



Overview of diagnostic tools and nano-based therapy of SARS-CoV-2 infection

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly infectious virus that belongs to the RNA virus family *Coronaviridae*, infects a variety of animal species, and replicates primarily in the lower and upper respiratory tract. A proportion of 90% of infections are uncomplicated, and 4–7% of people are hospitalized. The main focus is on people with comorbidities, which are risk factors for severe disease and lead to high rates of hospitalization and death. During the early stages of a pandemic, many diagnostic approaches such as biochemical, serological, and molecular investigations are used to detect SARS-CoV-2. This review emphasizes immunodiagnostic techniques, including virus neutralization assay, CRISPR, nicking endonuclease amplification reaction, lateral flow immunoassay, protein-peptide microarray, and chemiluminescence immunoassay; it also includes novel approaches such as next-generation sequencing. Nanotechnology is critical in the prevention, diagnosis, and treatment of SARS-CoV-2, with an in-depth review of its principles, usefulness, benefits, and drawbacks. The study sheds light on diagnostic methods that apply to future infectious viruses as well as SARS-CoV-2. Furthermore, the article explores diverse nano-based treatments, including vaccines, immunotherapy, and gene therapy, providing encouraging methods for the prevention and management of SARS-CoV-2.

Keywords SARS-CoV-2 · Serological · Nanotechnology · Nano-based treatments · Immunotherapy

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the new 2019 coronavirus disease (COVID-19), which belongs to the large RNA viruses, i.e., the *Coronaviridae* family, which affects a

variety of mammals (Nickbakhsh et al. 2020 and Bhattacharjee et al. 2023). The SARS-CoV-2 virus causes a wide spectrum of clinical symptoms, ranging from asymptomatic illness to severe pneumonia, short-lived flu-like symptoms, and acute respiratory distress syndrome. It is an extremely contagious virus that replicates mainly in the lower and upper respiratory tracts and is transmitted primarily by aerosols and droplets (Kang et al. 2020).

In general, 90% of infections are uncomplicated, have mild symptoms, or are oligosymptomatic, not requiring hospitalization. However, hospitalization is required in 5–10% of cases, including patients with comorbidities, such as hypertension, chronic heart or lung failure, diabetes mellitus, older age, and immunodeficiency (Salzberger et al. 2021 and Sahu et al. 2021). SARS-CoV-2 is structurally similar to SARS-CoV, middle respiratory syndrome coronavirus (MERS-CoV), and other coronaviruses of living organisms (Singh and Yi 2021).

Coronavirus consists of approximately 30,000 nucleotides and four structural proteins, namely the envelope protein (E), membrane protein (M), nucleocapsid protein (N), and

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spike protein (S) encoded by this gene, as well as many non-structural proteins (NSP) (Fig. 1). The viral capsid is a type of protein shell that contains a core capsid commonly referred to as a nuclear protein. It is linked to the positive single strand of the virus's RNA, allowing it to enter living tissue and transform it into a viral casting unit (Boopathi et al. 2019). SARS-CoV-2 induces the angiotensin-converting enzyme 2 (ACE2) surface receptor to enter the living tissue of the host cell (Sarma et al. 2021). Table 1 provides information about the variants of SARS-CoV-2 and their structural differences.

The viruses and their characteristics were obtained from a variety of patient samples, including nasal and throat samples, mucus, saliva, plasma, and serum. Numerous techniques are used to analyze SARS-CoV-2 in the initial phase of the pandemic, such as monitoring body temperature (fever), as this is associated with SARS-CoV-2 symptoms. However, even individuals undergoing thermal screening are positive in confirmatory analytical procedures (Schultz et al. 2023). There are several diagnostic approaches to detect the presence of SARS-CoV-2 that can make the results more accurate. Biochemical testing is among the methods that can improve diagnosis. The tests include C-reactive protein (CRP), complete blood count, and cytokines; however, a major drawback is that they are not highly specific (Ye et al. 2022). To determine the target component of SARS-CoV-2, both molecular and serological diagnostic methods are used. The most widely used molecular diagnostic tests for the diagnosis of SARS-CoV-2 are polymerase chain reaction (PCR), real-time quantitative reverse transcription PCR (qRT-PCR), computed tomography (CT), and reverse transcription loop-mediated isothermal amplification (RT-LAMP). In contrast to SARS-CoV-2, the detection of viral antibodies (IgM/IgG) and antigens in serological tests, such

as enzyme-linked immunosorbent assay (ELISA), lateral flow immunosorbent assay (LFIA), and biosensor-based diagnostics, as well as CRP in clinical samples, is used to determine the stage of the disease (Hazra and Patra 2023 and Xiao et al. 2023). The major drawback is that antibodies are slow to form in response to the SARS-CoV-2 virus (D. f. Li et al. 2023). In this regard, serological diagnostic systems provide not only extremely sensitive and specific reports but also faster reports, cost-effectiveness, simplicity, and ease of use, making them an excellent alternative for biosensor operations (Salahandish et al. 2023). After the onset of infection, IgM antibodies can be found in the acute phase. In secondary infection, higher levels of viral IgG and IgM antibodies can be detected, and the results of detecting the amount of IgM and IgG antibodies in the blood sample, along with viral RNA, can provide a minute indication of the extent of SARS-CoV-2 contamination and resistance in an individual. The diagnostic methods for SARS-CoV-2 are serologic and molecular systems using clinical specimens containing viral proteins or antigens, immunoglobulins/antibodies (IgM, IgG), and RNA unique to SARS-CoV-2 (Salahandish et al. 2023). Lymphopenia is an example of an immunological abnormality that can be used to predict the health status of a SARS-CoV-2-diagnosed individual. Table 2 provides an overview of existing methods for detecting SARS-CoV-2 and immunological techniques.

In addition, there are other promising techniques, such as CRISPR (clustered regularly interspaced short palindromic repeats), surface-enhanced Raman scattering, next-generation sequencing, vertical flow assay, amplicon-based metagenomic sequencing, nicking endonuclease amplification reaction (NEAR), virus neutralization test (VNT), and nano-based techniques, such as colorimetric assays, microfluidic devices, magnetic nanoparticle-based separation,

Fig. 1 Genomic surface protein structural representation of SARS-CoV-2

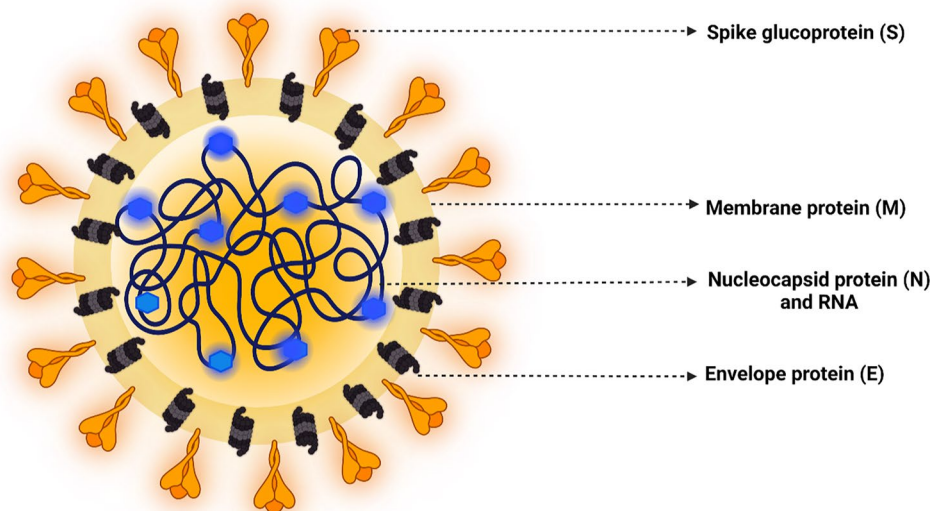


Table 1 Summary of important COVID-19 variants and their structural differences

COVID-19 variant	Infection ability	Structural differences	References
UK variant (B.1.1.7 or 20I/501Y.V1)	Transmission of infection is about 40–60%, and the mortality rate is 55%	It has 23 notable mutations, and its binding potency with the S protein is found to be increased in the N501Y mutated gene, which interacts with ACE2 receptors	Galloway et al. 2021 and Davies et al. 2021
South African variant (B.1.351/501Y.V2)	Transmission of infection increases in the age group of young and adults	It has 23 notable mutations with 17 amino acid changes, of which K417N, E484K, and N501Y occur on the S protein	Cascella et al. 2022
Brazilian variant (P.1 or 20 J/501Y.V3)	Transmission of infection is comparatively 2.2 times higher than other variants, and it simultaneously causes reinfection in COVID-19 patients	It has been found to show 35 mutations with 17 amino acids, of which K417N, E484K, and N501Y have shown notable mutation	Cascella et al. 2022
Brazilian variant (B.1.1.2.8)	The transmission of infection rate is not yet reported	It has only one notable mutation with the S protein, E 484 K, with no efficacy or effectiveness reported with respect to vaccines for future prospects	Voloch et al. 2021
US Midwest variant (20C-US or COH.20G/501Y)	Transmission of infection ability and virulence are not yet reported	It shows mutations on S protein (Q6778), N protein (D377Y), and M protein (A85S), but effectiveness against disease-causing factors has not yet been reported	Voloch et al. 2021
US San Francisco Bay area variant (B.1.427 and B.1.429)	The transmission rate is increased by 20% in the mode of transmission, with a decrease in the efficacy level with respect to therapeutic importance	It shows mutations in both variants related to S protein, with the following associated mutations: L452R, D614G, S131, and W152C	Voloch et al. 2021
US Southern California variant (CAL..20C)	The transmission of infection rate is not yet reported	Notable mutations associated with this variant are W152C, ORF1b: D1183Y, ORF1a:14205 Y, L452R, and S: S131	Zhang et al. 2021
Double-mutant variant (B.1.617)	Transmission of infection is very high, and resistivity toward the vaccine is found to be maximum	Notable mutations reported are E484Q and L452R, showing potent effectiveness with a double-mutation effect	Samal et al. 2107 and Vasireddy et al. 2021
Triple-mutant variant (B.1.618)	Transmission of infection is high, and knowledge about the vaccine scene is not fully elucidated	Notable mutations reported are the same as double-mutant variants; however, deletion of the chain of the amino acid causes it to be more effective in infection	Vasireddy et al. 2021
Indian variant Andhra Pradesh (N440K)	The transmission of infection rate is 10- to 1000-fold more effective toward ACE2 receptors	Mutation in the S protein has been reported, but it shows resistivity toward three monoclonal antibodies, such as C135 and REGN10987	Rani et al. 2021
Newest variant omicron (B.1.1.529)	Virulence and transmission studies reveal that this variant is somewhat similar in action to gamma, beta, and alpha variants	This type constitutes a very high mutation rate compared to other variants, with notable mutation for 32 amino acid residues: T478K, K417N, D614G, N501Y, and E484K. The mutation rate is 5.5 to 11 times higher with the receptor-binding motif (RBM)	Kannan et al. 2021

Table 1 (continued)

COVID-19 variant	Infection ability	Structural differences	References
Epsilon variant (B.1.427 and B.1.429)	Predicted to be approximately 20% more contagious than co-circulating progenitors and linked to two times greater elevations of upper-airway viruses	For the RBD mutation L452R, this form was the first to be published	Deng et al. 2021
Iota variant (B. 1.526)	The Iota strain was detected for the first time in New York. It reached a predominance of 5–10% in the United States in June 2021, although it was still infrequent outside of the United States	The identical NSP-6 mutation is expressed as in the gamma, beta, and alpha strains	Annavaajhala et al. 2021
Eta variant (B.1.525)	Nigeria has the highest frequency of infection; however, the Eta form is widespread in several nations at modest rates	The RBD alteration E484K is the main feature exhibited by all Eta variants	Ozer et al. 2021
Zeta variants (P.2)	An increased incidence of the Zeta variant occurred late 2020 and early 2021 in Brazil, although this incidence now seems to be declining	The RBD alteration E484K is the main feature exhibited by all Zeta variants	Ferrareze et al. 2021
Lambda variants (C.37)	Prominent in a majority of South American states	The NTD (N-terminal domain) deletion Δ 246–252 and the RBD (receptor-binding domain) spike alterations F490S and L452Q are unique to the Lambda variant (C.37)	Acevedo et al. 2021
Omicron BF.7 (BA.5.2.1.7)	The overall infection rate was 43.1%–75.7% (Beijing) but prior to China, the BF.7 variety has been spreading in the USA and Europe at an exceptionally high level since August 2022	R346T is the result of the Arg346 mutation in BF.7 spike glycoprotein variants which cause a high transmission rate	Chenchula et al. 2022
Omicron BA.5.7.1	Recent reports of BA.5.1.7 in the Inner Mongolia region's Yantai and Shaoguan cities. It is anticipated to emerge as a new dominant variation and may provide a bigger risk due to its rapid transmission frequency and immunity evasion	–	Suresh and Chattamvelli 2022

Table 2 Compilation of laboratory-based nucleic acid testing and immunodiagnostic approaches to SARS-CoV-2

