



Review Article

A comprehensive review of current microbiological detection methods of SARS CoV-2

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ABSTRACT

Introduction: COVID-19 the most recently discovered infectious disease caused by SARS CoV-2 novel corona virus is now a pandemic affecting many countries globally. Genetic sequencing was done from lung wash samples collected from a cluster of pneumonia cases in city of Wuhan, China in January 2020. From the genome sequence the novel regions of the genome was identified and new molecular diagnostic tools were developed from that.

Materials and Methods: A detailed internet search particularly at the following websites, World health organization, Indian council of Medical research, Centers for disease control & prevention, Foundation of Innovative new diagnostics were done. The latest information of testing strategy, test kits list and their performance are periodically updated by the above mentioned organizations & websites. Other authenticated links from where resources were collected are mentioned in the manuscript. Numerous diagnostic tests were used to identify COVID-19. Unless we know who is infected we cannot stop the pandemic. This article briefs overview of diagnostics used for detecting COVID-19, the most important being Molecular assay & Immunoassay, Sample collection & Lab biosafety for testing. A comprehensive review of advantages & disadvantages of closed and open molecular platforms, potential application of the assays used for detection, where to look for SARS-CoV-2 diagnostics performance data and full list of all molecular /immunoassay COVID-19 detection tests.

Conclusion: This comprehensive review might help the readers to understand the current microbiological detection methods and update on the same.

“The most effective way to prevent infections and save lives is by breaking the chain of transmission, and to do that you must test and isolate. We cannot fight a fire blindfolded. We cannot stop this pandemic if we don't know who is infected. We have a simple message for countries— test, test, test.”

” Dr. Tedros Ghebreyesus, WHO Director-General-media briefing on the 16th of March 2020,

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1. Introduction

The COVID-19 pandemic which started from Wuhan city of China now has spread globally to more than 180 countries with 5,819,962 confirmed cases as on 31st May.¹ The critical importance of diagnostic services in tackling this pandemic was emphatically proven because diagnostics are fundamental for Identification & control of an Outbreak. WHO also emphasized that diagnostic testing,

isolation, and contact tracing should be the backbone of the global response to COVID-19 as social distancing measures and hand washing alone will not stop the epidemic.² Eventhough currently there are legion of tests available globally for COVID-19, what tests to be used in COVID-19 response and what strategy is to be used to scale up the testing capacity is critical for correct identification followed by isolation of confirmed cases & contact tracing. Timeline of key dates of diagnostic response globally & in India are mentioned.

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1.1. Key dates in the diagnostic response (global) timeline^{3,4} include

1. 7 January 2020: The full genetic sequence of the novel coronavirus was published. This enabled the development of real-time PCR diagnostic (RT-PCR) tests by different countries and diagnostic companies.
2. 4 February 2020: The US Centers for Disease Control and Prevention's (CDC's) testing protocol [CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel] received Emergency Use Authorization from the Food and Drug Administration (FDA).
3. 4 February 2020: The Republic of Korea's Ministry of Food and Drug Safety approved the emergency use of RT-PCR kits for COVID-19.
4. 19 February 2020: FIND launched an expression of interest for test developers of in vitro diagnostics that detect SARS-CoV-2 nucleic acid (molecular tests) to undergo an independent evaluation, in collaboration with WHO.
5. 12 March 2020: FDA issues Emergency Use Authorization for first commercial molecular test for SARS-CoV-2.
6. 13 March 2020: FIND launched an EOI for test developers of immunoassays (manual ELISA, machine-based or lateral flow, rapid tests specific for SARS-CoV-2 antigen or antibodies) to be evaluated using a standardized, independent protocol, in collaboration with WHO.
7. 2 April 2020: FDA authorizes the first antibody-based test for COVID-19.
8. 7 April 2020: WHO lists two tests for emergency use: one RT-PCR test and one qualitative assay.
9. 12 April 2020: Close to 14 million SARS-CoV-2 tests have been performed worldwide (data from 184/195 countries) with around 1.8 million positive results reported on FIND's Test Tracker.

1.2. Key dates in the diagnostic response (India) timeline⁵⁻⁸ includes

A Total of 3126119 samples were tested for SARS CoV-2 (COVID-19) in India as on 26Th May 2020.

1. First case for COVID-19 in India was reported on 31'st December.
2. By 13 April 2020, there had been nearly 9,000 confirmed cases and over 300 deaths.
3. Rapid scaling up of PCR testing from mid-March, from 19 to over 669 (Government laboratories: 466 & Private laboratories: 203)⁹total Operational Laboratories reporting to ICMR as on 31/5/20 which includes 466 Real-Time (RT PCR) testing labs, 134 TrueNat testing labs & 55 CBNAAT Testing labs for COVID-19:

4. 17/03/2020: Advisory Strategy of COVID 19 testing in India (Version 2).
5. 04/04/2020: Advisory to start rapid antibody based blood test for COVID-19 17/04/2020: Protocol for using 'Rapid antibody test' in Hot area - epidemiological studies and surveillance for the State/UT.
6. 27/04/2020: Revised Advisory to state on Rapid Antibody Blood Test.
7. 09/05/2020: Advisory for use of Cartridge Based Nucleic Acid Amplification Test (CBNAAT) using Cepheid Xpert Xpress SARS-CoV2.
8. 18/05/2020: Revised Strategy for COVID19 testing in India (Version 5).
9. 19/05/2020: Revised Guidelines for TrueNat testing for COVID-19.

