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

CHAPTER

12

Current clinical testing approach of COVID

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12.1 Introduction

Human fight with microorganism is not new. Past centuries have been the witness to emergence of number of new micro-organisms. Every time this tiny nonvisible enemy came with new approach by changing their genetic features but the astute human has always conquered and uprooted it from the ground. Innumerable pandemics came in past century; humans confronted against all and surpassed every opponent whichever tried to cease human community. But the year 2019 was different and gave the most awful memories to human community by claiming more than 0.8 million lives across the world so far. The recent emergence of novel coronavirus in the human population has caused dramatic and appalling influence on world economy and prompted mobilization of public health authorities around the world. Having stuck almost the whole world and affecting nearly 213 countries, this vicious creature had a deadly impact on human community and has caused colossal damage to society, swallowed umpteen lives. The aftermath consequences are also equally harrowing that is, the psychological impact on survivors and the social and economic downturn are relatively more disgusting. A recent mathematical model suggested that undocumented infections might be major drivers of SARS-CoV-2 spread in the world [1]. The efficacy of the virus to get adapted in new environment by modifying itself through mutation make a constant threat to population which may turn illnesses into pandemic and testing became more crucial.

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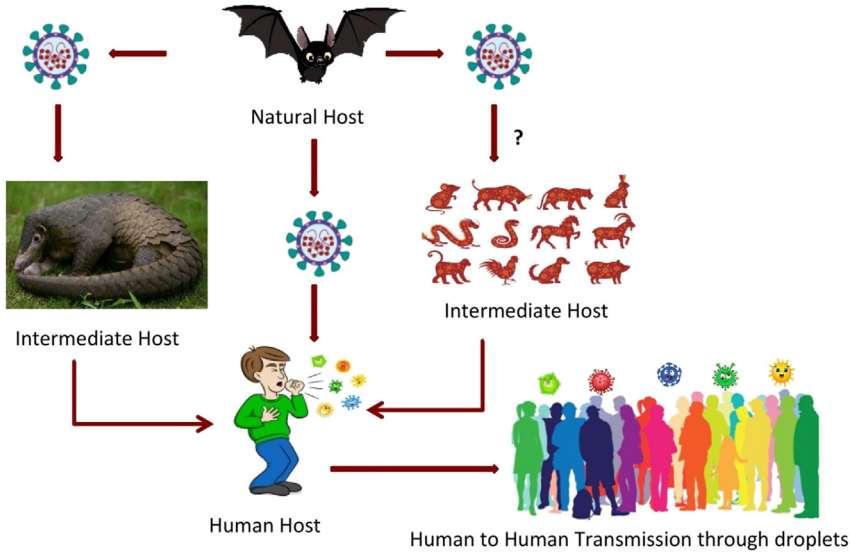

FIGURE 12.1 Mode of transmission of Coronavirus via different mediators (only alpha and beta virus have ability to transmit to humans).

Corona viruses belong to the *Coronaviridae* family in the *Nidovirales* order. They are large, enveloped, single-stranded RNA viruses which have the largest genome among all RNA viruses, typically ranging from 27 to 32 kb (Fig. 12.1) and approx. 50–200 nm in diameter. The subgroups of coronavirus family are alpha (a), beta (b), gamma (c), and delta (d). Among them, alpha- and beta coronaviruses infect mammals, gamma coronaviruses infect avian species, and delta coronaviruses infect both mammalian and avian species.

The genome is packed inside a helical capsid formed by the nucleocapsid protein (N) and further surrounded by an envelope. This N protein holds the RNA genome embraced with three structural proteins that is, M (The membrane protein), E (The envelop protein) and S (The Spike protein) which fabricates the viral envelope together [2]. M and E are responsible for viral assembly whereas the S spike protein is located on the surface of virus and plays a key role in virus entry into host cells. Viruses enter the host cells through Receptor-mediated endocytosis. The spike proteins responsible for the viral entry have N-terminal and C-terminal domains, and two major subunits S1 and S2 which are present in almost all coronaviruses [3]. One of these S1 or S2 subunits binds with the host receptors and acts as a receptor-binding domain (RBD). Among these structural proteins, these spikes form large protrusions from the virus surface, giving coronaviruses the appearance of having crowns (hence their name; corona in Latin means crown).

These viruses were thought to infect only animals until the world witnessed a severe acute respiratory syndrome (SARS) outbreak caused by SARS-CoV, at Foshan municipality, Guangdong Province, China, in November 2002 [4]. Only a decade later, in 2012 another pathogenic coronavirus, known as Middle East respiratory syndrome coronavirus (MERS-CoV) occurred in April 2012 in Jordan and has been diffused within and sporadically outside the Middle East regions [5] causing steady endemics in the countries. The SARS-CoV and MERS-CoV are prone to infect the lower respiratory tract, resulting in acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), septic shock and multiorgan failure, with high case fatality ratio (CFR). In 2019 emergence of new CoV outburst into pandemic has 80% sequence identity to SARS-CoV (with whom it is classified into the species severe acute respiratory syndrome-related coronavirus) [6] and 50% to MERS CoV. [VIII] SARS-CoV-2 belongs to the *Sarbecovirus* sub genus of the *Corona viridae family* and is the seventh coronavirus known to infect humans. The virus has been found 96% nucleotide identity with CoV isolated from bat origin, but not yet clear, that it directly infects human or through an intermediate host [7]. The SARS-CoV-2 full genome has been determined and published in GenBank [8]. Distinct features of SARS-CoV, MERS-CoV, and SARS Cov-2 are described in Table 12.1.

The phylogenetic analysis of SARS-CoV-2 shows similar Beta lineage as that of bat corona virus named as Bat CoV-RaTG13 [6]. Recent studies have suggested that bats may be the potential natural host of SARS-CoV-2 [7,9] and Malayan pangolin the potential intermediate host [10]. However, SARS-CoV-2 has been shown to have much higher human-to-human transmissibility [11]. As for the new virus, it is highly infectious and has already killed over 200,000 people with an estimated sCFR (symptomatic case fatality risk) of 1.4% (0.9–2.1%) [12] on comparison, fatality rate of SARS was roughly 10%, whereas, for MERS, it was approximately 36%, making it one of the deadliest human pathogens [13].

On the basis of recent studies most of the patients develop symptoms against infection of COVID-19 within 3−7 days [14] while clinical feature arises in 1–14 days. In general, the most common clinical manifestations are fever, dry cough, fatigue, sputum production, dyspnea, sore throat, headache, myalgia or arthralgia, and chills. Less common symptoms include nausea or vomiting, nasal congestion, diarrhea, hemoptysis, and conjunctival congestion [15]. In children, SARS-CoV-2 infection is generally mild and, in some case, asymptomatic; however, when presented, the main symptom includes fever (43% on admission, and 88.7% during hospitalization), cough (67.8%), diarrhea (3.8%), and fatigue [16,17]. More recently, several studies have been suggested that COVID-19 infection was associated with cutaneous manifestations in patients [18–20]. Major manifestations observed in COVID-19 patients including different types

TABLE 12.1 Comparative analysis of biological and historical features of SARS-CoV, MERS-CoV, and SARS-CoV 2.

Features	SARS-CoV [32,218–222]	MERS [223,224]	SARS-CoV-2 [7,143,225–229]
Genus	Clade I, lineage B	Clade II, lineage C	Clade I, lineage B
Emergence date	16 November 2002	4 April 2012	7 December 2019
Location of emergence	Guangdong, China	Zarqa, Jordan	Wuhan, China
Current status	Controlled	Sporadic	Still ongoing
Potential animal hosts	Bats, palm civets and Raccon dogs	Bats, Dromedry camels	Bats, Pangolins
Countries infected	26	27	213[a]
Total infected cases	8098	2519	102 million[a] (as of 31 January 2021)
Total attributed deaths	776	876	Above
Mortality rate	9.6%	35%	4.4%
Entry receptor in humans	ACE2 receptor	DPP4 receptor	ACE2 receptor
Sign and symptoms	Fever, cough and shortness of breath, ARDS	Fever, cough and shortness of breath, ARDS	Fever, myalgia, cough, shortness of breath, sneezing, headache, diarrhea, shivering, ARDS

of lesions such as purpuric, papulovesicular, livedoid, urticarial, maculopapular, and thrombotic ischemic [21]. Symptoms of severe COVID-19 disease include shortness of breath, loss of appetite, confusion, persistent pain, or pressure in the chest.

According to WHO criteria, a person is suspected of being infected with COVID-19 in three scenarios: (i) a patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, e.g., cough, shortness of breath) and a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset; (ii) a patient with any acute respiratory illness who has been in contact with a confirmed or probable COVID-19 case in the last 14 days prior to symptom onset; and (iii) a patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, for example, cough, shortness of breath, and requiring hospitalization) and in the absence of an alternative diagnosis that fully explains the clinical presentation. If a patient then tests positive with a

laboratory diagnostic, then the infection becomes a confirmed case of COVID-19, irrespective of clinical signs and symptoms [22].

12.2 Mechanism of infection: Key features and entry mechanism of human coronaviruses

The human-to-human spreading of the virus occurs due to close contact with an infected person through coughing, sneezing, respiratory droplets, or aerosols. These aerosols can penetrate the human body (lungs) via inhalation through the nose or mouth [23–26]. Animal models play a vital role to uncover the mechanisms of viral pathogenicity from the entrance to the transmission and designing therapeutic strategies. As the entire genome of the 2019-novel coronavirus is more than 80% similar to the SARS-like bat CoV, previously used animal models for SARS-CoV can be utilized to study the infectious pathogenicity of SARS-CoV-2. The sequence analysis revealed a mutation at spike glycoprotein in SARS CoV [27]. Thus, it could be another suitable option to develop spike glycoprotein targeting therapeutics against novel coronaviruses. Recently, mice models and clinical isolates were used to develop any therapeutic strategy against SARS-CoV-2 induced COVID-19 [28,29]. In a similar study, artificial intelligence prediction was also used to investigate the inhibitory role of the drug against SARS-CoV-2 [28]. SARS-CoV-2 infected patients were also used to conduct randomized clinical trials [28,30,31]. The rate of infection or the average number of people getting infected by an individual (R0) was 2.75 in the case of SARS pandemic in 2003. The R0 value of MERS, was around 1. The SARS-CoV-2 R0 value was estimated to be in the range of 1.5–3.5. Comparative study of SARS, MERS and SARS-CoV-2 has been displayed in Table 12.1. However, the difficulties arise for investigating therapeutic reagent against SARS-CoV-2 infection due to (1) basic mechanism of viral infection and incubation periods were uncertain, (2) most of the asymptomatic individuals are equally efficient in expansion of infection, (3) alteration in responsiveness of the society in proliferation of infection remains unanswered. In addition, there were no control measures for this spread [30]. With the ability to infect people through asymptomatic carriers, it can remain unnoticed and quickly disseminate itself, making the disease containment a confounding public health challenge [32]. It is now important that the scientists worldwide collaborate to design a suitable model and investigate the in vivo mechanisms associated with pathogenesis of SARS-CoV-2.

The respiratory droplets are the main routes of transmissions; SARS-CoV-2 can be transmitted to a healthy person if they encounter with infected person or any of his belongings, including clothes, doorknobs, etc.

Studies have been reported that aerosol transmission (Airborne transmission) is also possible for SARSCoV-2, but there is no clear study on neonatal infections (mother–to-child) [17,20,21,33,34]. During the initial phase of the COVID-19 outbreak, a dataset was obtained from 1099 patients with laboratory confirmed COVID-19 from 552 hospitals in 30 provinces of China on January 29, 2020. Only 2% of the patients had a history of contact with animals; more than three quarters have either visited the Wuhan city or are residents. Hence, the outbreak patterns or the source of infection could not be predicted from their study. The SARS-CoV-2 was detected in saliva, blood, sputum, and urine before the development of viral pneumonia, and some patients do not develop pneumonia at all. Asymptomatic persons are potential sources of SARS-CoV-2 infection, which control the transmission dynamics of the current outbreak [2,7,35,36]. Spikes comprises of three segments: (1) large ectodomain, (2) transmembrane domain, and (3) intracellular tail. The receptor-binding subunits S1 and S2 are placed in the ectodomain region. During the infection, the S1 binds with the host receptor, and S2 fuses the host and viral membranes, thereby releasing the viral genome into the cell. The spike protein is a clove-shaped trimer with three S1 heads and a trimeric S2 stalk [9]. During viral infection, spike protein (∼1300 amino acid residues) is cleaved by host proteases into receptor binding subunit S1 and membrane fusion subunit S2. During cell entry, the S1 subunit binds directly to the sugar receptors [37] and ACE2 of the host cell surface, and the S2 subunit undergoes conformational changes and obtains postfusion state [38]. During this state, the three pairs of heptads repeat region HR-N and HR-C in trimeric S2 form a six-helix bundle structure [39]. The buried hydrophobic fusion peptides become exposed and insert into the target host membrane. These fusion peptides and the transmembrane anchors are positioned at the end of a six-helix bundle structure, bringing the viral, and host membranes to fuse [39,40]. During this process, a large amount of energy is released, which accelerates the membrane fusion forward. Along with this, receptor binding and low pH can also trigger this membrane fusion. Since the spike protein has a good binding affinity for sugar receptors of human cells, it uses them as a mechanism of cell entry [3]. Notably, the SARS-CoV-2 has a higher affinity to human ACE2 than the SARS-CoV virus strain. The ectodomain of the SARS-CoV-2 spike protein binds to the peptidase domain (PD) of ACE2 with a Kd (equilibrium dissociation constant) of $\sim$ 15 nM [41]. Spike protein priming is done by transmembrane protease serine 2 (TMPRSS2), which is also essential for the entry of SARS-CoV-2 [42]. The glycoprotein spikes on the outer surface of coronaviruses are responsible for the attachment and entry of the virus to host cells. The receptor-binding domain (RBD) is loosely attached among virus; therefore, the virus may infect multiple hosts [43,44]. The spike protein of SARS-CoV-2 contains a 3-D structure in the RBD region to maintain the van der Waals

forces [45]. The 394-glutamine residue in the RBD region of SARS-CoV-2 is recognized by the critical lysine 31 residue on the human ACE2 receptor [46].

Throughout this viral entry process, the Lys31 and Lys 353 of human ACE2 receptors are termed as "hot spot" residues, which consist of a salt bridge buried in a hydrophobic environment and contribute critically for the virus and host cell receptor binding. Thus, this key residue comparison of SARS-CoV-2 with the civet and human SARs-CoV explains how actively SARS-CoV-2 is choosing and binding with the human ACE2 receptors, which is likely to cause the human-to-human transmission [46]. Hussain et al. reported that the mutations S19P and E329G in ACE2 disrupt the intermolecular interactions and have low binding affinity with viral spike protein. In addition to the variations in the viral spike protein, ACE2 allelic variants can also drive the potential resistance against SARS-CoV-2 infection [47].

A diagnostic test method should have sufficient sensitivity and accuracy to make appropriate clinical decisions rapidly during a pandemic [48]. The most effective way to curb outbreak of pandemic is early detection and isolate patients [49,50]. The laboratory-based diagnosis assumes a role for the clinical management of patients and the implementation of disease control measures. Here, we review the clinical features, laboratory methods, and imaging findings that are used for COVID-19 diagnosis.

12.3 Types of specimen

The right type of sample, appropriate collection procedure, and reliable transportation must be placed to minimize the risk of inaccurate results. The difference in the sensitivity of different types of swabs may depend on disease progression. Therefore, it is important to identify the appropriate type of sample considering the medical condition of the patient and diagnostic facility available for the test.

