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Review

A Review on Potential Electrochemical Point-of-Care Tests Targeting Pandemic Infectious Disease Detection: COVID-19 as a Reference

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Abstract: Fast and accurate point-of-care testing (POCT) of infectious diseases is crucial for diminishing the pandemic miseries. To fight the pandemic coronavirus disease 2019 (COVID-19), numerous interesting electrochemical point-of-care (POC) tests have been evolved to rapidly identify the causal organism SARS-CoV-2 virus, its nucleic acid and antigens, and antibodies of the patients. Many of those electrochemical biosensors are impressive in terms of miniaturization, mass production, ease of use, and speed of test, and they could be recommended for future applications in pandemic-like circumstances. On the other hand, self-diagnosis, sensitivity, specificity, surface chemistry, electrochemical components, device configuration, portability, small analyzers, and other features of the tests can yet be improved. Therefore, this report reviews the developmental trend of electrochemical POC tests (i.e., test platforms and features) reported for the rapid diagnosis of COVID-19 and correlates any significant advancements with relevant references. POCTs incorporating microfluidic/plastic chips, paper devices, nanomaterial-aided platforms, smartphone integration, self-diagnosis, and epidemiological reporting attributes are also surfed to help with future pandemic preparedness. This review especially screens the low-cost and easily affordable setups so that management of pandemic disease becomes faster and easier. Overall, the review is a wide-ranging package for finding appropriate strategies of electrochemical POCT targeting pandemic infectious disease detection.

Keywords: electrochemical biosensor; point-of-care testing; diagnostic tools; COVID-19; SARS-CoV-2 detection; portable analyzers; smartphone; surveillance

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1. Introduction

Precluding human-to-human transmission of a pandemic disease at the early onset of an outbreak crucially needs quick point-of-care testing (POCT) at all levels of the community in the format of a personalized or decentralized as (Figure 1) [1–4], because faster infectious disease diagnosis creates opportunities for faster treatment. POCT is a rapid diagnostic approach performable at homes, offices, or nearby clinics without the assistance of trained personnel using just low-cost and easily accessible/operated diagnostic devices or setup staff. Point-of-care (POC) testing simplifies the pandemic management to some extent, not only by minimizing the test load at the centralized laboratory but also by generating room for hastening the public health-related policymaking (Figure 1); this was agreed upon by the World Health Organization (WHO) global research and innovation forum sitting in an assembly at the Geneva, February 2020 where the experts included the rapid POC test at

the number one position in the list of eight research priorities to address the COVID-19 pandemic situation [5].

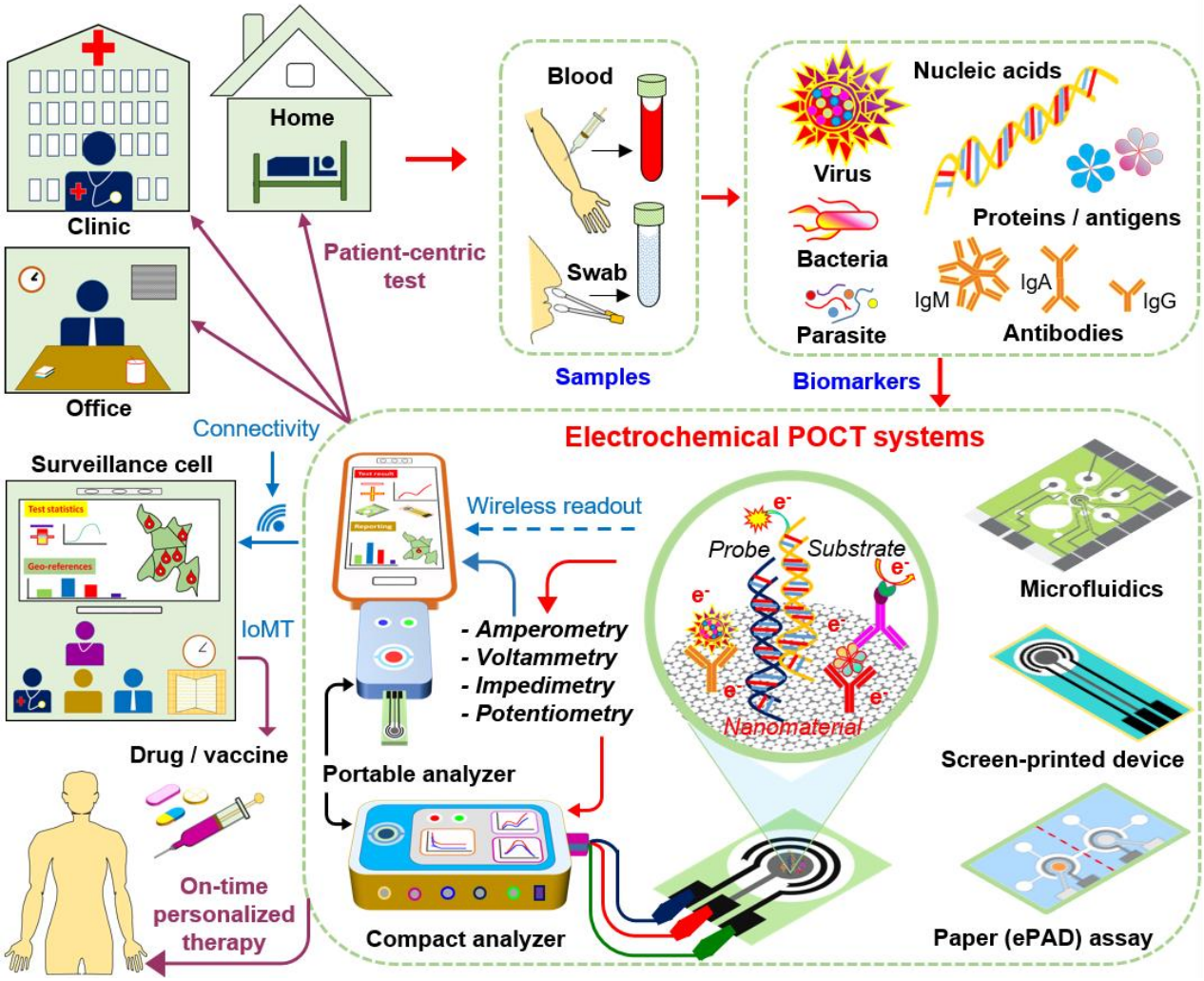

Figure 1. Schematic of electrochemical point-of-care testing (POCT) systems. POCT platforms (for example, compact/portable diagnostic analyzers, microfluidics, lab-on-chips/screen-printed devices, and paper-based assays) facilitate patient-centric (or decentralized) diagnosis of infectious diseases by detecting a variety of biomarkers (e.g., virus particles, bacteria, nucleic acids, proteins/antigens, and antibodies) at homes, offices, or nearby clinics without trained staff so that the on-time personalized therapy can be approached. The connection between POCT systems and surveillance cells enables epidemiological reporting during the pandemic epoch.

The rapid blowout of COVID-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [6,7], since its first appearance in Wuhan City (Hubei Province, China) in late December 2019 [8,9], impacted almost every corner of the globe (222 countries), accompanying millions of confirmed infections (404.9 M) and deaths (5.78 M) as of 11 February 2022 [10], coupled with the frequent lockdown of civilizations and enormous economic encumbrance, which mainly unveiled the diagnostic gaps to fight the new contagious pathogen.

SARS-CoV-2 has defeated other epidemic-causing coronaviruses, for instance, Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) in the race of community transmission [6–9,11–13]. Numerical simulations exposed the impact of delayed diagnosis that can increase the basic reproduction number (i.e., the average number of secondary cases generated by SARS-CoV-2), markedly upsurge the transmission risk, and lengthen the peak time and value of newly confirmed cases, thus producing an

enlarged number of total infections, enough to create an endemic of COVID-19 that cruelly exerts pressure for preclusion measures, i.e., isolation, quarantining, diagnosing, and treatment of patients [12,13]. As a consequence, WHO inclined to increase the frequency of diagnostic tests for defeating COVID-19 by promoting "test, test, test" to the respective authorities [14].

Relying on the sensitivity of PCR, WHO suggested adopting real-time reverse-transcription PCR (RT-PCR) tests at the onset of the COVID-19 outbreak in January [15,16] after the SARS-CoV-2 genome was explored [17,18]. However, the RT-PCR test procedure is multifaceted and pricey, and it is usually performed in centralized laboratories with highly expert personnel expending approximately 4–6 h of completion time. Additionally, the higher consumption of reagents may create a critical time delay to produce the report, when mass testing of people is approached. Furthermore, errors in preanalytical handling and testing stages resulted in some false-prone results [19]. Therefore, rapid, cost-effective diagnostic tests with high specificity and sensitivity that could provide a better room for screening true victims of SARS-CoV-2 infection, management of isolation, and quarantining were exigent to halt the COVID-19 outbreak [20].

Biosensors, which are represented as coherent analytical devices that use a biorecognition element to attach an analyte and adopt a transduction process to detect that signatory event, have significant clinical benefits due to their greater specificity, sensitivity, and low cost of deployment while performing POC diagnostic tests. This is especially apparent when the biorecognition event is transformed into electrical signals via electrochemical transduction techniques in the presence of suitable electrodes. POC electrochemical biosensors that use specific bioaffinity tests and produce sensitive, fast, easy, and affordable electroanalytical procedures could help patients recover faster by enabling early disease diagnosis and treating them quickly [21–24].

Utilizing the past diagnostic experiences in controlling a pandemic situation and balancing the gaps, several worthwhile electrochemical POC tests employing microfluidic labs-on-chip (LOCs), screen-printed electrode (SPE)-based strip devices, and/or electrochemical paper analytical devices (ePADs) have been projected for nucleic acid and immunological/serological testing for SARS-CoV-2 detection in the last 2 years (2020 and 2021) since the outbreak of COVID-19. Electrochemical diagnostic methods have received wide attention because of their simplicity, low cost, miniaturization, and sensitive analytical capabilities. Consequently, a few reviews on electrochemical biosensors were periodically published highlighting subjects such as overall perspective studies [25–27], a systematic review [28], emerging nanomaterials [29,30], immunosensors [31], detection and inactivation [32], artificial intelligence (AI) and the internet of medical thing (IoMT) [33,34]. Many of them covered electrochemical sensors from previous investigations and highlighted a few of the amazing electrochemical tests reported for SARS-CoV-2 detection. In addition, it is required to assess if the already reported electrochemical biosensors for COVID-19 diagnosis are adequate for usage in future POCTs. As a result, there is still room to track the development of POC electrochemical biosensors on the basis of POC attributes (discussed later), find gaps (if any), and articulate future directions for electrochemical POC diagnosis of infectious diseases in pandemic-like emergencies.

In this paper, therefore, we review the simple, low-cost, easily accessible, effortlessly performable, portable, and fast POC nucleic acid and immunocomponent-based electrochemical diagnostic systems with miniaturized chip-based (i.e., micro-device/LOC/SPE-based strip), paper-based (i.e., ePAD/μPAD), and other platforms (including nanomaterial-assisted sensors) that were recently adopted for diagnosis of COVID-19. Some useful strategies for detecting other infectious diseases biomolecules are also outlined to strengthen the current POCTs. We highlight portable analyzers and smartphone-integrated POC diagnostic tests, self/remote diagnosis, and disease surveillance (i.e., epidemiological reporting/contact tracing), which can simplify the readout of detection assays and surveillance in future episodes of a pandemic. Lastly, we summarize the contents and talk about the future preparedness regarding the development of efficient and fast POCT systems

targeting pandemic management. We believe that this article will be a worthy repository for the diagnostic seekers and developers to quickly determine the appropriate type/strategy of electrochemical POC diagnostics required to maintain efficient control over disease spread in upcoming pandemics (if any).

2. Basics of Electrochemical Biosensors

The electrochemical biosensors convert/transduce the biochemical reactions/interactions on analytical devices into the electrical outputs such as current, voltage, or impedance (Figure 1) [21,23,24]. Electrochemical biosensors have several merits such as simplification of instrumentation, higher sensitivity, affordability, and suitability for miniaturization, which generate ample opportunities for POC diagnostics [1,21,24,30,35].

The electrochemical biosensing platform usually comprises a three-electrode system, i.e., working electrode (WE), counter electrode (CE), and reference electrode (RE). A two-electrode configuration is also observed in potentiometry, where the signal is measured using an electrometer rather than a potentiostat [21]. Alternatively, field-effect transistor (FET)-based sensors own a three-terminal system, i.e., source, gate, and drain [36]. A surface modification with pathogen-specific bioreceptor proteins (e.g., antibodies, antigens, or cytokines) and/or sequence-specific complementary probes is usually performed on the working electrode to avail the biorecognition of the target antigens, antibodies, proteins, and genes/nucleotides. To improve the sensitivity, immobilization, and biorecognition processes in electrochemical assays, further modifications have also been attempted either for WE or affinity/redox reactions using various nanomaterials such as carbon, metallic, and other nanoparticles (NPs) [23,29,30]. The readout of electrochemical interactions of proteins (such as antibodies, antigens, or enzymes), nucleic acids, or cells are harnessed through the amperometry or voltammetry (such as cyclic voltammetry (CV), square-wave voltammetry (SWV), and differential pulse voltammetry (DPV)), as well as potentiometric or impedimetric methods (e.g., electrochemical impedance spectroscopy (EIS)) [21,35,37,38].

Amperometric systems record the current response from a redox reaction of an electroactive molecule when an electric potential is applied at the WE in the presence of an analyte. The current is dependent on the electron transfer on the electrodes in the vicinity of sample, thus correlating with the analyte's concentration [21,38]. In CV, cyclic ordered sweeping of electrical potential is applied to measure the current. In contrast, the DPV and SWV apply the potential pulses in periodic intervals, hereby ameliorating the assay time and sensitivity [21,38]. Coulometry is another electrochemical technique, which measures the charge as the integral of the current [21]. Potentiometric approaches read out the electrical potential with regard to the applied current [21]. The EIS technique determines the change in resistance/impedance resulting from the interruption of electron flow upon binding of biomolecules, which is promising for label-free electrochemical assays utilizing an extraneous redox probe (e.g., ferri/ferrocyanide redox couple) [21,38,39]. On the other hand, FET-based biosensors aid in pathogen detection by measuring the conductivity variation between the channels of the source and drain in response to the applied potential to the gate where biorecognition of the analyte is realized. FET can enable easier and mass fabrication of the device and contribute to high-sensitive detection of biomarkers through a label-free assay [21,36].

3. Attributes of POC Electrochemical Biosensors for Pandemic Situations

According to WHO's direction, diagnostic devices for application in resource-inadequate settings should be ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users) [1,2,40], which is equally important when designing POC tests for a pandemic situation, regardless of a country's status. The micro total analysis system (μTAS)/microfluidics/LOC platforms were improved with many POC techniques in the last decades to meet the ASSURED diagnostic demands through a small-scale complete analysis of biochemicals, offering the benefits of utilizing a low

reagent/sample volume, shorter detection time, high sensitivity, high-throughput process, portability, and disposability [1,41–44].

As a follow-through, utilizing novel nanomaterials, antibiofouling chemistries, and bioreceptors, many electrochemical biosensors have emerged for effective detection of nucleic acids, proteins (i.e., antibodies, antigens, cytokines, hormones, etc.), cells, viral particles, bacteria, ionic species, and others targeting POC diagnosis [21,35]. Despite lucrative advancements in electrochemical biosensing, which have resulted in a substantial improvement in sensitivity and specificity, the need for additional features that are particularly crucial for POC applications is growing.

The ASSURED policy favors the creation of reagentless, reusable devices that can perform real-time and continuous measurements. The test should be easy, with a minimum number of steps (preferably one), and performed by a non-expert [22,35,45]. Most importantly, the detection system, which includes the device, detector, and reader, should be portable so that electrochemical tests may be performed in POC situations (i.e., at or near patients' location). Accordingly, the device should be simple, affordable, and mass-fabricated. In the race of feasible microfluidic techniques, alongside polymer/glass/silicone-based chips, the application of plastic or paper-integrated electrochemical devices is expanding because of the ease of fabrication, inexpensiveness, and functions. Other forms of biosensors such as textile, paper/plastic hybrid, and nanomaterial-assisted biosensors are also promising for trending the low-cost, user-friendly POCTs. In this context, electrode construction onto plastic/film, flexible material, or paper substrates using techniques such as screen-printing, inkjet-printing, template filtration, or roll-to-roll printing are expected to simplify the mass production of electrodes, as well as POCT devices [21,35]. Furthermore, nanocomposites (made with new metal nanoparticles, metal oxide/sulfide, nanowires, quantum dots, nanorods, and other materials) used to make the sensor surfaces should be stable, conductive, biocompatible, affordable, and easily produced [23,29,30].

Any nucleic acid- or antigen/antibody-based test that is sensitive and specific to the target pathogen can be used for diagnostic purposes. However, because of its ability to provide genetic evidence of the pathogen, nucleic acid testing (NAT) has an advantage and could be applied as a confirmatory test for infectious diseases. In the unreachability of NAT, antigen testing could be employed as a reliable diagnostic method if an antigen-specific monoclonal antibody (mAb) is used as a capture probe. However, mAb production is not straightforward. Serological antibody testing, on the other hand, could be an alternative; nevertheless, it runs the danger of decreasing specificity because the human immune system produces various autoantibodies in response to every pathogenic attack [46,47]. Therefore, the serological test can be utilized as a supplement to NAT and for immunostatus monitoring [48]. Aptamers (short DNA/RNA oligonucleotide sequences) can, on the other hand, be advocated as capture probes (or bioreceptors) for quick diagnoses of pandemic infectious illness, rather than antibodies, because aptamer synthesis is less complex than mAb synthesis and can be designed for detecting the antigen, antibody, and nucleic acid sequences [35,49–51].

Amplification-free and reagentless electrochemical testing [22,35,45], rather than amplification-dependent and enzymatic reaction-based assays (such as in PCR-, isothermal nucleic acid amplification (iNAA)-, or affinity-based sensors), is promising for POC diagnosis during a pandemic because it can save test time and cost by reducing procedural steps and the cost of extra reagents (such as enzymes, primers, labels, and others). Furthermore, antifouling capabilities should be included in the devices so that they can execute analyses with unprocessed or sparsely treated samples in resource-limited conditions [30,52]. Moreover, multiplexed electrochemical testing is likely to improve diagnostic reliability when infections in near proximity may exhibit identical symptoms due to structural and functional differences in genomes [21,35,53].

One apparent limiting aspect in the on-site deployment of electrochemical biosensors is the necessity of a detector, such as a potentiostat, as well as a reader/displayer that is

miniaturized, cost-effective, and portable [22,35,54]. To support the portability, the analyzer should be either power-free or battery/smartphone-powered [54,55].

