COVID-9 Detection Strategies: Recent Advances and Future Prospects

Tina Shaffaf

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Abstract

COVID-19 pandemic has created a global medical and economic crisis. Having many unsuspecting asymptotically carriers interacting with others increases the risk of infecting healthy people which leads to problems such as overloaded clinics and hospitals. These conditions make tracing the asymptotic carriers of COVID-19 and detecting all infected individuals rapidly and accurately critical for the control and further prevention of this disease. Considering the long duration of vaccine development, their low efficiency for protection against some of the viral variants, and lack of any drugs for efficient COVID-19 treatment, diagnostic tests are essential for detecting the infection and limiting the viral spread. Therefore, how to efficiently screen for positive patients with coronavirus 2019 has become the primary task for epidemic prevention. Due to the critical roles of the diagnostic tools in fighting the coronavirus disease, a large number of techniques have rapidly. This work, as a comprehensive review, aims to cover not only the currently approved nucleic acid- and protein-based diagnostic technologies, but also the promising strategies for COVID-19 detection and also fighting future hazards. The goal is to bring together the most important advances from the broad discipline of biomedical engineering, enhancing their visibility through opinion and new articles, and providing overviews of the state-of-the-art in each field.

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List of Abbreviations

Abbreviation	Full form
AB	Antibody
ACE2	Angiotensin-converting enzyme 2
AuNI	Gold nano-island
AuNP	Colloidal gold nanoparticle
BRE	Biorecognition element
BSL-2	Biosafety level 2
CDC	Centres Of Disease Control and Prevention
CLIA	Chemiluminescence Immunoassays
CMIA	Chemiluminescent microparticle immunoassay
CMOS	Complementary metal oxide semiconductor
COVID-19	Coronavirus Disease 2019
CRISPR/CAS	Clustered regularly interspaced short palindromic repeats/CRISPR-associated
ddPCR	Droplet digital PCR
E	Envelope
ECLIA	Electrochemiluminescence immunoassay
EIA	Enzyme-linked immunoassays
EIS	Electrolyte–insulator–semiconductor
ELISA	Enzyme-linked immunosorbent assay
EUA	Emergency Use Authorization
FDA	U.S. Food and Drug Administration
FET	Field-effect transistor-based biosensors
FMIA	Fluorescent microsphere immunoassay
FMS	Fluorescent microsphere
gFET	Graphene FET
GISAID	Global initiative on sharing all influenza data
HoT	Hands-on time
Ig	Immunoglobulin
ISFET	Ion-selective field-effect transistor
IST	Immunochromatographic strip tests
IVDS	In vitro diagnostics
LAMP	Loop mediated isothermal amplification
LFA	Lateral flow assays

LFIA	Lateral flow immunoassays
LIS	Laboratory Information System
LNP	Lanthanide-doped polystyrene nanoparticle
LoC	Lab-on-a-chip
LoD	Limit of detection
M	Membrane
MCLIA	Magnetic CLIA
MIA	Microsphere Immunoassay
mNGS	Metagenomic next-generation sequencing
N	Nucleocapsid
NA	Nucleic acid
NAATS	Nucleic acid amplification tests
NGS	Next Generation Sequencing
NIIA	Non-Isotopic Immunoassay
nPCR	Nested PCR
NTS	Nanopore target sequencing
ORF1ab	Open reading frame ab
PoC	Point-of-care
PoN	Point-of-need
PPT	Plasmonic photothermal
qPCR	Real-time PCR (quantitative PCR)
RBD	Receptor-binding domain
RdRp	RNA dependent RNA polymerase
RT-LAMP	Reverse Transcription-LAMP
RT-PCR	Reverse Transcription- Polymerase Chain Reaction
RUO	Research use only
S	Spike
SARS-COV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SNR	Signal-to-noise ratio
TaT	Turn-around time
WBC	White blood cell
WHO	World Health Organization

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Chapter 1

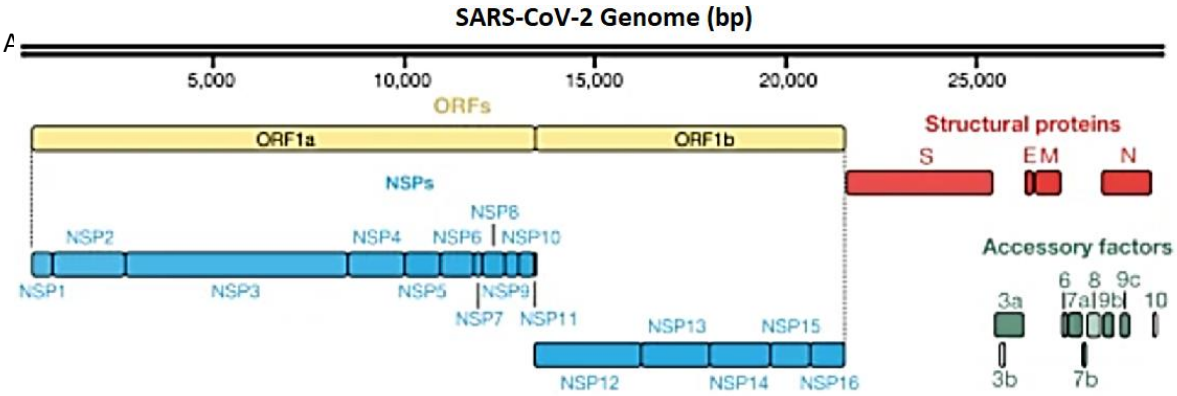
Introduction

The spread of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has resulted in a life-threatening novel respiratory disease worldwide [1]. As of August , 2021, the total number of the confirmed coronavirus disease 2019 (COVID-19) cases and the deaths have been reported at least 216,000,000 and 4,490,000, respectively [2]. To date, a few companies have developed vaccines to protect people from the infection caused by COVID-19. Vaccination is a very beneficial strategy to limit the viral spread; however, it takes at least a few months to develop an effective and safe vaccine after the initiation of the pandemic, and hundreds of thousands of lives would be threatened during this period. Another issue is the natural mutations resulting in some variation in the viral structural proteins which may result in the resistance of the virus to the vaccine which has taken a long time and labour to be developed and commercialized. Although these vaccines have successfully received U.S. Food and Drug Administration's (FDA) approval, their long-term effects, side effects, and the effectiveness of them are some other challenges requiring more time and evaluation after the initiation of vaccination in the real world. Another concern is that a considerable portion of the infected cases do not experience the typical known symptoms of fever, fatigue, and dry cough in the first stage and only symptomatic cases of COVID-19 are being identified and isolated [3]. Such pre-symptomatic or asymptomatic cases are not informed of the being carriers and accelerate the community spread by their presence in the society. Considering the critical and fatal effects of COVID-19, reliable rapid, accurate and affordable diagnostic tests are urgently required not only for detection of the infection in the earliest stages but also to monitor the disease and conducting convalescence studies to control this outbreak.

The genetic material of the coronavirus 2019 is a RNA including six open reading frames (ORFs) responsible for the production of the nucleocapsid (N), spike (S), membrane (M) and a small envelope (E) structural protein subunits; the rest of the genes produce 16 functional proteins such as RNA dependent RNA polymerase (RdRP) and helicase (Figure 1. *1*) [4]. N protein attaches to the coronavirus genetic RNA and forms nucleocapsid. Spike glycoprotein (S1 and S2 subdomains) are surface densely glycosylated proteins specifying the type of the infected host and are involved in various tendencies to different tissues [5]. Angiotensin-converting enzyme 2 (ACE2), expressed in human endothelial cells in the lung, intestine, heart and kidney, is a surface protein and a functional receptor for coronavirus [6]. This protein acts as a direct binding site for virus S protein, the receptor-binding domain (RBD) of the S1 subunit

of S protein directly contacts with the ACE2 receptor with a high affinity and has crucial roles in attachment and fusion of the virus through the ACE2-containing host cells [7]. M protein has the main role in forming new virus particles and could insert some proteins from the host to the viral envelope [8]. E protein is the smallest structural protein having roles in coronavirus assembly and pathogenesis [9]. The arrangement of these four proteins is different among coronaviruses but their presence is critical for infectious characteristics of SARS-CoV-2. After the insertion of the coronavirus into the human body, the immune system demonstrates a prompt defensive response to the viral antigens and produces specific antibodies to fight the disease [10].

From a diagnostic point of view, specific virus antigens or specific antibodies against these antigens are detectable in the specimens collected from COVID-19 positive patients e.g. respiratory swabs, saliva, blood, serum, stool, and other types of samples [11]. Considering the guidance published by the World Health Organization (WHO), FDA, and centres of disease control and prevention (CDC), nucleic acid (NA)-based tests are the main tests employed for detection of the virus in suspected cases and serological tests are beneficial for confirmation of the reported result as supplementary tests together with other clinical data. These tests are also more informative while performing for evaluation of convalescence status, immune response and employed as screening tools for testing the rate of serosurvey, prevalence [12, 13].



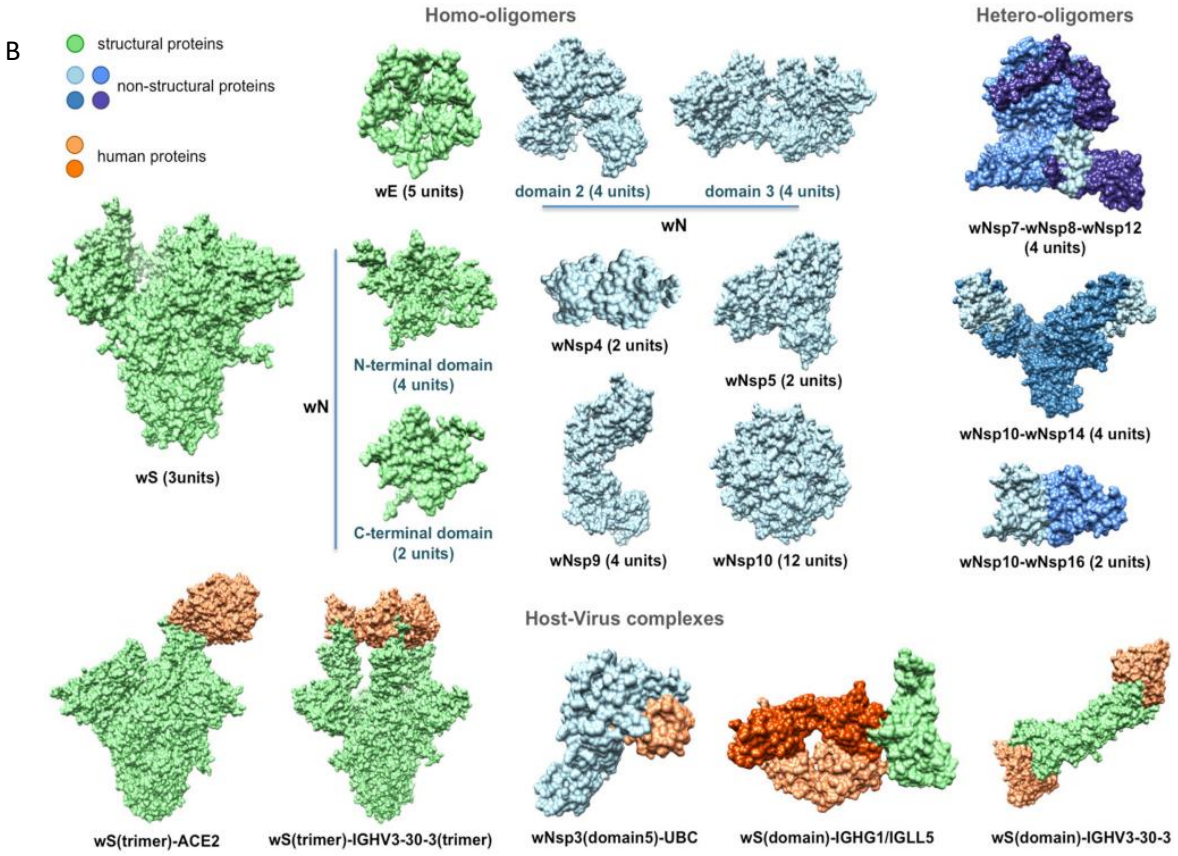


Figure 1. 1. A) Complete genome of severe acute respiratory syndrome coronavirus 2 [14], B) Structural characterization of SARS-CoV-2 proteins and their intra- and host–viral interactions with the number of the involved subunits [15].

Based on FIND, as of March 5, 2021, at least 1130 diagnostic tests have been submitted to various regulatory organizations in different countries, about 1030 tests have received emergency authorization and are manufactured [16]. Two main diagnostic categories are employed for sensing the coronavirus 2019; the first group targets viral nucleic acid and the second one captures specific polypeptides either in the virus structure or in the blood of the suspected people (summarized in Figure 1.2). The present research will provide a comprehensive review to discuss the current and promising technologies for COVID-19 detection and challenges associated with each technology

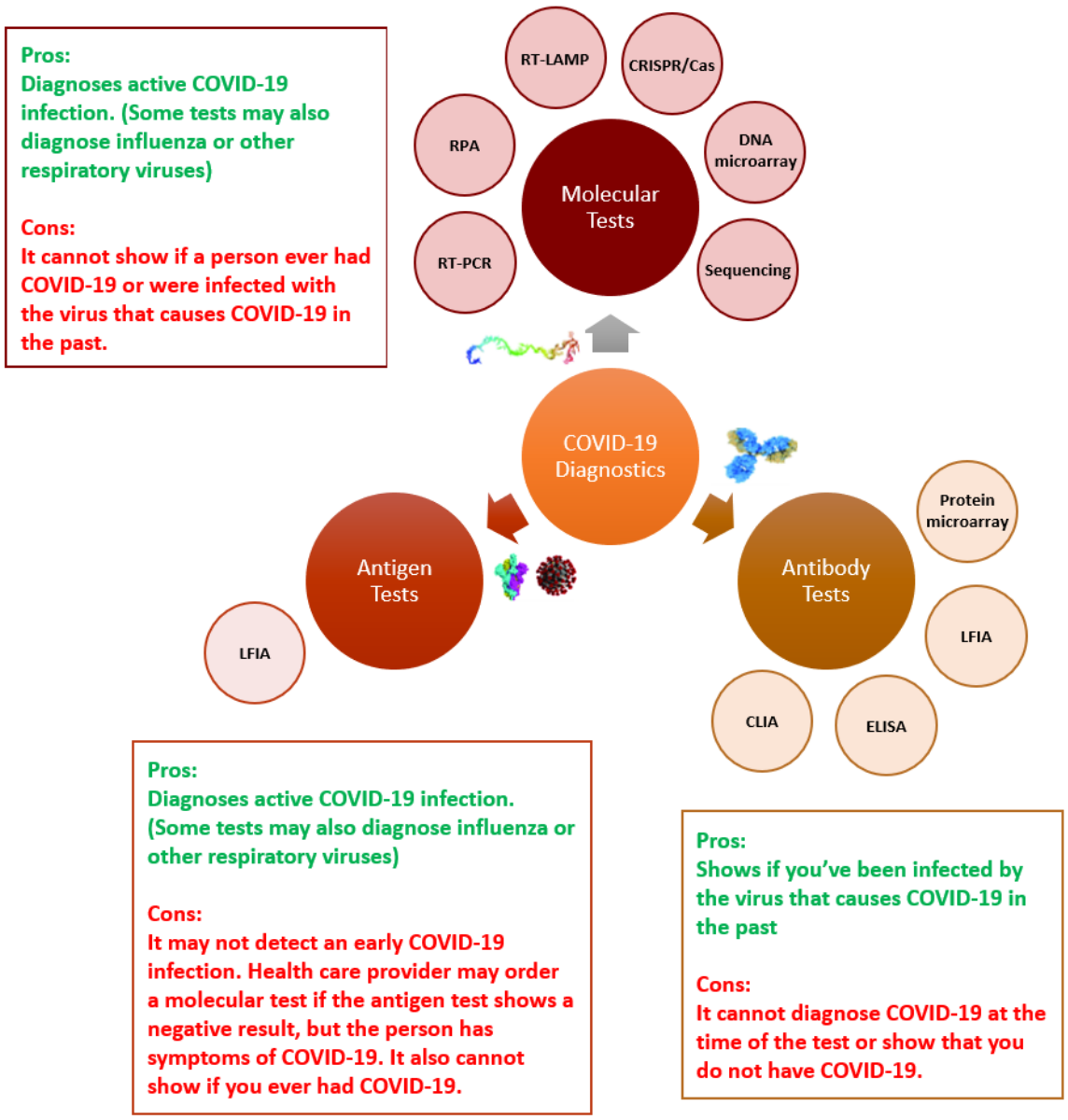


Figure 2. 2. The diagram summarizes the main strategies discussed in this research.

1.1. Research Plan

In this work, we aim to study and document all of the characteristics, advantages, disadvantages, potentials and weakest links of the diagnostic strategies. The goal is to bring together the most important advances from the broad discipline of biomedical engineering, enhancing their visibility through opinion and new articles, and providing overviews of the state-of-the-art in each field. We will put one step forward to improve human health or healthcare by encouraging the researchers and inspiring them to improve their innovative methodologies beyond conception. For this aim, the mechanisms of the detecting strategies and the take-home message are encapsulated for all audiences, the most likely productive avenues for future research will

be indicated and the challenges and limitations will be highlighted to be addressed by the engineers. We will open an avenue for preparing a reliable technology adaptable for future pandemics.

In this research, our plan was to divide the diagnostic strategies to two main categories of protein-based and nucleic acid-based technologies. We also reviewed the proposed and promising COVID-19 detection technologies for future diagnostic purposes. At least 400 papers and information sources will be reviewed in this comprehensive review.

One of the challenges associated with this literature review was that the diagnostic technologies are multidisciplinary and have different parts from detection to displaying the results, they have very different characteristics such as structure, specificity and sensitivity. Specificity of a test is equal to the portion of true positives divided by true positive + false negatives, demonstrating the ability of the test to report the correct results. On the other hand, specificity stands for the portion of true negatives divided by true negatives + false positives, showing the ability of the test to detect the healthy cases correctly [17]. Additionally, due to the prompt spread of the COVID-19, various technologies and tools were developed for detection of the SARS-CoV-2 and this critical situation resulted in issuing emergency use authorization by the agencies such as FDA. For this reason, many of these tools or technologies lack sufficient any clinical research and reports and there is very few information for the rest of the devices or assays. Some of the proposed technologies have not yet received FDA approval, and even some of the Emergency Use Authorization (EUA)-approved devices are withdrawn. Hence, gathering enough clinical reports and information is one of the most important challenges in this study. We will address these challenges by a comprehensive review and doing research not only based on the published articles but also from the main source of the approval agencies such as FDA and CDC. For clinical reports, we will try to find all of the present report articles and to discuss the technologies, we will try to find the papers which are cited in both clinical reports and the FDA website. In the next steps, we will focus on the complementary metal oxide semiconductor (CMOS) capacitive sensors and the surface functionalization strategies in order to investigate the effectiveness of such electrochemical biosensors for early detection of the diseases with high accuracy.

