

## Diagnosis of COVID-19: An Evolution from Hospital based to Point of Care Testing

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### Abstract

The ongoing COVID-19 pandemic necessitates the development of a diagnostic method which could help in rapid and economical diagnosis of COVID-19 disease. The current problem with the diagnosis of the disease is that most tests are laboratory based and time consuming. Real Time PCR (RT-PCR) is the only gold standard method for diagnosis of COVID-19 disease till now. As soon as WHO declared COVID-19 as a pandemic, scientists immediately started development of rapid kits for fast detection of disease and point of care tests so that community transmission of disease can be controlled. The rapid antigen antibody and many other rapid tests were developed in response to community transmission, and efforts to develop improved alternatives are ongoing. In this article we have reviewed all the methods and strategies available till date.

**Keywords:** COVID-19; RT-PCR; Olfactory test; LAMP/POC method; CRISPR-Cas

**Abbreviations:** RT-PCR: Real Time Polymerase Chain Reaction; MERS: Middle East Respiratory Syndrome; SARS: Severe Acute Respiratory Syndrome; ACE2: Angiotensin Converting Enzyme 2; OP: Oropharyngeal; NP: Nasopharyngeal; BAL: Broncho Alveolar Lavage; PPE: Personal Protective; ICMR: Indian Council of Medical Research; ELISA: Enzyme Linked Immuno Sorbent Assay; VTM: Viral Transport Media; CSIR: Council of Scientific and Industrial Research; DAE: Department of Atomic Energy; WHO: World Health Organization; FDA: Food and Drug Administration; LAMP: Loop-mediated Isothermal Amplification; EUA: Emergency Use Authorization; PoC: Point of Care

### Introduction

Public health threats from corona viruses are constant and long-term because corona viruses have potential to adapt in environments through mutation and recombination [1-3]. Corona viruses are a large family of viruses that are known to cause infection ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) [4].

Corona Virus Disease 2019 (COVID-19) is a recently emerged infectious disease caused by novel corona virus. It is also called the severe acute respiratory syndrome corona virus

2 (SARS-CoV-2). It is an ongoing pandemic which originated from Wuhan City of Hubei Province of China and spread rapidly to the rest of the world [5]. In the early stages of the pandemic, the highest number of new cases and deaths were reported from China, rapid and exponential increases in cases were soon reported in South Korea, Italy, Iran, United States of America and almost all countries across the world.

As of 15<sup>th</sup> March 2021, 11,92,20,681 confirmed cases of COVID-19 and 26,42,826 deaths have been reported globally [6]. The reported number of cases and deceased are possibly an underestimate of the actual numbers due to limitations of the surveillance system and proper testing. The World Health Organization (WHO) has declared this disease as a public health emergency of international concern.

The SARS-CoV-2 is an enveloped non-segmented positive sense single-stranded RNA virus with genome size ranging from 26-32 kb in length [7]. This new virus belongs to the genus *Betacoronavirus* (subgenus *Sarbecovirus*, subfamily *Orthocoronavirinae*) [8-10]. The spike like projections on the viral surface results in a crown like appearance under the electron microscope [11]. It spreads by human-human transmission and can infect humans by binding to Angiotensin-Converting Enzyme 2 (ACE2), same as earlier reported. SARS-CoV-2 can affect all age groups and spreads through respiratory droplets and direct contact. It is transmitted through droplets which are generated during coughing or sneezing by symptomatic or asymptomatic infected patients [12]. Higher viral loads have been reported in the nasal

cavity as compared to the throat, with no difference in viral load between symptomatic and asymptomatic people [13]. However Zhang, et al. have reported the presence of SARS-CoV-2 in fecal swabs and blood. This suggests the possibility of transmission via multiple routes [14]. The incubation period of this virus varies from 2 to 14 days. The symptoms are usually fever, cough, sore throat, breathlessness, fatigue and malaise. Few patients may complain of gastrointestinal symptoms [15]. Older persons and those who have pre-existing medical conditions such as diabetes, hypertension, heart disease, lung disease, cancer or cardiovascular disease are at a high risk of developing serious illness.

The pandemic spread of SARS-CoV-2, and reports of community transmission in almost all countries has driven research not only focused on the development of treatment and preventive (vaccine) strategies but also the development of laboratory testing strategies which can be rapid and economical, and can be implemented in community as a point of care testing. The whole world is looking for authentic, economic and quick method so that testing can be implemented in community for prevention of rapid community spread. At present, Real Time PCR based testing method is the most common method used for of testing SARS-CoV-2 in almost all the countries. This test needs to be conducted in labs with BSL-2 or higher safety levels and modern molecular biology facilities. Furthermore, the test takes 4-8 hours to complete, including collection of sample, transport and also processing. This places a significant burden on testing facilities, and as a result, countries all over the world are struggling to meet testing demands.

