CHAPTER 1

Updated insight into COVID-19 disease and health management to combat the pandemic

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1.1 Introduction to SARS-CoV-2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a pandemic potential zoonotic virus that belongs to the Coronaviridae family.[1−3] As of January 2021, more than 92 million people around the globe are actively battling the virus and over 2 million people have already succumbed to the disease so far (https://covid19.who.int/). Although the origin of this virus remains speculative, earliest reports of patient cases were linked to a live sea food market in Wuhan in the Hubei province of China.[3] The zoonotic virus is thought to originate in bats and has been transmitted to humans through an intermediate host animal such as palm civet cats or pangolins.[3] However, the intermediate animal host is yet to be established.

The contagious nature and the enhanced transmission rate of the virus are the primary causes for such rapid spread of the disease globally.[4,5] The human-to-human transmission of the virus primarily occurs through direct or indirect contact with infected respiratory water droplets.[6] Such contact is established either by inhalation or direct contact with a contaminated surface or infected body fluid such as saliva and urine.[6−8] The ability of the virus to remain active in suspension of infected respiratory droplets over a long period of time and to be carried over to long distances makes this pathogen airborne.[9,10]

Public health management strategies directly contribute to suppression of human-to-human disease spread. For instance, Taiwan's success in battling the first wave of viral infection is principally credited for the quick and strict observance of public safety measures such as use of facial masks, social distancing between individuals, and strict travel restrictions.[11] Another example is that of Hong Kong, home to a population of 7.5 million, which effectively curbed the spread of SARS-CoV-2 using strategies such as

3

prompt and swift surveillance of the disease progression, quarantine and social–distancing measures, closure of schools, and obligatory use of face masks.[12] Another example is that of Vietnam, home to 96 million people, which has surprised the world with less than 2000 COVID-19 cases in the country.[13] The success is primarily attributed to early risk assessment of the situation and placement of strict travel and border restrictions with foreign countries including quarantines apart from the standard health safety pro-cedures.[14] Such successful examples have inspired many nations around the globe with hope that are actively battling the virus outbreak. However, attempts to contain the spread of the virus worldwide has not been uniform among all the nations globally since not all nations are in the same phase of infection, and other reasons, including less stringent public safety measures, delayed governmental policies, and/or even absence of prior experience with such a coronavirus epidemic.[15] For instance, in Italy, the country was taken by surprise when the initial outbreak of the virus was reported. The level of governmental unpreparedness, a decentralized policy of following different health safety measures in different regions of the country, in addition to not-so-sophisticated health care facilities caused significant economic and human loss in the country.[16] The world bank forecasts a great economic dip in the world economy since the world war era as the aftermath of this pandemic.[17] Given the deep impact of the pandemic as the world enters into a great socioeconomic dip, the importance of health management strategies cannot be stressed enough.

In humans, COVID-19 presents with clinical symptoms ranging from mild in healthy adults to severe in individuals with immune–compromised or weakened immune system and the elderly.[18–20] Some of the clinical symptoms include fever, sore throat, fatigue, loss of smell and sense of taste for milder cases, while acute-to-severe respiratory distress syndrome (ARDS) is among the major complications that require medical attention.[18] The rapid global spread of the disease warranting increased hospitalization of COVID-19 patients and constant monitoring of the disease progression in patients has pressured the scientific community worldwide. Scientists and health care professionals around the globe are working at record speeds to understand the origin and nature of SARS-CoV-2 to search for suitable novel technologies for rapid virus detection and vaccines to contain the spread of the virus.[21,22] Currently, over 10 vaccines against SARS-CoV-2 are already available for public use, and many more are expected to be

commercialized in the first quarter of 2021.[23−25] Albeit the success and enthusiasm regarding the commercial availability of COVID-19 vaccines, concerns such as efficiency of the vaccine over time and its effects on pregnant women are currently being evaluated.[26]

Early detection of the virus and isolation and further quarantine are important steps of actions in the health care management strategies to control the COVID-19 pandemic.[27,28] Health care diagnostics and advances in biotechnology play an instrumental role in successful implementation of the health care management strategies.[29] Molecular biotechnological tools such polymerase chain reactions (PCRs) are the current global standard for detection of the virus in suspected individuals. Rapid detection of the virus in suspected individuals is of prime importance for efficient implementation of health management strategies aimed at containing the spread of the virus.

Keeping in mind all of the aforementioned facts, this chapter provides an overview of the epidemiology of coronavirus and the current classical health management strategies and issues to tackle this pandemic. The chapter particularly highlights the role of standard as well as novel biomolecular diagnostic techniques (with capacity to offer rapid and robust detection of SARS-CoV-2) as tools for successful implementation of such public safety measures issued by medical policy makers and governing authorities.

1.2 Overview of SARS-CoV-2 virus

SARS–CoV–2 is classified as a member of the Coronaviridae family.[30] The detailed classification of the virus is shown in Fig. 1.1. The newly identified SARS-CoV-2 is a bona fide human pathogen like some other famous members of this family, such as HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, and MERS-CoV.[31,32] In the past, members of the betacoronavirus genus have caused human life–threatening respiratory diseases.[33] Examples include the SARS outbreak in 2003 and the MERS outbreak in 2012 with a mortality rate of 10% and 35%, respectively.[34,35] Based on the mortality analyses of COVID-19 cases, the mortality rate is estimated to be between 0.4% and 9%.[36]

The members of coronaviruses are a group of large enveloped viruses that carry positive-sense single-stranded RNA (+ssRNA) as their genomes.[32,37] They are known to infect a wide range of host organisms from chicken

Category	Coronaviruses
Realm	*Riboviria*
Order	*Nidovirales*
Suborder	*Cornidovirineae*
Family	*Coronaviridae*
Subfamily	*Orthocoronavirinae*
Genus	*Betacoronavirus*
Subgenus	*Sarbecovirus*
Species	*Severe acute respiratory syndrome-related coronavirus*
Individuum	SARS-CoVUrbani, SARS-CoVGZ-02, Bat SARS CoVRf1/2004, Civet SARS CoVSZ3/2003, SARS-CoVPC4-227, SARSr-CoVBtKY72, SARS-CoV-2 Wuhan-Hu-1, SARSr-CoVRatG13, and so on.

