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.

Trends in Analytical Chemistry 133 (2020) 116072

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

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

Analytical insights of COVID-19 pandemic

Gayathri Chellasamy [1], Shiva Kumar Arumugasamy [1], Saravanan Govindaraju**, Kyusik Yun*

Department of Bionanotechnology, Gachon University, Gyeonggi-do, 13120, Republic of Korea

ARTICLE INFO

Article history:
Available online 17 October 2020

Keywords:
COVID-19
Genome
Diagnostic techniques
Treatment
Vaccines
Pandemic outbreak

ABSTRACT

Coronavirus disease-19 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (2019-nCoV or SARS-CoV-2). Genomic analysis has revealed that bat and pangolin coronaviruses are phylogenetically related to SARS-CoV-2. The actual origin and passage history of the virus are unknown, but human-human transmission of the virus has been confirmed. Several diagnostic techniques have been developed to detect COVID-19 in this prevailing pandemic period. In this review, we provide an overview of SARS-CoV-2 and other coronaviruses. The origin, structure, current diagnostic techniques, such as molecular assays based on oligonucleotides, immunoassay-based detection, nanomaterial-based biosensing, and distinctive sample based detection are also discussed. Furthermore, our review highlights the admissible treatment strategies for COVID-19 and future perspectives on the development of biosensing techniques and vaccines for the diagnosis and prevention of the disease, respectively.

1. Introduction

The pandemic outbreak of COVID-19 was caused by SARS-CoV-2, which belongs to the *Coronaviridae* family in the order *Nidovirales* and the subfamily of *Orthocoronavirinae*. Alpha-coronavirus (α-CoV), beta-coronavirus (β-CoV), gamma-coronavirus (γ-CoV), and delta-coronavirus (δ-CoV) are the four genera of coronaviruses in the subfamily *Orthocoronavirinae* [1,2]. Among these, α- and β-CoVs mainly infect mammals, while δ- and γ-CoVs infect birds. β-CoVs include SARS-CoV, the Middle East respiratory syndrome (MERS) virus (MERS-CoV), and the current SARS-CoV-2 (COVID-19). Globally, SARS-CoV and MERS-CoV have infected more than 8000 and 2428 individuals, respectively. The comparative analysis of SARS-CoV, MERS-CoV, and SARS-CoV-2 are presented in Table 1. Patients infected with these viruses develop pneumonia, followed by acute respiratory distress syndrome (ARDS) and renal failure [3]. Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 attacks the lower respiratory system, leading to viral pneumonia. Additionally, it affects the gastrointestinal (GI) system, liver, central nervous system, and kidney [4]. The incubation period of COVID-19 is 5–25 d, and its symptoms include fever and severe respiratory symptoms, such as cough, dyspnea, and muscle soreness. Headaches and diarrhea are reported in less than 10% of patients, with recent confirmation of loss of smell and taste [5,6].

Understanding the origin and transmission of SARS-CoV-2 is an important factor that can help in determining the preventive measures for COVID-19. In the case of SARS-CoV, researchers initially focused on palm civets and raccoon dogs. Samples from civets showed a positive result for viral RNA detection, implicating them as secondary hosts for SARS-CoV. For MERS-CoV, researchers predicted camels as the zoonotic source for viral infection and recently identified the same RNA sequence in *Pipistrellus* and *Perimyotis* bats. Before determining the genomic similarities between coronavirus and SARS-like bat viruses, some researchers suggested snakes as a possible host. However, after genomic identification, the bat virus was shown to have more similarities to the coronavirus [7] (Fig. 1). For COVID-19, researchers still do not have a precise theory explaining the natural origin of the COVID-19 pandemic. They have suggested bats and pangolins as the possible natural hosts of SARS-CoV-2 and that the virus crossed from these animals to humans, posing a risk to human health. In addition, the virus developed the ability to spread from human to human and cause more serious and life-threatening diseases [8]. Research is ongoing to identify the exact natural host, diagnostic techniques, and treatment strategies for COVID-19.

* Corresponding author.
** Corresponding author.
E-mail addresses: biovijaysaran@gmail.com (S. Govindaraju), ykyusik@gachon.ac.kr (K. Yun).
[1] G. C and S. A contributed equally to this work.

https://doi.org/10.1016/j.trac.2020.116072
0165-9936/

List of abbreviations

ACE2	Angiotensin Converting Enzyme 2
ARDS	Acute Respiratory Distress Syndrome
AuNIs	Gold Nanoislands
AuNPs	Gold Nanoparticles
BALF	Bronchoalveolar lavage fluid
CDC	Centers for Disease control and Prevention
CLIA	Chemiluminescence Enzyme Immunoassay
COVID-19	Coronavirus disease-19
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CSAb	SARS-CoV spike S1 subunit protein antibody
CT	Computed Tomography
ELISA	Enzyme Linked Immunosorbent Assay
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
Gr-FET	Graphene-Field Effect Transistor
ICG	Immunochromatographic strip
ICMR	Indian Council of Medical Research
Kd	Equilibrium dissociation constant
LAMP	Loop-mediated Isothermal Amplification
LFIA	Lateral Flow Immunoassay
LOD	Limit of Detection
LSPR	Localized Surface Plasmon Resonance
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
NIV	Non-invasive Ventilation
NP	Nasopharyngeal
Nt	nucleotides
ORF	Open Reading Frame
PPT	Plasmonic Photothermal
RBD	Receptor Binding Domain
RdRp	RNA Dependent RNA Polymerase
rRT-PCR	Real-Time Reverse Transcriptase-Polymerase Chain Reaction
RT-LAMP	reverse transcription LAMP
SARS-CoV-2/2019-nCoV	Severe Acute Respiratory Syndrome Coronavirus 2
sgmRNA	subgenomic mRNA
SPR	Surface Plasma Resonance
WHO	World Health Organization

Table 1
Comparison between SARS-CoV, MERS-CoV and SARS-CoV-2.

Coronavirus	SARS-CoV	MERS-CoV	SARS-CoV-2
Source	Bats	Camel	Under Debate
Identified	2010	2012	2019
Origin	Guangdong, China	Jeddah, Saudi Arabia	Wuhan, China
Reported Countries	29	27	205
Incubation time	4 d	4–5 d	4–14 d
Total cases (global)	8096	2519	8,804,719
Recovered (global)	7322	1653	4,657,677
Deceased (global)	774	866	463,510
Fatality	11%	34.3%	9%
Diagnosis Technique	PCR	RT-PCR	Nucleic Acid Testing (rRT-PCR), Antibody Testing
Vaccines	Phase 1 trial	Phase 1 trial	In development

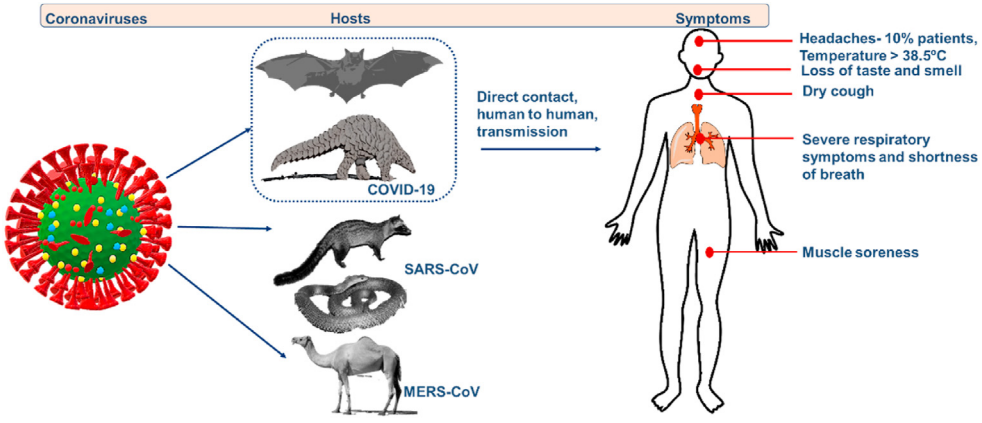

Fig. 1. Schematic diagram of coronaviruses hosts and symptoms.

Different testing strategies have been used by different countries based on the availability of reagents and laboratories. Real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) assay plays a main role in the diagnosis of COVID-19 from respiratory specimens. In addition, computed tomography (CT), serological testing, viral culture, immunoassay testing, laboratory specimen test, and thermal scanning have been performed to diagnose COVID-19 [9]. Various bodies, including the Centers for Disease Control and Prevention (CDC) of many countries and the World Health Organization (WHO), have presented different

measures for the prevention of the spread of COVID-19. Avoiding travel, social distancing, hand washing, and wearing of face masks are some of the preventive measures that have been recommended to the public [10]. To date, no antiviral treatment or vaccine has been developed for COVID-19. WHO announced that 12 potential drug trials are underway, including those for drugs already used for the treatment of HIV and malaria. Some other experimental treatments have been employed to reduce inflammation associated with COVID-19 [11]. In this review, we briefly discuss the origin of SARS-CoV-2, the genome organization, and its comparison with other coronaviruses. Here, we present the principle, design, accuracy, sensitivity, selectivity, and response time for currently available COVID-19 diagnostic tools.

2. SARS-CoV-2 and its genome

Coronaviridae viruses possess an envelope and crown-like viral particles around their genetic materials. Despite the efforts of researchers to treat infectious diseases, a new disease with high mortality has emerged and spread worldwide [12]. In the past two decades, the CoV family has caused serious illnesses, such as SARS in the years 2002–2003 and MERS in 2012. Now, the unexplained respiratory infection caused by a novel CoV family member (SARS-CoV-2) was reported in December 31, 2019, and was named COVID-19 by WHO. Since COVID-19 was identified to be highly contagious due to its spread by human–human transmission, Chinese researchers have rapidly sequenced its genome [13]. Genome sequencing of SARS-CoV-2 revealed that it has a single strand positive-sense RNA (+ssRNA) molecule of approximately 29,903 nt, containing genes in the order of 5′–3′ as follows: ORF1ab, spike (S), envelope (E), membrane (M), and nucleocapsid (N). The analysis shows that SARS-CoV-2 has terminal sequences that are typical of beta-coronaviruses, containing 265 nt at the 5′ end and 229 nt at the 3′ end. The ORF1ab gene codes for 16 predicted non-structural proteins, followed by 13 downstream ORFs. The predicted S, ORF3a, E, M, and N genes of SARS-CoV-2 are 3822, 828, 228, 669, and 1260 nt in length, respectively. In addition, SARS-CoV-2 carries an ORF8 gene of 366 nt between the M and N ORF genes [14]. The genome data of SARS-CoV-2 is publicly available to facilitate the search for the original host of this virus. Fig. 2 shows the structure of COVID-19 and its genome organization.

2.1. Proximal host genome of SARS-CoV-2: a comparative analysis

Rapid genome sequencing of SARS-CoV-2 at an early stage by Chinese researchers paved the way for the identification of the proximal host and tracing of the evolutionary relationship between SARS-CoV-2 and former coronaviruses. Phylogenetic analysis of coronaviruses shows that SARS-CoV-2 shares a highly conserved domain with SARS-CoV in nsp1 (LLRKNGNKG: amino acids 122–130). Further studies on evolutionary research suggested that SARS-CoV-2 shows 82.3% and 77.2% amino acid identity with the bat coronaviruses, SL-CoVZC45 and SARS-CoV, respectively [13]. A comparative study on the receptor-binding domain (RBD) of its spike protein and those of SARS-CoVs and bat SARS-like CoVs reported 73.8–74.9% and 75.9–76.9% amino acid similarity, respectively. In addition, the RBD of SARS-CoV-2 is only one amino acid longer than that of SARS-CoV. These findings suggest that SARS-CoV-2 has the ability to use human angiotensin converting enzyme 2 (ACE2) receptors for cell entry, similar to SARS-CoV. Another comparative study identified SARS-CoV-2 S protein to possess a furin-like cleavage site, which is absent in SARS-like CoVs [15].

