Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 7 Issue 8 August 2024

Review Article

A Comprehensive Review on Approaches for SARS-CoV-2 Detection Kits - An Indian Scenario

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DOI: 10.31080/ASMI.2024.07.1414

Received: June 26, 2024

Published: July 28, 2024

Abstract

The global outbreak of SARS-CoV-2 virus has taken more than 6.3 million lives and the number is increasing at a constant pace. Different methods for diagnosis of the virus including sensitive techniques like RT-PCR and LAMP as well as quick and cost-effective techniques including antigen detection technique have been developed globally. India has played a strong character in terms of COVID research to manage the pandemic situation. The presented review focuses on various diagnostic approaches with available kits (RT-PCR, LAMP and Antigen detection) developed by India and approved by ICMR till late 2022. In detailed methods and principles of each technique have also been discussed. It also represents the Next Generation Sequencing (NGS) techniques utilized for understanding different variants of the virus. The article not only dictates the Indian scenario of COVID diagnosis but also explains the research gaps and future aspects of the same.

Keywords: SARS-CoV-2; Diagnostic Kit; RT-PCR; RT-LAMP; ICMR; Next Generation Sequencing

Introduction

The ongoing COVID-19 breakout was initially discovered in December 2019 in Wuhan, China, a city in the Hubei province [26]. COVID-19 was designated as new pneumonia after a cluster of unusual pneumonia cases and attempts to identify the pathogen responsible for the occurrence [24]. Subsequent nucleic acid screening of identified pathogen panels using high throughput real-time polymerase chain reaction (RT-PCR) revealed negative findings, indicating that the culprit of pneumonia was unexpected [25]. However, advancement in themolecular methods and Next-generation sequencing (NGS) technology find out the culprit as a virus, that was recognized as Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2). The sequence of it was initially reported to local councils on 5 January 2020 before being made available on the open-access virology website virological.org on 11 January [23]. Moreover, within two weeks of the epidemic being declared, whole genome sequencing of the SARS-CoV-2 virusbecame available on the global public repository, Global Initiative on Sharing All Influenza Data (GISAID) (https://www.gisaid.org/) [4]. That helped a lot to scientific community to develop diagnostic kits including nucleic acid based RT-PCR kit as well as protein based rapid antigen test kit and supports the exhaustive research for the drug development as well as vaccine development against SARS-CoV-2.

The epidemic of the coronavirus swiftly spread over the globe and the World Health Organization (WHO) declared it as a global pandemic of 21stcentury. COVID has remained a threat to humanity, killing over 6.3 million people and infecting over 536 million people worldwide [21]. By the time academics, government officials, and private-sector entrepreneurs were working on strategies to relieve and tackle the issue at rapid scale and pace. Testing for SARS-CoV-2 in the community is a fundamental strategy that has been deployed internationally among the other methods used to restrict the spread of the infectious disease [18]. Availability of genome sequence on public domain leads many commercial manufacturers to generate a wide range of RT-qPCR kits and antigen based rapid test

kits in a reasonably short period of time. To handle the emergency and elevated demand for testing, regulatory agencies such as the US Food and Drug Administration (USFDA) adopted the Emergency Use Authorization (EUA) procedure for diagnostic use of kits, rather than the more conventional method of authorizing full approval or clearance for diagnostic assays [6].

The South Asian Association for Regional Cooperation (SAARC) is an organisation made up of eight nations: Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka. With 23.75% of the world's population living in the South Asian Region, COVID-19 infection has persisted in this region [13]. The testing capability of SAARC nations has drawn attention, with Bangladesh facing criticism for only having the capacity to test up to 15,000 people out of its 165 million residents [3]. India exhibits highest percentage of tests overall among the nations with the highest COVID-19 prevalence per 100 million individuals due to availability of different kits for the SARS-CoV-2 detection [17]. By adopting a worldwide pattern of fast escalator of diagnostic capability,1260 Indian diagnostic laboratories across the nation were approved by Indian Council of Medical Research (ICMR).

Currently, there are many diagnostic kits available globally that use the molecular based detection or antigen-antibody dependent principles [9]. The diagnosis of SARS-CoV-2 has become a lot simpler with the development of commercial kits and automated mechanization. Such changes have accelerated the diagnosis and surveillance by reducing the assay time [20]. There are many diagnostic kits available globally, which are approved by the US-FDA. However, the limitation of using such globally renowned kits is that they are expensive to the economically restrained laboratories in the developing countries. To overcome the limitations, local companies of such developing countries have launched various diagnostic kits for COVID-19 professing to detect SARS-CoV-2 at par with kits by global giants. Hence, the Indian made diagnostic kits need to be approved by ICMR before launching their product into the market.

With the SARS-CoV-2 pandemic as a background, this review intends to serve as an important source for learning about the development of various diagnostic kits in India in the context of infectious disease. We started by discussing the various COVID-19 diagnostic procedures, followed by the approaches of diagnostic kits developed in India and approved by ICMR. Moreover, the advance technique such as NGS plays a key role in the diagnosis and identifying different variants of SARS-CoV2. The sequencing approach and its output in India has also been discussed. We wrapped up with emphasizing the need of more diagnostic kits for SARS-CoV-2 and its other variants as well as highlighting the different approaches which are already in the pipeline for the development of other diagnostic kits.

Current testing methods for detecting viral infections

There are three main methods for detecting viral infections, which are depicted in Figure 1. The molecular assays detect the presence of viral nucleic acid sequences to identify the infected patients. The antigen tests are faster with only five to fifteen minutes of testing time for detection of viral protein. Limitation of these tests are its lower sensitivity and accuracy. On the other hand, the serological tests are used to detect the antibodies that the patient's body would typically release if infected with the virus. Butan infection happened months ago may also give positive result due to its less sensitivity. In further section, various RT-PCR kits and rapid antigen test kits developed by Indian manufacturer and approved by ICMR are discussed.

