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Review of Current Diagnostics Developed for COVID-19

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COVID-19 emerged in 2019 as a pandemic that affected the world in many aspects and it has been the biggest challenge for many countries in the past year. Due to the lack of approved treatment methods for the disease, and more importantly, to inhibit its spread, the critical task is to detect it fast and reliable. Many research groups and companies have been developing different methods and products for the diagnosis of COVID-19. Each method has advantages and disadvantages, while rapid, inexpensive, and high-throughput detection methods are needed. In this regard, significant progress has been achieved so far. In this article, we reviewed in-vitro Diagnostics (IVD) and Commercial Kits in three main categories including real-time reverse transcription-polymerase chain reaction kits, serology-based tests, and point-of-care diagnostic tests. In addition, familiarizing with coronavirus and its detection methods, genome study, Cell entrance and antigenicity, and Specimen, Lab Biosafety, and Authorization of Medical Devices for COVID-19 were discussed.

Keywords: COVID-19, rRT-PCR kits, Serology-based tests, Point-of-care testing

INTRODUCTION

The recent pandemic of coronavirus disease (COVID-19 or 2019-nCoV) has been infecting many people and caused severe health problems worldwide. Looking at some of the past pandemics, Influenza A Virus (H1N1) (2009), polio (2014), Ebola in West Africa (2014), Zika (2016), Severe Acute Respiratory Syndrome (SARS) (2003), and Middle East Respiratory Syndrome (MERS) (2012) [1], the role of viruses in shaping human life on earth could be fully understood. These non-live particles carry genomic information that can hijack its machinery and change it into a virus production factory upon its entrance to a cell. The

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severity of the disease caused by viruses is very different and can range from very mild to health-threatening. The outbreak caused by SARS was found to be the cause of 30-100 billion US$ cost to the world's economy [2] and affected more than 8000 people in the world [3], *i.e.*, it is necessary to learn from past experiences to get well prepared for the future.

Mathematical models using available statistics worldwide predict the potential for millions of infected people with a high number of deaths. A key to control this serious situation, preventing more infections, and even avoiding another pandemic is to develop fast, sensitive, and reliable detection methods. In case of a virus infection, many devices and kits have been developed so far using different approaches. Here, we summarized virus detection methods, and provided information about coronavirus, specimen, lab biosafety, and authorization of medical devices for COVID-19. Then, we discussed *in vitro* diagnostics and commercial kits developed for COVID-19 in three main categories containing Reverse transcription-polymerase chain reaction (RT-PCR) kits, serology-based tests, and POCTs that can be used onsite and in the field.

Conventional Viral Diagnosis Methods

Currently, viral detection methods are mainly based on either viral antigen or nucleic acid and antibody detection. There are also less frequently used methods are available, *e.g.*, based on cell culture or histology. In the cell culture methods, the standard cell lines become inoculated with the patient's specimen [4] and monitored *i.e.*, the detection is based on characteristic viral cytopathic effects. The advantage of cell culture is that many viruses could be detected. However, this method is not only labor-intensive and time-consuming but also requires skilled personnel [5].

Shell vial technique is an alternative method for cell culture in which centrifugation and immunofluorescence are used to shorten the required time for a positive test. This technique is more targeted, while has a limited range of detectable viruses [6]. In addition, virus exposure is more probable during cell culture due to the required steps in the techniques, *e.g.*, opening the containers, mixing, or pipetting. On the other hand, in histology and cytology methods, detection is based on morphologic features of the infected tissue specimens after special staining protocols [5]. Electron microscopy is an efficient method for viral detection, which is based on viruses' morphology determined with atomic resolution and quantification. Furthermore, in combination with confocal laser scanning microscopy to enhance resolution, electron microscopy-based detection methods enable live imaging viruses. However, this method is very expensive and required experienced personnel [7], which dramatically weakens its practicability.

Molecular assays: These methods are fast and easily automated and sensitive enough for clinical settings. They are especially beneficial when there is a delay in antibody production, *e.g.*, human immunodeficiency viruses (HIV) [8]. Nowadays, there are different nucleic acid amplification tests (NAAT), including polymerase chain reaction (PCR), quantitative PCR (q-PCR) for DNA viruses, and rRT-PCR for RNA ones. Conventional approval of COVID-19 cases is based on the detection of unique RNA sequences of the virus by rRT-PCR method. In addition, nucleic acid sequencing after NAAT for further confirmation is also applied. Other molecular assays are also available, such as nucleic acid sequence-based amplification, transcription-mediated amplification, and branched DNA probes [5].

Immunoassays: Using immunoassays to detect immunoglobulin M (IgM) as a sign of current infections, and immunoglobulin G (IgG) antibodies as a demonstration of a recent one, clinicians are able to monitor the status of patients' immune response [6].

a. Traditional assays include complement fixation, which is not often used nowadays, hemagglutination inhibition for viruses that have surface proteins capable of agglutinate red blood cells, and immunofluorescent assays, which are expensive and require expertise [5].

b. Solid-phase assays have an antigen or an antibody coated on a surface, such as non-competitive or competitive indirect enzyme immunoassays (EIAs) with immobilized viral antigens [5] and enzyme-linked immunosorbent assay (ELISA), which is considered as gold standard for protein detection [6].

Different variants of EIAs are now available with better sensitivity that could detect very small numbers of target antigens/antibodies.

c. Lateral flow immunochromatography: These tests have been recognized as point-of-care assays by Food and Drug

Administration (FDA). They are fast and could be operated by an untrained person. However, their sensitivity is lower, and the test results are qualitative rather than quantitative [9].

A biosensor is an analytical device that determines a biological event or quantifies a biomarker in medical detection. Point-of-care diagnostic platforms use detection methods that could be employed without the need for a medical infrastructure, *e.g.*, rural areas, or home settings. In the past decade, biosensors and point-of-care technologies emerged in medical diagnostic industries and became one of the main strategies in healthcare [10-14]. Towards this way, many advancements have been introduced employing different strategies, such as the use of nanomaterials and polymers for enhancing the sensitivity of biosensing systems [15-17]. The biosensor industry specialized in virus detection is in high demand due to the urgent need for stand-alone detection methods that could be used in resource poor settings without the need for sophisticated devices. Based on previous experiences and the strong potential of these devices with novel innovations, they could be used for the detection of COVID-19, which is the main target for this review.

COVID-19 Outbreak

Introducing the new virus. 2019-nCoV, identified first in China, is the third virus from zoonotic coronaviruses after Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome coronavirus (MERS-CoV) that has been transmitted to human and developed respiratory syndrome outbreaks [18]. Since the virus has been identified as the cause of pneumonia with clinical symptoms, *e.g.*, dry cough, dyspnea, and fever [19], it was called SARS-CoV 2, and later, the disease was named COVID-19 by World Health Organization (WHO) [20]. To date, two forms of the disease have been reported; one milder form that was resolved in most cases, and another one causing severe health problems such as organ failure, septic shock, and Acute Respiratory Distress Syndrome (ARDS) [21].

Global Response and Statistics

Undoubtedly, having more scientific insight will help fight better and more effective against COVID-19, *i.e.*, many publishers provide global open access for related articles and book chapters. As of December 27, 2020, more than 80 million cases have been confirmed globally. The ratio of deaths to the closed (recovered + death) cases is roughly 2.2%, but it varies across different regions and with time. In addition, there is a group of asymptomatic patients that might not be included in total cases, which makes the ratio more doubtful. Although the reported mortality is about 6.6%, which is lower than SARS, *e.g.*, 9.6%, it is more infectious. Furthermore, considering asymptomatic patients, the virus could be transmitted even more easily. Figure 1A represents the general scheme of spreading COVID-19.

Origin of COVID-19, Structure and Genetics

COVID-19 is a positive-stranded RNA virus belonging to the *Coronavirinae* subfamily, which shares more than 79% identity in its genome with SARS-CoV [22]. Coronavirus has one of the longest genomes in comparison to others. The organization of its positive-sense RNA genome and transcriptome has been studied in more detail by Kim *et al.* showing that there are at least 41 RNA modification sites, which increases the complexity of its transcriptome to a large extent [23]. Unlike other lineage B beta coronaviruses, there is a polybasic furin cleavage site at the junction of Spike protein (S protein) subunits [24] which can be important in determining infectivity and host range akin to the case of avian influenza [25]. In its mature form, COVID-19 has four structural proteins, including envelope (E) and membrane (M) proteins which are important in viral assembly [26], nucleocapsid (N) protein with a critical role in RNA synthesis, and spike (S) protein which interacts with a host cell receptor to initiate virus entry [27].

SARS and MERS, as well as the new type coronavirus, originate from bats but with different intermediate hosts, and the former has been transmitted *via* civet cat, and the latter from camel [28,29] and the intermediate host of the new one is still a controversial issue. The similarity between bat coronavirus and 2019-nCoV indicated a direct transmission to humans from bats, while there might be intermediate hosts [18].

