Evolution of SARS-CoV-2 and Diagnostic Techniques For COVID-19

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Abstract: *From the turn of the twenty-first century through 2021, RNA viruses have been the primary cause of most illness outbreaks around the planet. Recent reviews on SARS-CoV-2 have primarily focused on the virus's structure, outbreak progression, suitable precautions, management trials, and possible medicines. Nevertheless, this review aims to look at the historical evolution of all coronaviruses and to accompany viral epidemics, as well as COVID-19 diagnostics in the twenty-first century. Different RNA viruses that impact individuals have been compared based on their genome, structure, outbreak period, mode of dissemination, virulence, causative agents, and transmission. Due to the current mayhem caused by the fast-developing virus, SARS-CoV-2, its genome upgrades and infectivity are receiving extra attention. Finally, current diagnostic techniques include nucleic acid testing (natural time polymerase chain reaction and loop-mediated isothermal amplification), CRISPR-based diagnostics (CRISPR-based DETECTR assay, CRISPR-based SHERLOCK test, AIOD-CRISPR, FELUDA, CREST), chest radiographs (computed tomography, X-ray), and serological tests (Lateral flow assay, enzyme-linked immunosorbent assay. Clinical signs, contact tracing, and laboratory tests are essential parameters to consider when making a final diagnosis in the current bleak situation.*

Keywords: SARS-CoV-2, COVID-19, Diagnostics

1. Background

Coronaviridae is a coronavirus family that includes about 40 different species, most of which are known to cause disease in animals [1]. At the turn of the twenty-first century, a severe acute respiratory syndrome (SARS-CoV) outbreak was reported in China and Hong Kong (in 2003) [2]. The Middle East respiratory syndrome (MERS-CoV) outbreak in 2012 in the Middle East and the Republic of Korea [3] was the second. The outbreak's chain has persisted, and in December 2019, the globe witnessed the COVID-19 pandemic, which was caused by a novel coronavirus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and had a high mortality rate. Because of the high rate of mutation and short generation time that leads to fast evolution, these RNA viruses are highly infectious. RNA viruses have an ambiguous origin. Many pieces of evidence suggest that RNA viruses arose from a few DNA viruses and developed within some vertebrate hosts millions of years ago [4]. When gene sequences and the rate of evolutionary change (nucleotide substitution) were used to track the evolution of RNA viruses, it was discovered that the families of RNA viruses we know today appeared very recently, perhaps within the last 50, 000 years. [5].

Recent analyses of the new SARSCoV-2 virus have primarily focused on its structure, outbreak progression, appropriate precautions, management trials, and available treatments [6]. Nonetheless, this analysis aims to examine the history and evolution of all coronaviruses and viral outbreaks in the twenty-first century. We also compared the pathogenesis and epidemiology of the coronaviruses we studied, focusing on the diagnostic approaches for detecting different RNA viruses.

RNA viruses

Positive-sense RNA (ssRNA+) viruses, negative-sense RNA (ssRNA) viruses, and a third class that includes both positive and negative sense molecules, known as arenaviruses, are the three types of RNA viruses [7]. The replication in RNA viruses occurs by generating messenger RNA (mRNA) from their genome. The mRNA synthesizes numerous polyproteins cleaved into multiple proteins using viral or cellular protease enzymes. These viruses have the genetic codes for synthesizing an RNA-dependent RNA polymerase [8]. This enzyme then transcribes the +ve RNA strand and the complementary −ve RNA strands, which occur as intermediate products of genome replication. The second transcription step produces new genomic RNA molecules throughout this phase. [9]. Viruses having a continuous, single-stranded, ve-sense RNA genome must be duplicated to create protein and other viral components. Some retroviruses (HIV) follow reverse transcription to produce dsDNA to translocate into the host nucleus, integrate with its genome, and start replication to produce RNA [10].

The number, size, and position of viral genes in the RNA molecule, the number of polyproteins synthesized at the time of viral infection, and the presence of an envelope as a virion component all contribute to the classification of RNA viruses into different families. Picornaviridae, Astroviridae, Caliciviridae, Hepeviridae, Flaviviridae, Togaviridae, Arteriviridae, and Coronaviridae are some of the positive sense RNA virus families. The Rhabdoviridae, Bornaviridae, Paramyxoviridae, and Filoviridae families of negative-sense RNA, on the other hand, include Rhabdoviridae, Bornaviridae, Paramyxoviridae, and Filoviridae [11].

Pathogenesis of coronaviruses

Coronaviruses (CoVs) are RNA viruses with the best-understood genomic structure. RNA-based viruses, such as the coronavirus and influenza, mutate 100 times quicker

than DNA-based viruses; however, the coronavirus mutates more slowly than influenza viruses [12]. In 1962, CoVs were characterized as respiratory tract viruses found in samples taken from people who had respiratory tract infection symptoms. This is a giant virus family found in camels, cattle, cats, and bats, among other animals. CoVs are divided into four genera: alpha-CoV, beta-CoV, gamma-CoV, and delta-CoV (Family: Coronaviridae; Order: Nidovirales) [14]. Generally, gamma and delta CoVs infect birds, albeit some can infect mammals. Alpha and beta CoVs, on the other hand, are harmful to people and animals. Humans are susceptible to the SARS-CoV (beta coronavirus), 229E (alpha coronavirus), HKU1 (beta coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), and MERS-CoV (beta coronavirus). BetaCoVs, on the other hand, is the most relevant since they include highly pathogenic viruses in humans, such as COVID-19, MERS-CoV, and SARS-CoV [15]. Animal CoVs can be transferred to people on an infrequent basis, and as a result, the virus can spread among humans during outbreaks like MERS, SARS, and COVID-19 [16].

Evolution of the novel coronavirus

A novel coronavirus was discovered in Wuhan, China, in December 2019. The first cases of infection were discovered during a recent trip to Wuhan Huanan Seafood Wholesale Market. The preliminary examination and analysis indicated coronavirus features comparable to SARS and MERS. The novel coronavirus, on the other hand, has some different characteristics. SARS-CoV-2 is the name given by the World Health Organization to the recently found coronavirus. The sickness produced by SARS-CoV-2 is known as COVID-19 [17].

