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A Mini-Review On Emerging Trends and Recent Advances in Analytical Methods for Covid-19: Analytical Chemistry Perspective

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ABSTRACT: *In recent times SARS-CoV-2 pandemic, public health experts have slated testing, tracking infected people, as well as tracing their contacts as an efficient method to reduce the viral spread. Numerous diagnostic approaches are reported for detecting the coronavirus in public health, clinical and research laboratories. Most tests detect the infection directly by detecting the viral RNA while other test may indirectly detect the host antibodies in the course of detecting the infection. A diagnostic test during the pandemic should aid in making proper clinical decision within a short time frame. In this review, we describe the various techniques involved in sample collection from nasal, throat and sputum specimens, standard and developed methods for diagnosis of covid 19 such as the polymerase chain reaction (RT-PCR), CRISPR-based assays and Serological and immunological assays. General information on selected electroactive antiviral Covid-19 drugs have been highlighted including: Favipiravir (FAV), Remdesivir (REM), Lopinavir + Ritonavir (LOP + RIT), Hydroxychloroquine (HCQ) and Chloroquine (CQ), Ribavirin (RIB) and Sofosbuvir. This review presents the major standard, commercial and designed methods for detecting SARS-CoV-2 and their analytical performance. Also, chromatographic techniques have been highlighted as a rapid examination for SARS-CoV-2 identification, and some recent advances and emerging trends in analytical methods for covid-19 diagnosis have been addressed.*

KEYWORDS: COVID 19, antibodies, polymerase chain reaction, biosensors, nanomaterials, RNA

INTRODUCTION

The outbreak of coronavirus disease (COVID-19) in December 2019 in China follows numerous previous epidemics occasioned by respiratory viral infections which are highly

transmissible. For the first time, it was reported in Wuhan, China that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was the main cause of COVID 19 [1], which further resulted to global spread requiring urgent public health emergency.Experts in the field of Public health have pointed out some effective strategies to reduce viral spread such as testing large number of individuals as possible, tracking infected people, and tracing their contacts. Variably, this practice has been adopted globally by numerous government utilizing different testing methodologies.

The SARS-CoV-2 which is genetically similar to SARS corona virus and bat SARS-like corona viruses is a positive-sense single-stranded RNA β family [2, 3]. Each virion is in the range of 50–200 nm in diameter comprising of four structural proteins represented as E (envelope), M (membrane), S (spike), and N (nucleocapsid) [2]. The RNA genome of the virus is retained in the N protein, with collective creation of the virus envelope achieved by E, M and S proteins [2, 3]. Suggestion from recent studies implies that bats may be the potential natural host of SARS-CoV-2 [4, 5] and Malayan pangolin the potential intermediate host [6].

A large number of diagnostic methodologies have been employed for the detection of coronavirus in clinical, public health, and research laboratories. When direct tests are carried out, the infection is detected directly by detecting the viral RNA, whereas, when indirect testing is adopted, antibodies against the virus in a host that has been exposed is quantified. To make appropriate clinical decisions rapidly during a pandemic, diagnostic test method should be sufficiently sensitive and accurate [7]. Nucleic acid amplification by reverse transcription polymerase chain reaction (RT-PCR) is commonly employed for direct SARS-CoV-2 diagnosis [7]. Quantifying the antibodies against the SARS-CoV-2 is carried outusing Immunoassays. More recent methods involving the applicati of CRISPR (clustered regularly interspaced short palindromic repeats) have also been reported, as well as its incorporation into RT-PCR and immunoassay. Recently, many point-of-care (POC) and rapid test methods are available. The main diagnostic methods can be seen in Figure 1 below:

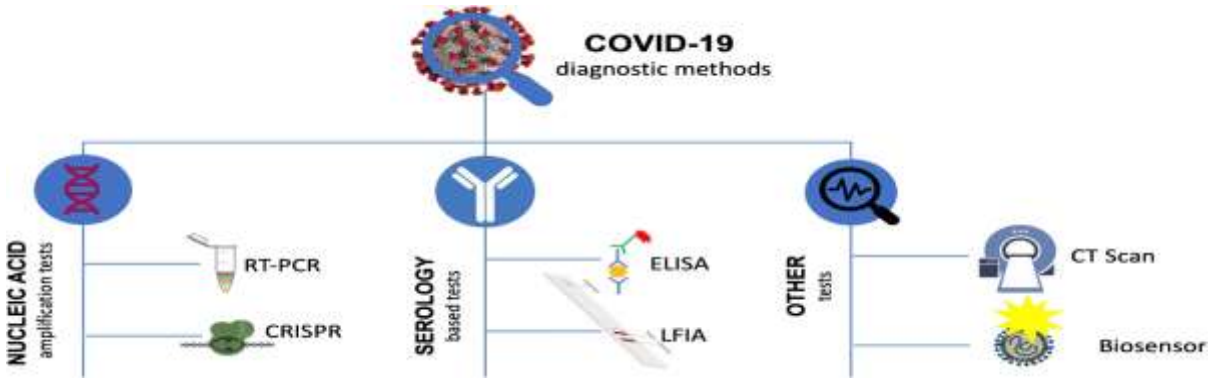

Figure 1. Main diagnostic methods reported for the detection of SARS-CoV-2 [3].

