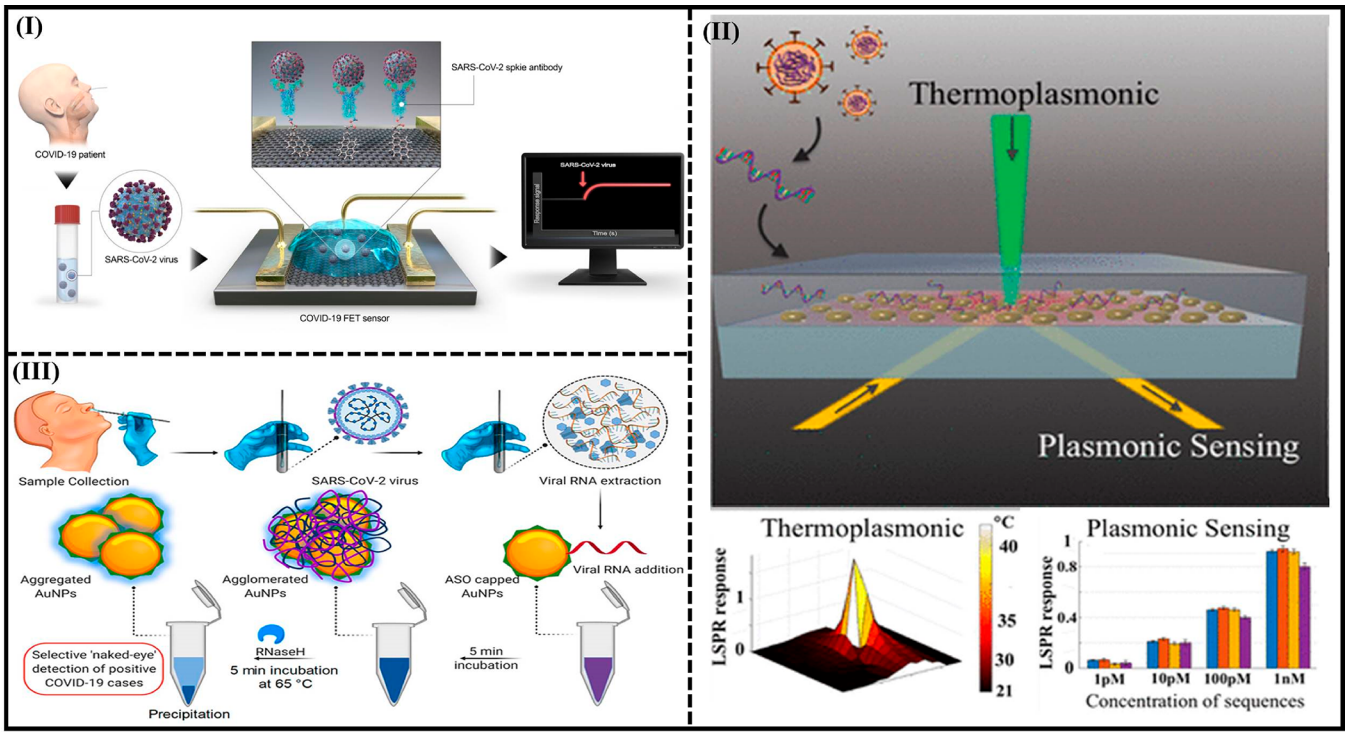

Figure 3. Representation of (I) FET-based biosensor. Reproduced from ref 59. Copyright 2020 American Chemical Society. (II) Combined plasmonic photothermal effect with localized surface plasmonic resonance-based dual-functional optical plasmonic biosensor. Reproduced from ref 62. Copyright 2020 American Chemical Society. (III) Naked eye detection of SARS-CoV-2 RNA mediated by the suitable designed ASO capped AuNPs. Reproduced from ref 64. Copyright 2020 American Chemical Society.

clinical settings is yet to be validated.[55] The physiological, anatomical, biochemical, serological, and viral component-based biomarkers along with their respective diagnostic approaches has been summarized in Table 1. In addition, we discuss briefly the underlying mechanism of diagnostic methods, time required, and cost, along with pros and cons of each methods in Table 1. Further details of biosensor-based diagnostic methods are described in a later section of this review.

NOVEL BIOSENSORS FOR POINT-OF-CARE DIAGNOSTICS OF COVID-19

Over the past decades, biosensor-based diagnostics have emerged as reliable and precise analytical tools, and are being explored for the detection of various infectious agents and other diseases in human beings. As currently used mainstream diagnostic methods are expensive and time-consuming, require trained personnel, and cannot be multiplexed, point-of-care high-throughput diagnostics are the need of the hour. They can provide rapid, cost-effective, highly specific and sensitive results and can be handled by a layman, which is the most pressing need in the current scenario of the SARS-CoV-2 pandemic. Being high-throughput, rapid, highly sensitive, cost-effective, and easy to use, with capacity of miniaturization and multiplexing biosensors, this holds promise to deal with the current pandemic situation. In this regard, considerable attention has been shown by the research community to develop electrochemical, optical, and microfluidics-based biosensors for the diagnosis of SARS-CoV-2 infection. In this section, we will be discussing the recently developed point-of-care biosensors for early detection of SARS-CoV-2.

