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Diagnostic Approaches for Covid-19: Present Status and Future Prospects

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Abstract: During this COVID-19 outbreak, diagnostic tests were crucial in controlling its spread. This pandemic, which was caused by the SARS-CoV-2 virus, has created an urgent need for rapid diagnostic testing to enable efficient treatment and control of COVID-19. Many institutes and companies are trying to develop effective methods for detecting COVID-19. The essential indicative instrument presently utilized is reverse transcription-polymerase chain response (RT-PCR), which can have great affectability. Tragically, execution costs, time is taken, and false-negative results have limited the use of RT- PCR. Lateral flow assays (LFAs) are a type of diagnostic test that is clinically sensitive enough, might bridge various inadequacies in the present RT-PCR system, especially in low- and middle-income countries. In basic hospitals and laboratories, AuNP-LFA is a viable technique for diagnosing COVID-19, especially in emergency settings where a large number of samples must be examined quickly. This review summarizes the different diagnostic approaches for detecting the SARS-COV-2. This review thus provides guidelines for the scientists and technicians engaged in detecting SARS-COV-2. An accurate and sensitive detection system would enormously benefit in controlling this pandemic.

Keywords: COVID-19; RT-PCR; CRISPR; pandemic; antibodies.

1. Introduction

Till now, we knew very little about these coronaviruses as scientists had not much interest in the common cold viruses. It is important to understand the emergence of COVID-19 for stopping future pandemics. In 2002 a beta coronavirus suddenly emerged known as SARS-COV [1]. It was reported initially in China, and then it caused a pandemic by spreading to 29 other countries. Out of 8,809 confirmed infected people, 813 people were killed. This outbreak came under control by following simple public health measures. Then in 2012, another beta coronavirus emerged named MERS-COV [2]. It has a high fatality rate, but luckily it remained limited to the Middle East because, in humans, it was not able to transfer efficiently. In 2016, an alpha coronavirus named Swine acute diarrhea syndrome coronavirus (SARS-COV) emerged [3]. It was a bat originating from China. SARS-COV-2 is the most recent coronavirus outbreak from Wuhan, a city in China.

Bats are considered the major reservoir of animal coronaviruses. In a study of 19,000 animals, 98% of coronavirus was detected in bats, and in about 9% of randomly studied bats, it was seen that they were infected with some other coronaviruses. The genetic sequence of these coronaviruses is similar to SARS-COV and SARS-COV-2 [4]. SARS-CoV-2 is a large, enveloped, positive-stranded RNA virus that infects various mammals and birds [5]. Coronaviruses are viruses with spike-like projections of glycoproteins on their surface that resemble a crown under an electron microscope, hence the name (Figure 1) [6]. An RNA-based metagenomic next-generation sequencing technique was used to characterize its entire genome, 29,881 bp long (GenBank no. MN908947) and encodes 9860 amino acids [7].

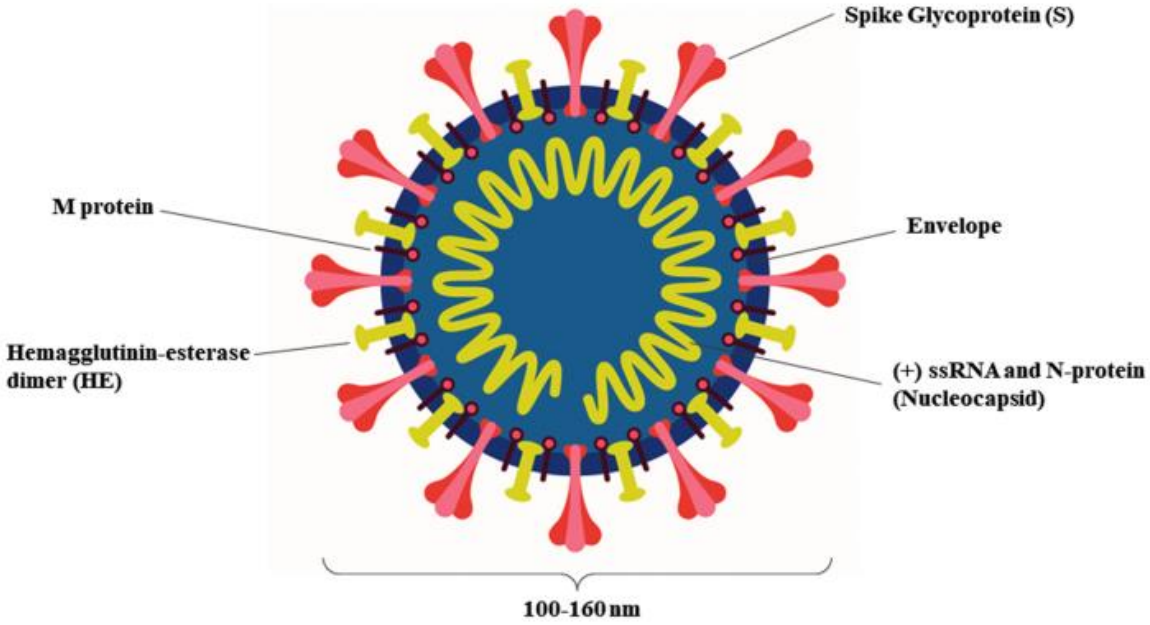

Figure 1. The general structure of the SARS-COV-2 virus shows spike-like projections of glycoproteins on their surface. It represents major structural proteins such as spike (S), membrane (M), nucleocapsid (N), and envelope (E) proteins.

Gene fragments produce both structural and nonstructural proteins. The structural proteins are encoded by the *S*, *E*, *M*, and *N* genes, while nonstructural proteins such as 3-chymotrypsin-like protease, papain-like protease, and RNA-dependent RNA polymerase are encoded by the ORF area. The membrane (M), the envelope (E), and the spike protein are structural proteins associated with the envelope (S). These structural proteins include the trimeric S proteins, which protrude from the viral envelope and are required for virus entry into the host cell [7,8]. Scientists have identified some coronavirus emergence hotspots, including Africa, the Middle East, South Asia, particularly China [9]. There is still a very high risk of future coronavirus outbreaks. Bat tourism, wet markets, environmental disturbances, and animal supplies for consumption are the major risk factors for causing zoonotic diseases [10,11]. Mishra *et al*. (2021) have recently reviewed how far or how close is the solution to the 2019-nCoV pandemic? [12].