Most countries are utilizing some type of clinical and epidemiologic information to determine who should have testing performed. However, reports of asymptomatic carriage as a source of transmission suggests the need to test as many as people in a country. At the very least, clusters within a community need to be identified for rapid detection of positive cases and to avoid further spread of disease. In the United States, criteria have been developed for Persons under Investigation (PUI) for COVID-19 [16]. According to the U.S. CDC, most patients with confirmed COVID-19 have developed fever and/or symptoms of acute respiratory illness (e.g., cough, difficulty breathing). If a person is a PUI, it is recommended that practitioners immediately put in place infection control and prevention measures. Initially, they recommend testing for all other sources of respiratory infection. Additionally, they recommend using epidemiologic factors to assist in decision making. Examples of epidemiologic factors that assist in the decision on who to test includes anyone who has had close contact with a patient with laboratory-confirmed COVID-19 within 14 days of symptom onset or a history of travel from affected geographic areas (presently China, Italy, Iran, Japan, and South Korea) within 14 days of symptom onset.

Both developing and developed countries are struggling to meet testing demands due to the lack of an available point of care test. More than 200 countries are effected and carrying out tests and there is huge variation in the number of tests are being conducted. In the early stages of the pandemic in India, only patients with specific symptoms of COVID-19 were being tested, however more recently community based sero surveys were also conducted [17].

Global reports of community spread underpin a need to have information on all available diagnostic tests across the world available. This review gives a brief insight of the tests already available and also tests that are in pipeline, based on the published/unpublished information of journals and also online information available till now. The public health prospective of all these tests are also discussed.

Diagnostic tests can be divided in to two broad categories, first the molecular based tests, which are more successful in hospitals and are time consuming and economically costly. The second category comprises antigen/antibody based tests which can be rapid, cost effective and most importantly may not require sophisticated instruments like Real time PCR. This second category of tests is more fitting to Public health specifically for Indian continents where there is a demand for rapid economic and bulk testing is. Here we discuss both the categories of testing currently available. Since test, track and treat is the only way to prevent spread of infection and save lives, it is imperative that testing should be made widely available to all symptomatic individuals in every part of the country. ICMR advises all concerned State Governments, Public and Private Institutions to take required steps to scale up testing for COVID-19 by deploying a combination of tests as advised above.

### **Real Time PCR Based Method**

Culturing of virus is not routinely performed due to lack of cell lines, time taken for getting result, labor-intensive and expertise requirements, and the lack of commercial antisera for culture confirmation. Molecular methods are widely used for diagnosis of COVID-19 disease. Among molecular methods, Real time PCR is considered the gold standard method and most is commonly used. This method which was originally discovered for diagnosis of corona virus is rapidly evolving based on research and application. Different countries have adapted this methodology for enhanced simplicity and cost effectiveness. For performing RT PCR method Oropharyngeal (OP) swabs were preferred than nasopharyngeal swabs in China during COVID-19 epidemic [18]. But US centre for disease control advice the use of Nasopharyngeal (NP) swabs. Collection of an OP specimen is not preferred, and, if collected, these samples should be combined in the same tube as the NP swab [19] (<https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html>. Accessed 16 March 2020).

Samples should be placed in a universal or viral transport medium. For the most sensitive detection of SARS-CoV, MERS-CoV, and SARS-CoV-2, the collection and testing of both upper and lower respiratory samples [sputum, Bronchoalveolar Lavage fluid (BAL)] is recommended [20]. However, the collection of sputum and particularly BAL via bronchoscopy increases biosafety risk to healthcare workers through the creation of aerosol droplets. Proper use of Personal Protective Equipment (PPE) by healthcare workers is important.

As far as PCR based tests are concerned, several RT-PCR protocols which are being carried out all over the world for detection of SARS-CoV-2 RNA have been posted by the World Health Organization [21].

