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

Chemical Engineering Journal 414 (2021) 128759

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Point of care detection of COVID-19: Advancement in biosensing and diagnostic methods

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ARTICLE INFO

Keywords:
COVID-19
Coronaviruses
Pandemic
Symptoms
Diagnosis
Biosensors

ABSTRACT

The recent outbreak of COVID-19 has created much inconvenience and fear that the virus can seriously affect humans, causing health hazards and death. This pandemic has created much worry and as per the report by World Health Organization (WHO), more than 43 million individuals in 215 countries and territories were affected. People around the world are still struggling to overcome the problems associated with this pandemic. Of all the available methods, reverse-transcriptase polymerase chain reaction (RT-PCR) has been widely practiced for the pandemic detection even though several diagnostic tools are available having varying accuracy and sensitivity. The method offers many advantages making it a life-saving tool, but the method has the limitation of transporting to the nearest pathology lab, thus limiting its application in resource limited settings. This has a risen a crucial need for point-of-care devices for on-site detection. In this venture, biosensors have been used, since they can be applied immediately at the point-of-care. This review will discuss about the available diagnostic methods and biosensors for COVID-19 detection.

1. Introduction

The social history of virus describes about the viral infections and their influence on the human is just not a recent happening, but existed even before in the prehistoric period. However, due to the explosion of the world population, viral infections have dispersed throughout the world. As declared by The World Health Organization (WHO), public health is facing serious threat due to the emergence of new viral strains that are drug-resistant and the entrance of new pathogens. World has witnessed several viral infections including dengue, cholera, severe acute respiratory syndrome, plague, avian influenza, ebola, chikungunya, middle east respiratory syndrome, nipah virus infection, and recently COVID-19. Of all these, the recent outbreak of COVID-19 has affected human life seriously not only with illness, but also with the social life by disturbing the world's economy. After the first appearance of the disease in China, WHO formally notified about the pneumonia cases that have infected the group of individuals, in a short time the virus was isolated and the genome was shared. WHO announced it as a global health threat and announced the name, COVID-19. Further investigations suggested that they were infected with corona virus. Immediately, corona invaded all the countries round the globe with the leading number of positive affected patients. At present (20th January 2020), 96,742,480 people have been infected and about 2,068,745 deaths were reported in around 215 countries and territories.

Corona virus is associated with Coronaviridae family, which is further linked to sarbecovirus subgenus and further included in Nidovirales order distributed in animals that can possibly be transmitted to the humans [1]. Historically, it is not the first time that respiratory infections in humans were observed due to coronavirus as six other corona viruses have also been identified and originated from the animals. The

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https://doi.org/10.1016/j.cej.2021.128759
Received 26 October 2020; Received in revised form 21 January 2021; Accepted 25 January 2021
Available online 31 January 2021

two betacorona viruses reported earlier were SARS-CoV and MERS-CoV that have caused epizootic disease: severe-acute respiratory syndrome [1] and Middle-East respiratory syndrome [2]. Over the past two decades, a large number of cases (approx. 10,000) were reported of which 10% death rates were recorded with the invasion of SARS [3] and 37% for MERS [4]. The previously reported corona viruses were severe, but not quite worst, with even more newly and extreme zoonotic incidents. Tiredness, fever (with or without cough) and flu, shortness of breath are some of the common symptoms of COVID-19 including some less common symptoms such as body aches and pains, conjunctivitis, headache, a rash on the skin, sore throat, diarrhoea and loss of taste or smell.

According to the data from Canters for Disease Control and Prevention (CDC) the death rate of patients affected with COVID-19 are comparatively more in males in the age group between 75 and 84 and in females with age 85 or above. The risk of death with people lesser than 45 years is minimum, while more deaths were reported due to COVID-19 in the age group of 65–74 years. Individuals infected with influenza and pneumonia showed the highest death rate in COVID-19 infected patients [5]. Further, immuno-assay detection methods may not be applicable to the patients who are immune compromised or deficient.

As regards the detection methods, the entire world relies on only one diagnostic test viz., RT-PCR, which offers many advantageous features such as specificity, sensitivity and reproducibility. The method can detect the virus at an early onset of infection and can be applied to immune-compromised or immune-deficiency patients, but it has limitations as it requires expertise, and is expensive with a bulky instrument. One major limitation associated with this method is that different temperatures are required at different reaction cycles, making it difficult to apply at the point-of-care. In order to circumvent this hurdle, a new technique viz., nucleic acid-based sequence-based amplification (NASBA) that requires the same temperature at different reaction cycles is available, which can be coupled with various other visual detectors to add a value to the device.

Even though the NASBA coupled with a visual detector was developed that led to commercialization, yet another technique, called clustered regularly interspersed short palindromic repeats (CRISPR) could be a boon to the diagnostics family of tools. The LAMP (loop mediated isothermal amplification) method was also coupled with CRISPR, which led to the development of lateral flow assay through CRISPR. These techniques have the potential advantages of sensitivity, specificity and affordability. This review presents a brief pathogenesis along with recent development in diagnostic methodologies as well as the pathogenesis of COVID-19. The recent literature search confirmed that nucleic acid-based methods are at the fore-front towards the diagnosis of COVID-19.

2. Transmission ofCOVID-19

2.1. Span journey of COVID-19

The primary spread of COVID-19 occurred in China on 29th of December 2019 as the Acute Respiratory disorder of an unknown origin was first registered [6]. Acute respiratory distress syndrome (ARDS) with the first 41 cases reported COVID-19 infections, since pneumonia was detected in one third of the patients needing intensive care, while about six patients have died initially [7].The risk assessment rate was very high not only in China, but also globally, and emergency was declared in many countries. The situation became worst with terribly positive cases rising, and the majority of cases occurred in the United States of America and other European countries [8] resulting in the lockdown. In India alone, 82 positive cases including 15 Italian tourists were reported, which gradually increased drastically [9].The toll rises as per the report by the WHO on 7th December 2020, total number of cases reported were over 65.8 million of which deaths tolled over 1.5 million. [10].

2.2. Signs, symptoms and transmission

People suffering from COVID-19 infection may or may not show symptoms of fever [11] though the virus produces large amounts cytotoxins similar to SARS and MERS-CoV [12] resembling in some respects to that of SARS-CoV [13]. Both COVID-19 and SARS-CoV show stability at low humidity conditions and low ambient temperature [14]. The incubation time for COVID-19 is 10 days on an average (maximum 14 days) from the infection point to the onset of the symptoms [15]. People infected with the virus show symptoms within 2–14 days such as fever, cough, shortness in breath, headache, loss of taste and smell, body pain and sore throat [16]. The challenging aspect of COVID-19 infection is that it is highly transmissible and can spread from one person to other even without symptoms, making the task difficult to track and isolate the potential carriers [17,18]. SARS-CoV-2 forms of transmission can be direct, indirect or close contact with the secretions of the infected person, such as respiratory secretions, droplets or saliva, which are released while coughing, sneezing or talking [19,20].

At the start it was believed that transmission can be only from symptomatic patients, but later the story was unfolded and it became apparent that transmission from the pre-symptomatic and asymptomatic people can be probable. Respiratory droplets >5–10 μm in dia droplet nuclei <5 μm (respiratory droplets can also evaporate and turn into nuclei of droplets) [21] containing the virus that can reach the healthy person's mouth, nose or eyes. Respiratory droplet transmission can be possible within the range of 1 m [21] while airborne transmission by an infected individual of virus-laden droplet nuclei, which are relatively small to stay airborne, can easily float up to a distance of 10 m in air, which can easily cause the infection [22]. In addition to sneezing and crying, there is a significant risk of COVID-19 transmission by the voice droplets [23]. Virus-laden aerosols, once airborne, can react with different elements of the environment, which can impair their longevity. In case of exposure to the toxic ambient component, the disease may become inactivated and no longer spread the infection or if exposed to a healthy component, the disease may not always spread, but sometimes it can transmit to others based on the period of dwelling. The half-lives of SARS-CoV and SARS-CoV-2 in aerosols are the same and are about 1.1 to 1.2 h, thereby making the social distancing necessary for the population [24].