Two major scenarios related to the origin of COVID-19 exist (i) natural selection in a host other than human before

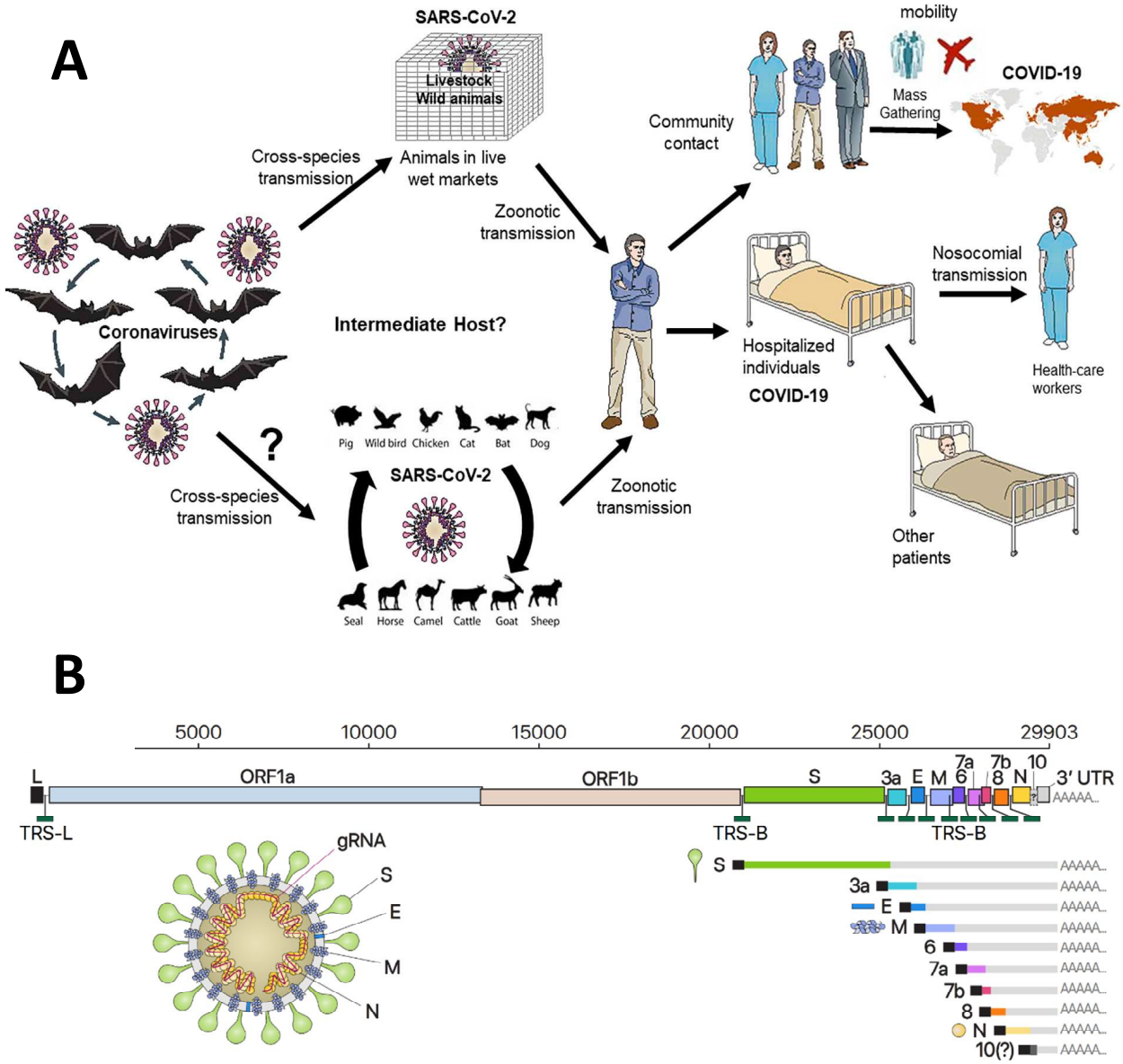

Fig. 1. A) The most probable routs of COVID-19 transmission from human to human and from animals to human. Reprinted with permission (28). B) Genome representation of Coronavirus. Reprinted with permission (23).

transmission and (ii) natural selection process taking place besides human-to-human transmission discussed in more detail in [30]. Accordingly, Fig. 1B represents the genome of COVID-19.

COVID-19 genome encodes S protein, E protein, M protein, and N protein that are the main candidates to use in serological test fabrication (Fig. 2A). N protein is the most abundant protein in coronavirus that is highly conserved and immunogenic. The immunogenicity of this protein is related to its high hydrophilicity. The N protein is composed of two separate domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), both capable of binding RNA *in vitro* using different mechanisms [31,32]. N protein is heavily phosphorylated and has two specific RNA

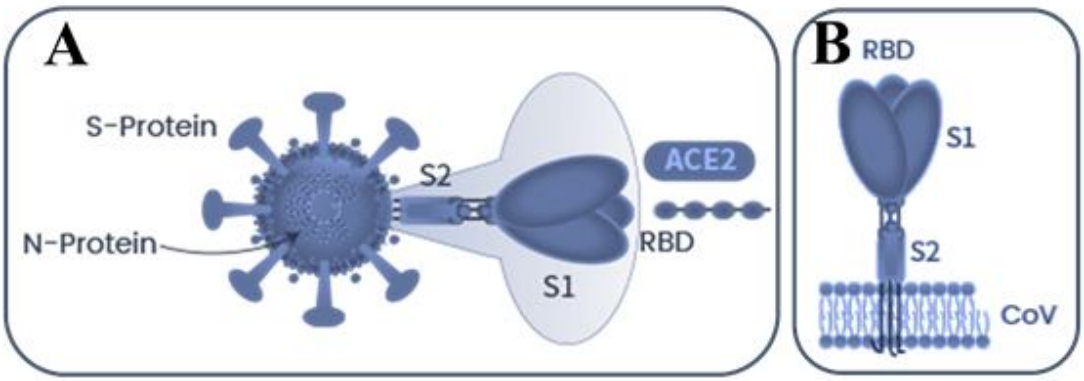

Fig. 2. A) The main candidate proteins of coronavirus for serological test development; B) Coronavirus spike protein and its subunits. Adopted from www.sinobiological.com.

substrates, including the transcription-regulating sequences (TRSs) and the genomic packaging signal, which bind specifically to the RNA binding domain. Moreover, the interactions between N protein, nsp3 (a key component of the replicase complex), and the membrane protein help in viral particle packaging *via* the replicase-transcriptase complex (RTC). Coronavirus S protein is a fusion protein responsible for virus attachment to the host receptor. S protein is highly N-glycosylated and is the main target for neutralizing antibodies and vaccines [32] (Fig. 2B).

As it is seen in Fig. 2B, the S protein has two functionally distinct subunits: the globular S1 subunit (large receptor-binding domain (RBD) for receptor recognition) and the highly conserved S2 subunit (the stalk-shaped part for membrane fusion) [33]. The S gene of COVID-19 has been reported as highly divergent to other coronaviruses, with less than 75% nucleotide sequence identity. The major differences are the three short insertions in the N-terminal domain with sialic acid-binding activity, and four out of five main residue alterations in the receptor-binding motif [33,34].

Cell Entrance and Antigenicity

Although their host cells are the same, unlike the previous viruses, COVID-19 grows better inside human airway epithelial cells rather than in standard tissue-culture ones [18]. New coronavirus uses the same receptor with SARS-CoV, *e.g*., human angiotensin-converting enzyme 2 (hACE2) receptor, to infect cells [22]. Wang et al. reported the crystal structure of the S protein complex of COVID-19 and hACE2, which is very similar to the complex formed by SARS-CoV with higher affinity due to slight changes in binding residues [35]. This phenomenon has not predicted from computational analyses, *e.g*., the binding sites have been evolved such that they are not man-made [30]. Genetic similarity to SARS-CoV rather than MERS-CoV increased the possibility of further adaptation to the human host via mutations akin to what observed for SARS-CoV due to error-prone RNA-dependent RNA polymerases [36]. Furthermore, the antigenicity of the COVID-19 S protein was evaluated using murine monoclonal and polyclonal antibodies produced against SARS-CoV S1 receptor binding domain. These antibodies were not able to form effective immune complexes that highlight the critical changes that alter the antigenicity of COVID-19 S protein [37].

Transmission and Prevention

In the beginning, it was assumed that human-to-human transmission is low [18], which was rejected very soon [35]. Based on the findings on SARS and MERS, transmission occurs with the help of respiratory droplets and contact [38] so that quarantining and making the contacts limited, especially with symptomatic people, is very useful in

prevention. It was strongly recommended for patients to wear face masks, but the real efficacy of masks in prevention was not completely understood, *e.g*., Leung *et al.* studied how virus shedding decrease with face masks. They found that surgical masks can effectively reduce viral load in aerosols and droplets [39]. Individuals without symptoms could still spread the virus, *i.e*., physical distance should be kept, and protective equipment should be worn.

Specimen, Lab Biosafety, and Authorization of Medical Devices for COVID-19

Specimen type and priority for COVID-19. One of the most critical factors for the diagnosis of COVID-19 is the specimen collected from patients. Providing specimen from a proper region is crucial to realize a rapid and accurate molecular diagnosis for COVID-19. Poor quality of patient sample could increase the risk for false results COVID-19 positive patients, which could lead to life-threatening results and the spread of the virus. Hence, most of the international healthcare organizations, including the Centers for Disease Control and Prevention (CDC) and WHO, have published guidelines and protocols for collecting, handling, and testing clinical specimens from persons for COVID-19 tests [40-43]. Like most viruses, the ideal specimen is commonly collected from diluted body fluids. According to CDC guidelines, the nasopharyngeal specimen is preferable for COVID-19 swab-based testing. Furthermore, there other alternatives such as oropharyngeal (OP) specimen, nasal mid-turbinate (NMT) swab, and anterior nares (NS) specimen [41]. CDC suggests testing the lower respiratory tract specimens if available. For patients who develop a productive cough, the preferable method is the use of sputum collected without induction [41]. Although the duration and frequency of shedding of COVID-19 virus in other body fluids are not precisely determined, regarding the reports on SARS and MERS infections, blood, stool, and urine specimens could be collected due to the current situation. In the case of deceased patients, autopsy material including lung tissue could be considered. On the other hand, for the patients alive, paired serum (acute and convalescent) could be the preferred specimen for retrospective serological assays. Moreover, it is essential to deliver specimens to the laboratory immediately after collection. The fresh specimens should be stored and shipped at 2-8 °C.

However, in case of a delay between sampling and storage processes, the use of a medium for viral transport, keeping the specimens frozen (-20 °C or ideally -70 °C), shipping them on dry ice, as well as avoiding repeated freezing and thawing are strongly recommended [44]. Table 1 presents useful information regarding specimen handling.

Lab Biosafety for COVID-19

All medical laboratories and research centers involved in handling and processing specimens associated with COVID-19 have been recommended to apply appropriate biosafety practices. Any COVID-19 test with clinical specimens should be performed in laboratories equipped with safe and appropriate equipment operated by trained staff. Specimen collection, storage, packaging and transportation should be handled based on the standard operating procedures. Although WHO has published an interim biosafety guideline to define structures for working with the potentially infected specimen, national guidelines on the laboratory biosafety rules have to be followed in all conditions. There is still limited information on the risk posed by COVID-19, but the risk assessment is needed for all procedures.