Figure 1.1 A detailed taxonomical classification of SARS-CoV-2 in the Coronaviridae family. *(The image is adopted from Gorbalenya AE, Baker SC, Baric RS, et al. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5(4):536–544. 2020/04/01. https://10.1038/s41564-020-0695-z with permission.)*

to humans.[37] Although the origin of SARS-CoV-2 remains unclear, SARS-CoV-2 shares over ∼96% genetic similarity with RaTG13, a coronavirus strain originally thought to be found among the bats that are trapped in the Yunan caves of People's Republic of China (PRC).[38–41]

Detailed structural studies using electron microscopy reveals that SARS-CoV-2 contains an icosahedral viral head structure and appears spherical with diameter in the range of ∼100−120 nm.[42,43] The virus possesses numerous envelope proteins (E) such as spike (S), the transmembrane glycoprotein (M and E), and the nucleocapsid protein (N), as shown in Fig. 1.2.[44] The uncanny resemblance of S protein on the virus envelope to
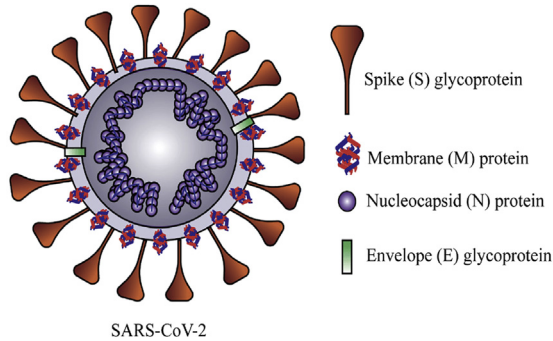
Figure 1.2 Virion morphology and RNA genomic structure of SARS-CoV-2. This structure of the virions of the members of betacoronavirus genus shows the arrangement of spike (S) glycoproteins, membrane proteins, nucleocapsid (M and N), and envelope glycoprotein (E). *(Image adopted from Sarkar C, Mondal M, Torequl Islam M, et al. Potential therapeutic options for COVID-19: current status, challenges, and future perspectives.* Front Pharmacol *2020;**11**(1428). 2020-09-15.* http://10.3389/fphar.2020.572870 *with permission.)*

that of the flares/corona of the sun or the crown of a queen, gives the virus its classical name of coronavirus. SARS-CoV-2 actively uses the S protein to engage with the host cell.[45] The S glycoprotein mediates viral anchoring on the host cell and fusion of the membranes for viral entry into the host cell. The virus uses human angiotensin converting enzyme 2 (ACE-2) to facilitate this viral entry.[46] Thus, current efforts to develop vaccines and antibodies to arrest the spread of the virus largely target the trimeric S protein.[43] Accumulation of point mutations in the S protein of the virus leading to the genomic evolution of SARS-CoV-2 has been elaborated in the following section.

1.2.1 Genomic evolution of SARS-CoV-2

SARS-CoV-2 carries +ssRNA with a genomic size of $\sim$ 30 Kb, one of the largest genomic size among the known RNA viruses.[43] A key feature of this family of viruses is that they possess large open reading frame (ORF1a and ORF1b) that occupies nearly two-thirds of the genome (from the 3′ proximal end of the genome) and encodes for nearly 16 nonstructural proteins while the rest of the genome (toward the 5′ proximal end) encodes for all the known structural proteins of the virus.[47−49]

SARS-CoV-2 appears to have a remarkable adaptation to its host organism.[50,51] One of the 16 nonstructural proteins is nsp 13 that encodes for an *exo-ribonuclease* that provides a proofreading activity for the virus, thereby

efficiently maintaining the infectivity and virulence of the virus.[52] Recently, comparative studies on SARS–CoV–2 strains isolated from patients in Wuhan (in January 2020) and that isolated from patients in Western countries (predominantly in the US, Spain, and Italy in July 2020) suggests that the virus has accumulated point mutations on its S protein, i.e., D614G, and is currently the predominant strain in the Western world, as shown in Fig. 1.3.[53] The study also demonstrated clinical evidence that the newly accumulated D614G mutation renders the virus more highly infectious than its original strain; however, the effect of this mutation on severity of the disease is not yet known.[53]

Currently, not much has been understood regarding the immunity achieved upon infection, although it appears that the severity of the infection is linked to the larger amount of the antibodies produced.[54] However, current scientific reports support the possibility of SARS–CoV–2 reinfection in individuals who have already had COVID–19, i.e., the antibodies produced by the body against this SARS–CoV–2 could last for a
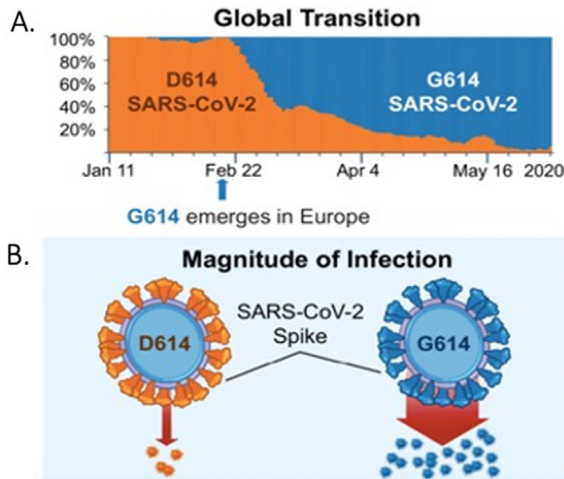

Figure 1.3 (A) Evolution of the virus: A mutation in spike protein of SARS-CoV-2 was first observed in Europe around mid-February and as of July 2020, we observe a global dominance of the mutated virus strain with a magnitude of infection over 9 times more efficient than its D614 strain. (B) Structural mutation in spike protein (from D614 to G614) showing the impact of the mutation in the viral replicability. *(Image reproduced with permission from Korber B, Fischer WM, Gnanakaran S, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus.* Cell *2020;**182**(4):812–827. e19. 2020/08/20. https://doi.org/10.1016/j.cell.2020.06.043.)*

month or less, thus leaving a previously affected individual once again vulnerable to the disease.[55,56] This appears to be a unique feature of SARS-CoV-2 compared to infections from other known infectious coronaviruses. In the past, researchers have observed that the antibodies created in the infected individuals protected them for a maximum period of three years.[57] Thus, a reinfection of masses and multiple waves of coronavirus infection is a great possibility until mass vaccination is initiated. A deeper understanding of the viral transmission, and the advantages and limitations of the current classical diagnostic tools and novel testing approaches are important to highlight the concern and importance of practicing public health safety measures during the current pandemic.