Methodology	Sample screening	Venue	Size of sample	Protocol for identification	Target identification	Detection limit	References
Reverse transcription polymerase chain reaction (RT-PCR)	Lacrimal discharge and tear secretion	Nucleic acid	9 individuals experienced serious complaints, and 21 individuals showed pervasive indications	-	Viral RNA	-	Xia et al. 2020
RT-PCR	Throat, nose, and sputum swab	Nucleic acid	-	-	N genes E genes RdRp-Hel RdRp genes RdRp-P2	8.3 duplicates created per response 3.9 duplicates created per response 11.2 duplicates created per response 3.6 duplicates created per response 21.3 duplicates created per response	Chan et al. 2020
Isothermal LAMP-based	Specimen of respiratory fluid	Nucleic acid	-	-	ORF1ab gene	17 duplicates/ μ L	Yu et al. 2020
Reverse transcription loop-mediated isothermal amplification (RT-LAMP)	Viral RNA	Nucleic acid	-	-	Primer sensor	100 duplicates created per response	Park et al. 2020
Lateral flow immunoassay (LFIA)	-	IgM/IgG antibody	Human plasma samples varied from 39 to 165	-	-	-	Adams et al. 2020
LFIA	Human plasma	IgM/IgG antibody	525 individuals were diagnosed, comprising 128 uninfected findings and 397 positive PCR-confirmed findings	Approximately 15 min	Spike protein	-	Li et al. 2020a
Enzyme-linked immunosorbent assays (ELISA)	Blood specimens from upper respiratory tract swabs in healthy volunteers	IgM/IgG antibody	A total of 90 samples were collected, 40 of which were positive and 50 of which were negative	90 min for monitoring 5–6 h	Spike protein	-	Adams et al. 2020
Sandwich ELISA	Sarcoidosis of the lungs following ablation	IgM/IgG antibody	-	-	Nucleocapsid protein	100 ng/mL	Li et al. 2020b
Proteome peptide microarray (PPM)	Serum specimens	Antibody	-	-	Antibodies	94 pg/mL	Hasan et al. 2023

Table 2 (continued)

Methodology	Sample screening	Venue	Size of sample	Protocol for identification	Target identification	Detection limit	References
CRISPR-Cas12	Saliva specimens	Nucleic acid	–	–	ORF1ab, ORF1b, and RdRp genes in RNA sequences	ORF1ab-10 duplicates/ μL	Lucia et al. 2020

gold nanoparticles, nucleic acid-based biosensors, antibody-based biosensors, and surface plasmon resonance (Truong et al. 2023 and Simon et al. 2023). Nano-based particles have been developed to target viral infections due to their unique physicochemical properties, such as nanoscale dimensions, easily accessible surface modifications, and greater surface proximity. Nano-based particles have been used as drug delivery systems for the therapy of coronavirus infections. Different types of nanoparticles (NPs) can be used as nanomedicines, such as nano-based vaccines, nano-based gene therapy, and nano-based immunotherapy against coronaviruses (McNamee et al. 2023 and Li et al. 2023). The current review mainly focuses on the various available diagnostic approaches, i.e., molecular, immunological, and platforms under development, as well as the future prospects with respect to techniques for the detection of SARS-CoV-2. In addition, a holistic overview of nano-based therapies against SARS-CoV-2 infections is provided.

The data for the current study were acquired from renowned academic databases, including Web of Science, Scopus, PubMed, and Google Scholar, and covers the publication period from 2020 to 2023. The following keywords used such as “SARS-CoV-2 and Diagnostic Tools”, “Nano-based Treatment”, and “Nano-based Vaccines” to get the most relevant articles. Initially, 692 articles were found based on our search keywords. We evaluated the information for preliminary assessment based on the abstract and title of each publication. We deducted all redundant and withdrawn articles, followed by the deduction of non-English-language, paid articles and research paper with no outcome. A considerable number of publications related to SARS-CoV-2 were found, and we limited ourselves to a selection of 200 articles.

Traditional laboratory diagnostic tools for SARS-CoV-2 recognition

Virus culture

Viral culture can be carried out using standard methods. Vero cells were used to inoculate nasopharyngeal and oropharyngeal samples, which were cultured at a temperature of 37 °C with 5% CO₂ in Dulbecco’s modified Eagle medium in combination with 2% fetal serum (bovine) (Park et al. 2023a). The particular cytopathic effects were detected after 3 days of inoculation. Later, real-time RT-PCR was utilized to confirm these effects. By inoculating bronchoalveolar lavage (BAL) samples, researchers from Wuhan, China, were able to isolate 2019-nCoV from human airway Vero E6, Huh-7 cell lines, and epithelial cells (Dastoorpoor et al. 2023). Although the isolation of viruses from human airway epithelial cell cultures is a time-consuming process, it has been proven to be extremely promising for

the analysis of human respiratory infections (Subramanian et al. 2023). Using Vero CCL-81 cells, an Indian team recently announced the first isolation of SARS-CoV-2 (Kumar et al. 2023). The inoculated cells were observed for particular cytopathic effects for SARS-CoV-2 using nasopharyngeal and oropharyngeal samples, then fixed, dried, and cut into segments for transmission electron microscopy using conventional methods. Coronavirus-specific morphology was discovered, as well as virus particle sizes ranging from 70 to 90 nm. The virus was also revealed to be present in a variety of intracellular organelles, including vesicles. The method's decision to choose real specimens relying on the spread of virus loads seen throughout prognostic testing has both strengths and weaknesses, where the clinical features and infections were not taken into consideration while choosing samples, and it was likely that the specificities found for PCR-positive samples may have been overstated since PCR-positive and PCR-negative samples were assessed independently (Park et al. 2023a).

Reverse transcription polymerase chain reaction (RT-PCR)

Currently, available SARS-CoV-2 pandemic diagnostic approaches focus on nucleic acid, protein-based, and antibody detections; however, RT-PCR has been accepted as the ideal reference for pinpointing viral nucleic acids (Du and Wang 2023). Nucleic acid assays provide superior precision and accuracy relative to currently accessible

serological investigations, enabling virus identification. In February 2020, the US Food and Drug Administration (FDA) authorized licensed laboratories to undertake SARS-CoV-2 diagnostic tests (World Health Organization 2020). The initial stage in the procedure is to isolate viral RNA and then convert it to cDNA (complementary DNA). The biological enzyme Taq DNA polymerase is used to expand the cDNA. The overall turnaround time can be as long as 2 days, with the danger of cross-contamination, which reduces specificity. In most cases, the tests are carried out in hospital laboratories (Islam et al. 2023). Variations in viral RNA sequencing can affect real-time RT-PCR findings, utilizing markers that target several virus-related genomic sections. Moreover, false-negative reports may occur as a result of virus progression (Bedoya-Joaqui et al. 2023). The challenges and weaknesses of RT-PCR techniques include specimen retention, inadequate extraction of nucleic acid, cost, and delayed results; even with these drawbacks, the RT-PCR technique is still the preferred tool for diagnosing SARS-CoV-2. Figure 2 shows a schematic workflow of the RT-PCR assay starting with the naso-/oropharyngeal swab, taken from the subject which can be stored at 2–8 °C for 3 days and followed by extraction and purification of RNA. The purified RNA is then transcribed reversibly to DNA using specific enzymes and markers in an RT-PCR machine. If a positive result from the amplification is obtained, then the SARS-CoV-2 virus is present (Cui et al. 2023).

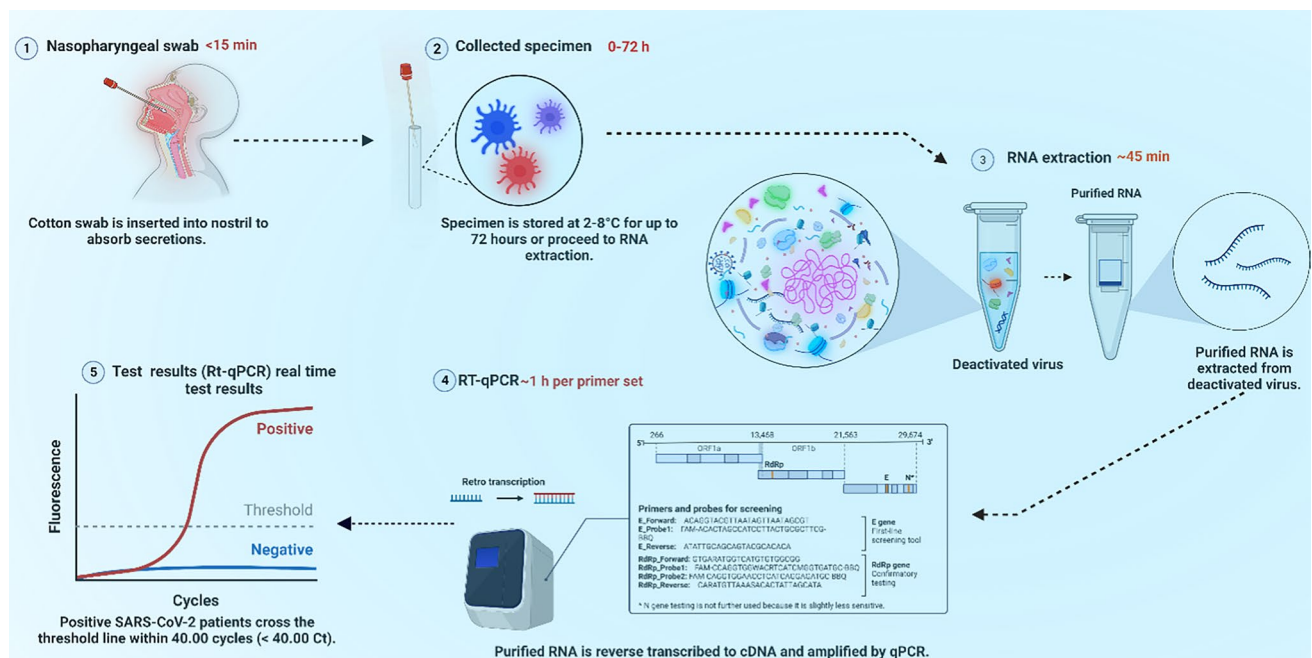


Fig. 2 Diagrammatic illustration of SARS-CoV-2 recognition by RT-PCR assay

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

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is an additional nucleic acid screening approach used to identify SARS-CoV-2. The RT-LAMP approach uses four to six oligos (oligonucleotides), DNA polymerase, and reverse transcriptase of a specific pattern to amplify nucleic acid in a single phase. Turbidity, fluorescence, and colorimetric measurements are employed to identify LAMP-based monitoring procedures. The methodology is lucid to construct and anticipate, and it produces negligible background distortion. Interpretation, experience, and reaction optimization are the key limits of LAMP testing (Moulahoum et al. 2021). The indication readout capabilities of EvaGreen were larger than those of SYBR® Green of the two fluorescent dyes examined (Zhu et al. 2020). RT-LAMP is a viral diagnostic system based on strips/paper incorporated as part of a microfluidic platform (Augustine et al. 2020). In the experiment, fluorescein was assigned to one primer set, and the reaction was catalyzed by labeled RT (Zhu et al. 2020). To develop a perceptible violet pigment with a leucocrystal violet dye, an alternate approach for LAMP reliably identified SARS-CoV-2, allowing for the detection of 100 duplicates per response. The limit of detection of the LAMP assay can be extended by deploying a closed-unit Penn-RAMP, which integrates RT-recombinant polymerase amplification and RT-LAMP in the same unit (Moulahoum et al. 2021 and Song et al. 2021). The strength of RT-LAMP is that LAMP is more proper than RT-PCR for

analyzing a pandemic because it has certain intrinsic features over RT-PCR, such as proliferation at a static temperature, absence of a thermal cycler, a rapid test response, and perhaps a broader diagnostic capability. The RT-LAMP assay sequence is illustrated schematically in Fig. 3. The results of the first three phases of the RT-LAMP technique can be used as a model for LAMP system response. In phase (i) of Fig. 3, LAMP reagents, such as avian myeloblastosis virus (AMV) transcriptase, polymerase (Bst 2.0), and deoxyribose adenosine triphosphate (dATP), are employed to create the amplification solutions. The interaction of LAMP reagents with fluorescein isothiocyanate (FITC)-labeled open reading frame 1a/b (F1ab) forward loop primer (LF) (F1ab-LF*) and biotin-labeled nucleoprotein (np)-backward loop primer (LB)(np-LB*) initiates the isothermal amplification in phase (ii). In phase (iii), traceable SARS-COV-2RT-LAMP solutions are offered. FITC/biotin-labeled F1ab-LAMP and FITC/biotin-labeled np-LAMP amplicons, as well as the results of labeling np-LB* and np-LF* or F1ab-LB* and F1ab-LF* for biotin and digoxigenin, are displayed in phase (iii). The F1ab primer set, on the other hand, is tagged with FITC, and the np-RT-LAMP product is measured with biotin, as well as digoxigenin, whereas the F1ab-RT-LAMP result is tagged with biotin and FITC (Song et al. 2021).

