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Laboratory-Based Diagnostic Tools for COVID-19: An Overview of Challenges and Limitations

Surabhi Shukla[1], Suruchi Singh[2], Namrata Khanna[3], Tanushri Chatterji[4]*, Upasna Yadav[5]*, Reeta Maurya[6], Sadanand Pandey[7]*

[1] *Department of Microbiology, Dr. Ram Manohar Lohia Institute of Medical Sciences (RMLIMS), Vibhuti Khand, Gomti Nagar, Uttar Pradesh, Lucknow- 226010, India*
[2] *Life Sciences, Tata Consultancy Services Limited, Hiranandani Estate, Thane (W), Mumbai-400607, India*
[3] *Department of Biochemistry, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Faizabad Rd, Atif Vihar, Uttar Pradesh, Lucknow–226028, India*
[4] *Department of Microbiology, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Faizabad Rd, Atif Vihar. Uttar Pradesh, Lucknow–226028, India*
[5] *School of Applied Sciences, Amity University Lucknow Campus, Malhaur (Near Railway Station). Uttar Pradesh, Lucknow-226028, India*
[6] *Department of Chemistry, College of Natural Science, Yeungnam University, 280 Daehak-Ro, Gyeongsan, Gyeongbuk, 38541, Republic of Korea*

Corresponding authors's e-mails: *tanushri@gmail.com* (TC); *upa_biotech@yahoo.com* (UY); *sadanand.au@gmail.com; spandey@ynu.ac.kr* (SP)

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Abstract

The spread of severe acute respiratory syndrome coronavirus-2 (SARS Cov-2) as a pandemic has been a catastrophic clinical situation afflicting millions and affecting the socioeconomic scenario across the world. These unprecedented circumstances have evoked the need for an early and accurate diagnosis, followed by immediate and effective treatment of the disease. A reliable, rapid, and correct diagnosis is required to prevent transmission and for early patient management. False-negative results hasten the spread of the contagion, while false-positive results cause nonessential therapy and may result in unwarranted agony to the individual. Therefore, detection of the virus should be through accurate, rapid, and convenient diagnostic tests. Various immunological and nucleic acid amplification-testing kits are currently in use. Reverse transcription-polymerase chain reaction (RT-PCR) is a promising technique for COVID-19 diagnosis, but it is not accessible at the primary hospital level. For accurate detection of the coronavirus, sample collection plays a crucial role. Usually, a nasopharyngeal swab is collected as a sample. However, in some instances, to confirm detection, sputum and bronchoalveolar lavage (BAL) samples may be obtained from the lower part of the respiratory tract. The purpose of this review is to provide a brief overview of the specimen selection and laboratory techniques available for detecting SARS Cov-2 so that medical professionals can strategize the setting up of sophisticated and well-equipped diagnostic centers.

Abbreviations

BAL: Bronchoalveolar Lavage; CDC: Centre for Disease Control and Prevention; COVID-19: Coronavirus Disease; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats- and associated Cas Proteins 13; Ct: Cycle threshold; CT: Computed Tomography; DMEM: Dulbeco's Modified Eagle's Medium; ELISA: Enzyme-Linked Immunosorbent Assay; HCPs: Healthcare Professionals; HE: Hemagglutinin Esterase; ICMR: Indian Council of Medical Research; IgM: Immunoglobulin M; IgG: Immunoglobulin G; LAMP: Loop-Mediated Isothermal Amplification; NA: Nasal Aspirate; nCoV: Novel Coronavirus; NGS: Next Generation Sequencing; NMT: Nasal Mid-Turbinate; NS: Nasal Swab; OP: Oropharyngeal; ORF: Open Reading Frames; PPE: Personal Protection Kit; RdRp: RNA-dependent RNA polymerase; RT-qPCR: Quantitative Reverse Transcription Polymerase Chain Reaction; RT-PCR: Reverse Transcription Polymerase Chain Reaction; SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus; SHERLOCK: Specific High Sensitivity Enzymatic Reporter Unlocking; WHO: World Health Organization.

1. Introduction

On December 31st, 2019, WHO reported cases of pneumonia with unknown etiology from the city of Wuhan in China. However, the causal agent was not confirmed until January 2020, when it was identified on the basis of its genetic constitution as the novel Coronavirus (nCov) or Coronavirus 2 (SARS-CoV-2) [1]. WHO named the disease COVID-19. The identified causal virus was given the name SARS-CoV-2 (severe acute respiratory syndrome Coronavirus 2) by the International Committee on Taxonomy of Viruses (ICTV) based on the fact that it was genetically related to the Coronavirus, which resulted in the SARS outbreak of 2003 [2]. COVID-19 is extremely contagious, and its lightning-fast spread affected millions worldwide. On 23rd April 2022, there were 505,817,953 cumulative cases of COVID-19 and 6,213,876 cumulative deaths reported globally [3]. In January 2020, COVID-19 was declared a Public Health Emergency of International Concern by WHO, as the number of cases rapidly increased worldwide [4]. In India, as reported by government agencies, the total number of cases is 43,052,425 and 5,22,116 deaths have been reported so far [5]. The COVID-19 infection has reported outbreaks not only in India but also in various developed and developing countries like the USA, Brazil, Spain, Britain, Italy, etc.

