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REVIEW

COVID-19 Pandemic: Review of Contemporary and Forthcoming Detection Tools

Mumtarin Jannat Oishee [1]
Tamanna Ali [1]
Nowshin Jahan [1]
Shahad Saif Khandker [1]
Md Ahsanul Haq [1]
Mohib Ullah Khondoker [2]
Bijon Kumar Sil [3]
Halyna Lugova [4]
Ambigga Krishnapillai [4]
Abdullahi Rabiu Abubakar [5]
Santosh Kumar [6]
Mainul Haque [7]
Mohd Raeed Jamiruddin [8]
Nihad Adnan [9]

[1]Gonoshasthaya-RNA Molecular Diagnostic and Research Center, Dhaka, Bangladesh; [2]Gonoshasthaya Samaj Vittik Medical College, Dhaka, 1344, Bangladesh; [3]Gono Bishwabidyalay, Dhaka, 1344, Bangladesh; [4]Faculty of Medicine and Defence Health, National Defence University of Malaysia, Kuala Lumpur, Malaysia; [5]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University, Kano, 700233, Kano, Nigeria; [6]Department of Periodontology and Implantology, Karnavati University, Gandhinagar, 382422, India; [7]The Unit of Pharmacology, Faculty of Medicine and Defence Health Universiti Pertahanan, Nasional Malaysia (National Defence University of Malaysia), Kuala Lumpur, Malaysia; [8]Department of Pharmacy, BRAC University, Dhaka, 1212, Bangladesh; [9]Department of Microbiology, Jahangirnagar University, Dhaka, 1342, Bangladesh

Correspondence: Mainul Haque
The Unit of Pharmacology, Faculty of Medicine and Defence Health, Universiti Pertahanan, Nasional Malaysia (National Defence University of Malaysia), Kem Perdana Sungai Besi, Kuala Lumpur, 57000, Malaysia
Tel +60109265543
Email runurono@gmail.com

Abstract: Recent severe acute respiratory syndrome 2 (SARS-CoV-2) known as COVID-19, presents a deadly challenge to the global healthcare system of developing and developed countries, exposing the limitations of health facilities preparedness for emerging infectious disease pandemic. Opportune detection, confinement, and early treatment of infected cases present the first step in combating COVID-19. In this review, we elaborate on various COVID-19 diagnostic tools that are available or under investigation. Consequently, cell culture, followed by an indirect fluorescent antibody, is one of the most accurate methods for detecting SARS-CoV-2 infection. However, restrictions imposed by the regulatory authorities prevented its general use and implementation. Diagnosis via radiologic imaging and reverse transcriptase PCR assay is frequently employed, considered as standard procedures, whereas isothermal amplification methods are currently on the verge of clinical introduction. Notably, techniques such as CRISPR-Cas and microfluidics have added new dimensions to the SARS-CoV-2 diagnosis. Furthermore, commonly used immunoassays such as enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay (LFIA), neutralization assay, and the chemiluminescent assay can also be used for early detection and surveillance of SARS-CoV-2 infection. Finally, advancement in the next generation sequencing (NGS) and metagenomic analysis are smoothing the viral detection further in this global challenge.

Keywords: SARS-CoV-2, COVID-19, severe acute syndrome, diagnostic, detection-tools, immunoassay, amplification, gene-sequencing, cell-culture, microscopy

Introduction

The outbreak of unknown severe pneumonia, first reported in December 2019 in Wuhan city of China, has turned into a global pandemic and a rapidly emerging crisis.[1] A novel strain of coronavirus, similar to the acute SARS-CoV of 2002–2003, was responsible for the current pandemic (COVID-19). Henceforth, it was named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2),[2–4] there is no universally approved treatment protocol is available other than symptomatic treatment, although FDA recently provided emergency use authorization to Remdesivir.[5] Concerted effort globally paves the way into vaccine development. Currently, FDA has approved Pfizer-BioNTech COVID-19 Vaccine and Moderna COVID-19 vaccine for emergency use authorization.[6–8] Following FDA approval, other countries have also approved the vaccine for mass roll-out.[9–11]

Early diagnosis is of prime importance for disease containment and reducing transmission by quick isolation of patients and supporting critical treatment.

Real-Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) has been the most widely implemented SARS-CoV-2 diagnostic tool.[12] An increasing urge for point-of-care tests predisposes the availability of several other diagnostic tools and techniques. Point of care tests are generally updated technologies that include both the rapid and laboratory-free diagnosis, which plausibly would meet the urgency of the ongoing situation.[13] A recently published review paper on COVID-19 diagnostics by Yüce et al focused on the principles of available molecular and serological diagnostic tests along with explaining Emergency Use Authorization-issued commercial test kits while another evaluated the two mainstream of diagnostics – molecular and serology in the light of only FDA approved kits.[14,15] Moreover, review on the suitable sampling site or solely on the principles of diagnostics have also been published.[16,17] However, a comprehensive review covering all the available in-use and potential technologies for SARS-CoV-2 detection along with their strengths and drawbacks as well as suitable sampling sites is required to fill the gap. Our current review is intended to elaborate the missing piece pertaining to all

the up-to-date FDA-approved kits and discuss the emerging technologies with the potentials as supporting diagnostic tools.

Methodology

A thorough search was performed using online databases such as Google Scholar, PubMed, ScienceDirect independently, using such keywords as "COVID-19", "SARS-CoV-2", "Novel coronavirus", "n-CoV", "Diagnosis", "Diagnostic" without any year restriction. Further, required information were also collected from authentic websites (ie, WHO, FDA, etc.). Only English literature was included. References were managed using EndNote software (Version X7).

Diagnostic and Detection Methods

The deployment of different diagnostic tests (Figure 1) varies depending on resource facilities, the urgency in obtaining results, and the type of methods available. Presently available test procedures are categorized in Table 1.

Cell Culture and Microscopy

Cell culture, followed by microscopic technique, has been instrumental in pathogen identification and detection of
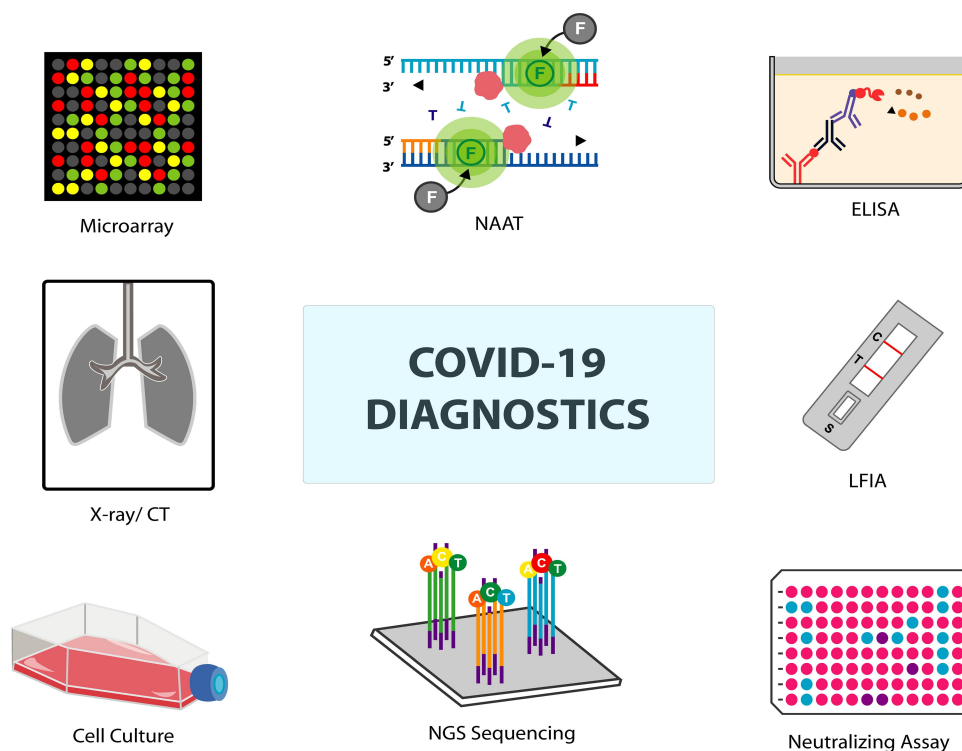
Figure 1 Graphical representation of various diagnostic assays of COVID-19.
Abbreviations: NAAT, nucleic acid amplification test; ELISA, enzyme-linked immunosorbent assay; CT, computed tomography; LFIA, lateral flow immunochromatographic assays; NGS, next-generation sequencing.

Table 1 Present COVID-19 Test Procedures

Mode of Detection	Detection Methods
Culture-based detection	• Virus propagation in cell lines
Radiology based technology	• X-Ray • Chest Computed Tomography
Molecular technology	• Real-Time RT-PCR • Isothermal amplification • CRISPR-Cas technology • Lab-on-chip
Immunoassay technology	• ELISA • Neutralization assay • Chemiluminescent assay • Lateral Flow Assay • Dip-stick
Technology under development	• Aptamer • Molecular imprinting technology (MIT) • Microarray • Biosensors • MALDI-TOF profiling
Sequencing technologies	• Sanger-sequencing • Next-generation sequencing • Nanopore sequencing

emerging diseases.[18–21] In the ongoing SARS-CoV-2 pandemic, these techniques are used for both diagnostics and research purposes.[22–27] In this approach, following the initial identification by sequencing, the novel virus's identity is confirmed through immunofluorescence microscopy using a cross-reactive viral antibody. Also, electron microscopy reveals its typical coronavirus-like morphology.[28,29] Microscopy and cell culture have also provided insights into its structural organization, cell tropism, pathogenesis, transmissibility and replication, and host–virus interaction.[23,30,31] However, WHO dictates that such culture and propagation of SARS-CoV-2 be carried out in biosafety level 3 laboratories only, making its wider use unrealistic in public and commercial settings.[32]

Radiology-Based Detection

Multiple studies are advocating radiologic imaging such as chest radiograph (CXR), chest ultrasound (US), lung ultrasound (LUS), and particularly chest computed tomography (chest CT) as complementary and, in some cases, as standard diagnosis method of COVID-19.[33–47] Patients who developed COVID-19 pneumonia shared similar lung abnormalities.[48] Peripheral ground-glass opacities, multiple

bilateral consolidation, crazy paving patterns, and reticular pattern are standard features found in chest CT of pneumonia patients.[41] Although China's health commission has defined radiologic features as a significant criterion for COVID-19 diagnosis, the scientific community remains uncertain.[39,49] A meta-analysis of chest CT's diagnostic accuracy for COVID-19 detection reported that it has an overall high sensitivity, especially in difficult epidemic situations.[38] Li and Xia said that CT-based findings could identify COVID-19 earlier than laboratory results with a 3.8% misdiagnosis rate.[40] The limitations of CT to be taken into consideration are: (i) CT features of COVID-19 patients can overlap with other infections including SARS-CoV, MERS-CoV, influenza, and H1N1, thus limiting its capacity in distinguishing between COVID-19 and other pneumonia, (ii) Typical CT manifestation of COVID-19 patients are only found in the later stage and mostly absent in earlier phase or asymptomatic patients, (iii) most of the studies supporting CT are based in China which warrants the need for more evidence-based studies in other regions.[49] Also, the success of the technique depends on the experience of radiologists. Conversely, the American College of Radiology (ARC) does not recommend CT to be used as a first-line test or for screening to diagnose COVID-19, and if used, should only be in cases where the patient has already been hospitalized with symptoms and clinical signs for CT.[50]