RT-PCR based detection of SARS-CoV-2

RT-PCR is extremely focused and sensitive method for the detection of virus. To test SARS-CoV-2 genetically, a variety of RT-PCR kits have been developed globally. A list of ICMR approved RT-PCR kits, which are developed by Indian companies are listed in Table 1. ICMR has approved around 140 RT-PCR kits from domestic region and majority of all follow a same protocol. If we look at the basics, the samples are collected by a nasopharyngeal swab or an or pharyngeal swab for the test. For Nasopharyngeal specimen the swab is inserted in the nostril and gently moved forward into the nasopharynx. Then it is rotated for a specified period time to collect secretions that contain the virus. Once the swabbing is applied, the swab is placed immediately into sterile tube containing a viral transport medium. Coronaviruses contain an extraordinarily long single-stranded RNA genome, and to detect these viruses with PCR, RNA molecules must be converted into their complementary DNA (cDNA) sequences by reverse transcriptase enzyme. After that, the newly synthesized cDNA can be amplified by standard PCR procedures. This approach is universally known as RT-PCR and to perform this method, high-quality viral RNA should be extracted.

Several RNA purification kits are available for convenient, fast, and effective isolation of RNA. Extraction of the viral RNA using commercial kits is an easy task, where sample is first added into

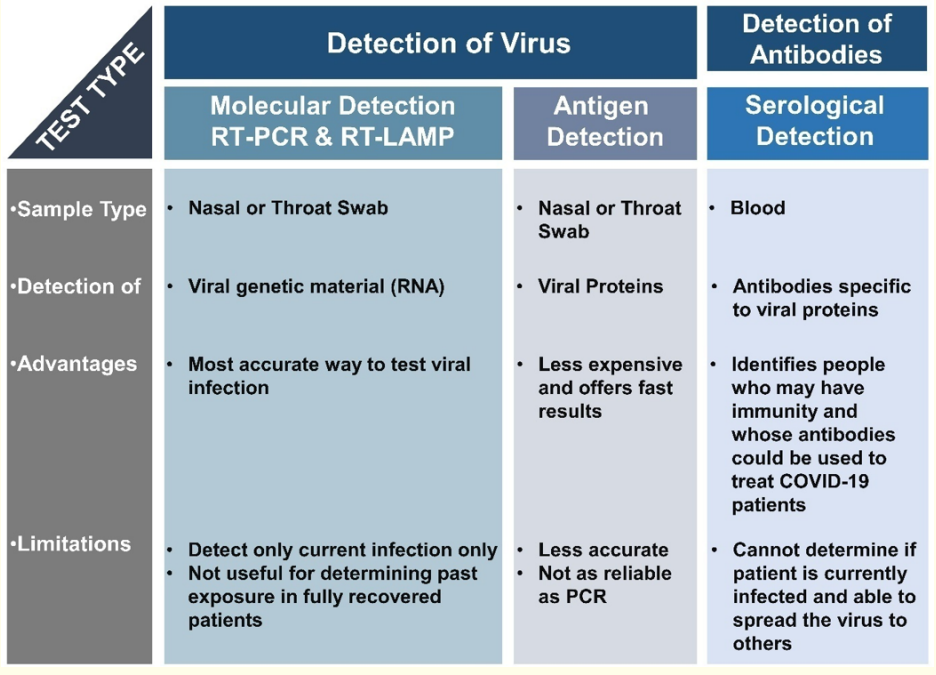

Figure 1: Main methods for the diagnosis of SARS-CoV-2 viral infection.

Sr. No	Name of the Kit	Developer
	TRUPCR SARS-CoV-2 RT-qPCR Kit	3B Black Bio Biotech India Ltd.
	InnoDetectTM CL COVID-19 Iso-	AB Diagnopath Manufacturing Pvt. Ltd.
	InnoDetect Covid-19 RT-PCR Kit	
	Accurex SARS COVID-19 RT-PCR	Accurex Biomedical Pvt Ltd.
	Novel Coronavirus (2019-nCoV) Nucleic Acid Detection Kit	
	GenePathDxCoviSpeedy	Achira labs Pvt Ltd.
	GENEPATH DX CoviQuick v2.0	
	Gene path DxCoVi Delta RT-qPCR	
	LyteStar 2019-nCoV RT-PCR Kit 2.0	ADT India Ltd.
	LyteStar 2019-nCoV RT PCR Kit 1.0	
	Holborn Wells Detect SARS-CoV-2	AffigenixBiosolutionsPvt. Ltd.
	RT-QPCR TEST Kit	
	AFFIGENIX COVID 19 TEST (ACT-	
	AffigenixCovid 19 test (ACT) kit	
	PATHOFIND COVID-19 Realtime	Ajay Bio-Tech (India) Ltd.
	COVID-19, ONE STEP MULTIPLEX	Alchem Diagnostics
	Ammagen RT-PCR Kit	Ammagenomics Medical Labs
	ANGPCR 2019-nCoV	Angstrom Biotech Pvt. Ltd.
	Aspen SARS-CoV-2 Nucelic acid detection kit (PCR- Fluorescent probe	Aspen Laboratories Pvt. Ltd.
	Aridia CoVid-19 Real Time PCR	Athenese-Dx Private Ltd.

	Aura's CORONAVIRUS (COVID-19)	Aura Biotechnologies Private Ltd.
	AvienCovi Quick Covid-19 Hid	AvienceBiomedicals Pvt Ltd
	AvienBio COVID-19 RT PCR Assay	
	One-Step RT-PCR Test for Covid-19	AxivaSichem Biotech
	GENEASY COVID-19 RT-PCR Kit	Bhat Biotech India Pvt Ltd
	COVID-19 TRIPLEX RT-PCR	Biogenix INC Pvt Ltd.
	Biogenix Covid-19 one step RT PCR	
	BIO COVID ID/COVID-19 qualitative	BioGenomics Limited
	BioCov-iD ERN Corona Virus Single	
	BioSci True Detect COVID-19 Real	Biosci Healthcare
	ConceptaCoViDx RT-qPCR Kit	BioSystems Diagnostics Private Limited
	CoviPlus RT-qPCR Kit	
	Concepta COVID-19 Multiplex RT	
	Bio Bee COVID19 Detection Kit	Bogar Bio Bee Stores Pvt Ltd.,
	Q Sens 2019-nCoV Detection Kit	Cancer Rop Co. Ltd (Anjanajyoti
	SARAGENE COVID-19 V.2 Real	CoSaraDiagnosticsPvt. Limited,
	SARAGENETM Corona Virus	
	DX-CoV-2 RT-PCR kit	Diasolex Solutions LLP
	XpertCovido 19-Fast RT-PCR kit	DNA XpertPvt. Ltd.
	Xperts Covido19 Multiplex RT-PCR	
	Global COVID 19 Kit	Equine Biotech
	DiagSure nCOV-19 Detection assay	GCC Biotech India Private Limited
	GenePathCoViDx One RT-qPCR	Gene Path Diagnostics
	Genes2me CoV Flu one step RT PCR	Genes 2 Me Private Limited
	Genes2Me RT-DIRECT Multiplex	
	COVIDtect Multiplex RT-PCR Kit for	
	VIRALDTECT II Multiplex real time	
	VIRALDTECT Multiplex Real-Time	
	Detection Direct 2 Gene SARS-CoV-2	Genestore India Private Limited
	DETECTION EXPERT SARS COV -	
	Detection Expert SARS-CoV-2rRT-	
	CoviNAT-Novel Coronavirus (SARS-	Genetix Biotech Asia Pvt Ltd,
	COVI-quick COVID-19 Real Time	
	COVISure (Lyo Mix) COVID19 Real	
	COVISure (version 2.0) COVID19	
	COVISure COVID-19 Real Time PCR	
	CoviSure Media Direct Extraction	
	GenosensnCOV 2019 Real time PCR	Genome Diagnostics Pvt. Ltd.
	GenoCoV	Genores Biotech Pvt Ltd.
	RT PCR SARS-CoV-2	Gland Pharma Ltd.
	Helini Coronavirus Real-Time PCR kit	Helini Biomolecules,
	Helini Coronavirus [COVID-19] Real	