COVID-19 infection manifested itself first as mild acute respiratory distress syndrome, and it was treated with less vigour than SARS and MERS. The China CDC reported a 2% mortality rate [18]. Time passed, and the unusual virus's fury was shown by its high infectivity. According to the WHO, more than 210 nations and territories were afflicted on August 9, 2021, with 202, 296, 216 confirmed cases and 4, 288, 134 reported deaths.

The genome of COVID-19

A single-stranded positive-sense RNA was discovered during the genomic study of the novel coronavirus. With a length of 30 kilobytes, this RNA virus is quite enormous. SARS-CoV, MERS-CoV, Bat-SARS-like (SL)-ZC45, Bat-SL ZXC21, SARS-CoV, and MERS-CoV are all beta coronaviruses [19]. Several proteins are encoded by genomic RNA, some of which are structural and others that are nonstructural. The envelope (E) contains 75 amino acids, the membrane (M) contains 222 amino acids, the spike (S) has 1273 amino acids, and the nucleocapsid (N) contains 419 amino acids [20]. Sixteen nonstructural proteins serve a variety of purposes. The bulk of nonstructural proteins makes up the replication transcription complex (RTC). The RTC has a bilayer membrane and several cleavage proteases. Inside the infected cell, RTC produces sub-genomic RNA (RNA segments). Each RNA segment shares a common 5′-leader and 3′-terminal sequence. The transcription mechanisms for genomic segments are known as regulatory sequences (TRS) [21]. TRS is separated from

the end of the transcription process by an open reading frame (ORF). Transcription termination is mediated by specific proteases such as chymotrypsin-like protease, main protease, papain-like proteases, and a unique COVID-19 protease named 3′–5′ exoribonuclease [22]. There are six ORFs in the coronavirus RNA. The first ORF, which makes about two-thirds of the RNA, encodes nonstructural proteins. The remaining one-third of ORFs encode structural and auxiliary proteins [23]. A common 5′-leader and 3′-terminal sequence can be found in each RNA segment. The regulatory sequences (TRS) are the transcription processes for genomic segments [21]. TRS is separated by an open reading frame (ORF), responsible for the transcription process's end. Specific proteases, including chymotrypsin-like protease, main protease, papain-like proteases, and a unique COVID-19 protease called 3′–5′ exoribonuclease, are involved in transcription termination [22]. The coronavirus RNA contains six ORF. The first ORF encodes the nonstructural proteins, which makes up two-thirds of the RNA. The structural and auxiliary proteins are encoded by the remaining one-third of ORFs [23].

When comparing the genome sequences of different CoVs, the region encoding nonstructural proteins had the highest similarity (about 54%). However, there was around 43% less similarity in the structural protein-encoding section in the structural protein-encoding section. This could mean that additional structural protein genomic sequence changes are possible, allowing for better adaptability to different hosts [24].

COVID-19 had a genome that was 96.5 percent comparable to bat CoV and 75.6 percent similar to SARS-CoV [25]. The new virus genome's resemblance to the bat corona genome raises the possibility of coming from bats. On the other hand, the first example denied any contact with bats and was only found in bats that had lately become available on the seafood market. As a result, intermediate hosts are most likely involved and must be identified as soon as possible [26].

Infectivity of COVID-19

COVID-19 has been proven to be a highly infectious virus. Early in the outbreak, the introduction reproduction rate (R0) was calculated and determined to be between 2.2 and 3.58. Several factors may play a role in its infectivity, one of which is the spike's structure, including a receptor-binding domain (RBD). COVID-19 uses the RBD in the spike to bind to a similar receptor in the host [27]. The highest compatibility with COVID-19 was discovered when the spike protein sequences of different CoVs were evaluated and aligned with the human angiotensin-converting enzyme-2 (ACE-2). COVID-19 RBD's strong affinity for human ACE-2 could indicate more intermediate hosts' participation, resulting in mutations and improved adaption [28].

The mechanism of transmission is the second important component in COVID-19 infectivity. The initial contaminated contact cases discovered in Wuhan, China, verified human-to-human transmission. The aerosol pathway is used to infect the respiratory system [29]. However, the virus was later recovered from the stool of some individuals, indicating that other modes of transmission, such as water-

borne and direct contact, are possible [30]. The new virus can survive on multiple surfaces for more extended periods. According to a comprehensive evaluation of multiple research, it can also survive for four days on wood, five days on steel, metal, glass, and paper, and nine days on plastic [31].

Based on the first 10 cases in China, the average incubation period for COVID-19 was 5.2 days, with a range of 4.1–7.0 days. As a result, the COVID-19 quarantine duration was increased to 14 days [32]. On the other hand, subsequent cases had a more extended incubation period. Compared to SARS-CoV and MERS, a study of 50 patients in Wuhan found a longer incubation duration. In the case of SARS-CoV2, it can take up to 24 days [33]. In China, a survey of 1099 patients in 552 hospitals found asymptomatic carriers with positive viral tests among the patients' contacts [34].

2. Diagnostic Techniques

Because coronavirus infections are very contagious, diagnostic procedures must be specific and not solely based on clinical manifestation. Auxiliary examinations are required in circumstances where symptoms are atypical for correct diagnosis. The epidemiological history, clinical manifestations, and laboratory investigation are used to make a clinical diagnosis of coronavirus infections.

Polymerase chain reaction (PCR) is a gold standard for the molecular diagnosis of viral infections because of its excellent sensitivity and specificity. There are currently no specific tests available for Zika infections. Clinical symptoms and epidemiological factors are used to make a diagnosis in most instances. The infected person's body fluids, such as blood, saliva, and urine, are collected 3–5 days after the onset of symptoms for PCR nucleic acid identification by reverse transcriptase-polymerase chain reaction (RT-PCR) (targeting the nonstructural protein five genomic regions) [35]. Screening with viral cultures is no longer advised [36].

Various serological assays are available, such as ELISA and Plaque Reduction Neutralization Assay [35]. Blood tests to detect the virus in the patient's blood diagnose Ebola fever [37]. PCR was also used to test for MERS. Throat swabs, sputum, tracheal aspirates, or bronchoalveolar specimens are collected and stored at 28 °C for 72 hours before being sent to reference laboratories for real-time reverse-transcriptase–polymerase-chain-reaction (rRT-PCR) assays [38, 39]. To do RT-PCR for SARS, at least two separate clinical specimens, nasopharyngeal and stool, must be collected. The results of RT-PCR testing are confirmed in all of these assays by evaluating viral load cycle-threshold values. In epidemic conditions, genomic screens of animal populations have mainly been employed to find new viruses.