1.2.2 Transmission of coronaviruses

The rapid spread of SARS-CoV-2 across the globe has put the spotlight on the role of SARS-CoV-2 transmission dynamics.[19] Reproduction number (or R_0) is a basic parameter that allows us to estimate a disease outbreak and intensity of the infection. R_0 is defined as an average number of secondary infections caused by an affected individual when the individual is introduced to a susceptible population.[58] The knowledge of R_0 of SARS-CoV-2 is a principle tool that aids to assess the current trends and to predict the future trends of viral infectivity, i.e., disease-spreading potential of the virus. The transmission rate of SARS-CoV-2 ranges between $R_0 = 2.2$ and $R_0 = 3.9$, i.e., 1 infected person could infect ~ 4 people in the vicinity, while the median R_0 of SARS-CoV-1 and the mean R_0 of MERS were between 0.58 and 0.69 respectively.[53] The higher the R_0 the stronger the human-to-human transmission.

Among the various viral human-to-human transmission routes, three principle routes of transmission have been identified by WHO:

1. Direct or close contact with diseased individuals is a major mode of viral transmission for COVID-19 disease spreading.[59] Presence of susceptible individuals within <1 m distance from the infected person facilitates close contact viral transmission via infected respiratory droplets.

2. Contact with contaminated surfaces: Studies show that SARS-CoV-2 are highly stable over plastic and stainless surfaces and remain viable for a period of at least 72 h.[60] Thus, direct contact with such contaminated surfaces carrying sufficient concentration of the virus induces the disease in susceptible individuals.

3. Airborne transmission occurs when a susceptible individual comes in contact with a suspended contaminated respiratory droplet. Liquid droplets of diameter <5 μm can remain suspended in air and are also capable of traveling up to six feet of distance in the course of air. Such suspended droplets are also called as aerosols.[10,61] An infected individual upon sneezing generates millions of respiratory droplets that could remain suspended in the air for a long period of time and in turn contaminate other individuals nearby. This kind of viral transmission is often observed in mass public gatherings or in crowded places, and in hospital settings where medical procedures were carried on COVID-19-positive patients. Scientists have demonstrated that SARS-CoV-2 remains stable and viable in the air for at least 3 h and over surfaces such as plastic/stainless steel for at least 72 h.[60]

Multiple other modes of human–to–human SARS-CoV-2 transmission such as mother–to–child and fecal route transmission have been documented. Overall, the probability of human–to–human transmission of this virus is very high and can occur upon direct or indirect contact with infected respiratory droplets or with contaminated surfaces.[62] An important point to note in this context is that the viral transmission risk is also dependent on other key factors apart from the R_0 factor such as identifying high–risk environments, effective contact tracing for constant monitoring of disease progression, and careful implementation of government health care policies.[63]

Worldwide, many nations have adopted different policies to implement classic health care and safety management measures such as use of facial masks, implementing social distancing, etc., to curb the spread of COVID-19. Most common national level health crisis management strategies include imposing nationwide lockdown, curfews that ban outside travels at certain hours, effective isolation and contact tracing, implementing social distancing, and bans on public gatherings and transportation.[15] Strict implementation of such public safety measures has reduced the infection rate by over 50%–60% in many Asian and European countries.[15] For instance, South Korea's success in COVID-19 management is mainly attributed to rapid implementation of strict government policies drafted in collaboration with its scientific community. The country was quick to issue stay-at-home orders, pursued extensive and efficient contact tracing to identify potential cases, and most importantly, provided rapid testing and secured required numbers of personnel to implement strict social crisis management strategies.[64] For a detailed

review on all the SARS–CoV–2 transmission modes and updates on detailed government policies to tackle the viral transmission, readers are directed to other excellent reviews.[65]

1.2.3 Clinical characteristics for COVID-19

Clinically, SARS–CoV–2 is infectious to human beings irrespective of age.[66] Younger adults often develop milder symptoms compared to older adults, particularly if the older adult has other existing medical conditions.[67] The median age of infection in adults is ∼47 years. People with COVID-19 most often develop mild-to-moderate symptoms without the need for medical assistance. Most often over 80% of the COVID-19 patients develop mild respiratory disease in the form of dyspnea (i.e., breathing difficulties). Based on the statements furnished by WHO, most common symptoms reported by COVID-19 patients include fever (i.e., over ∼99% of the patients develop fever at some stage during their infection period), dry cough, and tiredness. Classical symptoms exhibited by patients include fever above 38°C, fatigue, dry cough, sore throat, diarrhea, and the characteristic shortness of breath that results in hypoxia.[68] Individuals with weakened immune systems or with other chronic illnesses (∼10%−20% of the patients) experience severe hypoxia and often require the support of ventilators.[69−71] Body aches, sore throat, diarrhea, loss of taste and smell, skin rashes or finger/toe discoloration, and headache are among less common symptoms.[72] More serious symptoms include acute to severe breathing difficulties including shortness of breath, chest pain, and/or loss of speech or movement that require immediate medical attention.

Presentation of clinical symptoms occur on an average of 5−6 days postinitial viral exposure, while it could also take up to 11 days for the onset of the symptoms. In some cases, in spite of the infection, the individuals remain asymptomatic.[73] The viral transmission dynamics by such asymptomatic individuals is not very well understood currently.

SARS–CoV–2 primarily infects the human respiratory system and with potential to infect other organs of the body such as brain, liver, stomach, kidneys, etc.[74] The pulmonary infection causes diffuse alveolar damage, which is considered as the first sign of damage. Specific host immune response results in cytokine dysregulation causing massive infiltration of large macrophages and T-cells on the respiratory tract parenchyma and induces pneumocytic proliferation (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7152395/). Such massive infiltration of the immune cells can

be observed as patches on chest X-ray scans.[75] SARS-CoV-2 can further proceed and infect the gastrointestinal tract particularly targeting the enterocytes where the virus enters and replicates causing diarrhea.[71]

Currently, suspected individuals are recommended to undergo serological testing that checks for the presence of antibodies to the virus and molecular-based diagnostic testing such as polymerase chain reaction (PCR) for pathogen detection. SARS-CoV-2 positive individuals are advised to undergo imaging-based diagnosis to assess the lung infection.[76−78] In the following sections, various challenges and new age technologies for pathogen detection and monitoring of disease progression are elaborately discussed.

