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CHALLENGES AND PROSPECTS IN CURRENT DIAGNOSTIC TECHNIQUES AVAILABLE FOR CORONA VIRUS DISEASE-19 (COVID-19)

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ABSTRACT

Background: The current global pandemic (SARS Covid-19), was first detected in Wuhan, in December 2019. The increasing magnitude and significance of identifying the cases have accentuated the importance of diagnostic approaches to Covid-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Major challenges are being faced in various phases of laboratory diagnosis, from pre-analytical to post-analytical processes. Although the gold-standard method for testing is real-time reverse-transcription polymerase chain reaction (rRT-PCR), various limitations such as low sensitivity at early stages of infection, longer turn-around time (TAT) and influence of external factors have been reported in various studies. Choosing ideal targetsfor nuclei acid amplification tests (NAATs) are important to increase the sensitivity and specificity of the assays and lower the limit of detection.At present computed tomography (CT) of chest has been reported as a reliable diagnostic technique, even in rRT-PCR false-negative cases. Immunodiagnostic assays are being developed recently to overcome the short-comings in rRT-PCR method, to confirm the active cases, as well as determine the immune status of asymptomatic patients. Reverse-transcriptase loop-mediated isothermal amplification (RT-LAMP) based assays, have been suggested as rapid, cost-effective point-of-care tests (POCTs). Currently various diagnostic assays are under development based on isothermal amplification, CRISPR, nanotechnology, biosensors and AI (artificial intelligence), most with potential for POCTs. **Aim:** To identify and compare the relevance of various techniques and tests under development to detect Covid-19. **Methods:** Original articles, review articles, commentaries and short communications regarding the assays to detect Covid-19 were thoroughly examined to summarise the observations. **Conclusion:** Understanding the biological properties of the virus is crucial forthe development of new diagnostic approaches which can provide precise identification with high sensitivity, specificity, and short TAT, thus aiding in real-time patient management and controlling spread of infection.

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INTRODUCTION

The very first case of COVID-19 in humans (presenting with pneumonia of unknown etiology) was reported in Wuhan city ofHubei province, China in December 2019 (1). The etiological agent responsible was discovered as a novel coronavirus (2019-nCoV) belonging to the genera beta-coronaviruses of *Coronaviridae* family, renamed on 11 February 2020 as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (2). They are enveloped, non-segmented viruses with a positive-sense (single

stranded)ssRNA genome (30kb) with 14 Orfs coding for 27 proteins, which includes four structural proteins (S, E, M, and N) and RNA dependent RNA polymerase (RdRp). S gene codes for the spike surface glycoprotein which interacts with angiotensin converting enzyme 2 (ACE2) receptors, facilitating the entry of the virus into the host cell (3). Many studies have observed that mutations in the receptor-binding domain (RBD) such as L455Y, Q493N and N501T contributes to the improved and flexible binding of the spike glycoprotein to ACE2 receptors (4,5).Although the SARS-CoV-2 virus shows ~50% and ~80% similarity to Severe Acute Respiratory Syndrome (SARS-CoV) and Middle-East Respiratory Syndrome (MERS-CoV) genomes respectively, S gene has been reported to be divergent with only <75% resemblance) compared to other 3 structural proteins which are more conserved. Comprehending the biological characteristics of the virus plays a pivotal role in development of new diagnostic approaches and in control and management of the disease (3).

SARS-CoV-2 can be transmitted between humans most commonlyfromnose and mouth secretions, and less commonlyby indirect contact with fomites from infected symptomatic, pre-symptomatic or asymptomatic people (4,6,11). Although may studies (4,10) report insufficient evidences on vertical transmission Vivanti AJ et al, Shende P et al, and Kotlyar AM et al, have confirmed the transmission of the virus inutero (congenital/transplacental transmission), through virological and pathological investigations, and observation that the virus can survive, and replicate as well in the placenta, leading to induction of immune responses and fetal mortality (7,8,9).The other less frequently reported route of transmission are ocular and feco-oral routes (10). The basic reproduction number (R_0), which determines the virus transmissibility (of Covid-19)for India was estimated as 1.379,while it fluctuates from 2.24 to 3.58 globally (2,12,13). Incubation period ranges from a median of 5-6 days, and may extend up to 14 days according to recent reports (11,14).

The signs and symptoms of the disease varies from person-person, ranging from subclinical, mild-flu like symptoms to severe pneumonia, and/or multi-organ failure. Although the presentation may vary, hyposmia and dysgeusia are the most commonsymptoms encountered by patients of COVID-19 (3,11,14,21-25). Higher expression of ACE2 receptors in men, and absence of female sex hormones (estrogen receptors)may be some of the contributing factors to the high susceptibility of men to the disease (15,16,17,18). Comparatively low percentage of people complain with gastrointestinal symptoms such as diarrhoea and vomiting. ACE2 receptors are found abundantly in the alveolar epithelium of lung and brush-border epithelium of enterocytes in small intestine, showing consistency with the clinical presentation in patients (11,15,17). Diagnosis of both clinically suspected cases and subclinical cases are equally important to control the spread of infection (19,20,21,26). Challenges and limitations faced in laboratory diagnosis of Covid-19 spans from pre-analytical to post-analytical stages (Fig. 1), which, if not resolved, can have an impact on the accuracy of diagnosis and management of patients (2,24,27). Nucleic acid amplification tests (NAATs) are currently being used for diagnosis of Covid-19, which requires complete knowledge of the viral genome, so as to prepare primers and probe sequences (28,29,30). Despite the fact that new, rapid and more sensitive diagnostic approaches are being developed to diagnose Covid-19, real time reverse transcription-polymerase chain reaction (rRT-PCR) remains to be the gold standard technique.

Some of the major drawbacks faced in rRT-PCR include limit of detection, ruling out false-positive and confirming false-negative results, increased turn-around-time (TAT), requirement of trained laboratory personnel, and financial input. Rapid and accurate diagnostic assays for Covid-19 is necessary to contain the global pandemic (2,3,13,21,). As rapid and accurate diagnostic assays for Covid-19 is necessary to contain the global pandemic, this review focuses on discussing the diagnostic assays used currently, challenges faced and the future developments being undertaken to overcome the drawbacks.Review articles, commentaries, letters, retrospective and prospective studies on laboratory diagnosis were analysed to identify various the diagnostic approaches – both current and future developments for rapid and precise detection of SARS-CoV-2.