In pandemic-like emergencies, a smartphone analyzer/reader is anticipated to lead to POC diagnosis. In recent years, smartphones led to the development amazing applications as portable analyzers for optical, physical or electrochemical modules of POC diagnostic tests [54–56], and as media to derive and send test data for telediagnosis/remote self-tests [57,58] of infectious diseases. In the framework of the internet of everything (IoE) [59], real-time networking is becoming increasingly important in today's world for rapid disease surveillance and eHealth applications. Thus, the ASSURED criteria are expected to become a REASSURED policy (where RE means real-time connectivity) [40]. Via wireless connectivity (or internet data) and app software, smartphones can also support the management of isolation, quarantining, and medication through epidemiological reporting with the test results (i.e., evidence-based disease surveillance or contact tracing) during the pandemic sessions [33,34,58,60,61].

The next sections review the potential POC electrochemical diagnostic systems with miniaturized (micro-device/LOC/ePAD) platforms for applications in pandemic-like emergencies in relation to the diagnosis of COVID-19 and other infections, considering the aforementioned POC attributes.

4. Potential Electrochemical POC Tests

4.1. Chip-Based POC Electrochemical Tests

Electrochemistry, in recent times, paved the road to realizing smart next-generation and highly functional lab-on-chip or micro-biosensors [21]. One of the biosensors utilized for POC diagnosis of various contagious diseases is chip-based electrochemical biosensors. They are created by compacting traditional biochemical assays or incorporating innovative detection strategies onto microchip scales or microfluidic devices. Microfluidic chips consisting of polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), polytetrafluoroethylene (PTEE), or other polymers can provide cost-effective and highly specific electrochemical testing with reduced reagent volume usage [43,44]. However, they frequently necessitate the photolithography-dependent fabrication of thin-film electrodes, which is costly and requires complex capabilities. Screen-printed electrodes (SPEs), on the other hand, have given chip-based electrochemical sensing a new dimension and versatility [21]. SPEs are often easy to make, which has resulted in them being commercially accessible in a variety of forms and being frequently used for emergency POC applications. Furthermore, SPE-based biosensors can be readily coupled to portable analyzers, allowing for widespread use of SPE in the diagnosis of infectious disease such as COVID-19 via electrochemical POCT. Many notable chip-based electrochemical POCT technologies have been described for detecting SARS-CoV-2 nucleic acids, antigens, and antibodies with good sensitivity and specificity. Those chip-based electrochemical biosensors have been transformed into promising technologies for POCT of infectious diseases in emergency and resource-constrained settings when used in conjunction with a portable analyzer and smartphone reader.

4.1.1. Chip-Based Electrochemical Nucleic Acid Testing (NAT)

Because nucleic acid detection is a confirming test, PCR-based amplification and subsequent detection have been recommended for the diagnosis of infectious diseases such as COVID-19. GenMark Diagnostics (USA) reported the first electrochemical COVID-19 detection scheme named the *ePlex SARS-CoV-2 test* using "The True Sample-to-Answer Solution" ePlex instrument, which automated magnetic solid-phase viral nucleic acid extraction, RT-PCR-based complementary DNA (cDNA) amplification, and detection on a single microfluidic cartridge integrating the electrowetting-based digital microfluidics (a technique that manipulates the droplets on a hydrophobic printed circuit board (PCB) using an electric field) [62]. GenMark's eSensor technology enabled voltammetric (electrochemical) detection of COVID-19 relying on the competitive DNA hybridization technique. The

target DNA was hybridized to the target-specific (complementary) ferrocene-labeled signal and capture probes bound to gold electrodes. The limit of detection (LOD) of the test was 1×10^5 copies/mL (i.e, 0.17 fM) for the SARS-CoV-2 DNA, which was equivalent to the authorized RT-PCR for emergency use authorization (EUA). This detection platform is promising; however, it requires a longer assay time of 2 h. PCR-based electrochemical testing is not elaborated here because PCR-based tests are lengthy and require advanced facilities. Rather, isothermal nucleic acid amplification tests (iNAATs) and amplification/label-free nucleic acid testing are addressed because of the benefits of a shorter assay period and less reliance on more reagents and complex instrumentation.

Isothermal Nucleic Acid Amplification Tests (iNAATs)

The frequent alteration of reaction temperatures, lengthy steps, power supply, and bulky setup in PCR-based nucleic acid amplification processes are troublesome when carrying out rapid diagnosis. Nucleic acid amplification at a single or constant reaction temperature, a process called isothermal nucleic acid amplification (iNAA), is beneficial in terms of the complexity of instrumentation, as well as the cost and time of nucleic acid testing (NAT), and it is anticipated to reduce the community transmission of pandemic diseases [63,64]. Even though iNAATs were commonly seen for optical (fluorescent or colorimetric) transducing-based COVID-19 diagnosis, only a few iNAA-based productive electrochemical tests were conducted.

Rodriguez-Manzano et al. reported a reverse transcription loop-mediated isothermal amplification (RT-LAMP)-based and smartphone-integrated ion-sensitive field-effect transistor (ISFET) platform for quick POC detection of SARS-CoV-2 RNA (N gene) with an LOD of 10 copies of RNA per reaction in 20 min [65]. The complementary metal–oxide–semiconductor (CMOS)-based handheld LOC (i.e., microchip) platform was built using a 3D-printed disposable cartridge containing two microfluidic channels (Figure 2A). The reverse transcription loop-mediated isothermal amplification (RT-LAMP) experiment was carried out on a large array of over 4000 sensors that detect nucleic acid amplification by measuring protons released in the process associated with nucleotide incorporation during LAMP utilizing off-chip exacted RNAs. The voltage shift produced by pH changes was recorded and evaluated using a custom AndroidOS smartphone application. The battery-powered portable RT-eLAMP test was evaluated against RT-qLAMP using clinical samples (such as nasopharyngeal, throat, and nasal swabs) and found to have a sensitivity of 90% and a specificity of 100%. With secure cloud connectivity, the smartphone app may also help with real-time reporting and geotagging of tested cases to a monitoring cell, making this portable diagnostic tool suitable for epidemiological surveillance.

When iNAAT is dependent on a portable/integrated heater, it is more practical to execute iNAA-based electrochemical NAT in a POC setting if the heater can be excluded. Kim et al. recently demonstrated a quick and sensitive detection of SARS-CoV-2 using an electrochemical biosensor paired with body heat-based recombinase polymerase amplification (RPA) (Figure 2B) [66]. The glass/PDMS-based multi-electrode array microchips contained an RE, a CE, and five WEs for detection of two target genes (RdRP and N gene) by differential pulse voltammetry (DPV). The RPA process included the RPA amplicon hybridizing with thiol-modified primers bound on the working electrodes, resulting in a decrease in current amplitude as amplicons aggregated. The assay could be completed in less than 20 min at the human body temperature. The LODs for RdRP gene and N gene were 0.972 fg/μL and 3.925 fg/μL, respectively, which was comparable to the RPA assay outcomes acquired through gel electrophoresis. As target templates, the device utilized extracted N and RdRP genes. This platform is believed to have significant promise for on-site application if a direct sample and a portable detector can be employed.

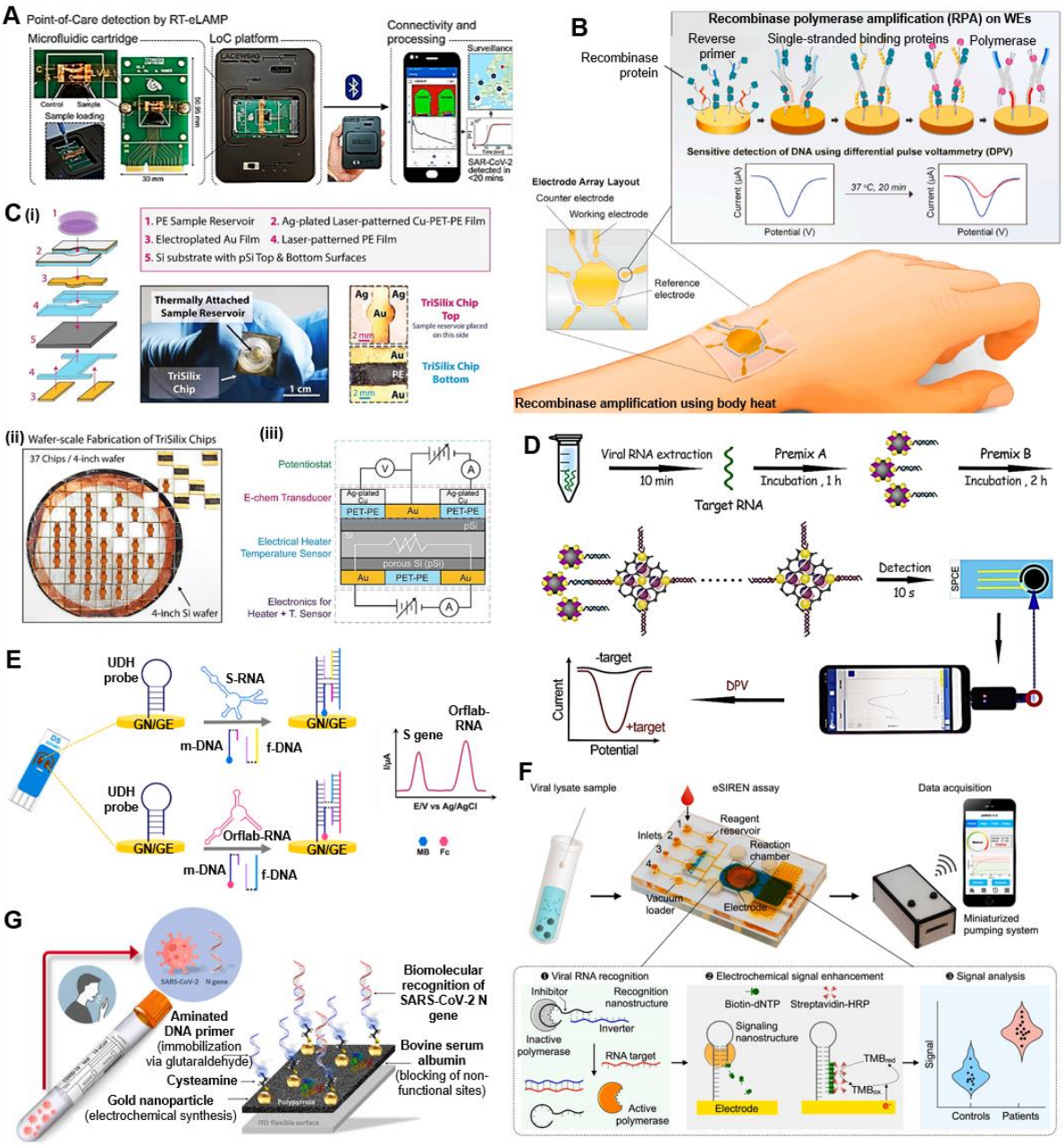

Figure 2. Chip-based POC electrochemical NAT. (**A**) Handheld LOC-coupled microfluidic RT-eLAMP and subsequent ISFET-based electrochemical detection of SARS-CoV-2 RNA. The platform enables smartphone-integrated real-time result visualization and geo-mapping. Reproduced with permission from [65]. Copyright 2021, American Chemical Society published under an ACS AuthorChoice License. (**B**) Illustration of electrochemical NAT incorporating body heat-based RPA and DPV-based amplicon quantification. Adapted with permission from [66]. Copyright 2021, Elsevier B.V. (**C**) Mass-fabricated TriSilix chip platform for nucleic acid amplification and detection. (i) Schematic of a TriSilix chip fabrication and images of the real device. (ii) Wafer-scale mass-fabrication of TriSilix (37 chips per 4 inch Si wafer). (iii) Drawing of the functional module of the TriSilix sensor. Reproduced with permission from [67]. Copyright 2020, Springer Nature under a Creative Commons CC BY License. (**D**) Schematic representation of nanocomposite-based and smartphone-assisted electrochemical platform for amplification-free detection SARS-CoV-2 RNA. Reproduced with permission from [68]. Copyright 2020, Elsevier B.V. (**E**) Schematic workflow of the amplification-free multiplex electrochemical NAT for detection of SARS-CoV-2 genes using four-way junction (4-WJ) hybridization. Adapted with permission from [69]. Copyright 2021, Elsevier B.V.

(**F**) Schematic illustration of the eSIREN platform integrating molecular nanostructures with microfluidic and pumping system for automated and direct detection of SARS-CoV-2 RNA. Adapted with permission from [70]. Copyright 2021, Elsevier B.V. (**G**) Illustration of the organic polymer conjugated ITO-based nanosensor for impedimetric diagnosis of COVID-19. Adapted with permission from [71]. Copyright 2021, Elsevier B.V. LOC, lab-on-chip; RT-eLAMP, real-time RT-eLAMP; ISFET, ion-sensitive field-effect transistor; RPA, recombinase polymerase amplification; eSIREN, electrochemical system integrating reconfigurable enzyme-DNA nanostructures; ITO, tin-doped indium oxide.

Another iNAAT termed rolling circle amplification (RCA) can be used to get a more sensitive NAT. Recently, Chaibun et al. presented an RCA–voltammetry biosensor for detecting the N and S genes of SARS-CoV-2 in clinical samples with a remarkable LOD of 1.0 copy/mL [72]. They performed the RCA in this study by sandwiching a redox-active label probe with RCA amplicons. To target the N and S genes, they created silicon nanoparticles and then functionalized those using methylene blue and acridine orange. The capture probe-conjugated magnetic bead particle (CP-MNB), silica–reporter probe (Si–RP), and target were mixed in a single hybridization stage, followed by a solitary washing step, in the single-step strategy. They tested 105 clinical samples and discovered 100% specificity and no significant cross-reactivity with influenza viruses. The electrochemical signal is measured by DPV on the test platforms using an SPCE and a palmsens4 potentiostat linked to a laptop. The test is appropriate for on-site usage; however, the assay duration is longer (~2 h).

In an emergency, a POC NAT device should be inexpensive and mass-produced. Nunez-Bajo et al. developed a silicon substrate integrated POC transducer (TriSilix) for chemically amplifying and quantitatively detecting pathogen-specific nucleic acid sequences in real time [67]. TriSilix was mass-fabricated (37 chip/wafer) in 7 h at a low cost (0.35 USD/chip) without the requirement for industrial processing employing metal-assisted chemical (wet) etching, electroplating, thermal bonding, and laser cutting. TriSilix is a multimodule nucleic acid amplification and detection device that includes an electrical (Joule) heater, a thermistor, and a label-free electrochemical sensor for identifying target NA using methylene blue as a redox-active reporter (Figure 2C). In detail, a substantial number of amplicons were generated in the presence of the targeted nucleic acid template utilizing RPA or PCR. Methylene blue subsequently engaged with the G-C base pairs and restricted the working electrode from functioning in electron-transfer processes, resulting in a reduced electroanalytical signal created by the redox-active reporter. The signal was measured in SWV method using a handheld potentiostat (PalmSens3). The TriSilix microchip is portable and simple to fabricate, and it can run up to 35 tests on a single 4000 mA h battery (a typical battery capacity of a smartphone). TriSilix has a detection limit of 20 fg and was effectively used to detect SARS-CoV-2 synthetic cDNA with good sensitivity against SARS-CoV (2003). Therefore, the TriSilix platform is prospective for high-throughput POC NAT.

Clustered regularly interspaced short palindromic repeats (CRISPR)-based assays can detect pathogens by programmed recognition and cutting of the pathogens' nucleic acid sequences with CRISPR-associated enzymes (such as Cas9, Cas12, and Cas13), which can be performed in small pots/tubes, microfluidic cartridges, and lateral flow strips with minimal equipment, making them promising for POC diagnosis of infectious diseases [73,74]. As a result, a number of CRISPR-assisted optical (fluorescent or colorimetric) sensors for the quick and selective detection of COVID-19 have evolved [75]. However, reading the assay result with those CRISPR procedures necessitated the use of powerful optical analyzers. CRISPR technique's practicality for POC applications can be increased by switching to electrochemical testing. CRISPR-assisted electrochemical tests, on the other hand, are uncommon. Recently, Puig et al. unveiled a CRISPR/Cas-based electrochemical platform that detects SARS-CoV-2 RNA on a gold chip with 100% accuracy in 1 h [76]. The sensor was built on gold electrodes with EDC/NHS-modified graphene oxide nanocomposite and target-specific ssDNA anchored by an amine-terminated peptide nucleic acid (PNA) linker,

allowing it to detect SARS-CoV-2 RNA at the single-molecule (or attomolar) level. Thirty clinical saliva samples were used to validate the test. Multiplexed high-precision detection of host antibodies (IgG and IgM) against spike S1, nucleocapsid, and spike-RBD was also achieved using the gold chip electrochemical biosensor. The sensor was powered by an HRP/TMB catalytic reaction, and the signal was determined by cyclic voltammetry using Autolab potentiostat. However, for the CRISPR assay, a LAMP-based 30 min nucleic acid amplification at 65 °C was required, which added a heating module to the diagnostic test. This problem can be remedied by incorporating an amplification-free CRISPR technology into an electrochemical assay [77].

Amplification-Free POC NAT

Despite the advantages of speed and single-temperature amplification over thermal cycler-dependent PCR, iNAAT's amplification process, which includes a heating module, enzymes, and primer sets, is still burdensome. Amplification-free NAT, in contrast, appears to be relatively straightforward in POCT.

Recently, the Zhao group from Singapore reported an amplification-free, sensitive, and rapid platform for electrochemical detection of SARS-CoV-2 ORF1ab RNA based on SPCE-based supersandwich assay and smartphone-assisted analysis [68]. They made the Au@SCX8-RGO-TB nanocomposite by forming the Au@Fe_3O_4 nanocomposite and GO, and then functionalizing them with hexane-1-thiol, *p*-sulfocalix [8] arene (SCX8), and toluidine blue (TB). Using the gold–thiol affinity, they first employed magnetic particles modified with a thiolated capture DNA probe (CP) to selectively extract the clinical RNA samples. The involvement of a thiolated specific CP, partly hybridized labeling probe (LP), and auxiliary probe (AP) in the vicinity of the Au@SCX8-RGO-TB nanocomposite improved sensitivity (sequence-specific detection) by allowing LPs and APs to hybridize several times. In clinical specimens (such as saliva, oral, throat swab, sputum, blood, plasma, urine, and feces), the proposed biosensor had an LOD of 200 RNA copies/mL (i.e, 0.33 aM), and the 85.5% of positive cases were detected in verified patients. DPV was used to read the electrochemical signal using a smartphone and a Palmsens Sensit Smart electrochemical workstation (Netherlands) (Figure 2D). As a result, this sensing platform may be converted into a portable electrochemical system that can produce results in seconds.

Recently, Kashefi-Kheyrabadi et al. reported an amplification-free multiplex electrochemical NAT biosensor based on four-way junction (4-WJ) hybridization for the detection of SARS-CoV-2 S and Orf1ab genes in clinical samples with a LOD of two or three copies/μL (∼4.98 fM), respectively in 1 h [69]. A 4-WJ structure was produced on double screen-printed gold electrodes (SPGEs) using a Universal DNA-Hairpin (UDH) probe hybridized with two adaptor strands and a SARS-CoV-2 RNA target (Figure 2E). To enable electrochemical analysis by EIS or SWV techniques, one of the adapter strands was modified with a redox mediator. The SPGEs also incorporated electrodeposited 3D gold nanoneedles, which improved the sensor's hybridization capacity and sensitivity. This single-step biosensor can distinguish single-nucleotide substitutions, making it helpful for on-site infection diagnostics with high specificity.