Apart from the direct source of transmission the virus can be transmitted from indirect routes as well during exhalation of the infected person where droplets released can be settled on the fomites, which is a common route for community spread [25]. As SARS-CoV-2 remains active on the surface with time duration depending on the surface such as stainless steel, plastics for about 7 days, while for cardboards it ranges to 24 h [26]. On a printing paper and tissue paper it can stay up to 3 h, for wood (treated) for days and for smooth surfaces such as glass and bank notes it can survive up to 4 days. The virus can even be detected on surgical masks (outer surface) post 7 days after the contact. Another source of transmission through indirect contact can be through faecal-oral routes. Studies have determined that SARS-CoV-2 can last for ten days in tap water if the temperature is 23 °C, but for 2 to 3 days in sewage water, which may cause transmission through air by the aerosolize faecal matter, especially during flushing. This possibility of infection is high in the environment of quarantine centres and hospitals where the same toilets are being used with the infected patients and uninfected ones. Water released during flushing can also lead this virus accessing sewerage systems. Even though such data are yet to be determined, apart from the survival rate on different surfaces, the presence of particulate matter in the environment can vary in transmission through such indirect routes [27].

Transmission risk factors include replication–competence of the virus, symptoms of cough and environmental factors related to infected persons. Based on the evidence of studies, the virus is rarely seen when cultured from the respiratory samples after 9 days of the progression and emergence of the symptoms in mild condition patients. About the role of

neutralizing antibodies as well as symptom resolution, WHO has recommended for releasing the patients from the isolation ward after 13 days, which is harmless based on clinical criteria and this is the minimum time for isolation [28].

2.3. Relationship between SARS-CoV and SARS-CoV-2

SARS-CoV-2 is a novel virus, which comes under the subgenus sarbecovirus that belongs to Betacoronavirus genus having some similarities to phylogenetics and genomics with SARS-CoV. Fig. 1 shows the structure of SARS-CoV-2 representing the spike glycoprotein, membrane protein, genomic RNA, nucleocapsid protein, envelope and hemagglutinin-esterase.

SARS-CoV is exceptional in pathogenesis, since it induces inflammation of the respiratory tract (in both upper and lower). The SARS-CoV-2 genome sequence is about 89% identical to SARS-like CoVZXC21 bat and 82% similar to human SARS-CoV [29]. SARS-CoV-2 needs receptor (ACE2) to enter into cell similar to SARS-CoV for infecting humans [30] which depicts similarities between SARS-CoV-2; the earlier reported SARS-CoV may be helpful for clinical diagnosis. As shown in Fig. 2, coronavirus (RNA virus) infects the individual host cells in a similar manner that is followed by all the RNA viruses by attaching to cell surface receptor (ACE2) and replicating inside the cell, thereby infecting other cells with an immediate exponential growth.

The cryogenic electron microscopy (cryo-EM) was used to study ACE2 structure when it interacts with its specific ligands such as transporter of amino acid B^0AT1, and determine whether ACE2-B^0AT1 complex would bind with receptor-binding domain (RBD) of SARS-CoV-2 [31]. Hitherto, these structures and interactions are not known. However, a comparison was attempted as to how SARS-CoV-RBD binding is different from that of SARS-CoV-2-RBD, which showed certain sequence changes making the interactions stronger in COVID-19. This structural study of ligands may play a promising role in the production of antibodies and targeting ACE2 or coronavirus spike protein to prevent the infection [31].

For the insight vision COVID-19 infection, spike protein associated with Receptor Binding Domain (RBD) present in SARS-CoV and Bat SARS-like Corona virus was compared with those in COVID-19 to find the extent to which it would cause the human infection. A close relationship was observed in SARS-CoV-2′s RBD sequences with that of SARS-CoV (73.8 to 74.9% of amino-acid identical) and in SARS-like CoV such as strains Rs4874, Rs7327 and Rs4231 (identical range was 75.9 to 76.9% amino acid); these strains are also capable of using human ACE 2 receptor for the cell entry [32]. It was also observed that only one additional amino acid was present in RBD of SARS-CoV-2 compared to SARS-CoV RBD [33]. Particularly, the structure of RBD containing the predicted protein structure and amino acid sequence showed strong similarities between SARS-CoV-2 and SARS-CoV, suggesting that SARS-CoV-2 would effectively use human ACE 2 receptor for entry into the cell, possibly promoting transmission from one human to another [34].

The genetics suggests that SARS-CoV-2 can be transmitted independently from animal to humans and is potentially a new coronavirus when examined for ORF1a, ORF1b, S genes and N genes. Fig. 3 shows the genomic organisation of SARS-CoV-2 representing structural and non-structural proteins, which can be targeted for antibody neutralization for vaccine development and as biological receptors for diagnostic applications.

3. Methods available for COVID-19 detection

3.1. Chest CT

The chest CT approach may not be ideal for COVID-19 detection even though it was used temporarily to diagnose during the shortage of kits in China [35]. The technique can be defined as imaging of different cross sections of chest through X-ray, which is non-invasive in nature, and X-ray measurements are done at different angles, while data need comprehensive examination by a physician. Chest CT may be used in COVID19 pneumonia to determine the extent of lung involvement and infection. The initial CT scans were analysed to examine: (a) existence of opacities in ground glass, (b) consolidated existence, (c) ground-glass impact on lobes affected (numbers) or consolidative opacities, (d) whole lung “total severity rating as well as degree of lobe involvement, (e) nodules present, (f) pleural effusions present, (g) thoracic lymphadenopathy (defined in short axis dimension as 10 mm lymph node size) if found and (h) involvement of chronic pleural disease such as fibrosis or emphysema. Certain abnormalities were observed (e.g., cavitation, reticulation, septal interlobular-thickening, calcification, and bronchiectasis). Ground-glass opacification was characterized as smoky increased lung attenuation with bronchial and vascular margin retention as well as consolidation with obscuration of vessels and airway margins as described by opacification [36].

The present international radiological guidelines do not suggest CT scans to diagnose COVID-19, including that of the Centers for Disease

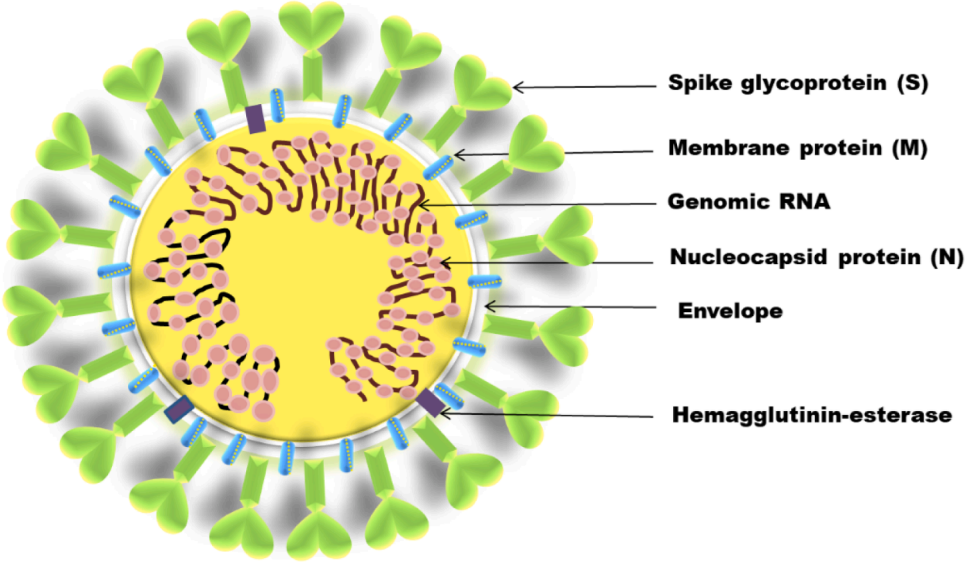

Fig. 1. Structure of SARS-CoV-2 representing spike glycoprotein, membrane protein, genomic RNA, nucleocapsid protein, envelope and hemagglutinin-esterase.