The use of some numbers of diagnostic methods have been approved by The US Food and Drug Administration (FDA) and World Health Organization (WHO), while some new methods

are receiving conditional approval under emergency use authorization (EUA) [8]. These diagnostic methods have variations in their respective batching capacity, requirement of infrastructure setting, throughput, turnaround times and analytical performance [7]. Before choosing a reliable and rapid diagnostic method to help make an siutable decision and timely public health actions, other than depending on the method and equipment itself, the result from a method also relies on sample/ reagent storage requirements, reagents used, sample collection protocol and potential for cross-contamination; all these factors must be highly considered [3].

Sample collection

For COVID 19 diagnosis, two types of sample specimens are being basically used. Serum samples are used for identification of antiviral antibodies and respiratory specimens are used for direct detection of virus [9]. Community surveillance may also involve direct detection of viral RNA in wastewater samples [10]. Stool and saliva samples have also been explored and require little technicality in sampling procedures than respiratory specimens [11]. The respiratory specimens [12] are most frequently collected from the upper respiratory tract (e.g., oropharynx or nasopharynx) and not usually from the lower respiratory tract (bronchoalveolar lavage fluid (BLF)). Ideally, within 7 days the upper respiratory specimens are collected in the acute phase of infection and the lower respiratory specimens are obtained from patients still symptomatic after more than a week [13, 14]. Other than throat and nasal specimens, sputum specimens are also collected for the diagnosis of COVID-19, achieved by discharging deep cough into a sterile container [14]. Immunoassay methods require Serum sampling. Volume of blood sample for immunoassays ranges from 5 to 10 mL for lab assays to capillary draws of 50–200 μL blood for lateral flow immunoassays (LFIA) [15]. The virus samples should be processed and tested as soon as possible. If immediate testing is not possible, the sample can be stored up to 72 h at 2–8 °C. However, for more than 72 h storage, the specimens should be frozen at -70 °C as soon as possible after collection [14]. However, repeated freezing and thawing of the specimen should be avoided [1]. Appropriate sample collection procedure, right type of sample and reliable transportation must be in place to minimize the risk of inaccurate results. Based on the purpose of test, as well as the stage of infection on a person, an appropriate sample should be collected. Zou et al. [16] have reported higher viral loads of SARS-CoV-2 from specimens collected from nose than from throat. Wang and his co-workers discovered higher positive rates with nasopharyngeal swabs than oropharyngeal swabs [9]. A case study with a pneumonia patient in Thailand showed a negative test with nasal or oropharyngeal swab samples but a positive test with bronchoalveolar lavage fluid [17]. To reduce the chances of virus detection error, sampling both nasal and oropharynx is recommended [13]. The sensitivity of nasal, nasopharyngeal (NP), and throat swabs was found to be 80%, 90%, and 87%, respectively [18]. Disease progression may likely be a determinant factor for the reason for variation in the sensitivity of different types of swabs. Therefore, it is necessary to identify the appropriate type of sample considering the medical condition of the patient and availability of diagnostic facility required to carry out the test [3, 19, 20].

COVID-19 Drugs

SARS-CoV-2 is highly contagious and it poses severe health risk globally. Recently, there has been breakthrough on various antiviral drugs formulation for this purpose, which have been

approved for various infections, and have been tested in worldwide [21-28]. Analytical methods play a crucial role in these studies, and electroanalytical methods are also of paramount too. A summary of general information on COVID-19 drugs selected for this review is discussed below.

Favipiravir (FAV): This drug is also known as 6-fluoro-3-hydroxy-2-pyrazincarboxamide. It is an inhibitor of RNA replication from an RNA template catalyzed by RNA polymerase enzyme [29]. In Japan, FAV was approved in 2014 as an antiviral for influenza viruses (avian flu and others). FAV has been employed to treat COVID-19 disease [21].

Remdesivir (REM):This new antiviral drug belong to the class of nucleotide analogues [30, 31]. The efficiency of REM has been ascertained via series of clinical trials [32, 33]. Its manifestation of exclusive efficiency and activity against COVID-19 in patients with mild and moderate symptoms makes it an option in this context.
The EU ab initio approved the use of REM against COVID-19 [33-37]. Moreover, its chemical structure contains a nitrile group, implying that excessive intake may be highly toxic to human [38]. REM has been previously used to treat Ebola virus patients in West African [33]. The mechanism of action of REM is based on viral RNA-dependent RNA polymerase inhibition. REM is a phosphoramidate prodrug of an analogue of adenine-C-nucleoside structure. REM is metabolized into its active form, which is a competitive inhibitor of RNA synthesis [39].