A dual probe-based amplification-free electrochemical sensing approach was proposed by Pang et al. for enhanced sensitivity detection of the SARS-CoV-2 nucleic acid sequence, in which the SPCE electrodes were modified with ssDNA capture probe using streptavidin/biotin and EDC/NHS chemistry, and the mimicked sample sequence was functionalized with FITC detector probe on which an anti-fluorescein antibody (HRP) tag was added [78]. For detection, the SPCE containing the dual probe-attached sample sequence was dipped in a solution of 3,3′,5,5′-tetramethylbenzidine (TMB) and H_2O_2, which generated a current signal to be measured by chronoamperometry method using an Autolab. The system is capable of detecting 1 pM of sample sequence in 1 h.

Zhao et al. developed an integrated platform (termed eSIREN) for the direct and automated detection of SARS-CoV-2 RNA (S gene) [70]. The system used a molecular circuitry that contained catalytic enzyme–DNA nanostructures for direct recognition of

target RNA sequences. An automated microfluidic system linked the molecular circuitry to the electrodes, to convert the target recognition into an electrical signal. They employed a complex of inhibitor and inverter DNA sequences as the recognition nanostructure to bind and inhibit the DNA polymerase enzyme, which was mixed with a clinical sample. The nanostructure detached and initiated strong polymerase activity when target RNA hybridized with the inverter sequence, elongating the self-primed signaling DNA nanostructure immobilized onto the electrode to encompass biotin-modified deoxynucleotide triphosphates (biotin-dNTPs) and streptavidin-conjugated horseradish peroxidase (HRP) (Figure 2F). Supplying the TMB substrate, the amperometric electrochemical current output was amplified and measured using the miniaturized potentiostat (PalmSens, EmStat3). The automated system enabled SARS-CoV-2 detection in 20 min with an LOD of seven RNA copies per µL (i.e., 11.62 fM) of extracted RNA samples or direct swab lysates. Thus, the system offered amplification-free highly sensitive detection of COVID-19.

However, the microfluidic system is reliant on an external pump (pressure) and power source. To regulate the solution supply in the microfluidic system, a LabVIEW interface and many solenoid valves are required. Furthermore, the gadget requires vacuum packing (and degassing) before it can begin processing the solution. The aforementioned difficulties may compromise the on-the-spot test's simplicity and cost.

It can be resolved by the active hydrophobic valve that can enable autonomous microfluidics when coupled with capillary-driven force [79,80]. On-chip automated, controlled, and multiplexed solution processing can be succeeded by switching the valve with low electric power or on-device produced potential [79,81]. In integration with a superabsorbent-based pump [82], this valve system enabled the automatic exchange of a series of solutions, which essentially fitted with the sequential steps of the immunoassay [80,82].

Thus, the assay on an automated microfluidic platform may create a great opportunity to produce portable POC devices for infectious disease detection. The use of a manual roller pump and a valve-free microfluidic device, on the other hand, can simplify solution processing for urgent bioassays [83]. As a consequence, no valve/electrode or external power-free pump components are required for the rapid stepwise assay.

Of late, field-effect transistor (FET) sensors have gained popularity for their quick sensing ability. A reduced graphene oxide (rGO)-FET in a microfluidic chip, using an elongated aptamer probe, enabled rapid and follow-through gene detection of H5N1 influenza virus with a LOD of 5 pM [84]. Using a graphene-based field-effect transistor (gFET), Hajian et al. were able to incorporate amplification-free CRISPR technology for DNA detection in 15 min [77]. They used graphene to immobilize the deactivated-associated protein 9 (dCas9). dCas9 can search the whole genome for a target sequence that matches the single-guide RNA. For Duchenne muscular dystrophy (DMD)-related mutations, the platform revealed an LOD of 1.7 fM.

Thus, amplification-free CRISPR has a lot of promise for easy-to-go and high-precision electrochemical diagnostics in a POC context.

A Chinese research team developed a graphene FET-based portable device with a low LOD of ~0.1 and ~1 fg/mL for RNA and antibodies (IgM and IgG), respectively [85]. A plug-and-play packaged biosensor chip and a piece of home-built electrical measuring equipment made up the detecting module. The sensor was constructed with PBASE in acetonitrile and subjected to either an ssDNA probe or an antigen protein. The degree of hybridization between the ss-DNA probe and viral RNA may be instantly translated to the current shift of the graphene channel without having to repeat the PCR procedure, which is a unique characteristic of this approach. This platform can detect SARS-CoV-2 antibodies (IgM and IgG) by substituting the ss-DNA probe with a specific antigen protein of SARS-CoV-2. They validated the tests with clinical samples (oropharyngeal swabs and serums). This detection scheme also has a fast diagnostic speed (about 10 min for RNA analysis and min for immunoassay), making it ideal for high-throughput POCT that must be performed quickly in emergency settings.

Label-Free Electrochemical NAT

POC diagnostics are also expected to use label-free electrochemical detection, which can simplify DNA/protein detection by omitting the use of a sandwiched assay or another signaling molecule apart from the probe and target [45,86].

Tripathy and Singh proposed a label-free electrochemical detection system for COVID-19-specific viral RNA/c-DNA [86]. As the reference and counter electrodes, the biosensor utilized platinum electrodes, while the working electrode was an AuNP-electrodeposited titanium substrate. At first, a complementary single-stranded probe was immobilized onto the gold surface utilizing the gold–thiol affinity. The capture probe hybridizes the target nucleotide upon availability on the biosensor surface. A USB was included in the handheld device to allow targeted DNA detection while the assay chamber was contained by a PDMS reservoir. Electrochemical data were analyzed using smartphones and software tools. The target DNA/RNA must be extracted from patients for sample processing, which is a disadvantage of this approach. DPV and EIS methods can be used to achieve an electrochemical signal of DNA sensors, which is currently under research.

In such an impedimetric (or EIS) platform, sensitivity can be improved by adding a conductive material such as polypyrrole (PPy) with AuNP. Using flexible electrodes on tin-doped indium oxide (ITO) substrate and a PPy–AuNP–Cys–Primer–BSA-based assay (Figure 2G), Avelino et al. realized SARS-CoV-2 N gene detection with an LOD of 258.01 copies/µL (i.e., 0.42 fM) in 15 min [71].

In a similar approach, Damiati et al. reported a flex printed circuit board (FPCB)-based genosensor for the quick, sensitive, amplification-free, and label-free detection of SARS-CoV-2 with a predicted LOD of ∼33 fg/mL (equal to around 5×0^5 copies/µL or 0.17 fM) in 30 min [87]. The sensor comprises a graphene working electrode and small easy-to-use reservoir chamber embedded into FPCB. Following streptavidin/biotin interaction-based ssDNA immobilization and analyte/probe hybridization, the sensor detects synthetic DNA strands mimicking the ORF1ab gene, which is then recorded using the DPV approach in the presence of a ferro/ferricyanide redox couple. The DNA sensor has sensitivity equivalent to isothermal nucleic acid amplification tests (iNAATs) and has the potential to be used for easy and inexpensive point-of-care diagnostics.

4.1.2. Chip-Based POC Electrochemical Immunoassays

Electrochemical immunoassays that can present momentous data about viral manifestation and diagnostic evidence are usually designed to quantitatively detect either antigens (i.e., structural or functional proteins of pathogens, viral particles) from invasive/noninvasive samples or antibodies (i.e., immunoglobulin G, M, or A) from blood/serum samples, based on the antibody–antigen interaction and fundamentals of biosensors (described above) [88–90]. Alongside, the target-specific antibodies and antigens, aptamers are also tailored to certain antigens or antibodies as the recognition elements (or capture probes). Electrochemical immunoassays for COVID-19 diagnosis are primarily focused on the detection of antigens (such as spike (S) protein, nucleocapside (N), ACE-2) followed by antibodies (IgG, IgM and others).

In addition toprotein-based detection, several other dimensional techniques were advanced in SARS-CoV-2, including molecularly imprinted polymer (MIP) [91,92], bioelectrically modified mammalian cell [93], exhaled breath condensate (EBC) [94], and carbohydrate [95]-based electrochemical immunosensors, which are promising for a new era of POC diagnostics.

Chip-Based Electrochemical Antigen Detection

In terms of confirming infection diagnosis, antigen tests still trail NAT, which were primarily put forth for electrochemical SARS-CoV-2 detection rather than antibody testing.

Beduk et al. presented a miniaturized laser-scribed graphene (LSG)-based disposable electrochemical biosensor with 3D Au nanostructures for COVID-19 diagnosis [96]. To produce electroactive groups on the gold-modified LSG surface, cysteamine and EDC:NHS

chemistry were used. With an LOD of 2.9 ng/mL, this sensor quantified S protein ranging 5.0 to 500.0 ng/mL. They used 23 clinical blood serum samples from COVID-19 patients to verify the assay. To make the POC diagnostic portable, easy-to-use, and accessible in distant situations, the system was combined into a handcrafted compact potentiostat and smartphone analyzer with bespoke KAUSTat software that measured the signal using the DPV method (Figure 3A). The test procedure and data management were also made more methodical using a smartphone. Because the device is inexpensive and requires just a 1 h incubation period, it may be used by patients in self-isolation to monitor their immune state.

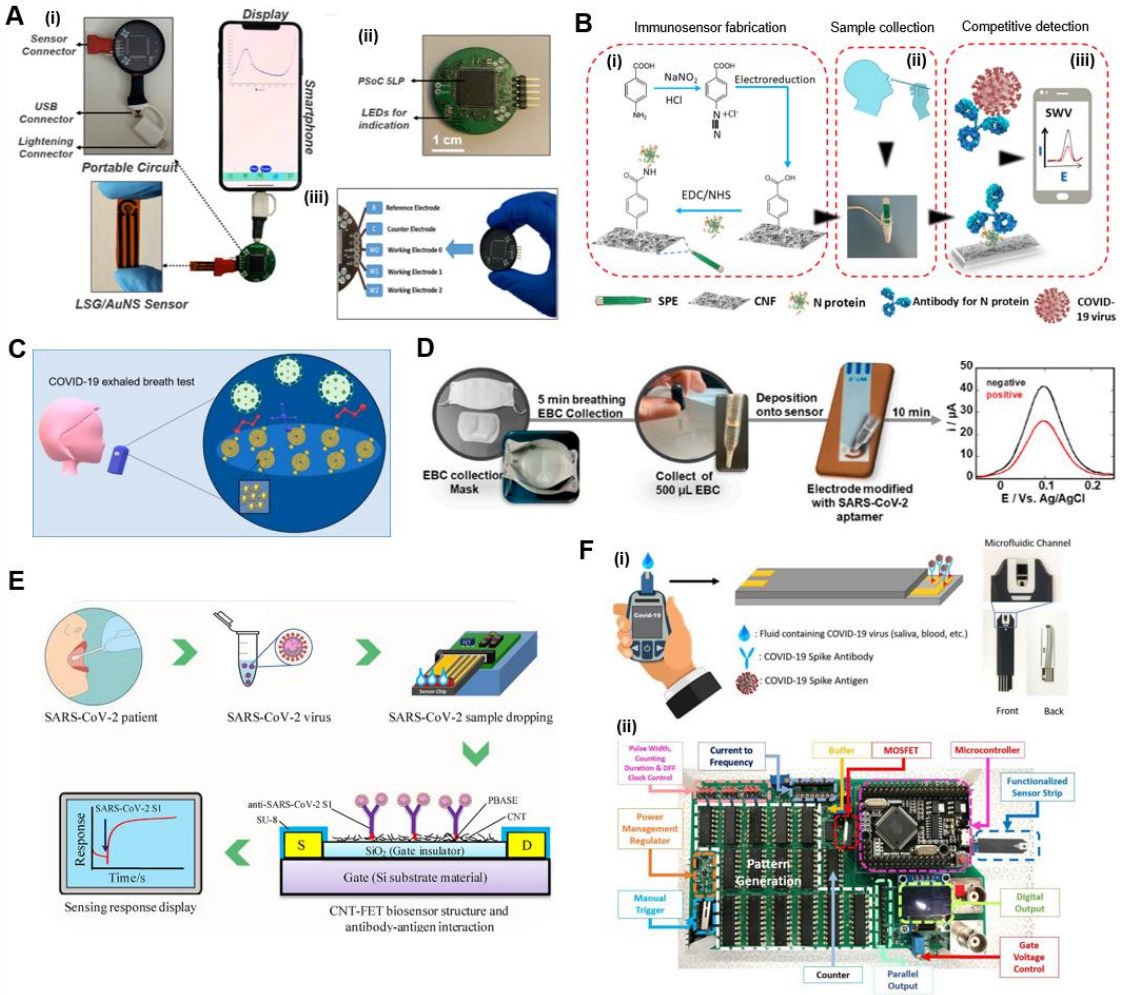

Figure 3. Chip-based potential electrochemical immunoassays—Part 1. (**A**) A laser-scribed graphene (LSG)-based disposable electrochemical biosensor for SARS-CoV-2 S protein detection. (i) Photographs of integrated portable hand-built POC potentiostat linked to a smartphone via a USB-C connection to enable signal readout using KAUSTat software. (ii) Image of the potentiostat. (iii) Pinpointing of the device components. Reproduced with permission from [96]. Copyright 2021, American Chemical Society. (**B**) Schematic presentation of cotton-tipped electrochemical immunosensor for SARS-CoV-2 N protein detection. (i) Carbon nanofiber electrode modification using electroreduction of diazonium salt and binding of viral antigen. (ii) Sample collection with the cotton-tipped electrode. (iii) Sensing scheme employing competitive assay and SWV method. Reproduced with permission from [97] Copyright 2020, American Chemical Society under ACS COVID-19 subset for unrestricted RESEARCH reuse. (**C**) AuNPs/organic ligand-based sensor for exhaled breath analysis of COVID-19 patient using the handheld analyzer. Reprinted with permission from [98]. Copyright 2020, American Chemical Society. (**D**) Exhaled breath condensate (EBC)-based COVID-19

diagnostic platform incorporating a mask-based EBC collection, extraction, and detection with an SPE sensor. Adapted with permission from [94]. Copyright 2021, Elsevier B.V. (**E**) Schematic outline of CNT-FET biosensor for SARS-CoV-2 S1 detection. Adapted with permission from [99]. Copyright 2021, The Authors. Published by Elsevier B.V. under the Creative Commons CC BY license. (**F**) Schematic drawing of Si MOSFET cartridge sensor for SARS-CoV-2 S protein detection. (i) Glucose test strip-based exterior sensor components. (ii) Printed circuit board (PCB) with a built-in microcontroller for digital signal readout. Reproduced with permission from [100]. Copyright 2021, the author(s). Published under an exclusive license by the AVS. CNT-FET, Carbon nanotube field-effect transistor; MOSFET, metal–oxide–semiconductor field-effect transistor.

Eissa et al. integrated a cotton tip in an electrochemical immunoassay for the SARS-CoV-2 N protein detection, in which the cotton tip served as both a detector and a sample/analyte collector [97]. The electrochemical sensor was constructed by diazonium electro-grafting the carbon nanofiber (CNF)-modified screen-printed electrodes followed by EDC:NHS-based functionalization and immobilization of the viral N protein. The viral antigen was detected through swabbing followed by a competitive assay against a set amount of N protein antibody in the solution (Figure 3B). The electrochemical response was measured using SWV, and the LOD for SARS-CoV-2 was reported to be 0.8 pg/mL. In spiked nasal samples, the sensor demonstrated remarkable selectivity and recovery percentage (91–95.5%) when evaluating cross-reactivity against other viral antigens such as HCoV and influenza A. Self-diagnosis can be envisioned by combining a portable potentiostat and a smartphone reader with this electrochemical platform.

In pandemic-like circumstances, mass-scale, on-the-spot primary testing of pretreatment-free samples using a reusable biosensor has great promise [101]. A unique portable handheld analyzer comprising a nanomaterial-based hybrid sensor array with multiplexed capabilities was reported for the detection and monitoring of COVID-19-specific volatile organic compounds (VOCs) from the exhaled breath (Figure 3C) [98]. AuNP-based chemiresistors were modified with different organic ligands and placed onto eight platinum interdigiated electrodes in the sensor arrays. VOCs are absorbed by and interact with the organic ligands when exposed to the sensor (nanomaterial film), causing the AuNP sensing layer to shrink or swell. The electric resistance changes as a result of the volume shift, which may be measured and shown digitally to support the diagnosis. More interestingly, the sensor chip is reusable, and it can be cleaned with alcohol and flushed with air after each usage to restore it to a working state. Furthermore, the presence of humidity in the breath might impair the sensor's sensitivity. For this reason, rather than being a replacement for pre-existing and established testing, the device might be viewed as a preliminary test (with 90% accuracy and 95% specificity) for COVID-19. The sensor's flexibility, on the other hand, is truly impressive and applicable in emergencies.

Daniels et al. introduced a COVID-19 self-diagnostic module that entailed collecting exhaled breath condensate (EBC) using a face mask and analyzing it using an aptamer-based electrochemical biosensor in a novel method to simplify sample collection and detection of SARS-CoV-2 S protein (Figure 3D) [94]. The EBC mask collector consists of a Teflon-based condensate-forming surface on which a superabsorbent polymer absorbed EBC and a polytetrafluoroethylene (PTFE) trap allow the exhaled vapor sample to be liquified. The mask is frozen for 30 min before use at 20 °C. After wearing the mask for 5 min, open-mouth breathing will assist in depositing the EBC in the cold trap, which may yield 200–500 µL fluid sample in 2 min. A pipette is used to collect the liquid sample, which is then transferred to the sensor surface for direct analysis. The EBC's efficacy was tested using RT-PCR for SARS-CoV-2 genes (E, ORF1ab) in specimens of seven COVID-19 patients. The electrochemical aptasensor, on the other hand, was created by immobilizing the S protein-specific aptamer on an Au electrode using thiol and EDC:NHS chemistry. The sensor enabled the detection of SARS-CoV-2 viral particles down to 10 pfu/mL (i.e., 16.6 zM) in cultured SARS-CoV-2 suspensions in 10 min. The current response of the sensor was recorded by the DPV method in presence of a ferrocenemethanol redox mediator

using a portable Sensit-Smart smartphone potentiostat (from PalmSens). The use of a simple sample collection approach and a portable analyzer (with a smartphone interface) has opened up a new avenue for quick self-diagnosis or individualized infectious status screening, maybe even at home.

Using Optimer (optimized aptamers) ligands and mass-fabricated thin-film gold electrode (TFGE) sensors, Lasserre et al. reported an impedimetric, low-cost, high-specificity mass testing platform for SARS-CoV-2 S1 protein detection in 15 min [102]. The SARS-CoV-2 S1 domain-specific DNA Optimer was synthesized from a pool of 10^{14} sequences, while the TFGE was made using an inexpensive glucose test strip material (i.e., polyester substrate). The sensor was produced by reducing the Optimer with TCEP (Tris(2-carboxyethyl)phosphine hydrochloride) and then co-immobilizing it with BSA onto a mass-fabricated TFGE. As a result, mass production of Optimer-based sensors is now possible. Furthermore, many CE-marked analyzers can interpret the test. All of these factors together make this platform suitable for POCT in pandemic-like situations.