1.2.4 Diagnostic detection of COVID-19

In addition to the ongoing struggle to contain the pandemic, the countries face major socioeconomic impact.[79,80] In an attempt to prevent further damage, many governmental authorities have strongly recommended coordination between the medical community and local public health authorities for developing COVID-19 health care management strategies including the use of novel diagnostic testing approaches for rapid pathogen detection.[81] Currently WHO recommended primary confirmatory diagnostic technique includes Nucleic Acid Amplification Tests (NAAT) such as real-time reverse transcriptase PCR (RT-PCR) for SARS-CoV-2 detection[82] (https://www.who.int/publications/i/item/10665-331501). A detailed overview on the principle and method of diagnosis using this technology can be found in Ref. [83] This section provides an overview of analytical issues at various stages of sample collection and processing. It further provides an overview of the primary COVID-19 diagnostic techniques highlighting their advantages and limitations.

1.2.4.1 Preanalytical issues impacting the diagnosis

As of January 2021, a typical COVID-19 diagnosis relies on genomic detection of SARS-CoV-2 from the respiratory tract samples such as nasopharyngeal (NP) swabs and/or an oropharyngeal (OP) swabs collected of suspected individuals.[84,85] The collected sample is placed into a viral transportation medium and is transported to nearby clinical laboratories for analysis.[86,87] Molecular diagnostic analysis based on nucleic acid amplification such as RT-PCR is performed to detect the pathogen in the collected sample.[88]

One of the most common issues regarding RT-PCR is the obtention of false-negative results, i.e., pathogens could go undetected in the patient

sample. The rate of obtention of false-negative results are in the range of ~2%−29%.[89] The false negative results pose a greater risk to society, since such individuals could further spread the disease adding to the difficulty in containing the virus outbreak.[90] To reduce such false negatives, technical issues must be well considered and addressed. For instance, technical issues such as stability of the RNA sample collected, the time of swab collection (i.e., the NP/OP swabs should be taken at the onset of the symptoms as viral loads tend to be higher in the region around this time), and swift swab collection procedures (i.e., collection of respiratory samples particularly NP/OP swabs requires proper prior training).[91]

Mishandling of such airborne pathogens during sample collection, handling, and transportation could be dangerous and would lead to a new outbreak of the disease among health care professionals. Such hazardous situations could be avoided by providing adequate formal training and sufficient supply of personal protective equipment (PPE) to all health care professionals dealing with COVID-19 samples and patients. The reuse of the sample processing kits should be avoided under all circumstances.[92] Thus, practicing safe and recommended methods of sample collection and maintenance of high hygienic standards in places of sample collection, manipulation, and storage are important steps toward ensuring safe diagnosis of COVID-19 in suspected individuals.

1.2.4.2 Primary diagnostic techniques for COVID-19 detection

Clinical diagnosis of COVID-19 disease is based on observation of symptoms, epidemiological history, and testing by standard molecular testing methods. Currently, the three most commonly used as molecular diagnosis for SARS-CoV-2 are RT-PCR, Loop-Mediated Isothermal Amplification (LAMP) and high-throughput Next Generation Sequencing (NGS) of the whole genome.[93] However, exploitation of NGS technology is limited due to its dependency on many instrumentations and the expenses incurred for such analyses. On the other hand, RT-PCR and RT-LAMP are cost-effective and straightforward technologies that allow the detection of pathogenic diseases. In addition, these techniques are portable, rapid, and robust in nature, requiring less instrumentation.[94] Currently, many new biosensors and nanomaterials-based techniques are being developed for the detection of infectious diseases particularly useful in the current scenario.[95−97] In the following sections, a brief overview of the three classical techniques listed above is presented.

1.2.4.2.1 RT-PCR

RT–PCR is one of the current standard method for COVID-19 detection. Respiratory samples such as NP/OP swabs collected from suspected individuals are processed to obtain the pathogenic genomic material, i.e., RNA as the source material, which is then subjected to nucleic acid amplification techniques such as RT-PCR. RT-PCR uses the reverse transcriptase enzyme to convert the RNA material obtained from the sample into complementary DNA (cDNA), which is further amplified using a DNA polymerase and primers to specific biomarker genes.[98] The technology uses fluorescent primers to amplify the genes under investigation. This facilitates real-time quantification of the relative expression of the biomarker's genes in the sample. Thus, higher expression of viral genes compared to various control samples is considered as a positive outcome for the assay. For SARS-CoV-2 diagnosis, viral genes such as N, E, S, and RdRp are being used as biomarkers.[99] SARS-CoV-2 positive samples are further confirmed by whole genome sequencing such as NGS technology.[82]

In spite of having multiple advantages (listed in the introduction of this section), there are some limitations in using the RT-PCR-based diagnosis.[100] As suspected cases of COVID-19 are surging, necessitating the rapid detection of the virus, limitations of RT-PCR based detection techniques for COVID-19 disease is more profoundly visible now than before.[101] Limitations in the detection technique include unreliable stability of the results, i.e., the efficiency of target gene and control gene do not remain identical at all times, obtention of false positive and negative results, and the unverified clinical significance of positive PCR amplicons. These are among the key issues that need to be addressed.[102,103] Multiple factors influence the molecular test, such as condition of the sample obtained, anatomical location from where it was extracted, the time of disease, i.e., the stage or the phase of the disease in the individual, and time spent for sample transportation.[104] In addition, the RT-PCR is time and labor consuming. Other methods of isothermal amplification of nucleic acids such LAMPs, antigen-based serological tests, and serum antibody tests could provide solutions to these issues.

