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Conventional and Nanotechnology-Based Sensing Methods for SARS Coronavirus (2019-nCoV)

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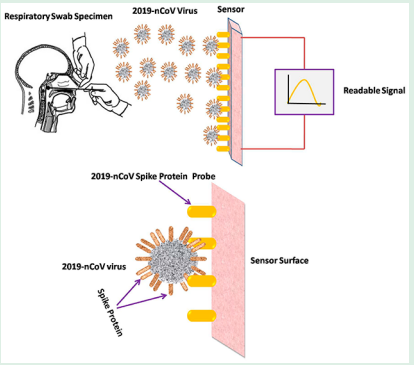
ABSTRACT: Ongoing pandemic coronavirus (COVID-19) has affected over 218 countries and infected 88,512,243 and 1,906,853 deaths reported by Jan. 8, 2021. At present, vaccines are being developed in Europe, Russia, USA, and China, although some of these are in phase III of trials, which are waiting to be available for the general public. The only option available now is by vigorous testing, isolation of the infected cases, and maintaining physical and social distances. Numerous methods are now available or being developed for testing the suspected cases, which may act as carriers of the virus. In this review, efforts have been made to discuss the conventional as well as fast, rapid, and efficient testing methods developed for the diagnosis of 2019-nCoV.Testing methods can be based on the sensing of targets, which include RNA, spike proteins and antibodies such as IgG and IgM. Apart from the development of RNA targeted PCR, antibody and VSV pseudovirus neutralization assay along with several other diagnostic techniques have been developed. Additionally, nanotechnology-based sensors are being developed for the diagnosis of the virus, and these are also discussed.



KEYWORDS: *biosensors, nanoparticles, virus detection, diagnostics, infection, healthcare*

1. INTRODUCTION

The invasion of novel coronavirus (2019-nCoV) infection first appeared in Wuhan city (Hubei, China) during late December 2019.[1−3] Until now the ongoing pandemic has affected nearly 218 countries and infected 88,512,243 with 1,906,853 deaths reported by Jan. 8, 2021; potential vaccine candidates have been developed by USA, China, Russia, and Europe; some of them are in trial phase III. It will still take more time for the vaccines to be available for the general public.[2] It is necessary to identify and isolate the infected persons, since many cases infected are asymptomatic or do not seem to show any symptoms of fever, cough, and so on, so contactless identification using thermal imaging or by thermometers may not be effective. It is therefore necessary to develop diagnostic techniques to identify and isolate the infected people from the general public to restrict the spread of the disease. For this purpose polymerase chain reaction (PCR), reverse transcription (RT-PCR) and antigen-based diagnoses are being adopted to detect the pandemic infections. These techniques can be divided into direct RNA and spike protein, indirect IgG and IgM, and virus-based detection.[3]

Conventional diagnosis methods suffer from being more time consuming to being less accurate, so it is important to develop more accurate, simple, facile, and accurate testing methods. For this purpose, newer techniques based on nanomaterials are considered. These include nanopore target sequencing (NTS) one-step reverse transcription loop-mediated isothermal amplification (RT-LAMP) integrated with nanoparticle-based biosensor (NBS) assay (RT-LAMP-NBS), field-effect transistor (FET) based biosensing device, graphene field-effect transistor (Gr-FET) with more specific antibody−antigen interaction of 2019-nCoV spike protein S1, dual-functional plasmonic biosensor using the combination of plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) sensing.

In this review, different procedures of sensing and detection of novel 2019-nCoV are discussed along with efficient sensing and detection techniques for 2019-nCoV. Sensing of different types of analytes is an important analytical tool for monitoring healthcare and the environment.[4−7] Development of sensing technology is therefore of utmost significance in the progress toward the diagnosis of different harmful elements including some biological pathogens.[8−24] The two basic components of a sensor are receptor, which interacts with the analyte, and a transducer, which converts physical or chemical interactions of

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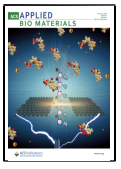

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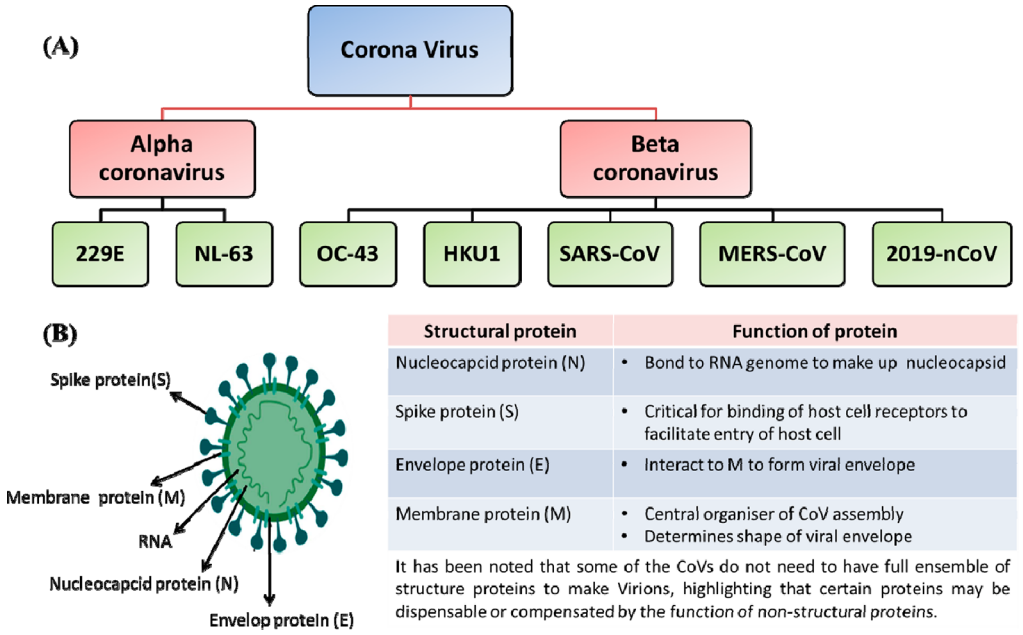

Structural protein	Function of protein
Nucleocapcid protein (N)	• Bond to RNA genome to make up nucleocapsid
Spike protein (S)	• Critical for binding of host cell receptors to facilitate entry of host cell
Envelope protein (E)	• Interact to M to form viral envelope
Membrane protein (M)	• Central organiser of CoV assembly • Determines shape of viral envelope

It has been noted that some of the CoVs do not need to have full ensemble of structure proteins to make Virions, highlighting that certain proteins may be dispensable or compensated by the function of non-structural proteins.