There are different types of assays to test specimens for COVID-19, *e.g*., an appropriate safety strategy should be applied for every process [45-47]. Sample collection and primary steps of specimen handling from cases with suspected/confirmed COVID-19 infection or screening specimens, good microbiological practices and procedures, and local guidelines for processing potentially infectious material should be carefully followed [47]. It is strictly recommended in WHO interim guidance that all operations of potentially infectious materials, especially the procedures including mixing, shaking, centrifuging, sampling, *etc.* should be performed by trained personnel in validated biological safety cabinets. All practices involving material with high concentrations of live viruses (virus propagation, isolation, neutralization, *etc*.) or large volumes of infectious materials require Biosafety Level (BSL)-3 or equivalent facilities with controlled ventilation system and exhaust high efficiency particulate air filter (HEPA) filters [48-50].
The non-propagative diagnostic laboratory work or non-culture procedures including, RNA extraction, sequencing,

Table 1. Specimen Collection Details, Storage, Shipment and Recommendations

Specimen type	Collection materials	Storage temperature	Recommended shipping temperature
Nasopharyngeal and oropharyngeal Swab	Dacron or polyester flocked swabs	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days
Bronchoalveolar lavage	Sterile container	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days
(Endo)tracheal aspirate, nasopharyngeal or nasal wash/aspirate	Sterile container	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days
Sputum	Sterile container	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days
Tissue from biopsy or autopsy (from lung)	Sterile container with saline or VTM	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days
Serum	Serum separator tubes	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days
Whole blood	Collection tube	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days
Stool	Stool container	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days
Urine	Urine collection container	2-8 ºC	2-8 ºC if ≤ 5 days -70 ºC (Dry ice) if > 5 days

PCR analysis and NAAT require BSL-2 or equivalent facility at a minimum.

Authorization for Medical Devices for COVID-19

Emergency Use Authorizations (EUA) have been issued from FDA related to COVID-19 pandemic situation for diagnostic, therapeutic, and protective medical products and devices. The guideline was latest updated on November 24, 2020, for clinical laboratories, commercial manufacturers, as well as food and drug administration staff.

Under defined emergency circumstances, even unapproved medical products or unapproved use of approved medical products may be allowed by FDA to be used in diagnose, treat, or prevent serious or life-threatening diseases or conditions when there is no adequate, approved, and available alternative (Section 564 of the Federal Food,

Drug, and Cosmetic Act (FD&C Act)). This guidance describes the agency's interpretation of the policy on regulatory issues for laboratories and manufacturers to speed up the use of tests for rapid and extensive testing capacity, particularly in the USA (51). CDC laboratories supported the FDA response regarding a EUA policy for COVID-19 that allows regulatory organizations to reinforce the public health protections against biological threats by simplifying the use of medical products for health emergencies [41].

Currently, various health categories such as personal protective equipment (PPE), *in vitro* diagnostics (IVD), molecular-based laboratory-developed tests, ventilators and other medical devices and therapeutics have been authorized for emergency uses for COVID-19. Local health authorities are asking manufacturers to provide quality management system documents, performance evaluation reports, labeling samples, testing reports, and attestation letters to issue EUA.

In Vitro Diagnostics (IVD) and Commercial Kits

IVD (devices or tests), including commercial test products and instruments, are used for the analysis of real samples, such as blood, plasma, urine, or tissue, to provide information about a specific condition of a patient. In the presence of rapidly spreading disease, detecting and isolating the infected individuals as early as possible is pivotal. It has been reported that COVID-19 patients express divergent and nonspecific symptoms that cannot be used for an accurate diagnosis. Hence, employing an appropriate diagnostic test is one of the key factors in tackling the spread of COVID-19. Testing is also needed to evaluate infection, mortality, and survival rates. Such data is critical for predicting the epidemiologic pattern of infection through the population and getting prepared for a potential crisis. Currently, diagnostic tests for determining infected people are conducted through three main methodologies: (i) testing the presence of the virus, *e.g*., molecular diagnosis *via* rRT-PCR and advanced sequencing, (ii) testing the effect of the virus, *e.g*., chest radiography and computed tomography (CT) scan and (iii) testing the presence of antibodies, *e.g*., serological tests [37,43]. In the following sections, rRT-PCR tests, serology-based tests and POCTs have been discussed in detail.

rRT-PCR Kits for COVID-19

Availability of the complete genome of COVID-19 early in the epidemic led to the development of various nucleic acid tests aiming at the detection of the genetic material of the virus which is the current primary method for diagnosing COVID-19. rRT-PCR stands for reverse transcription PCR that includes the reverse transcription of COVID-19 RNA into complementary DNA (cDNA) strands, followed by the amplification of specific regions of the cDNA. The design process involves two main steps: (i) sequence alignment and a primer/probe design, and (ii) assay optimization and testing with confirmation by nucleic acid sequencing when necessary [43,52].

Most of the PCR-based assays have been designed to a targeted number of SARS-related viral genome sequences that were reported to be conserved. The test can be performed in a singleplex format (two or three distinct assays) or multiplexed into a single reaction and amplification setup. The protocol of the first rRT-PCR assays was published on January 23, 2020. Bai *et al.* have reviewed the rRT-PCR programs and international primer/probe sets published by regulatory organizations [52]. The viral genes targeted so far include N, E, S and RNA-dependent RNA polymerase (RdRP) genes: N gene (the coding region for nucleocapsid protein) and E gene (envelope protein gene) are shared by all beta coronaviruses, and have been frequently used in the fabrication of screening tests [21,52]. However, RdRP and S genes have been reported as the confirmatory gene that is specific to COVID-19. Although the first kits were designed to detect N gene, the recent assays included RdRP and E genes due to higher analytical sensitivity for detection (technical limit of detection: 3.6 and 3.9 copies per reaction, respectively) [37]. It is preferred that the assay would be designed as a dual-target system with a primer/probe set for detecting coronavirus, including COVID-19 and a second primer/probe set for special detection of COVID-19. In a standard methodology, an internal control such as human housekeeping genes (*e.g*., ribonuclease P or β-Actin) and other spiked non-human genes (*e.g.,* MS2 phage genes) are investigated to ensure the quality of RNA extraction.

A comprehensive list of FDA-approved IVD and Research Use Only (RUO) products based on rRT-PCR for COVID-19 detection is presented in Table 2.

Table 2. The RT-PCR Kits Developed for COVID-19 Detection

Manufacturer	Product Name	Coronavirus screening gene(s)	COVID-19 confirmation Gene(s)	Internal control (IC)	Certification
Phosphorus Diagnostics LLC	Phosphorus COVID-19 RT-qPCR Test	-	N gene	RNase P gene	EUA-FDA CE-IVD
Fluidigm Corporation	Advanta Dx SARS-CoV-2 RT-PCR Assay	-	-	RNase P gene	EUA-FDA CE-IVD
Viracor Eurofins Clinical Diagnostics	Viracor SARS-CoV-2 Assay	Transcribed RNA	N gene	RNase P, MS2	EUA-FDA CE-IVD
University of Illinois Office of the Vice President for Economic Development and Innovation	covidSHIELD	-	SARS-CoV-2 RNA genomic regions	MS2	EUA-FDA CE-IVD
DxTerity Diagnostics, Inc.	DxTerity SARS-CoV-2 RT PCR CE Test	Synthetic SARS-CoV-2 RNA	-	RNase P gene	EUA-FDA CE-IVD
Gravity Diagnostics, LLC	Gravity Diagnostics SARS-CoV-2 RT-PCR for Use with DTC Kits	Sequence for the three SARS-CoV-2 Assay Targets	Sequence for the RNase P assay	RNase P gene, MS2	EUA-FDA CE-IVD
Assurance Scientific Laboratories	Assurance SARS-CoV-2 Panel DTC	-	human RNase P (RP) mRNA	-	EUA-FDA CE-IVD
Visby Medical, Inc.	Visby Medical COVID-19 Point of Care Test	-	RNA transcripts encapsulated in a phage protein	Human 18S ribosomal RNA	EUA-FDA CE-IVD
Thermo Fisher Scientific	TaqPath COVID-19, FluA, FluB Combo Kit	-	RNA control that contains targets specific to the SARS-CoV-2, influenza A, and influenza B genomic regions	MS2	EUA-FDA CE-IVD
Becton, Dickinson and Company (BD)	BD SARS-CoV-2/Flu for BD MAX System	-	RNase P gene	RNase P gene	EUA-FDA CE-IVD

Table 2. Continued

Bio-Rad Laboratories, Inc.	Bio-Rad reliance SARS-CoV-2/FluA/FluB RT-PCR Assay Kit	Inactivated influenza A, influenza B, RSV, and SARS-CoV-2 viruses	-	-	EUA-FDA CE-IVD
Yale School of Public Health, Department of Epidemiology of Microbial Diseases	SalivaDirect	s synthetic SARS-CoV-2 RNA	-	RNase P gene	EUA-FDA CE-IVD
Altona Diagnostics	RealStar SARS-CoV02 RT-PCR Kits	E gene	S gene	a heterologous amplification system	RUO
Seegene, Inc.	Allplex 2019-nCoV Assay	E gene-N gene	RdRNase P gene	MS2	EUA-FDA CE-IVD
Trax Management Services Inc.	PhoenixDx 2019-CoV	E gene	RdRNase P gene	RNase P gene	EUA-FDA CE-IVD
OSANG Healthcare	GeneFinder COVID-19 Plus RealAmp Kit	E gene-N gene	RdRNase P gene	RNase P gene	EUA-FDA CE-IVD
Fosun Pharma USA Inc.	Fosun COVID-19 RT-PCR Detection kit	E gene - N gene	ORF1ab	Lentivirus	EUA-FDA CE-IVD
Labs Inc.	Curative-Korva SARS-Cov-2 Assay	N1 gene	N2 gene	RNase P gene	EUA-FDA CE-IVD
GenoSensor, LLC	GS™ COVID-19 RT-PCR KIT	E gene - N gene	ORF1ab	GUSB gene-beta-glucuronidase	EUA-FDA CE-IVD
Maccura Biotechnology LLC	SARS-CoV-2 Fluorescent PCR Kit	E gene - N gene	ORF1ab	β-Actin	EUA-FDA CE-IVD
Atila Biosystems, Inc.	iAMP COVID-19 Detection Kit	N gene	ORF1ab	Gapdh	EUA-FDA CE-IVD
DiaCarta, Inc	QuantiVirus SARS-CoV-2 Test Kit	E gene - N gene	ORF1ab	RNase P gene	EUA-FDA CE-IVD
Becton, Dickinson & Company	BD SARS-CoV-2Reagents for BD MAX System	N1 gene	N2 gene	RNase P gene	EUA-FDA CE-IVD
Inbios International, Inc.	Smart Detect SARS-CoV-2 rRT-PCR kit	E gene - N gene	ORF1ab of RdRNASE P	RNase P gene	EUA-FDA CE-IVD
Gnomegen LLC	Gnomegen COVID-19 RT-digital PCR Detection Kit	N1 gene	N2 gene	RNase P gene	EUA-FDA CE-IVD