Electrochemical-Based Biosensor. In the current pandemic crisis, researchers around the world have been rushed to develop accurate and efficient novel biosensor-based approaches for early detection of SARS-CoV-2. Several advantages of electrochemical biosensors are high sensitivity, high specificity, ease of operation, cost-effectiveness, miniaturization, and rapid testing. They also have an ultralow limit of detection with minimum sample requirement without pretreatment, making them the most appropriate tools among other available diagnostic approaches. In addition, electrochemical biosensors could be easily integrated with the microfluidic platform and can be multiplexed, which further improves the sensitivity of the device.[56] Generally, electrochemical techniques such as amperometric, potentiometric, and impedimetric have been employed to assess the analyte by measuring the current and potential difference with the help of electrochemical devices. Based on measurement, the electrochemical devices can be categorized as amperometric (measure current), potentiometric (measure potential), electrochemical impedance spectroscopy (measure resistance), etc. Furthermore, numerous portable electrochemical biosensors for diagnosis of various diseases are now commercially available.[57] Recently, Mahari et al. have developed two electrochemical biosensors for detection of spike protein antigen of COVID-19 (SARS-CoV-2). They fabricated an electrochemical-based biosensor using fluorine-doped tin oxide, and the surface was modified by gold nanoparticles. Then, it was immobilized with monoclonal antibody, while the second biosensor was termed eCovSens, in which antibody was immobilized on a screen-printed electrode. The devices displayed a limit of detection in the 120 fM range in standard buffer and spiked saliva samples. Apart from this, the electrodes remain stable for up to 4 weeks, which could

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help in wide applicability of the diagnostic approach in distant remote areas, without affecting its sensitivity. Further, these electrochemical sensors provide results within 10−30 s and can be used directly in patient saliva samples.[58] In another approach, Seo et al. reported the field effect transistor (FET)-based biosensing device for detection of SARS-CoV-2 in clinical samples. They coated a graphene sheet transistor (FET) with an antibody specific to spike protein. They have tested performance of this sensor in spiked buffer, cultured virus and nasal swab clinical samples. This device showed a limit of detection up to 1 fg mL^{-1} in PBS and 100 fg mL^{-1} in clinical samples. In addition, this FET-based sensor detected spike protein of approximately 1.6×10^1 pfu mL^{-1} in culture medium and 2.42×10^2 copies mL^{-1} in clinical samples of SARS-CoV-2. This device is ultrasensitive toward analytes and needs no sample pretreatment or labeling, as well as being able to easily differentiate among different strains of Coronavirus such as SARS-CoV and MERS-CoV. Further details of these devices have been shown in Figure 3I.[59] Taken together, these experimentally proven electrochemical biosensors can not only detect analyte in ultralow quantity with high specificity and selectivity without the need of sample pretreatment, but also provide fast and cost-effective results when compared to existing RT-PCR based approaches.

Optical-Based Biosensor. Several advantages such as rapid visual diagnosis, real-time analysis, and less reagent requirement make optical biosensors a preferable method of choice. Optical biosensors can be categorized based on the optics principle as surface plasmon resonance (waveguide), Raman scattering (inelastic scattering of monochromatic radiation), fluorescence (emission of light at longer wavelength), and colorimetric (visible color change).[60] Several optical biosensors including surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) have been commercially available for a long time.[61] Further, several colorimetric lateral immune flow-based kits for detection of SARS-CoV-2 are available in the market, from which some selected examples are listed in Tables 2 and 3. In addition, we have discussed the colorimetric lateral immune flow-based POCT for COVID-19 diagnosis in the following section. In SPR, an enhanced plasmonic field is achieved by excitation of incident photons which resulted in oscillation of electrons on the metal surface. This further enhanced the electromagnetic field and light absorption properties of matter, which improves the device performance. In this technique, a shift in refractive index due to molecular interaction at a metal surface via surface plasmon wave is detected. It offers real-time, fast, label-free detection of target analyte. More recently, Qiu et al. have reported the combined plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR)-based technique for the development of a dual-functional optical-based plasmonic biosensor for diagnosis of COVID-19. They employed 2D gold nano-islands functionalized with DNA receptors which are complementary with specific regions of the SARS-CoV-2 genome and hence hybridize with it and produce a plasmonic signal. This ultrasensitive sensor displayed a lower limit of detection of 0.22 pM with high sensitivity to target the gene of SARS-CoV-2 in a multigene mixture. The details of this LSPR biosensor are shown in Figure 3II.[62] This sensor can also be used for environmental monitoring and can be employed to detect virus concentration in air of busy places like train stations or hospitals.[63] In another study, Moitra et al. developed an SPR-based biosensor of plasmonic gold nanocomposite for the diagnosis of SARS-CoV-2 with LOD of 0.18 ng μL^{-1}. Herein, thiol-conjugated antisense oligonucleotides were functionalized on gold nanoparticles which selectively bind to the N-gene of SARS-CoV-2. This antisense oligonucleotide upon binding with the target gene resulted in agglomeration of the nanocomposite, which was visually observed by the naked eye. This biosensor displayed higher specificity toward the SARS-CoV-2 gene when tested in a mixture of SARS-CoV-2 and MERS-CoV genome. Moreover, this biosensor can be modified with any other SARS-CoV-2 gene such as M-gene or S-gene, which can further improve its sensitivity and specificity to distinguish between different viral strains. The details of biosensors are shown in Figure 3III.[64] Recently, Djailleb et al. fabricated an SPR-based sensor for detection of antibodies against the nucleocapside protein of SARS-CoV-2 in human serum. For this purpose, they coated the SPR sensor with peptide monolayer and thereafter functionalized it with virus nucleocapsid recombinant protein. The label-free device detects antibodies up to ∼1 $\mu g\ mL^{-1}$ within 15 min and hence can be used for point-of-care testing.[65] At a glance, these SPR based optical sensors can not only detect infectious agent in ultralow concentration with high specificity and selectivity, but also detect the variants of viral strains. Further, with the current advancement in the field of nanofabrication they can also be integrated with microfluidic platform which can not only enhance the sensitivity of these devices but also help in the miniaturization process.[66]

OPTICAL LATERAL FLOW IMMUNOASSAY-BASED POINT-OF-CARE DIAGNOSIS FOR COVID-19

The genome-based diagnostic methods gave promising results for detection of COVID-19, but they can only be useful in the case of early infection (5−7 days window), because in the later period the chances of false negative results increases as the individual develops neutralizing antibodies against the viral particles. In that case, detection of serological markers such as IgG and IgM may provide valuable information regarding the extent of immunity developed by the individual; however, its response may vary from person to person. The IgM antibody appears faster following infection than IgG. Moreover, the serological test can be used for detection of both antibodies against viral antigen (antibody test) and viral protein/antigens, which induces the antibody response (antigen test).[67] The information on selected commercial antibody and antigen test kits has been described in Tables 2 and 3.