Nucleic acid testing
These tests are commonly used to detect viral infections because they detect specific nucleic acid sequences. They identify genetic material, which allows for early disease diagnosis compared to antigen or antibody detection, which requires a certain amount of time for these immunological components to arrive in the circulation. Because the amount

of genetic material to be identified is so small, it's always necessary to amplify it before detecting it. However, one key disadvantage of these assays is that they only identify viral RNA, not the live virus.

Real-timepolymerase chain reaction (RT-PCR)
COVID-19 is diagnosed via rRTPCR, similar to how other coronaviruses are diagnosed. Throat swabs, sputum, tracheal aspirates, and bronchoalveolar lavage specimens. On the other hand, the serum is not approved as a PCR sample [36]. Two technologies are employed to detect nucleic acids of the pathogenic virus in SARS-CoV-2: high-throughput sequencing and rRT-PCR [40]. These procedures, however, are not without flaws: high-throughput sequencing requires specialized equipment, instrumentation, and technical abilities and thus comes at a high cost. As a result, rRT-PCR is widely utilized and is seen to be a practical and uncomplicated method for detecting COVID-19 in COVID-19 patients' respiratory secretions and blood samples.

The nucleic acid of a new coronavirus is also detected in respiratory secretions or blood samples using real-time fluorescence RT-PCR [36, 41]. PCR is a technique for amplifying genetic material taken from these samples. Coronavirus conserved genetic codes can be discovered once enough genetic material has been acquired. The initial gene sequence is used to create detection probes. The CDC (Centers for Disease Control and Prevention) released these specific probe sequences [in the ORF1 (human RNA polymerase protein), E gene (Envelope protein), and N gene (Nucleocapsid protein) regions] and advised that they be used to detect SARS-CoV-2 [40, 42]. These primer and probe sequences were discovered to be 100% identical to other SARS-CoV-2 genome sequences available from the Global Initiative on Sharing All Influenza Data (GISAID). Three tests are included in many commercial PCR kits. Each assay targets a distinct gene in the virus; thus, the chances of all three genes altering simultaneously are slim. As a result, if one or both of these assays are positive, the results are considered inconclusive. As a positive control, SARS-CoV genomic RNA is employed. If both aims are optimistic, positive confirmatory results are obtained. If the test yields favourable findings, it is recommended that you repeat it. A one-step TaqMan-based fluorescent signal (RT-qPCR) test was also described to detect both the ORF1 and N gene sections of the viral genome separately [41]. An RT-qPCR (non-probes SYBR based fluorescent signal) showed positive results for SARS-CoV-2 at a high rate with saliva samples self-collected by patients in another investigation, suggesting that saliva could be a suitable non-invasive specimen for the diagnosis of SARS-CoV-2 [40].

Because the quality of available SARS-CoV-2-nucleic acid detection kits varies greatly, the reliability of PCR remains in question, and this test has a few flaws, as listed below:
- The test must be repeated at least 2 to 3 times in many situations due to the low detection rate for SARS-CoV-2. The detection rate is similarly low when the viral load is low, resulting in false-negative results. After repeated swab testing, patients with negative results were confirmed to be infected (RT-qPCR). According to the study, RT-PCR can only detect SARS-CoV in 50–79% of cases [40].

- The accuracy of test results is dependent on the collection of numerous clinical specimens, as one sample may not yield correct results.
- The sensitivity of the test is determined by the procedure utilized. With the protocol modification, there are differences in the outcomes.
- The test's sensitivity is found to be dependent on the type of sample. Pharyngeal swabs came back negative in a few cases, whereas bronchoalveolar lavage samples from the same patients came back positive [36]. According to one study, the clinical sensitivity of RT-PCR was 100 percent on swabs obtained on days 1–5 of symptoms, with no difference compared to swab and sputum samples taken simultaneously [43].
- The retention and operation of patient samples are associated with particular biological safety risks.
- After nucleic acid amplification, the steps for detecting nucleic acids are cumbersome and time-consuming.
- Results take a long time to arrive. After sampling, the findings can take up to a day to arrive.
- A disadvantage is the cost of the testing platforms.
- It necessitates specialized laboratory equipment as well as highly trained staff.
- Only a positive or negative diagnosis may be made; the severity and development of the disease cannot be determined.
- Toxic reagents cause a false-positive test.
- Cannot identify resolved infection, i. e., if a person has had an infection and cleared the virus, PCR will not detect this situation since PCR only detects the presence of an active virus.
- According to one research, only a few cases proved positive after two negative results in a row. It is still unclear if this is due to reactivation, reinfection, or simply a testing error [44].
- In the current outbreak condition, the supply of reagents is far insufficient compared to demand. The testing capacity of health care centres is insufficient to satisfy the needs of those waiting for SARS-CoV-2 to be detected by PCR. As a result of this flaw, many patients have gone undiagnosed, missing out on the opportunity for early isolation and treatment.
- Researchers at the University of Innsbruck (Austria), in partnership with Sinsoma GmbH (Völs, Austria), have developed a PCR test for SARS-CoV-2 identification that incorporates endpoint PCR and capillary electrophoresis [45].

Loop-mediated isothermal amplification (LAMP)
LAMP differs from RT-PCR in that viral DNA copies are made at a constant temperature of 60–65 °C rather than through a series of temperature changes, and the results are visible without using a machine. This test is quick, yielding findings in 2–3 hours. Compared to RTPCR, the amount of DNA generated in LAMP is substantially more significant. LAMP is a newer technology than RT-PCR, is technically straightforward, and can be performed in hospital laboratories, making it a more promising method for COVID-19 detection. Because there are ongoing clinical trials to support the test, there is not much data about its practical application at this time. LAMP can detect active

disease infections, allowing medical personnel to identify currently infected patients [46].