Coronaviruses have the ability to cross species barriers from wild animals to humans, and hence, there is a need to identify the exact host of SARS-CoV-2 to facilitate the development of effective therapy for COVID-19. Bats are considered the reservoir hosts of the previous SARS-CoV and MERS-CoV outbreaks, while their intermediary hosts are Palm civets and Dromedary camels, respectively. In the case of SARS-CoV-2, researchers believe that there are two possibilities for species transmission; it may have been directly transmitted from bats to humans, or indirectly through an intermediary host. Because bat CoVs show 96% similarity to SARS-CoV-2 at the whole-genome level, researchers believe that transmission might have occurred directly from the bat reservoir host [16]. Meanwhile, in another study, Guangzhou Chinese researchers suggested that pangolins long-snouted, ant-eating animals may also be a plausible host of SARS-CoV-2, with 99% sequence

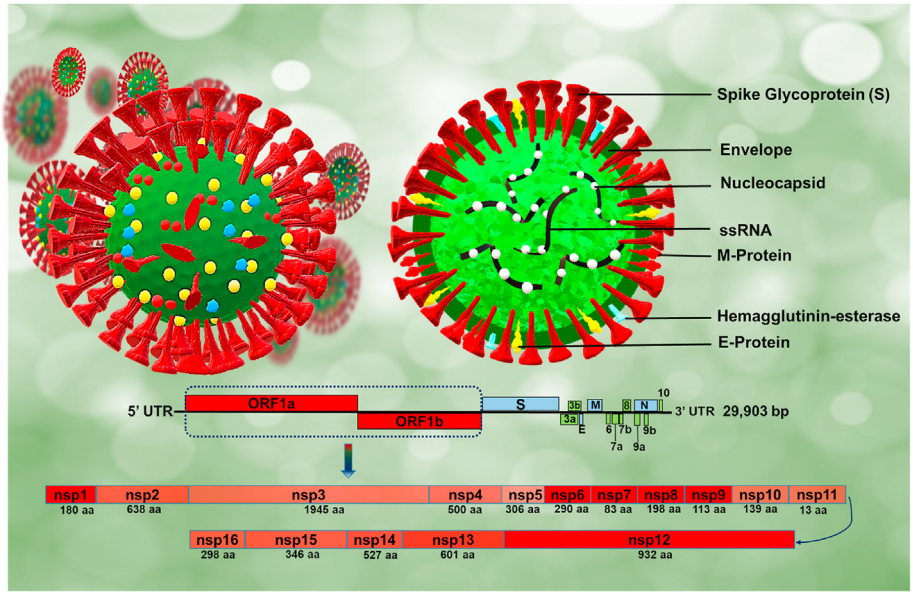

Fig. 2. Structure of COVID-19 and its genome organization.

similarity observed. However, this research is ongoing and has not yet been published. Another study on coronaviruses isolated from Malayan pangolins (*Manis javanica*) showed 100%, 98.2%, 96.7%, and 90.4% amino acid similarity with SARS-CoV-2 in the E, M, N, and S genes, respectively. SimPlot analysis of the whole genome sequence of the Pangolin-CoV showed sequence similarities of 80% and 98% with SARS-CoV-2 and bat SARSr-CoV RaTG13, respectively, except for the S gene, suggesting that pangolin coronavirus is capable of entering the human cells [17]. The findings of these studies suggest that both pangolins and bats are nocturnal animals and share overlapping ecological niches, which make pangolins a possible intermediary host for SARS-CoV-2. In addition, the genomic comparison suggests that SARS-CoV-2 might be a chimera of pre-existing coronaviruses (one close to pangolin CoVs and another close to RaTG13 bat CoVs). It is known that for recombination to occur, the two divergent viruses must have infected the same organism simultaneously, which has been described previously for SARS-CoV infection [18]. However, the organism in which this recombination takes place and the conditions under which this recombination happens are still unknown.

3. Detection techniques for COVID-19

The availability of the genetic information of SARS-CoV-2 and the detailed understanding of its biological properties have enabled researchers to develop detection techniques within a short period of the prevailing pandemic. Such developed detection techniques are categorized into four major classes i) Molecular assays based on oligonucleotides ii) Immunoassay-based detection iii) Nanomaterial-based biosensing and iv) Distinctive sample-based detection. Herein, the key elements emphasizing technique principle, processing, accuracy, and response time were analyzed and discussed. An outline of the diagnostic techniques for COVID-19 is presented in Fig. 3.

3.1. COVID-19 diagnosis based on molecular assays

The accurate diagnosis of COVID-19 on the basis of the expressed symptoms is difficult because the symptoms are nonspecific and similar to those of the common flu. Therefore, detecting SARS-CoV-2 at the molecular level would be more suitable for the accurate diagnosis of COVID-19 [19]. Molecular assays including rRT-PCR, loop-mediated isothermal amplification, and clustered regularly interspaced short palindromic repeats (CRISPR)-based techniques were designed and applied for the detection of SARS-CoV-2.

The most widely used gold standard technique for SARS-CoV-2 detection is rRT-PCR, which has the ability to amplify even a small amount of genetic material [20]. More advanced rRT-qPCR-based techniques have been developed by researchers in recent times. Ishige and team developed a multiplex rRT-PCR to detect two regions of the SARS-CoV-2 genome (E and N genes) and the human *ABL1* gene simultaneously, where *ABL1* serves as an internal control (IC) [21]. Compared with the general rRT-PCR, the multiplex assay provides higher sensitivity with a detection limit of > 25 copies/reaction. This technique of detecting SARS-CoV-2 does not involve the use of the most abundantly expressed genes, *ACTB* and *GAPDH*, as ICs because they prevent the amplification of less abundant targets and thus lowers the sensitivity of target detection. Thus, the multiplex rRT-PCR technique facilitates highly sensitive detection of SARS-CoV-2 RNA, with reduced cost and amounts of reagents. A study by Penarrubia et al. expresses the importance of multiple assays in RT-PCR targeting more than one region in the SARS-CoV-2 genome to mitigate the risk of sensitivity loss due to unknown genetic variability [22]. They suggested that viral mutation and recombination rates increase due to the genetic variability of the SARS-CoV-2 genome. Therefore, it is essential to perform multiple rRT-PCR assays targeting at least two regions to monitor genomic variations. Currently, several rRT-PCR-based COVID-19 kits are developed by different manufacturers, which have been

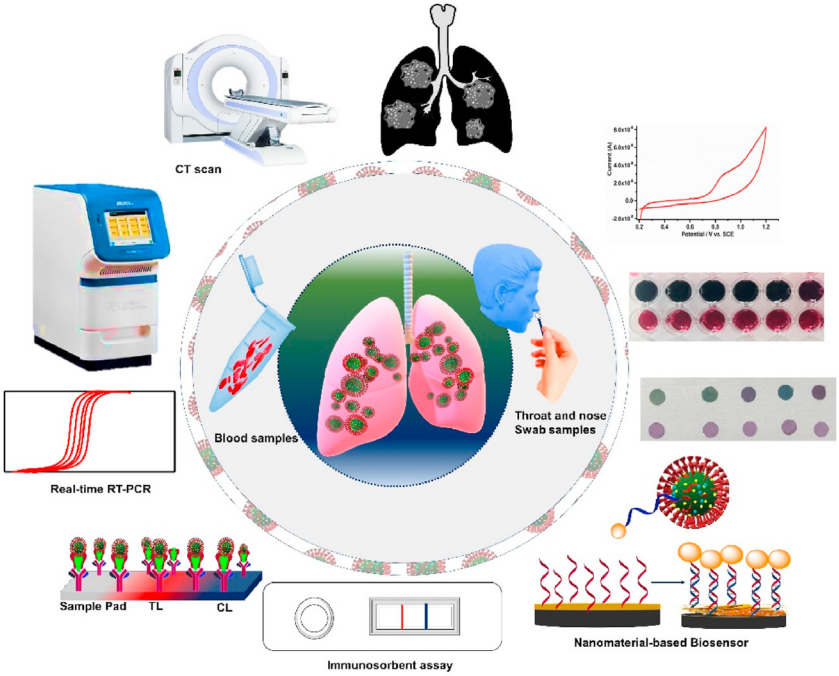

Fig. 3. Diagnosis techniques available for COVID-19.

commercialized. Van Kasteren research group carried out a study on COVID-19 diagnosing RT-PCR kits to assess their clinical and analytical performance [23]. The findings of this study showed that the rRT-PCR kits gave satisfactory PCR efficiency (≥96%) and the estimated 95% limit of detection (LOD95) varied within a 6-fold range between kits (3.8–23 copies/mL). In addition, they reported that the commercially available kits can be used for routine diagnosis of symptomatic COVID-19. Wang et al. developed a novel one-step single-tube nested quantitative real-time PCR for the sensitive detection of SARS-CoV-2 [24]. This technique targeted ORF1ab and N genes and showed detection sensitivity of 1 copy/reaction, with 100% specificity. It was officially approved by the National Medical Products Administration and used in the detection of COVID-19 nationwide. This method has the advantage of high specificity and sensitivity, with the ability to detect asymptomatic infected persons with low viral load.

Though RT-PCR-based detection is considered a standard technique, it faces several obstacles, such as time-consuming amplification process and high-cost reagents, and requires sophisticated thermal cycling equipment [19]. To overcome these obstacles, loop-mediated isothermal nucleic acid amplification (LAMP) was used as an alternative strategy for rapid and cost-effective detection of SARS-CoV-2. Park et al., designed and evaluated a one-step reverse transcription LAMP (RT-LAMP) method to detect SARS-CoV-2 [25], and the detection principle involved the use of leuco crystal violet method for colorimetric detection of SARS-CoV-2. They reported LOD of approximately 100 copies/reaction, with no cross-reactivity with other human coronaviruses. The research group of Baek reported a single-tube RT-LAMP assay to detect novel coronavirus N gene through colorimetric visualization [26]. Their method focused on targeting the N protein, which has the most abundant expression of subgenomic mRNA (sgmRNA) during infection, lacks the glycosylation site, and possesses distinctly unaltered immunological characteristics. It exhibited high sensitivity and specificity, with the ability to discriminate circulating human coronaviruses, including OC43, 229E, NL64, and MERS-CoV, as well as other respiratory viruses. Furthermore, this method yields rapid results within 30 min, with a detection limit of 10^2 RNA copies.

The other leading molecular technique for COVID-19 diagnosis is CRISPR-based detection, which offers precise and rapid detection of viral nucleic acid nearly at the attomolar (10^{-18}) level [27]. According to the study by Jinek et al., CRISPR-Cas technology relies on small RNAs for sequence-specific detection and silencing of foreign nucleic acids [28]. Kellner et al. described a protocol for the rapid detection of nucleic acids via fluorescence and colorimetric readouts using CRISPR-Cas13. This method provides results in <1 h with a setup time of less than 15 min [29]. Recently, Broughton et al. developed and validated a CRISPR-Cas12-based SARS-CoV-2 detection, combined with isothermal amplification [30]. The developed technique was rapid (30–40 min) with a LOD of approximately 10 copies/μL input. This method limits the need for expensive laboratory instrumentation, but involves routine protocols and commercially available off-the-shelf reagents. The advantages of CRISPR-Cas12-based detection system are rapid turnaround time, single nucleotide target specificity, integration with accessible and easy-to-use reporting formats, such as lateral flow strips, and no requirement for complex laboratory infrastructure. Huang and colleagues also demonstrated a diagnostic technology in their study for COVID-19 diagnosis based on CRISPR fluorescent detection system (CRISPR-FDS) [31,32]. CRISPR-FDS follows a simple three-step detection process, including RNA extraction, target amplification, and fluorescent signal detection, with the help of CRISPR Cas 12a/gRNA complex. This method allows easy detection of SARS-CoV-2 in fluorescent plate readers, with high sensitivity and detection limit of 2 copies/sample, within the sample-to-result time of ~50 min. This method of SARS-CoV-2 detection obtained comparable results with CDC-approved quantitative rRT-PCR assay; however, it failed to provide clear positive or negative results and was not able to quantify viral load.

