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Current Diagnostic Approach for COVID-19

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1.1 Introduction

The ongoing spread of coronavirus has presented a threatening scenario globally because of the non-availability of accurate and rapid detection methods. However, on 30 January 2020, World Health Organization (WHO) has declared “COVID-19” (coronavirus disease 2019) as the largest threat under “public health emergency of global concern,” as it is alone responsible for 250 000–260 000 deaths worldwide and across 3–3.5 million positive cases [1].

The detection and analysis procedure for this threatening virus started initially with a virus detection method, which somewhat has an advantage of non-detection of long culture cycles. Another way of detection is through the use of “nucleic acid profiling,” which [2] can rapidly, sensitively, and accurately detect the pathogens in confirmed COVID patients, but large amounts of genetic variations, mismatches in primers, probes, and some target sequences may result in interpretation of false results. Detection via genomic sequence analysis and the point-of-care diagnosis have become popular in the detection of emerging viruses for finally detecting the specific antibodies IgM and IgG related to COVID [3].

Section 1.2 describes and highlights the current diagnostics and treatment strategies for COVID-19.

1.2 Recommended Laboratory Diagnosis for COVID-19

1.2.1 SARS-CoV-2 Testing: Detection Approach by Screening Suitable Specimen Cultures

The first and foremost step in diagnosis and identification is related to the appropriate collection of suitable specimens, which [4] are being collected from the upper and lower respiratory tracts, WBC’s, and serum specimens. Furthermore, it has been mostly detected and screened from the swabs pertaining to nasopharyngeal area,

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oropharyngeal, sputum, stool samples, urine, saliva, conjunctival area, and rectal swabs [5].

It is recommended that the samples and swabs should be strictly collected from the lower respiratory tract, for confirmatory diagnosis, even if the upper respiratory swab analysis is negative for COVID-19, as the receptor "AEC 2" is actively distributed in the alveolar lining of epithelial cells. Various studies compared [6] the viral loads from the lower respiratory tract specimen for the suspected and confirmed COVID patients. The study further stated that the average viral load differed in different collected samples [7], as the viral load detected in sputum was higher around 17 420 ± 6925 copies/test than the nasal swabs (655 ± 502 copies/test) and throat swabs (2555 ± 1965 copies/test). In addition, high viral load was also recorded in swabs collected from [8] the lower respiratory tract. Most of the cases were examined and confirmed positive through isolation and culturing techniques from oral swab on the first day, followed by a five [9–11] day diagnosis of anal swabs, indicating a shift from early period diagnosis to late period diagnosis. However, in asymptomatic conditions, it can be detected by analysis of urine sample, with no urinary irritation symptoms. Recently, it has also been detected in samples of saliva. In addition, it has been detected in nasopharyngeal swab, conjunctival tear swabs, and [12] oropharyngeal swabs. However, there still exist glitches in terms of monitoring and isolation process to screen conjunctival secretions for confirmatory diagnosis. Currently, the [13] virus has not been traced in many samples such as cerebrospinal fluid, semen, pericardial effusion, female reproductive tract, etc.

1.2.2 SARS-CoV-2 Detection: The Nucleic Acid Approach

For successful diagnostic strategies, identification of some specific primers and probes is important to screen out the target sequences. These target sequences for COVID-19 involve the "envelope – E," "the nucleocapsid – N," "spikes – S," "RNA-dependent RNA polymerase," and "open reading frame – ORF." WHO further recommends [14] reverse transcription polymerase chain reaction (RT-PCR) as a routine recommendation but lacks suitability in terms of time consumption, requirement of expensive equipment and biosafety conditions.

1.2.2.1 COVID-19 Detection Approach Through Real-Time PCR

The target gene sequences for detecting CoV-2 vary globally from China (ORF's), the United States (3 N gene), Germany (RdRp, N, and E genes) to France (two targets in RdRp). Center for Disease Control and Prevention (CDC) established a RT-PCR process for the detection and analysis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with three specific primer sets to detect β forms of CoV-2 and the other two for SARS-CoV-2.