Two types of sample specimens are being primarily used for the diagnosis of COVID-19. Respiratory specimens are used for direct detection of virus and serum samples are used for identification of antiviral antibodies [51]. For specimen collection, the sensitivity of nasal, nasopharyngeal (NP), and throat swabs was found to be 80%, 90%, and 87%, respectively [18]. The respiratory specimens [52] are collected most frequently from the upper respiratory tract, that is, nasopharyngeal and oropharyngeal swab which is considered with regard to upper respiratory specimens, collected in the acute phase of infection—ideally within 7 days, and for lower respiratory specimens, sputum and endotracheal aspirate or bronchoalveolar lavage fluid (BLF) are considered [33] which should be obtained from patients remain symptomatic after more than a week [53,54]. A case study with

a pneumonia patient in Thailand showed a negative test with nasal or oropharyngeal swab samples but a positive test with bronchoalveolar lavage fluid [20]. A study reported that the highest rates of positive were found from bronchoalveolar lavage fluid (93%), followed by sputum (72%), nasal swab (63%), fibrobronchoscope brush biopsy (46%), pharyngeal swabs (32%), feces (29%), and blood (1%) [55]. Additionally, blood and stool specimen could be collected for clinical inspection which requires less challenging sampling procedures than respiratory specimens [56].

Serum samples are collected for immunoassay methods. Volume of blood sample for immunoassays ranges from 5 to 10 mL for lab assays to capillary draws of 50–200 μL blood for lateral flow immunoassays (LFIA) [57]. The virus samples should be processed and tested as soon as possible. If immediate testing is not possible, the sample can be stored up to 72 h at 2–8°C. However, for more than 72 h storage, the specimens should be frozen at -70°C as soon as possible after collection [53]. It is recommended to avoid repeated freezing and thawing of the specimen [33].

12.4 Clinical testing methods

It is of critical importance to rationally choose specific diagnostic methods to fight against viral outbreak, any negligence or compromise in the diagnosis may lead to devastating consequences. As part of the quality assessment of each assay, the FDA requires demonstration of specificity and exclusivity. Exclusivity means that no other viruses or bacteria from a specified list are detected by the test. Depending on the performance of the internal controls of the assays, the test results are reported as positive, negative, inconclusive, or invalid. Tests that detect 2 or more viral genes are interpreted differently in various assays. Some assays require that all viral genes be detected for the test result to be interpreted as positive, whereas others rely on the detection of 1 or 2 viral genes for a positive interpretation. These test results are positive during the incubation period, which is several days before the onset of symptoms of the disease and remain positive for the duration of symptoms. The tests detect parts of the viral RNA that can be present after the virus fragments. Therefore, the results can continue to be positive after the resolution of symptoms, even though a complete infectious virus may no longer be present [14,58–60].

Regulatory agencies such as the World Health Organization (WHO) and the US Food and Drug Administration (FDA) have approved the use of a number of diagnostic methods, while some new methods are receiving conditional approval under emergency use authorization (EUA) [61]. These diagnostic methods have varying throughput, batching capacity, requirement of infrastructure setting, analytical performance, and turnaround times [62]. In addition to depending on the equipment and

method itself, the result from a method also relies on sample collection protocol, reagents used, potential for cross-contamination, and sample/reagent storage requirements. These factors must be considered while selecting a reliable and rapid diagnostic method to help make an appropriate decision and prompt public health actions. Nucleic acid amplification using the reverse transcription polymerase chain reaction (RT-PCR) is the most widely used method for direct SARS-CoV-2 diagnosis [48]. Immunoassays are used to measure the antibodies against the SARS-CoV-2. CT imaging includes use of plain x-rays, computed tomography (CT) scanning, magnetic resonance imaging (MRI), nuclear scanning, and ultrasonography. Number of scientists also modified these traditional approaches and developed new accurate, error-free and faster techniques in order to avert any obstruction in spotting of this meticulous enemy. Despite of having these conventional procedures new emerging techniques like CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), RT-LAMP, DPCR, RCA, LFA, PLA have also been reported. Apart from laboratory-based CT imaging, RT-PCR, immunoassay and various emerging techniques, several point-of-care (POC) and rapid test methods have become available in the last few months. This Chapter aims to summarize the successive development of the current clinical approaches for SARS-CoV-2 diagnosis methods and provide recently presented information on various tests till date and compare their analytical efficiency in terms of limit of detection (LOD), sensitivity, and specificity. In addition, their limitations, ease of use, affordability, and availability of accessories have also been evaluated.

12.4.1 Molecular testing (nucleic acid test)

After identification of SARS-CoV-2 as the causative virus for this pandemic, the SARS-CoV-2 genome was quickly sequenced [63] from which unique sequences have been identified for COVID-19 diagnosis. PCR-based tests are widely used for the detection of viruses in human disease and are currently the most commonly used nucleic acid tests (NATs) performed in clinical laboratories. eRT-PCR-based diagnostic is highly sensitive, sequence specific, and useful in the early detection of COVID-19. Since the test accuracy varies depending on the disease stage and viral multiplication, the sensitivity can range from 71 to 98%, whereas the specificity is recorded to be 95% [64]. The same technique has been used in the diagnosis and surveillance of various other viral diseases including SARS-CoV and MERS-CoV [16,35,65]. The RT-PCR tests take less than an hour to a couple of days to give results, depending on the version of the PCR. The RT-PCR assay can be carried out in one- or two-step approaches. PCR instruments and techniques are in widespread use in both clinical and research laboratories and the basis of the tests is well known. The

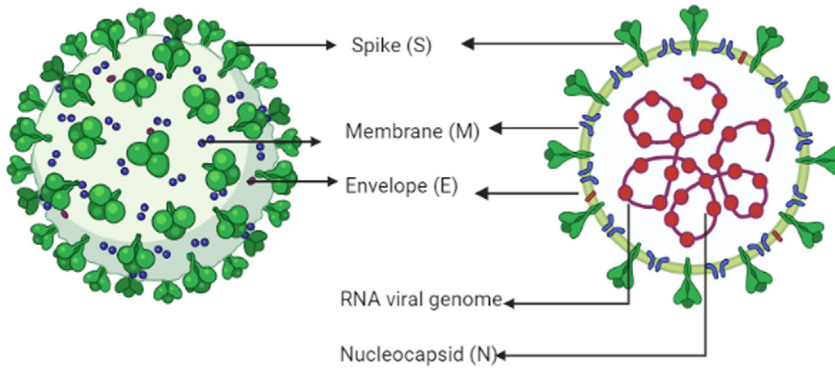

FIGURE 12.2 (A) Schematic presentation of Coronavirus showing viral structural proteins, spike (S), envelope (E), and membrane (M). (B) The single-stranded viral RNA is associated with the nucleocapsid protein having structural and accessory genes.

tests consist of nucleic acid extraction and purification from the human specimen using authorized extraction methods/instruments followed by real-time RT-PCR, where the RNA is reverse transcribed into cDNA and then amplified using the primer sets and detected using specific probes. Laboratory RT-PCR tests the RT-PCR assays in centralized laboratories are generally performed in 96-well plates for signal reading in batches. The high-throughput 384-well assay system using lower volume was reported recently with detection sensitivity down to 5 copies of viral genome per microliter [66] which yields 100% sensitivity and specificity. Real time reverse transcriptase polymerase chain reaction (rRT-PCR), or quantitative reverse transcriptase PCR (qRT-PCR), uses nucleic acid amplification test (NAAT) that has long been used routinely to amplify the DNA for the detection of viral RNA in clinical settings (Accelerated Emergency Use Authorization [[67] Summary; [68]]. Heat treatment prior to RNA extraction is not recommended as studies suggest that thermal inactivation in samples with low viral loads could result in potential false-negative nucleic acid test [69]. The SARS-CoV-2 genes most frequently used for detection so far include theORF1ab/RdRP gene, Nucleocapsid (N) gene, Envelope (E) gene, and Spike protein (S) gene (Fig. 12.2). One study reported that PCR amplification of the E and SARS coronavirus RdRp genes is 95% sensitive111. Virus-specific PCR-based tests required generating primers and probes unique to SARS-CoV-2 but not to the other closely related corona viruses [70–73]. In silico analysis using the many sequences available on publicly available databases (e.g., GenBank, the European Molecular Biology Laboratory (EMBL), Global Initiative on Sharing All Influenza Data (GISAID) to discriminate the SARS-COV-2 from other respiratory viruses is a hallmark widely employed to generate a specific primer for COVID-19 detection.

An amplification of each of these genes can be accomplished by the supply of proper forward and reverse primers. Studies also showed that the clinical sensitivity of a RT-PCR assay is under the influence of specimen type, amount of virus in a swab and the specimen collection time in relation to the onset of symptoms and the test result relies on sample collection, primers and probes used, analysis of fluorescence curves, use of suitable controls, and reliability of the temperature control. Respiratory specimens may contain different genera of coronaviruses along with other major viral pathogens. In the last six decades, before SARS-CoV-2, the human population was already infected with six other members (229E, OC43, SARS-CoV, NL63, HKU1, and MERS-CoV) of the CoV family [70]. False positive results occurring due to the cross reactivity with these viruses, human genome, and microflora can be obliterated with the sequence fidelity Fig. 12.3. Tsang et al. revealed that the saliva samples were more promising for use in RT-PCR [74], while Yam et al. concluded that testing more than one specimen could significantly maximize the sensitivity of the RT-PCR testing [73]. These findings suggest that it is rather important to apply nucleic acid-based kits with optimized conditions to maximize their diagnostic potency. Repeat testing, using various biospecimens (respiratory secretions, sputum, stool, rectal swabs, and serum), should be considered in patients with a high clinical suspicion of COVID-19 [51,59,72,75,76].

In a study of 205 patients, RT-PCR sensitivity was 93% for BLF, 72% for sputum, 63% for nasal swabs, and only 32% for throat swabs [51]. The presence of viral load that is below the assay's LOD (limit of detection) will also elicit false negative results. Therefore, a judicious way to increase viral load is to collect combined nose and throat swabs. Viral kinetics of SARS-CoV-2 showed that the viral load in respiratory specimens often peaks in the first week of illness and decreases as the disease progresses [75]. This posited appropriate sample collection times to enhance sensitivity. These findings are similar to a report that showed a 100% positive RT-PCR result by week 1 after onset of symptoms, followed by 89.3%, 66%, and 32% at week 2, week 3, and week4, respectively. By week 5, the positive detection rate plummeted down to only 5.4% [77]. In contrast to the widely used NP swabs, a study more recently showed an increase in the sensitivity by 13% when saliva samples were used [78]. Virus titers from saliva samples were found significantly higher than NP swabs and more importantly, unlike NP (nasopharyngeal) swabs, less temporal variation in viral titer was observed with longitudinally collected saliva samples. There are variants of RT-PCR methods that share the same mechanism while differing in the detection strategy. Real-time RT-PCR reads fluorescent signals during PCR amplification [68] to quantify the target, whereas nested RT-PCR uses two sets of primers to avoid nonspecific PCR amplifications [79].

The FDA has already issued several other molecular in vitro diagnostics under EUA [80]. In many protocols, RT-PCR assay of more than one gene

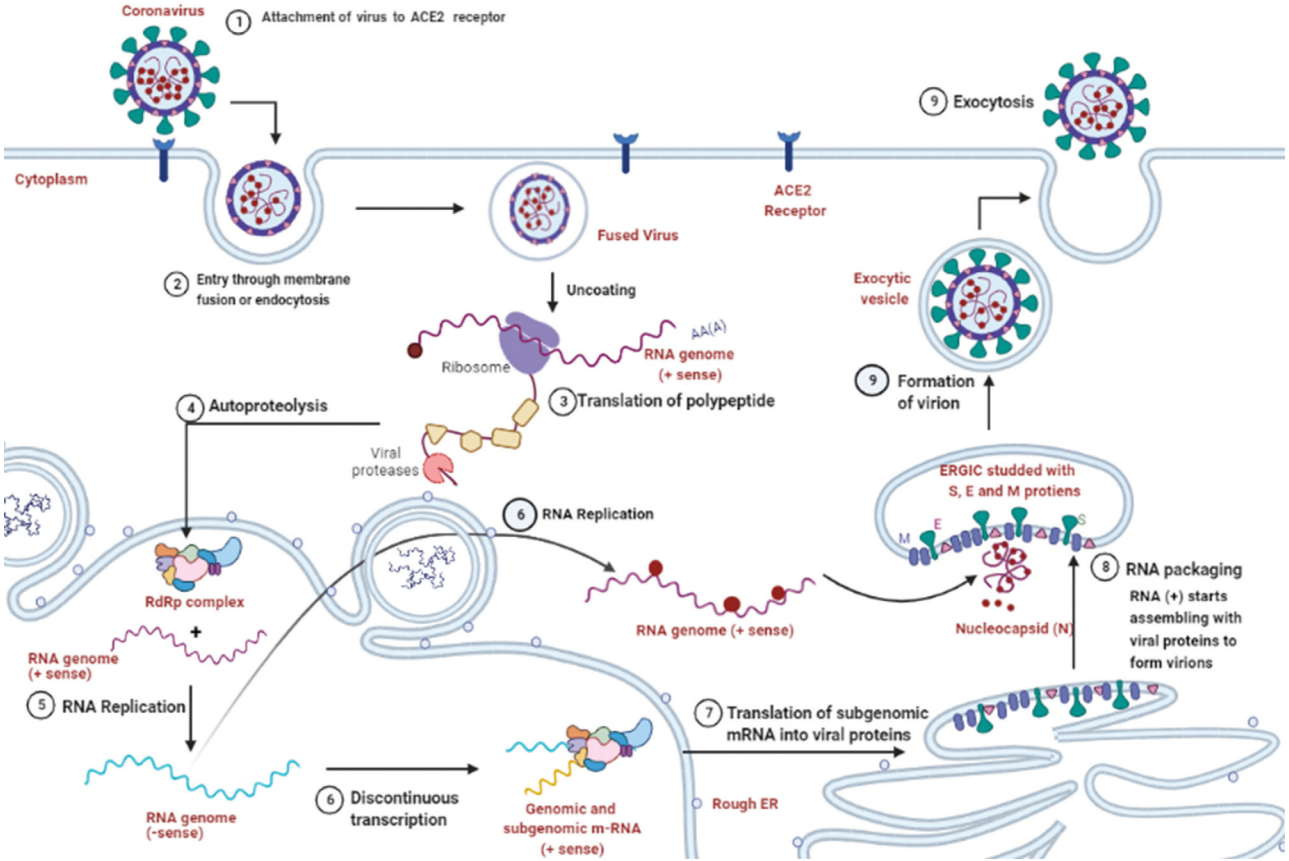

FIGURE 12.3 The schematic diagram of the mechanism of COVID-19 entry led to viral replication and viral RNA packing in the human cell.

target is performed for the positive authenticity of COVID-19. The CDC Real-Time RT-PCR Diagnostic Panel was the first to receive approval and used the N1 and N2 genome segment. The CDC generated the primers and probes and made the materials available for other laboratories to use with the same test. The US CDC real-time RT-PCR diagnostic panel under EUA targets two different loci of the N gene126 and considers positive results only when both gene targets (N1 and N2) are positive with LOD of 1 copy of RNA per microliter for both N1 and N2 primer sets [81]. However, sensitivity was affected by the use of different RNA extraction and purification protocols. If any of the two assays are negative, the result is inconclusive, and the assay has to be repeated following strict guidelines. Positive confirmation with a single gene target is possible if the amplicons are subjected to deep sequence analysis. In addition to these controls, the US CDC recommends the use of a human specimen control (HSC) [81] to ensure successful lysis and integrity of extraction reagents and to minimize false negative results by ensuring collection of enough human cellular material [2].

