Diagnostic Approach to a Patient with Coronavirus Disease

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4.1 Introduction

The highly contagious and infectious Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) has taken the world by a storm and has impacted the globe socially, mentally and economically in a big way. Social distancing, quarantine and contact tracing are the primary tools adopted for limiting the disease spread. Owing to a high rate of human-to-human transmission of the Coronavirus disease (Covid-19), the primary challenge in the containment of this pandemic is to identify asymptomatic carriers of the disease, which cause a rapid spread. Moreover, the pathogenic potential or reproduction number ($R_0$) of the SARS CoV-2 is $>2.5$ (up to 4 in some studies), reflecting the high number of secondary cases that can be infected by an individual, if not isolated at an early stage [1, 2]. The widespread use of accurate, rapid and convenient diagnostic methods can effectively aid in the early identification and elimination of the silent spread of the pandemic by asymptomatic carriers.

Structure of SARS CoV-2: SARS CoV-2 virus is an enveloped, positive-sense RNA (+ssRNA) virus of zoonotic origin, belonging to the beta-coronavirus family, and is found to be infectious to humans with a high fatality rate. It is typically spherical or pleomorphic in form, with a diameter of approximately 60–140 nm, and a single-stranded RNA genome of around 30 kb, which typically has an RNA-dependent RNA polymerase (RdRp) sequence. The viral genome and subgenomes may present six or more open reading frames (ORF). The first ORF (ORF1a/b) encodes 16 non-structural proteins (nsp1–16) involved in viral replication and encompasses around 66% of the entire genome. The remaining one third of the genome encodes the structural proteins of the virus.

SARS CoV-2 contains four structural proteins—envelope (E), spike (S), membrane (M) and nucleocapsid (N). The S, M and E proteins form the envelope of the
virus, while the N protein remains associated with the RNA forming the nucleocapsid inside the envelope. Polymers of S protein remain embedded in the envelope, giving it a crown-like appearance (hence the name *coronavirus*) (Fig. 4.1). Spike glycoproteins comprising of S1 and S2 subunits bind to receptors on the human cell surface called angiotensin-converting enzyme 2 (ACE-2), causing the infection [3]. Since the ACE-2 receptors are abundantly present in the epithelia of the lung and the small intestine, the commonest symptoms seen with the coronavirus disease relate to those of the respiratory and gastrointestinal tract. Two strains of the Covid-19 virus, namely, the L-type and the S-type, have been known, out of which the L-type (a mutated strain of the S-type) is more aggressive and contagious. The error prone nature of the viral replication process accounts for the easy mutation and recombination of this group of viruses, causing an adaptive evolution and sequence diversity. This would necessitate a long-term genomic surveillance of the SARS CoV-2, as it may cause a constant and long-term health threat, even if a vaccine is developed anytime soon.

### 4.2 Diagnostic approach to Covid-19

The primary transmission of the Covid-19 disease occurs via direct, indirect, or close contact with the respiratory droplets of the infected person. The incubation period of COVID-19, or the time between viral exposure to symptom onset in an individual, is 5–6 days on an average, but can be as long as 14 days [4]. Therefore, rapid and accurate identification of cases through appropriate testing and isolation of infectious cases is mandatory to prevent disease transmission. The diagnostic approach to the SARS CoV-2 may either involve detecting the viral RNA in the acute phase of infection via molecular assays, or by detecting the antibodies that may have developed as a result of viral exposure in the patient’s blood, which may be possible only after a few weeks of infection. All symptomatic patients, or individuals with any recent international travel history, or those in contact with confirmed or suspected patients, need to be tested for the virus. The World Health Organization

