Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Current trends in COVID-19 diagnosis and its new variants in physiological fluids: Surface antigens, antibodies, nucleic acids, and RNA sequencing

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ABSTRACT

Rapid, highly sensitive, and accurate virus circulation monitoring techniques are critical to limit the spread of the virus and reduce the social and economic burden. Therefore, point-of-use diagnostic devices have played a critical role in addressing the outbreak of COVID-19 (SARS-CoV-2) viruses. This review provides a comprehensive overview of the current techniques developed for the detection of SARS-CoV-2 in various body fluids (e.g., blood, urine, feces, saliva, tears, and semen) and considers the mutations (i.e., Alpha, Beta, Gamma, Delta, Omicron). We classify and comprehensively discuss the detection methods depending on the biomarker measured (i.e., surface antigen, antibody, and nucleic acid) and the measurement techniques such as lateral flow immunoassay (LFIA), enzyme-linked immunosorbent assay (ELISA), reverse transcriptase-polymerase chain reaction (RT-PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), microarray analysis, clustered regularly interspaced short palindromic repeats (CRISPR) and biosensors. Finally, we addressed the challenges of rapidly identifying emerging variants, detecting the virus in the early stages of infection, the detection sensitivity, selectivity, and specificity, and commented on how these challenges can be overcome in the future.

1. Introduction

COVID-19 is a highly infectious respiratory disease caused by the novel severe acute respiratory syndrome—coronavirus 2 (SARS-CoV-2). COVID-19 emerged in December 2019 (Wuhan, China) spreading the infection to 528 million people worldwide as of June 2022 and leading to more than 6 million deaths. There are five variants of concern (i.e. Alpha, Beta, Gamma, Delta, and Omicron) reported by the WHO. The SARS-CoV-2 virus is an enveloped virus with a single-stranded RNA genome encoding 16 nonstructural

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proteins, four major structural proteins known as a membrane (M), envelope (E), spike (S), and nucleocapsid (N), and several accessory proteins [1]. The predominant route of transmission is via droplets in respired air produced by coughing or sneezing. Several diagnostic methods exist but to conclusively identify a specific variant, sequencing of the entire SARS-CoV-2 genome or at least the S gene (full or partial sequencing) is required.

COVID-19 infection can be spread not only via symptomatic carriers but also silently via asymptomatic and presymptomatic individuals [2]. The average incubation period (time from exposure to symptom onset) of SARS-CoV-2 infection is 5 days with a maximum of 22 days [3]. A certain percentage of infected individuals show symptoms that may resemble a cold or flu, while others, especially young people, remain asymptomatic [4]. A small percentage of infected individuals develop life-threatening symptoms, including severe pneumonia and respiratory failure [5]. Currently, there is no fully effective treatment for SARS-CoV-2, although several anti-viral and antibody-based treatments exist as well as powerful vaccines that reduce the incidence of infection and dramatically reduce the severity of symptoms. These medicines, together with public health control measures during a "wave" of infections represent powerful strategies to control the spread of the virus. However, emerging viral variants could compromise the effectiveness of current vaccines (preliminary data suggest that BA.4 and BA.5 subvariants may lead to deeper lung infections and may trigger a summer wave of COVID) and rapid and accurate diagnosis of COVID-19 infection remains an important strategy for controlling the spread of the virus and emerging variants [6].

In early 2022, SARS-CoV-2 spread worldwide predominantly through the Omicron variant. This variant is a highly mutated form that has significantly higher transmissibility and has been classified as a variant of concern by the WHO. The viral load of SARS-CoV-2 or its newly discovered variants depends on the type of sample. These viruses usually fluctuate during the course of the disease, peaking in respiratory samples in the second week before gradually decreasing in the following days [7,8]. However, in severe cases, the amount of virus in the respiratory fluid is highest in the third and fourth weeks [9,10]. The average number of individuals (R_0 values) infected by a patient infected with Delta variant SARS-CoV-2 is 2–2.5, (B.1.617.2, discovered in late 2020). The Omicron variant (B.1.1.529, identified as a variant of concern on November 26, 2021), is more transmissible than Delta and causes less severe cases and hospitalizations [11].

Observational diagnosis of the COVID-19 disease is difficult because of the highly variable symptomatology [12]. Clinical symptoms are not sufficient to distinguish COVID-19 infection from other respiratory infections, so highly selective and reliable diagnostic tests are needed for accurate results. To date, several diagnostic methods based on viral antigens, whole virus, antibodies, and viral RNA have been developed for the SARS-CoV-2 detection in various body fluids [13]. A variety of immunoassays are considered standard diagnostic tests for the detection of viral antigens or specific antibodies produced by the host immune response against specific epitopes or antigens. Specific antigen detection can be used to detect a current infection, while antibody detection is used to detect a current or previous infection. The sensitivity of antibody-based tests depends on the infection status at the time of sample collection, e.g., the median time of IgG antiviral seroconversion is 6–14 days, and a high IgG antibody titer persists for at least 7 weeks [14].

Molecular diagnostic methods for the SARS-CoV-2 antigens have been developed. In particular, the SARS-CoV-2 antigen detection, including point-of-care, laboratory, and self-tests, is approved for COVID-19 diagnosis based on nasopharyngeal or nasal swab specimens [15]. The detection of SARS-CoV-2 nucleic acids, including real-time reverse transcription-polymerase chain reaction (RT-PCR) and other nucleic acid amplification tests (NAATs), is more sensitive compared with antigen-based assays [16]. Although RT-PCR is the gold standard method for diagnosing SARS-CoV-2 infection, false-negative test results can occur in 20% up to 67% of the patients, especially with the emergence of the new variants and again. The quality and timing of testing are important factors in determining the specificity of the test [4]. To date, several reports have been published on the development of different analytical methods for the detection of SARS-CoV-2 [17–21]. However, none of these articles comprehensively discussed the detection of SARS-CoV-2 in various body fluids.

In this review, we aim to provide a comprehensive overview of the origin, natural hosts, infection cycle, and modes of transmission of the SARS-CoV-2 virus, as well as current trends in the diagnosis of the SARS-CoV-2 virus. We discuss the detection of SARS-CoV-2 in various body fluids (i.e., blood, urine, feces, saliva, tears, and semen) and mainly focus on the targeted regions of the virus, detection methods, test medium, sensitivity, concentration range, and processing time of the SARS-CoV-2 detection techniques. The review is structured according to the target molecule (e.g., the whole virus or its antigenic proteins, the host antibody, and the viral gene). A further subdivision is based on the different methods of detection, including lateral flow immunoassay (LFIA) [22,23], enzyme-linked immunosorbent assay (ELISA) [24], biosensors (optical, electrochemical, and electronics) [25], reverse transcription-polymerase chain reaction (RT-PCR) [26,27], recombinase polymerase amplification (RPA) [28], reverse transcription loop-mediated isothermal amplification (RT-LAMP) [29], DNA microarray [30], and clusters of regularly interspaced short palindromic repeats [31,32]. We also discuss recent developments and challenges of SARS-CoV-2 detection techniques, such as early diagnosis of infection, limit of detection, analytical selectivity, and clinical specificity.

2. Coronavirus history, natural host, structure, and genome

COVID-19 pandemic began in late 2019 and is still considered a potentially deadly disease, especially for the vaccinated, in 2022. However, the number of new cases occurring each week has continued to decline since peaking in January 2022. On June 12, 2022, more than 540 million cases were reported. Bats and/or pangolins are the most likely reservoir from which SARS-CoV-2 originated [33]. However, there is no strong evidence of natural animal-to-human or animal-to-animal transmission of the COVID-19 virus [34]. Reverse zoonotic transmission to various animals (e.g. minks, lions, dogs, cats) has been reported [35]. Several investigations have shown that many species such as hamsters, cats, ferrets, bats, and monkeys can be easily infected with the virus while many others such as dogs, poultry, and pigs are resistant [35]. Studying the zoonotic capability of viruses in animals is crucial before they infect humans. Because the zoonotic potential of SARS-CoV-2 is not fully defined, approved SARS-CoV-2 vaccines or antivirals should be considered not only for humans but also for animals in close contact with people [34]. Fig. 1 shows the route of spread of various human coronaviruses (HCoV) in humans, their symptoms, and the natural and intermediate hosts of beta coronaviruses.

Coronaviruses are enveloped RNA viruses causing varying diseases in humans, ranging from the common cold (fever, cough, tiredness, loss of taste or smell) to severe respiratory illness (difficulty breathing, loss of mobility, confusion, chest pain) [36,37]. The overall mortality rate of SARS-CoV-2 seems to be lower than that of SARS-CoV and MERS-CoV. However, the transmissibility of COVID-19 is higher once it enters a community and some variants such as

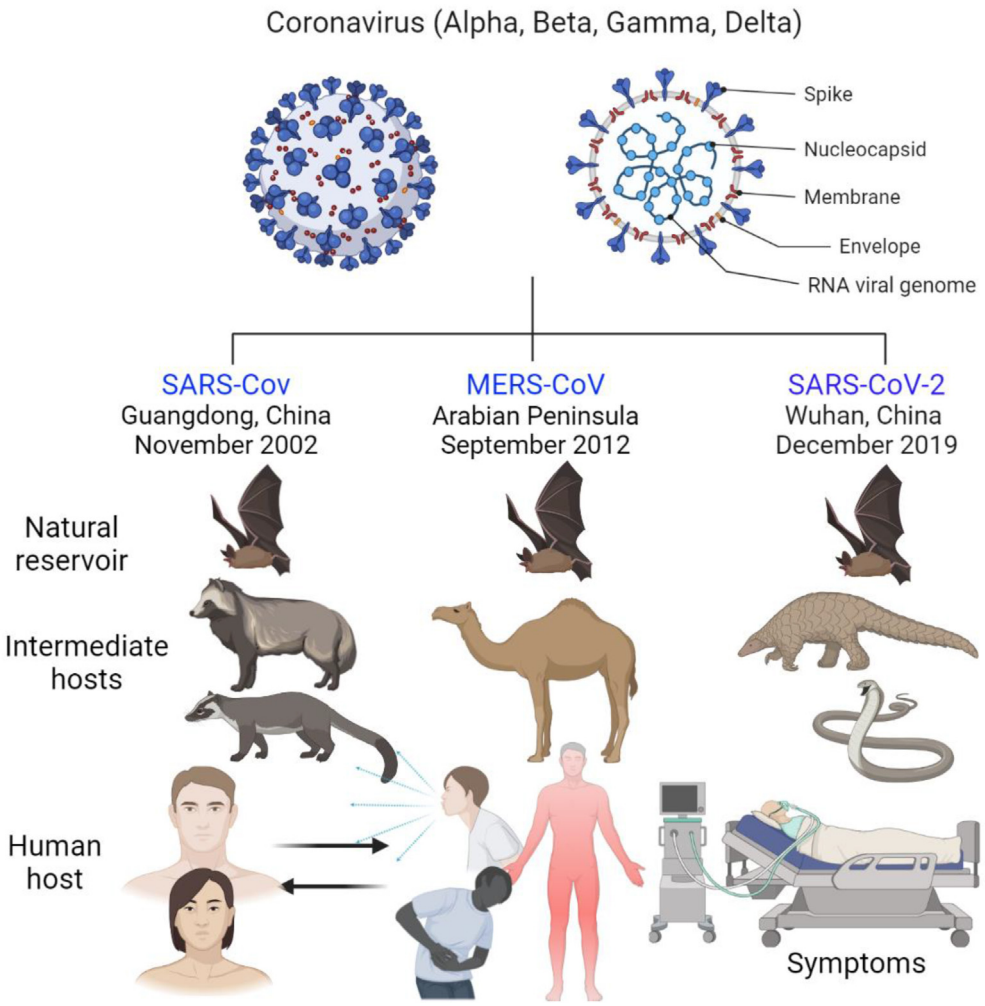

Fig. 1. Timeline of identified infectious human coronaviruses (HCoVs) with the symptoms they cause and their natural and intermediate hosts. The image was created with Biorender.

Delta showed higher transmissibility relative to the other variants [38]. HCoVs such as SARS-CoV, MERS-CoV, SARS-CoV-2, HCoV-HKU1, and HCoVOC43 are classified in the genus Betacoronaviruses, while HCoV-229E and NL63 belong to the Alphacoronaviruses [39]. The RNA sequence of SARS-CoV-2 is similar to that of MERS-CoV (~50%), and SARS-CoV (~79%) [40]. Pb1ab is the first open reading frame (ORF) from the end of 5' UTR of the whole genome length encoding non-structural proteins with the size of 29751bp (7073aa), 30119bp (7078), and 29844bp (7096aa), in SARS-CoV, MERS-CoV, and SARS-CoV-2 respectively [41,42]. The SARS-CoV has eight accessory proteins, while MERS-CoV contains only 5 and SARS-CoV-2 nine. Accessory proteins are not involved in viral replication but play an important role in the interactions between the host cell and the virus [43].

Coronaviruses are composed of 4 major structural proteins (in the 3′ terminus), namely the spike (S), nucleocapsid (N), transmembrane (M), and envelope (E) proteins (Fig. 2a) [44]. The S protein binds to the host cell ACE2 receptor [45], while the N protein plays an important role in viral genome replication and transcription [46]. Moreover, the M protein facilitates the viral fusion and assembly, while the E protein promotes the virion assembly [47], morphogenesis, and viral pathogenicity [48]. Coronavirus genomes contain the largest known viral RNA [49]. Two-thirds of the genome (in the 5' terminus) encodes for a replicase polyprotein called polyprotein 1 ab (pp1ab), which is comprised of 2 (ORFs), namely ORF1a and ORF1b [48]. These ORFs are then cleaved into 16 non-structural proteins (NSPs) by a viral or host protease enzyme that plays a role in genome replication and transcription (Fig. 2b) [50]. The structure of the spike protein in its closed configuration, in its original form, and its mutant form are given in Fig. 2c.

