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A comparison of conventional and advanced electroanalytical methods to detect SARS-CoV-2 virus: A concise review

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ABSTRACT
Respiratory viruses are a serious threat to human wellbeing that can cause pandemic disease. As a result, it is critical to identify virus in a timely, sensitive, and precise manner. The present novel coronavirus-2019 (COVID-19) disease outbreak has increased these concerns. The research of developing various methods for COVID-19 virus identification is one of the most rapidly growing research areas. This review article compares and addresses recent improvements in conventional and advanced electroanalytical approaches for detecting COVID-19 virus. The popular conventional methods such as polymerase chain reaction (PCR), loop mediated isothermal amplification (LAMP), serology test, and computed tomography (CT) scan with artificial intelligence require specialized equipment, hours of processing, and specially trained staff. Many researchers, on the other hand, focused on the invention and expansion of electrochemical and/or bio sensors to detect SARS-CoV-2, demonstrating that they could show a significant role in COVID-19 disease control. We attempted to meticulously summarize recent advancements, compare conventional and electroanalytical approaches, and ultimately discuss future prospective in the field. We hope that this review will be helpful to researchers who are interested in this interdisciplinary field and desire to develop more innovative virus detection methods.
1. Introduction

Coronaviruses (CoVs) are a highly contagious genus of respiratory viruses that can infect both animals (such as birds, cats, mouse, bats, pigs, and camels) and humans. They infect a variety of biological systems, including the respiratory, gastrointestinal tract and the central nervous system (CNS) (Wang et al., 2006; Chen et al., 2020a). It is well known that, viruses are nonliving creatures that can only multiply in the presence of a host organism. This ability is due to their structure, which consists of a protein capsid encapsulating deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) strands (Chen et al., 2020b). The respiratory virus infection symptoms include dry cough, fever, fatigue, loss of smell and sputum production. In recent years, acute respiratory infections caused by respiratory viruses (influenza virus and CoVs) have resulted in deaths and pandemics (Khoury et al., 2020; Zhang et al., 2020; Corman et al., 2018). In late December 2019, Chinese health officials identified a coronavirus that was linked near a seafood marketplace. The newly found beta coronavirus is named as “Severe Acute Respiratory Syndrome coronavirus 2” (SARS-CoV-2) (Gorbalenya et al., 2020). It has been found in the great majority of countries globally, prompting the “World Health Organization” (WHO) to declare the COVID-19 as a pandemic on 11th of March 2020 (Cucinotta and Vanelli, 2020). Preventative and regulator actions have been implemented based on the characteristics of respiratory virus transmission, such as the use of N95/KF94 masks to prevent the spread of respiratory viruses, sanitizing dirty surfaces to avoid harmful airborne spread contact (Pradhan et al., 2020; Yang et al., 2021). Until date, the ongoing pandemic has infected 539,893,858 people in approximately 220 nations, with 6,324,112 deaths reported by June 23, 2022 (Fig. 1) (WHO, 2022). However, because respiratory viruses have non-specific and broad symptoms, as well as rapid spread from positive patients (asymptomatic), early precise identification and isolation of infected individuals remain critical for monitoring the disease caused by respiratory viruses (Heraud et al., 2019; Zhang et al., 2021a, 2021b). To control the spread of the pandemic disease, diagnostic tools to detect and segregate affected people from the general public must be established. As a result, detecting respiratory viruses would be crucial.

The majority of widely adopted and current methods for detecting respiratory viruses are laboratory-based conventional techniques. The methodology involves, preliminary virus cultures, morphological identification, and serological exams to subsequent “reverse transcription-polymerase chain reaction” (RT-PCR), “immunochromatography” (IC), “enzyme-linked immunosorbent assay” (ELISA), and traditional diagnostic methods like computed tomography (CT) (Mardian et al., 2021; Sakurai et al., 2015; Dou et al., 2021). In practice, however, many traditional lab-based processes have drawbacks such as tedious sample preparation, expensive instrument costs, and the requirement for skilled operators. There is an increasing market demand for respiratory virus detection methods that are quick, simple, sensitive, and inexpensive. An electrochemical sensor employs an electrochemical perturbation technique to provide quantitative or semi-quantitative information on an analyte of interest. Advanced electrochemical techniques for sensing respiratory viruses have gained a lot of interest owing to their inherent strengths such as, sensitivity, selectivity, rapid response, portable apparatus, and moderate cost (Santhanam, 2021). However, progress in the development of electrochemical sensors for viruses has been modest for the past two decades.

Recently, there have been a few outstanding review studies available in the literature on the advantages of electrochemical sensors for COVID-19 virus detection. Kotru et al. (2021) looked through the literature to find the most promising biomarkers and the best electrochemical sensors for them. In particular, cannabidiol (CBD) is being discussed as a possible COVID-19 adjunct therapy because its medicinal characteristics may be beneficial in reducing the neurological or severe inflammatory damage produced by severe COVID-19 infection. Balkourani et al. (2021) studied the importance of transducer electrode nanomaterials to increase the performance of SARS-CoV-2 electrochemical biosensors, primarily focusing on gold (Au), carbon, and/or

![Fig. 1. Number of COVID-19 cases and deaths reported weekly to the World Health Organization (WHO). The number of affected cases globally reached over 539.8 million, with over 6.32 million deaths till June 23, 2022. Source gathered from the website of World Health Organization (WHO) on June 23, 2022 (https://covid19.who.int/).](https://covid19.who.int/)
graphene-based electrodes. Lopez and Mir. (2021) presented a comprehensive examination of commercialized genomic-based sensing and antigen/antibody identification approaches for COVID-19 monitoring. Alygizakis et al. (2021) summarized the significance of analytical approaches employed to detect SARS-CoV-2 in waste water, specially focusing on wastewater epidemiology (WBE). Ozer and Henry (2021) reviewed the recent developments in the paper-based devices (PADs) for virus detection, also the further improvements required for PADs for sensing viruses were discussed.

The goal of this comparative review is to abridge the aspects of conventional methods and electroanalytical methods to detect SARS-CoV-2 virus. The conventional methods like, RT-PCR, “loop mediated isothermal amplification” (LAMP), CT with artificial intelligence (AI), and serological survey methods are discussed, along with the advanced electrochemical sensing methods. To our knowledge, no review publication has looked into the detection of COVID-19 virus utilizing a combination of conventional and electroanalytical methods. This review is divided into four primary sections and subsections. To begin, we discussed about a quick summary of respiratory viruses and how they affect global activity. Later, we explained how SARS-CoV-2 differs from SARS-CoV, its brief history, symptoms, and transmission pathways. The fourth section is a detailed overview of the conventional methods for detecting the COVID-19 virus. In section five, the literature was rigorously reviewed to identify the promising electrochemical sensors described to date for the detection of the COVID-19 virus. Finally, both conventional and electrochemical approaches were discussed in terms of their benefits, disadvantages, and future prospects. We hope that this review will provide a quick overview for researchers working on novel ways for detecting the respiratory viruses, especially COVID-19 virus to fight against pandemic.

2. A brief overview of respiratory virus infection and its consequences

The twenty-first century has been noticeable by major epidemics, some of which considered as pandemics, such as those caused by historic diseases like cholera, plague, and yellow fever, as well as emerging diseases like “severe acute respiratory syndrome” (SARS), “middle east respiratory syndrome” (MERS), Zika, Ebola, influenza A, and recently COVID-19 (Ong et al., 2020). Respiratory viruses are the utmost communal cause of illness in humans, and they have a considerable impact on morbidity and mortality around the world (Weston and Frieman, 2019). “Acute respiratory infections” (ARI) reason for roughly one-fifth of all childhood demises globally, particularly among impoverished people in humid climates, where ARIs case-to-fatality ratios can be greater than in temperature countries (Boncristiani et al., 2009). Eight human respiratory viruses that are well-adapted to efficient person-to-person transmission and infect people of all ages have been found (Kakkola et al., 2021). The human respiratory syncytial viral (HRSV) has the potential to kill 14,000 persons over the age of 65 years in the USA each year (Azar and Landry, 2018). The COVID-19 is now being triggered by the SARS-CoV-2. The common human respiratory viruses, their syndrome, and main detection methods are briefly summarized in Table 1.