2. Epidemiology of the Disease

Coronavirus disease (COVID-19) is caused by SARS-COV-2 infection, spreading from one individual to another through close contact (inside around 6 feet or 2 meters). Infected people spread the disease when they cough, sneeze, breath, sing, or talk. Respiratory droplets

from the infected person cause the disease. These small droplets remain in the air for many minutes or hours and are transmitted through airborne transmission. It also spreads when an individual contacts a surface or article containing the virus and afterward contacts their mouth, nose, or eyes; however, the danger is low. The respiratory symptoms of COVID-19 are mainly determined. Still, many other systems can be affected by this virus, such as the digestive system that gives rise to gastrointestinal (GI) symptoms. GI symptoms such as diarrhea, loss of appetite, nausea, or vomiting occur in stomach pain as a prime complaint [13]. Most people only show mild to moderate symptoms of COVID-19.

But in some cases, severe medical complications occur like pneumonia, trouble in breathing, heart complications, blood clots, organ failure, acute respiratory distress syndrome, and other viral, fungal, and bacterial infections. Older people and people with previous medical conditions are at higher risk of developing severe illness from SARS-COV-2. Children mostly have mild illnesses and show similar symptoms to adults.

The most common symptoms of COVID-19 include difficulty in breathing, muscle pain, sore throat, conjunctivitis, nausea, diarrhea, skin rashes, vomiting, etc. People with heart disease, cancer, diabetes, obesity, high BP, asthma, weakened immune system, HIV, etc., need to be extra cautious with COVID-19 because they are at higher risk of developing severe illness from COVID-19. According to the WHO incubation period of COVID-19 is 1-14 days. According to high-quality studies, the meantime for incubation of COVID-19 ranges from 5.6 to 6.7 days. The incubation period increases by 1 day, increasing the mean age by 10 years [14].

3. Pathogenesis

SARS-CoV-2 is transmitted through aerosol particles and droplets from one person to another. It binds with the host receptor as it enters the body and is taken up into the host cell through endocytosis or membrane fusion. The life cycle of SARS-CoV-2 within the host (Figure 2) consists of five steps: attachments, penetration, biosynthesis, maturation, and release. The virus contains four structural proteins such as spike (S), membrane (M), nucleocapsid (N), and envelope (E) protein [15-17]. The S protein is present in the viral surface projected and is the most important for host attachment and penetration. The spike protein is made up of two functional subunits (S1 & S2). S1 subunit plays a key role in binding with the host cell receptor, whereas S2 subunit helps to fuse viral and cellular membranes [16]. Angiotensin-converting enzyme 2 (ACE 2) has been recognized as a functional receptor for coronavirus and is highly expressed by pulmonary epithelial cells, type 2 alveolar cells, myocardial cells, and urothelial cells of the bladder. ACE 2 was found to mediate the internalization of the virus [18]. As soon as SARS-CoV-2 binds with ACE-2, the S protein is cleaved by a protease enzyme in two steps sequential manner, resulting in activation of the spike protein.

The first cleavage maintains the S2 subunit at the attachment site, and the following cleavage may activate the S protein causing conformational changes leading to viral and host cell membrane fusion [19-22]. The activated S protein is initiated by TMPRSS2 and attaches to the ACE-2 receptor to enter the host cells [23]. Instantly after fusion, the membrane virus enters into the alveolar epithelial cell of pulmonary and releases viral content. After that viral RNA undergoes replication and formation of RNA by using pre-existing RNA through

transcription. The freshly produced RNA strands are then used for viral protein synthesis via translation [24,25].

