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SARS-CoV-2 Tests: Bridging the Gap between Laboratory Sensors and Clinical Applications

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ABSTRACT: This review covers emerging biosensors for SARS-CoV-2 detection together with a review of the biochemical and clinical assays that are in use in hospitals and clinical laboratories. We discuss the gap in bridging the current practice of testing laboratories with nucleic acid amplification methods, and the robustness of assays the laboratories seek, and what emerging SARS-CoV-2 sensors have currently addressed in the literature. Together with the established nucleic acid and biochemical tests, we review emerging technology and antibody tests to determine the effectiveness of vaccines on individuals.

KEYWORDS: *coronavirus, SARS-CoV-2, clinical tests, PCR, RNA, mutations, lateral flow devices, sensors, biosensors, electrical sensors, optical sensors, mechanical sensors*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported as a novel coronavirus in December 2019 in Wuhan city, Hubei province, China and determined to be the causative pathogen of the pneumonia outbreak first described in Wuhan.[1−3] The disease, designated as coronavirus disease 2019 (COVID-19), typically presents as a respiratory syndrome on a spectrum: from mild upper respiratory tract infection with symptoms of dry cough and fever, through to severe pneumonia, acute respiratory distress syndrome (ARDS), and mortality.[4,5] Transmission of the virus is predominantly thought to be airborne via aerosol or droplet acquisition when an infected patient talks, coughs, or sneezes within close proximity to an uninfected person.[6] As a direct result, the virus has spread rapidly throughout the global population, causing the COVID-19 pandemic; as of February 2021, SARS-COV-2 has caused 114 million cases worldwide of which 2.53 million suffered mortality and 64.3 million recovered.[7] In an infected patient, manifestation of infection can be asymptomatic and thus it can be difficult to diagnose active, transmissible infection.

■ SARS-COV-2

SARS-CoV-2 is an enveloped novel β coronavirus of the Order *Nidovirales* and Family *Coronaviridae*, containing single-stranded, positive sense RNA approximately 29.8–29.9 kb in length.[8,9] SARS-CoV-2 is supported by a nucleoprotein (N) and surrounded by a structure consisting of envelope small membrane proteins (E), spike glycoproteins (S), and membrane proteins (M);[10] see Figure 1. The virion is spherical and composed of a helical nucleocapsid that is between 60 and 140 nm in diameter within a distinctive envelope comprising the glycoprotein spikes (S) that range from 9 to 12 nm in length.[11] These spike proteins outside of the virion look like a solar "corona".[9]

The family of coronaviruses include those capable of causing severe disease, such as SARS-CoV, MERS-CoV, and SARS-CoV-2, and those causing mild illness, such as HKU1, NL63, OC43, and 229E.[12] SARS-CoV-2 shares 79.6% of its genetic sequence with SARS-CoV,[1] which was responsible for the Severe Acute Respiratory Syndrome (SARS) outbreak in 2003.[13] SARS-CoV-2 has a wider community transmission than previous coronavirus outbreaks, due to higher viral loads in the upper airway, a wider clinical spectrum of disease increasing asymptomatic and presymptomatic viral transmission, and enhanced binding capacity of the viral spike protein.[14]

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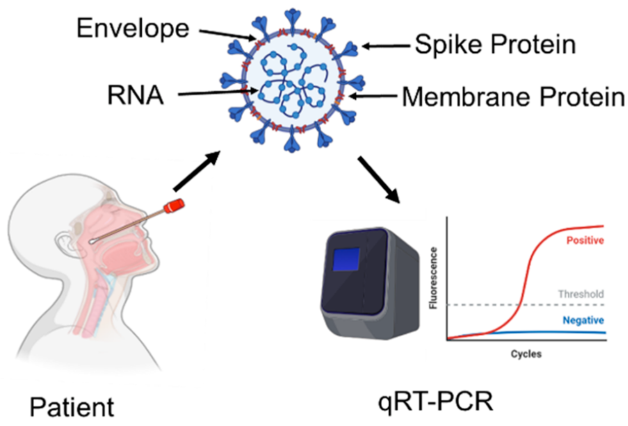

Figure 1. Structure of the SARS-CoV-2 virus particle and concept of qRT-PCR tests of a nasal swab.

Coronaviruses have genetically adapted and evolved to infect humans through genetic recombination events from animal hosts such as cattle, chickens, and, most commonly, bats.[1,15] Bats are considered to be the main natural reservoir for coronaviruses, and SARS-CoV-2 has 96.2% genetic similarity to bat β coronaviruses (SARSr-CoV; RaTG13); however, there is still significant debate as to how humans became infected with SARS-COV-2.[16] It is clear that the virus is zoonotic and the first cases were associated with the "wet" Huanan seafood and wildlife market in Wuhan City, China, of which there are considered to be two lineages—"S" and "L"; however, what is not clear is the exact origin or source. Many consider the virus to have genetically adapted and jumped the species boundary to infect humans from bats via an intermediate mammalian host.[1,4,16,17]

SARS-CoV-2 Mutation. RNA viruses are known to have high mutation rates which arise when they replicate; however, coronaviruses possess an ability to proof read any errors made during replication via a 3′ to 5′ exoribonuclease.[18] This has, however, not stopped the emergence of genetic variants of SARS-CoV-2 that could increase transmissibility of the virus.[19,20] There is evidence of increased mortality in patients infected with novel SARS-CoV-2 variants.[21,22] In response to the spread of the virus, there has been increased emphasis on tracking emergence of variants to generate useful epidemiological data through large scale pathogen genome sequencing.[20] This had proved important in other viral epidemics such as the Ebola outbreak in the Democratic Republic of Congo and influenza, and in the case of SARS-CoV-2, genomic sequencing globally has revealed that the virus has mutated from its original reference strain SARS-CoV-2 Wuhan-Hu-1.[5,8,23,24] Thus, genome sequencing has played a crucial role in aiding epidemiological understanding of SARS-CoV-2 variants and in tackling the disease. One such success story in the area is The Coronavirus Disease 2019 (COVID19) Genomics UK Consortium (COG) that was launched in March 2020 to sequence SARS-CoV-2 in up to 230,000 people with COVID-19 disease.[25] This consortium has already contributed to finding varying lineages of SARS-CoV-2 within the U.K., such as the D614G spike mutation, the globally concerning the B.1.1.7 lineage, and the variant of concern (VOC2020/1201).[26−28] Moreover, in South Africa, genome sequencing revealed a new SARS-CoV-2 variant B.1.351 which has caused further concern as the rise of such mutations may lead to increased transmissibility and virulence of the virus in the future.[29]

Quantification of SARS-CoV-2 Viral Load. Infectious viruses such as SARS-CoV-2 are usually quantified by measurement of viral RNA using molecular methods.[30] While this does measure the viral titer, this does not measure the exact number of infectious virions within a given sample.[31] The most accurate method of measuring infectious viral load is through laboratory viral cultivation methods such as plaque assays and tissue culture infectious dose (TCID)50 end point dilutions.[32]

Plaque assays are quantitative assays that detect the number of infectious SARS-CoV-2 in a given sample. The assay is conducted via preparation of cell monolayers (cell culture) which are infected with concentrations of the virus in a serial dilution, and the number of plaques formed after infection are counted in plaque forming units (PFUs).[33] A plaque is formed when a virus particle infects a host cell, replicates, and then lyses the cell, killing it. Plaques become visible after several replication cycles.[32] The tissue culture infectious dose assays measure the ability of the virus to induce cytopathic effects (CPEs) in cell culture after infection. The infection is conducted via serial dilution. TCID50 is the unit of measurement which determines the amount of virus required to induce 50% CPE effects in susceptible cells.[34] Due to a lack of laboratory resources in clinical settings, however, the majority of viral load quantification is conducted via molecular methods.[34,35]

SARS-CoV-2 Diagnostics. Genomic sequencing and tracking emerging strains of virus yields useful information that can be used to develop new diagnostics and aid treatment and triage of patients with COVID-19 disease. Controlling the pandemic is not limited to high-income countries who have been able to afford vaccine rollout, and thus a key part of this is ensuring that clinicians and healthcare workers can diagnose such strains rapidly, sensitively, and specifically in patients who have active SARS-CoV-2 and transmissible infection.[5] While this is more likely in high-income countries, developing a low-cost, easy to use diagnostic device that is able to directly detect SARS-CoV-2 at the molecular level for use in low- to middle-income (LMIC) settings is integral to diagnose patients with COVID-19 disease.[36] The ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end users) criteria for diagnostics for use in low-resource settings should be applied in the context of the COVID-19 pandemic.[37]

Current SARS-CoV-2 testing predominantly involves two pathways; see Figure 2. Table 1 summarizes the key characteristics and limitations of current testing strategies. The first is direct testing of the virus in respiratory samples, which can be conducted via viral culture, detection of a protein subunit or via detection and/or amplification of nucleic acids.[38,39] The second relies on detection of SARS-CoV-2 antibodies.

Real-time, reverse transcriptase polymerase chain reaction (RT-PCR) is considered the most sensitive and specific assay for detection SARS-CoV-2.[30,40] The assay will detect the genes that encode the proteins that the virus is composed of, alongside an RNA polymerase RdRp which is RNA-dependent and encoded within a large open reading frame, ORFab.[5] Importantly, the viral genomes are contained within the virus capsid and must be released via RNA extraction prior to molecular detection. This is a key step in the detection

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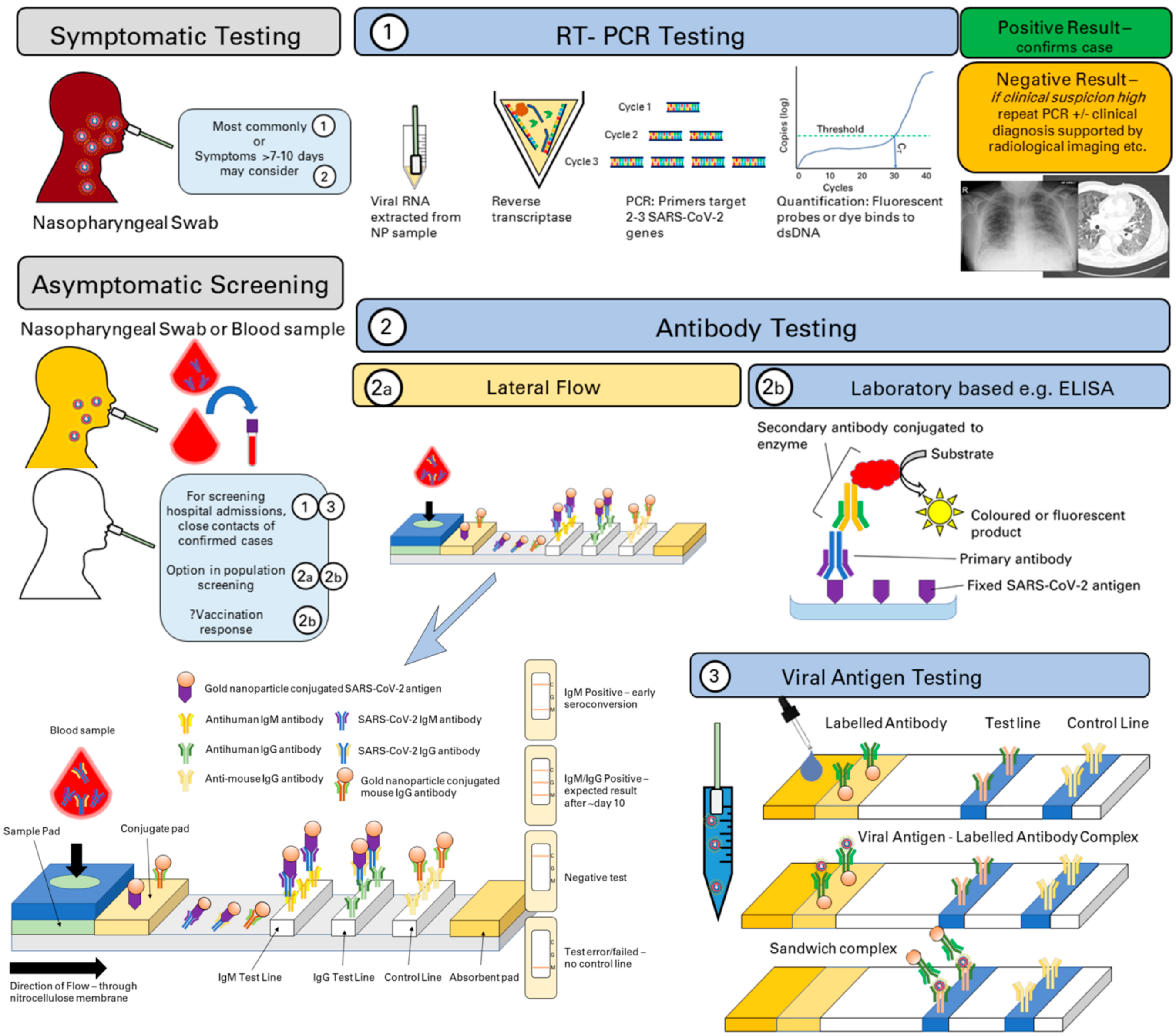