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**Fig. 4.1** Schematic of a coronavirus—this new virus probably looks a lot like this. (From Biowiki http://ruleof6ix.fieldofscience.com/2012/09/a-new-coronavirus-should-you-care.html)
(WHO) recommends the collection of upper respiratory specimens using nasopharyngeal or oropharyngeal swabs for the diagnosis of the Covid-19 in ambulatory patients. For severely ill patients, sputum, bronchoalveolar lavage (BAL) or tracheal aspirate from the lower respiratory tract yield better results. Collection and processing of respiratory specimens require compliance with the guidelines for aerosol-generating procedures and use of a biosafety level 2 (BSL-2) facility with stringent BSL-3 work practices. The efficacy of blood and stool samples in viral detection techniques, in addition to the respiratory specimens, is still unclear in the absence of sufficient data on viral shedding in these samples [5]. Correct handling of specimens during transportation is essential. The specimens should be stored and shipped at 2–8 °C until testing. In the event of a delay in testing, the use of viral transport media is strongly recommended. The specimens may be shipped on dry ice at −70 °C if further delays are expected [6].

*Chest CT in diagnosis of Covid-19*: In addition to the molecular assays for viral detection, imaging methods like the CT (computed tomography) chest were initially recommended as an auxiliary diagnostic method for Covid-19. It was claimed that the chest CT had a higher sensitivity for diagnosis of the corona virus disease and may be considered as a primary diagnostic tool for Covid detection, especially during the early course of the disease [7]. Typical CT findings included bilateral pulmonary parenchymal ground-glass and consolidative pulmonary opacities, progressing to ‘crazy paving’ patterns, and peripheral lung distribution [8]. Bai et al. reported the most discriminating features of Covid-19 pneumonia on chest CT to be a peripheral distribution (80%), ground glass opacity (91%), fine reticular opacity (56%), and vascular thickening (59%) [9] (Fig. 4.2). But these recommendations were not supported by major medical organizations and societies in view of the low specificity of the CT chest findings to the Covid-19 disease. The American College of Radiology recommends that CT scan should not be used as a first line test to diagnose the disease and should be reserved for hospitalized, symptomatic patients with specific clinical indications for CT [10]. The Society of Thoracic Radiology and American Society of Emergency Radiology, in their joint statement, said that chest CT scans should be restricted to patients who are tested Covid positive and are suspected to have pulmonary complications, and not as a routine screening tool for the disease.

![Fig. 4.2](image_url) (a) Peripheral patches of consolidations and ground glass haziness in left upper (A) and lower lobes (B and C). (b) Ground glass haze with interstitial thickening and subpleural reticulations bilateral lower lobes. (Image courtesy: Dr Anshu Mahajan)
The commonly available testing methods for Covid-19 are, therefore, classified into two major categories:

1. Molecular nucleic acid detection assays.
2. Serological or immunological assays.

4.3 Molecular Assays for Viral Nucleic Acid Detection

The evolution of SARS CoV-2 specific testing has been greatly facilitated by the availability of detailed genetic sequence of the virus, resulting in the development of various primers and probes needed for nucleic acid amplification. The various molecular assays available at present include:

1. Reverse Transcription-Polymerase Chain Reaction (RT-PCR).
2. Isothermal Nucleic Acid Amplification:
   (a) Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP).
   (b) Transcription-Mediated Amplification (TMA).
   (c) CRISPR-Based Assays.
   (d) Rolling Circle Amplification.
3. Microarray Hybridization Assay.
4. Amplicon-Based Metagenomic Sequencing.

4.3.1 Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RT-PCR assay is the gold standard for identification of SARS CoV-2 virus. It is a technique that combines reverse transcription of viral RNA template into
complementary DNA (cDNA) and the amplification of specific targets on cDNA inside a thermal cycler, using polymerase chain reaction (PCR). In real-time PCR techniques, the amplification in each PCR cycle is monitored real time using a fluorescent dye, enabling quantification, and hence is known as quantitative PCR, or qRT-PCR. The amplification curve is analysed to obtain a cycle threshold (Ct) value, with lower Cts indicative of abundant target nucleic acid in the sample.