CURRENT DIAGNOSTIC TECHNIQUES

The diagnostic approaches used currently are based on detection of amplified viral RNA from the clinical samples, which indicates the active stage of virus, antibodies (IgG and IgM) against the viral proteins in the serum, which can aid in monitoring the immune status of patients and abnormal features analysed in chests CT-scans by radiologists for an add-onto clinical diagnosis. Thus, the diagnostic approaches to COVID-19 can be stratified into clinical and paraclinical diagnosis (21,22,26,27). Clinical diagnosis involves, physical examination of the patient, clinical presentation, and radiological features, while para-clinical diagnosis involves molecular assays, immunodiagnostic assays, viral culture, (histopathology and autopsy findings), and genome sequencing. Genome sequencing can aid in studying mutations in the virus, designing primers and probes for NAATs, and can be used predominantly for research purposes (1,26,27,28,29).

Molecular diagnostic assays (MDA): The presently available different MDA are rRT-PCR, isothermal amplification techniques (RT-LAMP, NASBA; Nucleic Acid Sequence-Based Amplification), and CRISPR-based techniques. ICMR has approved different rRT-PCR assays with high sensitivity and specificity to detect SARS-CoV-2 virus, with minimal amount of challenges (2,21,30-32). Isothermal amplification techniques such as loop-mediated isothermal amplification (LAMP) has been reported to have higher efficiency and shorter turn-around-time (TAT) compared to PCR assays (1,3,29). Other potential isothermal amplification assays include PSR (Polymerase Spiral Reaction), HAD (Helicase Dependent Amplification), and TMA (Transcription Mediated Amplification) (19,33).

rRT-PCR (Real time Reverse Transcription-Polymerase Chain Reaction): rRT-PCR techniques are considered to be reliable, and the gold-standard method for diagnosis of SARS-CoV-2 RNA from clinical samples, even though viral isolation and culture are the conventional gold-standard methods, they are tedious and impractical methods which requires longer turn-around time in the present scenario for confirmation of diagnosis, and also presents a risk of spread of infection to healthcare workers. (33-37) .One of the challenges faced in this technique is the resolving of false-negative and false-positive results (38-41). Even though the sensitivity of an assay may be 90% or higher, the percentage of risk attributed to

false-negative results is significant, as it may relaxpatients from wearing of masks / maintenance of social distancing (42).

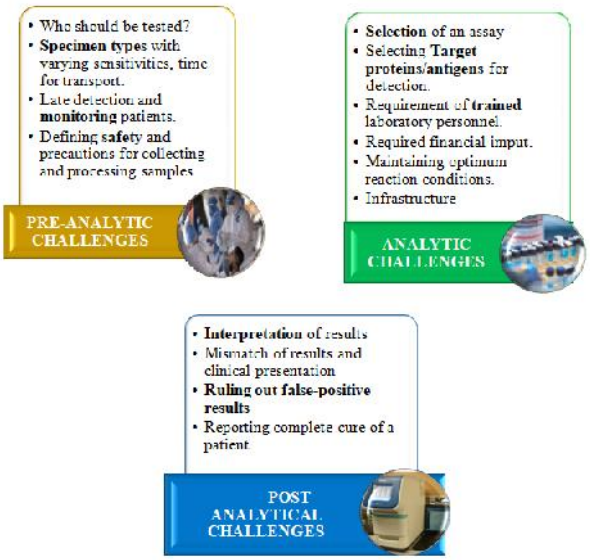

Figure I: Pre-analytic, analytic, and post-analytic challenges faced in the diagnosis of SARS-CoV-2 infection

Selection of specimen type: The nasopharyngeal swabs have been reported to have more sensitivity compared to other upper respiratory tract specimens, while sputum and bronchoalveolar lavage (BAL) are considered ideal samples for detection of viral loads in lower respiratory specimens (43,44,46).Tan W et al (43), had investigated the positive rates of SARS-CoV-2 isolation in various samples (1070 specimen) through rRT-PCR method, and found that bronchoalveolar lavage had the highest positive rates (93%), followed by sputum (72%), and nasal swabs (63%). Faeces (29%) and blood (1%) had the lowest positive rates, suggesting the rare incidence of systemic spread, while none of the urine samples tested to be positive. Zhang W et al (45) observed that oral swabs were more positive (53.3%), closely followed by blood (40%), anal swabs (26.7%), and serum (20%), among which anal swabs were found to be positive after day 5 showing a change in trend of high viral load from oral to anal swabs as time passes. Collection of the specimen should be performed using swabs while wearing PPE (personal protective equipment) (44).

Wolfel R et al. (47), analysed the viral load in various samples, and found that sputum samples, had the highest viral load (7×10^6copies per ml) which remained detectable for a long period of time (1 week). Nasopharyngeal and oropharyngeal swabs had a viral load of 6.76×10^5copies per whole swab, which gradually decreased after day 5, while urine and serum yielded no detection of virus. Although virus was detectable in fecal samples, the load was $<10^6$ copies/ml and viral isolation was unsuccessful (49,50,51). Some studies (27,33,48) have reported the detection of viral RNA in placental tissues, amniotic fluids, tears and conjunctival secretions also. Saliva has also been reported as a reliable specimen which can be used to gather information about the evolution of the disease apart from qualitative analysis (52,53). Rapid salivary test validated as a POCT by Azzi L et al (54) showed a high sensitivity of 93%, but a comparatively lower specificity of 42%, and was found to confirm/resolve the false positive/false negative results in the rRT-PCR testing of saliva and nasopharyngeal samples of the same patients.

Quite recently rectal swabs and stool samples have been evaluated for detection of viral RNA, and research studies show an increased sensitivity of the virus in asymptomatic cases and symptomatic patients for a longer time period, even when the viral loads had become undetectable in respiratory samples (after 3rd week of PSO; post symptom onset).This also points towards the possible feco-oral transmission (49-51,55).Further research into the sensitivity of viral RNA detection from stool samples can aid in reduction of transmission from patients to healthcare workers (29,51). Some of the samples that may be best to collect from COVID-19 patients are being researched in many studies. Yelin I et al (57)had observed that a single sample with the viral RNA can be frequently detected in a pool of up to 32 samples and a lower percentage of false-negatives on pooling. Pooling of samples can be used in monitoring of healthcare workers and determining the frequency of viral RNA carry-over among the population.