Lopinavir + Ritonavir (LOP + RIT)*:* LOP + RIT is an inhibitor of the human immunodeficiency virus (HIV) protease, whose main structure is different from the SARS-CoV-2 counterpart (3CLpro) [32, 33]. This state would affect the inhibition efficiency of LOP+RIT and raise doubt on the efficacy of LOP + RIT for COVID-19 treatment [32, 33], and WHO has discontinued LOP + RIT use in COVID-19 treatment [40].

Hydroxychloroquine (HCQ) and Chloroquine (CQ)*:* HCQ and CQ finds application in the treatment of malaria, rheumatoid arthritis and discoid lupus erythematosus.

More so, they have been applied as antivirals in COVID-19 disease treatment [42]. HCQ and CQ were thought to be inhibiting the pre-entry step of the viral cycle by interfering with viral particles binding to their cellular cell surface receptors. By insinuation the intracellular site of SARS-CoV-2 budding was determined by the localization of its membrane M proteins that accumulate in the Golgi complex beyond the site of virion budding, implying a possible action of HCQ or CQ at this step of the replication cycle of the virus [42]. A study on mortality outcomes with HCQ in COVID-19, posits that HCQ is associated with increased mortality in COVID-19 patients. Also, there is no benefit of CQ [21]. Therefore, WHO has discontinued HCQ and CQ use in COVID-19 treatment [43]. Currently, the use of HCQ in COVID-19 treatment was discontinued in some countries like Turkey [41]. Recently, HCQ has been analyzed by electroanalytical methods [21, 44]. These methods can be used in further studies, such as toxicological studies on patients who had previously used HCQ.

Ribavirin (RIB)*:* Some nucleotide analogues terminate RNA synthesis catalyzed by polymerases of coronaviruses. Molecular docking experiments, computational chemistry studies, including sequence analysis and modellings showed that some nucleotide analogue antiviral drugs can be used to prevent viral replication in infected cells. RIB is one of the RNA-dependent RNA polymerase enzyme inhibitors, which is utilized for the treatment of hepatitis C virus infections. RIB can also be used against other RNA viruses, such as coronaviruses and zika virus [21, 45].

Sofosbuvir (SOF): SOF is an alternative nucleotide derivative that is used against Hepatitis C Virus and also SARS-CoV-2. SOF competes with physiological nucleotide for RNA-dependent RNA polymerase enzyme active site, competitively inhibits viral RNA polymerase, and by the way, it prevents viral replication of SARS-CoV-2 in infected cells [21, 45].

STANDARD AND DEVELOPED METHODS FOR COVID-19 DIAGNOSIS

Analytical principle of RT-PCR technique

The polymerase chain reaction (PCR) is a simple approach that uses a DNA template to amplify in vitro unique DNA fragments. Traditional methods of replicating a DNA sequence in a living cell into a vector are often very time-consuming and labor-consuming, whereas PCR only takes a few hours to replicate the DNA sequences of interest [46]. Although most biochemical tests work with a large quantity of biological material, for example nucleic acid recognition with radioisotopes, the quantity is very small with PCR. Thus, in less time, PCR can perform accurate detection and greater amplification rates than other methods [46]. Reverse transcription PCR integrates two flexible and robust methodologies, reverse transcription and chain reaction of the polymerase, to create and amplify cDNA from RNA or mRNA transcripts. The acceptable reverse transcription PCR demands a significant fidelity of amplification. The RNA must be translated into cDNA first to obtain a thermostable DNA template for the polymerase to use in PCR in the test for RNA (Figure 2). This procedure is termed reverse transcription , hence the name of reverse transcription PCR . RT -PCR , a technology discussed in the work by Benda et al. (2021), excels at amplifying specific target RNA sequences . It allows for the monitoring of amplification progress during PCR reactions through the use of fluorescent reporter dyes or molecules . These dyes emit fluorescence as amplification proceeds , and the emitted fluorescence is detected and quantified accordingly . Then , through the fluorescent reporter dye /molecule , the amplification progress will be monitored during PCR reactions, and the amount of the emitted fluorescence will be detected [47].

The detection technique used is TaqManTM probes, which depends on the examination made of an oligonucleotide sandwiched between the two PCR primers marked with a fluorophore that is linked covalently to a quencher and a reporter. The emitted fluorescence is inhibited so long as the reporter and the quencher are close. Several reporter dyes are successfully used for labelling in TaqMan probes such as FAM, Cy5 and JOE [48-51]. The availability of genetic data allows the development of the primers and probes needed for the progress of SARS-CoV-2-specific testing [52]. RT-PCR depends on the capability to transmit a limited genetic viral quantity in a specimen and is known as the gold standard for SARS-CoV-2 virus recognition.

