ABSTRACT: Diagnosis of COVID-19 has been challenging owing to the need for mass testing and for combining distinct types of detection to cover the different stages of the infection. In this review, we have surveyed the most used methodologies for diagnosis of COVID-19, which can be basically categorized into genetic-material detection and immunoassays. Detection of genetic material with real-time polymerase chain reaction (RT-PCR) and similar techniques has been achieved with high accuracy, but these methods are expensive and require time-consuming protocols which are not widely available, especially in less developed countries. Immunoassays for detecting a few antibodies, on the other hand, have been used for rapid, less expensive tests, but their accuracy in diagnosing infected individuals has been limited. We have therefore discussed the strengths and limitations of all of these methodologies, particularly in light of the required combination of tests owing to the long incubation periods. We identified the bottlenecks that prevented mass testing in many countries, and proposed strategies for further action, which are mostly associated with materials science and chemistry. Of special relevance are the methodologies which can be integrated into point-of-care (POC) devices and the use of artificial intelligence that do not require products from a well-developed biotech industry.

KEYWORDS: SARS-CoV-2, point-of-care, COVID-19 diagnosis, biosensors, lateral flow devices, RT-PCR, surface plasmon resonance, nanoparticles

The COVID-19 pandemic has shown the relevance of developing new tools for diagnosis, especially with low-cost technologies that permit rapid assays within the so-called point-of-care (POC) diagnosis paradigm.1 The existence of well-established diagnostic methodologies for detecting viral genetic material2,3 and human antibodies using rapid tests4,5 has made it possible to achieve a relatively early detection of COVID-19 infection, in some cases with high accuracy. This has been instrumental for governments and societies to take proper actions to control spread and minimize the overall damage. Indeed, mass testing has been recommended from the early days of the pandemic by the World Health Organization (WHO)6 for the surveillance and control of the spread of the disease. There are, however, important challenges to be faced in terms of performance of the diagnostic tools for detecting both genetic material and antibodies, mostly due to the cost and testing speed. Even more importantly, only a few countries could fully benefit from the existing technology, either because
the methods are too expensive or not easily deployable in poorer settings. Overcoming these challenges by using efficient and ready-to-use biosensor workflow research products could rapidly address the outbreak, as we shall elaborate upon in this article.

The failure of many countries to implement mass testing during the COVID-19 pandemic has highlighted the need for extra efforts and investments in the research and technology development of disease diagnosis. This is crucial to guarantee the security of humanity in general and of nations in view of possible outbreak of other pandemics. Here, we propose three different strategies to be adopted by governments and the scientific community for future epidemics to ensure protection of the population: (i) dissemination of plants in the biotechnology industry or at least ensure means to adequately supply the diagnostic tools to all countries, (ii) development of low-cost alternatives for the detection of genetic materials and immunoassays, particularly within the paradigm of POC diagnosis, and (iii) development of diagnostic strategies based on pattern recognition methods, as this minimizes the limitations brought by the lack of biotechnology industry. To achieve these goals, we suggest readaptation of existing methodologies for the diagnosis of COVID-19. This will make it possible to monitor current and future infectious diseases and place humankind in better shape to combat upcoming outbreaks of pathogenic diseases.

In this review, we mainly focus on the genosensing and immuno sensing technologies which could be adapted to COVID-19 diagnosis, in addition to the molecular diagnosis and diagnosis exploiting pattern recognition. Attempts are made to provide a comprehensive review of the possibilities for diagnosis through illustration of the potential of such technologies. This review article is organized as follows. First, we comment on the structure, receptors, and possible targets of SARS-CoV-2 since an effective diagnosis requires understanding of the molecular machinery of the pathogens. The SARS-CoV-2 Diagnosis section introduces a brief discussion of the different detection techniques employed for COVID-19, with emphasis on the need to leverage distinct types of detection. An overview is presented of the challenges in detecting SARS-CoV-2 with nucleic acids-based and immuno-based techniques in the next two sections. Before concluding, we discuss emerging technologies that hold promise for the near future in the section Emerging Strategies for Diagnosis of COVID-19.

## STRUCTURE OF SARS-COV-2

Coronaviruses (CoVs) belong to the Coronaviridae family of enveloped positive-stranded RNA viruses that exhibit the largest RNA genome of all known viruses. This family is divided into four main genera: alpha, beta, gamma, and delta-CoVs.7,8 CoVs can be hosted by birds (gamma, delta-CoVs) or mammals (alpha, beta-CoVs), causing intestinal and respiratory illnesses.5,9 Before the emergence of the severe acute respiratory syndrome coronavirus (SARS-CoV-2) in the outbreak of Covid-19 in December, 201910 only six CoV
species were known to infect humans. Four of these, i.e., HCoV-NL63, HCoV-HKU1, HCoV-OC43, and HCoV-229E, are related to mild respiratory infections,\(^8,11\) while SARS-CoV and MERS-CoV cause severe respiratory illnesses. SARS-CoV caused an endemic in 2002–2003 in Guangang, China, and MERS-CoV emerged in Saudi Arabia in 2012.\(^8,11\) Both SARS-CoV and MERS-CoV spread to several countries, infecting thousands of individuals with respiratory and neurological diseases with a high mortality rate.\(^12\) The recent seventh member, SARS-CoV-2, is highly pathogenic. The disease it causes, referred to as COVID-19, is much more infectious and has spread to more than 200 countries in a time span of less than six months. Although the elderly are the most severely affected, with up to 50% of fatalities, a large number of hospitalizations have occurred for adult healthy patients with 2–11% fatality rate.\(^13\) As of 14th October, 2020, more than 38 million people were infected by SARS-CoV-2 with at least one million deaths confirmed.\(^14\)

The severity of the COVID-19 outbreak has led to a global mobilization by the pharmaceutical industry, governments, and academia to develop efficient diagnostics for mass testing, create a safe vaccine, and investigate treatments based on already approved medications.\(^15,16\) In all of these endeavors, structural studies of SARS-CoV-2 are proving essential.\(^17\)−\(^21\) All CoVs exhibit similar structures with their genomes arranged in a similar fashion, as illustrated in Figure 1A. The viral genome and the nucleocapsid protein (N) are complexed to form a helical case within the hemagglutinin–esterase viral membrane (this latter is only present in some beta-CoVs). The viral gene also encodes a spike protein (S), nucleocapsid (N), internal protein (I), small membrane envelope protein (E), and a membrane protein (M).\(^7\) In addition, a 5’-untranslated region (UTR), a 3’ UTR, nonstructural open reading frames (ORFs), and a conserved replicase domain (ORF1ab) (Figure 1B) exist in the viral genome.\(^22\) The spike protein of SARS-CoV-2 is divided into the subunits S1 and S2, with a functional polybasic furin cleavage at the S1–S2 boundary, which can improve infection in host cells.\(^11,23\) The spike protein receptor binding domain (RBD) localized in S\(^1\)\(^24\) has six amino acids (NS01, L455, Q493, F486, S494, and Y505)\(^11\) that are essential for binding on human ACE2 (Angiotensin-Convert ing Enzyme 2) receptors.\(^19\) Among these six amino acids, five differ from SARS-CoV to SARS-CoV-2.\(^11\) This interaction between the spike protein RBD and ACE2 receptors, which is crucial for the high contamination rate of SARS-CoV-2 in comparison to other human CoVs, has been studied in detail,\(^18,19,23−27\) and is a potential therapeutic target. The SARS-CoV-2 spike protein may be useful for neutralization immunoassays\(^28\) and a target for POC tests. The remaining structural proteins, N, E, and M, are mostly involved in the regulatory functions, RNA synthesis, protective function against the host immune system, and viral pathogenesis. These are more conserved proteins as compared to the S among the several human CoVs known so far. Moreover, structural similarities in SARS-CoV, MERS-CoV, and SARS-CoV-2 open the possibility for application or adaptation of existing diagnosis technologies and efficient treatment of COVID-19. On the other hand, SARS-CoV-2 exhibits a diverse gene position and has the chance of continued variation in the genome sequence due to the pandemic-scale spread of the disease.\(^29,30\) Previous studies on several genomic sequences of SARS-CoV-2 revealed approximately 4% genomic mutation of total 220 strains analyzed,\(^13\) suggesting the coexistence of different strains, which might be a new challenge for several diagnostic methods.

### SARS-COV-2 DIAGNOSIS

SARS-CoV-2 has a unique biological characteristic, which brings several challenges to the health systems globally, and resulted in a poor response to contain the pathogenic disease. After the genetic sequence of the virus was known, the disease could be diagnosed with molecular testing based on viral RNA, such as reverse transcriptase polymerase chain reaction (RT-PCR), which are laboratory based and required skilled persons for operating sophisticated equipment. Therefore, mass testing could not be performed in the beginning of the pandemic due

![Figure 2. SARS-CoV-2 most appropriate detection methods along the course of infection. This figure is an illustrative scheme and it should be mentioned that discrepancies exist in the literature, especially for the tails of the curves. We decided to keep the qualitative character until more data are collected and a consensus is established on the time dependences.](https://dx.doi.org/10.1021/acssensors.0c01382)
to the unavailability of testing facilities to the general public. Thanks to the research and development efforts of the clinical laboratory and academic researchers, several new and modified diagnostic tools were developed to make them available at varied locations. Indeed, today several types of diagnostic tools are available for detecting SARS-CoV-2.

Detection of SARS-CoV-2 has been made with various analytical techniques, either through quantification of nucleic acids or by measuring the immunoresponse of infected humans via antibody detection. The genetic-based techniques comprise RT-PCR, LAMP (Loop-Mediated Isothermal Amplification), CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), and genosensors, while immuno-based assays are mainly made with LFDs (Lateral Flow Devices), CLIA (Chemiluminescent Immunoassays), ELISA (Enzyme-Linked Immunosorbent Assays), and immunosensors. Detection in genosensors and immunosensors can be done with electrochemical and/or electrical measurements, as will be further discussed in the last two sections of this article. Even though the techniques mentioned are effective and sensitive, their specificity has been influenced by the choice of the principle of detection, viral load, and specific immuno-response of individuals. Indeed, one of the major challenges in the diagnosis of COVID-19 is the need to employ more than one detection strategy owing to the long incubation period of the virus. In addition to detecting genetic material of SARS-CoV-2, which can be performed at any stage, detection of antibodies through serological immunosensing needs to be carried out at different time points after infection. Figure 2 shows a schematic timeline with the most appropriate detection methods along the course of infection.

The profiles for IgG and IgM antibodies of SARS-CoV-2 have been discussed in a few works in the literature. They are similar to the ones obtained for SARS-CoV infection, although the time dependences are significantly different. For SARS-CoV infections, IgM peaks after 3 weeks from the onset of symptoms, while IgG peaks in the fifth week after onset of symptoms. In the case of SARS-CoV-2, the viral load peaks in approximately 5–7 days after the onset of symptoms. IgG and IgM antibodies have different profiles. The IgM level in the organism peaks within ~14 days after the onset of symptoms and rapidly decreases in the third week of infection. IgG, however, peaks between the second and third week of infection. Differently from IgM, the IgG level in the infected organism remains high until the fifth week of infection.

In the first days of infection, detection of COVID-19 is mainly performed by quantification of the SARS-CoV-2 viral load. Therefore, genetic-based techniques such as RT-PCR, LAMP, CRISPR, and genosensors are the most indicated (Figure 2, dark blue region, left). The peak of SARS-CoV-2 viral load coincides with the beginning of the immunoresponse to the disease through IgM and IgG production (middle region of Figure 2). In this case, in addition to detection of SARS-CoV-2 genetic material through LAMP, RT-PCR, CRISPR, and genosensors, immunological assays with high sensitivity can be applied for IgG and IgM, including CLIA, ELISA, LFDs, and immunosensors. After the third week of infection, IgG load reaches its maximum in the infected organism as the SARS-CoV-2 viral load remarkably decays. Hence, genetic-based techniques are no longer effective, and detection of SARS-CoV-2 is performed through IgG and IgM quantification.