SARS-CoV-2 variants of concern, including Alpha, Beta, Gamma, Delta, and Omicron have multiple clinically significant mutations in the spike gene responsible for ACE2 receptor recognition and entry into the target cell. The emergence of these new variants has been associated with a change in infectivity, severity, or sensitivity (resistance to neutralizing antibodies) of the SARS-CoV-2 and consequently the clinical outcomes [51]. Some of the SARS-CoV-2 variants are likely associated with resistance to current vaccines and the risk of reinfection. The delay in diagnosis and identification of the new variants leads to their global spread [52]. The variants of concern are distinguished from each other by multiple mutations in the viral RNA genome and by mutations in the S protein-coding sequence. False-negative results can occur in the detection of SARS-CoV-2 in both nucleic acid- and antigen-based tests. This may be the case if the mutations occur in the genomic region that the molecular assays are designed to detect, and such changes in the viral genome may translate into amino acid changes in the viral

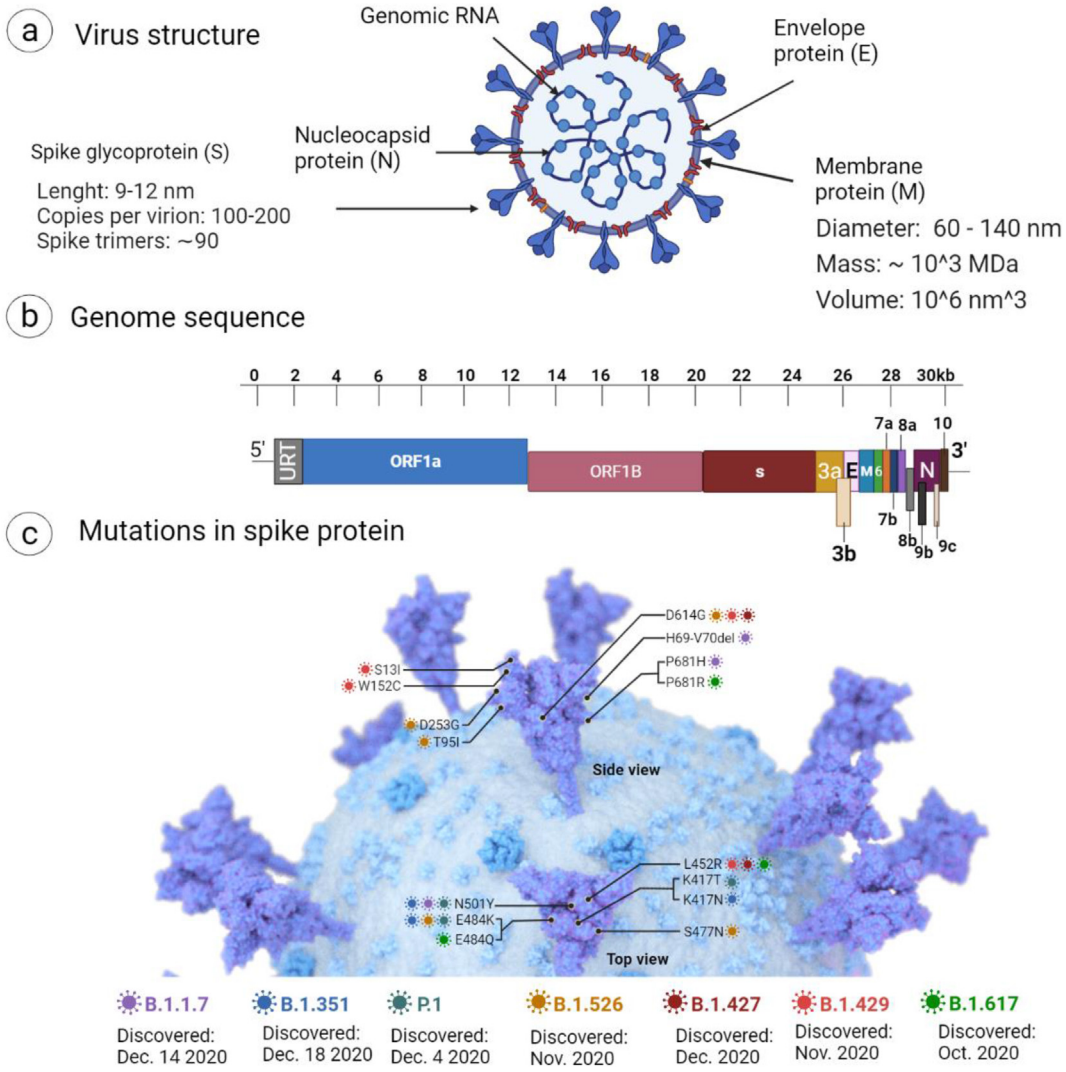

Fig. 2. Schematic representation of (a) cartoon model showing the structure of SARS-CoV-2, (b) genome structure and encoded proteins, and (c) mutation in spike proteins. The image was created with Biorender.

proteins or antigens and consequently affect the performance of the antigen tested [53].

3. Transmission routes and life cycle

Transmission of the SARS-CoV-2 virus occurs through direct and indirect routes. Direct transmission occurs through (i) aerosols in the form of droplet germs from respiratory air (responsible for most infections) [54], (ii) the body fluids and secretions, such as urine, feces, saliva, tears, semen, and (iii) mother-to-child. On the other hand, the indirect transmission takes place through fomites or surfaces close to infected persons or tools used by an infected patient [55]. As shown in Fig. 3, the infection cycle of the SARS-CoV-2 virus begins with binding to its complementary receptor ACE2, which is located on cells of the lower respiratory tract and is a known receptor for SARS-CoV-2 virus and regulates human-to-human transmission [56]**.** The virus uses its spike protein (S-glycoprotein) to attach to the ACE2 receptor on the host cell surface [57]. Upon binding to the ACE2 receptor, the virus entry to the host cell is facilitated by the transmembrane protease serine 2 (TMPRSS2) and cathepsin L. After entering the cell, the RNA viral genome is released into the cytoplasm, which is then translated into two overlapping polyproteins. The polyproteins are broken down into 16 non-structural proteins required for the completion of the life cycle in the target cell, including RNA-dependent RNA polymerase (RdRp). During replication, positive-sense RNA serves as a template for the synthesis of negative-sense RNA and sub-genomic RNA. The viral proteins resulting from the translation of the sub-genomic RNA are transferred to the ER-Golgi-Intermediate Compartment (ERGIC) to construct virion particles. Finally, the newly replicated positive-sense RNA is assembled into virion particles, which are then released from the cell membrane [58]. It is worth noting that, unlike SARS-CoV-2, the Omicron variant has more than 30 mutations that result in amino acid changes in the spike sequence, 15 of which are in the receptor-binding domains, the part of the spike protein that binds to human cells via the receptor ACE-2. Recent docking studies suggest that a combination of mutations in the receptor-binding domains may lead to a high binding affinity of this variant to human ACE2 [59].

4. Classification of diagnostic techniques

Diagnosis of SARS-CoV-2 typically follows three distinct stages. The first stage includes clinical signs that may include dry cough,

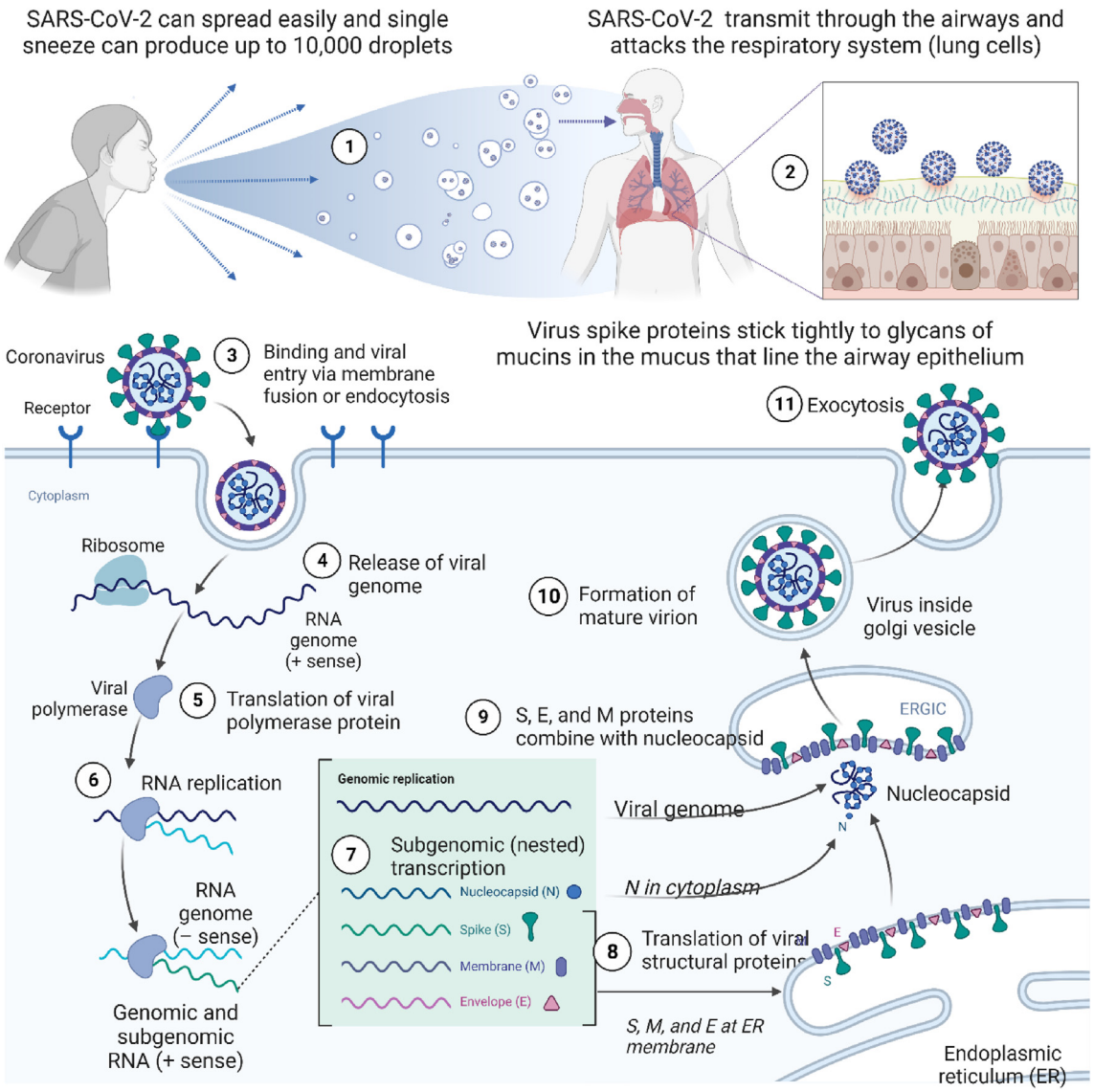

Fig. 3. Schematic representation of the spread, transmission, and life cycle of SARS-CoV-2 virus in a human host cell. Viral spikes bind to their receptor human ACE2 (hACE2) via their receptor-binding domain (RBD) and are proteolytically activated by human proteases. The image was created with Biorender.

fever [60], sore throat, muscle and chest pain, headache, confusion, dyspnea, disorientation, anosmia, and ageusia. These symptoms may reach a point where the patient's respiratory functions are impaired, while other organs such as the kidney, heart, and liver are also severely affected [61]. The second stage involves the detection of total viral load, viral antigens, antibodies, or other genetic materials in various body fluids (Fig. 4). The third stage aims to confirm the disease manifestations, which includes computed tomography of the chest (CT) or magnetic resonance imaging (MRI) [62]. Here, we focused on the second level of SARS-CoV-2 diagnosis, which targets various viral components as well as antibodies produced by the patient's body. We, therefore, reviewed the diagnosis of these targets under two main categories: (i) immunological assays, where we addressed the detection of antigens and antibodies in conjunction with various diagnostic techniques (e.g., LFIA, ELISA, biosensors, and spectroscopic methods), and (ii) nucleic acid assays, where we discussed the detection of viral nucleic acids in conjunction with a variety of detection tools (e.g., RT-PCR, RT-LAMP, microarrays, CRISPR, RNA sequencing, and biosensors).

5. Immunological assays: antigen and antibody-based techniques

5.1. Lateral flow immunoassay (LFIA)

Rapid antigen tests (LFIAs) rely on the detection of a specific antigen or antibodies and are considered common diagnostic tools due to their ability to be used near the patient which is in part due to the higher stability of the LFIA (antigen and antibodies) compared to molecular diagnostic assays (e.g., RT-PCR) [63]. This stability allows for more reliable point-of-need detection as they are less likely to degrade during transport and storage. Several studies suggest that salivary antigen testing has reasonable power to diagnose SARS-CoV-2 infection [64,65]. However, little is known about diagnostic accuracy in clinical practice, which can vary significantly from manufacturer claims. Manufacturers often state sensitivity of about 95% (94.3% in the case of the test mentioned below) [65]. These sensitivities need to be reassessed as new variants emerge. According to the National Institutes of Health (NIH),

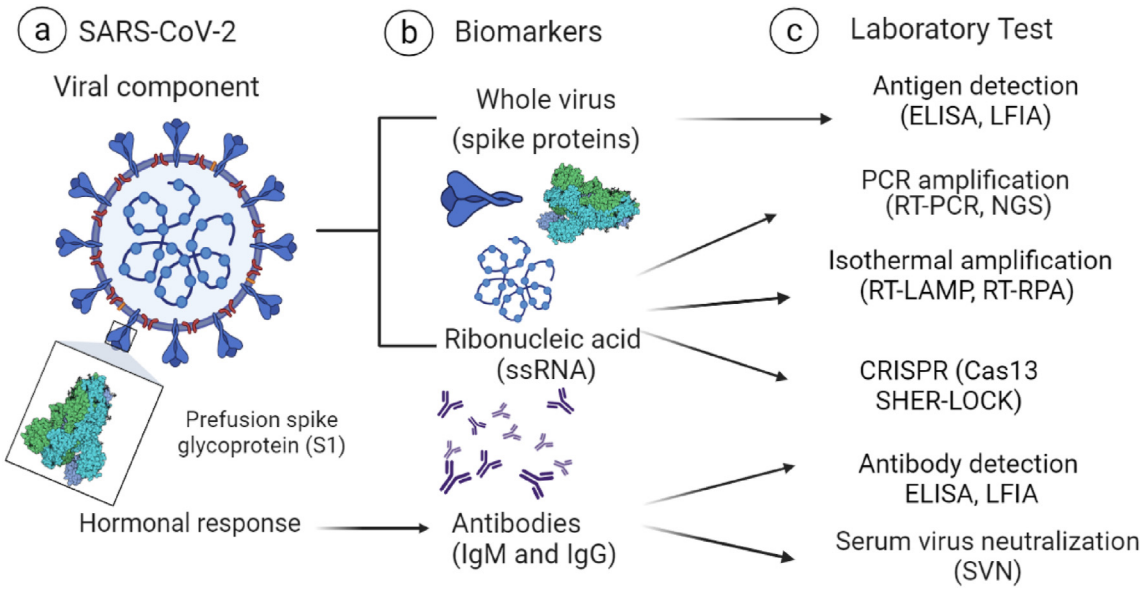

Fig. 4. Schematic representation showing the structure of SARS-CoV-2 virus and various detection methods: (a) viral components and human response; (b) biomarkers for detection; and (c) common laboratory tests. The image was created with Biorender.

rapid antigen tests continue to detect cases of the Omicron variant, although "reduced sensitivity" has been noted in early laboratory studies with some brands [66,67].