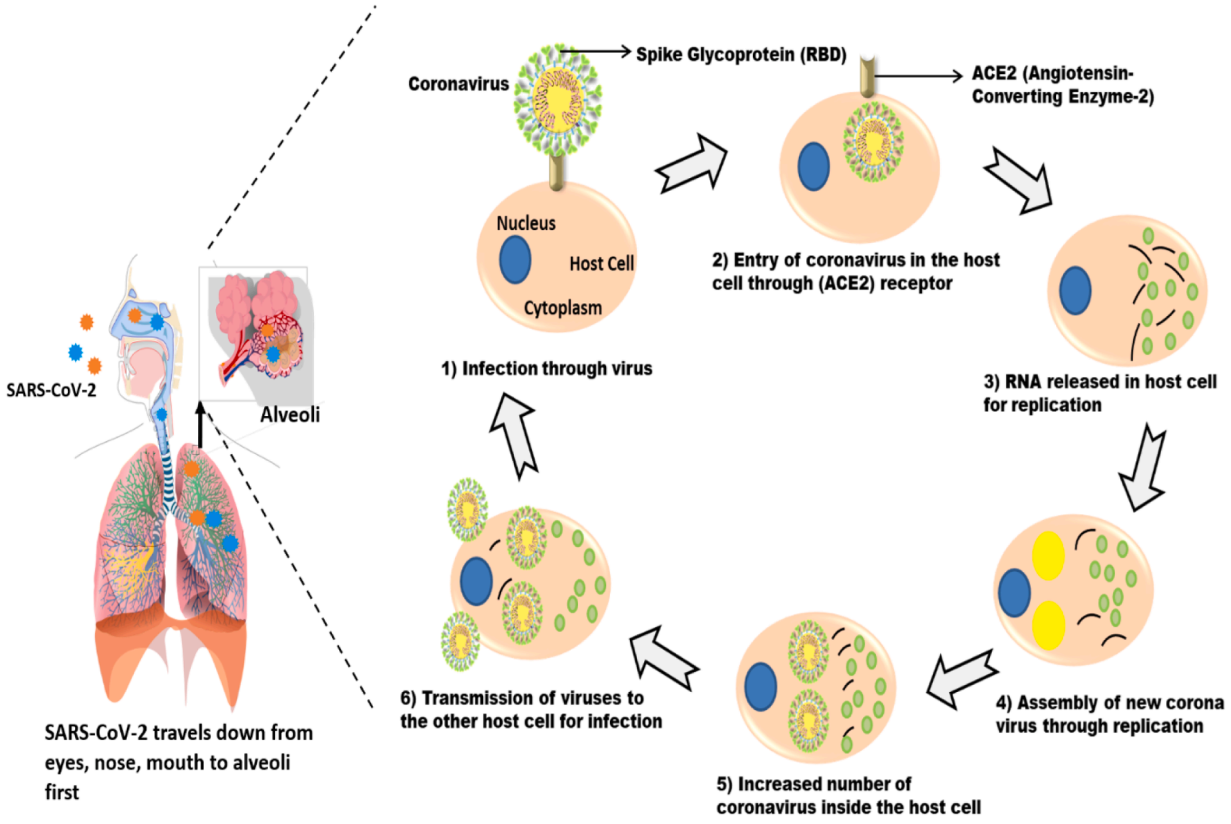

Fig. 2. Schematics showing the attachment of SARS-CoV-2 virus to the host cell, entry, replication and transmission.

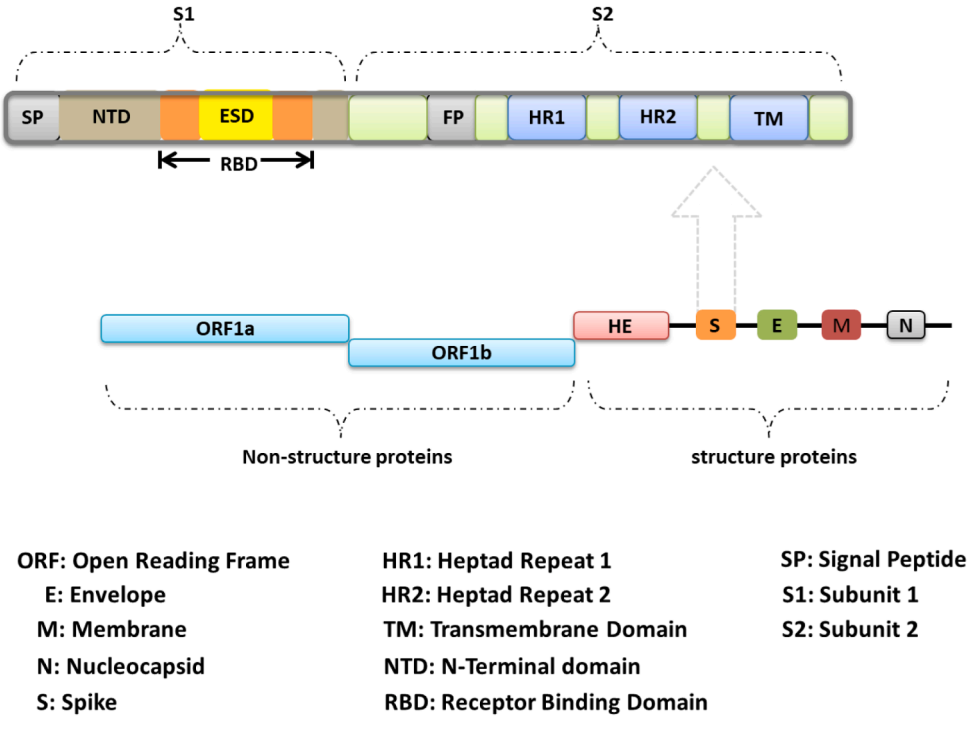

Fig. 3. Genome organization of SARS-CoV-2, representing structural and non-structural proteins.

Control (CDC), and the British Society of Thoracic Imaging (BSTI) as well as the American College of Radiology (ACR), but only relevant technique of diagnosis remains viral screening [37–39]. On the other hand, RT-PCR tests are not available in case of false-negative PCR assays, while in areas with a high prevalence of COVID-19, the CT scans can still be considered as a diagnostic tool [40–44]. As CT features tend to peak later (days 6–11) during the course of the disease although in both the early stage of COVID-19 [43] and the patients showing symptom in

chronic phase, CT scans can be helpful as they can reveal residual pneumonia [44,45]. Chest CT imaging characteristics typically differ based on scanning period, the level of the disease during follow-up, the age of the patient, immune status, underlying diseases and drug therapies [46]. If a patient with a background of COVID-19 exposure has a positive IgM and IgG, this means infection has started more than 14 days earlier and that immune system is aggressively developing antibodies. Chest CT can be effective in these symptomatic cases because it can show lung alterations; various treatments can also be used to treat long-term activation of immune response. Chest CT can be helpful in chronic respiratory symptoms or abdominal problems in COVID-19 patients presenting with laboratory alterations having the positive serology results despite the frequent negative RT-PCR results [47]. The disadvantages of this method are that it is expensive, has a high dose of radiation, and can sometimes be misunderstood, thereby contributing to mismanagement of the disease [48].

3.2. Metatranscriptomics next generation sequencing (mNSG)

The mNSG method is one of the alternative methods for the detection of causative virus. Transcriptomics has provided high-throughput novel conceptual perspectives into the expression of SARS-CoV-2 genes, the stoichiometry of the components of their genes, and potential molecular mechanisms of post-transcriptional changes, including regulation of gene expression of viral genes. Random primer metagenomic sequencing (sequence-independent single primer amplification [SISPA]) or metagenomic sequencing with spiked primer enrichment (MSSPE) have been used to classify SARS-CoV-2 using the small sample size in mNGS methods [49,50]. The opportunity to gain a glance is the main advantage of mNGS, since at a specific sampling location, environment of the patient to diagnose coinfections and assess other organisms that can influence the patient outcomes. As can be seen with the Middle East respiratory syndrome (MERS), understanding of the coinfection is important since it may contribute to exacerbation of COVID-19 to provide insights into the treatment of the patients [51]

The advantages of the above approach are that with RNA sequencing, relatively low-expressed genes can be classified, compiled and traced to biosynthetic processes including the entire *meta*-transcriptome containing RNAs that are not coded. The disadvantages are the instability of mRNA that can degrade the sample quantity even before sequencing as well as the difficulty in differentiating between microbial and host RNA. However, the existence of mRNA may not always imply the interaction between appropriate protein and its actual nature [52].