Immunoassays are cost-effective, sensitive, rapid, and selective, but they involve rigorous washing steps which affect automatization. The genetic material-based techniques frequently exhibit improved sensitivity and selectivity in comparison to immunological assays. This is a key advantage for detection of SARS-CoV-2, a virus with high structural similarity to SARS-CoV. Nevertheless, detecting nucleic acids often requires time-consuming analyses and highly skilled operators, being thus disadvantageous for mass testing in a pandemic outbreak. Therefore, there are challenges to be addressed in both types of detection for reaching an effective diagnosis, as will be further discussed in this review paper. Most of the techniques mentioned in this review have already been employed for SARS-CoV and/or MERS-CoV, including RT-PCR, ELISA, LAMP, CLIA, LFDs, immunosensors, and genosensors. Some have been implemented in POC devices for several pathologies, including LFDs, CLIA, immunoassays, genosensors, electrochemical immunosensors, and field-effect transistor devices. Herein, we will discuss important perspectives for adapting these existing technologies for COVID-19 detection.

From the need of different types of diagnostics, one may list the types of materials for the sensing units or test kits, most of which are products of the biotech industry. For detection of genetic material, the kits must contain DNA sequences (primers) which will function as biorecognition elements for different target genes responsible for proteins of the virus, such as ORF1ab, N protein, and S protein, for example. These are the cases of RT-PCR and LAMP assays. The primers are normally immobilized onto different materials depending on the technique and type of assay. As usual in any development of a diagnostic tool, the sensing platform is first validated with standard spiked samples before experiments are done with real samples (i.e., swabs, blood, serum, and plasma samples). The primers for RT-PCR and LAMP assays are produced by molecular biology methods. In the immunoassays, the biorecognition elements are frequently proteins (biomarkers) which will bind specifically to antibodies immobilized onto the sensing platform. These devices can be applied to a large number of samples, as blood, serum, plasma, urine, and saliva. There is a large variety of materials onto which these biorecognition elements can be immobilized, including polystyrene in ELISA assays, magnetic beads in CLIA, and nanomaterials in electrochemical and electrical immunosensors. As in the case of genetic-based platforms, immunosensors are validated in spiked samples prior to real sample analyses. The target antibodies and the biorecognition elements are produced by the biotech industry.

### GENETIC MATERIAL-BASED DETECTION TECHNIQUES

In genetic material-based techniques, SARS-CoV-2 is detected through quantification of its viral RNA. For RT-PCR and LAMP assays, RNA is quantified after its transcription to DNA. CRISPR assays, on the other hand, are based on Case 13 targeting enzyme activity. In this section, we shall discuss the operation principles of these techniques and their application to SARS-CoV-2. Unfortunately, genosensors for SARS-CoV-2 detections have not been developed yet. Because we believe that integrating genosensors in POC devices is a viable route
for mass testing of COVID-19, we shall discuss their earlier use for pathogens such as SARS-CoV.

**RT-PCR for SARS-CoV-2 Testing.** RT-PCR has been the most used technique for early diagnosis of SARS-CoV-2. RT-PCR, whose scheme to detect mRNA is shown in Figure 3, is already applied to detect several pathogens, including virus and bacteria.\(^{57,58}\) For COVID-19, this involves detection of the causative virus,\(^{53,59}\) similarly to its use in other acute respiratory infections. Diagnostics can be made with samples from different parts of the human body, including anal, nasopharyngeal, and oropharyngeal swabs and human fluids such as blood, blood serum, saliva, and urine.\(^{60,61}\) SARS-CoV-2 could be found in 78% of serum and 50% of plasma samples.\(^{22,62,63}\) Usually, PCR reactions are applied to DNA amplification and detection by direct reactions with *Taq* polymerase. However, since the genomic material of such viral pathogens as CoVs is RNA, a previous step for viral mRNA conversion to DNA is required. Therefore, the RT-PCR detection mechanism comprises two steps: (1) an RNA-dependent DNA polymerase (reverse-transcriptase) copies mRNA into complementary DNA (cDNA); and (2) *Taq* polymerase is applied to amplify the generated cDNA.\(^{58}\) Most RT-PCR tests for CoVs are performed with fluorescence measurements and are quantitative, which is why sometimes they are referred to as RT-qPCR. Briefly, cDNA polymerizes with a probe targeted with both fluorescent and quencher labels. After polymerization into double-stranded DNA (dsDNA), the quencher and fluorescent probes are separated and light emission from the fluorophore is observed upon light excitation.\(^{58}\)

RT-PCR detection is highly reproducible, sensitive, and selective. It is considered the most appropriate method for CoVs detection, including SARS-CoV-2.\(^ {58}\) However, it is time-consuming and expensive, requiring several steps to obtain the viral genome and post-sample treatments in a well-equipped laboratory. These requirements are especially limiting for the needed rapid, low-cost assays for SARS-CoV-2.\(^{58,59,64}\) Moreover, its reliability for SARS-CoV-2 has been questioned owing to conflicting results depending on the sampling specimens.\(^ {65,66}\) Indeed, false results from RT-PCR analyses were observed for pharyngeal swab specimens from Wuhan hospitals\(^ {67}\) probably owing to insufficient testing material collected from the patients, laboratory errors, or sample transportation issues.\(^ {7}\) In fact, to improve the reliability of the diagnosis, a recommendation was made to combine RT-PCR results with computer tomography images.\(^ {67}\) Furthermore, variable results from RT-PCR tests involving primers in ORF1ab and N genes were obtained due to variation in RNA sequences.\(^ {67}\) Thus, ORF1ab and N gene were not recommended for RT-PCR testing by institutions throughout the world.\(^ {22}\)

Many efforts have been made to improve RT-PCR in the context of COVID-19 detection. One such example is a high-throughput platform (Cobas 6800) for automated detection, which is helpful to cope with large numbers of samples without any fluctuations in the tests.\(^ {68}\) With this methodology, key steps in SARS-CoV-2 detection were fully automated, including nucleic acids extraction, purification, amplification, and detection. This facilitated sample-handling and data interpretation by people who are not familiar with RT-PCR assays. The samples can be inserted after a rapid processing, reducing the average total time of analysis to less than 30 min (including manual and hands-on steps).\(^ {68}\) Analytical validations regarding inter-run variability, limit of detection (LOD), and cross reactions with other pathogens were performed. For 25 mL of reaction volume, a LOD of 689.3 copies/mL with 95% of detection probability was reached, based on E gene targeting, without false positive results. It should be noted that test validation was made with spiked-in material, and therefore matrix effects from real samples could not be truly investigated.\(^ {68}\) These analytical results were consistent with those obtained by Corman et al., who also used RT-PCR for COVID-19 detection.\(^ {68}\) In this case, however, a workflow was established for situations in which viral genomic material of the virus is absent, which may be an alternative strategy for SARS-CoV-2 testing. The detection mechanisms were based on assays for E and RdRp genes. The latter assay contained specific probes capable of distinguishing SARS-CoV-2 from SARS-CoV, avoiding false positives that could occur due to structural similarities between CoVs. A LOD of 5.2 and 3.8 copies/reaction was obtained for E gene and RdRp, respectively. There was no cross reactivity with other human CoVs, 229E, KIU1, NL63, OC43, and MERS-CoV, showing the specificity of the assays.\(^ {67}\) To reduce the costs of RT-PCR assays, a new protocol was proposed for SARS-CoV-2 negative diagnostics with pharyngeal samples of asymptomatic volunteers with Trizol-RNA purification.\(^ {69}\) Although 3 of 12 volunteers involved in the work had to be restested, the accuracy and sensitivity limit reached with a known lentivirus was of 1–10 virus particles. These results were obtained after a primer validation step for RdRp, N, S and E genes, which is essential for a new RT-PCR assay. The time of analysis was 4 h, and the cost was estimated at less than US$ 15 per sample.\(^ {69}\) Hence, the issues to address for large-scale application of this protocol include the long time for analysis and the inaccuracy for detecting positive cases of SARS-CoV-2.

RT-PCR was also employed as the main diagnostic tool during MERS-CoV and SARS-CoV endemics.\(^ {71,72}\) and there-
fore was successfully adapted to SARS-CoV-2. For example, the protocol for MERS-CoV detection based on N gene targeting may complement other gene targeting, such as upstream MERS-CoV E gene (upE). A remarkable specificity was achieved, which was confirmed by the lack of false positive amplifications with other human CoVs. Although satisfactory analytical results were obtained, this study was limited by the low amount of available real samples, and therefore the latter data reflect mock specimens spiked with the virus. Furthermore, storage conditions, handling, and collection for all the spiked samples were not patterned, leading to the lack of reliability of the results. Hence, a stricter sample preparation protocol must be adopted if one decides to apply these assays for SARS-CoV-2 detection. In fact, important parameters optimized for other CoVs cannot be reproduced for a successful analysis of SARS-CoV-2.

| gene target | positive rate for SARS-CoV-2 | LOD (95% of detection probability) | type of samples | ref |
|-------------|-----------------------------|------------------------------------|-----------------|-----|
| RdRp, E     | 55%                         | Not informed                       | Faecal samples | 74  |
| ORF1ab, NP  | 40.98% (ORF1ab) and 39.80% (NP) | Not informed                       | Nasal and pharyngeal swabs, bronchoalveolar lavage fluid, sputum | 64  |
| Nsp2 protein| 39%                         | 1.8 TCID<sub>50</sub>/mL           | Urine, rectal swabs, RTS | 75  |
| E-Gene-LDT  | Not informed                 | 95.55 copies/mL                    | Oropharyngeal, nasopharyngeal swabs | 3   |
| Not informed | 3.33%                       | Not informed                       | Tears, conjunctival secretions, sputum | 76  |
| E           | Not informed                 | 27.6–32.2 (Ct, positive samples)   | Oropharyngeal swabs | 74  |
| E, S        | Not informed (single patient study) | Not informed                       | Semen, urine | 77  |
| RdRp helicase, N | 28.2% for RdRp helicase, 43.6% for negative RdRd-P2, 24.2% RTS and 8.5% NRTS | 11.2 copies/reaction for all genes | RTS, NRTS | 78  |
| CD4+, T lymphocytes, CRP, ESR, PCT | 16.7% (stool), 6.9% (urine), 21.8 (oropharyngeal, feces) | Not informed | Oropharyngeal swab, stool, urine, feces, and serum samples | 61  |

ESR: Erythrocyte sedimentation rate. CRP: C-reactive protein. PCT: Procalcitonin. CD4+: Cluster of differentiation 4. NP: nucleocapsid protein. RTS: Respiratory tract specimens. NRTS: nonrespiratory tract specimens. TCID<sub>50</sub>: 50% tissue culture infective dose. Ct: Threshold cycle.