3.2. Immunoassay-based detection

The detection of COVID-19 is based on the presence of specific immune proteins, which could be broadly classified into enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay (LFIA), and chemiluminescence enzyme immunoassay (CLIA). These approaches follow different strategies, but the basic principle is the antigen-antibody interaction. Although the techniques provide partial quantitative results, rRT-PCR is considered a standard analysis. However, to screen a large number of individuals for infection, alternative detection methods to the immunosorbent assay testing should be adopted. LFIA is more focused than ELISA as it requires trained personnel and sophisticated laboratories, whereas LFIA is simpler as well as more portable and rapid. For ELISA, samples are collected from nasopharyngeal (NP) sites and incubated in 96-well plates for 1 h; it is a time-consuming process and there is a high chance of producing false-positive responses upon cross-reactivity [33]. Zhang et al. demonstrated the procedure for COVID-19 detection using ELISA consisting of SARS-CoV-2 Rp3 N protein, antihuman IgG modified with horseradish peroxidase, and 3,3′,5,5′-tetramethylbenzidine [34]. The detection mechanism is based on peroxidase interaction with the adsorbed nucleoprotein that produces a color change in the plate. The specificity and sensitivity of such detection range from 81 to 100% after 5 days of infection. The performance of EUROIMMUN ELISA for semi-quantitative detection with respect to the S1 domain of the spike protein for both IgA and IgG antibodies was reported by Beavis et al. [35]. The samples were diluted and incubated in wells containing IgA/IgG antibodies, followed by incubation with the detection antibodies i.e. enzyme-labeled antihuman IgA or IgG, which catalyzed a color change. The results were determined as a ratio of excitation of the control or patient samples over the excitation of the calibrator. This ratio was inferred as follows <0.8 negative, > 0.8 < 1 borderline (but considered as positive), and >1.1 positive. The results showed a specificity of 90.5% for IgA antibody and 100% for IgG antibody after incubation for 4 days.

The use of immunochromatographic (ICG) strip to target IgG/IgM antibodies for the detection of viral loads in patients was investigated [36]. IgM antibody is the first line of defense produced by the immune system when the virus attacks, whereas IgG antibodies take almost 7–10 days after infection. The disease infection stages include the initial stage (from 1 to 7 days), intermittent stage (8–10 days), and late stage (>15 days). The positive response ratings were 3%, 26%, and 30%, respectively. Overall, the specificity and selectivity for the detection assay range from 97.1 to 100% depending on the duration of infection. Nicol et al. compared three immunoassays, ELISA (Euroimmun), CLIA (Abbott), and LFIA (NG Biotech) for the detection of SARS-CoV-2 [37]. In ELISA, the target analyte was spike protein, and for CLIA, the target was N protein. In ELISA, Euroimmun anti-SARS-CoV-2 IgG and IgA assays were utilized against the recombinant S1 structural protein, whereas the CLIA IgG antibodies of SARS-CoV-2 were solely utilized against the nucleoproteins in the serum or plasma. For LFIA, an immune colloidal assay for IgG and IgM antibodies of SARS-CoV-2 against nucleoprotein was performed. A comparative study was performed for samples collected after 14 days of infection, with IgG antibodies, and the results showed a specificity of 98% for CLIA and LFIA and 95.8% for ELISA.

3.3. Nanomaterial-based biosensing for COVID-19

Although RT-PCR and immunoassay-based techniques are the standard procedures for COVID-19 diagnosis, the use of nanoparticle-based biosensing for SARS-CoV-2 detection is considerably on the rise due to its precision and speed. Biosensors are emerging alternative tools to conventional laboratory equipment for medical and environmental analyses. The outstanding electrical and optical properties of nanomaterials are typically combined with those of biological or synthetic molecules by biosensors to selectively detect any kind of analyte [38]. Nanoparticles, including gold nanoparticles (AuNPs), carbon-based nanoparticles and other nanomaterials, play a significant role in the development of SARS-CoV-2 sensor. In particular, properties, such as quantum size effects, high adsorption, high surface-to-volume ratio, and high reactive capacity are important for the design of biosensing techniques. Moreover, the surface, size, and shape of nanomaterials can easily be tailored to enhance the detection limit and selectivity towards the analytes [39]. Based on these physicochemical properties, researchers have been developing accurate, rapid and cost-effective COVID-19 sensors. The schematic representation of nanomaterials-based biosensing techniques for COVID-19 is presented in Fig. 4.

Moitra et al. proposed a thiol-modified antisense oligonucleotides (ASOs) capped AuNPs based colorimetric assay to detect SARS-CoV-2 by the naked eye [40]. The ASOs are specific to the N gene (nucleocapsid phosphoprotein) of SARS-CoV-2, and the sensor was developed based on the agglomeration of ASO-capped AuNPs with the target RNA sequence of SARS-CoV-2. This phenomenon changes the surface plasmon resonance (SPR) signal and the addition of RNaseH led to the agglomeration of AuNPs. During the aggregation of AuNPs, the violet color changed to dark blue, which led to an increase in the absorbance at 660 nm with a redshift of 40 nm. The sensitivity of SARS-CoV-2 to ASO-capped AuNPs was monitored based on the increase in absorbance at 660 nm. The absorbance difference observed was due to thiol-modified ASOs with a comparative ratio of ASOs to the AuNPs, which play a major role in the detection of SARS-CoV-2 by the AuNPs. This technique could be used to detect COVID-19 cases within 10 min with a detection limit of 0.18 ng/μL of RNA. Moitra's biosensing system satisfies most of the assured criteria mentioned by WHO, which includes affordability, sensitivity, and low cost. Kumar et al. reported a similar rapid colorimetric assay for the detection of SARS-CoV-2 using NP RNA samples [41]. The sensor is based on the aggregation of colloidal gold nanoparticles, where the color changed from pink to blue during the reaction. This technique accomplished the detection of SARS-CoV-2 infection in human NP samples within 30 min with a detection limit of 0.5 ng. In this study, RNA-dependent RNA polymerase (RdRp) gene of SARS-CoV-2 was used as the target, which has good analytical capability as suggested by WHO. Mahari et al. developed an in-house built biosensing electrochemical device (eCoVSens) for the detection of COVID-19 antigen [42]. This device was fabricated based on the electrical conductivity changes during the interaction between COVID-19 monoclonal antibody immobilized on gold nanoparticles and specific antigen. The detection was performed in spiked saliva samples by eCoVSens with a detection limit of 90 fM.

Qiu et al. developed a dual-functional plasmonic biosensor platform (combination of plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) sensing transduction) as an alternative and promising technique for COVID-19 detection [43]. Gold nanoislands (AuNIs) with DNA receptor was used to detect SARS-CoV-2 virus through nucleic acid hybridization. The plasmonic resonance frequency generated thermoplasmonic heat on the same AuNIs chip, leading to better sensing performance. PPT heating utilized by the AuNIs matched the laser excitation wavelength at 532.2 nm. After the addition of a long pass filter, excitation wavelength did not affect the stability of real-time LSPR sensing transduction. The developed sensor platform exhibited enhanced accuracy, sensing stability, sensitivity, and reliability with a detection limit of 0.22 pM, allowing the detection of multiple genes of SARS-CoV-2 virus.

Seo et al. developed a field-effect transistor-based biosensor coated with a graphene sheet to detect SARS-CoV-2 with a specific antibody [44]. Antigen proteins, cultured virus, and NP swab specimens from patients with COVID-19 were used to determine the performance of the sensor. The LOD of SARS-CoV-2 varied depending on the samples: phosphate-buffered saline showed 1 fg/mL for SARS-CoV-2 spike protein, 100 fg/mL for clinical transport medium and SARS-CoV-2 in culture medium (limit of detection [LOD]: 1.6×10^1 pfu/mL) and clinical samples (LOD: 2.42×10^2 copies/mL). The performance of SARS-CoV-2 antibodies was verified using ELISA before immobilization. The antibody bound only to SARS-CoV-2, with a LOD of 4 ng/mL. The advantage of this sensing

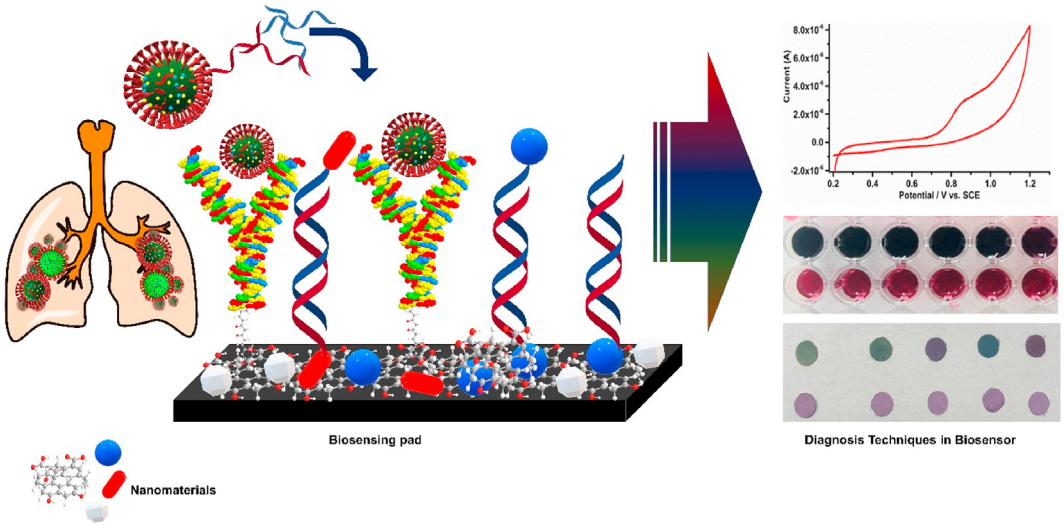

Fig. 4. Schematic diagram of Nanomaterials based Biosensing techniques for COVID-19.

platform is that pretreatment is not required for the samples. Moreover, the device provides high sensitivity as well as simple, rapid, and high response for COVID-19 detection.

Zhu et al. developed nanoparticle-based one-step reverse transcription loop-mediated isothermal amplification biosensor for the rapid and timely treatment of COVID-19 [45]. The sensing system was fabricated with two LAMP primer sets, opening reading frame 1a/b, and nucleoprotein genes of SARS-CoV-2, followed by amplification, and the results were compared with those of the lateral flow biosensor (LFB). Lateral flow biosensor visualization techniques were adopted for the diagnosis of COVID-19 by using multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP) assay. In LFB assay, the detection region is divided into two test lines (test line 1 and test line 2). The test line 1 consists of anti-FITC antibody for capturing ORF1ab-RT-LAMP products labeled with FITC and test line 2 is occupied by the anti-Dag antibody for capturing N-RT-LAMP products labeled with Dig. The other ends of LAMPs are functionalized with biotin and nanoparticles for naked observation of reactions. Based on this principle, the complete detection of SARS-CoV-2 was performed.

Zhang et al. established a coronavirus immunosensor using the combination of graphene-field effect transistor (Gr-FET) with antigen-antibody interaction [46]. The Gr-FET can rapidly identify and easily interact with COVID-19 spike protein. The study involved a direct comparison between SARS-CoV spike S1 subunit protein antibody (CSAb) and ACE2, which have affinity for RBD. Going by the results, the CSAb had higher affinity for the LOD at a concentration down to 0.2 pM. The developed sensor was able to easily identify and screen the neutralizing antibodies.