Collection, Transport and Processing: Collection, transport and processing of specimens can have a direct influence on the result, based on the influencing factors such as specimen quantity, expertise of the personnel collecting and processing, time of collection, transport conditions and time taken, as well as on the type of assay used. The collected specimens should be preferably transported to the laboratory in a universal viral transport medium (VTM), in refrigerated conditions (2-8°C) (20,38,57,58). Radbel J et al (60) establish the use of phosphate buffered saline (PBS) as a clinically important transport medium for short-term preservation of SARS-CoV-2 containing clinical specimens through their study. The collection of specimens is performed by twirling the swab with non-flocked synthetic fibres and synthetic nylon handles, three times (for 10seconds) from the required site. Processing of all respiratory specimens should be performed in bio-safety cabinet class II (BSC II) or higher which increase the cost of processing of samples. In order to perform rRT-PCR, the collected sample should be transferred to a lys is buffer to degrade the envelope coating of the virus and to prevent degradation of the viral RNA which increases the cost of consumables used for testing, compared to other techniques where direct sample can be used (20). In case of TrueNAT test, samples should be sent in viral lysis buffer and not in viral transport medium (21).

Target Selection: Selection of two targets is ideal to perform the assay, to increase the specificity of detection. The ideal design of a target involves one conserved region, and one specific region, to escape the effects of mutation so as to specifically target SARS-CoV-2 (1,3). The CDC recommends the use of N1 and N2 proteins, while WHO recommends RdRp, E, N, and S in different combinations (2). Most studies recommend the use of RdRp/Hel genes which has a high analytical sensitivity of 95%,lowest limit of detection and no cross- reactivity, which may aid in the reduction of false-negative cases. This assay was found to be comparatively better than RdRp-P2 assay, which has been reported cross-react with SARS-CoV in cell culture portraying reduced specificity (60-62). Use of dual targets E and RdRp genes, have been observed to improve the sensitivity of detection, and decrease the time period of detection as well.Use of E gene as a target, has been observed to perform better with higher sensitivity, significantly lower Ct values and for longer period of time compared to that of RdRp gene detection values (61).

Ct value: Interpretation of results varies within different countries and assay kits used. In patients with symptoms, the cycle threshold values can be detectable at a very early stage in the results (2,38). The number of cycles required for the fluorescence signal produced in a reaction to significantly cross the florescence threshold is defined as the cycle threshold (Ct) value. Ct values less than 40 are generally considered to be a positive result, which may vary with different manufacturer (63-69). ICMR (Indian Council of Medical Research) does not recommend relying on Ct values to determine the severity of the disease, while some studies have observed that low Ct values may indicate high viral loads can be used as an indication of transmissibility, and severity (68-71).The limit of detection of COVID-19 RT-PCR is 6.25cp/µl, as given by FDA. TRUPCR SARS-CoV-2 RT-qPCR Kit (2-tube assay), Helini Coronavirus Real-Time PCR kit (single tube assay), Meril COVID-19 One-step RT-PCR Kit (single tube reaction), are some of the rRT-PCR kits which detects E/N/RdRp genes, RdRp/ORF gene, N/ORF 1ab genes of SARS-CoV-2 respectively within a time period of 20-60 minutes with >95% sensitivity and specificity – approved by ICMR.

ISOTHERMAL AMPLIFICATION ASSAYS: The major advantage of isothermal amplification techniques over rRT-PCR technique is that it does not require the thermocycler. This technique is more useful to detect the viral RNA in crude samples, as they cannot be easily inhibited (19,72). They have a good potential for development to point of care tests (POCTs), as they are temperature independent unlike rRT-PCR.

TrueNAT RT-PCR: Truenat™ COVID-19 is a chip-based Real Time duplex Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the semi quantitative detection of SARS CoV-2 RNAin human oropharyngeal and nasopharyngeal swab specimen and aids in detection and confirmation of SARS CoV-2 infection and diagnosis of COVID-19. The test detects the E and Orf1a genes of the virus. Truenat™ COVID-19 runs on Truelab®Real Time Quantitative micro PCR Analyzers. Truenat™COVID-19 works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) based on Taqman chemistry. It enables same-day testing and reporting. This allows for faster patient isolation if required.

RT-LAMP: Reverse transcription loop mediated isothermal amplification (RT-LAMP) assay is specific, and efficient with simple protocol for sample preparation, and processing (3).They require a single temperature for the process, do not require any special expensive equipment such as thermocyclers to enhance the sensitivity of the reaction, and the visualisation of results is effortless. This has great potential to be developed as POCTs. Since RT-LAMP techniques can eliminate the step of purification of cDNA from reverse transcriptase, the reaction time can get reduced, enabling quick detection of the viral RNA (3,48). The challenge faced in the utilisation of LAMP assay is the requirement to optimise design of specific primers and conditions for smooth performance of the reaction. The assays have a TAT of approximately < 1 hour with a limit of detection of ~75 copies per µl (64,73,74). Zhang Y et al (74) explored the performance of calorimetric LAMP as a diagnostic assay for detection of SARS-CoV-2, and found it to be equally efficient as rRT-PCR, without any complicated instrumentation, useful in field testing type settings. Samples can be processed directly in this assay, skipping the RNA purification step. All these models have a great potential to be developed as POCTs. In India, Sree Chitra Tirunal Institute for Medical Sciences & Technology (75) developed LAMP diagnostic test for detection of SARS-CoV-2 and the kit is commercially called as 'Chitra Magna'. iLACO (isothermal LAMP based method for COVID-19) is a rapid calorimetric assay for detection of samples containing RNA and cDNA of SARS-CoV-2 within 20-30 minutes at 65°C. They can easily be used even in small laboratory settings, and has a lower limit of detection of up to 10 copies of Orf1ab target in the sample (76). POC RT-LAMP assays are assays under development which can deliver results within 15 minutes, eliminating the need of expensive equipment and fulfil the growing need for screening affected population (60).

NASBA and POCTs: Nucleic Acid Sequence Based Amplification, is one of the isothermal amplification techniques, currently developed and used for diagnosis of COVID-19. Unlike other NAATs, the final product in NASBA is single-stranded RNA with an opposite polarity to target sequence which is complementary (33,75). INSIGHT (Isothermal NASBA-Sequencing-based hIGH-throughput Test) – a barcoded isothermal NASBA assay, is a 2-stage testing strategy, which uses a combination of bed side diagnosis with whole genome sequencingfor COVID-19. It has a good sensitivity with a limit of detection of 50 copies per 20µl, which is comparable with qPCR. Additional research is required to develop this assay as POCTs (77).

CRISPR-BASED ASSAYS: It is a rapid detection assay with high sensitivity, efficient analysis, which does not require expert trained personnel or high financial input. Various clustered regularly interspaced short palindromic repeats (CRISPR) assays are being evaluated for their sensitivity and performance for nucleotide detection. CRISPR-Cas12a approach, DNA endonuclease-targeted CRISPR trans reporter (DETECTR) has currently been reported to distinguish clinical samples containing human papillomaviruses with 96% sensitivity (30,78).