Table 1. Various Types of RT-PCR Protocols for SARS-CoV-2 Detection in the Literature

Figure 4. Sensitivity susceptibility to the primer gene for LAMP analyses. (A,B) RT-LAMP sensitivity toward ORF1ab gene targeting for SARS-CoV-2 detection based on ORF1ab-4 primer; (D,E) sensitivity of the proposed RT-PCR assay for SARS-CoV-2 S protein targeting using primer set S-123; (C,F) Conventional PCR assay sensitivity concerning both ORF1ab and S genes targeting for SARS-CoV-2 detection. Reprinted with permission from ref 81. Copyright 2020, Elsevier Publisher.

https://dx.doi.org/10.1021/acssensors.0c01382
ACS Sens. XXX, XXX, XXX–XXX
infections. On the other hand, interference from other pathogens, e.g., other CoVs, may appear in case of simultaneous presence of these viruses in the sample. For instance, peaks of viral load for SARS-CoV, MERS-CoV, and SARS-CoV-2 are different. SARS-CoV and MERS-CoV viral load peaks were observed during the second week of infection, while the SARS-CoV-2 viral peak occurs in the first week of infection. Another important aspect concerns sampling specimens for RT-PCR analyses of viral RNA, which cannot be the same for the latter CoVs: SARS-CoV and MERS-CoV present a positive rate of infection of approximately 100% for LRT (lower respiratory tract) specimens. SARS-CoV-2, in its turn, was more detectable (higher positive rate for RNA) with URT (upper respiratory tract) specimens. This suggested that CoVs have different viral kinetics depending on the sampling specimens, and therefore, this is a new assay for SARS-CoV-2 detection has been recommended. It has been much less used than RT-PCR. LAMP was applied into DNA with DNA polymerase, but the selectivity is higher owing to the larger number of primers. RT-LAMP was applied for LAMP depends on using different primers and only shows optimum results at high temperatures, its application is limited and quite challenging under ordinary conditions. Besides, LAMP assays are not as useful as RT-PCR for mass testing because of the need of sophisticated (and, thus, expensive) thermal cycling equipment. Another challenge faced by scaling-up RT-LAMP assays regards the need of genetic primers for the nucleic acid amplification reactions, which are expensive and nontrivial reagents to be obtained and cannot be stock for a long time.

CRISPR-Based Biosensing Strategies. CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR-associated) is a powerful analytical tool to modify genomes and gene functions. A recent discovery of Cas13 as an RNA targeting enzyme in CRISPR-Cas has sparked excitement for next-generation biosensors with higher specificity, sensitivity, and rapid detection of nucleic acids. Cas13 is an RNA-guided RNase capable of producing multiple cleavages in the nonspecific target single-strand RNA (ss-RNA). To make it target specific, a CRISPR RNA (crRNA) is needed, and the resulting Cas13-crRNA complex has a higher target specificity for being guided to a RNA sequence of interest. Thus, RNA sensing of Cas13 with nonspecific endonuclease activity is used as a detection strategy, where this enzyme modified with an RNA guide sequence is activated after combining with the specific target. A fluorescent signal is produced as the activated enzyme interacts with a fluorophore quencher, which indicates the presence of RNA or DNA with high sensitivity and selectivity, up to fmol L\(^{-1}\). The collateral activity of the CRISPR Case family, particularly Cas13, is a triggered cleavage process with self-amplification ability, thus making the system highly sensitive and selective to detect nucleic acids. In addition to the fluoro sensor based detection, other strategies include visual detection of liquid—liquid phase separation in turbid solutions and lateral flow detection with antigen-labeled reporters. The CRISPR/Cas13 biosensing technology has been used to detect ss-RNAs of Zika virus, dengue virus, and micro-RNA (mi-RNA) in serum samples of brain-tumor patients. In the latter investigation, an integrated low-cost, portable CRISPR/Cas13a biosensor was used to measure the electrochemical signal from miR-19b (brain tumor marker) with detection limit of 10 pmol L\(^{-1}\) within a total processing time of less than 4 h. The technique can therefore be fast and low-cost, and does not require sophisticated laboratory equipment.

The sensitivity of the CRISPR/Cas13 method can be enhanced by introducing an isothermal preamplification step in a platform referred to as SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing). This system uses recombinase polymerase amplification (RPA) of RNA or DNA and depends upon the introduction of T7 promoter (RNA polymerase), enabling RNA transcription followed by detection with Cas13. A protocol using SHERLOCK technology has been published to detect target RNA sequences with synthetic S and ORF1a genes of SARS-CoV-2. RNA sequences at concentrations as low as 20 amol L\(^{-1}\) were detected using lateral flow detection readout. The CRISPR/Cas13a assay has been reported with faster analyses than RT-PCR. It can also be used as a benchmark for further advancements to provide a rapid, easy-to-handle, and sensitive system for mass testing.
One of the most prominent challenges for the application of CRISPR-based strategies is the noticeably high complexity of the technique. It requires an expert team and nontrivial reagents. Other difficulties include lack of standardization of tests, sequence limitations, and limited literature in CRISPR assays. Moreover, an additional step of DNA amplification is often needed to lower the LOD. Owing to these characteristics, CRISPR technologies are less economically viable than RT-PCR and LAMP assays.

**Genosensors.** Genosensors are a useful, cost-effective alternative to RT-PCR for detecting viral RNA fragments of specific sequences. Similar to RT-PCR, genosensing is based on nucleic acid testing via hybridization between a probe, usually a single strand oligonucleotide, and a complementary nucleic acid or RNA. Upon hybridization of the probe immobilized on the sensor surface, a fluorescent, electrical, or electrochemical signal is produced, thus allowing the biorecognition of the target RNA. In contrast to RT-PCR, the genosensing approach is free from amplification and separation steps, making it simple and easy to handle. It has been used in food analysis and environmental control, but only recently in POC diagnosis. Indeed, there is a demand for further developments of commercial products for mass testing, which may explain why genosensors for SARS-CoV-2 have not been reported yet. Because genosensors usually involve immobilization of specific probes for hybridization, these devices are of significant selectivity and rarely affected by interferents. This is relevant for the detection of SARS-CoV-2 in the presence of other CoVs, for example.

The prospects of employing genosensing assays for COVID-19 diagnosis are nevertheless promising because related technologies can be adapted. For instance, a genosensor device with a sandwich assay detected the Zika virus in samples of infected individuals, including saliva, serum, and urine in the presence of 1000-fold higher concentrations of dengue and chikungunya homologues. A miniaturized gold-based genosensor platform detected SARS-CoV with an electro-chemical technique with an LOD of 6 pmol L\(^{-1}\). The genosensor was sufficiently selective to distinguish 2-base and 1-base mismatches between complementary and unpaired hybrids. Both genosensors detailed in refs 44 and 107 contained biotin-labeled probes. For the SARS-CoV genosensor, optimized performance was reached with a judicious choice of matrices and approaches to immobilize the complementary strand to a 30-mer sequence that encodes specific SARS-CoV regions. It is in this aspect that materials chemistry and materials science may play a crucial role, since behind the simplicity of the genosensing methodology is the background knowledge acquired over decades of combining nanomaterials and biomolecules for sensing and biosensing (for a detailed review, see ref 108). Many are the examples of successful results with genosensors depending on leveraging nanomaterials for matrices and principles of detection. In the detection of a cancer biomarker, for instance, the organization of self-assembled monolayers was crucial to allow for distinguishing cell lines of neck and head cancer. For SARS-CoV-2 genetic material detection, a supersandwich-type genosensing device was developed for early detection of viral RNA without amplification steps. The LOD reached of 200 copies/mL was claimed as the lowest in the literature up to the present day. The detection assay allows the monitoring of the results through a smartphone. The development of these types of genosensing devices allow diagnosis with high sensitivity, which is suitable for mass-testing. On the other hand, the extensive preparation steps of the electrode and long incubation times can be considered time-consuming.

Therefore, this dependence on genosensor constituent materials should be considered for adapting current methodologies for COVID-19 diagnosis. While this dependence also applies to immunosensors, as discussed in the next section, the demand for rapid testing with genetic materials is a more urgent one while the COVID-19 pandemic lasts. On the other hand, despite its great selectivity, sensitivity, and other advantages, the scaling-up of genosensing technologies for quick mass COVID-19 technologies may face a number of challenges. As an example, the preparation of genosensing devices usually requires a long time and expensive reagents that cannot be stored at room temperature. Moreover, genosensing devices also demand non-ordinary transport conditions and normally present short shelf-lives.

In summary, the genetic materials-based diagnostics are rapid, sensitive, and specific and can enable the quantitative detection of SARS-CoV-2 RNA and hence early and large-scale detection of SARS-CoV-2. Nevertheless, their testing procedures are complex, in addition to their high cost and slow delivery to the end users. For example, building a RT-PCR processing lab may cost up to 15,000 USD to produce RT-PCR kits of 100 USD each, in addition to the high ratio of false positive and false negative results, which occurs most routinely in these diagnostics tests.

**IMMUNO-BASED TECHNIQUES**

In this section, operation principles and the applicability of immunologically based techniques, such as ELISA, CLIA, and Lateral Flow assays, will be described in the SARS-CoV-2 detection scenario. We will first discuss the use of POC assays for COVID-19 diagnosis, and highlight some of the challenges inherent in these assays. Some important aspects of immunology toward SARS-CoV-2 proteins will be discussed. As we did for genetic material-based techniques, perspectives will also be presented of the use of nanomaterials to improve performance. Emerging immunosensors for SARS-CoV-2 detections will be mentioned, in addition to existing immunosensors developed for other CoVs.

**Point-of-care (POC) immunodevices** provide a useful platform for SARS-CoV-2 detection. Most POC devices have used immunological testing mechanisms, since tests of nucleic acids (such as viral RNA, for example) in these platforms are not straightforward. It should be noted, however, that electrochemical and impedimetric genosensors are now also considered for POC purposes as we discussed previously. POCs have been used for real-time identification of CoVs and other pathogenic diseases, including SARS-CoV and MERS-CoV. A successful detection of CoVs requires a suitable target protein and its corresponding antibody. For SARS-CoV-2, the most studied protein is the S protein with 24.5% non-conserved amino acid sequence of SARS-CoV. Distinguishing between SARS-CoV and SARS-CoV-2 was possible owing to these divergences in amino acid sequences, especially the antigenicity differences in RBD attributed to the low similarity in the non-conserved amino acid sequences. Despite the differences in amino acid sequences, cross-reactivity between SARS-CoV-2 S protein is not inhibited by polyclonal anti-SARS-CoV S1 antibodies. The differences in amino acid sequences, cross-reactivity between SARS-CoV-2 S proteins antibodies is still...
possible in immunoassays. Indeed, SARS-CoV specific antibodies, such as the CR3022 human monoclonal antibody, bind to SARS-CoV-2 RBDs.\textsuperscript{119} Furthermore, polyclonal goat antibodies anti-MHV S AO4 could be used to detect three CoVs S proteins. This suggested the existence of a conserved epitope in SARS-CoV, MERS-CoV\textsubscript{s}, and SARS-CoV-2.\textsuperscript{119} The selective detection of these viruses using goat anti-MHV S antibodies AO4 by POC assays is not possible. Consequently, to avoid cross-reactivity and improve selectivity for COVID-19 diagnosis, specific recombinant antigens are needed. Therefore, some of the immunoassays for other CoVs based on S proteins should be carefully reviewed and readapted for SARS-CoV-2 diagnosis. An alternative protein that can be used for SARS-CoV-2 detections is N protein. There is evidence that SARS-CoV N and S protein was observed for positive COVID-19 serum samples regarding IgM and IgG responses.\textsuperscript{33} A combined detection of N and S proteins by their IgM and IgG appears to increase the SARS-CoV-2 detection rate in early combined detection of N and S proteins by their IgM and IgG activity involves SARS-CoV-2 S and N proteins,\textsuperscript{123} as confirmed by measuring their binding kinetics to S and N proteins.\textsuperscript{124} Most intensive care unit (ICU) patients had higher concentrations of S and/or N IgG antibodies, probably due to the longer-term viral infection. Detection of N protein-based IgM and IgG was more efficient for early identification of the infection, owing to the immunogenicity and intracellular accumulation before virus packaging (i.e., virus assembly).\textsuperscript{124} Some of these tests were less sensitive than S protein counterparts.\textsuperscript{121} Another important aspect of N and S proteins regards their cross reactivity. Some degree of cross reactivity of SARS-CoV N and S protein was observed for positive COVID-19 serum samples regarding IgM and IgG responses.\textsuperscript{33} A combined detection of N and S proteins by their IgM and IgG appears to increase the SARS-CoV-2 detection rate in early infections (up to 75\% of patients)\textsuperscript{33,124} Another antibody employed for SARS-CoV-2 detections in POC immunoassays is IgA, which can target the S protein RBD in immunoassays.\textsuperscript{32,125} Its kinetic response in COVID-19 patients, however, remains unknown.\textsuperscript{126} The concentration of IgA peaks during the third week of infection, being more persistent than IgM.\textsuperscript{127} Therefore, different POC assays exist for CoVs immunological testing (including SARS-CoV-2) with IgM, IgG, and IgA antigen—antibody interactions.\textsuperscript{125−130}

In contrast to the genetic material-based techniques, the immunosensing diagnostic tools could provide cost-effective diagnosis at primary health care units. Except for a few of these methods, such as ELISA which requires large readout devices, anticoagulants, and trained personnel, the majority of them do not need expensive laboratory infrastructure and a lot of reagents.\textsuperscript{131} Therefore, they can be easily implemented outside a laboratory. Although we could not find the price of single immunosensing devices in the literature, they are considered less expensive due to their simple assay protocols. These protocols include three or four steps: sample injection, reaction, visualization/interpretation of the results. Therefore, they are economically much more viable than molecular or nucleic acids-based techniques.