As far India is concerned, ICMR has established a fast-track mechanism for validation of non-US FDA EUA/CE IVD approved kits at ICMR NIV Pune. As on April, 2020 ICMR NIV Pune has completed evaluation of 20 non-US FDA EUA/CE IVD kits (Table 1). Only test kits with 100% concordance among true positive and

true negative samples have been recommended for commercial use in India [22]. There are multiple PCR based methods available, and these have been well documented. Among all recommended kits few were developed in India. Examples of Indian kits are My lab patho detect kit, BIO COVID ID/ COVID-19 qualitative PCR detection kit v. 2 from Biogenomics India and also COVID-19 Probe-free Real Time PCR Diagnostic Kit designed by IIT Delhi. Most of the available kits require real time PCR and major evolution in research of PCR kit was focused on time of test. The use of RT PCR kits developed in India provides two advantages. One is in house production and second is time to carry out test. A kit was also developed by Banaras Hindu University [23] which showed promising results. However, there are limitations to the utility of PCR based tests in a public health setting where resources are scarce and there is a need for a point of care test. For public health settings till now only serological tests are successful. PCR based tests cannot be taken into the field of public health unless there is portable instrumentation available.

S. No.	Name of Company	Name of Kit	Concordance among true negative (%)	Concordance among true positive (%)
1	Altona Diagnostics	RealStar SARS-CoV-2 RT-PCR kit 1.0	100	100
2	My Lab	Patho Detect	100	100
3	BGI	Real Time Fluorescent RT-PCR kit for detecting 2019-nCoV	100	90
4	Krishgen BioSystem	SARS-CoV-2 Coronavirus Real Time RT-PCR (RT-qPCR) Detection kit v1	100	80
5	ABI	Taqman 2019-nCoV Control kit v1	100	90
6	HIMEDIA	Hi-PCR Corona virus (Covid-19) Probe PCR Kit	100	5
7	HUWEL	Quantiplus Coronavirus (2019nCov) detection kit	100	40
8	IIT-Delhi	SYBR Green based One Step QRT PCR KIT	98	10
9	KILPEST (BLACKBIO)	TRUPCR	100	75
10	Genesig	Coronavirus(Covid-19) Genesig Real Time PCR analysis	100	84
11	Roche	LightMix Modular SARS and Wuhan CoV E gene	91	100

12	Roche	LightMix Modular SARS and Wuhan CoV N gene	93	67
13	Roche	LightMix Modular Wuhan RdRp gene	100	60
14	Seegene	Allplex 2019-nCoV assay	100	100
15	SD Biosensor	nCoV Real Time Detection kit	100	100
16	AmpliGene India Biotech	AmpE2 Covid -19 using real time PCR machine	Further validation needed	Further validation needed
17	AmpliGene India Biotech	AmpE2 Covid-19 using Ampligene device	Further validation needed	Further validation needed
18	Biogenomics	COVID-19 detection kit	82	100
19	COSARA Diagnostics	SARAGENE Corona Virus (2019 NCV) test kit	88	100
20	KILPEST	TRUPCR SARS-CoV-2RT-qPCR kit version-2	100	100

**Table 1:** List of 20 non- US FDA EUA/CE IVD kits validated by ICMR.

\* <https://www.investindia.gov.in/team-india-blogs/commercial-kits-nasalthroat-swab-based-diagnosis-covid-19-india>

### Abbott Molecular Test to Detect Novel Corona virus

Abbott developed a rapid testing kit for detection of Coronavirus (ID NOW™) that was approved by the FDA in September, 2020 [24]. Using this kit positive results will be confirmed within 5 minutes and negative in 13 minutes. Molecular technology is used, which is valued by clinicians and the scientific community for its high degree of accuracy. ID NOW is already the most widely available molecular point-of-care testing platform in the U.S. today. This kit is portable and can be used outside hospital settings [25].

Therefore, although real time PCR is the most accurate test, Abbott molecular testing is a promising candidate for point of molecular methods which still needs to be established globally.

### Serological Tests (IgM/IgG)

Serological methods have provided a ray of hope for rapid and economic detection as these tests can be carried out in the field. However, molecular assays can directly detect the viral genetic material and are available for the diagnosis of acute infection, and there is a lack of serological assays suitable to specifically detect SARS-CoV-2 antibodies. A serological Enzyme-Linked Immuno Sorbent Assay (ELISA) was developed using recombinant antigens derived from the spike protein of SARS-CoV-2. This assay is sensitive and specific [26].