Figure 1. (a) Classification of coronaviruses and (b) basic structure of a coronavirus. Reproduced with permission from ref 46. Copyright 2020 Nature Publishing Group.

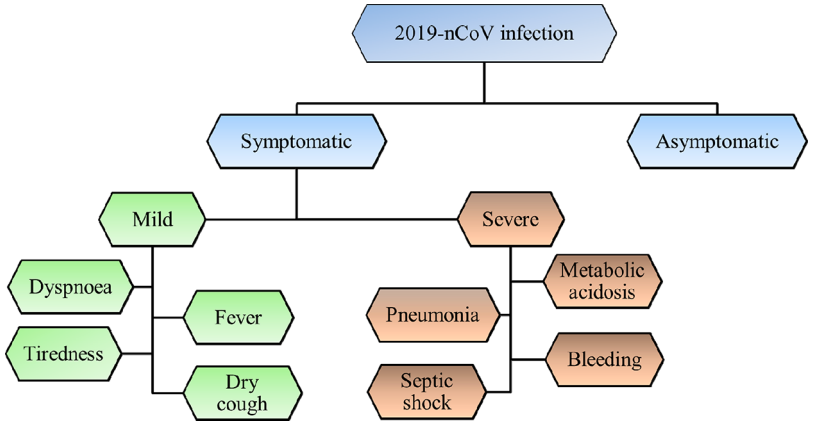

Figure 2. Types of infections of 2019-nCoV virus and their symptoms.

the receptor and the analyte into some reasonable signal, which can be processed and amplified to precisely quantify the analyte.[7,8,11−15,21,25−33] Sensors can be broadly divided into two types: chemical and physical sensors, which are based upon the property of the sensing material being harnessed depending on the type of the analyte.[8,25,34,35]

2. GENERAL ASPECTS OF 2019-NCOV

The novel 2019-nCoV virus belongs to the genus of Betacoronavirus, which is categorized as a subgenus of Sarbecovirus in the subfamily of Orthocoronaviridae, which belongs to the family Coronaviridae having suborder cornidovirinae and order Nidovirilae[34,36,37] The novel 2019-nCoV virus belonging to the class of coronaviruses is the seventh virus of this class, which has infected humans. The other coronaviruses that are known to infect humans are NL-63, 229E, OC-43, HKU1, SARS-CoV, and MERS-CoV.[38] These are divided into two types: α and β coronaviruses, among which 229E and NL-63 are α coronaviruses, while OC-43, HKU1, SARS-CoV, MERS-CoV, and 2019-nCoV are β coronaviruses[39] (Figure 1a). In humans, coronavirus leads to infections related to respiratory, gastrointestinal, heptic, and central nervous systems. Among the viruses, NL-63, 229E, OC-43, and HKU1 would cause only mild illness, sore throat, and cough, while SARS-CoV, MERS-CoVm, and 2019-nCoV cause serious symptoms including life threatening respiratory failure.[40−42] The infections and outcomes from the coronaviruses may range from mild, self-limiting to more severe symptoms, causing even death.[43]

The name coronavirus is derived from the Latin word *corona*, which literally means crown. The presence of crown-like structure on the surface of these viruses gives them its characteristic morphology. The coronavirus family consists of a single-stranded positive-sense RNA having the genome of length 30 kb and hence, they are the large RNA viruses.[36] Both structured and nonstructured proteins are coded in their RNA genome. All of the known coronaviruses have similar morphologies consisting of four main structural proteins, which are spiked, membrane, envelope, and nucleocapsid proteins[44] (Figure 1b). The structural proteins are responsible for the host cell infection and subsequent replication. In this regard, spike proteins attached to the host receptors and

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membrane proteins provide shape to the virion particles, while envelope proteins take a part during the congregation and release of particles; the nucleoplasid proteins bind the genome to the replication−transcription complex, which is necessary to replicate the genomic material.

Coronaviruses are well-known to infect birds and mammals, but their ability to mutate has facilitated their transmission from animals to humans.[42] SARS-CoV, MERS-CoV, and 2019-nCoV are more fatal to humans, and infections caused by these are all zoonotic in nature, which means that their mode of transmission can be traced back to animals such as bats. Among these, SARS-CoV and MERS-CoV have comparatively higher mortality rates of 10 and 35%, respectively, compared to 2019-nCoV having a mortality of 2%.[36] However, 2019-nCoV is more contagious as it can spread rapidly compared to SARS-Cov and MERS-CoV.[43] The clinical symptoms of 2019-nCoV infection (Figure 3) resemble those of SARS and MERS-CoV, which include fever, dry cough, dyspnoea, and tiredness.[36,45] The symptoms of upper respiratory track infection with 2019-nCoV such as sneezing, sore throat, and snot were rarely observed, which means that the virus mostly infects the lower respiratory tract.[46] Similar to SARS and MERS-CoV, there may be 20−25% chance of intestinal infections, resulting in diarrhea.[47] Under severe conditions, 2019-nCoV infection might result in pneumonia, septic shock, metabolic acidosis, bleeding, and coagulation dysfunction (Figure 2a).[43]

People suffering from 2019-nCoV infection may or may not show symptoms of fever,[48] and the virus produces large amounts of cytotoxins just like SARS and MERS-CoV,[49] resembling in some respects that of SARS-CoV.[43] Both 2019-nCoV and SARS-CoV are stable at lower ambient temperature and low humidity conditions.[50] The incubation time for 2019-nCoV virus is 10 days on an average (maximum 14 days) from the infection point to the onset of the symptoms.[46] One of the most challenging aspects of 2019-nCoV infection is that it is very contagious and spreads from person to person even without symptoms, making the task difficult to track and isolate the potential carriers of the virus.[48,50] On the other hand, people recovered from 2019-nCoV infection were suffering from other disorders such as Kawasaki disease. However, the blood plasma of people who have recovered has been used to cure other infected people with more severe symptoms.[51] However, it is still uncertain that to what extent the plasma is beneficial for the treatment of infected people.[52]

Many cases of asymptomatic spread of 2019-nCoV have been reported where the infected people have not shown any symptoms or signs of infection.[4] Among human beings, it can spread through sneezing droplets or close contact with an infected surface or with an infected person either during the incubation period or during symptoms.[4,37] The important safety precautions were suggested by the governments including screening of people at entry points, putting people on quarantine, who have travel history to infected areas, isolation of infected people, practicing social distancing, use of masks, and sanitizing of infected areas. However, individually, maintaining personal hygiene is highly recommended.