As stated by the Centre for Disease Control and Prevention (CDC), USA, the etiology of the COVID-19 virus is linked to a new virus entity, associated with the Coronavirus (CoV) family. The virus family comprises α, β, and δ strains of Coronaviruses. The novel coronavirus (nCoV) is reported to have six open reading frames (ORFs) and a plethora of additional genes. The 5′ terminal end of the virus consists of ORF1 and ORF2, constituting two-thirds of the overall genetic makeup. These ORFs are encoded by two polypeptides, *viz*. pp1a and pp1ab, and both are sequentially fragmentized into 11 and 16 proteins, respectively. On the other hand, the 3′ terminal end consists of the nucleocapsid (N), membrane protein (M), an envelope protein (E), and a spike (S) protein. The S gene helps SARS CoV-2 in detecting hosts and receptors, and their virion may possess hemagglutinin- esterase (HE) protein [6]. The virus can broadly affect numerous hosts, such as mammals and birds, besides humans. Coronavirus is classified as an RNA virus with a size ranging from 27 to 32 kb of positive-sense single-stranded RNA [7, 8].

Individuals with SARS Cov-2 infection may either be asymptomatic or exhibit acute manifestations of respiratory and digestive systems [7, 8]. The clinical indications generally reported for COVID-19 infected patients are primarily fever and cough, along with shortness of breath and myalgia. Severe manifestations include acute

respiratory distress syndrome (ARDS) and cytokine storm, leading to fatalities [9]. Other clinical implications associated with COVID-19 are diarrhea, fatigue, cough, anorexia, increased sputum production, dyspnea, and myalgia [10, 11]. The initial onset of infection does not indicate fever or imaging abnormalities [12].

In order to accurately detect the coronavirus, sample collection plays a crucial role. Usually, nasopharyngeal swab is collected as a sample. However, in some instances, to confirm detection, sputum, and bronchoalveolar lavage (BAL) samples may be obtained from the lower part of the respiratory tract [13].

A reliable, rapid, and correct diagnosis of SARS-CoV-2 is essential to prevent transmission, and for timely patient management, false negative test results can hasten the spread of the contagion, while false positive results may result in nonessential medication, causing unwarranted agony [14]. Therefore, detection of the virus should be through accurate, rapid, convenient, and consistent diagnostic tests. Various immunological and nucleic acid amplification testing kits are currently used to detect infection. **(**Figures 1, 2**)**.

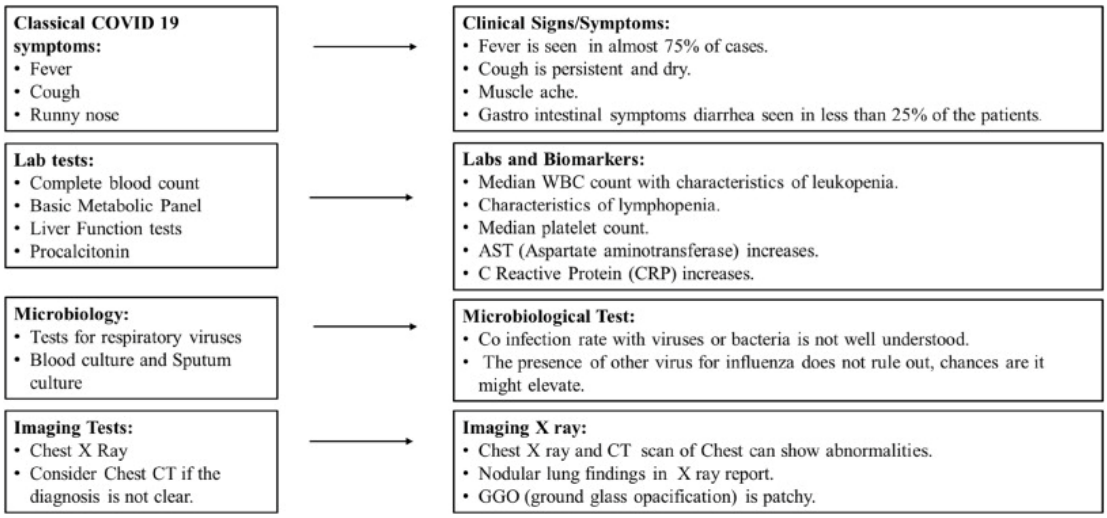

Figure 1. Clinical Manifestation followed with laboratory testing of SARS-CoV-2 [Adapted from WHO guidelines (15)]

The present review article explores the disposability and effectiveness of various diagnostic tools along with the selection of appropriate specimens that aim at an accurate diagnosis of SARS-CoV-2 cases. Reverse transcription-polymerase chain reaction (RT-PCR**)** is a promising technique for COVID-19 diagnosis. However, it is not accessible at the primary hospital level [15]. Due to the non-availability of RT-PCR at the primary level and for direct detection of the virus, a Rapid Card test or Rapid Diagnostic Test (RDT) is preferred for a preliminary diagnosis. RDT is based on the isolation of viral proteins. Like RT-PCR, RDT is also performed in sputum and throat swabs collected from the respiratory tract. In addition, human antibodies generated after infection are detected in serum samples. However, false negative results are common with RDT. Therefore, prudence should be exercised in interpreting the results, considering the negative predictive value of the test [16].