Owing to the advantages of biosensors such as fast results, low cost, and easy handling, there is considerable interest in developing a lateral flow immunoassay-based (LFIA) biosensor. To detect antibodies in patient samples, many LFIA require emergency approval from the government.[68] In LFIA, the immunosensor have four zones, such as sample pad, conjugate pad, detection zone (control line and test line), and absorption pad. Each zone/line was fabricated with their respective reagents and antibodies. Then, the clinical sample dropped on the sample pad and then flowed on the strip. The appearance of color on the test line and control line assessed by the naked eye or by a reader indicates the presence or absence of analyte. In addition, the multiplex analyte could be detected on this strip. More recently, a lateral flow assay-based colorimetric biosensor has been developed by Li et al. for the detection of IgG and IgM antibodies specific to SARS-CoV-2 in biological samples such as serum, plasma, and blood. It provides visual results within 15 min. Herein, the gold

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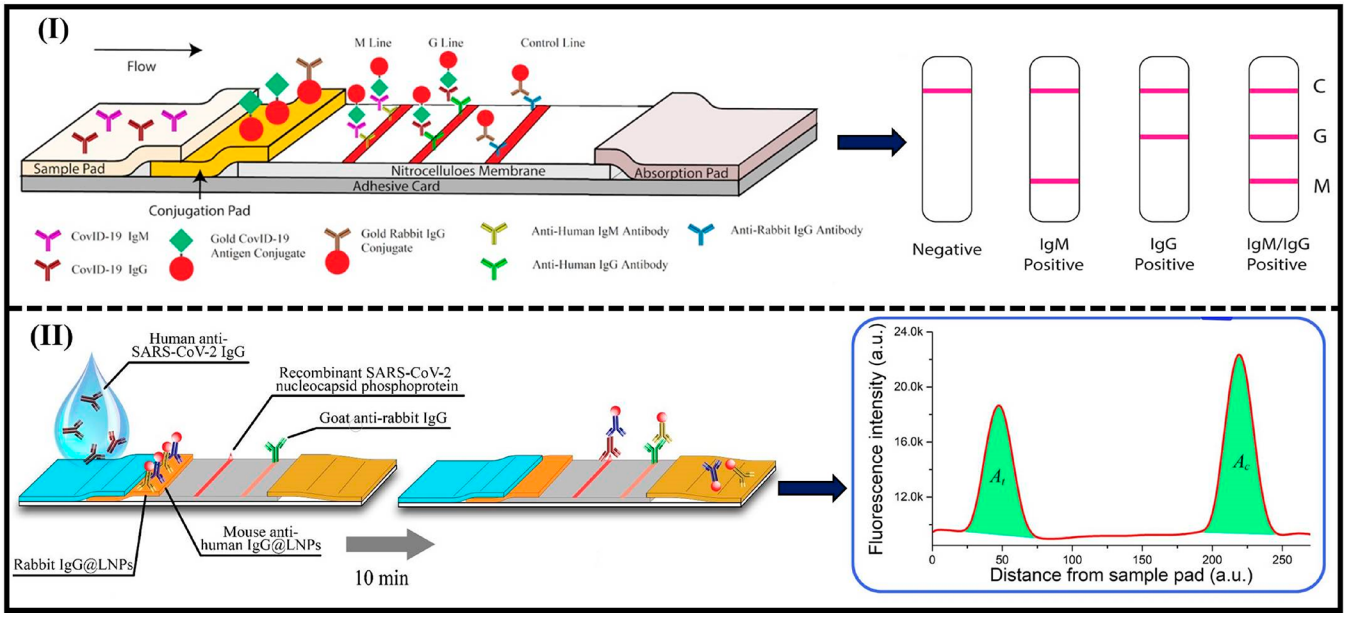

Figure 4. (I) Colorimetric biosensor for rapid diagnosis of SARS-CoV-2 IgM/IgG antibodies with testing result. Reproduced with permission from ref 69. Copyright 2020 WILEY-VCH Verlag GmbH & Co. KGaA. (II) Design and fabrication of LFIA with testing result. Reproduced from ref 70. Copyright 2020 American Chemical Society.