Table 2. Continued

Co-Diagnostics, Inc.	Logix Smart Coronavirus Disease 2019 (COVID-19) Kit	-	RdRNase P gene	RNase P gene	EUA-FDA CE-IVD
Scien Cell Research Laboratories	Scien Cell SARS-CoV-2 Coronavirus RT-qPCR Kit	N1 gene	N2 gene	RNASE PP30 gene	EUA-FDA CE-IVD
Luminex CoRNase Poration	ARIES SARS-CoV-2 Assay	N gene	ORF1ab	RNase P gene	EUA-FDA CE-IVD
Ipsum Diagnostics, LLC	COV-19 IDx Assay	N1 gene	N2 gene	RNase P gene	EUA-FDA CE-IVD
QIAGEN GmbH	QIAstat-Dx Respiratory SARS-CoV-2 Panel	E gene	RdRNase P gene	MS2	EUA-FDA CE-IVD
NeuMoDx Molecular, Inc.	NeuMoDx SARS-CoV-2 Assay	N gene	non-structural protein 2 (Nsp2)	Sample process control (SPC2)	EUA-FDA CE-IVD
Luminex Molecular Diagnostics, Inc.	NxTAG CoV Extended Panel assay	E gene - N gene	ORF1ab	MS2	EUA-FDA CE-IVD
Abbott Molecular	Abbott Real Time SARS-CoV-2 Assay	N gene	RdRNase P gene	unrelated RNA sequence is simultaneously amplified	EUA-FDA CE-IVD
BGI Genomics Co. Ltd.	Real-time fluorescent Kit for Detecting SARS-2019-nCoV		ORF1ab	β-Actin	EUA-FDA CE-IVD
Avellino Lab USA, Inc.	AvellinoCoV2 Test	N1 gene	N2 gene	RNase P gene	EUA-FDA CE-IVD
PerkinElmer, Inc.	PerkinElmer New Coronavirus Nucleic Acid Detection Kit	N gene	ORF1ab	MS2	EUA-FDA CE-IVD
Primerdesign Ltd.	Primerdesign Ltd. COVID-19 Genesig Real-time PCR Assay	-	ORF1ab	from non-biologically relevant exogenous source	EUA-FDA CE-IVD A
DiaSorin Molecular LLC	Simplexa COVID-19 Direct Assay	S gene.	ORF1ab gene	Internal Control RNA	EUA-FDA CE-IVD

Table 2. Continued

Quest Diagnostics Infectious Disease, Inc.	Quest SARS-CoV-2 rRT-PCR	N1 gene	N2 gene	RNA internal positive amplification control (RIPC)	EUA-FDA CE-IVD
Quidel CoRNase Poration	Lyra SARS-CoV-2 Assay	-	Non-structural polyprotein (pp1ab)	Process Control (PRC)	EUA-FDA CE-IVD
Laboratory CoRNase Poration of America (LabCoRNase P)	COVID-19 RT-PCR Test	N1 gene	N2 gene	Hs_RNASE PP30 Internal Extraction Control and positive for RNase P	EUA-FDA CE-IVD
Hologic, Inc.	Panther Fusion SARS-CoV-2	-	ORF1ab Reigon 2-ORF1ab Region 1	Internal Control-S (IC-S)	EUA-FDA CE-IVD
Thermo Fisher Scientific, Inc.	TaqPath COVID-19 combo kit	N gene	S gene-ORF1ab	MS2	EUA-FDA CE-IVD
Roche Molecular Systems, Inc. (RMS)	Cobas SARS-CoV-2	E gene	ORF1 a/b gene	non-Sarbecovirus related armored RNA construct	EUA-FDA CE-IVD
Wadsworth Center, New York State Department of Public Health's (CDC)	New York SARS-CoV-2 Real-time RT-PCR Diagnostic Panel	N1 gene	N2 gene	RNase P	EUA-FDA CE-IVD
Centers for Disease Control and Prevention's (CDC)	CDC 2019-nCoV Real-time RT-PCR Diagnostic Panel (CDC)	N1 gene	N2 gene	RNase P	EUA-FDA CE-IVD
Akron Children's Hospital	Akron Children's Hospital SARS-CoV-2 Assay	-	S gene-E gene	heterologous control	EUA-FDA CE-IVD
Alimetrix, Inc.	Alimetrix SARS-CoV-2 RT-PCR Assay	-	N1 and N2 gene ORF1ab	MS2- RNase P (RP	EUA-FDA CE-IVD
Aeon Global Health	Aeon global Health SARS-CoV-2 assay	N gene	S gene-ORF1ab	MS2	EUA-FDA CE-IVD
UMass Memorial Medical Center	UMass Molecular Virology Laboratory 2019-nCoV rRT-PCR Dx Panel	N1 gene	N2 gene	RNase P	EUA-FDA CE-IVD

Table 2. Continued

Tempus labs, Inc.	iC SARS-CoV2 Test	N gene	S gene-ORF1ab	MS2	EUA-FDA CE-IVD
Cuur Diagnostics	Cuur Diagnostics SARS-CoV-2 Molecular Assay	N gene	S gene-ORF1ab	MS2	EUA-FDA CE-IVD
SEASUN BIOMATERIALS, Inc.	AQ-TOP COVID-19 Rapid Detection Kit PLUS	N gene	ORF1ab	RNase P (RP)	EUA-FDA CE-IVD
University of California, Los Angeles (UCLA)	UCLA SwabSeq COVID-19 Diagnostic Platform	S2 gene	RPP30 gene	Synthetic internal control S2 gene	EUA-FDA CE-IVD

According to Table 2, many companies and laboratories have been developing rRT-PCR kits for the molecular diagnosis of COVID-19. As one of the pioneers, CDC announced receiving EUA-FDA for its real-time PCR diagnostic panel on February 4, 2020, according to the letter granting EUA Amendment(s) published on March 30, 2020, on the FDA website (https://www.fda.gov/media/134919/download). The kit was developed to target two regions of the N gene and used the RNase P gene as the internal control. However, shortly after its distribution, CDC faced negative reports regarding the performance issues of the kit owing to one of the reagents. CDC remanufactured the reagents with more robust quality control measures, and the kit is now available on the market. As mentioned before, the lower sensitivity of N gene compared to other target genes, including E, RdRP, and S has provoked other manufacturers to replace N gene with more sensitive alternatives for better diagnostic performance. Hence, as the target genes are very similar, it is strikingly the design of prime/probe sets that determine the superiority of these kits in case of sensitivity and specificity.

Furthermore, it should be noticed that up-stream procedures before rRT-PCR are as critical as the final assay. Any weakness in collecting/handling the specimens or employing poor technology for viral RNA isolation would prevent a correct and precise analysis. In other words, the pros and cons of the molecular diagnostic methodologies exist at the same time, where the susceptible nature of these tests (especially in a multiplex setup) results in error-prone platforms with a significant rate of false results. The reason for false-negative results by rRT-PCR could arouse from inappropriate extraction of nucleic acid from the specimen and insufficient sample for detection. In order to reduce false-negative results, alternative detection methods should be developed. For instance, specific biomarkers involved during the early stage of COVID-19 could be evaluated. Moreover, the number of false-positive results arising from cross-reaction could be eliminated by specifically identifying antibodies against COVID-19.

Despite all priorities, these tests have some known limitations that stand in the way of effective identification of infected people in all communities or screening programs in the future. Although PCR-based tests can detect virus RNA in the early period of the disease, test kits are not available for everyone and countries. Furthermore, these tests require trained specialists, laboratory equipment, and 1 to 3-hour test duration. Since all COVID-19 detection kits and panels have been developed for emergency use during the pandemic condition, there is not enough valid report on their performance in the evaluation of real samples. Future studies will certainly plot a clearer picture of these products and their performance.

Serology-based Tests for COVID-19

Serological tests, also known as antibody-based tests, are also employed for COVID-19 detection. Recently, increasing demand for serological tests for COVID-19 has resulted in the development of blood-based testing products that can be used to identify COVID-19 by evaluating patients' immune response to the infection caused by the virus rather than detecting the virus directly. These tests can give more detail into the prevalence of the disease in the population by identifying individuals who have developed antibodies to the virus [37].

Serological tests have been fabricated to detect IgM and IgG. However, antigen tests may additionally provide valuable information before or at the time of sampling for molecular screening [33,53]. After the exposure to an antigen, IgM can be measured earlier than IgG, which appears later but in a larger amount. These two immunoglobulins are the targets for different types of serological assays, including ELISA, Neutralization assay, and Rapid diagnostic test (RDT) [54,55].

RDT is considered as a point-of-care medical diagnostic test, which is quick and easy to perform, particularly for early detection or emergency screening. They are also suitable for the use in medical facilities with limited resources and provide same-day results, typically in 20 min [56,57]. Most of the COVID-19 RDTs are qualitative (positive or negative) lateral flow assays that are small, portable and designed for patient antibodies (IgG and IgM) or viral antigen.

In the beginning of the infection, when the immune response is being prepared, detection of antibodies might not be available, which limits the test's effectiveness. However, high viral loads in saliva samples in the first week after the symptoms showed a gradual decline with time, whereas antibodies produced against viral proteins offers a larger window of time for indirect detection of COVID-19 [53]. Zhao et al. have compared the sensitivity of different COVID-19 diagnostic tests days after the symptoms began (58). Their results are presented in Table 3 along with the variation within the levels of SARS-CoV-2 RNA and antigen, IgM and IgG after infection in Fig. 3. According to this figure, clinical significance of IgM/IgG serological test results could be interpreted with the PCR outputs [59].

Hence, even if the serological tests may not be able to involve in early COVID-19 detection or population screening, they have the potential to fight against COVID-19 by identifying individuals who overcame the infection and developed an immune response. In the future, the result of antibody-based testing could be used together with other clinical data to determine individuals who are no longer prone to infection. In addition, these tests could help to find convalescent plasma donors for producing possible COVID-19 treatment.

Table 4 shows the serological tests for detecting specific (IgM/IgG) or total antibodies against COVID-19. Furthermore, Fig. 4 represents the general idea of the lateral flow assay for detecting COVID-19 IgM/IgG.