RT-PCR is conventionally performed as a one-step or a two-step approach. Single step quantitative RT-PCR is the preferred diagnostic approach for the detection of SARS CoV-2, as it is quicker, with minimal handling of the viral sample and decreased bench time, and also has reduced chances of error and cross-contamination. Various molecular targets within the +ssRNA genome of the virus are used for RT-PCR, including the ORF1ab or ORF8 genes; envelope (E), spike (S) or nucleocapsid (N) proteins; or genes encoding RNA-dependent RNA polymerase (RdRp). The WHO has provided primers for the genes that encode the E, N and RdRp [11], although different targets may be preferred by different authorities. As per the standard protocol, a patient is confirmed of infection when both the selected target genes come to be positive. Further improvement in detection methods and better automation of the RT-PCR tests play an instrumental role in facilitating greater safety, lower costs and higher sensitivity.

The variable sensitivity and specificity of qRT-PCR remains one of the major challenges in the diagnosis of Covid-19. Multiple factors may contribute to the low sensitivity of the RT-PCR test, including:

1. Viral load kinetics:
   (a) **Sampling site:** Different anatomic sites have different diagnostic efficacy for SARS CoV-2 detection rates. The sensitivity of lower respiratory tract specimens like BAL and sputum is more as compared to other sites, including the upper respiratory tract [12]. Testing of specimens from multiple sites may improve the sensitivity and reduce false-negative test results.
   (b) **Sampling timing:** Viral load is shown to be greatest at the time of viral onset, the rate of positivity declining thereafter [13]. Prolonged viral shedding may however be seen in older patients and those with comorbidities [14].
   (c) **Sampling quality:** Poor quality of the specimen containing little patient material due to inappropriate collection techniques, improper transportation or handling of the specimen may yield false-negative results.

2. Technical reasons inherent in the test, e.g. **virus mutation** or **PCR inhibition**, may result in a false-negative test.

3. Mismatches between primers and probes and the target sequences can lead to decrease in assay performance and potential false-negative results, as different kit manufacturers use different viral genome sequence data [15]. Multiple target gene amplification, in the form of multiplex RT-PCR kits, is being used increasingly to avert this issue. An example of the multiplex PCR technique is the **TaqPath COVID-19 Combo kit**, developed by the ThermoFisher-Applied Biosystems, which contains three primer/probe sets specific to N, S and ORF1ab regions of SARS-CoV-2, making it highly sensitive in the detection of the Covid-19 virus.
If a negative RT-PCR result is obtained from a patient with a high index of suspicion for COVID-19 virus infection, particularly when only upper respiratory tract specimens were collected, additional specimens, including from the lower respiratory tract if possible, should be collected and tested [16].

Similarly, patients showing positive RT-PCR results after repeated negative tests and clinical recovery, should be interpreted with caution. A positive RT-PCR result may not necessarily mean the person is still infectious or that they still have any meaningful disease. The RNA could be from non-viable virus, and the amount of live virus may be too low for transmission [17].

Despite being widely used for the diagnosis of Covid-19 disease, the RT-PCR technique has its set of drawbacks, which include requirement of sophisticated lab equipment and technical expertise and the possible biological safety hazards during transport and sample processing [18]. Though most of these techniques yield results in less than 2–3 h, the need for sample transportation to the specific lab may make the overall process time-consuming.

### 4.3.2 Isothermal Nucleic Acid Amplification

It is a molecular amplification technique based on the synthesis of target DNA at a constant temperature of 60–65 °C, instead of thermal cycling used in RT-PCR techniques, making it faster and highly efficient. The cost effectiveness of isothermal platforms clears the way for their use in resource limited settings. They have a higher sensitivity and specificity in comparison to PCR, primarily due to the utilization of strand displacement amplification methods. These technologies have proved revolutionary, as they propose to improve the turnaround time to results and need minimal training to conduct at the community level, thereby helping to recognize both symptomatic as well as asymptomatic patients and preventing the disease spread. Swift collection of the upper respiratory swabs directly into a lysis buffer (containing an inactivating agent like guanidinium thiocyanate to inactive any viable virus and a non-denaturing detergent to prevent RNA degradation), combined with personal protective equipment (latex hand gloves, laboratory coat, appropriate face mask and eye goggle), makes isothermal nucleic acid amplification testing safe for non-laboratory personnel at community outreach clinics [19]. Several methods have been developed on the basis of the isothermal nucleic acid amplification technique, and these include:

- **Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP):** It is a rapid and simple test that combines reverse transcription and isothermal amplification, eliminating the need for RNA extraction, to achieve the detection of SARS CoV-2 virus in less than 30 min at a constant temperature of 65 °C. The LAMP method employs four sets of primers to target RNA encoding ORF1ab, S protein and two regions in N protein of the virus. The results of RNA amplification can be visualized by the naked eye and has a good sensitivity and specificity. The technology is therefore promising to be used for screening individuals for
SARS CoV-2 virus at the point of care testing [20]. A few currently available molecular assays that have come up using this user-friendly technology are ID NOW COVID-19 test from Abbott Diagnostics and iAMP COVID-19 detection kit from Atila Biosystems, Inc.

- **Transcription-Mediated Amplification (TMA):** It is an isothermal, autocatalytic target amplification method, which involves RNA transcription (via RNA polymerase) and DNA synthesis (via reverse transcriptase), to produce an RNA amplicon from a target nucleic acid. This method is easy to use and has a high throughput capability on a sensitive molecular detection platform. The Hologic Aptima SARS-CoV-2 Assay, utilizing TMA as a target amplification mechanism, has been shown to have a markedly higher analytical sensitivity than RT-PCR in the detection of SARS CoV-2 [21].

- **CRISPR-Based Assays:** CRISPR technology, popularly used as a genome editing tool, is adapted from the natural antiviral defence mechanisms of prokaryotes, like bacteria. Clustered regularly interspaced short palindromic repeats (CRISPR) are specialized stretches of DNA with two distinct characteristics – nucleotide repeats, and spacers or DNA bits interspersed among these repeats. In case of bacteria, the spacers are taken from viruses that attacked the organism previously and are used to detect and destroy DNA from similar viruses during subsequent infections. Once a spacer is incorporated and the virus attacks again, a portion of the CRISPR is transcribed and processed into CRISPR RNA, which helps Cas (or ‘CRISPR-associated’) proteins to act like a pair of molecular scissors, cutting strands of foreign pathogenic DNA. These Cas proteins or nucleases can be used as a tool for molecular testing due to their ability to specifically target viral RNA sequences, e.g. the Cas12 and the Cas13 families of nucleases are used for the detection of SARS CoV-2 viruses. Sherlock Biosciences have used the Cas13 enzyme that is effective in cutting out reporter RNA sequences in response to activation by the SARS CoV-2 virus. Similarly, Mammoth Biosciences have come up with a dipstick test (DETECTR assay) that depends upon the excising of fluorescent reporter RNA by Cas12a [22]. Both these tests are quick (require less than an hour), low cost, simple and reliable and provide a novel alternative for the portable, sensitive and specific detection of the Covid-19 virus [23, 24].

- While both SHERLOCK and DETECTR employ target amplification as the primary step, two amplification-free biosensing systems using CRISPR technology have recently been introduced: Cardean Transistors, 2020 using CRISPR Cas9, and Diagnostics with molecular, 2020 employing Cas13a. Both techniques promise a handheld corona virus detection device, which also have the potential to address potential mutations of SARS CoV-2 in a timely manner, owing to their multiplexed microfluidic chip technology.

- **Rolling Circle Amplification (RCA):** It is an isothermal nucleic acid amplification technique that can amplify target nucleic acid sequences with high fidelity and specificity by using strand displacing polymerases [25]. This enzymatic process is capable of amplifying the nucleic acid 10⁹-fold in each circle within 90 min. It requires only a few reagents and generates minimal false positive results and may prove to be a useful assay in the detection of SARS CoV-2 virus.
4.3.3 Microarray-Based Hybridization Techniques

A microarray is a laboratory tool used to detect gene expression. It has thousands of DNA fragments or oligonucleotides of known sequence (called probes or oligos) arrayed in a known sequence of rows and columns on a chip. Nucleic acid hybridization using microarray involves reverse transcription of viral RNA to cDNA, labelling of cDNAs with specific probes on the chip, their hybridization by the formation of hydrogen bonds between complementary nucleotide base pairs, and finally, washing away of non-specific bonding DNA sequences, generating a signal depending upon the amount of target sample bound to the probes. The advantage of microarray-based detection is that it can combine powerful nucleic acid amplification strategies with the massive screening capability of microarray technology, resulting in a high level of sensitivity, specificity and throughput capacity [26].