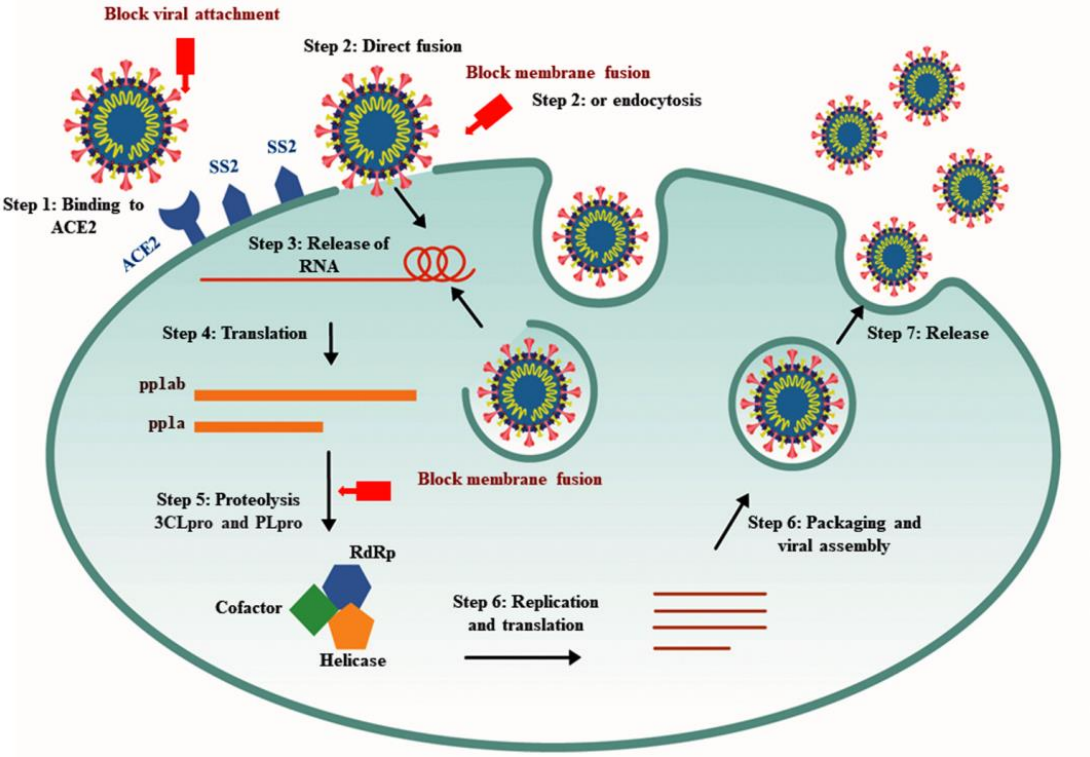

Figure 2. Different steps involved in the SARS-CoV-2 life cycle within the host cells. Life cycle mainly consists of 5 steps: (a) attachments, (b) penetration, (c) biosynthesis, (d) maturations and (e) release.

Now N protein binds with new genomic RNA whereas, M protein helps integrate the cellular endoplasmic reticulum. The newly synthesized nucleocapsids are then surrounded by the ER membrane and transferred to the lumen. The lumen is shifted to the cell membrane via Golgi vesicles and through exocytosis to the extracellular space. The newly formed viral particles are now ready to infect the nearby epithelial cells and supply fresh infective materials for community transmission [16].

4. Different Diagnostic Approaches

Coronavirus diagnosis is often based on a person's travel history to and from susceptible areas, as well as an examination of their clinical symptoms and a few auxiliary tests. Nabil *et al*. (2020) report point out several clinical approaches, side effects, and therapeutic agents that can help clinicians on the front line [26].

Clinical signs such as pneumonia caused by COVID-19 are highly unusual and look like pneumonia caused by other respiratory viruses. Despite the availability of several viral identification methods, each with varying degrees of specificity and based on single or multiple target molecules from the SARS-CoV2, a quick and sensitive diagnosis of COVID-19 remains elusive (Figure 3). Some methods have already been established and are considered Gold Standard procedures for this new virus, while others are presently being developed and validated for viral diagnosis, as mentioned below.

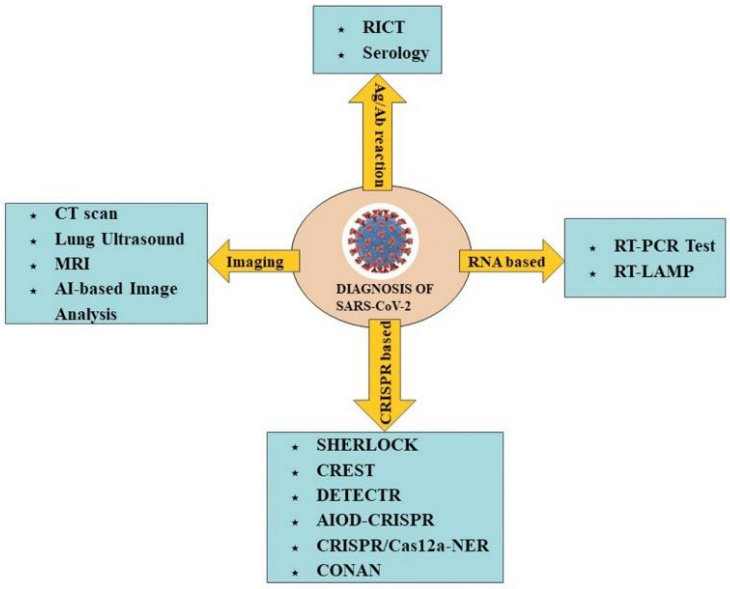

Figure 3. Diagnosis of SARS-CoV-2 by different methods. Major methods implied are Ag/Ab reaction, RNA based, CRISPR based, and Imaging techniques.

4.1. RT-PCR test.

COVID-19 is currently diagnosed using quantitative reverse transcription-polymerase chain reaction (rRT-PCR), a gold standard molecular diagnostic approach for several viruses (Figure 4). SARS-CoV-2 nucleic acids are detected in nasopharyngeal fluids using RT–PCR and the initial step in managing COVID-19 [27].

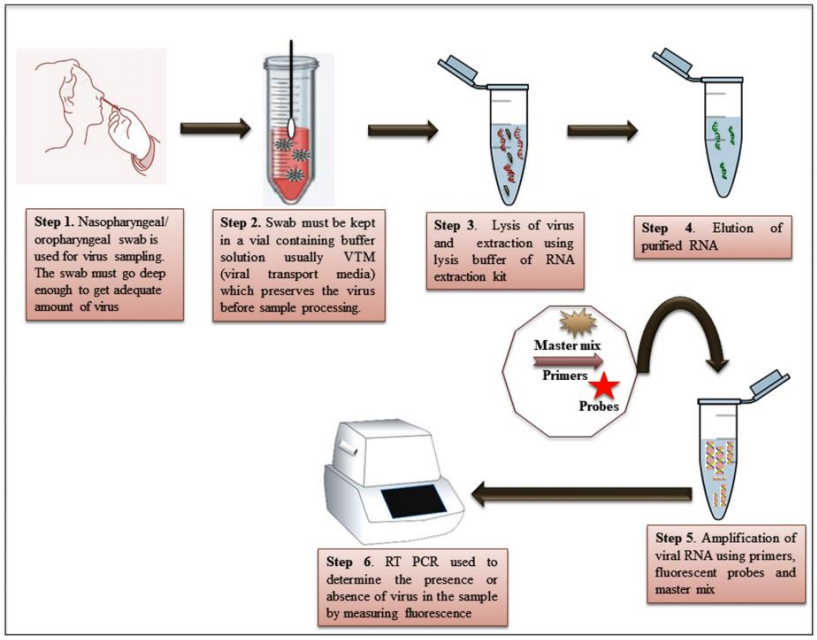

Figure 4. Steps involved in RT PCR for SARS-CoV-2 detection.