RTPCR testing for COVID-19 has been used for ocular secretions, saliva, or serum a few reports [53, 54].

Recently, the Rutgers Laboratory discovered an RTPCR assessment that collects saliva samples faster and far less painfully than most other sample collection approaches which reduces the risk to healthcare professionals, and may lead to a greater volume of experiments [55]. As the PCR progresses,the DNA amplification in RT-PCR is monitored in real-time by applying a dye or probe labelled with a quencher and a fluorescent substance, such as in the case of TaqMan tests [52]. Normally, RT-PCR is performed as a one- or two-step approach. One-step real-time RT-PCR utilizes a single tube having the required primers to complete the RT-PCR process. Two-step real-time RTPCR requires two tubes to separate between reverse transcription and amplification processes from the assay, but it provides additional flexibility and greater sensitivity than the one-step approach [56, 57]. In general, the one-step technique is the favoured method for SARS-CoV-2 detection. It is reliable to operate. It includes restricted handling of the sample, minimized test time and reduced errors. A notable example to examine the performance of the standard methods for COVID-19 diagnosis is themethod described by Radbel et al. [58]. The analysis of different samples such as OP and saliva testing compared to NP using the well-known RT-PCR method was reported. The contribution of this investigation is the use of phosphate-buffered saline (PBS) as a preservation medium instead of a viral transport medium (VTM) [59, 60]. The preparation in 2mL screw-top vials was contained in a Hanks' Balanced Salt Solution (HBSS), foetal bovine serum with a final percentage of 2%, 0.5 mg/mL of amphoteric B 100 mg/mL of gentamicin. According to the approved protocol by the Rutgers Institute Review Board (protocol number Pro2020000800), the respiratory excretions collected from 16 samples showed COVID-19 positive subjects within four days [59]. The collected specimens were kept in vials containing both preservation media, as well as VTM and PBS. The experiments were divided into two protocols, the first one with the standard preservation medium VTM and the second one with PBS. The accurate evaluation for detection of SARSCoV- 2, quantitative PCR (qPCR), was performed and compared the cycle threshold (Ct) values for three SARS-CoV-2 viral genes, specifically nucleocapsid (N), open reading frame 1ab (ORF1ab) and spike protein (S) genes. For the examination of positive control, bacteriophage MS2 (MS2) spiked into the samples was utilized. The viral RNA extraction was performed as the manufacturer described (PerkinElmer) on a Chemagic 360 instrument.

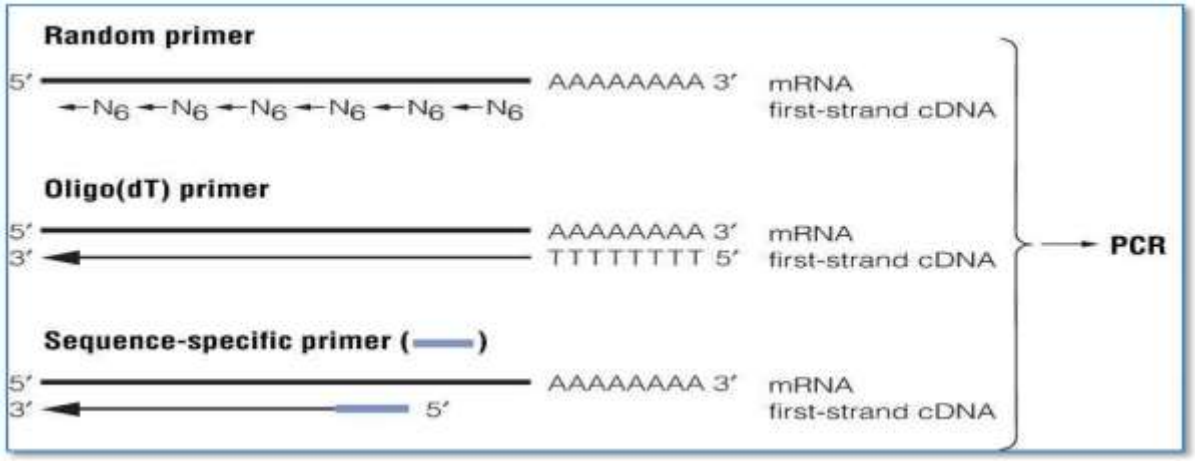

Figure 2. Diagram of RT-PCR approach (https://worldwide.promega.com.).