### Rapid Antigen Detection Test for COVID-19

Real time RT-PCR is the gold standard method for the

testing and diagnosis of COVID-19. There are various open and closed RT-PCR platforms (Open systems RT-PCR machines, TrueNat and CBNAAT) which are currently being used for diagnosis of COVID-19 in India. A limitation of these platforms is that they require specialized laboratory facilities in terms of equipment, biosafety and biosecurity. Minimum time taken for the test varies between 2-5 hours including the time taken for sample transportation. All these specifications limit the widespread use of the RT-PCR test. As these tests are time consuming, there is urgent need for a reliable point-of-care rapid antigen detection test with good sensitivity and specificity for early diagnosis of the disease.

No consistent antigen detection tests, which could be used as rapid point of care tests for quick detection of COVID-19 positive patients, are available. In view of this, an independent two site evaluation of the only available or stand-alone antigen detection assay available in India, Standard Q COVID-19 Ag detection kit was conducted with an aim to evaluate its sensitivity, specificity and feasibility of use as a point-of-care test for early detection of SARS-CoV-2.

The TrueNat system is a comprehensive assay for screening and confirmation of COVID-19 cases. Viral lysis buffer is used for collection of sample which minimizes the use of biosafety and biosecurity requirements. Three different types of TrueNat assays are currently available. Assay 1 includes TrueNat Beta CoV E gene Screening assay, followed by assay 2 which include TrueNat SARS-CoV-2 RdRp gene confirmatory assay. Assay 1 needs to be followed by Assay 2 for confirmation of the results. The third



assay is the TrueNat COVID-19 Multiplex assay which includes screening of both E gene and confirmatory Orf1a gene in a single test. This method can be used for testing all suspected COVID-19 cases. All negatives are to be considered as true negatives. All samples that test positive by this assay must be considered as true positives. The main advantage of this method is no further RT-PCR based confirmation is required for samples that are confirmed as true positives by the TrueNat assays [27,28].

### **Xpert Xpress SARS-CoV-2 Test**

The Xpert Xpress SARS-CoV-2 test is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized [29].

### **Standard Q COVID-19 Ag Detection**

The Q COVID-19 Ag detection kit is a chromatographic immunoassay based kit used for qualitative detection of specific antigens to SARS-CoV-2. This kit was developed by SD Biosensor, which is a South Korea based company, which has a manufacturing unit based in Manesar, Gurugram, India. This test kit comes with an inbuilt COVID antigen test device, viral extraction tube with viral lysis buffer and sterile swab for sample collection. Only a NP swab needs to be collected using the customized sample collection swab provided with the kit. No other sample (throat swab, bronchoalveolar lavage or sputum) should be used. After collection of sample, the swab should be immersed and squeezed in the viral extraction buffer. This buffer inactivates the virus thereby reducing biosafety and biosecurity requirements. Routinely used Viral Transport Media (VTM) is not recommended for sample transportation for this kit. Sample transferred in extraction buffer is stable only for one hour; therefore, the antigen test needs to be conducted at the site of sample collection in the healthcare setting. Transportation of the sample to the lab is not recommended. After transferring sample into the buffer, sample is mixed thoroughly, then the buffer tube cap needs to be replaced with a nozzle provided with the kit and 2-3 drops of the sample with buffer are put into the well of the test strip. As this test is qualitative the test result can be interpreted as positive or negative after 15 minutes of putting the sample into the well by appearance of test and control lines, which can be read with a naked eye, requiring no specialized equipment. Maximum duration for

interpreting a positive or negative test is 30 minutes. After that the test strip should be discarded. Recommended storage temperature for the test kit is 2° to 30° C [30,31].

### **CRISPR SARS-CoV-2 Test**

This test is a new diagnostic method for diagnosis of SARS-CoV-2 which is based on CRISPR-Cas9 technology, which is used to identifying and target SARS-CoV-2 genetic material. The test was developed by the Council of Scientific & Industrial Research (CSIR)-Institute of Genomics and Integrative Biology (IGIB), Delhi and has been validated by the Department of Atomic Energy (DAE) – National Center for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru. The test has been approved by DCGI for use in India. Testing is performed using a Thermal Cycler, as opposed to a qPCR machine, and which works by identifying the SARS-CoV-2 virus strain. No further confirmation by RT-PCR is required. BSL2 level is required for performing this test [32].