1.2. Organization of thesis

In the remaining of this thesis, we will discuss the current and promising nucleic acid-based tests, their characteristics and challenges for COVID-19 detection in chapter 2-4. These

chapters are taken from three review papers (below). Protein-based tests with their specifications will be explained and compared in Chapter 3 and the promising protein-targeting tools will be discussed. In the last chapter, the current diagnostic technologies as well as the promising strategies for COVID-19 diagnostics will be discussed as conclusion and future works.

Chapter 2 is taken from: Shaffaf, T.; Ghafar-Zadeh, E. COVID-19 Diagnostic Strategies. Part I: Nucleic Acid-Based Technologies. Bioengineering 2021, 8, 49. https://doi.org/10.3390/bioengineering8040049.

Chapter 3 is taken from: Shaffaf, T.; Ghafar-Zadeh, E. COVID-19 Diagnostic Strategies Part II: Protein-Based Technologies. Bioengineering 2021, 8, 54. https://doi.org/10.3390/bioengineering8050054.

Chapter 4 is partially taken from: Shaffaf, T.; Forouhi, S.; Ghafar-Zadeh, E. Towards Fully Integrated Portable Sensing Devices for COVID-19 and Future Global Hazards: Recent Advances, Challenges, and Prospects. Micromachines 2021, 12, 915. https://doi.org/10.3390/mi12080915.

Chapter 2

COVID-19 Diagnostic Strategies. Part I: Nucleic Acid-Based Technologies

Tina Shaffaf, Ebrahim Ghafar-Zadeh

This chapter is published in the Journal of Bioengineering as a review paper [18].

Generally, the nucleic acid (NA)-based tests could be performed either in a laboratory based or in a point-of-care (PoC) setting. PoC tests include portable and miniaturized devices from benchtop-sized analysers to small lateral flow strips; they are capable of performing tests at the same place where the samples are collected or near it. These tests have the advantage of requiring very few or no steps of sample preparation and reporting the results within minutes due to their shorter duration. These tests detect the presence of the SARS-CoV-2 virus in different facilities such as pharmacies, physician offices, and health clinics [7]. The presence and mass production of reliable, cost effective, portable, and scalable PoC tools increases the scope for easy and affordable diagnosis outside the library and in near patient or even at-home setting. By employing these techniques, it is possible to both lower the load of costly and complex diagnostic procedures and reduce time consumption during any outbreaks. The present review discusses the COVID-19 detecting technologies, their performance and challenges associated with each technology to encourage the researchers and inspire them for advancing their innovative methodologies beyond conception. In this chapter, we will discuss the current lab-based and PoC strategies for NA-based detection of COVID-19. We will also highlight the potential alternative techniques that can be implemented for cost-effective, accurate, and rapid detection of infection in the future.

2.1 PCR-based tests

2.1.1 RT-PCR

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is the most commonly used strategy for the detection of COVID-19 in the laboratories which has also been announced as the gold standard for testing SARS-CoV-2 infection by WHO [19]. The combination of this technique with real-time PCR (qPCR) is frequently used to improve the technical aspect of the detection [20]. The majority of developed and commercialized NAATs for COVID-19 detection are based on conventional laboratory-based qRT-PCR technology developed to target one or multiple genes in the new viruses RNA such as ORF1ab, RdRP, E, N and S genes [21].

Two main categories of RT-PCR are manual lab-based kits and PoC nucleic acid-based tests. In 2020, WHO has published seven manual confirmed laboratory-based rRT-PCR protocols established by different companies or institutes including the CDC. Dozens of companies have developed manual or semi-automated PCR-based tests that have received approval in the U.S. and other countries. These assays are the adapted version of the previous tests to target specific sequences in the novel virus genome. The tests may vary in the target gene(s), sensitivity, specificity, the limit of detection (LoD) and total turn-around time (TaT) [21]. The sensitivity and specificity of the RT-PCR-based tests are often comparable; however, some of them have the advantage of accepting more sample types due to their higher flexibility. As an example, mid-turbinate swabs are accepted by a few assays including Thermo Fisher Scientific's test [22]. Another important characteristic is the throughput of the instrument compatible with the test; performing the test for a higher number of the samples simultaneously is merit for some of the tests such as PerkinElmer® SARS-CoV-2 real-time RT-PCR Assay and TaqPath COVID-19 Combo Kit which perform up to 96 samples while Alinity m SARS-CoV-2 assay requires 2-3 hours to report the results for only 24 samples. Some of the FDA EUA approved manual qRT-PCR kits are explained and compared in Table 2. 1*Table 2.* ***1***. Although these kits benefit from high sensitivity and specificity, they require multiple hands-on steps and a long hands-on time (HoT) to reporting the final result. Hence, they are suitable for a Lab-based early detection setting but not for rapid and near-patient diagnostics [23].

On the other hand, fully automated or sample-to-answer assays are instrument-bast tests performing all of the steps automatically in a mobile- or in a facility-based platform which makes them suitable for PoC detection of infection. Compared with manual kits, they are operated faster by less technical staff and technical training, do not need infrastructural requirements, requiring only a few minutes of HoT which frees up the technicians and observing less contamination in the samples due to the fully automated system [24]. Some of the well-known companies have developed their previous sample-to-answer technologies to sense novel coronavirus 2019 and their assays are commonly being used such as Roche Cobas®SARS-CoV-2 assay, Xpert Xpress SARS-CoV-2 test, GenMark ePlex SARS-CoV-2 test, NeuMoDx™ SARS-CoV-2 assay, Abbott RealTime SARS-CoV-2 assay and Hologic Aptima® SARS-CoV-2 assay. Roche Cobas ® SARS-CoV-2 test is a laboratory-based kit performing 96 tests in ~3 hours. This assay targets ORF1 ab non-structural region, and a conserved part of the E-gene on SARS-CoV-2 RNA. An LoD of ≤10 copies/mL is achieved for this test using clinical samples. However, this test is not suitable for PoC detection due to some limitations such as a huge instrument and requirement for trained technicians [25, 26]. Cepheid

Xpert® Xpress SARS-CoV-2 rapid and qualitative test is the first automated assay authorized to be used in PoC setting while requires to be performed by trained technicians. This assay targets in E, RdRp, ORF1a and N gene sequences in viruses RNA [27]. Compared with Cobas ® SARS-CoV-2 test, this system is much smaller with a lower throughput of 1-80 modules, it has the advantage requiring shorter HoT and reporting the results within 45 minutes, the specificity and the sensitivity of this assay equal are 97.8% and 95.6% respectively with an LoD of 0.0200 PFU/mL [28]. Moran and colleagues evaluated Roche Cobas SARS-CoV-2 Assay with Cepheid Xpert Xpress SARS-CoV-2 tests and based on the evidence, high-throughput laboratory-based assays achieve lower LoDs and higher sensitivities compared with rapid mobile analyzers [29]. Xpert® Xpress SARS-CoV-2/Flu/RSV is another kit which is recently approved to track six respiratory viruses with LoD of 131 copies/mL for SARS-CoV-2 [30].

QIAstat-Dx Respiratory SARS-CoV-2 Panel is a rapid PoC test that has the advantage of requiring no PCR-trained laboratory technicians [31]. This respiratory panel has the advantage of simultaneous detection of 21 different pathogens including SARS-CoV-2 by targeting ORF1b and the E genes. Evaluation of this kit in comparison with the WHO-PCR workflow has demonstrated a sensitivity of 100% and a specificity of 93% with no cross-reaction [32].

ePlex SARS-CoV-2 Test is another automated lab-based or PoC test which targets only one sequence, SARS-CoV-2 N gene [33]. The throughput of the system varies from 3 to 28 samples. The literature has reported an LoD of 1,000 copies/mL for ePlex test which is considerably lower than the LoD stated by the company while submitting for EUA (100,000 copies/mL based on in vitro tests) [34]. As limitations of the ePlex test, the specificity of the test is decreased at high titers and cross-reactivity is observed with SARS CoV-1 [35].

GenomEra SARS-CoV-2 Test is a rapid multiplex RT-PCR assay targeting its RdRp and E genes [36]. The throughput of the system is lower compared with the other automatic tests, 1-4 samples, the HoT is about 5-10 minutes for four samples and the results are reported in 70 minutes. The benefit of this test is detecting pathogens without requiring the RNA-extraction step which significantly reduces the technicians' workload and TAT [37]. ANDiS® SARS-CoV-2 RT-qPCR Detection Kit amplifies ORF1ab, N and E gene using RT-qPCR method. In this kit, primers and probes simultaneously target SARS-CoV-2, Flu A and Flu B virus-specific. The kit is highly sensitive with an LoD of 5 copies/reaction, its performances were compared with Next Generation Sequencing (NGS) and the results demonstrated a sensitivity of 96% for SARS-CoV-2 RT-qPCR Detection Kit with a specificity of 100%. The final results of coronavirus detection will be available after a TaT of 60 min PCR program [38].

ARIES® SARS-CoV-2 Assay is by Luminex company, its HoT is about two minutes, the throughput is up to 12 samples and the results are delivered about two hours. This assay uses two ORF ab and N genes to detect SARS-CoV-2 [39]. The test has both sensitivity and specificity of 100%, these statistics require to be confirmed using clinical experiments though [40]. BD MAX™ automated system performing qRT-PCR Reagents including primers and probes based on CDC protocol requiring trained laboratory technicians. The test targets are targets N1 and N2 regions in the Nucleocapsid gene of SARS-CoV-2 genome with about a one-minute HoT per sample and a 15-minute HoT per run and a TaT of about three hours for up to 12 samples [41]. One of the concerns associated with this assay is its false-positive results. In July 2020, FDA has warned the clinical laboratory staff and health care providers a high risk of about 3% for obtaining false-positive result using BD SARS-CoV-2 Reagents for the BD Max System test and mentioned that the results of this test should be confirmed using a second authorized test [42].

Table 2. 1. A selected list of the FDA EUA approved Real Time PCR-based tests and their performance for COVID-19 detection.

Company	Test name	Target Gene(s)	Sensitivity	LoD	Specificity	Assay Time
Vela Diagnostics [43]	ViroKey™ SARS-CoV-2 RT-PCR Test	ORF1a, RdRp	97.2%	(ORF1a: 250 genome equivalents (GE)/mL, RdRp: 560 GE/mL	95.1%	3.5 h
Verily Life Sciences [44]	Verily COVID-19 RT-PCR Test	ORF1ab, N gene, S	100%	60 GE/mL	100%	(No info)
MiraDx [45]	MiraDx SARS-CoV-2 RT-PCR assay	N1, N2	96.90%	4000 copies/mL	100%	2-4 h
BayCare Laboratories, LLC [46]	BayCare SARS-CoV-2 RT PCR Assay	ORF1, E gene	88%	0.009 TCID50/mL	100%	(No info)
DxTerity Diagnostics, Inc. [47]	DxTerity SARS-CoV-2 RT PCR CE Test	N gene, E gene, ORF1ab	97.3%	50 copies/mL	90.0%	2-4 h
Texas Department of State Health Services, Laboratory Services Section [48]	Texas Department of State Health Services (DSHS) SARS-CoV-2 Assay	N gene and ORF1ab	100.0%	20 copies/mL	100%	(No info)
Yale School of Public Health, Department of Epidemiology of Microbial Diseases [49]	SalivaDirect	N gene (N1 region)	94.1%	6000 copies/mL	90.9%	~ 2 h
Solaris Diagnostics [50]	Solaris Multiplex SARS-CoV-2 Assay	N gene (N1 and N2 regions)	100%	10,000 copies/mL	100%	2-4 h

Alpha Genomix Laboratories [51]	Alpha Genomix TaqPath SARS-CoV-2 Combo Assay	ORF1ab, N, S	96.70%	4000 copies/mL	100%	2-4 h
George Washington University Public Health Laboratory [52]	GWU SARS-CoV-2 RT-PCR Test	N gene (N1 and N2 regions)	95.00%	12500 copies/mL	100%	(No info)
Wren Laboratories [53]	Wren Laboratories COVID-19 PCR Test	N1 of SARS-CoV-2, N3 of Sarbecovirus	100 %	10,000 copies/mL	95.0%	(No info)
Ethos Laboratories [54]	Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay	Orf1ab, N1, N2, N3, ORF1	98.10%	1 TCID50/mL	96.3%	2-4 h
Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute [55]	Cleveland Clinic SARS-CoV-2 Assay	E, RDRp	97.0%	10,000 copies/mL	100%	(No info)
ISPM Labs, LLC dba Capstone Healthcare [56]	Genus SARS-CoV-2 Assay	N (2 targets)	100.0%	40,000 copies/mL	100%	(No info)
Abbott Molecular Inc. [57]	Alinity m SARS-CoV-2 assay	RdRp, N	100%	100 copies/mL	100	< 115 min to 12 first results, 16 min thereafter

altona Diagnostics [58]	RealStar SARS-CoV-2 RT-PCR Kit U.S.	E, S	No info.	1.00E-01 PFU/ml	100%	4-6 h
Beijing Wantai Biological Pharmacy Enterprise Co. Ltd [59]	Wantai SARS-CoV-2 RT-PCR Kit	ORF1ab, N	100%	50 copies/mL	100%	2-4 h
bioMérieux SA	SARS-COV-2 R-GENE®	N, E, RdRP	100%	380 copies/mL	100%	<1 h
EUROIMMUN AG [60]	EURORealTime SARS-CoV-2	ORF1ab, N	100%	1 copy/µl	100%	
Sansure Biotech Inc. [61]	Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)	ORF1, N	94%	200 copies /mL	99%	1 h 15 min
SD Biosensor Inc. [62]	STANDARD M nCoV Real-Time Detection Kit	E, ORF1ab	No info.	0.5 copies /µL for upper respiratory specimens and 0.25 cp/µL for lower respiratory specimens	100%	6 h
Seegene Inc. [63]	Allplex™ 2019-nCoV Assay	E, N, RdRP	No info.	4,167 copies/mL	100%	1 h 50 min
Thermo Fisher Scientific [64]	TaqPath™ COVID-19 CE-IVD RT-PCR Kit	ORF1ab, S, N	100%	1250 copies/mL	97%	4 h

Spartan Biosciences Inc. in Ottawa has developed a laboratory-in-a-box technology and received Health Canada approval for its Spartan Cube Covid-19 System to be performed in hospitals or by healthcare Professionals using the Spartan Cube system which is the smallest DNA analyzer in the world which has recently received approval from Health Canada on January 22, 2021 [65]. NeuMoDx™ SARS-CoV-2 Assay [66] and AIGS assay [67] as two other PoC molecular tests and DiaSorin Molecular Simplexa™ COVID-19 Direct qRT-PCR assay [68] as a fully automated lab-based test are some other assays developed for PoC COVID-19 detection.

Even the fastest and the most accurate RT-PCR-based tests suffer from some shortcomings. There are mostly high costs for purchasing both the instrument and the required materials especially for high-throughput tests and for this reason, a vast majority of people do not have access to the equipped centres for such tests globally. While using the automated system, all of the inserted samples are consumed inside the instrument and there is no access to the extracted nucleic acid to perform additional tests especially when the result is negative and supplementary tests are required. Additionally, these systems are quantitative and do not detect the viral load in the sample for more specific analysis [35, 69].

2.1.2 ddPCR

droplet digital PCR (ddPCR) is one of the potential strategies to increase the sensitivity and accuracy of the conventional PCR tests by reducing the false-negative results especially when the patient is a weak positive with a low virus load [70]. Bio-Rad laboratories in a partnership with Biodesix Inc. have developed a Droplet Digital™ PCR (ddPCR™) technology for COVID-19 detection [71]. The Bio-Rad SARS-CoV-2 ddPCR™ Kit is an FDA EUA approved qualitative partition-based endpoint RT-PCR test targeting N1 and N2 regions in the viral N gene with 100% accuracy and an LoD of 625 copies/mL which means that this test is capable of detecting the viral genetic materials when there are 25 or more copies of them in 1 mL sample. Additionally, it demonstrated no cross-reaction with other pathogens [71]. The main disadvantage is the long duration since the results are reported 24-48 hours after sample collection which has limited applying ddPCR in medical laboratories, the duration of ddPCR workflow is approximately 2 hours higher than qRT-PCR (15% more time) and the average costs for the mandatory equipment and consumables for ddPCR is about 5-10% higher than qRT-PCR [72, 73]. Distinct clinical evaluations have obtained high accuracy as well as low LoD capable of detecting 2 viral copies/mL. The limit of detection is considerably low for this test in comparison with most of the other kits and it can detect the copy numbers of the virus

RNA as low as 2 copies in in one millilitre of the introduced sample [74]. This strategy has the potential of increasing the signal-to-noise ratio and requires lower amounts of materials which results in making it more cost-effective [75]. A comparison between the performance of RT-qPCR with ddPCR strategy has illustrated that qRT-PCR is not capable of detecting low viral loads owing to its insufficient sensitivity. Another important advantage of ddPCR over qRT-PCR is that using micro dilutions results in fewer effects interference of any reaction inhibitors on the system and the results are more repeatable and robust [73]. It can be concluded that ddPCR improves the diagnostic procedure for early detection of COVID-19 and also the effect of the therapeutic interventions and drug doses with high accuracy but this strategy is not suitable for rapid PoC detection of this disease [76].