	Hi-PCR® Covid-19 Detection Kit	HiMedia Laboratories Private Limited
	Hi-PCR COVID-19 Triplex Probe	
	Hi-PCR® Coronavirus (COVID-19)	
	Quantiplus Multiplex COVID-19	HuwelLifesciencesPvt. Ltd.
	QuantiplusCoV detection KIT Ver 2.0	
	Covid 19 Probe-free Real Time PCR	Indian Institute of Technology
	RT PCR Kit SARS CoV2	InnoDx Solutions Pvt. Ltd
	InnoDetect COVID-19 RT-PCR kit	
	identi SARS CoV-2 G2 RT PCR KIT	Jeev Diagnostics Pvt. Ltd
	JITM COVID-19 Detection Kit	JITM Skills Private Limited
	Karwa SARS-CoV-2 Nucleic Acid	Karwa Enterprises Private Limited
	TRUPCR SARS-CoV-2RT-qPCR kit	KILPEST (3B BlackBio Biotech India
	Accucare COVID-19 One-step RT-PCR	Lab Care Diagnostics (India) Pvt. Ltd.
	LS COVID-19 RT-qPCR kit	Lifespan Biotech Pvt Ltd,
	LS COVID-19 RT-qPCR Multiplex	
	Lipomic COVID-19 multiplex	Lipomic Healthcare Pvt. Ltd.
	Art Test COVID-19 kit	LLC Art Biotech
	MJ Coronavirus (COVID-19) RT-	M.J.BiopharmPvt. Ltd
	IMDX SARS-CoV-2 Multiplex	M/s Siemens Healthcare Private
	MagGenome COVID-19 single Tube	MagGenome Technologies Pvt. Ltd
	Genome Analyst SARS-CoV-2 detection kit	Med Achievers Private Limited, Noida
	COVID-19 RT-PCR kit	Medsource Ozone Biomedicals,
	OZOFIND SARS COV2 RT PCR KIT	
	Meril COVID-19 One-step RT-PCR Kit	Meril Diagnostics Pvt Ltd.
	One-Step COVID-19 RT-PCR Kit	
	Pathkits MDS COVID Detect RTPCR	MetaDesign Solutions Pvt Ltd.
	Veri-Q COVID-19 Multiplex	Mico Biomed Co., Ltd
	CoviSwiftTM COVID-19 S Plus	Mylab Discovery Solutions Pvt. Ltd.
	Patho Detect	
	Pathodetect (Two tube assay)	
	Pathodetect Coronavirus (COVID-19)	
	TruemixCovid 19	
	CoviDx Assure SARS-CoV-2 RT-PCR	NeoDx Biotech Labs Private Limited
	CoviDx Detect SARS-CoV-2 RT-PCR	
	CoviDX Direct Plex SARS-CoV-2	
	CoviDx mPlex-3NC SARS-CoV-2	
	CoviDxTM mPlex-3NR SARS-CoV-2	
	CoviDx mPlex-4R SARS-CoV-2 RT-PCR	
	COVSCAN-RT-qPCR Kit	NextGenInvitro Diagnostics Pvt
	SARS CoV-2 Triplex PCR Kit	Nucleus Diagnosys LLP
	COVID-19 RT-PCR Kit	Oscar Medicare Pvt. Ltd.
	Q-Line Molecular ER nCOV-19 RT-	POCT Services Private Limited
	Q-line Molecular Coronavirus	POCT Services Pvt. Limited
	POLYMED Q 2019 nCoV RT PCR	Poly Medicure Limited

	ProPCR COVID-19 RT-qPCR	Promea Therapeutics
	'R-Green Kit (SARS CoV-2 realtime	Reliance Industries Limited
	R-Green PRO-one: Taqman based	
	ViroDetect COVID-19 Real-Time	Shambhav Medical
	SN CoV Ensure One Step Multiplex	Shankaranarayana Life Sciences LLP
	MP RT-PCR Kit – Real Time PCR Kit	Sidak Life Care Pvt Ltd.
	IMDX SARS-CoV-2 Multiplex E/N	Siemens Healthcare Private Limited
	Chitra SARS CoV2 Multiplex RT-	SreeChitraTirunal Institute for Medical
	Stellence Covid-19 RT-qPCR Kit	StellencePharmsciences Pvt Ltd
	TATA MD CHECK RT-PCR XF	Tata Medical and Diagnostics Ltd
	TATA MD CHECK RT-PCR 3 GENE	
	TATA MD CHECK RT-PCR FAST	
	TATA MD CHECK RT-PCR FAST 3	
	TATA MD CHECK RT-PCR FLEX	
	Covi-Detect -2 Real Time PCR	TCM Limited
	GEN-COV Corona Virus Single Tube	Theragen Biologics Private Limited
	ErbaMDx COVID-19 RT PCR Kit	Transasia Diagnostics Solutions
	TrueScreen COVID-19 Multiplex Real	TranScience Innovative Technologies
	COVIDsure Multiplex Realtime RT-	Trivitron healthcare Pvt Ltd
	Covidsure Pro Multiplex RT-PCR kit	
	COVIDsure Direct Multiplex RT PCR	
	AMPLICHAIN SARS-CoV-2	Tulip mDiagnostics (P) Ltd.
	ViraGEN Molecular Diagnostics	uBio Biotechnology Systems Private
	SARS-CoV-2 Fluorescent PCR	VimekBioconcept Pvt Ltd
	CovTect COVID-19 Qualitative PCR	Vitane Biologics Pvt Ltd
	VITROMED Q 2019 nCoV RT PCR	Vitromed Healthcare
	SARS COV-2 qPCR Multiplex Kit	Yaathum Biotech Private Limited
	CoviGene COVID-19 RT-PCR,	Yashraj Biotechnology Limited