Figure 2. Summary of the main clinical testing strategies for SARS-CoV-2. (1) The current gold standard test in symptomatic patients is for a nasopharyngeal swab, on which RT-PCR is performed, using two to three primers targeting specific SARS-CoV-2 genes. Up to 40 cycles are performed; a lower cycle threshold (C_T) suggests a higher viral load. Cutoffs for a positive result vary between assays; typically 33−35 cycles are the threshold, with results reported as indeterminate if the threshold is reached after a greater number of cycles. (2) In patients presenting during or after the second week of symptoms, antibody testing may be a useful diagnostic tool. Antibody response can be assessed using either lateral flow tests or laboratory-based techniques such as ELISA, providing a quantitative result. (3) Antigen testing uses immunoassays to detect specific viral antigens. Nasopharyngeal swab samples are placed into the assays reagent. In lateral flow antigen tests, a sample is dropped onto the absorptive pad of the testing cassette and target viral antigens form a sandwich complex with colloidal gold or other labeled antibodies.

procedure and can increase the turnaround of results if being used at point of care (POC). However, a recent paper by Alexandersen et al.[5] found that detection of RNA from clinical samples may not be an appropriate indicator of active infection via RT-PCR as SARS-CoV-2 subgenomic RNAs could be detected from 11 days up to 17 days after initial detection of infection due to evidence of nuclease resistance and RNAs being protected by cellular membranes, which could lead to inconsistent PCR positive results.[5]

The viral load in patients increases within 5−6 days after onset of symptoms, with viral shedding occurring 2−3 days before the onset of symptoms.[4,41] The exact viral load in infected patients ranges between 641 and 1.43×10^{11} copies/mL, and a median of 7.99×10^4 in throat samples and 7.52×10^5 in sputum samples, respectively.[42] Thus, specimen collection from the nasopharynx, or an oropharyngeal swab must be conducted appropriately to capture the virus and subsequently detect it within the limit of detection (LoD) of the assay.[40]

Diagnostic laboratory testing to identify cases rapidly and track community spread are vital tools in gaining control of this current global health emergency. As of Feb. 1, 2021, over 1.3 billion tests had been carried out for SARS-CoV-2 (https://www.finddx.org/covid-19/test-tracker/).

Here, we review the current testing used in clinical practice and emerging diagnostic sensor technologies. We discuss the

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Table 1. Current Roles for SARS-CoV-2 Diagnostic Tests, Limitations, and Ideal Test Characteristics

	asymptomatic screening	symptomatic testing	convalescent testing and prevalence studies
primary objective	identify asymptomatic patients and inform inpatient and community isolation policies to halt viral transmission	diagnose active COVID infection, inform treatment decisions	identify infection rates at a population level assess population at risk of re-infection
characteristics of an ideal test	high sensitivity[a] rapid turnaround	high sensitivity	high specificity
main current testing strategy	rapid POC antigen testing ± RT-PCR	RT-PCR	antibody testing
time for result	5−20 min	40 min to 24 h (community transport dependent)	variable: lateral flow/POC testing, 15−20 min; blood sample, 1−24+ h
limitations of current test	low diagnostic accuracy necessitates RT-PCR if antigen test positive	false negatives during incubation phase false positives in convalescent patients	clinical uncertainty regarding future immunity and risk of re-infection

[a]Sensitivity is the ability of a test to correctly identify people with a disease, a highly sensitive test with a negative test result means a disease can be more confidently ruled out. Specificity is the ability of a test to identify patients with a disease. A highly specific test with a positive result means a patient can be more confidently diagnosed with the disease. While both characteristics are desirable for a diagnostic test, for the individual especially if presenting with acute symptoms a highly sensitive test may be more helpful as the consequences of incorrectly ruling out a diagnosis with a false negative result outweigh those of a false positive result. At a population level where the aim is to accurately identify only those who have or have had a particular disease, a more specific test is most desirable.

challenges that need to be overcome in order for laboratory sensors to become useful in the clinic and as rapid, accurate, and cost-effective (ASSURED) tests that are greatly needed for managing the pandemic.

■ CLINICAL ASSAYS

Quantitative Reverse Transcriptase−Polymerase Chain Reaction. Molecular tests rely on direct detection or amplification of nucleic acids. While the "gold standard" for SARS-CoV-2 molecular detection is quatitative reverse transcriptase−polymerase chain reactor (qRT-PCR), other molecular tests for SARS-COV-2 are also being developed and evaluated. Loop-mediated isothermal amplification (LAMP) has emerged as a viable alternative to qRT-PCR due its turnaround time from sample to result alongside maintaining its sensitivity and specificity without the use of a thermal cycler.[43] Lamb et al.[44] demonstrated detection of SARS-CoV-2 within 30 min using a modified reverse transcriptase LAMP assay which could be used at POC if required. Unlike qRT-PCR, the RT-LAMP method relies on a colorimetric readout, a thermal color change from red to yellow, when it is processed at 65 °C. This removes the need for more expensive processing equipment, using more simple methods to detect color change.[45] We also note here the use of colorimetric biosensors in association with nanoparticle interaction, which also provides a fast and reliable method of detecting SARS-CoV-2 with a red shift denoting the presence of viral surface proteins.[46]

The RT-LAMP assay was directly compared to qRT-PCR and performed optimally with a lower LoD of 0.08 fg, which is equal to 304 viral copies.[44] Lu et al.[47] also developed a RT-LAMP assay platform, named iLACO (isothermal LAMP-based method for COVID19) that is able to detect SARS-CoV-2 at a lower limit of 60 copies/μL in patient samples and, when compared to samples confirmed by qRT-PCR, demonstrated 97.6% sensitivity. A mismatch-tolerant RT-MALP assay has also been developed to detect SARS-CoV-2 where a high-fidelity DNA polymerase is used to remove the mismatched base pairs in the LAMP process.[47] The assay allowed improved accuracy of detection of 30 RNA copies of viral gene *RdRp* within approximately 40 min and showed 100% consistency when compared to the standard qRT-PCR assay. Finally, another RT-LAMP assay developed by Zhang et al.[48] demonstrated a LoD of ~120 copies or 4.8 copies/μL, which also shows the sensitivity and specificity of this assay. Thus, RT-LAMP assays are a suitable alternative to qRT-PCR assays.

Interestingly, combining RT-LAMP with CRISPR (clustered regularly interspaced short palindromic repeats) technology is another molecular diagnostic method that is gaining traction for detection of SARS-CoV-2. A study by Broughton et al.[49] demonstrated that SARS-CoV-2 could be detected by combining RT-LAMP with CRISPR-Cas12a in a lateral flow assay format within 40 min from respiratory swab RNA extracts. This CRISPR-Cas12 DETECTR technology demonstrated similar sensitivity and specificity to qRT-PCR assays, detecting 1×10^4 copies/mL; however, an RNA extraction step was required prior to utilization of the test.[49] CRISPR and Cas proteins identify and break down any foreign nucleic acids encountered by bacteria and archaea, acting as a form of adaptive immunity for these microorganisms. The degradation of these foreign nucleic acids is mediated via specific RNA molecules which activate the Cas nucleases to cleave any single-stranded DNA (ssDNA). Thus, the CRISPR Cas systems can be exploited for use in detection by use of ssDNA reporters that can detect this cleavage and generate a readout.[50] Huang et al.[51] also developed a similar qRT-PCR, CRISPR-Cas12a assay which was able to detect two copies of RNA per sample, which, when compared to the qRT-PCR, was unable to detect less than five copies of the RNA target.[51] Guo et al. have also developed an integrated viral nucleic acid detection platform- CASdetect (CRISPR assisted detection) for SARS-CoV-2.[52] The assay utilizes Cas12b-mediated DNA detection methods to detect a limit of 1×10^4 copies/mL of virus without cross-reactivity, similar to the previous studies described above. However, interestingly, authors recognized the need to liberate the RNA from the capsid and thus tested genome spin column extraction kits against lysis buffer kits, finding that lysis buffer kits would be more useful for POC testing (POCT) and spin columns useful for hospitals.[52] Authors also attempted to make the CASdetect more suitable for use at POC by developing a blue light box which could be

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used to determine the fluorescent signal generated in the presence of SARS-CoV-2.

Recently, Ning et al.[53] developed a chip-based CRISPR-Cas12a technique of saliva tests for COVID-19 detection. The system exhibits complete concordance with qRT-PCR results and is equipped with a smartphone-based microscopic device that may allow reduction of the infrastructure and expertise required to obtain ultrasensitive diagnostics tools.

POC and Lab-on-Chip Tests. Testing for SARS-CoV-2 within primary care and the community is essential to prevent further community transmission. This requires expansion of current testing capacity, and while this has been achieved somewhat by setting up SARS-CoV-2 testing laboratories,[54] this expansion requires accurate development and implementation of rapid POC tests for SARS-CoV-2. The diagnostic sensitivity of rapid POC molecular tests for SARS-CoV-2 must be comparable to those of gold standard qRT-PCR tests and have limited steps for ease of use (ASSURED diagnostics).[37] Another key stumbling block for development of such rapid POC tests is the first step of nucleic acid extraction, which can increase the duration of the POC itself. Moreover, high-quality clinical specimens are essential to enable detection of the virus in clinical samples.[38]

CovidNudge is an example of a novel, integrated gene detection system that utilizes RT-PCR in 90 min to detect SARS-CoV-2 without need for laboratory handling, thus negating risk to healthcare workers, and does not require sample preprocessing.[55] The assay is multiplexed to be able to detect seven viral targets (*rdrp1*, *rdrp2*, *e*-gene, *n*-gene, and n1, n2, and n3 genes) within a self-contained DnaCartridge that comprises a lab-on-chip device which enables a sample to result PCR reaction. The cartridge contains a staple preparation unit, with all buffers to extract and purify the RNA, and an amplification unit. The CovidNudge test was found to be able to detect SARS-CoV-2 with a specificity of 100%, and a sensitivity of 94% when compared to laboratory-based testing. However, the time taken for testing alongside the needed RNA extraction step, despite being integrated, may not be appropriate for use within a shorter period of time, such as within the time of a general practitioner's (GP's) appointment (~15 min) or when results are required immediately. This is where the majority of molecular POC diagnostics tests fall short.

Lab-on-chip technologies have also been used to detect SARS-CoV-2 and represent an efficient means of detecting the virus molecularly. Label-free electrochemical detection of SARS-CoV-2 was reported by Rodriguez-Manzano et al.,[56] where an ion-sensitive field-effect transistor (ISFET) was used and was comparable to testing with qRT-PCR; however, while the device was able to detect RNA within 20 min, the RNA from the samples had been extracted prior to testing and thus this does not represent a raw sample to result turnaround. Such devices are discussed in more detail in Virus Sensor Technologies.

Other promising POC technologies include use of nucleic acid and aptamer-based biosensors, such as plasmonics and localized surface plasmon resonance to generate detection signals. A good example of this is the use of dual function plasmonic biosensor to detect SARS-CoV-2 within clinical samples, as described by Qiu et al.[57] This biosensor is able to detect viral RNA sequences RdRp, ORF1ab, and the E genes from SARS-CoV-2 at a lower LoD of 0.22 pM in a multiplexed assay; the specifics of this device are discussed below. The use of plasmonics and DNA sequences to capture nucleic acid targets from varying microorganisms has been proven prior and represents a highly sensitive, specific, and efficient means of molecular detection of pathogens, but also uses microwave energy to liberate nucleic acids from the sample within seconds, rather than relying on other longer nucleic acid extraction methods. A POC device that could generate a highly sensitive and specific result, comparable to qRT-PCR, from raw sample, ideally within 15 min, would be immensely useful within the POC testing diagnostic sphere.

Despite the emergence of newer SARS-CoV-2 diagnostics, real-time reverse transcription has remained the gold standard worldwide in the diagnosis of COVID-19.[30,58−62] U.K. government data showed a total of 63.5 million RT-PCR tests for SARS-CoV-2 had been conducted between April 2020 and January 2021, with daily testing capacity rising 30-fold during this time, up to 800,000 tests a day (https://coronavirus.data.gov.uk/details/testing). The process involves reverse transcription of ribonucleic acid (RNA) and amplification of nucleic acid for subsequent analysis (NAATs). Corman et al. were the first team to finalize an international protocol and outline standards associated with the test.[30] They recommended using primers targeting one or several nucleic acids related to SARS-CoV-2.[30,58] These targets aimed to increase the sensitivity of the test to SARS-CoV-2 and minimize cross-reactivity with other known coronaviruses. The U.S. Centers for Disease Control and Prevention (CDC) assesses for two regions of the viral nucleocapsid gene N1 and N2 and for human RNase P gene (https://www.cdc.gov/), whereas, the World Health Organisation (WHO) tests for SARS-CoV-2 RNA-dependent RNA polymerase and envelope (E) genes.