There are a few drawbacks to using LAMP, such as:
- These assays are built on a more sophisticated basis than RT-PCR.
- Because it relies on catching and detecting the virus, it cannot detect a resolved infection. As a result, missing patients who have recovered are a possibility.
- Because viral density varies across the respiratory tract, many samples are required. Even if a person is sick, the virus may only be detectable in sputum or a nasopharyngeal swab, but not necessarily simultaneously.
- COVID-19 LAMP tests can only detect if a person is actively infected with this coronavirus.
- It cannot tell you about other diseases or symptoms, and it cannot tell you if you've already been infected or if you're immune to the virus.

CRISPR-based detection of COVID-19
Because of the limitations of current diagnostics, researchers are looking to innovative approaches such as Clustered Regularly Interspaced Palindromic Repeats (CRISPR) (CRISPR). Cas enzymes (CRISPR-associated enzymes) (which cleaves certain strands of nucleic acid that are complementary to the CRISPR sequence) and guide RNA are the two components of CRISPR (that recognizes the required sequence). CRISPR-Cas is a technique that allows scientists to break DNA at a particular place. CRISPR diagnostics take advantage of guide RNA's (gRNA) targeting ability. CRISPR-Cas components are modified in COVID-19 diagnostics, for example, by adding a fluorescent protein to the complex and causing it to emit a fluorescent signal in response to positive or negative detection of the target genetic sequence.

CRISPR based DETECTR assay
Recently a technology DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) is being adapted to detect SARS-CoV-2 [47]. In this technique, RNA extracted from nasopharyngeal or oropharyngeal swabs undergoes simultaneous, reverse transcription and isothermal amplification (using RT-LAMP), where viral N gene or E gene is amplified, followed by Cas12 detection of predefined coronavirus sequences. Cas12a-gRNA complex is designed to detect the N gene or E gene [48]. Cas12a-gRNA complex binds to the target sequence, due to which Cas12a is activated, and it starts cleaving reporter molecule, i. e., fluorescently labelled ssDNA. Later, fluorescence is visually detected. Different approaches like later-flow strips, agarose gel detection, and fluorescence visualization are used for visual detection.

CRISPR based SHERLOCK test
Specific High-sensitivity Enzymatic Reporter Unlocking (SHERLOCK) is being reworked to detect COVID-19 following the genomic characterization of SARS-CoV-2 [48]. Isothermal Recombinase Polymerase Amplification (RPA) amplifies the viral Orf1ab or S gene from extracted RNA. The Orf1ab gene or the S gene is detected using gRNA in this test. Cas13a binds to the target sequence with complementary crRNA (CRISPR RNA). When this happens,

the Cas13a enzyme is activated, and the surrounding RNA and fluorescent RNA molecules (added to provide detectable signal) are degraded, resulting in fluorescence. It is also equipped with several detecting methods, such as lateral-flow readout strips. DETECTR and SHERLOCK approaches based on CRISPR are demonstrated.

Other CRISPR techniques

CRISPR diagnosis aims to provide concrete, visual, speedier, more user-friendly, and less expensive alternatives to PCR. However, as compared to qRT-PCR, its detection sensitivity is lower. Researchers are constantly attempting to improve this technology by employing various strategies to overcome its limitations. For increased specificity, rapidity, and ultrasensitivity, AIOD-CRISPR (All-in-one dual CRISPR/Cas12a) employs two gRNAs [49]. FELUDA (FnCas9 Editor Linked Uniform Detection Assay) is a FnCas9-based nucleobase detection and identification tool that may be used in the field. It is vulnerable to the presence and location of DNA mismatches [45]. CREST (Cas13-based, Rugged, Equitable, Scalable Testing) is a Cas13a-based approach that combines the quality of PCR with CRISPR-based detection and is scalable, cost-effective, and easy to deploy [50].

However, these nucleic acid detection assays should not be used as confirmatory tests because patients with negative nucleic acid detection for SARS-CoV-2 may have positive chest CT findings. As a result, a clinically suspected patient who has a negative nucleic acid test but positive imaging data should be separated and treated as soon as possible.

Chest radiographs

Computed tomography (CT)

CT scans, which are more sensitive, are being proposed as a mandatory auxiliary diagnostic procedure by medical specialists. CT scans are more reliable than PCR since they are faster, take less time, and have a higher positive rate. In contaminated locations with a high frequency of infection. CT scans are thought to be helpful in the diagnosis of COVID-19, according to doctors. When RT-PCR findings are harmful to patients suspected of having COVID-19 symptoms, a combination of CT scan and repeated RT-PCR would be significantly more effective. Surprisingly, high-resolution chest CT is critical for early identification and monitoring of COVID-19 patients' illness progression, as this disease displays a variety of imaging presentations at different stages, all of which are primarily related to pathogenesis. Only a tiny percentage of patients have early-stage CT findings that are negative. According to several investigations, in the early stages of SARS-CoV-2 infection, viral pneumonia affects the terminal bronchioles and the pulmonary parenchyma around them. It then infiltrates the pulmonary lobules, and as it proceeds to the advanced stage, the alveoli become severely injured. Typical CT pictures show distinct imaging at different phases, making them more helpful in tracking illness progression.

- At this stage of COVID-19, the lesions are mainly localized and manifest as inflammatory infiltration limited to the sub-pleural or peribroncho-vascular regions of one or both lungs, with patchy or segmental pure ground-glass opacity (GGOs) and vascular dilation, as well as segmental/patchy bilateral pulmonary parenchymal ground-glass opacity (86–93 percent).

- A high length of pure GGOs, a few consolidated regions and GGOs around these lesions (a distinguishing hallmark of development), and involvement of numerous lobes with consolidative pulmonary opacities were seen on CT scans in the later stage (nearly 65 percent). Single or more lesions are also visible; vascular expansion in the lesion (71.3%), peripheral distribution (87.1%), and bilateral involvement (82.2%) are all visible. The lower lung (54.5 percent) has the most lesions, which are multifocal. Reticular marking was found in the peri-bronchovascular and sub-pleural regions and a crazy-paving pattern and interlobular septal thickening. Mediastinal lymph nodes and pleural effusion were seen in a few patients.

- COVID-19 had evolved to the point where CT imaging looked like other types of pneumonia. Lung whiteout is a feature of CT imaging that demonstrates the presence of disseminated lesions in both lungs at this stage— GGOs were discovered encircling consolidated lesions, which are typically accompanied by parenchymal bands and, on rare occasions, a minor amount of pleural effusion.