Table 1: List of ICMR approved RT-PCR kits for detection of SARS-CoV-2 developed by Indian companies.

a micro-centrifuge tube and then mixed with a lysis buffer. After lysis, a purification procedure is carried out by using a spin column based solid phase extraction method, in which the stationary phase consists of a silica matrix. Under optimal salt and pH conditions, RNA molecules bind to the silica gel membrane, while protein and other contaminants are not retained. The elution buffer is used to remove the viral RNA from the spin column at the last step of the procedure and a purified RNA. The next step is a preparation of the reaction mixture for PCR amplification, using a master mix which is a premixed concentrated solution of buffer, reverse transcriptase enzyme, nucleotides, forward primer, reverse primer, TaqMan probe, and DNA polymerase. Finally, to complete this reaction mixture, the RNA template is added, and the tube is mixed by pulse-vortexing. The reaction mixture is loaded into a PCR plate, the plate is placed in a PCR machine. Detection of SARS-CoV-2 using RT-PCR is by amplification of target sequences present in the RdRP gene, E gene, and N gene. Among the listed kits (Table 1), majority of kits developed based on the detection of either RdRP gene along with E gene or RdRP gene along with N gene of SARS-CoV-2, while limited number of kits were developed based on the detection of ORF1ab, S gene and N gene of the virus. However, all the listed kits use RNaseP gene as an internal control due to its presence in the human genome, and it is easily detectable. These assays can be carried out in a single step or in two steps separately. The one-step method is quicker and easier to use, however the two-step method is more

sensitive due to better target amplification. The RT-PCR initiates with reverse transcription, which forms cDNA that is primed with the PCR reverse primer and hybridizes to a complementary part of the virus RNA genome. Reverse transcriptase enzyme adds DNA nucleotides onto the 3' prime end of the primer for synthesizing complementary DNA to the viral RNA. The temperature as well as duration of this step depend on the primer, the target RNA and the reverse transcriptase used in the reaction. Next, an initial denaturation step is applied, causing denaturation of the RNA-DNA hybrids, which is required for the activation of DNA polymerase and a simultaneous inactivation of reverse transcriptase. PCR consists of a series of thermal cycles, with each cycle consisting of denaturation, annealing, and extension steps. Denaturation step consists of heating the reaction chamber to 95°C for denaturation of the double-stranded DNA template. In the next step, the reaction temperature is lowered to 55-65°C that allows annealing of the forward primer to its complementary part of the single-stranded DNA template. The annealing temperature relies directly on length and composition of the primers. In the extension step, the DNA polymerase synthesizes a new DNA strand complementary to the cDNA template strand by adding free nucleotides from the reaction mixture that in the 5' to 3' direction. After the first cycle, the double-stranded DNA target is obtained and the denaturation of this DNA yield in two single-stranded DNA molecules. The reaction temperature is again lowered to facilitate annealing of primers and TaqMan probe. The primers anneal to each of the single-stranded DNA templates while, TaqMan probe anneals the complementary part of the target DNA. TaqMan probe consists of a fluorophore covalently attached to the 5' end of the oligonucleotide probe which emits when it is excited by the light source of thermal cycler. Also, this probe consists of a quencher at the 3' end and a close proximity of reporter to the quencher prevents detection of its fluorescence. In the extension step, DNA polymerase synthesizes new strands and when the polymerase reaches a TaqMan probe, its endogenous 5' nuclease activity cleaves the probe to separate the dye from the quencher. With each cycle of PCR, more dye molecules are released, resulting in an increased fluorescence intensity that is proportional to the amount of amplicon synthesized. This method estimates the amount of a given sequence present in a sample. The number of double stranded DNA pieces is doubled in each cycle therefore, PCR can be used to analyze extremely small amounts of sample.

For the measurement of the fluorescence signal, a tungsten-halogen lamp, an excitation filter, mirrors, lens, an emission filter, and a charge-coupled device (CCD) camera are used. The fluorescent light emitted from the wells reflects off the mirror, passes through an emission filter and is detected by the CCD camera. The detected fluorescence light of each PCR cycle is converted into a digital data by the CCD camera. As this method monitors the progress of the PCR reaction in real time, it is known as real time PCR and routinely used in laboratories for detection of various viruses including SARS-CoV-2 [14].

Mutation Specific changes in SARS-CoV2 have an impact on the various properties of virus such as how easily it spreads, severity of the associate pathological conditions, or the effectiveness of the vaccine and therapeutic medicines. Since its first emergence in late 2019, several SARS-CoV-2 variants have been detected all around the world using NGS technology. Alpha variant (B.1.1.7 and Q lineages), Beta variant (B.1.351 and descendant lineages), Gamma variant (P.1, which is a descendant of B.1.1.28, and descendant lineages), Delta variant (B.1.617.2 and AY lineages), and Omicron variant (B.1.1.529 and BA lineages) are the PANGO (Phylogenetic Assignment of Named Global Outbreak) lineages correlated to these VOCs.

The major drawback of this gold standard technique (RT-PCR) is that it can only detect specific gene sequences, which are extremely short partial pieces of the viral genome that are amplified in the reaction and cannot be helpful to distinguish various variants of SARS-CoV-2. However, numerous kits were developed based on the RT-PCR assay for the detection of specific variants of SARS-CoV-2 (Table 2). TATA MD CHECK RT-PCR-OmiSure and KIRIDA Novus SARS-CoV-2 Omicron detection kit are approved by ICMR for the detection of Omicron variant of the virus. Moreover, Gene path Dx-CoVi Delta RT-qPCR kit was developed for the detection of Delta variant of SARS-CoV-2 and is now approved by ICMR for the diagnostic use. When it comes to assessing this deadly virus, there is a growing need to create new kits for detecting distinct variants, which will aid in the tracking of viral infection.