Antibodies are produced as an immune response to infection by a pathogen such as viruses, bacteria, fungi, etc. The presence of an antibody targeting specific species may indicate a topical (i.e., initial antibodies formed by the body when fighting a new infection) or past infection (i.e., antibodies formed after infection or immunization). Similarly, IgM, IgA, and IgG immunoglobulins are produced by the immune system to protect against the SARS-CoV-2 virus, which can serve as indicators of infection (Fig. 5). For example, the production of IgM initially increases in the first phase of infection and then rapidly decreases, while IgGs are produced in the second phase and remain in the blood after recovery [68]. LIFA antigen tests can detect SARS-CoV-2 infection in serum a few days after acute infection. However, they are not typically used to diagnose acute SARS-CoV-2 infection in the clinical setting. LIFA antibody testing can identify individuals with subsiding or past SARS-CoV-2 infection, which helps scientists and health care professionals better understand the epidemiology of SARS-CoV-2.

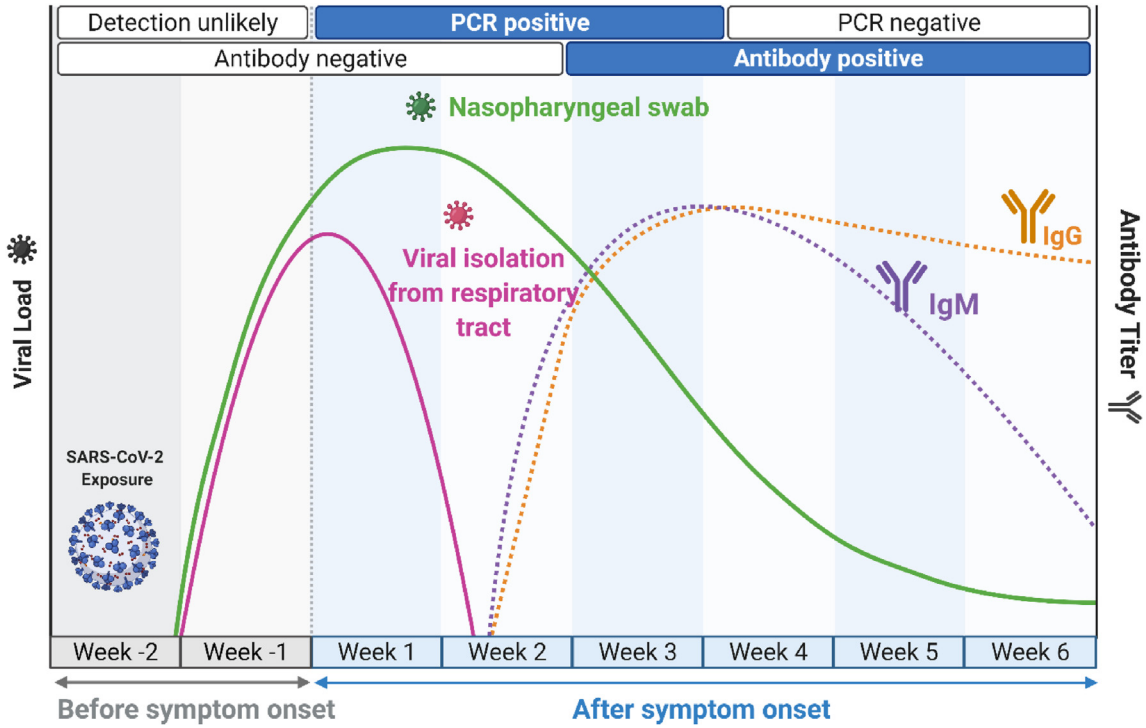

Fig. 5. Timeline for SARS-CoV-2 infection and COVID-19 positivity tests versus the molecular diagnostic assays (PCR). The image was created with Biorender.

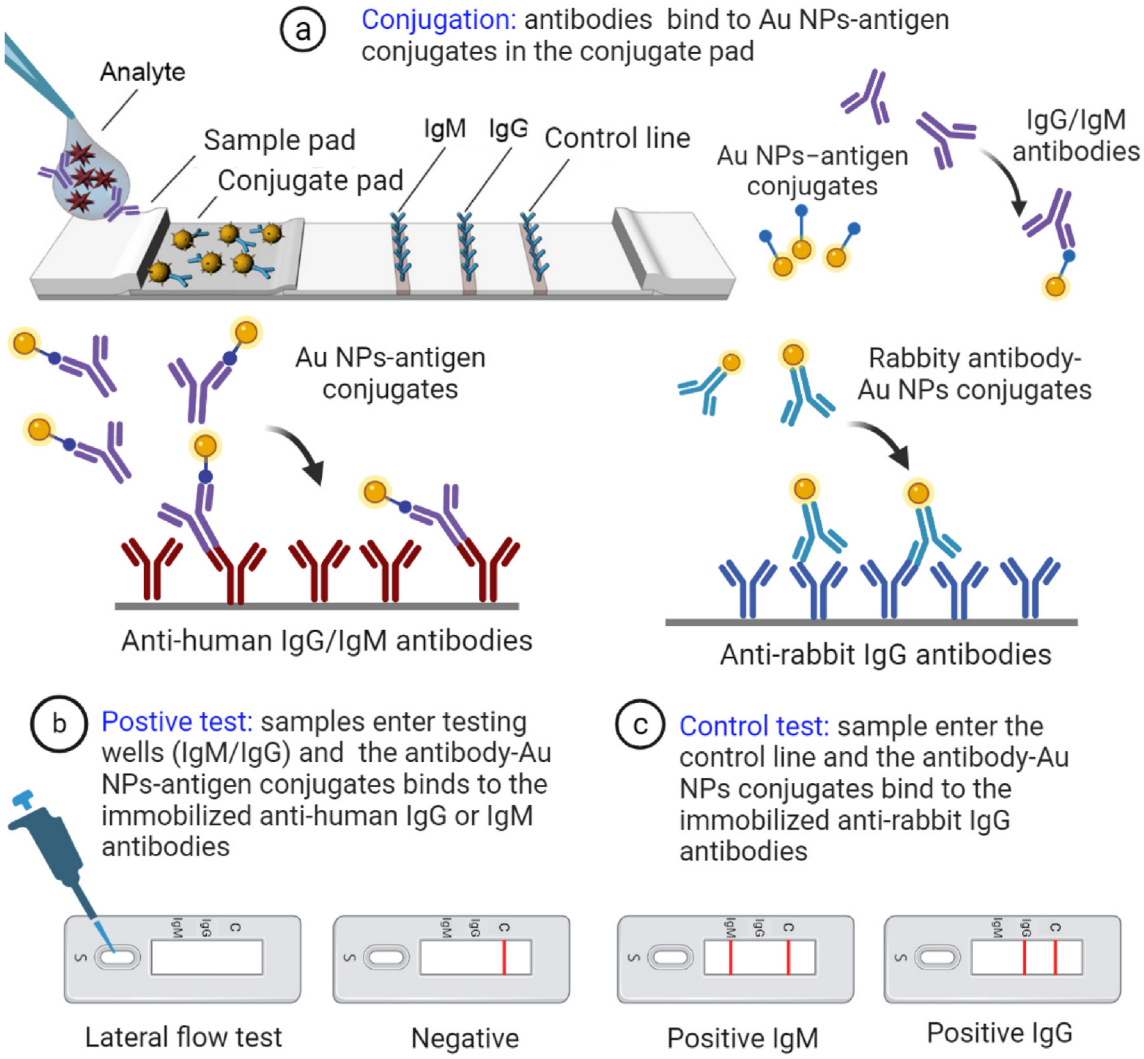

Fig. 6. Schematic representation of colorimetric LFIA of SARS-CoV-2 virus upon loading the sample and buffer solution to the nitrocellulose pad: (a) The antibodies specific to the virus (IgG, IgM) bind to the viral antigens and form antigen-antibody complexes. (b) When the antigen-antibody complexes flow to the secondary antibodies (antihuman IgG and antihuman IgM antibodies), the antigen-antibody complexes bind to the secondary antibodies and form sandwiches of antibodies (IgG, IgM) between the viral antigen and the secondary antibodies. (c) In a negative sample without SARS-CoV-2 specific IgG and IgM, only the control line is stained. The image was created with Biorender.

As shown in Fig. 6, LFIA tests use capillary force and antibody-antigen interaction to separate the components of a mixture (i.e., a sample). Two types of host antibodies (IgG, IgM) are transferred through the nitrocellulose membrane (adhesive pad) and interact with SARS-CoV-2 antigens and secondary antibodies immobilized on the surface. The analytical solution flows through the sample pad by capillary force and reaches the conjugation pad modified with labeled viral antigens. Typically, Au NPs modified with antibodies against human IgG (Au NPs-anti-IgG) are used for visual colorimetric readout from the conjugate pad. As the sample moves along the test strip under capillary force, the IgG binds to the Au NPs-anti-IgG in the conjugate pad. The complex formed is then transferred to the membrane, where it binds to the nucleocapsid protein and forms a colored line. Meanwhile, the unbound Au NPs–anti-IgGs bind to the polyclonal antibodies on the control line and form another colored line indicating a positive test result. In the absence of target IgGs, only one colored line is formed in the control area, indicating a negative test result.

LIFA antigen tests were found to be 10^3-fold less sensitive than virus culture, while it was 10^5-fold less sensitive than RT-PCR. The rapid antigen test detected between 11.1% and 45.7% of RT-PCR -positive samples from SARS-CoV-2 patients [69]. Recent studies evaluated the performance of the commercially available rapid antigen assay (BIOCREDIT COVID-19 Ag assay) for the detection of the SARS-CoV-2 virus and compared it with RT-PCR. This showed that the detection limits varied widely between the rapid antigen test, virus culture, and RT-PCR [69]. Corman and coworkers [70] compared the analytical sensitivity and specificity of antigen POC tests from seven vendors in a single-center laboratory study. Among 138 clinical specimens with quantified SARS-CoV-2 viral load, approximately 95% of test results were positive for six of seven antigen point-of-care tests, ranging from 2.07×10^6 and 2.86×10^7 copies per swab (RapiGEN BIOCREDIT COVID-19 Ag Kit) at 1.57×10^{10} copies per swab. The authors concluded that the sensitivity range of most rapid antigen tests overlaps with SARS-CoV-2 viral loads typically observed in the first week of infection (symptoms), which marks the infectious period for most patients [70].

5.2. Enzyme-linked immunosorbent assay (ELISA)

ELISA is a sensitive, high-throughput laboratory test that can

detect both viral antigens and host antibodies to a specific antigen. This test uses multiwall-plates coated with antibodies specifically directed against SARS-CoV-2 antigens (Fig. 7). By placing the patient's samples into the wells, the target SARS-CoV-2 antigens are captured. Next, enzyme-conjugated secondary antibodies are added for signal generation. ELISA can also be performed to detect antibodies to SARS-CoV-2 antigens as recognition agents. The selection of a viral antigen with high selectivity for the targeted antibody is critical for the construction of an efficient ELISA assay. Therefore, a comparison was performed between three ELISA sets using different viral antigens (N protein, S1 subunit, and RBD of S protein) [71]. The results suggest that the detection of N and RBD ELISA is more sensitive than the S1 ELISA test in patients with mild COVID-19 infection [71], but more data are needed to obtain a statistically robust result. The presence of IgG and IgM antibodies in the sera of 214 COVID-19 patients was investigated using N- and S-protein ELISA assays [72]. The results showed that S-protein ELISA tests had slightly higher sensitivity than N-protein ELISA tests. Further studies are needed, but it seems that the detection sensitivity for S and N proteins is not significantly different.

ELISA assays of SARS-CoV-2 take two to 4 h [73,74] with an analytical sensitivity in the picomolar (pM) range. The NovaLisa® SARS-CoV-2 (IgG/IgM/IgA ELISA kits) is intended for the qualitative detection of antibodies to the SARS-CoV-2 virus in human serum. The ELISA kits have high clinical sensitivity (the ability of a test to correctly identify patients with a disease) (100%) and specificity (98%) compared with RT-PCR. Domenico and colleagues constructed an ELISA assay for SARS-CoV-2 detection using antibody-functionalized PVDF (polyvinylidene difluoride) membranes. The easily transportable ELISA kit achieved a detection limit of 2 pg μL^{-1} [75]. In a study of 133 COVID -19 patients, including mild, severe, and critical cases, the results of RT-PCR and ELISA tests were compared [76]. Positive RT-PCR results were obtained in 66% of mild cases, 71% of severe cases, and 68% of critical cases, while the serological test showed the presence of IgM/IgG in 80%/93% of mild cases, 83%/100% of severe cases, and 73%/97% of critical cases. This result proves that ELISA for antibody detection is useful as an additional diagnostic tool for RT-PCR [76].

5.3. Biosensors

A biosensor is an analytical device in which a recognition element (e.g., an antibody, enzyme, nucleic acid, peptide, etc.) is connected to a transducer to realize a chemical binding event for quantitative detection of a specific analyte [77]. Biosensors can detect various targets, such as toxins in water [78], allergens in food [79], biomarkers for chronic diseases [80], blood glucose [81], bacteria [82], biofilms [83], and drugs [72]. They are also used to detect various respiratory viruses, which can provide miniaturized, low-cost, sensitive, portable, and rapid platforms compared to conventional laboratory-based methods [73]. Fig. 8 shows the sample collection and extraction steps for biosensor-based COVID-19 detection. After sample extraction from the patient, the specific binding event between the analyte and the receptor is detected by a transducer (e.g., optical, electrochemical, piezoelectric, etc.) [84]. Electrochemical biosensors have several positive attributes such as high sensitivity, ease of operation, cost efficiency, and the possibility of miniaturization, making them among the most suitable biosensors for POC applications as proven by the 4 billiuon glucose test strips sold annually.