A PCR assay with absolute exclusiveness to SARS-CoV-2 was made possible in the Charité protocol with the inclusion of additional probe (RdRp_P2) that anneals to only SARS-CoV-2 mRNA transcript. Nonetheless, this protocol does not discriminate between clades of the Sarbeco viruses like SARS-related CoVs from bats. This overlap is corroborated with the sim plot of SARS-CoV-2 showing more than 96% identity to a bat coronavirus [7]. The Charité protocol uses the E gene as the screening assay followed by confirmatory assay with the RdRp gene [82]. Further improvement in the sensitivity was found [65] while conducting a proficiency test for the sensitivity with E Sarbeco and RdRp genes using in vitro transcribed

RNA derived from SARS-CoV strain Frankfurt-1, where they found LOD of 5.2 and 3.8 copies per reaction, respectively, which was in good agreement with other participating laboratories. They also found that N gene assay was slightly less sensitive than the RdRp and the E Sarbecogene. However, a comparative study showed that the N2 primer/probe set developed by US CDC was highly sensitive comparable with the E Sarbeco set described in Charité assay at low viral copy number [83].

The protocol from Pasteur Institute [82] utilizes IP2 and IP4 gene targets as the first-line screening tool, while confirmatory testing utilizes the E gene target. Primer sets (IP2 and IP4), when used individually in an assay, can detect about 100 copies of RNA genome equivalent per reaction at 95% detection probability. A lower LOD of 10 copies was obtained with a multiplex assay using these primer sets [82]. The PCR assays developed by Pasteur Institute have been claimed that there is no cross-react with respiratory viruses like influenza A (H1N1, H3N2), enterovirus, adenovirus, human coronaviruses (HKU1, OC43, 22E, NL63), and MERS-CoV, indicating 100% specificity.

Assays from HKU (Hong Kong University) are specific to only subgenus Sarbecoviruses [84] where N gene was used as the first-line screening while the ORF1b as the confirmatory testing [85]. This assay did not distinguish between SARS-CoV and SARS-CoV-2 transcripts because SARS-CoV-2 shares a 79.6% sequence identity with SARS-CoV BJ01 [7], and many of the regions homologous with the primers are conserved nucleotide regions [13]. Primers based on the receptor binding domain of the S gene developed by Zhou et al. could discriminate SARS-CoV-2 from bat SARSr-CoV WIV1 [7].

Some studies also shown that primer/probe sets E Sarbeco from the Charité protocol and ORF1b-nsp14 from the Hong Kong University (HKU) protocol were the most sensitive with an LOD of 10 virus genome equivalents per microliter at 75% detection frequency [62].

The Chu et al. protocol recommends the N gene for screening, while ORF1b provides a confirmatory test [86]. They used the cloned DNA plasmid containing SARS-CoV ORF1b and N gene for one step real time RT-PCR approach to calculate the LOD of their assay. With a known titer of viral RNA, their preliminary study showed the LOD of <10 copies per reaction [84]. A dynamic range of$0.0002-20$ TCID50 (50% tissue culture infective dose) per reaction and detection limit below 10 RNA copies per reaction was obtained using RNA extracted from cells infected by SARS coronavirus as a positive control using one-step RT-PCR assays to detect two different regions (ORF1b and N) of the viral genome [84].

Scientists from Germany used the E gene for the first-line screening and the RdRP gene for confirmatory testing [85]. This method further increased sensitivity to detect as few as 5.6 RNA copies per reaction for the E gene and 3.8 RNA copies per reaction for the RdRP gene. In France, two RdRP genes were used for initial screening followed by the confirmatory E gene testing [85]. While in Japan, nested RT-PCR was used [85], which significantly reduced nonspecific target amplification, leading to decreased false positive results (i.e., increased specificity). In general, the sensitivity of these assays ranges from 3.8 to 10 RNA copies per reaction, with high specificities. In a public health emergency, highly sensitive methods are desirable. Indeed, the Chinese authority adopted Wang et al [87] approach for combining RT-PCR with other methods to diagnose SARS-CoV-2, in Wuhan by combining RT-PCR with CT scans [88].

Later, WHO developed a technical guidance including the protocols from different countries to aid COVID-19 diagnosis [85]. According to this compilation, in the US, CDC developed a real-time RT-PCR diagnostic kit with detection limits as low as $4-10$ RNA copies per μL.

In addition, rapid test design for the detection of viral protein is also in progress. The viral nucleocapsid (N) and spike (S) are the main immunogenic proteins. While nucleocapsid protein is the most abundant and 90% similar to SARS-CoV, viral spike (S) protein is divergent and elicits strong

immune response [89]. However, the S1 subunit of spike protein was found to be specific for SARS-CoV-2, but the S2 subunit of spike protein was conserved across coronaviruses [90]. Since many people have antibodies to the four endemic human coronaviruses, targeting specific part of the spike protein could avoid cross-reactivity.

12.4.2 Limitations

While having the advantage of assisting large mass of samples at a time, its turnaround time is measured in days. The standard RT-PCR test requires, high-cost facilities and machines and sample preparation and assay procedures need well-trained manpower. These shortcomings limit the wider use of the technology during viral pandemics. Since RT-PCR assays amplify specific target loci, but due to behavioral frequent change of SARS CoV, changes in its genome due to insertion or deletion, recombination, and interchange are swift [91,92] so even a single-nucleotide polymorphism due to mutation at the primer or probe binding site could vitiate the true RT-PCR result. The sensitivity may not be enough to detect early infections due to low concentrations of the virus, especially in asymptomatic cases and may result in false negative results. For areas afflicted with COVID-19, a negative PCR result does not imply the absence of the virus since a multitude of factors including viral mutation, PCR inhibition, improper handling of the sample, specimen collection time, low viral RNA, inappropriate shipment, or poor specimen quality can also lead to a negative result in an infected individual [93]. Although studies have shown that RT-PCR may be less sensitive than CT imaging at certain stages of COVID-19, its specificity makes it superior to other methods to detect SARSCoV-2.

12.4.3 Chest imaging

Imaging techniques such as chest X-rays, pulmonary computed tomography (CT) scans, and lung ultrasounds are important tools in the early diagnosis of pneumonia in patients with COVID-19. Apart from detecting the presence of the virus, chest CT scan can demonstrate the disease status and severity. During the early stage of pneumonia, there are multiple small patchy shadows seen with interstitial changes, which is unusual in the lung periphery [94,95]. Some cases could develop bilateral multiple ground-glass opacity, infiltrating shadows, and pulmonary consolidation with infrequent pleural effusion [96]. Chest CT imaging studies showed that chest CT images contained characteristic features for COVID-19 patients. The hallmarks of these CT images include ground glass opacities, crazy-paving pattern, consolidative opacities, septal thickening, and the reverse-halo sign [74,97–99]. These features demonstrate a highly organized

pattern of pneumonia. Reports indicate that COVID-19 patients submitted to chest CT scans on admission presented abnormal results (about 90%), showing bilateral multiple ground-glass and patchy opacity [100]. A study with 101 patients in China showed that COVID-19 pneumonia displayed typical imaging features, such as ground glass opacities (GGO) (86.1%) or mixed GGO and consolidation (64.4%), vascular enlargement in the lesion (71.3%), and traction bronchiectasis (52.5%). A recent study suggested that CT scan is more sensitive than the PCR procedure [101]. Importantly, it has a sensitivity that is greater than that of RT-qPCR (97.2 vs. 83.3%) for the diagnosis of COVID-19 patients [101].

However, studies on the sensitivity of CT imaging over RT-PCR, showed that CT imaging is more sensitive and reliable in detecting SARS-CoV-2 infections during certain stages of the COVID-19. Fang et al. studied 51 patients with COVID-19 symptoms based on their clinical manifestations and epidemiological histories [101]. They found that the chest CT scan was more sensitive (98%) than the RT-PCR method (71%). This study was limited by the number of subjects involved. However, another study involving more than 1000 patients reached similar conclusions [88]. Among 1014 patients, 59% were RT-PCR positive, from which 97% showed positive CT features. In some cases, chest CT imaging showed positive SARS-CoV-2 infection, while RT-PCR testing was negative [102]. In addition, 75% of RT-PCR negative patients showed positive CT features. To further validate this, Ai et al. studied multiple RT-PCR testing and serial CT imaging in a selected group. They found that 60−93% people who were RT-PCR negative showed initial positive CT images consistent with SARS-CoV-2 infections. From the patients in the recovery stage, 42% showed improvement in CT features before their RT-PCR results turned negative. According to these diagnostic studies, RT-PCR assays were not as sensitive and reliable as CT images in certain stages of the COVID-19.

Although various types of pneumonias have certain imaging features, COVID-19 and other viral pneumonias, bacterial pneumonia, and some lesions share some common imaging features [103]. In some cases, it is difficult to differentiate COVID-19 from them by imaging alone, and first clinical manifestations, contact history, and laboratory tests should also be considered to make the final diagnosis. In the initial screening, computed tomography (CT) examination is needed for the auxiliary diagnosis [104]. The diagnosis is then confirmed by the positive results of the nucleic acid amplification test (NAAT) of the respiratory tract or blood specimens using reverse transcription real-time fluorescence polymerase chain reaction (RT-PCR) [65]. Chinese researchers strongly recommend CT imaging as the main basis for the diagnosis of COVID-19 in the current situation [46]. An academician of the American Society for Radiation Oncology called for the immediate establishment of a CT-based diagnostic method for COVID-19 and improvement of the detection rate of the severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2) [105]. If a patient with clinically suspected COVID-19 has negative NAAT results but positive imaging results, the patient should be isolated and treated as soon as possible. The advantage of CT examination in the diagnosis of COVID-19 is obvious. Below, we analyze some typical patients who were examined and diagnosed by our institution.

12.4.4 Limitations

Although chest X-rays are less expensive and more convenient for follow up in pneumonia cases, the technique has low-resolution and projection overlapping, which could lead to many false-negative COVID-19 cases [106]. However, CT scans has drawbacks as the mechanism cannot differentiate pneumonia and other pulmonary anomalies from COVID-19 [107]. Despite its high sensitivity in diagnosing COVID-19, chest CT findings in COVID 19 share similarities with other viral pneumonia findings, resulting in false positives [108]. This is because features of the chest imaging from COVID-19 patients may overlap with other infections caused by influenza, H1N1, or SARS-CoV [109,110]. The caveat for the CT scans is that, at an early stage of infection, the lungs of a patient may not develop damaging features that can be picked up by CT scans, increasing its false negative rate.

Despite giving reliable results, the Centres for Disease Control (CDC) in the US does not currently recommend CT to diagnose COVID-19. Laboratory testing of the virus remains the reference standard, even if the CT findings are suggestive of SARS-CoV-2 infections [111].

12.4.5 Immunological test

Immunoassay is another widely used bioanalytical methods to diagnose and/or quantify an analyte using an antigen-antibody interaction. Sensitivity of the immunoassays for the presence of antibodies in human samples may depend on the viral titer and time of sample collected after viral infection; both factors impact circulating antibody concentration [72]. These methods detect viral protein antigens or serum antibodies in patients who have been exposed to the SARS-CoV-2. Several immunoassay kits are already on the market for emergency detection of COVID-19 specific antibodies. These antibody tests are important in detecting prior infections. In the SARS-CoV-2 infection, studies have shown that the seroconversion in the patient generally starts after a week of the first symptom [112]. In a study of postsymptomatic patients, Amanat et al. detected high IgA and IgM immune responses [113]. Most immune assays used for testing individuals for COVID-19 exposure detect specific antibodies (IgG and IgM) in the serum that react with SARS-CoV-2 proteins. Using recombinant

viral proteins, this immunoassay could detect antibodies as early as 3 days after the development of the first symptom. IgM is expressed earlier during an infection (~ 3–6 days) and IgG is the late antibody detectable only after ~ 8 days. IgG is generally more specific for a protein antigen than IgM and affinity generally increase with continued exposure to the (viral) antigen [114]. Liu et al. reported that the accuracy of the ELISA for IgG and IgM antibodies was more than 80% [115]. The efficacy of the immunoassay also depends on the specificity of the antigens used to capture the antibodies from the patients. Between the spike (S) proteins and nucleocapsid (N) proteins, the sensitivity of the S proteins is higher for the antibody capture. Among various spike proteins, the S1 protein has shown more capability to bind to SARS-CoV-2 antibodies [116].

Enzyme-linked immunosorbent assays (ELISAs) and lateral flow immunoassays (LFIAs) are the most widely practiced techniques to detect the antibodies against SARS-CoV-2 [117]. In a comparative study, both ELISA and colloidal gold immunochromatographic kits showed equal sensitivity with 100% specificity for the SARS-CoV-2 detection [86]. A two-stage ELISA protocol was described for measuring human antibody responses to the recombinant receptor-binding domain (RBD) of the spike protein or full-length spike protein of SARS-CoV-2 by Krammer's group from the Icahn School of Medicine in New York [118]. The first stage included a high through put screening of samples in a single-serum dilution against the RBD, followed by a second stage in which positive samples from the first stage underwent a confirmatory ELISA against the full-length spike protein.

Recently, a peptide-based magnetic chemiluminescence enzyme immunoassay was also developed for the diagnosis of COVID-19 [119]. The test has high throughput and can perform simultaneous clinical tests for other biomarkers, such as C-reactive protein (CRP), which should also be tracked in COVID-19 suspects [120].

ELISA and chemiluminescence assays based on the antibodies have shown a sensitivity of 70–95% and 82–97% respectively [121–123]. A peptide luminescence method was developed with recombinant S protein to detect IgM and IgG antibody. The total positive rate of detection was 81% with < 6% coefficient of variation. The immunoassay-based rapid diagnostic test (RDT) kits, depending on the vendor type, are reported to have sensitivity in the range of 60–80% and selectivity of 85–100% at a confidence interval of 95% [124]. The RDTs that are LFIAs have shown 86–88% sensitivity and 90–99% specificity to detect total antibodies [121,122,125,126]. A rapid (10 min) and sensitive LFIA that uses lanthanide-doped polystyrene nanoparticles to detect anti-SARS-CoV-2 IgG has also been reported [127]. A study by Bendavid et al. demonstrated 82% sensitivity and 99.5% specificity for detecting antibodies to SARS-CoV-2 tested in 3330 adults and children in Santa Clara County, CA, using LFIAs [57].

A test that detects both IgM and IgG produced a sensitivity, specificity, and accuracy of 57%, 100%, and 69% for IgM and 81%, 100%, and 86% for IgG, respectively. Combining the results from both IgM and IgG yielded a clinical sensitivity of 82% [86].

The Single Molecule Array (SIMOA) multiplexed method developed by Quanterix targets three immunoglobulin responses (IgG, IgM, and IgA) to four viral proteins (spike protein, S1 subunit, receptor binding domain, and nucleocapsid) in a single sample, thereby enabling the quantification of 12 antibody isotype-viral protein interactions. It gives a high-resolution profile of immune response of SARS-CoV-19 when compared with a traditional ELISA where only a single interaction can be interrogated. The SIMOA method demonstrated a sensitivity of 86% and a specificity of 100% during the first week of infection, and 100% sensitivity and specificity thereafter [128]. RDTs employing serological immunoassays are less complex, cost less than the molecular tests, and can give results in a short time period. Immunoassays are good tools to track and study past infections, especially in asymptomatic cases. Serological assays can be used to determine the infection rate and to estimate the population extent and prevalence of infection. Results from a serological survey can also be used to project mortality rates in a community. Furthermore, they are useful to characterize the immune response to the virus.

12.4.6 Limitations

Nevertheless, immunoassays are faster [129] and cheaper than the RT-PCR methods. They can be used for rapid screening of previous SARS-CoV-2 infections. This is particularly useful in the reopening stages of society at which people recovered from previous COVID-19 infections, and therefore immune to the virus, can safely re-engage with society. This method also has a unique advantage of identifying individuals who have strong immune responses against the virus and, therefore, can serve as potential donors for therapeutic and research purposes. However, immunoassays are not as specific, as the antibody tests may cross-react with other pathogens similar to human coronavirus and give false positive results [75,130].