RT-LAMP based detection of SARS-CoV-2

Various nucleic acid detection technologies are developed in recent years that provide quick diagnostic findings which based on diverse technological platforms to identify SARS-CoV-2. However, in comparison to RT-qPCR, these recently developed technologies have relatively poor diagnostic performance. The loop mediated isothermal amplification (LAMP) invented in 2000 [12], which is one of these approaches that enables quick and efficient gene detection [17]. LAMP has now been routinely used to identify a va-

Sr. No	Name of the Kit	Developer
	Gene path DxCoVi Delta RT-qPCR Kit v ME-2	Achira Labs Pvt. Ltd., Bengaluru, Karnataka
	TATA MD CHECK RT-PCR-OmiSure	TATA Medical and Diagnostics Ltd., Mumbai (Maharashtra), India
	KIRIDA Novus SARS-CoV-2 Real-Time PCR kit- Omicron detection kit	Kriya Medical Technologies Pvt. Ltd.

Table 2: List of ICMR approved RT-PCR kits for detection of specific variants of SARS-CoV-2 developed by Indian companies.

riety of diseases, including malaria, salmonella, influenza virus, dengue virus, Chikungunya virus and Zika virus [7].

Due to the high specificity, sensitivity, cost-effectiveness, and quick turnaround time, RT-LAMP is a potent alternative of RT-PCR [1]. The very first step in RT-LAMP is the sample collection and viral RNA isolation, same as the RT-PCR technique. In RT-LAMP reaction, there are set of total six primers comprising two outer, two inner, and two loop primers. As mentioned earlier, RT-LAMP does not follow higher temperature step for the denaturation process rather, it works on single temperature where two outer primers (forward outer and backward outer primer) are used for the strand displacement. The inner primers are specific to the genes, one for the sense and other is for antisense strand of the target gene. Last two primers are the loop primers (loop F and loop B) which quicken the amplification procedure. To carry out the reaction, the mixture of isolated pure RNA with all primers, buffer and enzymes is incubated at 60-65°C (varies based on manufacturer) for 60 minutes in heating block and then reaction is stopped by heating for 2 minutes at 80°C temperature. As the amplification of the genetic material is carried out at constant temperature, detection of the virus can be done anywhere without need of a specific instrument such as thermo cycler. LAMP colorimetric approaches detect turbidity due to accumulation of magnesium pyrophosphate or complex metric indicators, pH sensitive dyes, or DNA-intercalating dyes that are introduced into the reaction to give colour changes. Moreover, real time monitoring for the RT-LAMP amplification is performed by recording the optical density at 400nm at every 6 second in Loop-amp real-time turbid meter. Among the discussed approaches, colorimetric RT-LAMP tests are very appealing for point-of-care (POC) usage and deployment in low-resource settings due to their easy technical and instrumentation specifications [10]. Recently, many Indian companies have developed RT-LAMP assay for the detection of the SARS-CoV-2 which shows effectively results. The list of ICMR approved RT-LAMP kits, developed by Indian companies are listed in Table 3.

Antigen based testing detection of SARS-CoV-2

Sr. No	Name of the Kit	Developer
	COVIQWIK SARS-CoV-2 RT-LAMP	Acrannolife Genomics Pvt. Ltd.
	Direct Covid 19 LAMP Test Kit	Agappe Diagnostics Ltd.
	Agappe Real Time RT – LAMP	
	Karwa COVID-19 RT LAMP	Karwa Enterprises Private Limited
	SaralTest SARS-CoV-2-RT LAMP Kit	QRX Private Limited

Table 3: List of ICMR approved RT-LAMP kits for detection of SARS COVID-19 developed by Indian companies.

The rapid antigen test detects the presence of SARS-CoV-2 surface protein (antigen) in the upper respiratory tract of a patient using specific antibodies. This test is especially beneficial to identify a high level of viral infection. It is based on the principle of antigen antibody binding. Binding of antibody to antigen helps in deactivating or eliminating them from our body sometimes with the help of immune cells. Most rapid antigen kits target SARS-CoV-2nucleocapsid antigens using specifically developed monoclonal antibodies with specific epitope of nucleocapsid antigen. ICMR has approved 59 rapid test antigen kits for detection of SARS-CoV-2 developed by Indian companies, as listed in Table 3. Among 59 testing kits, nine kits are approved as a self-test kit.

A nasopharyngeal swab is taken from the suspect or patient and mixed with the buffer supplied with the kit to liberate viral antigens into the solution. In the next step, few drops of this sample solution are applied to the sample well of the detection chip. The chip is especially designed and contain key components or the sections such as conjugation pad, mobile gold-antibody, fixed antibodies for viral antigen and fixed antibodies for non-target antigen. The

sample is loaded on "s" (sample) point, and it moves to the conjugation pad where antibodies against the novel coronavirus antigen are present. At the "c" (control) point, test antibodies for non-target antigens normally present in the pharynx region are present. These antibodies are considered as control which are free to move through capillary action and conjugated with the gold nanoparticles helping in the visualization. The samples move laterally by capillary action towards conjugation pad and bind to their specific antibodies at point "t" (test). At these points if the antigens corresponding to the antibodies represent, they form a sandwich with antigens in between and appear as coloured lines. If both the test line and control line appear, it means the test is successful and patient is positive for novel coronavirus, while if only control line appears it means the run is successful and the patient is negative. As the rapid antigen test does not detect very low viral load, negative result needs to be further verified by RT-PCR test. Furthermore, if only the test line or no line appears, it suggests that the run is faulty, and the results are invalid, and the test needs to be repeated.

Role of NGS in detection of SARS-CoV-2

In the era of advance genomics and computational facility, researcher utilizes NGS not only to detect the virus but also to provide the genomic sequences [8]. Utilization of NGS technology for the identification of communicable diseases vows an objective stance that does not rely on culturing of pathogens [5]. NGS has already been documented in different case reports and previous study to recognize pathogenic organisms in samples collected from the respiratory system, nervous system, gastrointestinal system, and the eyes. The outbreak of the different variant of SARS-CoV-2 pushed many researchers to work hard and they are still working on new strategies to identify new variants. For the sequencing of SARS-CoV-2, three NGS techniques—Illumina, Ion torrent, and Nanopore WGS assay—are used. Figure 2 illustrates the key details for the mentioned three methods.

