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 2

State-of-the-art equipment for rapid and accurate diagnosis of COVID-19

Navchetan Awasthi[1,*], Swati Gupta[2,*], Amritanjali Kiran[3] and Rohit Pardasani[4]
[1]Harvard University, Cambridge, MA, United States
[2]Indian Institute of Science, Bengaluru, India
[3]Centre for Cellular and Molecular Platforms (C-CAMP), Bengaluru, India
[4]General Electric Healthcare, Bengaluru, India

2.1 Introduction

First reported to World Health Organization (WHO) as pneumonia of unknown cause and originating in Wuhan (China), COVID-19 on February 27, 2021, has infected at least 113,076,707 people and killed 2,512,272 people worldwide. It was declared as a pandemic in early March 2020, and it has affected more than 200 countries and territories across all continents around the world.

When the tests to identify the causative organisms started, the laboratories were unable to find any known pathogens. The metagenome sequencing of samples from patients revealed that it was a new virus, which was named 2019 novel coronavirus (2019 nCoV) initially, that showed more than 88% similarity to two bat-derived SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21) (Lu et al., 2020), and 96.2% genome similarity to BatCoV RaTG13 (Zhou et al., 2020), suggesting that the virus may have been originally present in bats. This virus was renamed from 2019 nCov to the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). It is capable of causing serious respiratory problems, and is now suspected to spread via multiple possible routes including direct, close, or indirect contact with an infected person, droplets, fecal–oral route, surfaces via fomite formation, and blood-borne (Chia et al., 2020; Liu et al., 2020; Luo et al., 2020; van Doremalen et al., 2020; Wang, Hu, et al., 2020) with an incubation period of 3–14 days. Although it has a lower severity, it has a higher reproduction rate than coronaviruses that have previously affected humans like MERS-CoV and SARS-CoV. It has a reproduction number (R_0) of >2.5, that is, an infected person on an average can infect 2–3 healthy people (Chen, 2020). This is a major cause of concern, especially with reports of a large number of asymptomatic patients who can still spread the virus. To enter host cells the SARS-CoV-2 virus uses ACE2 (angiotensin-converting enzyme 2) receptors, which are predominantly present in type 2 alveolar cells, and it affect the lungs. It is deadly in

* Authors Navchetan Awasthi and Swati Gupta contributed equally to this chapter.

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patients with preexisting conditions, such as cardiovascular disease or diabetes (Chen et al., 2020; Huang et al., 2020), and has thus shown more occurrence and higher mortality rate in people aged >60 (Harapan et al., 2020). The symptoms of being infected by coronavirus are very similar to common flu-like fever, cough, chest pain, and fatigue but get elevated to severe pneumonia, acute respiratory distress syndrome (ARDS), and septic shock that can lead to multiple organ failure (Chen et al., 2020; Zhu et al., 2020, p. 201).

Since no vaccine, antiviral drug, or cure is available patients currently are being treated only for symptoms. Some drugs like Remdesivir (anti-Ebola drug), hydroxychloroquine (Zhai et al., 2020), ribavirin, and other antiviral drugs are currently being tested (Shen et al., 2020), but they may have multiple side effects including acute kidney injury and liver damage (Nyarko, Boateng, Kahwa, & Boateng, n.d.). The only measure to control the pandemic in its current state is rapid, accurate detection and isolation of confirmed and suspected cases. Countries across the globe have observed 3–4 months of lockdown in order to prevent transmission via self- and institutional isolation. In spite of this, the sudden outbreak of cases, lack of proper healthcare facilities including isolation wards, ventilators, PPE kits, etc., and nonawareness of people has placed a huge burden on governing bodies, especially in developing and overpopulated regions, to control on the current situation. The economy is falling and the people are losing their livelihoods. Diagnosis has been a very important part of battling this pandemic. Scientists, industries, and other independent researchers have come up with numerous diagnostic kits that work on different principles. Some of them are standard kits already being used, while others are yet to be approved or are still in the testing phase. Each one of these has advantages and disadvantages for their use. We will discuss all of the diagnostic techniques in the next sections.

2.2 Types of diagnosis techniques

Various testing kits are available today for use in clinical settings. These use various methods to detect the presence or absence of SARS-CoV-19 or COVID-19. Some look for antibodies specific to the virus (ELISA), some match for a particular nucleic acid composition in the sample, while others check if there are visible pathological changes in body organs by imaging. There are also other tests that are currently being developed and not approved, including CRISPR-based tests. We have a long way to go to find faster and cheaper substitute testing procedures to cope with growing demand from hospitals as well as be prepared for another such outbreak in the future.

2.2.1 Viral RNA detection-based techniques

These methods use the RNA of the viral genome for the identification of infected individuals. The RNA might be used directly or converted to cDNA before the actual diagnosis. Since there is a similarity between different viruses, these methods can also help to see variations in different viruses and how they have evolved. The methods are mentioned below.