The use of multiple primer/probes in the RT-PCR technique means that it is unlikely to be significantly affected by mutations seen in SARS-CoV-2 genetic variants. For example, the TaqPath RT-PCT COVID-19 test uses three primer/probe sets to different genomic regions (Gene Orf-1ab, N protein, and S protein), so, unless mutations occur at all three regions, variants are still likely to be detected. Indeed, the change in the signature of the response to one of the three primers used helped to raise suspicion of the British variant (B.1.1.7).[63] Once mutated variants are identified, RT-PCR tests can be rapidly adapted to include primers sequences to detect the new strain.[64,65] However, the likelihood of false negatives would be expected to increase with increasing number of mutation sites in techniques targeting fewer genomic regions.

There have been multiple systematic reviews on sample collection; a meta-analysis from Mohammadi et al. concluded that sputum samples were the most sensitive for SARS-CoV-2 detection with nasopharyngeal second and oropharyngeal the least effective.[62] However, given the dry nature of cough and reduced sputum production, the nasopharyngeal swab has become the gold standard internationally. There have been concerns raised about the false negative rate of the RT-PCR method with samples from the nasopharynx. Various studies have put its sensitivity between 61 and 70%. These results have been explained by inconsistent sampling methods, a variety of different and sometimes not standardized kits, difficulties in preparation methods, and shortages of correct transport medium. RT-PCR testing remains the primary diagnostic test in confirming a diagnosis of COVID-19 in symptomatic individuals presenting to healthcare centers. Limitations in

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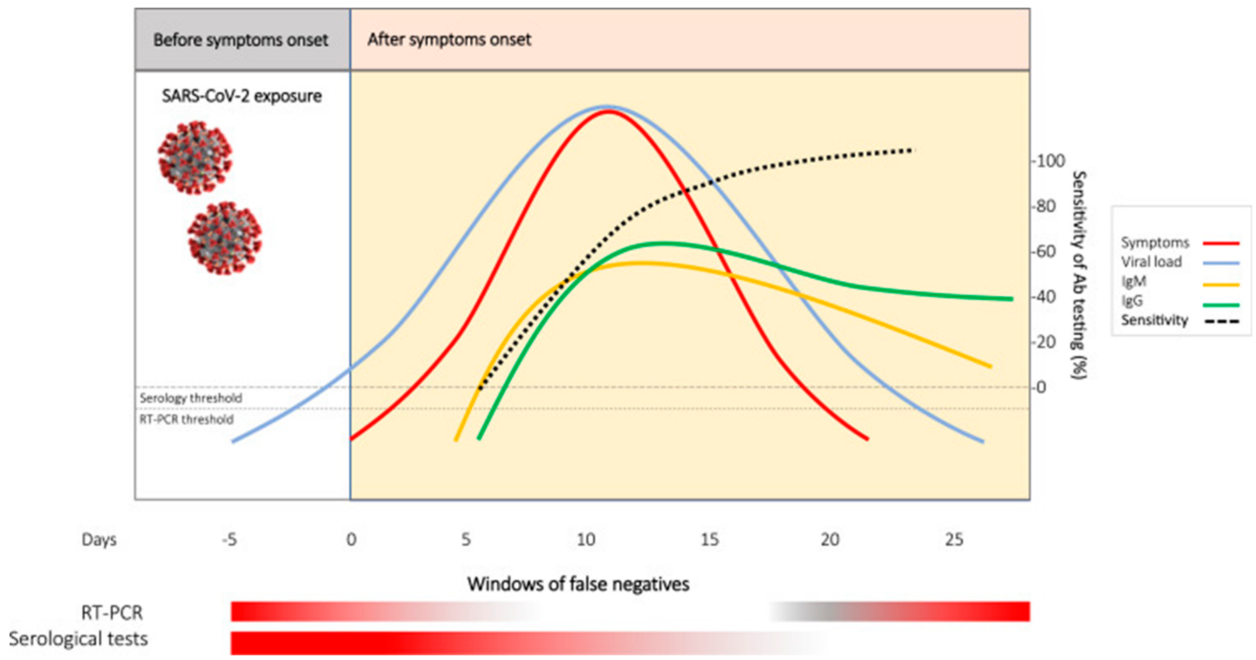

Figure 3. Time relationship between viral load, symptoms, and positivity on diagnostic tests. The onset of symptoms (day 0) is usually 5 days after infection. At this early stage corresponding to the window or asymptomatic period, the viral load could be below the RT-PCR threshold and the test may give false negative results. The same is true at the end of the disease, when the patient is recovering. Seroconversion is variable but may be as early as day 5 with a median between days 10 and 14; in the first week of the disease serological tests are more likely to give false negative results. The dotted black line in the graph illustrates the sensitivity of the chemiluminescent assay as derived from the data sheet of a commercial test (Abbott Diagnostics, USA). Ig, immunoglobulin; RT-PCR, reverse transcription-PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. Reprinted with permission from ref 58. Copyright 2020 Elsevier.

sensitivity result in the requirement for repeated testing in patients with presumed false negative results when there is a high clinical suspicion for disease and other supporting positive diagnostic features such as typical radiographic changes.

The other unclear variable is viral load. Most studies have concluded that it peaks within the first 2 weeks of infection. There is even less data and analysis of sensitivity in asymptomatic carriers or those with very few respiratory symptoms. This is a concerning pitfall as RT-PCR testing is being used to screen patients attending healthcare centers for elective procedures or with non-COVID-related acute presentations. With a high-community prevalence of asymptomatic infection and the possibility of transmission prior to symptom onset, there will be significant numbers of false negative tests among this patient cohort, leaving vulnerable hospitalized patients at risk of nosocomial infection.

There are also other pitfalls of this method. Despite the rapid emergence of miniturized RT-PCR testing capabilities to aid worldwide use of the test, some countries lack the laboratories and personnel to rely effectively and scale up to this form of mass testing. All of these factors have led to a shift in diagnostics to overcome these shortfalls.

Viral Antigen Testing. Coronavirus-19 antigens have been shown to be detectable in the serum, urine, and mucous membranes of patients with early COVID-19 infection. The presence of antigen, through rapid detection testing (RDT), holds promise as an effective strategy for the early diagnosis and isolation of confirmed cases.[66] Rapid COVID-19 antigen testing operates on the basis of lateral flow to detect viral antigen through immobilized coated COVID-19 antibody. This POC testing is appealing not only for its speed but also for its replicability and low cost. However, the use of RDT for other viral infections such as influenza has historically shown variable sensitivity and therefore been extrapolated to concern for rapid antigen testing in the diagnosis of COVID-19.[59] A recent Cochrane review conducted concluded that POC antigen testing for COVID-19 had an average sensitivity and specificity of 56.2% (95% CI, 29.5−79.8%) and 99.5% (95% CI, 98.1−99.1%), respectively.[67] The variability in the sensitivity of POC antigen testing has proven to be operator-dependent with lower results attained when testing is performed by nontrained personnel. Peto[68] suggests that further work is required to determine the level of training that nontrained personnel require to achieve optimal test performance. Furthermore, the false negative rates for rapid antigen testing are predominately confounded by the viral load variability during the course of a patient's COVID-19 illness.[58,69] Therefore, it is recommended that centers using large scale POC antigen testing to identify asymptomatic carriers should have all patients with negative results confirmed against COVID-19 RT-PCR to mitigate for potential false negatives,[70] limiting the clinical usefulness of such tests. Published data are lacking on the performance of lateral flow antigen tests in detecting novel strains, although a WHO statement reports no significant impact on performance (https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON305).

Serological Testing. Monitoring the humoral immune response to SARS-CoV-2 infection enables the diagnosis of COVID-19 in patients presenting during or after the second week of symptom onset. However, the clinical utility of such a test is limited, when RT-PCR testing is readily available and accessible within the first 5−7 days of symptoms and has a greater positivity rate (see Figure 3).[71] The low sensitivity of

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antibody testing in the first week of symptom onset means they have no role in early case identification to halt transmission. This reflects that the median time to seroconversion is between 12 and 13 days.[72] Antibody response remains detectable for about 4.5 months in a sampled healthcare worker population,[73] although symptomatic and more severe infections appear to produce higher antibody titers[74] and a prolonged immune response.[75]

In the subgroup of patients who present in their second week after symptoms onset, a not infrequent occurrence such as deterioration requiring hospitalization is common around 10 days; the adjunct of serological testing to RT-PCR improves diagnostic accuracy in COVID-19 diagnosis.[76]

Antibody testing for SARS-CoV-2 infection includes enzyme-linked immunosorbent assays (ELISA), chemiluminescence immune assays (CLIA), and lateral flow testing. The foundation for innovative diagnostics lists 625 immunoassay tests from 331 different manufacturers. 461 tests have received approval for use in the European Union (CE-IVD) and 42 U.S. Food and Drug Administration (FDA) Emergency Use Authorization (https://www.finddx.org/covid-19/pipeline). Clinical data assessing the diagnostic characteristics of these tests are available only for the minority,[77−82] and in some published data identification of the specific test used has been censored.[83]

Antibody testing typically identifies IgM and IgG antibody to SARS-CoV-2 either separately or in combination, IgM being the first level to rise and fall and IgG later to rise but sustaining a longer response.[84,85] Fewer tests use the IgA antibody. The majority of tests identify the antibodies made against the viral nucleocapsid (NC) protein, but antibodies against the spike protein may be more specific due to potential cross-reactivity with other coronaviruses of the NC protein.

A Cochrane review of 54 studies showed that the specificity of the antibody tests was consistently over 98% with similar results for IgA of 98.7% (95% CI 97.2−99.4), IgG of 99.1% (98.3−99.6), and combined IgA/IgG of 98.7% (97.2−99.4). The sensitivity of the tests showed greater variability; the combined IgM/IgG tests performing best at 22−35 days post symptom onset, 96.0% (90.6−98.3). POC serological testing had lower sensitivity than laboratory-based tests.[86,87] There are significant limitations in the studies assessing antibody diagnostic test accuracy. First, the majority of these studies were conducted in hospitalized patients and true COVID-19 cases defined on the basis of a positive RT-PCR positive test, risking inclusion of false positives and potentially underestimating test sensitivity.

The major use of antibody testing is in population level studies to assess infection prevalence and the impacts of public health policy;[88−90] thus most antibody tests are being carried out in a nonhospitalized and predominantly asymptomatic population. The characteristics of time to seroconversion and level of antibody response appear to be different between the severe infection hospitalized groups versus an asymptomatic population,[73,91] which makes the generalizability of the test characteristics assessed from hospitalized and symptomatic patient serum questionable.[92]

Most licensing of these tests for emergency use exemption requires a minimum standard of 98% specificity, but one study of 10 lateral flow antibody testing kits identified only 4 of these that met this standard.[93] When conducting mass screening of the population, specificity of greater than 98% may still not be sufficient for the test to be useful. The positive predictive value (PPV) of such a test when the prevalence is 10% is estimated at about 80%;[92] 1 in 5 positive results will be a false positive. Assuming a prevalence similar to that reported by the Office for National Statistics for the population of England, about 2%, the PPV of an antibody test approaches 50% (see U.K. Office for National Statistics, COVID 19 Infection Survery).

Antibody Testing Post Vaccination. Antibody testing has the potential role of testing vaccine effectiveness: identifying those who may require additional "booster" doses along with estimating the length of immunity conferred by vaccination. Future SARS-CoV-2 vaccine development and licensing may rely on this approach. However, this remains an area of much uncertainty and ongoing research. The mRNA vaccines (Pfizer-BioNTech[94,95] and Moderna[96]) and the Oxford/Astra-Zeneca vaccine[97,98] induce an antibody response to the receptor binding domain of the SARS-CoV-2 spike protein; antinucleocapsid (N) protein antibodies are only produced after native SARS-CoV-2 infection.[99,100] Measurement of the functional effectiveness of the antibody response to vaccination is via a plaque reduction neutralization technique (PRNT) which is labor intensive and expensive and requires level 3 biosafety as live virus is required.[101] Other surrogate/pseudotyped neutralization tests have been developed, but remain challenging to deploy on scale.[102] The correlation of quantitative antispike protein antibody levels to functional neutralization tests offers hope that antibody testing could be used reliably to assess vaccine response.[103,104] Results from a range of five quantitative serological assays showed good agreement in identifying those patients with a negative viral neutralization test (i.e., lacking functional protection postvaccination), but further standardization between serological assays and a larger data set is required before we can reliably interpret these results in a clinically meaningful way.[105] Laboratory-based serological testing using ELISA or chemiluminescence immunoassays (CLIAs) has been used in the majority of the studies investigating antibody response postvaccination; the utility of lateral flow tests or other POC modalities remains to be established.

