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Advanced high-throughput biosensor-based diagnostic approaches for detection of severe acute respiratory syndrome-coronavirus-2

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8.1 Introduction

In recent times, three coronaviruses have been emerged and pose a grave danger to the healthcare sector and mankind worldwide. These coronaviruses implemented severe acute illnesses and were named severe acute respiratory syndrome-Coronavirus (SARS-CoV), the Middle East respiratory syndrome Coronavirus (MERS-CoV), and SARS-CoV-2 (Yadav et al., 2021). In 2003, SARS-CoV emerged in China have flu-like symptoms that result in respiratory failure, pneumonia and lead to death in several cases. An outbreak of SARS-CoV affected 26 nations badly and led to 8000 demises globally (Zhong et al., 2003). In 2012, another zoonotic virus of the coronavirus family has emerged in Saudi Arabia namely MERS-CoV. It has shown that MERS-CoV has also caused fatality among humans and transmitted from dromedary camels to humans and human to human transmission also reported. The common symptoms associated with MERS-CoV are shortness of breath, coughing, pneumonia, diarrhea, and fever. It affected over 27 countries and led to 838 deaths worldwide (Alyami et al., 2020). Furthermore, in 2019, when unknown cases of pneumonia-like symptoms have been reported in Wuhan, China, and later these cases were identified as infected by the SARS-CoV-2 virus. SARS-CoV-2 is the pathogen that causes Coronavirus disease-2019 (COVID-19). Since its emergence, COVID-19 has rapidly

spread globally, and hence on March 11, 2020, WHO declared COVID-19 as a pandemic (Ji et al., 2020). The common symptoms of SARS-CoV-2 viral infection include high fever, loss of taste and smell, coughing, runny nose and difficulty in breathing, fatigue, etc. Moreover, these symptoms are not bound to happen owing to their asymptomatic nature (Ranjan et al., 2021). This pandemic led to an unnecessary lockdown and put a heavy burden on the socioeconomic conditions of developing as well as developed nations and resulted in unemployment, recession, and other negative impacts on mankind. As of date millions of deaths and billions of cases are reported worldwide. Lack of effective vaccine supply and the nonavailability of potential drugs resulted in the growing threat of this pandemic day by day. In addition, new variants/mutants of the virus also emerged which put an extra load on research and development and paved the way to a high mortality rate (Nicola et al., 2020).

Coronaviruses belong to the large family of viruses including four subdivisions like α -coronavirus, β -coronavirus, γ -coronavirus, and δ -coronavirus. SARS-CoV-2 is a positive single-stranded RNA virus having approximately 80–120 nm diameter with approximately 30,000 base chain length comes under genus β -coronavirus (Taha et al., 2020). As of date, seven coronaviruses' species have appeared. Out of these four HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 have low pathogenicity and cause mild respiratory illnesses similar to influenza-type symptoms (Chen et al., 2020). Despite, the remaining three coronaviruses, SARS-CoV, MERS-CoV, and SARS-CoV-2 trigger grave threats with serious respiratory illnesses, and out of these three, SARS-CoV-2 poses serious concerns due to its high infectivity and leads to a high rate of demises. SARS-CoV-2 comprises four distinct structural proteins named spike (S), envelope (E), matrix (M), and nucleocapsid (N) (Su et al., 2016). According to the phylogenetic study of SARS-CoV-2, it is 50% similar to MERS-CoV and 80% similar to SARS-CoV. Notably, SARS-CoV-2 shows a 96% genomic similarity with the bat-associated coronavirus RaTG13, and therefore this is the subject of debate that SARS-CoV-2 may originate from bat source (Paraskevis et al., 2020). The virus begins its process of infection through the binding of functional receptors on the membrane of the host cell. Therefore the entry and transmission of viruses are generally decided by functional receptors. The entry of SARS-CoV-2 in host cells by binding of angiotensin-converting enzyme 2 (ACE2) receptor via its spike protein with high affinity (Bian & Li, 2020). The rate of virus transmission mainly depends upon the parameter name as a basic reproduction number, that is, R_0 . It is explained as if the R_0 value is more than 1, then the virus leads to an epidemic. According to data reported, the R_0 value for SARS-CoV-2 ranges between 3.3 and 5.5 which is much higher than the early reported SARS-CoV having a range of R_0 between 2 and 5. Hence, SARS-CoV-2 has higher viral transferability (Zhao et al., 2020). In addition, mutations lead to new variants which are resulted in the rapid transfer of viruses from human to human and animals to humans. There are many such variants of SARS-CoV-2 that are reported that cause exponentially hikes in symptomatic and asymptomatic cases and high mortalities all over the world (Kim et al., 2020). High asymptomatic cases and a lack of high throughput testing modalities pose serious concerns to the healthcare industry. Therefore it is highly recommended for rapid and accurate detection techniques for early diagnosis of infected patients and to suppress the viral infection effectively. In addition, the development of rapid and reliable screening techniques for SARS-CoV-2 will also aid in the identification of negative cases

and circumvent needless home quarantines which negatively affected the socioeconomic conditions of individuals (Ye et al., 2020).

Currently, two main conventional diagnostic approaches include nucleic acid testing such as real-time RT-PCR, LAMP, CRISPR, and immunoassay-based tests such as ELISA, LFIA, etc. are utilized as gold standard tests for detection of SARS-CoV-2 (Carter et al., 2020). Although currently SARS-CoV-2 testing facilities are well organized but public health care organizations do not have many capabilities to test all the citizens. Consequently, the identification of symptomatic as well as asymptomatic cases should at the priority list to avoid extensive spreading of the virus. However, the new mutations of SARS-CoV-2 have posed challenges for these methods as the mutated virus escapes the RT-PCR tests and false-negative results have caused the transmission on a larger scale with an increase in the number of cases and deaths. Hence, there is an urgent need for optimization and the development of new methods with more reliable results and proof of concept (POC) detection to overcome the disadvantages of conventional techniques (Sastry et al., 2020).

Furthermore, the development of biosensors has achieved many advancements to fulfill the requirements of high-throughput diagnostics, enhanced performance, better accuracy, and rapid point of care testing (POCT). The high-throughput characteristic of a biosensor is responsible to handle the exceeding testing needs. The parallel multiplexed methodologies along with rapid AI-integrated response enables the execution of the vast number of tests and its analysis for the efficient management of infectious diseases, especially when the outbreak needs to be contained in densely populated regions.

In this book chapter, we have discussed high throughput diagnostic approaches for the detection of SARS-CoV-2 due to their high demand in the testing of viral infection of the ongoing COVID-19 pandemic.

8.2 Conventional diagnostic approaches for severe acute respiratory syndrome-coronavirus-2

The present diagnostic approaches include molecular testing such as RT-PCR, LAMP, CRISPR, and radiology-based tests such as CT scan, X-Ray, immunoassay-based tests such as ELISA, and others. Such conventional methods are laboratory-based assays that are highly specific, sensitive, and accurate but lack the rapid, high-throughput detection of samples. These are commonly used techniques that give reliable results in the current COVID-19 pandemic. However, the new mutations of SARS-CoV-2 have posed challenges for these methods as the mutated virus escapes the RT-PCR tests and false-negative results have caused the transmission on a larger scale with an increase in the number of cases and deaths. There is a need to improve optimize and develop new methods with more reliable results and POC detection to overcome the high demands (Kilic et al., 2020; Yuan et al., 2020). For instance, Jalali et al. (2020) proposed high-sensitive capillary electrophoresis coupled PCR-based model for the diagnosis of SARS-CoV-2. In PCR detection, firstly they extracted the RNA from the swab sample followed by the polymerization to form cDNA. Further, the samples flow through the capillary which was detected at a specific fluorescent signal. The advantage of the proposed method is that it could analyze

approximately 1000 samples in a single run. An RT-PCR test was employed by [Byrnes et al. \(2021\)](#) for multiplex diagnosis of SARS-CoV-2. They employed the CDC single plex target to amplification from viral transport medium with RT-PCR. The sensitivity and specificity of the assay were found to be 86.0% and 100% respectively after the diagnosis of 246 samples. [Shental et al. \(2020\)](#) developed a P-BEST method for analysis of SARS-CoV-2 in clinical samples even in asymptomatic patients. They developed the single-step detection strategy through a group testing approach. Thus the 48 pool samples are made from 384 clinical samples which were tested through PCR assay. Such a test reduces the total time consumption by eightfold.

With the enhanced sensitivity of assays, [Aynaud et al. \(2021\)](#) demonstrated the parallel investigation of RNA coupled to sequencing for COVID-19 screening (C19-SPAR-Seq), a next-generation sequencing (NGS) platform for multiplexed detection of SARS-CoV-2. They targeted the S, E, N, and RdRP genes of SARS-CoV-2 and had a specificity of 100% and a sensitivity of 91.0% and >95.0% for low and high viral load, respectively of the sensor. [Fig. 8.1](#) represents the application of C19-SPAR-Seq to detect SARS-CoV-2, (A) the five regions targeted for multiplex C19-SPAR-Seq indicated as RdRP (purple), S receptor-binding domain (Srbd) (red), S polybasic cleavage site (Spbs) (light red), E (yellow), and N (orange). (B) Detecting strategy for SARS-CoV-2; cDNA is synthesized using reverse transcriptase (RT) from RNA extracted from clinical samples, subjected to multiplex PCR, then barcoded, pooled, and analyzed by NGS. (C) A POC cohort ($n = 19$) was analyzed by C19-SPAR-Seq and read numbers for each of the indicated amplicons are presented in a heat map. The left panel shows control samples (HEK293T, synthetic SARS-CoV-2 RNA), while the right panel depicts unsupervised two-dimensional hierarchical clustering of results from negative (blue) and positive (red) patients. Ten thousand clinical samples could be analyzed through this assay in a single run that very efficiently minimize the diagnostics cost and time consumption and boost the diagnosis at bulk capacity. Similarly, [Bhoyar et al. \(2021\)](#) tested a total of 752 (positive and negative) clinical specimens on the NGS platform through the COVIDSeq approach for the detection of genes of SARS-CoV-2. This approach is highly sensitive and specific and examined some samples that are already positive are tested positive even they are found negative through the RT-PCR test. [Carrell et al. \(2020\)](#) proposed the high-sensitive colorimetric ELISA kit for antibody diagnosis against SARS-CoV-2 from a blood sample with ultra-low LOD of 2.8 ng/mL. The enzyme-labeled capillary channels of the transparent film were constructed where the flow of clinical samples are done. These channels provide the steps of automated flow, washing of samples, and addition of a reagent. Labeling of enzymes in channels could replace the widely used macrostructures and hence improve the sensitivity. Moreover, washing steps improve reproducibility and reduce false-positive results.