In context of variants of SARS-CoV-2, infection is not substantial for clinicians as the management around those patients regardless of whether they are infected with delta, alpha or omicron variant is

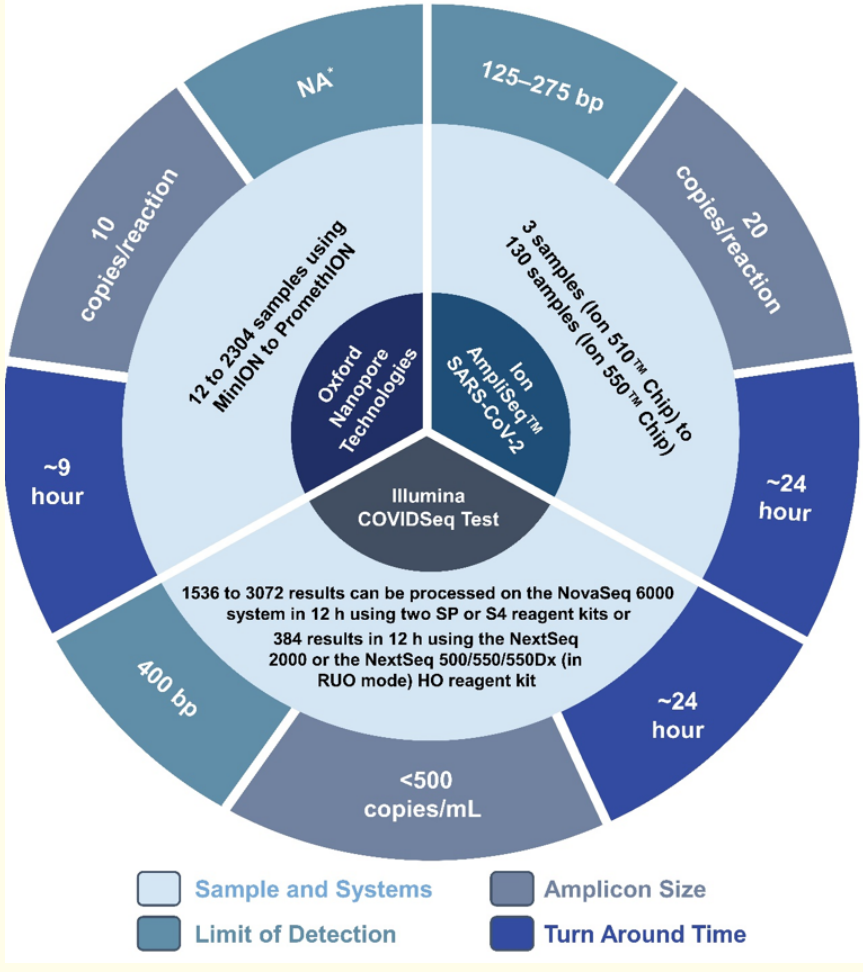

Figure 2: Graphical illustration of the key details for the COVID-19 sequencing methods (NGS).

usually going to be the same. But it is important for authorities to know the strains of virus entering into our communities, so that they can start to predict what the transmission is going to look like, how rapidly people might get infected and to be able to gauge things like a vaccination. At this point of time, sequencing comes into play that not only avails the knowledge of different strains but also creates a foundation for generation of effective therapies. Sequencing is not used so much as a diagnostic test, but it is used to help in verifying what type of SARS-CoV-2 variant is causing infectious in a population to track from an epidemiological perspective. Multi Institutional project which includes seven major institutes of India carried out sequencing of 752 samples through COVIDSeq protocol to develop high throughput detection of SARS-CoV-2. As per this research, COVIDSeq may be a high sensitivity test for SARS-CoV-2 detection with the added benefit of allowing genetic epidemiology of SARS-CoV-2 [2].

In Australia, agent-based modelling and genomic surveillance were found to be an effective combination for evaluating and preventing COVID-19 transmission [15]. A few freely accessible databases have also been created in support of these initiatives, such as the GISAID, which enables the open and quick exchange of SARS-CoV-2 genomic sequences. Among the South Asian countries, India plays major role in the sequencing of SARS-CoV-2 genome. India has the capacity to sequence 10,000 samples a week. The Indian SARS-CoV-2 Genomics Consortium (INSACOG) is a major example of this, which is a consortium of 38 government laboratory in the different states of India. Moreover, six private laboratories are also included in the 2021 to increase the number of sequencings of SARS-CoV-2 genome around India [19]. The National Centre for Disease Control (NCDC), Delhi is facilitating INSACOG, a multi-laboratory, multi-agency, Pan-India connection to observe genomic variants in the SARS-CoV-2 through a sentinel sequencing effort, which incorporates the Central Surveillance Unit (CSU) under the Integrated Disease Surveillance Program (IDSP). The consortium has created a powerful connection among clinical outcomes and genome sequencing. The INSACOG portal has results of genome sequencing updated on a real-time basis for everyone to see and use as they deem fit, which will be open for common public. The aim behind opening the public portal is not just to maintain transparency about variants in circulation, but also to prevent any kind of misinformation being spread about disease transmission.

For instance, study conducted in early 2020 showed that the spike (S) protein receptor binding domain (S1) had a structure that was notably identical to the earlier SARS-CoV virus, suggesting that the protein could probably engage the ACE-2 receptor [11]. Remarkably, based on the SARS-CoV-2 sequencing data from the same year, it was discovered that the S-protein amino acid includes a furin-like cleavage site and is responsible for effective transmission of the virus to humans. India has a remarkable contribution to the sequencing-based research. The furin cleavage site of spike (S) protein and the furin cleavage site on human epithelial sodium channel alpha subunit (ENaC-a) are similar, according to computational analyses performed by Anand and colleagues on 10,967 SARS-CoV-2 genomes that were made available in the public GISAID database [16]. Researchers from India, Yadav., *et al*. sequenced three early SARS-CoV-2 positive samples detected by RT-PCR testing in February 2020 using the Illumina MiniSeq technology. Using a variety of software tools that predict amino acid structures based on sequencing data, they used this data to discover anticipated linear and conformational B-cell epitopes as well as T-cell epitopes [22]. These epitopes are great candidates for development of antigen based diagnostic kit, vaccine development and need further research.