Previously, Vezza et al. demonstrated a mass-producible impedimetric sensor based on ACE2–spike protein interaction for SARS-CoV-2 detection with a sensitivity of 1.68 ng/mL [103]. Self-assembled monolayer (SAM) formation of 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol (PFDT) followed by functionalization via physisorption of ACE2 onto PFDT was used to create the sensors on thin-film Au electrodes bulk-produced on PCB substrate. The charge transfer resistance (R_{CT}) of the $[Fe(CN)_6]^{-3/-4}$ redox process rises when spike protein from the sample attaches to the ACE2, as assessed by the EIS technique. This platform is appropriate for mass testing during the pandemic period due to its capacity to mass-produce low-cost chips/electrodes and the ease of building sensor surfaces (like in glucose strips).

Virus detection could be enabled within a few seconds using an rGO-based FET sensor as reported by American scientists for the case of the Ebola virus, with a LOD of 1 ng/mL [104].

To speed up the detection of SARS-CoV-2 antigen, a few practicable FET-based electrochemical sensors were also explored.

Using a carbon nanotube field-effect transistor (CNT-FET)-based sensor, Zamzami et al. achieved significantly quicker (2–3 min) detection of SARS-CoV-2 S1 in fortified saliva samples with an LOD of 4.12 fg/mL [99]. The biosensor was created on a Si/SiO_2 surface using single-walled CNT printing and a PBASE linker to immobilize an anti-SARS-CoV-2 S1 in between the S-D channel region (Figure 3E). The CNT-FET sensor was incredibly sensitive and capable of distinguishing among SARS-CoV-2 S1, SARS-CoV-1 S1, and MERS-CoV S1 antigens, demonstrating strong SARS-CoV-2 S1 selectivity. For speedier POCT, this basic platform is intriguing.

A Korean research team presented a micro-fabricated graphene-based field-effect transistor (FET) biosensing platform for real-time detection of SARS-CoV-2 spike (S) protein with LOD of 242 virus copies/mL (0.42 aM) from unprocessed nasopharyngeal swab samples [105]. The anti-spike antibody was immobilized on the graphene sheet with the probe linker 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (PBASE) to construct the FET sensor that responded to 16 pfu/mL (26.59 zM) of cultured SARS-CoV-2 particles within 10 min. The biosensor was able to distinguish between SARS-CoV-2 and MERS-CoV on the basis of the S protein. However, the transporting medium could make noise in the signal readout and affect the LOD.

Xian et al. created an FET electrochemical framework for detecting SARS-CoV-2 S protein and cardiac troponin I (cTnI) simultaneously [100]. They developed an inexpensive disposable Si MOSFET cartridge sensor, with exterior sensor elements made from widely accessible glucose test strips that were biofunctionalized with SARS-CoV-2 antibodies or cTnI antibodies for this study (Figure 3F). Au-plated clusters were created in the working electrode that linked the MOSFET sensor's gate electrode. The device's LOD for cTnI was determined to be 100 pg/mL, whereas a meaningful result was reported for SARS-CoV-2 spiked protein at 100 fg/mL.

Typical screen-printed carbon electrode (SPCE)-based electrochemical sensors, on the other hand, continue to amaze with their ability to create simpler testing.

Mahari et al. presented a printed circuit board (PCB)-based home-built electrochemical eCovSens device that can diagnose SARS-CoV-2 S protein antigen in spiked saliva samples in 30 s with an LOD of 90 fM [106]. The biosensor was made by immobilizing SARS-CoV-2-specific monoclonal antibodies on an SPCE and compared with another sensor made with gold nanoparticles immobilized on fluorine-doped tin oxide (FTO). The device's performance was unaffected after 4 weeks of stability. The electrochemical readings were recorded using the DPV technique with a low-cost, portable, and battery-powered in-house made potentiostat, making this method potential for outdoor deployment.

Mojsoska et al. reported an SPE-based label-free electrochemical platform that used PBASE-functionalized graphene WE for SARS-CoV-2 S protein detection in saliva samples with an LOD of 20 µg/mL in 45 min [107]. The signal was obtained by CV and/or EIS from ferri/ferrocyanide redox couple using a portable Palmsens 4 potentiostat.

SPCE modification with Cu_2O nanocubes (Cu_2O NCs) can also improve the sensitivity as reported by Rahmati et al. for SARS-CoV-2 spike protein detection in clinical samples with an LOD of 0.04 fg·mL^{-1} in 20 min [108]. The sensor was made by attaching the IgG anti-SARS-CoV-2 onto the Cu_2O NCs-modified SPCE using the Prot A linker. The nanocubes' increased surface area offered more active sites for binding IgG, allowing more SARS-CoV-2 to be identified.

In POC electrochemical testing, multiplexed detection of different respiratory viruses is insufficient. Previously, Layqah and Eissa suggested a competitive assay-based simple electrochemical immunosensing technique for 20 min detection of MERS-CoV and HCoV [109]. The sensor works based on reduced peak current caused by the competitive binding of target antigens to specific antibodies immobilized on gold nanoparticle (AuNP)-modified microfabricated carbon array electrodes. The SWV readout showed the LODs as 0.4 and 1.0 pg/mL for HCoV and MERS-CoV, respectively. The system is simple, accurate, and specific, and it works well with spiked nasal samples; thus, it can be used for the detection of respiratory viruses (including SARS-CoV-2).

Li et al. proposed an SPCE-based multichannel electrochemical immunoassay (MEIA) system for the on-site and multiplex detection of SARS-CoV-2 and A(H1N1) virus [110]. The MEIA had eight channels and could identify several targets quickly on a single array. They coated the SPCE surface with the monoclonal antibodies specific to the influenza A(H1N1) hemagglutinin (HA) protein or the SARS-CoV-2 spike protein to bind the target antigens, which was subsequently coupled with an HRP-labeled detection antibody to create an immuno-sandwich complex that was determined by the amperometric process using portable Emstat Potentiostat (Palmsens) (Figure 4A). The MEIA had a wider linear range than ELISA and equivalent sensitivity for A(H1N1) HA and SARS-CoV-2 spike protein, according to the findings. The detection findings for A(H1N1) in 79 clinical samples revealed that the proposed MEIA platform had comparable sensitivity to ELISA (100% positive rate for positive samples) but greater specificity (5.4% false-positive rate for negative samples against 40.5% with ELISA). Thus, it has great promise for on-the-spot differential diagnoses of infected individuals.

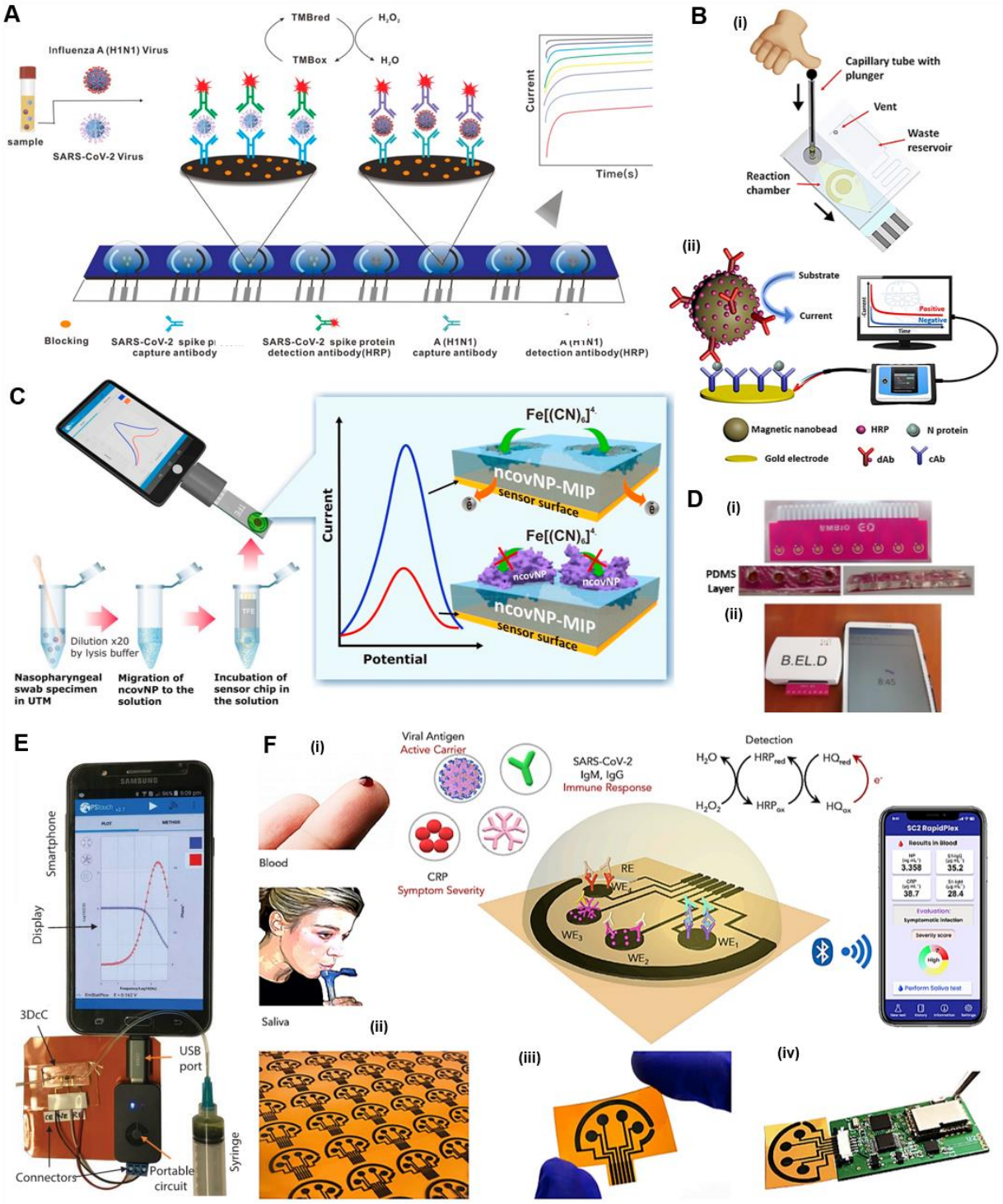

Figure 4. Chip-based potential electrochemical immunoassays—Part 2. (**A**) Schematic outline of the multichannel electrochemical immunoassay (MEIA) for antigen detection of SARS-CoV-2 and influenza A(H1N1) virus. Adapted with permission from [110]. Copyright 2021, American Chemical Society under ACS COVID-19 subset for unrestricted RESEARCH reuse. (**B**) Schematic drawing of the microfluidic magneto immunosensor chip and dually labeled magnetic enrichment for COVID-19 diagnosis using the smartphone-assisted potentiostat (i) and desktop PC-connected PalmSens4 analyzer. Reproduced with permission from [111]. Copyright 2021, American Chemical Society under ACS COVID-19 subset for unrestricted RESEARCH reuse. (**C**) Illustrated scheme of molecularly imprinted polymer (MIP)-based and smartphone-integrated disposable electrochemical biosensor for SARS-CoV-2 N protein detection. Adapted with permission from [92]. Copyright 2021, Elsevier B.V.

(**D**) Vero/anti-S1 cell-based electrochemical biosensor platform for bioelectric recognition assay (BERA). (i) Device with eight-channel SPGEs and PDMS wells. (ii) The potentiometer was connected to a tablet device for signal measurement. Reproduced with permission from [93]. Copyright 2021, the authors. Published by MDPI under Creative Common CC BY license. (**E**) The 3DcC electrochemical biosensor integrated with a USB-C-connected portable potentiostat and smartphone for detection of SARS-CoV-2 antibodies (S1 and RBD). Reproduced with permission from [112]. Copyright 2020, Wiley-VCH GmbH. (**F**) Schematic representation of the SARS-CoV-2 RapidPlex, a graphene-based electrochemical sensing and telemedicine platform for multiplex detection of antigens and antibodies, and dissemination of test results. (i) Sample collection, sensing mechanism and wireless telemedicine platform. (ii–iv) Mass-fabricated laser-engraved graphene-based flexible sensor arrays, photo of a single device, and image of PCB-connected sensor array for signal acquisition and wireless communication. Reproduced with permission from [113]. Copyright 2020, Elsevier Inc. SPGEs, gold screen-printed electrodes; 3DcC, 3D-printed COVID-19 test chip; PCB, printed circuit board.

Fabiani et al. demonstrated magnetic bead (MB) and carbon black (CB)-modified SPEs-based electrochemical immunoassay for detection of SARS-CoV-2 S and N protein in unprocessed saliva samples in 30 min, with LODs of 19 ng/mL and 8 ng/mL, respectively [114]. Precoating of MBs, immunoassay using MBs with ALP-labeled antibody, and electrochemical analysis employing CB-modified SPEs were all part of the experiment. The enzymatic byproduct 1-naphthol produced by ALP-mediated cleavage of 1-naphthyl phosphate was analyzed by the DVP technique using a portable analyzer (PalmSens3) connected to a computer. The platform is suitable for noninvasive sampling and easy-to-perform POCT.

The sensitivity of a magnetic bead-based sensor can be improved by doubly labeling nanobeads. Using the immunomagnetic concentration and signal enhancement of the dually labeled magnetic nanobeads, Li and Lillehoj succeeded in detecting SARS-CoV-2 N protein with LODs as low as 50 pg/mL and 10 pg/mL in whole serum and in fivefold diluted serum, respectively, in <1 h [111]. They used a microfluidic magneto immunosensor with screen-printed gold electrodes (SPGEs) that allows blood samples to be inserted into micro-flow channels using a syringe pump or a capillary plunger, as well as chronoamperometric current signal measurement using a PalmSens4 potentiostat connected to a desktop PC or a smartphone-integrated Sensit Smart potentiostat (Figure 4B).

Molecularly imprinted polymer (MIP) can be used to simplify electrochemical biosensing by eliminating the need for protein or aptamer-based capture probes.

Raziq et al. introduced a molecularly imprinted polymer (MIP)-based electrochemical sensor with an LOD of 15 fM for detecting SARS-CoV-2 N protein in nasopharyngeal samples [92]. A disposable sensor chip with an Au thin-film electrode (Au-TFE) connected the MIP (poly-*m*-phenylenediamine (PmPD)) to enable selective detection of the target analyte. The Au-TFE chip was attached to the PalmSens' portable potentiostat interfaced with a smartphone, and a DPV was utilized to determine the SARS-CoV-2 N protein concentration against a redox pair ferri/ferrocyanide (Figure 4C). The biosensor's portability allowed it to be used in POC situations.

In another approach, MIP synthesized from electropolymerized *ortho*-phenylenediamine (oPD) was integrated with macroporous gold SPE (MP-Au-SPE) for sensitive detection of SARS-CoV-2-RBD in the saliva solution with a LOD of 0.7 pg/mL [91]. The SARS-CoV-2-RBD molecules were incorporated in the MIP matrix as a template molecule during the production process. After that, the template molecules were extracted from the electrode, and cavities for target SARS-CoV-2-RBD were created using alkaline ethanol. The electron transfer resistance was measured using EIS after the template molecule was removed and the sample SARS-CoV-2-RBD was added, which corresponded to the amount of sample analyte.

Interestingly, bioelectrically modified mammalian cells may be used in high-specificity electrochemical antigen detection. Recently, Mavrikou et al. reported a bioelectric recognition assay (BERA) for the detection of SARS-CoV-2 S1 spike protein in 3 min using engineered monkey kidney cells with electroinserted anti-S1 antibody and an array of eight Au screen-printed electrodes [93]. The protein's binding to membrane-bound antibodies caused a selective and notable alteration in cellular bioelectric features, which was assessed using the potentiometric approach through BERA. To avoid sample diffusion among the electrodes, PDMS-based holes were used to separate the electrodes in the sensing strip. The BERA had an LOD of 1 fg/mL and exhibited no cross-reactivity with the SARS-CoV-2 N protein. The biosensor was also a ready-to-use system, with a portable read-out device (multichannel potentiometer (Embio Diagnostics Ltd., Strovolos, Cyprus) that could be controlled through a smartphone or tablet (Figure 4D). Thus, this new biosensor might be used for faster mass screening of infections.

Ghazizadeh et al. developed an intriguing carbohydrate-based double electrochemical sensing technique for spiked virus and SARS-CoV-2 detection, which was based on the development of natural bed-receptors by glycan-binding microparticle vesicle galactin-1 (MV-gal1) [95]. They employed an Au@Antibody-SARS-CoV-2 spike to capture the SARS-CoV-2 virus for specific detection of the SARS-CoV-2 antigen. The sensor was turned on by placing MV-gal1 on the SCPE/GNP, which raised the impedance owing to gal-1's charge being exchanged with $[Fe(CN)_6]^{-3/-4}$. By covering the glycosylation bond with the inactive SARS-CoV-2, the impedance of MV-gal1/SCPE-GNP was lowered, and the sensor was switched off. Dropping the Au@Anti-SARS-CoV-2 spike led the AuNP electrochemical amplifier to switch on the sensor once again. As a consequence, in less than 5 min, double sensing of SARS-CoV-2 antigen in a nasopharyngeal swab sample with great sensitivity (LOD: 4.57×10^2 copies/mL or 0.75 aM) was achieved. The sensor is very simple and inexpensive to be applied for POC diagnosis in pandemic-like emergencies.

When visual (or optical) detection is qualitative, it makes sense to put it into a quantitative test. Karakus et al. announced a COVID-19 diagnostic platform, which combines dual-response colloidal AuNP-based colorimetric and electrochemical biosensors to detect SARS-CoV-2 S antigen in 10 min with LODs of 48 ng/mL and 1 pg/mL for colorimetric and electrochemical techniques, respectively [115]. Due to monoclonal antibody–antigen contact, the thiol and EDC/NHS-modified AuNP aggregated quickly and permanently, changing color from red to purple, as seen with the naked eye or UV–Vis spectroscopy. Electrochemical detection, on the other hand, was accomplished by simply dropping the produced probe solution onto a commercially accessible, disposable screen-printed gold electrode with no electrode preparation or modification. The SWV technique was used to read the current response using a Metrohm Dropsens potentiostat. Both approaches were highly specific for identifying the SARS-CoV-2 spike protein against other antigens such as influenza A (H1N1), MERS-CoV, or *S. pneumoniae*. This simple platform proved that the electrochemical sensing approach is more precise and sensitive than the colorimetric system. As a result, this biosensing platform may simply be incorporated into a commercially available kit and repurposed for the detection of emerging infectious diseases.