Because this technique is well-established, it is simple to implement. Apart from designing and synthesizing a particular primer-probe, the rest of the process is similar to that of other viruses, with minor variations. After the initial sequencing data for the SARS-CoV-2 virus from China was published, possible diagnostic rRT-PCR tests were developed and made publicly available to researchers. Different agencies or manufacturers have chosen various sets of genes from among the several SARS-CoV2 genes (ORF-1a gene, ORF-1b gene, RdRp gene,

N gene, E gene, and so on), resulting in a broad range of sensitivity in each experiment [28]. When both of the designated target genes are positive, one patient is verified to be infected, according to the conventional protocol [29]. While some studies employed two single-step RT-PCR tests (premised on TaqMan technology) to identify and amplify two segments of any two genes, most often N or ORF1b from the viral genome, others used multiplex assays to amplify several genes in a single reaction. Although these techniques are extremely sensitive (almost 100 percent), confirmation takes longer since the test must be performed in a specialized facility. The tests must be carried out in two stages: the first is a screening assay that utilizes the SARS-CoV-2-specific E gene, and the second is a confirmation assay that uses the RdRp gene, the N gene, and the ORF-1b gene (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer%e2%80%93probes.html). These tests employed *in vitro* transcribed RNA with known copy numbers as the "positive control" material. As with other respiratory viruses, RNAse P gene detection is employed as an internal control to provide insight into the accuracy of specimen collection and extraction of RNA.

According to the WHO, the standardized testing procedure consists of five steps: (1) patient sample collection; (2) transporting obtained samples to the laboratory in an acceptable manner; (3) supplying clinical and demographic details to the laboratory; (4) testing of samples by the lab; and (5) proper and suitable test results preparation and reporting (https://apps.who.int/iris/bitstream/handle/10665/331509/WHO-COVID-19-lab_testing-2020.1-eng.pdf). Variation in viral RNA sequence can alter RT–PCR results using primers targeting distinct regions of the virus's genome. Furthermore, due to viral evolution, false-negative findings may arise [30]. Apart from the sensitivity issue, RT-qPCR has a number of disadvantages, including the potential of biological safety hazards during transportation and processing of samples, as well as nucleic acid extraction, and the need for intricate lab equipment such as biosafety cabinets, which are often only available in a few central laboratories [29,31]. Due to technical competence and sample transportation, the total process takes time, which is inevitable. These flaws could render the procedure less useful in the event of a health emergency, like the current global outbreak. Furthermore, PCR can identify the target virus and a number of other respiratory viruses, increasing false positive or negative results [32]. Despite these flaws, the RT–PCR method is still the gold standard for diagnosing SARS-CoV-2 [33].

4.2. RT-LAMP.

RT loop-mediated isothermal amplification is a nanotechnology-based approach with a relatively novel concept that is in the implementation process for COVID-19 detection. It's a molecular amplification method that can efficiently amplify any genetic materials in a short period [34]. Levels of turbidity or colorimetric or fluorescence metrics are used to detect LAMP-based diagnostic testing. This approach is easy to execute and visualize, producing minimal background interference. Instead of heat denaturation, as in conventional PCR procedures, at a particular temperature of 60-65°C, a specially designed primer sand enzyme (DNA polymerase) with strand displacement activity synthesizes target DNA [35]. LAMP is a user-friendly approach that may offer reliable, robust, and precise findings in less time than other traditional approaches. As a result, it has been fairly popular since its development, emphasizing microbial identification [36]. The primary limits of LAMP development are expertise, interpretation, and optimization of reaction [37]. EvaGreen's signal read-out capabilities were superior to SYBR Green among the two fluorescent dyes tested.

The RT-LAMP system is a lab-on-a-chip viral diagnostic device that uses paper/strips embedded in a microfluidic substrate [38]. Fluorescein is assigned to one primer set in the experiment, and the reaction is catalyzed by labeled RT [39]. An alternative method for LAMP accurately detects SARS-CoV-2, allowing 100 copies per response detection, using a leucocrystal violet dye to create a visible violet color. The LAMP assay's detection limit can be improved by using a closed-tube Penn-RAMP, which combines RT–recombinant polymerase amplification and RT-LAMP in the same tube [33].

4.3. Rapid immunochromatographic tests (RICT).

The World Health Organization currently recommends that COVID-19 be diagnosed by the laboratory utilizing molecular assays that target the RNA of the SARS-CoV-2 virus. This assures that test findings can be tracked, and the patients are recognized for isolation, treatment, and tracking of the pandemic. Unfortunately, due to present infrastructural constraints and supply shortages that restrict testing capacity, rapid antigen tests are being considered a way to improve laboratories'' laboratories' testing capacity to satisfy the most pressing medical and public health needs [40]. Rapid tests are non-automated tests that are mostly qualitative but quantitative. They have been tested for COVID-19 diagnosis and utilized for in vitro diagnostics (IVDs) of many diseases. These tests can produce results in 10-30 minutes, making them instantaneous compared to molecular tests, which can take up to 6 hours [28]. Antigen inside the samples combines antibodies mounted to a paper strip in a plastic container for this experiment (Figure 5).