PathogenDx’s novel DetectX-RV technology combines RT-PCR with powerful DNA microarray technology for multiplex testing in Covid-19. Following RNA extraction and PCR amplification, the resulting cDNA is labelled with a fluorophore and added to the DNA microarray containing 144 synthetic ssDNA probes. DetectX-RV supports rapid analysis, with results in 6–8 h, and the microarray design enables 12 individual specimens per slide, and up to 16 slides are tested simultaneously for improved throughput. The test is still in research phase and has not yet been approved by the Food and Drug Administration (FDA) for use in laboratory for diagnostic purposes.

4.3.4 Amplicon-Based Metagenomic Sequencing

These are two complementary techniques used to identify and sequence SARS CoV-2. First, an amplicon-based next generation sequencing enables complete genome sequencing via a highly multiplexed target enrichment panel, from RNA to sequence-ready libraries in a short time (<6 h). Secondly, a metagenomics approach helps in assessing the background microbiome, besides the SARS CoV-2 genome, helping in the identification of co-infections with other viruses or bacteria, thereby aiding in future treatment decisions and predicting patient outcomes. Based on these principles, Illumina has devised two workflows for sequencing SARS CoV-2 from clinical samples—one based on shotgun metagenomics and the other on target enrichment [27]. This dual technique of next generation sequencing provides many advantages. Specific amplicon-based sequencing of SARS CoV-2 helps in effective epidemiological studies and contact tracing. The use of metagenomics approach, e.g. sequence-independent single primer amplification (SISPA), provides a check on genomic divergence for amplicon-based approaches. This may help in identifying viral mutations and recombination, thereby influencing vaccine and antiviral efficacy [28].
4.4 Serological or Immunological Assays

Serological testing, or analysis of the patient’s blood or plasma for monitoring the immune response to the disease, plays an important role in the diagnostic, surveillance and epidemiological progress of the SARS CoV-2 disease. These immunoassays work on the principle of specific antigen-antibody reaction and mainly target the immunogenic proteins of the SARS CoV-2 virus—the S (spike) and the N (nucleocapsid) proteins. The S1 subunit-based immunoassay may be more specific than the entire S antigen for diagnosing SARS-CoV-2 infections. Also, the receptor-binding domain (RBD) located along the S protein that binds ACE-2 in humans is a target of interest to detect the presence of SARS CoV-2 specific antibodies. Exposure to the Covid-19 virus is determined by the detection of either IgM or IgG antibodies specific for these viral antigens. Rapid antigen tests, wherein the presence of viral antigens in swab samples is detected by the SARS CoV-2 antibodies, may be used in conjunction with the antibody detection tests in Covid-19.

SARS-CoV-2 infection follows a seroconversion timeline similar to other viral infections. The seroconversion rate and antibody levels rise quickly during the fortnight after symptom onset, and the cumulative seropositive rate is 50% on day 11 and 100% on day 39 [29]. The SARS CoV-2 specific IgM antibodies peak between 2 and 3 weeks after symptom onset, while the IgG antibodies peak after 17 days of infection. A high titre of total antibodies is independently associated with a more severe clinical disease. The characterization of antibody profiles suggests that any suspected individual with undetectable antibody levels against SARS CoV-2 after 20 days of symptom onset may be a true negative case. There is no specific chronological order in terms of IgM or IgG seroconversion, suggesting the importance to test for both IgM and IgG antibodies to confirm a positive infection [30]. Also, seroconversion does not imply a rapid decline in the viral load, and people may continue to remain infectious despite being truly positive in the antibody testing [31] (Fig. 4.3).