1.2.4.2.2 RT-LAMP assays

LAMP assays are a robust, highly sensitive, high accuracy, and rapid diagnostic tool for SARS-CoV-2 detection.[105–109] It is a popular choice point-of-care (POC) technique employed for rapid COVID-19 testing.[110] This promising nucleic acid LAMP amplification technique uses six

different target sequences for the same viral gene, which increases the sensitivity and specificity of the process.[111] Moreover, as LAMP does not require expensive instruments and reagents, it is highly cost–effective. The technique uses one single heat block for nucleic acid amplification process, and results can be obtained in less than 60 min.[111] These are the critical advantages of the technique that improve cost reduction and assist in rapid COVID-19 detection at this crucial time.[112]

Recently, researchers have developed colorimetry-based reverse transcriptase LAMP (RT–LAMP) technique for further rapid detection of COVID-19 that allows one-step reverse transcription, which can be detected by a color change and is visible to the naked eyes.[110] Yu et al. developed the RT–LAMP technique for the detection of genomic RNA from SARS-CoV-2 strain and showed that technique has a limit of detection (LOD) up to 100 copies/reaction, thus demonstrating that the technique has very high sensitivity for pathogen identification. They also experimented on various other strains of coronaviruses such as human coronaviruses (hCoV-229E, hCoV-OC43), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV2 (Fig. 1.4). They claimed that this RT–LAMP process is highly specific for detection of SARS-CoV-2, and due to the colorimetric method, this tool can be exploited as a potential POC test for COVID-19 detection in the near future.[113]

This technique, however, has a few limitations, such as requiring high sample purity, sophisticated sample processing steps, issues regarding result reproducibility, and in some cases, sample stability issues have also been reported.[114,115] Currently, research is aimed at developing the techniques suitable for POC testing.

1.2.4.2.3 NGS techniques

NGS is an emerging technology that can potentially overcome some of the limitations mentioned earlier and can efficiently cater to the growing diagnostic demand. For quite some time, NGS has been used as tool to study and understand the genomic details of a pathogen. For instance, at the initial stage of SARS-CoV-2 outbreak NGS technology was used for identification and discovery of the viral strain.[116−118] Early identification of this virus has helped to conduct rapid and promising advanced research on the virus, which has led to the rapid production of medicines and vaccines against viral infection.[119,120] Later, RT-PCR techniques helped to detect the specific sequences of the SARS-CoV-2 genome. Moreover, NGS tools
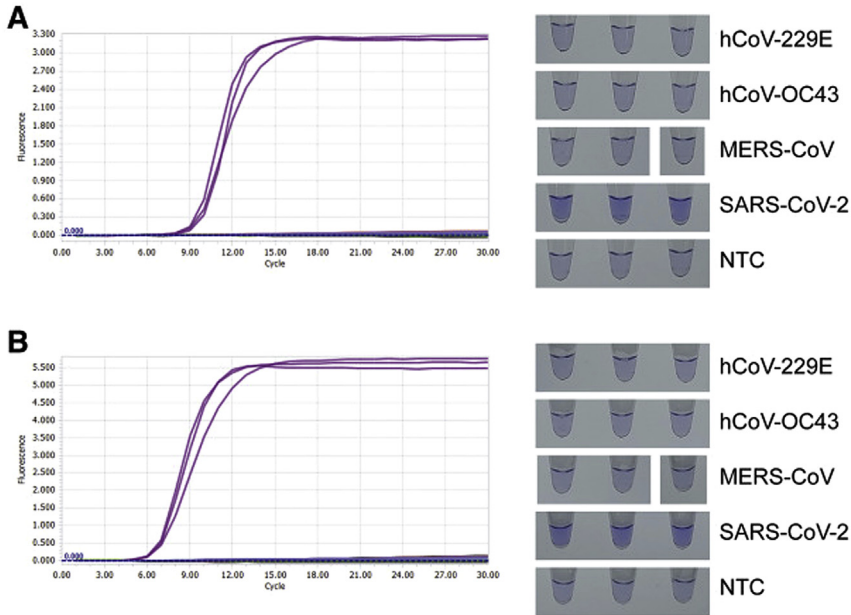
Figure 1.4 RT-LAMP assay for detection of SARS-CoV-2. Series of selectivity tests are conducted among human coronaviruses, MESR-CoV, SARS-CoV, SARS-CoV-2, and negative templates by colorimetric RT-LAMP process. (A) and (B) represents different sets of newly designs SARS-CoV-2 LAMP primers. *(This figure is reproduced with permission from Park G-S, Ku K, Baek S-H, et al. Development of reverse transcription loop-mediated isothermal amplification assays targeting severe acute respiratory syndrome coronavirus 2. J Mol Diagn 2020. 2020/04/07. https://doi.org/10.1016/j.jmoldx.2020.03.006.)*

have been instrumental for identification and detection of the mutation frequency in the virus and thus providing a detailed overview of the evolution of the virus over time.[121,122]

Nanopore sequencing is a third–generation sequencing technique that has been used for detailed viral genome analysis from various clinical specimens.[123,124] This technology also measures RNA molecules from the viral genomic sample without the necessity of generating cDNA for analysis. Such techniques offer complete genome information of the pathogen, thus providing novel research insights that help in understanding the nature of the pathogen.[117]

The use of computational tools for analyzing the sequencing data including the genome analyses using NCBI's Basic Local Alignment Search Tool (BLAST) is often time and labor consuming to generate whole outcomes.[125,126] Thus, such genome analysis techniques are highly expensive and time–consuming compared to simple RT-PCR, RT–LAMP, or other molecular diagnostic assays.

1.2.4.2.4 Serum-based antigen/antibody-based detection techniques

Antigen-based detection techniques employ patient serum and detect antigens present in patient samples. Many antigen detection techniques are highly sensitive, robust and rapid. Antigen-based detection techniques are currently available in the market. Many serology-based tests are highly compatible with high-throughput analysis, i.e., thousands of samples could be processed in very less time compared to conventional techniques.[127] The success of such serology-based tests and their commercialization will provide rapid answers to suspected COVID-19 patients, and subsequently contribute to increased efficiency in contact tracing and precautionary isolation approaches that will truly contribute to efficient containment of the disease.[128−130]

Although such techniques are highly specific, they are very sensitive to the mutations accumulated by the RNA, i.e., they cannot successfully identify the pathogen, should they evolve over time. In spite of the aforementioned issues, the capacity of the technology for rapid COVID-19 detection testing has powered the advancement of various serological approaches.[131] Many nations worldwide have exploited such serological methods for initiating surveillance of suspected individuals. For example, as of January 31, 2020, Singapore has employed more serological based SARS-CoV-2 testing than the traditional RT-PCR technique for mass COVID-19 testing in the country. They have been successful in identifying many positive cases using this approach.[132]

A major advantage of this technique is that many asymptomatic patients have also been diagnosed as COVID-19 positive since this technique involves detection of antigens and antibodies in the patient samples.[133] Hence, alongside molecular testing, application of serological testing is also an important assay.