8.3 Biosensors as proof of concepts for rapid detection of severe acute respiratory syndrome-Coronavirus-2

The POC-based biosensors have numerous advantages over the conventional techniques such as portability, POC detection, rapid diagnostics, ease of fabrication, simple processing, and high throughput. Several such biosensors include electrochemical biosensors,

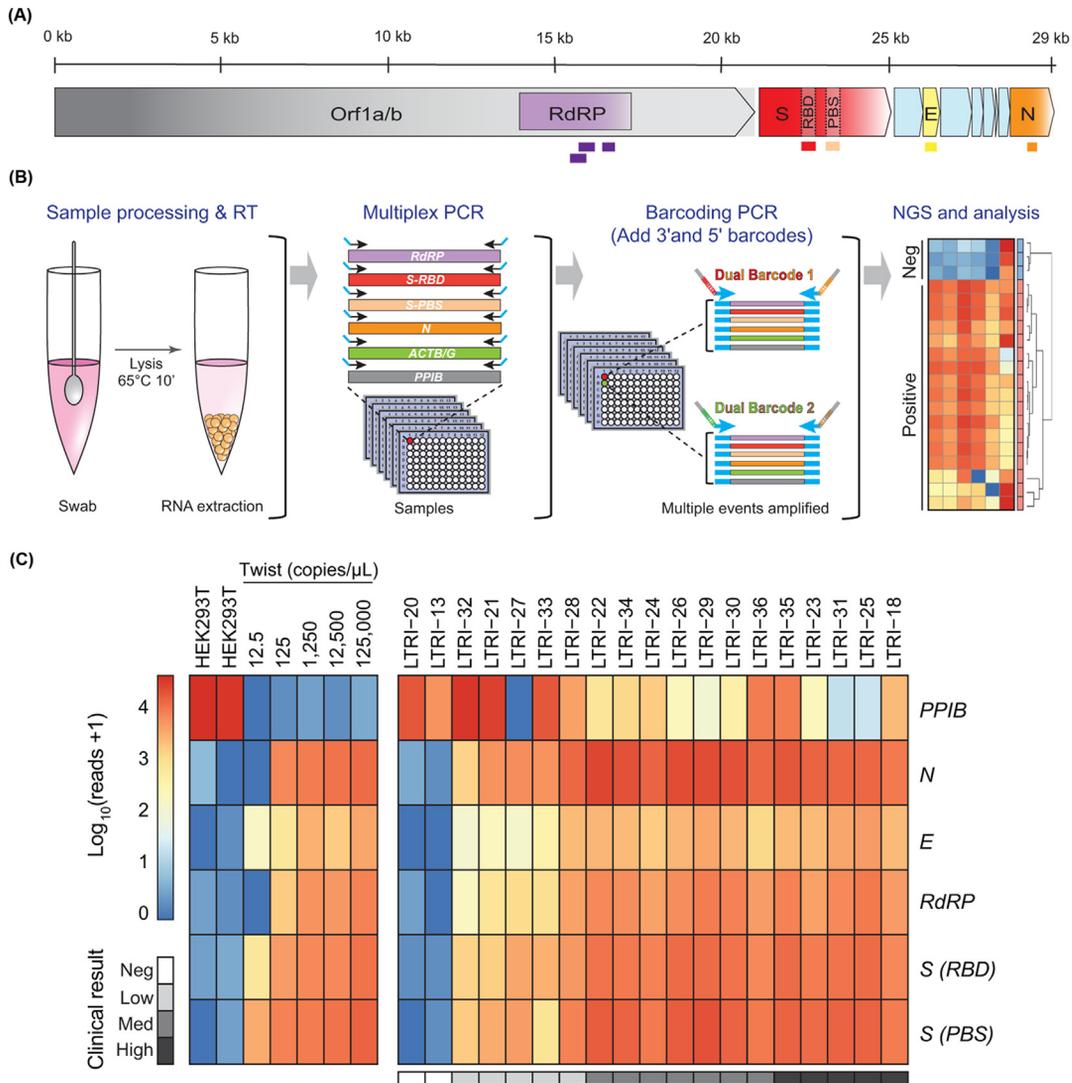


FIGURE 8.1 (A) Schematic representation of the severe acute respiratory syndrome-Coronavirus-2 with the five regions targeted for multiplex C19-SPAR-Seq. (B) Schematic diagram of the C19-SPAR-Seq strategy for detecting SARS-CoV-2. (C) Analysis of archival NASOP swab eluents by C19-SPAR-Seq. Source: Reprint with permission from Aynaud, M.M., Hernandez, J.J., Barutcu, S., Braunschweig, U., Chan, K., Pearson, J.D., Trcka, D., Prosser, S.L., Kim, J., Barrios-Rodiles, M., Jen, M., Song, S., Shen, J., Bruce, C., Hazlett, B., Poutanen, S., Attisano, L., Bremner, R., Blencowe, B.J., ... Wrana, J.L. (2021). A multiplexed, next generation sequencing platform for high-throughput detection of SARS-CoV-2. *Nature Communications*, 12(1). <https://doi.org/10.1038/s41467-021-21653-y>. Copyright 2021; Marie-Ming Aynaud et al.

optical biosensors, colorimetric biosensors, LFIA Strips, microfluidic biosensors, which are still in development for better performance (Parihar et al., 2020; Sharafeldin & Davis, 2021). For instance, Gorshkov et al. (2020) reported the homogeneous cell-based AlphaLISA sandwich assay for the diagnosis of N-protein of SARS-CoV-2. This assay should efficiently detect both recombinant and endogenous N-protein in viral lysate and tissue culture medium using two monoclonal antibodies. In this assay, the streptavidin-coated donor beads are excited at the light of a particular wavelength that generates reactive oxygen species (ROS) which activates the second antibody that binds to N-protein in samples and produces a luminescent signal. A homogeneous cell-based AlphaLISA sandwich assay is schematically represented in Fig. 8.2. AlphaLISA has low LOD up to pg/mL of antigen concentration and it can replace the conventional ELISA test which is tedious and have multiple steps washing process. Very recently, Du et al. (2021) reported the highly sensitive immunosensor for the detection of anti-SARS-CoV-2 IgG antibodies in serum and plasma samples. This high throughput immunosensor was made of the magnetic beads modified with recombinant spike protein 1 RBD which exhibits fluorescent signal and is measured through the Luminex 200 and MAGPIX devices. The clinical tests of 107 positive and 226 negative COVID-19 samples with the satisfactory result and even they examined that no cross-reactivity was found for IgG antibody detection for other viruses such as HIV, HAV, HBV, RSV, CMV, EBV, Rubella, Influenza A, and Influenza B.

Over these techniques, electrochemical-based biosensors gain much popularity in POC applications. An electrochemical electrode is fabricated by the nanostructured conducting materials which contain high ionic conductivity, large surface area as well as long-term stability. Afterward, the specific biomolecules (antibody or antigen) are immobilized on the nanostructured surface to target the selective analytes. When the clinical sample containing the target, analytes drop on the electrode surface, they display antibody-antigen chemistry, hence a small current is generated which is analyzed to confirm the presence of a disease-specific biomarker. Several advantages such as rapid diagnosis, high sensitivity, and specificity, portability, ultra-low detection limit, ease of fabrication, etc., of electrochemical biosensors make an excellent choice in biosensing application. Moreover, some biomarkers have an optically inactive property and are tough to detect though the optical biosensor could be easy to detect through an electrochemical biosensor. Moreover, it can be integrated or coupled with artificial intelligence (AI) that could offer the smart management of the COVID-19 pandemic (Kaushik et al., 2020; Ranjan et al., 2021). For instance, Vezza et al. (2021) developed the impedimetric biosensor of the ACE2 enzyme which selectively diagnosed the spike protein of SARS-CoV-2. Herein, electrode surface was prepared through the self-assembly monolayer (SAM) of 1H,1H,2H,2H-Perfluorodecanethiol on the gold surface, and then after an enzyme, ACE2 was deposited on the SAM. This sensor has an excellent sensitivity of 1.68 ng/mL with a LOD of 38.6 copies/mL. Similarly, a label-free biosensor was reported by Rashed et al. (2021) for the diagnosis of antibodies of SARS-CoV-2 within 5 minutes. They did the impedimetric detection through the commercially available ACEA Biosciences, 16-well plate xCELLigence system (RTCA S16).

In biosensor application, graphene and it is analogous such as graphene oxide (GO), reduced graphene oxide (RGO), and graphene quantum dots (QGDs)-based nanocomposites have great importance due to their advantageous properties. Since they have high conductivity, large surface to volume ratio, long-term stability, and possess oxygen

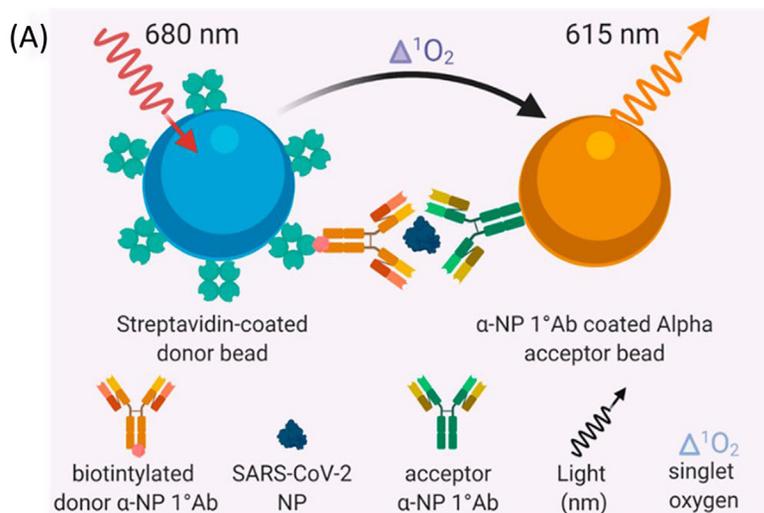
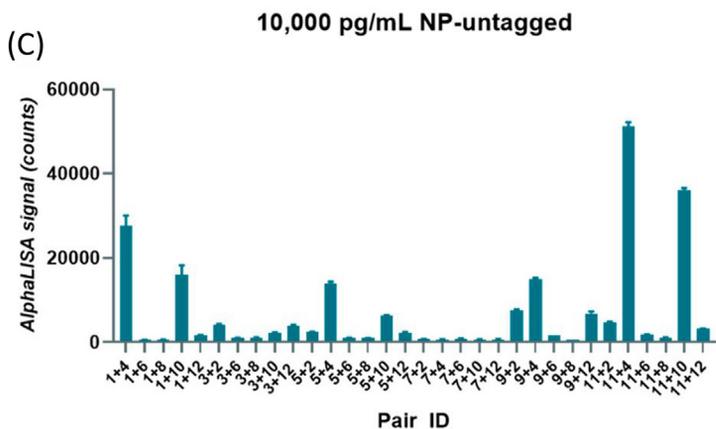
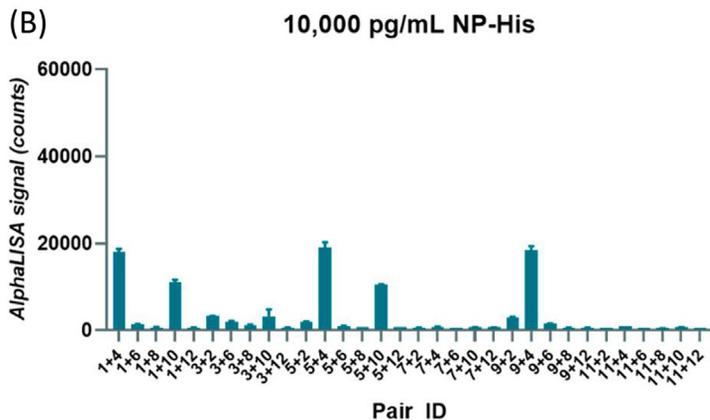