Like any other coronavirus, 2019-nCoV is also an enveloped virus containing positive-sense RNA (+ssRNA) having 29,900 nt.[36,37] It shares 79.5% similarity with SARS-CoV and 96% of its similarity with bat coronavirus bat-CoV-RATG13.[43] Its genetic properties significantly differ from SARSr-CoV and SARS-CoV with a close relationship with bat coronaviruses, viz., bat-CoV-RATG13 and bat-SL-CoVZC45,[53] which share 96 and 89% of the nucleotide sequence. Thus, from the protein sequence analysis, 2019-nCoV can be considered to be a SARS related SARSr-CoV coronavirus[43] and that 2019-nCoV, originating in bats, can be transmitted to humans. It can also be isolated from pangolins, where genetic sequence is 99% similar to 2019-nCoV found in infected humans. Hence, the transmission and evolution pathway of 2019-nCoV can be originated through bat-CoV to pangolins, which acts as an intermediate host after which it is infected to humans (Figure 2b).[35,43,54]

3. DIAGNOSTIC METHODS FOR 2019-NCOV VIRUS

Figure 3 represents some of the common methods used for the detection of pathogens. For detecting COVID-19 infected cases;

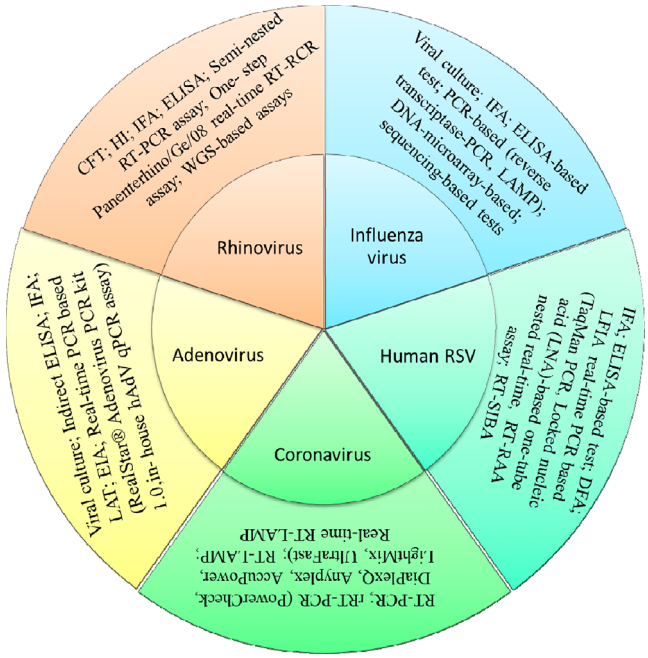

Figure 3. Approach toward diagnosis of respiratory viruses.

collection of appropriate specimen is crucial since, in some cases, detection can be wrong for samples collected from nasopharyngeal swab. Hence, collection of swab should be from lower respiratory tract such as sputum, bronchoalveolar lavage.[55] It is also necessary to control false negative or positive results, which may lead to epidemic or mental trauma and unnecessary treatment to patients.

As per the World Health Organization (WHO) guidelines, healthcare professionals should be equipped with a personal protective kit including gown, gloves, N95 mask, and eye protection glasses. As per recommendation from Centre for Disease Control and Prevention, samples collected must be from one of the following; nasopharyngeal specimen; an oropharyngeal specimen; nasal mid-turbinate swab; anterior nares; or nasopharyngeal wash/aspirate or nasal aspirate. A collected specimen should be placed in a tube containing viral transport medium, and if shipping is required, it must be packed in a triple packing system. Investigations have suggested that the virus, as well as in the respiratory system, can invade into the digestive system and hematologic system.[56] These studies suggest that the rate of lowest positivity was found in urine sample (11%) and highest in pharyngeal swab (78%), and intermediate in blood and anal swab (22% each). Studies have suggested positive results for different species with the highest probability in respiratory specimen. Hence to conclude the final results of a highly suspected infected individual, one must collect various types of specimens.

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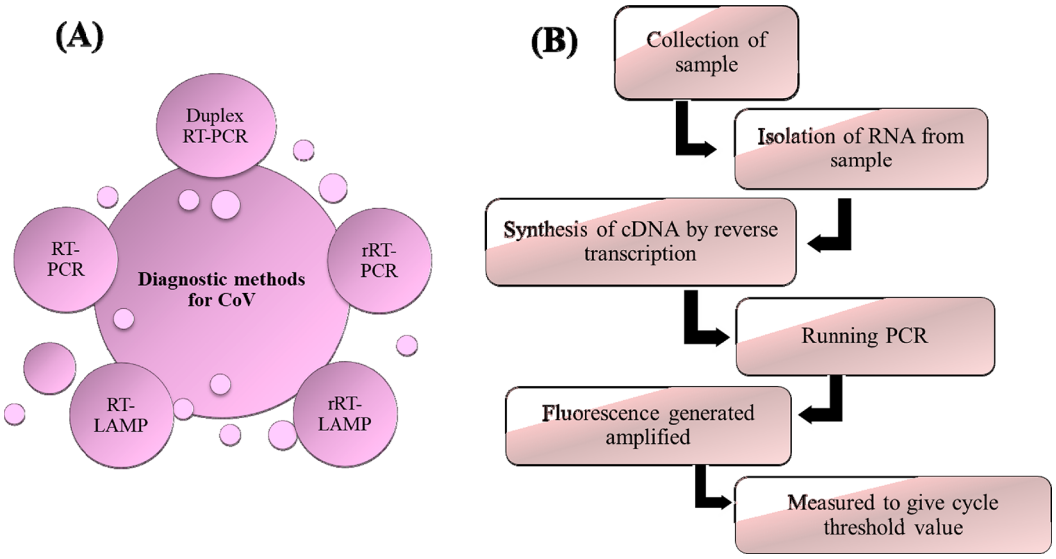

Figure 4. (A) Diagnostic methods for coronavirus detection; (B) schematic representation of step for PCR.