Mavrikou et al. reported the detection of SARS-CoV-2 S1 spike protein in membrane engineered mammalian cells bearing the spike S1 antibody [47]. The sensor was developed based on the considerable and selective changes in cell bioelectric properties, which were measured by using the bioelectric recognition assay. This advanced technique showed rapid detection lasting for approximately 3 min, with a detection limit of 1 fg/mL. Furthermore, the biosensor was configured as ready-to-us platform, which can be conceivably applied for the mass screening of SARS-CoV-2 surface antigens without prior sample processing.

Li et al. developed AuNPs, a nitrocellulose membrane, and a plastic pad-based sensor kit to detect IgM and IgG antibodies against SARS-CoV-2 [48]. The detection was based on the interaction of antigens with the antibodies and the complex flow through the membrane. As the antigens proceed further, they get immobilized by capturing antibodies, making the red/blue line visible red in case of only AuNPs and blue due to plasmon coupling. The response time for the confirmation of viral load takes only 15 min, thus reducing the exposure of the laboratory technicians to the virus. The sensitivity and specificity of the assay were 88.6% and 90.6%, respectively. The assay mechanism is based on the transport and hydration of the reagents to react with the analyte in the strip.

Tian et al. developed a technique for the quantification of nucleic acids based on circle-to-circle amplification and opto-magnetic analysis of nanoparticle assembly for the detection of synthetic complementary DNA of SARS-CoV-2 [49]. The proposed methodology detected the target RdRp coding sequence with a detection limit of 0.4 fM, and it took 100 min to complete the assay. The system involves two construction techniques—padlock probe ligation and rolling circle amplification method. Chen et al. reported lanthanide-based nanomaterials for the detection of SARS-CoV-2 using an immunodiagnostic method [50]. In their study, lanthanide-doped polystyrene nanoparticles could detect the anti-SARV-CoV-2 IgG in human serum within 10 min, and the proposed system was compared with the RT-PCR technique to validate the method for clinical applications.

Portable devices as well as ultra-sensitive and selective diagnosis methods are critical for the rapid diagnosis of SARS-CoV-2. Colorimetric and smartphone-based biosensors have potential applications in house-used point care devices. Several nanomaterial-based sensing kits have been developed in the current pandemic period; however, more reliable, rapid, low cost, and widely available diagnostic tools are still needed.

3.4. Distinctive sample-based detection

Ramos et al. performed clinical testing of different specimens to determine if the lower respiratory tract infection detected in bronchoalveolar lavage fluid (BALF) occurs in the absence of upper respiratory tract infection [51]. NP samples were collected from various patients under the age of 70 years. In addition, BALF samples collected from the upper and lower respiratory tracts were compared by RT-PCR analysis. The sensitivity of the confirmed cases was 19%, and this could be used as a further confirmation for the individuals who test negative during NP testing. Park et al. demonstrated a novel method for COVID-19 detection using rRT-PCR techniques from fecal samples of asymptomatic or mildly infected patients [52]. GI symptoms and shedding of virus in feces could also be helpful for COVID-19 detection. The sensitivity of this procedure was estimated to be 35% and it could persist for >50 days. The viral loads in respiratory and fecal samples are comparable. A similar study reported a detection rate of 33.7%, and the measurement of SARS-CoV-2 RNA was carried out in female patients having higher detection rate. Specific control strategies against fecal contamination are urgently needed [53].

A high binding affinity aptamer specifically aiming at SARS-CoV-2 RBD was identified using an ACE2 competition-based aptamer selection and machine learning screening algorithm. An aptamer-based approach targeting the spike glycoprotein was developed for COVID-19 detection, and the Kd values were 5.8 nM and 19.9 nM for CoV2-RBD-1C (51 nt) and CoV2-RBD-4C (67 nt) aptamers, respectively, upon molecular dynamics simulation [54]. Chest computed tomography (CT) images could also be used for the preliminary identification of COVID-19 in symptomatic or asymptomatic patients. Y. Soon et al., reported pulmonary lesions, which included patchy lesions as well as large and small nodular lesions. These lesions are not well-defined and the lower lung zones are patchy with an opacity of 80% [55]. The overall developed detection techniques for COVID-19 are summarized in Table 2.

4. Admissible treatment of COVID-19

The basic principle of treating patients positive for COVID-19 is to initially provide comfort to avoid worsening of their symptoms and prevent the emergence of secondary infections and potential complications from underlying diseases. In the most critical situations, patients are provided with organ function support, such as oxygen ventilation, in a timely manner [56]. Research on vaccines and drugs for coronavirus-related diseases has been ongoing since the SARS and MERS outbreak; however, no appropriate drugs or treatment regimens currently exist to specifically treat COVID-19. However, doctors have used various lines of treatment ranging from corticosteroid therapy, antibiotic therapy, antiviral therapy, antibody therapy, oxygen therapy, vaccines, and plasma transfusion [57]. The treatment strategy for COVID-19, lines of attack of different drugs, and coronavirus replication cycle are schematically presented in Fig. 5 [11].

Table 2
The overall developed detection techniques for COVID-19.

S. No	Technique	Design	Accuracy	Response time
1.	rRT-PCR	E and N genes of SARS-CoV-2 with human ABL1 gene	21 copies/reaction	NA
2.	RT-PCR	N genes of SARS-CoV-2	3.8–23 copies/mL	NA
3.	RT-PCR	T7 transcription	200 copies/mL or 10 copies/reaction	NA
4.	RT-LAMP	Leuco crystal violet method	100 copies/reaction	NA
5.	RT-LAMP	Expression of sub-genomic mRNA	102 RNA copies	30 min
6.	CRISPR	Viral nucleic acid detection via fluorescence and colorimetric assay	NA	<1 h
7.	CRISPR-Cas12	Isothermal amplification	10 copies/μL	30–40 min
8.	CRISPR-FDS	CRISPR Cas 12a/gRNA complex	2 copies/sample	<50 min
9.	ELISA	Rp3 nucleocapsid protein	81–100%	NA
10.	Euroimmun ELISA	S1 domain of spike protein	90.5%	NA
11.	LFIA	IgG/IgM antibodies	97.1–100%	15 min
12.	ELISA LFIA	Spike protein with IgG antibodies	98%	NA
13.	Colorimetry	Gold Nanoparticles capped with antisense oligonucleotides specific to Nucleocapsid protein	0.18 ng/μL	10 min
14.	Colorimetry	Gold Nanoislands (i) Spike protein (ii) Clinical transport medium (iii) Culture medium	1 fg/mL 100 fg/mL $1.6 * 10^1$ pfu/mL	NA
15.	RT-LAMP	F1ab-RT-LAMP and Np-RT-LAMP labeled with biotin and streptavidin conjugated nanoparticles	NA	NA
16.	Gr-FET	CSAb/ACE2 bindining to the graphene surface spiked with S protein	0.2 pM	NA
17.	Bioelectric Recognition Assay	Engineered Vero cells of mammalian spiked with S1 antibody	1 fg/mL	3 min
18.	LFIA	AuNPs with IgM/IgG antibodies	88.6%	<15 min
19.	C2CA	Magnetic Nanoparticles assembled to the RdRp gene	0.4 fM	100 min
20.	RT-PCR	Nasopharyngeal samples	19%	NA
21.	rRT-PCR	Fecal samples	35%	>50 d
22.	rRT-PCR	Fecal samples	33.7%	NA
23.	Aptamer	Spike glycoprotein with CoV2-RBD-1C and CoV2-RBD-4C	5.8 nM and 19.9 nM	NA
24.	CT images	Pulmonary lesions	80%	NA

4.1. Corticosteroid therapy

Glucocorticoid therapy ease body temperature, improve oxygenation, and prevent the onset of pneumonia, and hence, it was adopted during SARS and MERS outbreak, and it is being used in the current outbreak of COVID-19 as the first line of treatment. A recent study on patients treated with corticosteroids suggests that they induced secondary complications associated with fungal and bacterial infections due to exudate accumulation in the lungs. In addition, other complications observed were hyperglycemia, GI bleeding, venous thrombosis, hypertension, and hypokalemia. Therefore, the efficacy of glucocorticoids against COVID-19 needs further elucidation, and it is strongly recommended only as an early, low-dose, and short-term application to improve clinical symptoms [58]. Recently, a breakthrough in COVID-19 treatment was achieved by a small team at Oxford University, led by Prof Peter Horby, as dexamethasone, a steroid found to be effective in severe forms of COVID-19, was discovered [59]. Dexamethasone acts on the immune system to dampen its response and reduce the cytokine storm, thereby preventing the inflammation of the lungs and heart. Reportedly, dexamethasone reduces the mortality rate of patients who were on ventilator from 25% to 20%.

4.2. Antibiotic therapy

As the majority of patients are susceptible to secondary bacterial infections during first-line treatment with corticosteroids, doctors were urged to administer antibiotics such as moxifloxacin, levofloxacin, cephalosporins, hydroxychloroquine, azithromycin, and chloroquine to patients. Several antibiotic-based trials have been performed worldwide to determine an effective therapeutic regimen for COVID-19. China has used aminoquinolines, such as chloroquine and hydroxychloroquine, which have been able to reduce the viral load in patients with COVID-19. However, hydroxychloroquine is preferred over chloroquine because it is less toxic and more soluble than chloroquine, and it also has lower ocular toxicity [60]. Another comparative clinical trial focused on the treatment of patients with only hydroxychloroquine and later hydroxychloroquine with azithromycin. The study revealed that at day 6 post-inclusion, 100% of the patients treated with the combination of hydroxychloroquine and azithromycin were cured, compared with 57.1% of the patients treated with hydroxychloroquine only, and 12.5% of the control patients. These results suggest that the use of antibiotics against COVID-19 opens the possibility of combating viral infection in real time [61].

4.3. Antiviral therapy

The greatest hope for the treatment of COVID-19 is the administration of antiviral therapy, which is adopted by many countries around the world. Antiviral drugs are administered to infected patients as first-line treatment to reduce viral shedding in respiratory secretions [62]. Similar to the treatment of SARS and MERS, COVID-19 treatment also involves the use of antiviral drugs, such as remdesivir, as suggested by the FDA. Remdesivir is a promising antiviral drug that inhibits viral replication effectively and has been administered to patients since it passed the initial clinical trials [63]. Therapies without any supporting therapeutic evidence, including lopinavir, nitazoxanide, oseltamivir, baloxavir, interferon, ribavirin, or IVIG, have not been used for COVID-19 treatment [64].

4.4. Antibody therapy

SARS-CoV-2 enters the host cells through the binding of its S protein to angiotensin-converting enzyme 2 (ACE2) receptors, and

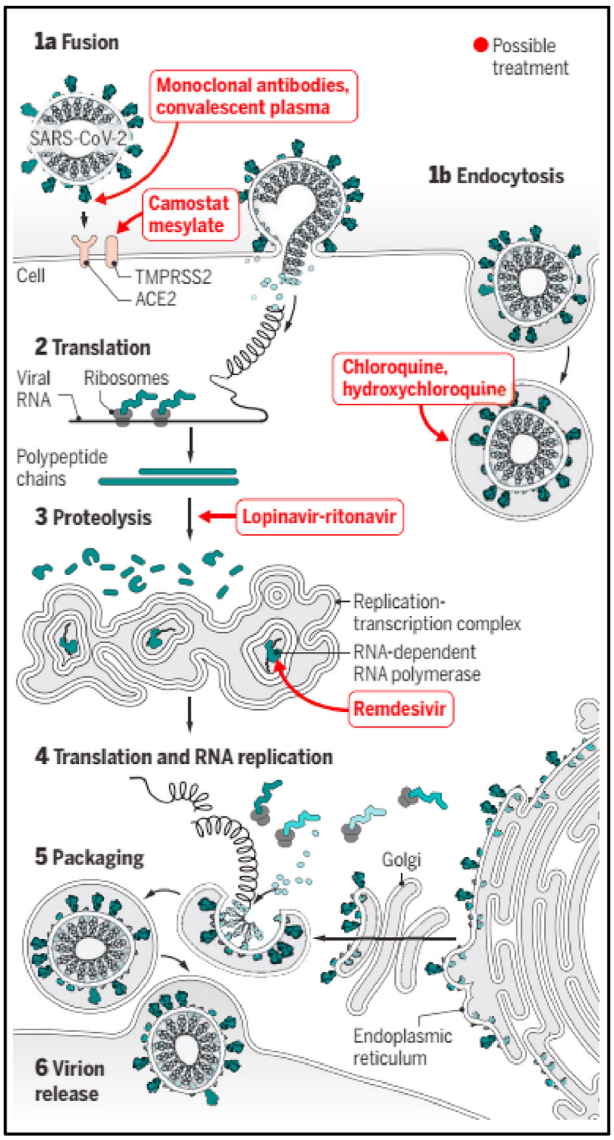

Fig. 5. The treatment strategy of COVID-19 and the lines of attack of different drugs, together with the coronavirus replication cycle (Resource: [11]).

hence, the development of neutralizing antibodies against these receptors has been adopted as a strategy to reduce the severity of the disease [65]. It has been demonstrated that the FDA-approved tocilizumab, a recombinant human IL-6 monoclonal antibody that effectively blocks the IL-6 signal transduction pathway, is effective in the treatment of patients with COVID-19 [10,66].