nanoparticles were modified with SARS-CoV-2 recombinant protein (Au-COVID-19) and rabbit-IgG (Au-rabbit-IgG). The modified gold nanoparticles were cast on the conjugate pad of the sensor surface, where M, G, and the control line were immobilized with anti-human-IgM, anti-human-IgG, and anti-rabbit-IgG, respectively. In the diagnostics step, the color visualization of pink or red occurs on either M line or G line or on both M and G line along with control line. It indicates the presence of SARS-CoV-2-IgM antibodies or SARS-CoV-2-IgG antibodies or both in the biological sample, respectively. This immunosensor displayed a quick result with high specificity (90.63%) and sensitivity (88.66%), respectively (Figure 4I).[69] Lateral flow immunoassays are easy to operate and results can be visualized by the naked eye; however, they give false negative and false positive results. This could be due to the availability of low concentration of IgG/IgM antibody in the blood during the initial stage of the primary immune response. This indicates the low concentration or disappearance of IgM after 2 weeks of infection. Recently, a lateral flow immunoassay-based fluorescent immunosensor was developed by Chen et al. for the diagnosis of anti-SARS-CoV-2 IgG in serum sample. Herein, they utilized lanthanide-doped polystyrene nanoparticle conjugates with rabbit IgG and mouse anti-human IgG for the construction of the conjugate pad of the immunosensor. The sample pad, T line, and C line were fabricated with human anti-SARS-CoV-2 IgG, recombinant SARS-CoV-2 nucleocapsid phosphoprotein, and goat anti-rabbit IgG, respectively. In this test, the concentrations of anti-SARS-CoV-2 IgG were measured by the ratio of fluorescence intensity of the test line and control line after interaction of coated antibodies with target analyte (Figure 4II).[70] Similarly, Feng et al. developed an immunosensor strip by fluorescent lanthanide compound for the diagnosis of IgG/IgM antibodies in a serum sample. For this purpose, they constructed the conjugate pad of an immunosensor surface by nucleocapsid protein and chicken IgY functionalized fluorescent Eu(III) microsphere, while anti-human-IgG, anti-human-IgM, and anti-chicken-IgY were coated on T1, T2, and C lines, respectively. This immunosensor exhibited high sensitivity of 98.72% and 96.68% for IgG and IgM, respectively.[71] Another lateral flow immunosensor was reported by Huang et al. for the diagnosis of IgM antibodies against SARS-CoV-2 in serum samples. Herein, they fabricated a conjugate pad of lateral flow chip by coating with anti-human IgM functionalized gold nanoparticles. The control line and test line were coated by goat anti-mouse IgG and SARS-CoV-2 nucleoprotein, respectively. When the serum sample dropped on the capture probe of the immunosensor, IgM present in blood was bound to AuNPs-(anti-human IgM) and then flowed on the biosensor surface. Target IgM against SARS-CoV-2 was captured on the test line, while AuNPs-(anti-human IgM) were captured at the control line. The appearance of red color on the control and test lines indicates the presence of IgM against SARS-CoV-2 virus in serum.[72] Similarly, Wen et al. reported the LFIA of colloidal gold nanoparticles for the diagnosis of SARS-CoV-2 within 20 min. They fabricated a conjugate pad of the sensor surface by a mixture of anti-human IgG modified gold nanoparticles and nucleocapsid protein and employed similar colorimetric testing for the diagnosis of SARS-CoV-2.[73] More recently, Zeng et al. reported LFIA for the detection of IgG/IgM antibodies in blood samples. In this sensor, they fabricated a chip of a conjugate pad by rabbit-IgG antibodies and SARS-CoV-2 antigen-labeled colloidal gold nanoparticles. Further, M line, G line, and C line were fabricated by anti-human IgM antibodies, anti-human IgG antibodies, and goat anti-rabbit IgG antibodies, respectively. The appearance of red color on M line with C line indicates the IgM antibodies and on G line and C line indicates the presence of IgG antibodies, although the appearance of both M line and G line with C line indicates the presence of both IgM/IgG antibodies in the sample. They observed sensitivity using this immunosensor of 82.35% and 61.76% for IgM and IgG, respectively. The sensitivity improved to 85.29% when both antibodies are present in the sample.[74] In another report, the colloidal gold nanoparticles

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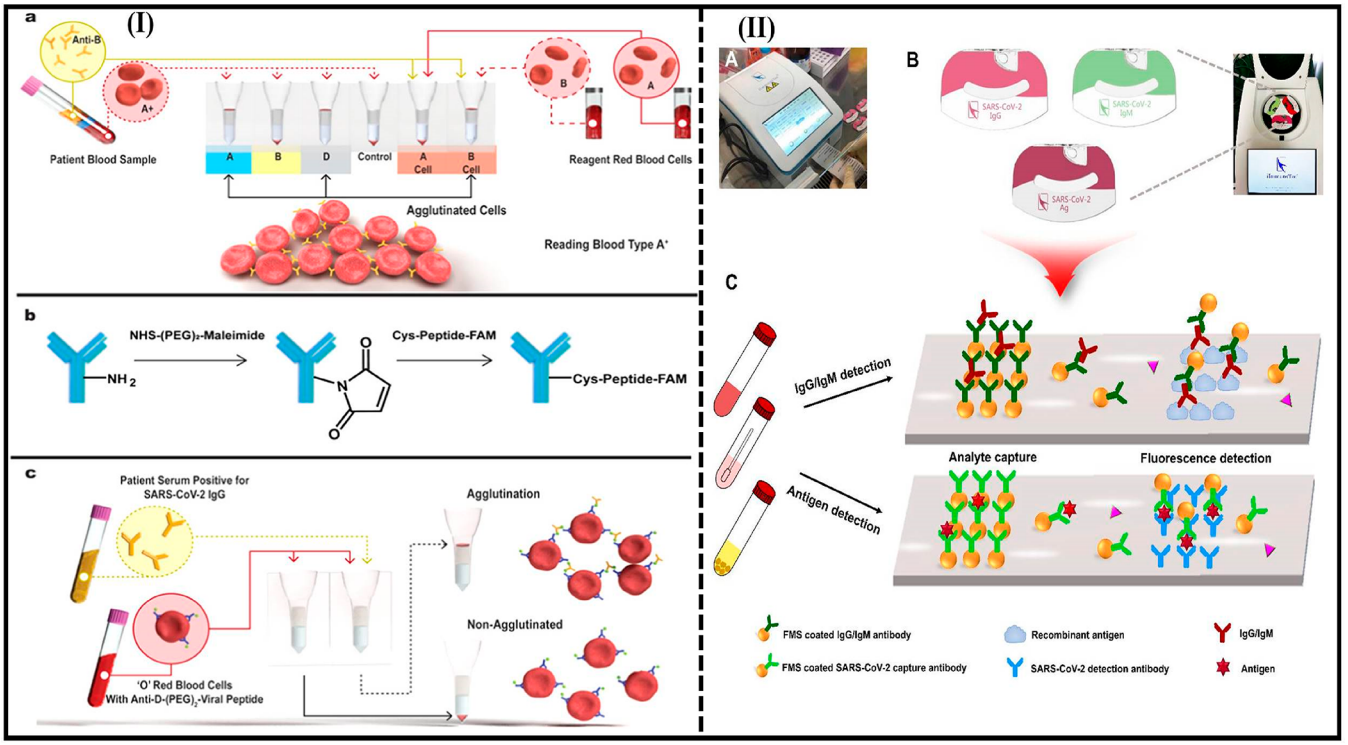

Figure 5. (I) Column agglutination test assay for SARS-CoV-2 diagnosis. (a) Typical blood typing assay. (b) Reaction scheme employed to produce the antibody−peptide bioconjugate in a two-step process. (c) Serology assay for visual detection. Reproduced from ref 77. Copyright 2020 American Chemical Society. (II) Photograph of (A) portable homemade fluorescent detection equipment and (B) immunoassay microchip ready to use. (C) Microfluidic fluorescent immunoassay for IgG/IgM/antigen detection of SARS-CoV-2. Reproduced from ref 83. Copyright 2020 American Chemical Society.