### **Pooled Samples for Molecular Testing of COVID-19**

As the number of COVID-19 cases is rising in India, it is necessary to increase the numbers of tests conducted by laboratories. Positivity rate in cases is still low. Hence, pooled samples may be helpful for screening. This involves the PCR screening of a specimen pool containing specimens from multiple patients, followed by individual testing (pool deconvolution) only if a pool screens positive. As all individual samples in a negative pool are regarded as negative, it results in significant increase in cost savings when a large proportion of pools test negative. This study was conducted at DHR/ICMR Virus Research & Diagnostic Laboratory (VRDL) at King George's Medical University (KGMU), Lucknow. It has been demonstrated that performing real-time PCR for COVID-19 by pooling 5 samples of TS/NS (200 ul/sample) is feasible when the prevalence rates of infection are low. All individual samples in a negative pool can be regarded as negative. Deconvoluted testing is recommended if any of the pool is positive. Pooling of more than 5 samples is not recommended to avoid the effect of dilution leading to false negatives. Recommendations: for sample pooling for real-time RT-PCR screening for COVID-19 are as follows (based on the KGMU study): 1. Use only in areas with low prevalence of COVID-19 (initially using proxy of low positivity of 5% for COVID-19 Preferable number of samples to be pooled is five, though more than two samples can be pooled, but considering higher possibility of missing positive samples with low viral load, it strongly discouraged to pool more than 5 samples, except for research purposes [33].

### **BioMedomics Rapid Kit (IgM/IgG)**

The BioMedomics Rapid IgM-IgG Combined Antibody Test for COVID-19 is immunochromatography based. The test card contains (1) colloidal gold-labeled recombinant novel coronavirus

antigen and quality control antibody colloidal gold marker, two detection lines (G and M lines) and one quality control line (C) fixed on a nitrocellulose membrane. M is fixed with monoclonal anti-human IgM antibody for detecting the novel coronavirus IgM antibody. G is fixed with monoclonal antihuman IgG antibody for detecting the novel coronavirus IgG antibody. The quality control antibody is fixed on the C line. When an appropriate amount of test sample is added to the sample well of the test cassette, the sample will move forward along the test card via capillary action. If the sample contains IgM antibody, the antibody will bind to the colloidal gold-labeled novel coronavirus antigen. The antibody/antigen complex will be captured by the anti-human IgM antibody immobilized on the membrane, forming a red M line and indicating a positive result for the IgM antibody. If the sample contains IgG antibodies, the antibody will bind to the colloidal gold-labeled novel coronavirus antigen and the antibody/antigen complex will be captured by the antibody immobilized on the membrane, forming a red G line and indicating a positive result for the IgG antibody. If neither antibody is present, a negative result is displayed [34].

### **CRISPR Technology Paper Kit by India**

In a major breakthrough, a team of Indian scientists have successfully developed a low-cost, paper-strip test which can detect the new Coronavirus within an hour and address India's urgent need for rapid-testing. The test uses the cutting-edge gene-editing tool- Crispr-Cas9 to target and identify the genomic sequences of the novel Coronavirus in the samples of suspected individuals [35].

### **Bosch Rapid Kit**

Bosch has developed a new rapid test for its Vivalytic analysis device to detect the SARS-CoV-2 pathogen. The test provides a reliable result in 39 minutes and is currently the fastest (PCR) test worldwide.

Bosch's new rapid test is predestined for decentralized use in mobile test centers at freeway service stations and airports. People who take the test can obtain a reliable result while at the testing site. Available now in Europe, the CE-approved test helps avoid time in quarantine, reduce pressure on diagnostic laboratories, and make travel and work safer again. "One of the keys to fighting the Coronavirus pandemic is to rapidly identify sources of infection. The development of the new Bosch PCR singleplex test is part of a research and development project funded by the German Federal Ministry of Education and Research (BMBF). The test has a sensitivity of 98 percent and a specificity of 100 percent. To develop it, the Bosch subsidiary Bosch Healthcare Solutions joined forces with the German biotechnology company R-Biopharm – a leading provider of highly sensitive manual PCR tests [36].

### **Ten Minute Finger Print and Prick Test**

#### **Finger print test**

This test is known as the COVID-19 IgM IgG Rapid Test and the kit is manufactured by BioMedomics. Results can be obtained within 15 minutes. Blood is used as a sample in this test.

The blood test is not being used in the UK, despite health bodies in China, Italy and Japan adopting it for rapid diagnosis of COVID-19 positive patients. On March 5, 2020, BioMedomics claimed its 'quick and easy' test was ready and being used in South Korea, Japan, Italy, China and some countries in the Middle East.