WHO recommends a strategy for COVID-19 testing and has defined four transmission scenarios.^{10,11} Each scenario gives the aim, how the testing will be done and what will be the actions taken based on the results.

2. Overview of Diagnostics for Covid-19

When we consider the role of diagnostics in the detection of infection, there are many important goals to consider like right clinical sample taken for the right test & right patient at the right time in the right setting. And of course right interpretation also matters. Basic diagnostic methods for infectious diseases typically fall into two different categories-Pathogen detection & Host biomarker detection as shown in Figure 1. Main differences between molecular (or PCR) assays and immunoassays, and advantages of each technology is shown in Table 1.

2.1. Role of genomic detection

Next-generation sequencing tests are a specific type of molecular test that can determine the sequence of the viral genome in a sample to track geographical and temporal variations for research purposes.

3. Specimen collection and Lab Biosafety

The World Health Organization (WHO)¹² and Centers for Disease Control and Prevention (CDC)¹³ recommend specimens from the lower respiratory tract, including sputum, bronchoalveolar lavage and tracheal aspirate, for the diagnosis of COVID-19 using the approved molecular testing methods. Specimen collection have to be done donning appropriate PPE¹⁴ and the same has to be doffed carefully & disposed as per bio medical waste management guidelines. Table 2 describes specimen type recommended and collection material & storage for the same. Laboratory biosafety for COVID-19 testing is shown in Table 3.

Table 1: Difference between molecular assay & immuno assay

	Molecular Assay	Immunoassay	
Principle	Amplifying genetic material	Antibody detection (Serological tests) Detect Antibodies –IgG/IgM or Both	Antigen detection Detects viral proteins/antigens
Techniques/ Available platforms	RT-PCR/ TRUNAAT/ CBNAAT (Manual/Automated)	ELISA	ELISA
Clinical Samples	LAMP Respiratory specimens	RDT/LFIA Serum/whole blood/plasma	RDT/LFIA Respiratory specimens
Sensitivity	Sensitive during First week after onset of symptoms	Sensitive from Second week of illness	Sensitive during First week after onset of symptoms
Applications for COVID-19	Diagnose/confirm active COVID-19 virus infection in individuals	A rapid simple test used for serological surveillance purpose	A rapid simple test used for triaging suspected cases at the point-of-care, or in community settings
	Monitor active COVID-19 virus infection	Neither WHO nor ICMR recommends this for diagnosis of COVID-19	Needs confirmation by RT PCR
TAT (Turn around time)	a. RT-PCR -Longer (4-5 hours/test) b. TRUNAAT-45 min c. CBNAAt-45 min d. LAMP- around 30 min	RDT: Less than 30 min ELISA;1-5 hours	RDT: Less than 30 min ELISA;1-5 hours
Infra structure	Lab based: Needs BSL 2 & Specific equipments	ELISA: Lab based. Needs BSL 2 & Specific equipments RDT: POC test	ELISA: Lab based. Needs BSL 2 & Specific equipments RDT: POC test
Cost	Expensive	Less expensive	Less expensive
Availability in India	Yes. Many ICMR approved Govt & Private labs. Testing done by RTPCR/Truenat/CBNAAT	Yes. But only for Serosurveillance purpose by ICMR as of now	Yes Available Negative test needs to be confirmed by RTPCR. Positive test can be reported

Table 2: Specimen types and storage for COVID-19

Specimen type	Collection materials	Storage & transport
Sputum (deep cough)	Sterile leak-proof container	Refrigerate and ship at 2–8°C up to 48 hours, if >48 hours freeze at –70°C and ship on dry ice
Bronchoalveolar lavage	2–3 ml in sterile leak-proof container	Refrigerate and ship at 2–8°C up to 48 hours, if >48 hours freeze at –70°C and ship on dry ice
Endotracheal or nasopharyngeal aspirate	2–3 ml in sterile leak-proof container	Refrigerate and ship at 2–8°C up to 48 hours, if >48 hours freeze at –70°C and ship on dry ice
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swab in viral transport medium in a sterile leak proof container	Refrigerate at 2–8°C up to 5 days, if >5 days freeze at –70°C and ship on dry ice
Venous blood	Serum separator tube	Store upright for at least 30 minutes after collection. Refrigerate and ship at 2-8 °C within 5 days