The SHERLOCK method developed by Sherlock Biosciences uses Cas13 that is capable of excising reporter RNA sequences in response to activation by SARS-CoV-2-specific guide RNA (19). SHERLOCK has also been developed as point of care test named STOP; SHERLOCK Testing in One Pot, which does not require extraction of RNA from samples, and works within three steps in 1 hour 30 minutes – lysis of viral RNA, detection of RNA by STOP reaction, and visual detection of results by paper-dipstick (32).The DETECTR assay by Mammoth Biosciences relies on the cleavage of reporter RNA by Cas12a to specifically detect viral RNA sequences of the E and N genes, followed by iso- thermal amplification of the target, resulting in a visual readout with a fluorophore. SHERLOCK in combination with HUDSON (heating un-extracted diagnostic samples to obliterate nucleases) has been used directly in the battle against SARS-CoV-2 (30,75). 'All-In-One Dual CRISPR- Cas12a' (termed 'AIOD-CRISPR') assay for low-cost, fast (typically 5–20 min), ultrasensitive, precise and visual detection of nucleic acid has been developed by the University of Connecticut Health Centre (32,36). Some of the factors that can hinder the precise detection of nucleotide include off-target effects of CRISPR and CRISPR/Cas effectors which tend to show tolerance to mismatches between the guide RNA and target nucleotides (36). Based on RPA (recombinase polymerase amplification), CRISPR-nCoV (CRISPR novel coronavirus), which detects Cas13 has been reported to have very low limit of detection (LoD) of approximately one copy per reaction with high sensitivity and reduced turn-around-time (TAT) (30,36).

Table I. Comparison of ICMR approved RT-PCR kits for detection of specific gene targets of SARS-CoV-2

S. No.	NAME OF THE KIT	SPECIMEN TYPE	CONCENTRATION OF MASTER MIX AND TEST SAMPLE	TARGET GENE (S)	TURN AROUND TIME (TAT)	SENSITIVITY	LIMIT of DETECTION (LoD)	SPECIFICITY/CROSS-REACTIVITY	RESULT	LIMITATIONS
1.	A*STAR FORTITUDE KIT 2.0	Nasopharyngeal swab	Master mix - 22.50µl; Test sample - 2.5µl	NR	~20 minutes	99%	25 copies/reaction	Does not cross-react with other CoV	Ct values and shape of amplification curve are taken into consideration	Cannot be used on specimen directly
2.	LyteStar™ SARS-CoV-2 RT-PCR Kit 1.0 S	Human respiratory specimens	Master mix - 20µl; Test sample - 5µl	E gene	~50 minutes	≥ 95%	2.72 copies/µl	NR	Ct values and shape of amplification curve are taken into consideration	NR
3.	AFFIGENIX COVID-19 TEST (ACT) KIT	Respiratory specimens/serum, plasma, blood and tissues	Master mix - 7µl; Test sample - 8µl	N /ORF 1ab gene	~20 minutes	NR	NR	NR	Ct values and shape of amplification curve are taken into consideration	Cannot be used on specimen directly
4.	RealStar® SARS-CoV-2 RT-PCR Kit 1.0	Human respiratory specimens	Master mix - 20µl; Test sample - 10µl	E/S gene	~25 minutes	95%	$1*10^{-1}$ PFU/ml	Does not cross-react with other CoV	Ct values are taken into consideration	Other sample types cannot be used/Cannot be used on specimens directly
5.	TRUPCR SARS-CoV-2 RT-qPCR Kit (2-tube assay)	Human respiratory specimens	Master mix - 20µl; Test sample - 5µl (in each tube)	E/N/RdRp genes	~20 minutes	82-96%	10 copies/µl	100%	Ct values and shape of amplification curve are taken into consideration	Not a quantitative test/Cannot be used on specimen directly/Validated with 2 systems only
6.	1copy COVID-19 qPCR Triplex Kit (single tube reaction)	Nasopharyngeal/Oropharyngeal swab	Master mix - 10µl; Test sample - 5µl	E/N gene	NR	95-100%	5 copies/reaction	100%	No mention on Ct values	Mutations can lead to false results
7.	Detection Expert 1S © SARS CoV-2 One Step rRT-PCR Kit	Human respiratory specimens		N1/N2/RNase P gene	NR	100%	100GCE/reaction	100%	Ct values are taken into consideration	Other sample types cannot be used/Cannot be used on specimens directly
8.	Helini Coronavirus Real-Time PCR kit (single tube assay)	No specific sample mentioned	Total components - 15µl; Test sample - 10µl	RdRp/ORF gene	NR	95%	0.65 copies/µl	Shows 100% homology to wide range of clinical reference sequences	Ct values are taken into consideration	Cannot be used on specimen directly
9.	Covidsure Pro Multiplex RT-PCR kit	Human respiratory specimens	NR	E/N/ORF 1ab genes	1 hour	100%	< 5 copies/µl	100%	No mention on Ct values	Cannot be used on specimen directly
10.	SARS-CoV-2 Fluorescent PCR Kit (for RUO)	Oropharyngeal swabs and sputum specimens	qRT-PCR mix - 20µl; Test sample - 20µl	E/N/ORF 1ab genes	2 hours	99.56%	1000 copies/ml	Shows 100% identity to all SARS-coronaviruses	Ct values are taken into consideration	Cannot be used for differential diagnosis of SARS-coronaviruses
11.	DiagSure nCOV-19 Detection assay (Taqman based) (single tube reaction)	Nasopharyngeal and throat swab samples	NR	ORF 1ab/N/RNase P genes	1.5 hours	NR	100copies/ml	NR	No mention on Ct values	Mutations in target sequence can lead to false results
12.	RealCycler CORO-G Real Time PCR Kits	Oropharyngeal, Nasopharyngeal and nasal swabs	NR	E gene		≥ 95%	1 copy/µl	NR	No mention on Ct values	Mutations in target sequence can lead to false results
13.	Meril COVID-19 One-step RT-PCR Kit (single tube reaction)	Sera/Nasopharyngeal and Throat swab/Sputum	NR	N/ORF 1ab genes	65 minutes	100%	< 5 copies/reaction	100% (No cross-reactivity detected)	No mention on Ct values	Mutations in target sequence can lead to false results
14.	SARS-CoV-2 Multiplex qPCR kit (for RUO)	NR	Master mix - 10µl; Test sample - 9µl	E/N/RdRp genes	15 minutes (PCR processing)	≥ 95%	0.7 copies/µl (RNA); 1.6 copies/µl (DNA Template)	No cross-reactivity reported	No mention on Ct values	Mutations in target sequence can lead to false results

Continue ….