The consequences of new respiratory viral infections in human beings are not insignificant. The SARS-CoV first observed in China in 2002 and hurrily spread to 29 countries before being halted by rigorous control measures (Gray and Abdelgadir, 2021). A new human adenovirus–14 –strain (Ad14) first looked in the united states (US) in 2006 and rapidly spread to few other nations, causing 1000 illnesses and 13 fatalities. A triple reassortant H1N1 influenza virus (swine-like) appeared in Mexico in 2009 and speedily spread over the world, caused 60.8 million illnesses and around 12000 fatalities in the US alone (Gibbs et al., 2009; Shrestha et al., 2011). It is proposed that, interactions between human beings, animals, and nature are intricately related to the emergence of new strains (Swelum et al., 2020). The majority of these newly emerged respiratory viruses are assumed to have evolved in animals before passage over to infect people and are from four different viral families (Coronaviridae, Orthomyxoviridae, Picornaviridae, and Adenoviridae) (Fuller et al., 2013; Alluwaithi et al., 2020). In addition to initiating high disease and death, human respiratory viruses have had a considerable influence on international travel, trade, and the economy (Domingo et al., 2014). According to a report published by World Bank in 2018, the SARS epidemic cost the affected countries $ 30–50 billion and $ 45–55 billion by the H1N1 pandemic (Gray and Abdelgadir, 2021; Berthe et al., 2018). The cost of a MERS-CoV occurrence in Republic of Korea between June 2015 and June 2016 was estimated to be $2.6 billion (Joo et al., 2019). At this point, it is evident that COVID-19 will have a greater economic impact than any recent respiratory disease pandemic.

3. The SARS-CoV-2 viral characteristics, transmission and its symptoms

The brief history of the COVID-19 virus, its characteristics, mechanism of transmission, and symptoms were addressed in this section. It is well known that, for the detection of COVID-19 virus, its structural proteins, genome sequences, and antibodies could be used as a target. We attempted to provide a concise summary of these topics here.

3.1. The journey of COVID-19 and its characteristics

In December 2019, many people with flu-like symptoms were admitted to hospitals in Wuhan, China. To recognize the infectious pathogen, patient’s samples were collected and screened for metagenomic RNA sequencing. According to the test reports it was confirmed that, the sickness was triggered by a new form of coronavirus (Song et al., 2021). On January 30, 2020, the “World Health Organization” (WHO) stated pneumonia caused by the recently recognized coronavirus was “2019-nCoV” acute respiratory disease”. The very next day, WHO again announced the novel coronavirus outbreak a public health emergency of international concern. The “International Committee on Taxonomy of Viruses” (ICTV) named this new virus as SARS-CoV-2 because its genetic resembles to “Severe Acute Respiratory Syndrome Coronavirus” (SARS-CoV). The SARS-CoV-2 and SARS-CoV belong to the

Table 1
The common human respiratory viruses, their principal syndrome and main detection methods.

| SI No | Virus                     | Principal Syndrome                                  | Main detection method |
|-------|---------------------------|------------------------------------------------------|-----------------------|
| 1     | Human Bocavirus (HBoV)    | URI, bronchiolitis, asthma                           | PCR                   |
| 2     | Human Rhinovirus (HRV)    | URI, asthma and COPD excretion, bronchitis, pneumonia | Culture, RT-PCR       |
| 3     | Human Metapneumovirus (HMPV) | URI, bronchitis, pneumonia                           | Culture, RT-PCR       |
| 4     | Human Coronavirus (HCoV)  | URI, bronchitis, pneumonia                           | Culture, RT-PCR       |
| 5     | Coronavirus associated with SARS-CoV (SARS-CoV) | SARS                              | Culture, RT-PCR       |
| 6     | Human Respiratory Syncytial Virus (HRSV) | URI, bronchiolitis, croup, bronchiolitis, pneumonia | Culture, antigen detection, RT-PCR |
| 7     | Human Parainfluenza Virus (HPIV) | URI, croup, bronchiolitis, bronchiolitis, pneumonia | Culture, antigen detection, RT-PCR |
| 8     | Adenovirus (ADV)          | URI, PCF, bronchitis, pneumonia                      | Culture, antigen detection, PCR |

URI- Upper respiratory infection, PCR- Polymerase chain reaction, COPD- Chronic obstructive pulmonary disease, RT-PCR- Reverse transcription-polymerase chain reaction, SARS- severe acute respiratory syndrome, PCF- pharyngococonjuctival fever.
identical clade. The infection brought by SARS-CoV-2 is termed as COVID-19 (Bedford et al., 2020). Chinese researchers proposed that SARS-CoV-2 may be raised from bats, but the intermediate host yet to be identified (Lau et al., 2020; Zhou et al., 2020). The genome of SARS-CoV-2 shares ~89% sequence homologous to SARS-like-CoVZXC21 and ~82% sequence homologous to human SARS-CoV (Chan et al., 2020).

Coronavirus is named after the Latin word corona, which means “crown” in English. These viruses have a distinct morphology due to the presence of a crownlike structure on their surface. Coronavirus genomes are “positive-sense single-stranded ribonucleic acid” (+ssRNA) with a 30 kb genome length, making them large RNA viruses (Madhugiri et al., 2016; Chauhan et al., 2020). The COVID-19 virus is composed of four key structural proteins, which are “spike” (S), “membrane” (M), “envelope” (E), and “nucleocapsid” (N) proteins as showed in Fig. 2A (Chauhan et al., 2020). The S proteins form trimers on the virion surface, making peplomers encased in an envelope that give the virion its unique “corona” or crown-like look (Pal et al., 2020). They are accountable for allowing viral entrance into the cell by binding to the host receptor via “angiotensin-converting enzyme 2” (ACE2) (Letko et al., 2020). The S glycoprotein has two functional components S1 and S2, the S1 facilitates cell attachment whereas S2 for membrane binding (Ou et al., 2020). The E protein is the smallest constituent in the COVID-19 virion responsible for virus replication and growth (Schoeman and Fielding, 2019). M protein is the abundant component in the virus structure, defining the viral envelope (Neuman et al., 2011). Finally, the N protein, is a structural protein that binds to the single-stranded RNA (ssRNA) to form a shell around the nucleic acid inside (McBride et al., 2014). The N protein involves in both the viral duplication and the response of the host cell to viral infection (Tai et al., 2020).

3.2. The transmission and cell entrance mechanism of SARS-CoV-2

SARS-CoV-2 can be transmitted in two main modes, they are contact transmission and droplet transmission (Song et al., 2021). The contact transmission occurs when a healthy individual inhale the droplets/air released by infected people when they talk, sneeze, cough, or breathe near to them. In addition to this, the droplets released from an infected person are adsorbed/deposited on the surfaces, infection can occur if a healthy individual touch the contaminated surface and subsequently touches his/her mucous membranes in the mouth, nose, and eyes (Gonçalves et al., 2021). The SARS-CoV-2 cell entrance mechanism consists of several phases, as showed in Fig. 2B (El-Aziz and Stockand, 2020): Viral entry proteins must fold into an energetically stable state before undergoing a conformational change that provides enough energy to overcome the virus’s inherent aversion to cellular membranes. As a result, the S protein switches to a metastable state before membrane fusion, which is prone to transition to a low-energy state (Jackson et al., 2022). To begin, the SARS-CoV-2 virus binds to ACE2 receptors on the host cell surface. ACE2 is typically expressed by epithelial cells of the alveoli, trachea, bronchi, and bronchial serous glands of the respiratory system (Liu et al., 2011; Wang et al., 2021a, 2021b). The virus penetrates and duplicates in these cells. The recently created virions are subsequently discharged into the environment and infect additional target cells.

Viruses get around immunity by modifying the residues that neutralizing antibodies identify. Because immune escape is only essential in the presence of immunological pressure, no escape variants
developed during the initial days of the pandemic, when the disease was less widespread and vaccination was not available (Jackson et al., 2022). However, additional neutralization-resistant variants have emerged in recent months. The Alpha, Beta, Gamma, and Delta variations, are less sensitive to neutralization by immune plasma generated from COVID-19 recovered patients or vaccinated persons (Jackson et al., 2022; Wang et al., 2021c; Edara et al., 2021). One of the possibilities is that, these aforementioned variants are appeared from previously infected or vaccinated persons is supported by studies in which escape variants were generated in the presence of neutralizing antibodies, convalescent sera, or sera collected from vaccinated persons (Jackson et al., 2022; Andreano et al., 2021; Baum et al., 2020). Some studies showed that, the SARS-CoV-2, like other CoVs, is sensitive to ultraviolet (UV) light and heat, which could help inhibit virus propagation. Furthermore, CoVs can be deactivated using a variety of solvents, including ethanol (70%) and ether (75%) as well as chlorine-containing disinfectants (El-Aziz and Stockand, 2020; Seyer and Sanlidag, 2020; Dhama et al., 2021).

