A review of diagnostic techniques for analysis of Covid-19 virus

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Abstract- SARS-CoV-2 emerged in Wuhan, China in 2019, causing a global pandemic. Early diagnosis and screenings are crucial, using molecular and serological assays. Measures like isolation, social distancing, and masks were recommended to curb the spread. This review article summarizes the effectiveness of different diagnostic methods used over the past two years, including their mechanisms, limitations, and accuracy. It also discusses the potential integration of artificial intelligence and deep learning algorithms for future improvements. Auxiliary methods like point-of-care devices, biosensors, and lateral flow devices have been utilized for quick qualitative diagnosis. Despite limitations, every development in such times can be a significant advancement when used appropriately.

Keywords: SARS-CoV-2, Lab diagnosis, Pandemic, Detection methods, Molecular assay, Serological methods.

INTRODUCTION

SARS-CoV-2 is an SS-RNA virus with a crown-like shape and spike proteins. Its genome contains ORF1ab polypeptides encoding two-thirds of the genome, while the remaining portion consists of surface (S), envelop (E), membrane (M), and nucleocapsid (N) proteins. The virus binds to ACE2 and CD209L receptors on host cells, primarily infecting the respiratory tract's epithelial membrane.

ACE2 alters the structure of the spike protein, and non-structural proteins are encoded by ORF1a/1b. The S2 region is involved in cellular and viral interactions, and viral replication is facilitated by RdRp at cytoplasmic membranes. Transcribed proteins enter the endoplasmic reticulum and Golgi apparatus, where the viral RNA is packed into the capsid. During exocytosis, portions of the genome are released.

Understanding the virus's genomes and proteomics is crucial for molecular diagnostics. RT-PCR, utilizing probes and primers, amplifies specific gene segments for detection.

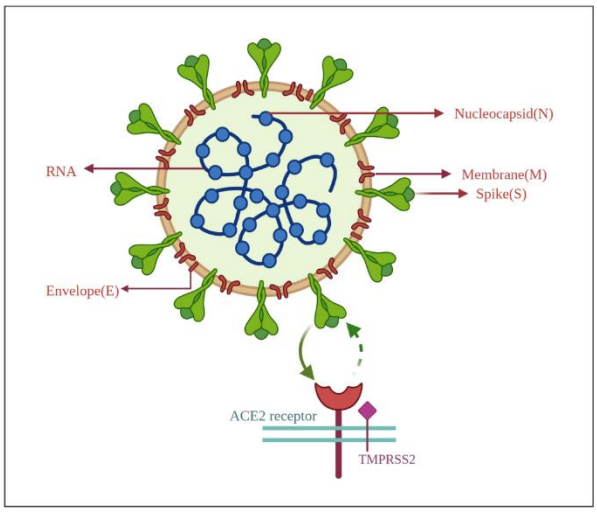

Fig 1 Schematic Structure of SARS CoV2 Virion

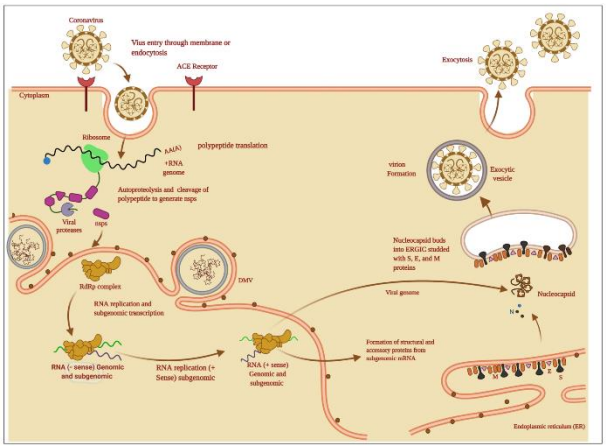

Fig 2 Infectious cycle of SARS CoV2 for entering into the host cell by attaching to ACE receptor and leaving the host cell.