Different countries have a [15–18] large number of qRT-PCR (quantitative reverse transcription polymerase chain reaction) protocols provided by the official WHO website, which play the principal role in the detection of SARS-CoV-2. In recent time, different countries are following different protocols of gene targeting for the detection of SARS-CoV-2, for example, France (two targets in RdRp aka

RNA-dependent RNA polymerase), Japan (pancorona and numerous targets, spike protein), the United States (three targets in N gene), China (N genes and ORF1ab), Thailand (N gene), and [19–21] Germany (RdRp, N, and E genes). Different institutes use different RT-PCR primers or tests for the detection of SARS-CoV-2. A new RT-PCR panel has been rooted by the CDC for the universal detection of SARS-like β-CoVs and specific detection of SARS-CoV-2. For the N gene [22–25], three sets of distinct primers were devised – two sets of probes or primers were specific for identifying SARS-CoV-2 and the last set was universally used for detecting all β-CoVs. COVID-19 must be confirmed as positive for all the three individual targets. The Charite (Germany) developed two nucleic acid tests for the detection of E genes of the bat-like β-CoVs, SARS-CoV-2, and [26] SARS-CoV. If both of the tests are positive, only then it could enter the next level/step of detection, which is for the RdRp gene and is called the SARS-CoV-2-specific RT-PCR test [27].

Despite the various protocols developed by numerous institutions for SARS-CoV-2 testing, it is still not crystal clear whether the outcomes of the [28–31] nucleic acid tests based on the different targets can be compared or not. Various RNA transcripts that were extracted from a COVID-19 patient by Chantal et al. were used to study the detailed analytical sensitivities of the four qRT-PCR assays rooted in Hong Kong, Germany, China, and the United States. According to a study, in all the primer–probe sets enforced in the qRT-PCR tests, SARS-CoV-2 could be identified; however, there was a significant disparity in the ability to find the positives and negatives with a lesser viral load and in the detection limit. HKU-ORF1 (Hong Kong), 2019-nCV_N1 (United States), and E-Sarbeco (Germany) were found to have the highest sensitivity primer–probe sets, while RdRp-SARSr (Germany) had the lowest sensitivity, which can be due to the mismatching in the reverse primer. Also, the sputum samples or nasopharyngeal swab from the [32–34] COVID-19 patients (Germany) were used for comparing the qRT-PCR tests in a commercial reagent and different polymerase chain reaction (PCR) systems. A clear discrepancy in the analytical sensitivities among different PCR systems was detected when the same probes and primers were used. The results concluded that when a one-step qRT-PCR system was used, the RdRp [35] target was less sensitive than the E gene target. However, the test evaluation was not crystal clear as it was disturbed by the high background nature of the E gene target. The sensitivity may be improved by the additional optimization of the E gene assay [36].

1.2.2.2 Detection Approach Through Nested RT-PCR

To detect the low-copy-number SARS-CoVs present in the early stage of the disease, real-time nested RT-PCR assay [37] is the perfect choice as it bridges the real-time instruments (time-saving) with the high sensitivity of the nested PCR. The identification of the SARS-CoV-2 with the help of nested RT-PCR has already [38] been verified in countries like Japan during the initial days of the pandemic. This technique had already detected 20–25 COVID-19-positive patients in Japan, as of the first week of February 2020. A new OSN-qRT-PCR assay (one-step nested real-time RT-PCR) was recently devised by Ji et al. for targeting the N genes and SARS-CoV-2 ORF1ab genes. This assay had a difference in sensitivity (1 copy/test

and 10-fold higher) with that of the commercial qRT-PCR assay (10 copies/test). The OSN-qRT-PCR confirmed the 14 samples with qRT-PCR negative, among the 181 clinical samples taken. Additionally, it also confirmed the seven samples as positive with qRT-PCR positive in the gray zone. In comparison with the qRT-PCR kit, it was clearly shown that nested RT-PCR analysis has both higher specificity and sensitivity, thus confirming that nested RT-PCR should be used for the clinical application for detecting [39–41] the SARS-CoV-2 whenever the viral load is low. However, there is a great chance of cross-contamination in nested RT-PCR, which may end in false-positive/negative results.