Specimen Type. RT-PCR and viral antigen testing is most commonly carried out using nasopharyngeal or combined nasal and throat swab specimens. A recent meta-analysis taking nasopharyngeal swabs as the gold standard showed combined nasal and throat swabs had a sensitivity of 97%.[106] Alternative specimen samples using saliva or nasal only swabs showed reduced sensitivities of 85 and 86%, respectively, therefore missing 15% of infected cases. Anal swabs and stool samples have also been investigated, detecting viral particles shed into the gastrointestinal (GI) tract.[107] GI specimens have the potential advantage of extending the detection window of infection beyond that of upper airway swabs. However, clinically the emergence of antibody testing has thankfully filled this niche.

Current diagnostic testing relies on specimens that are uncomfortable and in some, e.g., infants or people living with dementia, may be challenging to obtain. Additionally while self-testing has become the norm for many people, healthcare worker sampling is superior but incurs additional cost and a personal protective equipment burden. Superior diagnostic performance versus RT-PCR using less invasive samples such as saliva or tongue swabs should be the goal for novel diagnostic technologies if there is to be a paradigm shift in diagnostic pathways.

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Table 2. Electrical, Mechanical, and Optical Virus Sensors Perspective for SARS-CoV-2 Detection[a]

device	virus, biomarker, receptor	sensitivity	advantages	disadvantages
		Electrical Sensors		
silicon nanowire sensor (Patolsky et al.[115])	influenza type A, anti-influenza A antibody	starting from single viral particles	high sensitivity, rapid test (3−20 min)	no crucial disadvantages
silicon nanowire sensor to analyze exhaled breath (Shen et al.[116])	influenza A (H3N2 and H1N1)	29 virus particles/μL (in 100-fold diluted exhaled breath condensate)	high sensitivity, rapid test (~1 min)	relatively long specimen preparation
double-etched porous silicon (Gongalsky et al.[117])	influenza A, no markers used	low	reusable, rapid test (>5 min)	low sensitivity and big volume of specimen required
electrochemical sensor* (Si MOSFET) (Xian et al.[118])	SARS-CoV-2, SARS-CoV-2 spike antibody or cTnI antibody	100 fg/mL (7 fM)	high sensitivity, rapid test (within 1 min)	no crucial disadvantages
graphene-based field-effect transistor* (Seo et al.[119])	SARS-CoV-2, SARS-CoV-2 spike antibody	16−16000 pfu/mL for cultured samples; 1:1 × 10^5 (242 copies/mL) for clinical samples	high sensitivity, big dynamic range of viral concentration, rapid test (within 10 min)	no crucial disadvantages
graphene with gold nanoparticles* (Alafeef et al.[120])	SARS-CoV-2, oligonucleotides (ssDNA) targeting viral nucleocapsid phosphoprotein	231 copies/ μL and LoD 6.9 copies/μL	high sensitivity, rapid test (<5 min)	relatively high cost
tin-doped WO_3/In_2O_3 nanowire biosensors (Shariati et al.[121])	hepatitis B, single-stranded DNA oligonucleotides	0.1 pM to 10 μM with limit down to 1 fM	wide dynamic range of detection of viral concentrations, rapid test (within ~1 min)	no crucial disadvantages
biosensor based on In_2O_3 (Ishikawa et al.[122])	SARS virus N-protein, antibody mimic proteins	sub-nanomolar concentrations	high sensitivity, rapid test (~1−10 min)	no crucial disadvantages
		Mechanical Sensors		
microgravimetric immunosensor (Owen et al.[123])	influenza A, anti-influenza A antibodies	4 virus particles/mL	high sensitivity, rapid test (10 min)	no crucial disadvantages
quartz crystal microbalances (Cooper et al.[124])	type 1 herpes simplex virus, anti-gD IgG monoclonal antibody	from single virions level to over 6 orders of magnitude	fast detection, high sensitivity, wide dynamic range of viral concentration	40 min for specimen incubation
		Optical Sensors		
1D photonic crystal (Shafiee et al.[125])	HIV-1 binding to anti-gp120 antibodies	10^5 −10^8 copies/mL	relatively wide dynamic range	diffusion-limited
nanoplasmonic biosensor chip* (Huang et al.[126])	SARS-CoV-2 pseudovirus binding to SARS-CoV-2 mAbs (monoclonal antibodies)	370−10^7 particles/mL	high sensitivity, rapid test (15 min), portable (can use a microplate reader and mobile phone)	no crucial disadvantages
SERS-based LFIA with magnetic nanoparticles (Wang et al.[127])	influenza A H1N1 virus and human adenovirus simultaneously binding to their complementary antibodies	50−10^7 and 10−10^7 PFU/mL, respectively	high sensitivity and wide range of concentrations detected; simultaneous detection of different viruses possible	relatively long test (30 min)
plasmonic photothermal and localized surface plasmon resonance biosensor* (Qiu et al.[57])	SARS-CoV-2 nucleotide sequences binding to complementary nucleotides	0.22 pM to 1 μM	high sensitivity and wide range of concentrations detected	relatively sophisticated and skill-demanding methodology
WGM microsphere (Vollmer et al.[128])	influenza A virions	~10 fM	rapid test (within 1 min), high sensitivity; possible detection without specimen special preparation	slow for detecting low concentrations unless microfluidics integrated

[a]Asterisks (*) mark technologies demonstrated for SARS-CoV-2 detection.

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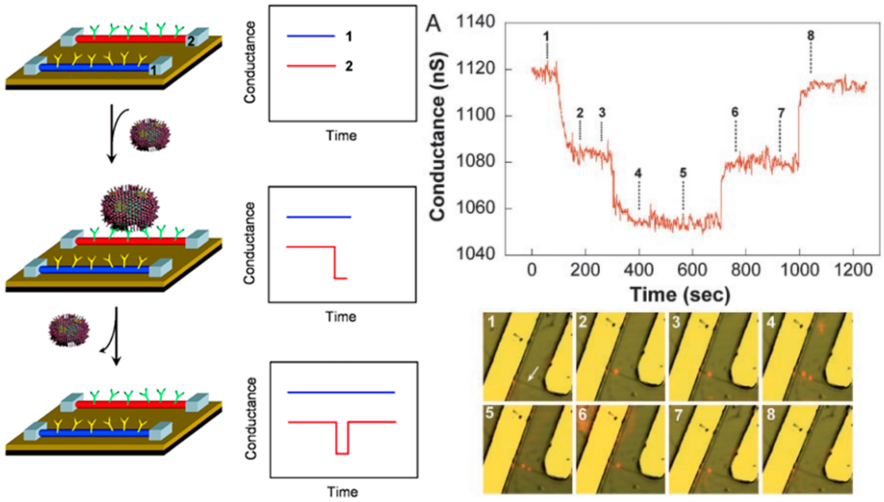

Figure 4. Nanowire-based detection of single viruses. (Left) Schematic shows two nanowire devices, 1 and 2, where the nanowires are modified with different antibody receptors. Specific binding of a single virus to the receptors on nanowire 2 produces a conductance change characteristic of the surface charge of the virus only in nanowire 2. When the virus unbinds from the surface, the conductance returns to the baseline value. (Right) Single virus binding selectivity. Simultaneous conductance and optical versus time data recorded from a single-nanowire device with a high density of anti-influenza type A antibody. Influenza A solution was added before point 1, and the solution was switched to pure buffer between points 4 and 5 on the plot. The bright-field and fluorescence images corresponding to time points 1−8 are indicated in the conductance data; the viruses appear as red dots in the images. Each image is 6.5 × 6.5 μm^2. Reprinted with permission from ref 115. Copyright 2004 National Academy of Sciences.

VIRUS SENSOR TECHNOLOGIES

The COVID-19 pandemic has revealed that novel SARS-CoV-2 sensors and detection methods are urgently needed, especially in developing countries where access to tests performed in clinical laboratories is limited. Factors such as cost, access, healthcare, complexity of assays, assay time, and difficulty in collecting the samples make widespread testing difficult. Electrical, mechanical, and optical biosensor devices can alleviate some of these problems, enabling more rapid and assured assays at a lower cost, with less sample preparation, meaning a clinical laboratory is not required. Biosensor devices are versatile and can be used for POC and home testing. As we show in Table 2, there are many different types of sensors that are useful for detecting virus material and viral particles, with potential for developing much-needed and improved sensing assays for SARS-CoV-2 detection. Recently, efforts have intensified to develop SARS-CoV-2 biosensor devices and to improve upon their accuracy and sensitivity. Current approaches show that it is possible to reduce the time for analysis and achieve a high detection sensitivity on platforms suitable for developing POC applications.

We identify three important steps for developing electrical, optical, and mechanical SARS-CoV-2 biosensor devices. The first step is the identification of a suitable biorecognition element, such as an antibody that is able to make a specific molecular interaction with the SARS-CoV-2 target. Examples include virus capsid protein, human angiotensin-converting enzyme 2 (ACE2)[108] and aptamers[109] that target SARS-CoV-2. The specific biomarker targets for detecting SARS-CoV-2 infection include human immunoglobulins and specific virus proteins such as the SARS-CoV-2 spike protein, which can be important to target mutations such as the D614G mutation.[109] The SARS-CoV-2 RNA genome currently serves as the key target for detecting the virus in most clinical assays that make use of RT-PCR.[110] In general, the analysis of protein samples typically requires a much larger amount of sample as compared to RT-PCR tests of the RNA genome.[110]

The second step in SARS-CoV-2 biosensor development is the identification of a suitable linker to immobilize the biorecognition element to the sensor. The biological recognition element has to be immobilized in such a way that the transducer produces a large signal output when the target biomarker/molecule interacts or binds to the bioreceptor. Ideally, bioreceptors are immobilized on the sensor in a stable, oriented, and reproducible way to enable a consistent sensor performance. Multiple linkers and various immobilization techniques for biosensors have been used in the past years: streptavidin−biotin interactions, electrodeposition, physisorption, and chemisorption (see, e.g., ref 111) are some of the examples. These immobilization techniques can provide dense and accessible monolayers of bioreceptors which are desired for more sensitive detection.

The third step in biosensor development is to optimize the signal readout and the signal analysis methods. Many different types of transducers find use for SARS-CoV-2 detection. They include plasmonic optical transducers such as surface enhanced Raman spectroscopy (SERS) based lateral flow immunoassays (LFIAs), mechanical transducers such as quartz crystal microbalances (QCMs), and electrical transducers such as nanowire or graphene-based field-effect transistors and electrochemical sensors. Various methods are employed to optimize their signals together with computational algorithms for improving the signal-to-noise ratio (SNR) and the LoD.[112] In the next subsections, we review the main optical, electrical, and mechanical virus sensor devices that are currently being investigated and that can be adapted for SARS-CoV-2 detection, before reviewing those sensors that have already been used for SARS-CoV-2 detection in the section SARS-CoV-2 Electrical, Mechanical, and Optical Detection Technologies.

Electrical Sensors. In this subsection, we review a class of sensors which operate by detecting virus biomarkers from changes in their electric characteristics, i.e., through current, conductance, resistance, or impedance measurements. We

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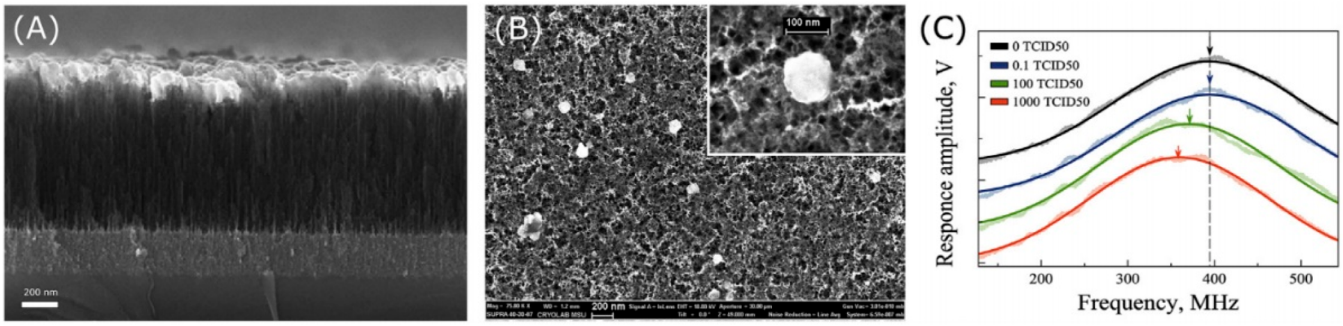

Figure 5. Double-etched porous silicon with gold electrode deposited: cross-section (A) and top view with a H1N1 virus particle (B). (C) Voltage amplitude versus frequency of the sensor depending on virus concentration. Reprinted with permission from ref 117. Copyright 2020 The Authors, published by Elsevier.

review the most recent advances in viral sensing, together with the seminal contributions to the field of electrical sensors.