conjugated with SARS-CoV-2 spike the antibody-based toroidal plasmonic metasensor described. They have detected up to femtomolar levels (LOD ~ 4.2 fM) of SARS-CoV-2.[75] To reap the advantages of optical-based biosensor methods, Hou et al. have used a chemiluminescence immunoassay-based method to detect the presence of IgM and IgG antibodies in patients' nasal and pharyngeal swab samples.[76]

Another technique, based on the column agglutination test, was utilized for detection of viral infection. In this technique, the reagent red blood cells (RRBC) were first coated with antiviral peptide specific for SARS-CoV-2, and then mixed with biological sample on the gel card. After interaction of antibodies present in the biological sample with RRBC coated with Anti-D(PEG) viral peptide, agglutination of antibodies with RRBC was observed. They were further collected and separated by a centrifuge from free cells.[77] For instance, Alves et al. developed a gel card agglutination assay for the diagnosis of SARS-CoV-2 in a blood sample. In this assay, they mixed the bioconjugate cells of anti-D-IgG with SARS-CoV-2 spike protein containing a blood sample. The anti-D-IgG interacts with peptide of spike proteins and is agglutinated, which results in the red color on the surface of the gel card, further confirming the presence of SARS-CoV-2. This technique can be used to assess more than 100 samples within an hour; however, trained personnel with equipment are the primary need to perform this test (Figure 5I).[77] In another approach, Mavrikou et al. reported a cell-based biosensor for the diagnosis of S1 spike protein of SARS-CoV-2 antigen within 3 min with a low limit of detection of 1 fg mL^{-1}. In this assay, cell-based probes were modified by the electro-insertion of human chimeric spike S1 antibodies for the selective targeting of S1 antigen. The interaction of antibodies present in clinical samples with antigen results in a change in membrane-engineered cell bioelectric properties, which were measured and confirm the presence of SARS-CoV-2.[78] In another report, Chen et al. exploited the advantage of high specificity and sensitivity of aptamer-based biosensors and developed a 5-biotinylated aptamer for early detection of the N-protein of SARS-CoV-2.[79] Although the sensitivity of LFIA is much lower than with RT-PCR based assays, the rapid results, low cost, and ease of handling with no sample pretreatment/labeling make this the method of choice for improved prognosis of COVID-19 disease worldwide. Therefore, numerous LFIA based kits are commercially available in the market.[68] We have discussed a few selected examples of commercially available LFIA in Tables 2 and 3.

MICROFLUIDIC INTEGRATED TECHNIQUE-BASED BIOSENSORS

A microfluidic-based platform allows the diagnosis of a clinical fluidics sample via microchannel based on their separation mechanism which offers fast detection and high sensitivity in terms of detection. It is highly specific and can detect up to a single cell in a microliter sample. Moreover, more than one biomarker simultaneously can be detected in a single chip, which can be easily integrated with other techniques such as electrochemical, fluorescence, and chemiluminescence into a portable device.[80,81] Several microfluidic biosensors have been developed and exploited for the detection of various infectious agents such as bacterial pathogens (e.g., *Streptococcus*, *E. coli*, *Haemophilus influenza*), foodborne pathogens (e.g., *Salmonella*, *Listeria*, Cholera toxin), and viral pathogens (e.g., Hepatitis C,

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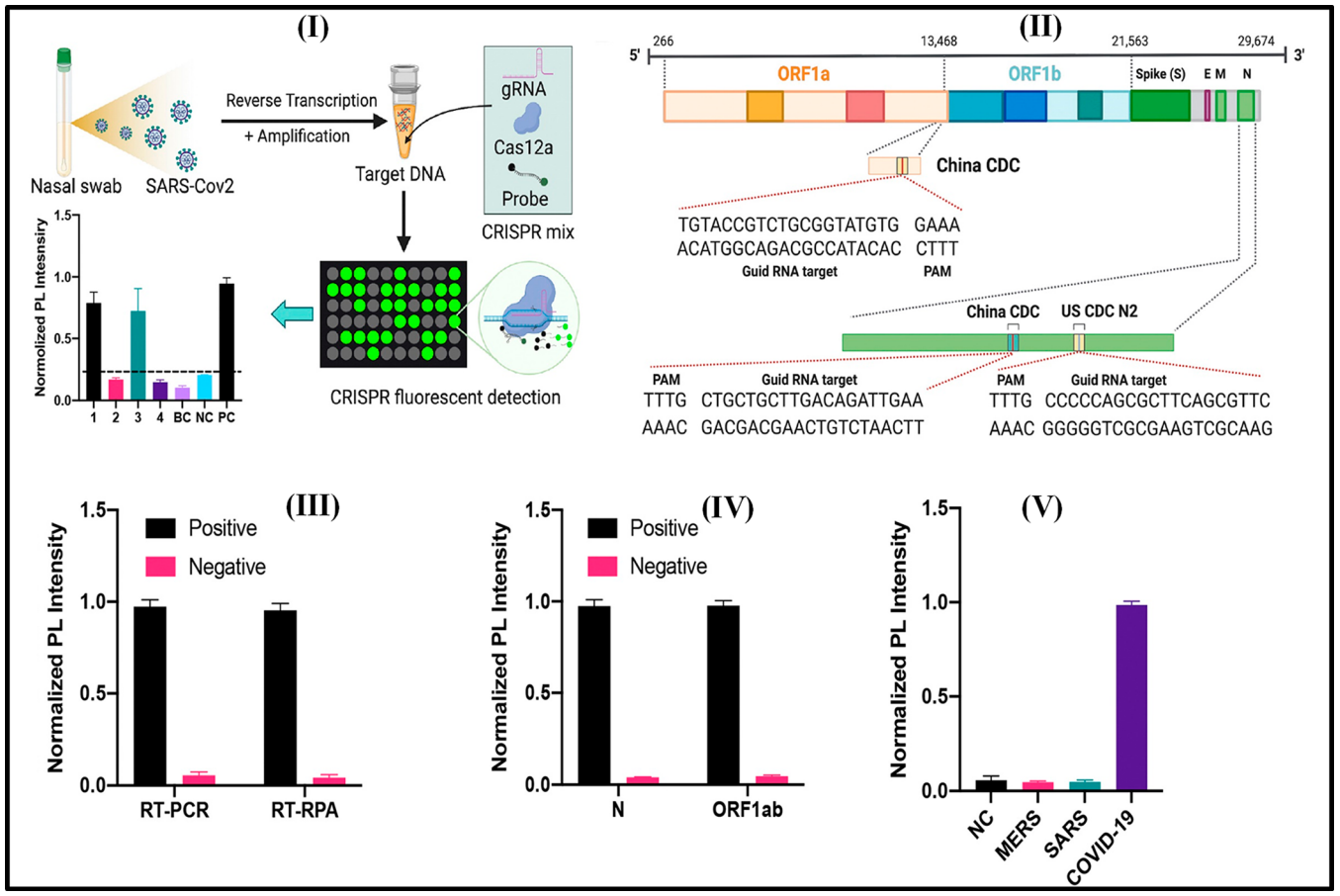