Nucleic Acid Amplification Tests

Generally, lack of understanding regarding the clinical manifestations, symptoms, and epidemiological features of SARS-CoV-2 hinders the containment and management efforts for the COVID-19 infection. However, such efforts can be better realized with a rapid, and accurate diagnosis. Molecular diagnosis by nucleic acid amplification of the virus paves the way. Specific probes and primers designed and updated with the enrichment of sequence data render nucleic acid amplification test (NAAT) an ideal mode of diagnosis. These tests supplement the demanding situation, including Real-Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR), isothermal amplification methods, and CRISPR-Cas based detection system.[51–53]

In addition to diagnose SARS-CoV-2 virus, FDA recently authorized emergency use tests. One of them is Abbott Diagnostics' ID NOW COVID-19, which detects the *RdRp* gene from nasal or nasopharyngeal swab (95% sensitivity and 100% specificity). FDA also approved TaqPath COVID-19 Combo kit (ThermoFisher-Applied

Biosystems), Smart Detect SARS-CoV-2 rRT-PCR Kit (InBios International, Inc.), Biomeme SARS-CoV-2 Real-Time rRT-PCR Test (Biomeme, Inc) which detect *Orf1ab, E, N, and S* gene (mentioned in Table 2). Besides several diagnostic kits like US Food and Drug Administration (USFDA) certified Sherlock Biosciences's CRISPR SARS-CoV-2 Rapid Diagnostic kit as the first CRISPR-based assay which is a combined approach done by using RT-PCR, CRISPR-based assay. Atila BioSystems Inc., on the other hand, has developed FDA approved iAMP COVID-19 detection kit, using isothermal amplification technology (Table 2).

Real-Time RT-PCR

Viral RNA is initially reverse transcribed into short cDNA using RNA-dependent DNA polymerase (RdDp), which is facilitated by appropriate primer specific for viral RNA genome sequences.[54] Real-time PCR technology allows monitoring the ongoing DNA amplification in real-time, yielding quantitative measurement of PCR amplicons; Amplification process repeats for around 40–45 cycles to detect viral DNA by using specific DNA probe tagged with a fluorophore and a quencher (TaqMan assays) or sequence nonspecific fluorogenic dye (SYBR Green).[55–57] While performing Real-Time RT-PCR, the total reaction is carried out in a single tube starting from cDNA synthesis to PCR amplification, referred to as a one-step procedure. Another way is a two-step procedure where cDNA synthesis and amplification are performed in separate tubes. Though the two-step procedure provides greater sensitivity, flexibility, and the advantage of multiple cDNA usage, the one-step approach is the preferred method for SARS-CoV-2 detection in diagnostic settings.[58] The technique is not tedious, involves less sample handling, thereby lowering the possibilities of cross-contamination between reverse transcription and PCR steps.[59,60]

For SARS-CoV-2 detection, several genomic regions are being used as targets for PCR amplification. This includes nucleocapsid (N), envelope (E), spike (S), genes, RNA-dependent RNA polymerase (RdRP), ORF1ab, or ORF8 regions.[61,62] For SARS-CoV-2 detection, several in-house and commercial assays have been developed based on either of these genes. Some manufacturers or authors suggest identifying one gene as a screening step and another gene detection as a confirmatory test.[62,63] On the contrary, three or more genes are suggested to be examined by others, requiring all genes to be detected for being optimistic.[64–66] For example,

researchers at the University of Hong Kong retained *N* gene identification as a screening procedure and *ORF1ab* detection as a confirmatory test.[67] The National Institute of Infectious Diseases of Japan recommends nested RT-PCR test targeting *ORF1a*, *S* gene, and *N* gene.[68]

Real Time-PCR by nasopharyngeal and respiratory samples is considered the gold standard for the qualitative detection of SARS-CoV-2 infection.[67] Several studies suggested that SARS-CoV-2 viral nucleic acid can be detectable at several locations, including sputum, nasopharyngeal swab, bronchial aspirates, bronchoalveolar lavage fluid (BAL), blood, anal swab, and urine.[12,69,70] In a study of 4800 cases in a single hospital in China, bronchoalveolar lavage fluid showed the highest rate of detection (100%) for the *ORF1ab* gene of SARS-CoV-2, followed by nasal and pharyngeal swabs samples (38.25%) and the sputum (49.12% positive rate).[71] However, another study conducted on sputum samples and nasopharyngeal swab samples collected from sixty-three subjects on the same day revealed that representatives from nasopharyngeal swab were distinct from sputum samples, which yielded some-false negatives. This may be because of specimen type and processing.[72] In addition to low viral count in the nasal and pharyngeal swab, variable and inherently unstable rRT-PCR test results make it difficult to diagnose SARS-CoV-2.[73] One of the most extensive head-to-head comparisons of nasopharyngeal (NP) and oropharyngeal/nares (OP/Na) swab for the detecting SARS-CoV-2 demonstrated that OP/Na sampling is a suitable alternative for COVID-19 detection in symptomatic ambulatory patients.[74] When the oral swab is negative during the late infection, identifying the virus in blood and anal swab may be an alternative strategy.[75] Wang et al detected SARS-CoV2 in multiple sites' specimens and found lower respiratory tract specimens positive in most cases.[16] A recent meta-analysis of 11 studies encompassing 3442 respiratory specimens reveals sputum testing having higher sensitivity for SARS-Cov-2 detection than the other respiratory sites.[76] Recently, saliva samples have also been claimed as a reliable tool for diagnosis by real-time rRT-PCR.[77] Furthermore, patients with mild COVID-19 symptoms may result in false-negative for nasopharyngeal samples.[78] The existence of SARS-CoV-2 at multiple sites necessitates specimen collection from different body locations at different stages of infection for diagnosis by rRT-PCR.[16,70] Suggested sampling sites and several studies' findings on suitable specimens for rRT-PCR are mentioned in Table 3.

Table 2 rRT-PCR Kits for SARS-CoV-2 Diagnostic

Test Kit Name	Manufacturer	Target	Sample Type	LOD	Sensitivity %	Specificity %	Approving Authorities	Reference
SalivaDirect	Yale School of Public Health, Department of Epidemiology of Microbial Diseases	*N1* gene	Saliva	6–12 copies/μL	94.1	90.9	FDA (EUA)	[242]
Biomeme SARS-CoV-2 Real-Time RT-PCR Test	Biomeme, Inc	*Orf1ab* and *S* gene	Nasopharyngeal, nasal, and oropharyngeal swab	1.8 copies/μL	96.9	98.3	FDA (EUA)	[243]
GWU SARS-CoV-2 RT-PCR Test	George Washington University Public Health Laboratory	*N* gene	Nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swabs	12.5 copies/μL	95	100	FDA (EUA)	[244]
Wren Laboratories COVID-19 PCR Test	Wren Laboratories, Inc	*N*-gene	Nasopharyngeal, oropharyngeal (throat), anterior nasal, and mid-turbinate nasal swabs	10 copies/μL	100	95	FDA (EUA)	[245]
SARS-CoV-2 Test Kit (Real-time PCR)	Xiamen Zeesan Biotech Co., Ltd	*ORF1ab* and *N* gene	Nasopharyngeal swabs, oropharyngeal swabs, anterior nasal and mid-turbinate nasal swabs	200 copies/mL	100	100	FDA (EUA)	[148]
CRL Rapid Response	Clinical Reference Laboratory, Inc.	*RdRp* gene	Saliva	0.25 viral copy/μL	100	100	FDA (EUA)	[148]
SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay	Sandia National Laboratories	*N* gene	Nasopharyngeal swab, anterior nasal swab, mid-turbinate nasal swab	6.25 copies/μL	96.67	100	FDA (EUA)	[148]
OraRisk COVID-19 RT-PCR	Access Genetics, LLC	*RdRp*	Nasopharyngeal swab and nasal swab specimens	15 viral copies/μL.	100	95.7	FDA (EUA)	[148]
In house RT-PCR protocol	Corman et al.	*E, RdRp*	Respiratory specimens	–	95	–	–	[62]
ID NOW COVID-19	Abbott Diagnostics (Worldwide)	*RdRp* gene	Nasal, throat or nasopharyngeal specimens	125 GE/mL	95	100	FDA (EUA)	[148]
ANDiS® SARS-CoV-2 RT-qPCR Detection Kit	3D Medicines	*ORF1ab, N,* and *E* genes	Respiratory specimens	5 copies/reaction	96	100	CE	[148]

(*Continued*)

Table 2 (Continued).

Test Kit Name	Manufacturer	Target	Sample Type	LOD	Sensitivity %	Specificity %	Approving Authorities	Reference
SARS-CoV-2 Real-Time PCR LAB-KIT	BIOMAXIMA S.A.	Orf1ab and N gene	Respiratory specimens, blood, urine, and stool	10 RNA copies	99	99	CE	[148]
Novel Coronavirus (SARS-CoV-2) Nucleic Acid Assay Kit (RT-PCR) (RUO)	Elabscience	ORF1ab and N gene	Respiratory specimens	1×10^3 copies/mL	100	100	CE	[148]
Smart Detect SARS-CoV-2 rRT-PCR Kit	InBios International, Inc.	E, N, and ORF1ab gene	Nasopharyngeal swab, anterior nasal swab, and mid-turbinate nasal swab samples	1.1×10^3 copies/mL	100	100	FDA (EUA)	[148]
ePlex SARS- CoV-2	GenMak Diagnostics (United States)	SARS-CoV-2 nucleic acid	Nasopharyngeal swab	1×10^5 copies/mL	95	100	FDA (EUA)	[148]
iAMP COVID-19 detection kit	Atila BioSystems, Inc.	ORF1ab and/or N gene	Nasal, nasopharyngeal, and/or oropharyngeal swabs	~2000 copies of viral RNA per swab.	>95	≥ 80	FDA (EUA)	[148]
CRISPR-based tests for SARS-CoV-2	Cepheid Sherlock Biosciences	ORF1ab and N gene	Nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate and BAL	6.75 cp/uL	100	100	FDA (EUA)	[148]
TaqPath COVID-19 combo kit	Rutgers Clinical Genomics Laboratory ThermoFisher-Applied Biosystems	ORF1b and N and S Gene	Nasopharyngeal swab (NP) and Bronchoalveolar lavage (BAL) samples	–	100	100	FDA (EUA)	[148]
BioFire RP2.1	BioFire	S and M gene	Nasopharyngeal swab	160 copies/mL	98	100	FDA (EUA)	[246,247]
Xpert® Xpress SARS-CoV-2	Cephid	N2 and E	Nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens	0.0050 and 0.0200 PFU/mL	99.5	95.8	FDA (EUA)	[248]
VitaPCR™	Credo Diagnostics	Specific N gene and a common conserved N gene	Nasopharyngeal sample	1484 copies/mL	99.3	94.7	–	[249]