Infection stages and testing types determine specimen quality for SARS-CoV-2 detection. Upper respiratory samples are recommended for testing active infection in symptomatic individuals, while lower respiratory samples are used for high viral load cases. Blood samples are not suitable for molecular assays but can indicate antibody response. Serological tests are employed for prior infections. Mutations of the virus, such as Delta and Omicron, have emerged, necessitating the

development of accurate and faster diagnostic tools. Latest technologies have improved performance, specificity, sensitivity, and turnaround time. This review summarizes COVID-19 screening, diagnostic, and follow-up methods, highlighting their advantages and limitations. All the images were created in biorender premium.

Various Testing Techniques of Covid-19

COVID-19 diagnostic tests are classified as molecular and serological. Imaging techniques such as CT scans, CXR, and ultrasonography help assess illness severity. Advanced technologies like AI and POC gadgets enhance diagnosis. Table 1 provides a summary of diagnostic tests.

Table 1. Overview of various diagnostic tests with advantages, and disadvantages along with their applications

Diagnostic technique	Advantage	Disadvantage	Application
NGS	Unknown virus detection viral mutation and evolution can be detected.	Long turnaround time Expensive and complicated	It is used for diagnosis, lineage tracking, and mutation discovery. Decision-making for clinical, infection control, and outbreak infection.
RT-PCR	High sensitivity and specificity	An experienced person is required; false positives can occur. Need a high viral sample to test (181)	Confirmation of Covid-19, discharge from hospital.
RT-LAMP	On-site detection, low cost, minimum steps	Low throughput	Diagnosis, epidemiological surveillance
ELISA	High throughput, low cost	Easily contaminated time-consuming, an active infection cannot be detected in most cases Ineffective in the early stages of infection, not approved by many authorities	Past infection, discharge from hospital, epidemiological surveillance, a complement to virus detection test
CLIA	Higher sensitivity than ELISA, reproducible, automated	Bulky equipment required	Past infection, discharge from hospital, epidemiological surveillance
LFIA	Low cost, simple operation	Low sensitivity	discharge from hospital, epidemiological surveillance

Molecular Tests

RT-PCR

RT-PCR, considered the gold standard for SARS-CoV-2 detection, detects genomic RNA and is recommended by WHO and CDC. Upper and lower respiratory tract or stool samples are taken. The process involves generating cDNA, amplifying it using gene-specific primers, and comparing positive and negative samples by fluorescence intensity. RT-PCR has high sensitivity (0.77) and specificity (0.988). Most countries target specific genes like RdRp, N, E, S, and ORF1b/ORF8.

It is a definitive, sensitive, and fast technique, but requires a well-equipped lab and skilled technicians. The test takes around 24 hours and is more costly than other methods.

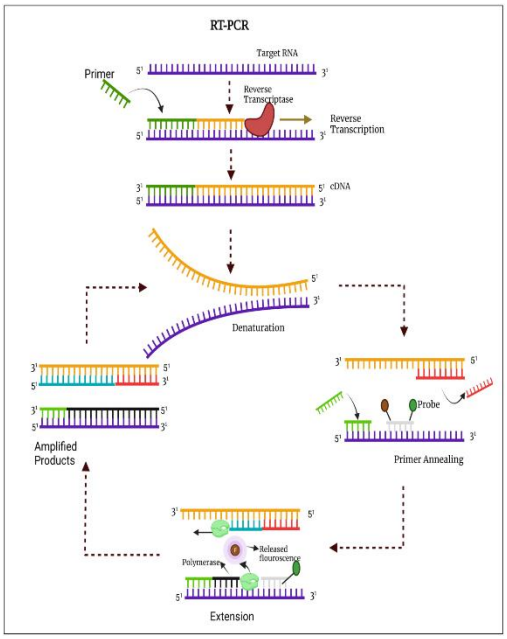

Fig 3 Typical Step by step procedure of RT PCR test for detection of the virus.