Figure 6. CRISPR-based fluorescent diagnosis system for COVID-19 (COVID-19 CRISPR-FDS): (I) CRISPR-FDS assay for detection of SARS-COV-2 RNA in clinical sample; (II) SARS-CoV-2 genomic map of COVID-19 CRISPR-FDS target sequence, and normalized CRISPR-FDS photoluminescent (PL) signal from SARS-CoV-2 RNA positive (10^9 copies/sample) and negative control (polyA carrier RNA) samples following (III) target amplification by RT-PCR or RPA, (IV) by RT-PCR for each assay target, and (V) by RT-PCR for related beta coronavirus species (10^9 copies/sample). Reproduced with permission from ref 89. Copyright 2020 Elsevier.

influenza, and dengue virus).[82] However, for detection of COVID-19 using microfluidic platform only a few attempts have been made so far. For instance, Lin et al. developed the quick multiplexed detection of IgG, IgM antibodies, and antigen of SARS-CoV-2 simultaneously in a single microfluidics chip. They fabricated a microfluidic channel and functionalized it via fluorescent microsphere-labeled capture antibodies. When a drop of sample was dropped on a microfluidic chamber, the fluid sample flowed through the microchannel due to capillary action, and IgG, IgM, and antigen present in the sample bind to the labeled antibodies. Due to this interaction, the florescence signal was generated and measured through the detector. The detail of this sensor has been shown in Figure 5II.[83] Recently, Broughton et al. suggested the potential integration of the CRISPR/CAS12-based diagnostic approach in the microfluidic platform.[84] To improve the nucleic acid amplification testing (NAAT) in order to improve its rapidity, sensitivity, and specificity, Tian et al. integrated RT-LAMP into reaction units of a microfluidic disc and constructed a fully automated centrifugal microfluidic system for sample-to-answer nucleic acid testing. This system provides results in ~70 min with limit of detection of 2 RNA copies per reaction, and throughput of this system is 21 reactions per disc. Moreover, this is an enclosed and automated microfluidic-based system which efficiently prevents contamination of samples and hence can be used for testing of infection in a less stringent environment.[85] Taken together, several advantages are offered by microfluidic biosensors such as multiplexing, miniaturization, and capability of integration with various techniques when compared to existing diagnostic approaches for COVID-19, indicating their suitability as a potential candidate for detection of viral infection.

CURRENTLY USED/UNDER TRIAL DIAGNOSTIC METHODS FOR SARS-COV-2 DETECTION (QRT-PCR, NGS, CRISPR, AND RT-LAMP)

One of the most widely accepted and followed diagnostic methods worldwide for detection of SARS-CoV-2 is reverse transcription−polymerase chain reaction (RT-PCR). In principle, this technique involves conversion of viral RNA into complementary DNA (cDNA) by the reverse transcriptase enzyme. This cDNA is further amplified to several copies until the detection limit is reached through repeated heating and cooling processes. The primer specifically designed for upstream oligonucleotide of the RdRp, envelope gene (E gene), nucleocapsid gene (N gene), and OrF gene has been used for designing RT-PCR based kits for detection of SARS-CoV-2 infection.[86] Commercially, the RT-PCR method, due to its high accuracy and sensitivity, has been exploited and developed worldwide for detection of COVID-19. We have summarized the information on some commercial RT-PCR-kits in Table 2. However, despite the promising results, the

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prolonged time and the need of costly instrument and reagents along with skilled personnel are a few limitations of this method, which encourages researchers to search for better options. Another technique based on genome sequencing is the next-generation genome sequencing (NGS) method, which is a high-throughput technology, has been successfully used recently for detection of the SARS-CoV-2 genome. In this context, Salis et al. used NGS for analyzing 20,000 biological samples in one run and found highly accurate results comparable to RT-PCR.[87] The advantageous feature of NGS is that we can simultaneously analyze thousands of samples. However, prolonged duration for the result output (~2 days) and need of an expensive centralized setup along with trained personnel limits its wide applicability. CRISPR is another genome-based detection method which has shown tremendous promise as a fast (~1 h) and accurate process for diagnosis of COVID-19. This technique utilizes the ability of CRISPR machinery to recognize the specific genetic sequence of the target organism, and then a guide RNA attaches onto a specific sequence of genome followed by recognition by CRISPR enzyme. The enzyme cuts the part and generates a signal either as fluorescence glow or as a dark band on strips; therefore, this method is sometimes referred to as "cut and detect". More recently, the US FDA has provided emergency authorization for the SHERLOCK coronavirus assay kit.[88] Broughton et al. exploited this technique and reported a lateral flow immunoassay of CRISPR-Cas12 based DETECTR technique which can detect up to 10 copies μL^{-1} of SARS-CoV-2 in a respiratory swab specimen.[84] Moreover, CRISPR-based fluorescent detection involves mainly three steps: RNA extraction, followed by target amplification and signal generation. Huang et al. reported CRISPR-Cas12a based fluorescence biosensor for the diagnosis of SARS-CoV-2 with low detection limit of 2 copies per sample in a nasal swab specimen (Figure 6).[89]