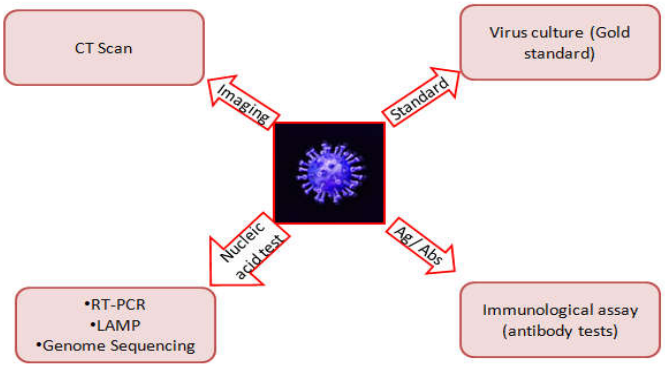

Figure 2. Diagnostic approaches for detecting Coronavirus

2. Pathophysiology of COVID-19 Infection

The SARS-CoV-2 invades the host cell through endoplasmic uptake, and the onset of infection results in elevated expression of ACE_2 receptors. The upper and lower respiratory regions, including the tissue of endothelium, myocardium, and gastrointestinal mucosa cells, depict type II pneumocytes. As the virus enters the respiratory system, it releases its genetic material (RNA) into the cytoplasm. On this site, replication of the viral genome and production of its proteins occur. The freshly composed nucleocapsid constituting the viral RNA and N protein is transferred to the endoplasmic reticulum-golgi intermediate compartment. Assembled and packaged newly synthesized viral progeny is imported in vesicles to the plasma membrane where they exit [17-20].

3. Types of Clinical Specimen and their Handling

CDC directs the collection of specimens from the upper respiratory tract, preferably nasopharyngeal swabs, for COVID-19 testing by RT-PCR [21]. If there is difficulty in collecting a nasopharyngeal sample, other specimens like oropharyngeal (OP), nasal mid-turbinate (NMT) swab, an anterior naris (nasal swab; NS) specimen, and nasal aspirate (NA) can be collected. The collected specimen is transported to the laboratory in a tube designed specifically for viral transport. Likewise, for performing the assay in serum, blood is drawn as per standard protocol given by the Infection Prevention and Control Guidelines of WHO. Health care professionals (HCPs) should use personal protective equipment (PPE) like masks (N95 preferred), gloves, shields, and glasses to protect the face and eyes while collecting the samples to avoid transmission of infection [22]. A flow diagram for handling the specimen as per the WHO guidelines is shown in Figure 3.

In contrast to the CDC, the WHO recommends a triple packaging system for the transportation of specimens to the reference diagnostic laboratories. The vial for the sample must be appropriately labeled, sealed, and externally covered with an absorbent material (primary container). The primary container is then kept in a secondary container. Thereafter, the secondary container is kept in an external container with frozen gel. The external container is a thermocouple box for maintenance of the storage temperature of the sample ($\leq$ 5 days: 4˚ C and $\geq$ 5 days: -70˚C) (Figure 4). Standard protocol [23] should be followed right from the process of specimen collection to packaging, labeling, and further shipping for accurate diagnosis.

Figure 3: Flow diagram depicting specimen handling for COVID-19 detection [Adapted from Holshue *et al.*, 2020 (21)]

Figure 4: Materials required for COVID-19 sample collection [Adapted from Holshue et al., 2020 (21)]

Various studies have reported that apart from affecting the respiratory tract, the virus can also invade the digestive and hematological systems. Peng *et al.* conducted studies on a variety of specimens collected for the detection of coronavirus RNA. The pharyngeal swab was reported with the highest positivity rate of 78%, followed by anal and blood samples with a 22% positivity rate, while positivity in urine was 11% [24]. Another investigation reported that stool, oropharyngeal, and nasopharyngeal samples tested positive for SARS-CoV-2, whereas urine and serum samples reported negative results. This study was published as a case report conducted on a single individual [25]. A study reported from China, conducted on 41 individuals who tested positive for SARS-CoV-2 infection, reported a high mortality rate of 15%. [26]. Another noticeable study from China confirmed the detection of the virus in blood and stool apart from samples of the respiratory tract, though the results were not conclusive [27].

According to a recent publication from Japan, when a viral infection is suspected, a negative PCR report from the throat swab cannot reliably rule out infection. In such cases, bronchoalveolar lavage (BAL) samples from the lower respiratory tract should also be tested for accurate diagnosis [28, 29]. Hence, the final diagnosis of SARS-CoV-2 infection is made only after running tests on different specimens. Many other specimens can be considered for diagnostic purposes, though the intensity of infection may vary with site or types of specimens. After a preliminary assessment, further tests need to be performed for confirmation, as they enhance the rate of detection and reduce false negative outcomes. Likewise, Peng *et al.* also recommended the necessity and utility of testing different samples for detecting COVID infection, also for monitoring progression of the disease, and establishing its prognosis [24].