3.1. RNA and Spike Protein-Based Sensing Strategies. At present, diagnostic tests involve nucleic acid amplification and immunological assay. The diagnosis methods include real-time polymerase chain reaction, reverse transcription real-time PCR (rRT-PCR), real-time loop-mediated isothermal amplification (RT-LAMP), and reverse transcription LAMP (rRT-LAMP) (Figure 4).[57−59] Nucleic amplification-based techniques to detect 2019-nCoV are often used, due to increase of pandemics, as a result of their high specificity and sensitivity. PCR-based techniques are one of the first choices when required to make a large number of copies of a specific DNA using small sample and amplify to significant quantity to be used for further investigations. In a RT-PCR technique, a combination of reverse transcription of RNA and PCR to amplify DNA is performed. One advantage of quantitative RT-PCR (qRT-PCR) over the conventional RT-PCR is that results are available in real time even when the process is still going on. Assays for 19-nCoV infection are mostly RT-PCR-based diagnostic methods; loop-mediated isothermal and multiplex isothermal amplification with microarray detection have emerged as alternative methods worldwide. LAMP has advantages of amplification using 4 to 6 primers that promises high specificity and selectivity under 63−65 °C (isothermal conditions).[60]

PCR-based assays are performed on molecular targets identified in viruses RNA. The group of coronaviruses related to SARS including 19-nCoV has various molecular targets within their RNA genome and positive-sense available for PCR assay. Such identified molecular targets in coronavirus RNA include transmembrane, helicase, nucleocapsid, envelope, and envelope glycoproteins spike. In addition, open reading frames ORF1a and ORF1b, Hemagglutinin-esterase, and RNA-dependent RNA polymerase are some of the structural protein encoding genes utilized in qRT-PCR for the diagnosis of 19-nCoV. Measurements are performed considering the cycle threshold (Ct), which is the cycle number required for signals from fluorescent measurement to be detectable, having crossed the threshold. A PCR positive result is reported clinically when a Ct value < 40 cycles is obtained for a specimen. False negative results may be reported in RT-PCR method due to inappropriate timing of sampling or sampling error.[61]

Considering the cross-reaction with other coronaviruses and potential genetic drift of 19-nCoV, at least two molecular targets should be included in the assay. Hence, according to WHO and the United States, E gene assay with a confirmatory assay using RNA-dependent RNA polymerase gene and N1 and N2 two nucleocapsid protein targets molecular assay can be utilized for screening.[62,63] Considering the three developed novel qRT-PCR methods that target nucleocapsid, envelope glycoprotein spike genes, and RNA-dependent RNA polymerase gene/Helicase, the lowest detection limit in vitro along with high specificity and selectivity was found in RNA-dependent RNA polymerase gene/Helicase.[64]

Wong et al.[65] proposed a method to determine the 19-nCoV in chest radiography and correlated it with qRT-PCR. Six patients (9%) showed abnormalities in chest radiography before eventually testing positive for COVID-19 with RT-PCR. Sensitivity of the initial RT-PCR (91%; 95% confidence interval: 83%, 97%) was higher than that of baseline chest radiography (69%; 95% confidence interval: 56%, 80%) (P = 0.009). Yip et al.[66] demonstrated the diagnostic performance of the hydrolysis probe free 19-nCoV-nsp2 assay. The investigations involved 96 SARS CoV-2 and 104 non-SARS-CoV genomes. Using GolayMetaMiner genome subtraction, an identified region was the 154-nt conserved sequence in the nsp2 gene, which was absent in human-pathogenic coronavirus and were not targeted in the qRt-PCR assay of 19-nCoV.

A duplex RT-PCR procedure was also developed, for testing and diagnosis of SARS and MERS coronaviruses,[66] which is based on the targeting of a spiked S2 gene of the virus by primers and probes.[67] Corman et al.[68] developed an RT-PCR testing procedure for 2019-nCoV detection, which was carried out in the absence of a physical viral genomic nucleic acid. The authors thus established a diagnostic workflow screening, which can be helpful at the time when 2019-nCoV isolates are not widely available to the international health community. The method developed was based on using synthetic nucleic acid technology, which relied heavily on the close genetic relationship of 2019-nCoV and SARS-CoV viruses.

The presently used clinical nucleic acid detection has limited sensitivity and its relationship to genetic variation is still not well understood. To overcome this problem, Wang et al.[69] carried out research to establish the reference sequences for 2019-nCoV, which was done by the retrieval of 95 full-length genomic sequences of the virus strains collected from the National Center of Biotechnology Information and GISAID databases. The reference sequence was finally established via carrying out multiple sequence alignment and phylogenetic analysis as well as analysis of variations in sequence across the 2019-nCoV genome. The homology was observed to be high among the strain of the viruses, which was 99.99% both at nucleotide and amino acid levels. However, variations in the open reading frame regions were found to be low and only 13 variation sites were found. These findings threw some light on the fact that there may be some selective mutations possible in the 2019-nCoV virus.

Determining the impending mutation of 19-nCoV, deep sequencing molecular methods that involve metagenomic next generation sequencing are to be developed, but in the current diagnosis of 19-nCoV it is impractical. These techniques have an advantage of rapid

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sequencing of the whole genome. Metagenomic next generation sequencing involves running of nucleic acid in a sample that may have different microorganisms and then comparing with a reference genome to recognize the proportion and type of microbe. This technique allows sequencing and identification of nucleic acid from multiple microbes to avoid bottleneck problems. However, RT-qPCR-based methods have promising characteristics to detect SARS and MERS, but the genomic similarity of 19-nCoV with other coronaviruses may lead to cross-reaction with other associated viruses. Hence, confirmatory tests are required. Commercially, innumerable efforts have been made to develop automated instruments involving the extraction of RNA, PCR assembly, and detection technique.

3.2. Indirect IgA-, IgG-, and IgM-Based Sensing. Immunological test unlike molecular techniques targets the presence of specific immune proteins. An infected person triggers an immune response producing antibodies against virus infestation, which are the measures in an immunological test, or the proteins of virus present in the sample can also be measured. These tests are very useful in understanding the current situation of the person being infected in the past or not. However, these tests lack the identification of active cases. In addition, immunoassays such as antibody test, wherein an immune response produces in an individual serum immunoglobulins such as IgA, IgG, and IgM, can also be measured. Formats such as ELISA and lateral flow have been the approved diagnostic tests for 19-nCoV.

The ability of serological testing in a short time to diagnose and to identify an active immune response against virus has attracted attention recently. Only a few have been approved under Emergency Use Authorization from the FDA of the hundreds of currently available serological tests in the market.[70] Per the literature, the most common antigen used for serological test is spike and nucleocapsid proteins viral antigens against which antibodies are raised.[71] Serology test results can help one to understand the real spread of virus in a population, even though an individual was untreated or asymptomatic. Immune sensing of 2019 nCOV was reported by Xiang et al.,[72] who observed specificity for the detection of IgM and IgG up to 100% and 95%, respectively, but sensitivity was 77.3% and 83.3%, respectively, in confirmed patients. Further, in results for suspected 19-nCoV cases, sensitivities for IgM and IgG were 87.5% and 70.8%, whereas specificities were 100% and 96.6%, respectively. Thus, to establish reliable diagnosis, the detection of both IgG and IgM with higher specificity is required.