The first electrical transducers reported in 2001[113] made use of conductance changes for real-time sensing of biological and chemical species. They were based on nanowire sensing elements made from silicon. This work set the stage for further developments and applications in this class of nanowire sensors, which is now being explored for SARS-CoV-2 detection.

Silicon-Based Electrical Sensors of Viruses. Work by Cui et al.[113] introduced the functional principles of how semiconductor nanowire sensors work in biosensing. Semiconductors exposed to different media can change their electrical properties, e.g., via shifts of the valence band away from the Fermi level, resulting in hole depletion and a reduced conductance.[114]

Binding of biomolecules to the surface of a nanowire leads to the depletion or accumulation of carriers in the "bulk" of the nanowire (versus only the surface region of a planar device). According to ref 113, the conductance response of silicon nanowire-based electrical sensors for biotin−streptavidin interaction can reach $\sim 10^{-8}$ siemens, which corresponds to a concentration range down to picomolar. This high sensitivity allows for single-virus detection, as we will discuss in the following sections. Also, the small size of a nanowire suggests that dense arrays of sensors could be prepared, facilitating real-time, rapid, and multiplexed detection of different biomarkers on integrated electrical sensor chips.

Lieber et al. used electrical biosensors based on nanowire field-effect transistors for direct and real-time detection of single particles of influenza A virus.[115] The sensor schematic is shown in Figure 4. The field-effect transistor with a nanowire-based sensing element, "gate oxide", demonstrated dependence of conductivity upon binding of single influenza A virus particles. Conductance changes up to −20 nS were observed for single influenza A virus binding events, producing a step in the conductance time trace easily visible against the noise of a few nS. Virus binding and unbinding events could be clearly observed from downward and upward steps in the conductance trace. To verify the binding/unbinding events, fluorescence imaging of single virus particles was performed in parallel and compared with the real-time sensing data, as shown in Figure 4. Results for the specific detection of influenza A virus and avian adenovirus group III were compared, with high selectivity demonstrated by using antihemagglutinin receptors for influenza A detection and antiadenovirus group III antibody for adenovirus detection. The antibodies were covalently attached to the nanowire sensor. For antibody immobilization, the nanowire device was exposed to an ethanol solution of 3-(trimethoxysilyl)propyl aldehyde, washed, and heated. After this step, the two sets of monoclonal antibodies were chemically linked via their amine groups with the aldehyde-terminated nanowire surfaces in a phosphate buffer solution. Device arrays for multiplexed experiments were made in the same way, except that distinct antibody solutions were spotted on different regions of the array.

Field-effect transistors, as well as three-electrode potentiometric and amperometric systems, have potentially wide application as sensors for the detection of pathogens because they are compatible with CMOS manufacturing techniques and, therefore, can be potentially integrated with other electronics and in portable devices. Already, this class of sensors has found use in combination with smart phones for detecting Zika virus.[129] Note that mobile phones are becoming one of the crucial nonpharmaceutical interventions, which have slowed down the epidemic in many settings; an interesting perspective article about the role of mobile phones in fighting the COVID-19 pandemic can be found in ref 130.

An important advance toward real-world applications of the nanowire sensor array was made by Shen et al.[116] In contrast to ref 115, they developed silicon nanowire sensors for influenza A (H3N2 and H1N1) virus in breath samples. This was possible by passing the liquid condensate obtained from exhaled breath onto the nanowires. The breath condensate samples were collected from a total of 19 human subjects with and without flu symptoms. The collection procedure involved the patient breathing through the mouth onto a cold hydrophobic surface for 5 min. The collected samples were further diluted for the experiments.

For sample delivery, they used a single channel with an inlet and outlet made of poly(dimethylsiloxane) (PDMS) and covering the entire nanowire sensor area (~4 μm wide). This breath-analysis sensor demonstrates a high sensitivity for detection down to 29 virus particles/μL of the 100-fold diluted condensate. The approach also demonstrated a high selectivity and accuracy: for 90% of cases, it was observed that samples tested positive or negative in accordance with the gold standard method qRT-PCR that was used as the control. The flu diagnosis with the electrical sensors took 2 orders of magnitude less time as compared to the RT-qPCR method.

Another class of silicon-based electrical sensors for virus detection is based on AC electrical impedance measurements. Influenza virus particles have been detected with double-etched porous silicon,[117] as illustrated in Figure 5. Porous silicon is a promising material for sensing due to its high surface area, which provides high surface/volume ratio for binding of viral particles to specific receptors, together with the tunability of pore morphology which can be important for the sample delivery. Fabrication of porous silicon is accessible with standard methods and mostly done by electrochemical etching.[117] The porous silicon is then covered with metal electrodes for impedance measurements with a vector network analyzer. The background conductance of the etched silicon is negligible, while impedance of the whole circuit is mostly governed by capacitance of the etched silicon and this provides enough sensitivity for the direct measurements of viral particle concentrations.

In experiments with the porous silicon sensors,[117] influenza virus strain A/PR/8/1934 (H1N1) stock solution, with initial concentration of about 10^7 TCID50, was used, which indicates 50% tissue culture infective dose in 1 mL. The stock solution was diluted in 0.9% NaCl to adjust the concentrations of viruses to 0.1 TCID50, 100 TCID50, and 1000 TCID50. The prepared 1 mL virus suspension was aerosolized with a nebulizer for 5 min into a closed volume in which the porous silicon sensors were placed; no biorecognition elements were used. Figure 5c shows voltage dependences on the AC oscillation frequency before and after adsorption of virions at different concentrations. The increase in the capacitance is caused by infiltration of viruses into the porous structure that leads to a shift of resonance frequency to lower values (about 30 MHz change). This sensor showed stable results after only 5 min exposure to the viral aerosol. A similar approach has potential for use in coronavirus detection. A simple advantage of this sensor is that it can be reused after washing in ethanol; measurements were reproducible at least 10 times.

Other Materials for Electrical Sensors of Viruses. Apart from silicon-based materials, tin-doped WO_3/In_2O_3 nanowires can serve as virus biosensors,[121] having detected hepatitis B virus. For this, single-stranded DNA oligonucleotides were covalently immobilized on the sensor to detect the hybridization of complementary DNA in a label-free approach through electrochemical impedance spectral measurements.

The same class of In_2O_3 sensors have been used to detect SARS-CoV virus N-protein.[122] Each of four major proteins (envelope protein, membrane protein, nucleocapsid (N) protein, and spike protein)[131] plays a role in the virus structure and is involved in the mechanism of the replication cycle. Long before the COVID-19 pandemic, authors of ref 122 introduced antibody mimic proteins in experiments of in vitro selection as a new class of biorecognition element for SARS biomarker N proteins in biosensors on the basis of the In_2O_3 nanowires. Upon binding of N proteins, changes in the electric current were registered. This platform was capable of specifically detecting the N protein at sub-nanomolar concentrations, in the presence of 44 μM bovine serum albumin as a background.

A gold nanoparticle-decorated graphene field-effect transistor has been proposed for the detection of biotinylated macromolecules with ultrahigh sensitivity and specificity.[132] The authors employed an avidin−biotin technology to demonstrate the specific detection of biotinylated proteins and nucleotides in the sub-picomolar range. The graphene/Au nanoparticles were fabricated on a Si substrate. Their sensing performance was characterized by real-time two-terminal electrical current measurement upon injection of the analyte solution into a silicon pool preattached onto the electrodes. The sensing capability of the composite was tested with the biotinylated protein A, with sensitivity of ∼0.4 pM achieved. By selection of corresponding linkers, this platform could be useful for coronavirus detection as well.

Mechanical Sensors−Quartz Crystal Microbalances. A QCM is a label-free technique that has been developed for several decades for biosensing applications. The key component of QCM is a thin quartz crystal that oscillates at a resonant frequency under applied voltage. In response to binding/unbinding events, the QCM frequency is decreased/increased in accordance with the Sauerbrey equation. These frequency changes are proportional to the mass of materials attached to the sensor. QCM sensors have potential for coronavirus detection with sensitivity down to units of virus particles per mL or nanograms per cm^2. QCM-D provides an additional characteristic for sensing, namely, the rate of dissipation. This characteristic is defined as the rate of oscillation coming to rest when the applied voltage was switched off. Thus, the dissipation rate could provide the rigidity of the materials attached; i.e., soft materials will tend to dissipate oscillations more readily.

Prior to the COVID-19 pandemic, QCM biosensors have been developed for the detection of influenza A (H_3N_2) virus particles.[123] The biosensor consists of a bioreceptor coupled to a QCM transducer that translates a biorecognition event into a measurable frequency-shift signal. Self-assembled monolayers of mercaptoundecanoic acid were formed on QCM gold electrodes to provide a surface amenable for the immobilization of anti-influenza A antibodies by using NHS/EDC coupling chemistry. Influenza A virions were aerosolized; for this, a nebulizer directly connected to a chamber housing the antibody-modified crystal, a so-called immunochip, was used. Upon exposure to the aerosolized virus, the interaction between the antibody and virus led to a change and damping of the oscillation frequency of the QCM sensor. The magnitude of the frequency change was directly related to the virus concentration. The LoD was estimated to be 4 virus particles/mL.

Another example of virus particle detection with QCM has been demonstrated by Cooper et al.[124] Type 1 herpes simplex virus has been chosen as the target. The virions were covalently attached to the microbalance surface and then detached by changing the amplitude of the QCM oscillations. Single virions were detected, on the basis of the registration of changes in the frequency of oscillations; however, the surface preparation is a complex multistage process. The microbalance surface was coated with a layer of chromium providing high-adhesive properties, a thick layer of gold for high conductance, and then a monolayer of mercaptoundecanoic acid to provide free carboxylic acid groups to the solution phase. These groups were converted to reactive *N*-hydroxysuccinimide esters and coupled to an anti-gD IgG monoclonal antibody to detect the 120 nm herpes simplex virus particle by binding the virus-specific membrane glycoproteins, including glycoprotein D (gD).

Photonic Sensors. The field of photonic biosensing has grown rapidly in recent years and presents many opportunities to expand COVID-19 testing rapidly and efficiently. Several biomarkers have been utilized for virus detection including viral genomic material,[57,133] surface membrane proteins,[126,134] and label-free detection of intact virions,[128,135] the former two of which require a receptor which differentiates viruses and immobilizes the target. A wide variety of sensing methods

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based on the effect of the interaction between target biomarkers and light are discussed, focusing on optical biosensors already proven to detect viruses including SARS-CoV-2 virions. Such methods provide direct, real-time measurements and high levels of sensitivity, as can be seen in Table 2.

Whispering Gallery Mode and Waveguide-Based Sensors. Individual intact viruses can be directly detected using whispering gallery mode (WGM) resonators (WGRs), which are facilitated by the trapping of light from a tunable laser by total internal reflection at a resonant wavelength in a spherical microcavity (Figure 6). Analyte binding perturbs the evanescent field of the mode resulting in a resonant wavelength shift. This reactive sensing mechanism has been used to detect the binding of single influenza A virions to the surface of a WGM silica microsphere resonator with LoD ∼ 10 fM, allowing an accurate lower bound estimate of virus mass and radius to be calculated.[128] Influenza A and SARS-CoV-2 virions have similar radii (∼100 nm); hence, this method holds potential for development in SARS-CoV-2 detection. Such a method has detected virions as small as MS2 virus, with a mass of ∼1% of influenza A through nanoplasmonic hybridization of the WGM at the microsphere surface. A plasmonic nanoparticle, such as a gold nanoshell, attached to the microresonator equator enhances sensitivity[136] via localized surface plasmon resonance (LSPR). The resonant oscillation of surface conduction electrons stimulated by photons increases electric field intensity, providing enhanced sensitivity within a small detection volume associated with the LSPR hotspot.[57,136] However, the ability to distinguish between viruses of similar mass, shape, and size usually requires functionalization with receptors to provide specificity.