2.1.3 nPCR

Nested PCR (nPCR) is an amplification-based technology employing more than one forward and reverse oligonucleotide primer set [77]. The sensitivity of the standard RT-PCR can be improved by applying an additional nested PCR on the primary amplicons [78]. BioFire® COVID-19 Test is one of the sample-to-answer automated assays taking advantage of nPCR technology for COVID-19 detection (Figure 2. 1) [79]. This nested multiplexed real-time RT-PCR test targets ORF1ab and ORF8 sequences and reports qualitative results in ~50 minutes [79] with 90% sensitivity, 100% specificity and an LoD equal to 330 copies/mL [80]. Another BioFire® authorized qualitative multiplex test is BioFire® Respiratory 2.1 (RP2.1) Panel detecting SARS-CoV-2 and 21 additional respiratory viruses and bacteria simultaneously, a high-throughput and automated assay with ~2 minutes HoT. The sensitivity and the specificity of the RP2.1 Panel are 97.1% and 99.3%, respectively and the estimated LoDs related to the panel for SARS-CoV-2 detection is 6.9E-02 TCID50/mL for heat-inactivated virus and 1.6E+02 copies/mL for the infectious virus [81]. Clinical evaluation of this panel yielded a slightly higher accuracy with 98% sensitivity and 100% specificity. These studies have demonstrated that this panel along with four other assays including BioFire® Defense COVID19, Roche Cobas and Cepheid Xpert Xpress were capable of detecting lower viral loads compared with ID NOW and Hologic Aptima tests (explained in the next sections) making them reliable tools even in the acute presentation phase of the infection low viral titre detection [82].

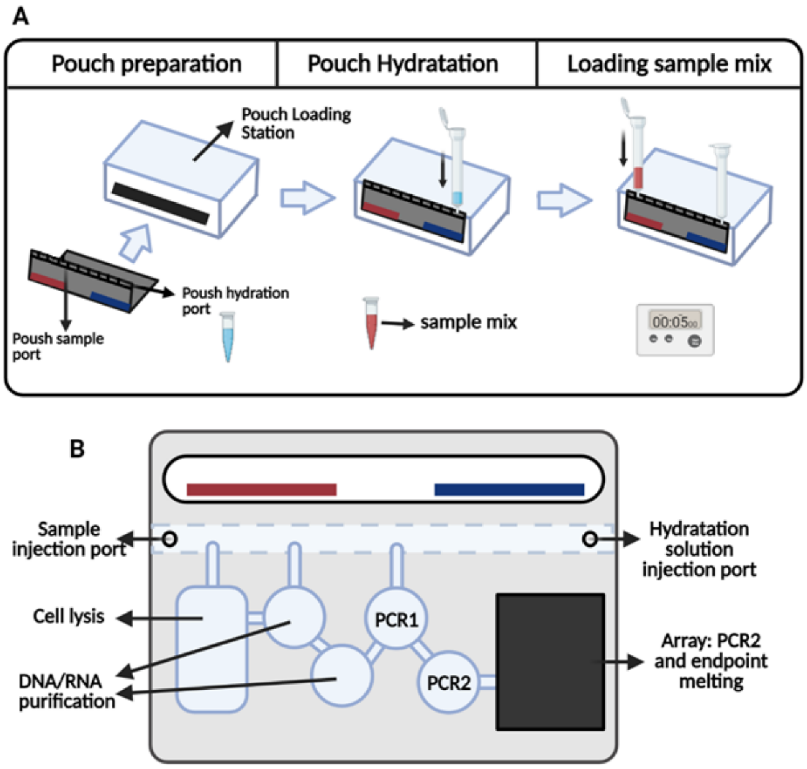


Figure 2. 1. (A) Schematic view of the BioFire® COVID-19 Test quick workflow including the insertion of the pouch into Pouch Loading Station with sample vial and hydration injection vial, pushing down to puncture seal and hydrating the solution, adding specimen to the sample injection vial, loading sample mix into the pouch in 5 s, discarding the vials, followed by inserting the pouch into the system and running the test. (B) Schematic view of FilmArray® system. After sample injection, the system lyses the sample by agitation, extracts and then purifies NA using magnetic bead technology and performs nested multiplex PCR: PCR1 a reverse transcription multiplexed reaction, PCR2 multiple singleplex second-stage PCR reactions amplifying PCR1 products.

SARS-CoV-2 TEM-PCRTM Test is another FDA EUA authorized assay developed by Diatherix Eurofins Laboratory. The technology underlying this qualitative assay is nested end-point PCR followed by hybridization [83]. Diatherix Eurofins Scientific takes advantage of a private technology, TEM-PCR™ (Target Enriched Multiplex PCR) which is based on target-specific nested primers. The main characteristic of this technology is the enrichment of different targets by using primer mixes. Clinical evaluations for this assay have reported an LoD of as low as 1 copy/µL ; sensitivity and specificity of the test are estimated 100% and 98% respectively and no false-positive was obtained [83]. The main weakness of this technology is an increased risk of contamination due to the sequence of manual steps, and this is the reason why this technique is not usually the preferred diagnostic test to be performed in clinical

laboratories. Additionally, the necessity of performing two manual steps of nPCR elevates the workload and HoT which makes it not applicable as a PoC test [84, 85].

2.2 Isothermal nucleic acid amplification-based tests

Due to the current limitations of PCR-based strategies, the development of other potential diagnostic strategies has drawn great interest globally. Isothermal nucleic acid amplification is an alternative strategy with a short TaT allowing amplification at a constant temperature and eliminates the need for a thermal cycler [86, 87]. Such advantages are the reasons now some detection methods are under development or have received approval based on this principle. This category includes the promising approaches with the potential of being used in combination with other systems as the amplification step to address the challenges [88, 89].

2.2.1 Loop mediated isothermal amplification (LAMP)

Loop mediated isothermal amplification (LAMP) is one of the alternative strategies for the quantitative detection of respiratory viral infection. The test is initiated by either inserting RNA samples and performing Reverse Transcription (RT)-LAMP, or inserting cDNA samples for LAMP amplification [90]. This technology employs 4 to 6 or even 8 primers to sensitively identify different regions within the target sequence [91]. LAMP is suitable and ready for rapid lab scale-up and high-throughput automation for pathogenic detection and some researchers have already used this strategy for COVID-19 as well (Table 2. 2). Lucira COVID-19 All-In-One Test Kit is a RT-LAMP-based assay the only prescription home testing kit given FDA EUA to be used in PoC and also at a home setting for suspected people older than 14 [92]. Such kits may act as game-changers in the near future as rapid PoC at-home tests; however, it is necessary to employ them in a non-clinical setting for more supporting research and collecting data hence the current information is not reflecting the use of the kits in the real world. The other potential drawbacks are sample-type limitation, being operator dependent and vague performance characteristics for at-home usage [93].

Abbott Diagnostics automated test, namely ID NOW COVID-19 assay, is one of the first PoC NA-based tests. This rapid and qualitative instrument-based test targets RdRp gene and provide positive results in as low as ~5 minutes and negative results in 13 minutes [94]. The sensitivity of this assay is measured 71.7% with an LoD of 125 copies/mL [94, 95]. This test is reported to achieve a high number of false-negative results, especially while performing tests in the two first weeks of the infection [96] and it has also been criticized for its false-negative rate [97]. The rate of the false-negative detections can be related to the sample type and/or its low viral

load; considering the LoD of the test, weak positives are not accurately detected in the early stages of the infection [98].

Gun-Soo Park et al. used an RT-LAMP assay for detection of SARS-CoV-2 with an LoD 100 copies/reaction in 30 minutes, but this test was not low enough for early detection of the infection as well. This problem could be resulted from inappropriate target selection and might be improved by choosing more appropriate target sequences for LAMP amplification [90]. Yan and colleagues have tried to increase the sensitivity of the LAMP test by targeting reliable targets in ORF1 ab and S genes in separated tubes with 5 and 6 primer sets respectively, the results were achieved in ~26 minutes with a LOD of 20 copies/reaction and no cross-reaction with other respiratory pathogens while the sensitivity and specificity of the test were 100 % for 130 clinical cases [99].

Compared with RT-PCR, LAMP is faster, targets multiple sequences within the target DNA with no expensive thermocyclers without facing supply chain issues. It also amplifies longer sequences with a comparable sensitivity with PCR, produces much higher amounts of DNA compared with qRT-PCR and its visual interpretation has made it independent of any costly instruments [100]. To improve the sensitivity, this technology is widely used in combination with other sensing strategies such as Clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins (CRISPR/Cas)-based systems as a pre-amplification step for boosting the accuracy of the test [101-103]. Due to its limited background in the literature compared with RT-PCR, LAMP technology is in the position of being assessed and improved in a clinical setting for COVID-19 detection [100].

2.2.2 Recombinase polymerase amplification (RPA)

RPA (Recombinase polymerase amplification) is another isothermal amplification strategy used for the detection of viral nucleic acid. The advantage of the high sensitivity of RPA is resulted from adding extra probes to the test [104]. RPA-based viral detecting assays are able to detect low concentrations of pathogenic RNA or DNA faster than PCR or other isothermal amplification techniques [105, 106]. This technology is known as a highly sensitive, specific, cost-effective, simple and compatible amplification method. The test is very fast, requires lower temperature compared with LAMP, there is no need for adding multiple primers or denaturation at the first stage. These characteristics along with amplifying low nucleic acid concentrations make this equipment-free technique suitable to be performed in a wide range of assays in PoC settings [107]. However, the necessity of manual intervention increases the HoT time and probability of contamination. Additionally, various results may be yielded in a user-dependent

manner and similar to the other tests, the performance of the RPA reagents could possibly change based on the storage circumstances [108, 109].

RPA has been used in combination with other technologies such as lateral flow immunoassay (LFIA) system for diagnostic purposes [110]. It has proven to be a potential strategy for pre-amplification of the target sequences prior to CRISPR-based nucleic acid detection to provide the required products for CRISPR/Cas biosensing systems which are explained in more detail in the next section [111].

Table 2. 2. A selected list of the developed LAMP-based tests and their performance for COVID-19 detection

Manufacture	Test	LoD	Sensitivity	Specificity	Target	Duration	Regulatory status
Lucira Health, Inc. [112]	Lucira COVID-19 All-In-One Test Kit	900 copies/mL	A single nucleotide mismatch is probable in one of the primers (Positive agreement:94%, Negative agreement:98%)	No cross-reaction	N	30 min	FDA EUA
Detectachem Inc. [113]	MobileDetect Bio BCC19 (MD-Bio BCC19) Test Kit	30% for 25 copies/mL and 100% for 75 copies/mL	Positive agreement; 97.7% Negative agreement: 100%	Cross-reaction with SARS-CoV	N, E	30 min	FDA EUA
SEASUN BIOMATERIALS, Inc. [114]	AQ-TOP COVID-19 Rapid Detection Kit PLUS	1 copy/μL	(No info)	Some primers have homology with other microorganisms	ORF1ab, N	20 min	FDA EUA
UCSF Health Clinical Laboratories, UCSF Clinical Labs at China Basin [115]	RT-LAMP	20000 copies/mL	95%	100.0%	N (N2 region)	45 min	FDA EUA

Abbott Diagnostics Scarborough, Inc. [116]	ID NOW COVID-19	125 copies/mL	71.7%	no cross-reactivity	RdRp	Positive results 15 min, Negative results 30 min	FDA EUA
Pro-Lab Diagnostics [117]	Pro-AmpRT SARS-CoV-2 Test	125 genomic equivalents/swab	96.60%	100.0%	ORF1ab	30 min	FDA EUA
Color Genomics, Inc. [118]	Color SARS Cov-2 Diagnostic Assay	0.75 copies/µl	100%	100%	ORF1a, E, N,	70 min	FDA EUA
SEASUN BIOMATERIALS [114]	AQ-TOP™ COVID-19 Rapid Detection Kit	7000 copie/ml	(No info)	(No info)	ORF1ab	Positive results 15 min, Negative results 30 min	FDA EUA
Atila BioSystems Inc. [119]	Atila iAMP® COVID Detection Kit	~2000 copies of viral RNA per swab	100%	99%	ORF1ab, N	75 to 90 min	FDA EUA

CapitalBio Technology [120]	Respiratory Virus Nucleic Acid Detection Kit	5×10^2 copies per reaction	(No info)	(No info)	(No info)	13 for respiratory pathogens simultaneously	CE-IVD

CE-IVD: approved CE Marking according to be sold in Europe

2.2.3 Transcription-mediated amplification (TMA)

Transcription-Mediated Amplification (TMA) amplifies the target sequences much more efficiently compared with RT-PCR-based assays without requiring a thermal cycler. The high rate of sensitivity can be related to either the extraction step or amplification step or both [121]. TMA amplification has an autocatalytic nature which is expected to efficiently generate more RNA amplicons than PCR-based assays [122]. The products of these technologies are detectable using colorimetric assay, fluorescent probes and gel electrophoresis [123].

Hologic, Inc. is one of the centres focusing on TMA strategy for detection. After the initiation of the COVID-19 outbreak, they have developed their proprietary technology for SARS-CoV-2 detection called Aptima SARS-CoV-2 assay [124]. This fully automated test is one of the commonly used tests which amplifies two conserved sequences in ORF1ab gene [125]. Analytical sensitivity and specificity of this test are measured 0.026 TCID50/mL and 100% respectively. Fully automated systems are used to perform this test which decreases the HoT time and contamination probability; however, it requires trained technicians and qualitative results are reported in up to 3.5 hours [125]. Studies have demonstrated comparable or even higher analytical sensitivity for COVID-19 detection using Hologic Aptima SARS-CoV-2 compared with RT-qPCR due to higher sensitivity of TMA which makes it helpful for high-throughput and rapid detection of infection in laboratories [126, 127].

2.3 CRISPR/Cas based tests

Clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas) systems are powerful and specific tools for biosensing nucleic acids and genome editing in different fields [128]. One of the promising applications for this system is DNA or RNA-targeting in which CRISPR/Cas system is used for signal generation in the detection step in combination with an additional step of pre-amplification step [129]. The prior pre-amplification procedure is mostly an isothermal amplification such as LAMP and RPA which increases the sensitivity of the assay by amplifying the target sequences and decreasing the LoD. The final step is signal reporting, the readout of these fluorescent signals is performed using agarose gel, quenched fluorescent, or visual detection when integrated with lateral flow assays (LFA) [129-131].

The COVID-19 detecting assays which employ CRISPR/Cas system are mainly all rapid, portable, sample tolerant, highly accurate even for the detection of single-base variations, simple to develop or redevelop, independent of any expensive instruments or traditional infrastructures necessary in traditional molecular laboratories and extremely low costs per

sample [132]. These tests require a combination of materials different from PCR, offering a potential alternative during chemical shortages. Another merit of this technology is the capability of being combined with a paper strip to detect the presence of SARS-CoV-2. Considering all of the aspects, CRISPR/Cas systems are novel and improving technologies suitable for large-scale screening in near-patient settings. However, they suffer from integrated sample preparation and its complications, limited target regions and issues for multiplexed sensing [133]. It should be noted that their applications are limited to lab-based diagnostic tests due to a series of manual steps of mixing and incubation. The necessity of an additional amplification step and sample pre-treatment increase the workload and total HoT from few minutes to hours are the other demerit to be eliminated in the future [134, 135].

The pioneer scientists in this field, Sherlock Biosciences and Mammoth Biosciences, along with other manufactures have developed their patented methodology for SARS-CoV-2 detection [136]. Each kit contains a specific Cas protein, isothermal amplification procedure and monitoring technology (Table 2.3). SHERLOCK (specific high sensitivity enzymatic reporter unlocking) is the first CRISPR-Cas13 platform designed in combination with RT-RPA [111]; SHERLOCKv2 is the revised platform of SHERLOCK which is capable of detecting more than one target sequence simultaneously [137]. SHERLOCK COVID-19 detection protocol is completed in 1 hour followed by a paper-based visual readout (Figure 2. 2). The assay is capable of detecting SARS-CoV-2 RNAs with an LoD of 10-100 copies/µL [138]. However, this lab-based method is complicated, includes two distinct steps of reaction with an increased probability of cross-contamination due to requiring sequential steps of manual fluid handling and opening the tubes. Hence, researchers have used STOP (SHERLOCK Testing in One Pot) strategy and simplified SHERLOCK assay to make it suitable for COVID-19 PoC testing outside the library. In this assay, RPA reaction is replaced with LAMP amplification targeting N gene. STOPCovid test turns result in 40 minutes when using fluorescence readout, and 70 minutes when with LF readout. The obtained LoD for this test is 100 copies/reaction for the strip-based setting [139]. STOPCovid.v2 is the new version of STOPCovid test adapted with a magnetic bead purification step for increasing sensitivity. This assay achieved positive results in 15-45 minutes with 93.1% sensitivity and 98.5% specificity. Although this simplified format requires further development, it is suitable to be performed in PoC setting [140].

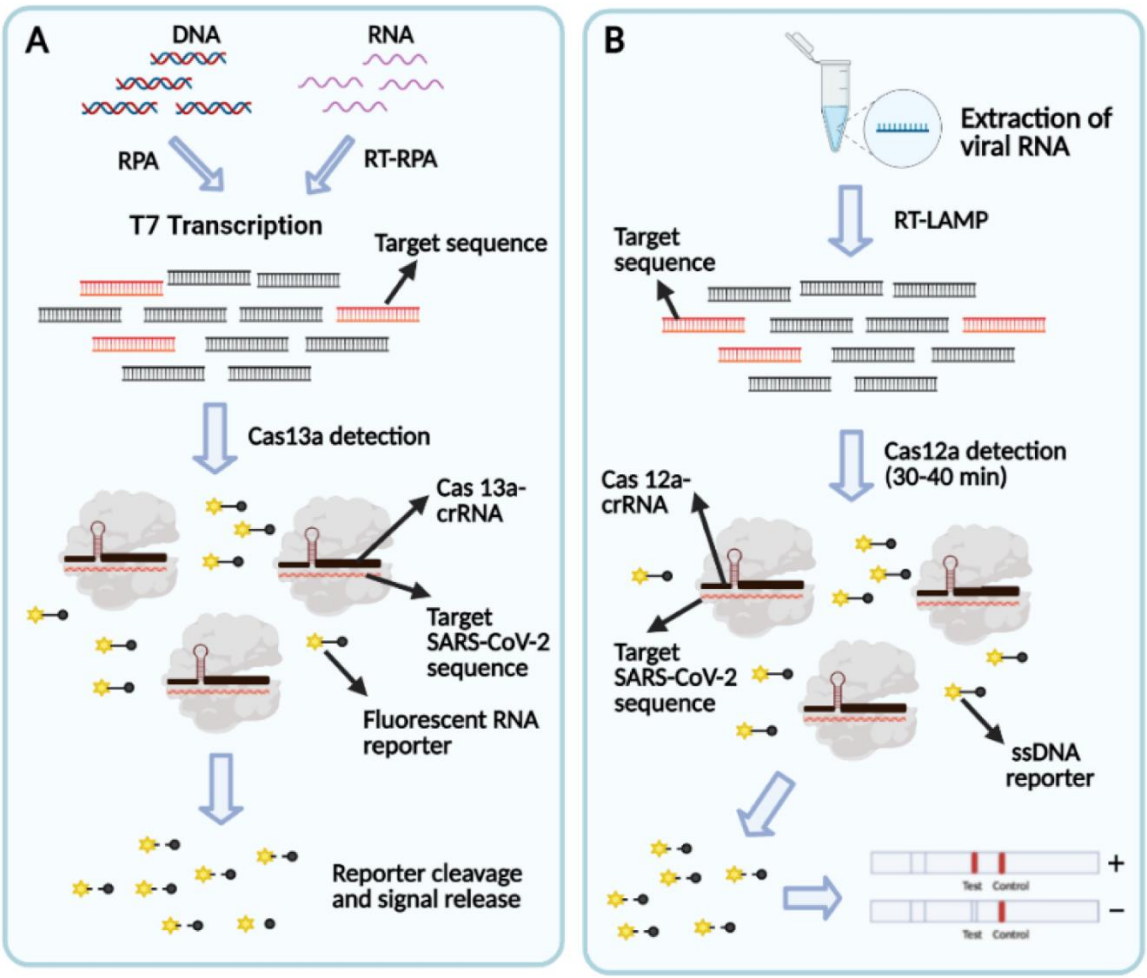


Figure 2. 2. (A) Schematic of SHERLOCK detection assay. A pre-amplification step is started with RNA or DNA sample inputs, the amplicons are converted to RNA using T7 transcription and then detected using Cas13−crRNA complexes. This detection is followed by cleaving and activating the fluorescent RNA reporters. (B) Schematic for SARS-CoV-2 detection using SARS-CoV-2 DETECTR. RNA extraction is followed by an RT-LAMP pre-amplification step and a subsequent Cas12-based detection, which is visualized by an LF reader.