RT-LAMP

The newest gene amplification test, RT-LAMP, utilizes six primers to detect specific gene areas in a single step. The separated RNA is heated with primers, Bst DNA polymerase, reverse transcriptase, and pH indicator dye, resulting in a color change due to DNA amplification. RT-LAMP has been enhanced by utilizing serial primers and magnetic beads to avoid RNA elution. It demonstrates similar sensitivity and specificity to RT-PCR. RT-LAMP is rapid, has minimal specimen transmission risk, and can be performed using a heating block at a constant temperature. It is suitable for on-site detection and requires fewer resources compared to RT-PCR

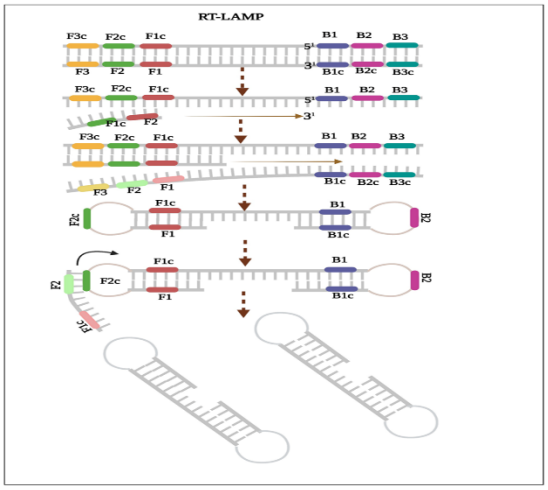

Fig 4 Amplification of nucleic acid through LAMP process.

Amplification techniques

Rolling Circle amplification (RCA)

RCA is an enzymatic isothermal viral detection technique that utilizes repetitive, incompatible DNA base pairs. It offers advantages over PCR by maintaining isothermal conditions more effectively and requiring fewer chemicals. This results in better clinical performance with fewer errors and contamination. The step-wise procedure of the test is illustrated in Figure 5.

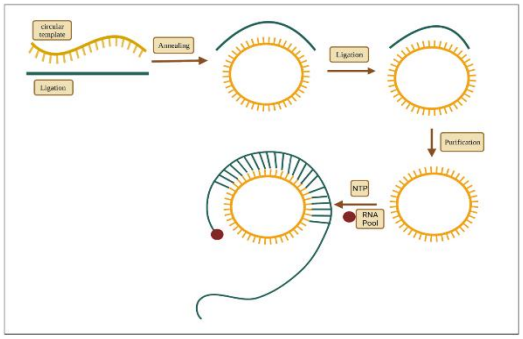

Fig 5 Stepwise mechanism of rolling circle amplification for detecting the viruses.

TMA and NASBA

The most common isothermal tests are transcription-mediated amplification and nucleic acid sequence-based amplification. NASBA has three enzymes compared to TMA's two. Both are more efficient than PCR and reduce errors.

In Fig 6 both TMA and NASBA are depicted.