3.3. SARS-CoV-2 infection symptoms

If a healthy being is infected with SARS-CoV-2, the first symptom should appear in five to six days (Li et al., 2020a, 2020b). Individual instances can take up to 40 days to incubate the virus, with 14 days being the normal incubation period (Wang et al., 2020b). In the COVID-19 infection is considered by high fever, dyspnea, dry cough, and weakness. In addition, sore throat, sneezing, and mucus were recorded as signs of COVID-19 infection in the upper respiratory tract (URT) rather rarely, showing that the virus mostly affects the lower respiratory tract (LRT) and there is a high chance of intestinal infections (Chen et al., 2020c). In severe circumstances, infection with Covid-19 can result in pneumonia, bleeding, metabolic acidosis, septic shock, and coagulation issues (Li et al., 2020b). The common symptoms of COVID-19 are showed in Fig. 2C (El-Aziz and Stockand, 2020).

Asymptomatic individuals are people who are infected with SARS-CoV-2 but do not show any symptoms (Gandhi et al., 2020). As a result, surveillance and case detection will necessitate significantly more resources and attentiveness than previously assumed; for example, temperature monitoring is insufficient to discover all SARS-CoV-2 infected individuals (Boyton and Altmann, 2021; Arons et al., 2020). A COVID-19 outbreak was reported in a US care facility in April 2020, with one positive case infecting 63 percent of residents, half of whom had asymptomatic infections that would have remained unknown if routine PCR testing was not performed (Arons et al., 2020). As a result, asymptomatic transmission was called the “Achilles’ heel” of SARS-CoV-2 infection control strategies (Gandhi et al., 2020). A recent study shows that, children recovered with SARS-CoV-2 infections showed Kawasaki like disease (Khan and Ullah, 2021). On the other hand, recovered people’s blood plasma has been utilized to treat infected patients with acute symptoms (Duan et al., 2020).

4. Popular conventional methods available to detect SARS-CoV-2

The development of effective procedures to detect SARS-CoV-2 in an infected person could be crucial, as observations can aid in the establishment of an early-warning strategy that can track the spread and severity of the virus at the community level (Gowri et al., 2021). In this part, we will go through some of the most popular conventional methods for detecting SARS-CoV-2. Viral or molecular tests, which are designed to detect the virus’s genetic material, are usually used to diagnose active infection. PCR, LAMP, serological survey and computed tomography (CT) with artificial intelligence (AI) are representative examples of conventional approaches. The electrochemical approaches for sensing the SARS-CoV-2 virus are summarized in the next section.

When handling test samples, utmost caution must be used to avoid the infection to the person who conducts the study. According to WHO procedures, healthcare providers should be equipped with “personal protective equipment” (PPE) kit such as a mask (preferably N95), gloves, gown, and eye glasses (WHO, 2020). PPE kit is unquestionably a barrier of safety for healthcare workers (HCWs) who are fighting the pandemic as a treasured asset to the country. However according to a report, the most usual complications related with using PPE kit is extreme sweating (100%), fogging of eye protection glasses (88%), breathlessness (61%), suffocation (83%), fatigue (75%), headache due to continued usage (28%), and marks on the skin after repeated use (19%) (Agarwal et al., 2020). According to “Centre for Disease Control and Prevention” (CDCP) recommendations, the health care professional must wear a PPE kit and collect nasopharyngeal swab (NP) specimen (Marty et al., 2020). A test sampling must be stored in a tube containing viral transport media and a triple packed, and/or packed in a biohazard bag if shipment is required (Bundgaard et al., 2021).

4.1. Methods based on PCR

This crucial molecular biological technique was first invented by Kary Mullis in 1986 (Mullis et al., 1986). PCR is utilized in a variety of circumstances, including pathogen detection (Qiu et al., 2020), food and water safety (Kim et al., 2019), and forensics (Gu et al., 2019). The use of PCR to detect SARS-CoV-2 is crucial in this pandemic. According to WHO laboratory testing guidelines, the routine confirmatory test for COVID-19 is RT-PCR, with the ORF1ab/RdRp, E, N, and S genes being targeted (Van-Kasteren et al., 2020). Dong et al. (2021), pictographically showed the procedure to detect SARS-CoV-2 with PCR as in Fig. 3. RT-PCR is one of the nucleic acid amplification strategy that converts RNA into complementary deoxyribonucleic acids (cDNA), which is then rigorously amplified for detection (Tombolou et al., 2011, 2022; Gerard et al., 1997). In the collected NPS samples, RT-PCR identifies the presence of viral nucleic acids. For effective marketing and utilization, PCR with extreme sensitivity is required in the identification of infection. In general, sensitivity is influenced by the extent of a PCR reactor and/or the available nucleic acid templates. It is well known that, in order to get the great sensitivity, the amplification period with a bulky reactor tends to be lengthy. A detailed review conducted by Dong et al. (2021) reviewed the numerous PCR-related approaches for pathogen identification in the situation of the current COVID-19 pandemic. The results of rapid PCR using microfluidics in the previous five years are summarized. The key challenges surrounding rapid PCR, such as reactor design and thermal cycling implementation, are thoroughly covered.

Many RT-PCR testing kits for the detection of COVID-19 infected individuals have recently become available on the market, but their effectiveness has yet to be independently validated, and Van-Kasteren et al. (2020) provided an excellent review of this important issue. The aim of the investigation was to assess the analytical and clinical demonstration of seven different RT-PCR kits (Manufactured by: Altona Diagnostics, KH Medical, BGI, CerTest Biotec, R-Biopharm AG, Primer-Design, and Seegene). All assays had a PCR efficacy of 96 percent, and the estimated “95% limit of detection” (LOD95) varied within 6-fold range. Authors noticed some slight differences in detection rates between kits while using clinical samples. Except for the SARS-CoV-2 E-gene, none of the experiments revealed cross-reactivity with other respiratory (corona) viruses, as was expected. Finally, the authors conclude that skilled molecular diagnostic laboratories can use all the RT-PCR kits evaluated in their study for COVID-19 diagnosis in patients.

As discussed earlier, RT-PCR has several merits and distinctive potentials, making it a promising in the diagnostic field. For example, RT-PCR can be employed to recognize the viral genome sequence during the initial phases of infection. Furthermore, RT-PCR is extremely specific, highly sensitive, and capable of quantifying a patient’s viral load (Alteri et al., 2020). RT-PCR technique also has drawbacks, such as the need of expensive chemicals, tedious procedures, contamination, possible false-negative or false-positive results, and complicated sample handling.
protocols. These restrictions have inspired scholars to look for alternate molecular diagnostic procedures that are quick, inexpensive, and easy to implement in low-resource labs. Since the invention of LAMP, a reliable replacement interface for the precise detection of low copy number nucleic acids in the diagnosis of numerous viral infections has become available.

4.2. LAMP method

LAMP reaction can be used to detect even a few copies of specific viral DNA sequence under isothermal (60–65 °C) conditions with the aid of primer sets (specifically designed). It is becoming more prominent in the diagnosis of numerous viral infections due to its simplicity (Notomi et al., 2000; Parida et al., 2005, 2007). The detection of RNA viruses necessitates the use of an additional reverse transcriptase enzyme, which is referred to as “Reverse Transcriptase LAMP” (RT-LAMP). This approach, unlike PCR, can be carried out in a low-resource setup by simply heating the reagent chemicals and samples in a sole reaction tube. In a pandemic scenario, RT-LAMP has many merits over RT-PCR (Dhama et al., 2014; Augustine et al., 2020). Augustine et al. (2020) summarized the key characteristics of RT-LAMP in comparison to RT-PCR as showed in Fig. 4. The key components of LAMP reaction mixes are a set of salts, nucleotides, 4 to 6 primers (2 loop and 2 outer primers, forward and backward inner primers), and DNA polymerase to catalyze the synthesis of complementary strands with strand displacement action (Augustine et al., 2020; Curtis et al., 2008). An additional component for detecting RNA viruses is a heat-stable reverse transcriptase enzyme (Augustine et al., 2020). Recently, few researchers reported using targeted primers for various structural protein encoding regions, such as the S-protein encoding S gene or the nucleocapsid encoding N gene, individually or in combination, with the goal of obtaining results with excellent sensitivity, high specificity, and negligible cross-reactivity in a shortest period of time (Augustine et al., 2020; Yan et al., 2020; Park et al., 2020).