3.3. Molecular technique

3.3.1. RT-PCR

Controlling the coronavirus infection and identifying the possible sources of virus are important, and hence, prompt diagnosis of the infected patient is necessary. Many of the early medical techniques depend on complex protocols such as serology, population of viruses and identification of antigens [53]. RT-PCR in real time is commonly used in diagnostic virology in emergency. A molecular diagnostic test has identified coronavirus as the causative agents of respiratory distress, making it necessary to identify the species. Real-time RT-PCR fluorescence can be done to identify SARS-CoV-2 if present in lower respiratory tract or in samples of throat swab and sputum. RT–PCR involves the conversion of viral RNA of SARS-CoV2 into cDNA by reverse transcriptase enzyme and then amplification of reverse transcribed cDNA. These primers are used for amplification, which is specific for SARS coronavirus.

RT–PCR offers many advantages and unique features making it a star in diagnostic area in the early stages of pandemic. For instance, RT-PCR identifies the viral genome sequence, so it can be applied during the early onset of the infection. Furthermore, RT-PCR is very specific, sensitive and also can be quantitative as it can also measure viral load of the patient [54]. The disadvantages of this method include use of expensive reagents and somewhat complex techniques. High precision between primers and models is often required to achieve high sensitivity. However, the technique has several limitations such as different temperature requirements for different reaction cycles and fast scanning of a huge number of samples. Therefore, real time RT-PCR assay (two-tube multiplex) and reverse transcriptase –loop mediated isothermal amplification (RT-LAMP) came into existence.

3.3.2. Loop mediated isothermal amplification (LAMP)

LAMP is similar to PCR wherein specific DNA sequences are amplified to detect the pathogen. The technique has now reached the technical maturity for the identification of SARS-CoV2 in the majority of research labs. In this approach, there is no requirement of different temperatures at different reaction cycles and hence, the method can be utilized at resource limited settings. LAMP reaction mix contains primers (2 inner primers and 2 outer primers), RNA extracted from patients' sample, reverse transcriptase, isothermal amplification buffer, nucleotides triphosphate, fluorescent dye and magnesium sulphate.

LAMP is a very specific technique because it utilizes four to six primers, which can locate six or four different regions of the viral genome. For the visualization of amplified sequence, some dyes are also added in the reaction mixture, which can bind the double-stranded (dsDNA). Zhao et al., [55] reviewed the use of LAMP as a colorimetric method for point-of-care virus detection and thus, LAMP coupled with visual detector is a promising technique for the diagnosis of COVID-19 [56].

3.4. Recent tools based on CRISPR

3.4.1. CRISPR-Cas13a (SHERLOCK)

Cas13a an RNases (single effector RNA guide ribonucleases) that are dependent on CRISPR (clustered regularly interspaced short palindromic repeats) can be enabled to close the "collateral" cleavage by non-target RNA until its RNA targets have been identified. This test paper produced by Zhang et al., (2020) lead to rapid detection of COVID-19 within one hour by utilizing SHERLOCK (Specific High Sensitivity Enzyme Reporter UnLOCKing) technique. An S and ORF1ab gene of SARS-CoV-2 was used in the detection, which includes three steps: 1) isothermal amplification of the sample of nucleic acid collected using the available recombinase polymerase amplification (RPA) kit with incubation of 25 min; 2) using Cas13 detection of amplified viral RNA sequence with 30 min incubation and 3) last stage is detection via visualisation by a commercially available dipstick (paper) within 2 min of incubation. The overall process of SHERLOCK is schematically illustrated in Fig. 4. Firstly, viral RNAs are reverse transcribed to cDNA and amplified through few sets of primers. Then amplified segments were converted into RNA. Cas13 along with the guided RNA targets the amplified RNA sequence; the activated Cas 13 involved in quenching the fluorescence of the reporter probe [57]. This method is now under clinical trial, but it has been already tested on many patients infected with COVID-19.

3.4.2. Lateral flow assay detection via CRISPR

DNA endonuclease targeted CRISPR *trans*-reporter (DETECTR) technique is another CRISPR Cas12 based assay for recognition of COVID-19. This performs RT-LAMP amplification (loop mediated) along with reverse transcription of RNA isolated from nasopharyngeal/oropharyngeal swabs in the universal transport medium (UTM), accompanied by Cas12 identification of predetermined coronavirus sequences, the cleavage of a reporter molecule confirms recognition of the virus. The DETECTR tool is based on isothermal multiplication of target DNA and Cas12-mediated collateral cleavage of single-stranded DNA (ssDNA) test. DETECTR system performs reverse transcription as well as DNA amplifications simultaneously with loop-mediated amplification

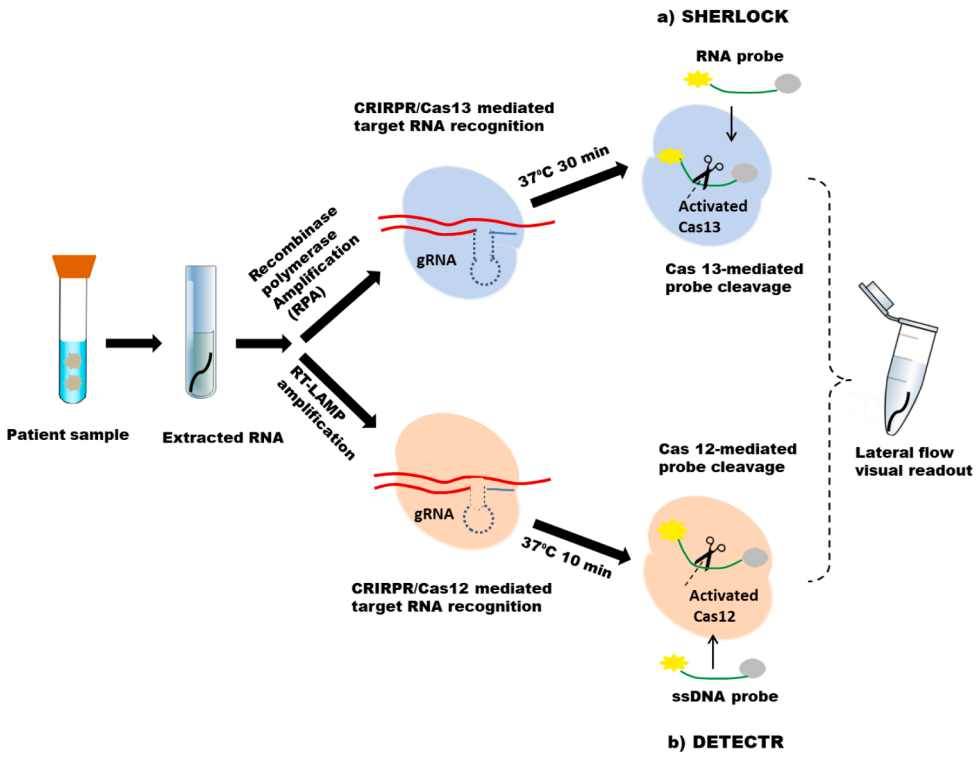

Fig. 4. General principle of SHERLOCK (top scheme) and DETECTR (bottom scheme) based on CRISPR.

(RT-LAMP). Primers are designed for Envelope and Nucleoprotein gene of SARS-CoV-2 in combination with proto spacer adjacent motif (PAM) that is the target sequence of enzyme Cas 12. Once Cas12 precisely recognizes and binds to target ssDNA, it will be allowed to fully degrade ssDNA molecules non-specifically. Although this method has high sensitivity and specificity, it can show some off-target effects in response to recent guidelines from the US FDA. The clinical validation of this procedure is underway in clinical Laboratory Improvement Amendments (CLIA) certified microbiology-laboratory, and this approach can be an effective point-of-care diagnosis for COVID-19 pandemic [58].

Initial investigations for COVID-19 serum biochemical test include creatine kinase, lactate dehydrogenase, electrolytes, renal and functioning of liver was also examined for complete blood count, and coagulation profiles were performed. The CRP (C-reactive protein) with 41.4 mg/L of blood (a comparative range from 0 to 6 mg/L) was identified such that the amounts of creatine-kinase, lactic-dehydrogenase and aspartate-aminotransferase were slightly increased in blood test of the patients who were infected with COVID-19. Detection of coronavirus in plasma was also done via total RNA extraction. The positive tests for RT-PCR were labelled as RNAaemia in plasma samples in real time and not viraemia [59].