Briefly, 4 mL of poly(A) RNA, 10 mL of proteinase K, 300 mL of lysis buffer, and 8 mL of MS2 were added into each sample. Then, 300 mL of each TaqPathCOVID-19C positive and nCOV negative controls were added, followed by 150 mL of magnetic beads and 900 mL RNA binding buffer. After washing the mixture of beads/RNA with 500 mL of both buffer 3 and buffer 4, respectively, the 50 mL of extracted RNA final volume sample was eluted through the elution buffer. The amplification of the extracted RNA was performed on the RT-PCR system to detect the viral genes mentioned above. This method received a great attention as the reaction was performed in 20 mL-volumes and ran through the following program: for 2 min the temperature was kept at 2 °C, then the temperature increased sharply to 53 °C for 10 min, after that 95 °C for 2 min, then 95 °C for 3 s, and finally 60 °C for 30 s. The fluorescence signal collected during the final 60 °C step was repeated for a total of 40 cycles. Tests were conducted in triplicate, and the lower limit of detection (LOD) of SARS-CoV-2 is 200 copies/mL. A strong correlation of the values was observed in both the transport media, and confirmed that either the VTM or PBS could be used, providing a new solution for preserving the media and storing the samples with efficacy [Leland 1992]. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) RT-LAMP was performed as an alternative approach to SARS-CoV-2 detection for fast and cost-effective research [60]. RT-LAMP uses four specific primers to the targeted gene/area to enhance the response and integrates a reverse transcription stage to detect RNA. To identify the amplification product, this technique measures the turbidity resulting from precipitated magnesium pyrophosphate in solution as an amplification byproduct. To record the process in real-time, the method uses intercalating dyes to calculate the turbidity or fluorescence. Because only heating and optical observation are needed for real-time RT-LAMP diagnostics, its versatility and responsiveness make it an excellent applicant for virus detection [46, 61].

CRISPR-based assays

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are a group of nucleic acid sequences contained in bacteria. A collection of bacterial enzymes, called CRISPR-associated enzymes, can recognize and cut these sequences identified by Cas9, Cas12 and Cas13. Some enzymes were designed to attack and cut the viral RNA sequence in Cas12 and

Cas13 [62]. These CRISPR-based systems do not need complex equipment, and are read utilizing strips of paper to confirm the location of the SARS-CoV-2 virus, avoiding missing of responsiveness or precision. These experiments are low-cost and could be completed in as little as one hour. Such assessments are immensely promising for the diagnostic process at the point of treatment [63].

Serological and immunological assays

Although viral RNA detection based on RT-PCR has also been commonly used in the diagnosis of COVID- 19, it would not be used to track the progression of the phase of the infection. It cannot be used to identify immunity and past diseases broadly [64]. The serological analysis is characterized as blood, serum, or plasma examination. The immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies in sputum and saliva can be checked. This assay performs a remarkable role in epidemiology and vaccines' progress, offering an evaluation of both the short-term antibody response, and the abundance and diversity of antibodies. After a few days of infection, IgM becomes observable in serum first, followed by IgG, which appears after a few weeks. So, IgM can be an early-stage disease indicator. In recent years, not only for the detection of antibodies themselves but also for applying antibodies to the detection of pathogen-based antigens, immunological tests' specificity and responsiveness have been improved [65]. These experiments have tremendous potential for COVID-19 epidemiology. Still, at least three circumstances will affect the test results: (1) a group of subjects with positive molecular and genetic assays for SARS-CoV-2 infections is seronegative due to delays in developing antibodies following infection. (2) Samples could be seropositive but negative for results of a molecular genetic assay representing recent, milder disease. (3) Restriction in precision and specificity of the assessment [66]. Enzyme-linked immunosorbent assay (ELISA) ELISA is a plate-based approach that evaluates and measures molecules such as proteins, antibodies, peptides and hormones. The test could be qualitative or quantitative, and the time for getting the results usually varies between 1 to 5 h. The plate micro-wells are normally coated with a viral protein as in the case of SARS-CoV-2, as seen in Figure 2. If antibodies are present in the patients' specimens, they directly bind to the viral protein coated on the microplate. The complex can be identified with a conjugated secondary antibody. After that, the enzymatic conversion of the substrate generates a measurable colour change. ELISA is fast, has the opportunity to verify different specimens, and can be optimized for enlargement automation but can vary intolerance and is appropriate for point-of-care assessments [67]. Different serological assays have been used to identify SARS-CoV-2; including ELISA, immune-fluorescent assay and the immunochromatographic assay [68, 69].

The fast and easy approach of SARS-CoV-2 serodiagnosis may aid in diagnosing the disease. Many reports of SARS infection showed that the IgM antibody was detected in serum over 3–6 days [70]. The immune-chromatographic approach is considered quick and efficient, commonly utilized in several diseases, including infectious diseases [71]. Its theory is straightforward and facile, and embraced by physicians, making it particularly appropriate for a severe epidemic situation, such as the COVID-19 pandemic caused by SARS-CoV-2, which to spread rapidly across the globe within two months. Accurate detection of the illness and isolation of patients is currently believed to be the best way to prevent and monitor COVID-19

spread. Therefore, a responsive immunofluorescent test approach has been progressed to detect specific IgM and IgG against SARS-CoV-2 in human serum rapidly, within 10 min. The SARS-CoV-2 recombinant nucleocapsid protein has been utilized as a catch antigen. Fluorescent microsphere Lanthanide, Eu(III), has been used to assess the solid phase immune-chromatographic experiment in qualitative/ semi-quantitative terms. The results revealed that the quick immune-assay is highly responsive and precise and was valuable for quick serodiagnosis of COVID-19 [72].