Lateral flow assays are used for emergency rapid and on-site detection of 19-nCoV. These essays work on detecting antigen of 19-nCoV or IgG and IgM antibodies, which are developed against 19-nCoV. This technique will help to understand the burden of infection, track asymptomatic patients, and so on.[73] One such point-of-care device available in the market for 19-nCoV IgG/IgM rapid testing is the IgM/IgG Rapid Test of Bio-Medomics.[74] However, sampling variability and low viral load in a infected person may be responsible for false negative result. WHO has recommended these immunodiagnostic tests for research laboratories but not for clinical decisions until evidence is supported. Although the limitation persists, these tests may play a critical role in tracing past infection. It may also provide support in choosing the convalescent plasma that can be used in further treatment of the infected person. Zhang et al.[75] investigated molecular and serological investigations of 2019-nCoV on infected humans by adopting multiple shedding routes. The nucleic acids of 2019-nCoV can be found in human samples such as sputum, nasopharyngeal swabs, secretions of the lower respiratory tract, blood, and feces.[37]

More emphasis is necessary for developing rapid testing techniques to provide results in a much lesser time such as rapid antigen tests, which are low-cost detection platforms, though these suffer from poor sensitivity for influenza viruses.[76] Hitherto, there is no possible random access diagnosis of 2019-nCoV and manual batch-wise testing is the only option though it is more tedious and takes longer time to obtain sample results.[77] Diao et al.[78] fabricated fluorescent immunochromatographic assay for sensing 2019-nCoV virus, which was rapid, simple, and quite accurate. The method was based on the detection of nucleocapsid protein of the virus for COVID-19 diagnosis in nasophyrangeal swab and urine samples. The sensing equipment consisted of a sample pad, conjugate pad, nitrocellulose membrane, and an absorbent pad along with a supporting plastic cassette. The testing method offered results within 10 min, detecting 73.6% of nucleocapsid protein from urine samples of human patients infected with 2019-nCoV.

3.3. Virus-Based Detection Methods. The ongoing development of a reference sequence for 2019-nCoV is quite helpful for biological studies as well as for identification, clinical monitoring, and intervention of any future infections of 2019-nCoV. Pesudo-virus is another useful tool for emerging and re-emerging viruses such as 2019-nCoV due to its versatility and safety. Due to highly contagious nature of 2019-nCoV and nonavailability of vaccines, it is necessary for handling this live novel virus under biosafety level 3 conditions, which has hindered the development of its vaccines.

Nei et al.[79] developed a VSV pseudovirus neutralization assay technique for the evaluation of neutralizing antibodies against 2019-nCoV virus under biosafety level 2 facilities. The assay was formulated using the optimized key parameters including cell types, cell numbers, and virus inoculum. Upon testing against 2019-nCoV pseudovirus, a high neutralizing potency was observed from 2019-nCoV convalescent patient sera. Thus, it can be considered as a promising therapeutic, where the detection limit was 22.1 and 43.2 for humans and mouse serum samples, respectively. Also, low coefficients of variation were observed at 15.9% and 16.2%, respectively corresponding to inter- and intra-assay analysis. Wang et al.[80] developed a nanopore target sequencing (NTS) for 2019-nCoV detection within 6−10 h. Parallel testing using qPCR kits and NTS was also carried out with 61 nucleic acid specimens from the suspected individuals. It was observed that NTS procedure was more effective and more sensitive than the mutated nucleic acid sequences or other respiratory viral infections.

Recently, a CRISPR-based detection system was developed for 2019-nCoV sensing by Brougton et al.[81] Sensing was somewhat rapid by this method taking <40 min with accurate results that are easy to implement. This approach was based upon CRISPR-cas12 lateral flow assay for identification of virus in respiratory swab samples. Sensing was validated by the devised reference and patient specimen, which included 36 people infected from 2019-nCoV and 42 infected from other viruses. This method was a better alternative to RT-PCR, which offered the faster detection with 95% positive predictive agreement with 100% negative predictive agreement.

Although by PCR it is possible to perform complicated and real-time investigations about viruses, but the method suffers from the risk of getting contaminated by the foreign nucleic acids. If in case some improper primers are selected, then it may badly affect the obtained results. For emergency situations, biorecognition may be necessary for the skilled workforce. Apart from PCR, the next generation sequencing is a highly selective and sensitive diagnosis method, but this requires trained personnel and is expensive since it requires complicated equipment.[82−86] Due to this reason, the method is less preferred for diagnosis of viral diseases. Enzyme linked immunoassays (ELISAs), which rely on antigen−antibody interactions, are more sensitive and give quicker results than the PCR and next generation sequencing. However, ELISA requires very specific and high affinity antibodies, which can sometimes increase the cost of the diagnosis. In the next section, more recent nanomaterial-based techniques are described, which tend to overcome the limitations of the above-mentioned diagnostic techniques.[87]

4. DEVELOPMENT OF NANOSENSORS FOR 2019-NCOV DETECTION

Nanotechnology has been a promising tool for the diagnosis and sensing of 2019-nCoV due to their size- and surface-dependent properties in detecting materials.[23,31,75,88] Surface modification is done by functionalizing the nanomaterial with a molecule or receptor, capable of selectively binding with the