An in-house built electrochemical biosensor called eCovSens was fabricated using a fluorine-doped tin oxide (FTO) electrode conjugated with gold nanoparticles (Au NPs) and SARS-CoV-2 monoclonal antibodies. The sensor detects SARS-CoV-2 spike antigen in the range of 1 fM to 1 μM with a LOD of 90 fM within only 30 s [85]. An impedimetric biosensor was developed for label-free and rapid detection of antibodies against the SARS-CoV-2 virus [86]. RBD was immobilized on an array of interdigitated electrodes in 16-well plates as a receptor moiety, which was then evaluated for detection of monoclonal antibodies against the SARS-CoV-2 virus. The electrochemical sensing platform was able to separate the positive clinical samples from the control samples and the results

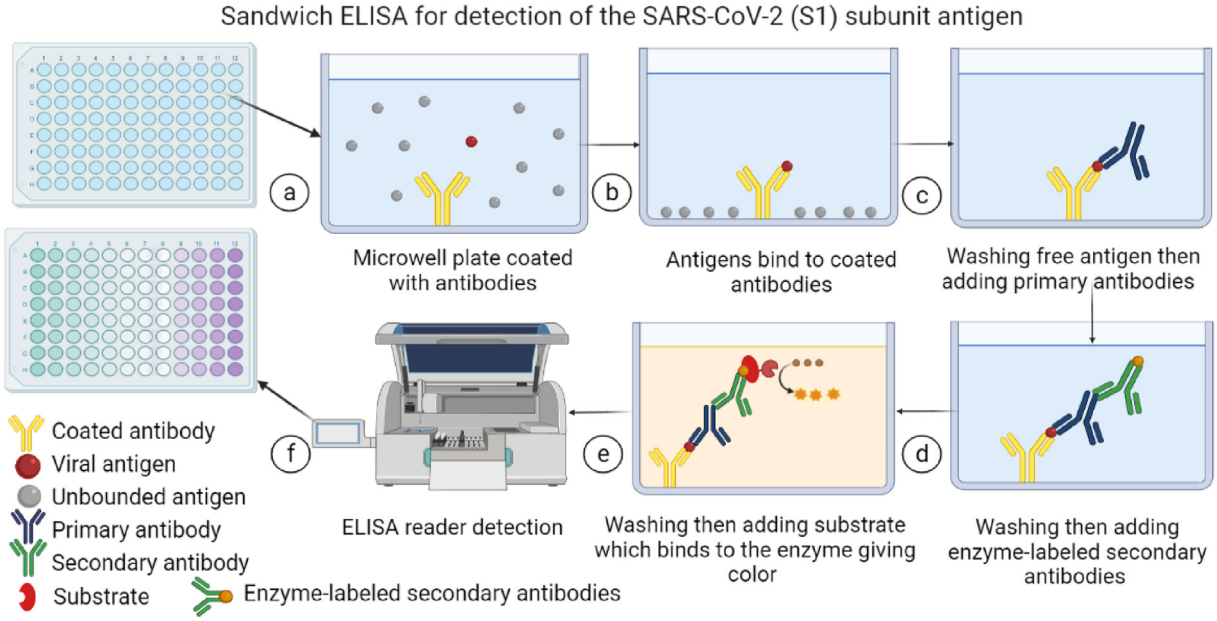

Fig. 7. Sandwich ELISA for the detection of SARS-CoV-2 antigens. (a) Microwell Plate coated with the capture antibody, (b) Addition of the patient sample containing the viral antigens, (c) Washing to remove bound antigens, then add primary antibodies, (d) Washing to remove the unbound primary antibodies, then the addition of the enzyme-bound secondary antibodies, (e) After washing to remove the unbound secondary antibodies, the substrate is added and converted by the enzyme into a detectable form by assuming a color that depends on the presence and concentration of the viral antigen; then the stop solution is added to terminate the enzyme-substrate reaction, and (f) The ELISA reader is used to detect the presence and concentration of the viral antigen in the sample. The image was created with Biorender.

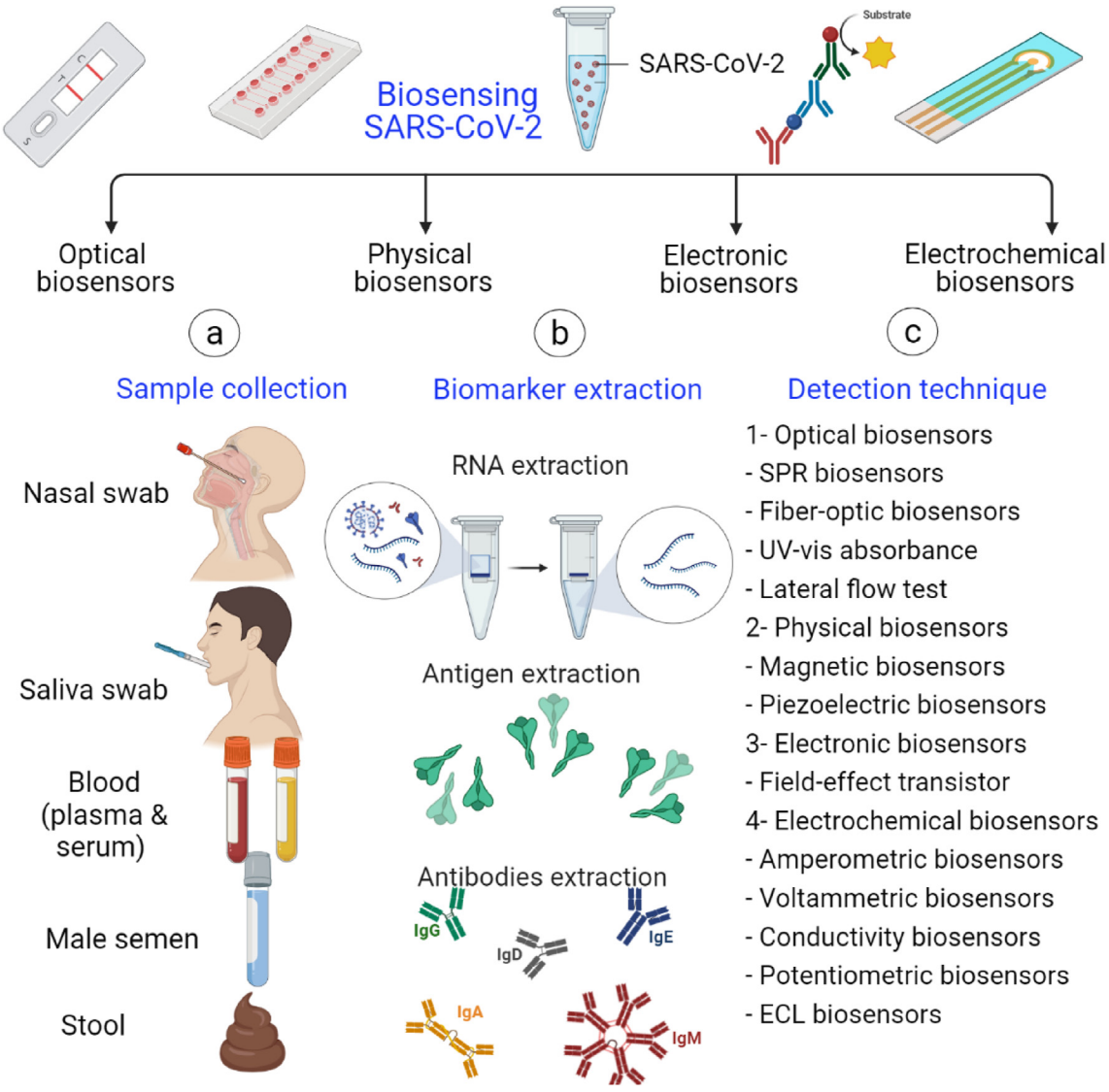

Fig. 8. Schematic representation of COVID -19 in the context of biosensor technologies: (a) sample collection, (b) extraction of antigens and antibodies; and (c) biosensor detection methods. The image was created with Biorender.

correlated with a standard ELISA assay [86]. The key advantage of this approach is that no luminescent or redox-active labeling is required. A field-effect transistor biosensor (FET) was developed for the detection of SARS-CoV-2 virus spike protein in a clinical sample [87]. The FET biosensor is based on graphene nanosheets conjugated with monoclonal antibodies on the gate electrode and achieved a detection limit of 100 fg mL^{-1}. The sensor was able to distinguish between healthy controls, MERS-CoV protein, and COVID-19 patients. Electrochemical transistor biosensor-based self-assembled monolayers (SAMs) were developed for rapid detection of SARS-CoV-2 antigens in complex body fluids (Fig. 9) [88]. The sensors combine a solution-processable conjugated polymer in the transistor channel on disposable gate electrodes. The sensor showed a sensitivity of over 8 orders of magnitude (attomolar to nanomolar) after a 10-min exposure of 5 μl of untreated samples (saliva and serum). The sensor was able to distinguish between MERS-CoV protein and SARS-CoV-2 with a wide range of viral loads [88].

A local surface plasmon resonance (LSPR) biosensor for S protein was constructed using monoclonal antibodies on a nanoplasmonic chip surface [89]. The biosensor used a uniform nanocup pattern on the sensor chip to improve the optical sensing performance and Au NPs functionalized with antibodies provided further signal enhancement. The sensor showed a linear detection range of 10^2-10^7 viral particles (vp) mL^{-1} with a detection limit of 370 vp mL^{-1} [89]. Another optical biosensor for S protein was developed using aptamers as receptors on a fiber optic sensing platform [90]. A nucleic acid receptor for RBD of spike glycoprotein was immobilized onto a gold-coated fiber optic probe using short PEG chains and biotin-streptavidin biorecognition chemistry. Upon binding of the virus particles, the refractive index of the gold surface changes, resulting in a red-shifted resonance signal. The aptamer optical probe achieved a LOD of 37 nM for protein S in PBS and 75 nM in diluted human serum. Elledge and colleagues developed a luminescent biosensor using a cleaved nanoluciferase enzyme consisting of two units (SmBiT and LgBiT) for anti-SARS CoV-2 detection [91]. Each of these fragments was coupled with viral antigens that bind to two adjacent binding sites (Fab arms) of the target antibody. Such a binding event brings the SmBiT and LgBiT fractions of the cleaved enzyme into proximity, resulting in the generation of a

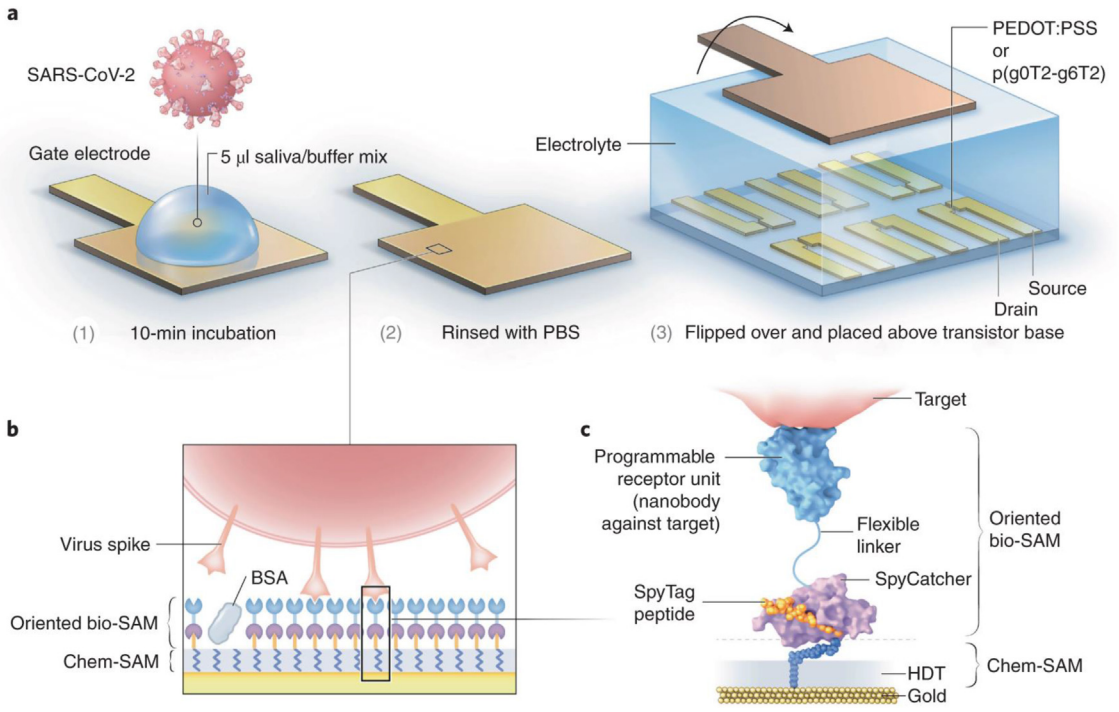

Fig. 9. Single-molecule detection of SARS-CoV-2 and MERS antigens using nanobody functionalized organic electrochemical transistors: (a) The electrode is exposed to a sample (saliva) in a buffer solution. (b) Functionalization of the gold electrode surface and bio-recognition of the SAM layers (Chem-SAMs and Bio-SAMs) bind to the antigens on the virus surface. (c) Molecular architecture of the composite layers and binding of the antigen to the modified gold electrode surface. © Nature, 2021 [88].

luminescent signal. The proposed enzyme-labeled sensor showed a sensitivity of 89% for antibodies to S and 98% for antibodies to targeting when 150 patient samples were evaluated [91].