Saliva testing

The presence of SARS-CoV-2 RNA in saliva studies, particularly in nasopharyngeal samples, is essentially irrelevant to the etiology of the disease. On the other hand, human

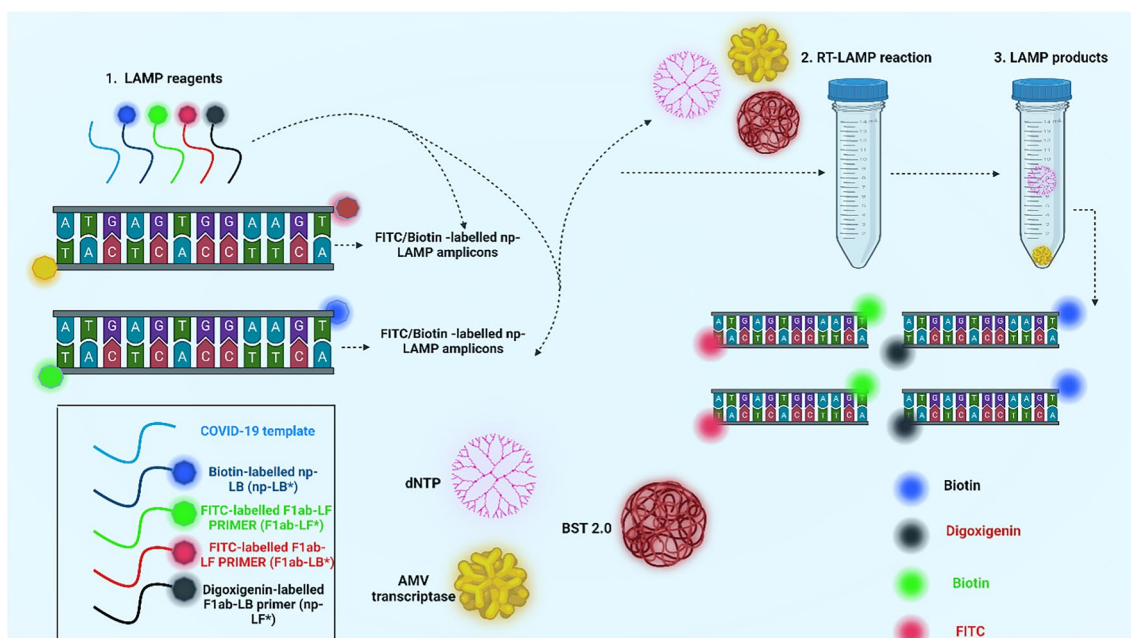


Fig. 3 Schematic representation of SARS-CoV-2 recognition by RT-LAMP assay

saliva is becoming increasingly popular as a diagnostic tool for the diagnosis of infection (Ning et al. 2021). Sputum is a noninvasive and easy-to-use sampling method. Unfortunately, a weakness in the test is that 72% of SARS-CoV-2 patients are unable to provide an adequate sample volume (Galderisi et al. 2023). Saliva is a valuable biological medium because it contains nucleic acids, proteins, hormones, and electrolytes derived from a variety of local or systemic sources. Saliva contains approximately 30% of the biomolecules that are also found in blood, which can be used in the diagnosis of diseases and infections caused by microbes and viruses (Miller et al. 2021). In addition, it has a benefit, as the saliva samples can be stored in emollient solutions and sent to the testing center many days later (Johannsen et al. 2021). The use of RT-PCR to detect respiratory infections in saliva, including two seasonal human coronaviruses, has resulted in matches (Bogdan et al. 2023). Figure 4 shows a schematic workflow of a saliva test. A sample of the subject's saliva is collected. The saliva sample is processed to obtain gene material (RNA) tagging with biomarker for identification and then run for the test. A positive result from the test for viral RNA indicates the sample of saliva contains the SARS-CoV-2 virus.

Fecal test

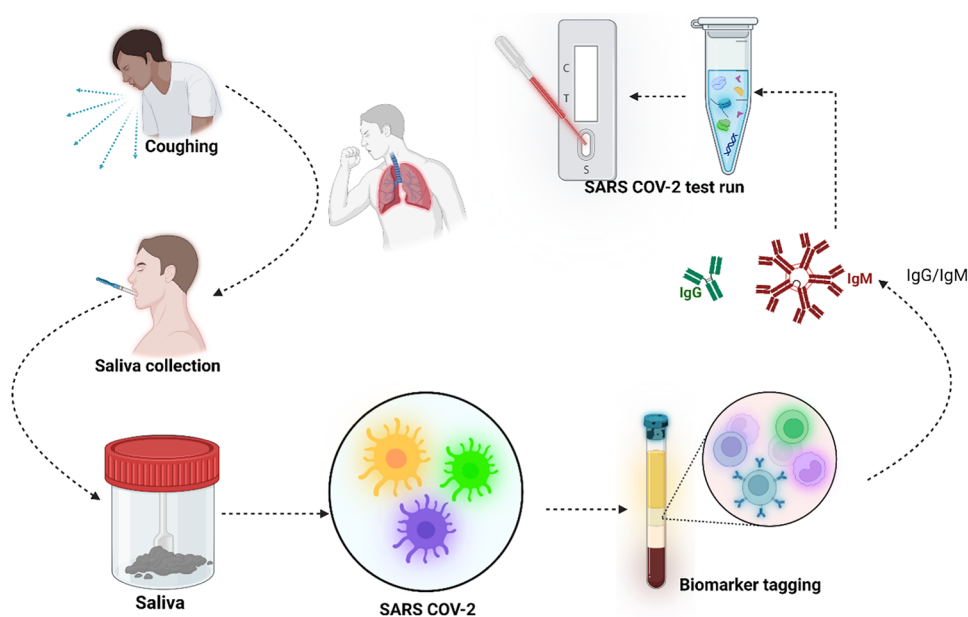
When nasopharyngeal samples were virus-negative, a high prevalence and viral persistence in feces were reported (Xiao et al. 2020). It is worth noting that the viral strain can be determined in stool specimens up to one month after the commencement of the infection. The dangers of healthcare workers being exposed to the feces of diseased patients are well-established, especially in operations that generate a

lot of aerosols. Whereas cough and fever are well-known symptoms, confirmed gastrointestinal symptoms suggest fecal–oral transmission pathways (Nobel et al. 2019). The European Centre for Disease Prevention has urged continued self-isolation based on the persistent virus detaching in feces and respiratory samples 14 days after release. According to studies, the live virus may also be isolated from feces samples, which suggests fecal–oral transmission. SARS-CoV-2 can be tracked through wastewater, which enables community monitoring and might be beneficial in tracking SARS-CoV-2 spread (Xiao et al. 2020).

Radiographic testing

The main diagnostics tool for SARS-CoV-2 is viral nucleic acid RT-PCR testing based on qualitative and quantitative analysis, although their sensitivity for nasal (63%) and oropharyngeal (32%) swab samples is still low (Wang et al. 2020a). As a result, suspected cases, whether or not they have been tested by RT-PCR, require further confirmation. The combinatorial radiographic and antigen-based molecular strategies are the leading methods of assessment of SARS-CoV-2 in research prospects (Hosseiny et al. 2019). Following the onset of breathing complications and the identification of SARS-CoV-2, a patient's first examination generally includes a radiographic imaging assessment, which includes diagnostics techniques such as chest X-ray (CXR) (Liu et al. 2019). However, the imaging characteristics of a conventional CXR are frequently non-specific. The disease's radiographic signs include extensive radiological spots on the lower-left corners and upper lobe of the lung. More well-defined radiographic characteristics appear as the illness progresses, increasing the authenticity

Fig. 4 Schematic representation of SARS-CoV-2 recognition by saliva testing



of a SARS-CoV-2 diagnosis. Whereas a CXR is the most important test for confirming lung illness, it does not rule out other diseases, especially when SARS-CoV-2 symptoms are present, because it is not specific. Meta-analyses of individuals with lower respiratory infections, particularly those admitted to an intensive care unit, support the utility of CXR (Johannsen et al. 2021).

Computed tomography (CT) and magnetic resonance imaging (MRI)

SARS-CoV-2 supplemental diagnostic testing confirms viral infection and allows for ongoing surveillance. For initial identification of SARS-CoV-2-related lung illness, conventional CXR has a sensitivity of almost 60% (Hasan et al. 2023). The bilateral lower zone, hazy opacities, and peripherally prominent consolidation are among the CXR abnormalities (Wong et al. 2020). CT scans also reveal septal thickening, as well as a ‘reversed halo’ pattern (Bernheim et al. 2020). Consolidated pulmonary opacities and bilateral lung parenchymal ground glass with a sometimes-rounded shape and marginal lung dissemination are observed on CT scans. Individuals infected with SARS-CoV and MERS-CoV present with lung engrossment with a peripheral preponderance. A computed tomography scan of the chest with pulmonary ground-glass opacification and association is more likely to divulge SARS-CoV-2 virus infection (Wang et al. 2022a). Chest CT scans might be utilized as an auxiliary assessment technique in conjunction with recurring RT-PCR tests to diagnose patients with negative RT-PCR test results. High-resolution CT scanning is essential for confirmatory analysis and evaluation of disease severity in individuals with possible SARS-CoV-2 infection (Hosseiny et al. 2019). In a survey of 1014 individuals carried out at Tongji Hospital in Wuhan, 59% (i.e., 601 patients) had tested positive by RT-PCR, whereas 88% (i.e., 888 patients) had tested positive by chest CT scans. Even though despite chest CT scans showed a sensitivity of 97% for SARS-CoV-2, 75% (308 of 413 patients) had tested positive by chest CT scans despite negative results from RT-PCR (Liu et al. 2019). The immunological activation of SARS-CoV-2 causes the synthesis of chemokines and cytokines, which typically leave inflammatory cells visible on CT scans as yellow discolorations. Healthcare practitioners should restrict employing MRI in SARS-CoV-2 individuals, according to the American College of Radiology. The American College of Radiology’s implemented protocols suggest that other imaging methods can also be utilized to scan persons who have tested positive or are suspected to have contracted SARS-CoV-2 infection. The main drawback is that disinfecting MRI scanners is time-consuming and involves of several challenges. HEPA (high-efficiency particulate air) filter methods, which

are commonly used to improve air interchange, are incompatible with MRI (Khalili et al. 2020).

Ultrasound

Patients with SARS-CoV-2 are also examined using pulmonary ultrasonography. Although lung ultrasound (LUS) does not appear to be specific for SARS-CoV-2 pneumonitis or pneumonia, it is recommended to identify the infection location. The merits of LUS are that it might be informative in the prompt detection of SARS-CoV-2 pneumonia as an expensive method of determining the site of infection; owing to its high sensitivity to positive terminal outflow, LUS results are more sensitive than CXR. LUS has several characteristics, including pulmonary consolidation in severe local illnesses. LUS revealed more apparent signs of SARS-CoV-2 pathogenesis in the posterior and inferior pulmonary regions in individuals with SARS-CoV-2 infection, similar to what was detected with CXR or chest CT. The infection usually spreads from the peripheral area to the center of the pulmonary tissues. LUS observations of pulmonary edema are frequently used by intensive care unit staff to position SARS-CoV-2 patients therapeutically (Poggiali et al. 2020).

Virus detection from breath

For the objective of monitoring SARS-CoV-2, the exploration of expelled breath may be a less intrusive form of analysis (Škapars et al. 2023 and Enoch Amor et al. 2023). However, screening for severe acute respiratory syndrome SARS-CoV-2 from expelled breath has proven to be quite complicated. Aspects of these investigations, which are especially significant, reveal that SARS-CoV-2 individuals breathe billions of severe acute respiratory syndrome coronavirus RNA molecules each hour (Ma et al. 2019). Expiratory breath exhibited a higher affirmative incidence (26.7%) than air (3.8%) and surface (5.4%) specimens, according to scientific interpretation. Moreover, it was indispensable to assemble the specimen for a prolonged period by adopting a distinctive methodology called expelled breath condensate (EBC) in an attempt to immediately pinpoint the virus from the expelled breath. Current investigations have shown that nonvolatile components, including viruses, bacteria, DNA, and RNA, may be readily detectable and observed by extracting and analyzing the aqueous fraction of the expelled breath (expelled breath aerosol (EBA) or EBC) (Lamote et al. 2020). Depending on these two factors, an EBC system can effectively capture various droplets: (1) the proportion of SARS-CoV-2 virus that is still alive after extraction and (2) the ratio of the percentage of particulates extracted to the total percentage of particulates in the air. Haick and colleagues conducted an introspective scientific analysis in Wuhan, China, for SARS-CoV-2 in March 2020, which

included testing with a breath analyzer device that follows the principle underlying chemo-resistive sensors made up of gold nanoparticles in combination with machine intelligence algorithms (Škapars et al. 2023 and Shan et al. 2020). In another experiment, investigators explored preliminary markers of enhanced generation of reactive oxygen species in SARS-CoV-2 swab specimens (Miripour et al. 2020). In this approach, exposing swab specimens to an electrochemical sensor nanostructured with carbon nanotubes revealed 97% real affirmative recognition readings in 30 s.

Immunological/serological techniques for SARS-CoV-2 recognition

Considering molecular approaches have limitations, novel methodologies that employ antibodies or antigens in inpatient specimens to identify SARS-CoV-2 have been developed.

Antibody testing

Capillary blood sampling is the most common sample source used in antibody screening. The specimens are analyzed for patient antibodies, viz. IgA, IgM, and IgG, that are sensitive to a viral epitope to confirm the existence of SARS-CoV-2. The most prevalent antibody candidates for detection are IgM, IgA, and IgG antibodies. As a result of viral multiplication, the viral load in the samples fluctuates with time. As a consequence, the quantity of viral infection is a constraint of serological approaches. SARS-CoV-2 screening is now carried out using a variety of point-of-care (POC) diagnostic methods. These techniques include quick enzyme-linked immunosorbent assays (ELISA), proteome peptide microarrays (PPM), and lateral-flow diagnostic tests. The main drawback is that the predictive value of the test (false-negative report) is commonly reported (Kohmer et al. 2021).