DETECTR (DNA endonuclease-targeted CRISPR trans reporter) is developed by Mammoth Biosciences and GSK to target SARSS-CoV-2 N and E genes with high sensitivity using CRISPR-Cas12 system and visual lateral flow strip [141]. The duration of the test is 30–40 minutes with an LoD of 70-300 copies/µL. This test is much faster compared with SHERLOCK COVID-19 assay but its sensitivity is lower specially for early detection of the disease which may result in more false-negative results; the evaluations have also demonstrated cross-reactions with SARS-like coronaviruses [142].

Many other research groups have focused on CRISPR-based solutions for the detection or other purposes to fight COVID-19 (Table 2. 3) [143-145]. Abbot et al. developed a CRISPR-Cas13-

based method termed PAC-MAN (prophylactic antiviral CRISPR in human cells) as an antiviral strategy for degrading RNA from Influenza A and SARS-CoV-2 viruses in human respiratory epithelial cells and successfully decreased the viral load in these cells by targeting at least 90% of the viral particles and showed to be a potential inhibitory technology [11]. Curti and colleagues evaluated a CRISPR-Cas12 based diagnostic tool with RT-RPA for SARS-CoV-2 sensing and reported a LoD of 10 copies/μL for ORF1ab [136]. One other proposed assay for SARS-CoV-2 and HIV qualitative detection is an all-in-one dual CRISPR-Cas12a (AIOD-CRISPR) assay which is validated using COVID-19 patients' samples. The duration of the test is just a few minutes with a sensitivity of 4.6 copies. After a 40-minute incubation at 37°C, 1.3 copies of DNA targets were detected with no cross-reaction with other tested respiratory viruses [146].

Table 2. 3. CRISPR-based tests for COVID-19 detection

Manufacture	Test	Technique	Detection	Test Format	Target	Time to Result	Analytical Sensitivity (LoD)	Specificity	Regulatory Status
Sherlock Bioscience [139]	Sherlock™ CRISPR SARS-CoV-2	RT-LAMP + CRISPR/Cas	Lateral-flow visual readout	Rapid PoC test	ORF1ab, N	1 h	6.75 copies/uL	No cross-reaction	FDA EUA
Mammoth Biosciences [142]	SARS-CoV-2 DETECTR Reagent Kit	RT-LAMP + CRISPR/Cas12	Lateral-flow visual readout	PoC High-throughput diagnostic	N and E	30-40 min	20-30 copies/µL	No cross-reaction	FDA EUA
Caspr Biotech [147]	Lyo-CRISPR SARS-CoV-2 Kit	RT-LAMP + CRISPR-Cas12	Fluorescence detection using reader	Semi-automated, High throughput (48 tests), using Lyophilized beads	Direct from Sample Kit: 2 regions in N and 1 region in orf1ab; Purified RNA kit: 1 region in N	~ 1 h	Direct from Sample Kit: 25 copies/µl; Purified RNA kit: 7.5 copies/µl	100%	In review for FDA EUA

2.4 DNA-microarray based tests

Microarray is a valuable technique for quantitative detection and genotyping the viral nucleic acid. Microarrays consist of thousands of DNA oligonucleotides as probes able to identify different nucleic acids simultaneously exhibiting a significantly higher specificity and sensitivity in comparison with the tests targeting only one sequence [148]. These tests are not commonly used for PoC purposes and their main application is achieving information about gene expression levels, genotyping, characterizing the DNA or RNA for detection of mutations and some other novel applications [149].

After the initiation of the COVID-19 pandemic, PathogenDx's novel DetectX-RV combined multiplex end-point RT-PCR with DNA microarray to improve the specificity of the test and create new possibilities for multiplex testing. This multiplex assay contains five primer sets targeting SARS-CoV-2 N1, N2, N3 genes and SARS-CoV N2 gene. The results are ready in 6-8 hours. Although this test has not received FDA EUA yet, it employs up to 12 specific probes which is a higher than most of the authorized tests with a throughput of 96 tests per kit. The TaT for microarray hybridization is one hour, the LoD of the test is observed between 50-250 copies/reaction for three low to high viral concentrations. However, like the other nucleic acid-based tests, microarrays may yield false results in various circumstances [150, 151]. Alimetrix, Inc. has developed the FDA EUA authorized Alimetrix SARS-CoV-2 RT-PCR Assay for COVID-19 detection. This assay combines RT-PCR and microarray technologies to target SAES-CoV-2 ORF1ab, N1 and N2 genes [152]. VereRTCoV™ SARS-CoV-2 Real-Time RT-PCR 2.0 Kit is another qualitative microarray-based test that combines microfluidics, molecular biology and microelectronics. In June, this test has received CE-IVD marking to be used in the clinical setting in Europe [153]. This test targets two sequences in the viral N gene and human RNase P gene including a long HoT and many manual steps making it unsuitable for PoC detection as a rapid near-patient test. The duration of the run is ~1.5 hours with a sensitivity of 2 RNA copies/reaction and no cross-reactivity with any human or other respiratory nucleic acid [154].

Lumex Instruments Canada is another company producing a research use only (RUO) Microchip RT-PCR COVID-19 detection system targeting N1 and N2 primer-probes target sequences in the viral N gene and human RNase P control gene. The results are achieved in 50 minutes, it requires low amounts of reagents, the LoD of the assay is 9×103 copies/mL and the costs are lower compared with PCR [155, 156]. Genomica SauÂ is a Spanish company that has

developed CLART®COVID-19 test based on their patented CLART® technology. In this assay, a multiplex-PCR amplifies the targets which are followed by inserting the products into a low-density microarray and hybridization with specific probes. The results are reported within five hours with 96% specificity and 98% sensitivity and the throughput is up to 96 samples [157]. When the aim of the research is investigating a few genes or mutations, microarrays compete with PCR-based tests. On the other hand, complex microarrays are capable of performing a large number of tests and provide huge information while PCR-based tests are not, in such cases the competition is between Microarray and NGS technique [158]. The main challenges of designing microarray-based tests are the presence of highly conserved sequences in coronaviruses RNA as well as the incidence of cross-reaction between coronaviruses genomes [159]. Although the costs are usually high for microarray-based tests, low-cost microarrays have also been developed to investigate coronavirus strains with a sensitivity comparable to qRT-PCR though [160].

2.5 Sequencing-based tests

Next-generation sequencing (NGS) is a high-throughput method for studying the whole genomes, some parts of the genetic material or the transcripts in the cells [161]. Three main strategies have been employed for SARS-CoV-2 RNA sequencing: Whole-genome sequencing, Direct RNA sequencing and Metagenomic sequencing. On January 10, 2020, researchers uploaded the full genetic sequence of SARS-CoV-2 from positive SARS-CoV-2 samples to the global initiative on sharing all influenza data (GISAID) platform and the sequences of thousands of SARS-CoV-2 genomes have been uploaded to this database [162]. GenBank and the Sequence Read Archive of the US National Centre for Biotechnology websites also have several full-length sequences of SARS-CoV-2 from different regions [163, 164]. Random-amplification deep-sequencing approaches have been playing a crucial role in identifying and direct investigating infectious MERS-CoV and SARS-CoV-2 viruses. Such deep-sequencing strategies such as NGS and metagenomic next-generation sequencing (mNGS) will remain beneficial for the determination of future mutations and variants of SARS-CoV-2 [165].

FDA has authorized the first NGS test, Illumina COVIDSeq Test, for coronavirus 2019 detection in June 2020 in order to generate information on viral genetic material, monitoring the mutations and finding the reason for the genetic variations which is critical for fighting the virus [166]. Illumina has developed a Shotgun metagenomic sequencing strategy using illumine sequencing systems for the qualitative detection of novel SARS-CoV-2 [167]. Thermo Fisher is another manufacture that has announced Ion AmpliSeq SARS-CoV-2 test for COVID-19

identification, sequencing, surveillance and epidemiology research. This panel covers more than 99% of SARS-CoV-2 RNA. The workflow of this assay is a fast targeted NGS reporting the results in 1 day, capable of detecting 20 viral copies in the sample [168]. Wang et al. have developed another nanopore target sequencing (NTS) for the detection of respiratory viruses including SARS-CoV-2 simultaneously in 6-10 hours. The LoD of this assay is 10 copies/mL with the capability of detecting more than one infection simultaneously. Some of the merits of this test are lower cost in comparison with whole-genome sequencing, rapid TaT in the same day, a wide range of detection and a lower rate of false-negative compared with RT-PCR. On the other hand, this test is limited due to failure in the detection of the nucleic acid fragments 300–950 bp in length which significantly decreases the sensitivity of the test. Additionally, although the test is faster compared to the other sequencing technologies, it is still longer than qPCR or other PoC tests which are only acceptable for lab-based purposes. The throughput of this system is low and the process includes opening the lid of the tubes increasing the probability of contamination [169]. Some other companies have developed sequencing-based tests for COVID-19 studies (Table 2. 4).

BillionToOne is a cancer molecular diagnostics company that has developed the sanger sequencing-based molecular diagnostic tool called qSanger-COVID-19 test using qSanger. This FDA EUA approved test is very similar to the traditional Sanger sequencing and significantly faster than PCR due to the throughput of 1,536 samples on qSanger. The workflow of qSanger COVID-19 assay includes reverse transcription and RT-PCR amplification of both SARS-CoV-2 target sequences and synthetic spike-in DNA in the master mix followed by sanger sequencing the products. The data obtained from the resulted chromatogram are used to report a positive or negative result for the COVID-19 suspected specimen [170]. Performing the test requires the presence of expert sanger sequencers in the laboratory [171].

Due to the nature of sequencing, this methodology is not suitable for PoC and fast detection of COVID-19 in most cases; mNGS is restricted by many factors including turnover time, high probability of contamination, requiring highly trained technicians and high costs which is the main barrier to introduce this technology as a diagnostic method [86]. Another limitation associated with such non-propagative diagnostic laboratory works is their mandatory conduction using biosafety level 2 (BSL-2) containment procedures and facilities. Performing NGS requires specific equipment and multiple manual interventions. Future simplification and automation may turn this strategy into a routine diagnostic plan by reducing the HoT, the potential contamination and human errors [172]. However, by emerging a portable and real-

time sequencing device by Oxford Nanopore, particularly MinION and Flongle adapter, this technology offers a way forward for PoC diagnostics and brings sequencing to the near patient setting. Oxford Nanopore company that has developed rapid sequencing tests for the detection of SARS-CoV-2 [173]. A nanopore sequencer can be used for sequencing the DNA or RNA molecules relying on the conversion of the electrical signal of the nucleotides which pass through a nanopore [174]. They have announced their LamPORE assay as a low-cost and rapid test for COVID-19 detection. This test is based on real-time nanopore sequencing technology combined with amplification of the viral RNA in the original sample for library preparation using LAMP, targeting E, N ORF1a and a control gene. It analyses a large number of samples simultaneously with a new barcoding approach and the results of 12 extracted RNA samples are reported in one hour [175, 176]. ARTIC network has also proposed a protocol for rapid nanopore whole-genome sequencing of SARS-CoV-2 with a reduced reagents cost, decreased HoT and employing 22 additional primers to achieve more coverage using the portable Oxford Nanopore MinION sequencer which is smaller than a cell phone [177]. This strategy requires 7 hours for the steps of reverse transcription, PCR, adding barcode, adding adapter, sequencing and analysis. This methodology is based on direct detection of the target virus via tiled, multiplex primers with high sensitivity with the advantage of using clinical samples directly as input compared with metagenomic approaches [178, 179].

Table 2. 4. A selected list of the sequencing-based targeting SARS-CoV-2

Manufacture	Test	Test Format	Target	Time to Result	LoD	Sensitivity	Specificity	Regulatory Status
IDbyDNA [180]	NGS-Based SARS-CoV-2 Detection test	NGS-based metagenomics	(No info)	(No info)	(No info)	(No info)	(No info)	Used in Indonesia
BGI Genomics [181]	DNBSEQ-T7 2019-nCoV	Combination of RT-PCR and meta- genomics detection (combinatorial probe anchor synthesis sequencing)	(No info)	Results in a few hours	(No info)	(No info)	(No info)	RUO
Helix OpCo, LLC [182]	Helix COVID-19 NGS Test	NGS	S gene	2-4 hours	125 genomic copy equivalents/mL	100.0%	100.0%	FDA EUA
BillionToOne [183]	qSanger-COVID-19 Test	Sanger Sequencing Combining the Sanger sequencing and the machine learning algorithm	N protein	(No info)	3200 copies/ml	(No info)	No cross-reaction is expected	FDA EUA
YouSeq [184]	SARS-COV-2 Coronavirus	Complete kit for amplicon based NGS Library	99.5% viral genome coverage	~9 hours	(No info)	(No info)	(No info)	RUO

	NGS Library Prep Kit	preparation, Amplicon based protocol						
Illumina Inc. [185]	Illumina COVIDSeq Test	NGS High-throughput Shotgun metagenomic sequencing	Detects 98 targets on SARS-CoV-2	1536 to 3072 results can be processed in 12 hours	(No info)	1000 copies/ml	(No info)	FDA EUA
Oxford Nanopore [186]	LamPORE COVID-19	NGS High-throughput-combines nanopore analyses with loop-mediated isothermal amplification	SARS-CoV-2 genes including E, N ORF1a	Under two hours	7–10 copies/µl	98%	100%	CE-marked
Oxford Nanopore [186, 187]	LamPORE	NGS High-throughput nanopore sequencing	Entire viral genome (>99%) using the ARTIC network	Provide a consensus viral genome in 7 hours	(No info)	(No info)	(No info)	RUO
Thermo Fisher [188]	Ion AmpliSeq SARS-CoV-2 Research Pane	Targeted sequencing by overlapping amplicons	Entire viral genome	14 hours	20 copies	(No info)	(No info)	RUO
Paragon Genomics Inc. [189]	CleanPlex SARS-CoV-2 Research and Surveillance NGS Panel	NGS Highly multiplexed amplicon-based target enrichment	Entire viral genome except for 92 bases at the ends using 343 primer pairs	5.5 hours with Less than 1 hour HoT	3.9 copies/reaction for the E gene assay and 3.6	E gene and RdRp gene assays (5.2 and 3.8 copies per	(No info)	RUO

					copies/reaction for the RdRp assay	reaction respectively)		
Fulgent Genetics/MedScan Laboratory [190]	COVID-19	NGS	(No info)	2-4 days	(No info)	(No info)	(No info)	FDA EUA
Guardant Health [191]	Guardant-19	Reverse Transcriptase PCR (RT-PCR) and NGS	N1 region of the SARS-CoV-2 N gene and human RNase P gene	2-4 hours	125 copies/mL	95%	98%	FDA EUA
Twist Bioscience [192]	NGS-based target capture for SARS-CoV-2 detection and screening	NGS-based target capture	Entire viral genome	(No info)	10 copies	Coverage of > 99.9% of the genome	(No info)	RUO
Clear Labs, Inc. [193]	Clear Dx SARS-CoV-2 Test	Automated (manual RNA extraction) and high-throughput (192), Multiplexed barcoded RT-PCR and targeted NGS	21 target genes	2-4 hours	2000 copies/mL	100%	Cross-reaction with SARS-CoV-1 in one sequence	FDA EUA

University of California, Los Angeles (UCLA) [194]	UCLA SwabSeq COVID-19 Diagnostic Platform	NGS High-throughput RT-PCR and Sequencing	S2 gene	12 hours	250 genome copy equivalents/mL	100%	Not expected	FDA EUA

2.6 Other biosensors

The current benchtop strategies require multiple steps of human intervention and set-up process. Due to the increasing demand for rapid, cost-effective, less complex and accurate tests for COVID-19 detection, novel technologies have attracted researchers' attention to make up for the shortcomings in a testing area [195]. One of the solutions can be employing lab-on-a-chip (LoC) strategies to perform all of the tests on one device. Such microfluidic-based biosensors with glass, polymeric, paper-like or silicon substrate can be used for on-site detection by acting as miniaturized laboratories and have the potential to be adapted for targeting NA from various pathogens [196].

Among biosensing technologies, field-effect transistor-based biosensors (FET) have demonstrated to be promising strategies based on their miniaturized size, fast and sensitive response and parallel sensing with the potential of being used in PoC setting [197]. A cleavage-based approach has been developed for sensing which combines powerful graphene FET (gFET) with a sensitive CRISPR/Cas system by immobilizing CRISPR/Cas on gFET to target specific sequences in a CRISPR-Chip [198]. In one of the proposed tests with this technology, the catalytically deactivated Cas9 (dCas9) CRISPR complex (dRNP) functionalizes the graphene and interacts with the target sequence by scanning the genetic material [197]. liquid-gate electrodes are in direct contact with the mixture of reaction buffer and the sample constantly. The current between two source and drain electrodes of the graphene channel is controlled by the applied voltage between source electrodes and liquid-gate. Immobilized dRNP is hybridized with negative-DNA alters the conductivity of the channel, counter accumulate to generate a stable neutral charge which produces an ion-permeable layer on the surface (Figure 2. **3**) [199]. Different ion-concentration between this permeable layer and bulk solution creates Donnan potential which changes the electrical field between gate electrodes and the source and the final result is the ability to sense the DNA [200].