Photonic crystals (PC) serve as optical sensing platforms with the advantages of low cost, high sensitivity, and short measurement time. Zhao and co-workers employed a PC biosensor modified with recombinant spike protein for IgG detection [92]. The analyst was sandwiched between the receptor S and secondary antibody conjugated Au NPs to form an immunocomplex which was then imaged with photonic resonator absorption microscopy (PRAM) for digital counting. The one-pot, 15-min long assay achieved a LOD of 26 pg mL^{-1} and a linear detection range of 0.1 ng mL^{-1} to 100 ng mL^{-1} for the diluted IgG spiked serum samples. Drain and colleagues [93] have developed a microfluidic immunoassay called LumiraDx to detect N for POC applications. Fluorescent latex particles conjugated with N-specific antibodies form complexes in the microchannels of the test strip after binding. Clinical tests performed with LumiraDx on 512 patients showed a clinical sensitivity of 98% and a specificity of 97% for nasal swabs and a sensitivity of 98% and a specificity of 98% for nasopharyngeal swabs within 12 days of symptom onset. Shan et al. [94] reported a nanomaterial-based sensor array with multiplexing capabilities for detecting and monitoring COVID-19 in exhaled air. The sensors are composed of different Au-NPs linked with organic ligands, resulting in a multilayered sensor layer that can swell or shrink upon exposure to volatile organic compounds (VOCs), leading to changes in electrical resistance. Training and test data showed 94% and 76% accuracy, respectively, in distinguishing patients from controls, and 90% and 95% accuracy, respectively, in distinguishing patients with COVID-19 from patients with other lung infections.

5.4. Spectroscopic methods

Rapid detection of viral infections using UV–Vis spectroscopy [83], Raman spectroscopy [95], and Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) [96,97] have been reported for predicting SARS-CoV-2 in blood, sera, plasma, infected cells, among others [98]. Zhang and coworkers [98] used ATR-FTIR for a SARS-CoV-2 detection in a time of 2–3 min using only ~3 µL of serum sample. The ATR-FTIR test can distinguish SARS-CoV-2 from normal controls, and common respiratory viral infections using the area under the characteristic curve (AUROC) of 0.9561 (95% CI: 0.9071–0.9774). Kitane and coworkers [99] reported a reagent-free quantitative spectroscopic detection of SARS-CoV-2 based on a multivariate analysis of FTIR spectra of RNA extracts. In agreement with RT-PCR, this technique achieves 98% accuracy, 97% sensitivity, and 98% specificity. Similarly, Carlomagno et al. [89] developed an innovative Raman spectroscopy-based approach to detect current and past SARS-CoV-2 infections using samples from SARS-CoV-2 patients with sensitivity and specificity greater than 95% [100]. Several structural details distinguish SARS-CoV-2 from the newly discovered variants and can be detected in the Raman and FTIR spectroscopy [101].

6. Nucleic acid targeting detection techniques

Nucleic acid-based assays directly detect the presence or absence of viral genomic RNA, allowing such a method to determine by quantification whether or not a person is currently infected and contagious. Nucleic acid-based methods allow specific and sensitive detection of viral genetic material extracted from the patient sample. The gold standard method for SARS-CoV-2 diagnosis is nucleic acid-based detection tests, including RT-PCR [22]. To establish the nucleic acid-based diagnostic tools for COVID-19 infection, high-throughput sequencing was initially used to identify SARS-CoV-2 virus infections. After the identification of the SARS-CoV-2 virus, the entire genome was sequenced, which enabled the development of specific nucleic acid-based detection

methods [102]. The following subsections summarize the existing examples of nucleic acid-based detection of SARS-CoV-2 in conjunction with RT-PCR, RT-LAMP, microarrays, CRISPR RNA sequencing, and biosensors. Interestingly, currently available RNA-based diagnostic kits continue to detect the Omicron variant. However, the U.S. Food and Drug Administration (FDA) has identified only three SARS-CoV-2 molecular tests that fail to detect the Omicron variant because these kits produce false-negative results.

6.1. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Polymerase chain reaction (PCR) is a molecular biology technique used to amplify the targeted DNA fragments. Since the genetic material of SARS-CoV-2 consists of positive-stranded RNA rather than DNA, a reverse transcription step is required to produce cDNA (complementary DNA) from the viral RNA, which serves as a template for PCR amplification (Fig. 10). After heat denaturation and the annealing step, the primer is extended (elongated) using DNA polymerase. Oligonucleotides with a fluorescent dye and a quencher at each end are used as detection probes to simultaneously monitor the level of amplification. The designed detection probes bind specifically to the amplified cDNA. During the synthesis of a new strand, DNA polymerase cleaves the fluorophore-labeled probe, resulting in the separation of the dye and quencher. This uncoupling event recovers the previously quenched fluorescence activity, which increases with the number of amplified target sequences [103].

The complete sequence of SARS-CoV-2 enabled the development of specific primers for spike protein sequence to detect and differentiate SARS-CoV-2 [104]. Since then, several other primers and probes targeting different genomic regions in the SARS-CoV-2 virus, including the RdRp gene, the E gene, the N ORF8, or ORF1b sites, have been developed for the detection and quantification of SARS-CoV-2 infection in different body fluids [88·89]. Although the RT-PCR represents the gold standard method for SARS-CoV-2 detection due to its high sensitivity, there are some limitations of real-time RT-PCR including false-negative results that may be caused by the variation of the viral RNA sequence, low viral load, poor or inappropriate sample collection [107,108]. To address these issues, test results with different primers for the same target gene should be compared and merged with a medical history and other clinical information to determine the patient's infection status [109]. Nevertheless, the high cost, the need for technical personnel and equipment, and the time-consuming procedure remain the major drawbacks of this technique.

The RT-PCR tests for SARS-CoV-2 are mainly applied to specimens obtained by swabbing the upper respiratory tract. However, in some studies, RT-PCR is also performed with ocular secretions, stool, and serum [110]. The different sensitivity rates obtained for RT-PCR can be explained by the differences in disease stage, viral copy number in patient samples, and mutation rate of the viral genome [108,111]. Table 1 lists the different sensitivity levels of RT-

Table 1
The sensitivity rate of SARS-CoV-2 virus detection in various body fluids is based on viral nucleic acid using RT-qPCR.

Body fluid	Numbers of samples	Positive rate	Reference
Nasopharyngeal swab	132	38.13	[116]
Sputum	132	48.68	[116]
Blood	132	3.03	[116]
Feces	132	9.83	[116]
Anal swabs	132	10.00	[116]
Saliva	12	91.7	[117]
Stool (Feces)	42	66.67	[118]
Stool (Feces)	10	100	[119]
Nasopharyngeal swab	10	100	[119]
Urine	10	0	[119]
Nasopharyngeal swabs	38	73.7	[120]
Ocular abnormalities	38	91.7	[120]
Tears	38	Low prevalence	[120]
Semen	38	15.8	[121]

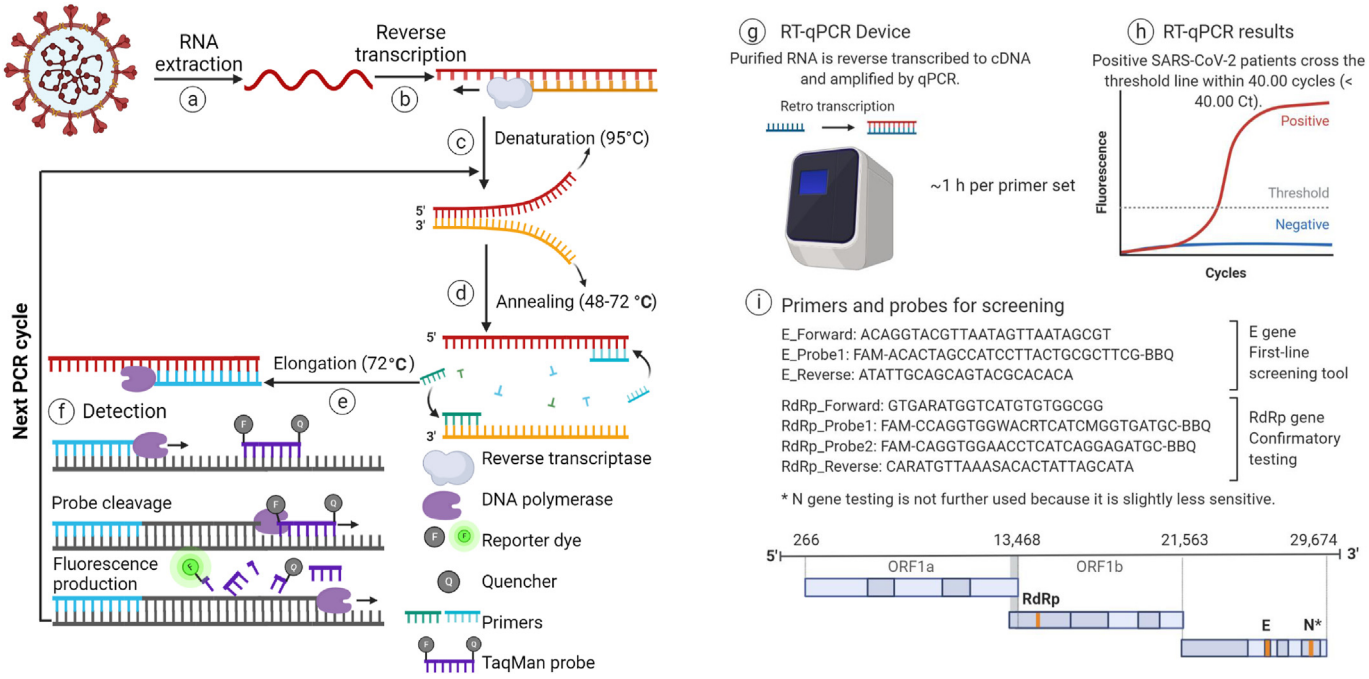

Fig. 10. Schematic representation of nucleic acid detection of SARS-CoV-2 by RT-PCR assay. (a) RNA extraction, (b) reverse transcription, (c–e) PCR amplification by (c) c-DNA denaturation, (d) primer annealing, (e) primer elongation by DNA polymerase enzyme, (f) detection steps with TaqMan probe, (g) RT-qPCR instrument, (h) signal results, and (i) primers and probes for screening. The image was created with Biorender.

PCR for the samples obtained from different body fluids. The positive ratio of SARS-CoV-2 from different samples was compared using three different fluorescent RT-PCR kits. Viral RNA was detected in oropharyngeal samples from 9 of the 19 patients, and viral nucleic acid was also detected in stool samples from eight of these nine patients. Significantly, no positive results were obtained in blood or urine samples. All three different kits used in this study showed the same results, and the positive rate of viral nucleic acid detection was only 47% in patients who required a secondary diagnostic method such as computed tomography (CT) [112]. The detection sensitivity of RT -qPCR diagnostic tests is reduced when the mutations occur at the sites where the primers and/or probes bind to the viral genome, and consequently, the diagnostic performance of the new variant may be compromised [113]. Some recent reports suggest that a high percentage of RT-PCR primer binding sites are mutated [114]. To date, hundreds of RT-PCR kits for SARS-CoV-2 diagnosis are available on the market, some of which are approved by the Food Drug Administration (FDA) or their country of origin authority for emergency use, but many of them also lack independent clinical performance evaluation [115].

The main challenge with nucleic acid detection methods based on reverse transcription (RT), such as RT-qPCR, is that these methods require a high-quality RNA template [122]. RNA is susceptible to degradation by ribonucleases (RNases) in the environment, which hinders the widespread use of reverse transcription-based bioassays. In addition, there is a risk of false-negative results if the conditions for the production of the RNA template for reverse transcription cannot be met. To avoid this drawback, a ligation and recombinase polymerase amplification (L/RPA) assay was developed for the rapid detection of SARS-CoV-2. However, ligase-based strategies usually have the problem of low efficiency in RNA templates. Therefore, Wang and co-workers [28] developed a L/RPA bioassay for the rapid detection of SARS-CoV-2 on the N and ORF1ab genes targeting the specific biomarkers. The authors overcame the problem of the low efficiency of the RNA template method by using a high concentration of T4 DNA ligase and taking advantage of the high sensitivity of recombinase polymerase amplification. By selecting ligation probes and optimizing the recombinase-polymerase amplification primers, the assay achieved a satisfactory sensitivity of 10 viral RNA copies per reaction, which was comparable to RT-qPCR. However, the developed L/RPA bioassay could be performed in less than 30 min using a simple procedure, so no complicated thermocycling equipment was required.

6.2. Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

RT-LAMP enables one-step reverse transcription and visualization by color change without complicated laboratory equipment. The RT-LAMP is an assay that combines a standard test LAMP with reverse transcription to detect viral RNA in as short as 20 min [123]. It can be used to detect the nucleic acid sequence of SARS-CoV-2 in

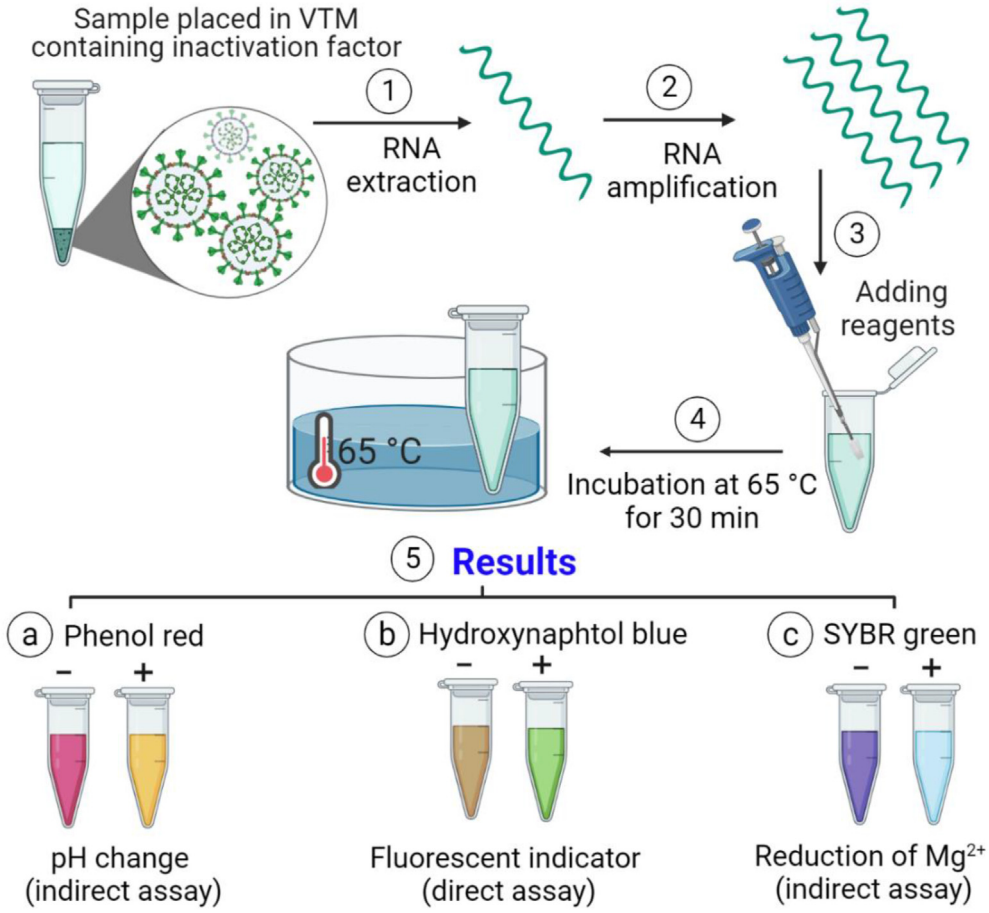

Fig. 11. An illustration shows the RT-LAMP procedure and results. (1) Collection of nasopharyngeal swabs or saliva samples, (2) extraction of viral RNA in 10–30 min, (3) RNA amplification, (4) addition of reagent and incubation at 65 °C for 30 min. Depending on the reagent and reaction conditions, different colors are observed: (5a) pH change due to phenol red, the medium is acidified after DNA amplification, (5b) hydroxy naphthol blue varies from purple to sky blue as a result of reduced Mg^{2+} in the amplified DNA, (5c) intercalating dyes such as SYBR green and displacement probes can be used as fluorescent indicators. The image was created with Biorender.