In the past, molecules like interferon, rheumatoid factor, and nonspecific IgM have been shown to cause problems in immunoassays [123], and levels of such potential interferents can be highly variable in COVID-19 patients. There are a few reports where affinity of antibodies used in a biosensor [131], is not sufficient for direct detection of small numbers of virus particles. Results may also be biased due to prior COVID-like illnesses that confound the specificity of the antibody response. One of the difficulties in validating an assay for antiviral antibodies is the availability of appropriate negative and positive controls. Other drawbacks of immunoassay include

changes in viral load over the course of infection [132], potential cross reactivity (less specific) [133], and low sensitivity with respect to nucleic acid-based methods. To improve the sensitivity, it is paramount to include other biomarkers of the early stage ofSARS-CoV-2 infection [120]. In addition, the complexity of interpreting results from diagnostics for antiviral antibodies, the antibody response in a patient depends on age, nutritional status, and existing medical conditions and medications [75,116]. Most of the patients develop antibodies only after the second week of infection, that is, in the recovery phase of COVID [134,135]. It loses the main course of period for probability to intervene the disease. WHO has not recommended the use of antibody-based POC systems in clinical decision-making [136].

12.4.7 Serological testing

Serological tests are fundamental to determine the acquired immunity of patients who had the disease and to establish the level of immunity in the general population. This assay overcomes two important limitations of SARS-CoV-2 PCR-based techniques. One that it could be done under biosafety level 2 and second is that no weight given to sampling issues. Serologic testing helps to determine the abundance of an outbreak or extent of infection in society under study. It provides more accurate data of infected population with SARS-CoV-2 and can also assess unknown cases which have not been identified through routine or active surveillance. To analyze the explosion of COVID-19 society-based serosurveys has been done to determine the specificity and sensitivity of SARS-CoV-2 ubiquity and transmission dynamics [137–139]. Moreover, serology can also be useful in situations where RT-qPCR is negative and there is a strong epidemiological link to COVID-19 infection [140]. In these cases, paired serum samples collected in the acute and convalescent phase can be of diagnostic value. Several studies have reported hematologic and blood chemistry alterations in patients infected by SARS-CoV-2 [141,142] which allows us to understand the pattern of infections and help us to opt better defensive measures. It also helps in recognizing asymptomatic individuals who are at higher risk of infections.

Major laboratory findings in COVID-19 patients identified by meta-analysis include leukopenia, leukocytosis, decreased albumin levels, increased levels of C-reactive protein, lactate dehydrogenase (LDH), creatinine kinase, and bilirubin, and a high erythrocyte sedimentation rate (ESR) [143]. Development of an impeccable antibody testing is a major challenge, and as of now, hundreds of trials are going on. There are important considerations like immune status, previous infections of infected person, and cross reaction and time of testing which could differ the test result for antibody testing.

Most serological assays are based on the SARS-CoV-2 nucleocapsid protein (N), transmembrane spike protein (S), or S receptor-binding domain (RBD) because of their high antigenicity [144–146]. As mentioned before, SARS-CoV-2 enters host cells using its spike protein and represents the main target of neutralizing antibodies produced by the host [147]. Several studies have demonstrated that serological tests using the S antigen are more sensitive than those using N-antigen-based antibody assays [148]. Instead of a single antigen, some companies have combined N- and S-based antigens to develop serological assays for SARS-CoV-2 detection [148]. Cross-reactivity between SARS-CoV-2 and SARS-CoV-1 has been reported against the N protein but not against the S1 subunit of the S protein [140]. Cross-reactive antibodies between SARS-CoV-2 and SARSCoV-1 have been detected in both the RBD region located in the S1 subunit and also in non-RBD regions [149].

In the past few months, a variety of serological tests have been designed to detect SARS-CoV-2, mainly neutralization tests, enzyme-linked immunosorbent assays (ELISA), immunofluorescence assays (IFA), chemiluminescence enzyme immunoassays (CLIA), and lateral flow assays (LFA). [148] and are used for the first-line screening of patient samples with COVID-19.

1. Neutralization tests specifies about vital and effective antibodies in the patient by measuring the proportion of viral growth inhibition by antibodies in the lab. This can be used with SARS-CoV-2 virus in a BSL-3 setting, or pseudo viruses that express certain SARS-CoV-2 proteins in a lower BSL setting.
2. Chemiluminescent immunoassay (CLIA) interprets the presence of antibody by exhibiting fluorescent signals during the interaction of viral protein with antibodies in infected patient.
3. Enzyme-linked immunosorbent assays (ELISAs): It is a most common test performed in labs to determine the antigen antibody interaction. Sandwich Elisa is carried out in which antibodies elicited from patients are “sandwiched” between the viral protein and detector antibody to identify active antibody against virus.
4. Lateral flow assays (LFAs) Mostly used in point of care settings providing the qualitative presence of antibodies via color reflection. In this sample from patient passed through a membrane coated with target antigen. Colored band appears if sample is having complementary antibodies interpreting the formation of complex between target antigens with specific antibody. These are similar to pregnancy tests.

However, a few antibody-based tests got emergency approval by the FDA, for instance, COVID-19 ELISA IgG Antibody Test from Cellex, which has a sensitivity of 93%, and qSARSCoV-2 IgG/IgM Rapid Test from Mount Sinai Laboratory with 92.5% sensitivity [13,150]. Another method includes

lateral flow chromatographic immunoassay that qualitatively assesses the presence of an analyte (e.g., antibody) from a patient's whole blood, serum, or plasma specimen. Recently, Abbott launched an IgG antibody test received CE mark with its 99.6% specificity [94]. TheElecsyAntiSARS-CoV-2 antibody test from Roche also received FDA approval that employs in-solution double-antigen sandwich format. This test can detect antibodies in human serum or plasma samples with specificity greater than 99.8% and sensitivity of 100% after 14 days of PCR confirmation [151].

The adaptive immune response to SARS-CoV-2 infection has also been studied [60,140,152,153] by Long et al. in 285 patients with COVID-19 in China. Seroconversion for IgM and IgG occurred simultaneously or sequentially, and the median day of seroconversion for both immunoglobulins was 13 days after COVID-19 onset. The seroconversion rate for IgG reached 100% 19 days after symptom onset [140].

In a related work, Zhao et al. showed that the median seroconversion times for total antibodies, IgM, and IgG were 11, 12, and 14 days after SARS-CoV-2 infection [154]. In addition, several studies have been used to evaluate the clinical sensitivity of serological tests with COVID-19 patient samples collected on different days after the onset of symptoms [155–158]. Pan et al. evaluated a commercial lateral flow immunochromatographic test targeting viral IgM or the IgG antibody and compared it with RT-qPCR. The sensitivity of the assay for SARS-CoV-2 detection was 11.1% in early-stage patients (1−7 days after onset), 92.9% in intermediate-stage patients (8−14 days after onset), and 96.8% in late-stage patients (more than 15 days after onset) [157]. In similar study, Tang et al. compared the diagnostic performance of two SARS-CoV-2 commercial serologic tests using 103 specimens from PCR-confirmed SARS-CoV-2 patients and 153 control specimens from different days after disease onset. The IgG antibodies bind to the recombinant SARS-CoV-2 antigen-coated gold nanoparticles (AuNP); the rabbit IgG gold-conjugates are used as control that binds to the anti-rabbit antibodies. The assay resembles a lateral flow pregnancy test but detects antibodies instead of a human glycoprotein.

The serological assays are relatively quick and easy to use which requires no highly trained staff and sophisticated equipment. Therefore, they are being increasingly used for diagnosis, contact tracing, herd immunity assessment, and vaccine efficacy evaluation. Serology testing emerges into a useful tool which may have several more applications in the future. It could be useful for complementing RNA tests, for the rapid identification of cases and for recommending quarantine to remarked raceme. It could be a reliable way to identifying the route of transmission to monitor the spread of the pandemic and facilitate epidemiological studies. Serology assay may also be useful to check immune status after vaccination. Seroepidemiologic studies can assist in the investigation of the ongoing pandemic and the retrospective assessment to determine the attack rate or the progress of

the pandemic through antibody detection. These studies can assist health authorities and governments in making sound decisions with respect to the implementation of public health measures during the course of the current pandemic

12.4.8 Emerging test

As discussed in Ideal Characteristics of Diagnostic Methods, diagnostic tests developed so far rarely meet all the ASSURED criteria. The most important features for the SARS-CoV-2 detection are sensitivity, specificity, and efficiency (throughput and cost-effective ness). In addition to the modification of conventional methods approaches like RT-PCR, isothermal amplification for nucleic acid targets, lateral flow based detection of nucleic acid targets sections, and immunoassay other emerging methods named CRISPR technology, differential diagnostics, AI assays have further been developed to fasten and specified diagnostic features. Besides all these techniques single molecule detection capability is used to improve the sensitivity [159–161].

12.4.8.1 Isothermal amplification for nucleic acid targets

Although RT-PCR is a widely used method in the confirmatory screening of COVID-19 infections (see Nucleic Acid Based Methods section), it is time-consuming and requires a sophisticated laboratory facility and trained personnel to operate [68]. Apart from conventional RT-PCR, additional molecular diagnostic tools are emerging for SARS-CoV-2.

Whole-genome sequencing of SARS-CoV-2 has the potential to overcome the limitations of RT-PCR. Genomic sequencing can detect fragments even when a complete genome is not present in the sample. Notably, the SARS-CoV-2 genome is free of repeats, making it susceptible to complete characterization using short sequence reads. The genome sequencing involves the construction of RNA library, next-generation sequencing (NGS) of the RNA construct, de novo assembly of the quality trimmed reads to generate a contig map and phylogenetic analysis [162]. The genome sequencing may overcome the false results arising from specious priming. However, due to cost, technical complexities associated with the instrument, data analysis, and higher turnaround time of 24–48 h, this method may not be a routine use for clinical diagnosis during pandemic [163].

Droplet-based digital PCR (dPCR) methods have also been sought as a more sensitive method to test for the RNA of SARS-CoV-2. This method, when tested in 77 patient samples, showed an improved sensitivity from 44 to 94% and the same specificity when compared with the RT-PCR method [164]. As the LOD reported for RT-PCR has been 2.1 copies/reaction for ORF1ab and 1.8 copies/reaction for N primer/probe set and in d PCR

method, it was found as 2 copies/reaction [165]. When tested in 103 fever-suspected patients, the d PCR method improved the sensitivity from 28.2% with RT-PCR to 87.4%.

Dual-functional plasmonic biosensor combined the plasmonic photothermal effect and localized surface plasmon resonance sensing transduction was reported as an alternative for the clinical diagnosis of COVID-19 [136]. Two-dimensional gold nanoislands functionalized with complementary DNA receptors were used to detect SARS-CoV-2 through nucleic acid hybridization. The biosensor was able to detect 0.22 pM and allowed precise detection of the specific target in a multigene mixture. Gold nanoparticles (AuNPs) functionalized with antisense oligonucleotides were developed for the simultaneous detection of two regions of the SARS-CoV-2 N gene [166]. In the presence of RNA target, AuNPs agglomerate, and the agglomeration is further enhanced by the addition of RNAse H enzyme to generate a distinguishable visible precipitate.

To simplify the testing procedures, isothermal nucleic acid amplifications have been developed. These methods do not require any thermal cycler to perform the amplification and, therefore, can be carried out in a simple water bath at a constant temperature of 40−65°C [167].

Reverse transcription loop mediated isothermal amplification (RT-LAMP) is an amplification approach in which RNA genome of SARS-CoV-2 is first reverse transcribed to cDNA and is then amplified using four to six target-specific primers. Prior to the LAMP amplification, a dumbbell-shaped single-stranded DNA (ssDNA) is formed through the annealing and the strand-displacing cycle on both ends of the target sequence with the help of the primers and a strand-displacing polymerase. The looped ssDNA on each end then serves as a seed for the LAMP amplification cycle [168–171]. As a result, the target sequence is amplified exponentially, which is detected by turbidometry [172] or fluorescence/colorimetry [171]. As an example, RNA extraction and LAMP amplification have been performed in the same tube [168,173]. This method has LOD ranging 80−500 SARS-CoV-2 RNA copies per milliliter, which is comparable to the RT-PCR assay.

Rolling circle amplification (RCA) [174] is another isothermal amplification method that gives sensitive detection of nucleic acids. In this method, a segment of the target genome is circularized and amplified by a highly processive strand displacing DNA polymerase. Wang et al. used this method to develop a highly sensitive and efficient assay for SARS-CoV [175]. Compared to the LAMP assay, the RCA method is simpler since it requires fewer steps and can be performed at room temperature. The method offers high sensitivity comparable to RT-PCR [176] since it amplifies the target sequence by ~10,000. In addition, it presents high specificity, as the RCA is initiated only after the formation of a circular template upon which a specific primer is hybridized [176]. Therefore, RCA reduces false-positive results often encountered in PCR-based assays.

A major difficulty in this method is that it requires a circular template whose preparation is dependent on the length of a linear template and the ligation efficiency of the DNA circularization. Inappropriate design of complementary sequences therefore results in failure of amplifications.

Penn-RAMP was developed by, El Tholoth et al.to improve the LOD. It is a two-stage closed tube test combines LAMP with recombinase polymerase amplification [177]. In the Penn-RAMP, each amplification was performed at a separate compartment in a single tube followed by mixing. The method demonstrated 10 times higher sensitivity than LAMP or RT-PCR alone. A new platform with engineered complementary recombinant RNA (crRNA) has been reported with LOD as low as ~700 fM cDNA from HIV, 290 fM RNA from HCV, and 370 fM cDNA from SARS-CoV-2 in 30 min without a need for target amplification. In this case, the isothermal amplification of SARS-CoV-2 RNA was performed using RT-LAMP, and the modified crRNAs were incorporated in a paper-based lateral flow assay that could detect the target within 40–60 min [178].

Some commercial COVID-19 diagnostic kits based on isothermal RT-LAMP assays are already on the market (see Table 12.1). Abbott ID Now is such an example. This method only requires 5 min to give positive results. Recently, however, issues on false negativity have been raised for the Abbott ID Now because of its relatively high LOD [179]. This may be attributed to the compromised performance of the RdRP target [180,181] used in this assay, which is found to be mutating and evolving [182].

12.4.8.2 Lateral flow-based detection of nucleic acids and proteins (LFA)

The nucleic acid based isothermal amplifications discussed above partially overcome the limitations of conventional RT-PCR assays, as they do not require sophisticated laboratory facilities while their turnaround time is short. However, these methods still require trained staff to operate various sample collection and processing steps. In LFAs, both nucleic acid detection methods and immunoassays can be utilized. The device is often made of papers with immobilized capture probes. Upon binding with nucleic acid targets, the probes give a visible signal [183–185].

Paper-based lateral flow assays have gained interest because of their low cost, easy manufacture, and full compatibility with POCT, which allow them to be conveniently performed by anyone at home. Byers et al. developed a 2D paper network to perform immunoassay for the detection of nucleic acids of SARS-CoV-2 with the POCT format [186]. Such methods still require initial nucleic acid extraction and amplification steps, the latter of which can be accomplished by the PCR or isothermal amplifications as discussed above. On the POCT platform, all those steps are integrated in a single device.

TABLE 12.2 Screening and confirmatory genes with their limit of detection of various research organizations during COVID-19 pandemic.