1.2.2.3 Detection and Analysis Approach via Droplet Digital PCR

For enhancing the accuracy of SARS-CoV-2 detection, sensitivity, and lower LOD (limit of detection), a technique called ddPCR (droplet digital polymerase chain reaction) has been implemented. By using the exact probe/primer sets issued by China CDC targeting ORF1ab or N gene, the utility of the ddPCR technique was studied by Suo et al. for the detection of SARS-CoV-2 RNA compared with the qRT-PCR. The importance [41–45] of ddPCR can be understood by the fact that 26 patients with negative RT-PCR results were re-confirmed as COVID-19 positive using this technique. There was a huge improvement in the accuracy and sensitivity from 47% and 40% of RT-PCR to 95% and 94% of ddPCR, respectively. Almost 43% of patients (42.9% to be exact as 6/14 patients) were tested positive by ddPCR within 5–12 days after the discharge. According to a study, a clear and large decrease can be observed in the number of false-negative results of qRT-PCR. The eight primers/probe sets [46] with the exact conditions and samples were used to further analyze the ddPCR and qRT-PCR performance. The results confirmed that qRT-PCR often gives us false results whenever the viral load is low as all the eight probes/primers that were used in qRT-PCR were not able to effectively [47–57] differentiate the positive and negative at a low viral load of 10–14 dilutions. qRT-PCR tests with false-positive results of US CDC-N1, N2, and China CDC-N probe/primer sets were identified. Although ddPCR was better than qRT-PCR in the overall performance, especially in the case of low viral load samples, however, it also had some limitations. Presently, ddPCR is more costly than qRT-PCR for each test performed by using consumables and suitable instruments [58–65]. Also, precise materials and gold standards still need to be effectively defined to ensure the commutuality between the molecular diagnostic laboratories.

1.2.2.4 Lab-on-chip Approaches Using Nucleic Acid as Chief Target Points

Loop-Mediated Isothermal Amplification Rapid amplification at a single temperature, which is highly effective and efficient in the rapid and safe diagnosis of coronaviruses, is the advantage of LAMP (loop-mediated isothermal amplification). The full LAMP primers that target the 5′ region of N genes and ORF1a genes of the SARS-CoV-2 and detected via colorimetric and visual RT-LAMP alongside a monetary RT-PCR assay were designed. In his experiment, total seven samples were taken among which six exhibited a visible [66] change in the color, thus depicting positive amplification, whereas one sample did not change its color and

remained pink and thus was confirmed as negative. The RT-PCR results and the colorimetric RT-LAMP analysis were 100% consistent with each other across a range of Cq values (cycle quantification value) and matched with the RT-PCR in the point-of-care settings and field without any calibrant instrumentation. An isothermal LAMP-based detection method was designed by Yu et al. for the ORF1ab gene and is known as the isothermal LAMP-based method for COVID-19 (ILACO). The comparison of 11 respiratory viruses' sequences (2 normal CoVs, 2 influenza viruses, and 7 similar CoVs) was done using ILACO, which ultimately showed the species specificity. Moreover, the sensitivity of Taqman-based RT-PCR and ILACOs' was comparable to each other, which can detect as low as 10 copies of SARS-CoV-2. Another extremely sensitive, point-of-care test based on LAMP and Penn-RAMP (rapid isothermal amplification assay), nested-like amplification assay, was [64] designed. For the testing of purified targets, LAMP and RT-PCR sensitivity was 10 times lesser than that of RAMP, and for testing the samples that are minimally processed, it was 100 times lesser than that of RAMP. The method of RAMP is perfect for home use, point-of-care, and in the clinic with the least trained people and minimal instrumentation. It can also lessen the false-negative results from the normal nucleic acid tests.

1.2.2.5 Analysis Through Nanoparticle Amplification Process

In the nucleic acid amplification system, there is an important application of nanoparticles for enhancing the specificity and sensitivity of SARS-CoV-2 detection. A naked-eye colorimetric method that is based on AuNPs [67] (gold nanoparticles) along with thiol-modified ASOs (antisense oligonucleotides) targeting the SARS-CoV-2 N-gene was developed. In the test performed by Parikshit, the LOD calculated was 0.18 ng/μl of the SARS-CoV-2 viral load. Moreover, in a one-step nanoparticle-based biosensor (NBS) that was coupled with RT-LAMP, the LOD found was 12 copies/test. When the RNA templates from non-COVID-19 patients were studied, it was found that the specificity of the assay among the laboratory-confirmed COVID-19 patients was 100% (96/96) and the analytical sensitivity of SARS-CoV-2 was also 100% (33/33). The nanoparticles have some different properties that provide them an extra advantage over the classical conventional methods that are laborious and more expensive. For the diagnosis of the SARS-CoV-2 disease in first-line clinical laboratories, the nanoparticle-based amplification is an effective technique, particularly in the areas that have limited medical resources. The only limitations of this technique are that the pretreatment steps are quite complex, and also it is way more expensive as compared to qRT-PCR. Also, a high risk of photobleaching is there [68] that might end in false-negative/positive results and can also decrease the sensitivity, as it uses the conventional organic carriers [69].