Table.1 RT-PCR results of various studies for diagnosis of SARs-CoV-2 infection

Target	No. of patients	Positive (%)	Sensitivity	Specificity	Reference
RNA-dependent RNA polymerase (RdRp)/helicase (Hel) gene	64	58 (91%)	91%	NA	Zhu *et al.,* 2020 [28]
Nonstructural protein 2 (nsp2) Real-Time RT-PCR Assay	14	14 (100%)	100%	NA	Jonsdottir *et al.*, 2016 [29]
Open reading frame 1ab (ORF1ab)	34	27 (79.4%)	79%	100	Hopkins, 2020 [30]
Open reading frame 1ab (ORF1ab), nucleocapsid (N) gene and envelope (E) gene	57	36 (63.15%)	71%	NA	Xiang *et al.*, 2019 [31]
Nucleocapsid protein (NP) genes	4880	1942 (39.8%)	NA	NA	Chen *et al.*, 2004 [32]

4. Laboratory Diagnosis of SARS-COV-2 Infection

4.1. *Virus Culture*

A standard methodology that can be adopted for performing virus culture was reported by *Kim et al*. In this method, virus from nasal and oropharyngeal samples was inoculated into Vero cells, which were cultured in 1 × Dulbecco's modified Eagle's medium (DMEM) augmented with 2% fetal bovine serum, at 37 °C with 5% CO_2 [26]. Cytotoxic effects, confirmed by RT-PCR, were observed after three days of inoculation. The morphology observed was

similar to that of SARS coronavirus, and the viral particle size ranged from 70 to 90 nm. Kim *et al.* further concluded that the virus could be observed in many intracellular organelles, chiefly vesicles [30]. SARS-CoV-2 was first isolated using Vero CCL-81 cells in India [31]. The inoculated cells from nasal and oral swabs were sectioned and subjected to transmission electron microscopy, as standardized by Kim *et al*. It is recommended to culture the virus in Level 3 of a biosafety facility.

Another research in China has also isolated the virus from epithelial cells of the respiratory tract and also from Vero E6 and Huh-7 cell lines through bronchoalveolar-lavage (BAL) inoculation [32]. The cultures of epithelial cells from the human respiratory tract are quite promising for the identification of respiratory pathogens in the human respiratory tract. However, the procedure requires expertise and a large workforce [33]. Cell culture is usually recommended for the isolation and identification of viruses; however, the same is not useful for diagnosis.

4.2. *Immunological/ Serological Assay*

Serological assays rapidly evaluate immune responses generated against infectious agents. The immunological/serological assay provides information regarding exposure to the SARS-CoV-2 virus. It assesses the antibodies generated as immune response or estimates the viral protein in the specimen from the respiratory tract. When a virus invades the body, it elicits an immune response by producing specific antibodies against that virus. The mechanism of action involved in the detection of the viral entity is depicted in Figure 5.

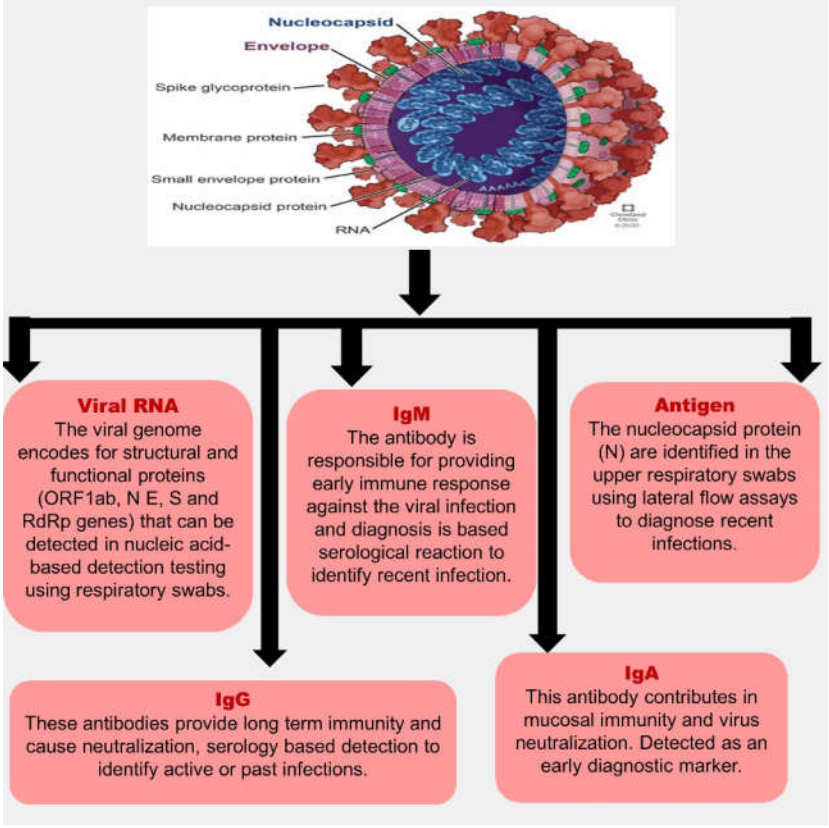

Figure 5. Generation of varied immune response against SARS-CoV-2 (Adapted from [20, 34])

However, as stated earlier, the antibody identification assay is not recommended for the detection of active cases of coronavirus infection, as it may give negative results. There are four types of serological assays [35] (Figure 6):

a) Rapid diagnostic test (RDT): qualitatively identifies antibodies against the virus present in serum.
b) Enzyme-linked immunosorbent assay (ELISA): quantitatively confirms antibodies against the virus present in serum.
c) Neutralization assay: detects the presence of active antibodies in serum that are able to inhibit virus growth *ex vivo* in a cell culture system.
d) Chemiluminescent immunoassay: provides quantitative presence of antibodies against the virus present in the patient's serum.