To increase the performance of the RT-LAMP reaction as a point of care test (PoCT), researchers applied various colorimetric detection approaches in which color-changing agents were added in the reaction mix. A reaction mixture incorporating a cresol red (pH-dependent indicator) dye was tested for rapid RT-LAMP based real time COVID-19 detection (Lu et al., 2020a, 2020b). The cresol red changes color from red to orange-yellow as an outcome of a +ve reaction (Tanner et al., 2015; Wu et al., 2021a). The pH of the LAMP reaction mix generally decreases in positive samples due to increased DNA polymerase activity (Augustine et al., 2020). In resource-constrained circumstances, a colorimetric RT-LAMP approach using phenol red (pH indicator), which alters color from pink to yellow at low pH (due to extensive DNA polymerase activity), can also be employed as a simple diagnostic method (Baek et al., 2020). As a result, the results could be seen with the naked eye. As a commercial alternative, Wu et al. (2021b) reported a dye combination for isothermal detection of SARS-CoV-2 with an enlarged colorimetric spectrum. Recently, Davidson et al. (2021) invented a paper-based device utilizing RT-LAMP to find SARS-CoV-2 in infected individuals’ saliva. The device could detect the virus in 60 min and had a 97% analytical sensitivity and a 100% specificity, with a detection limit of 200 genomic copies/μL of patient saliva. Even though LAMP has several advantages over PCR, one of the most critical issues to be solved is the complexity of designing the primers (Jia et al., 2019).
4.3. Serological test

Since the commencement of the COVID-19, serology (antibody) tests to identify past SARS-CoV-2 infection have been in more market demand. The initial scarcity of diagnostic tests, combined with asymptomatic infections, resulted in a high demand for serology tests to recognize previous infections (West et al., 2021a, 2021b). Serology tests are performed on patients to identify SARS-CoV-2 specific antibodies, the seropositivity (presence of SARS-CoV-2 specific antibodies) can specify previous infection (West et al., 2021b). Serology tests generally measures immunoglobulin M (IgM) and/or immunoglobulin G (IgG) antibodies. The IgM antibodies, which arise 5–10 days after infection, whereas, IgG antibodies will start to appear 7–10 days (or even more days) after infection (West et al., 2021a; Post et al., 2020). Serology (antibody) testing should not be performed to identify current infections, even if an individual who tests +ve with a serology test may still be infectious (West et al., 2021a). Infection with SARS-CoV-2 in the earlier was supposed to provide some resistance (immunity) against COVID-19. However, it is unknown how long immunity lasts and how many antibodies are required for immunity (To et al., 2021). This method has various advantages, including the need for a modest laboratory space for serological screening, which can be done at the point of treatment to reduce sample transfer and processing periods.

For a fast, easy, and sensitive diagnosis of COVID-19, examining specific antibodies to SARS-CoV-2 in patient blood is a suitable option. Before the creation of adaptive, high-affinity IgG responses, which are critical for long-term immunity (resistance) and immunological memory, IgM is thought to offer the early line of resistance during viral infections (Li et al., 2020c) [102]. In addition, the presence of IgM antibodies implies recent SARS-CoV-2 exposure, but the presence of COVID-19 IgG antibodies shows virus exposure some time ago. Based on these facts, Li et al. (2020c) [102] invented a point-of-care (PoC) lateral flow immunoassay (LFIA) test that can sense IgM and IgG in human blood in 15 min as showed in Fig. 5A. In this lateral flow test, a membrane strip is covered with 2 lines: gold nanoparticles (AuNPs) – antibody conjugates are on one line, while antibodies bind on the other. The patient’s blood sample is placed on the membrane, and the proteins are drawn through the membrane strip by capillary action. The antigen binds to the AuNPs – antibody conjugate as it passes through the first line, and the complex moves through the membrane. This strip has 3 distinguishing lines in total. When the test sample has moved through the cartridge, the control (C) line appears. A pink/red line in the M and G regions will prove the presence of anti-SARS-CoV-2 IgM and IgG. The test sample is negative if and only if the control line (C) is red. The existence of anti-SARS-CoV-2-IgM, and anti-SARS-CoV-2-IgG, or both antibodies in the samples is shown by the M or G lines turning red. The test is faulty if the C line does not display red, and it should be performed with a different cartridge (Fig. 5B). To demonstrate its clinical efficacy, this product was examined in eight hospitals and Chinese CDC divisions. The outcome showed that this rapid antibody test has an acceptable level of specificity and sensitivity. This device is suitable for usage in health centers, and laboratories. The test may also be used at companies, colleges, ports, seaports, and railway stations, among other places, making it a powerful tool in the fight against this pandemic.

4.4. Computed tomography (CT) with artificial intelligence

Computed tomography (CT) has recognized as an essential means for identifying and investigating potential COVID-19 infection in patients at an early stage (Wang et al., 2020a). Although a CT scan is an effective tool for diagnosing COVID-19, it is insufficient to notice COVID-19 alone owing to its less specificity (25%) and the difficulties that radiologists may experience in distinguishing COVID-19 from other viral pneumonias on chest CT scans (Alsharif and Qurashi, 2021). CT scan can be utilized to diagnose COVID-19, as well as to monitor the size of lesions and track any variations in patients who have negative RT-PCR assays and plain radiography imaging (Bai et al., 2020a; Raptis et al., 2020). According to several studies, nearly all COVID-19 patients had characteristic imaging findings on a chest CT scan (Caruso et al., 2020; Zhao et al., 2020; Huang et al., 2020; Xie et al., 2020). When compared to other examination methods, CT scan is currently preferred by the majority of clinics in China. This preference could be due to the availability of CT equipment in China (Lee et al., 2020). According to a study, a chest CT scan had a 97% sensitivity for COVID-19 when compared to RT-PCR as a reference (Ai et al., 2020). As discussed earlier, CT scan of COVID-19 infected patients exhibit symptoms that are similar to those of other pneumonia disorders, artificial intelligence (AI) could assist radiologists in distinguishing COVID-19 infection from other pneumonia related disorders.
Artificial intelligence is a branch of computer science concerned with the development of intelligent systems that can learn from data and make appropriate judgments and predictions (Collins et al., 2021). Recently, artificial intelligence has been used in a variety of areas, including industries, banking, agriculture, and healthcare (Wirtz et al., 2018; Liu et al., 2020; Khan et al., 2021). This developing technology is being employed in a variety of medical investigations and has been shown to improve scale-up, timeliness, reliability, and efficiency, as well as beat human in specific healthcare jobs (Coeckelbergh, 2010; Davenport and Kalakota, 2019; Bohr and Memarzadeh, 2020). AI-based methods, particularly deep learning models, are promising techniques for assisting radiologists in COVID-19 early detection. Additionally, it minimizes radiologist’s workload, enhances detection efficiency and accuracy, and delivers early and accurate remedy for COVID-19 patients (Khan et al., 2021; Albahri et al., 2020; Wang et al., 2021b; Gudigar et al., 2021). Bai et al. (2020b) did an excellent study to compare radiologist’s accuracy with and without AI in differentiating COVID-19 from non-coronavirus patients. They found that, with AI support, radiologists achieved high accuracy (90%), specificity (91%), and sensitivity (88%). This type of report has recently been published in a various journal (Jin et al., 2020; Chan et al., 2020; Xu et al., 2020; Huang et al., 2020). This shows that the AI system could play a role in improving radiologist’s everyday workflow and COVID-19 diagnostic outcomes.

5. Electroanalytical methods for sensing SARS-CoV-2

One of the most fascinating fields in electroanalytical research is the fabrication of electrochemical and/or biosensors (Ganesh et al., 2022a). When compared to the proposed conventional methods in section 4, electrochemical investigations offer important advantages such as ease of design, low cost equipment, high sensitivity and selectivity, accuracy, fewer power requirements, and ease of integration inside microfluidic devices (Unlüer et al., 2020; Cioates, 2020). Biosensors and/or electrochemical sensors are widely used in pharmaceutical, environmental, and agricultural studies (Ganesh et al., 2022a; Ganesh and Kim, 2022b; Rajaji et al., 2021, 2022; Kundu et al., 2019). Electrochemical transducers are now used in the majority of biosensors because they are simple to create, user friendly, measurement is unaffected by interferences, and need modest reaction volumes. Electrochemical detection methods can be categorized into the following three major categories based on the measured property; i) amperometry or voltammetry method, (measurement of current) ii) potentiometric method, (measurement of potential) and iii) impedance method (resistance and/or conductance measurement) as showed in Fig. 6 (Bobrinetskiy et al., 2021).

Amperometry methods are based on the measure of resultant current (I) generated from the oxidation and/or reduction of electrochemically active species. The electroactive molecules are interacted at the electrode surface when the constant voltage is applied, and the generated current is measured (Gaudin, 2019). An amperometric biosensor’s working electrode is grafted with the biosensing component and its role is to improve the selectivity of fabricated biosensor. Nanomaterials are also used as the sensitized element in the fabrication of composite electrodes when no bioreceptor is required to sense the target analyte. According to the principle of voltammetry, the working electrode is driven to operate within a given potential window where an electrochemical redox phenomenon occurs, and the resultant I is measured (Bard and Faulkner, 2000; Compton, and Banks, 2018). This generated I

![Fig. 6. Electrochemical (bio) sensors using different electroanalytical methods (amperometry, potentiometry and impedance methods) for target analyte sensing and its concentration determination. [Adopted from Ref. (Bobrinetskiy et al., 2021), Copyright 2021, by the authors, Licensee MDPI, Basel].](image-url)
is directly proportional to analyte concentration when “cyclic voltammetry” (CV), “linear sweep voltammetry” (LSV), “differential pulse voltammetry” (DPV) and “square wave voltammetry” (SWV) are used (Scholz et al., 2010).