Assay	Manufacturer	Gene target	Specimen	LoD			Approval	Reference
Lyra SARS-CoV-2 Assay	Quidel	Specific conserved non-structural polyprotein (pp1ab)	Nasal, nasopharyngeal (NP) or oropharyngeal (OP) swab	34,000 cp/mL	97	100	FDA (EUA)	[250]
Simplexa™ COVID-19 Direct Kit	Diasorin	ORF1ab and S gene	BAL, nasal swab, nasal wash/aspirate, NPS and saliva specimens	242 copies/mL	99.4	100	FDA (EUA)	[251]
Panther Fusion™ SARS-CoV-2	Hologic	ORF1ab Region 1 and 2	Nasopharyngeal, nasal, oropharyngeal swab specimens and LRT specimens	1×10-2 TCID50/mL	100	100	FDA (EUA)	[252]
LabCorp COVID-19 RT-PCR	LabCorp	N1, N2 and N3 gene	Nasal swab	3.125 cp/µL	100	100	FDA (EUA)	[253]
ARIES® SARS-CoV-2 PCR test	Luminex	ORF1ab and N gene	Nasopharyngeal swab	1500 copies/mL	100	100	FDA (EUA)	[254]
SARS-CoV-2 DETECTR Reagent Kit	Mammoth Biosciences, Inc.	N gene and E gene	Nasopharyngeal and oropharyngeal swab,	10 copies/mL	95	100	FDA (EUA)	[255]
Accula SARS-CoV-2 Test	Mesa Biotech	N gene	Nasal swab	150 copies/mL	68	100	FDA (EUA)	[256]
Fortitude COVID-19 RT-PCR Test	MiRXES	ORF1ab region 1 and ORF1ab region 2	Nasopharyngeal and oropharyngeal swabs, washes and aspirates	10 copies/reaction	100	100	–	[257]
QIAstat-Dx Respiratory SARS-CoV-2 Panel	Qiagen	Orf1b poly gen (Rdrp gene) and E gene	Nasopharyngeal swab	500 copies/mL	100	100	FDA (EUA)	[258]
Cobas® SARS-CoV-2 Test	Roche	ORF 1a/b and E gene	Nasal, nasopharyngeal, and oropharyngeal swab	25 copies/mL	100	100	FDA (EUA)	[259]
Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit	Sansure Biotech	ORF1ab and N gene	Nasal, Throat, and Nasopharyngeal swabs	200 copies/mL	94.34	98.96	FDA (EUA)	[260]
STANDARD M nCoV Real-Time Detection kit	SD Biosensor	ORF1ab gene and E gene	Nasopharyngeal swab, Oropharyngeal swab, Sputum	0.5 cp/µL	100	100	FDA (EUA)	[261]

(*Continued*)

Table 2 (Continued).

Test Kit Name	Manufacturer	Target	Sample Type	LOD	Sensitivity %	Specificity %	Approving Authorities	Reference
Allplex™ 2019-nCoV Assay	Seegene	E and N gene	Sputum, Nasopharyngeal swab, Nasopharyngeal aspirate, Bronchoalveolar lavage, Throat swab	5000 copies/mL	100	96.84	FDA (EUA)	[262,263]
Viracor SARS-CoV-2 assay	Viracor Eurofins Clinical Diagnostics	N1 and N2 gene	Nasopharyngeal swab, nasal swab, nasopharyngeal wash, nasal wash, oropharyngeal swab and bronchoalveolar lavage	73 copies/mL	100	100	FDA (EUA)	[264]

Abbreviations: S, spike; N, nucleocapsid; RdRp, RNA-dependent RNA polymerase; E, envelope; BAL, bronchoalveolar lavage; LRT, lower respiratory tract; LOD, limit of detection; FDA, Food and Drug administration; EUA, emergency use authorization.

The rRT-PCR testing for COVID-19 holds various advantages. Its sensitivity and specificity are a method of choice in the early stages of infection. It provides valuable information during the acute phase of the disease when a person has not yet seroconverted. False-negative results, low stability, tedious working procedures, and lack of trained personnel and proper facilities are some of the limitations inherent with the rRT-PCR detection technique.[79] Furthermore, test accuracy is affected by different variables such as specimen collection site, sampling time point, procedure, and viral load, ie, LOD (Limit of Detection) of tests. These reasons result in decreased rRT-PCR sensitivity by 20% to 70%.[71,80–83] The highest sensitivity, ie, lowest false-negative rate (20%), is observed if the test is done on day eight after exposure, which approximates to 3 days after symptom onset, as depicted in Figure 2.[82] Co-detection of other respiratory coronaviruses and low stability of RNA may account for false-negative results, too.[78,84]

Furthermore, the detection of multiple loci and differences in reagents' sensitivity to different genetic regions thwart the tests' accuracy and consistency.[85,86] However, more research needs to be addressed to attain an accurate portrait of sensitivity and specificity. To overcome the limitations, manufacturers are now improvising current rRT-PCR test procedures with increased fidelity, as mentioned in Table 2. Considering all the factors, the US Food and Drug Administration (FDA) has deduced that negative real-time RT-PCR test result does not necessarily substantiate the absence of SARS-CoV-2 infection and shall not be the only factor in patient management decisions.[87,88] Re-testing should be taken into consideration in consultation with public health authorities.[89]

Isothermal Amplification Technologies

Molecular diagnosis in the laboratory settings by conventional PCR methods needs to be swapped with Point-Of-Care (POC) tests to increase test frequency and eliminate prolonged turn-around time. Such a test facilitates rapid, robust detection onsite in a cost-effective and user-friendly manner. One such promising molecular point-of-care test method includes Isothermal Nucleic Acid Amplification Techniques.[90,91]

LAMP

Isothermal amplification methods allow the circumvention of the need for sophisticated thermal cyclic equipment, costly reagents and facilities, and time-consuming steps of real-time RT-PCR. Reverse transcription

Table 3 Recommended Plausible Sampling Sites by Several Studies for Real-Time RT-PCR

Sampling Sites	Sample Size/Study Participants	Findings	Reference
Sputum and nasopharyngeal swab	8274 patients	Nasopharyngeal swab samples with the more precise result than sputum	[72]
Pharyngeal swabs	610 patients	The variable and potentially unstable result from Pharyngeal swabs	[73]
Oral swabs, anal swabs, and blood samples	178 subjects	Blood and Anal swab positive when oral swab negative at the later stage of infection	[75]
Nasopharyngeal swabs, sputum, blood, feces, and anal swabs	132 patients	Rate of positive in sputum higher at an early stage, the digestive tract nucleic acid (fecal/anal swab) is found at a later stage of infection	[236]
Nasal swab, throat swab, sputum	76 patients	Sputum induction suggested being more helpful than throat swabs in convalescent patients	[237]
Bronchoalveolar lavage fluid, Pharyngeal swabs, blood, sputum, feces, urine, and nasal samples	1070 specimens from 205 patients	Bronchoalveolar lavage fluid specimens with the highest sensitivity	[16]
Urine, blood, anal swabs, and oropharyngeal swabs	74 patients	Sample collection from multiple sites suggested by the author.	[70]
Sputum, throat swab, urine, and stool	82 patients	Viral load in sputum higher than throat swab, load in anal swab less than respiratory samples.	[12]
Nasopharyngeal swabs, oropharyngeal swabs, and sputum	291 specimens from 43 patients	Sputum with the highest detection of SARS-CoV-2, Nasopharyngeal swabs, and oropharyngeal swabs with similar yield	[238]
Saliva	200 specimens	Saliva pooling as a plausible alternative method in ambulatory patients in a low prevalence setting.	[239]
Gargle lavage, Nasopharyngeal, and oropharyngeal swab	50 patients	Gargle lavage can be a viable alternative for swab sample	[240]
Upper respiratory tract samples, lower respiratory tract samples, blood, and others	113 studies with a median sample size of 15 patients in all studies	Viral load in sputum higher than the other upper respiratory tract sample at the early stage of infection	[241]

Loop-mediated isothermal amplification (RT-LAMP) is one such alternative rapid nucleic acid amplification method that can amplify DNA in 13–20 minutes by using DNA polymerase strand displacement activity at fixed temperature of 60–65°C while employing 4–8 specially designed primers.[92–95] Furthermore, reaction endpoint can be visually observed via photometry, where the turbidity is caused by precipitation of magnesium pyrophosphate or fluorescent dye.[96–98] The advantage of detecting RNA directly from unpurified samples while being a rapid, robust, and relatively simple test has made it a method of choice for diagnosis of SARS-CoV-2.[99–103] In one study, primers specific for nucleocapsid genes were found to detect 100 copies of RNA per reaction and were regarded as most sensitive. RT-LAMP test reported by Zhang et al virtually determined viral RNA at levels of approximately 4.8 copies per μL, which was comparable with rRT-PCR.[104] Recently, a novel real-time RT-LAMP assay has been developed with colorimetric versions performed in a single tube. The assay shows high sensitivity, a LOD of 118.6 copies/reaction, and reasonable specificity regarding common respiratory viruses.[93] Direct swab–to–RT-LAMP assay has also been developed faster and convenient, with less sensitivity and robustness.[105] Multiplex RT-LAMP coupled with lateral flow biosensor has been devised, exhibiting 100% sensitivity and specificity
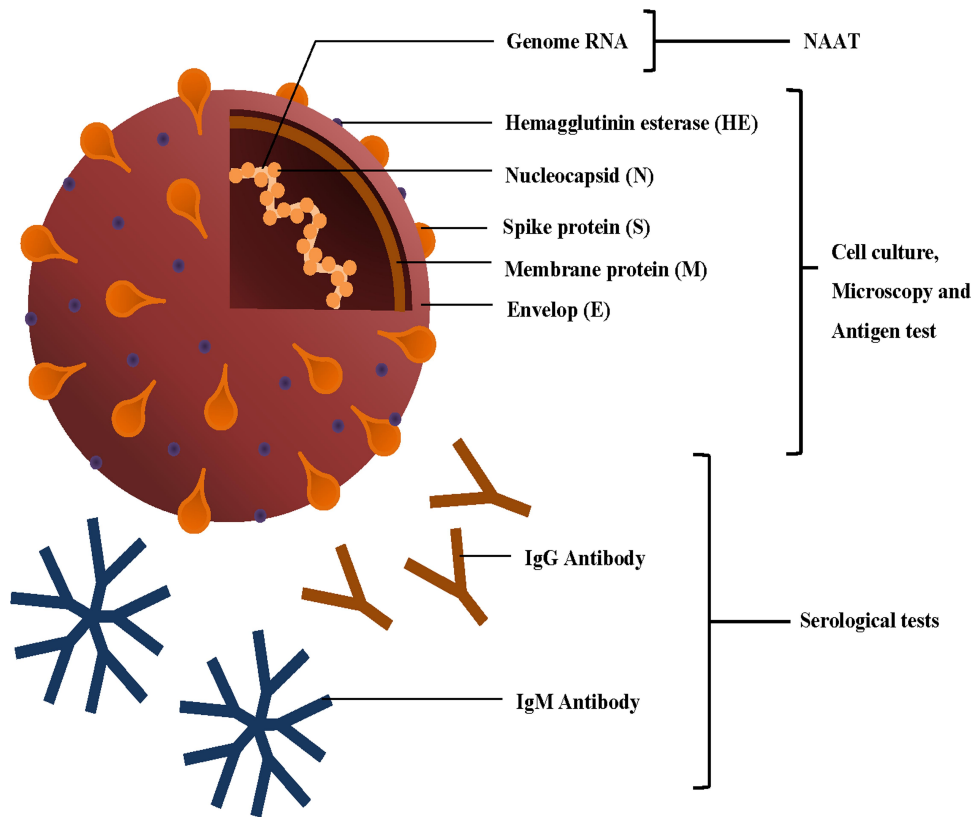
Figure 2 Simplified structure of SARS-CoV-2 and different tests based on viral RNA, proteins, and antibodies (ie, IgM and IgG) against viral antigens.