Cellex Inc. was among the first companies to acquire FDA and EUA approval for their qSARS-CoV-2 IgG/IgM Rapid Test. This lateral flow immunoassay is a qualitative test and intended for the differentiation of IgM and IgG antibodies to COVID-19 in samples of serum, plasma, or venipuncture whole blood. Test components are a conjugate pad that consists of recombinant antigens for COVID-19, gold nanoparticles, rabbit IgG-gold conjugates, and a nitrocellulose membrane strip that contains anti-human IgG, an IgM line (M Line) coated with anti-human IgM, and the control line (C Line) coated with goat anti-rabbit IgG. The test has a three-step procedure, and the result is ready within 15-20 min (www.cellexcovid.com).

Another FDA and EUA certificated product in this category was presented by Ortho Clinical Diagnostics, Inc. The company has developed VITROS Anti-COVID-19. The product tests the total antibodies to the COVID-19 (IgG, IgM, IgA and other isotypes). The test is implemented by VITROS Immunodiagnostic Anti-COVID-19. The assay has two main phases that started with the interaction of COVID-19 antibodies followed by the addition of the recombinant COVID-19 antigen with horseradish peroxidase (HRP) attachment. The luminescent signal generated during the reaction is measured. Later, the company announced the development of another ELISA-based product for the qualitative detection of IgG antibodies against COVID-19, which has also been EU authorized by the FDA (www.orthoclinicaldiagnostics.com).

POCTs for COVID-19

Point-of-care testing (POCT or bedside testing) is a kind

Table 3. Sensitivity of Diagnosis Tests for COVID-19 for Days after Symptoms (58)

Target of test	Days after symptom onset		
	1-7	8-14	15-39
RNA	67%	54%	45%
Total antibody	38%	90%	100%
IgM	29%	73%	94%
IgG	19%	54%	80%

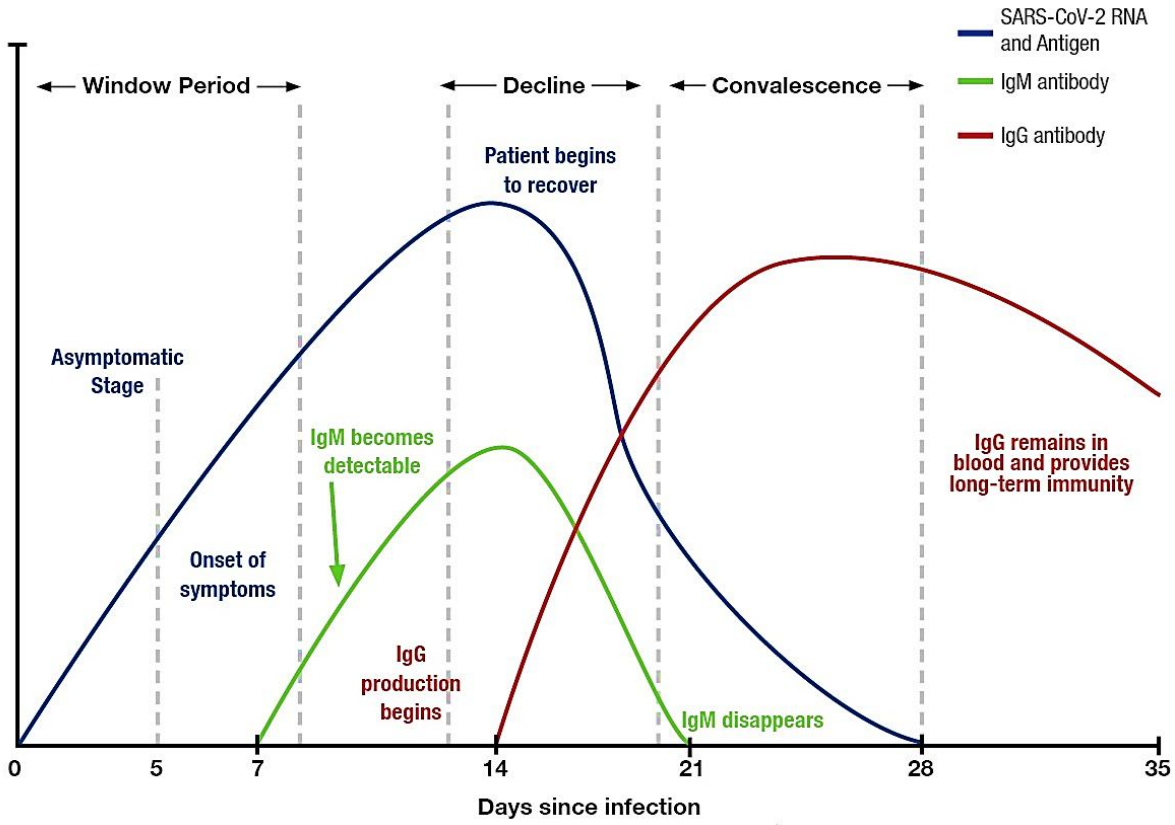

Fig. 3. Variation of the Levels of SARS-CoV-2 RNA and Antigen, IgM and IgG after infection (59). Reprinted with permission

of medical diagnostic test that can be used immediately at the location where a patient is present. These testing strategies are different from traditional diagnostic methodologies by eliminating the need for medical laboratory, trained personnel, transportation of specimens, and long test durations [57].

Table 4. The Serological Test Settings Developed for COVID-19 Detection

Manufacturer	Product Name	Antibodies	Product Certification
Abbott Laboratories Inc	SARS-CoV-2 IgG assay	IgG	US FDA EUA
Acon Biotech (Hangzhou) Co., Ltd.	Detects IgG/IgM antibodies to SARS-CoV-2	IgM/IgG	CE-IVD
Academia Sinica	Anti-SARS-CoV-2 nucleocapsid protein human IgM/IgG rapid detection kit	IgM/IgG	In development
Access Bio Korea, Inc.	CareStart™ COVID-19 IgM/IgG	IgM/IgG	In development
AccuBioTech Co. Ltd.	Accu-Tell COVID-19 IgG/IgM Rapid Test Cassette	IgG/IgM	CE-IVD
Acon Biotech (Hangzhou) Co., Ltd.	SARS-CoV-2 IgG/IgM Rapid Test	IgG/IgM	CE-IVD
AIVD Biotech Inc.	COVID-19 IgG/IgM Rapid Test (colloidal gold-based	IgG/IgM	CE-IVD
Anomalous Materials Pte Ltd	2019-nCoV IgG/IgM Rapid Testing Kit	IgG/IgM	CE-IVD
Assure Tech. (Hangzhou) Co., Ltd	COVID-19 IgG/IgM Rapid Test Device	IgG/IgM	CE-IVD
Atlas Medical	Atlas COVID-19 IgM Elisa Kit	IgM	CE-IVD
Attomarker Ltd	Quantitative Immuno-kinetic assay for Covid-19 IgG+IgM+IgA for a multiantigen panel with CRP	IgG/IgM/IgA	CE-IVD
Autobio Diagnostics Co. Ltd.	Anti-SARS-CoV-2 Rapid Test	IgG/IgM	
Avioq Bio-Tech Co., Ltd.	Novel Coronavirus (2019-nCov)Antibody IgG/IgM Assay Kit (Colloidal Gold)	IgG/IgM	RUO
Baiya Phytopharm, Co, Ltd.	Baiya Rapid COVID-19 IgM/IgG test kit	IgG/IgM	In development
Beijing Abace Biology Co., Ltd.	COVID-19 Antibody (IgG/IgM)Test Kit (Colloidal Gold Immunochromatography)	IgG/IgM	In development
Beijing Diagreat Biotechnologies Co., Ltd.	2019-nCoV IgG Antibody Determination Kit	IgG	CE-IVD
	2019-nCoV IgM Antibody Determination Kit	IgM	
Beijing Kewei Clinical Diagnostic Reagent Inc.	Kewei COVID-19 IgG ELISA Test Kit	IgG	In development
	Kewei COVID-19 IgM ELISA Test Kit	IgM	
	Kewei COVID-19 IgG/IgM Rapid Test Kit	IgG/IgM	
Beijing Tigsun Diagnostics Co. Ltd.	Tigsun COVID-19 Combo IgM/IgG Rapid Test (Lateral Flow Method)	IgG/IgM	CE-IVD
Biocan Diagnostics Inc.	Tell Me Fast Novel Coronavirus (COVID-19) IgG/IgM Ab Test	IgG/IgM	CE-IVD
Biogate Laboratories Ltd.	COVID-19 IgG/IgM Ab Rapid Test	IgG/IgM	RUO

Table 4. Continued

Manufacturer	Product Name	Antibodies	Product Certification
Biogenix Inc. Pvt Ltd.	SARS CoV-2 IgG/IgM Ab Rapid Test	IgG/IgM	RUO
BIOHIT HealthCare (Hefei) Co., Ltd.	SARS-CoV-2 IgM/IgG antibody test kit (Colloidal Gold Method)	IgG/IgM	CE-IVD
Biolidics Ltd	2019-nCoV IgG/IgM Antibody Detection Kit	IgG/IgM	CE-IVD
BIOMAXIMA S.A	2019-nCoV IgG/IgM Rapid Test Cassette	IgG/IgM	CE-IVD
BioMedomics, Inc.	COVID-19 IgM-IgG Dual Antibody Rapid Test	IgM-IgG	CE-IVD
BluSense Diagnostics ApS	ViroTrack COVID IgA/IgM/IgG/Total Ig Ab	IgA/IgM/IgG	
Boditech Med, Inc.	AFIAS COVID-19 Ab, IgM/IgG (automated)	IgG/IgM	RUO
Bright Line Research	Rona 19 Screen Coronavirus (SARS-CoV-2) IgG/IgM Rapid Test	IgG/IgM	In development
BTNX Inc.	Rapid Response COVID-19 IgG/IgM Test Cassette (Whole Blood/Serum/Plasma)	IgG/IgM	RUO
Calbiotech, Inc.	ErbaLisa COVID-19 IgG ELISA	IgG	US FDA EUA - CE-IVD
Cellex Inc.	Cellex qSARS-CoV-2 IgG-IgM Cassette Rapid Test	IgG/IgM	US FDA EUA - CE-IVD
Chembio Diagnostic System, Inc.	DPP COVID-19 IgM/IgG System	IgG/IgM	US FDA EUA - CE-IVD
Core Technology Co., Ltd.	COVID-19 IgM/IgG Ab Test	IgM/IgG	CE-IVD
DIA PRO Diagnostic BioProbes Srl	COV19M.CE - ELISA COVID 19 IgM	IgM	CE-IVD
DIA.PRO Diagnostic Bioprobes Srl	COV19CONF.CE - ELISA COVID 19 IgG Confirmatory	IgG	CE-IVD
DiaSorin Inc.	LIAISON SARS-CoV-2 S1/S2 IgG.	IgG	US FDA EUA - CE-IVD
Dynamiker Biotechnology (Tianjin) Co., Ltd.	2019 nCOV IgG/IgM Rapid Test	IgM/IgG	CE-IVD
Ebram Produtos Laboratoriais Ltd.	CORONAVÍRUS IgG/IgM (COVID-19)	IgM-IgG	Brazil ANVISA
Edinburgh Genetics Limited	Edinburgh Genetics COVID-19 Colloidal Gold Immunoassay Testing Kit	IgM-IgG	CE-IVD
Elabscience	SARS-CoV-2 (2019-nCoV) IgG/IgM Lateral Flow Assay Kit (Whole Blood/ Serum/ Plasma)	IgG/IgM	RUO