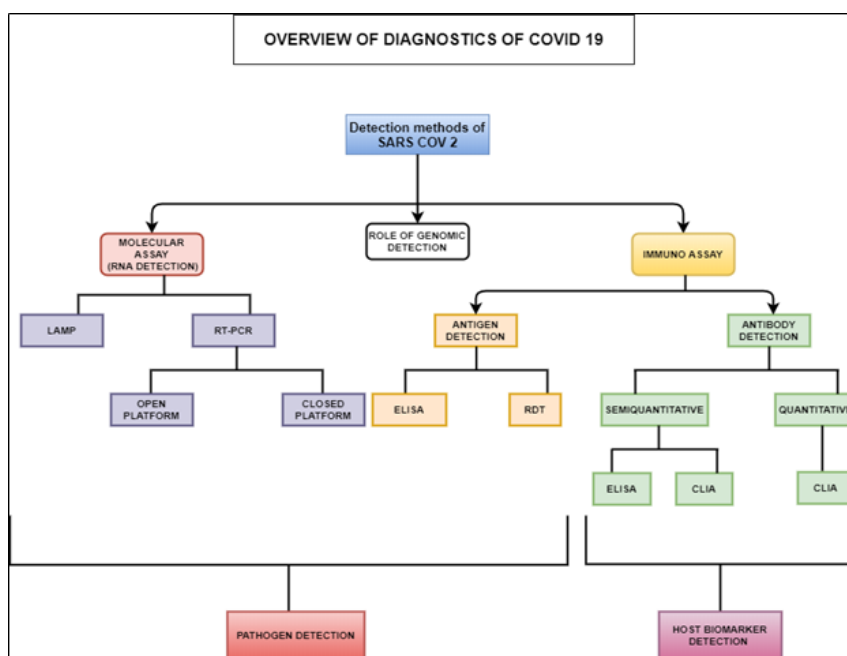


Fig. 1: Overview of diagnostics of COVID-19

RT-PCR-Real time Polymerase Chain reaction; LAMP: - Loop-mediated isothermal amplification; ELISA: Enzyme Linked Immunosorbent Assay; RDT: Rapid Detection tests; CLIA: Chemiluminescence immunoassay

Table 3: Lab biosafety for COVID -19 Testing¹⁴

Procedure	Recommended PPE	BSL
Specimen collection	Non-sterile gloves; single use only, Gown, - Eye protection, N95 respirator	NA
Specimen receipt and accession	Non-sterile gloves; single use only, Gown	NA
Specimen testing (non-propagative)	-Non-sterile gloves; single use only, Gown, N95 respirator mask	2
Specimen testing (propagative)	Non-sterile gloves; single use only, Gown, N95 respirator mask	3

BSL = Biosafety Level NA = not applicable

4. Molecular assay -RT PCR

4.1. The targets for molecular assay for SARS COV-2 is shown in Figure 2

Current testing modalities for laboratory diagnosis of COVID-19 by Real Time PCR

1. Initial Screening RT PCR involves detection of 'E' gene (coding for SARS-CoV-2 viral envelope).
2. Confirmation of samples positive in screening PCR involves detection of One of the following gene targets:
 - a. RdRp gene (coding for SARS-CoV-2 RNA dependent RNA Polymerase).
 - b. ORF gene (coding for SARS-CoV-2 Open Reading Frame).
 - c. N gene (coding for SARS-CoV-2 Nucleocapsid).

4.2. Principles of good molecular biology testing practice^{15,16}

Molecular labs should follow best practices to minimize nucleic acid contamination of the laboratory environment & best practices to minimize and detect contamination of samples in an analytical run. This could be achieved by proper design of a molecular lab. Contamination is a major concern because, they cause false positive results.

To reduce the likelihood of contamination and to protect the integrity of specimens, proper design of laboratory, and following general laboratory practices are important. Separation of pre- and post-amplification work areas and reagent preparation areas to prevent contamination is mandatory. Ideally, molecular laboratory should have three work areas that are physically separated and preferably at a distance from each other:

Area 1: reagent preparation room (positive air pressure relative to outside);

Area 2: specimen control/preparation, PCR set-up room (negative air pressure relative to outside); and

Area 3: nucleic acid amplification and amplification product analysis room (negative air pressure relative to outside).

These work areas must be arranged so that there is a unidirectional flow of personnel and specimens from “clean” areas to “dirty” areas, i.e. from Area 1 to Area 3 (this includes cleaning and maintenance personnel). See the Figure 2 for an example of work area layout.

4.3. *Molecular testing on open platforms:*^{17–19}

Test kits and protocols that are used on open PCR platforms require the end user to extract the viral RNA in a separate step to the PCR reaction. As these manual PCR kits and protocols are compatible with multiple PCR machines (thermocyclers), the end user may also choose which thermocycler to use. There are two types of manual PCR protocols that can be performed on open PCR machines: those that follow laboratory-developed (or in-house) protocols and those that follow commercial kit protocols.

4.4. *Advantages of tests for use on open platforms*

1. Tests may be well supported in-country by the manufacturer’s distributor network and service staff (if using commercial kits but does not apply to Local Developed Tests).
2. It may be easier to scale testing using multiple kits that are compatible with the same open system, as long as consistent quality can be guaranteed by the laboratory, which may mitigate any supply chain concerns.
3. Laboratory staff may already have been trained on testing, troubleshooting and maintaining open platforms.
4. Tests are usually less expensive than tests for use on closed platforms.