Conclusion and Future Aspects

Among all different obstacles humans have faced, COVID-19 is undoubtedly one of the most dangerous phases. A fight against this deadly virus is globally and researchers as first line defenders have played major role in not only detection of the virus but also in discovering new effective treatments. India is risen as a strong defender among Asian countries with accelerated diagnostic methods of the virus. NGS has played a key role in the situation by availing sequences of different strains of the deadly virus. The availability of sequence helps to generate new primers for sensitive diagnostic methods like RT-PCR and RT-LAMP techniques as well as serves enough knowledge of protein for development of rapid test kits. India has 137 ICMR approved RT-PCR kits which also includes identification of specific variant including delta and omicron. With 5 RT-LAMP kits and 59 antigen detecting kits, India is ahead of many countries in terms of COVID diagnosis. But, as the virus is mutating rapidly, new sequencing data and diagnosis kits for specific variants are urgent need. Several diagnostic kits (Table 5) including RT-LAMP, RT-PCR and antigen detection are under research which would be helpful in near future. A constantly gained knowledge of various variants helps not only in development of diagnostic techniques but also to understand the severity of the variant that can

lead to take early steps before the situation may worsen. The presented review gives a fair scenario of COVID diagnosis in India in late 2022 and represents the gaps in research that need to be filled.

Funding

This research received no external funding.

Sr. No	Name of the kit	Developer	Type of sample
	Accucare COVID-19 Antigen Lateral Test Device	LabCare Diagnostics Ltd., Valsad, Gujarat	Oropharyngeal and Nasopharyngeal swabs
	BIOCARD Pro COVID-19	Trivitron Healthcare Pvt. Ltd., Chennai, Tamil Nadu	Nasopharyngeal swab
	Sure status COVID-19 Ag Test (CIPtest COVID-19 Antigen Card Test)	Premier Medical Corporation, Valsad, Gujarat	Nasopharyngeal swab
	Angcard COVID-19	Angstrom Biotech Pvt. Ltd., Alwar, Rajasthan	Nasopharyngeal and Oropharyngeal swabs
	SENSIT Rapid COVID-19 Ag kit	Ubio Biotechnology Systems Pvt. Ltd., Kochin, Kerala	Nasopharyngeal swab
	COVID-19 Antigen Detection Test	Meril Diagnostics, Vapi, Gujarat	Nasopharyngeal swab
	Alpine COVID-19	Alpine Biomedicals Pvt. Ltd., Ambala (Haryana),India	Nasopharyngeal swab
	Oscar CORONA Rapid Ag Test kit	Oscar Medicare Pvt. Ltd., Delhi, India	Nasopharyngeal swab
	ImmunoQuick COVID-19	ImmunoScience India Pvt. Ltd., Pune, Maharshtra	Nasopharyngeal swab
	iNSTAXPLOR™ COVID-19	STRUmed Solutions Pvt. Ltd., Chennai, Tamil Nadu	Nasopharyngeal swab
	EzDx COVID-19 Rapid Ag Test	ADVY Chemical Pvt. Ltd., Thane, Maharashtra	Nasopharyngeal swab
	$Vitros SARS-CoV-2 Ag Test CLIA Kit Vitros 3600	Ortho Clinical Diagnostics, Mumbai, Maharashtra	Nasopharyngeal swab in VTM
	SAIC-19 Ag Kit	M/S Lorven Biologics Pvt. Ltd., Telangana	Nasopharyngeal swab
	FutureCare COVID-19	MedzomeLifesciences Pvt. Ltd., Solan, Himachal Pradesh	Nasopharyngeal swab
	Pathkits SIMPLE COVID-19	Pathkits Healthcare Pvt. Ltd., Gurugram, Haryana	Nasopharyngeal swab
	MyTest COVID-19 Ag Test	Biofootprints Healthcare Pvt. Ltd.	Nasopharyngeal swab
	CoviRAT COVID-19	Zephyr Biomedicals (Tulip Diagnostics) Goa	Nasopharyngeal swab
	Covid-19 Antigen Detection Test (FIA)	Meril Diagnostics Pvt. Ltd., Vapi (Gujrat), India	Nasopharyngeal swab
	One Step novel corona virus (COVID-19)	SidakLifecare Pvt. Ltd., Jhajjar, Haryana	Nasopharyngeal swab
	Xamin Covid-19 Ag Rapid Test Device	Diagnocure (INDIA), Solan, Himachal Pradesh	Nasopharyngeal swab

	DSI COVID-19	Dia Sure Immunodiagnostics LLP, Delhi, India	Nasopharyngeal swab
	Rapid Covid 19 Ag kit	Biolab Diagnostics India Pvt. Ltd., Mumbai, Maharashtra	Nasopharyngeal swab
	COVSCAN SARS-Co V-2 Antigen Detection kit	NextGen In Vitro Diagnostics (P.) Limited., Faridabad, Haryana	Nasopharyngeal swab
	COVID-19 Antigen Card Test	Oscar Medicare Pvt. Ltd., Haridwar, Uttarakhand	Nasopharyngeal swab
	AG-Q COVID-19 N	Agappe Diagnostic Pvt. Ltd., Kerala	Nasopharyngeal swab
	TRUSTline COVID-19	Athenese-Dx Private Limited, Chennai, Tamil Nadu	Nasopharyngeal swab
	YBIO Rapid Test	YuvRajBiobiz Incubator India Pvt. Ltd.	Nasopharyngeal swab
	One step COVID-19 (Cov-Ant)	PatanjaliPharma Pvt. Ltd., (IIT Mumbai), India	Nasopharyngeal swab
	COVID-19 Antigen Card	Diagnostic Enterprises Parwanoo, Himachal Pradesh	Nasopharyngeal swab
	Nulife COVID-19	NuLifecare, Noida, Uttar Pradesh	Nasopharyngeal swab
	ELISA based COVID- 19 MICROLISA SARS-CoV-2 detection kit	J. Mitra& Co. Ltd., New Delhi	Nasopharyngeal swab in VTM
	COVID-19 Antigen Rapid Test	Cadila Healthcare Pvt. Ltd., Ahmedabad, Gujarat	Nasopharyngeal swab
	INSTA COVID-19/ Ag One Step SARS-CoV-2	Seloi Healthcare Pvt. Ltd, Mumbai, Maharashtra	Nasopharyngeal swab
	InstaCOVID Ag kit	QAWACH Bio Pvt. Ltd., Mumbai, Maharashtra	Nasopharyngeal swab
	ErbaQik COVID-19	Transasia Bio-Medical Ltd.	Nasopharyngeal swab
	RapiCov rapid	Genes2Me Pvt. Ltd., Gurugram, Haryana	Nasopharyngeal swab
	PolyMed COVID-19	Poly Medicure Ltd., Faridabad, Haryana	Throat swab
	LordMed COVID-19	Lord's Mark Industries Pvt. Ltd., Mumbai, Maharashtra	Nasopharyngeal swab
	SARS-CoV2 rapid Test Kit	KoshBio Pvt. Ltd., Faridabad, Haryana	Nasopharyngeal swab
	Gazelle PathoCatch COVID-19 (FIA) test kit	Mylab Discovery Solutions Ltd., Pune, Maharashtra	Nasopharyngeal swab
	COVID-19 Ag Card Test- ver. 2	J. Mitra& Co. Ltd., New Delhi	Nasopharyngeal swab
	Elecsys SARS-CoV-2 Antigen Test-CLIA (Cobas e411)	Roche Diagnostics Pvt. Ltd., Maharashtra	Nasopharyngeal swab in VTM
	ImCOV-Ag (SARS-CoV2	IMGENEX India Pvt. Ltd., Bhubaneswar, Odisha	Nasopharyngeal swab
	COVID-19 Rapid Antigen Test kit	Recombigen Laboratory Pvt. Ltd., New Delhi	Nasopharyngeal swab