S. No.	Agency	Screening gene	Confirmatory gene	LOD
1.	CDC	N1-N2		1 micro lt
2.	Charite protocol	E gene	RdRp-P2	5.2 and 3.8 /rexn
3.	Pasteur Institute	IP2-IP4	E gene	10/rexn
4.	Chu et al.	N gene	ORF1b	<10
5.	HKU (Hon Kong University)			10/microlitre
6.	Germany	E gene	RdRp	5.6 & 3.8
7.	France	RdRp gene	E gene	

A novel method based on the Sanger sequencing was developed by the Chandler-Brown group that targets the SARS-CoV-2 N protein [187]. This method uses COVID-19 spike-in DNA as an internal control that provides quantification of the viral mRNA. The major advantages of this method are the omission of RNA extraction procedure so there will be no impedance in the testing capacity due to shortage of RNA extraction kits and potential for a very high performance of one million tests per day with proper customization of the sequencer.

12.4.8.3 Immunoassay

Similarly, Byers et al. developed a 2D paper network to perform immunoassay for the detection of nucleic acids of SARS-CoV-2 with the POCT format [186]. Although nucleic acid based lateral flow assays are sensitive, lateral flow immunoassays have gained interest in the massive surveillance of the COVID-19 pandemic because of their simplicity and cheap cost. An electrochemical ultrasensitive POC device named eCovSens detects the spike protein of SARS-CoV-2 within 10–30 s [188]. This device was fabricated by immobilizing antibody against S1 protein on screen-printed carbon electrodes. With spiked saliva samples, the LOD of the device was found to be 90 fM.

Currently, IgM/IgG rapid test kits are available for qualitative antibody testing of COVID-19. Many such commercial devices have already been developed (see Table 12.2). One problem associated with the immunoassay based lateral flow assay is the weak signal, which results in reduced sensitivity [183]. Various signal enhancement strategies therefore have been proposed. A promising signal amplification strategy in lateral flow assays is the use of colloidal gold nanoparticles conjugated with the probes. Upon binding with the target, the gold nanoparticles linked to the capture probe

aggregate to change the color, enhancing the signal [189]. Other signal amplification strategies include solvent evaporation for analyte preconcentrations nanoparticle, catalyzed nanoparticle labeled assays, and ion concentration polarization methods [190]. Due to the low-cost requirement of the POCT, detection in the LFA is usually achieved by visual inspection. To improve detection sensitivity, cameras in smartphones have been used [191]. These cameras are sensitive to subtle color changes and hence provide more effective color detection than traditional RGB sensors or the naked eye [192]. For improved readout of the results and data processing, a machine learning algorithm could also be used [193]. Smartphones can also be coupled with external adapters to integrate external biosensor platforms for more versatile POC testing [194].

The single molecule enzyme-linked immunosorbent assay (ELISA) has been developed to offer a detection limit of subfemto molar protein concentrations [195]. In this method, each microscopic bead decorated with specific antibodies is loaded into individual femtoliter wells. Sensing was accomplished by the ELISA on each bead, whereas the excellent concentration detection limit was achieved by a large array of such beads. To be applicable in clinical setting, however, this method requires special equipment, increasing its cost.

12.4.8.4 Proximity ligation assay (PLA)

The method utilizes two or more DNA tagged aptamers or antibodies for binding of multiple targets. [196] The DNA tags on the probes are amplified only when the two different targets are in close proximity. The multiple targets ensure the specificity of target detection. However, this method requires intact SARS-CoV-2 virus particles from which two different targets are present for positive detection. This demands stringent sample processing steps to increase the throughput.

12.4.8.5 Next generation sequencing [197] and DNA microarray [198]

In the case of COVID-19, evidence has suggested that the SARS-CoV-2 is rapidly evolving while infecting people. Therefore, it is critical to rapidly identify the genome of the causative agent [199]. The DNA microarray has been used in high-work rate identification of mutations in SARS-CoV-2 [200]. However, for these methods, the time limiting step becomes the sample collection, which must be performed one-at-a-time. In addition, these methods involve rather advanced equipment with high cost; therefore, they may not be appropriate for the economic and rapid screening in the COVID-19 pandemic.

12.4.8.6 CRISPR/Cas-based diagnostic methods

The clustered regularly interspaced short palindromic repeats (CRISPR)/Cas machinery has recently been adapted as a POC tool for the rapid detection of nucleic acids (DNA or RNA) [154,201]. Overall, this CRISPR machinery is programmed to cleave specific sequences in the DNA/RNA target where the results can be easily observed by combination with a lateral-flow strip. CRISPR gene editing tool has been employed to construct an accurate, faster, and simple-to-use SARS-CoV-2 detection test. A rapid (<40 min), easy-to-implement, and accurate CRISPR–Cas12-based lateral flow assay for the detection of SARS-CoV-2 from respiratory swab RNA extracts has been recently reported.

DNA endonuclease-targeted CRISPR trans reporter (DETECTR) developed by Mammoth Bioscience Company based on the CRISPR/Casto detect any RNA or DNA target, which has now been used to detect the SARS-CoV-2 RNA genome from respiratory swab RNA extracts [202]. It is based on CRISPR–Cas12 and it can distinguish SARS-CoV-2 with no cross-reactivity for related coronavirus strains using N gene gRNA within 40 min [202]. The result is visualized by using a FAM-biotin reporter molecule and lateral flow strips to capture labeled nucleic acids. The suitability of DETECTR technology for the detection of SARS-CoV-2 was evaluated using 78 patient specimens, including 36 patients with COVID-19 infection and 42 patients with other viral respiratory infections, and then compared with the CDC RT-qPCR as a reference method to confirm COVID-19 infection. Clinical sensitivity and specificity were 95% and 100%, respectively, for the detection of the coronavirus in 83 total respiratory swab samples [202]. The SARS-CoV-2 DETECTR test had 95% positive predictive agreement and 100% negative predictive agreement when compared with RT-qPCR results. The LOD of this method was 10 copies per microliter reaction.

Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK), was developed by Zhang's team on the basis of CRISPR/Cas-based platform that combined isothermal preamplification to detect strains of single-strand RNA viruses, identifies mutations and human genotype DNA, and distinguishes pathogenic bacteria [154,201]. More recently, using the same knowledge, they adapted a protocol using the SHERLOCK system for SARS-CoV-2 detection [203], which incorporates a thermostable Cas12b enzyme from alicyclobacillus acidiphilus and targets S and ORF1ab gene fragments of SARS-CoV-2 was described by Sherlock Biosciences. The test recently got FDA approval under emergency use authorizations [204]. The test can be conducted by extracting RNA from patient samples and can be read out in less than an hour using a dipstick, without requiring extensive instrumentation. This method was able to detect synthetic COVID-19 RNA sequence in a range between 10 and 100 copies per microliter and could be completed within 1 h [203].

FnCas9 Editor Linked Uniform Detection Assay (FELUDA) is a different approach taken to build a CRISPR-based tool in India. It is a highly accurate enzymatic readout for detecting nucleotide sequences [205]. The assay is quick to provide output and can be used in rapid diagnosis.

In other developments, Zhang's group [203] and Chiu's group [202] integrated the LAMP with the CRISPR-based SHERLOCK (see Table 12.1) [206] and CRISPR-Cas12 based methods, respectively, to detect the SARS-CoV-2 RNA with a detection limit as low as 10 copies/μL on a point-of-care testing (POCT) format. Taken together, these results highlight the great potential of CRISPR-based diagnostic methods as a rapid, specific, portable, and accurate detection platform for the detection of the SARS-CoV-2 genome in patient samples. Despite these promising results, CRISPR/Cas-based diagnostic methods are not widely used by diagnostic laboratories and need further implementation.

12.4.8.7 Artificial intelligence

Though relatively slow and expensive, computed tomography (CT) of the chest has also been explored in the diagnosis of COVID-19 as a complementary tool to molecular techniques when combined with medical history and clinical observations [46,187]. In a recent study, artificial intelligence (AI) algorithms were used to integrate chest CT findings with clinical symptoms, exposure history, and laboratory testing to rapidly diagnose patients who are positive for COVID-19 [207]. Moreover, the machine learning approach shows potential to predict criticality in patients [208]. The AI system had equal sensitivity as compared with a senior thoracic radiologist. In addition, it improved the detection of patients who were positive for COVID-19 via RT-PCR who presented with normal CT scans, correctly identifying 17 of 25 (68%) patients, whereas radiologists classified all of these patients as negative for COVID-19. When CT scans and associated clinical history are available, the proposed AI system can help to diagnose COVID-19 patients. The supplementary CT scan imaging may help to rule out negative RT-PCR results [88]. Some AI inspired mobile application-based tools are in development to preliminary detect suspected COVID-19 patients [13].

12.4.8.8 Differential diagnosis

Differential diagnosis is the process by which a single disease or condition is differentiated from those having similar clinical features. Patients with COVID-19 can have coinfection or superimposed infection by other viruses or bacteria simultaneously. Differential diagnosis is therefore important to differentiate SARS-CoV-2 induced infection from other viral or bacterial and mycoplasma pneumonia. Since the clinical manifestations of COVID-19 are similar to those of other respiratory diseases, differential

diagnosis is of paramount importance in assisting physicians in the therapeutic management of patients and health officials in establishing disease control policies.

In an effort to do so, respiratory pathogens in patients suspected of COVID-19 in Italy have been investigated [109]. Here, researchers tested the nasopharyngeal swabs of 126 suspected causes using SARS-CoV-2 RT-qPCR and a commercial multiplex respiratory cartridge that detects and differentiates viral and bacterial pathogens. Only 3 patients were confirmed to be infected with SARS-CoV-2, and 67 (53.2%) were positive for other respiratory pathogens, including 26 (20.6%) positive for influenza A and 10 (7.9%) positive for influenza B. Other pathogens detected in the patient samples were common cold CoV (H-CoV 229 E, H-CoV NL63, and H-CoV HKU1), rhinovirus, enterovirus, metapneumovirus, adenovirus, Mycoplasma pneumoniae, Streptococcus pneumoniae, and Legionella pneumophila [109]. These results highlight the diagnostic differential and demonstrate the importance of using a spectrum of molecular kits for the rapid detection of respiratory pathogens in order to improve the clinical management and treatment of patients infected with COVID-19. In another study, Yan et al. reported the cases of two patients in Singapore who initially had moderate symptoms including myalgia, a mild cough, and diarrhea and presented with thrombocytopenia and a normal chest radiograph [209]. Dengue was suspected, and a rapid serological test for dengue produced false-positive results. As patient symptoms worsened, they were later diagnosed with COVID-19 by RT-qPCR. Taken together, these findings suggest that special attention is needed in the differential diagnosis between arboviruses and SARS-CoV-2 infection, especially in countries where is there a circulation of DENV, ZIKV, and CHIKV. Moreover, coinfection with SARS-CoV-2 and other respiratory viruses, such as common cold CoV, influenza, and metapneumovirus, have been reported, highlighting the need for repeat testing based on clinical indications [210–213].

References

[1] R. Li, S. Pei, B. Chen, Y. Song, T. Zhang, W. Yang, et al., Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2), Science 368 (2020) 489.

[2] F. Wu, S. Zhao, B. Yu, Y.M. Chen, W. Wang, Z.G. Song, et al., A new coronavirus associated with human respiratory disease in China, Nature 265 (9) (2020) 579.

[3] F. Li, Structure, function, and evolution of coronavirus spike proteins, Annu Rev Virol (3) (2016) 237–261.

[4] N. Zhong, B. Zheng, Y. Li, L. Poon, Z. Xie, K. Chan, et al., Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February,, The Lancet 362 (9393) (2003) 1353–1358.

[5] N. Wang, X. Shi, L. Jiang, S. Zhang, D. Wang, P. Tong, et al., Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4, Cell Research 23 (8) (2013) 986.
[6] A.E. Gorbalenya, S.C. Baker, R.S. Baric, et al., The species Severe acute respiratory syndrome-related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2, Nat. Microbiol 5 (4) (2020) 536–544.
[7] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, et al., A pneumonia outbreak associated with a new corona virus of probable bat origin, Nature 270 (3) (2020) 579.
[8] Gen (Bank). (https://www.ncbi.nlm.nih.gov/genbank/)
[9] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, et al., Genomic characterization and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding, Lancet 395 (2020) 565–574.
[10] T.T.Y. Lam, M.H.H. Shum, H.C. Zhu, Y.G. Tong, X.B. Ni, S. LiaoY, et al., Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins, Nature 1 (6) (2020) 579.
[11] S. Sanche, Y.T. Lin, C. Xu, E. Romero-Severson, N. Hengartner, R. Ke, High contagiousness and rapid spread of severe Acute respiratory syndrome coronavirus 2, Emerging Infectious Diseases 26 (7) (2020).
[12] J.T. Wu, K. Leung, M. Bushman, et al., Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China, Nature Medicine 26 (4) (2020) 506–510.
[13] E. De Wit, N. Van Doremalen, D. Falzarano, V.J. Munster, SARS and MERS: Recent insights into emerging coronaviruses, Nature Reviews Microbiology 14 (8) (2016) 523–534.
[14] W. Guan, Z. Ni, Y. Hu, W. Liang, C. Ou, J. He, et al., Clinical characteristics of coronavirus disease 2019 in China, New England Journal of Medicine 382 (2020) 1708.
[15] WHO Report of the WHO-China Joint Mission on Coronavirus Disease (2019). (COVID-19) 2020. https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-oncovid-19-final-report.pdf (accessed April 10, 2020).
[16] I.M. Mackay, K.E. Arden, A. Nitsche, Real-time PCR in virology, Nucleic Acids Res. 30 (2002) 1292–1305.
[17] X. Wang, L. Tan, X. Wang, W. Liu, Y. Lu, L. Cheng, et al., Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously, International Journal of Infectious Diseases 107 (9) (2020) 94.
[18] Berenger, B.M., Fonseca, K., Schneider, A.R., Hu, J., & Zelyas, N. (2020).Sensitivity of nasopharyngeal, nasal and throat swab for the detection of SARS-CoV-2, preprint, Infectious Diseases [except HIV/AIDS].
[19] S.A. Irving, M.F. Vandermause, D.K. Shay, E.A. Belongia, Comparison of nasal and nasopharyngeal swabs for influenza detection in adults, Clin Med Res 10 (2012) 215–218.
[20] P. Winichakoon, R. Chaiwarith, C. Liwsrisakun, P. Salee, A. Goonna, A. Limsukon, et al., Negative nasopharyngeal and oropharyngeal swabs do not rule out COVID-19, Journal of Clinical Microbiology 58 (5) (2020) E00297–20.
[21] OASH. Nasal [anterior nasal] specimen collection for SARS-CoV2 diagnostic testing. https://www.cdc.gov/coronavirus/2019-ncov/downloads/OASH-nasal-specimen-collection-fact-sheet.pdf [accessed May 20, 2020].
[22] WHO Coronavirus Disease 2019 (COVID-19), Situation Report*83*; (2020 e).
[23] Q. Li, X. Guan, P. Wu, X. Wang, L. Zhou, Y. Tong, et al., Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia, New England Journal of Medicine (2020).