4.5. *Disadvantages of open platforms*

1. Strong quality control, quality assurance procedures and validation of the methods required in every laboratory to ensure high test performance & Quality.
2. Sample processing methods may not be specific for the specimen types recommended for COVID-19 diagnosis.
3. Well-trained staff are needed to perform the many manual steps and interpret results.
4. Open platforms require additional consumables, equipment or quality controls that may not be supplied with the machine, test or test component.

4.6. *Molecular testing using closed (proprietary) diagnostic platforms*^{17,18,20}

Use of closed/proprietary systems generally enables greater automation of workflow, and frequently includes extraction, amplification and output of results all on the same machine. A number of manufacturers have rapidly developed SARS CoV-2 tests to run on their existing closed platforms to take advantage of available machines and associated resources in laboratories. Example of some COVID-19 closed platform tests authorized for use are shown in Figure 4.

4.7. *Advantages of closed platforms*

1. Closed platform instruments are often already available in many countries, where they have been deployed for TB, HIV and other testing.
2. Closed platforms are well-supported by manufacturers or their representatives (e.g. manufacturers may have in-country service staff supporting the platforms).
3. Some closed platforms allow for high-throughput testing, which can help to rapidly scale up COVID-19 testing, whereas others may be more amendable to more decentralized testing.
4. Laboratory staff will be trained on testing, troubleshooting and maintaining closed platforms already in use.
5. Most, if not all, required reagents or consumables for closed platforms are directly provided by the test supplier.
6. Closed platforms require limited hands-on time and have largely automated result reporting and interpretation.
7. Internal quality controls for closed platforms are included in the platform or test.

4.8. *Disadvantages of closed platforms*

1. Due to the high global demand, stocks of COVID-19 tests for use on closed systems may be constrained.
2. Some closed platforms have limited throughput per instrument.
3. As the tests are end-to-end, if one reagent or component is out of stock, or the machine is out of order, no testing can be performed.

4.9. *What should one consider when selecting an open/Closed platform test?*

When implementing molecular tests for use on open/closed platforms, one should consider performing an assessment of the available platform tests at their setting including assessment of infrastructure/resources. Once the best combination of tests or test components is matched to the correct PCR and result visualization machine, then procurement and training of the staff needs to be considered.

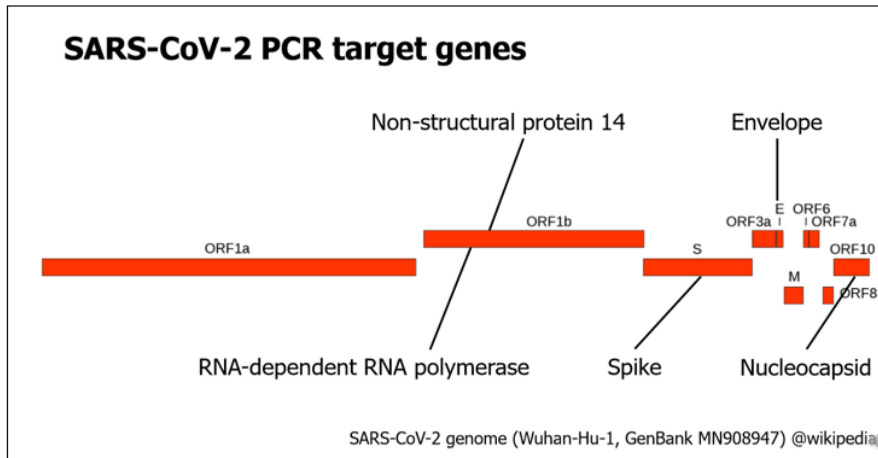
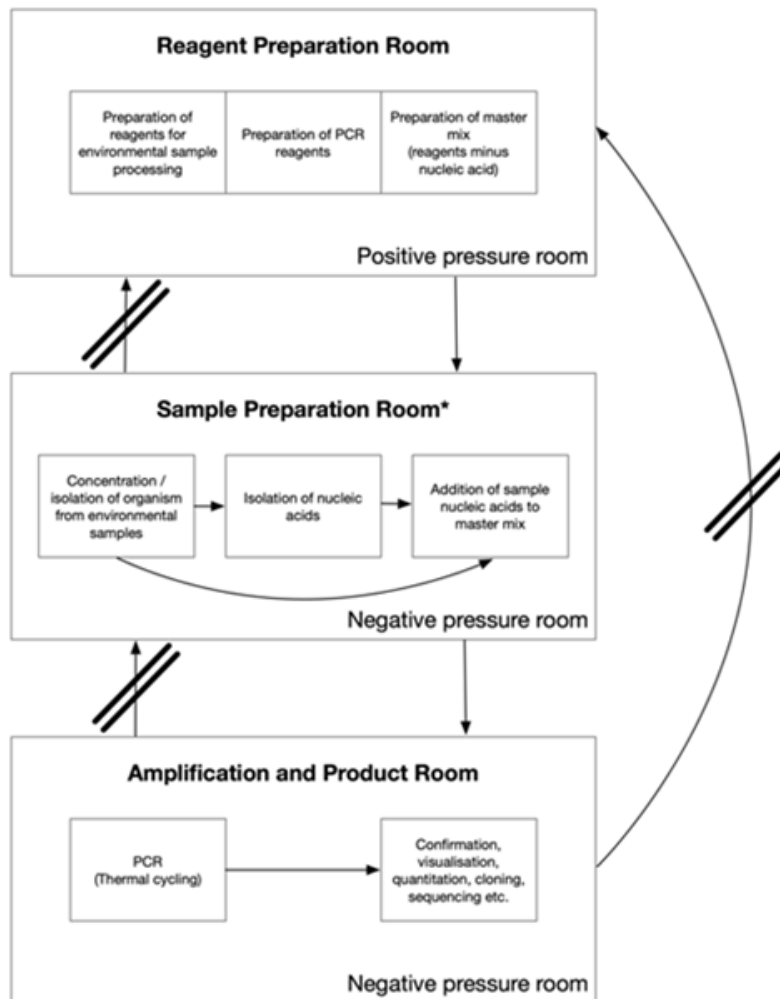