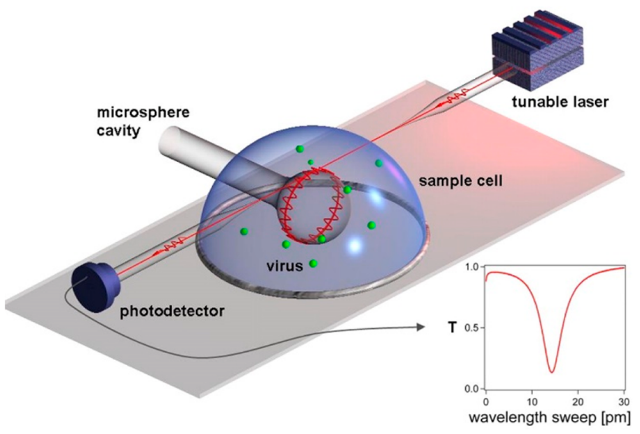

Figure 6. Experimental setup for a microsphere optical cavity, coupled to a tunable laser using a tapered optical fiber. Label-free detection of single influenza A virus particles was achieved using this WGM sensor. Inset graph demonstrates a resonant mode as a dip in the transmission spectrum. Single virus particles perturb the WGM resonance wavelength and shift the transmission dip. Reprinted with permission from ref 128. Copyright 2008 National Academy of Sciences.

Specific sensing using WGRs was demonstrated using thiol-modified nucleotides conjugated to gold nanorods on the surface of a WGM microsphere. The hybridization kinetics of nucleotides, including base mismatches in octamers, were detected with concentrations between 10 and 100 nM.[133] Cysteamine detection with LoD in the 100 aM range has been reported; hence, WGRs have potential as extremely sensitive photonic sensors.[137] The typical viral load in patient saliva has been reported to average between 10^5 and 10^6 SARS-CoV-2 virus particles per mL, corresponding to a concentration of ∼0.2−2 fM.[138] Currently, WGM-based techniques are often diffusion-limited; however, the integration of microfluidics using liquid core waveguides[139] has the potential to further lower LoDs and provides a gateway for transferring this technology into portable devices for POC testing. For example, Dantham et al.[136] utilized microfluidic channels to pump MS2 virus solution through a chamber containing a microsphere, and external referencing optofluidic microbubble resonator systems have been proven effective for specific detection of biomolecules such as D-biotin.[140]

Specificity and speed in detection are of the utmost importance if a sensor is to have potential use in widespread testing in a pandemic. A Mach−Zehnder-type optical waveguide made from sol−gel detected single influenza A virions with concentration of 100 μg/mL within 15 min.[141] The device used a single-mode fiber white light source coupled into the waveguide, with output power and wavelength monitored. Anti-H1N1/HA1 antibody was immobilized on one arm of the device, with the other arm left unfunctionalized. When influenza A virus particles were added to the arm hybridized with antibodies, virus−antibody binding resulted in a refractive index (RI) shift, with resulting power loss and wavelength shift detected by an optical spectrum analyzer. Wavelength and power of light passing through the unfunctionalized arm are unaffected, hence providing a reference measurement.[141] Reference measurements are useful to subtract background signals and reduce noise.[140]

U-bent optical fibers are another low-cost, portable method of photonic detection utilizing the change in RI resulting from a molecule binding to the sensor. The optical fiber is coupled to an LED or laser, with a spectrometer measuring the output light and subsequent increased absorbance upon molecule binding. The bend in the fiber increases the penetration depth of the evanescent field of light into the analyte, increasing detection sensitivity by an order of magnitude. Gold nanoparticles bound to the fiber surface further enhance sensitivity via the LSPR mechanism.[142] Huang et al.[134] used a similar approach to detect SARS-CoV nucleocapsid protein with LoD of 0.1 pg/mL. The LSPR electric field enhancement due to an AuNP on the fiber surface enhanced the fluorescence of a fluorophore bound antibody conjugated to the AuNP when excited by a laser beam. Using a low bandpass filter and photodetector, fluorescence intensity was measured when SARS-CoV nucleocapsid protein bound to its complementary antibody.[134] More recently, a plasmonic fiberoptic absorbance biosensor (P-FAB), with a similar design, has been developed on the basis of this technique.[143] This low-cost, portable system provides huge potential for point-of-care testing, as proposed by Murugan et al.[144] for COVID-19.

Surface enhanced Raman spectroscopy sensing. Aside from WGR and waveguide-based sensors, other optical mechanisms have proved effective as biosensing platforms. Raman spectroscopy is used to measure molecular vibrations, utilizing the inelastic scattering and consequential loss of energy of a visible or IR photon when it interacts with a molecule. Surface enhanced Raman spectroscopy (SERS) is a label-free technique that uses the plasmonic resonant oscillation of surface electrons in metallic nanostructures excited by the incident laser beam. This increases the electric field intensity, thus enhancing the intensity of the Raman

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spectrum of molecules adsorbed onto the sensor surface.[135] This technique has been demonstrated many times for virus detection: Shanmukh et al.[135] applied a variety of respiratory viruses to a silver nanorod array, with a 785 nm near-IR laser used for measurements, achieving LoD of 100 PFU/mL for respiratory syncytial virus. The relative intensity of peaks in the SERS spectra enabled the differentiation of influenza strains, with high reproducibility, and without the need for receptors to provide specificity because specific Raman bands arise from biomarkers such as nucleic acids and surface proteins that can identify a virus.[127,135]

More recently, SERS has been applied to enhance the sensitivity of lateral flow immunoassays, as can be seen in Figure 7, with the advantage of highly sensitive and real-time

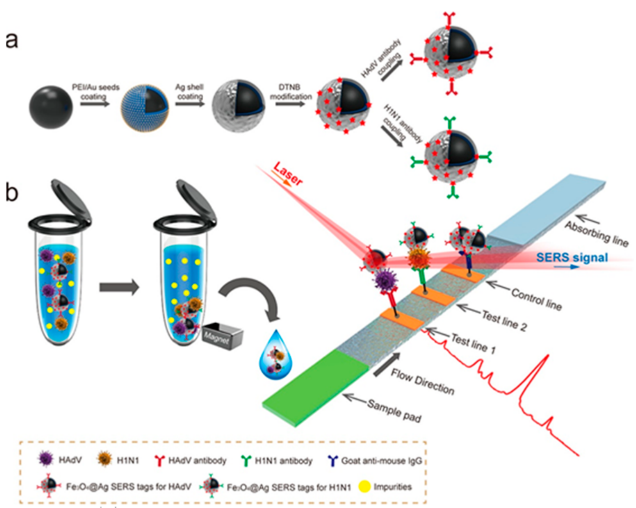

Figure 7. (a) Production method for Fe_3O_4@Ag magnetic nanoparticles functionalized with antibodies and (b) diagram of the Magnetic SERS LFIA with strips to detect H1N1 Influenza and Human Adenovirus.[127] A sample SERS spectrum is shown, which would only occur upon viruses binding to complementary immobilized antibodies. The spectrum intensity is proportional to virus concentration, with large differences in Raman intensity between strips with and without bound virus-antibody-nanoparticle complexes. Reprinted with permission from ref 127. Copyright 2019 American Chemical Society.

testing. Simultaneous detection of influenza A H1N1 virus and human adenovirus was achieved using a SERS-based LFIA, with magnetic SERS strips.[127] Unlike traditional SERS, a solution containing both viruses and their complementary antibodies conjugated to magnetic Fe_3O_4@Ag nanoparticles was prepared, with a magnet used to separate virions bound to their complementary antibodies from the sample solution. Once the virus−antibody−nanoparticle complexes were resuspended in 70 μL of running buffer, the enriched solution flowed over strips of immobilized antibodies specific to different viruses on the sensor. Viruses bound to their complementary antibodies on the strip surface, and the interaction of molecular bonds with IR light produced a SERS spectrum with intensity enhanced by the presence of Fe_3O_4@Ag nanoparticles. Sensitivity as low as 10 PFU/mL was recorded for human adenovirus, with simultaneous detection and differentiation of multiple viruses possible.[127] Moreover, a portable SERS multichannel LFIA was also developed by Xiao et al.,[145] with a similar Au nanotag−antibody−antigen−antibody sandwich utilized for SERS enhancement to detect three cancer markers down to 0.01 ng/mL concentration. The portability and ease of operation of this device opens avenues for its translation to POC testing.[145] SERS has been proposed for use in PDMS-based microfluidic devices that trap viruses, enabling rapid screening of asymptomatic patients;[146] hence, it has huge potential in future diagnostics.

Photonic Crystal-Based Sensors. A variety of periodic arrangements of dielectric materials at the nanoscale, called photonic crystals (PhCs), have been successfully utilized for virus sensing. One-dimensional PhC biosensors consist of periodic strips of dielectric material, which reflect a narrow resonant wavelength band when illuminated by a white light source, while other wavelengths pass through the device. This wavelength corresponds to that at which standing waves form in the device.[125] Shafiee et al.[125] utilized such a subwavelength grating coated in TiO_2 with immobilized anti-gp120 biotinylated antibodies to capture HIV-1 in serum and PBS samples with concentration between 10^5 and 10^8 copies/mL. Each binding event resulted in a resonant wavelength shift due to the virion altering the bulk RI. Similarly, Pal et al.[147] used a silicon 2D PhC coated with antibodies to detect human papillomavirus virus-like particles in 10% fetal bovine serum, with LoD of 1.5 nM. Such 2D PhCs have periodic variation in dielectric constant in 2D, so exhibit a photonic band gap (PBG). By changing the size of a single hole in the lattice, an optical cavity is created, with a resonant mode within the PBG. When light from a tunable laser source of wavelength ∼ 1.5 μm, at which silicon is transparent, illuminates the device, a dip at the resonant wavelength is observed in the transmission spectrum detected by a photodetector; this resonant wavelength shifts upon virus binding. PhCs tend to have smaller mode volumes than WGRs such as microspheres, making them more sensitive to environmental changes; multiple PhCs in series can also be used for reference measurements and multiple virus strain detection on a single device.[147]

The devices in refs 125 and 147 rely on diffusion of viruses to the sensor surface, which inherently increases measurement time; however, examples of PhCs with integrated microfluidics have been produced. Yanik et al.[148] developed an optofluidic nanoplasmonic biosensor, which detected viruses including pseudotyped ebola using antibodies immobilized on a nanoplasmonic array, with detection range of 10^6−10^9 PFU/mL. Plasmonic nanoholes couple normally incident light to oscillation of surface plasmons at resonant wavelengths, and an effect known as extraordinary light transmission results in increased transmission of other wavelengths, minimizing noise. Complex optical setups to couple light are not required due to the normal incidence of light. As for other devices, binding of viruses alters local RI, providing measurable red shifts in the resonant wavelength dip in the transmitted light detected using a spectrometer.[148]

Direct Imaging. The focus of this section is on direct sensing; however, it is also possible to use imaging to detect viruses and antibodies using techniques including fluorescence microscopy[149] and single-particle interferometric imaging.[150] Notably, a technique called plasmonic resonator absorption microscopy has been proven for detection of human IgG against SARS-CoV-2 with LoD of 100 pg/mL.[151] A 1D PhC coated with TiO_2 acts as a narrow bandwidth resonant reflector at 625 nm when submerged in aqueous media. Formation of a sandwich immunocomplex of COVID-19 IgG bound to antibodies immobilized on an AuNP, with the COVID-19 IgG then binding to spike proteins immobilized on

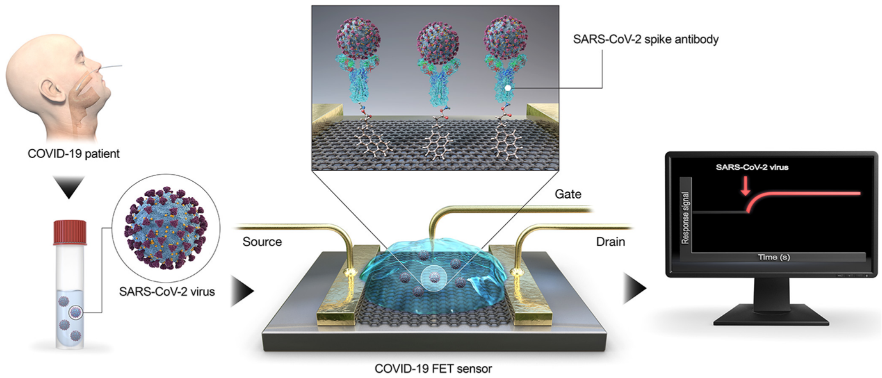

Figure 8. Schematic diagram of COVID-19 field-effect transistor (FET) sensor operation procedure. Graphene is a sensing material; SARS-CoV-2 spike antibody is conjugated onto the graphene sheet via 1-pyrenebutyric acid *N*-hydroxysuccinimide ester. Reprinted with permission from ref 119. Copyright 2020 American Chemical Society.

the PhC surface, increases light absorption when the surface plasmon resonant wavelength matched the PhC resonant wavelength. Reflected resonant intensity decreases in the locality of the AuNP; therefore, single particles can be observed and counted. Utilizing LED illumination and a 2D image sensor allows for portability of the device.[151] Such techniques for antibody detection will become increasingly important for testing vaccination efficacy.

Electrical virus biosensors have demonstrated a number of important advantages: direct instantaneous measurements of virus particles (in real time or within minutes); high levels of sensitivity down to single virus particles, i.e., using small amounts of analytes; chip-scale sizes; and compatibility with CMOS technology of integrated circuits.