1.2.2.6 Portable Methodology: The Concept of Benchtop-Sized Analyzer

One of the powerful, accurate, and highly sensitive methods for the rapid detection of SARS-CoV-2 has been provided by the [70] automated molecular diagnostic platform. This assay can easily achieve various technological innovations and

instant decisions, even without any point-of-care testing or any PCR training in the laboratory. It has been observed that for the detection of SARS-CoV-2, the performance of different portable benchtop-sized analyzers was inconsistent. The QIAstat-Dx Respiratory SARS-CoV-2 panel was evaluated by Benoit et al. for SARS-CoV-2 detection. The sensitivity of this platform (LOD at 1000 copies/ml) was comparable to that of RT-PCR. The overall percentage [71–74] recommended by WHO of QIAstat-Dx SARS and RT-PCR was 97%, with a sensitivity of 100% (40/40), and a specificity of 93% (27/29). There was no observed cross-reaction of any other bacteria or respiratory virus in this assay. According to the results, the sensitivity of the QIAstat-Dx Respiratory SARS-CoV-2 panel was comparable to that of the RT-PCR assay (Table 1.1).

According to a recent research, the PPA (positive percent agreement) between the ID NOW tests and an improved test developed by CDC laboratory is 94%, whereas, according to other assessments, the PPA of ID NOW is [75–79] lower (75–87%), when compared with laboratory-developed reference methods. The detection time for each sample is the fastest for ID NOW (~17 minutes) when compared with the ePlex assay (~1.5 hours) and the Xpert Xpress assay (~46 minutes), but the limitation is the decrease in clinical and analysis performance, with the lowest PPA and highest LOD. Moreover, this assay had a specificity of 100% as shown by a research; however, among the 46 SARS-CoV-2-positive samples taken, 13 were found to be false negative; thus, the sensitivity got reduced to 71.7%. All the false negatives were actually the weak-positive samples [80–84]. Thus, it is clear that for the samples with [75–79] average or high viral RNA load, ID NOW has fair performance but shows low sensitivity in the case of weakly positive samples.

Xpert Xpress point-of-care assay (Cepheid GeneXpert systems) was evaluated by Femke et al. to target the SARS-CoV-2 E-gene and N2-gene in the medical laboratories of the Netherlands. It can detect SARS-CoV-2 with an LOD of 8.26 copies/ml in these laboratories. However, the Xpert Xpress test was reported for targeting the SARS-CoV-2 E-gene and the N2-gene with an LOD of 100 copies/ml [85–89]. The various methods were used to identify the input concentration that ultimately resulted in this difference, and it requires more verification. Compared to the LOD of ePlex (1000 copies/ml) and ID NOW (20 000 copies/ml), Xpert Xpress had the lowest LOD (100 copies/ml) and highest PPA (98.3%) when compared to ePlex (91.4%) and ID NOW (87.7%) – according to Wei's study.

1.3 Antigenic Approach for COVID-19 Diagnosis

There are various virus-encoded proteins such as E, S, M, and N proteins in SARS-CoV-2. The main antigenic targets of SARS-CoV-2 antibodies are N and S proteins. The S protein is spliced into two different polypeptides (S1 and S2) in most of the CoVs by the action of a host cell furin-like protease. Although the S protein exists on the viral surface and is also essential for viral entry, still the protein that is the most abundantly expressed immune dominant protein that interacts with the RNA is the N protein, and the N protein is also more conserved than the S protein.

Table 1.1 Techniques incorporated for diagnosis and detection for COVID-19.

Techniques incorporated for diagnosis and detection	Method employed for the specific technique	Advantages offered by the technique	Disadvantages offered by the technique
Sequencing of genome	Nanopore-assisted and targeted sequencing	Accuracy is very high, variation can be monitored, turnaround time is rapid, and detection range is wide	Costly, skill is required, and delicate instruments are used
	Sequencing based on hybrid capture	Intra-individual variations can be detected easily, highly sensitive	Recombinant viruses or highly diverse viruses cannot be sequenced using this method, use of sophisticated instruments
	Amplicon sequencing	Sensitivity is high, convenient, highly economical, low viral load samples can be detected easily	Recombinant viruses or highly diverse viruses cannot be sequenced using this method, use of sophisticated instruments
Polymerase chain reaction	Nested RT-PCR	Higher specificity than RT-PCR, viruses with low copy number can be detected easily, highly sensitive, requires less time	The pretreatment steps are quite complex, requires highly skillful people
	qRT-PCR	Equipments are not expensive, time-saving, and highly sensitive	Frequent problems of false-negative, pretreatment steps are quite complex, requires highly skillful people
	ddPCR	Low viral load samples can be detected easily, does not depend on standard curve, sensitive	Exogenous contamination can be seen, much expensive than qRT-PCR
Immunological diagnostic tests	ELISA	Risk of infection is low, simple, and convenient, detection is quantitative	Sensitivity is less, may encounter cross-reactivity, time-consuming, use of highly expensive monoclonal antibodies