Fig. 2: Targets of molecular assay for SARS-CoV-2



* Sample / negative control preparation and positive control preparation should occur in physically separated work areas (biological cabinets) or different rooms

Fig. 3: Recommended work area layout for COVID-19 testing^{15,16}

Closed platform manufacturer	Test name	Specimen type	Platform	Target	Capacity	TAT
Abbott Laboratories	Abbott RealTime SARS-CoV-2 test	Nasal, nasopharyngeal and oropharyngeal swabs	Abbott m2000	RdRp and N genes	24–96 specimens	470 patient samples in 24 hours
	Abbott ID NOW™ COVID-19 test	Nasal, nasopharyngeal and throat swabs	Abbott ID NOW™	RdRp gene	Single test	5–13 minutes
Becton, Dickinson and Company (BD)	BioGX SARS-CoV-2	Nasopharyngeal and oropharyngeal swabs	BD MAX™ System	N gene	24 specimens	<3 hours
Cepheid Inc	Xpert® Xpress SARS-CoV-2	Nasopharyngeal swabs, nasal aspirates, and nasal washes	GeneXpert®	E & N genes	Single test	45 minutes
Hologic Inc	Panther Fusion® SARS-CoV-2	Nasopharyngeal and oropharyngeal swabs	Panther Fusion®	Orf1ab gene	1150 tests in 24 hours	2.4 hours
QIAGEN	QIAstat-Dx® Respiratory SARS-CoV-2	Nasopharyngeal swabs	QIAstat-Dx® Analyzer 1.0	Orf1b and Rdrp genes	Single test	1 hour
Roche Holding AG	cobas® SARS-CoV-2	Nasal, nasopharyngeal and oropharyngeal swabs	cobas® 6800/8800	Orf1ab gene	960 tests in an eight-hour shift (on the cobas® 8800)	3 hours
Seegene Inc	Allplex™ 2019-nCoV	Sputum, nasopharyngeal swabs and aspirates, bronchoalveolar lavage and throat swabs	Seegene NIMBUS & Seegene STARlet	RdRP and N genes	Up to 2256 samples in 24 hours	NA

TAT, turnaround time.

Fig. 4: Example of some COVID-19 closed platform tests authorized for use

5. Molecular Assay- Loop-Mediated Isothermal Amplification (LAMP)

Although real-time RT-PCR is sensitive and reliable, it is time-consuming (~2 h) and requires a specific detection device or instrument, which limits its broad application to current huge demand for the global pandemic of COVID-19.

LAMP It is a very sensitive, easy and time efficient method. (LAMP) is a rapid technology of DNA amplification which has been applied to pathogen detection such as virus, bacteria and malaria. The LAMP reaction generally

needs one constant temperature, and the target DNA can be amplified in 30 min. COVID-19 diagnosis kit for the rapid detection of SARS-CoV-2, using one-step reverse transcription and loop-mediated isothermal amplification (RT-LAMP) has been developed.^{21–23} But commercial kits based on this principle is not yet available in India

6. Immunoassays

{Current WHO recommendations do not support their use in clinical management, but encourage their inclusion in research projects to assess their performance and clinical

utility}.

Immunoassays are used in detection or quantification of viral proteins, antigens or antiviral antibodies produced by the immune response against a pathogen from clinical specimens. All immunoassays rely on the binding of an antibody to an antigen, and the ability to produce a measurable signal in response to the binding, either via chemically linking antibodies or antigens with some kind of detectable label. A variety of different labels and detection methods used are either emission of radiation, prediction of a color change in solution, or fluorescence.

6.1. Targets of immunoassays for SARS-CoV-2^{24–26}

Antigen tests for SARS-CoV-2 detect and/or quantify the nucleocapsid (N) or spike (S1 & S2) proteins of the virus, Spike protein S1 is specific. Antibody tests detect the antibodies (IgG, IgM or IgA) produced against the N or S viral proteins.