2.2.1.1 Next-generation sequencing

In this technique, the genetic constituent DNA/RNA from the sample is sequenced and may be compared to previously known pathogen sequences to identify the causative organism. For COVID-19, next-generation sequencing (NGS) was able to establish that this pandemic was being caused by some pathogen that has not been seen or treated yet, that is, it is novel (Massart et al., 2018). By sequencing the virus, we got to know about its constitution. SARS-CoV-2 is a coronavirus belonging to the *Coronaviridae* family. It shows a 79% sequence similarity to SARS-CoV that had previously infected the world (Zhang & Holmes, 2020). It has 27 proteins; the spike (S) protein that binds the host cells, nucleocapsid (N) protein, membrane (M) protein, and small envelope (E) protein are the prominent ones (Wu et al., 2020). NGS was also able to tell us the closest relatives of the virus and pointed out possible routes for its origin. One can get information about mutation and recombination rates, sites, and various strains of the virus.

Advantages

1. This test gives rapid results with high specificity.
2. The test has also been able to tell us about how the virus has mutated ($\sim$2.5 nucleotides per month) (Duchene et al., 2020) and it has been observed that different mutations caused different severity of symptoms in patients. Phylogenetic trees to showcase the mutation rate, the evolution of the virus, and the demographic patterns of viral strains across the globe are being monitored and studied (Meredith et al., 2020).

Limitations

This method suffers with the following limitations:

1. There is a requirement of costly equipment and the test runs on very specific machines. The chemicals used are also quite expensive.
2. The test is highly time-consuming.
3. The test results are low throughput.
4. Due to specific machinery usage, there is high expertise required to run the machinery.

Recently, FDA gave an emergency use authorization (EUA) to Illumina NGS for an NGS-based diagnostic kit, the first of its kind (First NGS, 2020). Called COVIDSeq, the test will be able to process around 3000 nasopharyngeal or oropharyngeal samples at a time with results within 24 hours. This test could suffer from the requirement of trained personnel to operate the machinery, and the lack of sites where the equipment is installed.

2.2.1.2 Real-time reverse transcriptase–polymerase chain reaction (RT-PCR)

Reverse transcriptase–polymerase chain reaction (RT-PCR) has been the gold standard molecular diagnostic test for numerous viral detections previously. It gives a qualitative detection signal for the nucleic acid being tested. Even with COVID-19, real-time RT-PCR was one of the first diagnostic tests to be approved for clinical diagnosis.

Polymerase chain reaction (PCR) is an amplification process that enables one to make multiple copies of a target gene. Here, the DNA is split into its two strands, the target gene selected with help of a primer, designed specially to identify that gene, and the DNA polymerase which makes copies of the gene continuously along the template segment. Coronavirus has RNA as its nucleic acid which needs to be converted to cDNA first for PCR, and so the reverse transcription is required as a preprocessing step. The process is thus called RT-PCR. The real-time RT-PCR is a variant of the same, which gives a diagnosis even in early infection stages and has higher sensitivity than conventional RT-PCR for detecting coronavirus (Corman et al., 2012; Noh et al., 2017).

This method is highly specific and sensitive with the requirement of specific primer-probes designed for the identification of proteins from viral RNA. To increase specificity and take into account mutation rates in the virus, patient samples are as a standard procedure tested for two target viral genome proteins, including the SARS-CoV-2 nucleocapsid protein (N gene), an envelope protein (E gene), RNA-dependent RNA polymerase (RdRp) genes, open reading frame-1 antibodies (ORF1ab), and target genes N1, N2, and N3; one as a screening assay and the other as a confirmation test. Samples can be collected from saliva, nasal swabs, bronchoalveolar lavage fluid (highest positive rates), pharyngeal swabs, sputum, blood, and feces, although each one has different rates of showing false negatives depending on patient condition and other physiological conditions (Wang, Hu, et al., 2020; Zhang, Du, et al., 2020).

The RT-PCR begins with the collection of patient samples, mostly from the upper and lower respiratory tract. The swab samples are collected and placed in buffer lysate and transported to laboratories. Here, the RNA is purified from the sample. If not being processed immediately, the samples are stored at 4°C. Depending on the method of amplification observation, different kinds of probes and fluorophores bind to target nucleic acid (SARS-CoV-2 RNA here). Primers now bind opposite strands and reverse transcriptase enzyme does primer extension and forms the cDNA. The first cycle thus forms cDNA, which is amplified in subsequent cycles of PCR by cDNA denaturation, primer annealing, and extension. The important thing is that the primer and probe are designed in such a way that only the target sequence is amplified. This amplification signal is quantified and thus helps to check for the presence of viral genetic material in the sample [see Fig. 2.1 (4a–7a)].