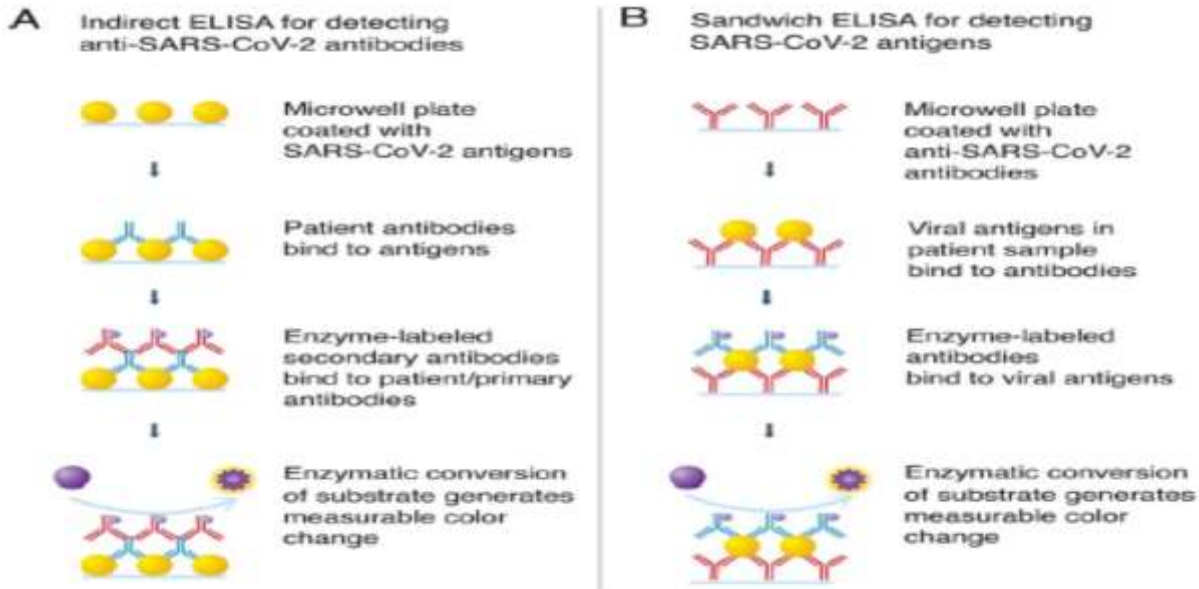

Figure 3. Two different ELISA assays to detect SARS-CoV-2 by indirect assay (A) or direct assay (B) [61].

SOME RECENT ADVANCES AND EMERGING TRENDS IN ANALYTICAL METHODS FOR COVID-19

The detection of minimal amounts of viral proteins constitutes one of the challenges of current analytical chemistry, requiring the application of ultrasensitive techniques since proteins, unlike viral nucleic acids, are not susceptible of being directly amplified. Both mass spectrometry (MS) and proteomic techniques [73-76] are called to play an important role in this direction, as well as in the characterization and quantitative measurement of viral proteins. MS detects in a robust way the N, S, and M, SARS-CoV-2 structural proteins [77, 78]. Nanotechnology's contribution to the analytical chemistry of COVID-19 has a promising future [76, 79]. It also plays a useful role in improving personal protective equipment [80] since many nanoparticles exhibit powerful antiviral properties. Nanomaterials have nanoscale physical, chemical, biological, mechanical and electrical features [81, 82], which cannot be found in the micro and macro scale domain. This is due to their high surface area, very small size, great stability and versatile chemistry of the modified and functionalized material's

surface. Pfizer-BioNTech and Moderna, the pioneers among COVID-19 vaccine developers, use nanoparticles [83] in its mRNA (messenger Ribonucleic Acid) vaccine delivery.