Potentiometric measurements involving calculating the electrical potential difference between two electrodes: a constant-potential reference electrode and a working electrode (indicator electrode) whose potential varies depending on the sample composition (Angeles et al., 2018). Potentiometric biosensors could use “ion selective electrodes” (ISEs) and “ion sensitive field effect transistors” (ISFET). A field effect transistors (FET) is made up of a semiconductor channel with two electrodes, and the current flowing through it is controlled by the electric field. The intrinsic charge of molecules collected by bioreceptors (as immobilized) could be detected using FET. The combination of a FET with an ion selective membrane (ISM), which covers the transistor gate, is known as an ISFET. If an enzyme layer is placed on the gate, the fabricated biosensor is known as enzyme-FET (ENFET) (Lee et al., 2009). ISFET-based biosensors can sense a wide range of chemical and/or biological species with excellent sensitivity and real-time label-free detection. They can also be combined into circuits for large production (Syu et al., 2018).

Electrochemical impedance (EIS) examines the response of a redox probe (electrochemical probe) to an applied voltage. Impedimetric method of sensing is based on a change in interfacial capacitance (C) and/or resistance (R) at the electrode’s surface (Stoynov and Vladikova, 2009). This change in interfacial R and/or C was produced by the formation of a complex among the bioreceptor (immobilized on electrode surface) and analyte solution (Mansoorifar et al., 2018). Impedimetric biosensors are less explored in the literature as compared to amperometric or potentiometric biosensors (Angeles et al., 2018). Electrochemical impedance, on the other hand, is a useful analytical tool for characterizing the surface of modified electrodes, batteries, thin film behavior, studying corrosion mechanisms, and electron transport kinetics (Meddings et al., 2020; Brett, 1990; Cesilis et al., 2016). The main disadvantage of this method is the time it takes to acquire an impedance spectrum when studying wide frequency ranges; although, it is a nondestructive and direct method.

Many electrochemical biosensors have been developed in recent decades using electroanalytical techniques and nanotechnology features. Nanomaterials can improve the sensitivity, response time, and selectivity of the fabricated electrochemical biosensor, making them more accessible for viral detection (Abad-Valle et al., 2005). Among the many nano materials, gold nanoparticles (AuNPs) are intensively described in the virus producers (Boopathi et al., 2021). Raziq et al. (2021) delivered a rapid and large-scale method to detect SARS-CoV-2 virus.

Rapid and large-scale method to detect SARS-CoV-2 virus is required to inhibit the transmission of the pandemic within and across communities and to reduce the outbreak, and using lower cost paper-based electrochemical sensors is an excellent idea. Alafeef et al. (2020) designed a fast, economic, simple-to-implement and quantitative paper-based electrochemical sensor chip for the digital identification of SARS-CoV-2 genetic material in less than 5 min duration. The authors designed a graphene-based electrochemical biosensor with an electrical readout device to sense SARS-CoV-2 genetic material. The biosensor’s high selectivity arises from the integration of a suitable design of thiol-modified antisense oligonucleotide (ssDNA) specific for SARS-CoV-2 nucleocapsid and four such ssDNA probes were designed to simultaneously target two different regions within the similar viral N-gene. The electrochemical assay was made substantially more sensitive by using thiol-modified ssDNA-capped AuNPs. The sensor’s response was tested with RNA samples taken from Vero cells infected with SARS-CoV-2 virus.

Graphene (Gr) is a great electrode material for electrochemical sensors because it has several appealing properties, such as a high surface area and superior conductivity. Recently, Zhang et al. (2021b) prepared Au/Gr nanohybrids as the electrode material and arginine as the functional monomer to build a unique MIP sensor for the SARS-CoV-2 N-protein. This proposed sensor showed a LOD of 3.0 fM in a linear range 10.0–200.0 fM. The sensors density repeatability (RSD of 3.92% for eight parallelly fabricated electrodes), stability (7.8% reduction after four weeks of storage), and selectivity make it an important application in the SARS-CoV-2 detection. Moreover, SARS-CoV-2 viral antigen detection using a cotton-tipped electrochemical immunosensor was reported by Eissa and Zourob, (2021). In this report, the virus N protein was immobilized on screen-printed electrodes modified with carbon nanofibers and subsequently functionalized with diazonium electro grafting. The detection was done using “square wave voltammetry” (SWV), and the LOD for the electrochemical biosensor was 0.8 fM concentration range, with a limit of detection (LOD) of 15 fM and limit of quantification (LOQ) of 50 fM. In addition, it was also able to distinguish ncovNP from interfering proteins (BSA, E2 HCV, CD48 and S1). The authors evaluated the performance of sensor in clinical samples (nasopharynx swabs) of COVID 19 patients made promising findings, validating the sensor’s ability to detect ncovNP in complicated biological conditions. This electrochemical sensing strategy could be a useful alternative for rapid COVID-19 screening as a portable diagnostic platform.
pg/mL for SARS-CoV-2, demonstrating that the sensor has a high sensitivity. The biosensor showed no substantial cross-reactivity with some other virus antigens like influenza A or HCoV, showing that the method is very selective. In addition, Zhao et al. (2021) reported a smartphone enabled detection of SARS-CoV-2 viral RNA by exploring the uniqueness of r-GO. This electrode material is synthesized by functionalizing the r-GO surfaces with p-sulfocalix-[8]-arene (SCX8) onto which Au@Fe$_3$O$_4$ NPs were deposited. The detection ability (artificial target) of the proposed sensor by DPV technique was nearby to 3 aM (aM = $10^{-18}$ mol/L) ranging from $10^{-17}$ to $10^{-12}$ mol/L. This is one of the fastest sensors with the response time of less than 10 s. However, in clinical samples analysis, the LOD observed was 200 copies/mL, achieving a 100% accuracy. The biosensor’s ability to be simply connected to a smartphone makes it more helpful as a diagnostic tool.

5.2. Electrochemical methods based on spike protein detection

The S protein helps viral entry into the host cell by allowing the virus to bind to the host cell’s surface receptors and then fusing the viral and host cell membranes. As explained earlier, S1 and S2 are the two domains of the S-protein. The “receptor binding domain” (RBD) of the S1 subunit is important for binding to host cell receptors, whereas the S2 subunit allows membrane fusion of virus and host cell membranes. Unlike nucleocapsid protein, which is highly preserved, S-protein, particularly its S1 component, is more alterable, sharing roughly 70% similarity with the individual SARS-CoV and hence may have fewer cross-reactivities with other coronaviruses (Wrapp et al., 2020; Ayankojo et al., 2022). As a result, the SARS-CoV-2 S-protein is an important target for diagnosis. Recently, Ayankojo et al. (2022) fabricated a thin-film metal electrode chip (Au-TFME), modified with a MIP film that has selectivity for S-protein subunit S1 (ncovS1) and used as a recognition element in the fabrication of an electrochemical sensor to sense SARS-CoV-2 S-protein. The chip is linked to a portable potentiostat, which detects the ncovS1-specific reduction in the intensity of charge transfer (CT) through the MIP film using a redox probe. The sensor’s performance and selectivity were tested in both buffer and NPS samples from COVID-19 patients with LOD value of 15 fM and 64 fM respectively. With a rebinding time of 15 min and a measurement time of 5 min, the sensor displays a quick diagnostic capability that is equivalent to existing antigen detection procedures. Furthermore, it shows strong discrimination against ncovNP, which may be present in COVID-19 patient samples. The sensor’s detection principle is based on measuring variations in CT among the Au-TFME and the Fe$^{2+}$/Fe$^{3+}$ redox probe via imprinted pathways formed inside the ncovS1-MIP film. When ncovS1 rebinds during sensor incubation in analyte solution, the CT is severely hindered by the non-conductive protein, resulting in a concentration-dependent contraction in the measured current peak (Fig. 8A). Even though the sensor shows practical discrimination against
advancements, this strategy is the first to be able to detect whole testing technologies have recently been revealed that represent major infected individuals in minutes. The authors tested the sensors long-term SARS-CoV-2 virus particles in undiluted saliva samples from COVID-19 the ferrocene label interacts with the electrode, and ferrocene is stability after 9 months of storage and found that they are still func the electrode surface. (C) Schematic representation of the RAPID detection method. [(A) Repeated with permission from Ref. (Ayankojo et al., 2022), Copyright 2021, Elsevier B. V. (B) Reproduced with permission from Ref. (Yousefi et al., 2021), Copyright 2021, American Chemical Society. (C) Reproduced with permission from Ref. (Torres et al., 2021), Copyright 2021, Elsevier Inc.].