FIGURE 8.2 Schematic diagram of homogeneous cell-based AlphaLISA sandwich assay for severe acute respiratory syndrome-Coronavirus-2 nanoparticle detection. Source: Reprint with permission from Gorshkov, K., Chen, C.Z., Xu, M., Carlos De La Torre, J., Martinez-Sobrido, L., Moran, T., & Zheng, W. (2020). Development of a high-throughput homogeneous AlphaLISA Drug Screening Assay for the Detection of SARS-CoV-2 Nucleocapsid. *ACS Pharmacology and Translational Science*, 3(6), 1233–1241. <https://doi.org/10.1021/acspsci.0c00122>. Copyright 2020 @ American Chemical Society.



functionalities which are favorable for the immobilization of biomolecules on the surfaces of the nanocomposites. Such biomolecules target the disease-specific biomarkers in the clinical sample to indicate the disease stage in the body (Vermisoglou et al., 2020). For instance, at the very early of COVID-19 pandemic, Seo et al. (2020) developed the graphene-based field-effect transistor (FET) biosensor for detection of S-protein of SARS-CoV-2 in Nasopharyngeal Swab. The FET sensor was constructed by the coating of a thin layer of graphene and further modified by a spike antibody specific to S-protein. FET biosensors have an excellent limit of detection of up to femtogram per microliter concentration. Moreover, it could easily differentiate the SARS-CoV-2 from their similar virus MERS-CoV. Recently, Fabiani et al. (2021) reported the electrochemical sandwich immunoassay to target S and N-antigen of SARS-CoV-2 in saliva. The sandwich assay was constructed by functionalization of antibody specific to SARS-CoV-2 on magnetic beads and alkaline phosphate labeled secondary antibody on carbon black surface. Magnetic beads have high surface areas which favor the immobilization of numerous biomolecules on the surface along it reduces the washing steps in the measurement. The sandwich assay integrated with the portable PALM SENS potentiostat device makes them easier for the interpretation of data and on-site detection. Fig. 8.3 shows the working of the immunoassay in a real sample. LOD of detection of the assay in untreated saliva was calculated to be 19.0 and 8.0 ng/mL for S and N antigens respectively. However, it has high specificity for SARS-CoV-2 antigen even if the negligible concentration of H1N1 influenza virus is present in the sample. Very recently, Torrente-Rodríguez et al. (2020) proposed a multiplexed RapidPlex electrochemical sensor which is simultaneously detected the four biomarkers, N-protein, IgG Ab, IgM Ab, and C-reactive protein (CRP) in saliva and blood samples. Herein, they constructed the sensor by laser-engraved graphene electrode followed by the modification with the mixture of antigen and antibodies respective to target analytes through the common linkers, 1-pyrene butyric acid, and 1H-pyrrole-1-propionic acid. N-protein and CRP were detected through sandwich assay while the antibodies through the indirect immunosensor. The cross-reactivity was also tested for SARS-CoV and MERS-CoV and observed no such characteristics for this sensor. Thus it is highly specific for SARS-CoV-2 as well as the rapid diagnosis and miniaturized nature of the sensor could help to tackle the management of the COVID-19 pandemic. Electrochemical biosensors are highly sensitive and specific but sometimes the presence of a negligible number of biomarkers in the clinical sample could hamper the actual result.

A new class of biosensors, known as optical biosensors, are utilized for the diagnosis of biomarkers in an enormous area. Since, they are highly sensitive, specific, rapid, and easy to detect the optically active biomolecules. Moreover, in most cases, the result is easy to observe through the naked eye, and no need for the requirement of costly instruments. The high intensity and effortless interpretation of results make them suitable choices in the biosensor platform (Lukose et al., 2021; Pashchenko et al., 2018). For instance, Azad et al. (2021) reported a nano-BiT-based assay for the detection of antibodies against SARS-CoV-2 RBD in the serum sample. In this assay, they are utilizing the small portion and large portion segment that can be used to tag the specific protein which results in the formation of the bioluminescent molecule for selective detection of antibodies. The LOD of this assay was 5.0 ng in 50.0 μ L of serum sample and has long-term stability in a wide temperature and pH range. In addition, it could be efficiently done simultaneous detection

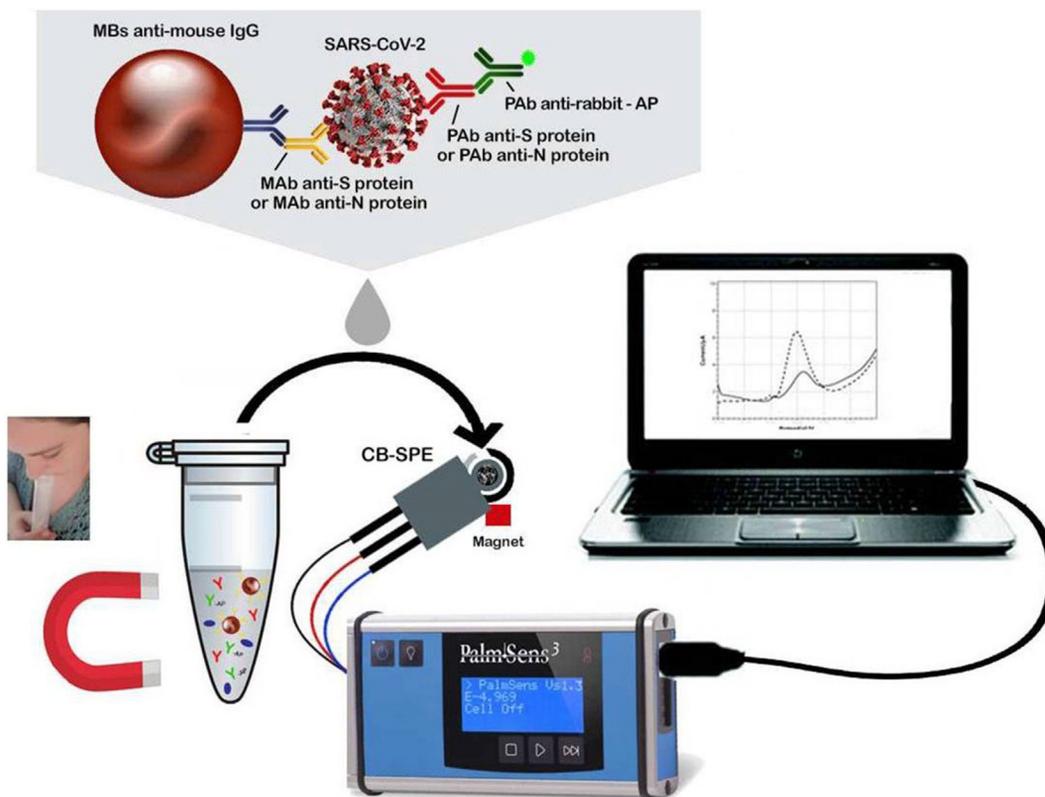


FIGURE 8.3 The Magnetic beads (MBs)-based assay for severe acute respiratory syndrome-Coronavirus-2 detection in untreated saliva. Source: Reprint with permission from Fabiani, L., Saroglia, M., Galatà, G., De Santis, R., Fillo, S., Luca, V., Faggioni, G., D'Amore, N., Regalbutto, E., Salvatori, P., Terova, G., Moscone, D., Lista, F., & Arduini, F. (2021). Magnetic beads combined with carbon black-based screen-printed electrodes for COVID-19: A reliable and miniaturized electrochemical immunosensor for SARS-CoV-2 detection in saliva. *Biosensors and Bioelectronics*, 171, 112686. <https://doi.org/10.1016/j.bios.2020.112686>. Copyright 2021 @ Elsevier.

in 384 samples in an hour. A lateral flow immunoassay was developed by Liu et al. (2020) for simultaneous diagnosis of IgM and IgG antibodies for SARS-CoV-2 within 15 minutes. The proposed assay is useful in symptomatic and asymptomatic patients as well as provides a rapid onsite detection platform. The assay was constructed by coating a layer of a mixture of gold nanoparticles (AuNPs) conjugated antigen and AuNPs conjugated rabbit IgG on a conjugate pad. While the two test lines, IgM and IgG, and control line by antihuman IgM, antihuman IgG, and antirabbit IgG respectively. The schematic view of (A) preparation, (B) assembled products, and (C) scheme of results is shown in Fig. 8.4

SERS-based POC biosensors are another choice in the sensing field, where the plasmonic nanoparticles are the good choice to make a sensor, where the scattering of light takes place after interaction with nanoparticles. Moreover, to further enhance the intensity of the scattered signal, nanoparticles are labeled by Raman dye, which dramatically enhances the

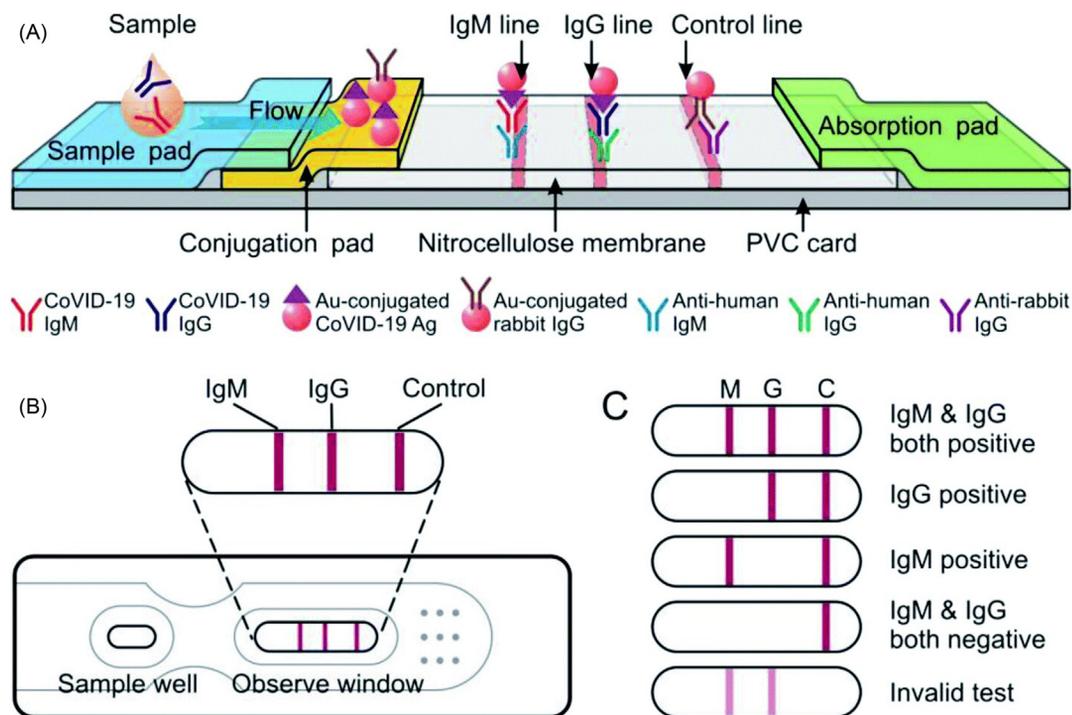


FIGURE 8.4 Schematic view of the Coronavirus disease-2019 IgM/IgG rapid test strip. (A) Preparation and principle of COVID-19 IgM/IgG rapid test strip. (B) Assembled products. (C) Scheme of test results. Source: Reprint with permission from Liu, C., Mao, B., Martinez, V., Chen, X., Li, Y., He, L., Chen, S., Guo, X., Shen, X., Bao, X., Shen, H., Lenna, S., Qian, P., Wu, L., & Li, C. (2020). A facile assay for rapid detection of COVID-19 antibodies. *RSC Advances*, 10(47), 28041–28048. <https://doi.org/10.1039/d0ra04107f>. Copyright 2020 @ Royal Society of Chemistry.