The S1 subunit in the S protein [90] is more eminently specific to SARS-CoV-2 and is less conserved, thus proving that for the COVID-19 serologic identification, the S1 subunit is much more specific as an antigen when compared with the S2 subunit or full-length S protein. Moreover, the cross-reactivity of the RBD (receptor-binding domain) with other CoVs is very less. Also, in comparison with the full-length S or S1 subunit, the RBD domain of the S1 protein is much more conserved. The targets used are the various forms of S protein (RBD or S2 domain, full-length S, S1 domain) and N protein.

The most frequently used technique for SARS-CoV-2 antigen detection is the immunochromatographic assay. Bioassay, Liming bio, Savant, and RapiGEN (the four lateral flow antigen detection kits) were analyzed and compared by Thomas et al. for SARS-CoV-2 detection. There was an observable difference in the test performances. Out of all the four tests, the test with the highest accuracy (89.2%) and κ coefficient of 0.8 was the Bioassay test, and because of the poor performance, the Liming bio test was discontinued while testing. The sensitivity of the other kits varied from 16.7% for Sarvant assay to 85% for the Bioassay test.

For the detection of SARS-CoV-2, various highly sensitive biosensor-based tests have been established when compared to the lower sensitivity of immunochromatography. To detect the SARS-CoV-2 S1 protein, a rapid, portable cell-based biosensor with human chimeric spike S1 antibody was developed by Sophie et al., which permits tests completed within three minutes with a 1 fg/ml detection limit and a 10 fg to 1 μg/ml semilinear response range. Furthermore, for targeting the SARS-CoV-2 S1 protein, eCovSens [91] (a biosensor device) was designed, who correlated it with another commercial potentiostat biosensor. In the saliva samples, eCovSens had an LOD of 90 fM, while the LOD for the commercial potentiostat biosensor was 120 fM. Thus, these are helpful for monitoring SARS-CoV-2 antigen on large scale, thus providing hope of eventual control of the pandemic.

1.4 Antibody Diagnostic Strategies for Detection of COVID-19

The antibody test for the diagnosis of a specific antigen has become a preference in detecting the rising titers of individual antibodies such as IgM, IgG, and IgA. In addition, these antibody productions can be an indicative strategy that relies on the appearance of different antibodies indicating different infection situations, such as the rise of IgM that is produced within [92] 4–7 days is helpful in determining the frequency of recurrence of the infection, while the rise of IgG (10–15 days) provides a sure reason for easy detectability (Figure 1.1) of the viral infection, respectively. Further IgA is a useful indicator of mucosal immunity and can be easily detected in mucus secretions within five to eight days of onset of infection.

In situations where RT-PCR fails to demonstrate the results, serum analysis can be conducted during the important phases such as the acute and convalescent phase, which support validated serological procedures for rapid analysis of COVID-19.