Enzyme-linked immunosorbent assays (ELISA)

Antibodies, antigens, hormones, proteins, and glycoproteins are all ubiquitously identified and characterized using ELISA (Kohmer et al. 2021). Three distinct types of ELISA techniques are deployed to pinpoint SARS-CoV-2. These techniques comprise indirect ELISA, direct ELISA, and sandwich ELISA. Antibodies/antigens are exploited in both indirect and direct ELISA to pinpoint viral infection. Viral epitopes are spotted in sandwich ELISA after they adhere to detection and capture antibodies. The individual SARS-CoV-2 antibodies/antigens from human specimens are immobilized overnight on 96-microwell plates in all three procedures. An enzyme–substrate coupling enables these antibodies/antigens to adhere to the polystyrene surface

(Sebbar and Choukri 2023). Antiviral antibodies from human specimens adhere to the surface when the pathogenic protein is expressed. A supplementary tracer antibody that gives a visual indication can be used to identify the antibody–protein aggregates (Kohmer et al. 2021). The median time required to obtain a response is 2–5 h. In contrast to IgA, IgM, and IgG antibodies, proinflammatory cytokines, such as interleukin-6 antibodies, are incorporated in ELISA to distinguish the existence of viral antibodies (Sebbar and Choukri 2023). The ELISA approach has significant advantages over nucleic acid analysis techniques to identifying SARS-CoV-2, which include high accuracy and sensitivity (Park et al. 2023b). Another feature of ELISA is that it takes less time to execute than other approaches to nucleic acid analysis. Furthermore, using gold nanoparticles (AuNP) with an ELISA assay enhances the efficiency of colorimetric antigen recognition for SARS-CoV-2 spike antigen (Bradley et al. 2023). An assessment of the several methods of ELISA-based techniques revealed that each has significant advantages in identifying the existence of viral antigens or antibodies in afflicted individuals. Direct ELISA minimizes the possibility of cross-reactivity with secondary antibodies because it only employs primary antibodies. Indirect ELISA is convenient, as it is very adaptable and may be used with various primary antibodies. Sandwich ELISA is the most sensitive of the three varieties (Yadav et al. 2023). Irrespective of its advantages, there are numerous pitfalls to adopting ELISA for viral antigen or antibody detection. For instance, some commercial diagnostic tools are designed for research study purposes and have never been used in a clinical context. Secondly, in an ELISA test, monoclonal antibodies are inadequate to capture the mutant SARS-CoV-2 virus (Žak et al. 2021).

Chemiluminescence immunoassay (CLIA)

CLIA is a method for identifying the prevalence of antigens or antibodies in human blood samples. Presently, protein-coated and magnetic microparticles are adopted in this methodology. Chemiluminescence immunoassays are identical to direct ELISA in terms of concept. The primary variation is that the outcomes are interpreted using luminescence. The CLIA methodology has numerous advantages. First, because of the strong signal magnitude and lack of interference emission, CLIA can achieve better specificity and sensitivity to SARS-CoV-2 than other serological procedures, such as ELISA and LFIA. CLIA has a cumulative sensitivity of 97.8% quantifying IgG/IgM antibodies. LFIA has a sensitivity of 66%, whereas ELISA has a sensitivity of 84.3% (Rahayu et al. 2023). The introduction of protein-coated and magnetic microparticles to CLIA improves the sensitivity of the technique. The high cost and requirements for a sophisticated apparatus and highly skilled specialists

to detect SARS-CoV-2 are a few of the key constraints of CLIA (Liu et al. 2020a).

Lateral flow immunoassay (LFIA)

The lateral flow immunoassay is a subjective separation technique used to detect the involvement of a specific biomarker in an unknown specimen. This procedure often uses fluids from nasal swabs, capillary blood sampling, urine, and saliva as specimen materials. These specimens are investigated the occurrence of the SARS-CoV-2 virus by screening the human antibodies (IgA, IgM, and IgG) that are sensitive to pathogenic antigens in several target sites. Spike (S) glycoproteins (receptor-binding area S1 and S2 subunits) and nucleocapsid (N) proteins are among the antigens that have been identified as targets (Wu et al. 2020). LFIA is usually designed as a band with an absorbent pad, a conjugate pad, a membrane zone, a sample pad, and a visualizing zone (Fig. 5). The mechanism of LFIA is dependent on capillary forces transferring a sample fluid across multiple regions of a band. A pathogenic antigen is coupled to fluorescent or gold particles to identify SARS-CoV-2-associated antibodies. The pathogenic antigen is effectively stabilized on the conjugate pad, and the antibodies in the human specimen adhere to it. SARS-CoV-2-targeting antibodies interact with the gold-conjugated antigen of SARS-CoV-2 if they are expressed in a specimen. Then, the antibody–antigen complexes flow to the device-capturing zone. The antibody–antigen complexes interact with additional antibodies in the capturing zone, triggering color development. The LFIA concept is used by a variety of sophisticated laboratories and organizations to manufacture kits. These systems exist

in a variety of packages, including casings and cassettes that are analogous to those employed in pregnancy testing (Ma et al. 2019). The LFIA implementation of antigen/antibody screening to discern the overall status of SARS-CoV-2 has significant potential. First, LFIA is equipped to generate results promptly. Generally, SARS-CoV-2 is identified by LFIA, which takes around 10–30 min. Secondly, two weeks after exposure to infection, LFIA accuracy and sensitivity for detection of SARS-CoV-2 improved. Thirdly, LFIA is low-cost, and the findings are accessible to the human eye (Kim et al. 2021).

Antigen testing

Antigen testing is a method used to detect SARS-CoV-2 infection. In this method, serological approaches and antigen tests primarily use nucleocapsid proteins (N) and spike glycoprotein (S). When identifying the SARS-CoV-2 spike (S) glycoprotein with the LFIA technique, Baker et al. suggest that glycans are used as the capture moiety (Baker et al. 2020). Nasopharyngeal and nasal swabs are the most commonly used sample sources. These samples are tested for specific antigens at different target sites (nucleocapsid protein and spike glycoprotein) to confirm SARS-CoV-2 infection. Antigen testing has several advantages, including high efficiency and sensitivity, immediate results, high specificity, and low cost (Mitchell and Ventura 2020).

Proteome peptide microarrays (PPMs)

The proteome peptide microarray approach is extensively applied to explore antibody–protein relationships in the

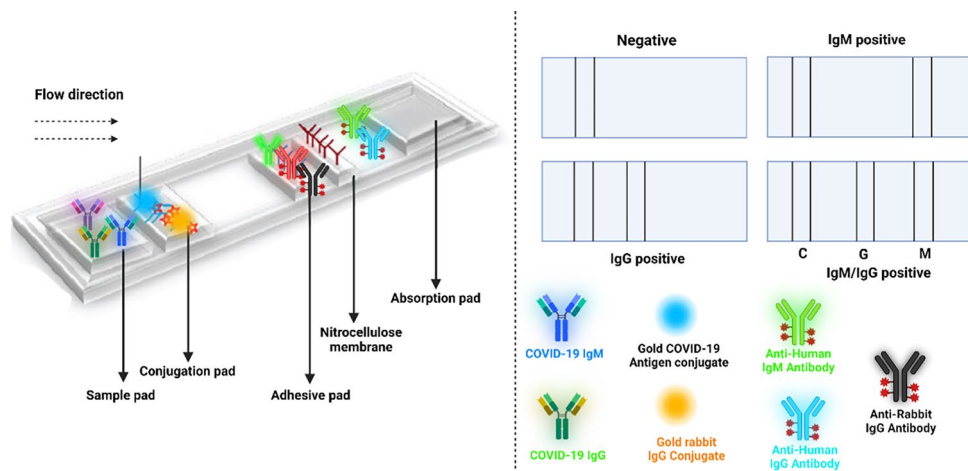


Fig. 5 LFIA for anti-SARS-CoV-2 antibody detection. The specimen material circulates horizontally down the band, which contains several zones, including a sample pad, an absorbent pad, adhesive pad, conjugate pad, and membrane pad. The conjugate pad comprises antibodies specific to the targeted molecule, as well as antibodies

complexed with signaling markers, i.e., gold and fluorescent particles. The screening columns (IgM and IgG columns) and the reference column are positioned on the nitrocellulose membrane. The final region is the absorption pad, which hinders liquid reflux

amino acid domain (Holenya et al. 2021). This approach provides insights into affinity mapping and viral protein motif recognition for antibody interaction toward SARS-CoV-2 domains. High-density proteome peptide microarrays have recently been exploited to investigate the interactions between antibodies in a patient's serum. This approach can aid in defining infection indications by aiding in the comprehension of antibody responsiveness to the SARS-CoV-2 protein. The underlying feature of the PPM approach is that it offers an exhaustive overview of antibody sensitivities to the viral protein (Wang et al. 2020b). The advantages and limitations of the above-mentioned methodology in the diagnosis of SARS-CoV-2 are depicted in Table 3.

Impending techniques for SARS-CoV-2 detection

Clustered regularly interspaced short palindromic repeats (CRISPR/Cas)

In contrast to established PCR approaches, the nucleic acid screening strategy based on CRISPR/Cas was designed using the attributes of affordability, accuracy, quickness, and sensitivity (Hillary et al. 2021). Wang et al. designed a technique for diagnosis with as few as ten duplicates of SARS-CoV-2 within 45 min that does not require specific equipment and demonstrated high accuracy with the qPCR technique (Wang et al. 2020d). Technologies based on CRISPR diagnostics (CRISPR-Dx) provide intriguing solutions for the detection of viruses. The CRISPR bacterial technology can distinguish and digest extraneous genetic substances. To support target-binding CRISPR RNA (crRNA), the CRISPR-associated (Cas) proteins, namely Cas13 and Cas12, exclusively bind to RNA and DNA strands (Casati et al. 2022). DNA endonuclease-targeted CRISPR trans reporter (DETECTOR) and specific high-sensitivity enzymatic reporter unlocking are the names of the Cas12- and Cas13-based detection systems (SHERLOCK), respectively. Utilizing SHERLOCK in lateral-flow configurations, information can be interpreted in less than 60 min to find viruses, pathogens, and tumors. Loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), which do not necessitate advanced technology, have been coupled with Cas-mediated nucleic acid identification to obtain considerable accuracy (Kevadiya et al. 2021).

Wang et al. successfully generated SARS-CoV-2 readily bounded, interspaced brief palindromic repetitions of RNAs, a single-stranded DNA reader, and Cas12a proteins. They further tagged the single-stranded DNA reader with a quenching green fluorescence marker readily fragmented by proteins of Cas12a under the existence

of nucleic acid of SARS-CoV-2 in the monitoring device, ensuring that the green illumination would be visible to the naked human eye by using a 485-nm wavelength light, facilitating on-site detection (Wang et al. 2020d).

An RNA-targeting molecule called Cas13 provocatively cleaves non-target nucleic acids in samples. In order to determine femtomolar quantities, the enzyme cleaves specific nucleotide molecules, such as various RNA molecules (collateral cleavage). A thermostatic pre-amplification stage has been employed in Cas13 and detected by the SHERLOCK method. This SHERLOCK CRISPR SARS-CoV-2 kit has been granted emergency use authorization (EUA) for the purpose of detecting SARS-CoV-2 and the test is having merits where currently, more than 160 viral variants can be detected using SHERLOCK-based multiplexed diagnostics. By using the CRISPR-Cas12a/guide RNA complex and a fluorescent sensor with RT-PCR or isothermal recombinase polymerase amplification, the targeted amplicons are obtained by employing viral F1ab and nucleocapsid area markers that capture two RNA variants. A negative RT-PCR report can provide a positive result in fluorescent detection based on CRISPR (Kevadiya et al. 2021).

The fabrication and validation of nucleic acid are based on Cas-13 techniques that use freeze-dried chemicals and swift specimen ablation at normal temperatures. The screenings, namely SHINEv.2 (for “streamlined emphasizing of diseases to manage epidemiological emergencies, version 2”), expedite the previously published RNA-extraction-free SHINEv.1 technique by doing away with the heating process and reagent storage (cold condition). By utilizing lateral-flow techniques and incubating in a thermal bath at 37 °C, SHINEv.2 can pinpoint SARS-CoV-2 in nasopharyngeal specimens in less than 1 h 30 min with 90.5% vulnerability and 100% accuracy (as compared to RT-qPCR). Additionally, SHINEv.2 enables the morphological distinction of the SARS-CoV-2 subtypes omicron (O), delta (δ), gamma (γ), beta (β), and alpha (α) and may be used without efficiency shortfalls by utilizing body temperature (Arizti-Sanz et al. 2022).

Earlier research, known as PAC-MAN (prophylactic antiviral CRISPR in humans), evidenced that virus-targeting crRNAs and Cas13d may restrict influenza-A virus (IAV) and SARS-CoV-2 sequencing. SARS-CoV-2 gene mutation escape has been demonstrated to be restrained by Cas13b, whereas Cas13a was recently found to block the influenza virus or SARS-CoV-2 in laboratory animals. The ability of CRISPR to attack numerous newly emerging SARS-CoV-2 and related coronavirus variants as a broad-spectrum antiviral is still uncertain, but it can be used as a pandemic preparation tool for future strains of concern (Kevadiya et al. 2021).