3.5. Serological tests

RT-PCR requires well-trained personnel, expensive equipments, and takes longer time, but leaving increasingly growing number of possible cases untested, thus creating a void in disease prevention efforts. In fact, moving to hospital environment for research raises the likelihood of infectious transmission that brings burden on the resource-limited healthcare system. For these reasons, an effective, fast, affordable, easy-to-use and responsive COVID-19 diagnostic device needs to be developed for use even by non-clinical individuals in their homes. Several ELISA manual kits are still commercially available for nucleocapsid and spike protein identification, but these are used mainly for testing purposes in research.

Several serological immune-based assays have been invented through IVD firms for the identification of antibodies against SARS-CoV-2 and viral-proteins present in serum or plasma. The most widely used biomarkers for commercial assays in the evaluation of SARS-CoV-2 infection in immunoassays (i.e., lateral flow immunoassay (LFIA) studies, manual ELISA, automatic chemiluminescence immunoassay (CLIA), and other forms) are immuno-globulins (IgM and IgG) generated from some suspected infections caused by virus post second week. The IgM can be found in samples taken from the infected patients from 10 to 30 days after infected with SARS-CoV-2, while IgG can be found later after 20 days of the progression. In comparison of IgG, the IgM reaction happens faster, but later fades away after declining. Conversely, IgG may be detected after the infection prevails for longer time and may have a defensive function to play. Several ELISA manual kits are still widely available for Nucleocapsid protein and Spike protein identification, but these are primarily used for testing purposes [60].

There are several advantages of this method such as requirement of small area of the laboratories for serological screening, which can be carried out at the place of treatment to reduce the transfer of samples and processing times. Furthermore, after the virus inactivation, ELISA can be carried out in one single location on an open bench, permitting it to be carried out by the personnel even with the least developed skills. ELISA (antigen-capture) can be carried out using the basic equipment, which can be developed with the least investment initially. Most significantly, ELISA (antigen-capture) would result in a high sensitivity for the diagnosis of CoV even during the initial infection stages [61]. The disadvantages of the method are that though these methods are highly sensitive, but they are not suitable for examining multiple samples because of their cost inefficiency and long period of time for data reporting. Moreover, such techniques require trained staff [62].

Similarities between SARS, MERS and SARS-CoV-2 have been found in the diagnosis and treatment. These viruses can be determined by RT-PCR testing of fluids from the respiratory tract of the patient or by analysing serum antibody and analysis of cell cultures from the respiratory fluids. All three viruses initially caused pneumonia, but running nose or gastrointestinal symptoms were rarely observed in those infected with COVID-19. These are the major differences between COVID-19

and other coronaviruses as the aforementioned symptoms are common in other two syndromes. Lung Radiography is important for the preliminary diagnosis and identification of the seriousness of the disease on a large scale. These viruses are similarly treated with the antiviral therapies, while COVID-19 has not yet been approved for any specific antiviral therapy though clinical trials are underway.

3.5.1. Lateral flow assay

The detection of antigen using lateral flow immunoassay (LFIA) for COVID-19 diagnosis can be attempted, which detects the presence of IgM and IgG in human serum and offers results within 15 min. In this assay, two lines are coated on a membrane strip (paper- like): the first line contains gold nanoparticles (AuNP)-antibody conjugates, while the second captures the antibodies. On the membrane strip samples from the patient, blood or urine is deposited, and through the capillary action on the membrane proteins are extracted. The (AuNP)-antibody conjugates interact with antigens, the matrix moves along the membrane when crosses the first line. A red or blue line can be visualised once it reaches the second line because the matrix gets immobilized through the capture of antibodies. Blue line depicts a clustered solution of AuNPs and because of the plasmon band coupling it shows blue color, whereas red colour is seen for the dispersed AuNP.

The principle of colorimetric detection using gold nanoparticles can be found in some review articles [63,64]. As shown in Fig. 5, antibodies coated with AuNPs combined with rabbit IgG were spread onto conjugation pads, which can bind with immobilized anti-rabbit IgG present on the control line. This has two separate lines for test with two mouse anti-human (monoclonal antibodies) different for both the immunoglobins IgG and IgM as well as SARS-CoV-2 antibodies combined with colloidal AuNPs (IgG and IgM both) spread onto conjugation pad, which binds particularly with SARS-CoV-2 surface antigen.

Various countries including the US, Germany, and China have produced this lateral flow assay kits in several *in-vitro* diagnostic companies, and hence, rapid testing could be used as a complement to the current RT-PCR assay, leading to a much better COVID-19 diagnosis and offers more details about suspect's immune-system. Nevertheless, clinical efficacy of rapid tests must be rigorously tested before they are approved for COVID-19 mass screening. IgM and IgG could only be detected in suspects around two weeks after the initiation of the infection; however, there is an enormous need to use other early-stage biomarkers of SARS-CoV-2 for better rapid testing of COVID-19 [65].

Another biosensor reported was that of standard Q COVID-19 IgM/IgG Duo Test Kit by SD biosensor, which is used as a Rapid immunochromatography test, intended to detect similar IgM and IgG to SARS-CoV-2 in humoral fluid qualitatively. This includes rapid analysis within 10 min for COVID-19 and requires 10 μl of the sample from the whole blood plasma, but it may not require any extra equipment. Its performance was characterized in South Korea during trial runs when the entire world was facing the dangerous pandemic. This has certain limitations as quantitative data cannot be determined, and the method was not reviewed by the US FDA [66].

3.5.2. Assay based on luminescence

DZ-Lite SARS-CoV-2 CLIA is an impressive change in the process of designing new methodology for detecting IgM and IgG tests found by Diazyme in the US, which was approved by the FDA. The molecular theory of this study is a chemiluminescence immunoassay (CLIA) with an outcome of 50 tests/h, operating on an integrated chemiluminescence analyser Diazyme DZ-Lite 3000 Plus. Likewise, for the detection of IgG and IgM in the sample of the patient, an automated CLIA test on MAGLUMI CLIA analyzers were performed in China that gave the results within 30 min. Thus, the key benefits of automatic COVID-19 assay based on CLIA analyzers relative to fast LFIA testing are high sample density that can be evaluated along with the ability to carry out further clinical studies for other biomarkers such as C-reactive protein (CRP), which still needs to be tracked in COVID-19 suspects [67].

4. Biosensors as point-of-care diagnosis

Point-of-care procedures are used to treat patients without sampling at the central hospitals, thereby allowing the populations without a laboratory system to identify the sick patients. Point of care is quick, cost efficient, and sturdy diagnosis, which is urgently required for COVID-19 for the early detection and this may be the only solution as of now. The biosensors act as a viable alternative tool for the diagnosis. Nanobiotechnology makes a promising analytical contribution, in particular developing biosensors for pathogenic microorganism detection. Various diagnostic tools available for the detection of gene/protein using different methods of diagnosis was given in Table 1.

Biosensors for virus detection have been reportedly tested using

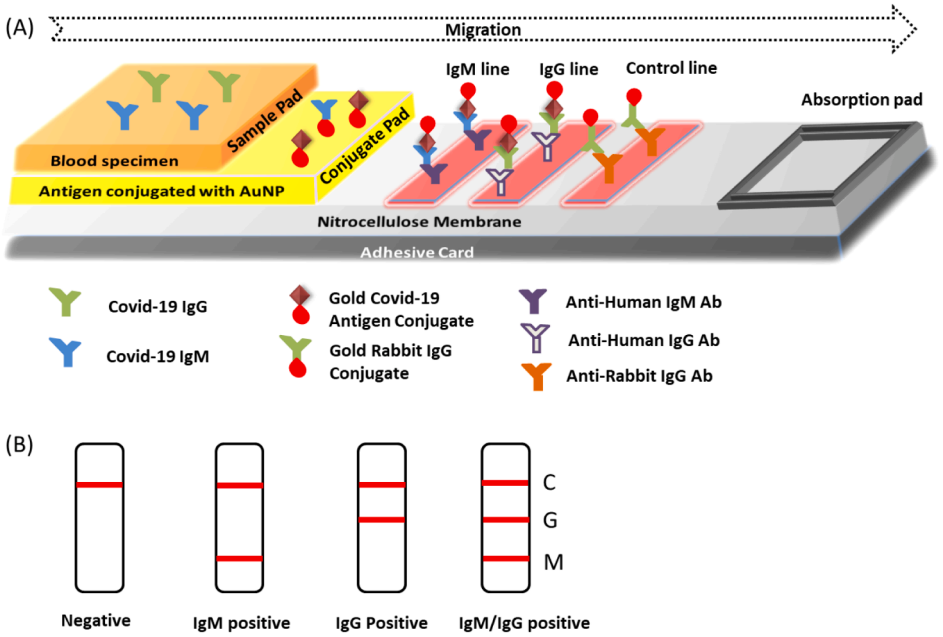

Fig. 5. Illustration representing IgG and IgM antibodies test for COVID detection.