[24] J. Parry, China coronavirus: Cases surge as official admits human to human transmission, British Medical Journal Publishing Group, 2020.
[25] L.T. Phan, T.V. Nguyen, Q.C. Luong, T.V. Nguyen, H.T. Nguyen, H.Q. Le, et al., Importation and human-to-human transmission of a novel coronavirus in Vietnam, New England Journal of Medicine (2020).
[26] J. Riou, CL. Althaus, Pattern of early human-to-human transmission of Wuhan 2019 novel coronavirus (2019-nCoV), December 2019 to January 2020, Euro Surveillance 25 (4) (2020).
[27] N. Nagata, N. Iwata, H. Hasegawa, S. Fukushi, M. Yokoyama, A. Harashima, et al., Participation of both host and virus factors in induction of severe acute respiratory syndrome (SARS) in F344 rats infected with SARS coronavirus, Journal of Virology 81 (4) (2007) 1848–1857.
[28] T.P. Sheahan, A.C. Sims, S.R. Leist, A. Schäfer, J. Won, A.J. Brown, et al., Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV, Nature Communications 11 (1) (2020) 1–14.
[29] Z. Wang, X. Chen, Y. Lu, F. Chen, W. Zhang, Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment, BioScience Trends (2020).
[30] M.L. Holshue, C. DeBolt, S. Lindquist, K.H. Lofy, J. Wiesman, H. Bruce, et al., First case of 2019 novel coronavirus in the United States, New England Journal of Medicine (2020).
[31] C.S. Ng, D.M. Kasumba, T. Fujita, H. Luo, Spatio-temporal characterization of the antiviral activity of the XRN1-DCP1/2 aggregation against cytoplasmic RNA viruses to prevent cell death, Cell Death and Differentiation (2020) 1–20.
[32] D.S.C. Hui, A. Zumla, Severe acute respiratory syndrome: Historical, epidemiologic, and clinical features, Infect Dis Clin N Am 33 (2019) 869–889.
[33] WHO Laboratory testing for coronavirus disease 2019 [COVID19] in suspected human cases: Interim guidance, 2 March 2020, World Health Organization. (2020 d). https://apps.who.int/iris/handle/10665/331329?show=full. Accessed 2 July 2020.
[34] L. Zou, F. Ruan, M. Huang, L. Liang, H. Huang, Z. Hong, et al., SARS CoV-2 viral load in upper respiratory specimens of infected patients, New England Journal of Medicine 382 (2020) 1177–1179. https://doi.org/10.1056/NEJMc2001737.
[35] C. Drosten, M. Seilmaier, V.M. Corman, W. Hartmann, G. Scheible, S. Sack, et al., Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection, The Lancet Infectious Diseases 13 (2013) 745–751.
[36] SARS-(CoV-2). possesses the typical coronavirus structure with spike protein and also expressed other polyproteins, nucleoproteins, and membrane proteins, such as RNA polymerase, 3- chymotrypsin-like protease, papain-like protease, helicase, glycoprotein, and accessory proteins.
[37] X. Huang, W. Dong, A. Milewska, A. Golda, Y. Qi, Q.K. Zhu, et al., Human coronavirus HKU1 spike protein uses O-acetylated sialic acid as an attachment receptor determinant and employs hemagglutinin-esterase protein as a receptor-destroying enzyme, Journal of Virology 89 (2015) 7202–7213.
[38] J.K. Millet, GR. Whittaker, Host cell proteases: critical determinants of coronavirus tropism and pathogenesis, Virus Research 202 (2015) 120–134.
[39] H. Hofmann, S. Pöhlmann, Cellular entry of the SARS coronavirus, Trends in Microbiology 12 (2004) 466–472.
[40] M. Gui, W. Song, H. Zhou, J. Xu, S. Chen, Y. Xiang, et al., Cryoelectron microscopy structures of the SARS-CoV spike glycoprotein reveal a prerequisite conformational state for receptor binding, Cell Research 27 (2017) 119–129.

[41] D. Wrapp, N. Wang, K.S. Corbett, J.A. Goldsmith, C.L. Hsieh, O. Abiona, et al., Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation, Science 367 (2020) 1260–1263.
[42] L.E. Gralinski, VD. Menachery, Return of the Coronavirus: 2019nCoV, Viruses 12 (2020) 135.
[43] S. Perlman, J. Netland, Coronaviruses post-SARS: Update on replication and pathogenesis, Nature Reviews Microbiology 7 (6) (2009) 439–450.
[44] V.S. Raj, H. Mou, S.L. Smits, D.H. Dekkers, M.A. Müller, R. Dijkman, et al., Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC, Nature 495 (7440) (2013) 251–254.
[45] X. Xu, P. Chen, J. Wang, J. Feng, H. Zhou, X. Li, et al., Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modelling of its spike protein for risk of human transmission, Science China Life Sciences 63 (3) (2020) 457–460.
[46] Y. Wan, J. Shang, R. Graham, R.S. Baric, F. Li, Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS, Journal of Virology (2020), doi:10.1128/JVI.00127-20.
[47] M. Hussain, N. Jabeen, F. Raza, S. Shabbir, A.A. Baig, A. Amanullah, et al., Structural variations in human ACE2 may influence its binding with SARS-CoV-2 spike protein, Journal of Medical Virology (2020). https://doi.org/10.1002/jmv.25832.
[48] M.P. Cheng, J. Papenburg, M. Desjardins, S. Kanjilal, C. Quach, M. Libman, et al., Diagnostic testing for severe acute respiratory syndrome–related coronavirus-2: A narrative review, Annals of Internal Medicine (2020). https://doi.org/10.7326/M20-1301.
[49] S. Knobler, A. Mahmoud, S. Lemon, A. Mack, L. Sivitz, K. Oberholtzer, Learning from SARS: Preparing for the next disease outbreak: Workshop summary, National Academies Press, United States, 2004, doi:10.17226/10915.
[50] WHO Managing epidemics: Key facts about major deadly diseases; (2018). https://www.who.int/publications/i/item/managingepidemicskeyfactsaboutmajordeadlydiseases.
[51] W. Wang, Y. Xu, R. Gao, R. Lu, K. Han, G. Wu, et al., Detection of SARS-CoV-2 in different types of clinical specimens, JAMA 323 (2020) 1843–1844.
[52] A. See, S.T. Toh, Respiratory sampling for severe acute respiratory syndrome coronavirus 2: An overview, Head & Neck 42 (2020) 1652–1656.
[53] CDC. Coronavirus disease 2019 [COVID-19] https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html. Accessed 28 Apr (2020).
[54] P.H.L.N. PHLN., Guidance on laboratory testing for SARS-CoV-2 [the virus that causes COVID-19], PHLN, 2020.
[55] W. Wang, Y. Xu, R. Gaoetal, Detection of SARS-CoV-2 in different types of clinical specimens, Journal of the American Medical Association 323 (18) (2020) 1843–1844.
[56] W. Feng, A. Newbigging, C. Le, B. Pang, H. Peng, Y. Cao, et al., Molecular diagnosis of COVID-19: Challenges and research needs, Analytical Chemistry 92 (2020) 10196–10209.
[57] E. Bendavid, B. Mulaney, N. Sood, S. Shah, E. Ling, R. BromleyDulfano, et al., COVID-19 antibody seroprevalence in Santa Clara County, California, medRxiv (2020). https://doi.org/10.1101/2020.04.14.20062463.
[58] J.A. Backer, D. Klinkenberg, J. Wallinga, Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20-28 January 2020, Euro Surveillance 25 (2020).
[59] Z. Yongchen, H. Shen, X. Wang, X. Shi, Y. Li, J. Yan, et al., Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients, Emerg Microbes Infect 9 (2020) 833–836.
[60] J. Zhao, Q. Yuan, H. Wang, W. Liu, X. Liao, Y. Su, et al., Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease, Clinical Infectious Diseases (2019). https://doi.org/10.1093/cid/ciaa344.

[61] U.S. FDA. FAQs on emergency use authorizations [EUAs] for medical devices during the COVID-19 pandemic. FDA. (2020). https://www.fda.gov/medical-devices/emergency-situationsmedical-devices/faqs-emergency-use-authorizations-euas-medicaldevices-during-covid-19-pandemic. Accessed 2 July 2020.
[62] C.B.F. Vogels, A.F. Brito, A.L. Wyllie, J.R. Fauver, I.M. Ott, C.C. Kalinich, et al., Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets, Nat Microbiol (2020) Epub 2020/07/12. pmid:32651556.
[63] D.L. Heymann, N. Shindo, COVID-19: What is next for public health? Lancet 395 (2020) 542–545.
[64] J. Watson, F. Whiting, J.E. Brush, Interpreting a COVID-19 test result, BMJ (Clinical Research Ed.) 369 (2020) 1–7.
[65] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, et al., Detection of 2019 novel coronavirus [2019-NCoV] by real-time RT-PCR, Euro Surveillance 25 (2020) 2000045.
[66] A.C. Nelson, B. Auch, M. Schomaker, D.M. Gohl, P. Grady, D. Johnson, et al., Analytical validation of a COVID-19 QRT-PCR detection assay using a 384-well format and three extraction methods, BioRxiv (2020). https://doi.org/10.1101/2020.04.02.022186.
[67] Accelerated Emergency Use Authorization (EUA) (Summary). COVID-19 RT-PCR Test (Laboratory Corporation of America), https://www.fda.gov/media/136151/download.109.
[68] S.A.& Bustin, R. Mueller, Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis, Clinical Science 109 (2005) 365–379.
[69] Y. Pan, L. Long, D. Zhang, et al., Potential false-negative nucleic acid testing results for severe acute respiratory syndrome coronavirus 2 from thermal inactivation of samples with low viral loads, Clinical Chemistry 66 (6) (2020) 794–801.
[70] S. Su, G. Wong, W. Shi, J. Liu, A.C. Lai, J. Zhou, et al., Epidemiology, genetic recombination, and pathogenesis of coronaviruses, Trends in Microbiology 24 (2016) 490–502.
[71] K.K. To, O.T. Tsang, C.C. Yip, K. Chan, T. Wu, J.M.C. Chan, et al., Consistent detection of 2019 novel coronavirus in saliva, Clinical Infectious Diseases (2020), doi:10.1093/cid/ciaa149.
[72] K.K. To, O.T. Tsang, W.S. Leung, A.R. Tam, T.C. Wu, D.C. Lung, et al., Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: An observational cohort study, The Lancet Infectious Diseases 20 (2020) 565–574.
[73] W.C. Yam, K.H. Chan, L.L.M. Poon, K.Y. Yuen, W.H. Seto, J.S.M. Peiris, Evaluation of reverse transcription-PCR assays for rapid diagnosis of severe acute respiratory syndrome associated with a novel coronavirus, Journal of Clinical Microbiology 41 (2003) 4521–4524.
[74] A. Bernheim, X. Mei, M. Huang, Y. Yang, Z.A. Fayad, N. Zhang, et al., Chest CT findings in coronavirus disease-19 (COVID-19): Relationship to duration of infection, Radiology 295 (2020) 200463.
[75] R. Wolfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M.A. M¬uller, et al., Virological assessment of hospitalized patients with COVID-2019, Nature (2020). https://doi.org/10.1038/s41586-020-2196-x.
[76] S. Zheng, J. Fan, F. Yu, B. Feng, B. Lou, Q. Zou, et al., Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, BMJ (Clinical Research Ed.) 369 (2020) m1443.
[77] A.T. Xiao, Y.X. Tong, S. Zhang, Profile of RT-PCR for SARS-CoV2: A preliminary study from 56 COVID-19 patients, Clinical Infectious Diseases (2020) 460. https://doi.org/10.1093/cid/ciaa.
[78] A.L. Wyllie, J. Fournier, A. Casanovas-Massana, M. Campbell, M. Tokuyama, P. Vijayakumar, et al., Saliva is more sensitive for SARS-CoV-2 detection in

COVID-19 patients than nasopharyngeal swabs, Med Rxiv (2020) 20067835. https://doi.org/10.1101/2020.04.16.
[79] H. Chen, J. Guo, C. Wang, F. Luo, X. Yu, W. Zhang, et al., Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women a retrospective review of medical records, Lancet 395 (2020) 809–815.
[80] CDC, U. Emergency use authorizations. FDA. 2020. https://www.fda.gov/emergency-preparedness-and-response/mcm-legalregulatory-and-policy-framework/emergency-use-authorization. Accessed 2 July 2020.
[81] CDC. CDC 2019-Novel coronavirus [2019-nCoV] real-time RTPCR diagnostic panel https://www.fda.gov/media/134922/download. Accessed 2 July (2020).
[82] WHOMolecularassaystodiagnoseCOVID-19: Summary table of available protocols, World Health Organization, 2020 chttps://.
[83] A.K. Nalla, A.M. Casto, M-L.W. Huang, G.A. Perchetti, R. Sampoleo, L. Shrestha, Comparative performance of SARS-CoV-2detection assays using seven different primer/probe sets and one assay kit, Journal of Clinical Microbiology 58 (6) (2020) E00557 –00520.
[84] D.K. Chu, Y. Pan, S.M. Cheng, K.P. Hui, P. Krishnan, Y. Liu, et al., Molecular diagnosis of a novel coronavirus [2019-NCoV] causing an outbreak of pneumonia, Clinical Chemistry 66 (2020) 549–555.
[85] WHO Summary of available protocols to detect SARS-CoV-2 (2020 b). https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.Pdfsfvrsn=de3a76aa_2.
[86] J. Xiang, M. Yan, H. Li, T. Liu, C. Lin, S. Huang, et al., Evaluation of enzyme-linked immunoassay and colloidal gold immunochromatographic assay kit for detection of novel coronavirus (SARS-Cov-2) causing an outbreak of pneumonia (COVID-19), medRxiv (2020), doi:10.1101/2020.02.27.20028787.
[87] C. Wang, P.W. Horby, F.G. Hayden, G.F. Gao, A novel corona virus outbreak of global health concern, Lancet 395 (2020) 470–473 a.
[88] Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W., et al. (2020).Correlation of chest CT and RT-PCR testing in coronavirusdisease2019(COVID-19) in China: A report of 1014 cases. Radiology, 200642.
[89] S.F. Ahmed, A.A. Quadeer, M.R. McKay, Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies, Viruses 12 (3) (2020) 254.
[90] N.M.A. Okba, M.A. Mu¨ller, W. Li, et al., Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients, Emerging Infectious Diseases 26 (7) (2020).
[91] D. Benvenuto, M. Giovanetti, A. Ciccozzi, S. Spoto, S. Angeletti, M. Ciccozzi, The 2019-new coronavirus epidemic: Evidence for virus evolution, Journal of Medical Virology 92 (2020) 455–459.
[92] J. Cui, F. Li, Z-L. Shi, Origin and evolution of pathogenic coronaviruses, Nature Reviews Microbiology 17 (2019) 181–192.
[93] False Negatives and ReinfectionsThe Challenges of SARSCoV-2 RT-PCR Testing, American Society for Microbiology, Washington,DC, USA, 2020 https://asm.org/Articles/2020/April/False-Negatives-and-Reinfections-the-Challenges-of.
[94] J.F.-W. Chan, S. Yuan, K.-H. Kok, et al., A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster, The Lancet 395 (no.10223) (2020) 514–523.
[95] C. Huang, Y. Wang, X. Li, et al., Clinical features of patients infected with 2019 novel corona virus in Wuhan, China, The Lancet 395 (10223) (2020) 497–506.
[96] D. Wu, T. Wu, Q. Liu, Z. Yang, The SARS-CoV-2 outbreak:wHatweknow, International Journal of Infectious Diseases 94 (2020) 44–48.