Table 3 Pros and cons of existing diagnostic techniques, along with immunological methodologies, for SARS-CoV-2

Methodology	Advantages	Limitations	References
Virus culture	Cell culture is beneficial for viral isolation and characterization	For diagnostic applications, cell culture for viral isolation is not suggested	Wang et al. 2020c and Mathuria and Yadav 2020
RT-PCR	Offers enhanced specificity and sensitivity for virus identification	Real-time RT-PCR findings employing primers targeting diverse viral genome areas can be influenced by variations in the viral RNA sequence. Furthermore, as a result of viral evolution, misleading negative outcomes may emerge. Sample storage, inadequate nucleic acid purification, cost, and delayed results are all disadvantages	Shen et al. 2020 and Smyrlaki et al. 2020
RT-LAMP	This method is easy to execute and visualize, and it produces minimal background interference	Interpretation, experience, and reaction optimization are key limitations	Jiang et al. 2020
Saliva test	Saliva is an important biological medium because it contains proteins, nucleic acids, electrolytes, and hormones from a variety of local and systemic sources. It is thought to contain approximately 30% of viral pathogens and biomolecules found in blood. Saliva samples can be stored in stabilizing solutions	Some patients are unable to produce sufficient sample volumes. This method also poses a risk to healthcare staff through sneezes or coughs, as well as viral particle transmission via aerosols	Nicola et al. 2020
Fecal test	The viral load in a stool sample can be identified up to four weeks after the commencement of the disease	The danger of healthcare staff being exposed to the feces of infected individuals and the risk of fecal–oral transmission	Huang et al. 2019
Radiographic testing	–	Sensitivity for nasal and oropharyngeal swab samples is still low	Liu et al. 2020b
Computed tomography and magnetic resonance imaging	Abnormalities are detected even in asymptomatic individuals with positive RT-PCR	Alternative imaging technologies can be used to screen individuals who are assumed to have SARS-CoV-2 infection or who have been confirmed positive. Cleaning MRI equipment takes a long time and presents substantial difficulties	Nasiri and Alavi 2022
Ultrasound	Cost-effective for early identification and to establish the location of infection	Unable to identify deep and intrapulmonary lesions	Abduljalil 2020
Antibody test	Finger pricks are an easy way to collect samples	Because of viral replication, the viral concentration in the specimens fluctuates over time	Baker et al. 2020
ELISA	When an infection occurs more than two weeks before testing, it has high sensitivity and specificity, and the processing time is lower in comparison to nucleic acid testing procedures	Commercial testing kits are mostly used in research applications and are not widely used in clinical settings. Monoclonal antibodies are unable to recognize the mutated virus	Žak et al. 2021
Chemiluminescence immunoassay (CLIA)	Can achieve greater sensitivity because of the high signal intensity and lack of interfering emissions	The high cost, complex instrumentation, and requirements for highly trained technicians associated with this technique	Bastos et al. 2020
LFIA	Provides results rapidly and at a low cost. The results are visible to the naked eye	It is less sensitive than other serological tests. Controlling the fluid velocity and capillary forces during the antigen-capture process might be difficult. The time required for analysis depends on the nature of the sample	Adams et al. 2020
Antigen testing	Provides rapid results with high sensitivity and specificity. It is an inexpensive process, and results can be detected with the naked eye	Difficult to perform due to the lack of widely available and affordable self-testing kits, and results are inaccurately interpreted due to reader error	Nguyen et al. 2020

Table 3 (continued)

Methodology	Advantages	Limitations	References
PPM	It provides a comprehensive visual representation of antibody behavior with respect to virus-infected proteins	Post-translational modifications or conformations may have altered the epitopes of numerous SARS-CoV-2 antibodies. Depending on which viral strain is present in the sample, the test results may vary	Wang et al. 2020b

Nicking endonuclease amplification reaction

Nicking endonuclease amplification reaction (NEAR) is used to exponentially amplify short oligonucleotides. Both nicking endonuclease enzymes and strand-displacement DNA polymerase (e.g., Bst polymerase) are used (Cao et al. 2022). The first step consists of combining a sample with nicking primers P1 and P2, each containing a restriction or stabilization site, a binding sequence, and a nicking site. The displacement extension, nicking, and primer-binding activities result in double-stranded DNA with restriction sites at both cleavages. Second, the restriction sites of the duplex are then cleaved by nicking enzymes, resulting in two free-ending templates that are unstable at 55 °C and ready for separation (NguyenVan et al. 2021). The advantage of this method is the amplification of the products, which is achieved by repeating the polymerization and single-strand cleavage on each template. These products also hybridize to the primers and support bidirectional amplification until the reaction mixture components are depleted. Thousands of copies can be made with a single restriction site, making NEAR a unique amplification technology with maximum efficiency. A key drawback of NEAR is the generation of non-specific yields that lower detectability and increase the diagnostic approach. Identifying the ideal reaction conditions (e.g., nicking enzyme content, Mg²⁺ content, and generated heat of reactivity) could help to solve this problem (Cao et al. 2022). ID NOW tests for influenza and group A streptococci are already on the market, favoring the rapid market introduction of ID NOW SARS-CoV-2. This assay was created to identify an array of RNA-dependent RNA polymerase (RdRp) segments in the SARS-CoV-2 genome, and the LOD result was 0.125 copies/mL; the assay has received FDA-EUA approval for SARS-CoV-2 (Nie et al. 2020).

Recombinase polymerase amplification

The principle of analogous DNA recombination is used to replicate double-stranded DNA in recombinase polymerase amplification (RPA) (Bai et al. 2022). These compounds look for homologous structures in the focused DNA and subsequently occupy appropriate locations. After the restriction enzyme has unpacked the nucleoprotein-bonded strand, the DNA polymerase displaces strand extension. Activated recombinases develop to produce fresh nucleoprotein strands for subsequent cycles, and the changed strand is maintained by uni-stranded binding proteins during this phase. Upon completion of this procedure, the targeted double-stranded DNA is extensively replicated (Jiang et al. 2022). Xia et al. described RPA primers targeting regions of the N gene to detect SARS-CoV-2. To enhance SARS-CoV-2 one-spot RNA reverse transcription, standard RPA solution was

combined with transcriptase and RNase inhibitors. Available fluorescent or lateral-flow probing tools were used to identify the enhanced targets. At an LOD of 0.2 copies per microliter, the response takes approximately half an hour to complete. However, instead of studying extracted viral RNA samples, the outcomes remain confined to utilizing artificial RNA (Xia and Chen 2020).

Virus neutralization test

The viral neutralization test (VNT) is a key indicator of a person's evolving antibody status against a target virus. An advanced kind of immunoassay used to identify antibodies capable of preventing viral reproduction *in vitro* is the live virus neutralization test (VNT). The live VNT is regarded as the gold-standard technique for evaluating neutralized antibodies (nAbs) and was utilized to establish a lower serum threshold value to screen against SARS-CoV-2 infection (Harcourt et al. 2020). Several types of neutralization tests are available, where studies have been performed with SARS-CoV-2. In the plaque reduction neutralization test (PRNT), colony-forming cells are quantified on an agar or carboxymethyl cellulose-coated cell layer, whereas neutralizing antibody titers are calculated using immunocolorimetric analysis in the focus reduction neutralization test (FRNT). A recent study evaluated the efficacy of FRNT and PRNT testing for specified RBD IgG reactions produced by SARS-CoV-2 individuals 6 days after PCR analysis and discovered a substantial association among the tests (Suthar et al. 2020). Additionally, pseudo-virus-based neutralization assays (PBNAs) have been developed by several researchers, employing pseudo-virus (PSV) as a preferable (biosafety level 2) alternative for the detection of SARS-CoV-2 (Wang et al. 2020e). PSV was created by integrating the S protein of SARS-CoV-2 in the envelope of a pseudo-type of vesicular stomatitis virus, further revealing that neutralizing antibody titers peaked 10–15 days following the initiation of the illness and then remained steady (Mitchell and Ventura 2020). In a study, almost 30% of recuperated subjects ($n=175$) had minute concentrations of neutralizing antibodies; this finding could have consequences for how serologic tests are applied and interpreted to detect previous SARS-CoV-2 infection. The main strength of this test is that it acts as a good indicator, and also helps to comprehend the prominence of protective immune response in asymptomatic and symptomatic cases (Guo et al. 2022).

Next-generation sequencing

Next-generation sequencing is a technology that, in conjunction with existing methods, is intended to simultaneously establish the pattern of numerous microscopic DNA extracts (Chiara et al. 2021). Next-generation sequencing

has proven effective in identifying and studying infections (SARS-CoV-2) when integrated with bioinformatics approaches (Hillary et al. 2021). This technology facilitates the retrieval of knowledge from a viral genome while also recognizing the occurrence of a pathogen in a specimen. This potentially efficient methodology was designed by Illumina (Carter et al. 2020). This technology having the advantage of indicating the appearance of several coronaviruses (SARS-CoV-2) variants in a specimen, as well as the presence of diverse pathogenic entities (Wang et al. 2020d).

Amplicon-based metagenomic sequencing

Amplicon-based metagenomic sequencing is an additional high-throughput sequencing approach for recognizing SARS-CoV-2. This approach, analogous to next-generation sequencing, recognizes and characterizes the microbiota in nasopharyngeal samples by integrating the base amplicon with metagenomic sequencing. By enhancing viral evolutionary analyses, genomic epidemiological investigations, and contact mapping, this amplicon-based sequencing approach advances the identification of SARS-CoV-2 infection. This approach is having the strength to be utilized in determining the extent to which a genomic expression has deviated (Moore et al. 2020).

Vertical flow assay

A vertical flow assay is an innovative technique for identifying SARS-CoV-2. This method is identical to a lateral flow assay, although devoid of a horizontal flow sequence, instead adopting a vertical flow sequence. These techniques encompass the development of antibody–antigen interaction, the adsorption of the capturing antibodies into a readout surface, and the adsorption of the tagged detecting antibodies to create a visual indication (Lei et al. 2022). Vertical flow assays furnish multiple features, including the utilization of extrinsic factors, viz. capillary and gravitational forces, and the ability to quickly duplicate the analysis. Furthermore, the detection advantage of a vertical flow assay is more rapid than that of a lateral flow assay, and the assay insights can be interpreted by inexperienced personnel (Kim et al. 2021).

Detection of SARS-CoV-2 by nano-based diagnostic techniques

Colorimetric assay

When the rate of SARS-CoV-2 infection is intense, a simple, rapid, and accurate “naked-eye” colorimetric SARS-CoV-2 diagnostic assay is required. Colorimetric assays make use of gold nanoparticle (AuNP) optical features and target

antisense oligonucleotides (ASOs), aiming at the phosphoprotein of SARS-CoV-2 altered with conjugated thiol (Duan et al. 2021). After RNA isolation, the detection is completed within 10 min. When the targeted SARS-CoV-2 RNA sequence is present, the ASO-modified thiol binds the AuNP combination, resulting in a shift in the surface plasmonic resonance, as shown in Fig. 6. The RNA strand is broken from the DNA-RNA fusion when RNase H is added, followed by the fabrication of a visible deposition aided by AuNP accumulation. In the manifestation of RNA of viral MERS-CoV, the LOD utilizing this approach was 0.18 ng/L of RNA with a SARS-CoV-2 viral load. A colorimetric analysis using a comparable method was also used to identify the occurrence of the Middle East respiratory syndrome coronavirus (MERS-CoV) (Moitra et al. 2020).

Microfluidic devices

Microfluidic devices offer an alternative way to conduct a proof-of-concept study where the chip is only the size of a palm inscribed with micrometer-sized networks and response compartments composed of paper, polydimethyl-sulfoxide, or glass, with benefits such as small size, rapid identification time, and low sample volume. The essential premise of these microfluidic chips is that they use capillary action and electrokinetic capabilities to mix and separate

liquid samples (Kumar et al. 2022). Multiple antibodies toward three sexually transmitted diseases can be identified of using microfluidic devices and a smartphone presentation connection, exhibiting 87% exactitude and 100% intensity. These devices can be adapted to detect the presence of RNA (SARS-CoV-2) or proteins, owing to their convenience and durability (Kumar et al. 2021a). Several modalities of study utilizing a microfluidic channel are shown in Fig. 7.