However, we cannot wholly rely on CT imaging. In some cases, it can be challenging to distinguish COVID-19 from other illnesses such as SARS, MERS, cytomegalovirus infection, influenza, adenovirus infection, and other viral and bacterial pneumonia based on a visual examination alone because they can have the same CT image. CT scans include some flaws, such as improper CT imaging hysteresis. Clinical signs, contact history, and laboratory findings must all be reviewed together to make a final diagnosis.

Chest X-ray

Posteroanterior and lateral views of chest X-rays are examined for architectural distortion, traction bronchiectasis, and pleural effusions, which may represent COVID-19 viral load and virulence [36]. To some extent, chest X-rays can be used to determine viral load. The emergency and non-emergency groups had statistically distinct viral loads and pathogenicity; thus, this X-ray will help identify the emergency type disease. As a result, the viral load may be used to determine the severity of COVID-19 pneumonia.

Serological tests

Serological tests employ blood samples and a patient's immune reaction to determine whether or not a person has been exposed to a particular virus. The extensive study will make these COVID-19 detection assays widely available. Unlike nucleic acid detection, these tests will show if a person has ever had an infection and then recovered from it. If improved in the context of COVID-19, these tests will aid in the research of pandemic prevalence in any population and the assessment of 'herd' immunity, which will aid in the decision of social distancing and quarantine measures. Because a person's immune response takes time to produce a detectable antibody response, these tests are limited in their application for SARS-CoV-2 identification. Serological testing will help determine the cause of the cases.

Antibodies are frequently formed against the virus's antigen, the most abundant protein. As a result, tests that detect antibodies against this protein would be more sensitive; consequently, understanding the critical viral proteins is critical (e. g., viral coat protein). There is, however, a chance that the antibodies will react with another coronavirus. Antibodies to specific proteins, such as the host-attachment protein RBD-S (Receptor-Binding Domain of S), would be more specific. As a result, combining the two antigens (RBD-S and viral coat protein) will yield a considerably more reliable test [51]. The SARS-CoV-2 spike protein has a few distinct regions, making it a possible antigen for COVID-19 diagnostics [52].

Detecting viral protein (Ag) is a recent strategy that uses monoclonal antibodies specific for viral protein and chromatography to visualize the results; nevertheless, these procedures require a high viral level to produce an accurate result. The study of the virus's essential proteins is required for antigen and antibody detection assays. So that monoclonal antibodies can be developed against them (for use in antigen detection tests), or these proteins can be employed in antibody detection assays themselves. The most challenging component is getting these critical proteins to express precisely. Serological testing employs the following methods:

Lateral flow assay
Antibodies, viral antigens, and tiny molecules are detected using a lateral flow assay (also known as immunochromatography). Antigens/Ag–Ab complexes/ antibodies are moved through a support medium such as micro-structured polymer, nitrocellulose paper, filter paper, or agarose. In response to the increased diagnostic demand for COVID-19 in the current pandemic, researchers are attempting to improve these tests. These tests are used to detect antibodies (IgM and IgG) and viral antigens in the case of COVID-19. A sample is taken from a drop of blood (finger prick), saliva, or nasal fluid. These tests detect infection by examining the patient's antibody response to the virus, but they have the disadvantage of being unable to differentiate between current and earlier infections. Lateral flow tests are quick, small, portable, and simple to use examinations that require expert workers and advanced labs [53].

The successful commercial launch of these tests will be an extraordinary achievement in containing this pandemic, as it will detect viruses straight from a single sample without any amplification, spending less time. Sona Nanotech Inc., a biotechnology company, based in Canada, claims to have developed a lateral flow test employing nanoparticles that can detect SARS-CoV-2 in 15 minutes. This quick-response lateral flow test is being developed to assess COVID-19 patients. This test is predicted to cost around $50 to avoid the high-cost difficulties. Sona Nanotech will merge its proprietary nanorod technology into a disposable lateral flow test platform that can be performed by anybody with no prior experience and requires no laboratory equipment. It will be a massive success in triage screening [54].

Despite these benefits, this high-end approach is not without flaws and has several disadvantages, including:

- Because the disease is new, there is not much information on its accuracy in detecting SARS-CoV-2.
- More testing is needed to determine whether the infection is current or previous.
- For large batches, it is costly and time-consuming.

Enzyme-linked immunosorbent assay (ELISA)
ELISA is a laboratory-based biochemical technique for detecting antigens and antibodies. Because it is done in a batch of 96 assays, it has a high throughput. This is a massive benefit in the current pandemic, COVID-19, because numerous samples must be examined in a short amount of time. Antibodies (IgM and IgG) generated against SARS-CoV-2 are identified in whole blood, plasma, or serum was taken from a patient. A viral protein of interest (e. g., Spike protein) is coated in 96 wells on a plate and allowed to cross-react with the collected samples; if the samples contain antibodies to the viral protein, they will bind together. Later, enzyme-labelled secondary antibodies were added, which attach to the Ag-Ab complex and produce a colour response or fluorescence readout (based on the label tagged with the secondary antibody). Aside from diagnosing COVID-19, ELISA gives critical data for viral infection control, such as determining the number of infected people in a population. For the diagnosis of COVID-19, ELISA-based IgM and IgG antibody testing have a 95% specificity. A dual ELISA test for COVID-19 that identifies specific IgA and IgG antibodies against the virus in the blood of infected patients is available commercially [53].

ELISA is a simple, rapid (1–3 h) and inexpensive technique that can test numerous samples simultaneously. It is not yet established for SARS-CoV-2 testing, despite being well documented and frequently utilized by researchers in various domains. However, the silver side is that numerous companies are working hard to commercialize them.

Chemiluminescent immunoassay (CLIA)
CLIA is also being tested to see whether it can be used to diagnose COVID-19. This is a quantitative test that works similarly to ELISA. This assay uses enzyme-labelled secondary antibodies that allow for a luminous readout using light. Magnetic or protein-coated microparticles are being used in modified versions of this test, such as a peptide-based luminescence immunoassay to detect IgG and IgM. When used in conjunction with PCR, this test is thought to improve the accuracy of COVID-19 diagnosis vastly [55].