4.5. Oxygen therapy

The last line of therapy provided to patients with COVID-19 at the severe stage of infection is oxygen therapy. Oxygen therapy includes the provision of nasal catheter or mask oxygen therapy during the early stage of respiratory distress (hypoxemia). High-flow nasal oxygen or non-invasive ventilation is provided if hypoxemia cannot be relieved by standard oxygen therapy. If respiratory distress worsens (within a short time, 1–2 h), endotracheal intubation and invasive mechanical ventilation are undertaken in the patient. If patient-ventilator asynchrony occurs during the time of ventilation, sedation and muscle relaxation strategies should be considered in patients [56].

4.6. Convalescent plasma transfusion

Treatment with convalescent plasma is believed to be the most consistent and responsive treatment for infectious diseases since the Spanish influenza epidemic to the more recent outbreaks of SARS and MERS. Thus, the same treatment has been used for COVID-19. A preliminary study suggests that treating patients with COVID-19 with convalescent plasma improves clinical outcomes [67]. However, the treatment requires a convalescent plasma donor, which has remained quite difficult, and the efficacy and safety of the procedure are still unclear and require further elucidation [68].

5. Vaccines

To date, there is no vaccine for COVID-19. The development of a vaccine against COVID-19 based on previous vaccines or strategies has been attempted. Antiviral vaccines are developed based on one of the following types of viral components: virus-like particles; inactivated or live-attenuated viruses; viral vectors; and viral proteins, mRNA, or DNA. To date, there are 363 patents in the Chemical Abstracts Service (CAS) content collection that are related to the development of vaccines against SARS and MERS. For SARS-CoV, vaccination involves increasing immune responses, particularly an antigen-specific $CD8^+$ T cell-mediated response, against the antigens of SARS-CoV. Chimeric nucleic acids are linked to antigenic polypeptides or peptides from SARS-CoV to enhance the immune response. The vaccination of mice by using the gene-gun delivery of DNA-coated gold nanoparticles caused an immune response. Viral infection is controlled by the inactivated or live-vectored strain of SARS-CoV (AY278741). Various other strains such as Tor2 (AY274119), Utah (AY714217), FRA (AY310120), HKU-39849 (AY278491), BJ01 (AY278488), NS1 (AY508724), ZJ01 (AY297028), GD01 (AY278489), and GZ50 (AY304495) have been used as inactivated or live-vectored vaccines to control the viral load in animal models [7]. Neutralizing antibodies have been developed against SARS-CoV by using recombinant protein from the Urbani (AY278741) strain of SARS-CoV [69,70]. Recently, a microneedle array-based vaccine delivery platform was developed by Eun kim et al., who evaluated the immunogenicity of trimeric MERS-S1 subunit vaccines delivered into mice by microneedle injection. All microneedle array-delivered vaccines generated a higher level of neutralizing antibodies [71]. This study confirmed the immunogenicity of microneedle array-delivered vaccines against coronaviruses.

In 2015, Novavax proposed a virus-like particle-based vaccine with an immunogenic composition of MERS-CoV nanoparticles containing one trimer of the S protein. It induced neutralizing antibodies in mice. In addition, sera (SAB-300 and SAB-301) prepared from vaccinated cattle and injected into Ad5-hDPP4-transduced BALB/c mice protected the mice from MERS-CoV. Based on their previous experience, Novavax announced on February 26, 2020, that it was beginning an animal testing of vaccines for COVID-19, MERS, and SARS [4]. More than 160 vaccines are under development by researchers worldwide to prevent COVID-19 in a safe and effective way. Of the vaccines, 135 are in the pre-clinical stage, 18 are in the safety testing and dosage stage (Phase I), almost 12 are in expanded safety trials (Phase II), and 4 vaccines are in phase III trial of large scale efficacy tests [72,73].

6. Strategies of various countries against COVID-19

Initially, upon the outbreak of COVID-19 in Wuhan, an outbreak of pneumonia cases was suspected based on clinical inspection through rRT-PCR and CT scans. Based on the sudden increase in the number of similar cases being reported, bronchoalveolar lavage

samples from patients were analyzed, and the results showed that the samples were positive for pan-betacoronavirus. The different types of specimens used for COVID-19 testing are presented in Table 3. Whole genomic sequences were obtained though Illumina and Nanopore sequencing. The genomic sequences were analyzed using bioinformatics tools, and the virus was classified under the family of coronavirus (2B lineage). The genetic configuration of the virus had a 96% similarity to that of BatCoV RaTG13, a bat SARS-like coronavirus strain, and it was documented in Global Initiative on Sharing All Influenza Data platform. As the next step in analyzing the cellular morphologies of the virus, human airway epithelial cells, Vero E6, and Huh-7 cell lines were infected with the virus. The isolated virus-infected cells were injected into human ACE2 mice and Rhesus monkeys to induce multifocal pneumonia with interstitial hyperplasia, and the virus was detected in the lung and intestinal tissues of the transgenic animals. Histopathological analysis of a human patient who died in Wuhan revealed bilateral diffuse alveolar damage and lung tissues displaying desquamation of pneumocytes, partially indicating ARDS [74,75]. Using the testing kit manufactured by China CDC, the targets for screening COVID-19 were the nucleoprotein (N) and ORF1ab genes. The primers used for COVID-19 detection are presented in Table 4.

The screened patients tested as positive (Ct < 37), negative (Ct ~0/Ct = 40), or suspicious (Ct = 37–40). In the case of a suspicious result, the test was repeated, and the sample was classified as positive if Ct < 40 [76]. A study from Wuhan comparing PCR technique with CT method demonstrated that CT is more effective than PCR in diagnosing COVID-19, despite its low specificity (overlapping with other cases of pneumonia) [77]. China has developed 23 diagnostic kits for COVID-19, which include 15 kits for nucleic acid testing (Novel coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit—Shanghai ZJ Bio-Tech) and 8 kits for antibody testing strategy (VivaDiag™ COVID-19 IgM/IgG Rapid Test—VivaChek Biotech, SARS-CoV2-IgM/IgG Antibody Rapid Test—Qingdao Hightop Biotech, SARS-CoV2-Antibody test—Guangzhou Wondfo Biotech, COVID-19 IgM/IgG Rapid Test Cassette—Hangzhou Clongene Biotech, COVID-19 IgM/IgG Rapid Test Cassette—Zhejiang Orient Gene Biotech, SARS-CoV-2 IgG/IgM Rapid Test Cassette—Hangzhou Alltest Biotech). BGI, a gene-sequencing company in Shenzhen, developed a kit based on the nucleic acid testing strategy, which has also been exported to other countries (Thailand, UAE, Egypt, Peru, and Brunei).

Following the reports of COVID-19 infections in China, South Korea prepared by developing an innovative strategy to deal with the outbreak. The Korean Center for Disease Control and Prevention stepped up promptly by urging biotech startup companies to develop test kits for COVID-19. Seegene (a diagnostic company) developed a test kit that condensed the result time from 1 d to 6 h by utilizing an artificial intelligence-powered automated production system. Another startup, MiCO BioMed, developed a test kit that could infer the test results within 1 h. Ahram Biosystems and Doknip Biopharm collaborated to develop a hand-held scan device that can detect COVID-19 within 30 min. The SolGent (DiaPlex Q Noble) kit utilizes almost 2 h for accurate interpretation of results. Optolane applied a semiconductor chip manufactured via MEMS (tiny chip embedded with 22,000 of 50 nm-sized well) to produce accurate results with minimal time, low viral count, and reduced cost. The Sugentech (SGTi-flex) COVID-19 IgM/IgG immunochromatographic kit determines the presence of viral protein from the whole blood/plasma/serum. This kit is accurate, and the results are obtained within 10 min. This kit detects the presence of specific proteins in symptomatic/asymptomatic patients. All diagnostic kits were approved by the Korean government under the Emergency Use Authorization scheme.

Korea's key strategy in controlling the spread of COVID-19 cases is speed, transparency, innovation, and voluntary civic participation. South Korea has conducted approximately <10,000 tests per day and has the capacity for testing 20,000 cases per day. Additionally, the test was offered free of charge, inclusive of drive-through testing for people showing symptoms and subsequent treatment for infected cases. Currently, Korea has conducted a large number of tests in its efforts to control and stabilize the number of COVID-19 cases.

Table 3
Specimens for COVID-19 testing (Source: [80]).

Types of specimens	Collection devices	Transport conditions	Storage conditions	Comments
Upper respiratory tract specimens: NP swab*, OP swab*, and NP aspirate	Dacron or flocked swabs in VTM	4 °C	Within 5 d: 4 °C Longer than 5 d: 70 °C	
Lower respiratory tract specimen: sputum*	Sterile container	4 °C	Within 48 h: 4 °C Longer than 48 h: 70 °C	
Lower respiratory tract specimen: bronchial washing*	Sterile container	4 °C	Within 48 h: 4 °C Longer than 48 hs: 70 °C	Pathogens might be diluted; however, the specimen can be subjected to diagnostic testing
Lower respiratory tract specimens: tracheal aspirate and transtracheal aspirate	Sterile container	4 °C	Within 48 h: 4 °C Longer than 48 h: 70 °C	
Lower respiratory tract specimen: lung biopsy	Sterile container with saline	4 °C	Within 48 h: 4 °C Longer than 48 h: 70 °C	
Serum†	Serum separation test tube (SST): adults and children, 3–5 mL; infants, 1 mL	4 °C	Within 5 d: 4 °C Longer than 5 d: 70 °C	For serological tests, a pair of specimens is collected Acute phase: within 7 d of symptom onset Convalescent period: 14 d after collection during acute phase Dispensing of serum into another container should be conducted in a Class II or higher BSC

Table 4
The primers used for COVID-19 detection.

Target	Forward primer	Reverse primer	Fluorescent probe
ORF1ab	CCCTGTGGGTTTTACACTTAA	ACGATTGTGCATCAGCTGA	5′-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3′
Nucleoprotein (N)	GGGGAACTTCTCCTGCTAGAAT	CAGACATTTTGCTCTCAAGCTG	5′-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3′

In Germany, the Robert Koch Institute in collaboration with other private independent laboratories has screened almost 85,063 people, and regular testing is still ongoing. The region has expanded its testing to almost 13,000 people per day using diagnostic kits. Bosch Healthcare Solutions developed the Vivalytic rapid test, a nucleic acid amplification test that can detect viral RNA under 2.5 h. The use of this test kit speeds up the identification and isolation of infected patients, and its main merit is that it can test for nine more respiratory diseases, including influenza A and B. This newly developed kit is the result of a collaboration between Bosch Healthcare and Randox Laboratories (Northern Irish Medical Company). The accuracy of the test results is 95%, and the kit also meets the WHO standards. Neighboring countries, such as Spain, Italy, France, Belgium, and the UK have screened almost 1,377,702 people by regular medical laboratory diagnosis and kits obtained from China. However, there are reports from the Spanish government that the use of kits from China has been banned because of their low reliability and less than 30% accuracy.