Table 4. Continued

Epitope Diagnostics, Inc.	EDI™ Novel Coronavirus COVID-19 IgM ELISA Kit	IgM	CE-IVD
	EDI™ Novel Coronavirus COVID-19 IgG ELISA Kit	IgG	CE-IVD
EUROIMMUN AG	Anti-SARS-CoV-2 ELISA (IgA)	IgA	CE-IVD
	Anti-SARS-CoV-2 ELISA (IgG)	IgG	CE-IVD
GA Generic Assays GmbH	GA CoV-2 IgG/IgM/IgG+	IgG/IgM	CE-IVD
Genitech NSAN Pharmaceutical Pvt. Ltd.	COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)	IgG/IgM	CE-IVD
Getein Biotech, Inc.	One Step Test for Novel Coronavirus (2019-nCoV) IgM/IgG Antibody (Colloidal Gold)	IgG/IgM	CE-IVD
Goldsite Diagnostics Inc	SARS-CoV-2 IgG/IgM Kit (manual)	IgG/IgM	CE-IVD
Hanghzhou AllTest Biotech Co., Ltd.	2019-nCoV IgG/IgM Rapid Test Cassette	IgG/IgM	CE-IVD
Hangzhou Biotest Biotech Co., Ltd.	COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)	IgG/IgM	CE-IVD
Hangzhou Clongene Biotech Co., Ltd.	2019-nCoV IgG/IgM Rapid Test	IgG/IgM	CE-IVD
Humasis	Humasis COVID-19 IgG/IgM Test	IgG/IgM	Korea MFDS EUA - CE-IVD
Hunan Lituo Biotechnology Co., Ltd.	COVID-19 IgG/IgM Detection Kit (Colloidal Gold)	IgG/IgM	CE-IVD
InTec Products, Inc.	Rapid SARS-CoV-2 Antibody (IgM/IgG)	IgG/IgM	CE-IVD
Jetta Labs LLP	OZO Diamond SARS-CoV2 (COVID-19) lgG/lgM Test (Latex Method)	IgG/IgM	US FDA EUA - CE-IVD
Liming Bio-Products Co., Ltd.	COVID-19 IgG/IgM Combo Rapid Test Device	IgG/IgM	CE-IVD
LOMINA AG	SARS-CoV-2(COVID19)IgM/IgG Antibody Fast Detection Kit	IgG/IgM	CE-IVD
LumiQuick Diagnostics Inc.	QuickProfile™ 2019-nCoV IgG/IgM Combo Test Card	IgG/IgM	CE-IVD
Mokobio Biotechnology R&D Center	SARS-CoV-2 IgM & IgG Quantum Dot Immunoassay	IgG/IgM	CE-IVD
MP Biomedicals	MP Rapid 2019-nCoV IgG/IgM	IgG/IgM	CE-IVD
Mount Sinai Laboratory	COVID-19 ELISA IgG Antibody Test	IgG	US FDA EUA
Nal von minden GmbH	NADAL® COVID-19 IgG/IgM Test	IgG/IgM	CE-IVD
NanoEntek Inc.	FREND™ COVID-19 IgG/IgM Duo	IgG/IgM	Korea MFDS EUA - CE-IVD
Nantong Egens Biotechnology Co., Ltd.	COVID-19 IgG/IgM Rapid Test Kit (Colloidal Gold)	IgG/IgM	CE-IVD
Naturitious LLC	Viralert COVID-19 IgG/IgM Antibody Rapid Test Kit	IgG/IgM	CE-IVD

Table 4. Continued

Ortho-Clinical Diagnostics, Inc.	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Reagent Pack	IgG	US FDA EUA - CE-IVD
Ortho-Clinical Diagnostics, Inc.	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack	Total Antibody	US FDA EUA - CE-IVD
PRIMA Lab S.A.	PRIMA COVID-19 IgG/IgM Rapid Test (For Professional Use)	IgG/IgM	CE-IVD
Qingdao Hightop Biotech Co., Ltd.	Hightop COVID-19 IgM/IgG Ab Rapid Test Kit	IgG/IgM	CE-IVD
Shandong ThinkLab Biotechnology Co., Ltd.	2019-nCOV IgM/IgG antibody test kits (Colloidal-gold Assay)	IgG/IgM	CE-IVD
Shanghai Outdo Biotech Co., Ltd	Novel Coronavirus (SARS-CoV-2) Antibody (IgM / IgG) Test	IgG/IgM	China NMPA EUA - CE-IVD
Shenzhen Bioeasy Biotechnology Co., Ltd.	Bioeasy 2019-nCoV Ab(IgG/IgM) GICA Rapid Test Kit	IgG/IgM	CE-IVD
Shenzhen Tailored Medical Ltd.	Novel Coronavirus (SARS-CoV-2) IgM/IgG Antibody Assay Kit	IgG/IgM	CE-IVD
Sichuan Xincheng Biological Co., Ltd.	SARS-Cov-2 IgG/M Antibody	IgG/IgM	CE-IVD
Tauschen Int. Ltd (Tauschen Groups)	COVID - 19 RAPID TEST KIT / Antibody IgG& IgM Test Kit	IgG/IgM	China NMPA EUA - CE-IVD
Tianjin Era Biology Technology Co., Ltd.	COVID-19 IgM/IgG Lateral Flow Assay	IgG/IgM	CE-IVD
U2USystems (India) Pvt. Ltd.	2019-ncoV IgG/IgM Test	IgG/IgM	CE-IVD
Vircell S.L.	COVID-19 VIRCLIA® IgM+IgA MONOTEST	IgM/IgA	CE-IVD
VivaChek Biotech (Hangzhou) Co., Ltd.	VivaDiag COVID-19 IgM/IgG Rapid Test	IgG/IgM	CE-IVD
Willi Fox GmbH	Willi Fox Covid-19 IgM/ IgG rapid test	IgG/IgM	CE-IVD
Wuhan UNscience Biotechnology Co., Ltd.	Covid-19 IgG/IgM Antibody Rapid Test Kit	IgG/IgM	CE-IVD
Xiamen Biotime Biotechnology Co., Ltd.	SARS-CoV-2 IgG/IgM Rapid Qualitative Test Kit	IgG/IgM	CE-IVD
Yuno Diagnostics Co., Ltd.	Novel coronavirus(SARS-CoV-2) IgG/IgM Antibody Combined Test Kits	IgG/IgM	CE-IVD
Zhuhai Livzon Diagnostics Inc.	Diagnostic Kit for IgM Antibody to Corona Virus(nCoV-2019)	IgG/IgM	CE-IVD
Zybio Inc.	SARS-CoV-2 IgM and IgG Antibody Assay Kit	IgG/IgM	CE-IVD
Abbott Laboratories Inc.	AdviseDx SARS-CoV-2 IgM	IgM	FDA EUA
Beckman Coulter, Inc.	Access SARS-CoV-2 IgM	IgM	FDA EUA
Genalyte, Inc.	Maverick SARS-CoV-2 Multi-Antigen Serology Panel v2	Total Antibody	FDA EUA

Table 4. Continued

Thermo Fisher Scientific	OmniPATH COVID-19 Total Antibody ELISA Test	Total Antibody	FDA EUA
ZEUS Scientific, Inc.	ZEUS ELISA SARS-CoV-2 IgG Test	IgG	FDA EUA
DiaSorin, Inc.	DiaSorin LIAISON SARS-CoV-2 IgM Assay	IgM	FDA EUA
NanoEntek America, Inc.	FREND COVID-19 total Ab	Total Antibody	FDA EUA
Nirmidas Biotech, Inc.	Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit	IgM, IgG	FDA EUA
Quotient Suisse SA	MosaiQ COVID-19 Antibody Magazine	Total Antibody	FDA EUA
Assure Tech. (Hangzhou Co., Ltd)	Assure COVID-19 IgG/IgM Rapid Test Device	IgM, IgG	FDA EUA
Jiangsu Well Biotech Co., Ltd.	Orawell IgM/IgG Rapid Test	IgM, IgG	FDA EUA
Shenzhen New Industries Biomedical Engineering Co., Ltd.	MAGLUMI 2019-nCoV IgM/IgG	IgM, IgG	FDA EUA
BioCheck, Inc.	BioCheck SARS-CoV-2 IgM Antibody Test Kit	IgM	FDA EUA
BioCheck, Inc.	BioCheck SARS-CoV-2 IgG Antibody Test Kit	IgG	FDA EUA
Sugentech, Inc.	SGTi-flex COVID-19 IgG	IgG	FDA EUA
TBG Biotechnology Corp.	TBG SARS-CoV-2 IgG / IgM Rapid Test Kit	IgM, IgG	FDA EUA
University of Arizona Genetics Core for Clinical Services	COVID-19 ELISA pan-Ig Antibody Test	Total Antibody	FDA EUA
Biocan Diagnostics Inc.	Tell Me Fast Novel Coronavirus (COVID-19) IgG/IgM Antibody Test	IgM, IgG	FDA EUA
BioCheck, Inc.	BioCheck SARS-CoV-2 IgG and IgM Combo Test	IgM, IgG	FDA EUA
Diazyme Laboratories, Inc.	Diazyme DZ-Lite SARS-CoV-2 IgM CLIA Kit	IgM	FDA EUA
BioMérieux SA	VIDAS SARS-CoV-2 IgM	IgM	FDA EUA
BioMérieux SA	VIDAS SARS-CoV-2 IgG	IgG	FDA EUA

POC technology is generally categorized into two main types. The first one is the technology which uses small benchtop analyzers such as blood gas and electrolyte analyzer systems, and the latter one is the handheld, single-use kits such as urine albumin, blood glucose and coagulation tests. In the presence of a rapidly spreading deadly disease, more attempts were given to developing kits than benchtop analyzers for the detection of COVID-19 detection. Producing accurate and reliable tests that can be applied at home settings could lower the need for clinic visits and unnecessary hospital admissions. One of the most desirable features of POCTs is their ability to provide rapid

results as they could be operated at the sites where the symptoms are observed. In response to the shortage of laboratory-based molecular testing capacity the presence of COVID-19 pandemic, diagnostic test manufacturers started to develop portable, rapid, and easy-to-use platforms.