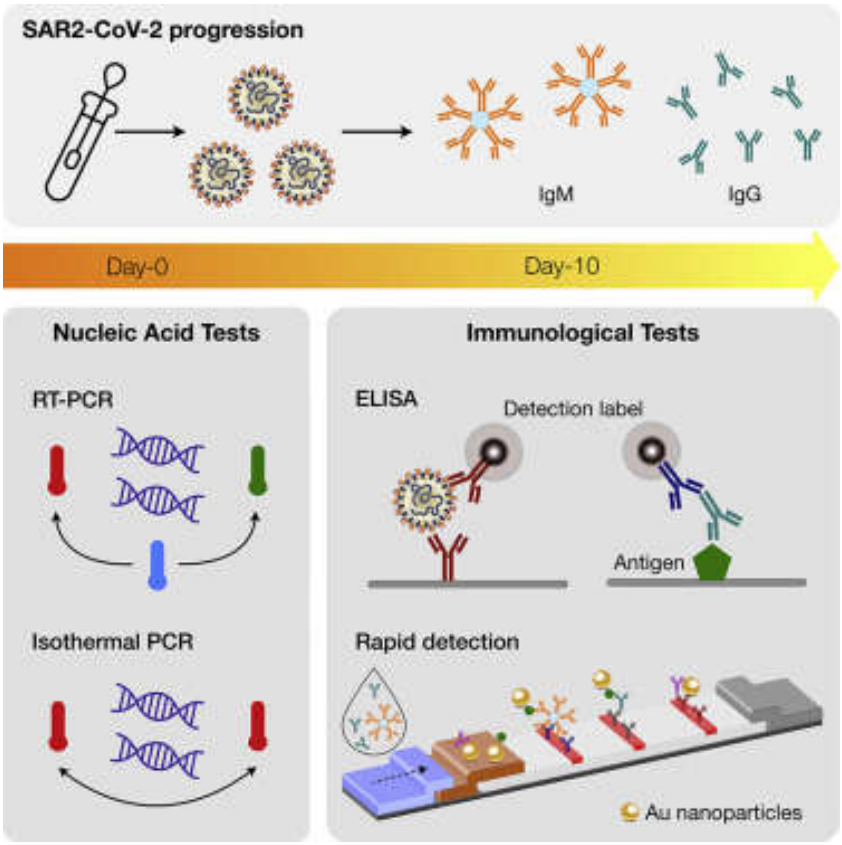

Figure 6. Efficacy of various serological tests for SARS-CoV-2 detection (Adapted from FDA statement, 2020 [38])

During the outbreak of the pandemic, multiple documents reported that the identification of viral-specific antibodies IgM and IgG are valid for assays in serum. To support the fact, Xiang *et al.* [36] conducted a study and verified that serological diagnosis of coronavirus based on IgM and IgG ELISA has high diagnostic specificity. In this study, it was observed that specificity and sensitivity for detection of IgM were 77.3% and 100%, respectively. Further, for IgG detection in COVID positive patients, the sensitivity and specificity were 83.33% and 95.0%, respectively. Likewise, in cases suspected of COVID-19, sensitivity and specificity for IgM were 87.5% and 100%, respectively, and for IgG, 70.8% & 96.6%, respectively. Hence, high specificity of both IgG and IgM is a reliable tool for the diagnosis of COVID-19 infected patients.

Earlier serological assays were employed in SARS and other Coronavirus epidemics and played a vital role in establishing their etiology [37, 38]. It was observed that immunohistochemistry (IHC) analysis is a significant tool for detecting antigens in pulmonary tissue. The presence of IgM and IgG antibodies in serum further confirms COVID-19 infection [32]. Additionally, recent research on family cluster cases of SARS-CoV-2 infections stated that testing in serum samples also assists in the early diagnosis of the infection by screening close contacts [39].

The lateral flow assay is employed for immunoassay in quick and point of care identification of COVID-19, particularly in emergencies. These assays focused on identifying the antigen of the SARS CoV-2 virus or for assessing IgG and IgM antibodies against the Covid-19 viral infection. Tang *et al*. advocated that the detection of IgM and IgG antibodies by rapid lateral flow assay plays an important role in confirming COVID-19 infections and aids in assessing the burden of infection along with detection of asymptomatic patients [13]. Rapid test kits detect antibodies and provide a speedy detection with 88.66% sensitivity [40]. Even though the test is rapid and low cost, some limitations have been reported. Immunological tests should not be used at the onset of COVID infection because at the initial stage, the immune response is not fully developed. As a result of variation in sample handling and viral loading, antibody testing may miss the infection in some cases [41, 42]. Despite limitations, rapid tests are significant for identifying individuals with a history of COVID infection. The test also helps in the selection of plasma from convalescing patients as a treatment alternative for COVID-19 positive cases [43].