sensitivity of the sensor, and could achieve the ultralow LOD and be detected even up to single molecules. For instance, Liu et al. (2020) developed the high-sensitive LFIA integrated with the SERS technique for simultaneous diagnosis of IgG and IgM in serum. They prepared the S-protein modified core-shell nanostructured silicon dioxide and silver nanoparticles ($\text{SiO}_2\text{@Ag-S protein}$) and further modified by Raman tag, 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) which was cast on the conjugate pad. However, the goat-antihuman IgG and goat-antihuman IgM were coated on two test lines which selectively capture the IgG and IgM respectively. The target IgG/IgM antibodies were detected by quantifying high SERS signal intensity on the test line. The detection ability of the SERS biosensor has 800 times greater than the AuNPs-based LFIA. In another study, for the diagnosis of SARS-CoV-2 specific antibody within 15 minutes, Kim et al. (2020) developed the cellulose membrane-based flow assay. Herein, they constructed the sandwich assay by immobilization of N-protein of SARS-CoV-2 that act as both capture and reporter agent. The target antibody capture between both antigens results in the production of blue color on the surface which indicates the presence of target analyte in the sample. On the other hand, a miniaturized colorimetric test kit results in easy detection and result in

observation through the eye as well as it is easy to use layman even at medical and home diagnosis. For instance, Ventura et al. (2020) constructed a highly sensitive colorimetric biosensor for simultaneous detection of the S, E, and M-protein of SARS-CoV-2 in nasal and throat swab samples. Fig. 8.5 represents the schematic of the colorimetric test. The colorimetric kit was constructed by specific antibody functionalized AuNPs that target the S, E, and M-protein. In a mechanistic study, when the functionalized AuNPs interact with the appropriate concentration of antigens that results in red shifting of excitation spectrum, and it was observed due to aggregation of nanostructures.

The microfluidic platform provides deals with the quantification of biomolecules at an ultra-low level at micro to picoliter of sample volume. They are utilized for high-throughput analysis of drug, environmental, different kinds of pathogen even at single-cell detection. Fabrication of most microfluidic devices is environment-friendly and single-use. Moreover, it has high sensitivity, selectivity, miniaturized nature, and is easy to integrate with other techniques such as electrochemical, SERS, SPR, etc., which further enhance their performance and detection ability and allow the multiplex detection of various analytes (Berlanda et al., 2021). For instance, Funari et al. (2020) developed the localized surface plasmon resonance (LSPR) technique coupled microfluidic device for diagnosis of antibodies specific to S-protein of SARS-CoV-2 in a minimal clinical sample in 30 minutes. They prepared the microfluidic chip by SAM of alkyl thiols on AuNPs followed by the immobilization of specific antigen onto it. In LSPR, they measured the change in the refractive index of functionalized nanocomposites when it displays antigen–antibody chemistry. Therefore it results in shifting of LSPR peaks to redshift that is directly proportional to the target analyte concentration. The proposed device has high sensitivity and LOD of ~ 0.5 pM in the plasma sample. In another report, Lin et al. (2020) reported a highly sensitive, portable microfluidic platform for simultaneous detection of IgG/IgM in serum as well as antigen-specific to SARS-CoV-2 in pharyngeal swab samples within 15 minutes. The detection was based on the fluorescence detection where they utilized the fluorescent microsphere (FMS) labeled capture antibody which binds to their specific analytes that measure through the fluorescent reader device. The (A) portable homemade fluorescence detection equipment, (B) immunoassay microchip, and (C) schematic of detection are shown in Fig. 8.6. Total 54 samples (both positive and negative) were tested and observed that it has excellent sensitivity and specificity for SARS-CoV-2. Swank et al. (2021) reported the sandwich-type microfluidic

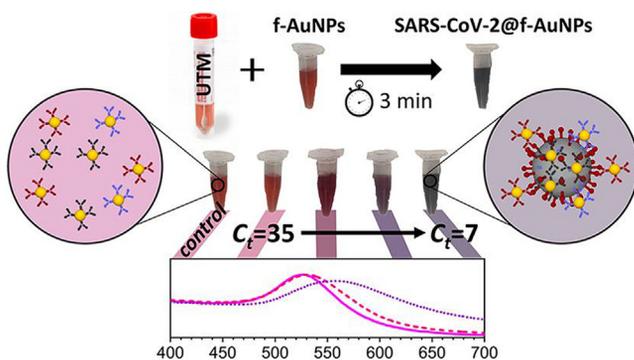


FIGURE 8.5 Schematic diagram of the colorimetric test for fast detection of severe acute respiratory syndrome-Coronavirus-2. Source: Reprint with permission from Ventura, B.D., Cennamo, M., Minopoli, A., Campanile, R., Censi, S.B., Terracciano, D., Portella, G., & Velotta, R. (2020). Colorimetric test for fast detection of SARS-COV-2 in nasal and throat swabs. *ACS Sensors*, 5(10), 3043–3048. <https://doi.org/10.1021/acssensors.0c01742>. Copyright 2020 @ American Chemical Society.

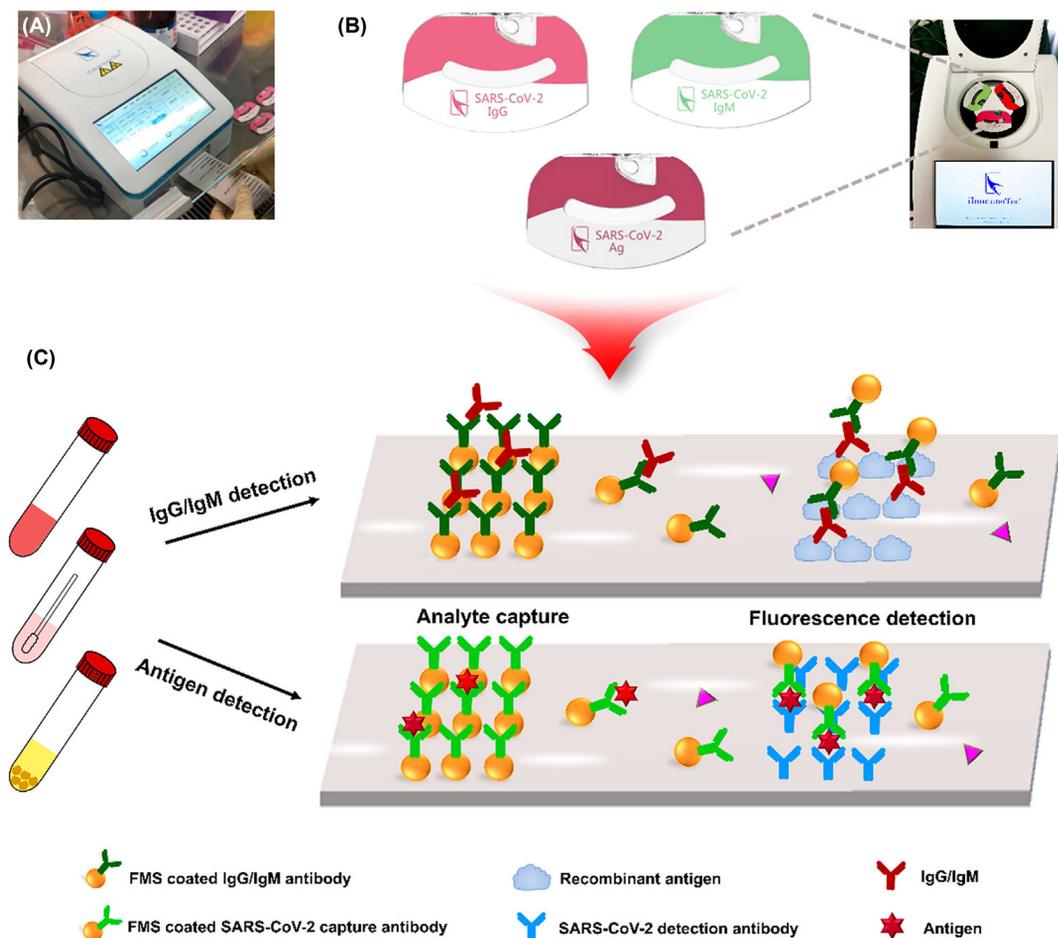


FIGURE 8.6 (A) Photograph of the portable homemade fluorescence detection equipment, (B) photograph of the immunoassay microchip ready to use, and (C) schematic illustration of the microfluidic fluorescence immunoassay for IgG/IgM/antigen detection of severe acute respiratory syndrome-Coronavirus-2. Source: Reprint with permission from Lin, Q., Wen, D., Wu, J., Liu, L., Wu, W., Fang, X., & Kong, J. (2020). Microfluidic immunoassays for sensitive and simultaneous detection of IgG/IgM/antigen of SARS-CoV-2 within 15 min. *Analytical Chemistry*, 92(14), 9454–9458. <https://doi.org/10.1021/acs.analchem.0c01635>. Copyright 2020 © American Chemical Society.

immunosensor to detect the IgG antibodies specific to SARS-CoV-2 in 1024 serum/blood samples on a single device with the LOD of 1.0 nM. The microfluidic device was made of the most commonly used PDMS where firstly channels are functionalized by SARS-CoV-2 antigen with his-tagged which capture the target IgG antibody from a clinical sample. Afterward, phycoerythrin labeled secondary antibodies flowed through the channels, and they are captured to IgG which makes it detectable in the microfluidic assay. They analyzed the 289 serum samples having high sensitivity and specificity of 98.0% and 100%, respectively.