requiring 1 hour to attain results.[106] Several studies comparing RT-LAMP with rRT-PCR demonstrated >90% sensitivity while being simple, reliable, and high-throughput than conventional rRT-PCR methods.[100,107–109]

Rolling Circle Amplification-Based Method

Rolling circle amplification (RCA) is another efficient isothermal DNA amplification diagnostic method that employs a target-mediated padlock probe (PLP) and high fidelity DNA polymerase with strand-displacing capacities phi 29 or *Bst* DNA polymerase.[110–112] The method is highly sensitive and robust amplifying signals by 10^9 fold for each circle within 90 min.[113,114] RCA and its variations are combined with different detection systems such as fluorescence detection, colorimetric assay, chemiluminescent assay, or electrical signal for target nucleic acid detection.[115,116] Its ultrahigh sensitivity at the femtomolar level and specificity discriminating even single-base variants and eliminating complex instruments make it an attractive molecular diagnostic tool.[117] Previously, RCA was performed in liquid and solid phases for SARS-CoV detection and applied to different biosensors to detect

other targets.[118–121] Recently, the Circular to Circular Amplification (C2CA) strategy, a modification of RCA, has been proposed as a promising technique to detect SARS-CoV-2 viral RNA. The proposed approach claims to achieve the detection limit at the sub-femtomolar level through simplified operation compared to previously reported C2CA-based sensors.[122]

Other Isothermal Amplification Methods

Other available isothermal amplification techniques include nucleic acid sequence-based amplification (NASBA), recombinase polymerase amplification (RPA), transcription-mediated amplification (TMA), multiple strand displacement amplification (SDA), isothermal helicase-dependent amplification (HDA), all of which can be used for nucleic acid detection.[123,124] These varieties of testing methodologies are easy to operate, requiring a heat-block or water bath, providing constant temperature.[125]

Nucleic acid sequence-based amplification (NASBA), known as self-sustained sequence replication (3SR), has already been deployed in detecting different bacteria, parasites, and viral agents, including SARS-CoV.[124,126–134] A testing approach has been proposed known as INSIGHT

(Isothermal NASBA-Sequencing based hIGH-throughput Test) for SARS-CoV-2 detection comprising testing at two stages, Nucleic Acid Sequence-Based Amplification (NASBA) at 41° C and Next Generation Sequencing (NGS) technologies.[135] This technique facilitates rapid fluorescence read-out, or lateral-flow read-out, specific to sequence at the first stage, further ascertained by NGS. This method provides high accuracy, detecting 10–100 copies/reaction in crude saliva lysate. Recently, transcription-mediated amplification (TMA) by Hologic Panther (non-Fusion) Aptima platform demonstrated higher analytical sensitivity (98%) and specificity (100%) for SARS-CoV-2 detection in nasopharyngeal swab compared to RT-PCR.[136]

A diagnostic test based on recombinase polymerase amplification (RPA), named Fast Isothermal Nucleic Acid Detection (FIND), has been developed for COVID-19 detection. RPA primers designed against both the *N* gene and the *S* gene of SARS-CoV-2 can detect viral RNA as low as two viral particles/µL.[137] Another sensitive approach introduced based on RT-RPA is reverse transcription–enzymatic recombinase amplification (RT-ERA) for SARS-CoV-2 identification at a low detection limit of 1 copy.[138] Recently, RT-RPA assay is reported having a 95% detection probability being one of the fastest detections by isothermal amplification method.[139] In addition to all, helicase-dependent isothermal amplification has also been successfully implemented in various diagnosis purposes, implementation of which is yet in its infancy for SARS-CoV-2 detection.[140]

CRISPR-Based Diagnostics

Clustered regularly interspaced short palindromic repeats (CRISPR) based nucleic acid detection can provide a rapid, inexpensive, ultrasensitive diagnostic tool in point-of-care diagnosis to detect pathogens and monitor the disease.[141,142] The CRISPR systems proposed in SARS-CoV-2 diagnosis utilize collateral catalytic activity of different Cas enzymes such as Cas 12, Cas-12a, Cas-13, and Cas-9 to detect target RNA. Labeled nucleic acid specifically amplified with conventional PCR or other isothermal amplification act as a substrate for these effector molecules. crRNA guided Cas enzymes, upon target recognition, cleave nucleic acid, and produce detection signals which can be read out through fluorescence visualization.[143–147]

SHERLOCK and DETECTR are two CRISPR-based SARS-CoV-2 commercial kits launched recently.[148] SHERLOCK (Specific High Sensitivity Enzymatic

Reporter UnLOCKing) technology targets the *S* and *Orf1ab* gene to identify the presence of COVID-19. The system uses Cas 13, catalytically inactive, in the presence of two or more mismatches in the crRNA target duplex. This makes it more specific for the target pathogen and eliminates any possibility of false positivity.[147] DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) is an assay developed by Mammoth Biosciences.[143] The gRNA of this system is designed to target the E region of three SARS-like coronaviruses (ie, SARS-CoV-1, SARS-CoV2, and bat SARS-like coronavirus) and the N region of SARS-CoV-2 specifically to distinguish this virus with no cross-reactivity from others and can detect ten copies of RNA/µL with 95% sensitivity and 100% specificity.[143] On the other hand, Lucia et al used the same enzyme system targeting *RdRp, ORF1b,* and *ORF1ab* genes of SARS-CoV-2.[149] Another CRISPR-Cas-based detection system reported is FELUDA, an acronym for FnCas9 Editor Linked Uniform Detection Assay.[146,150] FnCas9 is an ortholog that can recognize mismatches within DNA with high sensitivity. FELUDA uses this property for identifying conserved regions (NSP8 and Nucleocapsid phosphoprotein) of SARS-CoV-2.

Ding et al described a system that combines a pair of Cas12a-crRNA complexes and RPA amplification components within a single reaction to detect both HIV-1 and SARS-CoV-2, named All-in-One Dual CRISPR-Cas12a (AIOD-CRISPR) Assay.[144,151] The all-in-one reaction system without the prerequisite preamplification step makes it advantageous over other CRISPR-based detection systems. Another attractive point of this technique is the use of dual CRISPR RNA that enhances assay sensitivity. The test can detect as low as 1.2 copies of DNA targets and 4.6 documents of RNA targets in 40 min. The report claims that AIOD-CRISPR can be developed as one-step RT-AIOD-CRISPR with the addition of AMV reverse transcriptase to detect RNA targets without cDNA preparation.[144]

Microfluidic Biochip or Lab-on-a-Chip Technology

Various types of biochip or lab-on-chip-based techniques are used to detect pathogenic bacteria and viruses in POC settings.[152] Centrifugal microfluidic biochip or centrifugal microfluidic bio disk, a type of lab-on-a-chip technology known as lab-on-a-disc, is a handy diagnostic tool for virus detection. This type of microfluidic biochip can incorporate sample preparation, reactions, and detection

of nano-size molecules on a micron-scale chip. In this assay, molecules are separated by centrifugal force followed by viral lysis using chemical and physical methods to release genomic and proteomic materials. The viral RNA is then amplified using various amplification methods. Tools interpret results based on binary or quantitative assessment of the genomic contents. A change visualizes the interpretation within the reaction chamber (color change) or electrochemical assessment.[153] A recent study by Zhuang et al showed Filmarray®, a BioFire™ microfluidic assay device that combines nucleic acid extraction, purification, and PCR amplification a single chip, provide results with precise detections.[154] Previously, the device was used for Ebola Virus detection.[155] The FDA recently provided EUA with a COVID-19 test kit based on the microfluidic technology.[154]

Detection of SARS-CoV-2 by microfluidic technology has made significant progress with improved LOD, time, and speed along with high sensitivity and high specificity.[156] Microfluidic assays' advantages are primarily little reagents, and samples required to quickly obtain precise results, making it suitable for POC.[153,154] Meticulous sample preparation requiring enrichment and avoidance of contamination for accurate results are the limitations hindering its adaptation.[157]

Immunoassay-Based Detection

Immunoassay tests detect the presence of a particular antibody or antigen in the biological specimen, such as blood, serum, saliva, urine, sputum, and others from individuals. These tests are of utmost importance in defining a person's immune status (present or past infection) irrespective of symptoms development.[158,159] Although the ongoing pandemic rRT-PCR is considered the reference standard to diagnose COVID-19, serological analysis is crucial to identify patients with a viral load below the rRT-PCR detection limit.[160–163] Serosurveillance at the population level is critical for public health response in pandemic situations and the successful implementation of a vaccine program.[164] Furthermore, in the present pandemic, immunoassays play a pivotal role in measuring immune response, tracing contact transmission, and defining disease burden and disease scope.[161,165] A rapid antigen test with higher predictive value has been suggested for screening strategy.[166] Still, indirect diagnosis by antibody testing is more useful in this situation as it provides a more considerable window period than antigen testing in identifying the disease.[160] The new coronavirus generates

specific antibody responses against two major SARS-CoV-2 structural proteins: Nucleocapsid (N) protein and Spike (S) protein, subsequently making them important targets for developing serological testing.[167,168]

The spike is a class I glycoprotein, consisting of S1 and S2 subunit. S1 containing receptor-binding domain (RBD) is responsible for recognizing host ACE-2 receptors, while S2 is responsible for fusion.[167] Although hACE-2 is the entry receptor for SARS-CoV-2, an array of protease and kinase enzymes such as TMPRSS, TPC2, PIKFYVE, and CTSL are also significant for viral entry.[169,170] Alternative receptors, such as CD147 and integrins, have been reported as well.[160,171] Serum antibody response is mainly generated against N and different spike proteins.[160,164] The different dynamics of specific IgA, IgG, and IgM responses (Figure 3) have been reported with a recent study suggesting that IgA dominates early humoral response against SARS-CoV-2 with a significant contribution towards virus neutralization albeit at a low level, decreasing after only one month of presentation.[15,158,160,168,172] IgG and IgM seroconversions occur in the first week or within 20 days after symptom onset. Three types of seroconversions are observed: (i) IgG and IgM develop simultaneously, (ii) IgG comes earlier than IgM, (iii) IgM comes earlier than IgG.[158] While the IgM level reaches its peak in the second week and goes into decline, IgG continues its increase into the third week, attaining 100% seroconversion while persisting for three months.[160,164,168] If combined, IgG and IgM against both spike and nucleocapsid can detect up to 75% of patients within the first week.[168]

Four major types of immunoassays are utilized for serodiagnoses of SARS-CoV-2 are enzyme-linked immunosorbent assay (ELISA), automated chemiluminescence immunoassay (CLIA), lateral flow immunoassay (LFIA), and the neutralizing antibody assay. These assay methods target a particular protein antigen, total antibody, or all isotypes simultaneously and provide quantitative, qualitative, or semi-qualitative data.