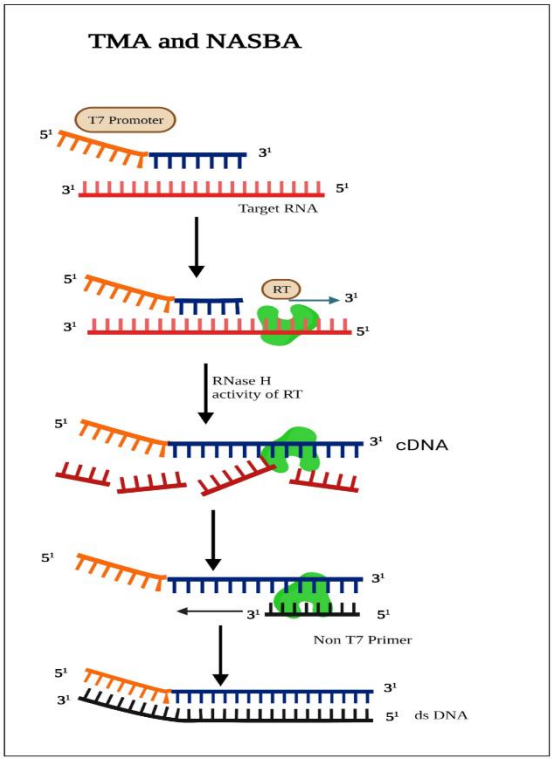

Fig 6 Process of TMA and NABSA

CRISPR diagnostics

CRISPR, initially discovered in bacteria, has been utilized for diagnosing viruses such as Zika and Staphylococcus aureus. POC-CRISPR, developed by Fan-En Chen et al. in 2021, combines Cas-assisted assay with sample preparation and readout in a thermoplastic cartridge. CRISPR/Cas is combined with RT-LAMP for viral RNA extraction, gene editing, and probe attachment. The DETECTOR technique utilizes Cas12 fluorescence recognition with lateral flow readouts.

CRISPR-Cas12 assays demonstrate high sensitivity, specificity, and accuracy compared to qRT-PCR. Cas13 is capable of cleaving single-stranded RNA, and electrochemical aptasensors have been employed for COVID-19 detection. Integration with smartphones allows for novel detection methods, and microfluidic chips combine isothermal amplification, CRISPR, and lateral flow. CRISPR/Cas12a-mediated techniques utilize aptamers for signal conversion. However,

inconsistent signal amplification poses challenges for early diagnosis and sample detection.

Serological assays

SARS-CoV-2 detection using RT-PCR becomes challenging after 14 days of infection due to low viral RNA count. Serological techniques, including antibody-based immunoassays, are used to identify antibodies and confirm SARS-CoV-2 infections. However, misinterpretation, false positives, and cross-reactivity are possible with recombinant antigens. Immunological assays show reliable performance and high sensitivity after 14 days of infection. Combining DNA and serology enhances accuracy, and the IgM-IgG combo assay is more sensitive than nucleic acid testing. Serological testing may require immunological monitoring

ELISA

ELISA tests detect immunoglobulins in serum to indicate past COVID-19 infection. WHO has authorized these tests for SARS-CoV-2 seroprevalence and antibody profiling.

Chemiluminescence immunoassay

CLIA and Lateral Flow immunoassays (LAF) are rapid point-of-care tests for identifying viral infections, including SARS-CoV-2.

Medical imaging techniques

Medical imaging confirms covid infection and studies lung damage. Interstitial alterations, pulmonary nodules, GGO(ground-glass opacities) are detected. CXR detects SARS-CoV 2 nodules, interstitial changes, and lesions. They're easy and available in many hospitals, but they can't detect low-density GGO. Chest CTs identifes reticular patterns, lung fibrosis, airway changes, paving patterns, GGO, etc.Early imaging can help with treatment and repeatability is high.Repetition might cause irreversible damage.Ultrasound detects B lines and pleural line irregularities.

It's inexpensive but doesn't assess the lung's deep field.

Recent advancements for fast detection

Electrochemical immunosensors

Recent methods combine electrochemical immunosensors with immunoassays for strong detection of SARS-CoV-2. Biosensing devices with graphene sheets or magnetic beads specific to antibodies have been developed. Paper-based SARS-CoV-2 diagnoses and AI-assisted CT scan analysis aid in early detection and treatment.

Deep learning neural networks algorithms

Next-gen point-of-care tests like EEVD and CHA-LFIA, and lab-on-chip devices provide sensitive biosensing for COVID-19 testing. Laser-scribed graphene (LSG) devices detect SARS-CoV-2 mutations. V-PLEX identifies serostatus and vaccine response. COVIDOT-TEST detects antigen-specific antibodies, and Smart gene offers RT-PCR-like performance. Quick and cost-effective, no extensive lab equipment needed.