In this section, we focus on the integrated molecular POC devices for the detection COVID-19. Abbott announced the development of the fastest available molecular POC test for the detection of COVID-19 via targeting RdRp gene, delivering positive and negative results in 5 and 13 min, respectively. The test runs on Abbott's ID NOW™ platform, providing results in a wide range of healthcare settings (www.abbott.com) (Fig. 5A).

Figure 5B shows the automated molecular test developed by Cepheid for the detection of COVID-19. The test is qualitative and uses the same design principles as current Xpert® Xpress Flu/RSV cartridge technology that has been designed for the detection of respiratory viruses. The current Cepheid POC test can provide the results in approximately 45 min with less than a minute of hands-on time to prepare the sample. The Cepheid Xpert® Xpress SARS-CoV-2 provides a single-use assay for one sample that runs on the GeneXpert® System. This system is composed of independent modules (1, 2, 4…) that allow random access to sample processing. These characteristics may suit POC needs. (www.cepheid.com)

Bosch Healthcare and Global Randox Laboratories came together to develop a POC coronavirus test entitled Vivalytic Viral Respiratory Tract Infection (VRI) Array (Fig. 5C). The test uses an array-based technology to simultaneously detect ten viral respiratory tract infections, including SARS-CoV-2, Influenza A and B, Sarbecovirus, and MERS. The array only requires 300 µl of nasopharyngeal swab sample and targets E gene for screening Sarbecovirus and ORF1ab for differential detection of SARS-COV-2. The VRI end-point PCR test is developed to be performed on the Vivalytic system, a fully automated, cartridge-based platform capable of both Hi-Plex and Lo-Plex testing. The cartridges used in this technology are fully sealed, which minimizes the contamination risk. The VRI Array is one of the world's first multiplex molecular diagnostic tests meeting SARS-CoV-2 testing recommendations of WHO and CDC. With the development of this fully automated multiplex array, the diagnosis could be accomplished rapidly at the point of care such as doctor offices (www.randox.com/bosch-coronavirus-vivalytic).

As a world leader in the field of *in vitro* diagnostics, bioMérieux and its subsidiary, BioFire Defense, has received EUA by FDA for BIOFIRE® COVID-19 test. Due to a large number of viral and bacterial pathogens that cause respiratory infections such as Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Bordetella pertussis, Chlamydophila pneumonia, and *etc*., BioFire RP2.1-EZ Panel makes it possible the detection and identification of 19 different respiratory targets including SARS-Cov-2 in approximately 45 min. This Panel must be used in association with CLIA-waived BioFire® FilmArray® 2.0 EZ Configuration System to run a multiplex PCR test to detect and differentiate 15 viral and four bacterial from just one nasopharyngeal swab sample and finally, an easy to read report indicates the positive or negative result. The automatic protocol of nucleic acid analysis makes this test a comprehensive, accurate, and rapid alternative method that minimizes the risk of missing real culprit (www.biofiredx.com).

Mesa Biotech is another company that received FDA EU authorization for their COVID-19 molecular POC entitled Accula SARS-CoV-2 Test. The test has been developed as a PCR-based qualitative test to investigate throat and nasal swabs, and can prepare results in 30 min. The test results are interpreted by the visualization of blue test lines on the detection strip in Test cassettes (www.mesabiotech.com).

Furthermore, there are several reports on academic-level based on CRISPR (clustered regularly interspaced short palindromic repeats) technology to COVID-19 pandemic. Morales-Narváez *et al*. have recently reviewed almost all detection strategies for CRISPR-powered COVID-19 testing, including SHERLOCK platform, loop-mediated isothermal amplification (LAMP), and DETECTR technologies [60].

GenMark Diagnostic developed ePlex Respiratory Pathogen Panel 2 (ePlex RP2 Panel) device for

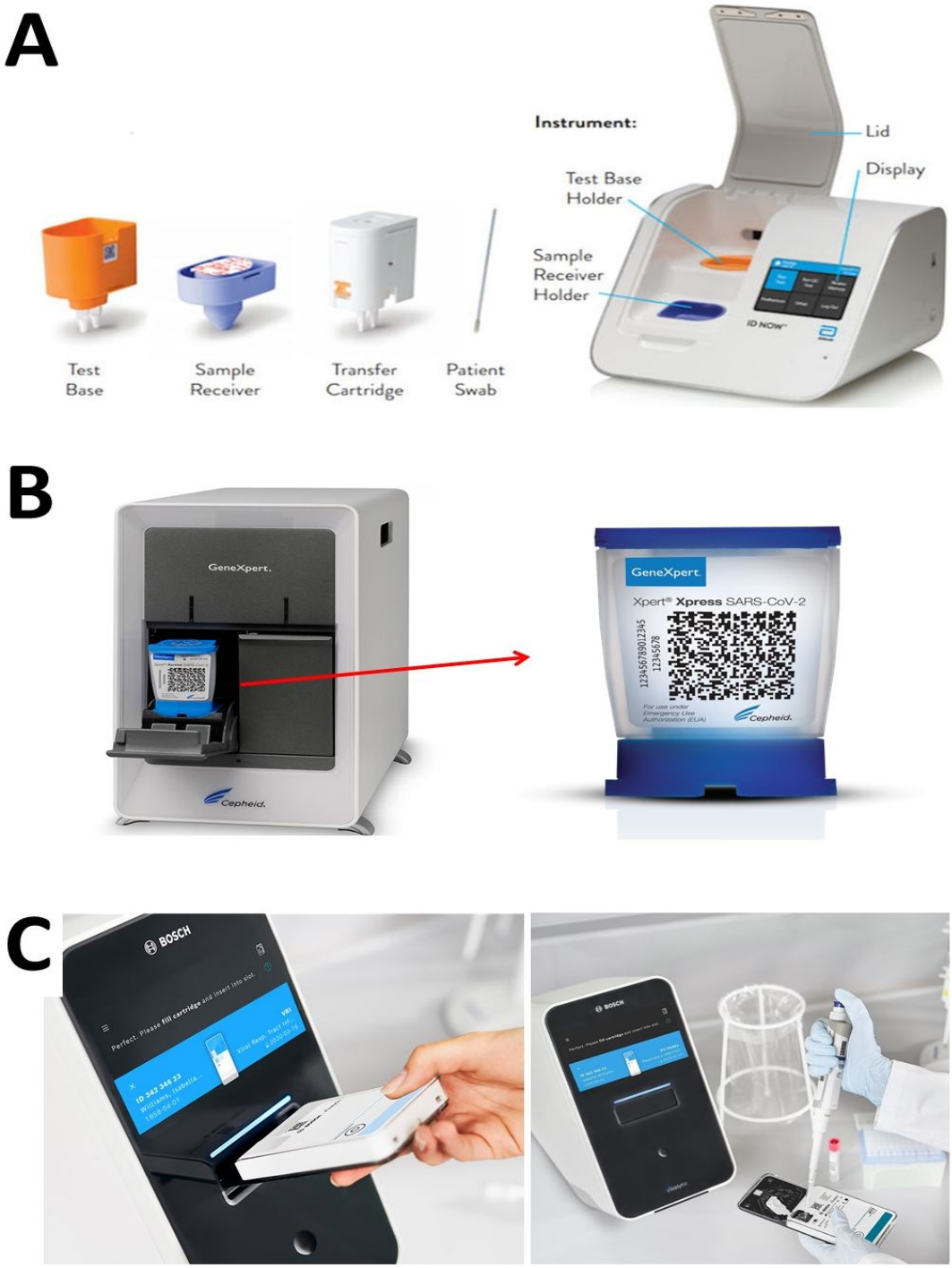

Fig. 5. A) Abbott's ID NOW™ platform. Adopted from www.abbott.com/coronavirus. B) Cepheid Xpert SARS-CoV-2 test. Adopted from www.cepheid.com/coronavirus. C) Vivalytic Viral Respiratory Tract Infection (VRI) Array. Adopted from www.randoxbiosciences.com.

simultaneous detection and differentiation of over 20 different respiratory pathogens that are often missed by traditional methods. The increasing trend of SARS-Cov-2 respiratory infection concerns people all around the world. The similarity of its symptoms to influenza, common cold, and co-infections increase the demands for designing a comprehensive panel to accurately and rapidly identify the pathogen. Detecting multiple respiratory viral and bacterial organisms' nucleic acids, a combination of electrowetting and GenMark's sensor technology in a single cartridge makes all aspects of testing including extraction, amplification, detection, and releasing of test results performed fully automated in less than 2 h. This panel test provides an extended overview of patients' clinical status and helps clinicians to choose a concise healthcare strategy, *i.e*., it maximizes Lab's efficiency and patient care by avoiding medical errors (www.genmarkdx.com) (Fig. 6A).

Oxford Nanopore Diagnostics has introduced LamPORE COVID-19, a fully automated and rapid assay to detect COVID-19 from oropharyngeal and nasopharyngeal swabs of patients. The test offers many advantages, *e.g*., it is based on amplifying three highly conserved genes (E gene, N gene and ORF1a) in positive samples and human actin as the negative control gene, a significant number of samples can be processed in 24 h (over 9.000 samples), and it is cost effective, reliable and accurate. The platform provides 99.1% sensitivity and 99.6% specificity. Its workflow is simple, and conducts a molecular test on RNA extraction of the collected samples, and incubates them after adding target gene reagents at a constant temperature. Then, samples (where genes are amplified to indicate positive or negative results) are sequenced. It is also important to note that all processes are performed automatically by means of GridION OND device, and there is no need for any manual intervention and interpretation. At the end of the process, results are reported in LIMS-compatible formats (www.oxfordnanoporedx.com) (Fig. 6B). LamPORE is CE marked for in-vitro diagnostic use.