In spite of all these advantages, these antigen-based detections have few limitations, such as the importance of time of sample collection, i.e., often antigen productions are low at the initial phase of the symptom onset, low sensitivity of the technique, potential false-negative results, chances of cross-contamination with closely similar antigens, requirement of trained personnel for sample obtention, requirement of a preenrichment processes.[134,135] Further development on ELISA-based detection is continuing and near future these problems will be resolved.

1.2.4.3 Novel Point-of-Care approaches and their limitations

Various biomolecular and immunoassays have been developed for the rapid detection of SARS-CoV-2. Assay selection is an important issue for efficient detection of SARS-CoV2. Few important POC immunoassays that are currently employed for the detection of the virus are lateral flow assays (LFAs), serological assays, and high–throughput immunoanalyzer assays.[136] The LFA process identifies antigens of SARS-CoV-2 by detecting antibodies such as IgM and IgG against COVID-19 disease.[137] This immunoassay, similar to qRT-PCR process, offers rapid and cost-effective detection of the virus. However, this assay is not as sensitive as other biomolecular assays (Table 1.1).[138–140] Few studies have showed nonspecific detection of IgM against COVID-19 in the immunoas-says.[141,142] Thus, the ongoing research, on the one hand, is looking for specific monoclonal antibodies against SARS-CoV-2, and on the other, is searching for other speedy antigen detection assays that can be reliably employed for the diagnosis of COVID-19.[143]

Assay selection for molecular detection of SARS-CoV-2 is also a current issue. Rapid amplification-based sequencing methods play a key role in the detection of the disease. The preliminary quick and robust detection is performed using molecular techniques such as qRT-PCR, RT-PCR, and RT-LAMP process.[149] A complete genome analysis is performed using NGS platforms in the second stage of further confirmation of the virus in positive samples.[150]

RT-PCR assay is the most commonly employed molecular technique in the first stage of the virus detection in patient samples.[6] Other molecular methods, such as LAMP, CRISPR-Cas9, and microarray-based techniques have shown great potential for the detection of the disease.[107,151–153] One of the major advantages of these molecular techniques is that the sample is amplified before analysis, and such amplification procedures significantly reduce the false-positive results. This helps to avoid amplicon contamination. These techniques are highly specific, and since only a few strains of coronaviruses that cause respiratory disorders are circulating at the moment, such high specificity is another added advantage for these techniques.[154]

The aforementioned advantages could also prove to be a limitation since both SARS-CoV and SARS-CoV-2 are a group of SARS-like bat origin coronaviruses.[155] Therefore, there is a possibility and risk for making nonspecific molecular observations in some samples. To overcome such issues regarding nonspecificity, the WHO and CDC recommends the use

Table 1.1 Advantages and disadvantages of different techniques to detect SARS-CoV-2.

Diagnostic technology	Detection limit (LOD)	Specificity	Response time	Specimen	Advantages	Disadvantages	Identification	References
RT-PCR	3.9 copies/reaction	99%	∼190 min	Samples from fecal	High specificity and sensitivity, used as gold standards procedure	Thermal cycler is needed, costly reagents and fully trained laboratory skilled personnel is needed and time consuming	RNA	94
Real-time RT-PCR	11.2 genomic RNA copies/reaction	Good specificity, tested with different other coronaviruses	Not mentioned	Nasal, oral and throat swabs	Good sensitivity and specificity	High-end sophisticated instrument is needed	Genomic RNA	144
One-step qRT-PCR	10 copies/reaction	Good specificity, checked with other strain of viruses	Not specifically mentioned	Nasal swabs and stool samples	Very specific and sensitivity wise is high with the vast capacity of samples experiments	Costly, laborious, and false-negative chances are there	RNA	145

Table 1.1 Advantages and disadvantages of different techniques to detect SARS-CoV-2.—cont'd

Diagnostic technology	Detection limit (LOD)	Specificity	Response time	Specimen	Advantages	Disadvantages	Identification	References
RT–LAMP	10 copies/ reaction	As good as RT-PCR process	∼40 min	Nasal, oral and lower respiratory swabs	No high-end instruments are needed, perfect for on spot examination, affordable, rapid and good sensitivity	False-negative chances and cross–contamination issues may occur	RNA	146
RCA	100 copies/ reaction	Good specificity	∼90 min	Respiratory swabs, and urine samples	Good specificity, rapid	Not so good sensitivity, difficult to prepare circular probe for the experiment	Genomic DNA	147
ELISA	Not specific	95%	240 min	Nasal, oral and lower respiratory swabs	Good for antibody testing	Costly, time consuming and specific instruments are needed and as compatible as PCR process	Antigen of SARS-CoV-2	148

of at least two molecular assays for testing for instance, the first-line screening with E gene assay followed by RdRp gene assay as confirmatory examination.[94]

1.2.5 Late detection issues

Generally, late detection of patients or asymptomatic individuals are problematic in terms of isolating infected patients from healthy populations. Many studies indicated that the viral loads of SARS-CoV-2 RNA is immensely high in the bronchoalveolar specimens, i.e., deep respiratory samples than Nasal/oral swabs.[156] Thus, the typical samples such as the sputum or bronchoalveolar specimens collected from lower respiratory tract contain the highest SARS-CoV-2 viral loads.[157] A recent study reported that the positive samples collected from bronchoalveolar lavage fluid showed the highest number of viral loads present in the sample.[156] The classic symptoms of COVID-19 can be two types, such as mild and severe. Mild symptoms are generally cough, fever, mild headache, sneezing, and fatigue.[158] However, these mild symptoms are similar symptoms of flu/influenza.[159] Sometimes, it may be confusing to differentiate between normal flu and COVID-19 because of such mild symptoms until severe symptoms such as lung congestion and shortness of breath appear.[160] By this time, it is often too late to treat the infected patient. Such patients must be admitted to a hospital, particularly to the intensive care unit (ICU) and may probably need life support. Alternatively, some studies have also reported patients with high viral loads of SARS-CoV-2 in their fecal material.[20,161] Therefore, for COVID-19 testing with swabs from respiratory tracts are not sufficient. Other studies have also reported SARS-CoV-2 viral loads from enterocytes in the digestive tract by means of electron microscopy.[81] Thus, for efficient sample collection and diagnosis, multiple swab samples should be obtained from the same suspected patients.[162,163]