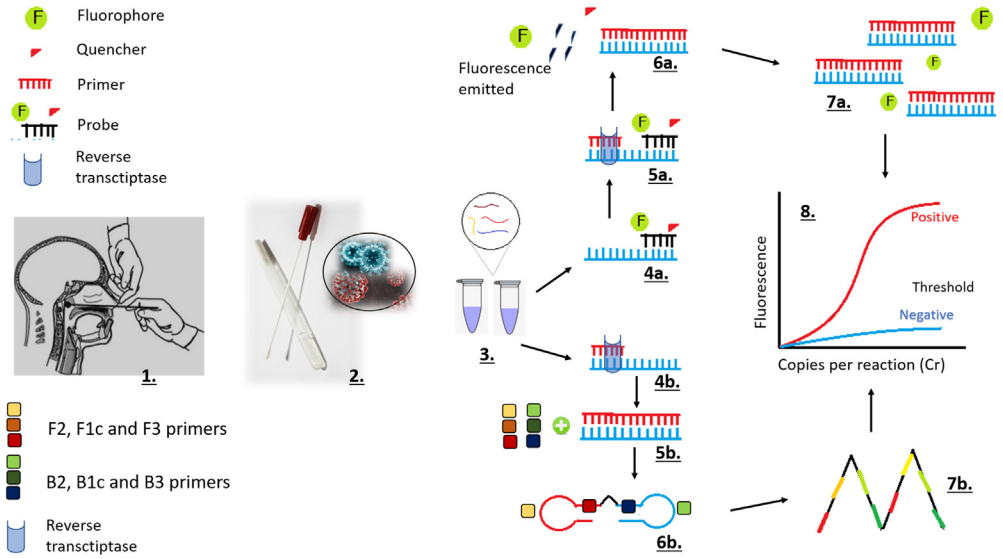

Figure 2.1 ***Image showing protocol for reverse transcriptase- polymerase chain reaction (RT-PCR) (4a–7a) and reverse tran-scriptional loop-mediated isothermal amplification (RT-LAMP) (4b–7b).*** Steps 1, 2, 3, and 8 are common to both procedures. (1) Shows a nasopharyngeal sample being collected from a patient. (2) and (3) Show extraction of viral RNA from swabs that are stored in appropriate buffer solutions while being transported or stored. RT-PCR. (4a) Shows attachment of a quencher molecule to the RNA. Primer binds to the target RNA only and reverse transcriptase does an extension using RNA as a template to form cDNA. This leads to the release of the quencher and fluorophore, as shown in (5a) and (6a). Finally, amplification cycles of PCR take place from the cDNA (7a) and the plot of fluorescence versus reaction cycle number gives us proof of infection or noninfection (8). RT-LAMP: (4b) shows the primer annealed to target RNA and reverse transcriptase starts extension. After cDNA formation, six primers (three forward, three backward) specially designed for target RNA are used for the procedure of LAMP (5b.). The extension starts with a stem–loop structure formation and then extends in a zigzag manner as shown in (6b) and (7b), respectively, at a constant temperature. As in RT-PCR, the plot in (8) determines the presence or absence of infection.

Numerous companies have already developed these kits, such as the Allplex 2019-nCoV assay by Seegene Inc. in Seoul, Korea [not yet FDA cleared but approved for detection of SARS-CoV-2 nucleic acid; Allplex 2019-nCoV Assay(1)[1]; Allplex 2019-nCoV Assay(2)[2]]; LightMix Modular Wuhan CoV RdRp-gene test, by Tib Molbiol GmbH, Berlin, Germany [FDA approved; Accelerated EUA Summary Sars COV-2 Test (Laboratorio Clinico Toledo)[3]]; COVID-19 RT-PCR test by LabCorp (FDA approved; LabCorp COVID-19 RT-PCR test EUA[4]); and others, some of which are

[1] https://www.fda.gov/media/137178/download.
[2] http://www.seegene.com/upload/product/IFU_FDA_COVID19_Seegene.pdf.
[3] https://www.fda.gov/media/139788/download.
[4] https://www.fda.gov/media/136151/download.

approved for commercial use while others are not. (PCR Kits approved for testing of COVID-19 as on July 6, 2020[5]; list of kits approved by FDA[6].) Each kit is developed such that it can test specific viral genes/proteins using a particular type of sample only, have different degrees of cross-reactivity, and have minor differences in protocols, mainly due to what reagents are used.

Advantages

1. This is a highly specific and sensitive method.
2. The test can give results using different types of patient samples.
3. It has been used previously for the identification of viral RNA, thus it is the gold standard protocol for testing.

Limitations

1. This test suffers from false negatives in results. The false negatives can occur due to many reasons in RT-PCR, for example, improper storage and/or transportation of samples, low viral load, and improper sampling (Huang et al., 2020; Lai, Wang, Ko, & Hsueh, 2020, p. 201).
2. Diagnostic kits for this method from different companies require primers and chemicals designed for their own protocol.
3. The method requires costly equipment and chemicals, and is laborious.
4. The sample collection, transportation, and extraction of nucleic acid processes make the testing procedure time-consuming as well as increase the chances of error creeping into the results.

2.2.1.3 Reverse transcription-loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification method, currently being used for diagnosis of SARS-CoV-2. It uses a set of four specially designed primers that bind to six locations on target DNA and amplification occurs rapidly and with high specificity, at a constant temperature using DNA polymerase (Notomi et al., 2000; Coronavirus disease COVID-19 outbreak, n.d.). Reverse transcription-loop-mediated isothermal amplification (RT-LAMP) is unlike RT-PCR in that it amplifies the target sequence at a constant temperature, removing the need for a thermocycler, and thus is faster, less complex, and cheaper. Once the primers are appropriately designed, this method can easily be set up with a simple water bath, reverse transcriptase, a DNA polymerase, and the primers. The reaction time varies from 15–40 minutes depending on the amount of RNA in the sample.

[5] https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2018/UploadPublic_NoticesFiles/PCR%20Kit-06-07-2020.pdf.

[6] https://hitconsultant.net/2020/04/23/in-depth-32-fda-approved-covid-19-testing-kits/#.Xz5zzOgzbIU.