Table 1
Various diagnostic tools available for the detection of gene/protein using different methods of diagnosis.

Test type	Test name	Gene or Protein for detection	Limit of detection	Sensitivity	Specificity	Country approved
Real Time RT-PCR	o Xpert Xpress SARS-CoV-2 test	N2 and E gene	250 copies/ml	100%	100%	Australia, USA, Canada, Singapore
	o Vita PCR SARS-CoV2 assay	Viral RNA	2.73 × 10^0	100%	100%	Canada
	o LYRA SARS-CoV2 assay	pp1ab	1.28×10^4 genome equivalent/mL	_	_	USA
	o Simplexa COVID-19 direct assay	ORF 1ab and S gene	500 copies/ml	_	_	USA
	o ARIES SARS-CoV2 assay	ORF 1ab and N gene	7.5×10^4 GCE/ml	_	_	
Multiplex Real time RT-PCR	o Bio-fire COVID-19 test	ORF 1ab and ORF 8	330 copies/ml	_	_	USA
	o Allplex 2019-nCoV assay	RdRp gene, N and E gene	500 copies/ml	100%	100%	Australia, USA, South Korea, Singapore
	o Taqpath COVID combo kit	S and N gene, ORF 1b	_	100%	100%	USA
Isothermal amplification (Nucleic acid)	o iAMP COVID-19 detection kit	N gene / ORF 1ab	4 Viral copies/μl	100%	99%	USA
	o ID NOW COVID-19	RdRP gene	125GE/ml	100%	100%	USA
	o Cue COVID-19 Test	N gene	_	95%	100%	USA
CRISPR- based	o CRISPR-based LAMP with lateral flow assay	RNA (E,N genes)	10 copy/μl	95%	100%	USA
	o SARS-CoV2 DETECTR	N genes and E genes	10 copies per μl input	_	_	USA
Lateral Flow Immunoassay (LFIA)	o National Bio Green Science, NBGC' Novel Coronavirus(2019-nCoV) IgM/IgG Antibody Rapid Test Kits	IgM and IgG	_	_	_	USA
	o STANDARD Q COVID-19 Ag test	IgM and IgG	_	88.66%	90.63%	USA, China
	o BioMedomics, COVID 19 IgM/IgG Rapid test	IgM and IgG IgG and IgM	_	_	97.8% (IgM) 99.6% (IgG)	Australia
	o Sure Screen Diagnosis, COVID-19 Rapid Test Cassette		_	96%		USA, Australia
	o Cellex, qSARS-CoV IgG/IgM Rapid Test				93.8%	
Luminescent assay	o Roche Diagnostics, Elecsys Anti-SARS-CoV-2	Total antibody against N protein	_	100%	99.8%	Switzerland
	o Siemens Healthcare, Atellica IM SARS-CoV-2 Total (COV2T)	Total antibody against RBD of S1 protein	_	100%	99.82% 99%	– China
	o Chemilum-inescence, detection kit	IgM and IgG				
ELISA	o KT-1033 EDI Novel coronavirus COVID-19 ELISA KIT	IgM/IgG	5 IU/mL	100%	100%	USA
	o Platelia SARS-CoV-2 Total Ab assay	Total antibody against N protein	_	92.2%	99.6%	_

Source collected from [68–73].

specific transducer as a better alternative to the traditional assays. Biosensors are the devices used for analysis by incorporation of a biological material, which may include directly tissues, nucleic acid, cell receptors, enzymes and proteins or derived samples such as engineered proteins, aptamers, recombinant antibodies, etc., that are closely linked or grouped within a microsystem of the physico-chemical transducers or transducer of different types such as piezoelectric, electrical, optical, and electrochemical [74].

Various biological elements can be used to develop biosensors such as antigen, antibody, and nucleic acids. Electrochemical immuno-sensors have now become the captivating option because of their high sensitivity, inexpensive nature and possibilities of their miniaturization. This bio-electroanalytical instrument is derived from the distinct of nanoparticles made of gold. Based on these ideas, many immuno-sensors have been designed to detect the viruses. Various immuno-sensors for the detection of virus causing influenza based on the electrochemical techniques have been documented in the literature using DPV (differential pulse voltammetry) [75].

Nanomaterials range from 1 to 100 nm size; when these particles are used in biosensors, various limitations and challenges can be controlled as these materials have nanoscale features such as conductance, properties including- thermal, chemical, unique optical, magnetic, and increased strength, which cannot be found in the other domain. Nanomaterials can be magnetic nanoparticles, noble metal nanoparticles, quantum dots, carbon nanostructures that have been used in sensor fabrications [76–82]. As regards the detection of COVID-19 biosensors based on gold nanoparticles (AuNPs), graphene, nanoislands (AuNIs) and nanowires are used [83]. Nanocarbon-based sensors can be operated very easily with portable detection facilities and high sensitivity these sensors have greater potential for the microbe detection [84–86].

5. Biosensors reported for earlier known coronavirus (SARS and MERS)

One of the widely explored biosensors is piezoelectric immuno-sensor for the bio-detection due to its specificity, simplifying procedure, sensitivity and speed. Crystals of piezoelectric are found on the surface of PZ immuno-sensor along with an antigen or immobilized antibody. These two biomolecules including antigen or antibodies (i.e., one freely present in the gas phase or solution, and another one is immobilized on the surface) are connected and used for real time bio-detection. The piezoelectric biosensor is a device to detect mass changes on its surface through resonant frequency where an increase in mass leads to a decrease in frequency [87].

The piezoelectric biosensor was used in the diagnosis for coronavirus related with SARS-CoV through sputum sample. This experiment consisted of binding of SARS-CoV horse polyclonal antibody from protein A to the surface with piezoelectric crystals. Shift in the frequency was recorded by changes in mass of the crystal through virus binding [88]. This approach could easily test SARS-CoV in a cost-effective manner as compared to the other SARS detection techniques.

Gold nanoparticles have also been explored in many immuno-sensing devices. These can act as an electron-conducting pathway between prosthetic groups and electrode surface, thus promoting the transfer of electrons between the redox proteins and the electrode surface. The AuNPs were largely exploited in many biosensors for detecting MERS-CoV and HCoV viruses. Layqah et al., [89] have designed a novel competitive immuno-sensor in the virus identification of MERS-CoV and HCoV by electrochemical techniques. Here, disposable array electrodes of carbon were utilized to prepare the electrodes with electro-deposition of AuNPs upon it. Array-electrode was used in the preparation and development of immuno-sensor as it allows simultaneous identification of coronavirus. The novel immuno-sensor that works on a competitive basis allows MERS-CoV to be identified in a single step, since it's sensitively and selectively can be utilized efficiently for the spiked nasal tissue. The electrochemical immuno-sensors are cost-effective, highly sensitive and allow device miniaturization as well as multiple samples screening with high performance on site [90].

In another approach, a localized surface plasmon resonance (LSPR) of AuNPs was used for the diagnosis of SARS-CoV. The optical immuno-sensor (LSPCF fiber) was used to improve the efficiency for the detection of nucleocapsid protein (N) diluted in serum as shown in Fig. 6, which is a biomarker of SARS-CoV within the limit of 0.1 pg/mL. In the early identification and diagnosis of clinical SARS coronavirus infection, this optical nanobiosensor produced amazing results.