The primary application of antibody testing in Covid-19 is to detect previous infections in individuals who had few or no symptoms, to guide serosurveillance and epidemiological studies, as well as to facilitate effective contact tracing in the community. These tests may also be used in the screening of eligible convalescent plasma donors from individuals who have recuperated from the infection, and in evaluating the immune response and effectiveness of candidate vaccines in their research phase. Also, the antibody tests may help in the diagnosis of COVID-19 in RT-PCR-negative patients who present later during disease course.

Serological antibody tests are fast, robust and easy to perform, but cannot detect the infection in early stage of the disease. There may be an inherent variability of the antibody response due to different genetic makeup of each individual, and cross reactivity to other common coronaviruses may limit the sensitivity and specificity of the antibody test [32]. Presently, the serological tests cannot be used as a definitive tool for determining protective long-term immunity in a recovered patient, as we do not yet have definitive proof of the same.
Fig. 4.3  The time relationship between viral load, symptoms and positivity on diagnostic tests. The onset of symptoms (day 0) is usually 5 days after infection (day-5). At this early stage corresponding to the window or asymptomatic period, the viral load could be below the RT-PCR threshold, and the test may give false-negative results. The same is true at the end of the disease, when the patient is recovering. Seroconversion may usually be detectable between 5–7 and 14 days after the onset of symptoms; therefore, in the first phase of the disease, the serological tests are more likely to give false-negative results. The dotted black line in the graph illustrates the sensitivity of the chemiluminescent assay as derived from the data sheet of a commercial test (Abbott Diagnostics, USA). Ig immunoglobulin, RT-PCR reverse transcription-PCR, SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.
The various techniques available for COVID-19 serology tests include the following:

1. Enzyme-Linked Immunosorbent Assay (ELISA).
2. Lateral Flow Immunoassay.
3. Luminescent Immunoassay.
4. Neutralization Assay.
5. Biosensor test.

### 4.4.1 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a plate-based ligand binding assay technique, to detect the presence of COVID-19 antibodies in a patient. The blood sample is placed inside the microtiter wells of an ELISA plate, which is coated with SARS CoV-2 specific antigens. The antibodies, if present in the sample, passively bind with these specific antigens, and an additional tracer antibody may be used to detect the bound antigen-antibody complex to generate a colorimetric or fluorescent-based readout. This technique needs sophisticated equipment and skilled technicians but can screen large number of specimens (up to 96 samples at a time) in less than 3 h.

A number of IgG/IgM ELISA testing kits have been approved for use under the FDA Emergency Use Authorization (EUA)—examples include the DEIASL019/020 SARS CoV-2 IgG ELISA kit by Creative Diagnostics; EUROIMMUN Anti-SARS-CoV-2 ELISA; KT-1032/33 EDI™ Novel Coronavirus COVID-19 ELISA Kit by Epitome Diagnostics; etc. The Center for Disease Control (CDC) also developed a SARS-CoV-2 serological kit (ELISA based) with a specificity of >99% and sensitivity of 96% [33], but this test awaits permission to be used as a diagnostic test. ELISA antigen tests may be developed in the future to detect current infections.

### 4.4.2 Lateral Flow Immunoassay (LFIA)

The LFIA rapid detection kits use the principle of immunochromatography, producing fast, qualitative results within 10–30 min without the need for specialized and costly equipment, and typically requires no sample or reagent preparation.

The LFIA kit is essentially a dipstick encased in a cassette, containing the capture reagents (either the viral antigen protein or monoclonal antibodies) immobilized on defined locations on a nitrocellulose membrane, as well as labelled detector antibodies that recognize the same target. A positive result, triggered by the antigen-antibody binding, is visible as a coloured line, much like the regular pregnancy kit. This rapid diagnostic test (RDT) has the potential to be deployed in large-scale serological surveys and can be used as a point-of-care (POC) test or self-test. However, at present, the WHO recommends the use of these tests in research settings only, until evidence supporting use for clinical decision-making is available [34, 35].
Lateral flow assay technology has been used to develop rapid antigen test kits, like the Standard Q COVID-19 Ag (SD Biosensor) and COVID-19 Ag Respi-Strip (Coris Bioconcept), which use nasopharyngeal swabs for sampling. The viral proteins in the swab samples bind to the specific monoclonal antibodies in the reagent strip to yield results in 30 min. These tests detect actively replicating viruses and therefore may be used to identify acute or early infection. In view of their high specificity but relatively low sensitivity, it is recommended that a patient who has tested negative for Covid-19 by rapid antigen test should be tested sequentially by RT-PCR to rule out infection, whereas a positive test should be considered as a true positive.