S-proteins from various SARS-CoV-2 viral variants, more research is needed to confirm its selectivity compared to all known virus strains and the accompanying selective detection mechanism.

In another report, Yousefi et al. (2021) described reagent-free electrochemical sensing method for detecting SARS-CoV-2 virus particles quickly, sensitively, and easily. It is constructed on the field-induced transport of a sensor complex on the electrode surface and the control of transport kinetics by viral component binding. Authors are able to predict variations in the kinetics in both simulations and tests, and establish the existence of the virus, despite the viral particles significant size and related hydrodynamic force. In this sensor, an antibody (analyte-recognizing) is coupled to a rigid, -ve charged linker made of DNA. To track the interface of the sensor with the electrode, a ferrocene (as redox probe) is connected to the DNA linker. Due to the –ve charges in the DNA linker, the sensor complex is drawn to the surface when a positive potential is applied to it. Electron transfer (ET) happens when the ferrocene label interacts with the electrode, and ferrocene is oxidized with a specific time constant. While multiple new SARS-CoV-2 testing technologies have recently been revealed that represent major advancements, this strategy is the first to be able to detect whole SARS-CoV-2 virus particles in undiluted saliva samples from COVID-19 infected individuals in minutes. The authors tested the sensors long-term stability after 9 months of storage and found that they are still functional. Furthermore, by simply varying the antibody used to fabricate the sensor, the testing method can be easily extended to detect other viral targets, and authors anticipate that this sensing interface will appeal a wide range of applications (See Fig. 8B) (Yousefi et al., 2021).

Idili et al. (2021) attempted to make a new sort of sensing technology capable of delivering an accurate quantitative response at the PoC, by developing an “electrochemical aptamer-based” (EAB) sensors. This approach uses the signal provided by a binding-induced conformational change of a redox reporter-modified aptamer on a gold (Au) electrode surface. The aptamer conformation changes when the target concentration changes, causing the redox reporter’s location relative to the electrode surface to alter, consequent in a quantitative electrochemical signal. The EAB sensor’s precise analytical response is combined with its short response time (few sec - 5 min) and relatively single step operation. The authors used two recently developed DNA aptamers (as recognition elements), designated 1C and 4C, that can identify the SARS-CoV-2 S-protein’s RBD. These aptamers were chosen because they exhibit three important characteristics for the construction of a sensitive EAB sensor. Initially, the variants were chosen in a working buffer that closely resembles physiological circumstances; thus, they may be used to quantify S-protein in biofluids. Second, the aptamers estimated dissociation constants (Kd) (5.8 ± 0.8 nM for 1C and 19.9 ± 2.6 nM for 4C) are identical to those of S-protein-binding antibodies that are commercially available. Third, the molecular dynamics (MD) technique was previously used to characterize the binding interactions among the target and the aptamers. The EAB sensor produced promising bioanalytical results for the identification of SARS-CoV-2 antigens. The picomolar quantities of the S-protein in serum, buffer, and 50 percent artificial saliva using the 1C version was detected. The aptamer’s fast binding kinetics and one-step operation allow for target identification in 15 s, making this EAB sensor model for increased testing at the PoC.

Rashed et al. (2021) demonstrated quick label-free electrochemical sensing of SARS-CoV-2 antibodies using a widely available impedance sensing method. A 16-well plate with sensing electrodes was pre-coated with the SARS-CoV-2 S-protein’s RBD and then tested with anti-SARS-CoV-2 monoclonal antibody CR3022 (0.1 μg/mL, 1.0 μg/mL, and 10 μg/mL) samples. The blinded testing was performed on six serum samples from COVID-19 and non-COVID-19 patients. The methodology was able to discriminate spikes in impedance measurements from a negative control for all CR3022 samples. In addition, all positive clinical samples were correctly distinguished from the negative control. The observed impedance values were accurate when compared to conventional ELISA test results, indicating a strong correlation. In another
report, Vadlamani et al. (2020) fabricated a cobalt-functionalized TiO$_2$ nanotubes (Co-TNTs)-based electrochemical sensor for affordable and quick detection of SARS-CoV-2 by sensing the spike present on the virus’s surface. An easy, low-cost, and single step electrochemical anodization process was employed for the synthesis of TiO$_2$ nanotubes (TNTs), followed by a cobalt functionalization of the TNTs platform using an incipient wetting method, which was coupled to a potentiostat for data gathering. This reported Co-TNTs-based sensor can detect the S-RBD protein of SARS-CoV-2 in less than 30 s (14–1400 nM, concentration range), using the amperometry electrochemical approach. Based on the impedance method of analysis, Torres et al. (2021) reported a real-time accurate portable impedimetric detection prototype 1.0 (RAPID 1.0) sensor that converts biochemical information from a specific molecular binding event involving the SARS-CoV-2 S-protein (binding element) and ACE2 (biological receptor) into an easily detectable electrical signal (EIS signal). As these two molecules bind, there is a change in an interfacial ET kinetics among the redox couple (Fe$^{3+}$/Fe$^{2+}$) and the conducting sites of the electrode. By monitoring the “charge-transfer resistance” (Rct), the semi-arc (the diameter) on the Nyquist plot, which relates with the number of targets bound to the receptive surface, one can quickly detect this change in an electrochemical signal. This device detected spike protein concentrations 10–1000 times lower than those reported in earlier studies (Rashed et al., 2021; Seo et al., 2020) highlighting the sensitivity of this method. Using EIS method, this RAPID 1.0 biosensor can detect SARS-CoV-2 in 4 min with a minimal sample volume. SARS-CoV-2 and UK variant B.1.1.7 are detected by the test in saliva and NPS or oropharyngeal samples (OP) with accuracy, specificity, and sensitivity at very less concentrations (1.16 PFU mL$^{-1}$) as schematically shown in Fig. 8C (Torres et al., 2021). Similarly, in the literature few other electrochemical based sensors based on spike protein detection are reported (Ali et al., 2021; Mahari et al., 2020; Mojoska et al., 2021; Nascimento et al., 2022; Abrego-Martinez et al., 2022; Zhao et al., 2022; Jiang et al., 2021; Aydn et al., 2021).

5.3. Electrochemical methods for simultaneous spike protein and nucleocapsid detection

The simultaneous electrochemical detection of S and N protein of SARS-CoV-2 was recently reported by Chaibun et al. (2021). In this excellent investigation, authors reported an ultrasensitive biosensor to sense SARS-CoV-2 protein (both S and N) constructed on a magnetic particle (MP)-based label-free simultaneous electrochemical detection approach. From RNA extraction to detection, the authors demonstrated the use of an electrochemical biosensor in combination with RCA to detect SARS-CoV-2 in a very sensitive and specific way. The viral N and S genes were detected in synthetic linear targets in addition to clinical samples (OP) with accuracy, specificity, and sensitivity at very less concentrations (1.16 PFU mL$^{-1}$) as schematically shown in Fig. 8C (Torres et al., 2021). In less than 2 h, the one-step sandwich hybridization technique could identify as low as 1 copy/μL of S and N genes. Sensor testing with 106 clinical samples, including 41 SARS-CoV-2 +ve samples and 9 samples + ve for other respiratory viruses, yielded a 100% concordance with quantitative reverse transcription PCR (qRT-PCR), with a good correlation among biosensor current peak and quantitation cycle (Cq) readings. Overall, the authors demonstrated the sensitivity of this method, the sensing mechanism based on the interruption of the redox active labels that may be detected by an electrochemical biosensor (based on DPV). In less than 2 h, the one-step sandwich hybridization technique could identify as low as 1 copy/μL of S and N genes. Sensor testing with 106 clinical samples, including 41 SARS-CoV-2 +ve samples and 9 samples + ve for other respiratory viruses, yielded a 100% concordance with quantitative reverse transcription PCR (qRT-PCR), with a good correlation among biosensor current peak and quantitation cycle (Cq) readings. Overall, the authors demonstrated the use of an electrochemical biosensor in combination with RCA to detect SARS-CoV-2 in a very sensitive and specific way. The viral N and S genes were detected in synthetic linear targets in addition to clinical samples owing to RCA’s strong amplification capability and the sensitivity of the electrochemical detection approach. From RNA extraction to detection, the entire experiment took less than 2 h to complete and did not require the need for a thermal cycler. The assay’s performance with clinical samples was analogous to RT-qPCR, with no false-positive results. This strategy could have a big impact in regions where early identification is needed to stop SARS-CoV-2 outbreaks from spreading. Recently, “Dual-mode lateral flow immunoassay” (LIFA) biosensor based on magnetic quantum dots (Wang et al., 2021a), and “magnetic particle spectroscopy” (MPS) bioassay method (Wu et al., 2021), for spike and nucleocapsid detections were reported.