1.2.6 Monitoring of severely affected COVID-19 patients

Continuous monitoring of critically ill COVID-19 patients is a great challenge. Critical patients have a high amount of SARS-CoV-2 RNA load in their body organs. Since higher viral loads have also been found in fecal material and in the respiratory tract of the infected patients, analyzing at least two samples from patients at the beginning of hospital admission is very crucial for patient monitoring.[156,162,164,165] Practicing standard hygienic practices such as thorough cleaning is mandatory, especially in the

clinical houses or hospitals, objects/articles that are related to COVID-19 patients, and the patient-frequented washrooms. Listed below are the CDC guidelines for maintaining high hygienic standards while handling COVID-19 patients, their clinical samples, and other related articles[166,167]:

(1) At the preliminary stage, the container containing the obtained patient sample should be sealed with a screw cap. It must inform the patient on the spot of examination centers.

(2) The container must have low risk of breakage and low chances of contamination, so it is suggested to carry a cotton swab inside a conical tube.

(3) The details of the individuals should be noted and recorded officially. The information should be written on the specimen container. For example, containers should have their name, number, and a specific barcode with date of collection.

(4) The outer surface of the container should be wiped with 70% ethanol and the container should be packed in a zipper bag before transportation.

(5) While transferring the specimens to the testing laboratories or hospitals, clinical staffs should wear PPE. The personnel handling the patient sample should have proper training. And a separate route should be used for such transportation.

(6) After transferring the specimen to the biosafety level 2 (BL2) laboratory, the personnel should change PPE, masks, and gloves. These articles should be disposed in a closed container after properly disinfecting.

(7) The laboratory bench should be cleaned with bleach or other strong disinfectants.

(8) The extraction of nucleic acids should be performed inside the higher biosafety cabinet (BSC) with standard protocol and precautions.

(9) A standard operating process should be used in all clinical laboratories. Currently real-time RT-PCR is being used for COVID-19 diagnosis.

(10) While using commercial kits, the instructions and all other manufacture information should be followed properly.

(11) To prevent the chances of cross-contamination during RNA extractions and pre-PCR process personnel need to take care of extra precautions and should follow the guidelines of biosafety rules. Personnel should wear gloves, masks, gown, and eye protection in a BL2 laboratory.

(12) At the end, all infectious waste such as residual specimens, contaminated reagents, PPE, tips, etc., should be properly disposed by following the institute recommended guidelines of handling infectious waste samples.

Further, the contaminated patient samples like swabs, stool cultures, surfaces, and instruments such as PCR machine, electron microscopy, and CT scanners have to be handled with extra attention, and proper sanitization protocols should be followed.[168] Some studies claim that handling respiratory samples are harder than handling stool samples from the patients because of the presence of higher viral loads in respiratory samples than in stools samples.[169] Currently, the preferred method for detecting SARS-CoV-2 is real-time PCR technique from a respiratory swab sample.[162,163,170]

1.2.7 Other health aspects due to COVID-19 and related health management factors

In a very broad spectrum, handling with proper management of COVID-19 patients and associated factors are very challenging and hectic. Implementation of good health management policies and recruitment of sufficient amount of manpower for implementing those measures together with the appropriate use of advanced technologies is crucial for efficient containment of the virus.

This pandemic situation has resulted in a high level of work stress among health care professionals who face the danger of being directly exposed to SARS-CoV-2 on a daily basis. The high-level work stress, long periods of confinement, and self-isolation approaches have negatively impacted mental health, giving rise to problems/issues such as depression and anxiety.[171] Pressure of loss of jobs due to economic recession and loss of loved ones due to COVID-19 bring greater risk of psychological stress in such individuals.[172] To reduce such mental pressures among health care professionals, equal work distribution should be made practical. The governing authorities and health care policy makers should undertake strong management factors with good leadership to support health care professionals and families. Fig. 1.5 shows few health managements factors that are necessary for maximal effective containment of virus. This section of the chapter also discusses other health management factors for other health issues caused by COVID-19.

1.2.7.1 Direct COVID-19 risk management

As mentioned earlier, early detection of COVID-19 with gold standard technologies are necessary alongside a few other issues that should be considered, for instance, postvaccination surveillance,[173] and the timely evaluation of preexisting medical risks that could trigger another disease outbreak causing further damage.[174] In general, the requirement of the
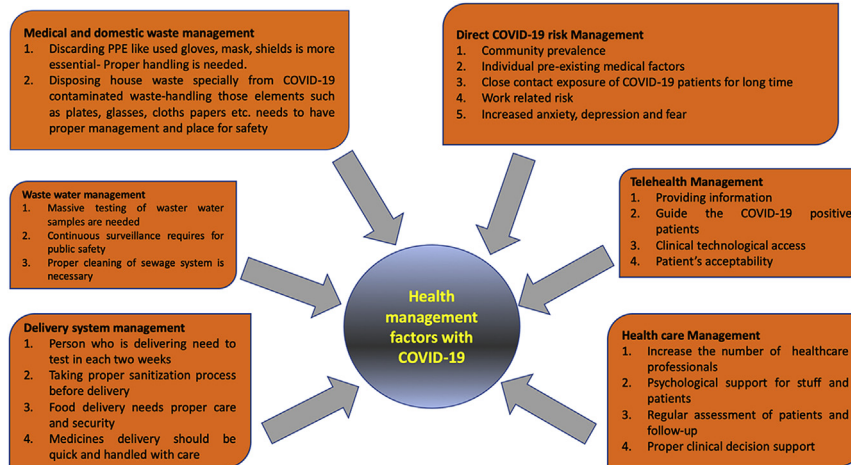
Figure 1.5 The framework of health management factors for taking good care of COVID-19 patients and environment at the same time during the pandemic.

physical presence of health care professionals in close proximity to the COVID-19 patients for treatments and other purposes poses a great risk and increases the chance of direct contamination with the virus manifold times. Thus, proper health care management is required to reduce such risks. For instance, the increase in the number of health care professionals dealing with COVID-19 patients and samples, and adequate production and supply of PPE are essential in addition to rapid COVID-19 testing on a daily basis.