**Enzyme-Linked Immunosorbent Assays (ELISA).** ELISA assays are popular immunoassays\textsuperscript{132} to detect pathogens via quantification of antibodies\textsuperscript{133} made to interact with antigens adsorbed on a solid support, e.g., polystyrene.\textsuperscript{133} In an ELISA assay, the sample is first incubated in a polystyrene tube coated with the antigen. Then, enzyme-labeled Ig antibodies are added to the tube. After antigen—antibody interactions and washing steps, the remaining enzyme provides a quantification of the specific antibody in the serum sample. Antibodies can also be detected by other similar ELISA strategies, in three different assays (competitive, inhibition, and double-antibody).\textsuperscript{133}

ELISA assays for SARS-CoV-2 have been developed for direct (sandwich) or indirect IgG and IgM detections,\textsuperscript{121,134−136} this latter also called as capture or sandwich approach. Figure 5 shows a detailed comparison of these two methodologies.\textsuperscript{57} Competitive ELISA is based upon the competitive binding of the sample analyte and a reference to the limited quantity of the adsorbed antibody.\textsuperscript{33} This latter approach is only adopted for small peptides containing a single recognizable binding epitope. For example, Liu and co-workers observed that ELISA assays for IgM detection of S protein of SARS-CoV-2 are more sensitive than for the N proteins.\textsuperscript{134} The sensitivity in IgG detection, on the other hand, was similar for S and N proteins. Therefore, the assays can be employed as a complementary test to RT-PCR, particularly for serum samples of COVID-19 patients after 10 days post-disease onset.\textsuperscript{134} The main advantages of ELISA assays, especially for COVID-19 diagnosis, are the high sensitivity, uniform testing for a wide range of patients, speed, and simplicity of operation.\textsuperscript{136} However, when direct antibody screening is employed to improve simplicity and rapidness of analyses, false positive results may be recorded. This is frequently related to interference factors. As an example, Wang and co-workers stated that interference in ELISA assays for IgM detections occurs.\textsuperscript{137} It was found that rheumatoid factors at mid-to-high levels can lead to false positive IgM results. These data were obtained through urea dissociation tests.\textsuperscript{137} Indirect antigen or
antibody screening, on the other hand, may be affected by nonspecific immobilizations.\textsuperscript{138} Besides these challenges, cross-reactivity between SARS-CoV-2 and SARS-CoV can also happen, specifically with the EUROIMMUN ELISA assay for IgG detections.\textsuperscript{139} Interference from other matrix components, such as hemoglobin, triglycerides, and bilirubin, was also evaluated, with no significant interference on the assay result.\textsuperscript{139}

Chemiluminescent Assays (CLIA). Strategies using antibodies include chemiluminescent immunoassays (CLIA) described in Figure 6A.\textsuperscript{140} CLIA are attractive due to possible automation, high specificity, low level of interferences, high stability of conjugates and reagents, reduced incubation time, compatibility with immunological assays protocols, and wide dynamic range.\textsuperscript{141} Validation of CLIA assays is frequently performed by comparing with results from ELISA. The large-scale application of CLIA is hampered, however, owing to

![Chemiluminescent Assays (CLIA). Strategies using antibodies include chemiluminescent immunoassays (CLIA) described in Figure 6A. CLIA are attractive due to possible automation, high specificity, low level of interferences, high stability of conjugates and reagents, reduced incubation time, compatibility with immunological assays protocols, and wide dynamic range. Validation of CLIA assays is frequently performed by comparing with results from ELISA. The large-scale application of CLIA is hampered, however, owing to](https://dx.doi.org/10.1021/acssensors.0b01382)
limited test panels, high costs, and nonlinear response to the analyte concentration due to complex chemical reactions. In CLIA assays, a luminescent label acts as indicator of the analyte concentration due to complex chemical reactions. For direct methods, acridinium and ruthenium esters are luminoephores, while enzymatic markers (alkaline phosphatases, horseradish peroxidase with luminol, and AMPPD) are applied for indirect methods. Antibodies may be labeled with different types of materials, including nanomaterials that possess increased surface electron density and surface area. This leads to signal amplification, as illustrated with the use of gold nanoparticles, magnetite nanoparticles, and Au–Ag alloy nanoparticles.

As occurs with ELISA assays, hemoglobin, triglycerides, bilirubin, and rheumatoid factor are matrix compounds which can interfere on CLIA results. In addition, because CLIA is an immunoassay, other compounds such as autoantibodies, endogenous compounds, and human anti-animal antibodies can also interfere and lead to false results. A detailed review on interference in immunoassays is given in ref 147.

Detection of SARS-CoV-2 using CLIA has been mostly performed by targeting N and S proteins through IgM and IgG antibodies. CLIA commercial assays had comparable sensitivity with ELISA and LFDs (lateral flow devices) POC devices, but less specificity. Montesinos and co-workers compared two automated assays for detecting SARS-CoV-2 in serum samples. Euroimmun IgG/IgA ELISA assays were more sensitive than IgG/IgM Maglumi CLIA assays, consistent with results for other pathogens. The results from a commercial CLIA assay for IgM and IgG are shown in Figure 6B, where the concentration of each antibody was monitored along the course of the infection, starting from the fever onset. Deviations in the results were less than 4% and 6% for IgM and IgG, respectively. The detection of SARS-CoV-2 through fully automated CLIA assays has been reported. The mass application of these automated assays is a challenge for low-budget locations, and there is the possibility of false negative results, especially at early-stage of infections. Therefore, other methodologies are required for the correct management and treatment of patients. Table 2 lists some ELISA and CLIA assays used for SARS-CoV-2 detection. Most of the articles do not present a LOD value or even the obtained cutoff for the studied assay. These latter parameters are essential to evaluate the minimum amount of analyte that can be detected by the assay. This issue is frequently related to misinterpretation of the meaning of sensitivity in the medical area. A thorough discussion of this topic is provided in ref 151.

Lateral Flow Devices (LFDs) for Point-of-Care Testing. POC immunoassays in LFDs provide quick, efficient, and inexpensive testing through immunochromatographic mechanisms. LFDs are paper-based strip devices comprising four main regions: (1) cellulose-based sample pad for sample dropping; (2) conjugate pad, generally made of glass fiber containing the labeled particles (generally colorimetric or fluorescent) conjugated to the analyte receptor; (3) nitrocellulose-based detection pad with test lines, and control lines—test lines contain biomolecules capable of binding to the analyte-label-conjugated particles (if the target analyte is present in the sample); a signal proportional to the analyte concentration is generated, while control lines are used to verify the test operation, capturing labeled-conjugated particles independently of the presence of the analyte; (4) absorption pad, also made of cellulose, which helps induce sample flow through the test strip. The limitations of POCs with LFDs are related to the low sensitivity due to the target analyte and cross-reactivity with other species in the sample matrix. Moreover, the viscosity of the liquid samples needs to be within a certain range, which prevents a wider use of such devices. Interference from matrix blood samples, as discussed in CLIA and ELISA assays, should also be taken into account for LFDs immunoassays. A number of other difficulties may exist for application of LFDs, which include denaturing of immobilized proteins (i.e., antigens), nonspecific adsorption, nanoparticle aggregation, and steric hindrance. The performance of the assays may also depend on the paper used in some LFDs. To exemplify, trapping of biomolecules on paper pores results not only in binding undesired effects, but also in flux rate changes. To cope with these problems, several pretreatments of the pads are carried out. An illustration of a LFD device likely to be used for SARS-CoV-2 is depicted in Figure 7.

Figure 7. Representation of a typical LFD assay that can be employed for SARS-CoV-2 testing. The design of commercial assays is the same as that represented above. The device comprises a sample pad in which IgM and IgG antibodies are immobilized. The conjugate pad contains gold nanoparticles (AuNP) conjugated to a SARS-CoV-2 antigen. In test lines, anti-human IgG and anti-human IgM are immobilized to interact with IgG-AuNP-antigen and IgM-AuNP-antigen complex. The control line contains nonhuman reactive anti-IgG or anti-IgM. The absorbent pad is useful for maintaining sample flow through the strip.

In spite of these limitations and challenges, LFDs have been applied for COVID-19 diagnosis in human blood and serum samples, in most cases to confirm suspected and asymptomatic cases. In comparison to ELISA and CLIA assays, LFDs diagnostics shows satisfactory sensitivity and accuracy for SARS-CoV-2 detection. The sensitivity with nine commercially available immunochromatographic gold-labeled LFDs for IgM and IgG detections ranged 72.7–100%, with results obtained in less than 20 min. Since LFDs are not quantitative for COVID-19, the sensitivity parameter adopted is different from those used in analytical chemistry. This difference was clarified by Lassaurière et al., who defined sensitivity as “the number of patients which were correctly diagnosed with SARS-CoV-2 infections by the studied POCs after a previous positive SARS-CoV-2 diagnosis by nucleic acids testing with respiratory samples.” Most LFD tests for SARS-CoV-2 are based on S or N proteins as antigens, but a large number of commercial LFDs assays do not specify the virus protein chosen as antigen. Although Whitman and collaborators reported the maximum sensitivity in their LFDs detections for COVID-19 diagnosis, they focused on the report of the “positivity” rate obtained, which increased significantly after 2 weeks of disease conditions.
Diagnosis of SARS-CoV-2 with LFDs has also benefited from recent advances in incorporation of nanoparticles. For instance, lanthanide-doped polystyrene nanoparticles were employed instead of conventional AuNP as fluorescent labels for detecting IgG through N protein immobilization. The assay was reproducible (coefficient of variation <15%) with detection results comparable to RT-PCR, showing the suitability for detection of suspicious and asymptomatic COVID-19 cases. However, in contrast to RT-PCR assays, LFDs assays are limited at the onset of infection, because production of IgG and IgM antibodies has not yet begun.

Colloidal gold nanoparticles were studied by Huang and co-workers on a simple and easy-to-handle LFD platform to detect SARS-CoV-2 N protein using an anti-human IgM–colloidal gold nanoparticle conjugate. Despite the similarity between these assays and the commercial LFDs for SARS-CoV-2 detection, selectivity and sensitivity achieved were satisfactory and up to 90% in both cases. However, this research is at an early stage of development. Indeed, there is no report of a well-established methodology using these innovative LFDs for COVID-19 diagnosis. This highlights another important challenge for the use of LFDs: the successful establishment of novel nanobiointerfaces for the development of new LFDs within an acceptable time frame. A possible alternative is to employ nanomaterials for antibody conjugates and colorimetric responses on test strips, which could be viable for COVID-19 diagnosis.

Some of LFDs for SARS-CoV-2 immunodetection are summarized in Table 3.