This method holds high promise for the detection of SARS-Cov-2, especially in developing countries. In addition to these advantages, the sensitivity for detecting another virus, SARS-Cov, which is quite genetically similar to SARS-CoV-2, was found to be comparable (Shen et al., 2020; Yu et al., 2020; Zhang, Odiwuor, et al., 2020) or even higher (Hong et al., 2004) to RT-PCR-based methods (Radiation Protection and Safety in Medical Uses of Ionizing Radiation, 2016). Zhang and Holmes (2020) showed that the method could use direct tissue or cell lysate in addition to purified RNA from samples. This method has previously been used in the detection of the Zika virus, dengue viruses, and Japanese encephalitis virus, MERS Cov, and SARS-CoV. For the latter two viruses, it showed no cross-reactivity with other viruses (Hong et al., 2004; Shirato et al., 2018).

The principle and procedure of amplification here is different from RT-PCR. Here, the four primers are designed such that two of them are inner primers that bind the target sequence internally while the other two are outer primers. This leads to the initial formation of dumbbell DNA that with the next cycling step converts to a stem–loop DNA structure, in which the amplification of target sequences takes place [see Fig. 2.1 (4b–7b)]. The procedure is introduced and explained thoroughly by Notomi et al. (2000).

Gel electrophoresis was initially used to study the amplification endpoint. Now, a fluorescence dye or measuring turbidity due to pyrophosphate precipitation is used for real-time analysis, making the process less time-consuming. Alterations in techniques to monitor output signal by use of quenching probes, pH indicators, or visualization strips have helped improve the performance of RT-LAMP by making it more specific, cheaper, and simpler (Jiang et al., 2015; Shirato et al., 2018).

Advantages

1. This method is cheaper and less complex machinery is used (i.e., no need for a thermal cycler).
2. Labor expertise for machinery or procedure is not required.
3. Sensitivity for detection was found to be comparable to the RT-PCR method.
4. It is less time-consuming.
5. This test has no cross-reactivity with other viruses in a few developed protocols.

Limitations

1. Designing six sequence-specific primers are time-consuming and laborious.
2. There is a lack of familiarity with the method as it is not widely used.

2.2.2 Serology/immunology-based diagnostics

Immunological/Serological diagnosis of COVID-19 is mostly achieved by detection of specific antibody IgM and IgG (primary antibodies generated in the human body toward SARS-CoV-2 infection) responses against the SARS-CoV-2 infection in

humans and utilizes antigen–antibody capture/sandwich direct or indirect methods. The methods like lateral flow assays are classified in serological assays and these tests provide advantages, such as easy operation with fewer infrastructure requirements, rapid test results, high sensitivity, and high specificity. These properties are suitable for processing high-throughput SARS-CoV-2 screening at point-of-care (POC) sites and hospital settings (Developing a National Strategy for Serology Antibody Testing in the United States, n.d.). The multiple methods of serological diagnostics are ELISA, gold colloidal methods, and lateral flow assay. Such diagnostic methods are classified as indirect diagnostics methods and can detect specific IgM and IgG antibodies in the blood (Li, Yi et al., 2020). These diagnostic methods, which are designed for SARS-CoV-2, use either nucleocapsid protein (N protein) or spike protein (S protein) as antigen. Serological assays are accurate, highly sensitive, and are efficient methods in detecting infectious pathogens and viruses, by the virtue of specific IgM and IgG antibodies being generated against the structural proteins of pathogens. SARS-CoV-2 viruses can be detected with enzyme-linked immunosorbent assay (ELISA), producing high-throughput results, and often they require less rigorous specimens (such as blood/serum collection) compared with RNA-based assays. Serology tests play a role in containing the COVID-19 by assisting healthcare professionals in identifying individuals who might have developed antibodies against SARS-CoV-2. Also, these test results can help in determining the donors for blood as named as convalescent plasma, which could be a possible treatment for the seriously ill patients from COVID-19.

2.2.2.1 Enzyme-linked immunosorbent assay

ELISA provides sensitive methods for detecting antigens or antibodies in body fluids and there are numerous clinical applications. ELISA was discovered independently by Engvall & Perlmann and Van Weeman & Schooners in 1971.

The principle of ELISA can be compared to the assays which utilize radiolabeled compounds. In ELISA, the enzyme labels are used in conjugation with antibody or antigen which has both immunological and enzyme-like properties. The antigen or antibody of particular interest is immobilized on a solid surface in the specific buffer which is then followed up by washing steps to wash off unreacted reagents and leave reacted reagents on the surface. After conjugation of antigen with the immobilized antibody and removing extra reagents through multiple wash-offs, the bound enzyme fraction is quantified by the addition of a nonchromatic substrate which gets converted to a chromatic product and changes its color. The amount of color produced provides an analysis of the concentration of antibodies present in the reaction titer (Engvall & Perlmann, 1971; Van Weemen & Schuurs, 1971). The representative images are shown in Figs. 2.2 and 2.3.