15	Real-Q 2019-nCoV Detection Kit	Human respiratory specimens	Master mix - 20µl; Test sample - 5µl	E/RdRp genes	~50 minutes	≥ 95%	6.25 copies/µl	No cross-reactivity reported	Ct values and shape of amplification curve are taken into consideration	Mutations in target sequence can lead to false results
16.	Xpert® Xpress SARS-CoV-2	Human upper respiratory specimens	Master mix - 6ml; Test sample - 300µl	E/N2 genes	NR	≥ 95%	0.005 (N2) and 0.02 PFU/ml	No cross-reactivity reported	Ct values are taken into consideration	Other sample types cannot be used/Cannot be used on specimens directly/Cannot rule out diseases caused by other bacterial or viral pathogens
17	EURORealTime SARS-CoV-2	Human upper respiratory specimens	Master mix - 10µl; Test sample - 10µl	N/ORF 1ab genes	~2 hours	95%	150 copies/ml	No cross-reactivity reported	Ct values are taken into consideration	Cannot rule out diseases caused by other bacterial or viral pathogens
18	NeoPlex™ COVID-19 Detection Kit	Human respiratory specimens	Master mix - 15µl; Test sample - 5µl	N/RdRp genes	2 hours	≥ 95%	50 copies/reaction	Might cross-react with SARS-CoV	Ct values and shape of amplification curve are taken into consideration	Mutations in target sequence can lead to false results
19	PowerChek™ 2019-nCoV Real-time PCR Kit	Human respiratory specimens	Master mix - 15.5µl; Test sample - 4.5µl	E/RdRp genes	~45 minutes	≥ 95%	4 copies/µl	No cross-reactivity reported	Ct values are taken into consideration	Performance established using NP swabs and sputum only/SARS like coronaviruses may cross-react with RdRp primer/Impact of vaccines, immunosuppressant drugs not evaluated/Cannot rule out diseases caused by bacterial and viral pathogens/Cannot be used on specimen directly/E gene signal could not differentiate SARS-CoV or SARS related coronaviruses
20	LabGun™ COVID-19 Assay	Nasopharyngeal and oropharyngeal swabs/sputum/BAL	Master mix - 16µl; Test sample - 4µl	E/RdRp genes	~50 minutes	≥ 95%	100 copies/reaction	No cross-reactivity reported	Ct values and shape of amplification curve are taken into consideration	Other samples are not evaluated
21	LabGun™ COVID-19 RT-PCR Kit	Nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swabs/nasopharyngeal wash/aspirate or nasal aspirate/sputum	Master mix - 15µl; Test sample - 5µl	E/RdRp genes	~50 minutes	≥ 95%	20 copies/µl	No cross-reactivity reported	Ct values and shape of amplification curve are taken into consideration	Does not reflect viral load in specimen/Performance evaluated with NP swabs and sputum only/Mutations may lead to false results
22	Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)	Nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate swabs/nasal washes and aspirates	Master mix - 30µl; Test sample - 50µl	N/ORF 1ab genes	~45 minutes	≥ 95%	200 copies/ml	No cross-reactivity reported	Ct values and shape of amplification curve are taken into consideration	Mutations in target sequence can lead to false results/Performance established with NP and OP swabs/the gene probes may detect bat and pangolin coronaviruses
23	STANDARD M nCoV Real-Time Detection kit	Nasopharyngeal, oropharyngeal, nasal and mid-turbinate nasal swab/sputum	Master mix - 20.5µl; Test sample - 10µl	E/ORF 1ab genes	~20 minutes	95%	0.5 copies/µl	Cross reactive with SARS-CoV and Sarbecovirus	Ct values are taken into consideration	Impact of vaccines, immunosuppressant drugs not evaluated/cross reactive with SARS-CoV
24	Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)	Throat swabs/Sputum	Master mix - 20µl; Test sample - 5µl	N/ORF 1ab genes	~30 minutes	95%	500 copies/ml	No cross-reactivity reported	Ct values and shape of amplification curve are taken into consideration	Mutations in target sequence can lead to false results
25	Real-time fluorescent RT-PCR kit for detecting 2019-nCoV	Throat swabs/BAL	Master mix - 20µl; Test sample - 10µl	ORF 1ab gene	~30 minutes	≥95%	100 copies/ml	No cross-reactivity reported	Ct values and shape of amplification curve are taken into consideration	Mutations in target sequence can lead to false results

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26	ProTect™ COVID-19 PCR Kit	Nasopharyngeal swab	NR	N gene	1 hour 15 minutes	≥ 95%	10 copies/reaction	No cross-reactivity reported	No mention on Ct values	NR
27	GeneFinder™ COVID-19 PLUS RealAmp Kit	Throat swab/Sputum/ BAL	Master mix - 15µl; Test sample - 5µl	E/N/RdRp genes	~30 minutes	95%	10 copies/reaction	Shows 100% homology to wide range of clinical reference sequences	Ct values are taken into consideration	Other samples are not evaluated
28	HELINI Coronavirus [2019-nCoV] Real-time PCR Kit	None specified	Master mix - 15µl; Test sample - 10µl	PAN coronavirus/2019-nCoV	~45 minutes	≥ 95%	1 copy/µl	Shows 100% homology to wide range of clinical reference sequences	Ct values are taken into consideration	Mutations in the target sequence can lead to false results
29	ViroQ SARS-CoV-2	Respiratory specimens (sputum/swabs)	Master mix - 17µl; Test sample - 5µl	E/RdRp genes	~30 minutes	100%	5 copies/20µl	98% (No cross-reactivity detected)	No mention on Ct values	Mutations in the target sequence can lead to false results
30	Coronavirus (COVID-19) genesig® Real-Time PCR assay	Nasopharyngeal and oropharyngeal swabs/sputum	Master mix - 12µl; Test sample - 8µl	NR	~15 minutes	≥ 95%	0.58 copies/µl	No cross-reactivity reported	Ct values and shape of amplification curve are taken into consideration	Performance validated in 3 PCR systems only/Cannot rule out diseases caused by other bacterial or viral pathogens
31	abTES™ COVID-19 qPCR I Kit	Nasopharyngeal and throat swab/sputum	Master mix - 15µl; Test sample - 5µl	N1/N2 gene	~15 minutes	≥ 95%	2.2 copies/µl (N1); 1.8 copies/µl (N2)	99.71-99.99% (No cross-reactivity detected)	Ct values and shape of amplification curve are taken into consideration	Mutations in the target sequence can lead to false results
32	AccuPower® SARS-CoV-2 Multiplex Real-Time RT-PCR Kit	Nasopharyngeal and Oropharyngeal swabs/sputum	Master mix - 10µl; Test sample - 10µl	E/N/RdRp genes	~30 minutes	95%	6 copies/µl (E); 2 copies/µl (N/RdRp)	No cross-reactivity reported	Ct values are taken into consideration	Impact of vaccines, immunosuppressant drugs not evaluated/Other samples - not validated
33	QuantuMDx SARS-CoV-2 RT-PCR Detection Assay	Human upper respiratory specimens	Master mix - 15µl; Test sample - 5µl	N/S/ORF 1 genes	~15 minutes	95%	10 copies/reaction	No cross-reactivity reported	No mention on Ct values	Other sample types are not validated/Cannot rule out diseases caused by other bacterial or viral pathogens
34	Liferiver Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit	Nasopharyngeal and orpharyngeal swabs/BAL/deep cough sputum	Master mix - 21µl; Test sample - 4µl	E/N/ORF 1ab genes	NR	≥ 95%	1000 copies/ml	98.1-100%	No mention on Ct values	NR