![Image](https://dx.doi.org/10.1021/acssensors.0c01382)

**Figure 8.** (A) Application of GFET-based electrical immunosensor with SARS-CoV-2 S protein antibodies immobilized onto graphene surface. The device was employed for SARS-CoV-2 detection in clinical samples from COVID-19 infected patients. Reprinted with permission from ref 182. Copyright, 2020, American Chemical Society. (B) Schematic representation of GFET immunosensor for RBD of S1 subunit. $V_{ref}$ shift due to antibodies immobilization in comparison to the bare graphene surface.

### Table 3. Commercial and Recent LFDs for COVID-19 Diagnosis

| immunoassay/test name | target | SARS-CoV-2 species | sample | sensitivity | ref |
|-----------------------|--------|--------------------|--------|------------|-----|
| LFD Avioq             | IgG/IgM| Recombinant SARS-CoV-2 antigen | Human serum | 68.8% | 125 |
| LFD/LNPs labeling     | IgG    | SARS-CoV-2 N protein | Human serum | Not informed | 163 |
| LFD                   | IgG/IgM| RBD of S protein    | Blood samples | 88.66% (positive results); 90.63% (negative results) | 128 |
| LFD                   | Ab/IgG/IgM| SARS-CoV-2 N and S proteins | Plasma samples | 97.5% (Ab); 86.3% (IgG) 88.8% (IgM) | 153 |
| LFD AutoBioDiagnostics| IgG/IgM| Not informed | Human serum | 93% | 130 |
| LFD DynamikerBiotechnology | IgG/IgM| Not informed | Human serum | 90% | 130 |
| LFD CTK Biotech       | IgG/IgM| Not informed | Human serum | 90% | 130 |
| LFD ArtronLaboratories| IgG/IgM| Not informed | Human serum | 83% | 130 |
| LFDs (DeepBlue, Bioperfectus, UCP) | IgG/IgM| Not informed | Human serum and plasma samples | 84.3–100% | 160 |

LNPs: Lanthanide-doped polystyrene nanoparticles. Ab: Total antibody. RBD: Receptor binding domain.
indeed been used for MERS-CoV but surprisingly, we have been able to find only one example for SARS-CoV-2, with the eCoVSens platform. A fluorine-doped indium–tin oxide electrode was modified with gold nanoparticles and COVID-19 monoclonal antibodies for detecting the S1 domain of S protein. Spiked samples were analyzed within 10–30 s, and the LODs of 90 and 120 fmol L$^{-1}$ were reached.

Among the immunosensors containing nanomaterials, of special relevance are the graphene field-effect transistors (GFETs) in which biosensing exploits changes in the electrical properties of the interface induced by analyte approximation and/or adsorption. High performance is expected owing to the high basal plane conductivity of graphene, and its dependence on surface changes. The first GFET developed to detect SARS-CoV-2 is illustrated in Figure 8A. Antibodies for the S protein were immobilized through 1-pyrenbutanoic acid and succinimidyl ester probe linkers. Detection was made by measuring the drain current with clinical samples prepared from nasopharyngeal swabs, under a constant bias of 10 mV. The device was capable of distinguishing infected from noninfected samples, with a detection limit of 0.42 × 10$^5$ copies/mL. The LODs for other samples tested were 1 fg/mL, 100 fg/mL, and 16 pfu/mL in phosphate buffer saline, clinical transport medium, and culture medium, respectively. Another GFET immunosensor applied to SARS-CoV-2 had antibodies from SARS-CoV S1 subunit immobilized onto graphene, as illustrated in Figure 8B. Using changes in the liquid gate voltage ($V_{gd}$), detection of RBD (Receptor Binding Domain of S1 subunit) of S protein could be achieved with a LOD of 0.2 pmol L$^{-1}$ with a measuring time of only 2 min, according to Figure 8B. The ACE2 enzyme could also be detected with S1 spike protein by monitoring the $V_{of}$ changes. This latter immunosensor is interesting for quantifications of infected cells, since ACE2, a human integral membrane protein, acts as receptor for SARS-CoV-2 binding and subsequent infection. Considering the strategies involving different targets for detection published so far for SARS-CoV-2, we believe that monitoring real-time ACE2–spike S1 protein interactions is useful for evaluating in vivo infections.

In spite of being promising, electrochemical and electrical immunosensors frequently face selectivity as the main challenge for their validation. This is normally addressed with functionalization strategies, as mentioned above related to specific antibodies for COVID-19 early and sensitive diagnosis. However, the need of additional preparation steps for functionalization and biomolecules incubation frequently increases the biosensor fabrication time, making them more expensive than other immunosensing devices.

On the other hand, the adoption of functionalization strategies for selectivity improvement is also useful for minimizing interference effects. These methodologies are known for their significant efficiency, and consequently, they are adopted not only in biosensing technologies, but also in a wider range of detection through electrochemical methods. For instance, a gold microelectrode presented remarkable antinterference activity toward As$^{3+}$ detections by using amino-functionalized graphene oxide as modifying agent. For SARS-CoV-2 diagnosis, interference from other CoVs with similar structures as well as other compounds from the sample matrix may be avoided by functionalizing the electrochemical device with recombinant antigens or their specific antibodies. This was the case of detection of SARS-CoV-2 S1 subunit antibodies using recombinant antigens on gold micropillar electrodes decorated with graphene oxide. The LOD was 1 pmol L$^{-1}$ with a detection time of 11.5 s with a smartphone-based interface, but these devices may not be suitable for mass-testing because of the target analyte chosen by the authors: specific antibodies for S1 subunit proteins of SARS-CoV-2, which are not produced by a human infected organism. Instead, perhaps an electrochemical sensing platform for detecting IgG and IgM antibodies may be a more efficient strategy.

### EMERGING STRATEGIES FOR DIAGNOSIS OF COVID-19

In this section, we wish to emphasize that the diagnosis of COVID-19 can be improved and extended if sensing strategies are explored, which can lead to low-cost methodologies for mass tests and/or increased accuracy. There are several such strategies, but for the sake of conciseness, we shall illustrate only three types: paper-based biosensing, plasmonic-enhanced biosensing, and use of pattern recognition methods (which may incorporate machine learning). All of these three strategies can be utilized not only for immunosensing and genosensing but also with alternative principles of detection in which specific biorecognition is not required.

#### Paper-Based Biosensors.

Paper-based biosensors (PBBs) are simple, inexpensive, and robust alternatives to assays, as has been proven for diagnosis of infectious diseases. These are miniaturized POC devices made of porous cellulose paper, allowing the detection of analytes through visual or electronic readouts. The detection scheme in which a biorecognition element is not required, in contrast to lateral flow and ELISA, may incorporate machine learning. All of these three strategies can be utilized not only for immunosensing and genosensing but also with alternative principles of detection in which specific biorecognition is not required.

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plotting, laser cutting, and photolithography. However, in addition to the expensive equipment required, preparation takes several steps and resolution is low. Therefore, the scaling-up of PBBs may be less advantageous than expected, especially in the context of COVID-19 pandemics, which requires fast and effective strategies.

**Plasmonic-Based Biosensors.** Plasmonic effects have been exploited for biosensing with various principles of detection (for a focused review, see ref 199), including surface plasmon resonance (SPR), and localized surface plasmon resonance (LSPR). LSPR, in particular, is promising owing to the enhanced electromagnetic field in the neighborhood of the nanostructures, which imparts higher sensitivity as demonstrated in LFD assays where the colorimetric signal of gold nanoparticles was amplified upon laser excitation. Another advantage is the possibility of using laser-reader systems with the standard LFDs architecture and operation mode. One should emphasize that LSPR can be useful for investigating interaction mechanisms during infection by SARS-CoV-2, including protein interactions with human receptors believed to be essential for the virus maintenance in human cells. Indeed, SPR can provide experimental evidence of phenomena related to docking studies.

Another advantage of LSPR biosensors is the cost of commercialization, as for example a home-built LSPR system based on white-light extinction would cost $25,000 or less. Compared with other more sophisticated LSPR equipment, this is a lower-cost possibility.

When plasmonic and thermal effects are combined, the sensitivity of the biosensing device for detecting nucleic acids can be increased considerably. The “thermoplasmonic” effect arises from the nonradiative relaxation of absorbed light in nanomaterials, thus generating an excessive localized heat energy that can be harnessed as a local heating source for controlled thermal processes. This provides a rapid alternative methodology for identifying DNA sequences and mutations. A similar methodology was adopted in dual-function biosensors for SARS-CoV-2 detection in synthetic viral oligonucleotide sequences identical to the one used in ref 53. The AuNIs (Au nanoislands) sensing chip functionalized with synthetic receptor oligonucleotide (RdRp SARS-CoV-2-C) was implemented into an LSPR detection system. Hybridization was ~8 times faster with the thermoplasmonic effect when RdRp SARS-CoV-2 genes were injected into the sensing chamber. The evaluation of this dual-plasmonic device for viral nucleic acids detection based on LSPR responses is illustrated in Figure 9. It should be noted that nonspecific binding of mismatching sequences was prevented, which demonstrates the impact of localized heating on the hybridization kinetics. Various challenges must be addressed to apply LSPR biosensor to COVID-19, including the difficulties in preparing robust, reproducible substrates. Furthermore, the devices should ideally be reusable and easy to functionalize and clean after analysis.

**Artificial Intelligence Based Methods.** The sensing strategies mentioned so far are all based on detecting specific targets requiring genetic sequence probes, proteins, and protein subunits. Because a considerable amount of data is generated, especially to account for the variability of the biological samples, statistical and computational methods can be utilized for the diagnosis, including machine learning, Internet of Things (IoT), deep learning, and other artificial intelligence (AI) approaches. AI can also be employed during COVID-19 pandemics for the development of personalized medicine. For example, Prasad and co-workers developed a guide for reinforced learning
algorithms to determine extubation time for each patient. The course of the treatment, evolution of symptoms, and day-to-day updates of the recuperation of patients can also be monitored through artificial intelligence algorithms.228 Another prospective application of artificial intelligence is in the development of safe vaccines and pursuing therapeutic drugs.229 Although artificial intelligence methodologies seem useful and efficient, several issues must be tackled for its widespread implementation. These include a speedy data collection, storage, and analysis, which requires specialized people and data centers. This may be difficult for some developing countries severely affected by SARS-CoV-2.

Perhaps the most important breakthrough will be reached when various types of data are integrated into a single system. This could be realized with computer-assisted diagnosis systems, as discussed in reviews dedicated to the use of Big Data concepts and computational methods in processing sensing data.221–228 IoT approaches are also useful for online data collection from sensing devices and interpretation of these data, as well as orienting medical decisions based on Big Data analysis models.221 The framework of these computer-assisted systems is entirely generic and can be applied to any type of task involving diagnosis, monitoring, or surveillance. More specifically for COVID-19, one may envisage a diagnosis strategy considering reports of symptoms, analysis of health parameters, and data from sensors which do not need to be specific for SARS-CoV-2 (i.e., sensors other than the immunosensors and genosensors discussed here). Hence, a somewhat accurate diagnosis could be achieved even if no specific tests are available. An added advantage would arise from feeding the results from a large number of people into a surveillance system to monitor the disease spread, which could be performed in a seamless manner in a computer system.

## CONCLUSIONS

Herein we have described the main strategies employed so far in SARS-CoV-2 diagnosis, which are basically categorized into methods to detect genetic material of the virus and immunoassays. Special emphasis was given to identifying the strengths and limitations of these methodologies. In particular, we elaborated upon the limitations in connection with the difficulties in performing mass testing in many countries, from which we identified three major challenges: (i) employ genosensors in POC devices to replace the sophisticated methods used (RP-PCR, LAMP, CRISPR) in detecting genetic material, since this is critical to diagnose individuals with no symptoms; (ii) improve the accuracy of the diagnosis based on immunoassays, which is by no means straightforward because distinct types of antibodies may be needed to detect owing to the time dependence of their concentrations along the infection course; (iii) use pattern recognition methods that do not require biotech products and the test kits, which is essential in poorer places.