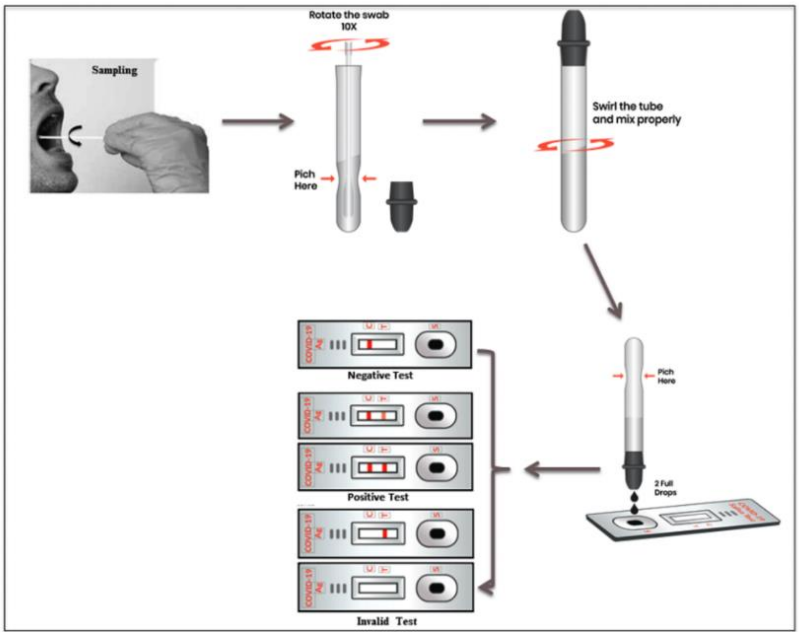

Figure 5. Rapid COVID-19 antigen test. These tests look for antigens which are protein markers found outside a SARS-CoV-2 virus. The sample is tested using nasopharyngeal and oropharyngeal swabs to detect the presence of SARS-CoV-2 nucleocapsid protein antigen on the viral surface.

Within half an hour, this reaction produces a discernible signal. Because the identified antigen is only expressed when the virus is actively replicating, the tests can be utilized to identify acute or early infection (Advice on the Use of Point-of-Care Immunodiagnostic Tests for COVID-19, World Health Organization, 2020). This assay detects the presence of SARS-CoV-2 nucleocapsid protein antigen from nasopharyngeal and oropharyngeal swabs on the viral surface. In this assay, specific monoclonal antibodies (MAb) bind to specific viral

antigens in fluid samples [41]. Calorimetric enzyme immunoassay improved chemiluminescent immunoassay, and the most contemporary, economical, quick, and user-friendly fluorescence lateral flow assay can all be used to detect it (LFA) [42]. The structural proteins of the coronaviruses, such as spike (S), membrane (M), envelope (E), and nucleocapsid (N), are the important foci in the SARS-CoV-2 virus for subsequent antigen-based assays [43].

Although antigen tests might identify viruses early in infection, their sensitivity may be limited compared to nucleic acid amplification assays, and they may cross-react with the other coronaviruses. Indirect antibody tests can cross-react with other coronaviruses and are therefore not useful for the early diagnosis of clinical cases [40]. However, they may be useful in determining individual immunity or addressing epidemiological issues, as mentioned later [44].

4.4. Serology.

Instead of the nasopharyngeal swab samples used in PCR tests, serological testing methods often employ blood samples to identify viruses. Antibodies or virus-specific antigens can be found in large and detectable concentrations in blood samples. Immunoglobulin G (IgG) and immunoglobulin M (IgM) are the two major types of antibodies in the blood that the test examines for (IgM) [45]. Antibodies produced are the body's ability to recognize previous infections and how it dealt with infections in earlier encounters so that the body can retaliate against the similar pathogen again. Within a few days, IgM emerges and acts as the first line of active defense, accompanied by IgG production to eradicate the infection. IgM and IgG are used to fight all illnesses [46]. To detect a specific disease, the body's immune response system might be used.

The COVID-19 blood test detects virus-specific proteins or antibodies for SARS-CoV2, but not for seasonal flu or other viruses, with proven SARS-CoV2 specific antibodies in case of antigen detection or confirmed SARS-CoV2 antigen in case of antibodies detection [28]. Because IgM and IgG antibody titers are only apparent roughly 6-15 days after disease starts, serological tests do not help diagnose acute cases in the first week of sickness [47]. SARS-CoV-2 exposure is determined by detecting IgM or IgG antibodies specific for various viral antigens, including but not limited to the spike glycoprotein (S1 and S2 subunits, receptor-binding domain) nucleocapsid protein [48].

4.5. Imaging techniques in COVID-19 diagnosis.

During the pandemic, the role of imaging techniques has evolved. CT scan could be a superior alternative to RT-PCR testing. The radiological tests have shown more sensitivity than the nucleic acid test. CT scans can show correlations between severe illness and a need for mechanical ventilation. Studies using artificial intelligence (AI) and Chest Computed Tomography (CT) to diagnose COVID-19 have been rapidly increased. Artificial intelligence can determine the diagnosis and prognosis of pneumonia caused by COVID-19 concerning CT-scan and radiography.

4.6. Chest X-ray (radiography) and CT-Scan.

Due to false-negative results of RT-PCR and less supply of COVID-19 detection kits, CT imaging is highly recommended for detecting COVID-19 patients. No abnormalities are seen in the early stages of COVID-19; therefore, radiography is not recommended as the first

method for diagnosing COVID-19 patients. In COVID-19 patients with mild infections, patchy shadows are seen in the subpleural area and the outer band of the lungs. Multiple and diffused consolidation shadows can be detected in severe cases, and they are present as "white lung" [49]. CT scan is a specialized imaging method that uses x-rays for creating 3D images of the chest [50]. Diagnosis of COVID-19 from cross-sectional images of CT-scan can be used by doctors for quick and efficient screening of COVID-19. CT scan is 97.2% sensitive in the detection of COVID-19. CT imaging has shown that lesions are majorly distributed in the subpleural region, and they rarely present along with the bronchial vascular bundles. Lesions are nodular, lumpy, or patchy. Mostly they have uneven density and a condensed bronchial wall.

Enlarged lymph nodes (mediastinal), air-filled bronchi, and pleural effusions are other associated symptoms [51]. In chest CT scans, radiologists analyze X-rays with abnormal presentations at different angles across the patient's chest. During different stages of infection of COVID-19, different CT scans are observed. 56% of COVID-19 patients showed normal CT-scan findings during the early stage of infection, and the lungs got involved after 10 days of infection. Findings of Chest CT-scan include multifocal ground-glass opacities and consolidations. Ground-glass opacity refers to the hazy appearance of the lungs caused by the filling of pulmonary airspaces with fluid or by the collapse of the airspaces. These abnormalities occur in the peripheral and basal areas of the lungs, and they are most common in the posterior lung bases. Lung consolidation and peripheral and bilateral ground-glass opacity are among the common features of COVID-19 infection. After 4 days of infection, ground-glass opacity becomes more evident. Once the infection progresses, an irregular paved stone pattern occurs, followed by consolidation of lungs. Due to false-negative results of RT-PCR, CT-scans became the method of choice with 86-98% sensitivity. The severity of the disease can be assessed by CT examination. COVID-19 patients with confirmed RT-PCR having normal CT imaging are usually mild cases, not severe cases, indicating that CT scans can help diagnose the severity of the disease [49].