	TRURAPID Covid-19	Kilpest India Ltd., Bhopal Madhya Pradesh	Nasopharyngeal swab
	COVID-19 Rapid Antigen Test kit	Nucleus Diagnosys LLP., Ahmedabad, Gujarat	Nasopharyngeal swab
	BioSci True-Detect COVID-19	BIOSCI Healthcare, Bhopal Madhya Pradesh	Nasopharyngeal swab
	Edge Xpress COVID-19	Edge Pharma Pvt. Ltd., Mumbai Maharashtra	Nasopharyngeal swab
	COVID-19 Rapid Ag Test Card	Reckon Diagnostics Pvt. Ltd., Vadodara, Gujarat	Nasopharyngeal swab
	Artron COVID-19 Antigen Test	Nanz Med Science Pharma Pvt. Ltd., Sirmour, Himachal Pradesh	Nasopharyngeal swab
HOME TESTING KITS			
	CoviSelfTM (PathoCatch) COVID-19 OTC Antigen LF device	Mylab Discovery Solutions Ltd., Pune, Maharashtra	Nasal swab
	CoviFind COVID-19 Rapid Ag Self -Test	Meril Diagnostics Pvt. Ltd., Vapi, Gujarat	Nasal swab
	ULTRA Covi-Catch™ SARS-CoV-2 Home Test	S.D. Biosensor Healthcare Pvt. Ltd., Gurugram, Haryana	Nasal swab
	CoviSelfTM (PathoCatch) COVID-19 OTC Antigen LF device (Home Test kit)	Mylab Discovery Solutions Ltd., Pune, Maharashtra	Nasal swab
	AbCheck Rapid Antigen Self- Test - Nasal	Nulife Care, Noida, Uttar Pradesh	Nasal swab
	NeoCheck - Covid-19 (Home Test kit)	NeoDx Biotech Labs Pvt. Ltd., Bengaluru, Karnataka	Nasal swab
	CoviEasy COVID-19 Rapid Ag Self -Test kit	Genes2Me Pvt. Ltd.	Nasal swab
	SureStatus COVID-19 Ag Card test (Home Test) kit	Premier Medical Corporation, Valsad, Gujarat	Nasal swab
	NeoCheck - Covid-19 Rapid Antigen Self -test Kit	NeoDx Biotech Labs Pvt. Ltd., Bengaluru, Karnataka	Nasal swab

Table 4: List of ICMR approved Rapid test antigen kits for COVID-19 detection developed by India.

Sr. No	Name of test	Type of test	Target of test	Name of manufacturer
	Quick COVID-19 Colorimetric LAMP PCR	Manual NAT	RNA	Aura Biotechnologies Ltd., Chennai, Tamil Nadu
	Quick COVID-19 Realtime Multiplex PCR	Automated lab-based, near-POC NAT or POC NAT, Manual NAT	RNA	
	DeQuanto SARS CoV-2 RTqPCR Kit	Automated lab-based, near-POC NAT or POC NAT, Manual NAT	RNA	DenovoBiolabs Pvt Ltd, Bengaluru, Karnataka
	Quantiplus CORONA Virus (2019nCoV) detection kit	Manual NAT	RNA	HuwelLifesciencesPvt. Ltd., GandipetHyderabad
	@sight COVID-19 IgG/IgM Rapid Test Kit	Rapid diagnostic tests	Antibody	Mediclone Biotech Pvt Ltd, Chennai, Tamil Nadu

	nCoVSENSe: IgM/ IgG test for spike and N-protein of SARS CoV 2 (manual)	Manual or automated immunoassays	Antibody	Module Innovations Private Ltd., Pune, Maharashtra
	EyeRa-Covid	Manual or automated immunoassays, Rapid diagnostic tests	Antibody	Prantae Solutions Pte Ltd., Bhubaneswar, Odisha
	SureStatus COVID-19 (SARS-CoV-2) EIA Test	Manual or automated immunoassays	Antibody	Premier Medical Corporation Pvt. Ltd, Valsad, Gujarat
	SureStatus COVID-19 (SARS-CoV-2) Card Test	Rapid diagnostic tests	Antibody	
	Nasal Flock Swab	Not mentioned	Not mentioned	SVS Industries, Ahmedabad, Gujarat
	ViSure transport kit	Manual NAT	Not mentioned	Trivitron Healthcare Pvt. Ltd, Ahmedabad, Gujarat

Table 5: List of COVID-19 detection kits under research in India.

Acknowledgments

We thankful to the Molecular Biology Department of Unipath Speciality Laboratory Ltd, Ahmedabad, Gujarat, India for the providing this work facility and resources.

Conflicts of Interest

The authors declare no conflict of interest.

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