Neutralization assay
The patient's antibodies are assessed for efficacy against SARS-CoV-2 in a neutralization assay. The patient's whole blood, plasma, or serum is obtained, and neutralizing antibodies (NAbs) are tested. NAbs are essential in viral clearance because they can prevent viral infection. Cells that allow SARS-CoV-2 to proliferate are cultivated and grown with decreasing concentrations of the patient's sample (antibodies) to see how many antibodies can stop viral growth [56]. A neutralization assay is also required to rule out antibodies that cross-react with another coronavirus. There is only a tiny amount of research on NAbs in COVID-19 patients. This test is currently a potential therapy in many countries since transfusion of convalescent plasma/serum

from recovered patients is an option. It is helpful to know whether or not the antibodies in convalescent plasma are effective. It is commendable that some researchers could design a sensitive plaque reduction neutralization assay in such a short period and concluded that a simple micro-neutralization assay had sufficient sensitivity for population studies [43].

Nano-sensors
In public settings, thermal screening guns are currently used to screen patients with a high temperature. Instead of broad thermal screening, nano-sensor diagnostic techniques that use nano-sensor technology to detect nucleocapsid protein specific for SARS-CoV-2 and offer specific results for this virus can be used. It is still in the research stage, but it has thrived and will yield a result in a short time [57].

Blood test
In the early stages of COVID-19 infection, laboratory findings suggested specific abnormal counts in blood cells and enzymes, including lymphopenia (70 percent), prolonged prothrombin time (58 percent), increased values of lactate dehydrogenase, liver enzymes, and muscle enzymes, and decreased or average white cell count or decreased lymphocyte count [6]. COVID-19 can also be detected using these types of blood testing as a diagnostic tool.

Viral sequencing
In addition to the verified presence of a virus, regular sequencing is strongly suggested by the WHO to monitor any mutations that could impair other diagnostic procedures [58]. With the advent of more lethal variants, viral sequencing is a critical step in accelerating our response to the COVID-19 pandemic.

Diagnostic prospective
Given the limitations of the currently available COVID-19 diagnosis, there is a pressing need to create in vitro diagnostic platforms that can detect COVID-19 accurately, quickly, and efficiently in the field. Different diagnostic procedures such as ELISA, Lateral Flow Assay, Improved Molecular Diagnostics (CRISPR), Colorimetric testing, Chemiluminescence Immunoassays, and Neutralization Assays are needed to design and validate sensitive and specific auxiliary tests. A greater understanding of the virus's pathophysiology, infectivity, and the life cycle is essential for improved diagnostics. Various investigations, such as identifying a link between viral quantity and illness severity, developing functional serological assays, and comparing molecular and serological assays, must be examined in the context of diagnosis.

A recombinant immunofluorescence assay was used in one investigation to determine the specific reactivity to SARS-CoV-2 recombinant spike protein [43]. However, developing serological diagnostics that can predict current infections in broad populations is still necessary. Rapid antigen detection and other studies should be used to rule out common respiratory pathogens and non-infectious disorders in suspected patients. Serum antibody testing should be undertaken in asymptomatic high-risk persons with a history of exposure to patients with COVID-19 pneumonia to aid in

the early diagnosis of the disease. Serum antibody testing should be undertaken in asymptomatic high-risk persons with a history of exposure to patients with COVID-19 pneumonia to aid in the early diagnosis of the disease. Additional tests, such as total blood cell count and regular microbiology, including molecular testing for other respiratory viruses, can be performed in hospital laboratories using universal precautions. Immunological detection assays that target viral antigens or antibodies against them should be adopted as soon as possible in laboratories.

3. Conclusions

The novel coronavirus (SARS-CoV-2) was discovered in China in December 2019. It is the seventh beta coronavirus to have been discovered. In the beginning, it was diagnosed as acute respiratory distress syndrome. It later manifested itself in various forms, ranging from asymptomatic individuals and moderate flu to the most severe form of acute respiratory distress sickness and respiratory failure. The high infectivity of this unique virus could be attributed to the spike's high compatibility with the binding site (ACE2 receptors) in human pneumocytes and the virus's lengthy life time on inanimate surfaces and long incubation period, and proven human-to-human transmission. The principal diagnostic tools are the lateral flow assay, enhanced molecular diagnostics (CRISPR), colourimetric tests, chemiluminescence immunoassays, neutralization assays, RT-PCR, and chest CT scan COVID-19; however, each has its own set of limitations. The use of just one or two methods affects the early detection and isolation of those affected. As seen by the resurgence of COVID-19 cases in various parts of the world, unidentified infectious persons constitute a severe hazard of monitoring, controlling, and halting the disease outbreak. Clinical signs, contact tracing, and laboratory tests are critical parameters to consider in making a definite diagnosis in the current bleak scenario.

References

[1] Coronaviridae Study Group of the International Committee on Taxonomy of Viruses (2020) The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 5 (4): 536–544. https: //doi. org/10.1038/s41564-020-0695-z

[2] Bell DM (2004) Public health interventions and SARS spread, 2003. Emerg Infect Dis 10 (11): 1900–1906. https: //doi. org/10.3201/eid1011.040729

[3] Hilgenfeld R, Peiris M (2013) From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses. Antiviral Res 100 (1): 286–295. https: //doi. org/10.1016/j. antiviral.2013.08.015

[4] Moya A, Holmes EC, González-Candelas F (2004) The population genetics and evolutionary epidemiology of RNA viruses. Nat Rev Microbiol 2 (4): 279–288. https: //doi. org/10.1038/nrmicro863

[5] Drake JW, Holland JJ (1999) Mutation rates among RNA viruses. Proc Natl Acad Sci U S A 96 (24): 13910–13913. https: //doi. org/10.1073/pnas.96.24.13910

[6] Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, evaluation, and treatment of

coronavirus (COVID-19). In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.