[97] W. Kong, P.P. Agarwal, et al., Chest imaging appearance of COVID-19 infection, Radiol. Cardiothorac. Imaging 2 (2020) e200028.
[98] F. Pan, T. Ye, P. Sun, S. Gui, B. Liang, L. Li, et al., Timecourseoflungchangesonchest CT during recovery from 2019 novel coronavirus (COVID-19) pneumonia, Radiology 295 (2020) 715.
[99] H. Shi, X. Han, N. Jiang, Y. Cao, O. Alwalid, J. Gu, et al., Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: A descriptive study, The Lancet Infectious Diseases 20 (2020) 425.
[100] J.J. Zhang, X. Dong, Y.Y. Cao, Y.D. Yuan, Y.B. Yang, Y.Q. Yan, et al., Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy 75 (2020) 1730.
[101] C. Long, H. Xu, Q. Shen, X. Zhang, B. Fan, C. Wang, et al., Diagnosis of the Coronavirus disease (COVID-19): RRT-PCR or CT? European Journal of Radiology 126 (2020) 108961.
[102] Y. Fang, H. Zhang, J. Xie, M. Lin, L. Ying, P.& Pang, et al., Sensitivity of chest CT for COVID-19: Comparison to RT-PCR, Radiology (2020) 200432.
[103] W. Wang, J. Tang, F. Wei, Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China, J Med Virol. 92 (4) (2020) 441–447.
[104] H.J. Koo, S. Lim, J. Choe, S.H. Choi, H. Sung, K.H. Do, Radiographic and CT features of viral pneumonia, Radiographics 38 (3) (2018) 719–739.
[105] M. Garg, N. Prabhakar, A. Gulati, R. Agarwal, S. Dhooria, Spectrum of imaging findings in pulmonary infections. Part 1: Bacterial and viral, Pol J Radiol 84 (2019) e205–ee13.
[106] W. Zhao, Z. Zhong, X. Xie, Q. Yu, J. Liu, Relation between chest CT findings and clinical conditions of coronavirus disease (COVID-19) pneumonia: A multicenter study, AJR, Am. J. Roentgenol. 214 (2020) 1072.
[107] X. Li, M. Geng, Y. Peng, L. Meng, S. Lu, Molecular immunepathogenesisanddiagnosis of COVID-19, Journal of Pharmaceutical Analysis 10 (2) (2020) 102–108.
[108] H.X. Bai, B. Hsieh, Z. Xiong, K. Halsey, J.W. Choi, T.M.L. Tran, et al., Performance of radiologists in differentiating COVID-19 from viral pneumonia on chest CT, Radiology 296 (2020) E46.
[109] L. Bordi, E. Nicastri, L. Scorzolini, A. Di Caro, M.R. Capobianchi, C. Castilletti, et al., On Behalf of Inmi Covid-Study Group and Collaborating Centers, Differential diagnosis of illness in patients under investigation for the novel coronavirus (SARS-CoV-2), Italy, February 2020, Eurosurveilance 25 (8) (2020), doi:10.2807/1560-7917.ES.2020.25.8.2000170.
[110] M. Hosseiny, S. Kooraki, A. Gholamrezanezhad, S. Reddy, R. Myers, adiologyperspectiveofcoronavirusdisease2019(COVID19): Lessons from severe acute respiratory syndrome and Middle East respiratory syndrome, AJR, Am. J. Roentgenol. 214 (2020) 1078.
[111] American Roentgen Ray Society. (Novel). coronavirus (COVID19) imaging features overlap with SARS and MERS: COVID-19's imaging features are variable and nonspecific, but the imaging findings reported thus far do show; Science Daily; https://www.sciencedaily.com/releases/2020/02/200228142018.htm
[112] Y. Gao, Y. Yuan, T.T. Li, W.X. Wang, Y.X. Li, A. Li, et al., Evaluation the auxiliary diagnosis value of antibodies assays for detection of novel coronavirus (SARS-Cov-2) causing an outbreak of pneumonia (COVID-19), medRxiv (2020), doi:10.1101/2020.03.26.20042044.
[113] F. Amanat, D. Stadbauer, S. Strohmeier, T.H.O. Nguyen, V. Chromikova, M. McMahon, et al., A serological assay to detect SARSCoV-2 seroconversion in humans, Nature Medicine 26 (2020) 1.

[114] C.A. Janeway Jr, P. Travers, M. Walport, M.J. Shlomchik, The distribution and functions of immunoglobulin isotypes, Immunobiology: The immune system in health and disease In, 5th ed, Garland Science, 2001.
[115] W. Liu, L. Liu, G. Kou, Y. Zheng, Y. Ding, W. Ni, et al., Evaluation of nucleocapsid and spike protein-based ELISAs for detecting antibodies against SARS-CoV-2, Journal of Clinical Microbiology (2020), doi:10.1128/JCM.00461-20.
[116] N.M. Okba, M.A. Mulle, W. Li, C. Wang, C.H. Geurtsvan Kessel, V.M. Corman, et al., SARS-CoV-2 specific antibody responses in COVID-19 patients, Emerging Infect. Dis. 26 (2020) 1478.
[117] X. Li, M. Geng, Y. Peng, L. Meng, S. Lu, Molecular immune pathogenesis and diagnosis of COVID-19, J Pharm Anal 10 (2020) 102–108.
[118] D. Stadlbauer, F. Amanat, V. Chromikova, K. Jiang, S. Strohmeier, G.A. Arun kumar, et al., SARS-CoV-2 seroconversion in humans: A detailed protocol for a serological assay, antigen production, and test setup, Curr Protoc Microbiol 57 (2020) e100.
[119] X.F. Cai, J. Chen, Q.X. Long, H.J. Deng, P. Liu, K. Fan, et al., A peptide-based magnetic chemiluminescence enzyme immunoassay for serological diagnosis of coronavirus disease 2019, The Journal of Infectious Diseases (2020). https://doi.org/10.1093/infdis/jiaa243.
[120] SK. Vashist, In vitro diagnostic assays for COVID-19: recent advances and emerging trends, Diagnostics 10 (2020) 202. https://doi.org/10.3390/diagnostics10040202.
[121] D. Lin, L. Liu, M. Zhang, Y. Hu, Q. Yang, J. Guo, et al., Evaluations of the serological test in the diagnosis of 2019 novel coronavirus (SARS-CoV-2) infections during the COVID-19 outbreak, European Journal of Clinical Microbiology & Infectious Diseases (2020) 1–7. https://doi.org/10.1007/s10096-020-03978-6.
[122] P. Zhang, Q. Gao, T. Wang, Y. Ke, F. Mo, R. Jia, et al., Evaluation of recombinant nucleocapsid and spike proteins for serological diagnosis of novel coronavirus disease 2019 [COVID-19], MedRxiv. (2020). https://doi.org/10.1101/2020.03.17.20036954.
[123] L. Zhong, J. Chuan, B. Gong, P. Shuai, Y. Zhou, Y. Zhang, et al., Detection of serum IgM and IgG for COVID-19 diagnosis, Sci China Life Sci 63 (2020) 777–780.
[124] G.J. Gorse, M.M. Donovan, G.B. Patel, Antibodies to coronaviruses are higher in older compared with younger adults and binding antibodies are more sensitive than neutralizing antibodies in identifying coronavirus-associated illnesses, Journal of Medical Virology 92 (2020) 512–517.
[125] Z. Li, Y. Yi, X. Luo, N. Xiong, Y. Liu, S. Li, et al., Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis, Journal of Medical Virology (2020). https://doi.org/10.1002/jmv.25727.
[126] Scott, A. (2020).Is a reagents shortage delaying European COVID-19 testing? https://cen.acs.org/analytical-chemistry/diagnostics/reagentsshortage-delaying-European-COVID/98/i13. Accessed 30 Apr
[127] Z. Chen, Z. Zhang, X. Zhai, Y. Li, L. Lin, H. Zhao, et al., Rapid and sensitive detection of anti-SARS-CoV-2 IgG using lanthanide doped nanoparticles-based lateral flow immunoassay, Analytical Chemistry 92 (2020) 7226–7231.
[128] M. Norman, T. Gilboa, A.F. Ogata, A.M. Maley, L. Cohen, Y. Cai, et al., Ultrasensitive high-resolution profiling of anti-SARS-CoV-2 antibodies for detecting early seroconversion in COVID-19 patients, medRxiv (2020). https://doi.org/10.1101/2020.04.28.20083691.
[129] C. Sheridan, Fast, portable tests come online to curb coronavirus pandemic, Nature Biotechnology 38 (2020) 515.
[130] WHO. Advice on the use of point-of-care immunodiagnostic tests for COVID-19. https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-testsfor-covid-19. Accessed 30 Apr (2020a).

[131] G. Seo, G. Lee, M.J. Kim, S-H. Baek, M. Choi, K.B. Ku, et al., Rapid detection of COVID-19 causative virus [SARS-CoV-2] in human nasopharyngeal swab specimens using field-effect transistor-based biosensor, ACS Nano 14 (2020) 5135–5142.
[132] R. Kassanjee, C.D. Pilcher, M.P. Busch, G. Murphy, S.N. Facente, S.M. Keating, et al., Viral load criteria and threshold optimization to improve HIV incidence assay characteristics-a CEPHIA analysis, Aids 30 (2016) 2361.
[133] J. Tate, G. Ward, Interferences in immunoassay, Clin. Biochem. Rev. 25 (2004) 105.
[134] N. Wang, S.Y. Li, X.L. Yang, H.M. Huang, Y.J. Zhang, H. Guo, et al., Serological evidence of bat SARS-related coronavirus infection in humans, China, Virol Sin 33 (2018) 104–107.
[135] W. Zhang, R.H. Du, B. Li, X.S. Zheng, X.L. Yang, B. Hu, et al., Molecular and serological investigation of 2019-NCoV infected patients: Implication of multiple shedding routes, Emerg Microbes Infect 9 (2020) 386–389 d.
[136] G. Qiu, Z. Gai, Y. Tao, J. Schmitt, G.A. Kullak-Ublick, J. Wang, Dualfunctional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection, ACS Nano 14 (2020) 5268–5277.
[137] E. Percivalle, G. Cambie, I. Cassaniti, E.V. Nepita, R. Maserati, A. Ferrari, et al., Prevalence of SARS-CoV-2 specific neutralising antibodies in blood donors from the Lodi Red Zone in Lombardy, Italy, as at 06 April 2020, Eurosurveillance 25 (24) (2020), doi:10.2807/15607917.ES.2020.25.24.2001031.
[138] S. Stringhini, A. Wisniak, G. Piumatti, A.S. Azman, S.A. Lauer, H. Baysson, et al., Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): A population-based study, Lancet 396 (2020) 313.
[139] X. Xu, J. Sun, S. Nie, H. Li, Y. Kong, M. Liang, et al., Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China, Nature Medicine (2020), doi:10.1038/s41591-020-0949-6.
[140] Q.X. Long, B.Z. Liu, H.J. Deng, G.C. Wu, K. Deng, Y.K. Chen, et al., Antibody responses to SARS-CoV-2 in patients with COVID19, Nature Medicine 26 (2020) 845.
[141] F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, et al., Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study, Lancet 395 (2020) 1054.
[142] W. Zhu, K. Xie, H. Lu, L. Xu, S. Zhou, S. Fang, Initial clinical features of suspected Coronavirus Disease 2019 in two emergency departments outside of Hubei, China. J. Med. Virol. (2020), doi:10.1002/jmv.25763.
[143] A.J. Rodriguez-Morales, J.A. Cardona-Ospina, E. Gutierrez-Ocampo, R. Villamizar Pena, et al., Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis, Travel Med Infect Dis 34 (2020) 101623.
[144] Y.W. Tang, J.E. Schmitz, D.H. Persing, C.W. Stratton, Laboratory diagnosis of COVID-19: Current issues and challenges, Journal of Clinical Microbiology 58 (6) (2020), doi:10.1128/JCM.00512-20.
[145] A.C. Walls, Y.J. Park, M.A. Tortorici, A. Wall, A.T. McGuire, D. Veesler, Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein, Cell 181 (2020) 281.
[146] M. Zheng, L. Song, Novel antibody epitopes dominate the antigenicity of spike glycoprotein in SARS-CoV-2 compared to SARS-CoV, Cell. Mol. Immunol. 17 (5) (2020) 536–538.
[147] K.H.D. Crawford, R. Eguia, A.S. Dingens, A.N. Loes, K.D. Malone, C.R. Wolf, et al., Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 spike protein for neutralization assays, Viruses 12 (5) (2020) 513.
[148] P.I. Kontou, G.G. Braliou, N.L. Dimou, G. Nikolopoulos, PG. Bagos, Antibody tests in detecting SARS-CoV-2 infection: A meta-analysis, Diagnostics 10 (5) (2020) 319.
[149] H. Lv, N.C. Wu, O.T. Tsang, M. Yuan, R. Perera, W.S. Leung, et al., Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections, Cell reports 31 (9) (2020) 107725.

[150] S.K. Lau, K.S. Li, A.K. Tsang, C.S. Lam, S. Ahmed, H. Chen, et al., Genetic characterization of Betacoronavirus lineage C viruses in bats reveals marked sequence divergence in the spike protein of pipistrellus bat coronavirus HKU5 in Japanese pipistrelle: Implications for the origin of the novel Middle East respiratory syndrome coronavirus, Journal of Virology 87 (15) (2013) 8638–8650.
[151] J.F.-W. Chan, K-H. Kok, Z. Zhu, H. Chu, K.K.-W. To, S. Yuan, et al., Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting, Wuhan. Emerging Microbes & Infections 9 (1) (2020) 221–236.
[152] S. Jiang, C. Hillyer, L. Du, Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses, Trends in Immunology 41 (5) (2020) 355–359.
[153] L. Ni, F. Ye, M.L. Cheng, Y. Feng, Y.Q. Deng, H. Zhao, et al., Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals, Immunity 52 (6) (2020) 971–977.
[154] J.S. Gootenberg, O.O. Abudayyeh, J.W. Lee, P. Essletzbichler, A.J. Dy, J. Joung, et al., Nucleic acid detection with CRISPR-Cas13a/C2c2, Science 356 (6336) (2017) 438–442.
[155] N. Kohmer, S. Westhaus, C. Rühl, S. Ciesek, H.F. Rabenau, Clinical performance of different SARS-CoV-2 IgG antibody tests, Journal of Medical Virology (2020), doi:10.1002/jmv.26145.
[156] I. Montesinos, D. Gruson, B. Kabamba, H. Dahma, W.S. Van den, S. Reza, Evaluation of two automated and three rapid lateral flow immunoassays for the detection of antiSARS-CoV-2 antibodies, Journal of Clinical Virology 128 (2020) 104413.
[157] Y. Pan, X. Li, G. Yang, J. Fan, Y. Tang, J. Zhao, et al., Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients, Journal of Infection 81 (2020) 28.
[158] M.S. Tang, K.G. Hock, N.M. Logsdon, J.E. Hayes, A.M. Gronowski, Clinical performance of two SARS-CoV-2 serologic assays, Clinical Chemistry 66 (2020) 1055.
[159] A. Kaur, K. Sapkota, S. Dhakal, Multiplexed nucleic acid sensing with single-molecule FRET, ACS Sens 4 (2019) 623–633.
[160] D. Koirala, P. Shrestha, T. Emura, K. Hidaka, S. Mandal, M. Endo, et al., Single-molecule mechanochemical sensing using DNA origami nanostructures, Angew. Chem., Int. Ed. 53 (2014) 8137–8141.
[161] D. Koirala, Z. Yu, S. Dhakal, H. Mao, Detection of single nucleotide polymorphism using tension-dependent stochastic behaviour of a single-molecule template, Journal of the American Chemical Society 133 (2011) 9988–9991.
[162] L. Chen, W. Liu, Q. Zhang, K. Xu, G. Ye, W. Wu, et al., RNA based MNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak, Emerg Microbes Infect 9 (2020) 313–319.
[163] J.-W. Ai, Y. Zhang, H.-C. Zhang, T. Xu, W.-H. Zhang, Era of molecular diagnosis for pathogen identification of unexplained pneumonia, lessons to be learned, Emerg Microbes Infect 9 (2020) 597–600.
[164] T. Suo, X. Liu, J. Feng, M. Guo, W. Hu, G. Dong, et al., ddPCR: A more accurate tool for SARS-CoV-2 detection in low viral load specimens, Emerg Microbes Infect 9 (2020) 1259–1268.
[165] L. Dong, J. Zhou, C. Niu, Q. Wang, Y. Pan, S. Sheng, et al., Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR, medRxiv (2020) 20036129. https://doi.org/10.1101/2020.03.14.
[166] P. Moitra, M. Alafeef, K. Dighe, M. Frieman, D. Pan, Selective naked eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped plasmonic nanoparticles, ACS Nano 14 (2020) 7617–7627.
[167] S.J.R. Silva, K. Pardee, L. Pena, Loop-mediated isothermal amplification (LAMP) for the diagnosis of Zika virus: A review, Viruses 12 (2020) 19.