Table II: Comparison of ICMR approved and validated ELISA kits for antibody detection

S. No.	NAME OF THE TEST	MANUFACTURING COMPANY	SPECIMEN TYPE (VOLUME)	Incubation time	ANTIGEN (S) USED	SENSITIVITY	SPECIFICITY	RESULT	LIMITATIONS
1.	COVID KAVACH Anti-SARS CoV-2 Human IgG ELISA	Trivitron Healthcare Pvt. Ltd., Mumbai (Maharashtra), India	Serum/Plasma	130 minutes	NR	98%	100%	Results read at 450nm/Qualitative detection of IgG antibody	NR
2.	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG)	Euroimmun US Inc., USA	Human serum/plasma	~ 150minutes	S1 domain of spike protein	90%	100%	Results read at 450nm; Reference wavelength - 620-650nm/Qualitative detection of IgG antibodies	Cross-reactivity observed with anti-SARS-CoV-1 IgG antibodies/Cannot be used to screen donated blood
3.	SARS-CoV-2 IgM/IgG	YHLO iFlash, China	Human serum	NR	NR	90% (IgM); 95% (IgG)	95% (IgM); 95% (IgG)	NR	NR
4.	VOXEL Anti-SARS COV-2 IgG Antibody detection kit	Voxtur Bio Ltd., Mumbai (Maharashtra) India	Human serum/plasma	NR	SARS-CoV-2 whole cell antigen	NR	NR	Qualitative detection of IgG antibody against SARS-CoV-2	NR
5.	ErbaLisa COVID-19 IgG antibody ELISA (simple one-step serum dilution)	Transasia Bio-Medicals Ltd., Mumbai (Maharashtra), India	Human serum (10µl)	50 minutes at room temperature	Recombinant spike subunit antigen	98.30%	98.10%	Results read at 450nm; Reference wavelength - 630nm/Semi-quantitative assay	NR
6.	ICMR-NIV Anti-SARS CoV-2 Human IgG ELISA COVID KAVACH – MERILISA	Meril Diagnostics Pvt. Ltd., Vapi (Gujarat), India	Human serum/plasma (5µl)	130 minutes at different temperatures	SARS-CoV-2 whole cell antigen	93.30%	100%	Results read at 450nm/Qualitative detection of IgG antibody against SARS-CoV-2	NR
7.	COVID KAWACH IGG MICROLISA	J. Mitra & Co. Pvt. Ltd., Delhi, India	Human serum/plasma	130 minutes	NR	96.33%	100%	Results read at 450nm/Qualitative detection of IgG antibody	NR
8.	Dia.Pro COVID-19 IgG ELISA	DIA.PRO Diagnostic Bioprobes Srl, Italy	Human serum/plasma	NR	Spike protein 1 and 2, nucleocapsid	NR	NR	NR	NR
9.	ELI*Safe* 19^{TM} antibody test kit	HIMEDIA Laboratories Pvt. Ltd., Mumbai (Maharashtra), India	Human serum/plasma	NR	NR	100%	99%	Qualitative detection of IgG antibody against SARS-CoV-2	NR
10.	SARS-CoV-2 IgM (CLIA)	Shenzhen Mindray Bio-Medical Electronics Co. Ltd., China	Human serum/Heparin plasma or EDTA plasma (10µl)	NR	SARS-CoV-2 whole cell antigen	NR	NR	NR	Heterophilic antibodies can cause interference

Table III: Comparison of ICMR approved and validated rapid test kits for detection of IgG/IgM against SARS-CoV-2