Another promising technique, Loop Mediated Isothermal Amplification (LAMP) based on molecular detection has the potential to replace the conventional PCR method. In principle, LAMP involves two enzymes, one to convert viral RNA to DNA and another to copy this DNA. Apart from enzymes, a set of six primers which are specific to a viral genome sequence are also required. Herein, DNA copying is similar to that in RT-PCR, but newly copied DNA strands form looped structures which are further amplified more efficiently and rapidly as compared to RT-PCR; hence, the technique is named LAMP. However, the accuracy of this technique is low, and one can perform only a few samples at a time. Further, RT-LAMP operates at 60−65 °C temperature; therefore, it should be maintained properly using a hot plate. Recently, Pelechano et al. evaluated LAMP testing in samples of 248 people with confirmed COVID-19 infection and found that this technique detects SARS-CoV-2 in 90% samples. They explained that this test is less accurate for some samples due to cross contamination.[90] In another study, a reverse transcription-loop mediated isothermal amplification (RT-LAMP) method was reported for detection of SARS-CoV-2. The advantages of this technique are that it is less time-consuming, works at constant temperature, and is less expensive. In this technique, results can be easily visible by the naked eye and observed through color change, which was dependent on pH change during the amplification process. Herein, during the process, protons are produced, which lower the pH of the reaction solution. A suitable pH indicator can show the change in color which indicates the positive or negative results observed through the naked eye. This method offers detection sensitivity of 80 copies mL^{-1} of viral RNA in the crude sample.[91] The similar strategy is employed by Baek et al., who reported the visual detection through RT-LAMP for detection of nucleocapsid gene of SARS-CoV-2 within 30 min. Herein, this report, the direct RNA amplification was done without RNA extraction. The pH indicator phenol red dye displayed a change in color from pink to yellow due to reaction, which indicates the presence of the SARS-CoV-2 virus. The experimental limit of detection of this technique was found to be 10^2 RNA copies. This test showed high specificity against SARS-CoV-2 viral RNA in a mixture of other viruses such as MERS-CoV, influenza virus, and HCoV.[92] Similarly, Yan et al. reported RT-LAMP for the diagnosis of SARS-CoV-2 in a swab sample. In this, a small amount of fluorescent calcein was added into the sample mixture, and the change of color from orange to green indicates the presence of target analyte. However, on the basis of colorimetric and turbidity examination of sample, they found that the sensitivity of this assay was 2×10^1 and 2×10^2 copies/reaction for orf1ab-4 and S-123 primer, respectively. One cannot deny the probability of varying results due to primer mutation, which limits the applicability of this assay.[93] In another study, Lalli et al. utilized RT-LAMP for early diagnosis of SARS-CoV-2 in a saliva sample. This assay displayed a detection limit ~10^2 copy of viral genome/reaction.[94]

COMMERCIALLY AVAILABLE BIOSENSORS FOR DETECTION OF SARS-COV-2

The higher mortality and morbidity associated with SARS-CoV-2 viral infection created a pressing need for the development of a rapid, cost-effective, noninvasive diagnostic approach. Researchers around the world are racing for the development of an appropriate diagnostic method. So far, 408 studies found on the Clinical Trials.gov website which is run by the US National Institutes of Health for recording all the diagnostic approaches either under clinical trial or recruiting for clinical trial for the detection of SARS-CoV-2.[95] Another Geneva, Switzerland-based platform named FIND is specifically gathering overall information on all commercially available or under development SARS-CoV-2 diagnostic tests. The FIND interface reported more than 429 diagnostic approaches to date. This provides a dual platform for researchers to directly submit their research or gather it from publicly available resources.[68] However, it is not verified independently. The US Food and Drug administration (FDA) has provided emergency use authorization to some COVID-19 diagnostic tests to minimize the shortages of testing. In addition, we have included information on some selected commercially available FDA-approved[96] and Indian Council of Medical Research (ICMR-India) approved[97] diagnostic kits which are available for emergency testing purposes in Table 3. Some of this information is in concordance with the selected commercial rapid test reported in recently published articles,[34,35] which have gathered excellent information about assay technique and developmental tests for SARS-CoV-2 diagnosis. However, by scrutinizing these Websites and reviews, we found that most of the commercially available diagnostic approaches for SARS-CoV-2 are RT-PCR, ELISA, and immunoassay-based, and only a few are biosensor-based approaches. Considering the advantages offered by biosensor-based diagnostic approaches over existing methods, we have

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Table 4. Currently Available/Ongoing Clinical Trials for SARS-CoV-2 Detection

S.No.	biosensor device for viral detection	ClinicalTrials.gov identifier/kit name	availability	company name	ref
1.	Rapid Detection of COVID-19 by Portable and connected Biosensor	NCT04367142	under clinical trial (Not yet recruiting)	University Hospital, Lille	95
2.	Sensor based vital signs monitoring of COVID 19 patients	NCT04335097	under clinical trial (recruiting)	Lars Wik, Ullevaal University Hospital	95
3.	Use of Remote Monitoring for COVID-19 Patient (RPM)	NCT04425720	under clinical trial (Not yet recruiting)	Montefiore Medical Center Bronx, New York, United States	[95]
4.	Remote Monitoring of Cancer Patients With Suspected Covid-19 (RECAP)	NCT04397705	under clinical trial (Not yet recruiting)	The Christie NHS Foundation Trust Manchester, Greater Manchester, United Kingdom	95
5.	En-Vision (enzyme-assisted nanocomplexes for visual identification of nucleic acids) Lab-on-Chip platform integrating PCR and microarray	-	March 2020	Veredus Laboratories, Singapore	98
6.	Lab-on-chip for the detection of 3 corona viruses: MERS-CoV, SARS-Covand 2019-ncov	VereCoV	March 2020	Veredus Laboratories, Singapore	99

tried our best to collect all currently available commercial or in-development biosensor-based approaches for SARS-CoV-2 and summarized the information in Table 3.