The United States of America also developed various diagnostic kits for COVID-19: Cobas® SARS-CoV-2 (Roche Molecular Systems Inc.), Panther Fusion® SARS-CoV-2 Assay (Hologic, Inc.), TaqPath COVID-19 Combo kit (Life Technologies Corporation), and Xpert® Xpress SARS-CoV-2 (Cepheid) based on nucleic acid testing, and the Onsite COVID-19 IgG/IgM Rapid Test (CTK Biotech Inc) based on antibody testing. Additionally, the United States of America, the United Arab Emirates, Romania, Colombia, and 43 more countries had requested South Korea to export diagnostic test kits and other quarantine-related goods amid the rapid spread of infection. A Singapore-based MedTech company (Biolidics) launched a rapid diagnostic test kit for COVID-19 (SARS-CoV-2 IgG/IgM) by customizing an antibody-based approach. Furthermore, the country has developed other diagnostic kits: VereCoV detection kit (RT-PCR and microarray-based) from Veredus laboratories, A*STAR and TTSH's Fortitude Kit 2.0 (RT-PCR), and enVision (enzyme-assisted nanocomplexes for visual identification of nucleic acid) from iHealthTech researchers. All the aforementioned kits were approved by Singapore's Health Science Authority [78].

A Taiwan healthcare company has developed a COVID-19 test kit (BodySphere) based on lateral flow chromatographic immunoassay to detect IgG/IgM antibodies from the blood/serum/plasma of infected patients. The results can be obtained within 2 min with 91% specificity and 99% sensitivity. This kit was approved by the FDA under its Emergency Use Authorization scheme [79]. MyLab Discovery Solutions, an Indian-based biotech company, developed the Mylab PathoDetect COVID-19 qualitative PCR kit, which was approved by the Indian Council of Medical Research. Using an antibody-based approach, another company named Bione also developed a COVID-19 screening kit, which is awaiting approval from the ICMR. The Serum Institute (Pune) in collaboration with Codagenix (a USA-based Company) is in the pre-trial stage of vaccine development for COVID-19. The various kits used in different countries for COVID-19 detection are summarized in Table 5.

Table 5
COVID-19 detection kits from various countries.

S. No	Country	Kit Name	Technique	Company
1.	China	Real-Time Multiplex RT-PCR kit	Nucleotide testing	Shanghai ZJ Bio-Tech
2.		VivaDig™ COVID-19 IgM/IgG Rapid Test	Antibody testing	VivaChek Biotech
3.		SARS-CoV2-IgM/IgG Antibody Rapid Test	Antibody testing	Qingdao Hightop Biotech
4.		SARS-CoV2-Antibody test	Antibody testing	Guangzhou Wondfo Biotech
5.		COVID-19 IgM/IgG Rapid Test Cassette	Antibody testing	Hangzhou Clongene Biotech
6.		COVID-19 IgM/IgG Rapid Test Cassette	Antibody testing	Zhejiang Orient Gene Biotech
7.		SARS-CoV-2 IgG/IgM Rapid Test Cassette	Antibody testing	Hangzhou Alltest Biotech
8.		2019-nCoV (SARS-CoV-2): RT-PCR detection kit	Nucleic acid testing	BGI Genomics
9.		COVID-19: Antibody detection kit	Antibody testing	BGI Genomics
10.	South Korea	Allplex™ 2019-nCoV Assay	Nucleic acid testing	Seegene Inc
11.		Veri-Q PCR 316	Nucleic acid testing	MiCo BioMed
12.		Palm PCRTM Ultra-fast COVID-19 fast POCT kit	Nucleic acid testing	Ahram Biosystems Inc
13.		PowerChek 2019-nCoV Realtime PCR coronavirus detection kit	Nucleic acid testing	Kogene Biotech
14.		DiaPlex Q Noble kit	Nucleic acid testing	SolGent Co Ltd
15.		SGTi-flex COVID-19 IgM/IgG immunochromatographic kit	Antibody testing	Sugentech
16.	Germany	Vivalytic rapid test	Nucleic acid testing	Bosch Healthcare Solutions
		Hi-Plex & Lo-Plex assay	Nucleic acid testing	Randox Laboratories
17.	United States of America	Cobas® SARS-CoV-2	Nucleic acid testing	Roche Molecular Systems Inc.
18.		Panther Fusion® SARS-CoV-2 Assay	Nucleic acid testing	Hologic Inc.
19.		TaqPath COVID-19 Combo kit	Nucleic acid testing	Life Technologies Corporation
20.		Xpert® Xpress SARS-CoV-2	Nucleic acid testing	Cepheid
21.		Onsite COVID-19 IgG/IgM Rapid test	Antibody testing	CTK Biotech Inc.
22.	Singapore	SARS-CoV-2 IgG/IgM test kit	Antibody testing	Biolidics
23.		VereCoV detection kit	Nucleic acid testing	Veredus Laboratories
24.		A*STAR and TTSH Fortitude Kit 2.0	Nucleic acid testing	–
25.		enVision	Nucleic acid testing	iHealthTech
26.	Taiwan	COVID-19 test kit	Antibody testing	BodySphere
27.	India	Mylab PathoDetect COVID-19 qualitative PCR kit	Nucleic acid testing	MyLab Discovery Solutions
28.		COVID-19 Screening kit	Antibody testing	Bione

7. Conclusion and future perspectives

This review provides an overview of coronaviruses and COVID-19 disease. It includes the details on the origin, transmission, and structure of the novel coronavirus, a comparative analysis of SARS and MERS coronaviruses, and an overview of the symptoms, diagnostic techniques, and available treatments for COVID-19. Currently, nucleic acid-based testing, antibody-based testing, and nanomaterial-based biosensing are used for the rapid diagnosis of COVID-19. Controlling the spread of COVID-19 and adopting measures to avoid the regeneration of coronaviruses are crucial for the development of vaccines. There is a need for the development of more reliable, rapid, low cost, and widely available diagnostic tools. The lack of vaccines and therapeutic agents against coronaviruses might be due to the unconcerned attitude of pharmaceutical companies towards outbreak consequences, as the need for vaccines and drugs only remains as long as the infection lasts. By the time a drug or vaccine is developed, there may not be any infected patients for clinically testing. The control of COVID-19 requires the rapid design, testing, production, and clinical translation of effective drugs and vaccines.

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.

Acknowledgment

This work was supported by the Gachon University research fund of 2019 (GCU-2019-0816). This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (2020R1G1A1102394).

References

[1] [a] B. Arinjay, K. Kulcsar, V. Misra, M. Frieman, K. Mossman, Bats and coronaviruses, Viruses 11 (2019). https://doi.org/10.3390/v11010041;
[b] Y. Dong, J.L. Leibowitz, The structure and functions of coronavirus genomic 3′ and 5′ ends, Virus Res. 206 (2015) 120–133. https://doi.org/10.1016/j.virusres.2015.02.025.

[2] A. Abdullah, J.A. Al-Tawfiq, A.A. Al-Rabeeah, F.A. Al-Rabiah, S. Al-Hajjar, A. Al-Barrak, H. Flemban, W.N. Al-Nassir, H.H. Balkhy, R.F. Al-Hakeem, H.Q. Makhdoom, Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study, Lancet Infect. Dis. 13 (2013) 752–761. https://doi.org/10.1016/S1473-3099(13)70204-4.

[3] L. Cynthia, Q. Zhou, Y. Li, L.V. Garner, S.P. Watkins, L.J. Carter, J. Smoot, A.C. Gregg, A.D. Daniels, S. Jervey, D. Albaiu, Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases, ACS Cent. Sci. 6 (2020) 315–331. https://doi.org/10.1021/acscentsci.0c00272.

[4] J.R.M. Alfonso, J.A. Cardona-Ospina, E. Gutiérrez-Ocampo, R. Villamizar-Peña, Y. Holguin-Rivera, J.P. Escalera-Antezana, L.E. Alvarado-Arnez, D.K. Bonilla-Aldana, C. Franco-Paredes, A.F. Henao-Martinez, A. Paniz-Mondolfi, G.J. Lagos-Grisales, E. Ramírez-Vallejo, J.A. Suárez, L.I. Zambrano, W.E. Villamil-Gómez, G.J. Balbin-Ramon, A.A. Rabaan, H. Harapan, K. Dhama, H. Nishiura, H. Kataoka, T. Ahmad, R. Sah, Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis, Trav. Med. Infect. Dis. 34 (2020) 101623. https://doi.org/10.1016/j.tmaid.2020.101623.

[5] G. Jean-Francois Gautier, Y. Ravussin, A new symptom of COVID-19: loss of taste and smell, Obesity 28 (2020). https://doi.org/10.1002/oby.22809, 848-848.

[6] S. Muhammad Adnan, S. Khan, A. Kazmi, N. Bashir, R. Siddique, COVID-19 infection: origin, transmission, and characteristics of human coronaviruses, J. Adv. Res. 24 (2020) 91–98. https://doi.org/10.1016/j.jare.2020.03.005.

[7] G.A. Kristian, A. Rambaut, W.I. Lipkin, E.C. Holmes, R.F. Garry, The proximal origin of SARS-CoV-2, Nat. Med. 26 (2020) 450–452. https://doi.org/10.1038/s41591-020-0820-9.

[8] https://www.finddx.org/wp-content/uploads/2020/04/FIND_COVID-19_GUIDE-24.03.2020.pdf. Source: FIND Because diagnosis matters, 24 March 2020.

[9] S. Catrin, Z. Alsafi, N. O'Neil, M. Khan, A. Kerwan, A. Al-Jabir, C. Iosifidis, R. Agha, World Health Organization declares global emergency, A review of the 2019 novel coronavirus (COVID-19), Int. J. Surg. 76 (2020) 71–76. https://doi.org/10.1016/j.ijsu.2020.02.034.

[10] K. Kai, J. Cohen, Race to find COVID-19 treatments accelerates, Science 367 (2020) 1412–1413. https://doi.org/10.1126/science.367.6485.1412.

[11] F. Yi, K. Zhao, Z.L. Shi, P. Zhou, Bat coronaviruses in China, Viruses 11 (2019) 210. https://doi.org/10.3390/v11030210.

[12] C. Marco, M. Rajnik, A. Cuomo, S.C. Dulebohn, R.D. Napoli, Features, Evaluation and Treatment Coronavirus (COVID-19), StatPearls Publishing, 2020. https://www.ncbi.nlm.nih.gov/books/NBK554776/.

[13] W. Fan, S. Zhao, B. Yu, Y.M. Chen, W. Wang, Z.G. Song, Y. Hu, Z.W. Tao, J.H. Tian, Y.Y. Pei, M.L. Yuan, Y.L. Zhang, F.H. Dai, Y. Lui, Q.M. Wang, J.J. Zheng, L. Xu, E.C. Holmes, Y.Z. Zhang, A new coronavirus associated with human respiratory disease in China, Nature 579 (2020) 265–269. https://doi.org/10.1038/s41586-020-2008-3.

[14] B. Coutard, C. Valle, X. de Lamballerie, B. Canard, N.G. Seidah, E. Decroly, The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade, Antivir. Res. 176 (2020) 104742. https://doi.org/10.1016/j.antiviral.2020.104742.

[15] Z. Peng, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li, C.L. Huang, H.D. Chen, J. Chen, Y. Luo, H. Guo, R.D. Jiang, M.Q. Liu, Y. Chen, X.R. Shen, X. Wang, X.S. Zheng, K. Zhao, Q.J. Chen, F. Deng, L.L. Liu, B. Yan, F.X. Zhan, Y.Y. Wang, G.F. Xiao, Z.L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (2020) 270–273. https://doi.org/10.1038/s41586-020-2012-7.