Concerted globally effort has paved the route [84] into vaccine development, in a record time. FDA has authorized, for emergency use, the two mentioned vaccines as well as Johnson & Johnson's Janssen single dose viral vector COVID-19 vaccine. In addition to these three, the European Medicines Agency (EMA) has also approved the use of AstraZeneca viral vector vaccine. It is hoped that the efficacy of vaccines will provide sufficient control [85] over the disease and reverse social life to normality soon or, at least, little by little. There are some opportunities for improvement in the development of new RT-PCR platforms [86], covering better tolerance of matrix effects and compatibility with simpler or minimal sample, e.g. dPCR or ddPCR (droplet digital PCR). Primers, probes and reagents are the same, but the bulk reaction solution is partitioned into a myriad of nanoliterized microdroplets yielding more precise measurements in comparison to traditional PCR [76]. The employment of microfluidic devices known as Lab-on-a-Chip (LOC) combined with POC, may lead to instruments [87] providing better healthcare solutions for the general population, in terms of cost-benefits, without having to resort to train professionals and complex tools to interpret the results. The development of systems with these characteristics that allow the detection of SARS-CoV-2 in biological fluids at concentrations of the order of femtomolar (fM), picomolar (pM) or nanomolar (nM) would undoubtedly constitute a great achievement. Saliva (properly treated if necessary) is the most widely used fluid for diagnosis carried out with most microfluidic studies. The importance of emerging techniques in detecting COVID-19 has been highlighted in a number of recent reviews [88]. These emerging techniques are of vital importance for COVID-19 detection, monitorization, diagnose, screening and surveillance. A number of approaches used either alone or in combination with others may be the subject of mention: (i) single stranded nucleic acid oligonucleotide bioinspired receptor molecule aptamer-based detection; (ii) molecular imprinting technology-based detection; (iii) protein parallel high-throughput multifunctional microarray tools; (iv) agglutination tests; (v) highly sensitivity and specificity paper-based devices multiple DNA targets detection; (vi) simultaneous targets multiplexed LFIA recognition; (vii) new biomarkers approaches [70, 76, 89, 90], e.g. breath analysis; (viii) decentralization setting, e.g. wearable sensors; (ix) robots based sample test and other robotic applications to detection, screening and diagnose. Some of these issues need to be the subject of further research and attention, but so far, they have a promising future in order to move from the laboratory scale to the real situation of commercialization. Next Generation Sequencing (NGS), such as amplicon based mutagenic NGS whole-viral genome profiling, allows the screening of a large number of samples. On the other hand, the use of pseudovirus expressing the S protein of SARS-CoV-2 instead of the virus itself is also a great opportunity to simplify and accelerate the development of assays [91] for viral proteins. The integration of technologies to smartphone mobile devices [**76**] provides new opportunities in different areas. In the field of medical diagnostics, they represent a paradigm shift [92] that allows the provision of mobile health care and personalized medicine. Both AI and IoT/IoMT (Internet of Things/ Internet of Medical Things) [93] may be combined with, e.g. immunosensing, to prevent and manage COVID-19, giving fast and inexpensive increasing specific and sensitive approaches to research the proper networking through the management of database, sharing, and analytics.

Novel systems to remotely detect COVID-19 based on AI and IoT/ IoMT and the combination of several smart medical sensors (pulse, thermal and blood sensors) are under scrutiny and study. These "on line" diagnostic platforms systems collect invaluable data from patients in a remote way [78, 93], uploading them to the Cloud. Thus, the physicians have access to them in real time. We are walking in the right direction towards the revolutionary paradigm of precision medicine. Currently, there is an urgent need to perform extensive diagnostic tests (easier, cheaper and more sensitive and accessible) in order to avoid the spread of the virus and decreasing the occurrence of unreported cases (i.e., asymptomatic cases).

In this regard, massive effort has been made recently developing potential biosensors for COVID-19 that increase the sensitivity of the methods devised [**76]**. A biosensor device includes a biomolecular recognition element to confer selectivity and a signal transduction element [94] to allow quantitative or semi-quantitative analysis. The interaction between the analyte molecules and the biorecognition element (eg a layer of bioreceptor molecules) in a generic receptor causes the transduction of a measurable physical–chemical change, such as current flow, heat transfer, mass change or refractive index, or more sophisticated measurable properties. The captured signals are later amplified and processed in a suitable way to proceed with the data analysis. Among the most common biosensors are the following: electrochemical biosensor, field-effect transistor (FET)- based biosensor, localized surface plasmon resonance (LSPR)-based biosensors, surface enhanced and Raman scattering-based biosensor. Electrochemical biosensors have been profusely used in the detection of viral RNA, proteins, small molecular antibodies, and whole virus particles. The target can be recognized using an antigen–antibody reaction, hybridization of RNA, DNA, or peptide nucleic acids or aptamers that bind to the target with high affinity and specificity [76]. The biological interaction of these processes is translated into electrical signals, measuring then electrochemical properties such as capacitance, charge accumulation, conductance, current, impedance or potential changes [95]. Mavrikou et al. [96] set up a membrane-engineered kidney cell, which modifies with the SARS-CoV-2 SpikeS1 antibody, with the aim of detecting the SARS-CoV-2 S1 antigen. Changes in the membrane potential are produced when interacting the antibody with the target protein. The set is manufactured on eight gold screen-printed electrode, being covered with a layer of polydimethylsiloxane (PDMS) with eight wells. The sensor achieves an excellent sensitivity. For the detection of SARS-2 in saliva, Fabian et al. [97] focuses on both S and N protein, preparing magnetic beads in combination with carbon black-based screen-printed electrodes. The technique includes: 1) pre-coating of magnetic particles, 2) solid support magnetic particle immunoassay with alkaline phosphatase (ALP) -labeled reporter antibody, and 3) electrochemical finish; 1-naphthol, resulting from enzymatic cleavage of 1-naphthyl phosphate by ALP, is analyzed by differential pulse voltammetry technique, using carbon black-modified screen-printed electrodes. Among the main advantages of the approaches are the short analysis time (<30 min), straightforward sampling (untreated saliva) and handling of use (printed sensor and portable potentiostat).