CURRENT CHALLENGES AND FUTURE PERSPECTIVE

The biosensor-based rapid diagnostic technologies have the potential to minimize the transmission of respiratory viral infection diseases. Since rapid and early detection of viral infection alerts the health care provider, the infected person may be quarantined for a limited period of time, during which the infection is cleared. This may prove efficacious to stop further disease spread by breaking the chain of community transfer, although some researchers learned serious lessons from past SARS-CoV and MERS outbreaks for the potential development of therapeutic intervention against these coronaviruses; therefore, better preparedness is needed for the current pandemic. However, the road of preparedness seems lagging so far in terms of diagnostic technology. In current scenario, Singapore, Taiwan, and Hongkong, despite their close proximity to China, have managed to limit the infection and mortality rate. They learned from earlier outbreaks and used mass testing along with digitalization and followed strict quarantine measures for individuals who tested positive, whereas several other countries faced community transmission due to the low amount of testing and lack of preparedness for SARS-CoV-2 infection. At present, a plethora of RT-PCR based diagnostic kits have been used clinically for the detection of SARS-CoV-2. The reason behind their mass use could be because they provide high specificity and sensitivity and already have clinical approval for viral testing. However, their limitations such as the need for expensive setup and reagents, trained personnel, and delay (4−5 h) in result output created a huge potential for other diagnostic approaches in the current review. The immunoassay-based diagnostic approach though offers several advantages such as providing rapid results (20−30 min), ease of handling, and no need for expensive setup or reagents, but they lack specificity and sensitivity, and therefore the chances of false positive and false negative reports increase, which can worsen the viral spread and related consequences.[34] Being high throughput, less expensive, and fast with capacity to be multiplexed, the biosensor-based diagnostic approaches have the potential to become a mainstream therapeutic modality. In addition, recent advances in microfluidic-based approaches led to a significant revolution in terms of integration of existing PCR-based methods into microfluidic platforms, which can be exploited to integrate low-cost devices for future diagnostic purposes. In this regard, Broughton et al. have shown potential for a CRISPR/CAS12-based diagnostic approach to be integrated in a microfluidic system.[84] Further, at the very beginning of the SARS-CoV-2 outbreak, Verdus laboratories in Singapore had announced the development of a lab-on-chip assay for detection of SARS-CoV, MERS, and 2019-nCoV; however, this approach is still under clinical trials.[98,99] The lab-on-chip platform used in the VereCoV detection kit was based on the integration of PCR and microarray techniques and capable of detecting MERS-CoV, SARS-CoV, and SARS-CoV-2 infection simultaneously with high specificity and sensitivity.[99] In Table 4, we enlisted biosensor-based approaches which are currently under clinical trial. Although the genetic makeup of SARS-CoV-2, SARS-CoV, and MERS-CoV is very similar, SARS-CoV-2 exhibits very high transmission rates in comparison to their counterparts which could be due to gene mutations in this virus. The mutations in the genetic makeup of viruses makes them more pathogenic and infectious; therefore, the role of viral diagnostic technology would be critical in this regard. Recent advances in POCT biosensing approaches such as capacity to multiplex and easy and high-throughput fabrication have the potential to surpass the need of time. However, high transmission rate, mutated pathogenic viral strain, lack of centralized lab facility, poverty, and ignorance in underdeveloped countries are current challenges faced around the globe. Therefore, there is a pressing need for diagnostic technology which can be easily deployed in decentralized laboratories; is cheaper, user-friendly, and rapid; and provides high sensitivity and specificity. The race for development of such diagnostic technologies has begun very early, and more than 300 diagnostic approaches are available; however, many new tests are still in developmental stages. Critically, funding sources, investment, appropriate collaborative work, mass device manufacturing, FDA approval, device disposability, and integration of diagnostic method with Internet Of Things (IOT)[100] to provide high-throughput information along with ease of utility in the community are a few major challenges that need to be addressed while designing an appropriate biosensor-based device. Further, recent advances in the field of technology development/transfer and its commercialization have the potential to surpass the need of time, but considering the current pandemic situation around the globe, especially in

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underdeveloped countries, requires new avenues in terms of resources and technology implementation.

CONCLUDING REMARKS

Taken together, in perspective of today's scenario of the SARS-CoV-2 pandemic, where millions of people worldwide are under constant threat of serious respiratory illness, rapid cost-effective and early diagnostic tools are a prime need. Biosensor-based point-of-care methods not only can be used as home diagnostic tools but can also be exploited for environmental monitoring in public places such as airports, railway stations, and hospitals. Further, sewage and wastewater analysis can also be done using sensors. Moreover, overall monitoring of the viral threat and implementation of preventive measures holds promise to curb the current pandemic situation. While optical sensors offer the advantage of rapid qualitative estimation of analyte, the electrochemical biosensors provide more accurate quantitative results. Besides this, microfluidic technology integrated with biosensor holds promise to provide a multiplexed (simultaneous detection of more than one biomarker) integrated platform for the assessment of disease condition. With technology advancement, the nanobiosensing field is continuously evolving, and currently developed advanced biosensors have the potential to combat present and future pandemics.

AUTHOR INFORMATION

Corresponding Authors

Avanish K. Srivastava − *CSIR - Advanced Materials and Processes Research Institute, CSIR-AMPRI, Bhopal, Madhya Pradesh 462026, India;* orcid.org/0000-0001-8330-981X; Email: director@ampri.res.in

Raju Khan − *CSIR - Advanced Materials and Processes Research Institute, CSIR-AMPRI, Bhopal, Madhya Pradesh 462026, India;* orcid.org/0000-0002-3007-0232; Email: khan.raju@gmail.com

Authors

Arpana Parihar − *Department of Genetics, Barkatullah University, Bhopal, Madhya Pradesh 462026, India;* orcid.org/0000-0003-3678-9405

Pushpesh Ranjan − *CSIR - Advanced Materials and Processes Research Institute and Academy of Scientific and Innovative Research (AcSIR), CSIR-AMPRI, Bhopal, Madhya Pradesh 462026, India;* orcid.org/0000-0002-2162-466X

Sunil K. Sanghi − *CSIR - Advanced Materials and Processes Research Institute, CSIR-AMPRI, Bhopal, Madhya Pradesh 462026, India;* orcid.org/0000-0001-9937-9564

Complete contact information is available at: https://pubs.acs.org/10.1021/acsabm.0c01083

Notes

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