[16] X. Kangpeng, J. Zhai, Y. Feng, N. Zhou, X. Zhang, J.J. Zou, N. Li, Y. Guo, X. Li, X. Shen, Z. Zhang, F. Shu, W. Huang, Y. Li, Z. Zhang, R.A. Chen, Y.J. Wu, S.M. Peng, M. Huang, W.J. Xie, Q.H. Cai, F.H. Hou, Y. Liu, W. Chen, L. Xiao, Y. Shen, Isolation and characterization of 2019-nCoV-like coronavirus from malayan pangolins, bioRxiv (2020). https://doi.org/10.1101/2020.02.17.951335.

[17] G.L. Rachel, R.S. Baric, Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission, J. Virol. 84 (2010) 3134–3146. https://doi.org/10.1128/JVI.01394-09.

[18] U. Buddhisha, P. Kadhiresan, H.N. Kozlowski, A. Malekjahani, M. Osborne, V.Y. Li, H. Chen, S. Mubareka, J.B. Gubbay, W.C. Chan, Diagnosing COVID-19: the disease and tools for detection, ACS Nano 14 (2020) 3822–3835. https://doi.org/10.1021/acsnano.0c02624.

[19] C.J. Linda, L.V. Garner, J.W. Smoot, Y. Li, Q. Zhou, C.J. Saveson, J.M. Sasso, A.C. Gregg, D.J. Soares, T.R. Beskid, S.R. Jervey, C. Liu, Assay techniques and test development for COVID-19 diagnosis, ACS Cent. Sci. 6 (2020) 591–605. https://doi.org/10.1021/acscentsci.0c00501.

[20] I. Takayuki, S. Murata, T. Taniguchi, A. Miyabe, K. Kitamura, K. Kawasaki, K.M. Nishimura, H. Igari, K. Matsushita, Highly sensitive detection of SARS-CoV-2 RNA by multiplex rRT-PCR for molecular diagnosis of COVID-19 by clinical laboratories, Clin. Chim. Acta 507 (2020) 139–142. https://doi.org/10.1016/j.cca.2020.04.023.

[21] P. Luis, M. Ruiz, R. Porco, S.N. Rao, M. Juanola-Falgarona, D. Manissero, M. López-Fontanals, J. Pareja, Multiple assays in a real-time RT-PCR SARS-CoV-2 panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak, Int. J. Infect. Dis. 97 (2020) 225–229. https://doi.org/10.1016/j.ijid.2020.06.027.

[22] P.B. van Kasteren, B. van der Veer, B.S. van den Brink, L. Wijsman, J. de Jonge, A. van den Brandt, R. Molenkamp, C.B.E.M. Reusken, A. Meijer, Comparison of commercial RT-PCR diagnostic kits for COVID-19, J. Clin. Virol. 128 (2020) 104412. https://doi.org/10.1016/j.jcv.2020.104412.

[23] W. Ji, K. Cai, R. Zhang, X. He, X. Shen, J. Liu, J. Xu, F. Qiu, W. Lei, J. Wang, X. Li, Y. Gao, Y. Jiang, W. Xu, X. Ma, A novel one-step single-tube nested quantitative Real-Time PCR assay for highly sensitive detection of SARS-CoV-2, Anal. Chem. 92 (2020) 9399–9404. https://doi.org/10.1021/acs.analchem.0c01884.

[24] P. Gun-Soo, K. Ku, S.H. Baek, S.J. Kim, S.I. Kim, B.T. Kim, J.S. Maeng, Development of reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays targeting SARS-CoV-2, J. Mol. Diagn. 22 (2020) 729–735. https://doi.org/10.1016/j.jmoldx.2020.03.006.

[25] B. Yun Hee, J. Um, K.J.C. Antigua, J.H. Park, Y. Kim, S. Oh, Y.I. Kim, W.S. Choi, S.G. Kim, J.H. Jeong, B.S. Chin, H.D.G. Nicolas, J.Y. Ahn, K.S. Shin, Y.K. Choi, J.S. Park, M.S. Song, Development of a reverse transcription-loop-mediated isothermal amplification as a rapid early-detection method for novel SARS-CoV-2. Emerg, Microb. Infect. 9 (2020) 998–1007. https://doi.org/10.1080/22221751.2020.1756698.

[26] C. Feiyun, H.S. Zhou, Diagnostic methods and potential portable biosensors for coronavirus disease 2019, Biosens. Bioelectron. 165 (2020) 112349. https://doi.org/10.1016/j.bios.2020.112349.

[27] J. Martin, K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna, E.A. Charpentier, A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity, Science 337 (2012) 816–821. https://doi.org/10.1126/science.1225829.

[28] K.J. Max, J.G. Koob, J.S. Gootenberg, O.O. Abudayyeh, F. Zhang, SHERLOCK: nucleic acid detection with CRISPR nucleases, Nat. Protoc. 14 (2019) 2986–3012. https://doi.org/10.1038/s41596-019-0210-2.

[29] B.P. James, X. Deng, G. Yu, C.L. Fasching, V. Servellita, J. Singh, X. Miao, J.A. Streithorst, A. Granados, A. Sotomayor-Gonzalez, K. Zorn, A. Gopez, E. Hsu, W. Gu, S. Miller, C.Y. Pan, H. Guevara, D.A. Wadford, J.S. Chen, C.Y. Chiu,