S. No.	NAME OF THE TEST	MANUFACTURING COMPANY	KIT STORAGE	SPECIMEN TYPE	SPECIMEN STORAGE	VOLUME	TEST RESULTS READ BEFORE	SENSITIVITY	SPECIFICITY	LIMITATION/PRECAUTIONS
1.	STANDARD™ Q COVID-19 Ag Test	SD Biosensor, South Korea / India	2-30	Nasopharyngeal swab	Up to 1 hour at room temperature/4 hours at 2-8	350 µl	15-30 minutes	96.52%	99.68% (May cross-react with SARS-CoV)	Quantitative value of SARS-CoV-2 antigen cannot be determined/Quality and concentration of collected specimen can affect results/Difference in sensitivities between adults and children may be observed
2.	ACCUCARE™ COVID-19 IgG / IgM Lateral Flow Assay Test Kit	LabCare Diagnostics Ltd., India (Supplied by MyLab Discovery Solutions)	2-30	Serum/Plasma/Whole blood	Serum can be stored up to 2-8 for 5 days/-20 for longer storage	10 µl	15-20 minutes	93.75%	96.40%	Limited to qualitative detection/Concentration of specimen can affect results
3.	BIOCARD Pro COVID-19 Rapid Antigen kit	Trivitron Healthcare Pvt. Ltd., India	4-30	Nasopharyngeal swab	Up to 1 hour at room temperature/4 hours at 2-8	NR	15 minutes	100.00%	99.40%	NR
4.	COVID19 Ag RespiStrip (Dipstick)	Coris Bioconcept, Belgium	4-30	Liquid sample/Flocked swab	NR	100 µl	30 minutes	60-85.7%	98.3-100% (May cross-react with SARS-CoV)	NR
5.	Vstrip COVID-19 Antigen Rapid Test	Panion & BF Biotech., Taiwan	15-30	Nasopharyngeal swab	Up to 1 hour at room temperature/4 hours at 2-8	NR	10 minutes	83.30%	98.10%	Quantitative value of SARS-CoV-2 antigen cannot be determined/Specimens collected after 5 days PSO are likely to be negative/Minor changes in target epitope region may cause failure in detection
VALIDATED BUT NOT APPROVED RAPID KITS										
6.	STANDARD™ F COVID-19 Ag FIA (Fluorescent immunoassay)	SD Biosensor, South Korea/India	2-30	Nasopharyngeal swab	Up to 1 hour at room temperature/4 hours at 2-8	NR	30 minutes	NR	NR	NR
7.	Makesure™COVID 19 IgM/IgG Rapid Antibody Test (Immunochromatographic assay)	HLL Lifecare Ltd., India	2-30	Serum/Plasma/Whole blood	Serum can be stored up to 2-8 for 5 days/-20 for longer storage	Serum/Plasma - 10µl; Whole blood - 20µl	20 minutes	NR	NR	Limited to qualitative detection of IgM/IgG antibodies to SARS-CoV-2
8.	SARS-COV-2 Ag rapid Test Kit	Formosa Biomedical Technology Corp.,Taiwan	NR	Nasopharyngeal/Oropharyngeal swab	Up to 1 hour at room temperature/4 hours at 2-8	NR	10-15 minutes	10 copies/reaction	NR	NR
9.	BIOCREDIT SARS-COV-2 ANTIGEN RAPID TEST CE IVD (Differential black gold conjugate technology)	Rapigen Inc., South Korea	1-40	Nasopharynx/Nasopharyngeal swab	Up to 1 hour at room temperature/4 hours at 2-8	90-150 µl	5-8 minutes	90.20%	98-100%	NR
10.	Camtech COVID-19 IgM/IgG Rapid Test Kit	Camtech Diagnostics, Singapore	2-30	Serum/Plasma/Whole blood	Serum can be stored up to 2-8 for 5 days/-20 for longer storage	Serum/Plasma - 10µl; Whole blood - 20µl	15-30 minutes	87.5-100%	100%	Limited to qualitative detection of IgM/IgG antibodies to SARS-CoV-2
11.	MERIL COVID-19 IgG/IgM Rapid Test	Meril Diagnostics Pvt. Ltd., India	NR	Serum/Plasma/Whole blood/Venous whole blood	Serum can be stored up to 2-8 for 5 days/-20 for longer storage	10 µl	20 minutes	97.20%	99.22%	Limited to qualitative differential detection of IgM/IgG antibodies
12.	ANGCARD COVID-19 IgG/IgM Rapid Test Cassette	Angstrom Biotech Pvt. Ltd., India	4-30	Serum/Plasma/Whole blood	Serum can be stored up to 2-8 for 5 days/-20 for longer storage	25 µL	15-20 minutes	97.40%	99.30%	Specimen with extremely high concentrations of red blood cells, fibrin should be re-centrifuged before use
13.	Is It Covid-19 IgM/IgG Rapid Diagnostic Test	M/s Medsource Ozone Biomedicals Pvt Ltd., India	NR	Serum/Plasma/Whole blood	Serum can be stored up to 2-8 for 5 days/-20 for longer storage	NR	NR	NR	NR	Limited to qualitative detection of IgG/IgM antibodies to SARS-CoV-2
14.	GenBody COVID-19 IgM/IgG	GenBody Inc., South Korea	2-30	Nasopharyngeal swab and oropharyngeal swab	Up to 24 hours at 2-8 / -20 for longer periods	~ 100 µl	15-20 minutes	IgM - 40.0%, IgG - 56.7%; LoD - 2.87 x 10^3 $TCID_{50}$/ml	IgM - 98.8%, IgG - 100%	Cross-reactive with SARS-CoV-1
15.	CORONA ANTIBODY DETECTION TEST	Oscar Medicare Pvt. Ltd., India	2-30	Serum/Plasma/Whole blood	Serum can be stored up to 2-8 for 5 days/-20 for longer storage	NR	5-20 minutes	97.66%	99%	Limited to qualitative detection of antibodies to SARS-CoV-2
16.	Panbio™ COVID-19 IgG/IgM Rapid Test Device	Abbott Rapid Diagnostics Division, Chicago	2-30	Serum/Plasma/Fingerstick whole blood/venous whole blood	Serum can be stored up to 2-8 for 5 days/-20 for longer storage	Serum/Plasma - 10µl; Fingerstick/Venipuncture Whole blood - 20µl	10 minutes	95.80%	94.00%	Limited to qualitative detection of IgG/IgM antibodies to SARS-CoV-2

CREST (Cas-13, Rugged, Equitable, Scalable testing) approach has been proposed by Rauch et a (79)l, which uses portable thermocyclers and LED visualizers (plastic filter-based) for detection, and the results can be uploaded and stored using smartphones, increasing their feasibility.

IMMUNODIAGNOSTIC ASSAYS: Immunodiagnostic assays, both antigen and antibody detection tests can be an essential supplement to RNA detection by rRT-PCR during the course of the disease. Combining RNA and antibody detections may significantly improve the sensitivity of diagnosis for COVID-19 patients (34). Total antibody is found to be more sensitive after 12-15 days of onset in patients confirmed with COVID-19 compared to IgM and IgG; whereas RNA is found to decrease from day 7 to day 15 or day 39.One of the challenging factors to overcome while developing serological assays is cross-reactivity to other corona viruses (48,80,81). Immunoassays that can detect antigens and antibodies have higher resistance to disintegration during collection, storage, and transportation compared to viral RNA, making these assays more reliable and feasible (20). A study performed using chemiluminescent immunoassay to detect IgG and IgM, indicated that there might be an association between time and speed of IgM production with severity of illness, as patients with mild symptoms showed specific antibodies 7days PSO, while patients with severe symptoms showed antibodies 12 days PSO (3). Study results of Liu et al (82) have confirmed that recombinant spike protein (rS) based ELISA showed a superior sensitivity in detection of IgM antibodies. Another study performed with ELISA and ICA/LFIA (Immunochromatographic assay/Lateral flow immunoassay) have found the sensitivities of both assays to be individually higher compared to qPCR (quantitative PCR) results on the same study population. LFIA dependent tests have shown to perform uniformly with venous blood, fingerstick blood and plasma with 88.6% specificity (83). The false negative results (challenge faced) produced may be due to variation in the immune response of different individuals.Immunoassays mostly used S1 subunit, N and RBD proteins as targets, among which RBD and N protein-based assays were comparatively more sensitive in patients with milder symptoms (2,84). RDT (Rapid diagnostic tests) for detection of IgM and IgG reports suggest that IgG has better chance of detection with prolonged time period, PSO. Sensitivity and specificity of the immunological assays are considered imperative factors in the practical application of these methods (85,86).