A blood sample is collected and injected into the analysis device which is small in size. Following the addition of, the waiting time for the result is 15 minutes. A positive result is indicated by the appearance of two lines on the kit, indicating that the sample contains antibodies against SARS-CoV-2, which are produced shortly after infection. If a single line appears it means the test is negative. Two lines closer together mean the person is positive for the later-stage antibodies, and three lines mean the patient is positive for both types of antibodies (IgG and IgM). A small study showed the test produced a correct response 80 per cent of the time. PHE confirmed it was not using the advanced blood test because it was not accurate enough, and are hoping to develop their own. The US Food and Drug Administration (FDA) is also yet to approve it. A former PHE strategist said he was 'not confident' the test could produce correct results and it is therefore unlikely to be rolled out. However, the method had a number of desirable features from the perspective of the need for a rapid point of care diagnostic.

#### **Finger prick test**

This test is known as the COVID-19 Rapid Test Cassette and the manufacturer of the kit is Sure Screen Diagnostics Derby United Kingdom. Total diagnostic time is 10 minutes. This company has created a test kit which can allegedly determine with 98 per cent certainty if a person is infected. Blood sample is collected via finger prick and then added to the screening device.

Results are displayed in a similar fashion to those of an at-home pregnancy test within minutes and could potentially save delays in diagnosis.

Sure Screen says its test has been validated and is already being used by private buyers in the UK, Ireland, Germany, Spain, Switzerland, Netherlands, Turkey, UAE, Kuwait and Oman. It is believed around 175,000 tests have been conducted with the Sure Screen kit so far [37].

#### **LAMP based kit**

Most current diagnostic tests that detect SARS-CoV-2 genetic material are PCR-based, due to its high sensitivity and

specificity. However, the method can be expensive, slow, and requires sophisticated equipment and well-trained personnel, making it unsuitable for point-of-care use.

Molecular tests based on alternative methods such as Loop-mediated Isothermal Amplification (LAMP) are being developed, which could offer a low-cost, fast and portable way to detect SARS-CoV-2 infection. LAMP (which is an acronym for Loop-mediated Isothermal Amplification) is a single tube technique for the amplification of DNA. It provides a low cost alternative to polymerase chain reaction (PCR) technology to detect certain diseases. It involves the design of assay primers and use of a strand-displacing polymerase to allow rapid amplification at a constant temperature without the need for thermal cycling (required for PCR).

LAMP reaction mixture consists of 6 primers which target 8 different regions on the genome of bacteria or viruses. LAMP is highly specific, have low cost, fast and portable test for the diagnosis of pathogenic organisms (bacteria/viruses).

Due to the large number of binding sites on target DNA/RNA, the LAMP method is inherently highly specific. No thermal cycling is required due to the isothermal nature of the reaction which facilitates the use of a small, portable, analyzer like HiberGene'ss HG Swift.

LAMP reactions very rapidly generate large quantities of amplified material when pathogenic bacteria/viruses are present in the patient sample; typically time to positive result is 15-25 minutes from the start of the reaction, a detection speed which outstrips standard automated PCR.

In addition, the ready-to-use freeze-dried LAMP reagents used in HiberGene tests are highly resistant to inhibition, allowing samples to be used without extraction. The standard sample handling protocol for most of 13 CE-marked infectious disease tests involves simple dilution and heat treatment steps for crude samples including stool, respiratory and genital swabs, before addition to the reaction mix [38].

### **Saliva based testing**

The US Food and Drug Administration has given Emergency Use Authorization to a fifth saliva-based test for COVID-19. This method includes low cost and non-invasive procedure and is developed by the Yale School of Public Health which requires minimal processing and retains much of the accuracy of traditional nasopharyngeal swabs. SalivaDirect kit was received Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration on August 15th, 2020.

The critical component of this approach is to use saliva instead of respiratory swabs, which enables non-invasive frequent sampling and reduces the need for trained healthcare professionals

during collection. This kit is simplified in many ways as it does not require any 1. nucleic acid preservatives at sample collection, (2) replacement of nucleic acid extraction with a simple proteinase K and heat treatment step is not required, and (3) testing specimens with a dualplex quantitative reverse transcription PCR (RT-qPCR) assay. The SalivaDirect kit has been validated with reagents and instruments from multiple vendors to minimize the risk for supply chain issues. SalivaDirect is highly sensitive with a limit of detection of 6-12 SARS-CoV-2 copies/ $\mu$ L. When comparing SalivaDirect to paired nasopharyngeal swabs using the authorized ThermoFisher Scientific TaqPath COVID-19 combo kit, we found high agreement in testing outcomes (>94%) [39].