### 4.4.3 Luminescent Immunoassay

Luminescent immunoassays are variations of the standard ELISA technique using chemiluminescence or fluorescence.

Chemiluminescence immunoassay (CLIA) is an assay that combines chemiluminescence technique with immunochemical reactions (or immunoassays). CLIA utilizes chemical probes which could generate light emission through chemical reaction to label the antibody. This technique has become popular due to its high sensitivity, wide dynamic range and complete automation to quantitatively measure antibodies in plasma of infected individuals. It does not require long incubations and is therefore faster than the conventional ELISA technique, with a high throughput. Diazyme DZ-Lite SARS CoV-2 IgG/IgM test (Diazyme Laboratories) and Maglumi COVID-19 IgG/IgM test (Snibe Diagnostics) are few examples of the available CLIA testing kits for Covid-19, that promise high throughput, high clinical sensitivity, rapid detection within 30 min and antibody detection with numerical results.

Fluorescence immunoassay (FIA) is an immunoassay technique in which antigen or antibody is labelled with a fluorescent dye for rapid detection. Bioeasy nCoV rapid antigen kit utilizes fluorescence immunochromatography for detecting the SARS CoV-2 antigen.

### 4.4.4 Neutralization Assay

It is a specialized type of immunoassay which detects only those antibodies that can block virus replication (called neutralizing antibodies), and not all antigen-antibody reactions. This helps in the identification of the virus serotype, as groups of viruses may share common antigens, but only a fraction of these would be targets of neutralizing antibody.

Fluorescence-based neutralization assay is a rapid, high throughput assay that rapidly and reliably measures neutralization of a reporter SARS-CoV-2 by antibodies from patient specimens. The presence of SARS CoV-2 neutralizing antibodies would predict protection from reinfection, thereby helping in large scale
serodiagnosis and vaccine evaluation, and identification of high neutralizing convalescent plasma for therapy. The test, however, needs to be conducted in a biosafety level 3 (BSL-3) containment [36].

4.4.5 Biosensor Test

Biosensors are a promising alternative and a reliable solution to clinical diagnosis and real-time detection of the SARS CoV-2 virus. The biosensor technology, known as localized surface plasmon resonance (LSPR) sensing, detects interactions between molecules on the surface of a constructed metallic nanostructure incorporating DNA probes that recognize specific SARS-CoV-2 RNA sequences, which registers these interactions as a local change in refractive index [37]. This technology is complex and expensive, but is quick, sensitive, automated and real time, and offers tremendous potential for the rapid medical diagnosis of Covid-19.

4.5 Conclusion

The global data reflects the continuing menace of the novel coronavirus SARS CoV-2 across the world, despite preventive and therapeutic advancements. The key to prevent the spread of the virus is to develop better methods for mass screening. While RT-PCR remains the current gold standard for detection of Covid-19, newer molecular tests like isothermal amplification, hybridization microarray and cutting edge CRISPR-based techniques offer faster, cheaper and reliable alternatives. Serological tests like ELISA, lateral flow assays and CLIA help in predicting the course, degree and durability of immune response to the SARS CoV-2 infection, paving way for the development of effective vaccines in the future. These tests may also aid in epidemiological research and will help in confirming if seropositivity equates to immunity. The development of efficient, cost effective point of care laboratory techniques with high sensitivity and specificity, which can be employed on a large scale, is the need of the hour. The validation of these tests across different populations will however be required before they can be routinely used for clinical decision-making.

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