Interpretation

RT-PCR results are interpreted using the CT value, where a value below 40 is considered positive and above 40 is negative. Low CT values indicate high viral load and increased transmission potential. ELISA and CLIA tests for IgM and IgG antibodies provide clear interpretation through color change or fluorescence intensity. Antibody rapid POC tests detect antibody presence or absence through color change or fluorescence, but the duration of protection and persistence of neutralizing antibodies is unknown. Diagnostic testing can be imperfect, and clinical evaluation and multiple tests are often necessary for accurate interpretation. Sensitivity and specificity are important metrics for diagnostic tests, with serological assays having a sensitivity of 95% and RT-PCR 80%, and both having high specificity.

False negatives:

The sensitivity of PCR tests depends on viral load and sample collection timing. False-negative results can occur, especially in the early stages of infection, with higher rates on day 1 and day 4 of infectivity. Testing after exposure may not indicate infection, and false negatives can pose a risk by failing to detect asymptomatic positive cases. Negative results do not guarantee the absence of infection. Factors such as previous exposure, travel history, and disease prevalence should be considered to assess the probability of infection. Respiratory samples have the highest viral load, and disease severity is linked to viral load. Age, sex, and other factors can also influence viral load. Careful evaluation of test results and consideration of other factors is important in determining the presence of illness.

Challenges and Future perspectives

RT-PCR is the gold standard for COVID-19 diagnosis, but false negatives are common. Confirmatory tests should be used if clinical suspicion is high. Serological tests face challenges such as antigen selection and antibody cross-reactivity. CRISPR/Cas systems have decreased sensitivity and specificity. POC tests offer faster and more user-friendly testing, but their specificity and sensitivity should be clinically approved. Accurate diagnosis is crucial for understanding the scope of the pandemic. Further research is needed for zoonotic disease diagnostics and the role of local microbiota. AI can assist in test evaluations. A consolidated list of diagnostic methods and their limitations is available in Table 2.

Table 2. Consolidated list of major diagnostic methods including their assay methods and limitations

S No.	Name of the test	Assay type	Accuracy	Duration of test result	Sampling Method	Limitations
1	Molecular tests	RT-PCR	96%	24 hrs	Nasopharyngeal Swabs.	Complex, expensive and slow to deliver
2	Molecular tests	Digital PCR	95%	1 hr	Nasopharyngeal Swabs.	complicated workflow, expensive instruments and consumables,
3	Molecular tests	Quantitative PCR	47%	2-4 hrs	Nasal or Nasopharyngeal Swabs.	No Quantification, less precession
4	Nucleic Acid test	LAMP	92.3-100%	1-2 hrs	Blood, urine or saliva.	Designing primer sets, and temperature.
5	Nucleic Acid test	RT-LAMP	92%	2 hrs	Nasopharyngeal Swabs.	Difficulty in multiplexing
6	Serological	ELISA	67-98%	3-5 hrs	Blood, plasma, or serum samples.	-
7	Serological	Lateral Flow Assay	95%	-	Blood or nasal samples.	Inability to the early disease
8	Nucleic Acid test	RT-LAMP	-	< 1 hr	oropharyngeal & nasopharyngeal swabs and urine samples	limited technical infrastructure
9	Deep Learning Methods	Convolutional Net	89.92%	-	Chest Radiographic Images	Availability of data
10	Deep Learning Methods	Automated Deep Convolutional Neural Network	98%	-	X- ray.	Less number of data sets
11	Nucleic Acid test	Transcription-mediated amplification (TMA)	94.7%	5 -6 hrs	Nasopharyngeal or oral swabs.	Less detection

CONCLUSIONS

In a pandemic, diagnostic tools must be speedy, affordable, and accurate. RT-PCR requires adjustments for detecting variants, while isothermal amplification and CRISPR/Cas systems overcome mutations and low viral loads. Point-of-care devices and serological assays aid in rapid testing and immune response tracking. Clinical testing is needed for novel approaches, which should address sample preparation and user safety. AI integration for data analysis shows promise. Molecular, radiological, and serological diagnostics are advancing, along with the development of bio detectors, electrochemical tests, and AI applications. Deep learning algorithms can predict viral mutations and contribute to effective pandemic management.

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