Chip-Based Electrochemical Antibody Detection

Ali et al. recently reported a 3D-printed COVID-19 test chip (3DcC), a smartphone-integrated and nanomaterial-based 3D electrochemical biosensor for the detection of spike S1 and RBD antibodies against SARS-CoV-2 in 11.5 s (Figure 4E) [112]. The biosensing platform is made up of 3D micropillar electrodes printed using an aerosol jet, nanoflakes of reduced graphene oxide (rGO) coated on the electrodes, and particular viral antigens immobilized on the rGO nanoflakes using an EDC:NHS chemistry. For electrochemical testing, the electrode is then integrated into a microfluidic device. Using PalmSens' Sensit Smart potentiostat and smartphone reader, the selective antigen–antibody interaction was assessed by impedance spectroscopy. For spike S1 and RBD antibodies, the LOD of the electrode was 1.0 pM and 1.0 fM, respectively. They discovered that the current

responsiveness of 3DcC electrodes was over 50 times greater than that of a 2D electrode with the same composition. The additional feature of this electrode was that it could regrow in 1 min and be used up to nine times without losing effectiveness. The portable device will be a useful tool for investigating immune status during infections and after recovery.

This is the era when both antigen and antibody detection on the same platform is immensely beneficial for the evidence-based disease diagnosis and monitoring of the suspected patients' immunostatus.

To this end, Torrente-Rodriguez et al. introduced the most amazing multiplexed, mass-fabricated, portable, wireless electrochemical testing platform called SARS-CoV-2 RapidPlex for the ultrafast (1 min) detection of S1-IgG and S1-IgM isotypes, antigen N protein, and inflammatory biomarker C-reactive protein (CRP) using a single device (Figure 4F) [113]. With the aid of a crosslinker chemical, four mass-fabricated laser-engraved graphene (LEG) working electrodes on polyimide substrate were immobilized with SARS-CoV-2 related N protein, antigen, and S1 for selective binding of immunoglobulin isotypes, antigen, and CRP, and detection using an amperometric technique (with a CHI820 electrochemical station). COVID-19-negative saliva and blood samples were effectively detected using the SARS-CoV-2 RapidPlex technology. This technology has the benefit of being simple to utilize for SARS-CoV-2 high-frequency home testing, as well as telemedicine diagnosis and remote monitoring.

Similarly, Peng et al. demonstrated a 13 -min serological testing platform (termed SPEED) for electrochemically detecting the SARS-CoV-2 spike RBD protein-specific IgG and IgM in serum samples [116]. They created a screen-printed carbon electrode (SPCE) on the PET sheet and combined it with a PalmSens EmStat3 Blue portable potentiostat and a smartphone reader. The sensor was produced by immobilizing anti- SARS-CoV-2 spike RBD protein via streptavidin–biotin chemistry, and the chronoamperometric signal was generated by the alkaline phosphatase (ALP) catalyzing the oxi-reductive reaction of *p*-aminophenyl phosphate (pAPP). The LODs for IgG and IgM detection were 10.1 ng/mL and 1.64 ng/mL, respectively, as per the SPEED. The sensor is inexpensive (2.10 USD for each test), quick, long-term (24 weeks) storable, and portable, making it promising for POCT.

On the other hand, commercial electrochemical immunoassays have rarely been reported. Roche Diagnostics presented the only FDA/EUA-approved electrochemiluminescence immunoassay (ECLIA) called Elecsys Anti-SARS-CoV-2 for the qualitative detection of SARS-CoV-2 N antigen-specific antibodies (with 99.80% overall specificity) in human serum and plasma (K2-EDTA, K3-EDTA, Li-heparin) in just 18 min [117]. In ECLIA, the SARS-CoV-2-specific recombinant antigen was labeled with a ruthenium complex that induced chemiluminescent emission after voltage application. Beforehand, microparticles were prepared following streptavidin–biotin chemistry and magnetically captured onto the electrode surface. The detection scheme was simple and rapid; however, it was reliant on a customized analyzer, which may be a limiting issue for resource-poor conditions.

Overall, the existing COVID-19 diagnosis chip-based electrochemical POC tests are remarkable. INAATs and amplification/label-free electrochemical NATs were advocated to help overcome the limitations of PCR. Electrochemical antigen tests were studied extensively to supplement rapid diagnostic outputs in comparison to NATs and antibody detection techniques. Additionally, using innovative nano-chemistry, multiple electrochemical formats were developed to provide easy, inexpensive, sensitive, and specific detection of SARS-CoV-2. The procedures mentioned here were simplified considering the urgent applications; as a result, they can be used in future POCTs with minor modifications. The chip-based potential electrochemical POC tests are summarized in Table 1.

Table 1. Chip-based potential electrochemical biosensors for COVID-19 diagnosis.

Bioprocessing Approaches	Analytes	Samples	Transducers	Analyzers	LOD	Assay Time	Ref.
A. Nucleic Acid Testing (NAT)							
ePlex SARS-CoV-2 test; Electrowetting-based digital microfluidic chip—automated, sample-to-answer platform—RT-PCR—competitive DNA hybridization	RNA	Nasopharyngeal	Electrochemical	GenMark's ePlex instrument	1×10^5 copies/mL	<2h	[62]
(i) Isothermal nucleic acid amplification (iNAA)-based electrochemical NAT							
RT-eLAMP assay; CMOS-ISFET-based portable microfluidic LOC; epidemiological reporting	RNA (N gene)	Clinical swab samples	Potentiometric	Battery-powered handheld analyzer; Smartphone	10 RNA copies/reaction	<20 min	[65]
Body heat-based on-chip RPA; microchip—Au thin film WE—thiol-modified primers—RPA amplicons—hybridization; $K_3[Fe(CN)_6]$	RdRP and N gene	Extracted sample	DPV	CHI 830B instrument (CH Instruments, USA)	0.972 fg/µL (RdRP gene) and 3.925 fg/µL (N gene)	<20 min	[66]
RCA-based single-step sandwich hybridization assay; SPCE—CP-MNB + RCA amplicons + Si-RP (SiMB and SiAO)	N and S genes	Nasopharyngeal and throat swab samples	DPV	Palmsens4 potentiostat with a laptop	1 copy/mL	<2 h	[72]
TriSilix chip; Si–Au, Ag-plated Cu-PET-PE; RCA/PCR; methylene blue redox reporter	cDNA	Synthetic sample	SWV	Handheld potentiostat (PalmSens3)	20 fg of genomic DNA	Real-time	[67]
CRISPR assay; Au Chip/GO-PNA-ssDNA-amplicon; EDC/NHS-antigen	RNA and IgG/IgM	Saliva	CV	Autolab	Attomolar	1 h	[76]
(ii) Amplification-free POC electrochemical NAT							
Supersandwich electrochemical assay; SPCE chip—Au@SCX8-RGO-TB nanocomposite—MB—capture DNA probe	RNA	Clinical samples	DPV	Sensit Smart workstation (PalmSens), Smartphone	200 RNA copies/mL	Few seconds	[68]
SPGE-Au nanoneedle-MCH-UDH probe; 4-WJ) hybridization; $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ redox probe	S and Orf1ab genes	Clinical samples	EIS, SWV	CH instruments	2 or 3 copies/µL	1 h	[69]
SPCE-streptavidin/biotin-EDC/NHS-ssDNA-FITC probe-HRP-TMB/H_2O_2	Mimicked sample se-quence	Mimicked sample sequence	Chronoamperometry	Autolab	1 pM	1 h	[78]
Automated microfluidics; amplification-free; eSIREN; inhibition interaction; SPE chip—inverter DNA sequences—RNA hybridization—biotin/dNTPs—streptavidin/HRP—TMB	RNA	Extracted RNA, Swab sample	Amperometry	Miniaturized potentiostat (PalmSens, EmStat3)	7 RNA copies/µL	<20 min	[70].

Table 1. *Cont.*

Bioprocessing Approaches	Analytes	Samples	Transducers	Analyzers	LOD	Assay Time	Ref.
Bifunctional G-FET Chip—PBASE linker—ssDNA probe or antigen; hybridization technique	RNA and IgG/IgM	Oropharyngeal swabs and serums	Electrical (FET)	Home-built detector	~0.1 and ~1 fg/mL for RNA and antibodies	10 and 5 min respectively	[85]
(iii) Label-free electrochemical NAT							
AuNP-electrodeposited titanium substrate—RNA/DNA hybridization; label-free	RNA/c-DNA	-	DPV/EIS	Smartphone analyzer	-	-	[86].
ITO–PPy–AuNP–Cys–primer–BSA; 4[Fe(CN)6]/K3[Fe(CN)6] redox probe	N gene	Synthetic sample	EIS	Autolab (Metrohm)	258.01 copies/µL	15 min	[71]
Flex PCB—graphene WE-streptavidin/biotin-ssDNA-gene—ferro/ferricyanide redox couple	ORF1ab gene	Synthetic DNA	DPV	-	5×10^5 copies/µL	30 min	[87]
B. Immunoassays (antigen/antibody testing)							
(i) Electrochemical antigen testing							
LSG/AuNS Immunosensor; (LSG/AuNS/Cys/EDC:NHS/anti-SARS-CoV-2/BSA)	Spike (S) protein	Blood	DPV, CV	Handmade potentiosta with smartphone	2.9 ng/mL	1 h	[96]
Cotton-tipped sensor; carbon nanofiber (CNF)-SPE—EDC/NHS—N protein; competitive assay	N protein	Spiked nasal sample	SWV	-	0.8 pg/mL	20 min	[97]
Pt interdigitated electrode AuNP—organic ligand—VOCs	-	Exhaled breath	Conductivity	Handheld custom-made analyzer	90% accuracy, 95% specificity	-	[98]
Face mask-based EBC collection; aptamer-SPE-Au electrode—thiol/EDC:NHS; ferrocenemethanol redox mediator	S protein	Exhaled breath condensate	DPV	Sensit-Smart smartphone potentiostat (PalmSens)	10 pfu/mL	10 min	[94]
Thin-film Au WE-optimer/aptamer-TCEP-BSA	S1 protein	Commercialized sample	EIS	-	80 ng/mL (tested conc.)	15 min	[102]
PCB/thin-film Au E-PFDT-ACE2–spike protein	S Protein	Recombinant protein	EIS	-	1.68 ng/mL	30 min	[103]
Si/SiO_2-CNT-FET-PBASE linker—anti-SARS-CoV-2 S1	S1 protein	Fortified saliva	FET	Keithley 3 probe station	4.12 fg/mL	2–3 min	[99]
Microfabricated graphene FET chip—PBASE linker—anti-S protein	Spike (S) protein	Nasopharyngeal swab and cultured particle	FET	Semiconductor analyzer and probe station	242 virus copies/mL; 16 pfu/mL of cultured particles	Real-time to 10 min	[105]
Si MOSFET with glucose strip—Au clusters—antibodies; dual detection	S protein and cTnI	Saliva and spiked sample	Pulse method	Custom-made analyzer	100 fg/mL	-	[100]

Table 1. *Cont.*

Bioprocessing Approaches	Analytes	Samples	Transducers	Analyzers	LOD	Assay Time	Ref.
SPCE-mAb or AuNP-FTO—antibody; $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$	S protein	Spiked saliva	DPV	Home-built eCovSens	90 fM	30 s	[106]
Graphene SPE—PBASE - monoclonal anti-spike antibody	S protein	Saliva	EIS/CV	PalmSens 4	20 µg/mL	45 min	[107]
SPCE-Cu_2ONCs-ProtA—BSA—IgG	S protein	Saliva, artificial nasal swab	EIS	µ-Autolab type III	0.04/fg mL	20 min	[108]
Multichannel immunoassay (MEIA); SPCE—mAb—HRP—TMB	Spike protein	Clinical samples	Amperometry	Emstat Potentiostat (Palmsens)	0.15 ng/mL for SARS-CoV-2 1.12 unit/mL for A(H1N1)	1 min	[110]
Carbon black—SPE—MBs—ALP—pAb	S and N protein	Saliva	DPV	Portable PalmSens3, computer	19 ng/mL (S), 8 ng/mL (N)	30 min	[114]
Microfluidic chip—SPGE—cAb—dually labeled MB—dAb-HRP—TMB	N protein	Whole serum	Chronoamperometry	PalmSens4, Sensit Smart potentiostat, Smartphone	50 pg/mL and 10 pg/mL in whole serum and 5-fold diluted serum	<1 h	[111]
AU-TFE—MIP(PmPD)—ncovNP; ferri/ferrocyanide redox pair	N protein	Nasopharyngeal swab	DPV	EmStat3 Blue and Sensit Smart (PalmSens), Smartphone	15 fM	45 min	[92]
MP Au-SPE—MIP (oPD)—SARS-CoV-2-RBD; $Fe(CN)_6$ 3-/4-redox probe	RBD protein	Saliva solution	EIS	-	0.7 pg/mL	20 min	[91]
Bioelectric recognition assay (BERA); eight Au SPE—mammalian cells -electroinserted or membrane-bound antibodies—antigen	S1 protein	Synthesized samples	Potentiometry	Multichannel potentiometer (Embio Diagnostics Ltd., Cyprus), smartphone reader	1 fg/mL	3 min	[93]
SCPE/GNP-MV-gal1—spiked virus or protein; $[Fe(CN)_6]^{-3/-4}$ redox probe	Virus/antigen	Nasopharyngeal swab	EIS	SP-300 Instruments (SP-300) Texas, USA	4.57×10^2 copies/mL	~5 min	[95]
AuNP and/or SPE—thiol-EDC/NHS –BSA—mAb	S protein	Recombinant protein	Colorimetric and SWV	Metrohm Dropsens potentiostat	48 ng/mL (colorimetric), 1 pg/mL (electrochemical)	10 min	[115]

Table 1. *Cont.*

Bioprocessing Approaches	Analytes	Samples	Transducers	Analyzers	LOD	Assay Time	Ref.
			(ii) Electrochemical antibody testing				
3DcC chip—rGO nanoflakes—AuNPs micropillar—EDC:NHS—S1 and RBD	Spike S1 and RBD antibodies	Readymade antibod-ies	EIS	Sensit Smart workstation (PalmSens), Smartphone	1.0 pM for spike S1 and 1.0 fM for RBD Ab	11.5 s	[112]
Laser-engraved graphene (LEG) electrodes on polyimide—PBA—DMF—EDC:NHS—MES—capture protein—HRP–TMB; multiplex detection; telemedicine	N protein, S1-IgG, S1-IgM, and C-reactive protein	Saliva and blood	RapidPlex Amperometric	CHI820 electrochemical station, smartphone	-	1 min	[113]
SPCE-streptavidin/biotin-anti-SARS-CoV-2 spike RBD protein-ALP/pAPP	IgG and IgM	Serum	Chronoamperometry	PalmSens EmStat3 Blue, smartphone	10.1 ng/mL (IgG), 1.64 ng/mL (IgM)	13 min	[116]
Elecsys anti-SARS-CoV-2 assay; microparticles—streptavidin/biotin—magnetic capture—electrode—recombinant antigen labeled with a ruthenium complex	N protein-specific antibodies	Serum and plasma	Electro-chemiluminescence	Cobas e analyzer	Specificity: 99.80%; Sensitivity: 85.3%	18 min	[117]

N.B.: Readers are requested to look up the meanings of abbreviations used in the table in the texts that relate to specific references. Because there are so many abbreviations, only those that are not addressed in the texts are listed here. Abbreviations: Si-RP, silica-reporter probe; SiMB, silica–methylene blue; SiAO, silica–acridine orange; mAb, monoclonal antibody; pAb, polyclonal antibody; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, *N*-hydroxysulfosuccinimide; MES, ethanesulfonic acid hydrate; DMF, *N*,*N*-dimethylformamide; TCEP, Tris(2-carboxyethyl) phosphine hydrochloride; MCH, 6-mercapto-1-hexanol.

4.2. Paper-Based POC Electrochemical Tests

In recent years, microfluidic paper-based analytical devices (PADs) have been extensively researched for quick diagnosis of contagious diseases, with the potential to replace traditional lab tests and chip-based biosensors. Electrochemical paper-based analytical devices (ePADs) (an electrochemical sensing variation of PADs) are significantly simpler, more cost-effective, and faster than other devices, and they have excellent fitness for POCTs because of their ease of manufacturing, accessibility, disposability, and portability [21,22,118–120]. They use the capillary effect to let a biological sample diffuse without the use of additional components such as pumps or valves, as well as external power/pressure supplies. Screen/stencil/inject-printing, drop-casting, pencil/pen-drawing, wire-fitting, vacuum-filtering, pyrolyzed paper, and reagentless laser-scribing have all been used to produce electrode designs on PADs [21,22,118].

The folding or stacking properties, often known as the "origami" approach, have evolved appealingly to simplify electrochemical devices and procedures, resulting in a user-friendly POCT [21,120]. Here, we go through some possible paper-based electrochemical POC diagnostic tests that could be used in a crisis.

4.2.1. Paper-Based Electrochemical Nucleic Acid Testing (NAT)

Paper-based electrochemical NATs were few and far between for COVID-19 detection. Therefore, relevant NAT schemes designed for other diseases are presented targeting POC diagnostics in a pandemic.

Alafeef et al. developed a low-cost electrochemical paper-based analytical device (ePAD) for amplification-free sensitive detection of the SARS-CoV-2 N gene in clinical samples within 5 min [121]. They made an electrode out of a thick coating of graphene on

a piece of paper. To achieve higher specificity in the test scheme, four antisense oligonucleotides (ssDNA) functionalized gold nanoparticles (AuNPs) were targeted to two sections of the same SARS-CoV-2 N gene at the same time (Figure 5A). Individually, graphene and AuNPs increased the sensor's conductivity and sensitivity, allowing for an LOD of 6.9 copies/µL (i.e., 11.45 fM) when testing clinical specimens (nasopharyngeal and nasal swab and saliva samples). A handmade circuit was used to measure the electrochemical (current–voltage) signals. They tested 48 clinical samples to verify the biosensor and found no cross-reactivity with SARS-CoV, MERS-CoV, or negative samples, making this technique reliable for rapid infection identification in emergency scenarios.