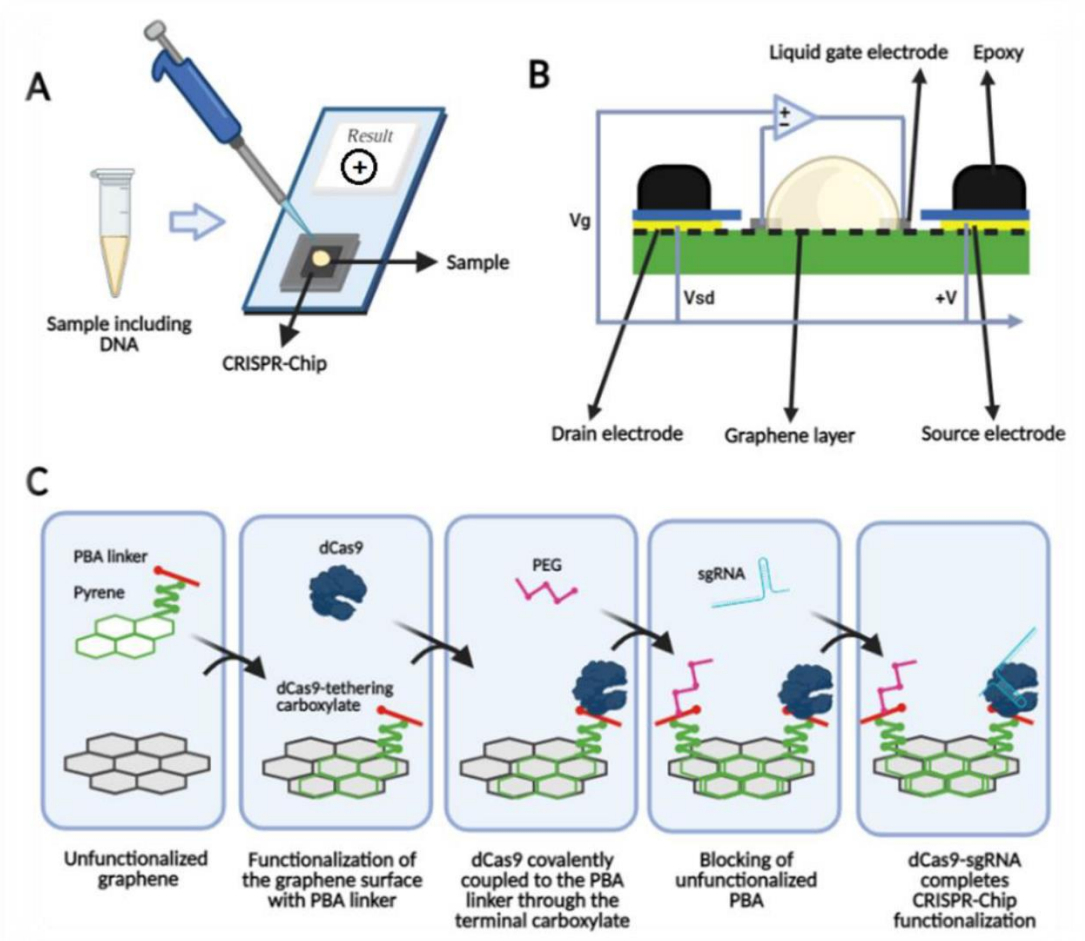


Figure 2. 3 *The schematic view of CRISPR–Chip functionalized with CRISPR–dCas9. (A) the CRISPR–Chip reports the results in 15 min; (B) the process of functionalization of the graphene surface; and (C) the components of the CRISPR–Chip including a liquid gate directly in contact with the sample, and three terminal gFETs utilizing functionalized graphene between source and drain electrodes.*

Smartphones have recently been integrated with microfluidic biosensing technologies for optical detection and analysis of the signals. These smartphone-assisted sensors are promising devices to be used for the detection of the infection in the early stages of the disease [201]. Another portable device that can improve the traditional PCR tests in using dual heating elements for amplification of nucleic acid. Designing the devices with built-in dual heaters yields the significant advantage of reduced cost and size of the instrument [202]. Such all-in-one microfluidic PCR systems can be employed for on-site detection of the pathogens in a PoC setting [203].

Jing Wang et al. have introduced a sensor-based optical detection test. They suggested a dual-functional plasmonic biosensor combining plasmonic photothermal (PPT) heating effect and localized surface plasmon resonance (LSPR) sensing transduction as a promising solution for

COVID-19 detection. In this technology, two-dimensional gold nanoislands (AuNIs) are functionalized with thiol-cDNA (RdRp-COVID-C) ligands as reporters DNA and target sequences from SARS-CoV-2 genetic material. 2D AuNIs successfully generated local PPT heat and transduced the hybridization (Figure 2. 4). the results were high sensitivity and accuracy of the dual-functional LSPR biosensor allowing specific detection of SARS-CoV-2 with LoD down to the concentration of 0.22 pM. This diagnostic platform is proposed as a potential clinical test besides PCR-based diagnostics [204].

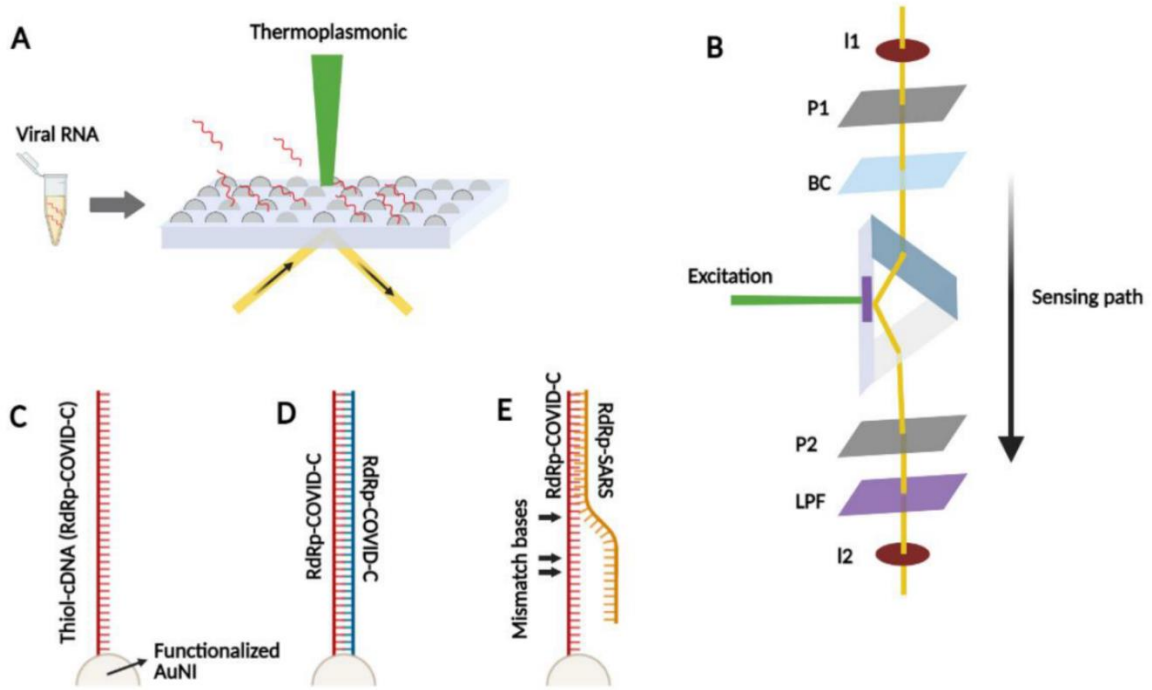


Figure 2. 4. *The schematics of the developed dual-functional plasmonic biosensor. (A) the overall structure of the sensor combining the PPT effect and LSPR for detection of the virus genetic material. (B) The dual-functional PPT enhanced LSPR biosensing system including the aperture-iris (I1/I2), the linear polarizers (P1/P2), the birefringent crystal (BC), and totally reflected at the interface of AuNI-dielectric for LSPR detection. A laser diode (LD) generates the PPT effect on AuNIs. (C) AuNI functionalization based on the reaction with thiol-cDNA ligands. (D) Illustration of the hybridization between two complementary strands, the reporter DNA, and the target sequence in SARS-CoV-2 genome. (E) Specific hybridization of the functionalized thiol-cDNA and inhibiting partial adhesion of RdRp-SARS sequence with two mismatches.*

Luminostics Inc. is a company developing PoC tests. In their products, they utilize reporters based on Luminostics' unique technology using smartphone's optic, an iOS/Android application and an affordable reusable adapter [205, 206]. They have also developed their technology for the detection of respiratory infectious pathogens. In this PoC NA-based test, LAMP is used for the generation of 109 copies in less than 60 minutes and a readout instrument

for end-point fluorescent detection of the emission from a EvaGreen DNA intercalating dye in the microfluidic chip RT-LAMP assay. This test could detect the virus from the nasal swabs' media. The current device detects and five respiratory pathogens and could be referred as a model system for infectious diseases including such as COVID-19. Briefly, on-chip detection initiates after insertion of the controls, targets primers and LAMP reaction mix followed by heating at 65°C. Then, the chip is inserted in the cradle for imaging and the results are reported in 30 minutes (Figure 2. 5). The sensitivity of this inexpensive portable test for early detection of EHV1 was reported 5.5×104 copies/mL corresponding to about 18 copies per reaction which is adequate and comparable with PCR-based assays [207].

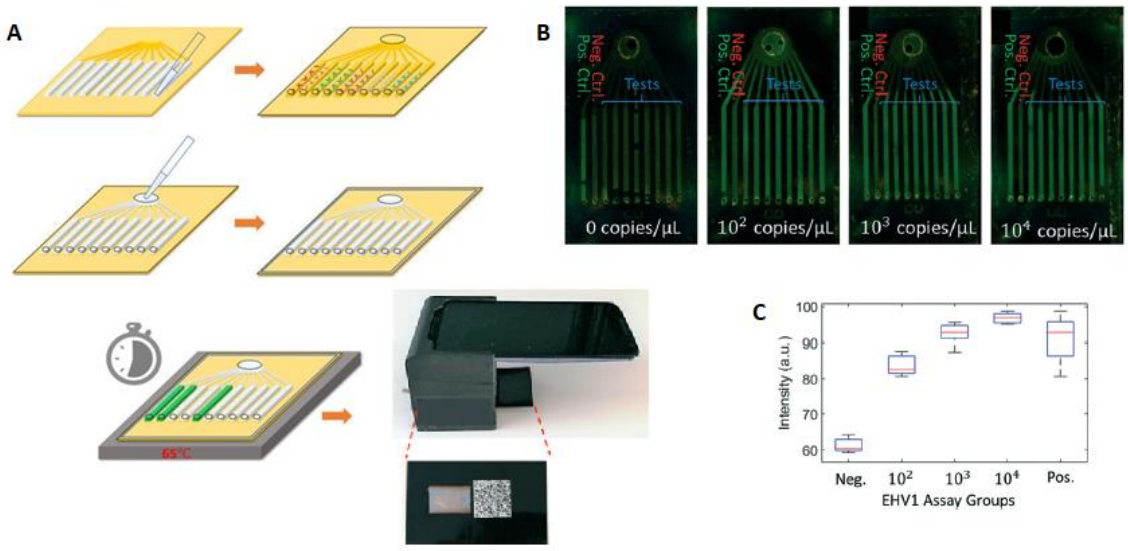


Figure 2. 5. The workflow of the LAMP-based on-chip detection, A) Deposition of the primers and controls, injection of LAMP reaction mix, heating the chip at 65°C and insertion into the cradle for end-point fluorescence imaging for 30 minutes. B) different concentrations of EHV1 templates were amplified on the chips (from left to right, channel 1: positive control, channel 2: negative control and channel 3–10: EHV1 primers). C) the average intensity reported for the channels for each assay [207].

Taken together, the current methods such as sequencing and PCR are time-consuming and there is always a probability to provide false results. Additionally, they are incapable of fulfilling the current challenges and demands for more accurate and direct PoC detection of the pathogens in the current and future pandemics. There are many emerging strategies in this category including aptamer-based bio-navigate, electrochemical and optical biosensor, DNA hydrogel formation by isothermal amplification of complementary target (DhITACT-TR) chip-based, Surface plasmon resonance (SPR), junction-gate field-effect transistor (JFET) or metal–oxide–semiconductor field-effect transistor (MOSFET) , graphene-FET, Ag/Au based electrochemical biosensor and surface plasmon platforms which have recently gained the attraction of the

researchers to hopefully overcome the shortcomings, develop more promising detection kits and pave the way of more rapidly controlling the viral spreads the future hazards [208]. These novel technologies have demonstrated to be successful in detecting the pathogens such as SARS or MERS viruses previously, they are readily available for mass production as cost-effective and miniaturized detecting devices with very low detection limits and high sensitivities which could be either combined with the current strategies or developed for on-sight detection of the pathogens from sample preparation to signal detection [209]. These integrated microsystems have an auspicious future in the early detection of diseases, particularly viral pandemics like the current global health burden.

2.7 Discussion

In this chapter, we discussed the current techniques and promising alternatives which have been developed or are in development for COVID-19 detection, or they have the potential to be setup to control and harness any probable viral spread during the pandemic outbreaks. Although NA-based tests are the gold standard and reliable for early detection, their applicability is limited due to many challenges. Evidence demonstrates that rapid evolution and genetic diversity have been affecting SARS-CoV-2 genotype from the initiation of the pandemic producing variations that may be located in the sequences complementary to the designed primers and probes in NA-based tests [165, 210]. Hence, the targets should be precisely designed to minimize the probability of occurring mismatches, increase the sensitivity and specificity of the test and reduce false results. The presence of mismatches between the primers and their target ends in a reduction in reporting false-negative results, especially in the mutant and novel variants of coronavirus 19. Some of the current kits may not be able to detect the mutant viruses such as U.K. variant in the samples [211-213]. Hence, it is critical to recognize these evolutionary hotspots and avoided targeting them when designing the test. The optimum primer designs include more than one gene on virus RNA targeting, at least one conserved or species-specific sequence along with at least one SARS-CoV-2-specific sequence to increase the accuracy of the test [162, 214].

Additionally, employing high-quality primers and probes directly increases the accuracy of the RT-PCR amplification [165]. Contamination is another issue that may occur during any steps of the test resulting in a false positive. It should be highlighted that although some manufactures claim their test to be 100% accurate, none of the developed tests assays is capable of achieving specificity and sensitivity of 100% owing to a variety of limitations including errors caused

during sample collection, sample transportation, using reagents lacking the standards and technical or executive faults [215].

The lesson is learned from COVID-19 and we should be prepared to face future viral attacks since it takes at least a few months to develop a functioning vaccine for a novel pathogenic disease. Hence, vaccination is not the sole solution and it is critical to standardize diagnostic technologies to be adapted for targeting new microorganisms rapidly and reliably during the early months of their spread when any vaccines have not received approval yet. To overcome the current challenges of NA-based tests including the complexity, high costs, long durations, requiring trained laboratory personnel, cross-contamination, false results, shortage of the required materials and consumptions and more importantly lack of reliable miniaturized and fully automated at-home tests for self-assessment or disease follow-up, researchers have proposed alternatives to more promising state-of-the-art methodologies such as electronic biosensors with excellent accuracy for monitoring human cells as well as pathogens. Innovations in modern medical technology are highly desired to enable the rapid selection of effective drugs for the treatment of infectious diseases. For most infectious diseases, early and effective treatment is crucial to avoid costly and perhaps even lethal complications. Sensitive and scalable PoC tests would increase the scope for COVID-19 diagnosis to be made in the community and outside the laboratory setting. Development and mass-production of cost-effective, easy-to-use, accurate and fast PoC and point of need (PoN) diagnostic devices would potentially eliminate the current workload and labour for the technicians, fewer hospitals and health care providers will be engaged with the disease and the doctors will be free to take care of the patients with other crucial requirements than COVID-19.

As mentioned before, due to obtaining unsatisfactory results from the current detection tools, variety of alternative sensors might be useful for prompt control of the viral spread in the future pandemics in the integrated fully automated systems. The authors would suggest developing novel low-cost micro fabricated fully automated devices for sample preparation and detection. The mass production of such technologies can lower the costs and make it affordable for many people. To date many efforts have been made to miniaturize the lab instruments used for molecular analysis using standard technologies such as Complementary metal-oxide semiconductors (CMOS). CMOS technology is a useful integration strategy and can lead to integration of the biosensors and microfluidic systems in one single chip in order to develop of portable low-cost PoC devices in the urgent pandemic situations and being economically produced in series right into the market. CMOS technology has proven to be useful in detection

of the pathogens. There are various types of CMOS-based electrochemical biosensors including potentiometric [216, 217], voltammetric [218], impedimetric [219] and capacitive [220] sensors which are useful for the NA-based diagnosis of infectious diseases. In one research, Malpartida-Cardenas et al. coupled 64×64 arrayed electrochemical ion-selective field-effect transistors (ISFETs) fabricated in unmodified CMOS technology with LAMP for p.falciparum malaria diagnosis and artemisinin-resistance detection [216]. Hsu et al. have also reported an Electrolyte–insulator–semiconductor (EIS) sensor array using a polar-mode measurement method for the detection of Zika Virus oligonucleotides [221]. Hence, CMOS has demonstrated great advantages for biosensing DNA and can be adopted for COVID 19 or the other pandemics. Additionally, Microfluidic technologies have been greatly grown in the fold of bioengineering and proposed many solutions for DNA and RNA sample preparation [222, 223]. These advances are also potential to be adopted for COVID 19 diagnostics. There are many other microfabrication technologies that can be adequately selected and adopted for infectious detection purposes using molecular technique. These methods can be standardized for low-cost PoC purposes after receiving FDA.

Taken together, more promising PoC and Point-of-need (PoN) diagnostic tests should be employed to overcome the limitations and shortcomings of the current strategies. Developing such biosensors will ease the way of real-time monitoring of the cells with cost-effective, rapid and accurate Home-Use tools shortly to be prepared for future pandemics. This review paper can help researchers in further evolving the biosensors for limiting the growth of life-threatening pandemics.