A novel and automated kit received FDA EUA authorization in recent days. Detectachem Inc. commercialized MobileDetect Bio BCC19 (MD-Bio BCC19) Test Kit, which performs qualitative molecular detection of RNA up to 96 specimens of COVID-19 patients in 30 min. Nasopharyngeal, anterior nasal swabs, mid-turbinate nasal swabs, and oropharyngeal swabs are combined with target reagents, and tests run on MD-Bio heater to utilize a reverse transcription loop-mediated isothermal nucleic acid amplification (RT-LAMP) strategy. The automated procedure eliminates the need for a separate RNA extraction step. After the amplification step, a visual color shift enables manual interpretation of results, *e.g*., positive and negative results are indicated by yellow and red color, respectively. A free mobileDetect app is added to this device to enhance the instant result reporting by scanning a QR code for up to 8 samples (www.detectachem.com).

The Novel clear Dx SARS-CoV-2 from Clear Labs received the emergency use authorization as the first nanopore sequencing-based test from FDA. The platform has a strong potential for next generation DNA sequencing *in vitro* diagnostic tests. Rapid results provided in hours, fully automated protocols, and deep monitoring of gene mutation and characterization enables rapid diagnosis as well as pharmaceutical research for inventing drug-treatment of COVID-19. Combination of the latest technology in automation such as Robotic pipetting, multi-target analysis of arrays of pathogens, better accuracy, proving environmental maps, and predictive risk assessments by employing Artificial Intelligence (AI) and machine learning make this PCR kit a significant asset in the market (www.clearlabs.com) (Fig. 6C).

Aptima SARS-CoV-2 assay is designed by Hologic in the USA to detect isolated and purified RNA from upper respiratory specimens from individuals whose symptoms meeting COVID-19 epidemiological criteria. This assay enables the testing of over 1000 samples in 24 h. Target capture and Mediated Amplification technologies, as well as Dual Kinetic Assay, are combined to develop Aptima SARS-CoV-2 assay. The assay is performed on Panther and Panther Fusion system fully automated nucleic acid analysis based on real-time PCR *in vitro* diagnosis (www.hologic.com).

Becton and Dickinson (BD) introduced The BioGX SARS-CoV-2 Reagents for BD MAX™ System to run a real-time PCR for detecting nucleic acids from SARS-CoV-2 nasopharyngeal and oropharyngeal swab sample. BioGX SARS-CoV-2 Reagents contain multiplexed sets of

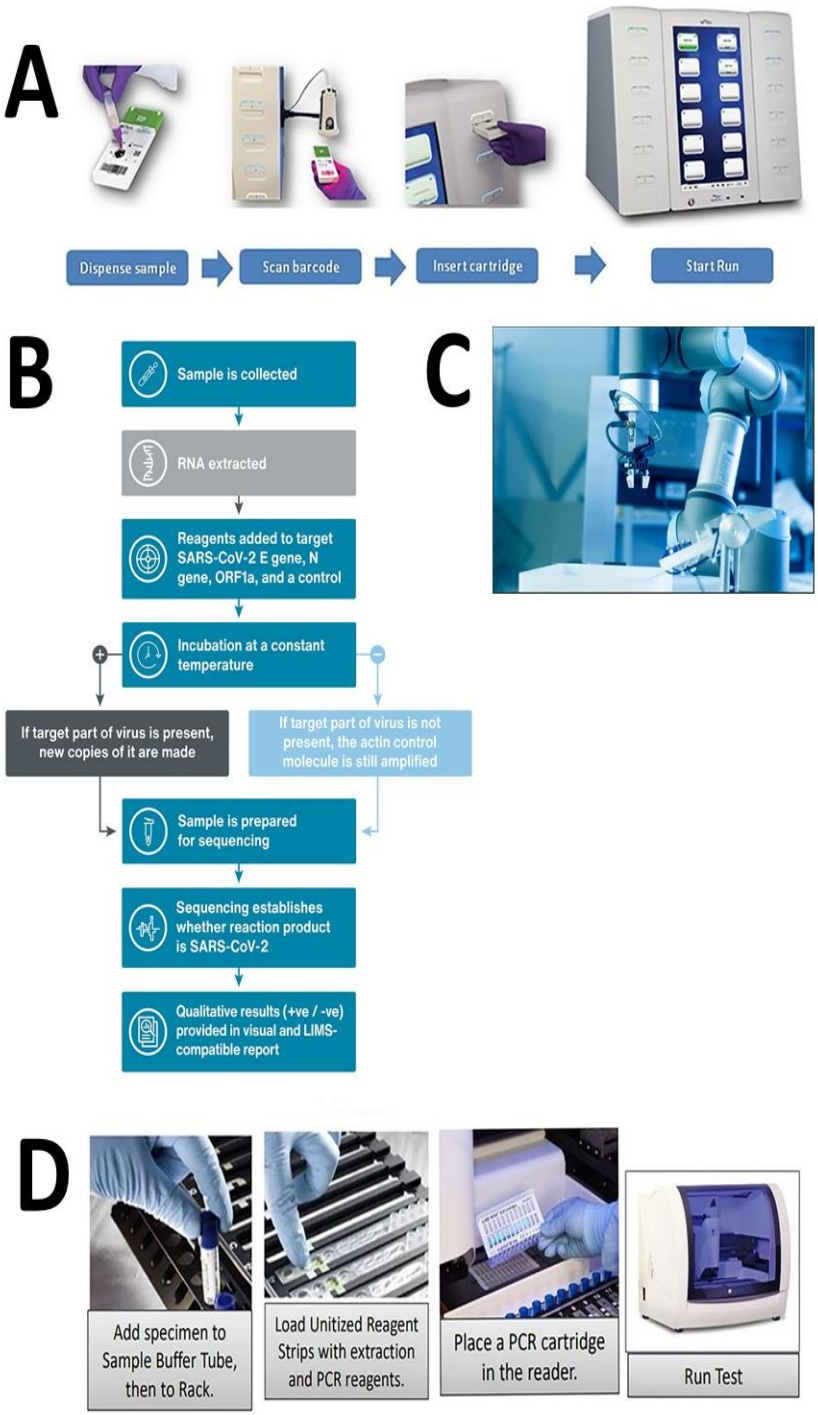

Fig. 6. A) ePlex Respiratory Pathogen Panel 2 (ePlex RP2 Panel) device. Adopted from www.genmarkdx.com. B) LamPORE COVID-19 platform. Adopted from www.nanoporetech.com. C) Clear Dx SARS-CoV-2 platform. Adopted from www.clearlabs.com. D) The BioGX SARS-CoV-2 Reagents for BD MAX™ System. Adopted from www.bd.com.

primers and probes for amplifying the unique N1 and N2 regions of nucleocapsid phosphoprotein gene by targeting SARS-CoV-2 coronavirus RNA. Extraction, isolation, and purification of SARS-CoV-2 nucleic acid are performed by the use of BD MAX ExK TNA-3 kit and then followed by reverse transcribed into cDNA and PCR amplification and detection using BioGX SARS-CoV-2 Reagents for BD MAX System (www.bd.com) (Fig. 6D).

Pros and Cons of COVID-19 Detection Methods

rRT-PCR is currently known as the 'gold standard' method for detection of COVID-19 as it is quantitative and specific [61,62].

The method and its kit are minimally invasive and ideal for social distancing. While the main test (rRT-PCR) is performed at the laboratory, sampling does not require a sophisticated infrastructure. Moreover, studies have shown that using rRT-PCR kits for COVID-19 with deep nasal swabs has fewer false negatives compared to other methods. The method is able to provide test results within a day. However, during the pandemic conditions, due to the limits in accessing real-time machines and related equipment, test results could be determined in one to two weeks, which is not preferred in the presence of a deadly disease [61].

As explained before, there are two main types of serology tests developed for COVID-19. Most serology tests determine COVID-19 specific antibodies (IgM and IgG) in the blood. Therefore, these methods are more invasive than molecular ones. It is important to note that after infected by COVID-19, it takes several days or weeks to develop antibodies, while these molecules are typically not stable in blood and naturally vanish several weeks after recovery. Therefore, antibody tests are not suitable tools for early diagnosis, while can be used to show the infection history of the patient. Likewise, there is no clear evidence to suggest that the presence of these antibodies can protect people from the second COVID-19 infection [63].

Another serology-based test is the ones designed to detect COVID-19 surface antigens. These tests are rapid and need a few hours to provide the result. However, they have a high false-negative rate due to low sensitivity [61]. To overcome the disadvantages of rRT-PCR, digital PCR can be used as they have higher sensitivity which could reduce the number of false results. In addition, high-throughput gene sequencing, LAMP method, CRISPR/Cas-based methods, and POCs endure limitations as they are not scalable and lack practical reliability [61,62].

CONCLUSIONS

Coronaviruses could emerge in different forms, e.g., SARS-CoV, MERS-CoV and COVID-19, with rapid spread and high death rates that became massive viral outbreaks in history. Identified first in China, considering COVID-19's ability to mutate as well as to infect different species, *e.g.,* human, dog or cat, developing accurate diagnostic technologies that provide rapid test results is very critical to stopping these diseases before turning to a pandemic. With a new world after COVID-19, precautions such as hand and respiratory hygiene, masks, face shields, social distancing, and lockdowns became part of our life. The public health officials are also taking measures, *e.g.*, performing an enormous amount of COVID-19 tests mostly based on PCR for producing highly accurate results or rapid screening tests based on lateral flow, and following the strict procedure in the intensive care unit. As the entire genome of COVID-19 has been released, PCR technologies have already been adapted and employed to diagnosis this disease with a high success rate.

Currently, many countries are developing their own PCR kits through state or private sectors. A nasopharyngeal sent for PCR test could provide an accurate result, while the need for a developed infrastructure and long test duration extend the time for diagnosis in the presence of a large number of patients. With this in mind, determining viral proteins and their detection techniques dramatically speed up the diagnostic processes dramatically. Multiple international efforts on these technologies have been developed, *i.e.,* lateral flow tests, based on virus-antibody interaction that triggers a color change. Despite shorter test durations as well as eliminating the need for sophisticated devices, these screening tests for early COVID-19 diagnosis still need more work due to low accuracy.

Future COVID-19 research with new findings on the disease mechanism as well as an immune response to such disease will bring new modalities to stop COVID-19, *e.g.,*

vaccines. Moreover, our response to such virus pandemic should be frequently revised at the society, hospital, laboratory, and individual levels.

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Conflicts of Interest/Competing Interests

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