The generation of antibodies occurs in serum after 1–3 weeks of the onset of infection and has been reported to be produced in both the symptomatic and

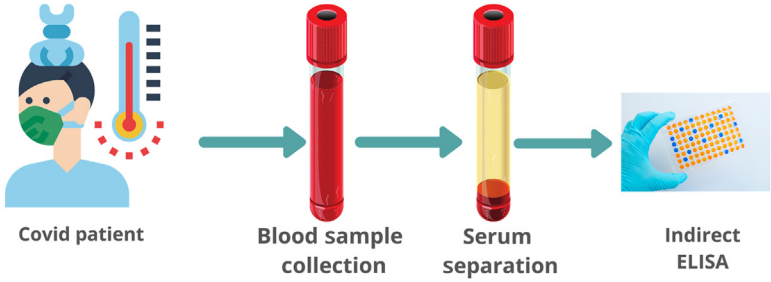

Figure 2.2 Representation of indirect enzyme-linked immunosorbent assay (ELISA) method used to test COVID-19-infected patients. Blood samples are collected from infected patients and are then centrifuged to separate serum and blood cells. The serum is further used as a testing sample in ELISA.

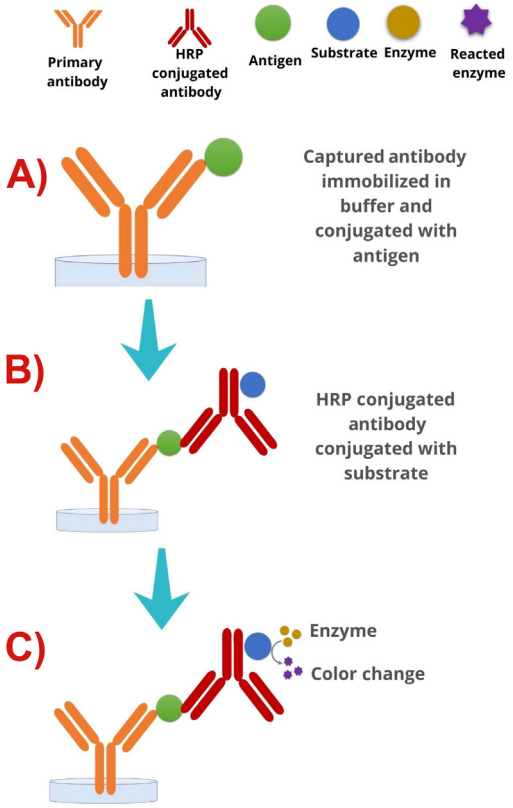

Figure 2.3 Cartoon representation of sandwich ELISA method. (A) Primary antibody immobilized in the suitable buffer on the ELISA plate, after appropriate washes, the antibody is conjugated with specific antigen. (B) A secondary antibody conjugated with horseradish peroxidase along with conjugated substrate is added in the buffer as mentioned in (A) and the reaction mixture is washed to remove any undesirable or unreacted components. (C) In the final step, the enzyme is added, which upon reacting with the substrate changes its color. The higher the concentration of antibodies in the solution, the stronger is the color change.

asymptomatic patients (Li, Yi, et al., 2020; Rothan & Byrareddy, 2020). It has been observed that the IgG and IgM antibodies are produced simultaneously, as reported by the calculation of seroconversion profiles of the patients infected by SARS-Cov-2 (Sethuraman, Jeremiah, & Ryo, 2020; Zhao et al., 2020).

These antibodies are produced against the structural proteins of viruses, such as spike antibody and nucleocapsid antibody (Zhao et al., 2020). The IgM antibody disappears with time, however IgG remains detectable in most of the patients (Tay, Poh, Rénia, MacAry, & Ng, 2020). The spike protein is classified as a transmembrane glycoprotein which is made up of the regions: S1 and S2. The identification and binding of viral receptors (ACE2) on the host cells are helped by the S1 region. The viral fusion and entry into the human body are aided by the S2 region (Ou et al., 2020; Walls et al., 2020). The major part of the S1 region is composed of the receptor-binding domain (RBD) which binds directly to the ACE2 receptor and is highly immunogenic. The S1-RBD in the SARS-CoV-2 has both unique and conserved sequences compared to other beta-coronaviruses (Ni et al., 2020).

Advantages

1. It can help clinicians in providing primary treatment to the patients due to its virtue of identifying specific infections.
2. It can be easily operated at POC sites and can be used as a high-throughput screening system.
3. This method is highly sensitive and specific toward capturing antigen or antibody.

Limitations

1. The clinical implementation of quantitative or semiquantitative results is unknown and cannot be indicated as degrees of immunity.
2. It cannot analyze protection from reinfection and cannot be compared to other SARS-CoV-2 antibody assays.
3. A positive result does not indicate a previous SARS-CoV-2 infection. The clinician will have to review other information, such as clinical symptoms and history, and the disease prevalence in the native region.
4. The negative result for a patient will indicate the absence of detectable anti-SARS-CoV-2 antibodies.

2.2.3 Point-of-care diagnostics

2.2.3.1 Chip-based biosensors for rapid diagnosis

Biosensors, mainly chip-based biosensors, are POC rapid diagnostic devices which are used for the detection of infectious pathogens. Materials like PDMS or poly(methyl methacrylate) (PMMA) are used to develop these biosensors (Choi, 2020; Zhang, Ding, et al., 2017). In the precise design of the biosensor and manipulation, which is

mostly automatic, the flow of the liquid through the chamber requires only a minimal amount of sample and the analysis is through high-throughput methods (Choi, 2020; Dincer, Bruch, Kling, Dittrich, & Urban, 2017). The chip biosensors that are made up of PDMS are frequently used by virtue of their high biocompatibility, transparency, and low cost (Nasseri et al., 2018). These biosensors are used in both nucleic acid detection and antibody detection. For example, a recent study revealed the development of a sample-to-answer "lab-on-a-disc" biosensor (Loo et al., 2017). These devices comprise multiple channels that help in automated extraction of nucleic acid, isothermal amplification (LAMP), and signal detection in real time. The double-stranded DNA amplification is measured by a fluorescent dye. The greater the fluorescence the higher is the amount of target in the reaction mixture. The biosensors can heat and lyse the sample for process extraction and amplification of the nucleic acids. The entire process is rapid and mostly takes 30 minutes to 2 hours. The platform is easy to use, however, the biosensors mostly remain large in size, which further leads to room for improvement. These biosensors-based devices could be customized for the detection of SARS-CoV-2 viruses (Nurul Najian, Engku Nur Syafirah, Ismail, Mohamed, & Yean, 2016).