1. Spike protein (S) is the most exposed viral protein of SARS-CoV-2, which attaches to a specific receptor on human cells, allowing the virus to infect the cell. The S protein consists of S1 and S2 subunits.
 - S1 seems to be the most variable antigen, making it a good candidate to differentiate between other coronaviruses (e.g. the SARS coronavirus, SARS-CoV).
 - However, the S2 subunit shares similarity in antibody epitopes (region of an antigen recognized by an antibody) with S2 from SARS-CoV.⁷
2. Nucleoprotein (N) is the most abundant viral protein produced and shed during infection with SARS-CoV-2. It is highly immunogenic and can be found in blood and urine.⁸
3. Matrix protein (M) is the most abundant protein inside a SARS-CoV-2 virus

In SARS-CoV-2, published evidence shows that viral load peaks at 3-5 days and that both IgM and IgG start to increase around 10 days after the onset of symptoms. Most patients produce antibodies in response to the virus (known as seroconversion) within the first three weeks.

6.2. Types of SARS-CoV-2 immunoassays^{24–26}

The most common types of immunoassays developed for COVID-19 are either enzyme-linked immunosorbent assays (ELISAs) or lateral flow immunoassays (LFIs), also known as rapid diagnostic tests (RDTs).

ELISAs are one of the most frequently used types of immunoassays. Some key features of ELISAs mentioned in Table 1. Labs can use commercial reagent kits or in-house protocols; and have high throughput – run on 96 or 324-well plates;

All laboratory procedures for ELISA must be performed in a facility using procedures equivalent to a BSL-2, be based on a risk assessment, and conducted by trained personnel. Initial processing of samples from patients suspected of having COVID-19 infection, as well as any aerosol-generating procedures such as vortexing and ELISA plate washing, must be done in a biological safety cabinet. Refer to TABLE on Laboratory biosafety for COVID-19 testing.

LFIs or RDTs allow for the rapid detection of SARS-CoV-2 antigens or antibodies using a test strip. RDTs use the same principles of antigen-antibody binding as ELISAs but are generally less sensitive. When a sample is added to the test strip, the antigen or antibody migrates up the test strip, where it is captured by a detector antibody. The antibody for detection can be conjugated to gold nanoparticles or latex beads. An example of RDT in use is shown in Figure 5. Some key features of RDTs in use are mentioned in Table 1. One can perform the test with limited technical expertise. They can be run on different sample types: nasopharyngeal swab/respiratory specimens for rapid antigen detection and serum/plasma/whole blood, and other fluids for antibody detection.

RDT antibody tests could be very helpful in research studies to understand, at a community level, the population attack rate, the case fatality rate, and to understand transmission patterns in specific subgroups, such as children, teachers, and frontline medical staff. It is not known how long the antibodies detected by these RDTs will persist, nor whether they offer immunity to reinfection. As molecular testing is not always available, especially outside large urban areas, there is substantial pressure to use these tests.

Testing of samples using RDTs outside a laboratory setting should follow standard precautions to provide a barrier between the specimen and personnel during specimen manipulation. Although the use of immunoassays is not currently recommended for routine diagnosis, Table 4 shows where immunoassays for COVID-19, especially RDTs, can potentially be used.

Other potential diagnostic applications for immunoassays include aiding in the estimation of the disease stage (acute, early, convalescent); assessing the immune status of healthcare and other essential personnel; assessing convalescent plasma and vaccine efficacy (more suitable for ELISAs versus RDTs); and Guiding identification of low-risk people to exit quarantine (e.g. lifting lockdown measures).

The following Figure 6²⁷ adapted from the Institute of Tropical Medicine, Antwerp, describes general performance requirements for antigen and antibody tests according to different testing scenarios:



Fig. 5: Example of an RDT in use

Table 4: Potential applications for COVID-19 immunoassays²⁶

Application	Antigen test	Antibody test
Screen potential COVID-19-infected individuals	Yes	Yes
Aid in the diagnosis of suspected cases when PCR is not available	Yes	Yes (for later-stage infection)
Aid in the diagnosis of of COVID-19 in a PCR-negative individual that has strong clinical indications of COVID-19 infection	Yes	Yes (for later-stage infection)
Monitor active COVID-19 infection	Yes	No
Support seroprevalence studies to understand extent of exposure to COVID-19 in a population	No	Yes

7. Sars-Cov-2 Diagnostics: Performance Data

Test performance is an important component that should be considered when selecting specific diagnostic products and determining the most appropriate implementation approaches. Some important performance characteristics of molecular assays & Immunoassay include:

1. Analytical sensitivity – also known as limit of detection, the lowest concentration of viral RNA that the test can detect.
2. Analytical specificity - or cross-reactivity – describes whether the test will detect other viruses or other infections that are not SARS-CoV-2, which can lead to false positive results.
3. Clinical sensitivity - the ability of a test to correctly identify COVID-19 positive individuals.
4. Clinical specificity - the ability of a test to correctly identify COVID-19 negative individuals.
5. A note on test utility.

The clinical utility of any test is affected by the prevalence or the probability of disease, in a population (even where the sensitivity and specificity of the test remain the same). Given a positive or negative result, the probability of having or not having COVID-19 (known as positive predictive value [PPV] and negative predictive value [NPV] respectively), will differ depending on the population tested.