For all the challenges above, we envisage that new developments will be required in materials, especially nanomaterials. From our survey, we noted that the main emphasis has been placed on the definition of target biomolecules, but strategies to enhance performance using nanomaterials have been limited. For detection of genetic material, for instance, developing low-cost alternatives to the expensive methods should be prioritized, and this depends on novel uses of nanomaterials. One should aim at POC devices which can fulfill all the stringent requirements of low-cost, rapid tests and easy deployment in any setting. Also relevant to the chemistry and materials community is the possible extension of well-established electrochemical, electrical, and plasmonic effects to diagnosis of SARS-CoV-2. In this review, we have mentioned a few examples in which graphene and metallic nanoparticles were incorporated into the sensing devices, and we can foresee that much more can be done by exploiting the whole portfolio of biosensing strategies in which nanomaterials are used.

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### Notes

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

The authors gratefully acknowledge the São Paulo Research Foundation (FAPESP) for the financial support of the research projects under the grant numbers: 19/15333-1, 19/12053-8, 18/11071-0, and 18/22214-6. Coordinating Agency for Advanced Training of Graduate Personnel (CAPES), MeDiCo Network CAPES-Brazil grant number: 88881.504532/2020-01 and for PNPD program, grant number: 88887.358060/2019-00. National Council of Scientific and Technological Development (CNPq) project number 305486/2019-5.

### VOCABULARY

Biosensors: Biosensors are devices that are able to transduce a biological event into a measurable analytical signal. Proteins, biological structures and microorganisms can be detected through biosensing strategies. Antibody: An antibody is a protein produced by the immunological system for the defense of their host organism from external pathogens. They do it with high specificity, by binding to specific parts of the targets. Point-of-care devices: These are devices that enable medical diagnosis at the time and place of patient care. They are suitable for quick and mass testing, without long sample preparation steps and reactions. Genosensors: A genosensor is a specific type of biosensor dedicated to the detection of DNA (i.e., genetic material). The event that enables detection consists of hybridization of DNA specific probes that are complementary to the DNA (or RNA) target sequence to be detected.
Electrochemical sensors: Devices that can give information on the composition of a system based on electron-transfer reactions into a sensing transducer (electrodes). The signal obtained can be used for quantification and other analytical purposes.

Analytical chemistry: A branch of chemistry dedicated to the determination of composition of matter and its quantification. In many cases, analytical chemistry deals with these issues through the development of robust instrumentation, implementation of statistics and innovative sensing strategies.

**REFERENCES**

(1) Sheridan, C. Fast, Portable Tests Come Online to Curb Coronavirus Pandemic. Nat. Biotechnol. 2020, 38, $15$.

(2) Li, Y.; Yao, L.; Li, J.; Chen, L.; Song, Y.; Cai, Z.; Yang, C. Stability Issues of RT-PCR Testing of SARS-CoV-2 for Hospitalized Patients Clinically Diagnosed with COVID-19. J. Med. Virol. 2020, 92, 903.

(3) Nörz, D.; Fischer, N.; Schultze, A.; Kluge, S.; Mayer-Runge, U.; Aepfelbacher, M.; Pfefferle, S.; Lütgéheftmann, M. Clinical Evaluation of a SARS-CoV-2 RT-PCR Assay on a Fully Automated System for Rapid on-Demand Testing in the Hospital Setting. J. Clin. Virol. 2020, 128, 104390.

(4) Abbas, I. The Promise and Peril of Antibody Testing for Coronavirus COVID-19. JAMA - J. Am. Med. Assoc. 2020, 323, 1881.

(5) Li, Z.; Yi, Y.; Luo, X.; Xiong, N.; Liu, Y.; Li, S.; Sun, R.; Wang, Y.; Hu, B.; Chen, W.; Zhang, Y.; Wang, J.; Huang, B.; Lin, Y.; Yang, J.; Cai, W.; Wang, X.; Cheng, J.; Chen, Z.; Sun, K.; Pan, W.; Zhan, Z.; Chen, L.; Ye, F. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. J. Med. Virol. 2020, 92, 1518.

(6) Strategic Preparedness and Response Plan on COVID-19; World Health Organization, 2020; p 20.

(7) Weiss, S. R.; Leibowitz, J. L. Coronavirus Pathogenesis, 1st ed.; Elsevier Inc., 2011; Vol 81. DOI: 10.1016/B978-0-12-385885-5.00009-2.

(8) Corman, V. M.; Muth, D.; Niemeyer, D.; Drosten, C. Hosts and Coronaviruses. Nature Rev. Microbiol. 2019, 17 (3), 181–192.

(9) Cui, J.; Li, F.; Shi, Z. L. Origin and Evolution of Pathogenic Coronaviruses. Nat. Rev. Microbiol. 2020, 18, 163–188.

(10) Wu, F.; Zhao, S.; Yu, B.; Chen, Y. M.; Wang, W.; Song, Z. G.; Hu, Y.; Tao, Z. W.; Tian, J. H.; Pei, Y. Y.; Yuan, M. L.; Zhang, Y. L.; Dai, F. H.; Liu, Y.; Wang, Q. M.; Zheng, J. J.; Ju, X.; Holmes, E. C.; Zhang, Y. Z. A New Coronavirus Associated with Human Respiratory Disease in China. Nature 2020, 579 (7798), 265–269.

(11) Andersen, K. G.; Rambaut, A.; Lipkin, W. I.; Holmes, E. C.; Garry, R. F. The Proximal Origin of SARS-CoV-2. Nat. Med. 2020, 26 (April), 450–452.

(12) Mahase, E. Coronavirus Covid-19 Has Killed More People than Polio. Weekly Epidemiological Update 2020, 8 (March), 1–14.

(13) Mahase, E. Coronavirus Covid-19 Has Killed More People than Polio. Weekly Epidemiological Update 2020, 8 (March), 1–14.

(14) Singhal, T. A Review of Coronavirus Disease-2019 (COVID-19). Indian J. Pediatr. 2020, 87 (4), 281–286.

(15) Weekly Epidemiological Update; October 2020. DOI: 10.30895/2312-7821-2020-8-1-3-8.

(16) Amanat, F.; Kramer, F. SARS-CoV-2 Vaccines: Status Report. Immunity 2020, 52 (4), 583–589.

(17) Elkilik, A. A.; Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenovir against SARS-CoV-2 RNA Dependent RNA Polymerase (RdRp): A Molecular Docking Study. Life Sci. 2020, 253, 117592.

(18) Walls, A. C.; Park, Y. J.; Tortorici, M. A.; Wall, A.; McGuire, A. T.; Veesler, D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 2020, 181 (2), 281–292.

(19) Chan, J.; Ge, J.; Ju, Y.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L.; Wang, X. Structure of the SARS-CoV-2 Spike Receptor-Binding Domain Bound to the ACE2 Receptor. Nature 2020, 581, 215.

(20) Yan, R.; Zhang, Y.; Li, Y.; Xia, L.; Guo, Y.; Zhou, Q. Structural Basis for the Recognition of SARS-CoV-2 by Full-Length Human ACE2. Science (Washington, DC, U. S.) 2020, 367 (6485), 1444–1448.

(21) Tahir ul Qamar, M.; Aalqathani, S. M.; Alamri, M. A.; Chen, L.-L. Structural Basis of SARS-CoV-2 3CLpro and Anti-COVID-19 Drug Discovery from Medicinal Plants. J. Pharm. Anal. 2020, 10, 313.

(22) Garry, R. F. The Proximal Origin of SARS-CoV-2. JAMA - J. Am. Med. Assoc. 2020, 323, 686–688.

(23) Zhang, H.; Penninger, J. M.; Li, Y.; Zhong, N.; Slutsky, A. S. Angiotensin-Converting Enzyme 2 (ACE2) as a SARS-CoV-2 Receptor: Molecular Mechanisms and Potential Therapeutic Target. Intensive Care Med. 2020, 46 (4), 586–590.

(24) Afzal, A. Molecular Diagnostic Technologies for COVID-19: Limitations and Challenges. J. Adv. Res. 2020, 26, 149.

(25) Phan, T. Genetic Diversity and Evolution of SARS-CoV-2. Infect. Genet. Evol. 2020, 81, 104260.

(26) Pachetti, M.; Marini, B.; Benedetti, F.; Giudici, F.; Mauro, E.; Storici, P.; Masciovecchio, C.; Angelletti, S.; Ciccoricco, M.; Gallo, R. C.; Zella, D.; Ippodrino, R. Emerging SARS-CoV-2 Mutation Hot Spots Include a Novel RNA-Dependent-RNA Polymerase Variant. J. Transl. Med. 2020, 18 (1), 1–9.

(27) Li, G.; Fan, Y.; Lai, Y.; Han, T.; Li, Z.; Zhou, P.; Pan, P.; Wang, W.; Hu, D.; Liu, X.; Zhang, Q.; Wu, J. Coronavirus Infections and Immune Responses. J. Med. Virol. 2020, 92 (4), 424–432.

(28) Kong, Q.; Liu, B.-Z.; Deng, H.-J.; Wu, G.-C.; Deng, K.; Chen, Y.; Liao, P.; Qiu, J.; Lin, Y.; Cai, X.; Wang, D.-Q.; Hu, Y.; Ren, J.; Tang, N.; Xu, Y.; Yu, L.; Mo, Z.; Gong, F.; Zhang, X.-L.; Tian, W.; Hu, L.; Zhang, X.; Xiang, J.; Du, H.; Liu, H.; Lang, C.; Luo, X.; Wu, S.; Cui, X.; Zhou, Z.; Zhu, M.; Wang, J.; Xue, C.; Li, X.-F.; Wang, L.; Li, Z.; Wang, K.; Niu, C.; Yang, Q.; J.; Tang, X.-J.; Zhang, Y.; Liu, X.-M.; Li, J.-J.; Zhang, D.-C.; Zhang, F.; Liu, P.; Yuan, J.; Li, Q.; Hu, J.-L.; Chen, J.; Huang, A.-L. Antibody Responses to SARS-CoV-2 in Patients with Coronavirus COVID-19. Nat. Med. 2020, 26, 845.

(29) Sutharan, N.; Jeremiah, S. S.; Ryo, A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA - J. Am. Med. Assoc. 2020, 323, 2249.

(30) Li, G.; Chen, X.; Xu, A. Profile of Specific Antibodies to the SARS-Associated Coronavirus. N. Engl. J. Med. 2003, 349 (5), 508–509.
(36) Wo, P. C. Y.; Lai, S. K. P.; Wong, B. H. L.; Chan, K. H.; Chu, C. M.; Tsoi, H. W.; Huang, Y.; Peiris, J. S. M.; Yuen, K. Y. Longitudinal Profile of Immunoglobulin G (IgG), IgM, and IgA Antibodies against the Severe Acute Respiratory Syndrome (SARS) Coronavirus Nucleocapsid Protein in Patients with Pneumonia Due to the SARS Coronavirus. Clin. Diagn. Lab. Immunol. 2004, 11 (4), 665–668.

(37) Liibana, S.; Brandão, D.; Alegret, S.; Pividori, M. I. Electrochemical Immunosensors, Genosensors and Phagosensors for Salmonella Detection. Anal. Methods 2014, 6 (22), 8858–8873.

(38) Zhang, L.; Hao, M.; Zhang, K.; Zhang, R.; Lin, G.; Jia, T.; Zhang, D.; Chang, L.; Xie, J.; Li, J. External Quality Assessment for the Molecular Detection of MERS-CoV in China. J. Clin. Virol. 2016, 75, 5–9.