Gold nanoparticles (AuNPs)

The use of AuNPs in biopharmaceutical and clinical contexts has advanced significantly. AuNP innovations have the advantages of being sensitive, expeditious, simple, and versatile, in addition to permitting quantitative analysis with outstanding combinatorial abilities. The sensitivity ranges of AuNP-based technologies for the recognition of antibodies and nucleic acid are equivalent to or superior to those of commercial ELISA kits that are not based on AuNP and RT-PCR. As a result, AuNP-based SARS-CoV-2 screening is a plausible substitute for RT-PCR, presenting opportunities to address this major unmet biological need, particularly in venues with inadequate facilities (Wang et al. 2022b). Research conducted *in vivo* and *in vitro* revealed that the respiratory syncytial virus was irreparably deformed by AuNPs with extended chains of mercaptoethanesulfonic acid (MES)

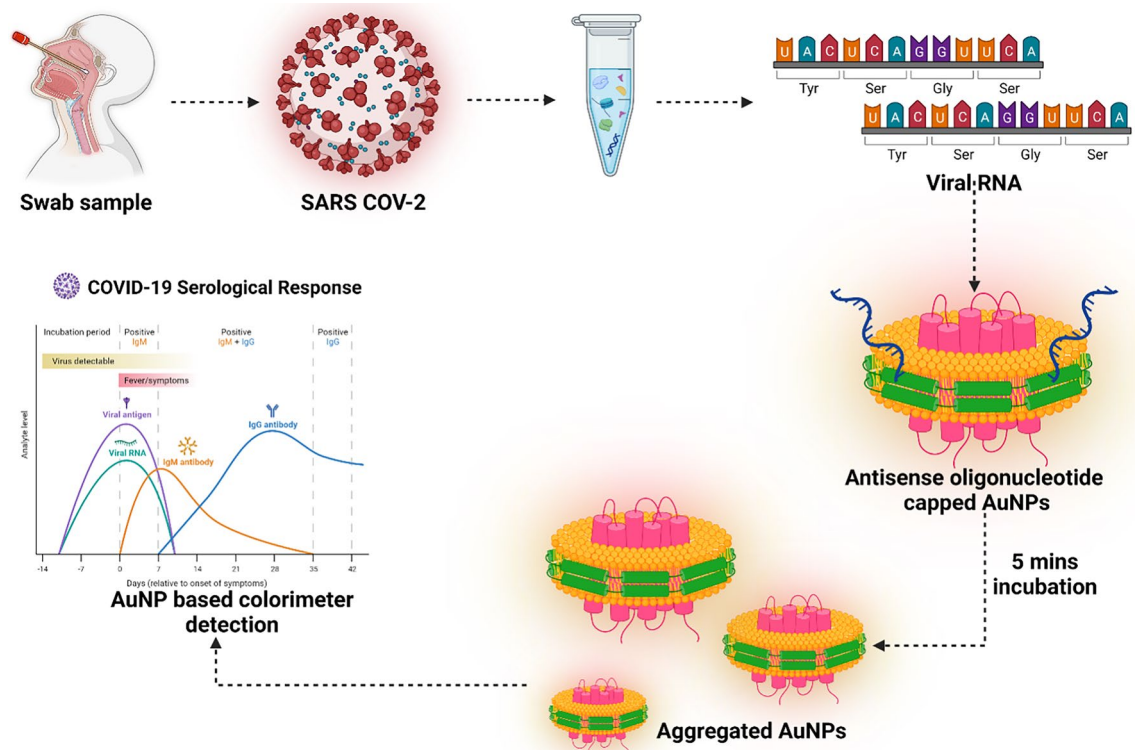


Fig. 6 SARS-CoV-2 detection utilizing gold nanoparticles (AuNPs) in a swab sample

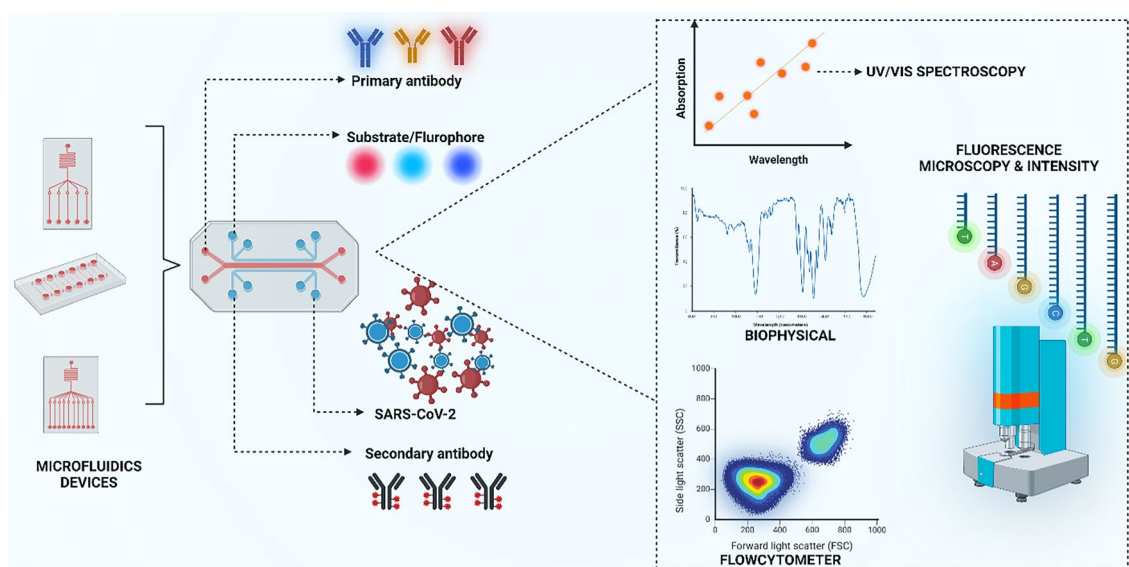


Fig. 7 Microfluidics approaches for coronavirus detection utilizing antibodies against SARS-CoV-2

and sulfonate undecanesulfonic acid (MUS). A molecular interaction exposes the combination of AuNPs and MUS, which is connected to the virus in a multivalent association. The structure of the capsid also disintegrates as a result. This multivalent bonding consequently offers a compelling plan of approach for COVID-19 treatment. In one study, AuNP was also used against an RNA virus, which may be used to treat SARS-CoV-2. Based on the immunochromatography of gold nanoparticles, a commercialized SARS-CoV-2 antibody testing kit was developed using the colloidal gold method. Although the test has to be validated on entire viral RNA sequences from human specimens, it may ameliorate the present stress on PCR-based diagnostics (Asdaq et al. 2021).

Magnetic nanoparticle-based separation

Molecular analysis for SARS-CoV-2 detection begins with the isolation of nucleic acids from a medical specimen, which is a slow and tedious process. Magnetic nanoparticles encapsulated in carboxyl polymer (pcMNP) were produced to facilitate the extraction of viral RNA for the diagnosis of SARS-CoV-2 RNA (Zhao et al. 2020). RT-PCR can also be performed with pcMNP–RNA complexes, providing a number of benefits over standard column-based extraction methods, including the ability to adhere viral RNA, as well as enhanced sensitivity. In addition, the pcMNP–RNA complex generated during MNP extraction can be used with a number of isothermal amplifying methods, such as LAMP. As a result, this technology may be utilized to create proof-of-concept systems (Khizar et al. 2022).

Surface-enhanced Raman scattering (SERS)

Surface-enhanced Raman scattering spectroscopy takes place in a robust investigative platform with the aim of molecular characterization (especially detection of viral antigens and DNA sequences), which can be extremely convenient for diagnosing applications when integrated with the intrinsic chemical and optical attributes of nanoparticles (Cha et al. 2022). SERS improves conventional fluorescence-based screening techniques with respect to specificity, sensitivity, and screening of distinct constituents in a blend, which is becoming increasingly relevant for therapeutic diagnosis. However, no outcomes have been reported to date with respect to the ability of SERS to recognize SARS-CoV-2 (Berry et al. 2021).

Surface plasmon resonance (SPR)

Surface plasmon resonance is a versatile visual platform that is often used to analyze the refraction index alteration of plasmonic components in real-time surfaces (Bahl et al. 2021). The term LSPR (localized surface plasmon resonance) refers to a photon-driven combined alternation of transmission band electrons on the planes of relatively small plasmonic sensible configurations (such as metal NPs). The plasmonic field exhibits remarkable reactivity, with an alteration in refractive index and molecular adherence of the LSPR sensing systems in the proximity of tiny structures (nanoscale range). As a result, LSPR may be used as a typical system for label-free, real-time diagnosis of very small amounts of samples (Qiu et al. 2020). A rapid and effective multi-use biosensor based on LSPR was devised recently

by combining plasmonic sensing attributes and photothermal abilities to identify the viral SARS-CoV-2 nucleic acid (SC-NA) on a singular plasmonic nanoabsorber chip made of gold nanoislands (AuNI) (Jiang et al. 2021a). Ultimately, this setup allows for the label-free and real-time exposure of any nucleic acid sequence, especially E genes, RdRp-COVID, and ORF1ab COVID from SARS-CoV-2. Moreover, this biosensor of LSPR can determine the availability of samples at quantities greater than 0.22 pM and provides a simply realizable diagnostics phase, which, when combined, could increase diagnostic accuracy and minimize reliance on PCR-based assays (Qiu et al. 2020).

Biosensors

Biosensors represent a rapid, high-efficiency diagnostic technology. Qiu et al. designed a double plasmonic biosensor that identifies SARS-CoV-2 by integrating the localized surface plasmonic resonance with the plasmonic photothermal effect (Qiu et al. 2020). The researchers employed a biosensor based on a field-effect transistor that can recognize SARS-CoV-2 from a human specimen (Seo et al. 2020).

Nucleic acid-based biosensors

A double-function plasmonic biosensor was created for the sensitive detection of SARS-CoV-2 nucleic acid. Plasmonic photothermal (PPT) response and local surface plasmon resonance sensing transduction are combined in this biosensor. A cDNA receptor for the E gene, ORF1ab, or RdRp is immobilized via gold-thiol linkage on an assimilated chip derived from two-dimensional gold nanoislands (AuNIs). This approach applies the concept of RNA conjugation with a LOD of 0.22 pM to ascertain SARS-CoV-2 RNA. The device's increased sensitivity is due to an improvement in the hybridization kinetics of the complementary strands resulting from the heat-generating capability of the local PPT of the AuNI plasmonic chips (Qiu et al. 2020). The action of biosensors in clinical specimens is still being investigated. Another test combines a nanoparticle-based LFIA with multiplex analysis, LAMP amplification, and reverse transcription (SARS-CoV-2 RT-LAMP-LFB). With no cross-reactivity, this LOD platform was estimated to yield 12 replicas per response. The SARS-CoV-2 N genome, two LAMP primer groups, and ORF1 antibodies were all amplified and identified simultaneously using coated polymer of streptavidin nanoparticles (Pouresmaieli et al. 2021).

Antibody-based biosensors

A novel biosensor based on antibodies was purportedly applied to diagnose the spike protein of SARS-CoV-2. As antigens, nasopharyngeal samples were taken from clinical

patients and used to encapsulate the respective viral antibody on graphene sheets of a field-effect transistor (FET) (Kang et al. 2021). The biosensor-developed limit of detection (LOD) was reported to be 100 and 1 fg/mL in a basic transport vehicle and saline solution. The virus was furthermore discovered in a growing medium, showing a detectable limit of 1.6×10^1 pfu/mL according to this sensor. Recent research found that with a limit of detection of 2.42×10^2 copies/mL, the SARS-COV-2 FET sensor can categorize diseased and fit individuals (Chiara et al. 2021). An ELISA kit based on recombinant S and N proteins was used to detect IgM and IgG antibodies, which were produced and tested on patients. A positive rate of 80.4 and 82.2% for N protein and S protein, respectively, were detected by these kits (Liu et al. 2020c).

Commercial kits based on the above techniques

Various COVID-19 commercial testing kits from different countries have been summarized based on their techniques or principles of detection like RT-PCR, RT-LAMP, biosensor, CRISPR, antigen testing, antibody testing, LFIA, ELISA, and CLIA by also highlighting the various target proteins which are responsible in aiding in the detection of SARS-CoV-2 and are being listed in Table 4.

Nano-based vaccine candidates against coronaviruses

Vaccination is a well-established biomedical approach to life-threatening infectious conditions; however, rapid and complex genetic alterations in SARS-CoV-2 viruses have made vaccine research and deployment challenging (Kurup and Schnell 2021). To mitigate these challenges, a plethora of attempts have been made to design vaccines that elicit a robust immunological response. Considering breakthroughs in vaccine innovation, vaccination prevalence remains constrained. Through the continuing advancement of nano-sciences, the concept of nanomaterials for vaccine development appears enticing, offering phenomenal potential, owing to the inimitable attributes of nanoparticles that render them excellent vectors for vaccine conveyance by safeguarding vaccines from immature deterioration while promoting the vaccine's optimum consistency, augmenting cellular transport via endocytosis mechanisms, improving depot response, and eliciting both cellular and humoral immunity. SARS-CoV-2 viral components (proteins and RNA) can undergo structural analysis and analogs to form virus-like particles, which can be utilized as nano-vaccines. The viral components can also be separated and inactivated. The

Table 4 Summary of COVID-19 commercial testing kits

Technique	Commercial kit	Target protein	Manufacturer	References
RT-PCR	Fosun COVID-19 RT-PCR kit	E, N, ORF1ab	Fosun, (Shanghai, China)	Garg et al. 2021
	Allplex 2019-nCoV assay	E, N, RdRP	See gene (Seoul, Korea)	Garg et al. 2021
	Patho Detect RT-PCR kit	E, RdRP	Mylab (Maharashtra, India)	Garg et al. 2021
RT-LAMP	SENSObiz COVID-19 (SARS-CoV-2) LAMP Assay	ORF1ab, RNaseP, Spike (S) protein, and Nucleocapsid (N) protein	NANOBIZ TECHNOLOGY (Ankara, Turkey)	Artik et al. 2022
	Veriplex™ RT-LAMP	Membrane (M) protein	SNPSig NOVACYST GROUP (Camberly, UK)	Rödel et al. 2020
	Biospeedy SARS-CoV-2 Variant Plus Kit	ORF1ab, RNaseP, Spike (S) protein, and Nucleocapsid (N) protein	BIOEKSEN R and D Technologies (Istanbul, Turkey)	Artik et al. 2022
Biosensor	USB Electrochemical Biosensor Prototype	Antigenic viral gene targeting S protein	Innovative Sensor Technology IST AG Biosensors (Ebnat-Kappel, Switzerland)	El-Sherif et al. 2022
CRISPR	All In One Dual CRISPR Cas 12a (AIOD-CRISPR)	Detector of Nucleic acid	Sigma-Aldrich (Darmstadt, Germany)	Ding et al. 2020a
Antigen testing	GB SARS-CoV-2 Multiplex	Targeted genes of RdRP and ORF1b	GENERI BIOTECH Ltd. (Kralovehradecky Kraj, Czech Republic)	Dankova et al. 2021
Antibody testing	cPass SARS-CoV-2 Neutralization Antibody Detection Kit	Inhibit RBD domain ACE-2 receptor interaction	GenScript (Piscataway, NJ, USA)	Papenburg et al. 2021
LFIA	Ig-M and Ig-G qSARS-CoV-2 Ig-M/Ig-G Rapid Test ²⁰	Spike protein	Cellex Inc. (Morrisville, NC, USA)	Yüce et al. 2021
	Ig-M and Ig-G Anti-SARS-CoV-2 Rapid Test ²⁰	Spike protein	Autobio Diagnostics Co Ltd. (Zhengzhou, China)	Yüce et al. 2021
ELISA	COVID-19 ELISA Ig-G Antibody Test ²⁰	Ig-G mediated target	Mount Sinai Laboratory (New York, NY, USA)	Yüce et al. 2021
CLIA	LIASON SARS-CoV-2 S1/S2 Ig-G ²⁰	Ig-G mediated target	DiaSorin Inc. (Saluggia, Italy)	Yüce et al. 2021

inactivated components are incorporated into nanoparticles (NPs) to form vaccine-loaded NPs. Additionally, the inactivated components can directly be used as a vaccine, which is depicted in Fig. 8 (Butkovich et al. 2021 and Gale et al. 2021). Importantly, NPs serve as adjuvants or immunostimulatory molecules, significantly improving the sensitivity of antigens (SARS-CoV-2). It has also been demonstrated that certain nano-vaccines promote increased polyclonal antibody response (Gale et al. 2021). Table 5 provides a comprehensive list of nano-vaccines in the pipeline.