Chapter 3

COVID-19 Diagnostic Strategies Part II: Protein-Based Technologies

Tina Shaffaf, Ebrahim Ghafar-Zadeh

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From a diagnostic point of view, specific virus antigens or specific antibodies against these antigens are detectable in the specimens collected from COVID-19 positive patients e.g. respiratory swabs, saliva, blood, serum, stool, and other types of samples [11]. Considering the guidance published by the WHO, FDA, and CDC, NA-based tests are the main tests employed for detection of the virus in suspected cases and serological tests are mostly qualitative tools beneficial for confirmation of the reported result as supplementary tests together with other

clinical data [12, 13]. As the protein-based COVID-19 diagnostic strategies have demonstrated a broad range of accuracy, to measure the characteristics of each developed kit, i.e., sensitivity and specificity, their results are evaluated with a reference method. This reference panel is established by FDA and consists of standardized materials. The main technology for evaluation of the specific kits is RT-PCR using nasopharyngeal samples. The related kits are distributed between the different companies and they are requested to perform tests using both the reference RT-PCR kit and their developed device and report the results back to the FDA [225].

3.1 Serodiagnosis of SARS-COV-2

Serological tests have been serving as one of the main categories to detect infectious diseases over time by targeting the specific antibodies (Ab) or so-called immunoglobulins (Ig) secreted in the human body. These systemic immunoglobulin are secreted from white blood cells (WBCs) particularly B cells (lymphocytes) as protective proteins during the infection [226, 227]. Each antibody contains 4 structural proteins Two heavy (H) chains and two light (L) chains when the N-terminuses of all of the chains are the antigen-binding site, specific to a particular antigen and different from the other antibodies. There are different isotypes of antibodies including immunoglobulin M (IgM), IgG and IgA which are distinguished by their specific regions in their heavy chains C-terminus. IgG as the most frequent antibody is secreted in the blood (serum), IgM is another antibody isotype in the blood while IgA is abundant in both blood and other liquids in human body such as saliva and breast milk. The expression of the specific antibodies binding to SARD-CoV-2 antigens is upregulated after the viral infection [228]. More specifically, IgM experiences an elevated expression in the moderate phase of the disease until about week two of the infection and begins to decline and almost disappears until week seven, while IgG expression is upregulated in the late phase from week two-three and remains high even beyond seven weeks (the exact durations are unknown) [229, 230]. IgM detection has low sensitivity in the early stage of infection which results in requiring repeated sample-taking every day. On the other hand, IgG is not preferred for screening the infection, but for patients' follow-up, self-healing and convalescence status as well as the determination of the immune response of asymptomatic cases [231]. Compared with IgM and IgG, IgA has gained less attention for diagnostic purposes; however, the evidence suggests that IgA upregulation takes place earlier even before IgM and systematic studies on the IgA in COVID-19 patients are still lacking [232]. The first generations of the tests targeting SARS-CoV-2 antibodies applied SARS-CoV antigens to detect the SARS-CoV-2 specific Abs due to the

absence of the specific antigens of the new coronavirus at that time. This problem was addressed after achieving more information regarding the new virus during the next month's [233].

All of the serological assays rely on capturing specific antibodies in a mixture using an antibody-antigen attachment [234]. Based on the literature, the majority of the COVID-19 specific antibodies are against SARS-CoV-2 N antigen making them the most sensitive targets for serodiagnosis. The most specific antibodies are against the S1 domain of S protein. For this reason, S1 is suggested as the most specific viral target while S2 has demonstrated to have cross-reactions with SARS-CoV-1 specific antibodies. In the early days after infection, the sensitivity of serological tests is low and results in a false negative. The amounts of the antibodies are not high enough to be detected in the samples collected from the patients in the early phases of the infection while after about 10-15 days and in symptomatic cases, the tests demonstrate higher sensitivity and specificity to the desired antibodies [235]. Noteworthy, the limitation of the serological tests which detect the specific antibodies is the probability of demonstrating cross-reaction due to the presence of pre-existing antibodies or for other reasons. Vaccination or prior infection with the SARS-CoV-2 could result in activating the immune system followed by secretion of long-term persistence of a portion of antibodies such as SARS-CoV-2 specific IgG [236]. The results of such tests strongly depend on affecting factors e.g., sample type, patient situation and disease phase at the time of collecting sample. Generally, antibody tests are very informative and important owing to their ability for past infection detection but they are not reliable for the early detection of COVID-19 in the first stages of the disease because seroconversion occurs after symptom appearance. These tests are more informative while performing for evaluation of convalescence status, immune response and employed as screening tools for testing the rate of serosurvey and prevalence [237].

By the date, hundreds of serological tests have been developed and received FDA EUA approval to be performed for COVID-19 detection to target one specific Ab or a different combination of them in the blood or blood products of the suspected individuals either in a lab-based or in a POC setting [238]. Considering the fact that many of these technologies have been developed very recently, the provided information regarding the tests' performance and accuracy are mostly based on both the companies and the FDA reports. By passing of time and the availability of more reports, we will cover the clinical reports of these tests along with their applicability in the real world in our future research.

3.2.1 Laboratory-based non-isotopic immunoassays (NIIA)

Enzyme-linked immunoassays (EIA) are NIIA assays that are suitable for lab-based detection or measurement of specific antibodies in the blood and serve as the main strategies which regularly assist laboratory scientists for COVID-19 screening. Generally, the main drawbacks limiting the clinical application of the immunoassays are high costs, their complexity and long duration making them not applicable as a simple and rapid POC and near-patient tool [239].

EIA variants including particularly Chemiluminescence Immunoassays (CLIA) and Enzyme-linked immunosorbent assay (ELISA) are frequently being employed for COVID-19 detection, they are also the routine serological methods for convalescence prediction and quantifying materials including antibodies and hormones. All of the immunoassays in this category take advantage of the affinity between antigens and specific antibodies with enzyme-based labels usually immobilized on the microplate surface [240].

I. Chemiluminescence immunoassays (CLIA)

CLIA is one of the most popular immunoassays which utilizes chemiluminescent or light-emitting labels for the detection of biomolecules present in blood, serum or plasma in a total duration of about 1-2 hours [241]. In this technology, recombinant antigens labelled with chemiluminescent materials or luminescent substances form complexes with the specific antibodies when the positive sample is introduced to the microwell, followed by instrument-based detection for light-emitting signal measurement [242]. CLIA assays benefit from the advantages of automation, requiring a low amount of antigen and shorter sample-to-result time compared with some other serological strategies. This technology is known as a sensitive method to detect the small amounts of proteins with a high throughput; although, there might be some problems during the measurement or compound solubility. The main drawbacks of this strategy are being complex and costly compared with other serological tests [240]. The proposed CLIA-based diagnostic tests for SARS-CoV-2 detection have mainly used viral N or S antigens or a combination of them [243]. Based on the literature, the RBD domain of S, and N antigen are the most preferred choices to be used for the detection of IgM/IgG or total antibody. The highest reported accuracy is related to the RBD-based CLIA detecting IgG antibody in the blood samples [244]. It should be highlighted that many factors such as the time of sample collection and disease phase affect the performance of these assays which might not be mentioned in the published articles. Although not being applicable in the POC setting, the main advantage of the fully automated versions of CLIA over rapid serological tests is the ability of high-throughput sample analysis [245].

Many researchers and manufacturers have developed diagnostic tests base on CLIA variants, electrochemiluminescence immunoassay (ECLIA) and chemiluminescent microparticle immunoassay (CMIA), to fight the current pandemic (Table 3. 1). The main difference between ECLIA and CLIA is the chemiluminescence generation technique, electrochemical reactions in ECLIA and chemical reactions in CLIA. Infantino et al. evaluated the clinical accuracy of the Shenzhen YHLO Biotech CLIA kits for SARS-CoV-2 antibodies IgM and IgG. IgG demonstrated a lower cut-off for anti-SARS-CoV-2 antibodies [246]. Long and colleagues have studies IgM and IgG using a combination of S and N antigens in 363 samples from COVID-19 positive patients and observed a medium IgG- and IgM positive serostatus at day 13 after the symptom onset and a 100% seroconversion for IgG at day 20 [247]. Cai et al. proposed a Peptide-based Magnetic CLIA (MCLIA) for serological detection of COVID-19 and evaluated it with 276 sera samples from confirmed COVID-19 patients. the positive rate was 71.4% for IgG and 57.2% for IgM [248]. Lin and colleagues developed a chemiluminescence-immunoassay method using magnetic beads and recombinant nucleocapsid antigen for the detection of COVID-19. The test obtained a sensitivity of 60.76% for IgM and 92.25% for IgG. The specificity of the test for IgM and IgG was 92.25% and 97.5% respectively. They concluded that IgG CLIA is more accurate compared with IgM CLIA and suitable to be performed with RT-PCR to improve clinical detection [249].

Another group employed the same assay with fewer specimens and reported low sensitivity of 48% but 100% specificity for IgM test while IgG detection illustrated 89% sensitivity and 91% specificity respectively. By combining IgM and IgG testing, they achieved the highest accuracy for COVID-19 detection [250]. Ma et al. evaluated using RBD antigen and N antigen using CLIA and demonstrated a higher accuracy for the RBD-based CLIA detection. besides, targeting IgA showed to be more sensitive and specific compared with IgM or IgG solely detection [244].

Table 3. 1. Selected approved CLIA-based tests as Lab-based and high throughput COVID-19 detection strategies based on FDA Serology Test Performance [251].

Company	Test	Technology	Target	Antigen	Sensitivity (Day 15 after Symptom Onset)	Specificity	Throughput
Ortho Clinical Diagnostics, Inc.	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack	CLIA	Total Antibody	S	100%	100%	150 tests/h with one result in 48 min
Beckman Coulter, Inc.	Access SARS-CoV-2 IgG	Automated CLIA	IgG	S	96.8%	99.6%	50–200 tests/h
Babson Diagnostics, Inc.	Babson Diagnostics aC19G1	Fully Automated CLIA	IgG	S	100%	100%	440 tests/h
Ortho Clinical Diagnostics, Inc.	VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Reagent Pack	CLIA	IgG	S	90.0%	100%	150 tests/h
Siemens Healthcare Diagnostics Inc.	ADVIA Centaur SARS-CoV-2 Total (COV2T)	Automated-Semi-quantitative CMIA	Total Antibody	S	100%	99.8%	240 samples/h with one result in 18 min
Siemens Healthcare Diagnostics Inc.	Atellica IM SARS-CoV-2 Total (COV2T)	Automated CMIA	Total Antibody	S	100%	99.8%	440 tests/h with one result in 10 min

DiaSorin	LIAISON SARS-CoV-2 S1/S2 IgG	Complex Automated CLIA	IgG	S (S1/S2)	97.6%	99.3%	170 tests/h and 35 min time to first result
Vibrant America Clinical Labs	Vibrant COVID-19 Ab Assay	CLIA	IgM and IgG	S and N	98.1%	98.6%	24–36 h
SNIBE Diagnostic	MAGLUMI 2019-nCoV IgM/IgG	Automated CLIA	IgM and IgG	S and N	64.3%	100%	30 min for one test
Diazyme Laboratories, Inc.	Diazyme SARS-CoV-2 IgM CLIA test	CLIA	IgM	S and N	94.4%	98.3%	50 tests/h
Diazyme Laboratories, Inc.	Diazyme DZ-Lite SARS-CoV-2 IgG CLIA Kit	Automated CLIA	IgG	S and N	100%	97.4%	50 tests/h
Roche Diagnostics	Elecsys Anti-SARS-CoV-2	ECLIA	Total Antibody	N	100%	99.8%	300 tests/h
Abbott Laboratories Inc.	Architect SARS-CoV-2 IgG	CMIA	IgG	N	100%	99.6%	100 samples in 70 min
Abbott Laboratories Inc.	Alinity i SARS-CoV-2 IgG	CMIA	IgG	N	100%	99.0%	4000 tests in 24 h, with a 29 min time to first result

II. Enzyme-linked immunosorbent assay (ELISA)

ELISA is one of the most frequently used methods for serological detection of the infection with about 1-5 hours. ELISA, as the manual version of the automated CLIA, is a microwell/plate-based assay employing immobilized capture antigen, and a secondary/tracer antigen which targets specific antibodies in the serum, plasma and/or whole blood samples [252], resulting in fluorescence or visible colour change in a chromogenic substrate by enzymatic activity qualitatively or quantitatively [252, 253]. The advantages of ELISA are being sensitive and specific, detecting both current and previous infection, lower costs compared with CLIA, high-throughput, requiring simple facilities and determining selective isotype and antibody titers. ELISA has many capabilities such as being performed as a multiplexed or microarray-based test for parallel detection of various antibodies in a single sample [254]. On the other hand, some challenges such as sample preparation, requiring high sample volume, probability of false positives, antibody variability, manual procedures and high workload, probable cross-reactions along the duration of the tests have limited their applications, especially as rapid POC tests. Requiring trained personnel and delivery of the samples to the specialized labs make these tests, even more, time and labour-consuming and costly [240]. Although laboratories widely employ ELISA for coronavirus 2019 detection, only a few numbers of commercialized kits are based on this strategy which may be resulted from these limitations.

For ELISA-based COVID-19 detection, the researchers represent the immune response of the human body to the SARS-CoV-2 infection, indicating prior or recent infection. based on the literature, IgM and IgG are recognized to be more up-regulated compared to IgA and act as better targets in COVID-19 investigations. However, studies have demonstrated that IgA is also increased in response to SARS-CoV-2 infection and some tests developed to target IgA in blood or serum samples as well [255].

Although a few numbers of the assays employ full-length S antigen as capture molecules in the test, it is commonly referred to use specific shorter peptides from this protein such as RBD domain. A research group developed two different versions of ELISA assay for the detection of S-specific antibodies using full-length S protein and RBD domain. When evaluated the tests, the data revealed that the reactivity of both of the antigens was high, with a significantly higher reactivity for S antigen [256]. Okba et al. also evaluated the effectiveness of S antigen and its S1 and RBD domains using different ELISA kits. They observed that RBD and N proteins achieved the best results for the samples from mild patients and when they were tested using samples from the patient on day 14 after symptom onset, RBD ELISA had 100% sensitivity for

IgG and 94% sensitivity for IgM while for N protein, the specificity of targeting IgG and IgM was 94% and 88% respectively. They also observed that when the capture molecule is S1 and targets are IgA and IgG, IgA ELISA demonstrates a better sensitivity and IgG displayed a better specificity [257]. In another study, Zhang et al. simultaneously targeted IgG and IgM against SARS-CoV-2 and demonstrated that anti-S antibodies are more appropriate to be detected compared with anti-N antigens [258].

Companies such as Bio-Rad Laboratories, DRG Diagnostics GmbH, Euroimmun, IBL International and Epitope Diagnostics have developed manual ELISA tests for COVID-19 detection. Various FDA EUA approved, and CE marked ELISA kits have been commercially available targeting IgM, IgG, IgA, IgG/IgM or total antibody in the collected specimens which their names and characteristics are listed in Table 3. 2. Only one of these authorized kits has targeted total neutralizing antibodies against SARS-CoV-2 RBD in human serum and plasma using blocking ELISA [259]; cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is a 96-well format ELISA test which has an unknown sensitivity, and cross-reactions may occur for various causes such as the pre-existing antibodies [260].

Lassaunière et al. validated three ELISAs with serum samples and demonstrated the best performance from Wantai SARS-CoV-2 Total Antibody ELISA with 100% specificity and 90% sensitivity, Euroimmun IgA ELISA showed 93% specificity and 90 sensitivity and Euroimmun IgG ELISA displayed the lowest sensitivity of 65% with 96% specificity [261]. Beijing Wantai Total Ab ELISA is reported to achieve higher sensitivity (94.5% claimed by manufacture) compared with Beijing Wantai IgM and IgG ELISAs with 83% and 65% sensitivities, respectively which was similar to the lower sensitivity of 65% for Euroimmun IgG ELISA as well. It can be concluded that total antibody ELISA tests display a better performance with higher accuracy and less cross-react compared with IgG ELISA, IgM ELISA or IgA ELISA [261].

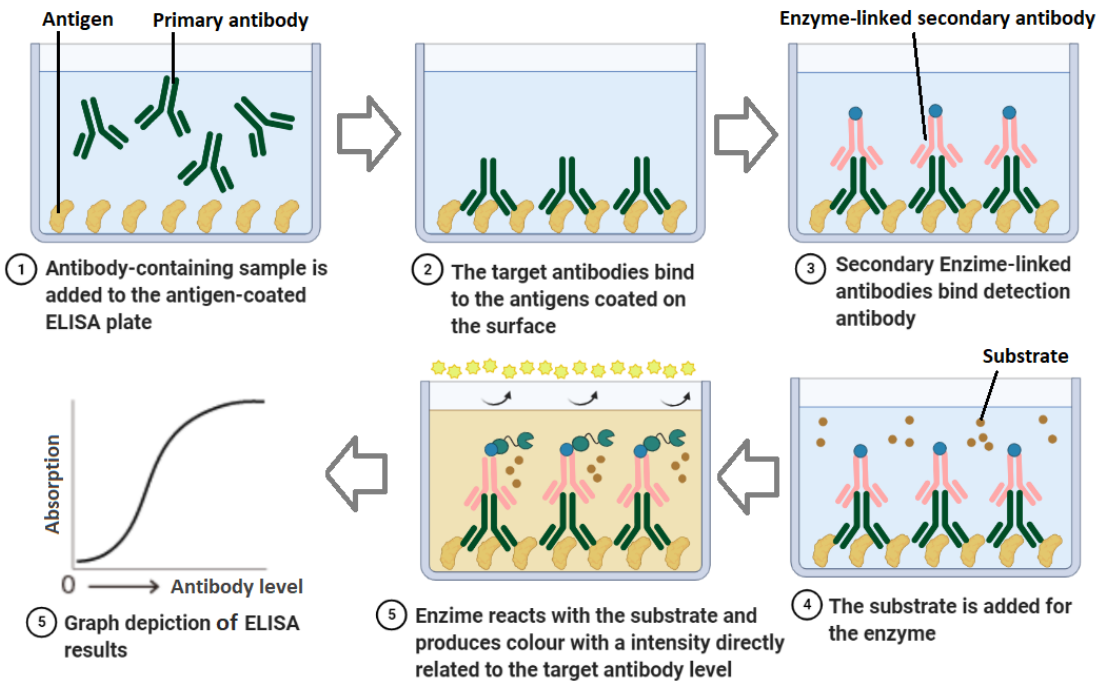

Figure 3. 1. Schematic of ELISA technique for indirect detection of SARS-CoV-2. SARS-CoV-2 specific antibodies are added to the well and adhere to the immobilized viral antigens. Primary antibodies attach to the target antibodies, and a secondary enzyme-linked tracer antibody reacting with a chromogen is added and produces colour change. The intensity of the colour correlated with the concentration of the antigen in the sample.