Commercially manufactured serology tests to detect SARS-CoV-2 antibodies are available through healthcare providers and professional laboratories and many of them have been recently certified by the US Food and Drug Administration. EUA issued Anti-SARS-CoV-2 ELISA (IgG) developed by EUROIMMUN US Inc. is a widely used ELISA kit to detect SARS-CoV-2 antigen S1 specific IgG, showed sensitivity 50–100% sensitivity and 98.5% specificity. Several other serology tests were approved by FDA like VITROS Immunodiagnostic Products Anti-
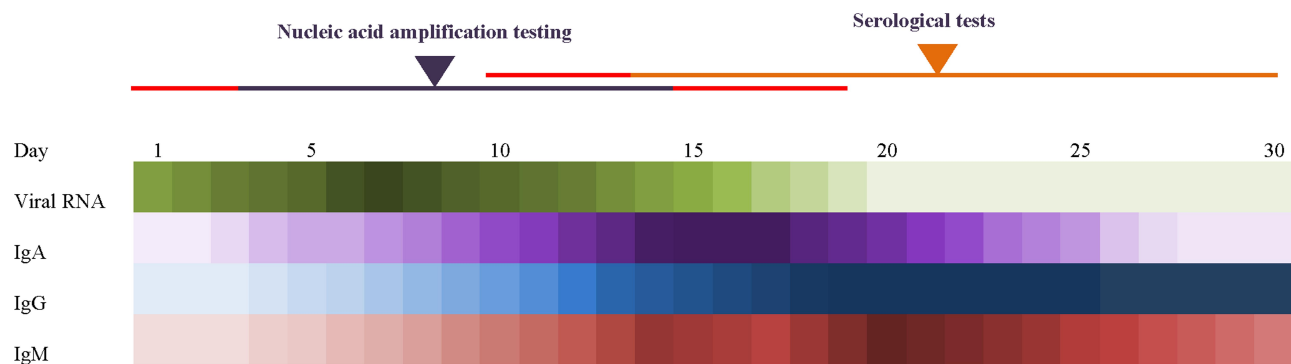
Figure 3 Expression of viral RNA load and antibody titer (IgA, IgM, and IgG) in patients over 30 days.[82,158,172,176,236]

SARS-CoV-2 IgG Reagent Pack (Ortho Clinical Diagnostics, Inc.) adopting CLIA technique, RapCov Rapid COVID-19 Test (ADVAITE, Inc.) following lateral flow technique, etc.[173,174]

BD Veritor™ System (Becton, Dickinson, and Company) adopting Chromatographic Digital Immunoassay, LumiraDx SARS-CoV-2 Ag Test (LumiraDx) pursuing microfluidic technique and most widely used Sofia SARS Antigen FIA manufactured by Quidel Corporation following lateral flow assay, are some FDA accredited serological antigen tests. All of them identify SARS-CoV-2 nucleoprotein showing sensitivity 95–100%.[173]

Enzyme-Linked Immunosorbent Assay

ELISA is broadly used as a serological test to detect COVID-19 positive patients. These methods use an antigen-antibody-substrate binding concept to detect specific IgA, IgM, and/or IgG against S (mainly RBD) and/or N viral proteins of SARS-CoV-2 as these are highly immunogenic, being the target of many neutralizing antibodies.[175–177]

Commercial ELISA kits incorporating the double-antibody sandwich immunoassay principle are used to detect SARS-CoV-2 IgM and IgG.[176,178] Table 4 lists examples of available kits for both research use only (RUO) and in-vitro diagnostics (IVD). However, only a few have received Emergency Use Authorization (EUA) from the US Food and Drug Administration (FDA). IgG and IgM ELISA kits diagnose the body's immune status and the past infection, respectively, whereas IgA ELISA kits detect early disease.[75,163,179]

In addition to antibody detection kits, many commercial kits for detecting SARS-CoV-2 pathogens have been developed. A commercial ELISA kit designed by Sino Biological for SARS-CoV-2 antigen detection is based on the principle of SARS-CoV-2 Spike RBD protein detection by capturing it with HRP labeled anti-SARS-CoV-2 Spike RBD monoclonal antibody.[180,181]

Serum investigation using ELISA provides convenience for determining the actual morbidity and infection status in the population. Furthermore, the ELISA technique's fast, accurate, and highly sensitive results can also be used to map antibody reaction kinetics against SARS-CoV-2.[86,182,183] Though ELISA kits can test multiple samples in a single run, they lack point-of-care applicability. The nonspecific binding of the antibody or antigen to the plate may lead to false high-positive results.[184]

Chemiluminescence Immunoassay (CLIA)

CLIA is a variation of enzyme-linked immunosorbent assay employed in serodiagnosis of SARS-CoV-2. Due to its high concordance with ELISA, the capacity to test a large volume of sample, high detection sensitivity (100%) for total antibody, this automated system is an attractive choice of the assay in outbreak situations.[176,185] Numerous studies have used CLIA to assess dynamic antibody response against SARS-CoV-2.[158,185–193] Table 4 lists currently available commercial and laboratory-developed chemiluminescence assays. RBD, S, N, S+N, and S1+S2 are used as capturing agents to detect specific serum antibodies in COVID-19 patients.[185,187–195] Studies validated and assessed these serological assays' clinical performance by analyzing the humoral response against SARS-CoV-2. Inadequate sample size, the anomaly of sample distribution, lack of data on cross-reactivity, the inconsistent time interval of sample collection is some of the limiting factors of these studies.[185,189–191,193]

Zhang et al used a commercial CLIA to study 736 subjects to observe the rising level of serum immunoglobulins along with disease progression.[193] Receiver operating

Table 4 Immunoassay Based Kits for SARS-CoV-2 Diagnostic

Test Name	Manufacturer	Capturing Agent	Target	Sample Type	Sensitivity %	Specificity %	Reference
Elisa kits ((for research) purpose)							
SARS-CoV-2 IgG ELISA Kit	Creative Diagnostics	SARS-CoV-2 whole virus lysate antigen	IgG	Serum, plasma	100	100	[198,266]
SARS-CoV-2 IgM ELISA Kit		Anti-μ chain monoclonal antibody	IgM		100	100	
SARS-CoV-2 Antigen ELISA Kit		SARS-CoV-2 Antigen ELISA Kit	SARS-CoV-2 N protein	Serum	No Data	No Data	[266]
In-house indirect ELISA for IgM, IgA, and IgG antibodies	In-house	Purified rNs	IgM	Serum	No Data	No Data	[163]
			IgA				
			IgG				
In-house ELISA-IgG Test	In-house	The rAg of SARS-CoV-2 Rp3	IgG	Serum	No Data	No Data	[75]
GR COVID-19 IgG Rapid ELISA Test kit (Nucleocapsid)	In-house	N protein	IgG	Serum	94.9	97.1	[267]
GR COVID-19 IgG Rapid ELISA Test kit (Spike & RBD)	In-house	S1 protein	IgG	Serum	94.9	100	[268]
		RBD protein			96.2	99	
KT-1032 EDI™ Novel Coronavirus COVID-19 IgG ELISA Kit	Epitope diagnostics	N protein	IgG	Serum	95	99	[198]
KT-1033 EDI™ Novel Coronavirus COVID-19 IgM ELISA Kit		Anti-human IgM specific antibody	IgM		95	98–100	

Category	Test	Manufacturer	Target	Antibody	Specimen	Sensitivity (PPA)	Specificity (NPA)	Ref
Elisa kits (for diagnostic purpose)	Coronavirus COVID-19 IgG ELISA Assay	Eagle Bioscience, Inc.	N protein	IgG	Serum	100	100	[266]
	Coronavirus COVID-19 IgG ELISA Assay	Eagle Bioscience, Inc.	N protein	IgG	Serum	100	100	[266]
	Novel Coronavirus COVID-19 IgM ELISA Assay	IBL International	Anti-human IgM specific antibody	IgM	Serum	45	100	[266]
	Anti-SARS-CoV-2 ELISA IgA	Euroimmun	S1 domain	IgA	Serum	50–100	92.5	[179]
	Anti-SARS-CoV-2 ELISA IgG			IgG		34–100	98.5	
	EDI™ Novel Coronavirus COVID-19 IgG ELISA Kit	Epitope Diagnostics	SARS-CoV-2 N	IgG	Serum	98.4 (PPA)	99.8 (NPA)	[269]
	EDI™ Novel Coronavirus COVID-19 IgM ELISA Kit		Anti-human IgM specific antibody	IgM		92.2 (PPA)	100 (NPA)	[269]
Lateral Flow system	RapCov Rapid COVID-19 Test	ADVAITE, Inc.	–	IgG	Whole blood	90.0	95.2	[270]
	VivaDiag COVID-19 IgM/IgG Rapid Test	Everest Links Pte Ltd	–	IgG/IgM	Serum/whole blood	18.4	91.7	[271]
	DPP® COVID-19 IgM/IgG System	Chembio Diagnostics Systems Inc.	SARS-CoV-2 N	IgG	Serum/plasma/whole blood	92	93.3	[272]
				IgM		82	91.7	
	COVID-19 IgG/IgM Rapid Test	CTK Biotech	–	IgG / IgM	Serum/plasma/whole blood	97.1	97.8	[273]
	COVID-19 IgM/IgG Rapid test	BioMedomics	–	IgM / IgG	Serum/plasma/whole blood	88.66	90.63	[274]
	qSARS-CoV-2 IgG/IgM Rapid Test	Cellex Inc	SARS-CoV-2 N & S	IgM, IgG	Serum/plasma/whole blood	93.8	96	[15]
	COVID-19 IgG/IgM Point of Care Rapid test	Aytu Biosciences/Orient Gene Biotech	–	IgG/IgM	Serum/plasma/whole blood	IgG: 96.7 / IgM: 86.7	IgG: 98 / IgM: 99	[198]
	COVID-19 IgG/IgM LF	AdvaGen Biotech, Brazil	–	IgG/IgM	Serum/plasma/whole blood	IgG: 100 / IgM: 85	99	[198]

(Continued)

Table 4 (Continued).