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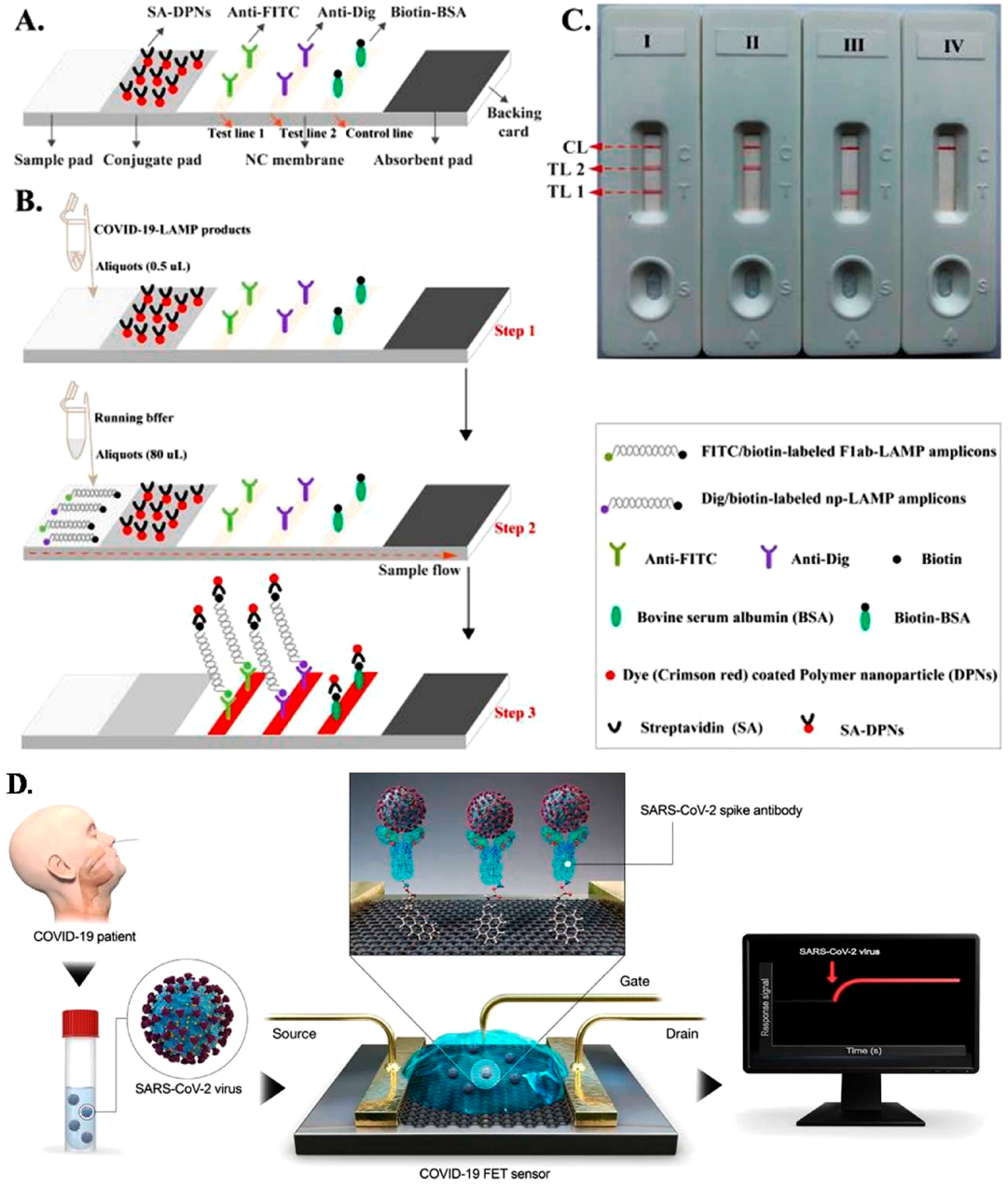

Figure 5. (A–C) Working principle of NBS coupled RT-LAMP diagnosis of 2019-nCoV virus and (D) schematic illustration showing the working of 2019-nCoV FET biosensor. Panels A–C reproduced with permission from ref 100. Copyright 2020 Elsevier. Panel D reproduced with permission from ref 38. Copyright 2020 American Chemical Society.

target. Surface activation can be done by manipulating the electronic structure of the nanomaterial either by doping or loading with other nanoparticles having different electronic structures.[14,15,17,89,90]

Zero-dimensional quantum dots, spherical nanoparticles,[91] one-dimensional CNTs,[4,76] metal,[92] metal oxide nanotubes and rods,[78,79] and 2D nanomaterials such as graphene[78,79,93] have also been widely used for sensing applications.[5,35,94–99] Apart from the above-mentioned diagnostic methods, development of nanosensors to detect 2019-nCoV virus can be very helpful to identify and isolate the infected individuals and offer infected patients the required treatment on time.

Per the development of nanosensors and diagnostic methods using nanomaterials, Zhu et al.[100] devised a rapid and precise testing method for 2019-nCoV that was based on one-step reverse transcription loop-mediated isothermal amplification (RT-LAMP) integrated with nanoparticle-based biosensor (NBS) assay (RT-LAMP-NBS). For this purpose, streptavidin-coated polymer nanoparticles immobilized with rabbit antifluorescein antibody, sheep antidigoxigenin antibody, and biotinylated bovine serum albumin were used for the NBS fabrication. F1ab and nucleoprotein (np) genes of 2019-nCoV were simultaneously amplified and detected by two LAMP primer sets in a one-step and single-tube reaction (Figure 5A–C). The one end of np and F1ab labeled with biotin was further bound to NBS. Here, NBS interpreted the results similar to the colorimetric method. The diagnostic method showed 100% sensitivity for 2019-nCoV in samples of infected patients with 100% specificity by testing of noninfected patients. This method is advantageous as it requires less time

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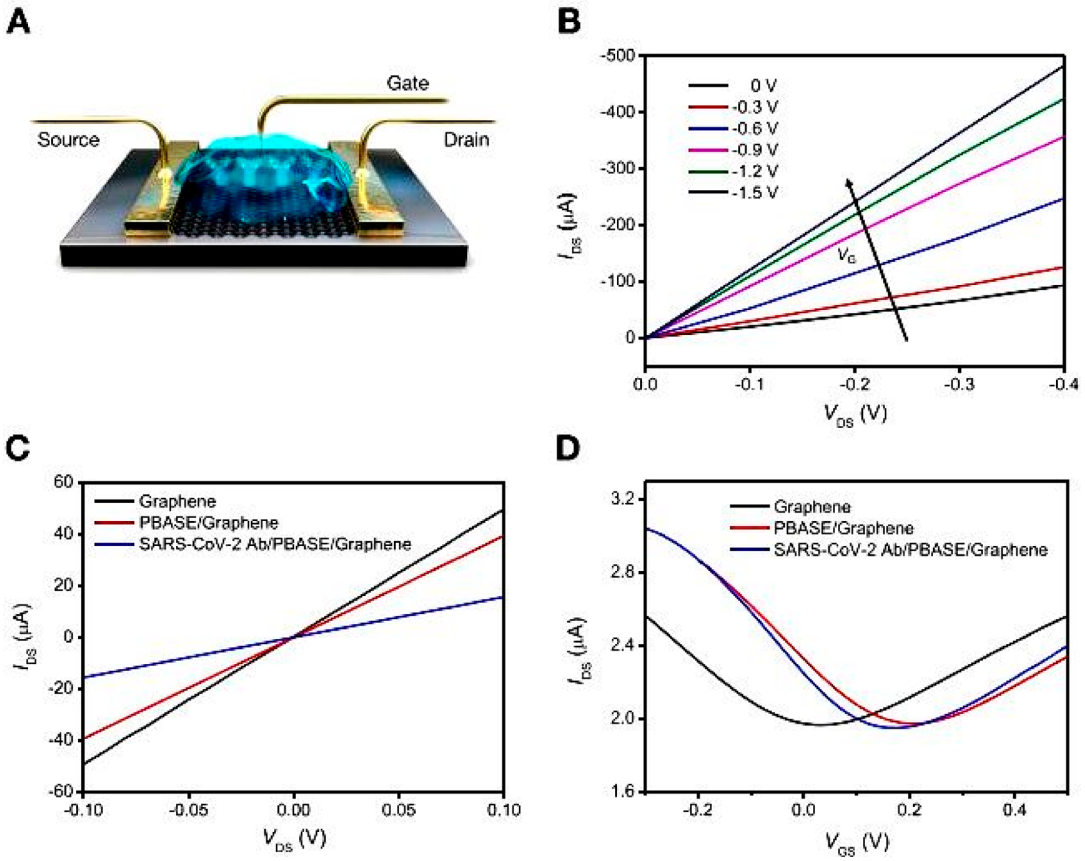

Figure 6. (A) Schematic illustration of FET sensor terminals, (B, C) *I*–*V* characterization, and (D) transfer curves. Reproduced with permission from ref 38. Copyright 2020 American Chemical Society.

and can offer results in 1 h; this can be useful in field laboratories since diagnostic results can be easily interpreted.