Mechanical sensors have several advantages over their electrical counterparts; e.g., the authors of ref 124 claimed that sensitivity of such sensors approaches the single-virion level, while the ability to detect particles spans over 6 orders of magnitude. Also, they can be reusable if washed between assays. However, the incubation time for the virus (herpes simplex) was found to be 40 min, which is longer in comparison to measurements taken with other sensor systems. In this regard, photonic and plasmonic sensors represent the most powerful instrumentation with exceptional characteristics: rapid real-time tests and highest sensitivity, down to attomolar concentrations.

While all of these techniques provide promise for more sensitive and rapid detection methods in the future, they have not yet been translated for SARS-CoV-2 detection specifically. An ever-increasing number of papers are being published upon this however. In the next subsection, we analyze several successful examples of bridging the gap between sensors demonstrated to date and their applications in SARS-CoV-2 sensing.

SARS-CoV-2 Electrical, Mechanical, and Optical Detection Technologies. Last year, graphene for SARS-CoV-2 detection has been shown as a promising material for sensor transducers. Graphene is a two-dimensional sheet of hexagonally arranged carbon atoms. Graphene-based electrical virus sensors have garnered great attention because graphene has exceptional properties: high electric conductivity, high carrier mobility, and large specific area. Thus, graphene could be an alternative, e.g., for silicon nanowire based field-effect transistors, providing a potentially lower concentration response because the whole sheet of graphene is a sensitive element. Graphene-based coronavirus detection was demonstrated by Seo et al.[119] They introduced a COVID-19 diagnostic method using graphene-based field-effect transistors (Figure 8) and demonstrated the ability to make highly sensitive and instantaneous measurements using small amounts of analyte of SARS-CoV-2 in cultured and clinical samples.

The sensor was fabricated by coating the gate of the transistor made from graphene sheets with antibodies that were immobilized using 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (PBASE) and that were specific against the SARS-CoV-2 spike protein. For this, graphene was transferred to a SiO_2/Si substrate and soaked in 2 mM PBASE in methanol and then rinsed several times with PBS and deionized water. To immobilize the receptor, the device was exposed to 250 μg/mL SARS-CoV-2 spike antibody. Virus infection experiments were performed from African green monkey kidney Vero E6 cells infected with a clinical isolate of SARS-CoV-2 and with clinical samples collected via nasopharyngeal swabs from COVID-19 patients and healthy subjects. All samples were inactivated by heating at 100 °C for 10 min and were stored at −80 °C for further use. The inactivated viral samples were applied to the graphene sensor's surface. The graphene sensor responded to virus concentrations as low as 16 pfu/mL, and the response was linear up to virus concentrations that were 1000-fold larger. The same experiments were carried out using clinical samples. Sensing signals were obtained using nasopharyngeal swab specimens from COVID-19 patients, and the signals were clearly differentiable from those obtained from control samples of noncovid subjects. In addition, the sensor responded to patient samples diluted to as much as 1:1 $\times$ 10^5 (242 virus copies/mL). Furthermore, this sensor was specific and able to distinguish the SARS-CoV-2 antigen protein from MERS-CoV.

A graphene-based multiplexed, portable, wireless electrochemical platform for ultrarapid detection of SARS-CoV-2 has been developed in ref 152. The platform uses capture antigens and antibodies immobilized on mass-producible, low-cost graphene electrodes. This multiplexed platform tracks the infection progression by diagnosing the stage of the disease, allowing for the identification of individuals who are infectious, vulnerable, and/or immune. Differential pulse voltammetry and open-circuit potential−electrochemical impedance spectroscopy techniques have been employed to electrochemically detect biomarker targets such as the viral antigen nucleocapsid protein and IgM and IgG immunoglobins, as well as

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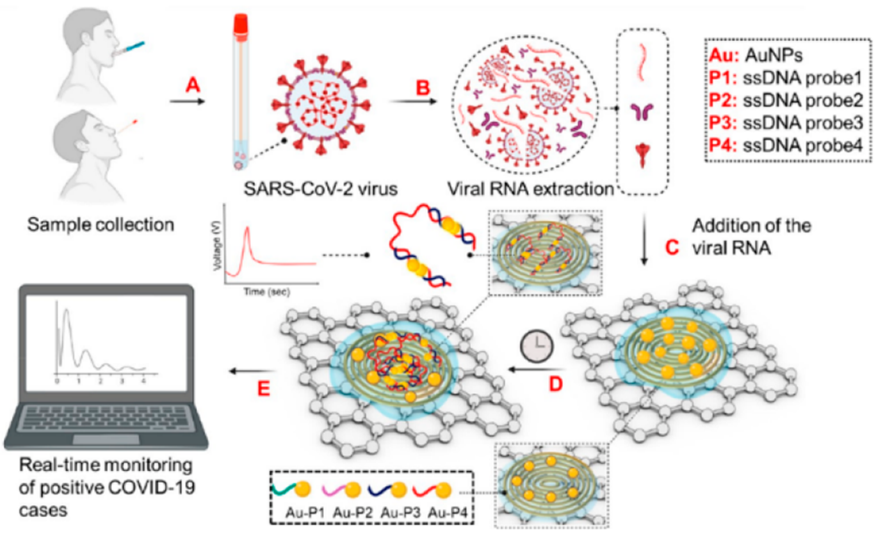

Figure 9. Schematic representation of the operation principle envisaged for the COVID-19 electrochemical sensing platform assembled on paper using graphene- and ssDNA-capped gold nanoparticles as the transducers, wherein step A: the infected samples will be collected from the nasal swab or saliva of the patients under observation; step B: the viral SARS-CoV-2 RNA will be extracted; step C: the viral RNA will be added on top of the graphene-ssDNA-AuNP platform; step D: incubation of 5 min; and step E: the digital electrochemical output will be recorded. Reprinted with permission from ref 120. Copyright 2020 American Chemical Society.

inflammatory biomarker c-reactive protein (CRP) in blood and saliva samples from COVID-19 positive and negative subjects.

A SARS-CoV-2 biosensor using graphene with gold nanoparticles has been developed by Alafeef et al.[120] This biosensor uses gold nanoparticles, capped with highly specific antisense oligonucleotides (ssDNA) targeting the RNA of the viral nucleocapsid phosphoprotein N-gene (Figure 9). The sensing probes were immobilized on a paper-based electrochemical platform. The biosensor coupled with an electrical readout setup selectively detected the presence of SARS-CoV-2 genetic material in Vero cells and clinical samples. The specific RNA−DNA hybridization led to a change in charge and electron mobility on the graphene surface, which resulted in an instant change in sensor output voltage. The thiol-modified ssDNA-capped gold nanoparticles improved the sensitivity of the electrochemical assay. The sensor response has been validated against RNA samples obtained from Vero cells infected with SARS-CoV-2, while SARS-CoV and MERS-CoV RNA has been used as one of the negative controls. This sensor also demonstrated good performance characteristics such as less than 5 min incubation time, a sensitivity of 231 copies/μL, LoD of 6.9 copies/μL, and almost 100% accuracy. Notably, the sensor is less affected by the genomic mutations of the virus because the ssDNA-conjugated gold nanoparticles simultaneously target two separate regions of the SARS-CoV-2 RNA sequence.

One other design for an electrical biosensor for COVID-19-related viral testing is an array consisting of eight gold nanoparticles linked to organic ligands.[153] This was integrated with electronic circuitry and an advanced apparatus that collects a 2−3 s exhaled breath sample. As the breath passes through the array of nanoparticles, a mixture of COVID-19-related volatile organic compounds reacts with the sensor causing changes in the electrical resistance. This produces a set of electrical resistance signals as a function of time. We do not analyze this method in detail because further validation studies, in differentiating patients with/without COVID-19 and other lung infections, are needed.

Another technology for SARS-CoV-2 detection, quartz crystal microbalances, can be proposed and use the same principle of sensing as was described—the sensor frequency can be changed under attachment of viral particles. For this, the recognition layer should be prepared on the sensor surface. A recent example of a proposal for implementation of engineered surfaces for SARS-CoV-2 detection was described by Pandey.[154] The author proposed self-assembled monolayers of hydrophobic and negatively charged groups which were intended to provide specific and strong interactions with spike proteins of coronavirus. On the basis of the biophysical chemistry of the spike protein, mixed COOH and CH_3 groups appear to be the most appropriate for the strong and specific attachment/binding of the spike S1 protein. To improve the specificity, antibodies (antispike glycoprotein such as immunoglobulin (Ig) and camelid heavy-chain antibody (VHH)) can be immobilized on the modified surfaces.

Optical biosensors based on surface plasmon resonance phenomenon have been used to detect a variety of respiratory viruses including SARS-CoV using genetic material as a biomarker.[155] Crucially, Qiu et al.[57] presented a plasmonic photothermal (PPT) and localized surface plasmon resonance (LSPR) biosensor shown in Figure 10a, capable of detecting SARS-CoV-2 nucleotide sequences at concentrations of 0.22 pM. In addition to the enhanced sensing capability enabled by LSPR discussed previously, PPT facilitates distinction between two oligonucleotides differing by a few base pairs, enabling distinction between selected SARS-CoV and SARS-CoV-2 sequences. When gold nanoislands functionalized with complementary nucleotides were irradiated by a 532 nm laser, hot electrons were generated whose energy dissipated rapidly as heat via the thermoplasmonic effect. Nucleic acid strands have a melting temperature, T_m, at which the DNA hybridized strands dissociate. Strands that are complementary have a higher T_m than those which differ by a few base pairs. Thus, the temperature increase produced by PPT means nucleotides with base pair sequence differing from the receptor nucleotide refrained from binding.[57] This provides the

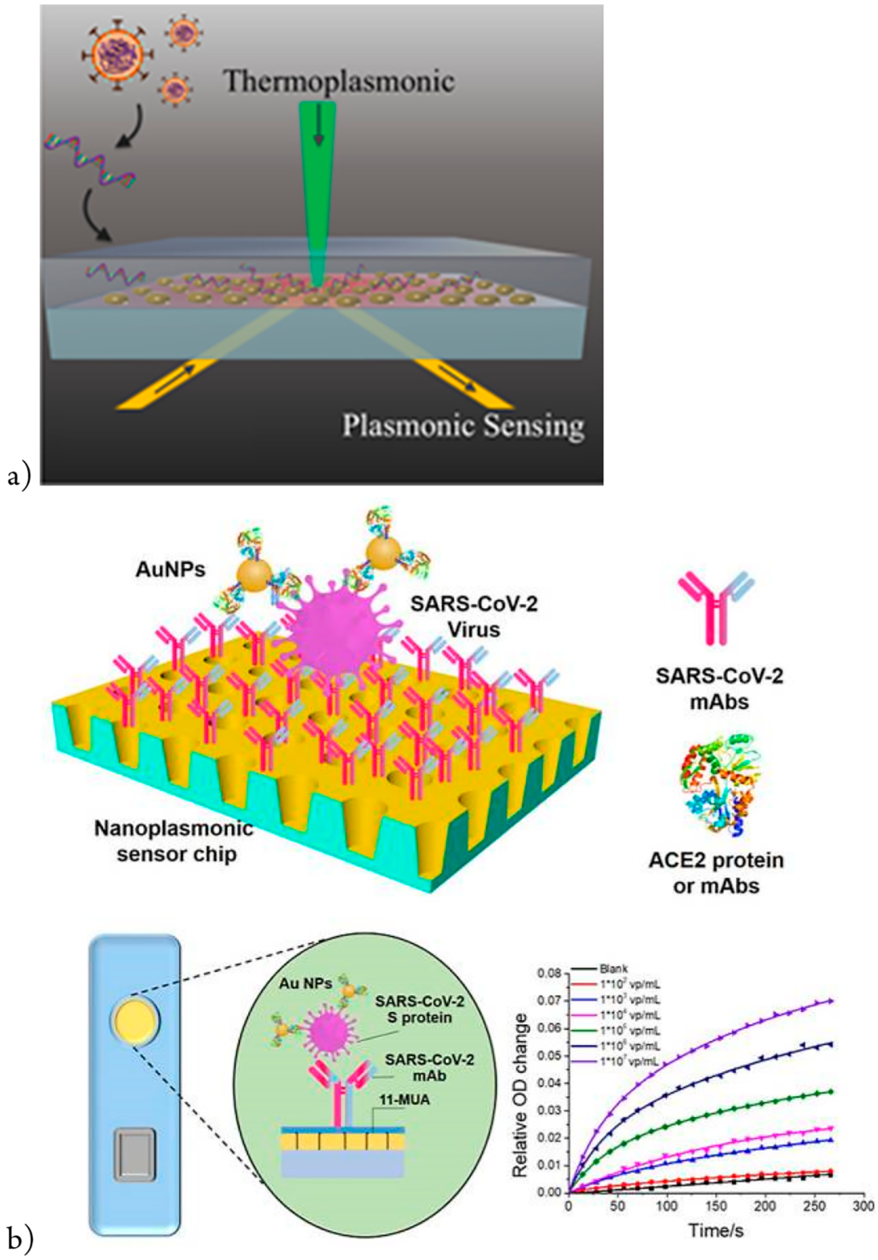

Figure 10. (a) Schematic of the LSPR and PPT biosensor. Gold nanoislands hybridized with nucleotides are irradiated by two sources for thermoplasmonic heating and plasmonic sensing, enabling detection of complementary nucleotide binding. Reprinted with permission from ref 57. Copyright 2020 American Chemical Society. (b) Nanoplasmonic sensing chip hybridized with SARS-CoV-2 monoclonal antibodies, to which a SARS-CoV-2 virion can bind, in itself bound to ACE2 protein conjugated to AuNPs for plasmonic enhancement. A cartridge containing such a chip for POC testing is shown, alongside a graph of relative optical density change demonstrating the wide range of concentrations this device can detect. Reprinted with permission from ref 126. Copyright 2021 from Elsevier.

potential for use distinguishing the mutant SARS-CoV-2 strains already discussed. However, conventional SPR equipment can be expensive and difficult to transport, making widespread POC use difficult, although it still has potential for laboratory-based processing of tests.