2.2.3.2 Paper-based biosensors for rapid diagnosis

Paper-based microfluidic devices have gained attention for use in POC testing as compared to other applications because they are cheap and biodegradable, have easy fabrication and simple functionalization, and require the least modification (Chan et al., 2016; Choi, Yong, Choi, & Cowie, 2019). Owing to these features, these rapid POC can provide onsite testing in remote settings (Tang et al., 2017).

For the detection of COVID-19 lateral flow test strips are being used. These test strips can detect IgG and IgM in the blood, plasma, and serum of human patients.

The paper-based biosensor strip consists of a sample pad which is used to inoculate the samples, a conjugate pad conjugated with COVID-19 antigen or antigens from the infectious pathogens/viruses, and gold nanoparticle-conjugated antibodies or antibodies derived from rabbit, nitrocellulose membrane coated with antirabbit IgG called the control line, antihuman IgG coated test line, antihuman IgM coated test line, and absorbent pad which absorbs the waste generated by the paper-based strip (Li, Yi, et al., 2020; Sheridan, 2020).

The IgM or IgG antibodies react with conjugated antigens which form a complex, and then the complex enters the nitrocellulose membrane which further reacts with the anti-IgG/IgM in their predetermined test lines. Further, the gold-rabbit IgG reacts with antirabbit IgG and produces a visible blue/purple color. In a detection kit, the results are inferred as follows: a positive IgM or positive at both the test lines detects a primary or acute infection, and only a positive IgG refers to a secondary or later stage of infection (Li, Yi, et al., 2020).

2.2.4 Clustered, regularly interspaced, short palindromic repeats

CRISPR/Cas is a gene-editing tool. It is composed of a protein that is designed to degrade a target sequence and specifically reach the target site with help of a guide RNA.

Clustered, regularly interspaced, short palindromic repeats (CRISPR)-based SARS-CoV-2 tests have not yet been approved, but claim to be very fast (~30 minutes reporting time) and cheap for the detection of the virus. The automated ID NOW COVID-19 assay32 by Abbott Laboratories, Lake Bluff, IL, United States is one such kit and claims to give results in <13 minutes. It has also been approved for use but has been found to have false negative rates of ~15%. Abbott is launching a molecular POC test to detect novel coronavirus in as little as 5 minutes. Another is SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) that is CRISPR/Cas13-based (Gootenberg et al., 2017; Zhang, Abudayyeh, & Gootenberg, n.d.).

Advantages

1. It is a very fast detection method.
2. In this method, there is an absence of any homology with another human coronavirus, which reduces the chances of nonspecific identification.

Limitations

1. The test can give results with high false negative rates.
2. The method is still under the microscope as to whether it can be used as a standard testing method or not.

Other than diagnoses, the CRISPR technology is being studied for the treatment of COVID 19 using its gene-editing capabilities. One such example is the PAC-MAN system that uses the CRISPR-Cas13d system to target the conserved sequences in the SARS-CoV-2 genome, for degradation.

2.2.5 Imaging-based techniques

Various techniques utilizing medical image physics have also been used for the detection of COVID-19. Generally, the diagnosis is confirmed by laboratory testing by identifying the viral RNA using RT-PCR. When RT-PCR is negative, not available in the presence of symptoms, or the results are delayed, chest imaging has been utilized for diagnosing the probable causes. Imaging can also complement the clinical evaluation and for managing patients who are currently diagnosed with COVID-19. Various modalities such as chest X-rays, computed tomography (CT) scans, and ultrasound imaging have been used for imaging the lung, as well as for segmentation and classification purposes (Radiation Protection and Safety in Medical Uses of Ionizing Radiation, 2016). The recommendations for using medical imaging modalities are set by the WHO and are presented in Table 2.1 and suggested to be used as guidelines

Table 2.1 The various conditions and the recommendations given by the World Health Organization for the assessment of COVID-19 symptoms.

Condition	Reverse transcriptase-polymerase chain reaction	Chest imaging
Asymptomatic contact of patients with COVID-19	Diagnosis is confirmed	Not required
Symptomatic patients with suspected COVID-19	Available and timely done for confirming the diagnosis	Not required
Symptomatic patients	1. Not available 2. Available but delayed 3. Negative but high clinical suspicion	Required
Patients with suspected or confirmed COVID-19 with moderate to severe symptoms and not hospitalized	Required	Required in addition to clinical and laboratory assessment
Patients with suspected or confirmed COVID-19 with moderate to severe symptoms and hospitalized	Required	Required in addition to clinical and clinical assessment
Hospitalized patients with COVID-19 whose symptoms are resolved	Required	Not required

for the diagnosis of COVID-19. These recommendations are conditional and expert opinion should be used in combination with these for proper and accurate diagnosis of COVID-19 (Coronavirus disease COVID-19 outbreak, n.d.).