oropharyngeal swabs, saliva, nasopharyngeal swabs, and serum [124]. RT-LAMP is based on the mechanism of autocyclic strand displacement of DNA synthesis under isothermal conditions. This method bypasses the PCR high-temperature melting step by using a strand displacement DNA polymerase linkage with 4–6 specially designed primers against specific coronavirus genomes, including the ORF1ab gene, the S gene, the RdRp gene, and the N gene, to achieve highly specific DNA amplification, e.g., 10^9 to 10^{10}-fold amplification in 15 min–60 min at ~65 °C [125]. After amplification, viral DNA can be detected by the naked eye or photometrically, as magnesium pyrophosphate is produced and precipitated in the reaction mixture, resulting in femtomolar turbidity of the amplification product [126]. The amplified genome can be analyzed visually using metal-sensitive indicators, pH-sensitive dyes, gel electrophoresis, turbidity measurements after magnesium pyrophosphate-induced precipitation, and fluorescent dyes (Fig. 11) [127].

RT-LAMP is particularly advantageous for the detection of viral nucleic acids because it can be performed with the naked eye [127]. Of note, RT-LAMP has higher specificity and sensitivity, with no false-positive results reported [120]. In addition, the reagents are relatively inexpensive and can be stored at room temperature. Moreover, RT-LAMP can detect viral RNA as low as 480 RNA copies without interference [103]. The major challenge for RT-LAMP assays is that they are not as useful for mass testing as RT-PCR bioassays. Huang and co-workers [128] recently developed a rapid SARS-CoV-2 diagnostic kit that is performed in one-step RT-LAMP, which enables to detection of the virus in 30 min. The whole reaction can be performed in only 30 min at a constant temperature of 65 °C. Such a test can identify virus-infected patients at an early stage, with a detection limit of 80 copies of viral RNA per mL of sample. The performance of the RT-LAMP assay needs further investigation when testing SARS-CoV-2 variants. For example, the Omicron variant contains a mutation in a region of the E gene that is targeted by the RT-LAMP -primers in some commercial kits. Given the location of this mutation and the general tolerance of RT-LAMP to point mutations, the manufacturers do not expect significant effects on test performance with the Omicron variant (B.1.1.529). In 2022, Yajuan Dong and his research group developed a high-fidelity DNA polymerase medicated probe-based multiplex system (HFman probe), which is a specific RT-LAMP assay for the detection of two different genes (ORF and E) of SARS-CoV-2 with 94.5% sensitivity, 100% specificity, and 96.8% consistency against RT-qPCR assay on purified RNA [29].

6.3. Microarray-based diagnostics

DNA microarray is a molecular biology technique that can quickly and efficiently analyze the expression of a specific gene. To detect

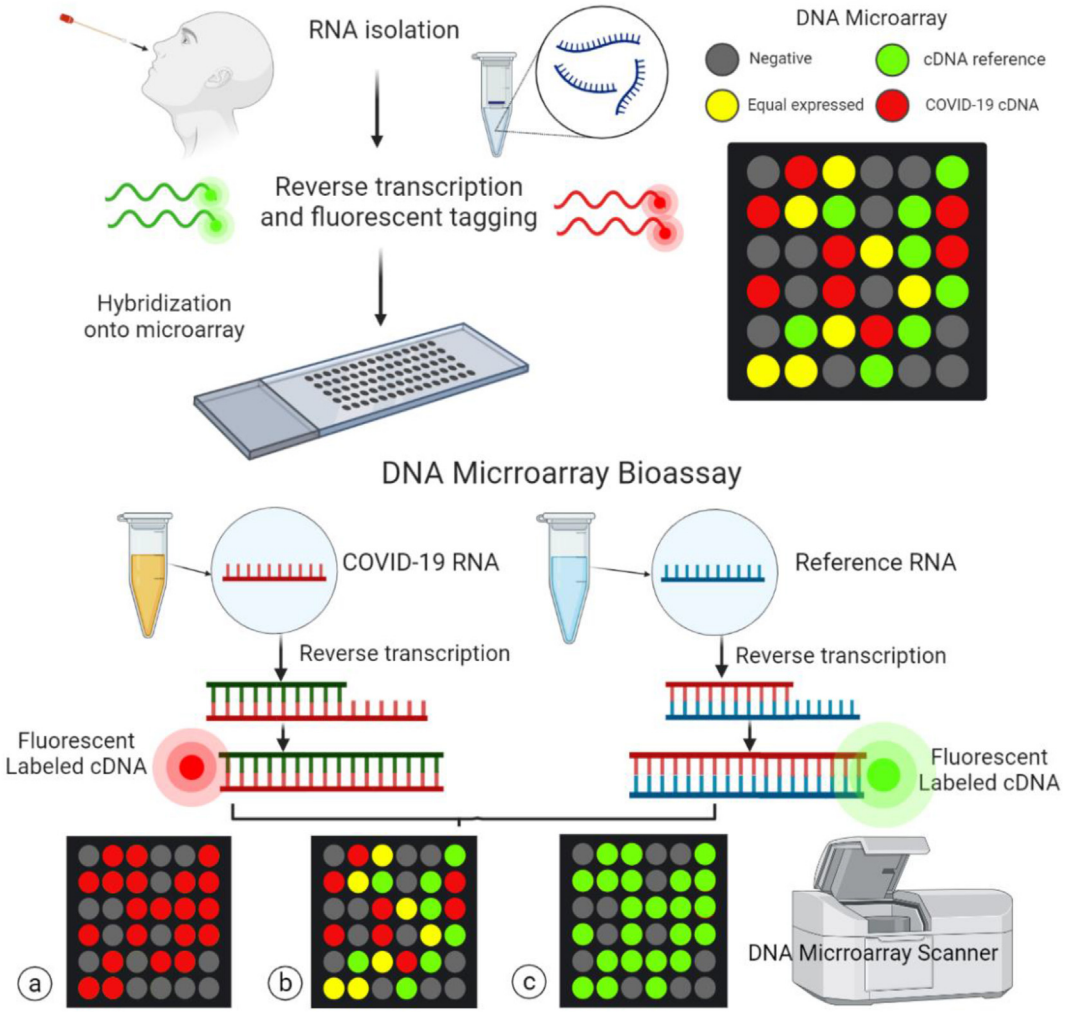

Fig. 12. Nucleic acid hybridization using DNA microarray. Fluorescent labeled viral and reference cDNA are placed into the microarray wells functionalized with specific DNA probes. (a) COVID-19 cDNA is indicated by the red fluorescence, (b) Overlaid fluorescence pattern, and (c) Reference cDNA is indicated by the green fluorescence. The image was created by Biorender.

viral RNA by microarray-based assays, viral RNA is first converted into cDNA [129]. Solid-phase oligonucleotides fixed on microarray plates are selectively hybridized with the desired cDNAs (Fig. 12). The speed, accuracy, and specificity of the microarray detection technique make it a superior tool for the detection of SARS-CoV-2 [130]. The microarray assays were used to detect SARS-CoV-2 in the patient samples using 60-mer oligonucleotides against the sequence of TOR2 [131]. Because rapid and unpredictable mutations occur in SARS-CoV-2, a new microarray technique was developed to detect 24 single nucleotide polymorphisms and mutations in the gene encoding the spike (S) protein of SARS-CoV-2 with 100% accuracy [132]. Recently, new microarray assays for the detection of various mutant SARS-CoV-2 viruses have been presented [129]. For example, Damin and coworkers [30] established a SARS-CoV-2 microarray for the detection of viral RNA in nasopharyngeal swabs with a sensitivity that matches the RT-PCR. RNA was extracted from the collected samples and cDNA was synthesized using reverse transcriptase. After PCR amplification of the cDNA and denaturation, the amplicons were spread on the microarray of N1 and N2 virus and human RPP30 target oligonucleotides. Such an assay could distinguish a false-negative clinical sample, and because of its high sensitivity, it allows the detection of viral markers with a lower number of PCR cycles compared with RT-PCR. Unfortunately, this method does not allow the diagnosis of some viral genes in volume-limited samples and is relatively expensive [133].

6.4. Clustered regularly interspaced short palindromic repeats (CRISPR)

CRISPR technology, which evolved from the adaptive immunity method of prokaryotes to prevent interference with exogenous DNA, opened a new field for genome engineering and diagnostic tools [134–137]. The exploration of CRISPRs and CRISPR-associated genes (CRISPR-Cas), which are present in most archaeal and many bacterial genomes, led to the development of CRISPR-Cas systems [131,132]. CRISPR-Cas nucleases are classified into 2 classes, 6 types and 33 subtypes [138]. Among the various CRISPT-Cas, Cas13, which was discovered in 2015 and classified as class 2, type VI [139], is used in various biotechnological applications, including specific RNA editing, knockdown, and transcript tracking, and nucleic acid detection [140–143]. The CRISPR-Cas13 system is relatively simple in design, and its activity requires only one molecule of Cas13 enzyme and one crRNA molecule [144]. In addition to specific cleavage of target RNA, a very important property of Cas13 is its collateral activity, i.e., cleavage of non-target RNA upon recognition of the target RNA [145]. Fig. 13 shows a schematic representation of the nucleic acid detection of SARS-CoV-2 using CRISPR/Cas assays.

Abudayyeh et al. demonstrated that rapid nonspecific collateral RNase activity of other RNAs in the *in vitro* reaction mixture occurred after specific RNA target recognition by CRISPR-Cas13

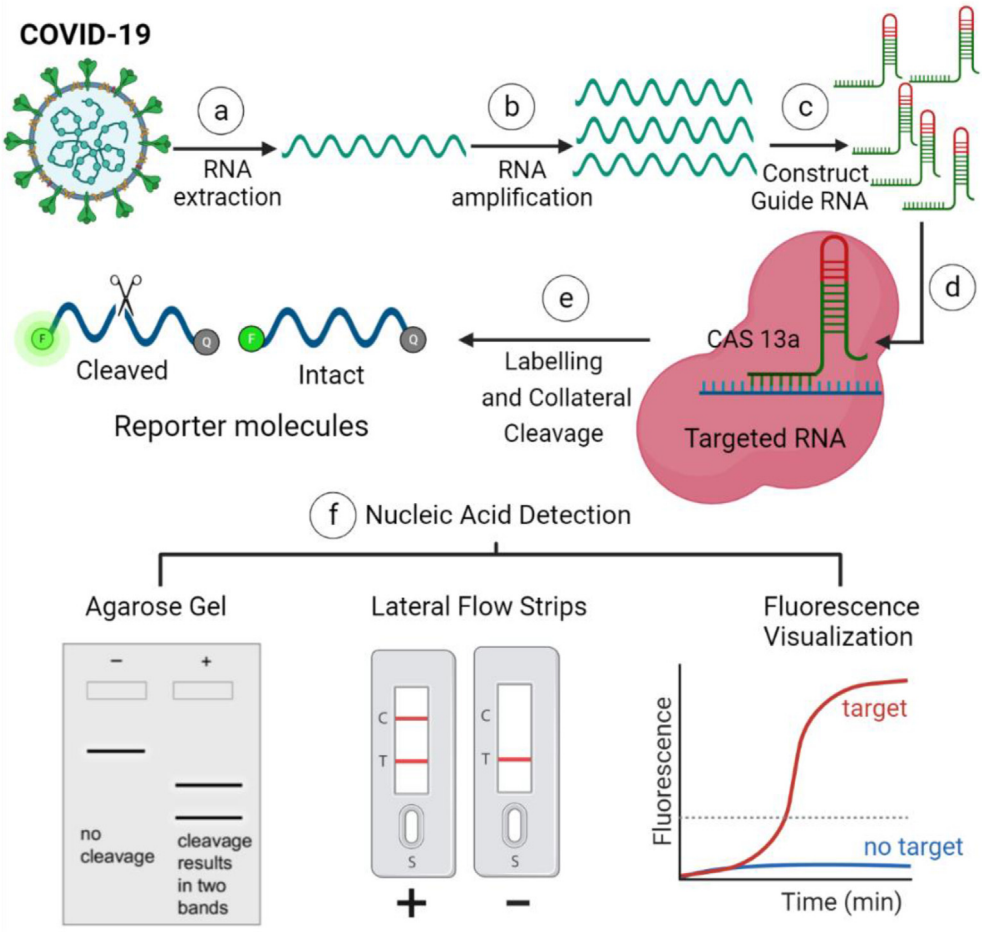

Fig. 13. Schematic representation of nucleic acid detection of SARS-CoV-2 using CRISPR/Cas assays. (a) RNA is extracted from patient spacemen. (b) DNA must be amplified from the extracted nucleic acid. (c) Construct the guide RNA. (d) Cas13 uses the guide RNA to find its target. (e) Label the target RNA by reporter molecules that fluoresce when cleavage occurs between fluorescence and quencher. (f) The detection of nucleic acid using agarose gel, lateral flow strips, and fluorescence visualization. The image was created with Biorender.