Test Name	Manufacturer	Capturing Agent	Target	Sample Type	Sensitivity %	Specificity %	Reference
SARS-CoV-2 rapid test of IgG-IgM combined antibody kit	China	COVID-19 rAg (MK201027)	IgG/IgM	Serum/plasma/ whole blood	88.66	90.63	[200]
Gold nanoparticle lateral flow system	–	SARS-CoV-2 N	IgM	Blood	100	93.3	[201]
Gold nanoparticle lateral flow system	–	SARS-CoV-2 N	IgM	Blood	100	93.3	[174,175]
ALLTEST 2019-nCoV IgG/IgM Rapid Test	Hangzhou ALLTEST Biotech Co., Ltd., China	SARS-CoV-2 N	IgG/IgM	Blood	IgG:>99.9 IgM:90.9	IgG: 98 IgM: 96	
Dynamiker 2019-nCoV IgG/IgM Rapid Test	Dynamiker Biotechnology (Tianjin) Co., Ltd., China	SARS-CoV-2 N	IgG/IgM	Blood	IgG: 89.2 IgM: 87.8	IgG: 100 IgM: 100	
ASK COVID-19 IgG/IgM Rapid Test	TONYAR Biotech Inc., Taiwan	SARS-CoV-2 Spike	IgG/IgM	Blood	NA	NA	
Wondfo SARS-CoV-2 Antibody Test	Guangzhou Wondfo Biotech Co., Ltd., China	–	IgG/IgM	Blood	86.4	99.6	
Wantai SARS-CoV-2 Ab ELISA	Beijing Wantai Biological Pharmacy Enterprise	SARS-CoV-2 RBD	IgM / IgG	Serum	93	100	[175]
2019-nCoVAbTest (Colloidal Gold)	INNOVITA Biological Technology Co., China	Mouse anti-human monoclonal IgG antibodies / Mouse anti-human monoclonal IgM antibodies	IgG / IgM	Serum/plasma/ whole blood	87.3	100	[275]
COVID-19 IgG/IgM Rapid Test Cassette	Zhejiang Orient Gene Biotech Co., China	Anti-human IgG antibodies / Anti-human IgM antibodies	IgG / IgM	Serum/plasma/ whole blood	97.2 / 87.9	100 / 100	[275]
Sofia SARS Antigen FIA	Quidel Corporation	No Data	SARS-CoV-2 NP	Nasopharyngeal and nasal swab	100	99.4	[173]

CIA system		Monoclonal anti-SARS-CoV-2 antibody	NP	Nasopharyngeal swab	80 (PPA)	100 (NPA)	
VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack	Ortho Clinical Diagnostics, Inc.	–		Nasopharyngeal swab	80 (PPA)	100 (NPA)	[276]
Anti-coronavirus (SARS-CoV-2) IgM antibody detection kit	Bioscience Diagnostic Technology	–	IgM	Serum	82	93.85	[277]
SARS-CoV-2 IgG antibody detection kit		–	IgG		86	96.92	
ND	Shenzhen YHLO Biotech.	S+N	IgG	–	100	97	[193]
		S+N	IgM		100	97	
ND	Shenzhen YHLO Biotech	S+N	IgM		48	100	[187]
		S+N	IgG		89	91	
Diazyme SARS-CoV-2 assay	Diazyme	S+N	IgG at 15 days		100	99	[191]
		S+N	IgM at 15 days		100	99	
The MAGLUMI™ 2000 Plus	SNIBE	S+N	IgG		100	88	[190]
		S+N	IgM		100	88	
The MAGLUMI 2019-nCoV IgG and IgM	SNIBE	S+N	IgG		100 >10–21 days	ND	[185]
		S+N	IgM		60	ND	
LIAISON®SARS-CoV-2	DiaSorin	S1+S2	IgG		95 >15days	97–98	[192,195]
Abbott Architect SARS-CoV-2 IgG	Abbott	N	IgG		100 >17days	100 >17days	[196]
Abbott Alinity i SARS-CoV-2 IgG	Abbott	N	IgG		100	99.6	[198]

(*Continued*)

Table 4 (Continued).

Test Name	Manufacturer	Capturing Agent	Target	Sample Type	Sensitivity %	Specificity %	Reference
In-house Lab developed kit	Division of Life Sciences and Medicine, University of Science and Technology of China	RBD	IgG	–	97	100	[189]
		N	IgG		96	100	
		RBD	IgM		97	92	
		N	IgM		78	95	
		RBD	IgA		99	98	
		N	IgA		90	85	
In-house Lab developed kit	Department of Clinical Laboratory, Xixi Hospital of Hangzhou	N	IgM		82	81	[188]
In-house Lab developed kit	Department of Clinical Laboratory, Xixi Hospital of Hangzhou	N	IgG		82	97	
Synthetic peptide-based MCLIA	Lab developed	S	IgG		74	ND	[194]
Synthetic peptide-based MCLIA	Lab developed	S	IgM		54	ND	[194]
Celer One Step COVID-19 Test	Celer Biotechnologia S. A.	Anti-μ chain antibody	IgG/IgM	Whole blood, human serum and plasma	86.43	99.57	[278]
BELTEST-IT COV-2	Pharmact	IgG	IgG	Whole blood, human serum	98.2	99.7	[279]
		IgM	IgM		98.1	99.5	
COVID-19 Ag Respi-Strip	Coris BioConcept	–	SARS-CoV-2 Ag	Nasopharyngeal swab	57.6%	99.5%	[280]
STANDARD Q COVID-19 Ag Test	SD Biosensor Inc.	Monoclonal anti-SARS-CoV-2 antibody	SARS-CoV-2 Ag	Nasopharyngeal swab	84.97	98.94	[281]
BD Veritor™ System	Becton, Dickinson, and Company (BD)	NP-specific antibodies	SARS-CoV-2 N	Nasal swab	100	97.5	[173]
Microfluidic Immuno-fluorescence assay — LumiraDx SARS-CoV-2 Ag Test	LumiraDx	N/A	SARS-CoV-2 N	Nasal swab	97.6	96.6	[173]

Abbreviations: rNP, recombinant nucleoprotein; RBD, receptor-binding domain; rAg, recombinant antigen.

characteristic (ROC) curve analysis gave a value of 0.988 and 1.000 for IgM and IgG, respectively, indicating higher diagnosis accuracy. The kit detected SARS-CoV-2 with high sensitivity and specificity with no cross-reaction from other respiratory viruses. Suhandynata et al executed a longitudinal study to evaluate Diazyme SARS-CoV-2 assay efficiency to analyze the seroconversion rate of IgM and IgG in acutely ill patients.[191] Seropositivity was observed following confirmed positive PCR results. A low sensitivity was observed in the first week, but a better one (94.4%) for both immunoglobulins (Ig) was reported in the second week. After 15 days from symptom onset, IgG's sensitivity increased slightly (94.7), and IgG/IgM reached 100%, though a reduction was observed for IgM (89%). The report noted high specificity for three different panels of Ig and no cross-reaction with other pathogens.

Two different studies assessed the SNIBE "MAGLUMI" CLIA System's analytical performance.[185,190] Padoan et al reported that the assay's reproducibility was not consistent with the manufacturer's claim. Still, detection sensitivity was 100% and 88% for IgG and IgM, respectively, at the later phase of infection (>12 days for IgG).[190] The study suggests that a cut of value refinement may increase IgM sensitivity, and IgG+IgM combinations may increase detection sensitivity at the early stage of infection. Antibody kinetics was consistent with other data. Lippi et al utilized the same system to perform a comparative analysis between MAGLUMI CLIA and Euroimmun Anti-SARS-CoV-2 IgA and IgG ELISA.[185] The study reports 90% similarities between these assays, regardless of using different immunoglobulin targets. Jin et al used CLIA to compare serological and molecular diagnostic values and dynamic variance in serum antibody.[187] When compared to molecular detection, IgG and IgM detection specificity was 90%. A combination of serology and molecular diagnosis revealed a dilutive difference in immunoglobulin titer before and after conversion to virus-negative status. Bryan et al did a performance analysis on the Abbott CLIA kit for the Abbott ARCHITECT platform that revealed an optimum threshold index for 100% sensitivity and specificity for the commercial kit.[196] A comparative analysis on RBD and N based CLIA demonstrated that RBD as an immobilized antigen works better than N.[189] The study insisted on the importance of IgA in the serological analysis of COVID-19. Inclusion of IgA with IgG in serological testing yield a higher accuracy (sensitivity, specificity, and overall agreement elevate to 99.1%, 100%, and 99.7%, respectively) than the current reference standard (RT-PCR, >70% sensitivity) and the presence of IgA correlates with

severity of the disease. Lin et al used N antigen-based CLIA to detect IgM and IgG reactivity, which showed higher sensitivity (82%) than the same N-based ELISA system while the specificity for IgG and IgM detection was 98% and 81%, respectively.[188] Cai et al have reported a peptide-based Magnetic Chemiluminescence Enzyme Immunoassay (MCLIA) to detect IgG and IgM with a 71% and 57% sensitivity, respectively.[194] If both are combined, detection sensitivity is enhanced to 81%. The study screened 20 different biotinylated synthetic peptides from orf1a/b, nucleocapsid (N), and spike (S) proteins and demonstrated a better result for spike antigen. Luciferase immunoprecipitation system (LIPS) assay, a liquid phase immunoassay technique, has also been highly sensitive in detecting the SARS-CoV-2 antibody. The advantage of this assay is that protein remains in its native form.[196]

A chromatographic digital immunoassay was developed to detect SARS-CoV-2 antigen (ie, nucleocapsid proteins) directly from the nasal swab with 96.6% specificity and 97.6% sensitivity.[197] Another FDA-approved kit based on Microfluidic immunofluorescence assay also detects the viral nucleocapsid antigen; however, it cannot differentiate between SARS-CoV and SARS-CoV-2 antigens.[198]

Lateral Flow Assay

The lateral flow immunoassay technique has been a popular diagnostic tool in recent years for its low-cost, low limit of detection, rapid, sensitive, and specific diagnostic capabilities.[199] In the rapidly emerging SARS-CoV-2, lateral flow technique can play a crucial role in the faster diagnosis of suspected patients and proper isolation. Thousands of lateral flow assay kits have been developed since the emergence of SARS-CoV-2. Still, critical assessment of the sensitivity and specificity limits their use, as being a POC, false-negative results would augment the catastrophic situation by further disseminating this virus. A lateral flow technique was developed to detect antibodies, eg, both IgM and IgG in whole blood/serum/plasma against the SARS-CoV-2 virus had a sensitivity and 88.66% specificity 90.63%, respectively.[200] Another research group developed a lateral flow assay based on colloidal gold nanoparticle to identify human IgM against SARS-CoV-2 infection. The nucleoprotein of SARS-CoV-2 was coated on the device strips, and compared to the rRT-PCR result, the test has a sensitivity of 100%, although the specificity was 93.3%. Small sample requirements (10–20 μL) and the quick result (15 min) made this technique unique.[201] A