Since the availability of the machines for diagnosis using CT or X-ray imaging has been an issue, ultrasound imaging has also been utilized, as it is a cost-effective technique for the diagnosis of lung abnormalities. Artificial intelligence (AI)-based models are found to be effective in many cases and perform similar to the expert's opinion. Many AI-based strategies employed deep learning techniques for the classification of lung ultrasound into three classes: healthy, pneumonia, and COVID (Born et al., 2020). Recently many AI-based techniques have been developed for faster and accurate diagnoses when the volume of data to be examined is high or when the experts' opinions vary to give an initial diagnosis. Deep learning has also been utilized in many other areas, such as segmentation, reconstruction, classification, and other image analysis tasks (Awasthi et al., 2019; Awasthi, Jain, Kalva, Pramanik, & Yalavarthy, 2020; Deep Learning in Medical Imaging, 2017; Gibson et al., 2018).

The various imaging modalities used are discussed below and the respective advances in AI applied to the modality have also been discussed for the same:

2.2.5.1 Chest X-ray

X-ray is one of the cheapest ways of analyzing broken bones, fractures, tumors, lung infections, and pneumonia. It is safe, easy to perform, and less harmful compared to CT analysis for the diagnosis of soft tissue inside the human body (Narin, Kaya, & Pamuk, 2020). The WHO has recommended using medical scans for diagnosis when the initial diagnosis using RT-PCR is not satisfactory and the symptoms point toward the further analysis of the same. ResNet-50, InceptionV3, and Inception-ResnetV2 models were proposed for an end-to-end detection of COVID-19 and it was shown that ResNet50 gave the best model accuracy for the classification of the chest X-ray (CXR) between the normal and the COVID patients. The model gave an accuracy of 98% as compared to 97% using the InceptionV3 and 87% using the Inception-ResNetV2 architecture (Narin et al., 2020). Another architecture that uses a capsule net consisting of capsule layers used a much lower number of parameters and achieved an accuracy of 95.7%, sensitivity of 90%, and specificity of 95.8% (Afshar et al., 2020). Another work toward explainable learning of CXR achieved an accuracy of 97% on 6523 chest X-rays in 2.5 seconds (Brunese, Mercaldo, Reginelli, & Santone, 2020). The model first discriminates between healthy and any pulmonary disease X-ray, and further predicts which areas of the chest are affected in the case of disease prediction. The work tries to explain why a region of interest is classified as COVID-19, pushing it toward explainable architectures.

2.2.5.2 Computed tomography

CT can also complement the RT-PCR test whenever there is a shortage of tests and the number of people for whom tests are required is large. When the viral load is not sufficient, RT-PCR can be negative and CT can be used to complement the test and test for abnormalities in the chest (Ai et al., 2020; Fang et al., 2020; Hani et al., 2020). It was found that during the early days of symptoms, the CT scan was normal but as time progresses the CT scan shows various symptoms including greater total lung involvement, linear opacities, consolidation, peripheral and bilateral disease, and a crazy-paving pattern (Bernheim et al., 2020). After the initial advantages of doing a CT, various other deep learning-based techniques were utilized for classification as well as segmentation for utilizing in CT scans. These techniques generally assist the radiologist in making an assessment. A fully automatic method was developed for the detection of COVID from other community-acquired pneumonia and lung diseases and an AUC of 0.95 was obtained on the test dataset (Li, Qin, et al., 2020). Another study found that the misclassification rate of CT is as low as 3.9% and hence can be utilized for the diagnosis of COVID-19 for the management of patients (Li & Xia, 2020). An extensive analysis of the chest CT over a number of days was performed to see the progression of the disease and it was shown that the temporal changes follow a specific pattern (Wang, Dong, et al., 2020).

2.2.5.3 Ultrasound

The previous techniques utilized, such as CT, can identify the changes in the lung but these remain hidden in a large percentage of radiographs. The lung ultrasound can identify the lung tissue correlating with histopathologic findings, which can also be identified by CT. Lung ultrasound can also help in the detection of ARDS before the development of hypoxemia (Soldati et al., 2020). Lung ultrasound has further capabilities, such as safety, portability, and ease of follow-up examinations. In addition, the ultrasound can be performed at the bedside, which helps in limiting the transmission to the patients or the healthcare practitioners. Many AI-based methods were developed for segmentation in ultrasound images and classification of abnormalities among the various pathologies, such as COVID-19 and pneumonia, compared to the healthy volunteers. POCOVID-net was proposed for the classification of ultrasound into different modalities, such as pneumonia, healthy, and COVID-19, by utilizing the VGG architecture, while another work tries to reduce the model size by utilizing the mobile architectures (Born et al., 2020). Roy et al. (2020) proposing a transformer-based model after undertaking localization and classification for ultrasound lung images and showing comparisons among different architectures. Thus various imaging modalities are currently used for the detection of COVID-19 and segmentation, as prescribed by the WHO for different cases, as mentioned in Table 2.1.