[7] Zanotto PM, Gibbs MJ, Gould EA, Holmes EC (1996) A reevaluation of the higher taxonomy of viruses based on RNA polymerases. J Virol 70 (9): 6083–6096. https: //doi. org/10.1128/JVI.70.9.6083-6096.1996

[8] York A, Hengrung N, Vreede FT, Huiskonen JT, Fodor E (2013) Isolation and characterization of the positive-sense replicative intermediate of a negative-strand RNA virus. Proc Natl Acad Sci U S A 110 (45): 4238–4245. https: //doi. org/10.1073/pnas.1315068110

[9] Dietzgen RG, Kondo H, Goodin MM, Kurath G, Vasilakis N (2017) The family Rhabdoviridae: mono- and bipartite negative-sense RNA viruses with diverse genome organization and common evolutionary origins. Virus Res 227: 158–170. https: //doi. org/10.1016/j. virusres.2016.10.010

[10] Menéndez-Arias L, Sebastián-Martín A, Álvarez M (2017) Viral reverse transcriptases. Virus Res 234: 153–176. https: //doi. org/10.1016/j. virusres.2016.12.019

[11] Ward CW (1993) Progress towards a higher taxonomy of viruses. Res Virol 144 (6): 419–453. https: //doi. org/10.1016/s0923-2516 (06) 80059-2

[12] Duffy S (2018) Why are RNA virus mutation rates so damn high? PLoSBiol 16 (8): e3000003. https: //doi. org/10.1371/journal. pbio.3000003

[13] Hamre D, Procknow JJ (1966) A new virus isolated from the human respiratory tract. Proc Soc Exp Biol Med 121 (1): 190–193. https: //doi. org/ 10.3181/00379727-121-30734

[14] Chu DKW, Hui KPY, Perera RAPM, Miguel E, Niemeyer D, Zhao J, Channappanavar R, Dudas G, Oladipo JO, Traoré A, Fassi-Fihri O, Ali A, Demissié GF, Muth D, Chan MCW, Nicholls JM, Meyerholz DK, Kuranga SA, Mamo G, Zhou Z, So RTY, Hemida MG, Webby RJ, Roger F, Rambaut A, Poon LLM, Perlman S, Drosten C, Chevalier V, Peiris M (2018) MERS coronaviruses from camels in Africa exhibit region-dependent genetic diversity. Proc Natl Acad Sci U S A 115 (12): 3144–3149. https: //doi. org/10.1073/pnas.1718769115

[15] Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, Liu W, Bi Y, Gao GF (2016) Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 24 (6): 490–502. https: //doi. org/10.1016/j. tim.2016.03.003

[16] Subissi L, Posthuma CC, Collet A, Zevenhoven-Dobbe JC, Gorbalenya AE, Decroly E, Snijder EJ, Canard B, Imbert I (2014) One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. Proc Natl Acad Sci U S A 111 (37): 3900–3909. https: //doi. org/10.1073/pnas.1323705111

[17] Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y (2020) Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. Int J Infect Dis 94: 91–95. https: //doi. org/10.1016/j. ijid.2020.03.017

[18] World Health Organization. Coronavirus disease (COVID-19): situation report, 67.2021. https: //apps. who. int/iris/handle/10665/331613. Accessed August 9 2021.

[19] Chen Y, Liu Q, Guo D (2020) Emerging coronaviruses: genome structure, replication, and pathogenesis. J Med Virol 92 (10): 2249. https: //doi. org/10.1002/jmv.26234

[20] Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J, Quan L, Xia Z, Tan W, Cheng G, Jiang T (2020) Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 27 (3): 325–328. https: //doi. org/10.1016/j. chom.2020.02.001

[21] Fahmi M, Kubota Y, Ito M (2020) Nonstructural proteins NS7b and NS8 are likely to be phylogenetically associated with evolution of 2019-nCoV. Infect Genet Evol 81: 104272. https: //doi. org/10.1016/j. meegid.2020.104272

[22] Ceraolo C, Giorgi FM (2020) Genomic variance of the 2019-nCoV coronavirus. J Med Virol 92 (5): 522–528. https: //doi. org/10.1002/jmv.25700

[23] Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Sourvinos G, Tsiodras S (2020) Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infect Genet Evol 79: 104212. https: //doi. org/10.1016/j. meegid.2020.104212

[24] Kandeel M, Ibrahim A, Fayez M, Al-Nazawi M (2020) From SARS and MERS CoVs to SARS-CoV-2: moving toward more biased codon usage in viral structural and nonstructural genes. J Med Virol 92 (6): 660–666. https: //doi. org/10.1002/jmv.25754

[25] Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, Yuen KY (2020) Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect 9 (1): 221–236. https: //doi. org/10.1080/22221751.2020.1719902

[26] Rothan HA, Byrareddy SN (2020) The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun 109: 102433. https: //doi. org/10.1016/j. jaut.2020.102433

[27] Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, Zhu J, Zhang Q, Wu J, Liu L (2020) Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. J Med Virol 92 (6): 595–601. https: //doi. org/10.1002/jmv.25726

[28] Preprints. The disruption of SARS-CoV-2 RBD/ACE-2 complex by Ubrogepant is mediated by interface hydration.2021. https: //www. preprints. org/manuscript/202003.0466/v1/download. Accessed July 28 2021.

[29] Riou J, Althaus CL (2020) Pattern of early human-to-human transmission of Wuhan 2019 novel coronavirus (2019-nCoV), December 2019 to January 2020. Euro Surveill 25 (4): 2000058. https: //doi. org/10.2807/1560-7917. ES.2020.25.4.2000058

[30] Zhang J, Wang S, Xue Y (2020) Fecal specimen diagnosis 2019 novel coronavirus-infected pneumonia. J Med Virol 92 (6): 680–682. https: //doi. org/10.1002/jmv.25742

[31] Derraik JGB, Anderson WA, Connelly EA, Anderson YC (2020) Rapid review of SARS-CoV-1 and SARS-CoV-2 viability, susceptibility to treatment, and the disinfection and reuse of PPE, particularly filtering facepiece respirators. Int J Environ Res Public Health 17 (17): 6117. https: //doi. org/10.3390/ijerph17176117

[32] Leung C (2020) The difference in the incubation period of 2019 novel coronavirus (SARS-CoV-2) infection between travelers to Hubei and nontravelers: the need for a longer quarantine period. Infect Control Hosp Epidemiol 41 (5): 594–596. https: //doi. org/10.1017/ice.2020.81

[33] Jiang X, Rayner S, Luo MH (2020) Does SARS-CoV-2 has a longer incubation period than SARS and MERS? J Med Virol 92 (5): 476–478. https: //doi. org/10.1002/jmv.25708