8.4 Recent advances in high-throughput biosensor-based diagnostics

In the past few years, there has been tremendous R&D in the field of biosensors to improve their performance and make them commercially viable for clinical samples. The research for high-throughput of such biosensors has been carried out significantly since the COVID-19 pandemic has affected the global population. The high testing demand due to numerous cases and transmission of the infection has attracted the research community to develop high-throughput detection devices to compete with the diagnostics such that the early treatment and management of the pandemic could be carried out effectively. The recent advancements in the field of biosensors have made use of analytical as well as digital techniques to improve the performance of the biosensors. The early and accurate detection of the infection is of utmost priority along with rapid testing at the point of care settings (Cui & Zhou, 2020). Kohmer et al. (2020) evaluated the total six commercial biosensors, where four automated immunoassays [Abbott Architect i2000 (N protein-based)], Roche Cobas e 411 analyzer (N protein-based), LIAISON XL platform (S1 and S2 protein-based), VIRCLIA automation system (S1 and N protein-based), and two ELISA immunoassays [Euroimmun SARS-CoV-2 IgG (S1 protein-based) and Virotech SARS-CoV-2 IgG ELISA (N protein-based)] kits for rapid detection of IgG antibody against SARS-CoV-2 in serum or plasma samples. The overall sensitivity and specificity were examined after founding the first PCR positive sample in 49 days' time frame on both S and N-protein but mainly on N-protein-based assay. Similarly, these authors also evaluated the performance of six immunoassays such as Xpert Xpress SARS-CoV-2 (Cepheid) and Vivalytic VRI Panel (Schnelltest COVID-19) (Bosch) POCT with four high-throughput immunoassays such as Cobas SARS-CoV 2 test (Roche), the Allplex 2019-nCoV Assay (Seegene), the SARS-CoV-2 AMP (Abbott) Kit, and RealStar SARS-CoV-2 RT-PCR Kit 1.0 (Altona) and one laboratory-developed a test using a SARS-CoV-2 RdRP gene-specific primer and probe set for the detection of SARS-CoV-2 RNA. They observed that the N-protein-based immunosensor has high sensitivity and reliable accuracy (Kohmer et al., 2020). Similarly, Prince et al. (2020) tested the four automated SARS-CoV-2 IgG immunosensors such as Architect SARS-CoV-2 IgG (Abbott Laboratories Inc.), Liaison SARS-CoV-2 S1/S2 IgG (DiaSorin Inc.), Vitros anti-SARS-CoV-2 IgG (Ortho Clinical Diagnostics), and Anti-SARS-CoV-2 ELISA (IgG) (Euroimmun Inc.) for detection of IgG antibody against N or S-protein of SARS-CoV-2 on approximately 1200 serum samples. Authors found excellent performance about their sensitivity, specificity, and stability in various groups of samples. In another study, Theel et al. (2020) evaluated the performance of four high-throughput serologic tests, Abbott Laboratories (Abbott Park, IL), Epitope Diagnostics, Inc. (San Diego, CA), Euroimmun (Lübeck, Germany), and Ortho-Clinical Diagnostics (Rochester, NY) for diagnosis of anti-SARS-CoV-2 IgG antibody using 56 patient's serum samples. These assays have excellent accuracy, sensitivity, and sensitivity as the sensitivities of the Abbott, Epitope, Euroimmun, and Ortho-Clinical anti-SARS-CoV-2 IgG assays were 97.3%, 73%, 94.6%, and 97.3%, and specificity 99.6, 99.6%, 98.0%, and 99.6%, respectively.

8.4.1 Molecular-based high-throughput biosensor diagnostics

Brown et al. (2020) reported a biosensor comprising a FRET-capable pair of fluorescent proteins gripped by a protease cleavable linker for the 3-chymotrypsin-like cysteine

protease from SARS-CoV-2. In protein-based biosensors, there is no need for expensive chemical reagents to perform routine molecular detection. A protein-based FRET-biosensor along with a high-throughput screen and EC_{50} assay is utilized to identify inhibitors of 3CLpro from SARS-CoV-2. The assay achieved the standards for a reliable high-throughput, low levels of disparity between repeats, and reasonably limited compounds interfering with the FRET signal. They validated the eCFP-Venus biosensor as being robust cheap, accessible, amenable, robust screen, with a high level of correlation between repeats to high-throughput screening, and identified several new inhibitors of SARS-CoV-2 3CLpro. In another study, Pfefferle and group (Pfefferle et al., 2020) demonstrated good analytical performance of an adapted SARS-CoV-2 assay on swab samples. For a 25 μ L reaction volume of SARS-CoV-2 RNA, they reported LoD of 5.2 copies per reaction corresponding to 208 copies/mL resulting in a 95% detection probability. The used spiked-up control material (purified RNA) rather than using purified target RNA directly which caused a difference in nominal analytical performance. The assay relied on spiked-in material and not on clinical SARS-CoV-2 samples to validate the performance of the assay. However, the assay designed for high-throughput molecular testing could be beneficial in the management of the ongoing outbreak by allowing quick and reasonable screening of large numbers of patients. Huang and team (Huang et al., 2020) have developed a CRISPR-based assay that can meet all these criteria. Target amplicons produced by standard RT-PCR are detected by this assay which utilizes a custom CRISPR Cas12a/gRNA complex and a fluorescent probe. A limit of detection of 2 copies/sample and response time of approximately 50 minutes was achieved by the sensitive and robust assay for the detection of SARS-CoV-2 samples. A CRISPR-based Fluorescent Diagnosis System for COVID-19 (COVID-19 CRISPR-FDS) is represented in Fig. 8.7. These CRISPR assay diagnostic results revealed better analytical sensitivity and more robust performance than other reported CRISPR-based assays. CRISPR-FDS thus yields sensitive and accurate results and streamlines high-throughput workflow appropriate for usage in clinical laboratories, and possibly relevant to point of care settings with the apt equipment.

8.4.2 Optical-based high-throughput biosensor diagnostics

During this ongoing threat of SARS-CoV-2, medical diagnostics need alternative approaches to deal with the high demand for throughput devices. In this regard, fluorescence (FL) detection of biomarkers is a potential method in biosensing. Ultra-sensitive and high-throughput *meta*-surface fluorescence biosensors exhibit high applicability for the detection of nucleic acids in target analytes. These all-dielectric *meta*-surface biosensors consist of silicon-on-insulator nanorod arrangement and have an excellent fluorescence emission that is improved by potential electromagnetic resonances. Moreover, the *meta*-surface fluorescence biosensors show high performance due to the direct detection procedure. For the feasibility study, Iwanaga (2021) experimented on the *meta*-surface biosensors and exhibit the fluorescence detection of single-stranded oligo DNAs which are partly alike to SAR-CoV-2 RNA designated by national infection institutes. Furthermore, without any amplification techniques they are succeeded in high-throughput detection of a nucleic acid target at a low concentration level, that is, 100 amol/mL. Due to the high demand of the

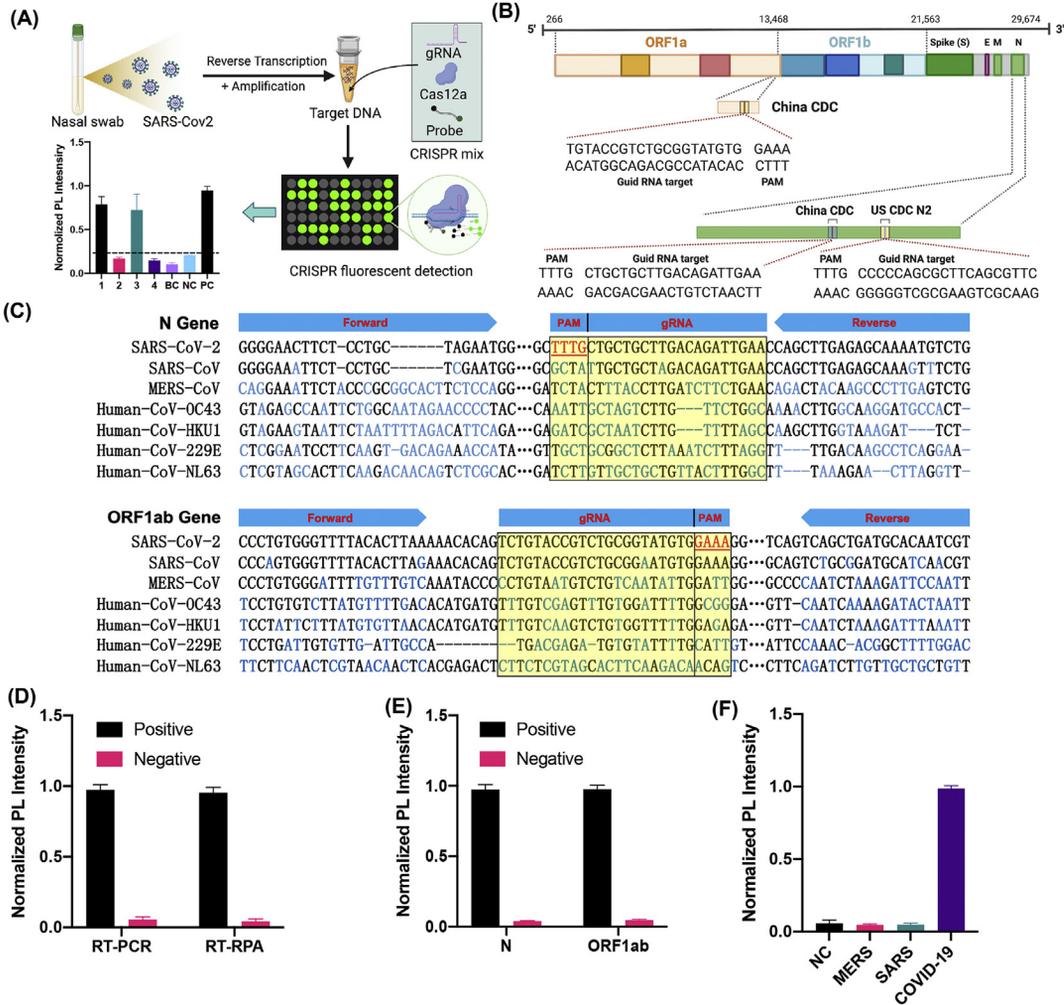


FIGURE 8.7 (A) Schematic illustration of a CRISPR-based Fluorescent Diagnosis System (CRISPR-FDS) assay for detection of severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) RNA in clinical samples. (B) SARS-CoV-2 genome map of Coronavirus disease-2019 (COVID-19) CRISPR-FDS target sequences, and (C) sites in ORF1ab gene and the N protein gene that are detected COVID-19 CRISPR-FDS. Normalized CRISPR-FDS photoluminescent (PL) signal from SARS-CoV-2 RNA positive (109 copies/sample) and negative control (polyA carrier RNA) samples following (D) target amplification by reverse transcription-polymerase chain reaction (RT-PCR) or RPA, (E) by RT-PCR for each assay target, and (F) by RT-PCR for related beta coronavirus species (109 copies/sample). Bar graph data represents the mean \pm SD, of three experimental replicates. Source: *Reprint with permission from Huang, Z., Tian, D., Liu, Y., Lin, Z., Lyon, C.J., Lai, W., Fusco, D., Drouin, A., Yin, X., Hu, T., & Ning, B. (2020). Ultra-sensitive and high-throughput CRISPR-powered COVID-19 diagnosis. Biosensors and Bioelectronics, 164 Copyright 2020 @ Elsevier.*