### **Olfactory Test**

There are some reports of loss of smell function (Anosmia) with COVID-19 infection. Smell test was designed to identify/diagnose asymptomatic COVID-19 positive individuals. A panel of five different odorants belonging to Indian households with unique and mutually exclusive odors were used to develop a prototype kit to test this hypothesis (loss of smell). The developed prototype kit was tested at 2 centers (N=49 and 34) with slight modifications. Simultaneously, the kit was also tested on 55 (N=35 and 20) healthy controls. Results indicate that otherwise asymptomatic COVID-19 positive individuals have a quantifiable deficit in smell sensation. Interestingly, variable sensitivity to different odorants was observed in different patients. None of the healthy controls reported difficulty in sensing any of the odorants, whereas, some of healthy controls did misidentify the odorants. Overall, this present study provides preliminary data that loss in smell sensation for various odorants can be exploited as a quick and affordable screening test to identify infected cases among at risk individuals [40].

### **Rapid Point-of-Care (PoC) Antigen Detection Test (for diagnosis along with RT-PCR)**

Since the entire public health machinery is focused to test, track and treat COVID-19 patients, it is imperative to explore the existing antigen-based assays as point-of-care tests for early detection of SARS-CoV-2. Such tests, if reliable would be valuable at field level for early detection of infection and quick containment. Availability of antigen-based detection tests is very limited all across the world. Most of such tests have relatively moderate sensitivity but high specificity. However, manufacturers of all antigen-based tests are encouraged to approach ICMR for validation and inclusion of their test in the wider testing approach of the country. A positive test should be considered as a true positive whereas all symptomatic individuals testing negative through the rapid antigen test should be confirmed with a real-time PCR test. ICMR and AIIMS, Delhi independently evaluated the stand-alone rapid point of care antigen detection assay which does not require a specialized machine and can be interpreted with a naked eye.

The test is a promising tool for quick diagnosis of SARS-CoV-2 in field settings. The assay is known as Standard Q COVID-19 Ag kit and has been developed by SD Biosensor with manufacturing unit at Manesar, Gurugram. On validation, the test has been found to have a very high specificity with moderate sensitivity. It is now recommended to use Standard Q COVID-19 Ag detection test as a point of care diagnostic assay for testing in the containment zones as well as hospitals in combination with the gold standard RT-PCR test [41].

### **Rapid point of care RT-PCR test**

Real time Polymerase Chain Reaction (RT-PCR) based testing is mostly used testing because of its sensitivity and specificity. But this testing usually requires a centralized laboratory and significant infrastructure. Rapid point of care RT-PCR test, the DnaNudge® platform CovidNudge test came as answer to this as this strategy does not require any laboratory handling or pre-processing of samples. Nasopharyngeal swabs are inserted directly into a cartridge which contains all reagents and components required for RT-PCR reactions, including multiple technical replicates of seven SARS-CoV-2 gene targets (rdrp1, rdrp2, e-gene, n-gene, n1, n2 and n3) and human ribonuclease P (RNaseP) as positive control. The CovidNudge platform offers a sensitive, specific and rapid point of care test for the presence of SARSCoV-2 without laboratory handling or sample pre-processing. The implementation of such a device could be used to enable rapid decisions for clinical care and testing programs [42].

### **LAMPORE Method**

This method which combines the rapid target-specific amplification provided by LAMP, a method of transposase-based library preparation, and real-time nanopore sequencing and data analysis. The resulting combination, LamPORE, is rapid, sensitive and highly scalable and here we demonstrate LamPORE's efficacy for detecting the presence or absence of SARS-CoV-2 RNA in clinical samples. Studies using much larger sample sets have been recently conducted to establish diagnostic performance claims [43].

## **Discussion**

Real Time RT-PCR is suggested as the gold standard test for detection of COVID-19. This test requires specialized laboratory setup with specific biosafety levels, and takes an average of 4-5 hours after the receipt of the sample to get results. The main advantage of this platform is accuracy of detection and its ability to run up to 90 samples in a single run. In view of the specialized laboratory requirements, this test cannot be performed at every district level lab as molecular virology facilities may not be available. However, wherever available, it is advised to use real time RT-PCR as the frontline test for diagnosis of SARS-CoV-2.

Alternative systems that have been deployed for COVID-19 disease are TrueNat and CBNAAT. These platforms have widespread availability even at district and primary health center level as these platforms are widely used for diagnosis of Tuberculosis and other infectious diseases. These platforms have a quick turnaround time (30-60 minutes) but only 1-4 samples can be tested in a single run, limiting the maximum numbers that can be tested to 24-48 samples / day. All COVID-19 tests conducted through RT-PCR, TrueNat and CBNAAT are reported on ICMR data entry portal which helps in drawing the National estimates on numbers of tests conducted, numbers of positives, tests conducted per million population etc.