CT-scan may have a limitation of low specificity in the COVID-19 diagnosis as it may overlap with other viral infections (like influenza pneumonia, SARS, and adenovirus pneumonia), bacterial pneumonia, mycoplasma pneumonia [52]. Peripheral distribution GGOs and reticular interlobular septal thickening are more common in COVID-19 pneumonia as compared to viral infections, and chances of having pleural effusion, central distributions, and lymphadenopathy are less [53]. Patchy shadows distributed along the bronchi and sometimes merging to form large lobe lesions or consolidation are seen in bacterial pneumonia. Different viral pneumonia has similar CT features making a differential diagnosis of these viral infections difficult. Due to this, nucleic acid testing, viral gene sequencing, or other methods are required to confirm the diagnosis [49].

4.7. Lung ultrasound.

Ultrasound is a common imaging method that can be used to systematically and rapidly examine organs. It can be done at the bedside without the use of radiation. Ultrasonography is useful in determining the direction of treatment (mainly hemodynamic therapy) of critically ill patients. In COVID-19 patients, pulmonary lesions appear in the peripheral and subpleural areas of the lungs. Rapid examination of illness can be done by ultrasound. Critical Consultation Ultrasonic Examination (CCUE) is advised for COVID-19 patients having symptoms of dyspnea or circulation fluctuation for accurate analysis of possible complications.

Progression of lung disease is suggested by an increase in the area of consolidation and the number of B-lines. Heart injury is indicated by abnormalities in segmental wall motion and decreased ventricular function, and changes in heart size and ventricular wall thickness [54]. In critical cases, respiratory support is important but when to switch from non-invasive ventilation to invasive mechanical ventilation is an issue that needs a doctor's attention. After invasive ventilation, the lung recruitment maneuver process can be monitored with the help of ultrasound, and lung injuries by mechanical ventilation can be prevented when oxygenation cannot be improved by mechanical ventilation. Extracorporeal membrane oxygenation (ECMO) is a good option. In the ECMO process, ultrasound enables visual management. ARDS is a common complication in COVID-19 patients, followed by secondary infection and acute heart injury.

In COVID-19 patients, troponin levels are elevated during the early onset stage, indicating viral myocardial damage, which needs to be confirmed by hemodynamic changes. Hemodynamics can be quickly evaluated by echocardiography. Around 20% of severe COVID-19 patients have reported coagulation abnormalities. There is a risk of lower-limb deep vein thrombosis for the patients staying in bed for 3 days or more with the symptoms of swelling or discomfort in bilateral lower limbs. The subpleural abnormalities can be detected by ultrasound, but ultrasound cannot detect deeply located lung lesions. Ultrasound may not be able to make a pneumonia differentiation diagnosis. It is recommended to diagnose critically ill patients who cannot move, and for whom CT examination is not possible. For the mild COVID-19 cases, ultrasound may not be useful. Ultrasound can be a good supplementary method for pulmonary and cardiac evaluation [49]. Ultrasound examination of the hospitalized patients detects thrombus, and its scope and nature can be defined. Timely intervention can be done in this way for patients who are at risk of developing embolism.

4.8. MRI.

Non-invasive MRI can be used to detect COVID-19 in pregnant women and children as this imaging technique visualizes soft tissues without radiation. But the scanning time is long, and its cost is high compared to CT scan. SARS-COV-2 infection mainly spreads in the lungs, but autopsies have shown that the infection also causes damage to the heart, vessels, kidney, liver, and other organs [55]. SARS-COV-2 host cellular receptor ACE-2 is present in multiple organs. Increased ACE-2 expression at mRNA and protein level is seen in patients with basic heart failure disease and can increase the risk of heart attack and cause more critical conditions. MRI of cardiac has shown cardiac involvement such as acute myopericarditis with systolic dysfunction in COVID-19 patients. For perfect visualization of functional and structural information of soft organs, MRI can be used. It can be used to understand the mechanism of COVID-19 infection better so that the vulnerability of different organs can be studied. MRI can be used to study the organ damage and mechanism of the disease [56].

4.9. AI-based COVID-19 analysis.

During this COVID-19 pandemic, when resources are becoming insufficient in managing COVID-19 disease, AI can play an important role. Diagnostic tools using computers are developed based on machine learning technology. CT scans involve a lot of manual work and are time-consuming. AI will help in reducing the workload of radiologists. AI models not only detect abnormal or infectious areas but can also help in diagnosing COVID-19 in a

convenient manner [56]. 3 types of AI strategies are: Detection of lesions using AI for fast COVID-19 screening; Use of AI for in COVID-19 diagnosis by using partial or whole lung in CT-scan; Prediction of COVID-19 outcomes (severity of COVID-19) using AI.

AI models have shown promising results in small datasets (<300 patients). However, the performance drops in larger datasets with more than 1000 patients. Larger scale studies need to be taken into account as they may show the ability of AI that it can achieve in COVID-19 diagnosis.

4.10. CRISPR-based diagnosis.

The CRISPR-based diagnostics of novel COVID-19 has recently become a promising technology for identifying DNA & RNA in a point of care (POC) testing format. This approach removes the need for expensive lab equipment and provides cost-effective diagnoses having high sensitivity and specificity. Here we will describe how the CRISPR systems can diagnose COVID-19 viruses. Different novel detection methods have been developed based on CRISPR system, which includes SHERLOCK, All-In-One-Dual CRISPR-Cas12a (AIOD-CRISPR), DETECTR, Cas13-based Rugged Equitable Scalable Testing (CREST), Cas3 Operated Nucleic Acid Detection (CONAN).