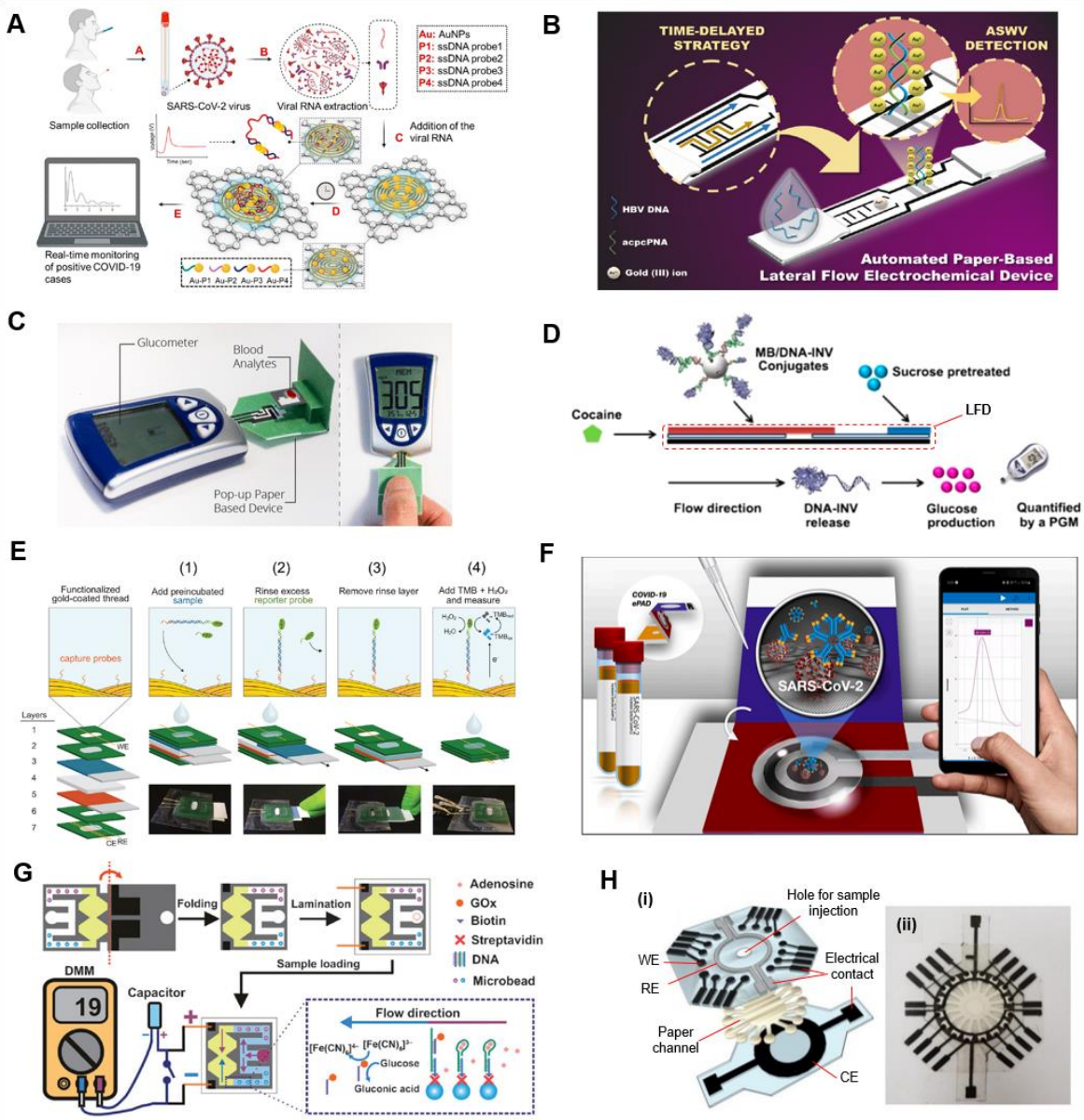

Figure 5. Paper-based potential electrochemical assays. (**A**) Schematic illustration of the paper-based electrochemical immunosensing platform for amplification-free SARS-CoV-2 RNA detection in nasal swab or saliva samples. Adapted with permission from [121]. Copyright 2020, American Chemical Society under ACS COVID-19 subset for unrestricted RESEARCH reuse. (**B**) Schematic presentation of the electrochemical lateral flow assay (eLFA) device for single-step HBV DNA detection following the metallization assay. Adapted with permission from [122]. Copyright 2020, American Chemical Society. (**C**) Photographs of 3D popup ePAD interfaced with a commercial glucometer. Adapted with permission from [123]. Copyright 2016, American Chemical Society. (**D**) Illustrated

presentation of electrochemical cocaine detection using lateral flow dipstick (LFD) and personal glucose meter (PGM). The platform ran on a competitive assay mediated by the aptamer and DNA–invertase conjugate-functionalized MBs. Reproduced with permission from [124]. Copyright 2016, American Chemical Society. (**E**) Schematics of the components, fabrication, and operation of the thread-electrode integrated ePAD for RPA-based NAT. Steps of the operation and assay—(1) addition of labeled sample, (2) rinsing of reporter probe, (3) removal of washing layer, and (4) application of TMB + H_2O_2 enzymatic substrate. Reproduced with permission from [125]. Copyright 2021, the authors. Published by American Chemical Society under Creative Common CC BY license. (**F**) Schematic drawing of the smartphone-integrated COVID-19 ePAD platform for antibody detection. The sample processing and reagent mixing were performed by folding the paper device. ePAD, electrochemical paper-based analytical device. Reproduced with permission from [126]. Copyright 2020, Elsevier B.V. (**G**) The operating principle of oPAD-based electrochemical aptasensing platform. The data reading is performed by a digital multimeter (DMM) from the electrochemical assay on the SPCE (bottom). Adapted with permission from [127]. Copyright 2012, Wiley-VCH Verlag GmbH & Co. KGaA. (**H**) The μMULTI-μEPAD platform for the multiplexed electrochemical test. (i) Schematics of device layers showing electrodes and other components. (ii) Photography of amassed μMULTI-μEPAD. Reproduced with permission from [128]. Copyright 2019, Elsevier B.V.

In an attempt to simplify NAT, the Chailapakul group from Thailand developed a paper-based automated electrochemical LFA (eLFA) for amplification-free and one-step detection of hepatitis B virus (HBV) [122]. The eLFA device was made by screen-printing three electrodes on a PVC substrate and placing them on a plastic supporting sheet, and then adding the wax-printed pad, sample pad, and adsorbent pad. The target DNA from serum arriving through the non-delayed channel was promptly hybridized by a pyrrolidinyl peptide nucleic acid (acpcPNA) probe immobilized on the test line (TL). A time-delayed route was used to provide an Au^{3+}-containing fluid to TL, allowing Au^{3+} to bond to hybridized DNA and create an electrical signal following the electrodeposition (i.e., metallization) process (Figure 5B). A portable PalmSens 4 potentiostat was used to measure the electrochemical output through anodic stripping square wave voltammetry (ASWV) approach. With a LOD of 7.23 pM HBV DNA, the one-step detection technique is rather easy and has the potential for quick POC NAT in pandemic-like emergencies.

A popup ePAD based on acpcPNA probe and target DNA hybridization was previously described by the same group for label-free HBV DNA detection with a LOD of 1.45 pM in 45 min [129]. On the back of said ePAD, the acpcPNA was covalently linked to cellulose paper. The compactness of the electrodes and reaction area on popped up paper, which can support sample processing and detection stages by easy folding, was one of the device's advantages. The device was folded into a tight paper form once the incubation process was completed, and ready to connect to an analyzer through contact pads on paper. DPV was employed to read the signal in presence of [Fe $(CN_6)^{3-/2-}$]. The popup ePAD showed the promise for POC application, but the accessibility through a portable analyzer or smartphone still has to be worked out.

The Whitesides group earlier solved this issue by combining a portable analyzer with a 3D popup ePAD and utilizing amperometric analysis with a commercial glucometer (Figure 5C) [123]. They detected beta-hydroxybutyrate (BHB), a biomarker for diabetic ketoacidosis, with an LOD of 0.3 mM in 2 min. The 3D popup ePAD's folding/unfolding mechanism aided fluid distribution timing and routing, as well as control over sequential assay steps and switches in electrical connection. The platform may be used to replace plastic test strips and allow for POCT on site or at home.

However, the use of a glucometer-like inexpensive and easily accessible analyzer in NAT or protein (antigen/antibody) testing has yet to be fully investigated for emergency use.

While multiphase reagent processing (such as immobilizations, hybridizations, washings, or enzymatic reactions) on an electrochemical device is challenging, end-users will

find it more convenient if the reagent processing can be automated and biomarker detection can be performed with glucose as the assay's end product and measured by a personal glucose meter (PGM) [130]. To address this constraint, Tsinghua University researchers integrated the lateral flow device/dipstick (LFD) and PGM into electrochemical biosensing for quantitative detection of cocaine and streptavidin with LODs of 7.7×10^{-6} M and 0.2×10^{-6} M cocaine and streptavidin, respectively [124]. To detect cocaine, for example, a DNA sandwich structure was formed on magnetic beads (MBs) and mounted on the reaction pad of the LFD by simultaneously hybridizing DNA–invertase, biotin–DNA, and cocaine aptamer. Upon dipping the LFD in sample (cocaine) solution, the DNA–invertase conjugate was released from the DNA sandwich structure due to the cocaine aptamer's target-specific structure switching, migrated to the reaction pad via a filter, and hydrolyzed the embedded sucrose to convert it to glucose, which was then electrochemically measured with PGM (Figure 5D). The cocaine concentrations in the solutions were correspondingly reflected by the glucose content. Because aptamers for particular DNA or antibody targets may be designed, the LFD-PGM platform can be a potent tool for detecting different targets by simply modifying specific recognition sites on the LFD strips. In low-resource situations and emergencies, the LFD-PGM system can be adopted for easy, rapid, and on-site POCT.

Despite the advantages of ePAD-based amplification-free NAT, isothermal amplification-based NAT has the potential to increase high-specific detection with a small number of target analytes, which was not explored for COVID-19 (until November 2021). Alternatively, the requirement for electrode printing on a paper substrate may result in additional costs, time, and resources. To address these issues, recently, Khaliliazar et al. reported a 3D microfluidic paper-based electrochemical device integrated with silver and gold thread-electrodes for RPA-based NAT of the toxic microalgae *Ostreopsis* cf. *ovata* [125]. The gold electrodes were functionalized with a thiolated capture probe before integrating into the devices. They incorporated a sandwich hybridization test that combined sample incubation, washing, and detection procedures utilizing moveable filter paper layers to enable time-sequenced responses (Figure 5E). Glass fiber substrates are used to store RPA reagents and perform isothermal amplification (at 37 °C) in the gadgets. The electrochemical output from the catalytic reaction was measured by chronoamperometry. The assay can be completed in 95 min with a LOD of 1 ng/µL DNA. The thread electrodes in paper-based devices might supplement single-use NATs for infectious illness diagnosis in the POC settings.

4.2.2. Paper-Based POC Electrochemical Immunoassays

Yakoh et al. recently reported a screen-printed electrochemical paper-based analytical device (ePAD) for label-free detection of SARS-CoV-2 specific immunoglobulins (IgG and IgM) that discarded the requirement of additional antibodies [126]. To construct the sensor, the spike protein receptor-binding domain (SP RBD) was immobilized onto the working electrode using GO-EDC/NHS chemistry. The sample and reagents were processed by folding the paper device (Figure 5F). The ePAD worked by reducing the current response due to the presence of SARS-CoV-2 antibodies that interrupted the redox conversion of the redox indicator ($[Fe(CN)_6]^{3-/4-}$) or created immuno-complexes. Square wave voltammetry (SWV) was performed using a portable and wireless PalmSens potentiostat (with a smartphone interface) to analyze the signal output. The ePAD was shown to be three orders of magnitude more sensitive than the colorimetric LFA in detecting SARS-CoV-2 antibodies with an LOD of 1 ng/mL in under 30 min. When the ePAD was tested against six other viruses, no cross-reactivity was observed.

Torres et al. followed the trend by attempting a simple, affordable, and smartphone-interfaced ePAD platform termed real-time accurate portable impedimetric detection prototype 1.0 (RAPID 1.0) for the SARS-CoV-2 S protein detection in 10 µL clinical samples with an LOD of 2.8 fg/mL in 4 min [131]. The ePAD sensor was produced by screen-printing conductive carbon and Ag/AgCl inks for WE/CE and RE on a phenolic paper circuit board or filter paper, respectively, and then immobilizing the SARS-CoV-2 ACE2 receptor using glutaraldehyde functionalization. The sensitivity and the anti-biofouling property

were improved by adding a Nafion permeable membrane. The electrochemical data were measured using smartphone-coupled Sensit Smart (PalmSens) potentiostats employing EIS against ferricyanide/ferrocyanide redox probe. RAPID 1.0 had 100% specificity and ~85% sensitivity for nasopharyngeal/oropharyngeal swab samples. The RAPID 1.0, in conjunction with telemedicine tools, might play a critical role in pandemic management by providing rapid, low-cost, and remote diagnostics.

Following a similar strategy, Ehsan et al. demonstrated a paper-coupled impedimetric electrochemical biosensor for label-free detection of SARS-CoV-2 spike protein in nasopharyngeal samples in 5 min [132]. For batch-fabricated electrode structuring, the inks of graphene/carbon composite, carbon, and Ag/AgCl were screen-printed on the paper substrate for the WE, CE, and RE, respectively. The electrode was modified utilizing PBASE or ProtA to immobilize the S1-specific IgG antibody, as a substitute to the recurring EDC/NHS chemical anchoring technique. The sensor showed remarkable analytical properties, such as a quantification limit of 0.25 fg/mL and linearity range up to 1 µg/mL for S and RBD protein. This demonstrates the power of paper-integrated electrochemical devices to deliver highly specific and sensitive results with LODs lower than those of ELISA. The electrochemical data were derived in EIS and CV methods using a battery-powered portable potentiostat/impedance analyzer (PalmSens4 or SensIT BT), and then shared to a laptop/smartphone using Bluetooth connectivity, which made this platform appealing for low-cost and fast POC diagnostics.

Li et al., on the other hand, suggested employing a µPAD electrochemical biosensor with zinc oxide nanowires (ZnO NWs) on the working electrode to improve label-free detection of SARS-CoV-2 [133]. While targeting HIV detection, the LOD of this EIS-based sensor was determined to be 0.4 pg/mL for p24 antigen. Testing various concentrations of SARS-CoV-2-specific IgG antibody proved the effectiveness of SARS-CoV-2 detection.

4.2.3. Complementary POC ePAD Approaches

Electrochemical components on origami paper and portable analyzer cum transducer can simplify the electrochemical POC device, thereby reducing the fabrication cost and increasing the portability. Liu et al. reported a self-powered origami paper (oPAD)-based electrochemical aptasensor coupling a portable digital multimeter (DMM) transducer for sensitive and rapid detection of adenosine (Figure 5G) [127]. The device is a single piece of printed paper folded into a three-dimensional (3D) conformation and laminated in plastic. The oPAD contained two microfluidic channels—one for aptamer immobilized microbeads to trap a target and release a GOx-labeled DNA strand, and another for control having only microbeads. GOx catalyzed the oxidation of glucose, which converted $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ resulting in a voltage due to the concentration difference in the two channels. The voltage charged a capacitor, which was discharged through the DMM when the switch was off. The capacitor amplified the current signal, which was measured with DMM. The detection could be completed in 10 min (with a LOD of 11.8 µm) depending on the fluid connection in the channel. The system was also capable of doing both fluorescence and electrochemical detection. The device is very simple, inexpensive, and operable without external power, making it ideal to be applied in POC diagnosis.

The detection of infectious agents could be possible within a few minutes if a potentiometric sensor on a simple printing paper strip is designed. A potentiometric aptasensor-on-paper was recently applied for the detection of the whole virus [134]. The paper sensor performed like a P–N junction based on two separate wet areas with dissimilar electrochemical potentials close to silver-paint electrodes painted on the printing paper strip. Zika viruses were immobilized on paper and bonded to the selective aptamers simply by putting the sample on the presoaked paper in aptamer solution, which made a concentration gradient along the paper strip and produced negative potential due to the small residual negative charge of Zika virus. The signal acquisition was achieved using a widely accessible simple multimeter. The sensing approach is pretty simple and rapid with approximately 5 min

detection time and a detectable signal limit of 1.2×10^6 Zika; which seems prospective for rapid POCT of pandemic diseases.

Despite numerous notable initiatives by ePAD to combat COVID-19, multiplex testing, which is required for assessing several biomarkers/analytes or strain discrimination, has remained a limiting issue. A 16-microfluidic-channel-based ePAD (named MULTI-μEPAD) was recently published by the Fatibello-Filho group for multiplex glucose detection in human urine samples [128]. The three-layered device contained 16 WEs and four REs on the top, 16 craft-cut Whatman filter paper-based microfluidic channels in the middle, and a circular CE at the bottom (Figure 5H). The device fabrication eliminated the necessity of wax patterning for flow channels. The carbon electrodes were fabricated on the polyester substrate using a stencil/screen printing technique. The WEs were reconfigured with chitosan/carbon black film and glucose oxidase, as well as ferrocene-carboxylic acid as a redox mediator, to conduct glucose detection. The device had an LOD of 3×10^{-5} mol·L^{-1} for glucose determination. The device, interestingly, works with a variety of electrochemical measuring techniques, including CV, SWV, DPV, and chronoamperometry. If improvised, the MULTI-μEPAD could be an effective tool in emergency settings for POC multiplex, high-throughput detection of protein (antigen/antibody) or nucleic acid linked with contagious diseases.

Overall, despite their huge potential, ePADs were reported for COVID-19 diagnosis far less frequently (Table 2). Replicability, LOD, specificity, storability, and multiplexing capacity must all be improved.

Table 2. Paper-based potential electrochemical biosensors for COVID-19 diagnosis.

Bioprocessing Approaches	Analytes	Samples	Transducers	Analyzers	LOD	Assay Time	Ref.
ePAD with graphene—AuNPs—ssDNA—two genes; Hybridization technique	N gene	Nasopharyngeal, nasal Swab and saliva	Electrochemical	Home-built circuit	6.9 copies/μL	<5 min	[121]
ePAD-SPE; GO—EDC/NHS-SP RBD; label-free	IgG and IgM	Clinical sera	SPV, EIS	Wireless PalmSens potentiostat, Smartphone	1 ng/mL	30 min	[126]
ePAD—carbon and Ag/AgCl inks—gluteraldehyde—ACE 2 receptor	S protein	Nasopharyngeal/ oropharyngeal swab	EIS	Sensit Smart (PalmSens) potentiostats, smartphone	2.8 fg/mL	4 min	[131]
μPAD-SPE—graphene/carbon WE—PBASE or ProtA—IgG antibody; label-free	S, RBD protein	Nasopharyngeal Swab	EIS, CV	PalmSens4 or SensIT BT, smartphone	0.25 fg/mL (limit of quantification)	5 min	[132]
μPAD—ZnO NWs WE—capture probe—blocking agent; label-free detection	P24 antigen for HIV, IgG for SARS-CoV-2	Spiked serum	EIS	Autolab	0.4 pg/mL (HIV)	~20 min	[133]

4.3. Nanomaterials/Nanochemistry-Assisted Electrochemical Tests

Alongside the POC electrochemical biosensors based on chip or paper devices, the development of nanomaterial/nanochemistry-assisted biosensors and different forms of electrode-based biosensors have been researched for COVID-19 diagnosis, which are discussed here along with a few other relevant platforms.