ELISA assays have been improvised/modified in different studies which have results in successful increase in accuracy, sensitivity and validity of the assays (2). Combining ELISA with RT-PCR (Detection of both RNA and antibodies) have also shown increased sensitivities of up to 99.4% compared to 67.1% when performed in the absence of ELISA (2,87,88). Antibodies to N protein was found to decrease very early, thus is recommended to test for both S & N protein. High specificity is given by S protein as SARS-CoV-2 exhibits novel epitopes. ELSIA was reported to have 96% sensitivity to N protein for 15-30 days PSO. LFIA was demonstrated to have 57% clinical sensitivity, 100% specificity, and 69% accuracy for IgM and 81% sensitivity, 100% specificity, and 86% accuracy for IgG, respectively, whereas a test that detects both IgM and IgG has a sensitivity of 82% (2,3,83,84,88,89). COVID KAVACH Anti-SARS CoV-2 Human IgG ELISA, COVID KAWACH IGG MICROLISA, and ELI*Safe* 19™ antibody test kits are among the few ELISA tests approved by ICMR for diagnosis of SARS-CoV-2. BIOCARD Pro COVID-19 Rapid Antigen kit, STANDARD™ Q COVID-19 Ag Test, BIOCREDIT SARS-COV-2 ANTIGEN RAPID TEST CE IVD (Differential black gold conjugate technology) are among the few rapid detection tests (RDTs) approved by ICMR for Covid-19 diagnosis.

NON-MICROBIOLOGICAL INVESTIGATIONS

RADIOLOGICAL DIAGNOSIS: The false negative results in rRT-PCR has highlighted the importance of other diagnostic techniques and management criteria. CT-scan (Computed Tomography-scan) is a rapid, practical diagnostic tool, with high sensitivity, which can be used to follow-up patients before PCR tests turn negative. Studies have reported a higher sensitivity for CT-scans compared to PCR due to misdiagnosis and false negative results (10,40).But some of the major challenges faced are low specificity (25%), financial input, and requirement of technical expertise (1,58,80). COVID-19 pneumonia CT findings are ground glass opacity (GGO), fine reticular opacity, reverse halo sign, vascular thickening (20,21).According to most studies, GGO is the most predominant sign in symptomatic COVID-19 patients (2,10,20,21). Chest CT abnormalities have been detected in patient prior to PCR detected in endemic areas (20). About 75% of RT-PCR negative patients had positive chest CT findings. Although the specificity is found to be low 25% due to other related aetiologies causing similar CT findings, sensitivity 97% and accuracy 68% have found to be higher compared to PCR. many studies report a need for repetition of rRT-PCR for avoiding misdiagnosis (21,90,91). CXR (Chest radiography) is another suggested tool to decrease cross contamination by CT suites, while a retrospective study reports a low sensitivity of CXR 69% compared to PCR 91% and CT 97% (21). Further evidence is needed to validate the diagnostic technique. The features depend on stage of infection PSO. CT scans were more frequent in early stages of the disease (0-2days) with maximum lung involvement 10 days PSO (10). Some of the major challenges faced are low specificity (25%), financial input, and requirement of technical expertise (1,58,80).

CONCLUSION

The pandemic caused by SARS-CoV-2 is a new disease; thus, exploring of all opportunities is crucial to find the most effective means of diagnosis, and strategies to prevention and treat the increasing number of cases. New technologies, including molecular techniques, immunodiagnostic assays, POCTs, and radiology techniques have been developed as a benefit of these efforts, also including artificial intelligence, nanotechnology, and biosensors for clinical applications. Rapid TAT and feasibility are some of the major objectives for newer assays being developed. Stringent measures of prevention and detection should be followed which could help improving the detect ability of the virus. Most importantly, the combination of clinical presentation of patients, clear patient history, physical examination of patients, radiological diagnosis with appropriate laboratory tests are still the most potent arsenal against the disease.

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GLOSSARY OF ABBREVIATIONS:

SARS Covid-19	Severe Acute Respiratory Syndrome Corona VIrus Disease-2019
SARS-CoV-2	Severe Acute Respiratory Syndrome-Corona Virus-2
rRT-PCR	Real-time Reverse Transcription-Polymerase Chain Reaction
TAT	Turn Around Time
NAATs	Nucleic Acid Amplification Tests
CT	Computed Tomography
RT-LAMP	Reverse-Transcriptase loop-mediated isotheral amplification assay
POCTs	Point-of-Care tests
CRISPR	Clustered Regularly Interspaced Short Palindromic Sequences
AI	Artificial Intelligence
RNA	Ribo Nucleic Acid
RdRp	RNA dependent RNA polymerase
ss	Single stranded
ACE-2	Angiotensin Converting Enzyme – 2
RBD	Receptor-binding domain
SARS-CoV	Severe Acute Respiratory Syndrome-Corona Virus
MERS-CoV	Middle-East Respiratory Syndrome-Corona Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MDA	Molecular Diagnostic Assays
NASBA	Nuclei Acid Sequence-Based Amplification
LAMP	Loop-mediated isothermal AMPlification
PCR	Polymerase Chain Reaction
PSR	Polymerase Spiral Reaction
HAD	Helicase Dependent Amplification
TMA	Transcription Mediated Amplification
BAL	Bronchoalveolar Lavage
PSO	Post Symptom Onset
VTM	Viral Transport Medium
PBS	Phosphate Buffered Saline
BSC II	Bio-Safety Cabinet class II
CDC	Centers for Disease Control and Prevention
WHO	World Health Organisation
Ct	Cycle threshold
ICMR	Indian Council of Medical Research
qPCR	Quantitative polymerase chain reaction
iLACO	Isothermal LAMP based method for COvid-19
INSIGHT	Isothermal NASBA-Sequencing based hIGH-throughput Test
DNA	DeoxyriboNucleic Acid
DETECTR	DNA Endonuclease-Targeted CRISPR Trans Reporter
STOP	SHERLOCK Testing in One Pot
HUDSON	Heating Un-extracted Diagnostic Samples to Obliterate Nucleases
AIOD-CRISPR	All-In-One Dual Clustered Regularly Interspaced Short Palindromic Sequences
RPA	Recombinase Polymerase Amplification
CRISPR-nCoV	Clustered Regularly Interspaced Short Palindromic Sequences-novel COrona Virus
CREST	Cas-13, Rugged Equitable, Scalable Testing
LED	Light Emitting Diode
rS	Recombinant Spike protein
ELISA	Enzyme-Linked ImmunoSorbent Assay
ICA	Immuno Chromatographic Assay
LFIA	Lateral Flow Immuno Assay
RDT	Rapid Diagnostic tests
GGO	Ground Glass Opacity
CXR	Chest radiography

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