One of the earliest expressed proteins found in SARS-CoV is nucleocapsid (N) protein that is important in coronavirus identification. The N protein was diagnosed in the serum of patients infected with SARS as early as with even in one day of the incubation [91]. Thus, identification of N proteins present in SARS-CoV is a beneficial technique for diagnosing and tracking the disease behavior and enables the early development of a fast and accurate diagnostic test. The optic immuno-sensors (LSPCF fiber) have been one of the best methods with respect to their efficacy and low limit of detection (LOD). However, LSPR has some limitations such as decreased mass transportation and the cost.

Field effective transistor coupled with nanoparticles was also explored for the detection of SARS-CoV. FET based biosensors offered many advantageous features such as specificity, sensitivity, real time application, label free detection and can be inserted as an electronic chip. FET based biosensors were also utilized for wearable diagnostics as it is a potential approach for future generation of clinical diagnostics. In any case, integration of nanomaterials will further increase the significant value of FET based biosensors.

6. Biosensors reported for COVID-19

6.1. Field-effect transistor

The biosensor reported by Zhang et al., [92] could achieve the diagnosis of COVID-19 virion digitally for quick screening. In this approach, two receptors viz., SARS-COV spike S1- subunit protein antibody (CSAb) or angiotensin converting enzyme 2 (ACE2) antibody specific SARS-CoV-2 spike S1 subunit protein (containing RBD) was immobilized onto highly sensitive graphene-field effective transistors to produce an immuno-sensor. The transistor modified with CSAb antibody could detect (real time) with a detection limit of 0.2 pM within the reaction time of 2 min. Gr-FET immuno-sensor was prepared by immobilizing either CSAb or ACE2 (both binds particularly to S protein RBD) onto graphene surface. Both the receptors showed high affinity towards antibodies, but CSAb showed higher affinity compared to ACE2.

Experimental data revealed that antibodies have the great potential for neutralizing the spike protein of SARS-CoV-2. Therefore, neutralizing antibodies can be significantly utilized in the prevention of healthy cells getting infected with the coronavirus. Thus, these findings have significance in rapid and simple detection as well as for developing new

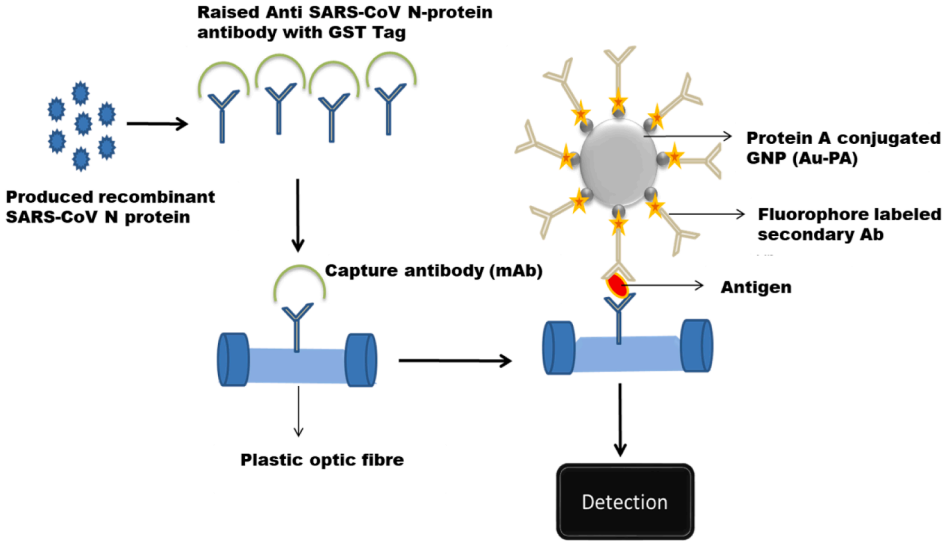

Fig. 6. Schematics of optic biosensor showing the production of antibodies by immunization of mice with glutathione S-transferase (GST) tag nucleocapsid protein and produced antibodies act as the capture antibody and secondary antibody binds with AuNPs.

vaccines, medicines and therapies to counteract COVID-19. Applications of label-free Gr-FET automated bio-sensing technology was also used to detect COVID-19 spike-protein S1 (which includes RBD) with a sensitivity compared to ELISA assay, thus avoiding any specifications of complex enzyme labelling procedures or bulky/costly optical instruments. FET based devices mostly utilize antigen/antibodies for the biological recognition. However, a complete design for technical maturity is still to be witnessed in Bio-FET based devices.

6.2. Nucleicacid-based biosensors for COVID-19

Identification of nucleic acid is an essential need for various applications such as nutritional processing and in clinical diagnosis by extracting the nucleic acid from a pathogen. In the case of immunodeficiency or immuno-compromised patients, nucleic acid-based detection methods play an important role. The nucleic acid-based amplification techniques have certain limitations and challenges. The analytical complications cover the amount of viral infection or sample selection timing in response to the disease advancement. Pre-analytical problems such as insufficient collection of respiratory samples or inappropriate handling of samples may lead to false negative results. A manual method for extracting respiratory samples using swabs is RT-PCR which is vulnerable to mistakes contrary to the major developments in analytical areas such as automatic handling and RT-PCR assay sensitivity. In addition, there are chances of obtaining false-negative results in case of faulty design of the primer/probe set in the RT-PCR assay for human RNase P gene [93]. Geno sensors that are compact tools and simple to manage are ultrasensitive and fast with a minimal false positive. Recently, various nucleic acid-based detection methods have been identified for the detection of COVID-19. Localised surface plasmon resonance (LSPR) combined with the nucleic acid as the biological recognition element offers many advantageous features, which make them highly sensitive, specific and reproducible.

6.2.1. Optical biosensors

The plasmonic biosensor has successfully shown high sensitivity, quick and trustworthy for the diagnosis in COVID-19 identification. Qiu et al has developed a dual functional LSPR biosensor which showed high sensitivity to SARS-CoV-2 sequences even at a lower level of detection limit of 0.22 pM [94]. Results show that reliable identification of the same target in a multigene mixture can be achieved. In this approach, two techniques were combined viz., plasmonic photothermal and LSPR to develop the sensing interface in the diagnosis of COVID-19 of which nucleic acid hybridization technique was exploited. The probe receptor in combination with the surface of gold nanoislands (AuNI) was used for the identification of SARS-CoV-2RNA.

The hybridization reaction between the probe and complementary sequence of viral RNA was performed under heat, which was generated by the plasmonic photothermal due to the illumination at plasmonic resonance frequency. When a laser of certain wavelength excites the same nanostructure on electrode, it induces localized heat and the change was monitored real time via a spectrophotometer. The developed interface can easily distinguish between SARS–CoV and SARS-CoV-2. Using such a setup, LSPR sensing unit was able to detect the viral sequences in real-time and without labelling including RNA dependent RNA polymerase (RdRp), gene based-RdRp-SARS-CoV-2, ORF1ab-2 SARS-CoV-2, and E genes of SARS-CoV-2. Most notably, in situ photothermal modification of AuNI chips vastly enhanced the accuracy of hybridization kinetics and nucleic acid recognition. Using the *in-situ* photothermal enhancement, the associated sequences such as SARS-CoV and SARS-CoV-2 RdRp genes were specifically differentiated. In the context of COVID-19 outbreak, this potential dual-functional LSPR biosensor may provide a robust and convenient diagnostic tool to boost diagnostic precision in clinical research and alleviate the burden of using PCR-based protocols.

Recently, a team of researchers from Empa, ETH Zurich and Zurich University Hospital has developed a biosensor for the detection of COVID-19 [95]. Zhu et al., [96] alternatively designed an optical biosensor, which could demonstrate two varied effects viz., thermal and optical for secure and accurate identification of the virus. The system is based on a glass substrate built on tiny gold structures, the so-called gold nanoislands. Artificially generated DNA receptors matching the SARS-CoV-2 RNA sequences were imprinted on the nanoislands. Consequently, the sensor receptors with the complementary DNA sequences to the unique virus RNA sequences can accurately recognize the virus based on the LSPR detection technique. The technique was tested for a very closely related virus, i.e., SARS-CoV in order to show the accuracy of the as-developed plasmonic biosensor to detect the existing SARS-CoV-2 virus. The test was successful as the data revealed that sensor could easily differentiate between the two viruses carrying very similar RNA sequences. Furthermore, the test can be completed within a few of minutes. However, in our opinion, this approach still needs further development; in this regard, efforts are underway to improve the reliability of the test for COVID-19 diagnosis.