4.11. SHERLOCK.

This is the first extensive and significant biosensing technique for identifying DNA/RNA dependent on CRISPR-Cas13 named "Specific High Sensitivity Enzymatic Reporter Unlocking" (SHERLOCK). This approach can identify both nucleic acids (RNA & DNA) with higher sensitivity. In 2017 Gootenberg and associates were able to detect tumor DNA mutation, human DNA genotype, ZIKA virus strains and differentiate pathogenic microorganisms using the SHERLOCK biosensing system [57].

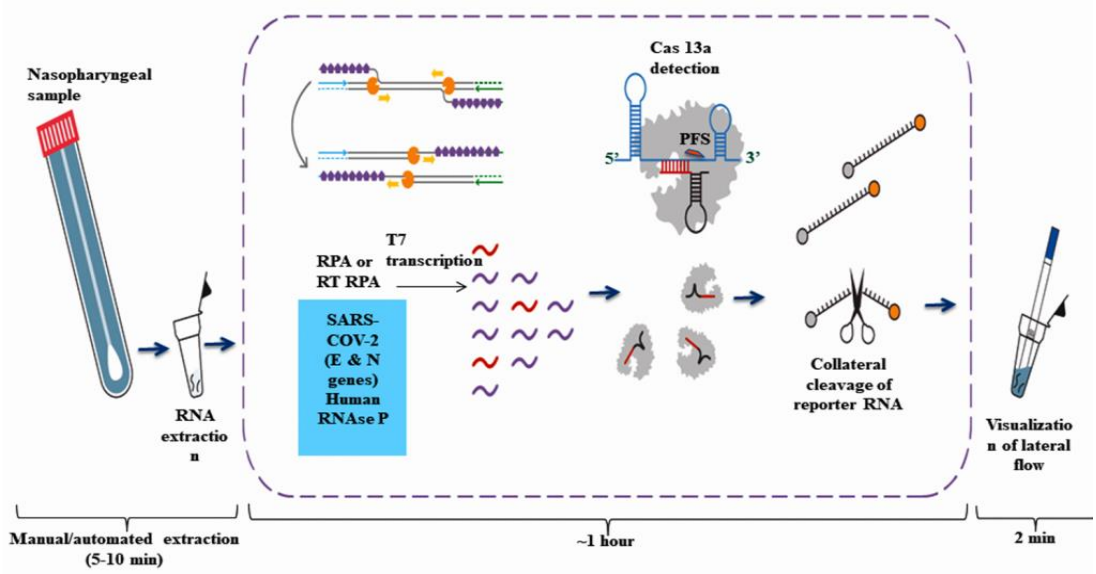

Figure 6. SHERLOCK system integrating RPA pre-amplification, colorimetric visualization, and Cas13a detection.

In this method, Recombinase Polymerase Amplification (RPA) amplifies the DNA molecule, followed by T7 RNA polymerase transcription. The RNA detection is being done by Cas13a ortholog. As the Cas13-gRNA complex captures and cuts off the RNA target, a

collateral breakdown activity liberates a signal by the reporter RNA molecules into the solution [57]. The sensitivity of the SHERLOCK system is boosted by the RPA enzyme from pmol/L to umol/L levels [10]. The *S* gene and *Orf1ab* genes were collected as two targets to find out the COVID-19 RNA in nucleic acid extractions of samples. To increase the assay specificity, primer and LwaCas13a-CRISPR gRNAs were arranged to find COVID-19 RNA in samples carrying the lowest off-targets of the similar human respiratory viral genome [58] (Figure 6).

The sensitivity of the STOPCovid is almost the same as reverse transcriptase quantitative PCR. Using a dipstick, the test result can be obtained in 70 minutes, whereas, by utilizing fluorescence, the test result can be obtained within 40 minutes. However, a plate reader is needed to find out the fluorescence reading, which is unfavorable for Point of Care Technologies (POCT). Therefore, Gootenberg and his colleagues developed a second version of SHERLOCK (SHERLOCK v2) to identify various diseases from a sample, making the technology more appropriate for POCT [59]. In this updated version of SHERLOCK, the orthogonal collateral activity of Cas12a or Cas13a with or without Csm6 was determined [59].

4.12. CREST.

Rauch and her associates in 2020 discovered a Cas13a-based, rugged, equitable scalable testing (CREST) platform to develop a cost-effective, sensitive, and easy-to-handle COVIN-19 detection method. This method combined with a PCR step, an amplification step, and an enzymatic signal amplified by a fluorescence detector. To avoid the requirement of priecy equipment, this technique uses affordable, Bluetooth entitled, battery-operated thermocyclers (DIY-Bio miniPCR mini16). These PCR machines can work with cost-effective solutions. P51 fluorescence visualizer (powered by a 9V battery) cardboard used to visualize positive results as a scalable and easy-to-interpret tool.

LOD of the CREST method was found to be 10 copies of the target RNA molecule per µl. Sample revealed that this method is sensitive as reverse transcriptase-qPCR. CREST instrument cost was found 30-50 times cheaper than RT-qPCR [60].

4.13. DNA endonuclease targeted CRISPR trans reporter.

COVID-19 was detected from RNA extraction of the patient samples using the Cas12-mediated DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR). This approach uses reverse transcription and isothermal amplification through RT-LAMP to amplify isolated RNA from nasopharyngeal or oropharyngeal tissues [61].

Predetermined coronavirus sequences were recognized and cleaved by Cas12. Afterward, the sequences are recognized by the breakup of the reporter molecule. To resolve this, primers were created to multiply the *E* & *N* gene of the COVID-19 virus. The amplified product overlaps with the *E* gene region and the N2 region in the *N* gene [60,62]. This method was devised to detect in almost half an hour having a reaction of RT-LAMP at 62°C for 20 min and Cas12 detection at 37°C for 10 min having visualization on a highly sensitive dipstick. The LOD for DETECTR assay was observed from 1-10 copies per microliter (Figure 7) [63].