CRISPR–Cas12-based detection of SARS-CoV-2, Nat. Biotechnol. (2020) 1–5. https://doi.org/10.1038/s41587-020-0513-4.
[30] H. Zhen, D. Tian, Y. Liu, Z. Lin, C.J. Lyon, W. Lai, D. Fusco, A. Drouin, X. Yin, T. Hu, B. Ning, Ultra-sensitive and high-throughput CRISPR-Powered COVID-19 diagnosis, Biosens. Bioelectron. 164 (2020) 112316. https://doi.org/10.1016/j.bios.2020.112316.
[31] M. Supratim, P. Chandra, Clinically practiced and commercially viable nanobio engineered analytical methods for COVID-19 diagnosis, Biosens. Bioelectron. 165 (2020) 112361. https://doi.org/10.1016/j.bios.2020.112361.
[32] B. Christian, M. Egger, K. Wiesinger, C. Gabriel, M. Clodi, T. Mueller, B. Dieplinger, Evaluation of the EDI enzyme linked immunosorbent assays for the detection of SARS-CoV-2 IgM and IgG antibodies in human plasma, Clin. Chim. Acta 509 (2020) 79–82. https://doi.org/10.1016/j.cca.2020.05.047.
[33] Z. Wei, R.H. Du, B. Li, X.S. Zheng, X.L. Yang, B. Hu, Y.Y. Wang, G.F. Xiao, B. Yan, Z.L. Shi, P. Zhou, Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes, Emerg. Microb. Infect. 9 (2020) 386–389. https://doi.org/10.1080/22221751.2020.1729071.
[34] B.G. Kathleen, S.M. Matushek, A.P.F. Abeleda, C. Bethel, C. Hunt, S. Gillen, A. Moran, V. Tesic, Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies, J. Clin. Virol. 129 (2020) 104468. https://doi.org/10.1016/j.jcv.2020.104468.
[35] P. Yunbao, X. Li, G. Yang, J. Fan, Y. Tang, J. Zhao, X. Long, S. Guo, Z. Zhao, Y. Liu, H. Hu, Y. Li, Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients, J. Infect. 81 (2020) e28–e32. https://doi.org/10.1016/j.jinf.2020.03.051.
[36] N. Thomas, C. Lefeuvre, O. Serri, A. Pivert, F. Joubaud, V. Dubee, A. Kouatchet, A. Ducancelle, F. Lunel-Fabiani, H.L. Guillou-Guillemette, Assessment of SARS-CoV-2 serological tests for the diagnosis of COVID-19 through the evaluation of three immunoassays: two automated immunoassays (Euroimmun and Abbott) and one rapid lateral flow immunoassay (NG Biotech), J. Clin. Virol. 129 (2020) 104511. https://doi.org/10.1016/j.jcv.2020.104511.
[37] W. Carsten, M. Carriere, L. Fusco, I. Capua, J.A. Regla-Nava, M. Pasquali, J.A. Scott, F. Vitale, M.A. Unal, C. Mattevi, D. Bedognetti, A. Merkoci, E. Tasciotti, A. Yilmazer, Y. Gogotsi, F. Stellacci, L.G. Delogu, Toward nanotechnology-enabled approaches against the COVID-19 pandemic, ACS Nano 14 (2020) 6383–6406. https://doi.org/10.1021/acsnano.0c03697.
[38] M. Govindhan, M. Sasidharan, V. Ganesan, Electrochemical sensor and biosensor platforms based on advanced nanomaterials for biological and biomedical applications, Biosens. Bioelectron. 103 (2018) 113–129. https://doi.org/10.1016/j.bios.2017.12.031.
[39] M. Parikshit, M. Alafeef, K. Dighe, M.B. Frieman, D. Pan, Selective naked-eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped plasmonic nanoparticles, ACS Nano 14 (2020) 7617–7627. https://doi.org/10.1021/acsnano.0c03822.
[40] K. Vinod, S. Mishra, R. Sharma, J. Agarwal, U. Ghoshal, T. Khanna, L.K. Sharma, S.K. Verma, P. Mishra, S. Tiwari, Development of RNA-based assay for rapid detection of SARS-CoV-2 in clinical samples, bioRxiv (2020). https://doi.org/10.1101/2020.06.30.172833.
[41] M. Subhasis, A. Roberts, D. Shahdeo, S. Gandhi, eCovSens-ultrasensitive novel in-house built printed circuit board based electrochemical device for rapid detection of nCovid-19 antigen, a spike protein domain 1 of SARS-CoV-2, bioRxiv (2020). https://doi.org/10.1101/2020.04.24.059204.
[42] Q. Guangyu, Z. Gai, Y. Tao, J. Schmitt, G.A. Kullak-Ublick, J. Wang, Dual-functional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection, ACS Nano 14 (2020) 5268–5277. https://doi.org/10.1021/acsnano.0c02439.
[43] S. Giwan, G. Lee, M.J. Kim, S.H. Baek, M. Choi, K.B. Ku, C.S. Lee, S. Jun, D. Park, H.G. Kim, S.J. Kim, J.O. Lee, B.T. Kim, E.C. Park, S.I. Kim, Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor, ACS Nano 14 (2020) 5135–5142. https://doi.org/10.1021/acsnano.0c02823.
[44] Z. Xiong, X. Wang, L. Han, T. Chen, L. Wang, H. Li, S. Li, L. He, X. Fu, S. Chen, M. Xing, H. Chen, Y. Wang, Multiplex reverse transcription loop-mediated isothermal amplification combined with nanoparticle-based lateral flow biosensor for the diagnosis of COVID-19, Biosens. Bioelectron. 166 (2020) 112437. https://doi.org/10.1016/j.bios.2020.112437.
[45] Z. Xiaoyan, Q. Qi, Q. Jing, S. Ao, Z. Zhang, M. Ding, M. Wu, K. Liu, W. Wang, Y. Ling, Z. Zhang, W. Fu, Electrical Probing of COVID-19 Spike Protein Receptor Binding Domain via a Graphene Field-Effect Transistor, 2020. arXiv preprint arXiv:2003.12529.
[46] M. Sophie, G. Moschopoulou, V. Tsekouras, S. Kintzios, Development of a portable, ultra-rapid and ultra-sensitive cell-based biosensor for the direct detection of the SARS-CoV-2 S1 spike protein antigen, Sensors 20 (2020) 3121. https://doi.org/10.3390/s20113121.
[47] L. Zhengtu, Y. Yi, X. Luo, N. Xiong, Y. Liu, S. Li, R. Sun, Y. Wang, B. Hu, W. Chen, Y. Zhang, J. Wang, B. Huang, Y. Lin, J. Yang, W. Cai, X. Wang, J. Cheng, Z. Chen, K. Sun, W. Pan, Z. Zhan, L. Chen, F. Ye, Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis, J. Med. Virol. (2020). https://doi.org/10.1002/jmv.25727.
[48] T. Bo, F. Gao, J. Fock, M. Dufva, M.F. Hansen, Homogeneous circle-to-circle amplification for real-time optomagnetic detection of SARS-CoV-2 RdRp coding sequence, Biosens. Bioelectron. 165 (2020) 112356. https://doi.org/10.1016/j.bios.2020.112356.
[49] Z. Chen, Z. Zhang, X. Zhai, Y. Li, L. Lin, H. Zhao, L. Bian, P. Li, L. Yu, Y. Wu, G. Lin, Rapid and sensitive detection of anti-SARS-CoV-2 IgG, using lanthanide-doped nanoparticles-based lateral flow immunoassay, Anal. Biochem. 92 (2020) 7226–7231. https://doi.org/10.1021/acs.analchem.0c00784.
[50] R.J. Kathleen, S.G. Kapnadak, B.F. Collins, P.S. Pottinger, R. Wall, J.A. Mays, G.A. Perchetti, K.R. Jerome, S. Khot, A.P. Limaye, P.C. Mathias, A. Greninger, Detection of SARS-CoV-2 by bronchoscopy after negative nasopharyngeal testing: stay vigilant for COVID-19, Respiratory Medicine Case Reports 30 (2020) 101120. https://doi.org/10.1016/j.rmcr.2020.101120.
[51] P. Soo-kyung, C.W. Lee, D.I. Park, H.Y. Woo, H.S. Cheong, H.C. Shin, K. Ahn, M.J. Kwon, E.J. Joo, Detection of SARS-CoV-2 in fecal samples from patients with asymptomatic and mild COVID-19 in Korea, Clin. Gastroenterol. Hepatol. (2020). https://doi.org/10.1016/j.cgh.2020.06.005.
[52] W.C.S. Martin, J. Huang, C. Lai, R. Ng, C.K.L. Chan, P.K.S. Chan, Detection of SARS-CoV-2 RNA in fecal specimens of patients with confirmed COVID-19: a meta-analysis, J. Infect. (2020). https://doi.org/10.1016/j.jinf.2020.06.012.
[53] S. Yanling, J. Song, X. Wei, M. Huang, M. Sun, L. Zhu, B. Lin, H. Shen, Z. Zhu, C. Yang, Discovery of aptamers targeting receptor-binding domain of the SARS-CoV-2 spike glycoprotein, Anal. Chem. 92 (2020) 9898–9900. https://doi.org/10.1021/acs.analchem.0c01394.
[54] Y. Soon Ho, K.H. Lee, J.Y. Kim, Y.K. Lee, H. Ko, K.H. Kim, C.M. Park, Y.H. Kim, Chest radiographic and CT findings of the 2019 novel coronavirus disease (COVID-19): analysis of nine patients treated in Korea, Korean J. Radiol. 21 (2020) 494–500. https://doi.org/10.3348/kjr.2020.0132.
[55] B. Li, D. Yang, X. Wang, L. Tong, X. Zhu, N. Zhong, C. Bai, C.A. Powell, R. Chen, J. Zhou, Y. Song, X. Zhou, H. Zhu, B. Han, Q. Li, G. Shi, S. Li, C. Wang, Z. Qiu, Y. Zhang, Y. Xu, J. Liu, D. Zhang, C. Wu, J. Li, J. Yu, J. Wang, C. Dong, Y. Wang, Q. Wang, L. Zhang, M. Zhang, X. Ma, L. Zhao, W. Yu, T. Xu, Y. Jin, X. Wang, Y. Wang, Y. Jiang, H. Chen, K. Xiao, X. Zhang, Z. Song, Z. Zhang, X. Wu, J. Sun, Y. Shen, M. Ye, C. Tu, J. Jiang, H. Yu, F. Tan, Chinese experts' consensus on the Internet of Things-aided diagnosis and treatment of coronavirus disease 2019, Clinical eHealth 3 (2020) 7–15. https://doi.org/10.1016/j.ceh.2020.03.001.
[56] Z. Pan, Y. Ding, X. Wu, J. Long, Y. Zhong, Y. Li, The epidemiology, diagnosis and treatment of COVID-19, Int. J. Antimicrob. Agents 55 (2020) 105955. https://doi.org/10.1016/j.ijantimicag.2020.105955.
[57] W. Yin, W. Jiang, Q. He, C. Wang, B. Wang, P. Zhou, N. Dong, Q. Tong, Early, Low-Dose and Short-Term Application of Corticosteroid Treatment in Patients with Severe COVID-19 Pneumonia: Single-Center Experience from Wuhan, China, medRxiv (2020). https://doi.org/10.1101/2020.03.06.20032342.
[58] Fergus Walsh, https://www.bbc.com/news/health-53096736, Accessed on 20 June 2020.
[59] S. Zahra, M. Shabani, S. Shokouhi, A. Saffaei, Aminoquinolines against coronavirus disease 2019 (COVID-19): chloroquine or hydroxychloroquine, Int. J. Antimicrob. Agents 55 (2020) 105945. https://doi.org/10.1016/j.ijantimicag.2020.105945.
[60] G. Philippe, J.C. Lagier, P. Parola, V.T. Hoang, L. Medded, M. Mailhe, B. Doudier, J. Courjon, V. Giordanengo, V.E. Vieira, H.T. Dupont, S. Honore, P. Colson, E. Chabriere, B.L. Scola, J.M. Rolain, P. Brouqui, D. Raoult, Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, Int. J. Antimicrob. Agents (2020) 105949. https://doi.org/10.1016/j.ijantimicag.2020.105949.
[61] M. Oriol, B. Clotet, Use of antiviral drugs to reduce COVID-19 transmission, Lancet. Glob. Health. 8 (2020) e639–e640. https://doi.org/10.1016/S2214-109X(20)30114-5.
[62] W. Manli, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro, Cell Res. 30 (2020) 269–271. https://doi.org/10.1038/s41422-020-0282-0.
[63] http://www.med.umich.edu/asp/pdf/adult_guidelines/COVID-19-treatment.pdf (Accessed on 03 April 2020).
[64] V. Balachandar, K. Jayaramayya, M. Iyer, A. Narayanasamy, V. Govindasamy, B. Giridharan, S. Ganesan, A. Venugopal, D. Venkatesan, H. Ganesan, K. Rajagopalan, P.K.S.M. Rahman, S.G. Cho, N.S. Kumar, M.D. Subramaniam, COVID-19: a promising cure for the global panic, Sci. Total Environ. 725 (2020) 138277. https://doi.org/10.1016/j.scitotenv.2020.138277.
[65] Z. Chi, Z. Wu, J.W. Li, H. Zhao, G.Q. Wang, The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality, Int. J. Antimicrob. Agents 55 (2020) 105954. https://doi.org/10.1016/j.ijantimicag.2020.105954.
[66] Z. Bin, S. Liu, T. Tan, W. Huang, Y. Dong, L. Chen, Q. Chen, L. Zhang, Q. Zhong, X. Zhang, Y. Zou, S. Zhang, Treatment with convalescent plasma for critically ill patients with SARS-CoV-2 infection, Chest 158 (2020) e9–e13. https://doi.org/10.1016/j.chest.2020.03.039.
[67] D. Kai, B. Liu, C. Li, H. Zhang, T. Yu, J. Qu, M. Zhou, L. Chen, S. Meng, Y. Hu, C. Peng, M. Yuan, J. Huang, Z. Wang, J. Yu, X. Gao, D. Wang, X. Yu, L. Li, J. Zhang, X. Wu, B. Li, Y. Xu, W. Chen, Y. Peng, Y. Hu, L. Lin, X. Liu, S. Huang, Z. Zhou, L. Zhang, Y. Wang, Z. Zhang, K. Deng, Z. Xia, Q. Gong, W. Zhang, X. Zheng, Y. Liu, H. Yang, D. Zhou, D. Yu, J. Hou, Z. Shi, S. Chen, Z. Chen, X. Zhang, X. Yang, The feasibility of convalescent plasma therapy in severe COVID-19 patients: a pilot study, Proc. Natl. Acad. Sci. U.S.A. 117 (2020) 9490–9496. https://doi.org/10.1073/pnas.2004168117.
[68] B. Himani, A. Roberts, L. Vogel, K. Subbarao, B. Moss, Neutralizing antibody and protective immunity to SARS coronavirus infection of mice induced by a soluble recombinant polypeptide containing an N-terminal segment of the spike glycoprotein, Virology 334 (2005) 160–165. https://doi.org/10.1016/j.virol.2005.01.042.

[69] K.W. You, F. Kien, A. Roberts, Y.C. Cheung, E.W. Lamirande, L. Vogel, S.L. Chu, J. Tse, J. Guarner, S.R. Zaki, K. Subbarao, M. Peiris, B. Nal, R. Altmeyer, Antibodies against trimeric S glycoprotein protect hamsters against SARS-CoV challenge despite their capacity to mediate FcγRII-dependent entry into B cells in vitro, Vaccine 25 (2007) 729–740. https://doi.org/10.1016/j.vaccine.2006.08.011.
[70] K. Eun, G. Erdos, S. Huang, T.W. Kenniston, S.C. Balmert, C.D. Carey, V.S. Raj, M.W. Epperly, W.B. Klimstra, B.L. Haagmans, E. Korkmaz, L.D. Falo Jr., A. Gambotto, Microneedle array delivered recombinant coronavirus vaccines: immunogenicity and rapid translational development, EBioMedicine 55 (2020) 102743. https://doi.org/10.1016/j.ebiom.2020.102743.
[71] World Health Organization, DRAFT Landscape of COVID-19 Candidate Vaccines, World, 2020.
[72] Jonathan Corum, Denise Grady, Wee Sui-Lee, Carl Zimmer, The New York Times. https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html. (Accessed 20 July 2020).
[73] https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf (Accessed on 24 February 2020).
[74] http://www.chinacdc.cn/en/COVID19/202002/P020200310326208577015.pdf. (Accessed 6 February 2020).
[75] S. Tao, X. Liu, J. Feng, M. Guo, W. Hu, D. Guo, H. Ullah, Y. Yang, Q. Zhang, X. Wang, M. Sajid, Z. Huang, L. Deng, T. Chen, F. Liu, K. Xu, Y. Liu, Q. Zhang, Y. Liu, Y. Xiong, G. Chen, K. Lan, Y. Chen, ddPCR: a more sensitive and accurate tool for SARS-CoV-2 detection in low viral load specimens, Emerg. Microb. Infect. 9 (2020) 1259–1268. https://doi.org/10.1080/22221751.2020.1772678.
[76] A. Tao, Z. Yang, H. Hou, C. Zhan, C. Chen, W. Lv, Q. Tao, Z. Sun, L. Xia, Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases, Radiology (2020) 200642. https://doi.org/10.1148/radiol.2020200642.
[77] https://www.hsa.gov.sg/announcements/regulatory-updates/hsa-expedites-approval-of-covid-19-diagnostic-tests-in-singapore-via-provisional-authorisation. (Accessed 24 March 2020).
[78] https://www.taiwan-healthcare.org/biob2b/breaking-news?articleTypeSysid=A. (Accessed 9 March 2020).
[79] H.H. Ki, S.W. Lee, T.S. Kim, H.J. Huh, J. Lee, S.Y. Kim, J.S. Park, G.J. Kim, H. Sung, K.H. Roh, J.S. Kim, H.S. Kim, S.T. Lee, M.W. Seong, N. Ryoo, H. Lee, K.C. Kwon, C.K. Yoo, Guidelines for laboratory diagnosis of coronavirus disease 2019 (COVID-19) in Korea, Ann. Lab. Med. 40 (2020) 351–360. https://doi.org/10.3343/alm.2020.40.5.351.