DNA-based vaccines

Entos Pharmaceuticals, a Canadian healthcare research organization, introduced a DNA vaccination utilizing its Fusogenix nanomedicine system (Muthiah et al. 2022). The Fusogenix system uses a proteolipid vehicle that integrates a neutral lipid biomaterial containing fusion technology of tiny permeability protein molecules to enable optimal merging and effectual transport of genomic cargo immediately to the cytoplasm of targeted cells. Entos Pharmaceutical intends to introduce a pancoronavirus vaccine design

that could encrypt numerous autoantigens of SARS-main CoV-2 immunogenic molecules, enabling a substantial and formidable defensive immunological response against the virus's complex architectural subunits. In vivo preclinical investigations were undertaken, leading to the advancement of Covigenix (Fusogenix DNA) vaccines targeting SARS-CoV-2, as shown in Fig. 9A, which exhibited significantly higher neutralizing antibody responses, regulated CD4 T cell immunity, high sensitivity, and effectiveness. Subsequent confirmations of this vaccine candidate, i.e., its immunogenicity, safety, and effectiveness, have been accomplished satisfactorily in phase 1 and 2 human clinical studies, with the vaccine candidate advancing to phase 3 investigations (Muthiah et al. 2022).

RNA-based vaccines

The vaccine potential, i.e., mRNA-1273, for the therapeutics of SARS-CoV-2 designed by Moderna in coordination with the National Institute of Allergy and Infectious Diseases (NIAID), a division of the National Institutes of Health (NIH) in the United State of America, is a marketed

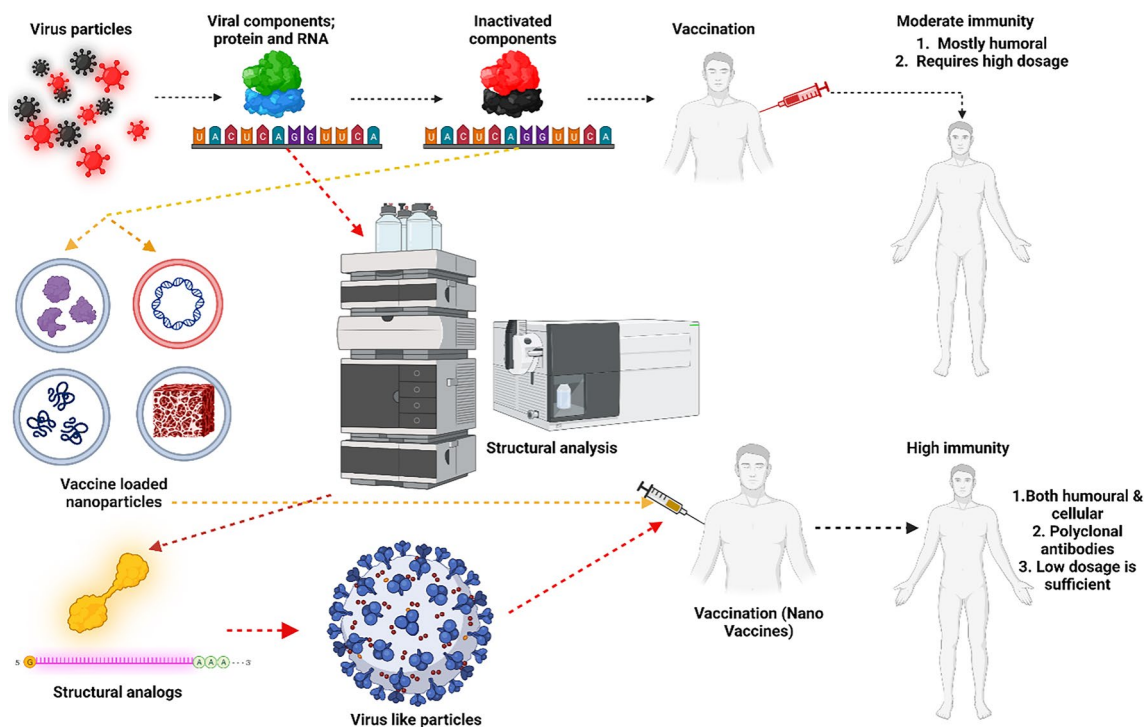


Fig. 8 Graphic depiction of the creation and delivery of traditional vaccines and nano-based vaccines

accessible SARS-CoV-2 booster and was the first to be evaluated in individuals (Fig. 9B) (Szabó et al. 2022). An mRNA version of the novel SARS-CoV-2 consists of prefusion-stabilized spike protein embedded within an experimental lipid nanoparticle matrix consisting of the exclusive ionizable SM-102, lipid, and three conventionally provided lipids, i.e., polyethylene glycol 2000 dimyristoyl glycerol, distearoyl phosphatidylcholine, and cholesterol. Moderna successfully developed an mRNA candidate vaccine entrapped in cationic phospholipid NP_s targeting the SARS-CoV-2 viruses using an identical approach. The vaccines feature entire epitope viral (S/S1/S2) proteins from a virus that created a significant concentration of neutralizing antibodies in rodents (Szabó et al. 2022).

Virus-like particle (VLP) vaccines

Virus-like particles are an intriguing, innovative strategy for vaccine design, as they imitate the original hierarchy of viruses and suggest a pathway to the immunological response for rapid identification and consequent modulation. Medicago, a biopharmaceutical firm based in Canada, has accomplished the preliminary process of researching a vaccine candidate by efficiently manufacturing VLPs of the SARS-CoV-2 virus, utilizing its plant-based VLP synthesis method (Kang et al. 2021). *Nicotiana benthamiana* plant are exploited by the firm as mini biofactories to establish

virus-like particles and restorative molecules for vaccine manufacturing. The vaccine has been in phase 3 clinical testing since 20 March 2021 (England et al. 2023).

Nano-based immunotherapy against coronaviruses

Immunomodulatory drugs in nanomaterial form have achieved encouraging results in the context of regulating immune system activity and minimizing immunomodulation-related damage (Bonam et al. 2021). Importantly, nanomaterials have the competence to assimilate numerous antigens on their interface for more robust immune system stimulation. As a result, nanoparticles can perform as prospective immunological adjuvants, as well as pharmaceutical vehicles. Carbon nanotubes, dendrimers, inorganic nanoparticles, liposomes, and polymer-based materials have all been explored as promising immunological venues to date (Andresen and Fenton 2021). Nanoparticles, including liposomes and poly (lactic-co-glycolic acid), can trigger CD4⁺/CD8⁺ T lymphocyte cells and facilitate antigen cross arrangement, resulting in efficacious antigen conveyance (Shinn et al. 2022). However, inorganic nanoparticles, especially gold nanoparticles, can connect with dendritic cells, elevating the performance of proinflammatory markers (viz., tissue necrotic factor- α , interleukin-6, interleukin-1,

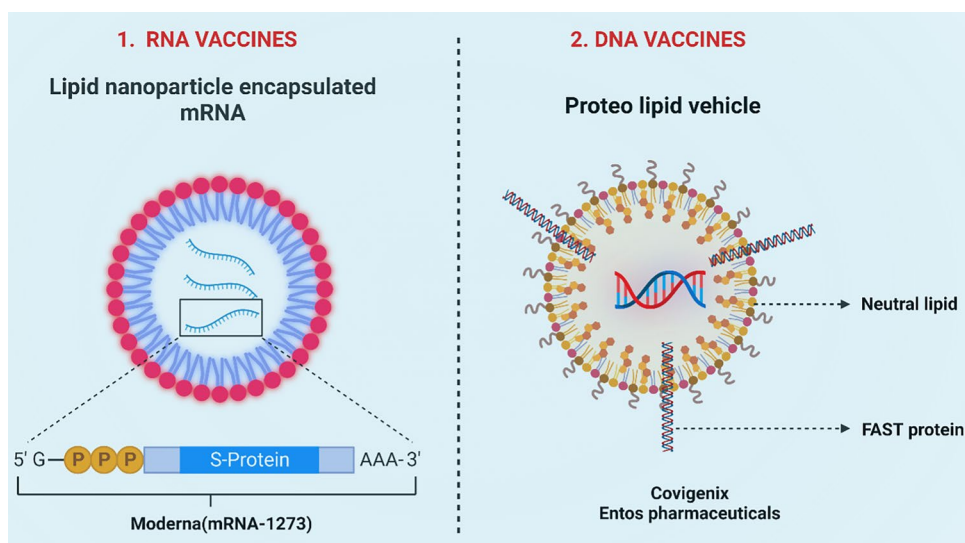
Table 5 Nano-based vaccine candidates against coronaviruses

Vaccine	Candidate vaccine	Nanoparticle nature	Antigen	Step of progress	Manufacturer	References
DNA	DNA-based nanoparticle aerosol vaccine	–	–	Preclinical step	Penn State University (State College, PA, USA)	Walach et al. 2021
	Covigenix	Proteolipid vehicle	Numerous epitopes	Phase 1 and 2 clinical investigation in humans	Entos Pharmaceuticals (Edmonton, AB, Canada)	Shafaati et al. 2022
	Nasal vaccine relying on DNA	Virus-like particle	–	Preclinical step	University of Waterloo (Waterloo, ON, Canada)	Singh et al. 2021
RNA	mRNA-1273	Lipid nanoparticle	S protein	Accepted for usage	The National Institute of Allergy and Infectious Diseases (North Bethesda, MD, USA) in collaboration with Moderna Inc. (Cambridge, MA, USA)	Szabó et al. 2022
	Self-replicates RNA	Leading NP _s non-viral delivery system	S protein	Phase 1 and 2 human clinical trials	Arcturus Therapeutics (San Diego, CA, USA) and Duke-NUS Medical School (Singapore)	Szabó et al. 2022
Virus-like particle (VLP)	Plant-based VLP production platform technology	VLP	S protein	Phase 1 human clinical trial	Medicago (Quebec, QC, Canada), GSK (Brentford, UK), DY-NAVAX (Emeryville, CA, USA)	Maharjan and Choe 2021
DNA	INO-4800	–	Plasmid DNA encode S protein	Phase 3 human clinical trial	Inovio Pharmaceuticals (Plymouth Meeting, PA, USA)	Kumar et al. 2021b
RNA	BNT162b1 and BNT162b2 (Nucleoside modified mRNA)	Lipid nanoparticle	S protein receptor binding domain trimerized	Accepted for usage	BioNTech (Mainz, Germany) and Pfizer (New York, NY, USA)	Mulligan et al. 2020
	IC- SApNP vaccine technology platform	S protein-based, self-assembled protein nanoparticle scaffolding	S protein	Preclinical step	Ufovax (Philadelphia, PA, USA)	Muthiah et al. 2022
	NVX-Co V2373 with Matrix-M adjuvant	Recombinant protein nanoparticle	S protein	Phase 3 human clinical trial	Novavax (Gaithersburg, MD, USA)	Muthiah et al. 2022

Table 5 (continued)

Vaccine	Candidate vaccine	Nanoparticle nature	Antigen	Step of progress	Manufacturer	References
Vector-based vaccines	ChAdOx1	–	S protein	Phase 3 human clinical trial	AstraZeneca (Pune, India) + University of Oxford (Oxford, UK)	Kyriakidis et al. 2021
	Adenovirus Serotype 5 vector (Ad5)-nCoV	–	S protein	Phase 3 human clinical trial	Beijing Institute of Biotechnology (Beijing, China) + CanSino Biologicals (Tianjin, China)	Kyriakidis et al. 2021
Inactivated-based vaccines	JNJ-78436735	–	S protein	Phase 3 human clinical trial	Johnson & Johnson Pharmaceuticals (New Brunswick, NJ, USA)	Kyriakidis et al. 2021
	Sputnik V/Gam-COVID-Vac	–	S protein	Phase 3 human clinical trial	Gamaleya Research Institute (Moscow, Russia) + Health Ministry of the Russian Federation (Moscow, Russia)	Kyriakidis et al. 2021
	Corona Vac	–	S and N protein	Phase 3 human clinical trial	Sinovoc Research & Development Co (Beijing, China)	Kyriakidis et al. 2021
	Covaxin/BBV152	–	Activated CD4 + and CD8 + cell response	Phase 3 human clinical trial	Bharat Biotech (Hyderabad, India) + Indian Council of Medical Research (New Delhi, India) + National Institute of Virology (Pune, India)	Kyriakidis et al. 2021

Fig. 9 Diagram of the several COVID-19 vaccination proposals based on DNA and RNA. **1** The mRNA -1273 (Moderna) vaccine is a comprehensive pre-fusion-stabilized spike protein of SARS-CoV-2 that is encoded by an mRNA-based vaccination that is embedded in lipid nanoparticles. **2** Covigenix (Entos Pharmaceuticals) is a plasmid DNA vaccine that manifests key antigenic determinants from SARS-CoV-2



interleukin-12, and interferon- α) while suppressing the expression of anti-inflammatory mediators (viz., interleukin-10 and tissue growth factor- β_1) (Dykman et al. 2020). AuNPs also triggered immunological activation in T cells and augmented dendritic cell phagocytic action. Despite breakthroughs in the clinical use of NPs in immunotherapy, basic research on the use of nanoparticle-based immunotherapy targeting SARS-CoV-2 is still limited (Yang et al. 2020).