In an effort to ramp up testing capacity, ICMR has approved a total of 1000 COVID-19 testing labs in both public (730) and private sector (270). This includes RT-PCR labs (557); TrueNat Labs (363) and CBNAAT Labs (80). However, in spite of these developments, access to testing still remains a huge challenge in a large country like India. There is a definite need to increase the outreach of testing by introducing rapid point of care diagnostic tests. Also, there is value in conducting serosurveys with IgG based antibody tests in certain situations. In view of this, it is now suggested to include additional testing methods to improve the access and availability of testing in various parts of the country.

SD biosensor developed a Standard Q COVID-19 Ag detection assay which was evaluated independently by the Indian Council of Medical Research, Delhi and All India Institute of Medical Sciences, Delhi. This test has a very high specificity (i.e. ability to detect true negatives). Specificity of this test ranged from 99.3 to 100% at the two sites. ii) and sensitivity of the test (i.e. ability to detect true positives) ranged from 50.6% to 84% in two independent evaluations, depending upon the viral load of the patient. Higher viral load correlated with higher sensitivity. Standard Q COVID-19 Ag detection assay by SD Biosensor is the standalone antigen detection test which is available in India and has been validated. In view of its high specificity while relatively low sensitivity, ICMR recommends the use of Standard Q COVID-19 Ag detection assay as a point of care diagnostic assay for testing in the containment zones or hotspots (to be performed onsite under strict medical supervision and maintaining kit temperature between 2° to 30° C.). Use of the rapid antigen test is recommended in A & B categories above subject to the following conditions: i) Suspected individuals who test negative for COVID-19 by rapid antigen test should be definitely tested sequentially by RT-PCR to rule out infection, whereas a positive test should be considered as a true positive and does not need reconfirmation by RT-PCR test. ii) Samples (only nasopharyngeal swabs) to be collected by a trained healthcare worker following full infection control practices including use of proper PPE. iii) The test should be conducted on-site under strict medical supervision and within one hour of



sample collection in extraction buffer. iv) All testing results using the standard Q COVID-19 Ag detection assay must essentially be entered on the ICMR COVID-19 portal and also communicated to the state authorities and officials of the Integrated Disease Surveillance Programme (IDSP) on a real-time basis.

IgG antibodies usually start appearing after two weeks of onset of infection, once the individual has recovered after infection, and last for several months. Thus, the IgG test is not recommended for the detection of acute infection. However, detection of IgG antibodies for SARS-CoV-2 may be useful in some situations, for example serosurveys to understand the proportion of population exposed to infection with SARS-CoV-2 including asymptomatic individuals. Depending upon the level of seroprevalence of infection, appropriate public health interventions can be planned and implemented for prevention and control of the disease. Periodic serosurveys are useful to guide the policy makers. Survey in high risk or vulnerable populations (health care workers, frontline workers, immunocompromised individuals, individuals in containment zones etc) to know who has been infected in the past and has now recovered. It is strictly advised to use IgG based ELISA and CLIA assays only to conduct serosurveys. ICMR has validated and approved IgG ELISA kits for COVID-19. In addition, USFDA approved IgG ELISA and CLIA kits are also available and can be used.

Serological assays allow us to study the immune response(s) to SARS-CoV-2 in a qualitative and quantitative manner. Furthermore, serosurveys are needed to determine the precise rate of infection in an affected area, which is an essential variable to accurately determine the infection fatality rate. Serological assays will also allow for the identification of individuals who mounted strong antibody responses and who could serve as donors for the generation of convalescent serum therapeutics. Lastly, serological assays will potentially enable identification of individuals who have developed immunity, or have yet to be exposed to the infection [26].

## Conclusion

Since test, track and treat is the only way to prevent spread of infection and save lives, it is imperative that testing should be made widely available to all symptomatic individuals in every part of the country and contact tracing mechanisms for containment of infection further strengthened. ICMR advises all concerned State Governments, Public and Private Institutions to take required steps to scale up testing for COVID-19 by deploying combination of various tests as advised above.

Different methods for detection of COVID-19 disease are reviewed in this article. There is still significant scope for research and development of rapid and accurate molecular testing methods for the community. Even for hospitals, multiplex PCR can be

developed for the identification of not only corona viruses but also multiple other viruses like H1N1 at a time.

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