8. Where to find data on Molecular/immunoassay Test Performance

FIND (Foundation for innovative new diagnostics) gives performance data on molecular & Immunoassay regarding detection of COVID-19²⁸ and is in the process of performing an independent evaluation of a large number of different SARS-CoV-2 tests including immunoassays. The website will make the data publicly available when the evaluations are complete. FIND is working closely with WHO on this. List of validated test kits for both Molecular assay and antibody assay are published by ICMR.^{29,30}

8.1. List of molecular and immuno assay test kits available from FIND website

The complete list of US-FDA and/or CE-approved SARS-CoV-2 real time PCR kits and immunoassays are available at FIND COVID-19 website.^{31,32} The status of the Tests if they are commercialized, in development or withdrawn, type of the test if it's a Manual /automated/POC molecular test, Antigen/Antibody RDT, which regulatory is performing the tests are published in the FIND COVID-19 diagnostic pipeline website.

Testing scenario		Performance requirements of antigen tests	Performance requirements for antibody tests
1. Epidemic settings	Triage of <i>symptomatic</i> individuals	High SN (to obtain high NPV) Moderate SP (to avoid unnecessary quarantine; fewer false positives)	<7 days from symptom onset: not recommended ≥7 days: High SN, moderate SP, if PCR confirmation available
	Moderate prevalence	<i>Alternative:</i> RDT with low SN and high SP can be used in sequential testing algorithm (where negative result retested by NAAT or Ab test [if NAAT not available])	<i>Potential:</i> High SN, high SP to rule out other viruses (if PCR not available). If test <7 days from symptom onset, potentially consider quarantine of those with negative tests and re-test 3–5 days later
	Triage of <i>asymptomatic</i> contacts of COVID-19 individuals	High SN (to increase NPV) High SP (to increase PPV)	Not recommended
	Low prevalence		
2. Endemic setting	Diagnosis of symptomatic persons	High SN High SP	<7 days from symptom onset: Not recommended >7 days: High SN High SP
	Variable prevalence		
3. Surveillance*	Variable to low prevalence	High SN High SP	High SN High SP
4. Detection of previous exposure	Variable prevalence	Not valid for antigen test	High SN High SP

Ab, antibody; Ag, antigen; NAAT; nucleic acid amplification test; NPV; negative predictive value; PPV, positive predictive value; RDT, rapid diagnostic test; SN, sensitivity; SP, specificity. *Mainly using ELISA.

Fig. 6: Performance requirements for antigen and antibody tests according to different testing scenarios;

9. Conclusion

Diagnostic tests play an important role in the COVID-19 pandemic response. We need to understand the attributes and limitations of each type of test and use the right test for the right patient at the right time in the right setting. This is a rapidly evolving area as many countries are trying to scale up community testing for COVID-19. Many tests are now commercially available but their performance is not clear. FIND the Foundation for Innovative New Diagnostics, is evaluating their test performance and WHO will soon issue recommendations on the desired test performance characteristics for each use case.

Summary of the links from where information collected

1. Indian Council of Medical Research. COVID-19.
2. Ministry of Health and Family Welfare Government of India. <https://www.mohfw.gov.in/#>.

3. Foundation for Innovative New Diagnostics. COVID-19 diagnostics resource centre.
4. World Health Organization. Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans.
5. World Health Organization. Country and technical guidance - coronavirus disease (COVID-19).
6. World Health Organization. Coronavirus disease (COVID-2019) daily situation reports.
7. Africa Centres for Disease Control and Prevention. Latest updates on the COVID-19 crisis from Africa CDC.
8. US Centers for Disease Control and Prevention. Coronavirus (COVID-19) updates.
9. London School of Hygiene & Tropical Medicine. COVID-19 updates.
10. European Centre for Disease Prevention and Control. COVID-19 updates.

11. National Institute for Communicable Diseases. Division of the National Health Laboratory Service South Africa. COVID-19.

10. Source of Funding

None.

11. Conflict of Interest

None.