(39) Huang, J. C.; Chang, Y. F.; Chen, K. H.; Su, L. C.; Lee, C. W.; Chen, C. C.; Chen, Y. M. A.; Chou, C. Detection of Severe Acute Respiratory Syndrome (SARS) Coronavirus Nucleocapsid Protein in Human Serum Using a Localized Surface Plasmon Coupled Fluorescence Fiber-Optic Biosensor. Biosens. Bioelectron. 2005, 29 (2), 320–325.

(40) Shirato, K.; Yano, T.; Senba, S.; Akachi, S.; Kobayashi, T.; Nishinaka, T.; Notomi, T.; Matsuyama, S. Detection of Middle East Respiratory Syndrome Coronavirus Using Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP). Virol. J. 2014, 11 (39), 1–11.

(41) Kumar, V.; Shin, J. S.; Shie, J.-J.; Ku, K. B.; Kim, C.; Go, Y. Y.; Huang, K.-F.; Kim, M.; Liang, P.-H. Identification and Evaluation of Potent Middle East Respiratory Syndrome Coronavirus (MERS-CoV) 3CLPro Inhibitors Vathan. Antiviral Res. 2017, 141, 101–106.

(42) Abad-Valle, P.; Fernández-Abedul, M. T.; Costa-García, A. Genosensor on Gold Films with Enzymatic Electrochemical Detection of a SARS Virus Sequence. Biosens. Bioelectron. 2005, 20 (11), 2251–2260.

(43) Liu, Q.; Cheng, S.; Chen, R.; Ke, J.; Liu, Y.; Li, Y.; Feng, W.; Li, F. Near-Infrared Lanthanide-Doped Nanoparticles for a Low Cost Nanomaterial-Based Electrochemical Immunosensor on Paper for High-Sensitivity Early Detection of Pancreatic Cancer. Sens. Actuators, B 2020, 305, 127516.

(44) Narang, J.; Singhal, C.; Mathur, A.; Sharma, S.; Singla, V.; Pandir, C. S. Portable Bioactive Paper Based Genosensor Incorporated with Zn-Ag Nanobiomes for Herpes Detection at the Point-of-Care. Int. J. Biol. Macromol. 2018, 107, 2559–2565.

(45) Chowdhury, A. D.; Takeamura, K.; Li, T. D. C.; Suzuki, T; Park, E. Y. Electrical Pulse-Induced Electrochemical Biosensor for Hepatitis E Virus Detection. Nat. Commun. 2019, 10 (1), 4–7.

(50) Gao, A.; Lu, N.; Wang, Y.; Li, T. Robust Ultrasonic Tunneling-FET Biosensor for Point-of-Care Diagnostics. Sci. Rep. 2016, 6 (March), 1–9.
Evaluation of a Quantitative RT-PCR Assay for the Detection of the Coronavirus Disease 2019 (COVID-19). Kim, D.; Chang, H.; Kim, V. N.; Lee, C. J.

ACS Sensors pubs.acs.org/acssensors

2020, 25 (9), 1–5.

Won, J.; Lee, S.; Park, M.; Kim, T. Y.; Park, M. G.; Choi, B. Y.; Kim, D.; Chang, H.; Kim, V. N.; Lee, C. J. Development of a Laboratory-Safe and Low-Cost Detection Protocol for SARS-CoV-2 of the Coronavirus Disease 2019 (COVID-19). Exp. Neurobiol. 2020, 29 (2), 107–13.

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.; Li, A. Classiﬁcation Techniques and Test Development for COVID-19 Diagnosis. ACS Cent. Sci. 2020, 6 (5), 591–605.

Al Johani, S.; Hajeer, A. H. MERS-CoV Diagnosis: An Update. J. Infect. Public Health 2016, 9 (3), 216–219.

Poon, L. L. M.; Chan, K. H.; Wong, O. K.; Yam, W. C.; Yuen, K. Y.; Guan, Y.; Lo, Y. M. D.; Peiris, J. S. M. Early Diagnosis of SARS Coronavirus Infection by Real Time RT-PCR. J. Clin. Virol. 2003, 28 (3), 233–238.

Lu, X.; Whitaker, B.; Sakhvhit, S. K. K.; Kamili, S.; Rose, L. E.; Lowe, L.; Mohareb, E.; Essalal, E. M.; Al-saouti, T.; Haddadin, A.; Erdman, D. D. Real-Time Reverse Transcription-Pcr Assay Panel for Middle East Respiratory Syndrome Coronavirus. J. Clin. Microbiol. 2014, 52 (1), 67–75.

Wu, Y.; Guo, C.; Tang, L.; Hong, Z.; Zhou, J.; Dong, X.; Yin, H.; Xiao, Q.; Tang, Y.; Qiu, X.; Kuang, L.; Fang, X.; Mishra, N.; Lu, J.; Shan, H.; Jiang, G.; Huang, X. Prolonged Presence of SARS-CoV-2 Viral RNA in Faecal Samples. Lancet Gastroenterol. Hepatol. 2020, 5 (5), 434–435.

Yip, C. C. Y.; Ho, C. C.; Chan, J. F. W.; To, K. K. W.; Chan, H. S. Y.; Wong, S. C. Y.; Leung, K. H.; Fung, A. Y. F.; Ng, A. C. K.; Zou, Z.; Tam, A. R.; Chung, T. W. H.; Chan, K. H.; Hung, I. F. N.; Cheng, V. C. C.; Tsang, O. T. Y.; Tsui, S. K. W.; Yuen, K. Y. Development of a Novel, Genome Subtraction Derived, SARS-CoV-2 Speciﬁc Viral RNA in Faecal Samples. Lancet Gastroenterol. Hepatol. 2020, 5 (5), 434–435.

Chan, J. F. W.; Yip, C. C. Y.; To, K. K. W.; Tang, H. C.; Wong, S. C. Y.; Leung, K. H.; Fung, A. Y. F.; Ng, A. C. K.; Zou, Z.; Tsoi, H. W.; Choi, G. K. Y.; Tam, A. R.; Cheng, V. C. C.; Chen, K. H.; Tsang, O. T. Y.; Yuen, K. Y. Improved Molecular Diagnosis of COVID-19 by W. Rapid Detection of SARS-CoV-2 Using Reverse Tran-
Electrochemical Genosensors in Food Safety Assessment.

Afkhami, A.; Arduini, F.; Bagheri, H. An Overview to Electrochemical Biosensor for the Detection of Escherichia Coli from Real Samples. Wrapped Multi-Walled Carbon Nanotube Modified Electrochemical Biosensor for Direct Detection of Specific RNA Sequences Derived from Avian Influenza Viruses Present in Biological Samples. Acta Genosensor. 2020, 11 (1), 1 DOI: 10.1038/s41467-020-15629-9.

Talanta 2017, 166, 27–35.

Haveri, A.; Smura, T.; Kuivanen, S.; Österlund, P.; Hepojoki, J.; Ikonen, N.; Pitäkkaasi, M.; Blomqvist, S.; Rönköö, E.; Kantele, A.; Strandin, T.; Kallio-Kokko, H.; Mannonen, L.; Lappalainen, M.; Bras, M.; Jiang, M.; Siré, L.; Salminen, P.; Puimalainen, T.; Sane, J.; Melin, M.; Vapaalhti, O.; Savolainen-Kopra, C. Serological and Molecular Findings during SARS-CoV-2 Infection: The First Case Study in Finland, January to February 2020. Eurosurveillance 2020, 25 (11), 1–6.

Flego, M.; Di Bonito, P.; Ascone, A.; Zamboni, S.; Carattoli, A.; Grasso, F.; Cassone, A.; Cianfiglia, M. Generation of Human Antibody Fragments Recognizing Distinct Epitopes of the Nucleocapsid (N) SARS-CoV-2 Protein Using a Phage Display Approach. BMC Infect. Dis. 2021, 5, 1–8.

Zheng, M.; Song, L. Novel Antibody Epitopes Dominate the Antigenicity of Spike Glycoprotein in SARS-CoV-2 Compared to SARS-CoV. J. Med. Virol. 2020, 92, 1671.

Diagnostics 2020, 10, 319.

Bagos, P. G. Antibody Tests in Detecting SARS-CoV-2 Infection: A Meta-Analysis. Diagnostics 2021, 11, 3274.

Zhong, N.; Chen, L. Kinetics of SARS-CoV-2 Specific IgM and IgG Responses in COVID-19 Patients. EMBO Rep. 2020, 21, 120648.

Trends. 2017, 20, 134–143.

Powder. 2017, 20, 120609.

Zhao, H.; Liu, F.; Xie, W.; Zhou, T.-C.; OuYang, J.; Jin, L.; Li, H.; Zhao, C.-Y.; Zhang, L.; Wei, J.; Zhang, Y.-P.; Li, C.-P. Ultra-sensitive Supersandwich-Type Electrochemical Sensor for SARS-CoV-2 from the Infected COVID-19 Patients Using a Smartphone. Sens. Actuators, B 2021, 327, 128899.

Molecular Findings during SARS-CoV-2 Infection: The First Case Study in Finland, January to February 2020. Eurosurveillance 2020, 25 (11), 1–6.

Montesinos, I.; Gruson, D.; Kabamba, B.; Dahma, H.; Van Den Wijnngaert, A.; Reza, S.; Carbonne, Y.; Vandenberg, O.; Gulbis, B.; Wolf, F.; Rodriguez-Villalobos, H.; Colvyn, N. Kinetics of SARS-CoV-2 Specific IgM and IgG Responses in COVID-19 Patients. EMBO Rep. 2020, 21, 120648.

Van Amerongen, A.; Veen, J.; Arends, H. A.; Koets, M. Lateral Flow Immunoassays. In Handbook of Immunoassay Technologies: Approaches, Performances, and Applications; 2018; pp 157–182. DOI: 10.1007/978-3-030-21676-2_00007-4.

Shajarijoubi Jaberi, S.; Dharia, A.; Mandaza, P.; OuYang, J.; Jin, L.; Li, H.; Zhao, C.-Y.; Zhang, L.; Wei, J.; Zhang, Y.-P.; Li, C.-P. Ultra-sensitive Supersandwich-Type Electrochemical Sensor for SARS-CoV-2 from the Infected COVID-19 Patients Using a Smartphone. Sens. Actuators, B 2021, 327, 128899.

Van Amerongen, A.; Veen, J.; Arends, H. A.; Koets, M. Lateral Flow Immunoassays. In Handbook of Immunoassay Technologies: Approaches, Performances, and Applications; 2018; pp 157–182. DOI: 10.1007/978-3-030-21676-2_00007-4.

Electrochemical Paper Based Nano-Genosensor Modified with Reduced Graphene Oxide-Gold Nanostructure for Determination of Specific RNA Sequences Derived from Avian Influenza Viruses Present in Biological Samples. Acta Genosensor. 2020, 11 (1), 1 DOI: 10.1038/s41467-020-15629-9.

Zheng, M.; Song, L. Novel Antibody Epitopes Dominate the Antigenicity of Spike Glycoprotein in SARS-CoV-2 Compared to SARS-CoV. J. Med. Virol. 2020, 92, 1671.

Bagos, P. G. Antibody Tests in Detecting SARS-CoV-2 Infection: A Meta-Analysis. Diagnostics 2020, 10, 319.

Flego, M.; Di Bonito, P.; Ascone, A.; Zamboni, S.; Carattoli, A.; Grasso, F.; Cassone, A.; Cianfiglia, M. Generation of Human Antibody Fragments Recognizing Distinct Epitopes of the Nucleocapsid (N) SARS-CoV-2 Protein Using a Phage Display Approach. BMC Infect. Dis. 2021, 5, 1–8.
(130) Lassaunière, R.; Frische, A.; Harboe, Z. B.; Nielsen, A. C.; Fomsgaard, A.; Krogfelt, K. A.; Jørgensen, C. S. Evaluation of Nine Commercial SARS-CoV-2 Immunossays. medRxiv 2020, 1.