CRISPR-Cas12 based lateral flow assay was developed, which uses respiratory swab instead of blood. A positive result detects the *N* gene or *E* gene in the extracted viral RNA used in this technology. It is a faster visual alternative method to rRT-PCR assay with a 95% positive and 100% negative predictive agreement.[144] Additionally, four different lateral flow techniques (ASK COVID-19 IgG/IgM Rapid Test, ALLTEST 2019-nCoV IgG/IgM Rapid Test, Wondfo SARS-CoV-2 Antibody Test, and Dynamiker 2019-nCoV IgG/IgM Rapid Test) showed 100% sensitivity and specificity for IgM and IgG antibody against SARS-CoV-2 detection after three weeks of symptom onset.[174]

Recently, FDA has approved a few SARS-CoV-2 rapid antigen detection kits such as Sofia SARS Antigen Fluorescent Immunoassay (FIA) for quicker diagnosis of COVID-19. It uses a lateral flow method coupled with an advanced immunofluorescence technique for the detection of SARS-CoV-2 antigen in nasal or nasopharyngeal swabs. Within 15 minutes, the result can be obtained with a 96.7% favorable percent agreement (PPA) and a 100% negative percent agreement (NPA).[173,202]

Neutralization Assay

Neutralization assays can detect antibodies that inhibit viral infection, thus neutralizing them.[203] The results are observed by detecting the cytopathic effects of viral replication in cell lines. It plays a crucial role in the clinical use of convalescent plasma and, in the long run, vaccine development. Recently two types of neutralization assays have been reported, pseudovirus neutralization assay and micro-neutralization assay for SARS-CoV-2.

Pseudovirus-Based Neutralization Assays (PBNA)

Pseudoviruses are recombinant viral particles containing the core or backbone and envelope proteins acquired from two different viruses.[204] They are crucial virologic tools for studying emerging and reemerging viruses.[204] Pseudotyping involves exchanging a viral attachment protein with a different virus, for example, vesicular stomatitis virus G glycoprotein (VSV-G), the most common glycoprotein used in the retroviral and lentiviral vectors. Pseudotyping has many advantages: efficient delivery to a wide range of cell types increased vector stability and increased infectious titer.[205,206] A pseudovirus neutralization assay developed by Nie et al detects SARS-Cov-2 constructed pseudoviruses with spike genes from Wuhan-Hu-1 (GenBank) strain. The results showed high potency of neutralization against SARS-CoV-2 pseudovirus when the convalescent sera of COVID-19 patients were tested. PBNA are advantageous over the virus-based approaches due to their versatility and safe-handling and easy treatment. The versatility of pseudovirus is accomplished by virus pseudotyping with proteins.[204,207] PBNA is a sensitive, precise, reproducible, and less labor-intensive method. The result, however, may vary with variation in the pseudovirus inoculum. Fixing the dose of the pseudoviruses is essential to yielding comparable results between different labs.[208]

Microneutralization Assay

Microneutralization assay is an essential test in the field of immunology, vaccine development, and epidemiological studies.[209–211] Neutralization assay is one of the most widely used methods for detecting virus-specific neutralizing antibodies.[212] This assay depends on cell culture, having the ability to detect biologically active antibodies.[213] SARS-CoV-2 viruses are cultivated in a particular cell line (ie, VERO cell lines, VERO E6 cell lines, and Huh-7 cell line set) heat-inactivated human serum samples of different dilutions are incubated with viruses harvested from those cell lines.[211] The dilution provides comparability of different sera to neutralize the viruses. The titer of neutralization is expressed as the reciprocal of the highest dilution, which blocks virus infection. The colorimetric analysis is done after three days of incubation. Maximum serum dilution is considered the neutralization titer showed by an optical density (OD) value more significant than the cut-off value. After four days of incubation, CPE (Cytopathic Effect) read-out is taken as the neutralization titer is considered the maximum serum dilution, which protects more than 50% of CPE cells. Manenti et al and Amanat et al developed micro-neutralization assays to detect SARS-CoV-2 viruses for research purposes. Manenti et al used commercial SARS-CoV-2 antigen for the detection of neutralizing antibodies. On the other hand, Amanat et al used commercial SARS-CoV-2 viruses to establish microneutralization assay.[214] The assay's main advantage is quantifying the antibody titer that specially neutralizes or blocks the SARS-CoV-2 viruses.[215] Microneutralization assay's main advantage is to quantify the antibody titer that essentially neutralizes or block the SARS-CoV-2 viruses.[211] This is also used to produce active and precise neutralizing antibodies at a high concentration and development of the vaccine. The major

drawback of this method is transfection, which kills the majority of the cell. However, a successful assessment requires 90% to 95% viable cells. Moreover, the technique is time-consuming, making it inconvenient to tackle the emergency.[215]

Sequencing-Based Methods

The advent of Next-generation sequencing (NGS) technologies facilitates effective and high-throughput screening of large numbers of samples.[216] The technology can identify a diversified array of microbes in a single sample without any previous knowledge. This makes it ideal for the rapid diagnosis of COVID-19 patients by detecting SARS-CoV-2 along with background microbiomes. Metagenomic NGS provides a novel solution for detection, typing, mutational analysis, epidemiological and surveillance studies, and comprehension of the host immune response against the SARS-CoV-2.[84,216,217]

MinION based sequencing is being used for the identification of SARS-CoV-2 within 10 hours in nasopharyngeal swabs of infected patients by the ISARIC 4C consortium.[218] It employs two complementary techniques, an amplicon-based system and a metagenomic approach, to identify and sequence of SARS-CoV-2. The former method, a set of primers targeting conserved regions, was exploited to amplify the SARS-CoV-2 genome with an approximately 200 base pair overlap allowing sequence assembly for about 1000 base-paired sequential fragments. For the metagenomic approach, amplification by sequence-independent single primer amplification (SISPA) was used.[218]

Illumina has recently devised two methods for the SARS-CoV-2 detection, namely shotgun metagenomics approach and target enrichment approach. The shotgun approach includes sample preparation, library preparation, sequencing, and analysis, while the target enrichment approach is used to reduce the amount of data to be analyzed. The latter can identify coronavirus and other respiratory viruses in a sample using the Respiratory Virus Oligo Panel.[219] The two approaches mainly differ in the number of reads required per piece for analysis. The former requires a minimum of 10 million reads per sample, while the latter requires 0.5 million reads to achieve acceptable results.[219]

Ion Torrent, a next-generation sequencing developed by ThermoFisher Scientific, based on amplicons ranging from 125 bp to 275 bp in length, is a fast and rapid system circumventing coronavirus typing.[220] Additionally,

Nanopore technologies provide a platform for metagenomics and meta-transcriptomic analysis of SARS-CoV-2, providing valuable information regarding co-infections that may aggravate the COVID-19 outcomes. These technologies include the base reading of DNA by retrieving changes in current as DNA passes through the tiny, small pores providing robust, fast, and effective diagnosis compared to POC applications.[220]

Forthcoming Technologies Under Development
Aptamer-Based Detection

Aptamers have recently been used as targets for rapid viral detection and therapeutic purposes and have been advocated to have great potential in coronavirus pandemic.[221,222] These short artificial nucleotides can bind to SARS-CoV-2 RBD with high affinity employing an ACE2 competition-based selection strategy. The inhibitory capacity of anti-RBD aptamer has the potential to be a treatment of COVID-19. Furthermore, Chen et al designed a DNA aptamer-based on SARS-CoV nucleoprotein to detect SARS-CoV-2 nucleoprotein.[223] In the study, the aptamer's binding affinity was evaluated with enzyme-linked aptamer binding assay (ELAA). Though the biomolecule's small size makes it a stable target with minimum cross-reactivity, its diagnostic performance is yet to be evaluated.[223]

Another RNA aptamer-based rapid detection system is SENSR, a ligation-dependent molecular technique utilizing SplintR ligase and T7 RNA polymerase for target RNA amplification and transcript detection by fluorescence read-out.[224] The method allows detecting a broad range of pathogens, including the novel coronavirus, with a low detection (0.1aM) in a one-step reaction.[224]

Molecular Imprinting Technology (MIT)-Based Detection

Molecular imprinting polymer (MIP), which utilizes molecular imprinting technology (MIT), can be employed as a diagnostic tool by binding to predictable structure with high affinity and specificity, analogous to the "Lock and Key" model of enzyme-substrate recognition.[225] A rapid point-of-care detection kit based on MIP has been proposed, which utilizes a combination of MIP-based sensor and SARS-CoV-2 specific aptamer to detect SARS-CoV-2 with high specificity.[225] Similarly, Puoci et al also developed a hemocompatible MIP-based "monoclonal type" antibody that can selectively bind with SARS-CoV-2

RBD, rendering them useful as sensors for COVID-19 diagnosis and therapeutics.[226]

Microarray

A microarray is a multifunctional tool employed for retrospective Search of SARS-CoV-2 in Human Fecal Metagenomes in diagnostics, research, and epidemiological studies. The technique allows studying the genomic behavior of pathogen, immunogenic response to different disease phases, antigen–antibody interactions, cross-reactivity between species, and target proteins used as biomarkers in a single platform.[227–229] Wang et al described a proteome microarray that mapped out SARS-CoV-2 specific antibody response at the amino acid level.[227] Array analysis revealed that commercial antisera against SARS-CoV proteins could be used as a marker for SARS-CoV-2.[227] A comparative study between respiratory viruses, including SARS CoV-2, used microarray to define the differences in pathogen-specific antibody profile and optimal antigen selection for diagnosis and vaccine development. Cross-reaction with other beta coronaviruses may decrease test specificity when S2 is used as a target antigen but combining S2 with N enhances its predictive value of diagnosis.[229]

Biosensors

Biosensors are advanced rapid diagnostic techniques for biomolecules, such as protein, glucose, and other analytes or pathogens, detection.[230] These are highly portable, simple, and of low cost.[230] Several studies provided evidence for the convenience of the technology in SARS-CoV-2 detection. Field-effect transistors (FET) are such devices fabricated as a biosensing detector of SARS-CoV-2.[231] The device's surface was primarily coated with graphene and then conjugated with the anti-spike antibody of SARS-CoV-2 via a molecular probe linker (eg, 1-pyrene butyric acid N-hydroxysuccinimide ester). This could successfully detect 1 fg/mL and 100 fg/mL of SARS-CoV-2 spike protein in phosphate buffer saline and clinical transport medium, respectively. The device had a limit of detection (LOD) of 1.6×10^1 pfu/mL and 2.42×10^2 copies/mL, respectively.