Another design, for a nanoplasmonic biosensor chip, has been used to detect SARS-CoV-2 pseudovirus with concentration as low as 370 vp/mL (vp = virus particles)[126] as shown in Figure 10b. A silicon plasmonic nanocup array chip coated with titanium and gold was produced, with SARS-CoV-2 monoclonal antibodies immobilized on the surface. AuNPs labeled with ACE2 protein were bound to SARS-CoV-2 virions, which subsequently bound to immobilized antibodies on the chip. In principle, the chip works as a label-free LSPR and photonic crystal biosensor with extraordinary optical transmission providing high sensitivity to local RI changes. Coupling between AuNPs and nanoplasmonic substrate enabled low-concentration measurements with enhanced optical signal intensity. A generic microplate reader at 640 nm wavelength was used to detect the change in resonant wavelength with virus binding via transmission measurements, as shown in Figure 10b. This makes for a far simpler design than that of Qiu et al.[57] Measurements could be taken in 15 min and processed using a smart phone connected to a POC

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device, providing easily transportable, low-cost, rapid detection which could be advantageous in resource-limited environments.[126] Moreover, Yoo et al.[156] recently produced a reusable SPR biosensor chip, which uses magnetic particles as a solid substrate for SPR. Once a sensing measurement is complete, a magnet removes these particles. This enables the device to be reused, with H1N1 influenza virus nucleoprotein detected more than 7 times without significant changes in signal. Such a technique has the potential to reduce the cost of SPR technology,[156] allowing widespread use in clinical settings.

The first steps toward devices for SARS-CoV-2 new generation sensing have already been taken. One example of biosensor hardware has been described by Xian et al.[118] They proposed a low-cost COVID-19 electrochemical sensor based on a Si metal-oxide-silicon field-effect transistor (MOSFET). As an example, the authors report detection of 100 fg/mL (7 fM) SARS-CoV-2 spike protein in saliva. They have succeeded in making use of a disposable sensor fabricated in-house for COVID-19 detection alongside a Si MOSFET-based sensor unit. Notably, electrochemical sensors exploiting potentiometry, voltammetry, coulometry, and electrochemical impedance spectroscopy have great potential for coronavirus detection via coronavirus-related proteins and detection of viral DNA.[157]

■ DISCUSSION

In the clinic, the standard assay used to identify an active infection of SARS-CoV-2 is a quantitative RT-PCR test. qRT-PCR LoD ranges between 60 and 300 viral copies/mL. qRT-PCR sensitivity is adequate for detecting most cases when the patient is infectious to others where one encounters a median viral load of 8×10^4 copies/mL. However, qRT-PCR tests encounter a window of false negatives early in the infection cycle when the viral load is low, and also later in the infection cycle when the virus may no longer persist in the upper airway. Overall, the success of a qRT-PCR test in detecting a positive SARS-CoV-2 case in the clinic is about 60%, leading to repeat testing to confirm cases if clinical suspicion for disease is high.

qRT-PCR false positives are often seen in patients who continue to shed viral RNA although viable viral particles cannot be cultured. Viral shedding usually occurs after COVID symptom onset with the obvious exception of asymptomatic patients. It is normal for this to occur for a mean of 17 days after infection, but continues in some patients for several weeks. This can cause confusion as one obtains technically "correct" positive test results, but these patients do not need to be isolated.[13] False negatives—anecdotally repeating three or four qRT-PCR tests in cases where clinical suspicion was high for a positive result—were not uncommon during the initial outbreak. These appear less common now as staff perhaps became more familiar with sampling, qRT-PCR tests improved by using multiple primers, and so on and our understanding of the time course of viral shedding has progressed. Evidence published early in the COVID-19 pandemic seems to support this with 30−60% positive results from RT-PCR in patients with suspected COVID-19. qRT-PCR seems to offer the most flexible testing platform that could adapt to mutant strains—although adaptable over weeks/months time frame—otherwise whole gene sequencing is required.

Molecular sensor tests identifying viable viral particles could overcome the problem of persistent positive results. Also, sensors could offer the multiplexed detection of mutants or one could offer specialized kits/sensors devices for each mutant. SPR and PPT devices[57] that detect specified nucleotide sequences using nucleotide hybridization have the potential for mutant strain detection focused on certain nucleotide sequences. However, SPR devices will most likely be used in a laboratory environment, rather than POC application. In future, if different treatments or differences in prognosis become apparent between mutant strains, a testing platform to differentiate and quantify the presence of viral strains in real time would be an invaluable clinical tool. High sensitivities for the detection of viral particles were already demonstrated with photonic devices, down to 10s−100s PFU/mL.[126,127]

A qRT-PCR replacement sensor test would need to offer faster turnaround times to enable the more rapid and repetitive testing that will be needed. Less invasive sampling by using saliva or even breath samples instead of the nasopharyngeal swab, which is uncomfortable, could improve sensor test uptake in and outside the clinic. Rapid sensor tests should ideally match or exceed the current LoD of qRT-PCR to enable the early detection of infection and the detection of infection in prolonged and asymptomatic cases. A number of photonic devices show promise for achieving this. They are potential candidates for replacing laboratory qRT-PCR tests, especially at the point of care; see Table 2. Already, optical sensor devices can provide extremely low limits of detection (nanoplasmonic devices achieve 370 particles/mL; graphene-based devices achieve 242 virus copies/mL), fast run time (many photonic-based sensors operate within 15−30 min on site), and a high dynamic range of detection (aM to μM). Plasmonic devices seem most promising for achieving high portability. The nanoplasmonic biosensor chip[126] requires only a point-of-care microplate reader and a smart phone to perform testing.

Lateral flow viral antigen tests currently lack diagnostic accuracy to be a single test in a diagnostic pathway. Lateral flow serology tests have only limited clinical value because they identify cases only after risk of infectious transmission. The result of these tests is highly dependent on the developed immunity and the length of the immune response. The often high rate of false positive (and negative) test results needs to be addressed. Laboratory-based serological tests have similar issues to lateral flow tests, but improved diagnostic accuracy, and there is supporting evidence that they have benefits in public health.[158]

Developing novel sensor-based lateral flow test devices to check the effectiveness of vaccines could become extremely useful. However, more evidence is needed that such tests can identify spike protein antibodies which seem to correlate best with viral neutralizing studies. Here, more clarity is needed from the manufacturers of the lateral flow tests on validation data. Lateral flow tests and other POC tests can have a rapid turnaround and would be ideal antigen tests. Most lateral flow tests have a lower sensitivity, suggesting that improvement on the scale of orders of magnitude in LoD is needed for many of the devices to become deployed in the clinic. The currently high LoD together with a limited specificity of these tests means that they are not yet fit for this purpose.

Other sensor techniques that directly detect virus particles could become important. Already, SERS lateral flow immunoassay with magnetic nanoparticles have demonstrated LoDs of 50 and 10 PFU/mL for influenza and adenovirus, respectively.[127] The intensity of the output spectrum is proportional to viral load, so identification of early stage infection may be possible. These sensors could be used to identify positive cases

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in the incubation period and could open potential for even more successful treatment pathways. The sensors would allow drugs preventing viral entry into cells to become of use at the early stage of the infection in a way similar to postexposure prophylaxis in HIV infection.

Private healthcare companies are charging £65 for an antibody test and £125 for a qRT-PCR test. The cost of a lateral flow antibody test is approximately $5 USD (e.g., BinaxNOW COVID-19 Ag Card). Ideally, to ensure future ASSURED sensing throughout the world, we need more affordable tests, with the potential for free tests to be made available in third world countries. Sensor approaches such as reusable SPR chip[156] with magnetic nanoparticles that can be removed after each measurement could help reduce the cost of using SPR for POC, with chips useable for different target viruses. Fortunately, antibodies are robust, can be stored at 2–30 °C, and are stable for use at room temperature 15–30 °C, so portability of sensors can be high.

A key challenge in developing in vitro novel diagnostics tests, especially at POC, is adoption of the technologies by stakeholders such as the National Health Service (NHS) in the U.K. or by the Food and Drug Administration in the USA. For U.K. adoption, the Medicines and Healthcare products Regulatory Agency and the National Institute for Health and Care Excellence must be informed of the technologies, with the aim of the in vitro diagnostic device (IVD) being externally validated to determine whether it meets appropriate sensitivity and specificity. This includes meeting the minimum requirements of the positive predictive value—an essential measurement of diagnostic capability and negative predictive value tested against an appropriate patient population to achieve regulatory conformity and expedite NHS adoption of the technology. The time taken for IVD adoption within the NHS has been approximately 10 years (https://www.bivda.org.uk/).

For the CE Marking to Directive 98/79/EC on in vitro diagnostic medical devices, contact with the MHRA is required for the Registration of the Device and accessories and then to move toward adoption via the Health Technologies Adoption Programme (HTAP), now coordinated by NICE. Assessing the Technology Readiness levels (TRL) of the IVD from 1 to 9 is of importance throughout before use in clinical practice and commercial translation, which also requires commercial partners and a business plan for implementation. Significant funding is also required to achieve commercial translation in order to reach TRL9.

Clinical necessity has driven rapid development of diagnostic testing for SARS-CoV-2. However, what we have seen has been rapid upscaling and adaptation of existing technologies rather than a diagnostic revolution. It is understandable that with timelines in adopting novel diagnostic technologies historically averaging 10 years, research and development funding has focused on established diagnostics rather than novel sensor technologies.

There is much optimism but uncertainty as to the impact of vaccination programs on the SARS-CoV-2 pandemic. Further outbreaks and possible seasonal waves are anticipated, and as such there may be an ongoing need and sufficient time for sensor diagnostics to develop to be ready to be integrated into clinical testing pathways. However, after the initial surge to establish testing pathways and investment in infrastructure, we may see a fatigue and inertia to innovation with the status quo viewed as adequate. Indeed existing RT-PCR techniques sets a high bar to overcome with high diagnostic accuracy, increasingly rapid turnaround times, scalability, falling costs, and detection at low viral loads. Sensor diagnostics will need to offer a paradigm shift or fulfill a critical niche to supplant molecular or immunoassay testing. Examples of such niches are in patients with suspected false positive RT-PCR tests during their convalescence; rapid testing for mass screening, e.g., crowds; testing using less invasive specimens; and to identify patients with or at risk of persistent COVID-19 symptoms ("post-COVID-syndrome"). Further enhancing the uptake of sensor-based COVID-19 screening methods may require the introduction of assays that are higher-throughput, especially when compared to RT-PCR. A typical strategy here is to adapt fabrication methods for sensors in array format and finding ways for integrating them with suitable high-throughput microfluidics.

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Notes

The authors declare no competing financial interest.

VOCABULARY

Biosensor, an analytical device, used for the detection of a biological/chemical substance that combines a biological component (receptor) with a physicochemical transducer; coronaviruses, the family of viruses that can cause illness in humans and animals, seven different types having been found to infect people, including SARS-CoV-2 responsible for the COVID-19 pandemic; COVID-19, the contagious disease caused by SARS-CoV-2; lateral flow devices (LFDs), one of the tools that can be used to support the diagnosis of COVID-19, the LFD detects a COVID-19 antigen that appears in the sample of a person that is or was infected with SARS-CoV-2; PCR, the polymerase chain reaction used to rapidly synthesize copies of a specific DNA, allowing one to take a sample composed of few DNA molecules and amplify a specific DNA

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sequence; SARS-CoV-2, the severe acute respiratory syndrome coronavirus 2

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