Microsphere Immunoassay (MIA) is one of the ELISA variants using fluorescent material-labelled secondary antibody and magnetic carboxylated microspheres-virus antigen particle conjugates for detection of the antibodies in serum [262]. This technique implements both flow cytometry and ELISA and includes two main parts. MIA is newer and more accurate than ELISA; however, it requires expensive instruments and materials and is usually more time-consuming [263]. In one research, semi-quantitative MIA (FDA approved) was evaluated. An important observation was that using this technique, SARS-CoV-2 antibodies were detected in 26.3% of the patients when they were at the hospital, but this number increased to 100% after 21 days from symptom initiation. Hence, this technique is reliable for the evaluation of immune response in convalescent and symptomatic patients but not for early detection of COVID-19 [264]. It is demonstrated that comparing MIA and ELISA are considerably more sensitive and specific than lateral flow immunoassays (LFIA) [262]. Conversely, Crook evaluated the sensitivity and specificity of two SARS-CoV-2 antibody tests using ELISA and LFIA and observed a higher sensitivity for LFIA devices compared with ELISA (65-85% and 55-70% respectively) and 93-100% specificity for LFIA versus 95-100% specificity for ELISA test

[265]. Rosenberg et al. tried to estimate SARS-CoV-2 cumulative incidence by conducting a developed and validated SARS-CoV-2 IgG MIA-based test. In summary, magnetic beads were coupled with viral N antigen. Labelled goat anti-human IgG secondary antibody was employed for microsphere-bound IgG antibodies detection using median fluorescence intensity (MFI). The test has 99.75% specificity and 87.9 sensitivity [266]. Fong et al. developed a microsphere-based antibody assay (MBA) for detection of anti-RBD and anti-S-specific IgGs and validated it using 294 serum samples. This test achieved 100% specificity for anti-NP IgG and 98.9% specificity for anti-RBD IgG. MBA seropositive rate for COVID-19 convalescence was 79.5% for anti-RBD IgG and 89.8% for anti-NP IgG with a shorter duration compared with EIA [267].

Table 3. 2. Selected FDA EUA authorized Lab-based ELISA tests for COVID-19 detection [251].

Manufacture	Test	Target Antigen	Antibody	Technique	Sensitivity	Specificity
Mount Sinai Laboratory	Mt. Sinai Laboratory COVID-19 ELISA Antibody Test	Full length S antigen	IgG	High Throughput 2-Step direct ELISA	92.5%	100%
InBios International, Inc.	SCoV-2 Detect IgG ELISA	SARS-CoV-2 S antigen	IgG	High Throughput ELISA	100%	100%
InBios International, Inc.	SCoV-2 Detect IgM ELISA	S antigen	IgM	High Throughput ELISA	96.7%	98.8%
Siemens Healthcare Diagnostics	Dimension Vista SARS-CoV-2 Total antibody assay (COV2T)	S antigen	Total Antibody	Fully automated, fvfRapid High Throughput ELISA (10 min for one result, 440 assays per hour)	100%	99.8%
Quanterix Corporation	Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test	S antigen	IgG	High Throughput, Automated Paramagnetic Microbead-based Sandwich ELISA. 96 tests in 2 h and 45 min	100% (LoD: 0.77 µg/mL)	99.2%
Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.	WANTAI SARS-CoV-2 Ab ELISA	S antigen (RBD domain)	IgG	High Throughput ELISA	96.7%	97.5%
Emory Medical Laboratories	SARS-CoV-2 RBD IgG test	S antigen (RBD domain)	IgG	High Throughput ELISA	100%	96.4%

Siemens Healthcare Diagnostics	Dimension EXL SARS-CoV-2 Total antibody assay (CV2T)	S antigen (RBD domain)	Total Antibody	High Throughput ELISA	100%	99.9%
GenScript USA Inc.	cPass SARS-CoV-2 Neutralization Antibody Detection Kit	S antigen (RBD domain)	Total Neutralizing Antibodies	High Throughput Blocking ELISA (92 samples in 1 h)	100%	100%
EUROIMMUN US Inc.	Anti-SARS-CoV-2 ELISA	S antigen (S1 subunit)	IgG	High Throughput ELISA	90.0%	100%
Luminex Corporation	xMAP SARS-CoV-2 Multi-Antigen IgG Assay	S antigen (S1 subunit and RBD domain) and N antigen	IgG	Multiplex, microsphere-based and high-throughput FMIA (96 samples per run in each 3 h)	100%	99.2%
Bio-Rad Laboratories, Inc.	Platelia SARS-CoV-2 Total Ab assay	Recombinant N antigen	Total Antibody	High Throughput Semi-quantitative ELISA	92.2%	99.6%
Wadsworth Center, New York State Department of Health	New York SARS-CoV Microsphere Immunoassay for Antibody Detection	N antigen	Total Antibody	High Throughput MIA (FMIA)	88.0%	98.8%

3.2.2 Lab-based fluorescence immunoassays (FIA)

FIA is a quantitative fluorescent-labelled immunoassay, a biochemical technique that detects the attachment of the capture antibody and the desired analyte [268]. This technology employs a fluorescent material that emits energy or light as a fluorescent signal. In this technique, fluorescent dyes such as FITC provide the signal and a microplate fluorometer measures it [269]. Fluorescent microsphere immunoassay (FMIA) is used as a quantitative or semiquantitative method for COVID-19 diagnosis. Compared with ELISA, FMIA has the advantage of being more accurate and cost-effective, detects the infection using both serum and non-serum samples and there is no need for cycles of dilution for performing a semi-quantitative test [270]. This technology requires a small volume of sample and simultaneously detects multiple targets [271]. However, the number of developed tests using FMIA is lower than other techniques.

FMIA employs beads or microspheres (Luminex) coated with antigen, the wells house hundreds of distinct sets of beads for the detection of unique antigens. Each microparticle is coloured with a unique combination of two various fluorescent dyes with various ratios as reporters. Additionally, two distinct lasers excite the beads during the test [272]. A FMIA-based test developed by Wadsworth Center, New York SARS-CoV Microsphere Immunoassay, has successfully received FDA EUA authorization for antibody Detection. This test uses the full-length N protein as the target antigen for the detection of total antibodies. The sensitivity and specificity of the test are estimated 88.0% and 98.8%, respectively for performing the test on day 25 after the onset of the disease. The important fact is that the sensitivity will be lower for the tests at earlier days of the infection [273]. Another FDA-approved FMIA test is developed by Luminex Corporation namely xMAP SARS-CoV-2 Multi-Antigen IgG Assay. This assay targets IgG antibodies against three different SARS-CoV-2 antigens including N, S1 and RBD polypeptides. The throughput of the test is 96 and reports the results in 3 hours using plasma or serum samples. PPA for this assay for serum samples while using MAGPIX® (NxTAG®-enabled) system is 71.4% and 96.2% for the samples collected on days 8-14 and >14 from symptom onset respectively while the NPA is 100% [274].

GenBody, Inc. has developed a manual Colloidal Gold Nanoparticle-Based FIA-based immunoassay, GenBody FIA COVID-19 IgM/IgG, which detects IgG and IgM in the samples collected from the patients. The sensitivity of the test is 50% at Day 1~6, 91.7% at Day 7 and after that and its specificity is 97.5%. The duration of the test is 10-15 minutes and employing the reader is optional for monitoring the results [275, 276]. iChroma COVID-19 Ab is another

FIA developed using fluorescent-labelled conjugated. The assay demonstrated no cross-reaction with other respiratory pathogens and reported the results in 10-15 minutes with a sensitivity of 95.8% -97.0% [277].

3.2.3 Rapid serological lateral flow-based tests

Considering the critical and fatal effects of COVID-19, rapid and accurate diagnostic tests are critical not only for detection of the infection at the earliest stages but to monitor the disease and perform convalescence studies to control any outbreaks [278, 279]. Compared with lab-based diagnostic strategies, rapid serological assays have gained much attention since they are applicable in the near-patient or even at home for prompt and simple wide screening and detection of the specific antibodies against the pathogens. [280]. However, several intrinsic shortcomings are currently associated with them such as poor clinical accuracy, high dependence to the disease stage, lack of the ability to distinguish the neutralizing antibodies and reporting false results. Although the rapid diagnostic tests require an upgrade to overcome these limitations, they play a fundamental role in diagnostic and epidemiologic studies to be adapted as reliable tools to be employed in the future pandemic.

Almost all of the rapid serological tests for SARS-CoV-2 detection are cassette-based devices relying on LFIAs or so-called Immunochromatographic strip tests (IST). Lateral Flow Assays (LFAs) are paper-based platforms for POC detection of infectious diseases such as COVID-19 as portable, fast, user-friendly and easily operated tests without requiring complex instruments and technical training. Detection using LFAs is based on specific protein-protein interactions using a chromatographic system [238]. One of the advantages of this assay is the ability to implement multiple tests and control bands simultaneously to rapidly detect multiple analytes in a single sample [281]. Qualitative or semi-quantitative rapid serological in vitro diagnostics (IVDs) are significantly cost-effective and deliver the results within 5-30 minutes. Such devices do not require to be performed by trained laboratory staff and are applicable in hospitals, emergency rooms and other patient care settings [172].

In LFIA strips, the first step is using a colorimetric method to detect the presence of the specific analyte(s). For this aim, biorecognition elements are fixed in the test line(s), and a control line to confirm the validity of the test (Figure 3. ***2***) [282, 283]. LFIA coloured signal reporters or reader-based qualitative and semi-quantitative reporters including carbon material, fluorescent particles, Quantum Dots (QDs), enzyme, liposome, magnetic nanoparticles or other nanoparticles detect the presence of specific antibodies [282]. Qualitative LFIAs achieve more accurate results due to adapting the signal transducer. Various transducers like electrical, optical

and magnetic readers are employed in these systems to produce digital signals by transforming the labels of the captured particle [284]. However, these quantitative tests are not able to detect multi targets simultaneously. Moreover, they are scanner-based and require expensive instruments for monitoring which is not suitable for a POC test [285].

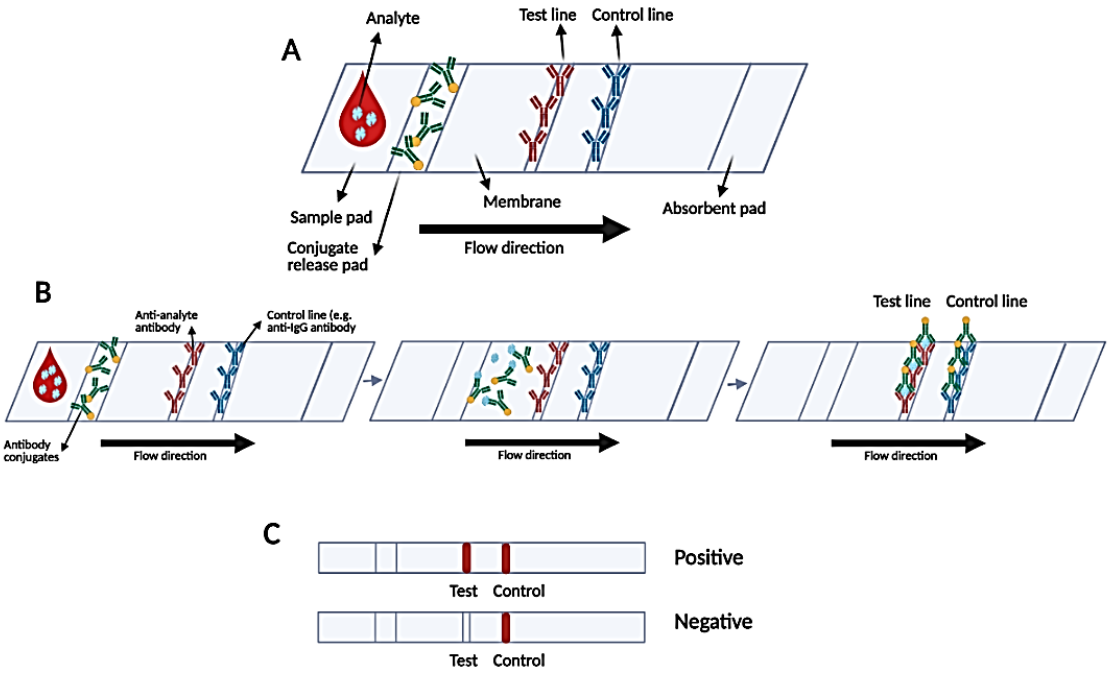

Figure 3. 2. (A) Schematic of LFIA test strip; (B) mechanism of LFIA operation and (C) possible visual positive and negative results [282].

For POC detection of the infection, the potential monitoring strategy is relying on the colour change visible to the naked eyes. Hence, colloidal gold nanoparticle (AuNP) is usually the preferred reporter which is widely observed in the developed and commercialized rapid tests due to its long-term stability, easy operation, rapid onsite detection, low costs, no requirement for complex and expensive instruments and eye-reading results, being highly biocompatible and negligible biological toxicity. However, AuNP-based LFIAs are not capable of performing quantitative measurements. One other drawback of these strips is the lower sensitivity compared with reader-based reporters [286]. The overall structure and workflow of the LFIA rapid tests for COVID-19 detection are almost similar with some differences such as the type of the reporter, capture molecule(s) and selected target(s) have a crucial impact on the sensitivity and accuracy of the device [287].

Almost all of these tests require a small amount of 10-20 µl of the sample from suspected patients and the results are reported in about less than 30 minutes (Table 3. 3). Cellex qSARS-CoV-2 IgG/IgM Rapid Test, the first FDA EUA approved rapid serological cassette-based test,

is consisted of a burgundy-coloured conjugate pad containing SARS-CoV-2 recombinant S and N proteins as antigens conjugated with colloidal gold (SARS-CoV-2 conjugates) and rabbit IgG-gold conjugates. NC membrane strip houses an IgG line (G Line) coated with 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. MyBioSource is another company developing SARS-CoV-2 IgM/IgG Antibody Assay Kit targeting total antibodies against the N protein of SARS-CoV-2 [288]. One of the rapid serological tests with good performance is developed by BioMedomics targeting IgG/IgM antibodies with 10 µL of serum/plasma or 20 µL finger-pricked blood in as low as 10 minutes [289]. One other prospective assay is Pharmacy AG SARS-CoV-2 rapid providing the results in 20 minutes [290]. Panagiota I. Kontou and colleagues evaluated IgG and IgM tests based on ELISA, CLIA, FIA and LFIA in COVID-19 positive samples in a systematic review. The results illustrated that tests using S antigen are more sensitive than N antigen-based ones capturing antibodies. It was also demonstrated that IgG tests have better performance and show a better sensitivity when the patients are in the latest days after symptom initiation. However, the combination of both antibodies achieved the best result. ELISA- and CLIA-based tests achieved the best sensitivity of about 90%-94%, this value ranged from 80% to 89% for LFIA and FIA [233].

Table 3. 3. Selected rapid LFIA serological tests for COVID-19 detection and their performance.

Company	Assay	Target	Capture Protein	Technology	Sensitivity (Day 15 after Symptom Onset)	Specificity	Time to Result
ADVAITE, Inc. [291]	RapCov Rapid COVID-19 Test	IgG	Recombinant SARS-CoV-2 N antigen	Colloidal Gold Nanoparticle-Based LFIA	90%	95.2%	Visual Read/ 15 min
Beijing Wantai Biological Pharmacy Enterprise Co., Ltd [292]	Wantai SARS-CoV-2 Ab Rapid Test	Total Antibody	Recombinant SARS-CoV-2 S antigen	Colloidal Gold Nanoparticle-Based LFIA	98.8%	100%	Visual Read/ 10–20 min
Salofa Oy [293]	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test Cassette	IgM/IgG	Recombinant SARS-CoV-2 S antigen	Colloidal Gold Nanoparticle-Based LFIA	93.3%	98.8%	Visual Read/ 10–20 min
Xiamen Biotime Biotechnology Co., Ltd. [294]	BIOTIME SARS-CoV-2 IgG/IgM Rapid Qualitative Test	IgM/IgG	Recombinant SARS-CoV-2 S antigen	Colloidal Gold Nanoparticle-Based LFIA	100%	96.2%—Cross-reactivity with HIV+	Visual Read/ 15 min

Healgen Scientific LLC [295]	COVID-19 IgG/IgM Rapid Test Cassette	IgM/IgG	Recombinant SARS-CoV-2 antigens (S, S1 subunit)	Colloidal Gold Nanoparticle-Based LFIA	100%	97.5%	Visual Read/ 10–15 min
Hangzhou Laihe Biotech Co. [296]	LYHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit	IgM/IgG	Recombinant SARS-CoV-2 antigens (S, S1 subunit)	Colloidal Gold Nanoparticle-Based LFIA	100%	98.8%	Visual Read/ 10 min
Hangzhou Biotest Biotech Co., Ltd. [297]	RightSign COVID-19 IgG/IgM Rapid Test Cassette	IgM/IgG	Recombinant SARS-CoV-2 antigen (S, RBD Domain)	Colloidal Gold Nanoparticle-Based LFIA	100%	100%	Visual Read/ 10–20 min
Megna Health, Inc. [298]	Rapid COVID-19 IgM/IgG Combo Test Kit	IgM/IgG	Recombinant SARS-CoV-2 N antigen	Colloidal Gold Nanoparticle-Based LFIA	100%	95.0%	Visual Read/ 10–20 min
Biohit Healthcare (Hefei) Co. Ltd. [299]	Biohit SARS-CoV-2 IgM/IgG Antibody Test Kit	IgM/IgG	Recombinant SARS-CoV-2 N antigen	Colloidal Gold Nanoparticle-Based LFIA	96.7%	95.0%	Visual Read/ 10–20 min

Assure Tech (Hangzhou) Co. Ltd [300] [52]	Assure COVID-19 IgG/IgM Rapid Test Device	IgM/IgG	Recombinant SARS-CoV-2 S1 and N antigens	Colloidal Gold Nanoparticle-Based LFIA	100%	98%	Visual Read/ 20 min
Cellex, Inc. [301]	Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test	IgM/IgG	Recombinant SARS-CoV-2 S and N antigens	Colloidal Gold Nanoparticle-Based LFIA	93.8%	96.0%	Visual Read/ 15–20 min
TBG Biotechnology Corp. [302]	TBG SARS-CoV-2 IgG / IgM Rapid Test Kit	IgM/IgG	Recombinant SARS-CoV-2 S and N antigens	Colloidal Gold Nanoparticle-Based LFIA	99.8%	99.8%	Visual Read/ 15 min
Biocan Diagnostics Inc. [303]	Tell Me Fast Novel Coronavirus (COVID-19) IgG/IgM Antibody Test	IgM/IgG	Recombinant SARS-CoV-2 S and N antigens	Colloidal Gold Nanoparticle-Based LFIA	96.2%	99.4%	Visual Read/ 10 min

Although reporter nanoparticles in most of the rapid tests are gold, some researchers and companies have developed strips using different detection techniques. DPP COVID-19 IgM/IgG test introduced by Chembio Diagnostics reporting the results in 15 minutes requiring optical readout using MicroReader 1 and 2 analyzers [304]. The FDA EUA had revoked the EUA of this test due to the effectiveness for IgM, the sensitivity of 50% and 93.3% for IgM and IgG respectively [259]. Chen and colleagues are another group developed a test using LFIA that using lanthanide-doped polystyrene nanoparticles (LNPs) for anti-SARV-CoV-2 IgG detection in human serum [106].