6.2.2. RT-LAMP based biosensors

The LSPR technique offers many advantageous features such as fast detection, high sensitivity and low sample/analysis volume, but the approach is very expensive and there still some limitations associated with the mass transport. Therefore, other approaches may be needed to develop to meet the demands of the growing disease epidemics. As introduced previously in molecular technique section, Loop-mediated isothermal amplification (LAMP) method is considered as a boon to the diagnostic world nowadays. In this approach, isothermal conditions are required for the amplification of desired gene sequence unlike the PCR, which requires variable temperature, thus limiting its application in resource limited settings. However, the major disadvantage associated with the method is that amplified sequence cannot be seen with the naked eye. If the technique is coupled with some colorimetric sensing probes or device, then it will add much value to the approach.

In efforts to solve the above-cited problems, recently a new method was discovered in which RT-LAMP can be coupled with the biosensor design based on the use of colored nanoparticles for visual detection. For accurate diagnosis of COVID-19, a one-step involving the single tube reaction RT-LAMP-NBS (reverse-transcription loop mediated isothermal amplification paired with biosensors based on nanoparticles), named as COVID-19 RT-LAMP-NBS was successfully developed. The overall diagnostic procedure from the selection of samples to the analysis of the findings would take around a total of 1 h, including 3 min for sample collection, 15 min for the extraction of RNA, 40 min for RT-LAMP amplification and less than or about 2 min for the interpretation of the results. However, this method requires very basic apparatus (such as heating block) to carry forward the steady temperature of 63 °C for only 40 min. Its working includes using two engineered LAMP priming sets, SARS-CoV-2 genes F1ab [(open reading frame) ORF 40 1a/b] and NP (nucleoprotein). At the same time, 'one-step' and 'one-tube' reactions can be multiplied and observed; thus, the colorimetric nanoparticles-based biosensors (NBS) can be easily viewed using the sensing results easily. TheCOVID-19 RT-LAMP-NBS was susceptible to 12 copies (for each target detection) per reaction, and non-COVID-19 templates provide no cross-reactivity. The experimental susceptibility of SARS-CoV-2 in oropharynx swab samples was found to be 100% among the clinically verified COVID-19 patients and precision of the results was also 100% when clinical samples from non-COVID-19 patients were examined.

In view of the above characteristics, COVID-19 RT-LAMP-NBS testing is easy, economical and technically a much simple method that offers practicality measurements for the field and clinical laboratories, and is more impactful in resource constrained settings. Two targets (F1ab and np genes) could be amplified simultaneously and identified in 'one-step' RT-LAMP reaction, further improving the precision of the assay. The as-developed single-step COVID-19 RT-LAMP-NBS assay provides an attractive diagnostic tool for detecting COVID-19. Moreover, virtually

equipment-free COVID-19 RT-LAMP-NBS model makes it accessible to resource-limited laboratories (e.g., field laboratories) and the findings of the analysis as well as its interpretation is simple. High precision, sensitivity, and viability, cost efficiency and easy to handle make this test a valuable diagnostic tool for use in actual fields, hospitals, public health and primary-care-laboratories, especially in less economically stronger regions.

6.2.3. Miscellaneous biosensors

A biosensor called CANARY™ developed by PathSensors Inc., (in March 2020) that can detect the novel COVID-19 was used in scientific laboratory tests (in May 2020), and further confirmation of the data is awaited on the latest SARS-CoV-2 device (in June 2020). However, the laboratory validations by the third party have shown that CANARY™ products are highly sensitive and effective for commercial use, while SARS-CoV-2 biosensor is available in the market. PathSensors anticipates its new applications such as quick sample screening, but the use of this system depends after checking environmental swabs and track airflow in sensitive spaces such as hospitals, schools, and food facilities. PathSensors provides three instrumentation frameworks for use, which produces test results usually in less than 5 min and expects that after testing for SARS-CoV-2, the device can be a powerful tool to counter COVID-19 spread [97].

Recently, Abbott ID Now TM developed COVID-19 detection kit, which was also based on LAMP and can be used to detect COVID-19 within 5 min, but its availability is limited. In this approach, fluorescently labelled molecular beacons are utilized for the identification of amplicons. The primers are used to identify the RNA dependent RNA polymerase (RdRp) viral gene. The developed kit can analyse the swabs of the mouth, nasal, nasopharyngeal, etc. The kit consists of 24 tests, including both positive and negative controls, swab collecting samples, and pipettes. The method was approved by the FDA EUA and can be a fantastic development in the international market [98].

As many dreadful viruses have RNA as a genetic material, high-throughput RNA sequencing method has been utilized for the diagnosis of COVID-19. In this approach, transposons are identified, which can fragment DNA-RNA heteroduplex. Researchers at the Peking University (China) constructed a tool for a quick analysis and rapid detection of COVID-19 by building a library of (transcriptomes) sequencing HEteRo RNA-DNA-hYbrid (SHERRY). This protocol offers a clear and precise approach to characterize and quantify the RNA. The authors used Tn5 transposase, which binds and cuts [99] dsDNA at random as well as to fragment and prime the RNA/DNA hetero-duplexes produced by the reverse transcription directly. The amplification of prime fragments was done using PCR.

Mahari et al., [100] recently created an in-house biosensor system (eCovSens) generated along with gold nanoparticles (AuNPs) and COVID-19 antibody electrode with fluorine doped tin oxide (FTO) electrode which is highly specific for SARS-CoV-2 spike antigen detection. These FTO-Immuno-sensors recognize COVID-19 antigen at optimum conditions ranging from 1 fM to 1 μM concentrations. In a standardized buffer, this eCovSens unit can diagnose SARS-CoV-2 antigen at 10 fM concentration. This system reveals the outcomes easily within 10–30 s.

7. Conclusions

In the current scenario, diagnosis is a major problem arising in densely populated nations such as India, Brazil, Mexico and China. Once the disease gets detected then management and transmission of the disease can be controlled with a high efficiency. Therefore, there is an urgent need for developing a device for easy identification at the point of care, so patients can easily self-isolate or quarantine themselves, which would greatly prevent further transmission, thereby reducing the burden on health professionals and the risk associated with the infection can be minimised. The major cause of infection in health professionals is when they diagnose the patients with the infected samples.

The immuno-sensors can be the alternatives for the point of care devices if their major limitations can be avoided i.e., cross reactivity. In any case, immuno-sensors offered many advantageous features such as good specificity, high sensitivity and direct antigen detection. Still early biomarkers for the development of interface in immuno-sensors are yet to be documented. In our opinion, nucleic acid-based detection is the best alternative as the problem of cross-reactivity and early detection can be avoided. Also, NASBA, if coupled with the visual detector, can prove to be a good diagnostic device. Further miniaturization can play a pivotal role in fabricating portable diagnostic tool for COVID-19.

From the literature, one can conclude that nucleic acid-based method is often at the forefront in the pandemic era, since it offers many advantageous features compared to the immuno-assay techniques. Firstly, nucleic acid method offers high specificity and hence, less chances of cross reactivity compared to immuno-based methods. Secondly, nucleic acid-based method has the early detection advantage as it is employed for the detection of virus, while immuno-assay is employed for the identification of biomarkers such as antibodies IgG/IgM. Thirdly, nucleic acid-based methods are also applicable to immuno-compromised or deficient persons, but immuno-assay is not applicable to the same.

In nucleic acid-based assay methods, samples need to be transported to diagnostic site, which is a major problem, but this can be overcome by developing lateral flow assay using CRISPR, which has the great potential of converting into the point of care devices. A new diagnostic method is needed to be developed, which can cover all the setbacks associated with the previous methods to offer high potential of applying at the point of care. Diagnosis can alleviate the major problem of COVID-19 pandemic as if more detection needs to be done in the early stage, and then COVID-19 infected person can be isolated and quarantined. In any case, in order to win over this tough battle against the pandemic, combined and specialized efforts between medical fraternity, clinicians, scientists, biologists, engineers and chemists is necessary. In parallel, advancement in biosensing field and biosensors that can be miniaturized and applied on site is necessary for combating the deadly epidemic.

Declaration of Competing Interest

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

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