Seo et al.[38] recently developed a field-effect transistor (FET) based biosensing device for the clinical sensing of 2019-nCoV virus. The sensor was fabricated by conjugating FET graphene sheets by particular antibodies against the spike protein of 2019-nCoV (Figure 5D), and its performance was investigated using antigen protein and cultured virus, nasopharyngeal swab samples from infected patients. The conjugation of spike protein of the virus on graphene sheets was investigated by electrical measurements using graphene sheet conjugated with antibodies and sample drop as gate terminal and the remaining two terminals as source and drain (Figure 6A). Transfer curves (Figure 6D) of the sensor reveal that conjugating graphene sheets with antibodies caused a negative shift in the curves due to the positive charge of antibody causing an n-doping-like effect on graphene sheets after their immobilization. This way, the sensor was quite sensitive as a diagnostic tool for the detection of 2019-nCoV and it exhibited no cross-reactivity with MERS-CoV antigen.

In a similar report, Zhang et al.[92] incorporated sensitive graphene field-effect transistor (Gr-FET) with more specific antibody–antigen interaction of 2019-nCoV spike protein S1 subunit protein antibody (CsAb)-2019-nCoV spike S1 subunit protein with receptor-binding domain (RBD) antigen interaction and developed an immune sensor for the detection of 2019-nCoV. In this case, the graphene surface of Gr-FET was functionalized with CsAb receptor acting as a binding site to the S1 subunit protein of 2019-nCoV. For comparison, another immune sensor was fabricated by functionalizing the surface of Gr-FET with ACE2 (human angiotensin-converting enzyme-2) receptor.

Qiu et al.[101] prepared a dual-functional plasmonic biosensor using the combination of plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR) sensing transduction for the detection of 2019-nCoV. Two-dimensional gold nanoislands (AuNIs) were prepared by self-assembly of thermal dewetted Au nanofilm. Heat from the thermoplasmonic process was generated when AuNIs were irradiated with resonant plasmonic frequency. The AuNIs chips were integrated with a laser diode, which was used for PPT heating by illuminating AuNIs at an angle incident to the normal, resulting in the formation of a dual-functional plasmonic biosensor. In the PPT process, plasmonic nanoparticles during LSPR released heat energy due to their large optical cross-sections, since it is a nonradiative relaxation of absorbed light, also called a thermoplasmonic process.

In the thermoplasmonic process, direct absorption of irradiation leads to nonradiative radiation by the excited hot electrons[102] due to thermal energies quickly released or dissipated by the highly photoexcited electrons at ambient conditions. The increase of temperature from thermoplasmonic effect caused variations in the refractive index of the surrounding environment of AuNIs, which was examined from the LSPR detection (Figure 7B,C). To make the biosensor for 2019-nCoV virus detection, the AuNIs' surface was functionalized with the RdRp-COVID-C sequence (Figure 7A), which is a complementary DNA receptor, responsible for very good selectivity toward the virus detection by nucleic acid hybridization of the selected sequences. The hybridization

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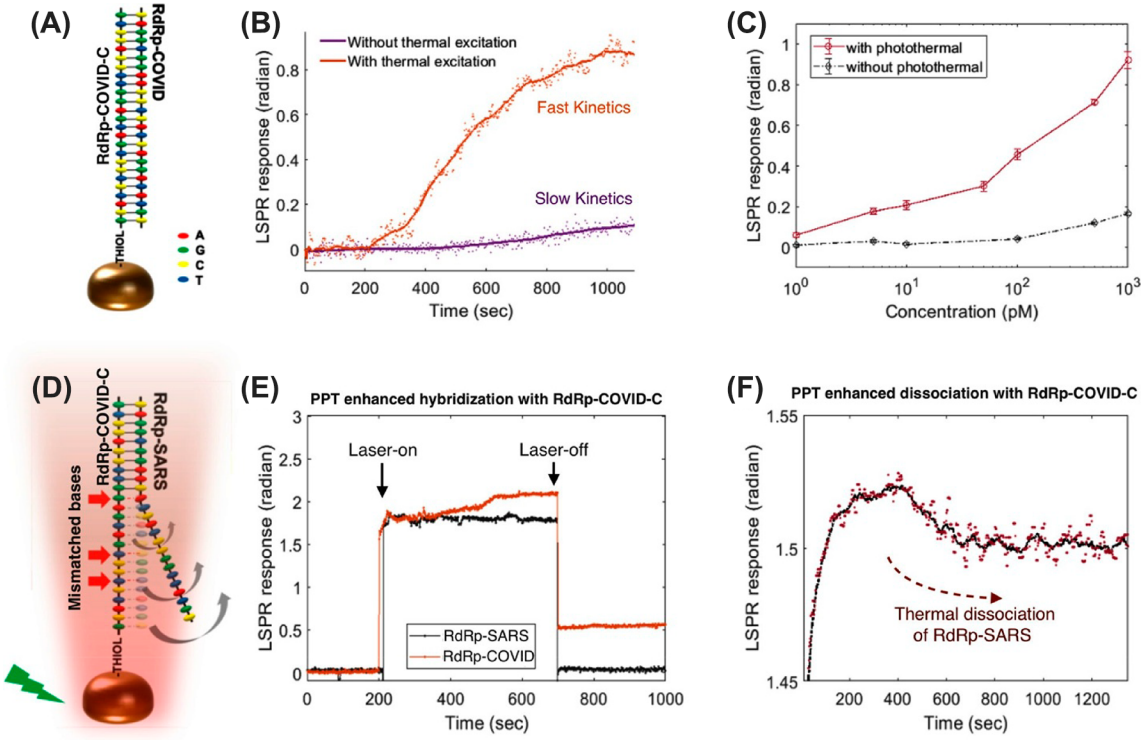

Figure 7. (A) Schematic representation of hybridization of RdRp of 2019-nCoV and complementary DNA. Real-time hybridizations of RdRp of 2019-nCoV and complementary DNA (B) with and without thermoplasmonic enhancement and (C) at different concentrations of RdRp of 2019-nCoV. (D) Schematic representation of inhibited hybridization of partially matched sequences. (E) Discrimination of two similar sequences by PPT heat. (F) Dissociation of RdRp-SARS sequence from immobilized RdRp-COVID C. Reproduced with permission from ref 101. Copyright 2020 American Chemical Society.