[140–145]. Similarly, the collateral activity of Cas13 was used to increase fluorescence after cutting RNA labeled with a fluorophore quenching reporter [146]. Gootenberg et al. [147] established the highly sensitive enzymatic reporter unlocking system (SHERLOCK) by modifying the CRISPR-Cas13 method by adding an isothermal amplification step using recombinase polymerase amplification (RPA), which showed sensitivity up to 100% and specificity of ~93% with a negative predictive value of ~92.9% when it was used to detect S1, ORF3 and ORF8 regions of the SARS-CoV-2 virus from the nasopharyngeal swabs [147]. While the T7 RNA polymerase step facilitates the transcription of amplified DNA into RNA [143]. The SHERLOCK opens up new possibilities for the rapid and sensitive detection of DNA or RNA molecules, including the detection of nucleic acids of specific viruses such as Zika and dengue viruses [143–146,148]. In addition, this method has been used as a COVID-19 diagnostic tool using Cas13, which can excise reporter RNA sequences in response to activation by SARS-CoV-2-specific guide RNA [149].

To modify the SHERLOCK system, the technique of heating unextracted diagnostic samples to eliminate nucleases (HUDSON) was developed to minimize the number of steps and the cost of the assay. Heating and chemical reduction have inactivated both the virus and nucleases in clinical specimens so that they could be used directly, without nucleic acid extraction, for detection with the SHERLOCK system [141]. To further simplify virus detection, including SARS-CoV-2, the STOP (SHERLOCK testing in one pot) assay was recently developed. The assay can be performed in less than 1 h with minimal equipment and combines viral RNA extraction, isothermal amplification, and SHERLOCK detection [150]. Another modification of CRISPR assays is diagnosis with enzymatic coronavirus reporting (DISCoVER). This assay combines HUDSON with LAMP amplification and T7 RT for stronger amplification of the target RNA [151].

Fozouni et al. presented several advances in the Cas13 diagnostic system, including increased sensitivity, viral load quantification, and simple and inexpensive signal detection [152]. Combinations of crRNAs allowed more Cas13a to be activated per target RNA, resulting in increased sensitivity (~100 copies μL^{-1}) without requiring amplification of the target RNA. The omission of amplification allowed direct quantitative monitoring of viral load because the signal was a direct translation of the amount of target in the sample. Another advancement is the use of cell phone cameras instead of specialized laboratory equipment to quantify the fluorescent signal generated by the collateral activity of Cas13. Another approach is to cut the reporter RNA with Cas12a to detect viral genomic sequences of the N gene and the E gene, and then amplify the target isothermally, resulting in visual analysis with a dye [153].

CRISPR assays are preferred because they do not require complicated instrumentation and viral detection can be easily read with paper strips without compromising sensitivity or specificity [106]. However, CRISPR assays require expert personnel and nontrivial reagents. Other difficulties include lack of assay standardization, sequence limitations, and limited literature on CRISPR assays. In addition, an additional step of DNA amplification is often required to lower the detection limit. Because of these features, CRISPR assays are less economical than RT-LAMP and RT-PCR [154]. The US Food and Drug Administration (FDA) has approved the first CRISPR-based detection kit against SARS-CoV-2, providing results in approximately 1 h [155]. A major drawback of this technique is the nonspecific binding of the single guide RNA to the genome, which leads to inaccurate results [155].

6.5. RNA sequencing

Next-generation sequencing (NGS) is a high-throughput sequencing technique based on capillary electrophoresis. First, the genomic strand is fragmented, and then the fragments interact

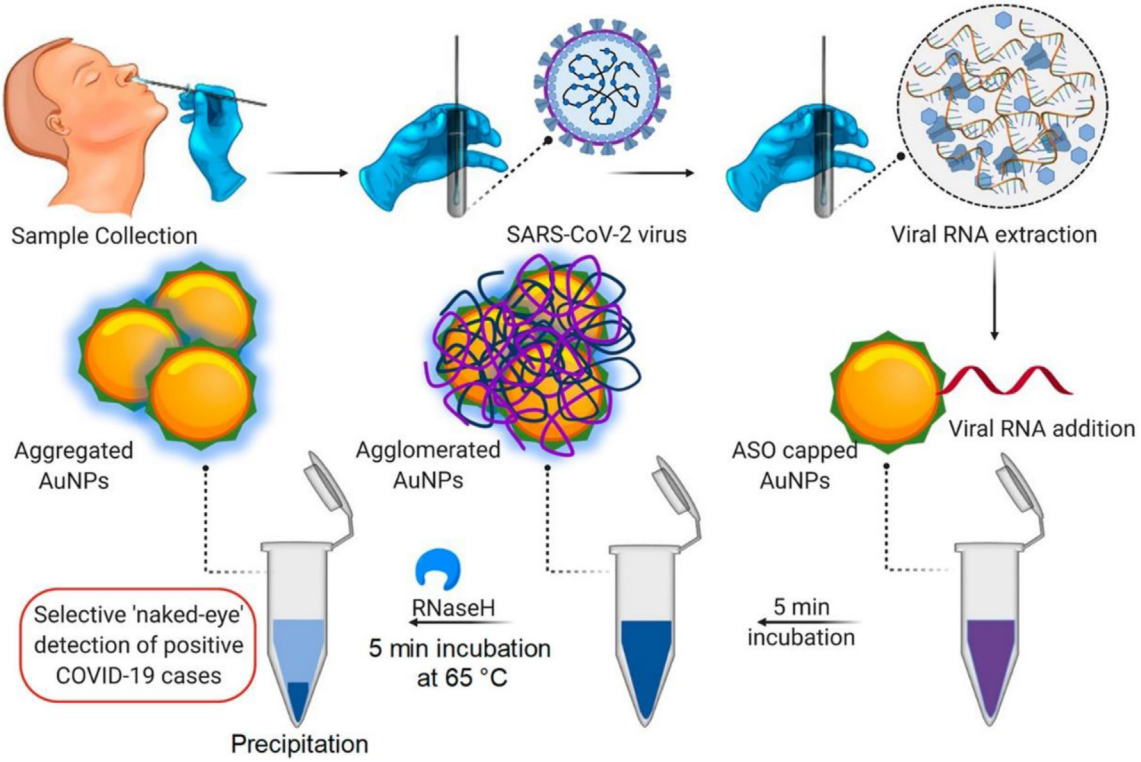

Fig. 14. Detection of SARS-CoV-2 RNA by antisense oligonucleotide (ASO)-capped Au NPs which allow the viral detection via naked-eye [159]. © American Chemical Society, 2021.

Table 2
Summary for different laboratory based diagnostic techniques.

Detection technique	Sample	Analyst	Duration	Advantages	Disadvantages	Ref
ELISA	Whole blood, plasma, serum	Viral antigen, host antibody	1−3 h	- Able to test multiple samples - Relatively simple and cheap	- Requires medical staff and special equipment - Multistep process	[164]
LFIA	Whole blood, sweat, urine, serum, and saliva	Viral antigen, host antibody	15 min	- Simple and rapid - Low-cost - No need for washing steps	- High ratio of false-negative results	[162]
RT-PCR	Nasopharyngeal swab, stool, sputum	Viral RNA	<2 h	- High sensitivity and selectivity - Early detection of low viral titers	- Time-consuming - False readings due to cross-reactivity or low viral load	[36,105,165]
RT-LAMP	serum, stool, Oropharyngeal swabs, saliva, nasopharyngeal swabs	Viral RNA	<1 h	- No false-positive results reported so far - Faster than conventional RT-PCR - Relatively cheap and stable reagents - Results are seen by the naked eye	- Difficult to optimize its primers and reaction conditions	[166,167]
Microarray techniques		Viral RNA	10 min	- High output technique - Fast detection	- Expensive oligonucleotides needed	[133]
CRISPR	Broncho alveolar lavage fluid, and nasopharyngeal swab	Viral RNA	<40 min	- Easy-to-perform and low cost. - User-friendly paper strips to detect the virus	- Non-specific binding may cause inaccurate readings	[106,168]
Next-generation Sequencing	Nasopharyngeal Swab	Viral RNA	1−2 days	- High sensitivity and specificity - Identification of a novel strain is possible	- High expertise and advanced equipment needed	[156]
FTIR spectroscopy	Serum Saliva	Function groups of different biomolecular compounds.	Several min	- Label-free sample - Low cost. - Rapid test. - No reagent is required. - High sensitivity. - Non-invasive analytical technique	- Lower specificity compared to the antibodies-mediated detection, - Can't be used as a diagnostic technique	[96,97]
Raman spectroscopy	Saliva Serum	RNA Immunoglobulins (IgM and IgG).	Several minutes	- High sensitivity - High selectivity - Early detection in asymptotic patient	- Requirement of additional laboratory-based assays.	[169,170]
UV−vis spectroscopy	Saliva	Spike protein (Antigen detection)	Few min	- High selectivity - High sensitivity due to surface Plasmon resonance (SPR)	- Requirement of additional laboratory-based assays	[95]
Electronic Biosensor (Transistor based biosensor)	Nasopharyngeal swab	Spike protein (Antigen detection)	Few min	- High sensitivity - High selectivity - Label-free sample - Rapid	- Large specific surface area of graphene	[87]
Piezoelectric biosensors	Sputum swabs, nasopharyngeal swabs, and throat swabs	Spike protein (Antigen detection)	Few min	- Rapid - Cost-effective - High sensitivity - High Specificity - Label-free simple	- Inaccurate values for some samples	[171,172]
Optical biosensors	Saliva	Spike protein Antigen detection	10−30 s	- No cross-reactivity - High sensitivity - Rapid detection - In-house built electrochemical device - Cost-effective	–	[85]
	Serum and saliva	Immunoglobulin's antibodies (IgG, IgM, and IgA)	–	- Rapid detection - Cost-effective - High throughput	- Require a long incubation time of up to 3 h for the saliva sample	[173]
Potentiometric biosensors	Saliva	Spike protein Antigen detection	10 min	- Sensor is reusable - Rapid test - Cost effective	–	[174]
Amperometric biosensors	SARS-CoV-2-S is produced in mammalian hamster CHO cells.	SARS-CoV-2-S is produced in mammalian hamster CHO cells.	Few min	- High sensitivity - Rapid - Simple test - Cost-effective	- Inconsideration of the interference of other similar biomolecules in complex biological fluids	[175]
Voltammetric biosensors	Nasopharyngeal swab	Nucleocapsid protein Antigen detection	15 min	- Cost-effective - Rapid response - Label-free sample - High sensitivity	- Cross-reactivity with SARS-COV	[176]

Table 2 (*continued*)

Detection technique	Sample	Analyst	Duration	Advantages	Disadvantages	Ref
Chemiluminescent biosensors	Serum	Immunoglobulin antibodies (IgG, IgM, IgA)	Few min	- High specificity - Highly sensitive and specific	- The low no. of a participant in the group make it difficult to build any definitive conclusions - The antibody/antigen combinations affected by the severity of the symptoms	[177]
Electrochemiluminescent biosensors	Serum	Spike protein Antigen detection	–	- High sensitivity - High specificity - It allows the detection of SNPs in the viral RNA sequence	- Require competing finances to be applied	[178]
Fluorescence biosensors	Nasopharyngeal samples	Spike protein and nucleocapsid protein (Antigen detection)	10 –20 min	- Used for quantitative detection - Good stability - High reproducibility in a wide range. - Use a smartphone App for online detection of COVID-19 patients	- Require competing finances to be applied	[179]
Colorimetric immunoassay	Nasal swabs and throat swabs	Spike protein, envelope protein, and membrane protein Antigen detection	3 min	- Able to detect a low concentration of the viral load - Rapid - High sensitivity to the viral virion	–	[180]

with the oligonucleotides of the known sequences. The bases of each fragment are identified by the signals they emit using luminescent agents. Several NGS-based test kits are now available for COVID-19 diagnosis. For example, the Chinese company BGI Biotechnology has introduced a sequence detection kit that can detect SARS-CoV-2 and other coronaviruses and respiratory diseases [156]. In a study of 129 patients with suspected COVID-19 infection, NGS was used to confirm the false-negative or false-positive results of RT-LAMP and RT-qPCR tests on 329 nasopharyngeal swabs [157]. NGS is a rapid and accurate tool for obtaining comprehensive genetic information, allowing a detection limit as low as 10 copies·mL^{-1} for SARS-CoV-2 and serving as a reference test for COVID-19, especially for samples with low virus content [158]. On the other hand, it requires expensive chemicals and equipment, which limits its application as a POC diagnostic tool.

6.6. Biosensors

Biosensors for viral gene analysis serve as a faster and less expensive alternative to conventional RT-PCR. In particular, colorimetric methods that allow selective detection by the naked eye without advanced instrumentation are desirable for viral diagnostics in POC applications. Such an assay has been developed using Au NPs in combination with N-directed antisense oligonucleotides (ASOs-Au NPs) [159]. The ASOs-Au NPs agglomerated in the presence of viral RNA, resulting in a shift in the SPR reaction. Moreover, the addition of RNase H, which cleaved the phosphodiester bonds of viral RNA, produced a visually observable precipitate in the sensor solution, while the ASO strands remained unchanged. The ASOs-Au NPs-based biosensor was able to detect the N gene in a dynamic range of 0.2–3 ng μL^{-1} with a LOD of 0.18 ng μL^{-1}. Similarly, ASOs-Au NPs were also used to construct a paper-based electrochemical genosensor, which was tested on SARS-CoV-2 infected cells and clinical samples [160]. The electrochemical measurements performed with a self-developed circuit resulted in a LOD of 6.9 copies·μL^{-1}. In another study, the N gene was detected using surface-enhanced infrared absorption spectroscopy (SEIRA)-based biosensor with an ssDNA probe as the receptor. The thiolated probe DNA was immobilized on Au NPs substrate to enhance the infrared absorption of the receptor-target complex [161]. Selective binding of the N gene to the probe DNA was detected by SEIRA spectra, which were statistically analyzed by principal component analysis (PCA) to detect the positive and negative SARS-CoV-2 cases. According to the recombinase-polymerase amplification method, the proposed sensor could detect viral RNA in samples as few as 2.98 copies μL^{-1} within 30 min.