Nano-Based Gene Therapy for coronaviruses

Small interfering RNA (siRNA) is efficacious in inhibiting the replication of RNA viruses, i.e., coronaviruses (Liu et al. 2022). The success of siRNA-based therapeutics is entirely reliant on the precise tailoring of the virion genetic pattern and the delivery of restorative siRNA to the target cells (Nooraei et al. 2021). From this perspective, nontoxic, biomaterial nanoparticles made of nanohydrogels, iron oxide NPs, dendrimers, lipids, silica, AuNPs, polymers, or lipid/polymer hybrid NPs are seen as potential siRNA delivery carriers. By hindering enzymatic breakdown, these nanocomposites can improve siRNA longevity (Idris et al. 2021). Lipids, polylactic-co-glycolic acid (PLGA), and polymer nanocomposites are appropriate for delivering antiviral siRNA in respirable form and aerosol-based cardiopulmonary administration of antiviral siRNA (Wu et al. 2021). Moreover, cholesterol-loaded lipid NPs were recently reported to achieve good performance in conveying SARS-CoV-2 vaccines relying on mRNA (Le et al. 2020). Nanocomposites based on spermine-liposome conjugate and histidine-lysine copolymer have also been validated for delivery of siRNA to appropriate sites in the SARS-CoV gene (Eygeris et al. 2020).

Nanosponges

A new method of developing treatments focuses on the impaired recipient tissues instead of the pathogenetic component. Cellular nanosponges were designed as therapeutic protection against coronavirus infection. These nanosponges are made from membranes extracted from mammalian cells that are inherently SARS-CoV-2 targets. Two categories of cellular nanosponges are generated using plasma cell membranes: either human lung epithelial type-II cells or human macrophages. The same protein receptors, both known and unknown, that are required for SARS-CoV-2 to infiltrate cells are present in each of these nanosponges.

We have learned that SARS-CoV-2 is a distinct virus that is responsible for the recent worldwide epidemic, and details about it are being discovered day after day. The virus has been proven to be rapidly evolving since the initial case that was identified at the end of 2019. The advancement of medicines and preventative measures is facing significant difficulties as a result of this high rate of mutation. Both Epithelial-NS and $M\phi$ -NS revealed a concentration-dependent neutralization of SARS-CoV-2. The nanosponge platform has a distinct advantage over other SARS-CoV-2 treatments that are under research since the nanosponges are possibly viral and mutation-insensitive. Once primed with the nanosponges, SARS-CoV-2 is neutralized and rendered incapable of infiltrating tissue. Importantly, the nanosponge platform is resistant to viral modifications and potential viral species, as the virus continues to attack its targeted host cell, and the nanosponges are ultimately capable of ejecting it. With regard to mutation and other recently developed coronaviruses, microsponges offer a wide-ranging defense system that is mutation-resistant. Additional testing in suitable animal studies is necessary to confirm the effectiveness of cellular nanosponges for the management of SARS-CoV-2

infections. Such testing is currently being carried out and will eventually lead to clinical studies. The antiviral effectiveness of such nanosponges can be enhanced by optimizing the core composition (Zhang et al. 2020).

Nanodecoys

Angiotensin-converting enzyme 2 (ACE2), a carboxypeptidase that is prevalent in all body tissue and is located in a variety of cells, is essential for viral entry into infected cells. Through the association of the spike protein with ACE2, SARS-CoV-2 selectively targets type II pneumocytes that exhibit ACE2 in the pulmonary tract and goblet secretory cells in the nasopharynx membrane (Ziegler et al. 2020). Resident lung epithelial and mesenchymal cells, with both type I and II pneumocytes, make up lung spheroid cells (LCS); these cells generate ACE2 as resident lung cells, and nanovesicles of the LCS membrane are created as ACE2 nanodecoys. Therefore, by serving as cell mimetics, these nanodecoys adhere to the spike (S) proteins of SARS-CoV-2 and cause a phagocytic reaction from macrophages, which leads to eradication of the virus (Jiang et al. 2021b). A recent study reported that nebulized treatment was used to provide such LSC nanodecoys to mice, where they lasted in the respiratory organs for more than 72 h after treatment. Moreover, SARS-CoV-2 analogs were rapidly eliminated from the lungs after inhalation of LSC nanodecoys, and no cytotoxicity was indicated. Four doses of these nanodecoys administered through inhalation enhanced viral elimination and lessened lung damage in cynomolgus macaques exposed to live SARS-CoV-2. The findings imply that LSC nanodecoys could be used as a medicinal therapy to combat SARS-CoV-2 (Li et al. 2021).

Exosomes

Exosomes are a form of extracellular vesicular particles that are produced spontaneously in the body system, making them intrinsic and perfect for delivery of vesicles for drug targeting (Stefańska et al. 2023 and Popowski et al. 2020). Particles are more effective in attacking similar recipient cell tissues because they possess and assert the proteins, RNAs, and lipids of their original cell, as well as the original cell's surface receptors and proteins (García-Fernández and Fuente Freire 2023). A study reported a vaccine comprising a modified SARS-CoV-2 receptor-binding domain (RBD) coupled with exosomes derived from the lungs, which, in comparison with liposomes, improved the accumulation of RBD in all the pulmonary airways lined with mucus and in the lung tissue. After a trial, the vaccine effectively eliminated SARS-CoV-2 pseudo-virus in mice by eliciting RBD-specific antibodies (IgG), mucosal IgA reaction, and CD8 + and CD4 + T

cells with a Th1-like cytokine production pattern in the mouse airway. Upon treatment with active SARS-CoV-2, double doses of the vaccination in hamsters suppressed inflammation and lessened chronic pneumonia. VLPs that are inhalable, durable at room temperature, with long shelf lives, and potential cost savings as a result of reduced shipping make the modified vaccine more widely available and a potentially viable vaccination alternative (Wang et al. 2022c).

Future perspectives

An increased focus on the novel, moveable diagnostic technology has been sparked by the plethora of the SARS-CoV-2 outbreak and its effects on testing accessibility. It is important to have diagnostic testing tools with high accuracy, fast developing, cost-effective antigen and serological tests, which are up to global standards and with the employment of new biosensors, nano-based therapy, electrospinning, and electrospun nanofibers which can prevent and reduce the spread of the virus.

High accuracy

Under ideal conditions and when performed by trained clinicians, most nucleic acid amplification tests have a diagnostic sensitivity and specificity of approximately 95% or greater. However, in practice, sensitivity drops to 60–70%, requiring retesting, which wastes time in symptomatic patients. To improve accuracy, several countries have introduced dual testing of pharyngeal/sputum and nasopharyngeal samples. Further studies are needed to assess the efficiency of swab testing and RNA yield (Serrano et al. 2020).

Developing fast, cost-effective antigen tests

For several reasons, including reducing the need for time-consuming RT-PCR, rapid antigen-based SARS-CoV-2 assays are needed. The development of nucleic acid amplification tests (NAATs) is a plausible and reasonable option, given their high analytical sensitivity and rapid development time. On the other hand, NAATs are often highly processed, susceptible to spoilage, and exorbitant (Chen et al. 2020). Antigen-based screening could represent a specialized option for cost-effective identification in outpatient clinics. Similar methods have already been developed for certain infections, such as influenza (Gentilotti et al. 2022). A SARS-CoV-2 antigen test can be performed as a triaging method to reduce the need for molecular testing.

Establishing effective serological tests

Current serological tests have many variations with generally low reactivity and accuracy. When comparing different test kits, virtually all of which are based on the lateral flow assay design, some perform significantly better than others as a result of the affinity compounds used. To increase the accuracy of diagnosis, researchers must first identify and synthesize the most immunogenic, high-affinity viral antigens (Whitman et al. 2020). Investigation of interference problems is also necessary to determine how medications, drugs, and coagulation states affect serologic test results. The demand for serologic testing will increase as SARS-CoV-2 enters its flattening phase. These tests can be used by those who are cured or asymptomatic to generate information for public pronouncements; community-wide serologic surveillance could allow governments to discover the true prevalence of the disease (Lassaunière et al. 2020).

Setting up global standards

The analytical validity of novel SARS-CoV-2 tests has led to their acceptance, as tested against the manufacturers' simulated samples. Independent testing has revealed significant differences in performance. Global reference standards (e.g., viral antigen, antibodies, viral nucleic acids, and pseudoviruses) need to be developed to allow for objective comparison between tests (Uğur and Özdemir 2023). Recommendations (e.g., on cost, sensor specifications, and accuracy) for various test targets need to be developed; detection of acute infections in healthcare facilities and long-term care facilities is required, as well as home-based surveillance with demographic surveys. These activities will help both the clinical and scientific sectors and drive technological advances (Ghaffari et al. 2021).

New biosensors

New diagnostic procedures should be developed more quickly. Novel sensor techniques, namely optical resonators, nanoplasmonics, and ion-gate transistors, offer exceptionally high sensitivity and could be utilized to directly detect viruses. A transistor based on graphene with a LOD of 2.4×10^2 viruses/mL and a plasmonic photothermal device that recognizes the RdRp target down to 0.22 pM has been reported. These techniques must be further explored to overcome present NAATs and provide rapid on-site diagnoses (Qiu et al. 2020 and Seo et al. 2020).

Nano-based therapy

The search is currently on for a vaccine against SARS-CoV-2 that is trustworthy, stable, effective, and long-lasting.

Nanotechnology-based vaccines have been shown to elicit significantly more robust immunoreactivity than various forms of coronavirus vaccines, as previous research has demonstrated. Therefore, further analysis of the use of nanotechnology in the analysis of SARS-CoV-2 is needed to develop an inventive, nano-based vaccine in appropriate animal studies to potentially induce long-term immunity (Gale et al. 2021). Promising alternatives for dissemination of effective drugs against coronavirus include magnetic nanoparticles (MNPs), AuNPs, and silver nanoparticles (AgNPs), as well as their associated compounds (Dykman et al. 2020). Several nanoparticle vaccines have demonstrated the ability to induce a strong autoimmune response. In addition, further research on the interface of virions with recipient cells is needed before smart nanoparticles can be used to target altered variants of the highly infectious SARS-CoV-2 (Bonam et al. 2021).

Electrospinning and electrospun nanofibers

Continuously non-woven nanofibers can be synthesized from a variety of polymeric composites using the basic, adaptable, and affordable method of electrospinning. With the help of polymeric compositions and electrospinning variables, the length and shape of nanofibers can be altered. As a result, electrospun polymer membranes are typically preferable for air-filtering processes and can be used as materials for protective masks against viruses or bacteria. Furthermore, the electrospinning method has been used for drug delivery, and scientists are attempting to design scaffolds that can deliver drugs against SARS-CoV-2 while regulating the amount of a drug absorbed by the body system, which is essential for usage as a quick absorbent. Moreover, this novel formulated nanotechnology has still not been advanced for application with potentially developed therapeutics (Castillo-Henríquez et al. 2020 and Ding et al. 2020b).

Conclusions

The identification of SARS-CoV-2 RNA in the upper respiratory tract, particularly nasopharyngeal specimens, is commonly achieved through polymerase chain reaction assays. Although SARS-CoV-2 immunity testing techniques are developing, precautions while interpreting results are advised because very few test kits on the market have been validated. Rapid testing and ELISA antibody analysis show a typical antibody response, with IgM production occurring initially, then IgA and IgG. On the other hand, limited information is known about how long protective immunity lasts. T cell response assessment test kits are presently unavailable for diagnostic use. Numerous diagnostic techniques are available, each with unique benefits. These

techniques include chemiluminescence immunoassay, lateral flow immunoassay, antigen testing, and protein-peptide microarray. Innovative techniques such as CRISPR, AuNPs, and nano-based technologies have the potential to identify more infectious viruses in addition to SARS-CoV-2. NPs based on immunotherapy are becoming an exceedingly prominent treatment alternative; however, there are still issues with maximizing benefits and reducing side effects. Effective immunotherapy requires a thorough understanding of immune responses and immunity regulation. When compared to traditional antiviral medications and immunizations, nano-based therapeutic agents particularly siRNA antiviral treatment offer benefit in terms of rapid response, effectiveness, and low RNA consumption.

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Declarations

Conflicts of interest On behalf of all authors that there is no conflict of interest.

Human and animal participants Not applicable.

Informed consent Not applicable.

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