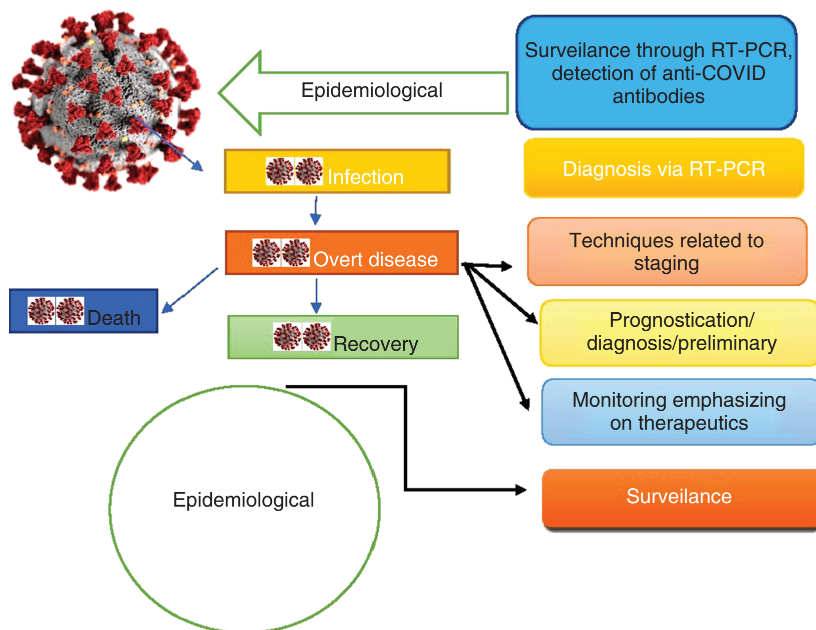

Figure 1.1 Epidemiological surveillance, monitoring, and prognostication of COVID-19. Courtesy of Alissa Eckert, MSMI, Dan Higgins, MAMS.

These antibody tests fall under two important diagnosis mechanisms, known as the "laboratory analysis and the point-of-care tests."

1.4.1 Enzyme-Linked Immunosorbent Strategies: The Vircell and Euroimmun ELISA

A study conducted highlights [93] the performance of different assays with a recombinant tagged N protein and S proteins corresponding to Vircell COVID-19 IgG and Euroimmun SARS-CoV-2 IgG.

The reports clearly indicated around 75–80% sensitivity in the case of Vircell enzyme-linked immunosorbent assay (ELISA) and around 50–60% in the case of Euroimmun ELISA on the 5–10th day of confirmation of COVID-19 by PCR. In addition, on the subsequent days, the sensitivity parameter was increased to 100% and 94% both in the case of Vircell and Euroimmun ELISA, respectively. Similarly, another research evaluated two specific diagnostic kits based on the N and S protein, where the sensitivity was reported around 90–95%. However, the overall sensitivity of both N- and S-based ELISA was around 65–70%, respectively.

1.4.2 Immunoassay-Based Detection Approach: Immunofluorescence and Chemiluminescence Assay

The first trial for detection through immunofluorescence technique was reported in Finland where the detection of SARS-CoV-2 IgM and IgG antibodies in serum was confirmed in a COVID-positive case [94].

The strategy involved the continuous dilution of patient's serum and a long incubation period in vero cell lines of 30 minutes for IgG and 2 hours for IgM. The sensitivity of IFA and the respective neutralization tests at different stages of COVID infection were recorded around 75–76%, which increased up to 100% by the 10th and 12th day of infection. Although this is a promising strategy for diagnostics, but in non-fluorescent cases, it may give false assumptions and results, research is needed to upgrade the specificity and sensitivity of the diagnostic strategies related to IFA. The advantage of quantitative detection and analysis belongs to the most popular diagnostic strategy known as the "chemiluminescence immunoassay (CLIA)." The recent research employs the benefit of detection against a specific target ranging from an ORF to N and S proteins. The individual sensitivity of both the antibodies (IgM and IgG) was recorded around 55–71%, whereas a combined sensitivity percentage increased to 82%. A similar research was carried out with four rapid tests known as "three LFA test and ELISA targeting IgM and IgG," for detecting COVID-19.

1.5 Point-of-care/Lab-on-chip Approaches: The LFA (Lateral Flow Assay)

The lateral flow assay (LFA) focuses on both in vitro semiquantitative and qualitative analysis and detection of SARS-CoV-2 IgM and IgG antibodies in plasma, serum, and venous blood samples [84–87].

Recent research studies highlight the working of three LFA tests known as the Quick Zen a Labo On Time and Avioq and in addition two [95] quantitative for the detection of SARS-CoV-2 IgG, IgM, and IgA antibodies in serum samples confirmed by RT-PCR. The test was recorded with 100% specificity in the analysis process, and further sensitivity of all these tests ranged from 90% to 95%, respectively. A related research also confirmed the three important factors (specificity, sensitivity, and seropositivity) in the diagnosis of COVID-19 patients. The recorded data show the sensitivity percentage of about 90% for IgG, followed by 91% in IgM and around 98% when seen in combination. A comparison between sensitivity percentage clearly depicts that a decrease has been witnessed in terms of sensitivity in IgG antibody as compared to IgM.