References

- World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard; 2020. Available from: <https://covid19.who.int/>.
- World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19; 2020. Available from: <https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19>.
- Future Learn: Online Courses and Degrees from Top Universities. [Internet]. Key dates in the diagnostic response timeline; 2020. Available from: <https://www.futurelearn.com/courses/covid-19-diagnostics-and-testing/1/steps/765091>.
- Foundation for Innovative New Diagnostics. [Internet]. Sars-cov-2 test tracker; 2020. Available from: <https://www.finddx.org/covid-19/test-tracker/>.
- Indian Council of Medical Research. [Internet]. COVID-19; 2020. Available from: <https://www.icmr.nic.in/content/covid-19>.
- Indian Council of Medical Research. [Internet]. Information of Testing Strategies; 2020. Available from: <https://www.icmr.gov.in/cteststrat.html>.
- Indian Council of Medical Research. [Internet]. Advisory to start rapid antibody based blood test for COVID-19; 2020. Available from: https://www.icmr.gov.in/pdf/covid/strategy/Advisory_Antibody_Testing_04042020.pdf.
- Indian Council of Medical Research. [Internet]. Revised Guidelines for TrueNat testing for COVID-19; 2020. Available from: https://www.icmr.gov.in/pdf/covid/labs/Revised_Guidelines_TrueNat_Testing_19052020.pdf.
- Indian Council of Medical Research. [Internet]. List of COVID19 testing Govt & Private labs; 2020. Available from: https://www.icmr.gov.in/pdf/covid/labs/COVID_Testing_Labs_30052020.pdf.
- World Health Organization. [Internet]. Laboratory testing strategy recommendations for COVID-19; 2020. Available from: https://apps.who.int/iris/bitstream/handle/10665/331509/WHO-COVID-19-lab_testing-2020.1-eng.pdf.
- Bedford J, Enria D, Giesecke J. COVID-19: towards controlling of a pandemic. *Lancet*. 2020;395:1015–8.
- World Health Organization. [Internet]. Country & technical guidance-coronavirus disease (COVID-19); 2020. Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus2019/technical-guidance>.
- Centers for Disease Control and Prevention. [Internet]. Interim guidelines for collecting, handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19); 2020. Available from: <https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html>.
- World Health Organization. [Internet]. Modes of transmission of virus causing COVID-10: implications for IPC precaution recommendations. Scientific Brief. 29 March 2020; 2020. Available from: <https://www.who.int/publications-detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations>.
- Dos, Molecular DF, Testing. World health organization. [Internet]. Dos and don'ts for molecular testing; 2018. Available from: <https://www.who.int/malaria/areas/diagnosis/molecular-testing-dos-donts/en/>.
- Future Learn: Online Courses and Degrees from Top Universities. [Internet]. Principles of good molecular biology testing practice; 2020. Available from: <https://www.futurelearn.com/courses/covid-19-diagnostics-and-testing/1/steps/765112>.
- World health organization. [Internet]. Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans; 2020. Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance>.
- Foundation for innovative new diagnostics. [Internet]. Sars-cov-2 diagnostic pipeline; 2020. Available from: <https://www.finddx.org/covid-19/pipeline/>.
- Future Learn: Online Courses and Degrees from Top Universities. [Internet]. Molecular testing on open platforms: assay selection and instrument compatibility; 2020. Available from: <https://www.futurelearn.com/courses/covid-19-diagnostics-and-testing/1/steps/765113>.
- Future Learn: Online Courses and Degrees from Top Universities. [Internet]. Overview of molecular testing using closed (proprietary) diagnostic platforms; 2020. Available from: <https://www.futurelearn.com/courses/covid-19-diagnostics-and-testing/1/steps/765116>.
- Park GS, Ku K, Baek SH. Development of Reverse Transcription Loop-Mediated Isothermal Amplification Assays Targeting Severe Acute Respiratory Syndrome Coronavirus 2. *J Mol Diagn*. 2020;(20):30090–8.
- Yan C, Cui J, Huang L, Du B, Chen L, Xue G, et al. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loopmediated isothermal amplification assay. *Clin Microbiol Infect*. 2020;26:773–9.
- Huang WE, Lim B, Hsu CC, Xiong D, Wu W, Yu Y, et al. RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microb Biotechnol*. 2020;13(4):950–61.
- Okba N, Müller MA, Li W, Wang C, Geurtsvankessel CH, Corman VM. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. *Emerg Infect Dis*. 2020;26(7). doi:10.3201/eid2607.200841.
- Petherick A. Developing antibody tests for SARS-CoV-2. *Lancet*. 2020;395(10230):1101–2. Available from: [https://dx.doi.org/10.1016/s0140-6736\(20\)30788-1](https://dx.doi.org/10.1016/s0140-6736(20)30788-1).
- Future Learn: Online Courses and Degrees from Top Universities. [Internet]. Quick guide to immunoassays; 2020. Available from: <https://www.futurelearn.com/courses/covid-19-diagnostics-and-testing/1/steps/765128>.
- Institute of Tropical Medicine, Antwerp. [Internet]. Guidance on the use of covid-19 rapid diagnostic tests. Antwerp; 2020. Available from: <https://www.itg.be/E/Article/guidance-on-the-use-of-covid-19-rapid-diagnostic-tests>.
- Foundation for innovative new diagnostics. [Internet]. FIND SARS-CoV-2 Diagnostics: Performance Data; 2020. Available from: <https://www.finddx.org/covid19/dx-data>.
- Indian Council of Medical Research. [Internet]. COVID-19 Diagnostic kit evaluation. Performance evaluation of commercial kits for real time PCR for COVID-19 by ICMR identified validation centers; 2020. Available from: https://www.icmr.gov.in/pdf/covid/kits/RT-PCR_Tests_Kits_Evaluation_Summ_30052020.pdf.
- Indian Council of Medical Research. [Internet]. COVID-19 Diagnostic kit evaluation. List of antibody (IgM, IgG) based rapid tests; 2020. Available from: https://www.icmr.gov.in/pdf/covid/kits/Antibody_based_tests_14052020.pdf.
- Foundation for innovative new diagnosticS. [Internet]. SARS-COV-2 DIAGNOSTIC PIPELINE; 2020. Available from: <https://www.finddx.org/covid-19/pipeline/?section=immunoassays#diag.tab>.
- Foundation for innovative new diagnostics. [Internet]. SARS-COV-2 Diagnostic Pipeline; 2020. Available from: <https://www.finddx.org/covid-19/pipeline/?section=molecular-assays#diag.tab>.

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