4.14. AIOD-CRISPR.

The AIOD (all-in-one dual CRISPR-Cas12a) -CRISPR is a visual-based assay for identifying DNA and RNA that is highly sensitive, specific, and rapid. In this method, all

chemicals are thoroughly combined in a single reaction tube and incubated at 37°C for isothermal nucleic acid amplification using LAMP 8 and RPA. This single-pot reaction system removes the requirement of transferring the pre-amplification and the final product separately. In addition, ssDNA-FQ reporters are added to AIOD-CRISPR to increase the detection signal. The reaction mixture's real-time fluorescence and color change detection creates an AIOD-CRISPR method for point-of-care detection. In association of reverse transcriptase of Avian Myeloblastosis Virus (AMV) with AIOD-CRISPR examine drives the advancement of a One-step RT-AIOD-CRISPR to distinguish the RNA like HIV-1 and SARS-CoV2 RNAs. RT-AIOD-CRISPR allows the identification of RNA in the absence of cDNA. Furthermore, modified AIOD-CRISPR allows the detection of SARS-CoV2 & HIV-1 without prior amplification. The limit of detection of AIOD-CRISPR was found to be 4.6 and 1.2 copies for RNA and DNA samples, respectively, in 40 min of incubation [64].

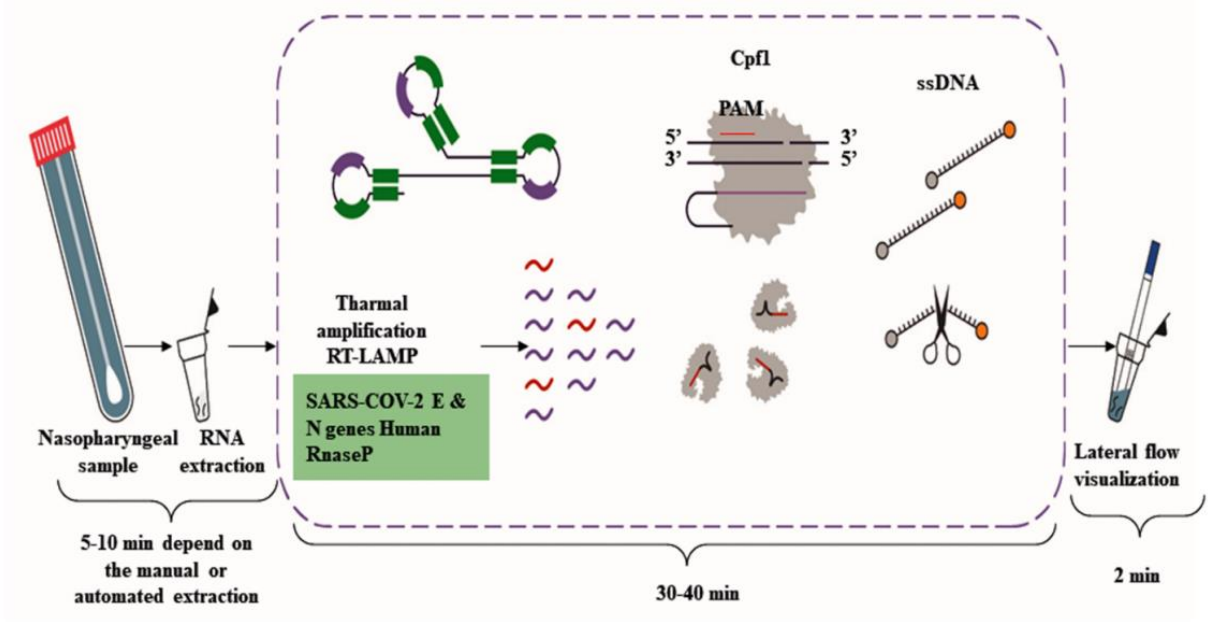

Figure 7. DETECTR system integrating LAMP pre-amplification, colorimetric visualization, and Cas12a detection.

4.15. CRISPRCas12a-NER.

This is another CRISPR-based stage that contains Cas12a effector, specific crRNA planned for SARS-CoV-2, and an ssDNA reporter. Cas12a, in association with a nucleic acid of SARS-CoV-2, separates an ssDNA reporter labeled with an extinguished green fluorescence bringing about the release of green fluorescence, which can be seen with the naked eye under 485 nm light.

In this technique, reverse transcript recombinase supported multiplication was utilized to pre-amplify the nucleic acid of SARS-CoV-2 out of 30 min at 39°C and recognition by Cas12a in 15 min. It has been found that the sensitivity of the CRISPR/Cas12a-NER varied out of the four genes. The highest sensitivity was reported with the E gene having LOD of 10 copies of nucleic acid [17].

5. Conclusions

In the present scenario, the COVID-19 pandemic is still spreading. Although the regulatory agencies have approved a few vaccines, these vaccines are insufficient to vaccinate

the world's 7 billion population. Therefore, early diagnosis and social distancing are the only methods to end the spread of COVID-19. In this investigation, we have focused on summarizing different classical and modern diagnostic technologies for COVID-19. Even though the RT-PCR technique is the gold standard for detecting COVID-19, this technique still has some limitations like lengthiness and lack of robustness. The other nucleic acid detection techniques, such as reverse transcriptase-LAMP and CRISPR, and immunological assay-based techniques, are currently emerging through the approval phase. Based on the antibody, serological diagnostics can be widely used with molecular techniques to upgrade detection accuracy. Different imaging techniques such as CT scan, lung ultrasound, MRI, etc., can be used to manage COVID-19 disease. CT scan examinations have been demonstrated as a productive clinical device in checking movement and reducing lung injuries in COVID-19 disease. The combination of artificial intelligence and CT imaging not only counter the limitations in the medical field but also support rapid detection of COVID-19. Besides, there is a critical requirement for developing successful drugs and a vaccine to manage the life-threatening pandemic.

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Conflicts of Interest

The authors declare no conflict of interest.

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