1.6 Miniaturization Detection Approach: Combining Microarray with Microfluidic Chip Technology

The concept of lab-on-chip technology has provided a strong microarray and microfluidic platform of technology where miniaturization can lead to automation and portability, high sensitivity, and high-throughput analysis in nano-based strategies.

The whole idea depends on the incorporation and integration of specific functions into small platforms known as "chips" for pathogen detection and diagnosis. Literature reports regarding a 65-microarray antigen concept for diagnosing

respiratory viruses related to various species of SARS. It additionally includes the coated [96] antigens corresponding to the S, N, S2 MERS-CoV protein. This platform provides advantages related to low cost, high sensitivity, and high specificity and proves to be a potential valuable tool for sero-survillence of COVID-19 patients. The techniques allow easy and specific detection of COVID-19 but lacks in expressing in some important mammalian cells that generally needs to be optimized and standardized.

1.7 Neutralization Detection Approaches Toward COVID-19

The gold standard evaluation strategies include the discoveries related to viruses, their pathogenesis, and their ability to induce infection. In this context, neutralization tests have been recommended to evaluate the serum capacity derived from COVID-19-infected person to reduce down the CPE effects (cytopathic effects) [97] caused by SARS-CoV-2. A strong positive correlation is being reported between the neutralizing titers of antibodies and total CoV IgG (anti-S1 IgA, IgG, and IgM) antibodies. Neutralization assay stands as a specific choice of test to monitor patient immunity to the virus. However, when compared with serological tests, neutralization tests are marked as laborious and tedious and have a limitation of being SARS-CoV-2 restricted to only biosafety level 3 or BSL 2, respectively.

1.8 Genomic Sequencing Detection Approach: The Amplicon, Hybrid Capture, and Meta-transcriptomic Strategy

A powerful tool known as "genomic sequencing" for analyzing the evolution of virus, correlating genetic association to different diseases, tracing the outbreaks of diseases, and finally developing new strategies, therapies, and vaccines is the need of the hour. The first genomic sequence of SARS-CoV-2 was done by combining a meta-transcriptomic technique with Sanger's sequencing method [11, 33]. Research studies carried out by Lu et al. reported around 10 genome sequences of SARS-CoV-2 from targeted patients including BALF and culture samples with the help of meta-transcriptomic sequencing. The genome sequences in the study were similar; having 99% sequence similarity, the 10 genome sequences were nearly identical, displaying more than 99.98% sequence identity [13].

The genome analysis of the nasopharyngeal and oropharyngeal found somewhat identical to each other and similar to the SARS-CoV-2 sequences. The method involving the amplicon sequencing method and hybrid capture sequencing includes very high sensitivity with low accuracy. Further, these techniques cannot be applied to recombinant or hybrid viral strains because of the unavailability of probes and primers. Significant increase is being witnessed for the SARS reads, indicating

enrichment efficiency that ranges from 5710- to 5595-fold in amplicon and hybrid sequencing patterns. However, lower frequency of sequencing for lower viral loads has been displayed by the alleles identified by hybrid capture sequencing when compared to amplicon and meta-transcriptomic sequencing [46, 98–109].

1.9 Conclusion

The global spread of COVID-19 has not only restrained the economic security but has also led to the health risks and threats to acerbate at a high rate. The detection and analysis thus become priority to diagnose the infection at an early stage and finally control its spread and transmission. RT-PCR routine confirmation has been widely accepted as the gold standard test for the screening and identifying SARS-CoV-2, which emphasizes the identification of conserved regions pertaining to the viral genome. However, some loopholes have been encountered regarding various mismatches in RNA primers, probes that can lead to poor performance ratios, and finally can result in negative or false results. In addition, serological tests can result in highlighting outbreaks and assessment of the percentage of viral attack in terms of antibody titer evaluation (IgG and IgM). However, these serological methods highlight diverse seroconversion processes that are not reliable for early detection. Further the use of immunological based strategies and molecular diagnosis are not suitable or not preferred for lab-on-chip or point-of-care detection strategies because of time consumption, expensive inputs, and strict biosafety regulations. Many upcoming strategies and methodologies that offer safety, quick diagnosis, efficacy, and sensitivity is into limelight (benchtop analyzers and lateral flow process). The demand of the present and future era is to provide and standardize some synergistic combinations of the techniques so that various advantages can be combined up for monitoring, detecting, screening, and diagnosing the current COVID-19.

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