large number of tests to occur, *meta*-surfaces are pretreated with biotin trappings similar to the microplates are precoated with anti-biotin Abs in commercial immunoassay kits. In addition, the processing time and the signal-to-noise ratio will be enhanced by the utilization of optimizations concerning reagents in the MF protocol. Hence, with the modification of FL probes into RNA sequence, the viable alternative to the antigen kits of SARS-CoV-2 for fast screening is the *meta*-surface nucleic-acid sensors to match the RNA targets. Additionally, these sensors will also be predicted to detect any other unknown viral infection rapidly without the production of respected antibodies. In another study, researchers Wang et al. (2021) reveals a spectrum-based SPR imaging sensing system capable of high scanning of wavelength with the help of an acousto-optic tunable filter (AOTF) and implemented the cost-effective, freckle-free halogen lamp for the source of SPR excitation. Herein for data processing, they developed a novel four-parameter-based spectral curve readjusting (4-PSCR) method that is more accurate and rapid than the other conventional data curve fitting method like the polynomial fitting method. For PoC they also performed an SPR high-throughput detection of SARS-CoV-2 spike protein with high accuracy and demonstrated the LOD of spike protein to be 0.2 $\mu\text{g}/\text{mL}$. These results reveal the high possible applicability of this configuration in the screening of COVID-19. Although the LOD of this configuration is still a little higher than other traditional methods like ELISA, rapid test strips, FET-based biosensors but this SPR-based biosensor has its kind of distinctive advantages of real-time screening and high-throughput detection abilities. As an alternative to conventional PCR-based tests for screening of SARS-CoV-2, another research group Qiu et al. (2020) developed a dual-functional plasmonic biosensor by a combination of plasmonic photothermal (PPT) effect and LSPR sensing transduction on a single cost-effective two-dimensional gold nanoisland (AuNI) chip for promising applications. In this method, the sensitive detection of selective sequences of SARS-CoV-2 with the help of the AuNIs functionalized with complementary DNA receptors via nucleic acid hybridization. The plasmonic resonances of PPT and LSPR can be excited at two distinct wavelengths by utilizing two different incident angles which remarkably improved the stability, sensitivity, and reliability of the sensing platform. A real-time and label-free detection of viral sequences including RdRp-COVID, ORF1ab-COVID, and E genes from SARS-CoV-2 are achieved through this LSPR sensing technique. Furthermore, the thermo-plasmonic heat is produced on the same AuNIs chip when illuminated at their plasmonic resonance frequency for better biosensing. The capability of localized PPT heat to increase the in-situ hybridization temperature and provide an easy route to differentiate the two similar gene sequences. This developed dual-functional LSPR biosensor is highly sensitive toward the selected SARS-CoV-2 sequences with LOD of 0.22 pM and also provides precise detection of a target in a multigene mixture with high specificity. This proposed dual-functional LSPR biosensor can come up with an authentic and easy-to-implement high-throughput diagnostic technique to enhance the accuracy of diagnostics in clinical tests.

8.4.3 Microfluidic-based high-throughput biosensor diagnostics

The “ASSURED” (affordable, selective, sensitive, user-friendly, rapid and robust, equipment-free, and deliverable) criteria for biosensing and POCT is fulfilled by

microfluidic devices which have the benefits of high accuracy, portability, rapid reaction time, high reproducibility, low sample volume, simple function, and high throughput processing. Multiplexing, miniaturization, and the capability of integration with various techniques are several advantages of these biosensors (Aziz et al., 2021). A high-throughput microfluidic device was reported by Rodriguez-Moncayo et al. (2021) that can evaluate antibody reaction against four SARS-CoV-2 antigens with high throughput. This platform processes IgG and IgM levels against four SARS-CoV-2 proteins in semiautomatic mode. Multilayer soft lithography was employed to make the control layer and flow layer microfluidic devices that facilitated automation and fluid control. Mechanically induced trapping of molecular interactions (MITOMI) technique is used to power the device, here MITOMI functions as a fluorescence biosensor where indirect immunoassays are performed in parallel. Affordable large-scale diagnostic testing is achievable due to the high throughput abilities along with the low sample requirement of the device makes it an attractive platform to perform and diagnose the antibody response of a mass population to SARS-CoV-2. Without considerably modifying the device in its existing design 50 serum samples in parallel can be processed and can be easily scaled up to process a larger number of samples. Fig. 8.8 shows the microfluidic device: (A) The tip of a pencil is shown for reference. Closeup shows an array of microchambers (blue) surrounded by valves (red) and a MITOMI button valve (red circle) in the center. The bottom inset shows top-view photographs and a cross-sectional view of the actuation of the MITOMI button. An indirect immunoassay is performed on the surface of a glass substrate; d_{Ab} = detection antibody, Ig = serum antibodies, and Ag = antigen. (B) Schematic flow showing antigen immobilization of the four antigens, followed by (C) injection of the 50 samples and (D) immunoassay quantitation by introducing next-generation Optosience fluorescently labeled secondary antibodies. Solid or transparent red rectangles denote the closing or opening of the microvalves, respectively. Bottom schematics show how MITOMI is used to perform an indirect immunoassay. S = spike; S1 = subunit S1; RBD = region binding domain; N = nucleocapsid. (E) fluorescence micrographs of an array of biosensors for samples collected prepandemic and a COVID-19 positive case. The high-throughput, multiplexing capabilities, higher sensitivity, and specificity of the microfluidic device compared to other serological assays make it advantageous in diagnostics.

Further, Xing et al. (2020) exhibit a high-throughput, multiindex nucleic acid isothermal amplification analyzer (RTIsochip-W) that is capable of detecting 19 common respiratory viruses, including SARS-CoV-2, from 16 samples in a single run with the help of a centrifugal microfluidic chip and response time of 90 minutes. The RTIsochip-W system has the merits of good repeatability, durable robustness, and high specificity. Based on extensive trials, the RTIsochip-W system delivers a potent platform for the COVID-19 pandemic management. The high sensitivity, excellent repeatability demonstrated in the widespread clinical tests verified that the RTIsochip-W system can be utilized as a powerful nucleic acid testing tool in the diagnosis and screening of respiratory viruses, including SARS-CoV-2, ICUs, medical quarantine zones, and many other healthcare settings.

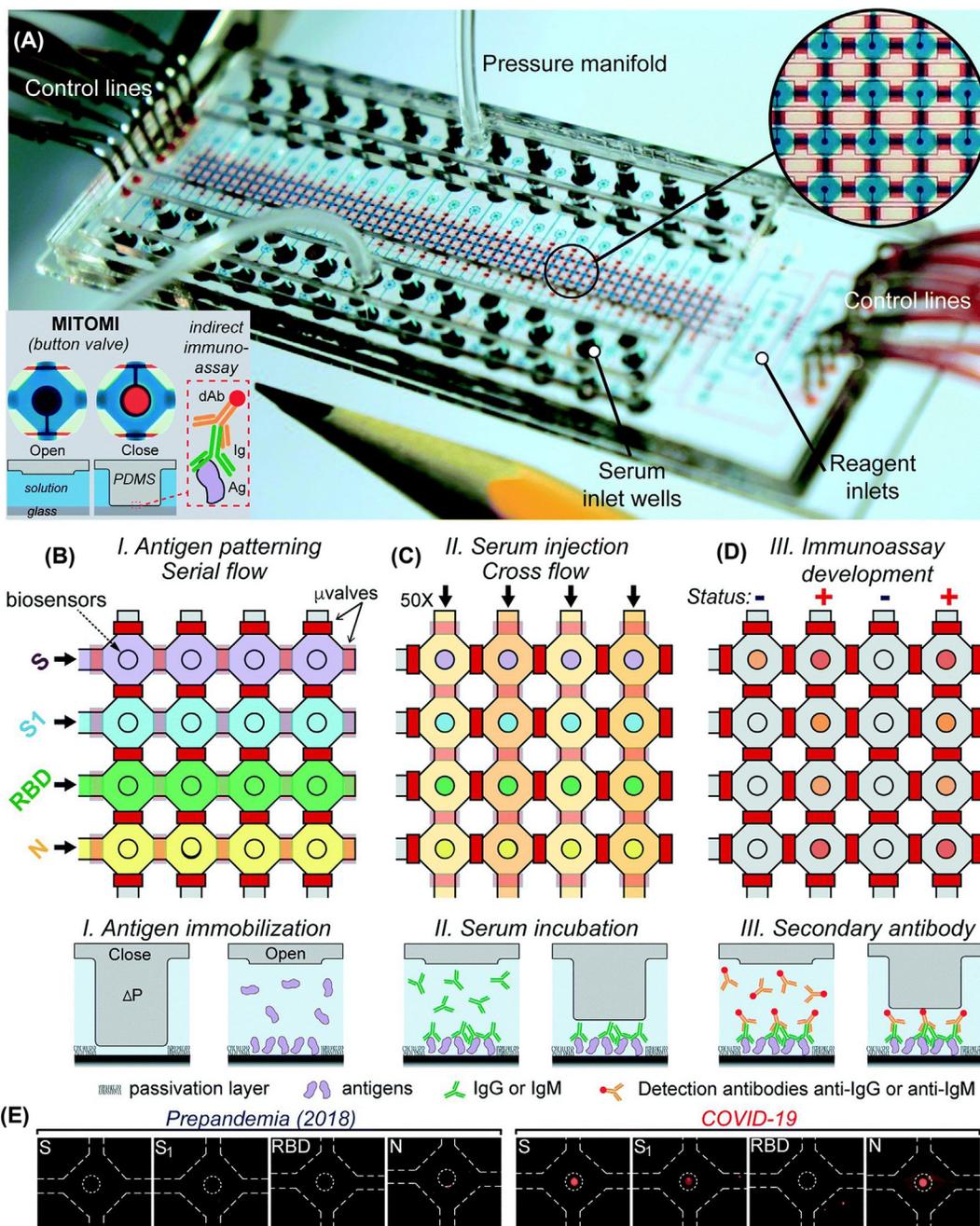


FIGURE 8.8 Microfluidic device for Coronavirus disease-2019 (COVID-19) antibody detection. (A) Photograph of the device indicating its different components. (B) Gross microfluidic assay schematic flow (C) injection of the 50 samples and (D) immunoassay quantitation by the introduction of fluorescently labeled secondary antibodies. (E) Typical fluorescence micrographs of an array of biosensors for samples collected before the pandemic (left) and a COVID-19 confirmed case (right). Source: Reprint with permission from Rodriguez-Moncalvo, R., Cedillo-Alcantar, D.F., Guevara-Pantoja, P.E., Chavez-Pineda, O.G., Hernandez-Ortiz, J.A., Amador-Hernandez, J.U., Rojas-Velasco, G., Sanchez-Muñoz, F., Manzur-Sandoval, D., Patino-Lopez, L.D., May-Arrijoja, D.A., Posadas-Sanchez, R., Vargas-Alarcon, G., & Garcia-Cordero, J.L. (2021). A high-throughput multiplexed microfluidic device for COVID-19 serology assays. *Lab on a Chip*, 21 (1), 93–104. <https://doi.org/10.1039/d0lc01068e>. Copyright 2020 @ Royal Society of Chemistry.

8.5 Conclusion and future perspectives

The ever-growing field of biosensors has witnessed tremendous change since the invention of the first biosensor and it is still becoming better and better with new developments in terms of its working principle, material requirement, and even integration with digital technologies. The fabrication process optimization, detection methodology improvements, data accuracy, and signal processing enhancements have efficiently assisted in the broader domain of diagnostics. With advancements in the healthcare industry and recent pandemics, the need for quick management of the outbreak has been supported with the help of high-throughput diagnostic devices. Various conventional and POC-based approaches have come forward to enhance the performance of the biosensors as well as diagnostic tools to make them more efficient and with high throughput. With an emphasis on viral infections, SARS-CoV-2 has been challenging for the diagnostics field with issues of inaccuracy, delayed, low-throughput, false results that lead to the spread and increased mortality rate of the virus. Ongoing research and developments in device fabrication techniques have helped in handling the outbreak and detecting the viral infection with better accuracy, faster response time, and high throughput. The available research on such high-throughput devices has been described in detail in the above report to contribute to the management of the pandemic. There are many more improvements and research needed in the diagnostic domain for the efficient control of SARS-CoV-2, that would involve the use of AI, IoT-based tools, integration of multiple detection technologies, combining diverse fields of medicine, biology, material science, engineering for the development of more advanced high-throughput biosensor-based diagnostic approaches. The use of high-throughput equipment would enable quicker and more accurate screening of COVID-19 patients that will help the medical experts to investigate and provide early treatment with precision. The potential of researchers and the manufacturing industry needs to be exploited for the growth and economic production of commercial devices that can be used by the common man and reach the global population to control the global pandemic. Innovative approaches to device fabrication and integration hold the key to tackling the spread and control transmission of viral infections in the future.