[34] Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia Z (2020) Review of the clinical characteristics of coronavirus disease 2019 (COVID-19). J Gen Intern Med 35 (5): 1545–1549. https: //doi. org/10.1007/s11606-020-05762-w

[35] Del Rio C, Malani PN (2020) COVID-19—new insights on a rapidly changing epidemic. JAMA 323 (14): 1339–1340. https: //doi. org/10.1001/jama.2020.3072

[36] Torres M, Hansen KN, Jerrard D (2015) Ebola: a review for emergency providers. Emerg Med Clin North Am 33 (2): e1–18. https: //doi. org/10.1016/j. emc.2014.12.001

[37] Jung IY, You JB, Choi BR, Kim JS, Lee HK, Jang B, Jeong HS, Lee K, Im SG, Lee H (2016) A highly sensitive molecular detection platform for robust and facile diagnosis of Middle East respiratory syndrome (MERS) corona virus. Adv Healthc Mater 5 (17): 2168–2173. https: //doi. org/10.1002/adhm.201600334

[38] Mackay IM, Arden KE (2015) MERS coronavirus: diagnostics, epidemiology and transmission. Virol J 12: 222. https: //doi. org/10.1186/s12985-015-0439-5

[39] Li X, Geng M, Peng Y, Meng L, Lu S (2020) Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal 10 (2): 102–108. https: //doi. org/10.1016/j. jpha.2020.03.001

[40] Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, Ng DYM, Wan CKC, Yang P, Wang Q, Peiris M, Poon LLM (2020) Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clin Chem 66 (4): 549–555. https: //doi. org/10.1093/clinchem/hvaa029

[41] Menachery VD, Yount BL Jr, Debbink K, Agnihothram S, Gralinski LE, Plante JA, Graham RL, Scobey T, Ge XY, Donaldson EF, Randell SH, Lanzavecchia A, Marasco WA, Shi ZL, Baric RS (2015) A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med 21 (12): 1508–1513. https: //doi. org/10.1038/nm.3985

[42] Shu Y, McCauley J (2017) GISAID: Global initiative on sharing all influenza data—from vision to reality. Euro Surveill 22 (13): 30494. https: //doi. org/10.2807/1560-7917. ES.2017.22.13.30494

[43] Sethuraman N, Jeremiah SS, Ryo A (2020) Interpreting diagnostic tests for SARS-CoV-2. JAMA 323 (22): 2249–2251. https: //doi. org/10.1001/jama.2020.8259

[44] BioRxiv. Rapid, field-deployable nucleobase detection and identification using FnCas9.2021. https: //www.biorxiv. org/content/10.1101/2020.04.07.028167v2. Accessed July 21 2021.

[45] Kashir J, Yaqinuddin A (2020) Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19. Med Hypotheses 141: 109786. https: //doi. org/10.1016/j. mehy.2020.109786

[46] Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, Miao X, Streithorst JA, Granados A, Sotomayor-Gonzalez A, Zorn K, Gopez A, Hsu E, Gu W, Miller S, Pan CY, Guevara H, Wadford DA, Chen JS, Chiu CY (2020) CRISPR-Cas12-based detection of SARS-CoV-2. Nat Biotechnol 38 (7): 870–874. https: //doi. org/10.1038/s41587-020-0513-4

[47] BioRxiv. An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12.2021. https: //www. biorxiv. org/content/10.1101/2020.02.29.971127v1. Accessed July 21 2021.

[48] Ding X, Yin K, Li Z, Lalla RV, Ballesteros E, Sfeir MM, Liu C (2020) Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. Nat Commun 11 (1): 4711. https: //doi. org/10.1038/s41467-020-18575-6

[49] Rauch JN, Valois E, Solley SC, Braig F, Lach RS, Audouard M, Ponce-Rojas JC, Costello MS, Baxter NJ, Kosik KS, Arias C, Acosta-Alvear D, Wilson MZ (2021) A scalable, easy-to-deploy protocol for Cas13-based detection of SARS-CoV-2 genetic material. J Clin Microbiol 59 (4): e02402-02420. https: //doi. org/10.1128/JCM.02402-20

[50] Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, Fan Y, Zheng C (2020) Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis 20 (4): 425–434. https: //doi. org/10.1016/S1473-3099 (20) 30086-4

[51] Yang Z (2009) The pattern of co-existed posttranslational modifications— a case study. J Biomed Sci Eng 2 (1): 63–69. https: //doi. org/10.4236/jbise.2009.21011

[52] Subbaraman N (2020) Coronavirus tests: researchers chase new diagnostics to fight the pandemic. Nature. https: //doi. org/10.1038/d41586-020-00827-6

[53] Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, Lan Z, Law S, MacLean E, Trajman A, Menzies D, Benedetti A, Ahmad Khan F (2020) Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 370: m2516. https: //doi. org/10.1136/bmj. m2516

[54] Maksimović M, Omanović-Mikličanin E, Badnjević A (2019) Nanofood and internet of nano things. Springer Nature, Bern

[55] Cai XF, Chen J, Li HuJ, Long QX, Deng HJ, Liu P, Fan K, Liao P, Liu BZ, Wu GC, Chen YK, Li ZJ, Wang K, Zhang XL, Tian WG, Xiang JL, Du HX, Wang J, Hu Y, Tang N, Lin Y, Ren JH, Huang LY, Wei J, Gan CY, Chen YM, Gao QZ, Chen AM, He CL, Wang DX, Hu P, Zhou FC, Huang AL, Wang DQ (2020) A peptide-based magnetic chemiluminescence

enzyme immunoassay for serological diagnosis of coronavirus disease 2019. J Infect Dis 222 (2): 189–193. https: //doi. org/10.1093/infdis/jiaa243

[56] BioxRiv. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications.2021. https: //www.medrxiv. org/content/10.1101/2020.03.30.20047365v2. Accessed July 21 2021.

[57] Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, Dittrich S, Yansouni CP (2020) Diagnostic testing for severe acute respiratory syndrome-related coronavirus 2: a narrative review. Ann Intern Med 172 (11): 726–734

[58] World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, March 2 2020.2021. https: //apps. who. int/iris/handle/10665/331329. Accessed July 21 2021.