2.2.6 Clinical data to predict progression

The severity and progression of SARS-CoV-2 (COVID-19) varies a lot across different patients (Viral and Host Factors Related to the Clinical Outcome of COVID-19 | Nature, n.d.). The limited availability of healthcare resources (ICU beds, ventilators, etc.) makes it necessary that reliable tools for triaging are in place to ensure the efficient utilization of resources and prevent casualties (Afshar et al., 2020). Researchers have proposed different AI-based solutions that use clinical data of COVID-positive patients to assess the severity of the disease and also predict the progression. The clinical data used in such solutions comprise demographic information, medical history, lab tests, and vitals, apart from biomarkers derived from X-ray, CT, and ultrasound imaging (Haimovich et al., 2020; Ji et al., 2020; Shi et al., 2020).

Before we look briefly into various solutions that aim to track or predict the severity of COVID-19, it is pertinent to discuss ARDS, as this is one of the critical conditions that originate from COVID-19. ARDS is a kind of failure of the respiratory system that is marked by a sharp rise of widespread inflammation of the lungs. Its symptoms include shortness of breath, rapid breathing, and skin turning bluish (Fan, Brodie, & Slutsky, 2018). The patients who survive ARDS often experience a decreased quality of life (Acute Respiratory Distress Syndrome, 2018).

ARDS could be caused by sepsis, trauma, pancreatitis, aspiration, and pneumonia (here COVID-19 pneumonia). It diminishes the lungs' capacity to exchange oxygen and carbon dioxide. According to the Berlin definition of ARDS, the severity of ARDS can be classified into three categories based on oxygenation levels (Fan et al., 2018):

1. Mild: PaO_2/FiO_2 of 201−300 mmHg and PEEP $\geq$ 5 cm H_2O
2. Moderate: PaO_2/FiO_2 of 101−200 mmHg and PEEP $\geq$ 5 cm H_2O
3. Severe: PaO_2/FiO_2 $\leq$ 100 mmHg and PEEP $\geq$ 5 cm H_2O

(PaO_2/FiO_2 = ratio of partial pressure arterial oxygen and fraction of inspired oxygen; PEEP = positive end-expiratory pressure.)

The patients suffering from ARDS are primarily treated by putting them on mechanical ventilation along with treating the underlying cause of syndrome (Fan et al., 2018). This explains the rising need for ventilators during the COVID-19 pandemic (A Framework for Rationing Ventilators and Critical Care Beds During the COVID-19 Pandemic, Critical Care Medicine, JAMA, & JAMA Network, n.d.). Patients suffering from ARDS have a mortality rate between 35% and 50% (Fan et al., 2018).

Though there is evidence that ARDS originating from COVID-19 is different from typical ARDS to some extent, it satisfies the Berlin definition and there is a need to track the respiratory deterioration in patients suffering from COVID-19 (Gattinoni, Chiumello, & Rossi, 2020). Thus many of the AI-based solutions have as their theme the monitoring and prognosis degradation of respiratory physiology.

Ji et al. (2020) have proposed a method that predicts whether or not the patients are under high risk of progression of COVID-19. The approach described for the task is a univariate and multivariate analysis using Cox regression. The authors have studied the progression of disease in 208 patients and have identified parameters that are indicators of the prospective severity of COVID-19. As per the regression model, four factors—comorbidity, older age, lower lymphocyte count, and higher lactate dehydrogenase—are significant factors that indicate a higher risk of deterioration. These four factors have been summarized in acronym CALL (comorbidity, age, lymphocyte, LDH) and the approach has been named CALL score, a quantitative assessment of progression risk on a scale of 4−13. As per the results the model achieved an area under receiver operating characteristic (AUROC) of 0.91 and has the potential to improve the therapeutic effect and reduce mortality through the efficient utilization of resources.

The discussion in Haimovich et al. (2020) concerns a severity scoring method to predict respiratory collapse in patients admitted to the emergency department (ED) and diagnosed with COVID-19. The study was performed on 1792 COVID-19 patients, out of which 35% had a respiratory failure in ED, while 12.3% of the remaining met the same situation within 24 hours of hospitalization. The outcome of the

study and data modeling is a simple bedside scoring system, the quick COVID-19 severity index (qCSI), composed of respiratory rate, oxygen saturation, and oxygen flow rate. In the same work, a machine learning model has also been proposed which has additional parameters: aspartate transaminase, alanine transaminase, ferritin, procalcitonin, chloride, C-reactive protein, glucose, urea nitrogen, white blood cell count, and age. The qCSI scores have been shown to be quite effective in predicting respiratory decompensation while using only bedside respiratory exam parameters.

The brief overview of the two works discussed above is just to illustrate the nature of the work that is being done using statistics and machine learning on clinical data for the assessment of severity/prognosis of COVID-19. The work in this domain is continuing as the pandemic continues to threaten human lives. Since the efficiency of such models is heavily dependent on the availability of data, we can hope that the continuous accumulation of data in hospitals will strengthen these prognosis tools.

2.3 Conclusion and further readings

This chapter discusses the possible methods and available diagnostic systems to test the undetermined trajectory of the COVID-19 infection in humans worldwide. All the diagnostic systems have their own advantages and disadvantages for helping us to take one step closer toward curbing the present pandemic situation. The guidelines have been provided by the World Health Organization, the United States Center for Disease Control and Prevention, and several other national and international organizations, which keep being updated on an ongoing basis.

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