[168] W.E. Huang, B. Lim, C. Hsu, D. Xiong, W. Wu, Y. Yu, et al., RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2, Microbial Biotechnology 13 (2020) 950.
[169] G. Park, K. Ku, S. Baek, S. Kim, S. Kim, B. Kim, et al., Development of reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays targeting SARS-CoV-2, The Journal of Molecular Diagnostics 22 (2020) 729.
[170] L. Yu, S. Wu, X. Hao, X. Dong, L. Mao, V. Pelechano, Rapid detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform, Clinical Chemistry 66 (2020) 975.
[171] Y. Zhang, N. Odiwuor, J. Xiong, L. Sun, R.O. Nyaruaba, H. Wei, et al., Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP, medRxiv (2020), doi:10.1101/2020.02.26.20028373.
[172] X. Yuan, Y. Wang, K. Meng, Y. Zhang, H. Xu, W. Ai, LAMP real-time turbidity detection for fowl adenovirus, BMC Vet. Res. 15 (2019) 256.
[173] M. Jiang, W. Pan, A. Aratehfar, W. Fang, et al., Development and validation of a rapid single-step reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) system potentially to be used for reliable and high-throughput screening of COVID-19, medRxiv (2020), doi:10.1101/2020.03.15.20036376.
[174] D. Liu, S.L. Daubendiek, M.A. Zillman, K.& Ryan, E.T. Kool, Rolling circle DNA synthesis: Small circular oligonucleotides as efficient templates for DNA polymerases, Journal of the American Chemical Society 118 (1996) 1587–1594.
[175] B. Wang, S.J. Potter, Y. Lin, A.L. Cunningham, et al., Rapid and sensitive detection of severe acute respiratory syndrome coronavirus by rolling circle amplification, Journal of Clinical Microbiology 43 (2005) 2339–2344.
[176] W. Zhao, M. Ali, M. Brook, M.A.Y. Li, Rolling circle amplification: Applications in nanotechnology and biodetection with functional nucleic acids, Angew. Chem., Int. Ed. 47 (2008) 6330–6337.
[177] M. El-Tholoth, H.H. Bau, J. Song, A single and two-stage, closed-tube, molecular test for the 2019 Novel Coronavirus (COVID19) at home, clinic, and points of entry, chem Rxiv (2020) 10.26434/chem rxiv. 11860137. v1.
[178] L.T. Nguyen, B.M. Smith, P.K. Jain, Enhancement of trans-cleavage activity of Cas12a with engineered CrRNA enables amplified nucleic acid detection, Bio Rxiv (2020). https://doi.org/10.1101/2020.04.13.036079.
[179] A. Basu, T. Zinger, K. Inglima, K. Woo, O. Atie, L. Yurasits, et al., Performance of the rapid nucleic acid amplification by Abbott ID NOW COVID-19 in nasopharyngeal swabs transported in viral media and dry nasal swabs, New York City Academic Institution. bioRxiv. (2020), doi:10.1101/2020.05.11.089896.
[180] M.J. Loeffelholz, D. Alland, S.M. Butler-Wu, U. Pandey, C.F. Perno, A. Nava, et al., Multicenter evaluation of the cepheid Xpert Xpress SARS-CoV-2 test, Journal of Clinical Microbiology (2020), doi:10.1128/JCM.00926-20.
[181] B. Visseaux, Q.L. Hingrat, G. Collin, D. Bouzid, S. Lebourgeois, D.L. Pluart, et al., Evaluation of the QIAstat-Dx Respiratory SARS-CoV-2 Panel, the first rapid multiplex PCR commercial assay for SARS-CoV-2 detection, Journal of Clinical Microbiology (2020), doi:10.1128/JCM.00630-20.
[182] M. Pachetti, B. Marini, F. Benedetti, F. Giudici, E. Mauro, P. Storici, et al., Emerging SARS-CoV-2 mutation hot spots include a novel RNAdependent-RNA polymerase variant, Journal of Translational Medicine 18 (2020) 1–9.
[183] K.M. Koczula, A. Gallotta, Lateral flow assays, Essays in Biochemistry 60 (2016) 111–120.
[184] J. Reboud, G. Xu, A. Garrett, M. Adriko, Z. Yang, E.M. Tukahebwa, et al., Paper-based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities, PNAS 116 (2019) 4834–4842.

[185] D. Zhang, D. Broyles, E.A. Hunt, E. Dikici, S. Daunert, S.K. Deo, A paper-based platform for detection of viral RNA, Analyst 142 (2017) 815–823.
[186] K.M. Byers, A.R. Bird, H.D. Cho, J.C. Linnes, Fully dried two-dimensional paper network for enzymatically enhanced detection of nucleic acid amplicons, ACS Omega 5 (2020) 4673–4681.
[187] D. Chandler-Brown, A.M. Bueno, O. Atay, D.S. Tsao, A highly scalable and rapidly deployable RNA extraction-free COVID-19 assay by quantitative Sanger sequencing, bioRxiv (2020). https://doi.org/10.1101/2020.04.07.029199.
[188] S. Mahari, A. Roberts, D. Shahdeo, GS. eCov, Sensul tra sensitive novel in-house built printed circuit board-based Review of analytical performance of COVID-19 detection methods 47electrochemical device for rapid detection of nCovid-19 antigen, a spike protein domain 1 of SARS-CoV-2. bioRxiv for Enzymatically Enhanced Detection of Nucleic Acid Amplicons, ACS Omega 5 (2020) 4673–4681.
[189] R. Tanaka, T. Yuhi, N. Nagatani, T. Endo, K. Kerman, Y. Takamura, et al., A novel enhancement assay for immune chromatographic test strips using gold nanoparticles, Analytical and Bioanalytical Chemistry 385 (2006) 1414–1420.
[190] B. Giri, A perspective on the sensitivity of paper-analytical devices for bioanalysis, J. Appl. Bionalysis 2 (2016) 6.
[191] A.W. Martinez, S.T. Phillips, E. Carrilho, S.W. Thomas, H. Sindi, G.M. Whitesides, Simple telemedicine for developing regions: Camera phones and paper-based microfluidic devices for realtime, off-site diagnosis, Analytical Chemistry 80 (2008) 3699–3707.
[192] A. Priye, S.W. Bird, Y.K. Light, C.S. Ball, O.A. Negrete, R.J. Meagher, A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses, Scientific Reports 7 (2017) 1–11.
[193] B. Berg, B. Cortazar, D. Tseng, H. Ozkan, S. Feng, Q. Wei, Cell phone based hand-held microplate reader for point-of-care testing of enzyme linked immunosorbent assays, ACS Nano 9 (2015) 7857–7866.
[194] T. Laksanasopin, T.W. Guo, S. Nayak, A.A. Sridhara, S. Xie, O.O. Olowookere, smartphone dongle for diagnosis of infectious diseases at the point of care, Science Translational Medicine 7 (2015) 273re1.
[195] D.M. Rissin, C.W. Kan, T.G. Campbell, S.C. Howes, D.R. Fournier, L. Song, et al., Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations, Nature Biotechnology 28 (2010) 595.
[196] C. Greenwood, D. Ruff, S. Kirvell, G. Johnson, H.S. Dhillon, S.A. Bustin, Proximity assays for sensitive quantification of proteins, Biomol. Detect. Quantif. 4 (2015) 10–16.
[197] M.L. Metzker, Sequencing technologies the next generation, Nature Reviews Genetics 11 (2010) 31–46.
[198] M.J. Khan, A.C. Trabuco, H.L. Alfonso, M.L. Figueiredo, W.C. Batista, S.J. Badra, DNA microarray platform for detection and surveillance of viruses transmitted by small mammals and arthropods, PLoS Neglected Trop. Dis. 10 (2016) E0005017.
[199] Z. Shen, Y. Xiao, L. Kang, W. Ma, L. Shi, L. Zhang, et al., Genomic diversity of severe acute respiratory syndrome−Coronavirus 2 in patients with coronavirus disease, Clinical Infectious Diseases 2020 (2019), doi:10.1093/cid/ciaa203.
[200] W. Long, H. Xiao, X. Gu, Q. Zhang, H. Yang, G. Zhao, et al., A universal microarray for detection of SARS coronavirus, Journal of Virological Methods 121 (2004) 57–63.
[201] J.S. Gootenberg, O.O. Abudayyeh, M.J. Kellner, J. Joung, J.J. Collins, F. Zhang, Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6, Science 360 (6387) (2018) 439–444.
[202] J.P. Broughton, X. Deng, G. Yu, C.L. Fasching, V. Servellita, J. Singh, et al., CRISPR-Cas12-based detection of SARS-CoV-2, Nature Biotechnology 38 (2020) 870.

[203] Zhang F., Abudayyeh O., Gootenberg J., A protocol for detection of COVID-19 using CRISPR diagnostics 2020 a. https://www.broadinstitute.Org/files/publications/special/COVID19%20detection%20(updated).pdf.
[204] J. Joung, A. Ladha, M. Saito, et al., Point-of-care testing for COVID-19 using SHERLOCK diagnostics, MedRxiv (2020) 2020.
[205] M. Azhar, R. Phutela, A.H. Ansari, et al., Rapid, field-deployable nucleobase detection and identification using FnCas9, BioRxiv 2020 (2020).
[206] M.J. Kellner, J.G. Koob, J.S. Gootenberg, O.O. Abudayyeh, F. Zhang, SHERLOCK: Nucleic acid detection with CRISPR nucleases, Nature Protocols 14 (2019) 2986–3012.
[207] X. Mei, H.-.C. Lee, K. Diao, M. Huang, B. Lin, C. Liu, et al., Artificial intelligence–Enabled rapid diagnosis of patients with COVID-19, Nature Medicine (2020) 1–5. https://doi.org/10.1038/s41591-020-0931-3.
[208] A. Wu, Y. Peng, B. Huang, X. Ding, X. Wang, P. Niu, et al., Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China, Cell Host Microbe (2020).
[209] G. Yan, C.K. Lee, L.T.M. Lam, B. Yan, Y.X. Chua, A.Y.N. Lim, et al., Covert COVID-19 and false-positive dengue serology in Singapore, The Lancet Infectious Diseases 20 (2020) 536.
[210] J. Chaung, D. Chan, S. Pada, P.A. Tambyah, Coinfection with COVID-19 and Coronavirus HKU1 - the critical need for repeat testing if clinically indicated, Journal of Medical Virology (2020), doi:10.1002/jmv.25890.
[211] E. Cuadrado-Payan, E. Montagud-Marrahi, M. Torres-Elorza, M. Bodro, M. Blasco, E. Poch, et al., SARS-CoV-2 and influenza virus co-infection, Lancet 395 (10236) (2020) 84.
[212] F. Touzard-Romo, C. Tape, J.R. Lonks, Coinfection with SARS-CoV-2 and human metapneumovirus, R I Med. J. 103 (2) (2013) 75–76 (2020).
[213] D. Wu, J. Lu, X. Ma, Q. Liu, D. Wang, Y. Gu, et al., Coinfection of influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-COV-2), Pediatric Infectious Disease Journal 39 (2020) 79.

Further Reading

[1] E.I. Azhar, D.S.C. Hui, Z.A. Memish, C. Drosten, A. Zumla, The Middle East respiratory syndrome (MERS), Infect Dis Clin N Am 33 (2019) 891–905.
[2] M.E. Killerby, H.M. Biggs, C.M. Midgley, S.I. Gerber, J.T. Watson, Middle East respiratory syndrome coronavirus transmission, Emerging Infectious Diseases 26 (2020) 191–198.
[3] S. Shalhoub, AS. Omrani, Middle East respiratory syndrome, BMJ (Clinical Research Ed.) 355 (2016) i5281.
[4] World Health Organization. MERS situation update, January (2020 c). 2020. http://www.emro.who.int/pandemic-epidemic-diseases/mers-cov/merssituation-update-january-2020.html. Accessed 13 July 2020.
[5] G.M. Leung, A.J. Hedley, L.M. Ho, P. Chau, I.O. Wong, T.Q. Thach, et al., The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: An analysis of all 1755 patients, Annals of Internal Medicine 141 (2004) 662–673.
[6] W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M.A. Berne, et al., Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, Nature 426 (2003) 450–454.
[7] W. Ren, W. Li, M. Yu, P. Hao, Y. Zhang, P. Zhou, et al., Full-length genome sequences of two SARS-like coronaviruses in horseshoe bats and genetic variation analysis, Journal of General Virology 87 (2006) 3355–3359.

[8] M. Wang, M. Yan, H. Xu, W. Liang, B. Kan, B. Zheng, et al., SARS-CoV infection in a restaurant from palm civet, Emerging Infectious Diseases 11 (2005) 1860–1865.
[9] World Health Organization. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. (2003). https://www.who.int/csr/don/2003_07_05/en/. Accessed 13 July 2020.
[10] G. Lu, Y. Hu, Q. Wang, J. Qi, F. Gao, Y. Li, et al., Molecular basis of binding between novel human coronavirus MERSCoV and its receptor CD26, Nature 500 (2013) 227–231.
[11] Z.A. Memish, S. Perlman, M.D. Van Kerkhove, A. Zumla, Middle East respiratory syndrome, Lancet 395 (2020) 1063–1077.
[12] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, et al., SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Cell. 181 (2020) 271–280.
[13] X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, et al., Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV, Nature Communications 11 (2020) 1620.
[14] World Health Organization Statement on the second meeting of the International Health Regulations (2005). Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). 2020. https://www.who.int/news-room/detail/30-01-2020 statement on the second meeting of the international health regulations (2005) emergency committee regarding the outbreak of novel coronavirus (2019-ncov). Accessed 13 July 2020.
[15] World Health Organization. Coronavirus disease 2019 (COVID-19) Situation Report –*175*. (2020 a). https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200423-sitrep-94-covid-19.pdf?sfvrsn=b8304bf0_4. Accessed 13 July 2020.
[16] World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. (2020 b). https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-mediabriefing-on-covid-19%2D%2D-11-march-2020. Accessed 13 July 2020.
[17] https://labblog.uofmhealth.org/rounds/how-scientists-quantifyintensity-of-an-outbreak-like-covid-19. Accessed 30 June (2020).

Non-Print Items

Abstract

December 2019, when the world was busy in welcoming New Year, unaware of the fact that the spark of COVID-19 has been ignited in Wuhan, China, will outbreak into wildfire to scorch the entire World. Nobody could have imagined that a single cell microorganism which even does not have any kind of cellular structure could be such a huge threat to human society. WHO and Public Health of Emergency of International Concern (PHEIC) declared COVID-19 as highly contagious within a month after reporting of the first case on January 11, 2020. As it started spreading across the globe, it was declared as a pandemic by WHO on March 12, 2020. Several scientists of government and nongovernment organizations started working toward the prevention and treatment of this novel disease. Its high rate of transmission, global spread, and high mortality rate started raising concerns worldwide. But as the disease was spreading at an extremely high rate through person-to-person contact, the main challenge was to develop fast and accurate diagnostic methods. Diagnostic tests during such pandemic are crucial as they help to evaluate the effectiveness of the prevention, treatment, and population-wise containment measures. This chapter discusses the various clinical methods that are currently being used worldwide to detect the presence of deadly Corona virus. The procedures of the tests are detailed and are compared based on their specificity, sensitivity, limit of detection (LOD), reliability, and affordability.

Keywords

COVID-19; SARS-CoV-2; RT-PCR; Corona virus; CRISPR