5.4. Immunoglobulin detection

Immunoaassays detect antibody binding to a specific target and can be used to detect the existence and concentration of specific antibodies in order to establish if a patient has previously been infected and to analyze the disease’s prevalence. As previously stated, ELISA is the standard diagnostic method for precise and quantitative antibody detection for most common pathogens, including SARS-CoV-2. However, due to tedious sample process and incubation phases, this method takes hours to complete (Warni et al., 2017). Electrochemical biosensors have proven to be an effective alternative to ELISA procedures. Recently, Peng et al. (2022) presented a portable “serological testing platform for rapid electrochemical detection of SARS-CoV-2 antibodies” (SPEEDS). This reported method is built on a cost effective electrochemical immunosensor that utilizes ELISA to determine serum IgG/IgM against the SARS-CoV-2 S-protein. Here the whole assay took approximately 13 min on SPEEDS platform, and the electrochemical immunosensor may be mass-produced at an affordable price. The manufactured ready-to-use immunosensor chips can be stored at room temperature for at least 24 weeks without performance degradation. The authors attained measurement ranges of 10.1 ng/mL – 60 μg/mL and 1.64 ng/mL – 50 μg/mL for human monoclonal anti-SARS-CoV-2 IgG and IgM, respectively, this covers a typical antibody levels in convalescent sera and sera of COVID-19 infected patients (both mild and severe infection). As shown in Fig. 9a, this SPEEDS platform contains a custom-made electrochemical immunosensor and a commercially available handheld potentiostat (PalmSens, EmStat3 Blue). The immunosensor comprises of three electrodes (a carbon working, Ag/AgCl reference, a carbon counter) screen printed on a polyethylene terephthalate (PET) film to form a three-electrode electrochemical cell. This can be put directly into a chip slot of a handheld potentiostat for electrochemical signal reading, and the testing data is sent to a smartphone through Bluetooth. An electrochemical ELISA is used to detect SARS-CoV-2 antibodies on the immunosensor, as shown in Fig. 9b and c. Streptavidin was initially immobilized on the immunosensor’s working electrode (WE) via physical absorption, then the capture probe was immobilized via streptavidin/biotin binding (Fig. 9b). The capture probe was the biotinylated SARS-CoV-2 S-RBD protein, which has a high affinity for SARS-CoV-2 IgG and IgM antibodies. In every test, the IgG or IgM antibodies of SARS-CoV-2 in the sample were captured on the working electrode (WE) by the RBD probe (Fig. 9c). Later, the alkaline phosphatase (ALP)-labeled anti-human detecting antibody, exactly against IgG or IgM antibody, was then added to attach to the captured IgG or IgM antibody. Finally, the electrochemical substrate of ALP, p-aminophenyl phosphate (pAPP), was introduced to the immunosensor in order to react with ALP, for subsequent chronoamperometry (CAMP) measurement as in Fig. 9d. A higher IgG or IgM antibody concentration results in a higher density of immobilized ALP on the WE, and thus a higher CAMP current. As a result, the CAMP current can be used to determine the concentration of SARS-CoV-2 IgG or IgM antibodies. This immunosensor’s sensitive and fast IgG and IgM detection meets the demand for quick COVID-19 serological testing for PoC diagnosis and population immunity screening.

Recently, Yakoh et al. (2021) demonstrated that a paper-based electrochemical platform may be used to diagnose COVID-19. In this method, the sensing mechanism based on the interruption of the redox probe (Fe$^{3+}$/Fe$^{2+}$) conversion caused by formation of an immunocomplex involving the immobilized S-protein of SARS-CoV-2 and the captured immunoglobulins produced in response to SARS-CoV-2 infection in humans. Because of the label-free electrochemical technology used in this study, no antibodies are required. SARS-CoV-2 antibodies were detected quickly (30 min) and sensitively, with a detection limit of 1 ng/mL. Also, this paper-based technology has an 100% sensitivity and 90% specificity for detecting targeted antibodies in patients (clinical samples). Here, the researchers demonstrate a concept for lab scale paper based electrochemical sensors for SARS-CoV-2 antibodies and
antigens. Furthermore, their method necessitated a lengthy process of surface functionalization, sample preparation, and testing, making it unsuitable for PoC testings. Therefore, to overcome these issues, Kumar et al. (2021) reported a new PoC electrochemical ELISA technology that uses disposable screen-printed electrodes functionalized with SARS-CoV-2 Spike glycoprotein S1 to allow for quick and reliable quantification of total IgG and IgM concentrations in clinical samples. This new technique has been thoroughly validated over multicentric evaluation at four centers. When compared to three FDA-approved laboratory standard procedures (Siemens COV2T S1RBD assay, DiaSorin Liaison® SARS-CoV-2 S1/S2 IgG assay, Vitros CoV2G IgG assay) this reported assay attains 100% sensitivity. Overall, the authors showed an assay with high sensitivity and specificity, making it a good fit for epidemiological examinations and antibody quantification in COVID-19 immunization campaigns.

In another interesting research carried out by Rahmati et al. (2021) designed a label-free electrochemical sensor that detects IgM/IgG antibody using the S-protein as a specific-receptor. The "screen-printed
carbon electrode” (SPCE) was modified with Ni(OH)$_2$NPs, flowed by immobilization of S-protein (specific-receptor) for ultra-sensitive selective quantitative analysis. Briefly explaining, initially the bare SPCE was electrochemically activated by dropping 6 μL H$_2$SO$_4$ (2 M) over the surface and then applied a 1.5 V voltage for 150 s using a CAMP approach, followed by drying under nitrogen atmosphere. Secondly, the CV approach was used to achieve controlled in-situ electrodeposition and the formation of Ni(OH)$_2$NPs on SPCE. Here, the electrodeposited Ni(OH)$_2$NPs on the SPCE surface served as a substrate for loading spike protein molecules. Following that, 6 μL of spike protein in “phosphate buffer solution” (PBS) (pH = 7.4) was cast onto the Ni(OH)$_2$NPs@SPCE surface for 2 h to immobilize it via the amine groups in the protein structure via the formation of a Ni(OH)$_2$NPs-NH$_2$ covalent link. The electrode was then washed with PBS to remove any non-bonded molecules before being dried in N$_2$ gas. In the final stage, authors covered the electrode surface with 6 μL of bovine serum albumin (BSA) (1%) solution for 30 min. As a result, the active sites that are available will be inhibited, and non-specific adsorption will be evaded. The resulting BSA/S-protein/Ni(OH)$_2$NPs@SPCE biodevice was then rinsed with PBS and used in IgG/IgM detection assays, either immediately or stored (at 4 °C) till use. The authors schematically displayed the entire procedure of manufacturing BSA/S-protein/Ni(OH)$_2$NPs@SPCE biodevice and detecting IgG or IgM antibodies as in Fig. 10A for easy understanding to readers. When the IgM/IgG specifically bound to the S-protein during testing, the Fe$^{2+}$/Fe$^{3+}$ ET might be disrupted due to ET site blockage (Fig. 10 B), resulting in a reduced CV response, as shown in inset CVs of Fig. 10 A. A few other noteworthy studies on IgG and/or IgM detection have been reported in the literature (Li et al., 2021; Hashemi et al., 2021).

6. Challenges and future prospective

The global community has recently faced major public health challenges and emergencies as a result of respiratory virus infections. Because these viruses can rapidly spread across huge populations in a short period of time, highly accurate diagnostic approaches that are streamlined and can be carried out in an integrated manner are critical. The COVID-19 pandemic describes the world’s biggest challenge at the moment, through the most essential emphasis being sensitivity and specificity enrichment, to which present technologies should concentrate for early detection of COVID-19 infection or future pandemic strains. The development of simple, economic, easy-to-use, and quick-response procedures that precisely satisfy the potential to be integrated into PoCT for COVID-19 diagnosis is in great demand on the market. As a result, the current research activities related to SARS-CoV-2 detection by conventional methods and advanced electrochemical approaches are described thoroughly in this review. The research suggests that further work is needed to tackle amplification and sensitivity difficulties when it comes to identifying SARS-CoV-2 using conventional methods. PCR offers a number of advantages and distinguishing characteristics that make it a viable diagnostic tool (Tombolougou et al., 2021, 2022). For example, during the early phases of infection, RT-PCR can be utilized to detect the viral genome sequence. RT-PCR is also capable of assessing a patient’s viral load and is highly specific and sensitive. However, the RT-PCR technology has a number of disadvantages as well, including the use of expensive chemicals, time-consuming procedures, complicated methodologies, inability to process more number of samples speedily, and varying temperature need for different reaction cycles. In comparison to the RT-PCR approach, LAMP has demonstrated to be a promising tool for SARS-CoV-2 identification due to its speed of analysis, utilization of isothermal conditions, and high amplification. Despite the fact that LAMP has various advantages over PCR, one of the most important problems to be addressed is the difficulty/complexity of designing the primers.