Many electrochemical systems require multistep procedures and labeling; therefore, easy pathogen detection strategies are essential [135]. We have developed several electrochemical assays for the detection of bacteria and viruses. Earlier, we developed a

neutralizer displacement assay for sensitive detection of *E. coli* using nanostructured microelectrodes [136]. For rapid detection of bacteria (within 2 min), we lysed bacteria near the sensor so that the slow-moving bacterial mRNA could quickly hybridize to the probe immobilized onto the sensor surface, which increased sensitivity, thereby enabling rapid detection of bacteria [137,138]. We also flourished the solution-based circuits for multiplexed pathogen detection in ~2 min [139]. The multiplexed chip was able to detect nine different bacteria, including antibiotic-resistant strains. We further developed a passive flow-driven, fully integrated, automated, and PCR-free nucleic acid detection approach for infectious disease (hepatitis C virus) diagnosis from whole blood in ~30 min [140]. All of these methods were very simple and did not need any kinds of labels. However, they require hexamine ruthenium in the readout solution for electrochemical signal generation and ferricyanide for signal amplification. Requiring external reagents for readout makes the methods not appropriate for direct in vivo analysis. Very recently, we developed reagent- and wash-free detection of the SARS-CoV-2 virus directly using a molecular pendulum [141]. Double-stranded DNA linkers, immobilized onto the sensor surface by thiol–gold chemistry, containing an antibody for the target of interest and a redox probe for signal readout, behave like an inverted pendulum under the influence of applied positive potential. The movement of the molecular pendulum is slower when it is bound to target analytes (Figure 6A). The time require for the pendulum to reach the sensor surface can be determined using chronoamperometry. This molecular pendulum sensor is able to monitor target proteins in vivo in the oral cavity of sedative mice [142]. Moreover, our sensor can directly detect SARS-CoV-2 in saliva samples obtained from COVID-19 positive patients in 5 min (with an LOD of 1 pg of spike protein and 4×10^3 particles per mL).

In an alternative approach, the configuration change of a nanoswitching-based biomaterial can offer more selectivity in sensing systems. Idili et al. recently suggested an electrochemical aptamer-based (EAB) sensor for SARS-CoV-2 spike protein detection, in which binding-induced conformational change of a redox reporter (Atto MB2)-modified aptamer on a gold electrode surface generates quantified signals (Figure 6B) [143]. This reagentless, single-step sensing technique can test serum and artificial saliva in 5 min with clinical sensitivity and specificity, making it ideal for quick, selective, and high-frequency testing; nevertheless, further research is needed on practical adaption.

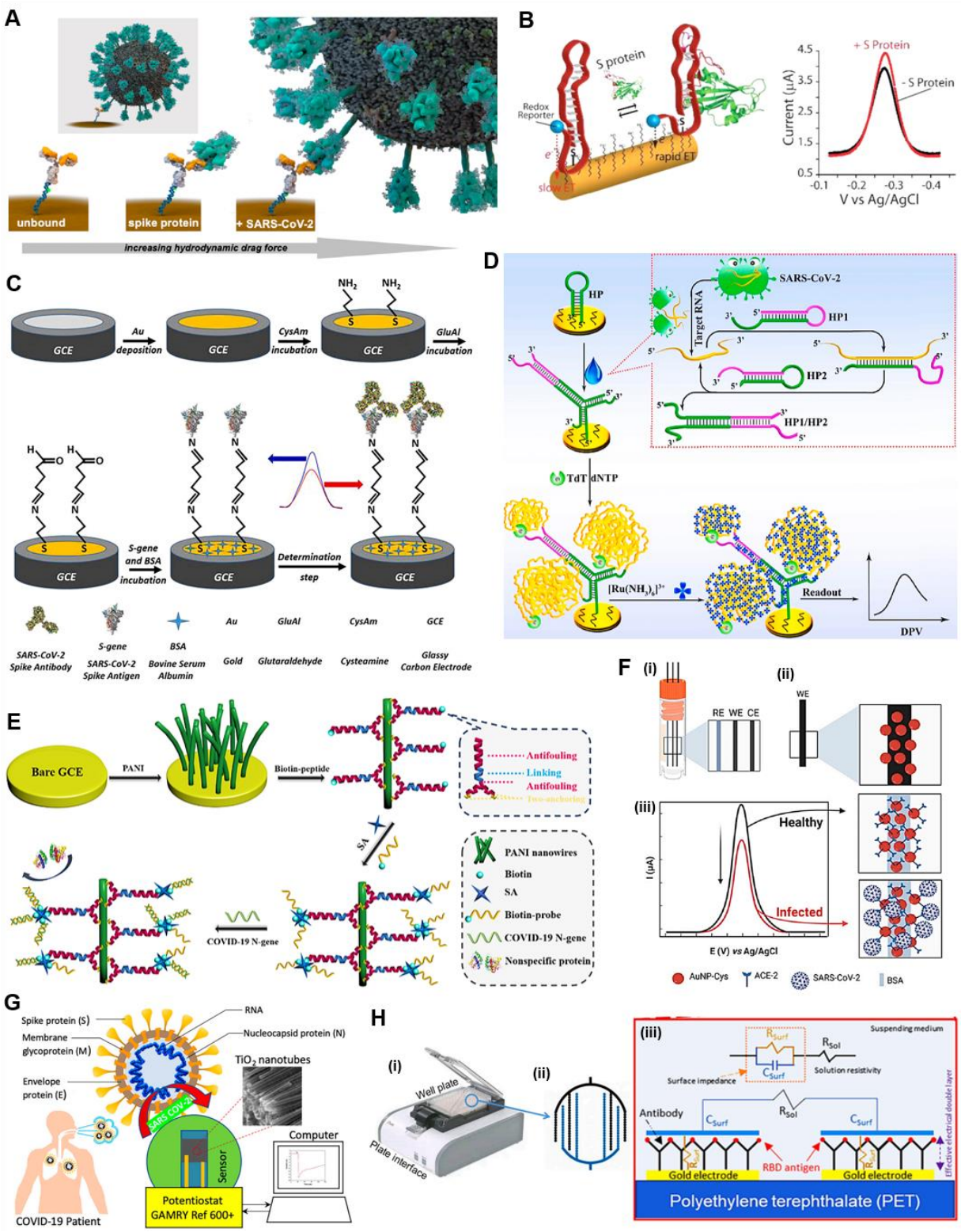

Figure 6. Miscellaneous potential electrochemical assays. (**A**) Schematic of the reagent-free POC electrochemical sensing platform for detection of SARS-CoV-2 viral particles using the molecular pendulum. The sensor undergoes a large change in hydrodynamic diameter when it binds to the spike protein and a viral particle, and then generates signal bending toward the electrode surface as a function of the time to respond. Reproduced with permission from [141]. Copyright 2021, American Chemical Society under ACS COVID-19 subset for unrestricted RESEARCH reuse. (**B**) Schematic of the electrochemical aptamer-based (EAB) sensors employing the binding-induced structural change of

a covalently attached and redox reporter (Atto MB2)-modified aptamer that exchanges electrons with the interrogating electrode and produces the electrochemical signal. Reproduced with permission from [143]. Copyright 2021, American Chemical Society under Creative Common CC BY license. (**C**) Procedures for making the electrochemical biosensing platform with GCE/Au/CysAm/GluAl/S gene/BSA for SARS-CoV-2 spike antibody detection. Adapted with permission from [144]. Copyright 2021, Elsevier B.V. (**D**) Principle of the sensitive electrochemical NAT platform for SARS-CoV-2 RNA detection based on the catalytic hairpin assembly circuit and terminal deoxynucleotidyl transferase-mediated DNA polymerization. Adapted with permission from [145]. Copyright 2021, Elsevier B.V. (**E**) Schematic of the antifouling electrochemical genosensor development process aimed at SARS-CoV-2 N gene detection. Adapted with permission from [146]. Copyright 2021, American Chemical Society under ACS COVID-19 subset for unrestricted RESEARCH reuse. (**F**) Schematic of low-cost electrochemical advanced diagnostic (LEAD) platform for SARS-CoV-2 S protein detection. (i) Graphite pencil electrodes (GPEs) for LEAD. (ii) AuNPs/Cys/glutaraldehyde-based GPE functionalization. (iii) EDC/NHS-mediated surface modification to enable selective binding of the analyte and generate a signal. Reproduced with permission from [147]. Copyright 2021, National Academy of Sciences; distributed under Creative Commons Attribution License 4.0 (CC BY). (**G**) Illustration of co-functionalized TiO_2 nanotube (Co-TNT)-based system for SARS-CoV-2 detection. Adapted with permission from [148]. Copyright 2020, the authors. Published by MDPI under Creative Common CC BY license. (**H**) Well-plate-based electrochemical impedance sensor. (i) and (ii) Images of a well-plate platform with electrode layout. (iii) Drawing of an impedance circuit model of the protein/antibody in solution. Reproduced with permission from [149]. Copyright 2020, Elsevier B.V.

Guo et al. introduced nanobody-functionalized organic electrochemical transistors (OECT) for the label-free detection of SARS-CoV-2, MERS-CoV, and green fluorescent protein (GFP) in 5 µL of untreated saliva and nasopharyngeal swab samples in ~10 min with an LOD of 23 fM [150]. The sensor was formed by immobilizing a spike protein-specific nanobody tagged with the SpyCatcher protein on gate (Au) electrodes through a SpyTag/SpyCatcher linker. Electrochemical signals were characterized by EIS/CV using Autolab potentiostat in the presence of $[Fe(CN)6]^{3-/4-}$. The gadget can detect single molecules to nanomolar levels of particular antigens in complex physiological fluids, making it an attractive candidate for high-sensitivity POCT.

The flexible OECTs can be applied for quick (5 min) POCT of SARS-CoV-2 IgG antibody in serum and saliva samples with an LOD of 10 fM utilizing a portable meter, Bluetooth transmission system, and smartphone interface, as recently described by Liu et al. [151].

The sensitivity of electrochemical sensors can be enriched by combining activated graphene and gold nanomaterial.

Hashemi et al. employed highly active graphene oxide (GO) and gold nanostars (Au NS) to create a label-free, ultraprecise fast electrochemical diagnostic tool for identifying monoclonal IgG antibodies against SARS-CoV-2 S1 protein in blood samples from COVID-19 patients in 1 min [152]. 8-Hydroxyquinoline, 1-ethyl-3-(3-dimethylaminopropyl), carbodiimide, and *N*-hydroxysuccinimide made up the GO on glassy carbon electrode (GCE). EIS was performed for signal generation. Even in samples with high contents of interfering compounds/antibodies, the nanosensor demonstrated excellent LOD and sensitivity of 0.18×10^{-19}% (V/V) and 2.14 µA·% $(V/V)\cdot cm^{-2}$, respectively, and it established a strong correlation with the gold standard (ELISA) in terms of specificity/selectivity. The G-Au NS sensor is also applicable for working electrode improvement of SPCE.

Otherwise, as revealed by Liv et al., enhanced functionalization of GCEs can provide excellent sensitivity. They achieved an LOD of 0.03 fg/mL for the detection of SARS-CoV-2 spike antibodies in synthetic and spiked real samples using the GCE modification, which included gold clusters (Au), mercaptoethanol (CysOH), S gene, and BSA (i.e., GCE/Au/CysOH/S gene/BSA) [144]. By substituting CysOH with a combination of cysteamine (CysAm) and glutaraldehyde (GluAl) (Figure 6C), the LOD could be reduced to

0.01 ag/mL (Liv et al., 2021). The modification system was thought to be applicable to commercial SPEs, expanding the breadth of the system's inclusion in POCT.

Efforts have also been undertaken to increase the sensitivity of electrochemical NAT for COVID-19 detection, which might be used in future POC applications.

The electrochemical NAT can be enhanced by triggering the catalytic hairpin assembly circuit, followed by terminal deoxynucleotidyl transferase-mediated DNA polymerization as reported by Peng et al. for monitoring SARS-CoV-2 RNA (26 nt long ORF1ab fragment) with a linear detection range of 0.1–100 pM and an LOD of 26 fM (Figure 6D) [145]. The process resulted in a huge number of extended single-stranded DNA molecules. Due to electrostatic adsorption, these negatively charged DNA products could hybridize with enormous positively charged $Ru(NH3)_6^{3+}$ electroactive molecules. After adding $Ru(NH3)_6^{3+}$, the electrochemical signals in DPV-based measurement were significantly enhanced.

Fan et al. presented an entropy-driven amplified electrochemiluminescence (ECL) approach for detecting the SARS-CoV-2 RdRp gene in a serum sample in 45 min with an LOD of <2.67 fM [153]. In the device, a DNA tetrahedron (DT) was formed on the electrode's surface to produce programmable structures in which target DNA might be incorporated. Afterward, at the vertices of the DT, an entropy-driven amplification reaction was carried out using $Ru(bpy)_3^{2+}$-modified S3 connected to a linear ssDNA capturing probe, which increased the ECL intensity and, hence, the sensor's selectivity. This allowed for a high-specificity and -sensitivity nuclease (enzyme-free) amplification reaction for NAT.

The voltammetric response/sensitivity in NAT may be improved by utilizing a redox probe in the silver ion-hexathia-18-crown-6 (HT18C6(Ag)) complex and a surface modifier such as SiQDs@PAMAM, as demonstrated by Farzin et al. for label-free and amplification-free detection of SARS-CoV-2 RdRP gene in sputum sample with a LOD of 0.3 pM [154]. Herein, to immobilize the probe sequences (aminated oligonucleotides), the HT18C6(Ag)-embedded carbon paste electrode (CPE) was further modified using chitosan and PAMAM dendrimer-coated silicon quantum dots (SiQDs@PAMAM). For the first time, the silver ions (Ag^+) in the HT18C6(Ag) were employed as a redox probe, and the declining current intensity was measured using the DPV method to represent the target sequence amount.

Alternatively, the sensitivity can be improved by enhancing the conductive surface and antifouling properties. Song et al. reported an antifouling electrochemical biosensor for detecting the SARS-CoV-2 N gene with a LOD of 3.5 fM, in which electrochemically polymerized polyaniline (PANI) nanowires were crafted onto glassy carbon electrode (GCE) to mount the inverted Y-peptides with antifouling attributes and two anchoring branches (Figure 6E) [146]. Different methodologies were used to investigate the antifouling efficacy against complex biological medium and protein. The SRSA-CoV-2 N gene-specific probe was anchored on the biotinylated peptide using a streptavidin linker. Even with a combination of 100 times greater interfering molecules, this genosensor maintained a high level of selectivity. In human serum samples, the biosensor exhibited excellent recoveries of up to 98.03–101.63%.

In a separate technique, Hwang et al. presented a recyclable label-free DNA capacitive biosensor for detecting the SARS-CoV-2 RdRp gene with a sensitivity of 0.843 nF/nM [155]. The sensor was made with platinum/titanium interdigitated electrodes on a glass substrate to detect the hybridized DNA complex of analyte and probe using capacitance–frequency measurements, and it could be recycled up to five times. The sensor was straightforward and may produce results in a matter of seconds; however, hybridization took around an hour.

In addition to expensive and time-consuming thin-film or screen-printed electrodes, thread- [125] or paint-based electrodes [134] (described earlier) can simplify the electrochemical testing system.

Recently, Lima et al. introduced a graphite pencil electrode (GPE)-based low-cost electrochemical advanced diagnostic (LEAD) platform that detected SARS-CoV-2 S protein with a LOD of 229 fg/mL within 6.5 min, at 1.50 USD per unit (Figure 6F) [147]. The

LEAD was made up of immobilized human ACE-2 onto the GPE that had been modified by glutaraldehyde–AuNP Cys–EDC/NHS chemistry, and it was packed in a plastic vial. The LEAD had an amazing performance index when analyzing clinical saliva (100% sensitivity, 100% specificity, 100% accuracy) and nasopharyngeal/oropharyngeal (88.7% sensitivity, 86% specificity, 87.4% precision) samples. No cross-reactivity with other viruses was discovered, and the test had a 5 day shelf life when kept at 4 °C. The current signal was derived by the SWV method using a lab-oriented Autolab potentiostat. If a portable potentiostat with a smartphone reader interface is incorporated, the ultracheap and easy electrochemical testing platform offers huge potential for self-diagnosis.

Ramanujam et al. adopted a novel electrochemical sensing strategy that included a rotating disc electrode (RDE) and electrochemical nickel hydroxide oxidation for SARS-CoV-2 S1 protein detection in saliva samples in 100 ms [156]. A Ni disc was used as the WE, a concentrically surrounding Pt ring was used as the CE, and a Pt foil served as the pseudo-RE in the RDE configuration. The sensor works on the basis of nickel oxyhydroxide (NiOOH) formation from the electrochemical oxidation of nickel hydroxide produced when nickel is oxidized by an alkaline medium. The process mimicking the SARS-CoV-2 binding onto host cells is used to detect the virus. The sensor can differentiate SARS-CoV-2 from other viruses like HIV. An activation step (CV) precedes the testing stage (chronoamperometry) in this technique. This platform is calibration-dependent, although it has the advantage of being able to be controlled remotely.

Vadlamani et al. disclosed a Co-functionalized TiO_2 nanotube (Co-TNT)-based electrochemical sensor for the quick and low-cost detection of S-RBD protein of SARS-CoV-2 in nasal swab and saliva samples in 30 s with a LOD of 0.7 nM [148]. TNTs were produced on Ti sheet via a simple and inexpensive single-step electrochemical anodization procedure, and Co functionalization was performed with the nascent wetting method. The upward-facing Co-TNT side serves as a WE, while the bottom-facing Ti side acts as a CE, with copper wiring on the top and bottom of the customized chip, respectively (Figure 6G). They recommended that in situ anodization synthesis and longer Co-TNT lengths might boost the biosensor's LOD even further. They also stated that SARS-CoV-2 was successfully detected in exhaled breath. A custom-built Co-TNT-packed printed circuit board arrangement with a Gamry reference 600+ potentiostat is used to monitor current amperometrically.

Increased levels of reactive oxygen species (ROS) in sputum samples, in addition to nucleic acid and protein markers, can be used as a prognostic indicator for COVID-19 infection. Miripour et al. used a needle tip electrode-based electrochemical sensor to detect ROS in a direct sputum sample in real time (30 s) with a 94% accuracy and 92% sensitivity [157]. The sensor was made by generating multiwalled carbon nanotubes (MWCNTs) on the tips of steel needle electrodes, and it worked on the basis of an ROS/H_2O_2-based electrochemical detection concept. The ROS release from virally infected lung epithelial host cells was correlated utilizing cyclic voltammetry response on a custom-built analyzer. This noninvasive testing tool has the potential to reduce the burden of CT scan-based diagnosis by prescreening respiratory illnesses.

Similarly, a low count of lymphocytes (such as $CD3^+/CD4^+/CD8^+$ T cells) could be a prognostic parameter for respiratory infectious diseases such as COVID-19 [158]. A few years ago, the Bashir group of the University of Illinois at Urbana-Champaign (Urbana, Champaign, IL, United States of America (USA)) introduced simplified and high-potential microfluidic biochips for counting $CD4^+$ and $CD8^+$ lymphocytes in 20 min from a single drop of whole blood with a view to POC diagnosis of HIV [159]. The chip enables on-chip preparation of samples such as erythrocyte lysis, quenching for leukocyte storage, and counting cells with the differential electrical counting method according to the Coulter counting principle that measures the impedance variation during the passing of particles or cells through a small orifice over an electrode. This approach presented accurate $CD4^+$ and $CD8^+$ lymphocyte counting over a dynamic range of 40 to 1000 cells/μL. The system can be easily compacted in a handheld, portable instrument for easy and faster POCT services.