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References

- Alyami, M. H., Alyami, H. S., & Warraich, A. (2020). Middle East respiratory syndrome (MERS) and novel coronavirus disease-2019 (COVID-19): From causes to preventions in Saudi Arabia. *Saudi Pharmaceutical Journal*, 28 (11), 1481–1491. Available from <https://doi.org/10.1016/j.jsps.2020.09.014>.
- Aynaud, M. M., Hernandez, J. J., Barutcu, S., Braunschweig, U., Chan, K., Pearson, J. D., Trcka, D., Prosser, S. L., Kim, J., Barrios-Rodiles, M., Jen, M., Song, S., Shen, J., Bruce, C., Hazlett, B., Poutanen, S., Attisano, L., Bremner, R., Blencowe, B. J., & Wrana, J. L. (2021). A multiplexed, next generation sequencing platform for

- high-throughput detection of SARS-CoV-2. *Nature Communications*, 12(1). Available from <https://doi.org/10.1038/s41467-021-21653-y>.
- Azad, T., Rezaei, R., Singaravelu, R., Jamieson, T. R., Crupi, M. J., Surendran, A., Poutou, J., Taklifi., Cowan, J., Cameron, D. W., & Ilkow, C. S. (2021). A high-throughput nanoBiT-based serological assay detects SARS-CoV-2 seroconversion. *Nanomaterials*, 11(3).
- Aziz, A., Asif, M., Ashraf, G., Farooq, U., Yang, Q., & Wang, S. (2021). Trends in biosensing platforms for SARS-CoV-2 detection: A critical appraisal against standard detection tools. *Current Opinion in Colloid & Interface Science*, 52, 101418. Available from <https://doi.org/10.1016/j.cocis.2021.101418>.
- Berlanda, S. F., Breitfeld, M., Dietsche, C. L., & Dittrich, P. S. (2021). Recent advances in microfluidic technology for bioanalysis and diagnostics. *Analytical Chemistry*, 93(1), 311–331. Available from <https://doi.org/10.1021/acs.analchem.0c04366>.
- Bhoyar, R. C., Jain, A., Sehgal, P., Divakar, M. K., Sharma, D., Imran, M., Jolly, B., Ranjan, G., Rophina, M., Sharma, S., Siwach, S., Pandhare, K., Sahoo, S., Sahoo, M., Nayak, A., Mohanty, J. N., Das, J., Bhandari, S., Mathur, S. K., & Sivasubbu, S. (2021). High throughput detection and genetic epidemiology of SARS-CoV-2 using COVIDSeq next-generation sequencing. *PLoS One*, 16(2). Available from <https://doi.org/10.1371/journal.pone.0247115>.
- Bian, J., & Li, Z. (2020). Angiotensin-converting enzyme 2 (ACE2): SARS-CoV-2 receptor and RAS modulator. *Acta Pharmaceutica Sinica B*, 11(1), 1–12. Available from <https://doi.org/10.1016/j.apsb.2020.10.006>.
- Brown, A. S., Ackerley, D. F., & Calcott, M. J. (2020). High-throughput screening for inhibitors of the SARS-CoV-2 protease using a FRET-biosensor. *Molecules (Basel, Switzerland)*, 25(20). Available from <https://doi.org/10.3390/molecules25204666>.
- Byrnes, S. A., Gallagher, R., Steadman, A., Bennett, C., Rivera, R., Ortega, C., Motley, S. T., Jain, P., Weigl, B. H., & Connelly, J. T. (2021). Multiplexed and extraction-free amplification for simplified SARS-CoV-2 RT-PCR tests. *Analytical Chemistry*, 93(9), 4160–4165. Available from <https://doi.org/10.1021/acs.analchem.0c03918>.
- Carrell, C., Link, J., Jang, I., Terry, J., Scherman, M., Call, Z., Panraksa, Y., Dandy, D.S., Geiss, B.J. and Henry, C., 2020. Point-of-Need Disposable ELISA System for COVID-19 Serology Testing.
- Carter, L.J., Garner, L.V., Smoot, J.W., Li, Y., Zhou, Q., Saveson, C.J., Sasso, J.M., Gregg, A.C., Soares, D.J., Beskid, T.R., & Jervey, S.R. (2020).
- Chen, Y., Liu, Q., & Guo, D. (2020). Emerging coronaviruses: Genome structure, replication, and pathogenesis. *Journal of Medical Virology*, 92(4), 418–423. Available from <https://doi.org/10.1002/jmv.25681>.
- Cui, F., & Zhou, H. S. (2020). Diagnostic methods and potential portable biosensors for coronavirus disease 2019. *Biosensors and Bioelectronics*, 165, 112349. Available from <https://doi.org/10.1016/j.bios.2020.112349>.
- Du, J., Chu, E., Zhang, D., Lu, C. M., Zhang, A., & Sha, M. Y. (2021). A high-throughput Anti-SARS-CoV-2 IgG testing platform for COVID-19. *Journal of Virological Methods*, 287. Available from <https://doi.org/10.1016/j.jviromet.2020.114009>.
- Fabiani, L., Saroglia, M., Galatà, G., De Santis, R., Fillo, S., Luca, V., Faggioni, G., D'Amore, N., Regalbuto, E., Salvatori, P., Terova, G., Moscone, D., Lista, F., & Arduini, F. (2021). Magnetic beads combined with carbon black-based screen-printed electrodes for COVID-19: A reliable and miniaturized electrochemical immunosensor for SARS-CoV-2 detection in saliva. *Biosensors and Bioelectronics*, 171, 112686. Available from <https://doi.org/10.1016/j.bios.2020.112686>.
- Funari, R., Chu, K. Y., & Shen, A. Q. (2020). Detection of antibodies against SARS-CoV-2 spike protein by gold nanospikes in an opto-microfluidic chip. *Biosensors and Bioelectronics*, 169. Available from <https://doi.org/10.1016/j.bios.2020.112578>.
- Gorshkov, K., Chen, C. Z., Xu, M., Carlos De La Torre, J., Martinez-Sobrido, L., Moran, T., & Zheng, W. (2020). Development of a high-throughput homogeneous AlphaLISA Drug Screening Assay for the Detection of SARS-CoV-2 Nucleocapsid. *ACS Pharmacology and Translational Science*, 3(6), 1233–1241. Available from <https://doi.org/10.1021/acscptsci.0c00122>.
- Huang, Z., Tian, D., Liu, Y., Lin, Z., Lyon, C. J., Lai, W., Fusco, D., Drouin, A., Yin, X., Hu, T., & Ning, B. (2020). Ultra-sensitive and high-throughput CRISPR-powered COVID-19 diagnosis. *Biosensors and Bioelectronics*, 164.
- Iwanaga, M. (2021). High-sensitivity high-throughput detection of nucleic acid targets on metasurface fluorescence biosensors. *Biosensors*, 11(2), 33. Available from <https://doi.org/10.3390/bios11020033>.

- Jalali, S., Patel, V. K., Prasad, V., Sapre, A., Dasgupta, S., & Bhadra, B. (2020). Detection of SARS-CoV-2 using high-throughput PCR. *Journal of Applied Biology and Biotechnology*, 8(3), 1–5. Available from <https://doi.org/10.7324/JABB.2020.80301>.
- Ji, T., Liu, Z., Wang, G., Guo, X., Akbar Khan, S., Lai, C., Chen, H., Huang, S., Xia, S., Chen, B., Jia, H., Chen, Y., & Zhou, Q. (2020). Detection of COVID-19: A review of the current literature and future perspectives. *Biosensors and Bioelectronics*, 166, 112455. Available from <https://doi.org/10.1016/j.bios.2020.112455>.
- Kaushik, A. K., Dhau, J. S., Gohel, H., Mishra, Y. K., Kateb, B., Kim, N. Y., & Goswami, D. Y. (2020). Electrochemical SARS-CoV-2 sensing at point-of-care and artificial intelligence for intelligent COVID-19 management. *ACS Applied Bio Materials*. Available from <https://doi.org/10.1021/acsabm.0c01004>.
- Kilic, T., Weissleder, R., & Lee, H. (2020). Molecular and immunological diagnostic tests of COVID-19: Current status and challenges. *iScience*, 23(8), 101406. Available from <https://doi.org/10.1016/j.isci.2020.101406>.
- Kim, S. J., Nguyen, V. G., Park, Y. H., Park, B. K., & Chung, H. C. (2020). A novel synonymous mutation of SARS-CoV-2: Is this possible to affect their antigenicity and immunogenicity? *Vaccines*, 8(2). Available from <https://doi.org/10.3390/vaccines8020220>.
- Kohmer, N., Westhaus, S., Rühl, C., Ciesek, S., & Rabenau, H. F. (2020). Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays. *Journal of Clinical Virology*, 129. Available from <https://doi.org/10.1016/j.jcv.2020.104480>.
- Lin, Q., Wen, D., Wu, J., Liu, L., Wu, W., Fang, X., & Kong, J. (2020). Microfluidic immunoassays for sensitive and simultaneous detection of IgG/IgM/antigen of SARS-CoV-2 within 15 min. *Analytical Chemistry*, 92(14), 9454–9458. Available from <https://doi.org/10.1021/acs.analchem.0c01635>.
- Liu, C., Mao, B., Martinez, V., Chen, X., Li, Y., He, L., Chen, S., Guo, X., Shen, X., Bao, X., Shen, H., Lenna, S., Qian, P., Wu, L., & Li, C. (2020). A facile assay for rapid detection of COVID-19 antibodies. *RSC Advances*, 10(47), 28041–28048. Available from <https://doi.org/10.1039/d0ra04107f>.
- Lukose, J., Chidangil, S., & George, S. D. (2021). Optical technologies for the detection of viruses like COVID-19: Progress and prospects. *Biosensors and Bioelectronics*, 178, 113004. Available from <https://doi.org/10.1016/j.bios.2021.113004>.
- Nicola, M., Alsafi, Z., Sohrabi, C., Kerwan, A., Al-Jabir, A., Iosifidis, C., Agha, M., & Agha, R. (2020). The socio-economic implications of the Coronavirus and COVID-19 pandemic: A review. *International Journal of Surgery*.
- Paraskevis, D., Kostaki, E. G., Magiorkinis, G., Panayiotakopoulos, G., Sourvinos, G., & Tsiodras, S. (2020). Full-genome evolutionary analysis of the novel Coronavirus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *Infection, Genetics and Evolution*, 79, 104212. Available from <https://doi.org/10.1016/j.meegid.2020.104212>.
- Parihar, A., Ranjan, P., Sanghi, S. K., Srivastava, A. K., & Khan, R. (2020). Point-of-care biosensor-based diagnosis of COVID-19 holds promise to combat current and future pandemics. *ACS Applied Bio Materials*, 3(11), 7326–7343. Available from <https://doi.org/10.1021/acsabm.0c01083>.
- Pashchenko, O., Shelby, T., Banerjee, T., & Santra, S. (2018). A comparison of optical, electrochemical, magnetic, and colorimetric point-of-care biosensors for infectious disease diagnosis. *ACS Infectious Diseases*, 4(8), 1162–1178. Available from <https://doi.org/10.1021/acsinfecdis.8b00023>.
- Pfefferle, S., Reucher, S., Nörz, D., & Lütgehetmann, M. (2020). Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV-2 using a high throughput system. *Eurosurveillance*, 25(9). Available from <https://doi.org/10.2807/1560-7917.ES.2020.25.9.2000152>.
- Prince, H. E., Givens, T. S., Lapé-Nixon, M., Clarke, N. J., Schwab, D. A., Batterman, H. J., Jones, R. S., Meyer, W. A., Kapoor, H., Rowland, C. M., Haji-Sheikhi, F., & Marlowe, E. M. (2020). Detection of SARS-CoV-2 IgG targeting nucleocapsid or spike protein by four high-throughput immunoassays authorized for emergency use. *Journal of Clinical Microbiology*, 58(11). Available from <https://doi.org/10.1128/JCM.01742-20>.
- Qiu, G., Gai, Z., Tao, Y., Schmitt, J., Kullak-Ublick, G. A., & Wang, J. (2020). Dual-functional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome Coronavirus 2 detection. *ACS Nano*, 14(5), 5268–5277. Available from <https://doi.org/10.1021/acs.nano.0c02439>.
- Ranjan, P., Singhal, A., Yadav, S., Kumar, N., Murali, S., Sanghi, S. K., & Khan, R. (2021). Rapid diagnosis of SARS-CoV-2 using potential point-of-care electrochemical immunosensor: Toward the future prospects. *International Reviews of Immunology*, 40(1–2), 126–142. Available from <https://doi.org/10.1080/08830185.2021.1872566>.