Although, WHO suggests using serological tests only in the absence of molecular testing, the former tests were used during this pandemic due to burgeoning cases [16, 39]. In COVID-19 infection, the immunological diagnosis is still in an early phase of development. A few diagnostic tests endorsed by the Food and Drug Administration of America are not used solely for detecting COVID-19 infection [43]. In addition, WHO directs that immune diagnostic tests should be done only in research settings and not for making clinical decisions until there is proven evidence [44].

4.3. *Nucleic Acid Testing Assay*

The PCR assay targets RNA-dependent RNA polymerase (RdRp) and host RNase P RNA (RPR) to detect the SARS-CoV-2 genome in nasopharyngeal samples.

For performing the PCR test, molecular target genes are detected within the RNA of Coronaviruses; and also in components like helicase (Hel), nucleocapsid (N), transmembrane (M), envelope (E), and envelope glycoprotein spike (S). Hemagglutinin-esterase (HE), open reading frames ORF1a and ORF1b, and RNA-dependent RNA polymerase (RdRp) [13].

In RT–PCR assay, the viral RNA is estimated as the cycle threshold (C_t), which is the number of cycles needed by the fluorescent signal to exceed the threshold and become detectable. RT-PCR tests are almost 100% specific, but false negative results may also be observed due to sampling error or wrong timing of sample collection [45]. When respiratory infection is acute, consistent usage of RT-PCR is recommended to identify causative viruses in respiratory secretions (Figure 7).

Currently, the real-time reverse transcription–polymerase chain reaction (real-time RT-PCR) is recognized as the gold standard technique for identifying, following, and examining the Coronavirus. Real-time RT-PCR detects the existence of distinct target genes. A value less than 40 Ct in real-time PCR is clinically positive. Nowadays, many fluorescent dyes are used as markers for the identification of specific target genes, while previously

radioactive isotopes were employed as markers. Real-time RT-PCR assays minimize the odds of getting a false positive result, as both amplification and analysis are performed in a controlled environment [45].

With the emergence of highly contagious Coronavirus, the need for a reliable and rapid detection test is paramount. Real-time RT-PCR facilitates analysis of results in real-time when the reaction is in process, making it more advantageous than traditional RT-PCR, which gives results after completion of the process. Real time RT-PCR is extensively used to detect Coronavirus because the test is rapid and reliable. However, molecular tests are still considered a "gold standard" for specific diagnoses [39]. Major molecular diagnostics being developed are centered on real-time RT-PCR assays. Loop-mediated isothermal amplification (LAMP), clustered regularly interspaced short palindromic repeats (CRISPR), and multiplex isothermal amplification followed by microarray detection are a few other techniques being developed [13]. According to WHO, the E gene assay followed by another confirmatory assay, which employs the RdRp gene, can be used for first-line screening of corona cases [47]; in the USA, the CDC emphasized use of two nucleocapsid protein targets [N1 and N2] for molecular assay. An investigation in Hong Kong compared the RdRp/Hel assay with the reported RdRp-P2 test and found that the RdRp/Hel assay had higher sensitivity and specificity and the lowest limit of detection *in vitro* [48].

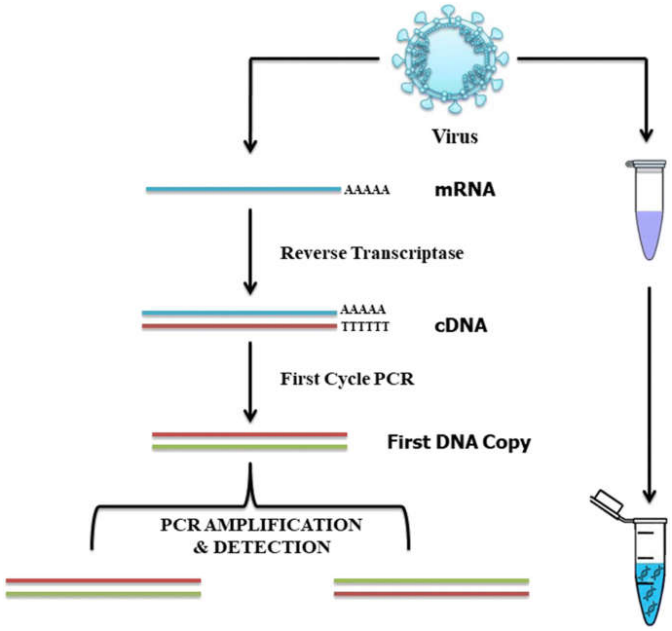

Figure 7. Schematic representation of RT-PCR technique for diagnosis of SARS-CoV-2 (Adapted from [46])

Two molecular targets are selected to evade a possible genetic drift of the coronavirus and to prevent its cross-reactivity with other endemic coronaviruses. However, a perfect design includes one conserved and one specific region in order to mitigate the effects of genetic drift, especially when the virus spreads to new populations [13]. Most researches on RT-PCR assay employed a combination of at least two target assays to diagnose COVID-19 infection. A group of German investigators used envelope and RNA-dependent RNA polymerase for the detection of virus [47]. In another study, the nucleocapsid was used for detecting the virus, followed by confirmation through ORF 1b [48], while a study by CDC, USA, preferred two loci in the nucleocapsid gene for detection of the virus [25]. A group of researchers from Turkey developed a multiplex rRT PCR by simultaneously targeting one human gene (RP) and two viral genes (RdRP and E) [49].