High-throughput, accurate, and scalable testing with POC devices is essential to curb the transmission of infections in a pandemic. To support this issue, Rashed et al. devised a label-free impedimetric biosensor that can detect SARS-CoV-2 antibodies in blood samples in as little as 5 min [149]. They processed selective capture of anti-SARS-CoV-2 monoclonal antibody in a 16-well plate using immobilized SARS-CoV-2 RBD. The interdigitated electrodes were built beneath the wells of the sensor (Figure 6H). The antigen–antibody interaction was evaluated using impedance/capacitance measures. To compute the LOD of the biosensor, the investigation requires further samplings. The platform also supports simultaneous detection utilizing four 384 well plates, allowing the system to assess more than 1000 samples simultaneously, which is the platform's significant advantage for high-throughput testing.

Kiew et al. presented a completely different EIS-based sensor that detects possible inhibitors of SARS-CoV-2 S protein–ACE2 binding on the basis of an ACE2-coated palladium nano-thin film (Pd-NTF) [160]. The key sensing element was the ACE2–Pd–NTF electrode, which was utilized to track changes in the binding of SARS-CoV-2 S protein to ACE2 following exposure to modulating agents. This method was particularly effective in detecting pharmacological inhibitors of SARS-CoV-2 ACE2 binding.

The overall efforts to simplify and improve the electrochemical sensor platform were commendable. We assembled a list of initiatives (for SARS-CoV-2 detection) that employ simple production processes to improve current biosensors (e.g., sensitivity and simplicity of use) (Table 3).

Table 3. Other notable and nanomaterial-aided electrochemical biosensors for COVID-19 diagnosis.

Bioprocessing Approaches	Analytes	Samples	Transducers	Analyzers	LOD	Assay Time	Ref.
Reagent-free sensing with a molecular pendulum; electrode-tethered sensors; Au thin-film WE—thiolated probe/PBS/TCEP—antibody-conjugated complementary probe/MCH—analyte	Spike protein, viral particles	Saliva sample	Chronoamperometry	Epsilon BASi potentiostat	1 pg of spike protein, 4×10^3 particles per mL	5 min	[141]
reagentless, single-step sensing; Au WE-MCH-Aptamer-Atto MB2	S protein	Serum and artificial saliva	SWV	-	Clinical range	5 min	[143]
OECTs (PEDOT:PSS Channel)-AuE-SpyTag/SpyCatcher linker-spike protein-specific nanobody; $[Fe(CN)6]^{3-/4}$ redox probe	Spike proteins	Untreated saliva and nasopharyngeal swab	EIS/CV	Autolab	23 fM	~10 min	[150]
Flexible OECTs (PEDOT:PSS Channel)-AuE- MAA-GOPS [(3-glycidyloxypropyl) trimethoxysilane]–spike protein–BSA	IgG	Serum and saliva	EIS	Portable meter (PolyU), BlueTooth, Smartphone	10 fM	5 min	[151]
GCE or SPCE-GO/gold nanostars (Au NS)—antigen; label-free	monoclonal IgG antibodies	Blood plasma	CV, EIS	-	0.18×10^{-19}% V/V	1 min	[152]
GCE/Au/CysOH/S-gene/BSA or GCE/Au/CysAm/GluAl/S gene/BSA	Spike antibody	Synthetic and spiked real sample	SWV, CV	Autolab	0.03 fg and 0.01 ag/mL respectively	35 min	[144]
HP/MCH/AuE-RNA—dNTP-$Ru(NH3)_6^{3+}$	RNA (26 nt long ORF1ab fragment)	Serum and saliva	EIS	CHI660D Potentiostat	26 fM	2 h	[145]
Au WE—EDC/NHS—S3 DNA-$Ru(bpy)_3^{2+}$	RdRp gene	Serum	ECL	CHI 660 E	<2.67 fM	45 min	[153]

Table 3. *Cont.*

Bioprocessing Approaches	Analytes	Samples	Transducers	Analyzers	LOD	Assay Time	Ref.
Carbon paste electrode (CPE)/(HT18C6(Ag))//chitosan/SiQDs@PAMAM—ssDNA	RdRP gene	Sputum	DPV	PGSTAT 302N workstation (Autolab)	0.3 pM	25 min	[154]
GCE-PANI nanowires—inverted Y-peptides-streptavidin/biotin—capture probe	N-gene	Serum	DPV, CV	CHI 660 E	3.5 fM	1 h	[146]
Platinum/titanium interdigitated electrodes on glass; APTES-ssDNA probe; label-free	RdRp gene	Synthetic sample	Capacitance–frequency	-	0.843 nF/nM	Few seconds	[155]
Graphite pencil electrode (GPE)–gluteraldehyde– AuNP Cys–EDC/NHS chemistry ACE2	S protein	Saliva, nasopharyngeal/oropharyngeal swab	SWP	Multi Autolab	229 fg/mL	6.5 min	[147]
Rotating disc electrode (RDE); Ni disc WE/Pt ring CE/Pt foil RE-NiOOH-protein	S1 protein	Saliva	Chronoamperometry	Gamry Reference 600+ Potentiostat	-	100 milliseconds	[156]
Ti foil WE/Pt CE with Cu wiring—Co-functionalized TiO_2 nanotubes (Co-TNTs)—capture protein	S-RBD protein	Nasal swab and saliva	Amperometric	Co-TNT packed PCB with a Gamry reference 600+ potentiostat	0.7 nM	30 s	[148]
Needle-tip electrode—MWCNTs—ROS/H_2O_2	ROS	Sputum	CV	Custom-built device	94% accuracy and 92% sensitivity	30 s	[157]
16-well plate with interdigitated electrode beneath the wells—coated with RBD protein	Monoclonal antibody	-	Impedance	Impedance analyzer (Agilent 4294A)	-	5 min	[149]

5. Smartphone-Assisted Evidence-Based Epidemiological Reporting (or Surveillance)

While the smartphone has demonstrated its utility as a portable diagnostic analyzer, developing specialized app software and/or artificial intelligence (AI)-aided framework for the smartphone that targets the reporting of POC diagnostic data and patient's geolocation to a pandemic monitoring cell can make the smartphone an excellent surveillance tool. As a result, evidence-based epidemiological databasing may be reinforced and integrated with national pandemic disease management and policymaking i.e., contact tracing, isolation/quarantining, and telemedicine [33,34,58,60,61]. In this wisdom, highly sensitive electrochemical POCT paired with a smartphone and internet connectivity can serve as the REASSURED diagnostic platform, which can provide high-precision evidence-based epidemiological reporting via real-time geo-mapping (Figure 1) [31,33]. However, when compared to symptomatic testing and visual POC tests, epidemiological reporting of POC electrochemical tests has received less attention.

Previously, smartphone cameras and app analyzers were employed in microfluidic and LFIA systems to present optical results of nucleic acid and protein detection enabling epidemiological reporting of infectious diseases [161,162]. In the case of COVID-19 surveillance, a platform that performs both POCT and epidemiological reporting was little explored during the outbreak. DetectaChem Inc. integrated such a feature with its MobileDetect App that can provide the facility of reporting the colorimetric antigen test results (negative/positive) along with GPS location, date, time, and other information to the pandemic surveillance center from the user's smartphone, thus assisting in tracking the disease and assessing the hotspots [163]. The Finland-based ArcDia company afforded real-time COVID-19 surveillance using its mariCloud™ service that automatically transmitted the antigen-based SARS-CoV-2 detection result employing its mariPOC® Respi test [164]. Recently, Ghosh et al. reported a smartphone-interfaced automatic microfluidic

chemiluminescence-based POCT system that can detect infectious diseases such as malaria, HIV, or Lyme (Ghosh et al., 2020), which can be applied for coronavirus [165]. The smartphone used here can send the test result to the doctor using the app. The epidemiological data reporting to the surveillance center can be also harnessed using the same facilities.

For real-time epidemiological reporting and geo-locating the patient(s) to a surveillance center, Rodriguez-Manzano et al. interfaced with a smartphone and cloud system with RT-LAMP-based electrochemical (ISFET) nucleic acid testing of SARS-CoV-2 [65]. Torrente-Rodriguez et al. dazzled with a smartphone-integrated wireless electrochemical testing device with multiplexed antigen/antibody detection (of SARS-CoV-2) and telemedicine capabilities to enable ultrarapid (1 min) at-home testing and distant monitoring [113]. Torres et al. envisioned a smartphone-interfaced ePAD platform as a more convenient and faster method of remote diagnostics and telemedicine [131].

In combination with POC clinical or self-diagnostic testing, a smartphone interface as a portable analyzer and reporting tool can provide factual data on transmission risk through epidemiological reporting of true test results during surveillance. Consequently, electrochemical POCTs should be portable, inexpensive, and accessible in communities with limited diagnostic resources, as well as coupled with smartphone-based analysis and internet connectivity to aid surveillance in pandemic-like situations.

6. Summary and Perspectives

In this report, the trends and technological gaps of COVID-19 diagnosis by POC electrochemical sensors were investigated, highlighting toward rapid detection strategies with affordable devices, specific, and user-friendly applications for the early management of pandemic infectious diseases.

In response to the rapid and accurate diagnosis of COVID-19 with either nucleic acid (i.e., molecular) or immunological tests, scientists made stunning progress on POCTs by promoting several chip-based, paper-coupled, and/or nanomaterial-assisted improved electrochemical biosensors (Tables 1–3). SPE-based plastic (e.g., PET or polyester) or paper devices have been widely used in molecular/immunological POCT systems to facilitate inexpensive electrochemical diagnostics. However, the development of those quick POCT systems was inadequate and often unaffordable in terms of worldwide demand, cost, and feasibility for field deployment. Thin-film electrode-integrated microfluidic electrochemical platforms have received less attention than SPE-based devices. Microfluidic devices with micro-flow structures and components hold promise for high-precision electrochemical analysis with less reagent volume [21] and should be expanded in the future.

To escape the shortcomings of long turnaround time, thermal cycler requirements, and some false-prone readouts of RT-PCR, the application of iNAATs (except a few RT-LAMP, RT-RPA, and RCA) and CRISPR-based electrochemical assays has not been advanced so far for COVID-19 diagnosis. In the future, a CRISPR-based electrochemical sensing scheme can be projected to offer a new dimension of rapid and high-specificity (or single-nucleotide-discriminatory) diagnostics [76,77].

The POCT devices that run sample-to-answer program into sealed cartridges can return with a faster diagnosis. For example, carrying out iNAA and a subsequent electrochemical assay using the same paper/plastic device (with the assistance of a portable/integrated heater or water bath) is quite attractive and affordable for POCT application and, therefore, suitable for self-diagnosis during a pandemic. Another aspect of iNAA is the use of body temperature for nucleic acid amplification (by RPA) to circumvent the necessity of an external/additional heater, which can be integrated into a wearable electrochemical device to enable a rapid and user-friendly self-diagnostic NAT platform [66,166].

To skip the complexity of iNAATs, sample preparation should be simplified. Single-stepped magnetic bead-based or chemical-induced nucleic acid extraction and/or purification can be attempted. Nucleic acid extraction-free (i.e., nontreated sample-based) POCT systems, in that sense, can be kept on the front line for on-the-spot diagnostic tests. It is attractive that scientists prioritized amplification-free NATs during the pandemic period,

as opposed to the iNAATs' dependency on a heating module, enzymes, and primer sets, which could be improved further to ensure more streamlined POCTs in the future. Because most amplification-free diagnostic tools involve single-stranded oligonucleotide targets, it is still vital to look into a straightforward DNA denaturation procedure.

Furthermore, to make DNA/protein detection easier, numerous label-free, reagentless, highly sensitive electrochemical detection schemes for COVID-19 diagnosis have been developed, which do not require sandwiched/hybrid assays or specific signaling probes/enzymes.

Additionally, multiplex electrochemical NAT should be emphasized to realize fast serotyping of numerous strains and discriminatory detection of related pathogens, which is essential throughout the pandemic age.

Several enhancements for electrochemical components based on nanomaterials or nanocomposites were also presented, which are attractive to be used in future POC diagnostics (Tables 1–3). Furthermore, the considerable scope remains to improve the architectures of sensing surfaces regarding better sensitivity and novel detection strategies. In this respect, nanomaterials such as carbon (e.g., SWCNT, MWCNT, and graphene) and noncarbon (e.g., metallic, silica, indium tin oxide (ITO), organic polymers, and nanowire) NPs should be researched further and continued until the electrochemical diagnostic test becomes stipulated and pared down enough for the end-users.

More attention is needed for ensuring the reproducibility and stability of the electrochemical biosensors through the shelf-life extension of bioreceptors and the reinforcement of the electrode surface modification. Another challenging issue for electrochemical biosensors could be the lower affinity (i.e., receptor–analyte binding) interaction that can be expanded by bioengineering the receptors or employing multiple receptors [167,168].

On the other hand, the developmental trend of electrochemical immunoassays was mainly focused on the rapid detection of SARS-CoV-2 specific antigens (such as S, N, and ACE2 proteins) followed by serological testing of immune markers, e.g., IgG/IgM (Tables 1–3). However, antibody-based detection can assist in complementing the nucleic acid tests and compromise absolute specificity [48,169]. To escape this shortcoming, production of the antigen-specific monoclonal antibodies (mAb) and their application in the POCT system are crucially important; which can be worthwhile for virus load and immune status testing during or after the pandemic period, as previously reported for the SARS-CoV outbreak [170,171]. SARS-CoV-2-specific mAB production has been less explored, and few studies were in the pipeline [172,173]. Instead, other immune-signatory (indirect) biomarkers such as thrombocytopenia (low platelet count) [158,174], lymphocytopenia (low lymphocyte count) [158], cytokines (e.g., interleukin-6) [175], and serum amyloid A (SAA) [176] can be pursued to prospective diagnosis. Even so, those biomarkers may only meet the specificity halfway. In contrast, an aptamer-based sensor can resolve the specificity-related issue to some extent with its genetic material-assisted biomarker detection ability; however, studies have focused little on SARS-CoV-2 electrochemical detection. Accordingly, it can be desired to observe the tremendous success of aptasensors in the near future because of their merits over antibodies.

The current systems lack multiplexing capability and automation of solution (i.e., sample or reagent) processing, and they are more often burdened with a longer time for sensing surface construction. The advancement of microfabricated electrochemical sensing platforms under the shade of nanotechnology-governed microfluidics can mitigate these issues and support the low-cost biosensor by enabling low-volume reagent consumption [21,35]; therefore, they should be projected for future applications.

Technically, POC assay devices should be simply fabricated and spontaneous so that fewer manual interventions are required for conducting the assay, and the diagnosis proceeds faster [21,80,83,177,178]. In that respect, simplification in the structure and function of the microfluidic components (i.e., micropump, valve, and others) is necessary. Moreover, self-powering by the chip should be the future focus to discard reliance on external power/pressure sources needed for automatic solution processing in microfluidic

devices [70,81,127]. Otherwise, manual pumping can be applied in microfluidic systems to run urgent stepwise bioassays [83].

To minimize the operational steps and achieve faster detection, utilization of freeze-dried/lyophilized reagents on the integrated device should be considered.

It is impressive that the use of miniaturized commercial potentiostats (such as PocketStat, DropStat, EmStat, Sensit smart, or others) and smartphones as portable readers/displayers has expanded dramatically in favor of quick and easy COVID-19 diagnosis (Tables 1–3). However, while a single test is rationally administered to aid in the urgent diagnosis of an individual in a pandemic situation, the POCT, even with commercial miniaturized analyzing devices, may not be economical due to higher manufacturing costs. In this respect, portable analyzers should be affordable enough to be used for on-the-spot situations, with the goal of self-diagnosis at home or in the workplace rather than in clinics, especially in the developing world with resource inadequacy.

Several attempts have already been made to build low-cost customized electrochemical analyzers [22], and these efforts have continued for COVID-19 diagnosis (Tables 1 and 2); however, improvements are still needed to make them practical for single use or multiple use while conducting other important tests in the near future. For example, Bezuidenhout et al. described a low-cost potentiostat based on inkjet-printed paper that is both simple and accessible for electrochemical measurements in POC situations [179]. If a paper-based potentiostat with an accessible smartphone displayer can be constructed for single use or adopted for glucometer-like multiple tests, then electrochemical tests, such as colorimetric LFA, can be augmented for infectious disease detection in an emergency. Other than Clark-type glucometers (for glucose/lactate monitoring), commercially produced small analyzers are still struggling with the cost of single/regular electrochemical measurements. Therefore, connecting electrochemical biosensors made using SPEs or electrochemical LFA with amperometric transduction employing glucometer-like electrochemical integrators, which are extensively used in clinics and by the community, is a viable technique.

As another approach, glucose-based biomarker (DNA or protein) detection paired with a glucometer [124,130] may be coupled with smartphones for wireless signal transmission, simplifying and expanding the tests necessary in pandemic-like circumstances. Interfacing internet connectivity with the reader can unlock much greater potential. Remote testing, geo-tagging, and telemedicine may all be accomplished as a result, which is crucial for evidence-based pandemic surveillance [33,34,58,60,61].

Biomarker test-based epidemiological reporting, which has previously been shown to be successful in managing community transmission of infectious diseases [34,55–58], received less attention during COVID-19, despite the fact that it should be prioritized in order to combat the pandemic.

Within the framework of smartphone-integrated self-testing and epidemiological reporting, it is time to consider minimum-stepped plastic/μPAD-based NAT for diagnostic reporting alongside the immunological/serological tests. On this point, a microfluidic saliva sample test can be encouraged to abate the risk of exposure to any assistants or health workers and reduce the discomfort of the self-testers while using nasopharyngeal and oropharyngeal swabs. Furthermore, POC microfluidic diagnostics using direct clinical samples such as saliva, whole blood, plasma/serum, or swabs must be thoroughly explored to overcome the challenge of extracting/purifying the genetic material and protein, and delay in diagnosis.

In the near future, an automated single POCT platform integrated with both nucleic acid and immunological tests embedding a built-in or portable analyzer like a smartphone will be highly anticipated to realize fast and accurate (self-)diagnostic output.

7. Conclusions

Electrochemical sensing tools have benefits over other bio/chemical sensing systems in terms of downsizing, batch production, sensitivity, and integration of the distinct components. Moreover, electrochemical techniques can analyze unpurified and opaque

samples. The electrochemical sensing strategies/techniques described in this report for POC COVID-19 diagnosis, as well as for other targets, are simple, user-friendly, easy-to-construct, comparatively inexpensive, and rapid. The major emphasis is put on the tests that can be performed on the spot or at small clinics near the patients using the least number of devices and completed in the shortest possible turnaround time (Table 1–3) so that a rapid detection scheme can be priorly designed, developed, and implemented to fight against the pandemic situation.

Despite some pros and cons, the progress in developing POC electrochemical diagnostic tests for COVID-19 detection was noteworthy in contrast to the short period available for designing and implementing the tests. In that respect, COVID-19 has provided a lesson to the global community to add value to POCT research, which should be the flagship priority for policymakers at national and international levels. A reliable, rapid, and low-priced on-the-spot test is inevitable for arresting the racing pandemic situation. The future of POCT or diagnostics is expected to be progressively digitalized and connected, which could offer fast-tracked healthcare services during pandemics. On this issue, automatic or smartphone-coupled easy-to-perform electrochemical self-diagnosis connected to healthcare services is highly prospective, which could be governed through centralized health management policy.

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