Table 1. Top-Performing Various COVID-19 Diagnostic Tools Based on Independent Evaluations of Sensitivity/Specificity

test name	test result time	gene or protein for detection	sensitivity (%)	specificity (%)	ref
Xpert Xpress SARS-CoV-2	results within 45 min	N2 and E gene	100	100	104
Accula SARS-CoV-2	results within 30 min	N gene	100	100	104
MiRXES FORTITUDE KIT 2.0	result within 90 min	viral RNA	99	100	104
STANDARD Q COVID-19 Ag	result within 15−30 min	IgM and IgG	100	84	105
Roche Cobas SARS-CoV-2	result within 3−8 h	viral RNA	95		106
VitaPCR COVID-19 assay	result within 20 min	viral RNA	100	100	107
MAGLUMI 2019-nCoV IgG	result within 30 min	IgM and IgG	98.8	95.1	108
NG-Test IgG COVID All-in-one Lateral Flow Immunoassay	result within 15 min	IgG and IgM	97	100	108
Autobio Diagnostics, Anti-SARS-CoV-2 Rapid Test	results < 15 min	IgG and IgM	99	99	109
Architect SARS-CoV-2 IgG Assay		IgG	97.2	100	110

was possible due to Au−S bond formation between the complementary DNA receptor and AuNIs. The LSPR response of the prepared AuNI dual-functional biosensor was increased by the addition of Rd-Rp-COVID genes due to the hybridization between the genes and AuNIs; however, hybridization has significantly increased with the thermoplasmonic heating.

Recently, cheap, sensitive cobalt-functionalized TiO_2 nanotubes (Co-TNTs)-based electrochemical sensors have been developed for the rapid detection of 2019-nCoV virus by Vadlamani et al.[103] The rapid detection of virus was carried out via sensing the spike protein present on the surface of the virus. A facile low cost one-step electrochemical anodization was used for the preparation of TNTs followed by wet cobalt functionalization. The sensor showed a high sensitivity even at very low concentrations of 14−1400 nM.

The nanotechnology-based diagnostic methods using nanomaterials are simple and facile compared to the conventional PCR and antibody-based techniques. The nanotechnology-based techniques can be more portable and accurate, although conventional-based rapid antigen detection gives quick results, but they suffer from false positive and false negative types of inaccuracies. Nanotechnology can lead to further advancements in testing and diagnosis of novel 2019-nCoV. The relationship between the nanotechnology and the conventional diagnostic methods is that with the help of nanotechnology, conventional PCR and antigen diagnosis can be further improved and made more accurate and fast for the viral diagnosis. However, the main disadvantages and limitations of nanotechnology-based sensing techniques is that these are still under development and the cost of sensor device fabrication needs to be further reduced to be available to the general public. The development of low cost and flexible nanomaterials

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is still important for the fabrication of portable, accurate, and rapid sensors that are very much important for on-time diagnosis of 2019-nCoV virus. Table 1 shows better performing various COVID-19 diagnostic tools based on independent evaluations of sensitivity/specificity.

5. CONCLUSIONS AND FUTURE PROSPECTS

Members of the 2019-nCoV related coronaviruses family, including SARS and MERS-CoV, are highly contagious and they spread quite rapidly. Until now, no proper treatment or vaccine has been available to treat 2019-nCoV. Its highly contagious nature and nonavailability of appropriate vaccines demand newer dimensions to handle this novel virus under biosafety level 3 conditions. Efforts are underway to find vaccines and medicines to prevent its spread. All we need is to maintain social distancing, personal hygiene, use of face masks, and sanitization of surfaces and to identify and isolate infected persons while visiting the affected places.

Like many other coronaviruses (SARS and MERS), 2019-CoV virus can be diagnosed via RT-PCR, PCR (rRT-PCR), RT-LAMP, and rRT-LAMP techniques. Additionally, other sensing methods have been reported to develop a reference sequence of the virus for its identification, formation of VSV pseudovirus neutralization assay, fluorescent immune−chromatographic assay, use of synthetic nucleic acid having genetic sequences close to 2019-nCoV and SARS-CoV, nanopore target sequencing (NTS), CRISPR-based detection system, and FET-based biosensing. The nanopore technology is already employed for DNA sequencing and analysis. It is also very promising for protein and peptide sequencing.[111] It can help in the electrical sensing of individual amino acids. It has been reported that the combination of N-terminal derivatization of amino acids with nanopore technology can result in their effective differentiation.[112] This can be very much helpful in rapid and accurate determination of novel viruses such as SARS CoV2. Some of these diagnostic methods may offer results in a shorter time with better accuracy and sensitivity.

Development of a reference sequence for 2019-nCoV would overcome the limitations of low sensitivity of the synthetic nucleic acid technique. Also, developing a reference sequence can be of much significance for future biological studies, clinical monitoring, investigations regarding future infections of 2019-nCoV, and analysis of sequence variations along the 2019-nCoV genome. Much research is to be done as many available diagnostic tests suffer either from low sensitivity, specificity/selectivity, or both and sometimes offer false-positive or false-negative results. These issues must be overcome to develop potentially successful diagnostic/detection methods that can be specific and sensitive and should not give false-positive or false-negative results.

Apart from the human body, viruses can also be found in pet animals. The survival times of viruses on different surfaces, in air, and in water are also a matter of concern, though the virus may have a lesser lifetime in these lifeless media. To be safe, it is necessary to detect viral presence on different surfaces, in air, and in water such that they can be further treated or sanitized to remove viral contamination. Many efforts are needed to develop methods of its effective diagnosis, particularly applying nanotechnology tools since nanomaterials have a high aspect ratio.

Recent reports of conjugating graphene sheets of FET sensors with the antibodies that are specific against the spike protein of 2019-nCoV virus are more effective, and hence, further research efforts should be directed toward the fabrication of such devices, which can provide specific results with better sensitivity and can detect small viral concentrations to detect even asymptomatic cases. We are hoping for a better future in this direction to win over the battle against 2019-nCoV virus.

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Notes

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