Furthermore, in comparison to RT-PCR, this kind of biosensors can present a competitive cost per analysis. Sampling and sample handling are critical points for POCT devices; manual operations, even minimal, pose serious inconveniences for their acceptance. In another attempt,

Seo et al. [98] use an anti-spike antibody binding to SARS-CoV-2 virus particles to make up graphene-based FET biosensors. The FET is covered with a saline buffer to keep an efficient gating effect, which uses the electrical signal transduction process. The aqueous solution-gated FET system detects SARS-CoV-2 on the basis of channel surface potential changes and the corresponding effects produced on the electrical response. These FET biosensors can respond to 16 pfu/mL (plaque-forming unit/mL) of virus particles in phosphate-buffered saline within 10 min.

Furthermore, the biosensor clearly discriminates between patient and healthy people samples. AuNPs attached to the antibodies, biomolecules, proteins, and aptamers can also increase the LSPR and SERS (surface enhanced Raman spectroscopy) signals, and the energy transfer between the fluorophore and AuNPs. A number of nanomaterial-based biosensors allows, actually, the detection of SARS-CoV-2 and related viruses [99]. For example, Qiu et al. [100] applied a dual-functional plasmonic biosensor utilizing plasmonic photothermal (PPT) effect and LSPR sensing transduction for the detection of various viral sequences genes from SARS-CoV-2. The aforementioned device is able to distinguish between SARS-CoV and SARS-CoV-2 viruses. The sensor shows a LOD of 0.22 pM. Most recently Liu et al. [101] developed a surface-enhanced Raman scattering-based lateral flow immunoassay (SERS- LFIA) to detect IgM and IgG simultaneously. To fabricate SERS, tags are modified with dual-layers of Raman dye, Silica core is completely coated with silver shell (SiO2@Ag), which shows superb SERS signal, decent monodispersity, and excellent stability. The anti-human antibodies immobilized capture SiO2@Ag- spike S protein anti-SARS-CoV-2 IgM/IgG complexes. The proposed technique presents a good performance under clinical environment and it is cost-effective. Colorimetric biosensors have also displayed potency in detecting SARS-CoV- 2. These biosensors show interesting properties such as rapid response, accuracy and cost-effectiveness. Moitra et al. [102] use thiol-modified antisense oligonucleotides (ASOs) capped AuNPs (Au-ASOs) for the specific detection of N- gene from nasal swab. The test allows detecting SARS-CoV-2 infection within 10 min. The detection mechanism involves the agglomeration of Au-ASOs architecture with target SARS-CoV-2 RNA, leading to red-shift. The cleavage of RNA strands from RNA-DNA hybrid is done by the addition of RNaseH (Ribonuclease H), a precipitate is finally obtained in the solution due to the additional agglomeration of AuNPs, being visible to naked-eye.

The biosensor shows a LOD of 0.18 ng/mL for SARS-CoV-2 RNA and no cross-reactivity being apparent with MERS-CoV viral RNA. Altogether, it is evident that up to now neither methods are ideal approaches for COVID-19 diagnose, but they should complement each other [103] in a reasonable way. Overall, the main advantages of biosensors are the cost-benefits relationships, reduced sample size and good reproducibility, as well as a rapid detection and high sensitivity.

CONCLUSION

The real-time reverse transcriptase polymerase chain reaction (RT-PCR) among other COVID 19 methods of diagnosis remains the gold standard method to detect SARS-CoV-2 occasioned

by higher sensitivity and specificity as well as its potency in detecting viral nucleic acids in respiratory specimen. During pandemic emergencies, shortage of resources such as PCR kits is likely to occur. Therefore, having multiple options for the diagnostic methods is paramount. Optional testing platforms and accessories that could be locally manufactured, even in a small scale, are equally essential. Such platforms would be appropriate in resource-limited settings too. The currently practiced RT-PCR methods are expensive, and therefore many middle and low-income countries may not be able to afford enough number of COVID-19 tests for the purpose of larger population screening. Important gaps remain in screening asymptomatic persons in the incubation phase. Precise determination of live viral shedding among patients in the convalescence phase to inform de-isolation decisions is also a big challenge requiring urgent attention. Finally, there is need for more studies to be carried out to enhance innovation of new selective, quick, and cost-effective COVID 19 diagnostic methods that can supply quick and effective test results in much lesser time frames.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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