Qui and co-workers combined LSPR transduction with plasmonic photothermal effect (PPT) to develop an optical biosensor with enhanced sensitivity [162]. Succinimide ester-functionalized Au NPs were modified with DNA receptors complementary to RdRp, ORF1ab, and E genes of SARS-CoV-2. Localized plasmonic photothermal heat energy accelerated hybridization, resulting in enhanced phase response. The dual-functional biosensor achieved a detection limit of 0.22 pM and a recovery rate of 96% when tested with multigene mixtures [162]. Fig. 14 shows the detection of SARS-CoV-2 RNA using antisense oligonucleotide (ASO)-capped Au NPs, which enable naked-eye virus detection [159].

7. Comparison of different diagnostic techniques

Clinical and nonclinical diagnostic methods based on antibody-antibody detections have been partially effective in satisfying the increasing demand for fast detection and slowing down the further spread of SARS-CoV-2 [163]. However, a negative serological test based on the antibody cannot exclude a previous infection with SARS-CoV-2. Furthermore, optimal specimens for antibody testing are acute and convalescent possibly two to four weeks after the acute phase. Currently, RT-PCR is the most widely used method worldwide for SARS-CoV-2 detection. Biosensors, RT-LAMP, NASBA, CRISPR-Cas-based detection, and digital PCR are techniques that are being used, with many of them awaiting diagnostic approval by the relevant authorities. Optical and electrochemical biosensors can serve as non-invasive, extremely sensitive rapid detection platforms with sensitivity down to 1 fM concentration in a few minutes. These biosensors can be manufactured on a mass scale

Table 3
Summary for commercial SARS-CoV-2 diagnostic kits classified based on the detection method.

Method	Commercial Name	Measurement	Target	Specimen	Time	Accuracy	Sensitivity	Specificity	Manufacturer website
	Absoludy COVID-19 Ag (Manufactured by Absology Co., Ltd.)	Semiquantitative	Antigen	Nasopharyngeal swab, Oropharyngeal swab	5 min	97.9%	98%	99%	www.absology.co.kr
	COVID-19-CHECK-1 test (Manufactured by VEDALAB, France)	Qualitative	Antibody, IgG, IgM against N-protein	Plasma, Serum, Whole blood	10 min	94.42% (IgM + IgG)	60.87% IgM 91.3% IgG	–	www.vedalab.com
	A&B RAPID TEST COVID-19 IgG/IgM (Manufactured by A&B Professional)	Qualitative	Antibody IgG, IgM	Plasma, Serum, Whole blood	10 min	97.8% IgM 99.6% IgG 98.2% (IgG + IgM)	91.8% IgM 100% IgG 94.1% (IgG + IgM)	99.2% IgM 99.5% IgG 99.2% (IgG + IgM)	www.aebrapidtest.com
Chromatography (Lateral flow immunoassay)	COVID-19 PRESTO (Manufactured by AAZ-LMB)	Qualitative	Antibody, IgG, IgM against N-protein	Plasma, Serum, Whole blood	11 min	100% IgG 100% IgM	100% IgG 100% IgM	100% IgG 100% IgM	https://www.covid19aaz.com/
	SC2Flu Triplex Fast Test (Colloidal Gold) For SARS-CoV-2 & influenza A/B antigens (Manufactured by AMPER INC)	Qualitative	N-protein Antigen	Nasal swab, Nasopharyngeal swab	15 min	100%	94.12%	100%	http://www.amperbio.com
	Coronavirus Ag Rapid Test Cassette (Manufactured by Healgen Scientific)	Qualitative	Antigen	Nasal swab, Nasopharyngeal swab	15 min	98.73% Nasal swab 99.42% Nasopharyngeal swab	97.25% Nasal swab 98.32% Nasopharyngeal swab	100% Nasal swab 99.6% Nasopharyngeal swab	https://www.healgen.com/
	VISION® COVID-19 AG RAPID TEST (Manufactured by Vision Biocenology)	Qualitative	N- protein Antigen	Nasal swab, Nasopharyngeal swab	15 min	98.47%	97.57%	99.6%	https://visionbiotechnology.com
	Rapid COVID-19 Antigen Test (Colloidal Gold)/Saliva (Manufactured by Anbio (Xiamen) Biotechnology)	Qualitative	Antigen	Saliva	15 min	99.52%	99.06%	100%	www.anbio.com
	SARS-CoV-2 IgG II Quant (Manufactured by Abbott Ireland Diagnostics Division)	Quantitative	Antibody, IgG against S-protein	Plasma, Serum	29 min	99.37% IgG	99.37%	99.55%	www.corelaboratory.abbott
ELISA	COVID-19 TEST RAPIDO ANTIGENE RICOV4 (Manufactured by Beijing North Institute of Biotechnology CO.,Ltd)	Qualitative	Antigen	Nasal swab, Nasopharyngeal swab, Oropharyngeal swab	20 min	97.4%	94.7%	99%	–
	Anti-SARS-CoV-2 RBD ELISA (Manufactured by AUTOBIO DIAGNOSTICS., LTD)	Quantitative	Antibody	Plasma, Serum	40 min	99.6%	97.89%	99.71%	https://www.autobio.com.cn/
	Kewei COVID-19 total antibody ELISA Test Kit (Manufactured by Beijing Kewei Clinical Diagnostic Reagent Inc)	Qualitative	Antibody	Plasma, Serum	60 min	96%	94.71%	99.6%	https://en.keweidiagnostic.com/
	COVID-19 IgA (Manufactured by DIA.PRO Diagnostic Bioprobes Srl)	Semiquantitative	IgA	Plasma, Serum	105 min	100%	100%	98%	https://www.diapro.it/products/covid-19-iga-elisa/
	COVID-19 IgG Confirmation (Manufactured by DIA.PRO Diagnostic Bioprobes Srl)	Quantitative	Antibody, IgG	Plasma, Serum	105 min	–	100%	100%	https://www.diapro.it/
Chemiluminescent Immunoassay	Anti-SARS-CoV-2 RBD CLIA Microparticles (Manufactured by AUTOBIO DIAGNOSTICS)	Quantitative	Antibody	Plasma, Serum	17 min	99.29%	79.49%	99.78%	https://www.autobio.com.cn/
	SARS-CoV-2 IgG (CLIA) (Manufactured by MEDCAPTAIN MEDICAL TECHNOLOGY CO., LTD)	Qualitative	Antibody, IgG	Plasma, Serum	25 min	96.5%	96.9%	–	https://www.medcaptain.com/
	SARS-CoV-2 IgG II CLIA Microparticles (Manufactured by Autobio Diagnostics Co., Ltd)	Qualitative	Antibody, IgG	Plasma, Serum	40 min	–	100% (≥15days after infection)	99%	https://www.autobio.com.cn/

	LIAISON® SARS-CoV-2 Ag (Manufactured by DiaSorin S.p.A)	Quantitative	Antigen	Nasal swab, Nasopharyngeal swab	42 min	99%	99%	98%	https://www.diasorin.com/
	RevoDx SARS-CoV-2 qPCR Kit (Manufactured by İDİL BİOTECH ARAŞTIRMA SAN. VE TİC. LTD. ŞTİ)	Qualitative	Nucleic acid	Nasopharyngeal swab, Oropharyngeal swab	47 min	–	–	≥99%	http://www.idilbiotech.com/page/covid-19/
RT-PCR	Freeze-dried Novel Coronavirus (COVID-19) Nucleic Acid Detection Kit (Fluorescence PCR method) (Manufactured by BIOTEKE CORPORATION (WUXI) CO., LTD)	Quantitative	Nucleic acid	Anterior nasal swab, Nasal aspirate, Nasopharyngeal swab, Oropharyngeal swab, Sputum	60 min	–	95%	98%	https://www.bioteke.cn/
	MutaPLEX® Coronavirus Real-Time-RT-PCR-Kit (Manufactured by Immundiagnostik AG)	Quantitative	Nucleic acid	Mid-turbinate swab, Nasal swab, Nasopharyngeal swab, Oropharyngeal swab, Saliva, Sputum	65 min	–	100%	100%	https://www.immundiagnostik.com/
	REALQUALITY RQ-2019-nCoV (Manufactured by AB ANALITICA)	–	Nucleic acid	Nasopharyngeal swab, Oropharyngeal swab	100 min	99%	98%	100%	https://www.abanalitica.com/
	AddMedi SARS-CoV-2 RT-qPCR Kit (Manufactured by Addbio Meditek Co.)	Qualitative	Nucleic acid	Bronchoalveolar lavage fluid, Nasopharyngeal swab, Oropharyngeal swab	109 min	–	96%	100%	https://www.addbiomeditek.com/
	CBDNA RT-LAMP RAPID TEST (Manufactured by Centrum Badań DNA ul. Ściegiennego 20 60–128 Poznań)	Qualitative	Nucleic acid	Nasopharyngeal swab	20 min	–	100%	100%	https://www.cbdna.pl/
RT-LAMP	Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo-Kit (Manufactured by Genomtec S.A., Poland)	Qualitative	Nucleic acid	Nasopharyngeal swab, Oropharyngeal swab, Saliva	40 min	–	93,75%	100%	https://genomtec.com/en/products/#evagreen
	LoopDeetect COVID-19 IC (Manufactured by LoopDeeScience)	Qualitative	Nucleic acid	Nasopharyngeal swab	45 min	–	95%	95.5%	https://www.loopdeescience.com/en/press-release-04-16-2021
	Dr Vida pocket for COVID-19 (Manufactured by STAB VIDA)	Qualitative	Nucleic acid	Nasal swab, Nasopharyngeal swab	50 min	–	95%	98%	https://www.stabvida.com/
CRISPER	Fosun SARs-CoV-2 CRISPR (Manufactured by Fosun Diagnostics (Shanghai) Co.Ltd)	–	Nucleic acid	–	–	–	–	–	https://covid-19-diagnostics.jrc.ec.europa.eu/devices/detail/2717
Digital-PCR	Cue's COVID-19 Diagnostic Test (Manufactured by cue health Inc.)	Semiquantitative	–	Nasal swab	20 min	–	98.7%	97.8%	cuehealth.com
	Dr. PCR™ Di20K COVID-19 Detection kit (Manufactured by OPTOLANE Technologies, Inc)	Qualitative	Nucleic acid	Nasopharyngeal swab, Oropharyngeal swab	60 min	–	100%	100%	https://www.optolane.com/
Nephelometry	Automatic Immunoassay System - HP-AFS/1 (Manufactured by Shijiazhuang Hipro Biotechnology)	Quantitative	N-protein Antigen	Anterior nasal swab, Nasal swab, Nasopharyngeal swab	6 min	90%	90%	95%	https://www.hipro.us/
	POCT Immunoassay System - HP-083/4 (Manufactured by Shijiazhuang Hipro Biotechnology)	Quantitative	IgG, N-protein Antigen	Anterior nasal swab, Nasal swab, Nasopharyngeal swab	6 min	90%	92%	97%	https://www.hipro.us/

(millions) to detect the SARS-CoV-2 viral load in different samples, even if the infected person is asymptotic [163]. The advantages and disadvantages of the currently existing techniques for SARS-CoV-2 detection are summarized in Table 2.

Although real-time RT-PCR is sensitive and reliable, it is time-consuming (~2 h), which limits its broad application to the current huge demand for the global pandemic of COVID-19. To address this challenge, rapid, sensitive, and simple-to-operate diagnostic kits would be highly desirable. Studies have attempted to design various effective test kits against SARS-CoV-2. Ideally, the test kits should be mobile without the need for any complicated instrument and the test result should be readable by the naked eye or perhaps a smart phone which has the advantage of creating an electronic record of the test that can be tied to an individual. Therefore, these tests can be easily used at airports, hospitals, and medical centers in rural areas. Recently, WHO reported that the widely available tests can detect individuals infected with any currently circulating variant including Omicron. However, some PCR tests will not be able to distinguish between the different variants. In Dec 2021, Roche's (ROG.S) and TIB Molbiol has developed three new test kits to help researchers study the Omicron variant. These testing kits, made only for research use, can differentiate between unique mutations in Omicron compared to other variants.

8. Global COVID-19 diagnostics market

The uncontrolled spread of the SARS-CoV-2 worldwide affects the growth of the diagnostics market [181]. The global diagnostics market size of SARS-CoV-2 is projected to reach USD 11.40 billion by 2027, with a CAGR of 7.9% from 2020 to 2027. The RT-PCR assay kits are estimated to have a maximum revenue share of 67% in 2020 [182] while ELISA and LFIA hold the second and third market sizes [181]. Swab tests are estimated to have a maximum revenue share in 2020. In particular, the nasopharyngeal swabs are estimated to dominate the revenue share with more than 45% in 2020 (USD 1.8 billion revenue) [182]. The hospital's segment dominates around 46% of the SARS-CoV-2 test kits market share in 2020. Some examples of commercial rapid tests for SARS-CoV-2 are also listed in Table 3.

9. Challenges and future perspective

Diagnosis of SARS-CoV-2 and its variants is challenging because different detection methods must be combined to identify the different stages of infection. By the end of 2021, numerous diagnostic techniques had been developed to detect SARS-CoV-2 virus infection: (i) by viral nucleic acid using RT-PCR, RT-LAMP, microarray assay, and CRISPR-Cas-based platforms, and (ii) by viral antigens or antibodies produced by the immune system using ELISA, LFIA, and biosensors. However, there are still limitations and challenges in the developed SARS-CoV-2 diagnostic techniques that need to be overcome, such as (1) the variation of the sensitivity of viral antigen- or antibody-based techniques over the infection period; (2) the need to identify epitopes of SARS-CoV-2 proteins and their newly discovered variants on monoclonal and polyclonal antibodies and serum from SARS-CoV-2 patients; (3) the high cost and long duration of detection of genetic material (RT-PCR and ELISA) in the early stages of infection; (4) the need to identify genetic material and develop accurate kits that distinguish between MERS-CoV, SARS-CoV-2, and the newly discovered variants, which can be performed at each stage, especially in the early stages of infection; and (5) the need to use more than one detection method for accurate diagnosis over an incubation period of the virus. These challenges can be overcome by developing efficient, portable, and ready-to-use biosensors. Such instruments, combined with the rapid detection capabilities of biosensors, could pave the way for highly efficient, simple, and reliable virus diagnosis that would play an important role in pandemics.

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.

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