Due to a lack of diagnostic tests and the predominance of asymptomatic illnesses, there was a strong demand for serology tests to detect previous infections. Patients are given serology tests to see if they have SARS-CoV-2 specific antibodies; seropositivity (the existence of SARS-CoV-2 specific antibodies) can suggest past infection. Serology (antibody) tests, should not be used to diagnose current infections, even if a person who tests +ve for a serology test is still infectious. However, serology testing may be employed in a variety of venues, including businesses, institutions, ports, seaports, and train stations, making it a significant tool in the fight against the pandemic. In addition, CT scan is an excellent tool for detecting COVID-19, it is insufficient to detect COVID-19 alone due to its low specificity (25%) and radiologist’s difficulty distinguishing COVID-19 from other viral pneumonias on chest CT scans. To address this issue, researchers suggested artificial intelligence-based technologies, particularly deep learning models, have recently been suggested as promising techniques for supporting radiologists in COVID-19 early detection. Furthermore, it reduces radiologist burden, improves detection accuracy and efficiency, and provides COVID-19 patients with timely and accurate treatment. When compared to these widely used conventional methods, electrochemical approaches attracted researchers in to develop quick, sensitive, and cost-effective SARS-CoV-2 sensing platforms. Electrochemical biosensors possibly will be a very promising SARS-CoV-2 detection method for: i) accuracy, ii) selectivity iii) economic, iii) sensitivity, iv) rapid, and v) portable detection, operating as a supplement to the present conventional diagnostic methods for regulating the spread of COVID-19. During our literature review, we discovered that a variety of electrochemical-based sensors to detect SARS-CoV-2 have been established and used in a relatively short period of time. Electrochemical sensors for sensitive detection, and quantification of viruses have advanced due to the implementation of innovative materials and developments in instrumentation technology. Electrochemical sensors continue to play a significant role in health-care systems, owing to their benefits of simple design, high analytical sensitivity, ease of integration within devices, robustness, low power consumption, and economical, as compared to other technologies with improved smartness and capability. In some research papers it is showed that, the generated electrochemical signal as a result of the biosensor’s interaction with SARS-CoV-2 can be read out using a smartphone (via Bluetooth connection with device), making it a point-of-care testing device. Multiple procedures are involved in the preparation of electrochemical biosensors, from cleaning the electrode to modifying/immobilizing using modifier/bioreceptor. More research is needed to make these numerous stages easier and more convenient.

Electrochemical biosensor responses can be recorded using antibody interactions with redox probe (most commonly [Fe(CN)$_6$]$_3$/$^{4-}$), as well as N and S proteins in the presence of a virus. Individual targets like IgG and/or IgM antibodies, N and/or S protein are frequently utilized in the literature to identify SARS-CoV-2. Antibody-based identification using IgG and IgM antibodies, needs longer to produce after an infection has occurred. As a result, while it may not be the best technique for detecting SARS-CoV-2 quickly, it can still be useful in tracking infections. From the literature we can see that, amperometry, EIS, and DPV are the most commonly used electroanalytical techniques for the SARS-CoV-2 detection. Meanwhile, there is concern regarding the ‘real’ current response of the sensor when employing the amperometric approach in conditions with high virus concentrations, because diffusion processes may be present. The EIS, SWV, and DPV are highly sensitive and consistent detection methods, especially for the target analyte’s low concentration levels. However, depending on the virus concentration, the optimum operating conditions (such as Hz, pulse width, scan rate etc.) must be determined each time. We hypothesize that, the use of nanotechnology, molecular imprinted polymers, and advanced electrochemical technologies will be critical in the design of rapid, low-cost, disposable electrochemical sensors for respiratory viruses, particularly SARS-CoV-2. We hope that by referring to the current state of the literature, many more cost-effective, selective and sensitive approaches to detect SARS-CoV-2 and its mutant are undoubtedly on the way in the
near future, which will not only bring improved choices for the health care workers to screen and early diagnose COVID-19, also make the communal in advance for any fatal viruses that may arise globally in the future.

7. Conclusions

This review covers the techniques and methods used to detect SARS-CoV-2 virus. To begin, a brief summary on the respiratory viral infections and its consequences were discussed, followed by an explanation of the SARS-CoV-2 virus’s features, transmission mechanisms, and symptoms. As a gold standard, RT-PCR tests are widely accepted because they can provide numerous benefits, making them life-saving diagnostic techniques. However, time-consuming process, tedious multiple steps, use of expensive chemicals, varying temperature necessities for different reaction cycles, and requirement of skilled workers are the few disadvantages of this method. In comparison to the RT-PCR method, LAMP has proven to be a promising technique for SARS-CoV-2 identification due to its speed of analysis and utilization of isothermal conditions. Despite the fact that LAMP has various advantages over PCR, one of the most significant issues that must be addressed is the complexity of designing the primers. In addition, serology testing’s and CT with artificial intelligence are also considered as significant conventional methods for the identification of COVID-19 infection. On the other hand, in a relatively less duration of time, the electrochemical-based sensors to sense SARS-CoV-2 have been developed and many researchers explored the uniqueness of nanomaterials, molecularly imprinted polymers, and electroanalytical techniques to fabricate a sensing device. The details of sensor fabrication procedures, electrochemical response mechanism, and their limit of detection, linear range, and their point of care applications have been also reviewed. Overall, we presented an overview of detecting SARS-CoV-2 viruses by conventional methods and advanced electroanalytical techniques. We believe that advances in electrochemical sensor technologies across various disciplines will cover the way for a new generation of highly precise, selective, and reliable electrochemical biosensors for the detection of respiratory viruses. We hope that this comparative review will aid researchers in the development of high-quality, affordable, PoC electrochemical sensing devices for respiratory viruses, SARS-CoV-2 in particular.

Author contributions

Pattan-Siddappa Ganesh: Conceptualization, Literature review, Data curation, Methodology, Writing- original draft preparation, Writing- review & editing.

Sang-Youn Kim: Conceptualization, Supervision, Funding acquisition, Project administration, Writing- original draft preparation, Writing- review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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List of abbreviations

ACE2 Angiotensin converting enzyme 2
ad14 Adenovirus 14 strain
AI Artificial Intelligence
ALP Alkaline phosphatase
ARIs Acute respiratory infections
AuNPs Gold nanoparticle
BSA Bovine serum albumin
CAMP Chronoamperometry
CBD Cannabidiol
CDCP Centre for disease control and prevention
cDNA Complementary deoxyribonucleic acids
CNS Central nerve system
Co-TNTs Cobalt-functionalized TiO₂ nanotubes
COVID-19 Coronavirus disease- 2019
CoVs Coronaviruses
Cq Quantitation cycle
CT Computed tomography
CV Cyclic voltammetry
DPV Differential pulse voltammetry
DVA deoxyribonucleic acid
E envelope
EAB Electrochemical aptamer-based
ELISA Enzyme-linked immunosorbent assay
ENFET Enzyme-FET
HCWs Healthcare workers
IC Immunochromatography
ICTV International committee on taxonomy of viruses
IgG Immunoglobulin G
IgM Immunoglobulin M
ISEs Ion selective electrodes
ISFET Ion sensitive field effect transistors
ISM Ion selective membrane
K₀ Dissociation constants
LAMP loop mediated isothermal amplification
LFIA Lateral flow immunoassay
LOD Limit of detection
LOD95 95% Limit of detection
LOQ Limit of quantification
LSV Linear sweep voltammetry
M membrane
MERS Middle east respiratory syndrome
MIPs Molecularly imprinted polymers
MPS Magnetic particle spectroscopy
N nucleocapsid
NPs Nanoparticles
NPS nasopharyngeal swab
PADs Paper-based devices
pAAP p-Aminophenyl phosphate
PBS Phosphate buffer solution
PCR polymerase chain reaction
PFU Plaque-forming units
PHEIC Public emergency of international concern
PoCT Point of care test
PPE Personal protective equipment
qRT-PCR Quantitative reverse transcription PCR
RAPID 1.0 Real-time accurate portable impedimetric detection prototype 1.0
RBD Receptor binding domain
RCA Rolling circle amplification
Rct Charge-transfer resistance
RNA Ribonucleic acid
RT-PCR Reverse transcription-polymerase chain reaction
S spike
SARS Severe acute respiratory syndrome
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