(131) European Commission. Current Performance of COVID-19 Test Methods and Devices and Proposed Performance Criteria; 2020.

(132) Noh, J. Y.; Yoon, S. W.; Kim, Y.; Van Lo, T.; Ahn, M. J.; Jung, M. C.; Le, T. B.; Na, W.; Song, D.; Le, V. P.; Haam, S.; Jeong, D. G.; Kang, J. K.; Kim, H. S.; Lee, J. H.; Lee, J. W.; Lee, K.; Li, H.-w.; Li, Y.-a.; Huang, S.-z.; Gao, L.; Xu, F.-h.; Zhang, Z.-y. Performance Evaluation of a Chemiluminescence Microparticle Immunossay for CK-MB. J. Clin. Lab. Anal. 2018, 32 (6), e22426.

(147) Tate, J.; Ward, G. Interferences in Immunoassay. Clin. Biochem. Rev. 2004, 25 (6), 105–120.

(148) Caini, S.; Bellerba, F.; Corso, F.; Díaz-basabe, A.; Napolitani, G.; Faccioti, F.; Raimondi, S.; Palli, D.; Mazzarella, L.; Pellici, P. G. Meta-Analysis of Diagnostic Performance of Serological Tests for SARS-CoV-2 Antibodies and Public Health Implications. medRxiv 2020, DOI: 10.1128/MD.3557987.

(150) Soleimani, R.; Khoussaji, M.; Gruson, D.; Rodríguez-Villalobos, H.; Berghmans, M.; Belkhir, L.; Yombi, J. C.; Kabamba-Mukadi, B. Clinical Usefulness of Fully Automated Chemiluminescent Immunossay for Quantitative Antibody Measurements in COVID-19 Patients. J. Med. Virol. 2020, DOI: 10.1002/jmv.26430.

(151) Mattioli, I. A.; Crespi, F. N. Problems of Interpreting Diagnostic Tests for SARS-CoV-2: Analytical Chemistry Concerns. An. Acad. Bras. Cienc. 2020, 92, 1–3.

(152) Zhao, J.; Yuan, Q.; Wang, H.; Liu, W.; Liao, X.; Yu, S.; Wang, X.; Yuan, J.; Li, T.; Li, J.; Qian, S.; Hong, C.; Wang, F.; Liu, Y.; Wang, H.; He, Q.; He, B.; Zhang, T.; Ge, S.; Liu, L.; Zhang, J.; Xia, N.; Zhang, Z. Antibody Responses to SARS-CoV-2 in Patients of Novel Coronavirus Disease 2019. Clin. Infect. Dis. 2020, 1–22.

(153) Lou, B.; Li, T.; Zheng, S.; Su, Y.; Li, Z.; Liu, W.; Yu, F.; Ge, S.; Zou, Q.; Yuan, Q.; Lin, S.; Hong, C.; Yao, X.; Zhang, X.; Wu, D.; Zhou, G.; Hou, W.; Li, T.; Zhang, Y.; Zhang, S.; Fan, J.; Zhang, J.; Xia, N.; Chen, Y. Serology Characteristics of SARS-CoV-2 Infection since the Exposure and Post Symptoms Onset. Eur. Respir. J. 2020, 56, 2000763.

(154) Wang, Z.; Li, H.; Li, J.; Yang, C.; Guo, X.; Hu, Z.; Chen, Z.; Wang, S.; Liu, J. Elevated Serum IgM Levels Indicate Poor Outcome in Patients with Coronavirus Disease 2019 Pneumonia: A Retrospective Case-Control Study. SSRN J. 2020, DOI: 10.2139/ssrn.3557987.

(155) Long, Q.; Deng, H.; Chen, J.; Hu, J.; Liu, J.; Biao, L.; Lin, Y.; Yu, L.; Mo, Z.; Xu, Y.; Gong, F.; Wu, G.; Zhang, X.; Chen, Y.; Li, Z.; Wang, K.; Zhang, X.; Tian, W.; Niu, C.; Yang, Q.; Xiang, J.; Du, H.; Liu, H.; Lang, C.; Luo, X.; Wu, S.; Cui, X.; Zhou, Z.; Wang, J.; Xue, C.; Li, X.; Wang, L.; Tang, X.; Zhang, Y.; Qiu, J.; Liu, X.; Li, J.; Zhang, D.; Zhang, F.; Cai, X.; Wang, D.; Hu, Y.; Ren, J.; Tang, N.; Liu, P.; Li, Q.; Huang, A. Antibody Responses to SARS-CoV-2 in COVID-19 Patients: The Perspective Application of Serological Tests in Clinical Practice. medRxiv 2020, DOI: 10.1128/mediarxiv.202038018.

(156) Quesada-González, D.; Merkoçi, A. Nanoparticle-Based Lateral Flow Biosensors. Biosens. Bioelectron. 2015, 73, 47–63.

(157) De Puig, H.; Bosch, I.; Gehrke, I.; Hamad-schifferli, K. Challenges of the Nano-Bio Interface in Lateral Flow and Dipstick Immunoassays. Trends Biotechnol. 2017, 35 (12), 1169–1180.

(158) Borse, V.; Srivastava, R. Process Parameter Optimization for Lateral Flow Immunossensing. Mater. Sci. Energy Technol. 2019, 2 (3), 434–441.

(159) Zainol Rashid, Z.; Othman, S. N.; Abdul Samat, M. N.; Ali, U. K.; Wong, K. K. Diagnostic Performance of COVID-19 Serology Assays. Malays. J. Pathol. 2020, 42 (1), 13–21.

(160) Whitman, D. J.; Hiatt, J.; Mowery, C. T.; Shy, B. R.; Yu, R.; Yamamoto, T. N.; Rathore, U.; Goldberg, M. G.; Whitty, C.; Woo, J. M.; Gallman, A. E.; Miller, T. E.; Levine, A. G.; Nguyen, D. N.; Bapat, S. P.; Balcerak, J.; Bylsma, L.; Lyons, A. M.; Li, S.; Wong, A. W.; Gillis-Buck, E. M.; Steinhardt, Z. B.; Lee, Y.; Apathy, R.; Lilje, B. M.; Smith, J. A.; Zheng, T.; Boothby, I. C.; Isaza, E.; Chan, J.; II, D. D. A.; Lee, J.; Macrae, T. A.; Kyaw, T. S.; Wu, D.; Ng, D. L.; Gu, W.; York, T.
L.; Li, P.; Yu, L.; Wu, Y.; Lin, G. Rapid and Sensitive Detection of Amplification. Prussian Blue Nanoparticles Mediated Signal Generation and Cascade A Sensitive Lateral Flow Immunochromatographic Strip with Adv. 15057. Balasubramanian, K. Selective Electrochemical Functionalization of A. Test Performance Evaluation of SARS-CoV-2 Serological Assays. Ernst, J. D.; Wu, A. H. B.; Lynch, K. L.; Bern, C.; Hsu, P. D.; Marson, J. A. Test Performance Evaluation of SARS-CoV-2 Serological Assays. medRxiv 2020, 1 DOI: 10.1101/2020.04.25.20074856.

(161) Qu, Z.; Wang, K.; Alfranca, G.; de la Fuente, J. M.; Cui, D. A Plasmonic Thermal Sensing Based Portable Device for Lateral Flow Assay Detection and Quantification. Nanoscale Res. Lett. 2020, 15 (5), 1 DOI: 10.1186/s11671-019-3420-3.

(162) Tian, M.; Xie, W.; Zhang, T.; Liu, Y.; Lu, Z.; Li, C. M.; Liu, Y. A Sensitive LFlow Immunochromatographic Strip with Prussian Blue Nanoparticles Mediated Signal Generation and Cascade Amplification. Sens. Actuators, B 2020, 309, 127728.

(163) Chen, Z.; Zhang, Z.; Zhai, X.; Li, Y.; Lin, L.; Zhao, H.; Bian, L.; Li, P.; Yu, L.; Wu, Y.; Lin, G. Rapid and Sensitive Detection of Anti-SARS-CoV-2 IgG Using Lanthanide-Doped Nanoparticles-Based Lateral Flow Immunoassay. Anal. Chem. 2020, 92, 7226.

(164) Huang, C.; Wen, T.; Shi, F.; Liang, X.Y.; Jiao, Y.-J. Rapid Detection of IgM Antibodies against the SARS-CoV-2 Virus via Colloidal Gold Nanoparticle-Based Lateral-Flow Assay. ACS Omega 2020, 5, 12550.

(165) Mohammadniaei, M.; Koyappayil, A.; Min, J.; Lee, M.-H.; Sun, Y. Electrochemical Detection of Multiple RNA Targets Using MXene/Duplex-Specific Nuclease: A Path towards Simultaneous Detection of SARS-CoV-2 and H1N1 Influenza Virus. In Biosensors for pandemics; 2020; p 39.

(166) Mavrikou, S.; Moschopoulou, G.; Tsekouras, V.; Kintziou, S. A Rapid and Sensitive Bioelectrochemical Biosensor for the Detection of the SARS-CoV-2 Spike Protein Based on Membrane-Engineered Cells. In Biosensors for pandemics; 2020; p 24.

(167) Hernandez, C. A.; Osma, J. F. Equivalent Electrical Model Circuit for COVID-19 Electrochemical Detection. In Biosensors for pandemics; 2020; p 33.

(168) Bouchiat, V.; Guerra, M.; Tripisky, A.; Bourrier, A.; Othmen, R. Graphene-on-Polymer Films for Low Cost Flexible & Disposable Biosensors. In Biosensors for pandemics; 2020; p 21.

(169) Iost, R. M.; Crespihlo, F. N. Layer-by-Layer Self-Assembly and Electrochemistry: Applications in Biosensing and Bioelectronics. Biosens. Bioelectron. 2012, 31 (1), 1–10.

(170) Iost, R. M.; Sales, F. C. P. F.; Martins, M. V. A.; Almeida, M. C.; Crespihlo, F. N. Glucose Biochip Based on Flexible Carbon Fiber Electrodes: In Vivo Diabetes Evaluation in Rats. ChemElectroChem 2015, 2 (4), 518–521.

(171) Kaya, S. I.; Karadurmus, L.; Orselikay, G.; Bakırhan, N. K.; Oztan, S. A. Electrochemical Virus Detection with Nanobiosensors. In Nanosensors for Smart Cities; INC; 2020; pp 303–326. DOI: 10.1007/978-0-1-819870-4-00117-7.

(172) Macedo, L. J. A.; Iost, R. M.; Hassan, A.; Balsubramanian, K.; Crespihlo, F. N. Bioelectronics and Interfaces Using Monolayer Graphene. ChemElectroChem 2019, 6 (1), 31–59.

(173) Macedo, L. J. A.; Lima, F. C. D. A.; Amorim, R. G.; Freitas, R. O.; Yadav, A.; Iost, R. M.; Balsubramanian, K.; Crespihlo, F. N. Interplay of Non-Uniform Charge Distribution on the Electrochemical Modification of Graphene. Nanoscale 2018, 10, 15048–15057.

(174) Yadav, A.; Iost, R. M.; Neubert, T. J.; Baylan, S.; Schmid, T.; Balsubramanian, K. Selective Electrochemical Functionalization of the Graphene Edge. Chem. Sci. 2019, 10 (3), 936–942.

(175) de Souza, J. C. P.; Iost, R. M.; Crespihlo, F. N. Nitrated Carbon Nanoblisters for High-Performance Glucose Dehydrogenase Bioanodes. Biosens. Bioelectron. 2016, 77, 860–865.

(176) Luz, R. A. S.; Crespihlo, F. N. Gold Nanoparticle-Mediated Electron Transfer of Cytochrome c on a Self-Assembled Surface. RSC Adv. 2016, 6 (67), 62585–62593.
Future with Ubiquitous Sensing and Intelligent Systems. *ACS Sensors* 2018, 3 (8), 1433–1438.