RT-PCR is the most reliable tool for a definite diagnosis of COVID-19, but reports suggest a lower sensitivity in comparison to chest CT examinations. Though the chest CT cannot differentiate between pneumonia caused by COVID and pneumonia resulting from other viral infections [50].

LAMP is another molecular technique that could serve as a possible substitute to the RT-qPCR for detecting COVID-19. LAMP is a nucleic acid amplification technique in which DNA amplification occurs in isothermal conditions. This method is rapid and highly specific and can be utilized for the diagnosis of COVID-19 without necessitating any special equipment and trained analysts. In the future, the point-of-care device based on LAMP is a potential diagnostic technique for detecting COVID-19 infection [51]. Recently, Zhang *et al.* gave a CRISPR-Cas13 (clustered regularly interspaced short palindromic repeats- and associated Cas proteins13) based SHERLOCK (Specific High-Sensitivity Enzymatic Reporter Unlocking) protocol, which is an accurate and rapid method for confirming the Coronavirus infection [52]. Kim *et al*., in their research, formulated the next generation sequencing (NGS) library through amplification of full-length genes from isolates, employing primers and cDNA specific for the coronavirus [30].

The divergence in the genome of the SARS-CoV-2 virus led to the emergence of new variants. Recently identified Omicron or B.1.1.529 is becoming a matter of concern due to its high transmission rate. Previously identified variants were Alpha, Beta, Gamma, and Delta, which were predominant globally. The leading cause of diversification in the viral genome arises due to mutations. The acquisition of mutations occurs due to addition and/or deletion and is deciphered by genome sequencing. Hence, next-generation genome sequencing has the potential to identify the variants of SARS-CoV-2 [53]. In future, the development and acceptance of these technologies will provide innovative, better, accurate, and quick diagnostic tools. Further developments may reduce the need for expensive equipment and specialized training.

4.4. *Computed Tomography (CT) Scan*

A chest CT scan is done to diagnose and manage COVID-19 symptoms [54]. COVID infection may cause inconsistent images in the scan, such as ground glass opacification (GGO), crazy paving pattern (GGO accompanied by thickening of the interlobular or intralobular septum), consolidations (increased parenchymal opacity obscuring underlying vessels), and linear opacification. Song F. *et al.* investigated 51 CT scans, of which 86% expressed bilateral pneumonia affecting both the lungs. Individuals less than 50 years of age indicated more GGO, while older patients had symptoms similar to pneumonia along with consolidations. These symptoms may vary with age and different stages of the disease, so it is not a reliable tool for confirming a final diagnosis [55,56].

According to Pan *et al.*, pneumonia progresses in 4 stages, from 0 to 26 days during COVID infection. In stage I of disease progression (0 to 4 days), the Ct score of the rtPCR test was low, and GGO with a slightly crazy paving pattern were observed in the CT scan. In stage II (5 to 8 days), the Ct score increased, and a clear crazy paving pattern was seen. The Ct score and crazy paving pattern enhanced further in stage III (9 to 13 days), accompanied by the emergence of consolidations. In the final stage IV (4 to 26 days), the Ct score and crazy paving pattern started waning, accompanied by a decrease in consolidations [57].

4.5. *Biosensors as Diagnostic Aids*

Biosensors are devices used to recognize analytes of biological importance. A bio recognition element targets the biomolecule, and a transducer converts this connection into a quantifiable signal. Transducers are mostly optical,

piezoelectric, or electrochemical-based systems, while bio recognition elements can be antibodies, nucleic acids, proteins, receptors, cells, and tissues [58].

Nanoparticles can be employed as transducers because of their stability in media and their compatibility with biofluids. The chemistry of nanoparticles facilitates conjugation and amplifies signals tremendously. Carbon-based nanoparticles (graphene and nanotubes), metallic nanomaterials like gold and silver, microgels, nanogels, and photonic crystals are a few commonly used transducers [59].

Aptamers have been widely acclaimed as effective diagnostic tools for detecting viruses. Aptamers are ssDNA or ssRNA that bind and detect many viral targets with great sensitivity and specificity. They can detect genes, proteins, and other viral markers with high accuracy, and such sensors can precisely detect infected host cells and active viruses [60]. Aptamer-based sensors are preferred because they are stable over a wide temperature range and easily modified and synthesized through the systematic evolution of ligands by exponential enrichment technique (SELEX). Research by Chen *et al.* observed N-protein of the virus in serum by using DNA-aptamer-based sensing methods [61].