- Rashed, M. Z., Kopeček, J. A., Priddy, M. C., Hamorsky, K. T., Palmer, K. E., Mittal, N., Valdez, J., Flynn, J., & Williams, S. J. (2021). Rapid detection of SARS-CoV-2 antibodies using electrochemical impedance-based detector. *Biosensors and Bioelectronics*, 171. Available from <https://doi.org/10.1016/j.bios.2020.112709>.
- Rodriguez-Moncayo, R., Cedillo-Alcantar, D. F., Guevara-Pantoja, P. E., Chavez-Pineda, O. G., Hernandez-Ortiz, J. A., Amador-Hernandez, J. U., Rojas-Velasco, G., Sanchez-Muñoz, F., Manzur-Sandoval, D., Patino-Lopez, L. D., May-Arriola, D. A., Posadas-Sanchez, R., Vargas-Alarcon, G., & Garcia-Cordero, J. L. (2021). A high-throughput multiplexed microfluidic device for COVID-19 serology assays. *Lab on a Chip*, 21(1), 93–104. Available from <https://doi.org/10.1039/d0lc01068e>.
- Sastry, S. R., Pryor, R., Raybould, J. E., Reznicek, J., Cooper, K., Patrick, A., Knowlson, S., Bailey, P., Godbout, E., Doll, M., Stevens, M. P., & Bearman, G. (2020). Universal screening for the SARS-CoV-2 virus on hospital admission in an area with low COVID-19 prevalence. *Infection Control and Hospital Epidemiology*, 41(10), 1231–1233. Available from <https://doi.org/10.1017/ice.2020.358>.
- Seo, G., Lee, G., Kim, M. J., Baek, S. H., Choi, M., Ku, K. B., Lee, C. S., Jun, S., Park, D., Kim, H. G., Kim, S. J., Lee, J. O., Kim, B. T., Park, E. C., & Kim, S. I. (2020). Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. *ACS Nano*, 14(4), 5135–5142. Available from <https://doi.org/10.1021/acsnano.0c02823>.
- Sharafeldin, M., & Davis, J. J. (2021). Point of care sensors for infectious pathogens. *Analytical Chemistry*, 93(1), 184–197. Available from <https://doi.org/10.1021/acs.analchem.0c04677>.
- Shental, N., Levy, S., Wuvshet, V., Skorniakov, S., Shalem, B., Ottolenghi, A., Greenshpan, Y., Steinberg, R., Edri, A., Gillis, R., Goldhirsh, M., Moscovici, K., Sachren, S., Friedman, L. M., Neshet, L., Shemer-Avni, Y., Porgador, A., & Hertz, T. (2020). Efficient high-throughput SARS-CoV-2 testing to detect asymptomatic carriers. *Science Advances*, 6(37). Available from <https://doi.org/10.1126/sciadv.abc5961>.
- Su, S., Wong, G., Shi, W., Liu, J., Lai, A. C. K., Zhou, J., Liu, W., Bi, Y., & Gao, G. F. (2016). Epidemiology, genetic recombination, and pathogenesis of Coronaviruses. *Trends in Microbiology*, 24(6), 490–502. Available from <https://doi.org/10.1016/j.tim.2016.03.003>.
- Swank, Z., Michielin, G., Yip, H. M., Cohen, P., Andrey, D. O., Vuilleumier, N., Kaiser, L., Eckerle, I., Meyer, B., & Maerkl, S. J. (2021). A high-throughput microfluidic nanoimmunoassay for detecting anti-SARS-CoV-2 antibodies in serum or ultralow-volume blood samples. *Proceedings of the National Academy of Sciences of the United States of America*, 118(18). Available from <https://doi.org/10.1073/PNAS.2025289118>.
- Taha, B. A., Al Mashhadany, Y., Hafiz Mokhtar, M. H., Dzulkefly Bin Zan, M. S., & Arsal, N. (2020). An analysis review of detection Coronavirus disease 2019 (COVID-19) based on biosensor application. *Sensors*, 20(23), 6764. Available from <https://doi.org/10.3390/s20236764>.
- Theel, E. S., Harring, J., Hilgart, H., & Granger, D. (2020). Performance characteristics of four high-throughput immunoassays for detection of igg antibodies against SARS-CoV-2. *Journal of Clinical Microbiology*, 58(8). Available from <https://doi.org/10.1128/JCM.01243-20>.
- Torrente-Rodríguez, R. M., Lukas, H., Tu, J., Min, J., Yang, Y., Xu, C., Rossiter, H. B., & Gao, W. (2020). SARS-CoV-2 RapidPlex: A graphene-based multiplexed telemedicine platform for rapid and low-cost COVID-19 diagnosis and monitoring. *Matter*, 3(6), 1981–1998. Available from <https://doi.org/10.1016/j.matt.2020.09.027>.
- Ventura, B. D., Cennamo, M., Minopoli, A., Campanile, R., Censi, S. B., Terracciano, D., Portella, G., & Velotta, R. (2020). Colorimetric test for fast detection of SARS-CoV-2 in nasal and throat swabs. *ACS Sensors*, 5(10), 3043–3048. Available from <https://doi.org/10.1021/acssensors.0c01742>.
- Vermisoglou, E., Panáček, D., Jayaramulu, K., Pykal, M., Frébort, I., Kolář, M., Hajdúch, M., Zbořil, R., & Otyepka, M. (2020). Human virus detection with graphene-based materials. *Biosensors and Bioelectronics*, 166, 112436. Available from <https://doi.org/10.1016/j.bios.2020.112436>.
- Veza, V. J., Butterworth, A., Lasserre, P., Blair, E. O., MacDonald, A., Hannah, S., Rinaldi, C., Hoskisson, P. A., Ward, A. C., Longmuir, A., Setford, S., Farmer, E. C. W., Murphy, M. E., & Corrigan, D. K. (2021). An electrochemical SARS-CoV-2 biosensor inspired by glucose test strip manufacturing processes. *Chemical Communications*, 57(30), 3704–3707. Available from <https://doi.org/10.1039/d1cc00936b>.
- Wang, X., Zeng, Y., Zhou, J., Chen, J., Miyam, R., Zhang, H., Qu, J., Ho, H. P., Gao, B. Z., & Shao, Y. (2021). Ultrafast surface plasmon resonance imaging sensor via the high-precision four-parameter-based spectral curve readjusting method. *Analytical Chemistry*, 93(2), 828–833. Available from <https://doi.org/10.1021/acs.analchem.0c03347>.

- Xing, W., Liu, Y., Wang, H., Li, S., Lin, Y., Chen, L., Zhao, Y., Chao, S., Huang, X., Ge, S., Deng, T., Zhao, T., Li, B., Wang, H., Wang, L., Song, Y., Jin, R., He, J., Zhao, X., & Cheng, J. (2020). A high-throughput, multi-index isothermal amplification platform for rapid detection of 19 types of common respiratory viruses including SARS-CoV-2. *Engineering*, 6(10), 1130–1140. Available from <https://doi.org/10.1016/j.eng.2020.07.015>.
- Yadav, S., Sadique, M. A., Ranjan, P., Kumar, N., Singhal, A., Srivastava, A. K., & Khan, R. (2021). Sens based lateral flow immunoassay for point-of-care detection of SARS-CoV-2 in clinical samples. *ACS Applied Bio Materials*, 4(4), 2974–2995. Available from <https://doi.org/10.1021/acsabm.1c00102>.
- Ye, F., Xu, S., Rong, Z., Xu, R., Liu, X., Deng, P., Liu, H., & Xu, X. (2020). Delivery of infection from asymptomatic carriers of COVID-19 in a familial cluster. *International Journal of Infectious Diseases*, 94, 133–138. Available from <https://doi.org/10.1016/j.ijid.2020.03.042>.
- Yuan, X., Yang, C., He, Q., Chen, J., Yu, D., Li, J., Zhai, S., Qin, Z., Du, K., Chu, Z., & Qin, P. (2020). Current and perspective diagnostic techniques for COVID-19. *ACS Infectious Diseases*, 6(8), 1998–2016. Available from <https://doi.org/10.1021/acsinfecdis.0c00365>.
- Zhao, S., Lin, Q., Ran, J., Musa, S. S., Yang, G., Wang, W., Lou, Y., Gao, D., Yang, L., He, D., & Wang, M. H. (2020). The basic reproduction number of novel Coronavirus (2019-nCoV) estimation based on exponential growth in the early outbreak in China from 2019 to 2020: A reply to Dhungana. *International Journal of Infectious Diseases*, 94, 148–150. Available from <https://doi.org/10.1016/j.ijid.2020.02.025>.
- Zhong, N. S., Zheng, B. J., Li, Y. M., Poon, L. L. M., Xie, Z. H., Chan, K. H., Li, P. H., Tan, S. Y., Chang, Q., Xie, J. P., Liu, X. Q., Xu, J., Li, D. X., Yuen, K. Y., Peiris, J. S. M., & Guan, Y. (2003). Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet*, 362(9393), 1353–1358. Available from [https://doi.org/10.1016/S0140-6736\(03\)14630-2](https://doi.org/10.1016/S0140-6736(03)14630-2).