Similarly, another research group developed a biosensor technique to detect SARS-CoV-2 spike protein (S1) based on bioelectric recognition assay.[232] This novel biosensor detected SARS-CoV-2 within 3 min with a LOD of approximately 1 fg/mL, without any cross-reactivity to SARS-CoV-2 nucleocapsid protein. The portable read-out

system of this ready-to-use biosensor platform can be controlled with a smartphone or tablet. Murugan et al proposed another portable field-deployable biosensor based on plasmonic fiber-optic absorbance. The design demonstrated a wash free, one-step detection of SARS-CoV-2 proteins using saliva samples where the LOD was 10^{-18} M (attomolar).[233] SARS-CoV-2 can be efficiently diagnosed using the Fiber Optic Surface Plasmon Resonance (FO-SPR) biosensor system. The FO-SPR, employs both immunoassay and aptamer-based strategy to the biosensor-based technique.

Other/Combined Diagnostic Methods

Rocca et al detected SARS-CoV-2 from nasopharyngeal swabs by combining multivariate analysis to MALDI-TOF Mass Spectrometry. It can be used as a complementary detection tool.[234] A surface-enhanced Raman scattering (SERS) based diagnosis combined with multivariate analysis was studied.[235] The method includes silver nanorods, which were functionalized on a silicon surface, and angiotensin-converting enzyme 2 (ACE2) was conjugated over the silver-nanorod SERS (SN-SERS) decorated as ACE2@SN-SERS array. The binding of the spike protein RBD of SARS-CoV-2 with the surface ACE2 ultimately generates SERS signals. SARS-CoV-2 was detected by identifying a potential spectral intensity where most peaks interestingly show a shift from 1189 to 1182 cm^{-1}.[235] These technologies (Table 5) thus hold a promising opportunity to explore the impediments faced in detecting SARS-CoV-2.

Summary and Future Perspective

Present worldwide circumstance draws attention to the fact that laboratory diagnosis is crucial in public health response to curb SARS-CoV-2 infection which has been continuously emphasized by the health authorities around the world. Various testing systems have been developed, compared and contrasted to assess the test eligibility for successful disease control, but a single quick, accurate and cost-effective method with adequate sensitivity has been difficult to ascertain with all benefits and drawbacks of each strategy. The current review looks at the diagnostic technologies that either have already been implemented or are under development. COVID-19 diagnostic systems are based on four major principles: Cell culture and microscopy, imaging

Table 5 Proposed Technologies Under Development for SARS-CoV-2 Diagnosis

Technology	Manufacturer	Capturing Agent	Target	Reference
Aptamer based detection	Laboratory developed	Anti-RBD aptamer	RBD	[282]
		DNA aptamer	N	[223]
		RNA aptamer	RNA transcript	[224]
Molecular Imprinting Technology	Laboratory developed	Plastic monoclonal type antibody	Spike protein	[283]
Microarray	Laboratory developed	SARS-CoV 2 proteome	IgG + IgM	[227]
		Coronavirus + respiratory virus antigen	IgG/IgA	[229]
Biosensor	Laboratory developed	Cell-based biosensor + Spike S1 antibody	S1	[232]
		FET + Anti-spike protein	Spike protein	[231]
		P-FAB + anti-N protein	N protein	[233]
Surface-enhanced Raman scattering (SERS)	Laboratory developed	SN-SERS+ ACE2	RBD	[235]

Abbreviations: FET, field effect transistor; P-FAB, plasmonic fiber optic absorbance biosensor; ACE 2, angiotensin-converting enzyme-2.

systems, nucleic acid-based detection, and immunoassay, though each has its advantages and disadvantages (Table-6). While cell culture and microscopy are important for a deeper understanding of the evolving novel coronavirus and its relationship with the host cell, these systems entail expensive criteria, which hardly makes it an optimal cost-effective diagnostic method. Imaging examinations have been very useful at the onset of the pandemic in detecting unknown pneumonia, but common respiratory viral pneumonia features make this a conditional approach for determining disease seriousness rather than a standard diagnostic method. Most commercial systems for COVID-19 detection are mainly based on the molecular and immunological techniques, with rRT-PCR being used in the front line. rRT-PCR is a very sensitive and specific technique that can identify viral RNA from very small sized sample input. But expensive instruments and facilities, trained human resources and a definite window period to achieve accurate results make it a little disadvantageous for an emergency situation. In contrast, isothermal NAATs and CRISPR technologies are getting utilized for advancement of rapid, POC testing that may reduce the dependency on rRT-PCR tests. Immunoassays are the other diagnostic tools that are dominating the COVID-19 diagnostic market after the gold standard rRT-PCR. Numerous commercial and laboratory-based immunoassays have been reported, but many of these reports lack the relevant validation data, which is critical for defining test sensitivity and specificity. Despite the drawbacks, immunoassays offer a powerful tool for dramatically reducing COVID-19 cases. It has the advantage of identifying both symptomatic and asymptomatic patients. Therefore, it can be used in conjunction with or complementary to RNA-based tests to increase diagnostic sensitivity. Moreover, immunoassays can be useful for contact tracing, studying seroprevalence and dynamics of an immune response against the virus. The world has moved toward vaccination, and more than four vaccines have already been in clinical use. Immunoassays will be required to evaluate these vaccine's effectiveness. Hence, it is essential to assess these immunoassay kits properly before approving for a community. Besides these conventional methods, microarray, aptamer, MIP and biosensor-based methods still under development should be evaluated to determine their effectiveness in urgent conditions. Even though vaccination programs have started, and reports have shown a slight reduction in disease severity, SARS-CoV-2 being a positive-strand RNA virus creates the continuing possibility of a recurrence of the previous situation. Before an efficient, commercially viable vaccine is successfully introduced, rapid POC tests based on molecular and immunoassay are still of great importance to deal with the challenges of COVID-19 disease containment. Further insights are yet to be addressed to unveil the epidemiological, immunological, genomic,

Table 6 Advantages and Disadvantages of Diagnostic Methods Currently Available or Proposed for Detecting SARS-CoV-2

	Categories	Sensitivity	Specificity	Early Phase Detection	Late Phase Detection	Expense	Sample Collection and Processing	Reagents and Materials	Facilities Required	Comments
Cell Culture and Microscopy	Cell Culture and Microscopy	+++	+++	++	+	+++	Critical	Expensive	BSL-3 facilities	Not suitable for diagnostic purpose
Radiology-based detection	Radiology-based Detection	±	±	–	+	+	N/A	Large and expensive equipment	Trained personnel	Might be ambiguous with respect to other respiratory diseases
NAAT-Based Technologies	Real-Time RT-PCR	+++	+++	+++	±	++	Crucial and tedious	Expensive equipment as well as reagents	Trained personnel and BSL-2 facilities	Sensitivity starts to fall after about 8 days of exposure but gold standard method
	Isothermal Amplification Technologies	+++	+++	++	±	+	Depends on the method applied	Large scale production can reduce the cost	Trained personnel and BSL-2 facility	Further study required to make it a suitable for POCT
	CRISPR-Based Diagnostics	+++	+++	++	±	+	Depends on the method applied	Large scale use might reduce cost	POCT	Can detect mismatches within DNA with high accuracy
	Microfluidic Biochip or Lab-On-A-Chip Technology	+++	+++	++	±	+++	Sample processing required	Small amount of sample and reagents required, but production cost is high	POCT	Performed on a single chip with high accuracy
	Next Generation Sequencing	+++	++++	N/A	N/A	+++	Tedious	Expensive reagents and equipment required	Trained personnel	Genomic identification and suitable for genomic epidemiological studies

							Multiple samples can be tested at the same time	Expensive reagent and equipment required for CLIA	Trained professional and BSL-2 facility	Effective for large scale screening in short time
Immunoassay	ELISA/CLIA	++	+++	++	±	++	Multiple samples can be tested at the same time	Expensive reagent and equipment required for CLIA	Trained professional and BSL-2 facility	Effective for large scale screening in short time
	LFIA	+	+++	+	—	+	Minimum	Minimum reagent	POCT	Risk of false negative results
	Neutralization assay	+++	+++	++	±	+++	Sample processing required	Expensive reagent and equipment required	Trained professional and BSL-2 facility	Time consuming
Technology Under Development	Aptamer	++	++	Not enough Data	Not enough Data	±	Sample processing required	Large scale use will reduce cost	Can be a POCT	Diagnostic performance needs to be evaluated
	Molecular imprinting technology (MIT)	+	+	Not enough data	Not enough data	Not enough data	Sample processing required	Expensive reagent	Can be a POCT	Diagnostic performance is to be evaluated
	Microarray	++	++	Not enough Data	Not enough Data	—	Sample processing required	Expensive reagent and equipment required	Qualified personnel and advanced facility	Time Consuming
	Biosensor	++	++	Not enough Data	Not enough Data	+	Sample processing required	Expensive reagent	Can be a POCT	Diagnostic performance is to be evaluated

Notes: "+" Positive/Good; "++" Better; "+++" High; "N/A" Not Applicable; "±" Variable; "—" Negative.

and proteomic features of the novel virus that might bolster the implementation of techniques under development for COVID-19 diagnosis.

Article Highlights

- Multi-faceted pathophysiologic behavior of SARS-CoV-2 makes it difficult for the physicians to draw a universal outline to manage the patients with COVID-19.
- Early diagnosis and isolation of the carriers from their neighbor are believed to flatten the disease mortality curve.
- Choosing rapid and accurate diagnostic kits, and their widespread implementation is critical for policymakers to rein in the pandemic as long as an effective and safe vaccine has not been established and provided to the masses.
- In this COVID-19 outbreak, initial detection and characterization were performed in cell culture laboratories followed by whole genome sequencing, but according to WHO, culturing of SARS-CoV-2 has only been approved in BSL-III laboratories, making cell culture-based detection mostly impractical and expensive, prohibiting its implementation in resource lacking countries.
- Radiological tools such as X-ray, CT-scan are effective for the diagnosis as well as prognosis.
- Nucleic acid amplification tests (NAATs), such as rRt-PCR, isothermal amplification techniques, CRISPR-Cas, and micro-fluidic systems, on the other hand, are considered the most convenient tools for diagnostic purposes.
- The advantage of immunoassay kits is that they can cover both the patient and viral windows using antibody and antigen tests.
- Prospective techniques such as aptamer, biosensors, microarrays, MALDI-TOFs, and others, alone or in combination, show promising outcomes in COVID-19 diagnosis.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Disclosure

Mr Md. Ahsanul Haq reports a patent 10202006327W pending; Prof. Dr. Bijon Kumar Sil report a patent 10202006327W pending; Dr Mohd. Raeed Jamiruddin reports a patent 10202006327W pending to Intellectual Property Office of Singapore; Dr Nihad Adnan report a patent 10202006327W pending; The authors report no other conflicts of interest in this work.

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