Table 2. Current laboratory testing methods for the coronavirus

Technique available	Working Principle	Advantages	Time required	Disadvantages
Next-generation sequencing (NGS)	Whole-genome sequencing	Very sensitive and specific; gives all required information; Can identify the new strains	1 to 2 days	Expert analysts, sophisticated equipment and a well-equipped lab required high cost
RT-PCR	Nucleic acid testing assay (detection by use of a primer-probe)	Quick results; Greater sensitivity; Requires minimal DNA in a single-step; Ratified methodology	3 to 4 hours	High cost as consumables and lab equipment is expensive, complicated and time-consuming detection
LAMP	Nucleic acid testing assay (diagnosis confirmed by more than two sets of primers)	Accurate and repeatable; single working temperature	1 hour	Very sensitive; false positive results common due to cross-contamination
Serological tests (conventional)	Antigen/Antibodies IgG/IgM	Proven sensitivity and specificity	4 to 6 hours	Results reported 3-4 days after infection; False positive results common
Rapid tests (serological)	Antigen/Antibodies IgG/IgM	Point of care test (POCT); done at or near the place of sample collection	15 to 30 minutes	Results reported after 3-4 days after infection; False positive results common
Computed Tomography (CT) scan	Chest scan	Enhanced sensitivity merged with RT-PCR results	1 hour	Cannot differentiate pneumonia in corona with that caused by other viral infections and the hysteresis of abnormal CT
Isolation and purification of virus	In vitro isolation and propagation of live virus	High (100%) specificity Gold standard	5 to15 Days	Sensitivity low as 100% isolation is not possible
Biosensors	Bio recognition element targets the biomolecule and a transducer measures the signal	Rapid, sensitive and scalable, single step identification	< 40 minutes	Cannot meet challenges like rapid mutations and demands for rapid detection in mass populations

5. Conclusion

Scientists and clinicians around the globe are striving toward preventing and controlling the spread of COVID-19. There is an immediate need for *in vivo* studies on the replication, spread, and pathogenesis of the coronavirus in suitable animal models [6]. In the present scenario, to prevent community transmission, timely diagnosis is crucial for the identification of positive cases. Several technologies are available for better diagnosis, and a few are summarized in Table 2 and Figure 8. The collection of an appropriate specimen at the right time is essential for proper and early diagnosis. Due to the high risk of infection, the challenges are to keep Health Care Professionals safe by providing appropriate PPE kits. RT-PCR assay remains the molecular test of choice for etiologic diagnosis of COVID-19 cases, while antibody-based immunological tests are used as supplementary tools for screening the whole community and conforming with the molecular assay. Both RT-PCR and immunological assays assist in diagnosing and controlling this unprecedented outbreak of infection that has severely affected lives and the global economic scenario. The rapid test kits are required for a quick diagnosis and are convenient for detection in bedridden individuals. To summarize, appropriate use of available tests alone or when merged with other tests can timely detect the virus and save several lives through pertinent treatment. Future prospects involve the treatment of rapidly transmitting and infectious viral entities of SARS-CoV-2. In this context, the potentiality of naphthalimide derivatives was observed using the computational study. The compound was synthesized and characterized using the techniques UV-Vis, FTIR, ^{1}H NMR, fluorescence spectroscopy, and elemental analysis. The antiviral properties were observed via molecular docking. The study investigated that the newly synthesized compound has efficacy against two vital proteins, including spike proteins of the SARS-CoV-2 [62].

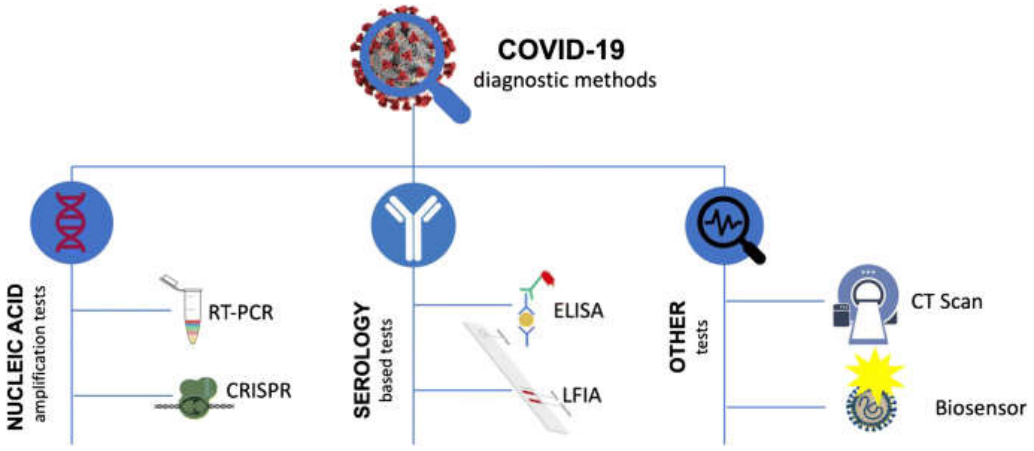

Figure 8. Varied diagnostic strategies of COVID-19 (Adapted from [63])

In order to increase testing facilities, the Medical Council of India (MCI) has asked the medical colleges admitting 50 to 250 MBBS students per year to set up a Bio-Safety (BS) level 2 laboratory as a basic requirement for conducting tests of infectious diseases like COVID-19 [64].

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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