1.2.7.2 Medical and domestic waste management

The requirement of proper environmental waste management strategies is a highly significant, growing concern as each day passes during this pandemic. Due to large-scale disposing of huge amounts of medical and domestic waste on a daily basis, environmental pollution is becoming a more serious problem. Medical and domestic waste in many countries such as China, USA, Spain, and Italy has increased in excessive quantities.[175] Reports indicate that in Wuhan, over 240 metric tons of contaminated medical waste are being generated on a daily basis.[176] Household waste has also increased due to online shopping and home delivery options, which are not getting recycled.[177,178] Because of the fear of further spreading of the virus, used and contaminated articles such as gloves, masks, expired medications, and other contaminated PPE cannot be recycled.[178] In future, researchers need to focus on more recycling and disposing processes of medical and domestic waste.

1.2.7.3 Telehealth management

Telehealth management is one of the best approaches to guide and advise patients. This approach optimizes in mitigating the risk of exposure, and thus is an excellent initiative toward reducing the spread of the virus. In the past, telehealth had been suggested due to a huge number of patients queries and the phone consulting facilitated appropriate medical care without the need for huge patient lines and/or waiting time.[179] The main aim for implementing telehealth management is to provide access to health care systems from rural areas.[180] However, in the recent scenario of COVID-19, telehealth management is proving to be a great boon. This management is used across all the medical specialties for maintaining patient access to care while successfully maintaining physical distance from the patients.[181]

For example, hospital admission formalities of a COVID-19 patients such as information about the patient history and the current status of COVID-19 disease can be done using telehealth systems. In general,

through this management process, consultants will directly discuss with patients and provide them the required services during the telehealth consulting sessions. This way, telehealth care reduces by half the number of patients visiting the medical center and successfully avoids the exposure of low-risk patients to the virus in hospital settings.[182] The study by Aziz et al. provides a new norm for handling medical visits for pregnant women under the current COVID-19 circumstances. The authors demonstrated that medical consultations of pregnant women who are in <11 weeks of gestation, i.e., the first doctor visit could also be done virtually where patient's clinical history can be taken. Genetic screening and clinical requirements could be reviewed. At a later stage, around 11—14 weeks of gestation, physical examination and prenatal blood examinations could be performed. The following 4 weeks, patient could continue to meet their doctors virtually depending upon the examination reports. Further medical visits of the patient up until the time of delivery could be virtual or on site depending on the patient's condition, while physical presence would be required for an ultrasound examination.[182] By following such practices, the exposure of high-risk and low-risk patients can be significantly reduced, which will play a major role in exposure prevention.[183]

1.2.7.4 Wastewater management

Many findings have reported that traces of SARS-CoV-2 is found in wastewater along with particles of disinfecting elements.[184] The byproducts of disinfectants cause toxicity in wastewater and discharges into rivers and oceans, which is spoiling the environment and public health indirectly.[185] The record from UN's world water development showed the 80% of wastewater is polluting the environment without enough treatment process.[186] One of the studies by Wang et al. stated that SARS-CoV strain was found in medical waste, domestic sewage, and tap water, which has led to fast spread of the virus.[187] Hence, the cleaning and treatment process and proper waste management is highly necessary for containment of the COVID-19 situation. To reduce the chances of virus and the quantity of disinfectant in wastewater, large-scale waste management strategies and treatment plants could be set up.[188] In such situations, the industrial partners and governmental agencies should come forward and work together for the establishment of a central wastewater treatment setup that will benefit everyone.

1.2.7.5 Other health management factors

Besides the aforementioned health management issues and strategies, there are a few other factors on which we must focus. For instance, increasing home confinements have drastically increased the concept of online food and article shopping. With increased home delivery of food, groceries, and other products, proper sanitization protocols and minimal contact with people should be strictly followed by the delivery and transportation system to minimize the risk for further virus spread. The CDC has laid proper guidelines that should be followed to minimize such risks. In addition, as side effects to some health management strategies, other mental health issues such depression, anxiety, and other disparities have been reported in some people due to long periods of nationwide lockdowns, self–isolation, and quarantine rules.[189,190] Therefore, a good balance in implementing the health management strategies considering all of these factors is necessary to successfully overcome this pandemic.

1.3 Conclusions

This chapter provides an overview of the COVID-19 disease caused by SARS–CoV–2, the responsible viral agent for the currently ongoing pandemic and world health crisis that has killed millions of people worldwide. A brief history, evolution, transmission of this virus, and comparisons of various diagnostic technologies are reported in this chapter. In addition to this, preventive measures by CDC and WHO are listed along with many health management factors that are discussed in this chapter. SARS–CoV–2 is a zoonotic virus and is thought to have spread from the Wuhan's live seafood markets in the Hubei province of China. The salient features of this virus such as the high replication rate, very high human–to–human transmission capacity, the potential to infect most body parts, and the persistent virulence of the virus over contaminated surfaces has made it one of the deadliest pathogens known to mankind. Therefore, understanding the origin and nature of the virus, the route of transmission, mode of interaction with host, and pathogenesis are essential for developing better medications, effective therapeutics, and vaccines to overcome this pandemic.

Among the various health management strategies implemented by various nations across the globe, lessons should be learned from nations that have been successful in implementing the public health management policies. Few health care management factors listed in this chapter such as

adequate support to health care professionals and provisions for health care facilities, appropriate use of diagnostic tools and advances in technologies for monitoring the disease spread, and proper establishment of waste management are essential to contain the virus spread. Thus, ensuring a proper health care risk assessment, rapid establishment of the governmental health care management strategies upon careful consideration of a wide range of health care factors mentioned in this chapter could help to achieve success in containing the current pandemic, further preventing more human loss.

Since COVID-19 first emerged in December 2019, the scientific communities, public authorities, private enterprises, and people around the globe have relentlessly contributed to the efforts initiated by their countries during the pandemic. We hope that vaccines and better medications should soon reach all the people and that the world returns to normalcy.

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