Until now, rapid strips have been widely adapted for novel coronavirus detection; however, this strategy requires to be modified in the future to be more accurate and reliable. The investigations demonstrate that the accuracy of the LFIA tests is lower compared with qRT-PCR tests. As a critical shortcoming, many reports illustrate that many of the developed LF tests suffer from sensitivity lower than 70% [305-308]. FDA EUA given to some of the rapid LFIAs are even revoked due to low accuracy and false-negative results such as Autobio Diagnostics Co. Ltd.'s Anti-SARS-CoV-2 Rapid Test and Chembio Diagnostic Systems, Inc.'s DPP COVID-19 IgM/IgG System [259]. Generally, due to their lower sensitivity and a higher rate of cross-reactivity with other respiratory pathogens resulting in false-negative and false-positive results respectively, rapid LFIA tests are better to be conducted as supplementary tests to confirm RT-PCR results especially when the sample is reported negative [259]. In some cases, rapid LFIAs are comparable with the automated complex CLIA serological assays such as LIAISON SARS-CoV-2 S1/S2 IgG and compete them due to their low costs and simplicity and similar accuracy.

3.2.4 Protein microarray

Antibody microarrays, or so-called antigen microarrays, belong to the category of protein microarrays with the unique capabilities and taking advantage of a novel promising proteomic technology performing high throughput, multiplex and miniaturized tests to target low-abundant analytes in the samples [309]. ELISA and LFIA are capable of targeting single or a few proteins; conversely, protein microarrays provide a proteome-wide characterization of the present antibodies in response to SARS-CoV-2 antigens [310]. This strategy is preferred for profiling antibodies by enabling antibody screening using some or all of the proteins present in SARS-CoV-2 particles with a high resolution [311]. SARS-CoV-2 genome encodes 28 proteins including 5 structural, 15 nonstructural and 8 accessory proteins; specific polypeptides from these 28 proteins can be employed for the fabrication of SARD-CoV-2 specific arrays [5].

The main limitation of the protein microarrays is the higher turn-around time (TaT) than most of the serological tests and the total duration of this test takes less than 24 hours including sample preparation to data analysis [312]. Since the initiation of the COVID-19 pandemic, researchers and manufacturers have developed lab-based microarrays to screen and capture the SARS-CoV-2 specific antibodies. Jiang and colleagues proposed an antibody microarray to profile the SARS-CoV-2 specific IgG/IgM convalescence responses. firstly, the oligonucleotides related to all of the SARS-CoV-2 proteins including RBD of S1 subunit were obtained from GeneBank, synthesized and cloned in E.coli BL21. Then, a total of 18 proteins including proteins extracted from the sequences of N gene, S gene or other ORFs of viral RNA including E gene and nsp genes were spotted on the PATH substrate slide and formed a 2x7 subarray format. The test was evaluated using 29 serum samples collected from recovered patients and the results clearly illustrated that S1 and N protein are suitable for detection, S1 with higher sensitivity than N protein. The responses of the antibodies to ORF9b and NSP5 proteins were also significant. The data provides insights in the field of vaccine development as well as diagnostics and therapeutics [313]. Wang et al. developed a SARS-CoV-2 proteome microarray by immobilized 15 amino acid-long peptides with 5-amino acid overlap to cover all the proteomes. The processing time is estimated 1.5 hours for this array with an LoD of 94 pg/mL. This peptide-based SARS-COV-2 proteome microarray was capable of profiling antibodies and epitopes related to COVID-19 [311].

The developed and commercialized antibodies for CODIV-19 produced by this company and other manufactures along with their main characteristics are presented in Table 3. 4. Quotient Limited company has announced a novel antibody array developed for COVID-19 detection. MosaiQ ™ COVID-19 Antibody Magazine FDA EUA approved commercialized device to detect IgG and IgM antibodies against the Spike S1 protein secreted in response to SARS-CoV-2. MosaiQ platform allows disease screening of patient blood and produces a comprehensive result in about 35 minutes. The inputs for this array are anticoagulated blood samples centrifuged and loaded with the throughput of 3000 tests in 24 hours. with the presence of SARS-CoV-2 specific IgG and IgM antibodies, they will bind to the appropriate probes and the positive results are visualized and interpreted by the instrument camera as black spots for COVID-19 samples. Each microarray generates a reaction on 132 printed biological markers. The required sample volume is as low as 5 µl for each test and after reporting the first result, the other results will be available every 24 seconds [314, 315]. The performance of this microarray for detection of COVID-19 specific antibodies has recently been evaluated using serum samples from Blood Donation Screening Laboratory and demonstrated high clinical

accuracy; before the day from symptom onset, the sensitivity of IgG detection was 71%-80% while this rate was increased to about 100% after day 15 which was superior to some other high-throughput available antibody assays such as EuroImmun (sensitivity: 71%), Abbott (overall sensitivity: 78 %) or Roche (overall sensitivity: 76 %). The specificity of the MosaiQ® test was also evaluated 100%, and higher than the three other well-known tests [316, 317]. This chemiluminescence-based kit is one of the most expensive and complex automated COVID-19 detecting technologies while offering the throughput of thousands of samples per day with a short duration of the test. The fact is that such complex and expensive have demonstrated comparable accuracies with simple and low-cost tests which require no trained laboratory personnel. For the tests with comparable sensitivity and specificity, the rapid tests with a simple workflow and lower cost compete with the complex and costly tests requiring trained laboratory staff [318].

Table 3. 4. The developed protein microarray-based tests for COVID-19 detection.

Manufacturer	Test	Target	Microarray Content	Sensitivity	Specificity	Format	Regulatory Status	Note
Quotient Limited SAÂ [236]	MosaiQ ™ COVID-19 Antibody Microarray	IgG, IgM directed to SARS-CoV-2 S protein	SARS-CoV-2 S protein antigens	Varies based on the phase of the disease (71–100%)	99.8%	High-throughput automated Immunoassay-Antibody employing enhancement reagent to enable silver to nucleate on the gold nanoparticles	FDA EUA—CE-IVD	35 min for the first microarray, 24 s for each next microarray.
PEPperPRINT GmbH [319]	PEPperCHIP® SARS-CoV-2 Proteome Microarray	IgG, IgA, and IgM	The whole proteome of SARS-CoV-2 (GenBank ID: MN908947.3) translated into overlapping peptides	(No info)	(No info)	Manual-One single peptide array	CE-IVD	For vaccine development, or screen viral antigens to find and characterize immunodominant epitopes for in-vitro diagnostics research

PEPperPRINT GmbH [320]	PEPperCHIP® SARS-CoV Antigen Microarray	SARS-CoV-2 specific Antibodies	S, N, M and E antigens	(No info)	no cross-reactivity	Manual-Containing three array copies per microarray, with 998 antigen specific peptides printed in duplicate	CE-IVD	including a two-day experimental workflow
PEPperPRINT GmbH [321]	PEPperCHIP® Pan-Corona Spike Protein Microarray	Antibodies against S antigen	S proteins derived from seven coronaviruses translated into overlapping peptides	(No info)	(No info)	One array with 4564 peptides in duplicate	RUO	For Serum antibody fingerprint analysis, Immune monitoring and Epitope studies
Nirmidas Biotech, Inc. [322]	pGOLD™ COVID-19 IgG/IgM Assay Kit	IgG and IgM against S1 subunit and rbd domain of S	Three SARS-CoV-2 specific antigens	Sensitivity > 87% for IgM 5 days post symptom, ~100% for IgG and IgM 15 days post symptom onset	>99.5	Automated semi-Quantitative Microarray Based High Throughput ELISA-like COVID-19 array	RUO	48 samples with controls in each run, read by western blot reader or Nirmidas' MidaScan™instument

Sengenics Corporation Pte Ltd [323]	ImmuSAFE™ Respiratory Virus Protein Microarray	SARS-CoV-2 specific Antibodies	Multiple SARS-CoV-2 proteins, N from 5 other human Coronaviruses as well as Influenza A and B HA antigen subtypes	(No info)	(No info)	Manual or automated single and double-colour fluorescently-labelled antibody assay	RUO	The key application is for research and development purposes
Sengenics Corporation Pte Ltd [324]	ImmuSAFE™ COVID+ Biochip Test	SARS-CoV-2 specific Antibodies	Multiple SARS-CoV-2 specific domains (N and S) including full-length and numerous truncated versions	(No info)	(No info)	Single-colour fluorescently-labelled antibody assay, and Dual-colour fluorescently-labelled antibody assays for quantitative analysis	RUO	24 arrays per slide (24 samples per slide)—Key applications are vaccine clinical trials and seroprevalence research studies.

Sinommune™ Antigen Multiplex Microarray is another serosurveillance array developed collaboratively by Sino Biological and Nanoimmune Inc. for COVID-19 detection. This microarray consists of nitrocellulose slides containing single array pads with hundreds of spots including pre-printed recombinant antigens which are absorbed onto the 3D nc slide. This array includes 65 viral antigens including S1, S2, S1+S2, HE, N, S RBD and Plpro antigens specific for SARS-CoV-2 and other selected 5 groups of coronavirus family for investigating their reaction with SARS-CoV-2 specific IgG antibody [325]. ImmuSAFE™ is another company that has developed a patented technology to develop three different antibody microarrays using multiple domains, full-length and numerous truncated versions of SARS-CoV-2 S and N proteins. The chip determines different IgG, IgA, IgM antibodies, and IgG1-4 subclasses. ImmuSAFE™ are also capable of assessing the response of the patient to the vaccines by differentiating the antibodies secreted in response to the vaccine, or they are a result of a previous infection.

3.2 Rapid antigenic tests

Antigenic assays are the second category of protein-based tests which are newly released to the market. This strategy relies on capturing the specific virus antigens in a mixture using an antibody-antigen attachment to detect the presence of the viral particles directly [326]. Currently, LFIA is the preferred technology for the development of rapid POC and at-home antigenic tools. The antigenic tests are the only tools that have received to be used at home even without a prescription or requiring assistance from a specialist [327].

The clinical performance of these tools is hugely dependent on various factors and the patient's situation. The best time window for sensing the viral particles is the first week after the infection. The viral load is elevated at this time and antigen tests demonstrate their best performance. This amount decreases during the time which results in dropping the accuracy of the antigen testing in the next stages of the disease [328]. Another factor is the sample type which could directly affect the test results. Most of the current antigen-detecting tests are based on nasopharyngeal specimens which is similar to the gold standard RT-PCR tests and the measurement of the accuracy is less complicated [329]. However, the rest of the kits test nasal samples with a few ones detecting the antigens in salivary specimens. The variation in the sample type increases the complexity of the evaluating procedure using the gold standard which recommends using nasopharyngeal swabs [330].

The most frequently present protein in SARS-CoV-2 structure is the N protein, an evolutionary conserved and highly immunogenic phosphoprotein. S protein, specifically in the S1 RBD

subdomain, is another immunogenic protein on the viral particle surface with rare changes in the amino acids. All of the antigen detecting tests apply antibodies specific to SARS-CoV-2 proteins, more frequently N antigen, as capture molecules. Rapid antigen tests have some advantages over PCR such as lower costs and faster speed. They are highly specific for SARS-CoV-2 but demonstrate a low sensitivity; this is one of the main current limitations of the antigenic tests explaining why they may not detect all of the active coronaviruses [331].

As of February 15, 2021, the number of the developed antigenic tests have been far less than the serological tests (Table 3. 5) [259]. A portion of the developed antigenic tests employ AuNPs as the reporter for visual detection of the infection. Conversely, the rest of these kits require a specific instrument for the detection step. Although the visual detection is simpler and cost-effective requiring less equipment, the reader-based tests have the advantage of getting the results automatically from the analyzer and releasing the results sending messages and posting the results to the patient file by integrating Laboratory Information System (LIS) to their detecting system [332].

On May 8, FDA authorized the first antigenic test developed by Quidel Corporation, Sofia SARS Antigen FIA to perform tests in authorized laboratories and also POC settings. This test is a cassette-based LF immunofluorescent sandwich assay that detects viral N protein. Sofia2 or Sofia analyzer is required for qualitative detection. The results are reported in 15 minutes with 87.5% sensitivity. The test detects both SARS-CoV and SARS-CoV-2 but is not capable of differentiating them from each other [333]. Sofia 2 Flu + SARS Antigen FIA is an automated test reporting the results of influenza A, influenza B, and COVID-19 in a POC setting in 15 minutes. This sandwich immunofluorescent test should be performed using Sofia 2 instrument and is capable of detecting N antigens from other pathogens in direct swab specimens. However, the test does not distinguish SARS-CoV-2 from SARS-CoV infected samples [334].

The third FDA EUA authorized antigenic test is BD Veritor System for Rapid Detection of SARS-CoV-2 developed by Becton, Dickinson and Company (BD). This test employs chromatographic digital immunoassay and detects viral N protein in the samples taken from the patients in the first five days of symptom initiation. The assay monitoring is not visual and depends on the reader. This test is a rapid (approximately 15 minutes) chromatographic digital immunoassay for the direct and qualitative detection of SARS-CoV-2 antigens in nasal swabs. The sensitivity of this test is 84% and has a 100% specificity for COVID-19 detection [335, 336].

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Table 3. 5. A list of the developed Antigen-based tests for COVID-19 detection.

Manufacturer name	Test Name	Technology	Target Antigen	Sensitivity in Symptomatic Patients	Specificity	Detection	Test Duration
Abbott Diagnostics Scarborough, Inc. [337]	BinaxNOW COVID-19 Ag Card Home Test	Colloidal Gold Nanoparticle-Based LFIA, Prescription Home Testing	N antigen	97.1%	98.5%	Visual read + submitting the result via the NAVICA mobile application	15 min
Ellume Limited [338]	Ellume COVID-19 Home Test	Fluorescent LF, Over the Counter (OTC) Home Testing, Screening	N antigen	95%	97%	Instrument Read (smartphone-based)	15 min
Access Bio, Inc. [339]	CareStart COVID-19 Antigen test	Colloidal Gold Nanoparticle-Based LFIA	N antigen	88%	100%	Visual read	10 min
Princeton BioMeditech Corp [340]	Status COVID-19/Flu	Colloidal Gold Nanoparticle-Based LFIA, Multi-analyte	N antigen	93.9% (LoD: 2.7 × 103 TCID50/mL)	93.9%	Visual Read	15 min
Celltrion USA, Inc. [341]	COVID-19 Antigen MIA	Magnetic Force-assisted Electrochemical Sandwich	S antigen (RBD domain)	94.4% (LoD: 3.0 × 101 TCID50/mL)	100%	Instrument Read	10 min

Quanterix Corporation [342]	Simoa SARS-CoV-2 N Protein Antigen Test	Immunoassay (MESIA) High throughput Paramagnetic Microbead-based Immunoassay	N antigen	97.70% (LoD: 0.31 TCID50/mL)	Cross-reaction with SARS-CoV	Instrument Read	80 min
Luminostics, Inc. [343]	Clip COVID Rapid Antigen Test	LF immunoluminescent assay	N antigen	(LoD: 0.88 × 102 TCID50/mL)	Cross-reaction with SARS-CoV	Instrument Read (smartphone-based)	30 min
Abbott Diagnostics Scarborough, Inc. [344]	BinaxNOW COVID-19 Ag Card	Colloidal Gold Nanoparticle-Based LFIA	N antigen	97.1%/22.5 TCID50/mL	98.5%	Visual Read + submitting the result via the NAVICA mobile application	15 min
LumiraDx UK Ltd. [345]	LumiraDx SARS-CoV-2 Ag Test	FIA	N antigen	97.6% /32 TCID50/mL	96.6%	Instrument Read	12 min
Becton, Dickinson and Company (BD) [346]	BD Veritor System for Rapid Detection of SARS-CoV-2	Chromatographic digital immunoassay	N antigen	84%	No cross-reaction	Instrument Read	15 min
Quidel Corporation	Sofia SARS Antigen FIA	FIA	N antigen	87.5%	Cross-reaction with SARS-CoV	Instrument Read	15 min

Quidel Corporation [347]	Sofia 2 Flu + SARS Antigen FIA	FIA	N antigen	(No info)	Cross-reaction with SARS-CoV	Instrument Read	15 min

Lin et al. have developed a POC microfluidic immunoassay for detection of IgG/IgM/ SARS-CoV-2 Antigen simultaneously in 15 minutes and evaluated its clinical performance using 28 healthy and 26 COVID-19 samples (Figure 3. ***3***). They combined various biomarkers as targets to increase the accuracy of the assay. This sample-to-answer test requires 10 µL and 70 µL dilution buffer as indicated. While the sample is COVID-19 positive, SARS-CoV-2 biomarkers attach to the capture antibodies which are coated with fluorescent microsphere (FMS) and the formed complex is immobilized on the fluorescence test region via a second interaction due to antigen-antibody interaction. After a 10-minute duration, the portable fluorescence analyzer reports the results. The achieved cut-off was 100 (T value) for antigen detection, and 200 for sensing each IgG and IgM antibody. It was also observed that serum samples have a significantly fluorescent value compared with a pharyngeal swab. The proposed assay was then tested with samples from patients in 1−7 days onset and over 14 days after symptom separately which demonstrated a growth in the T value while the time changed [348].

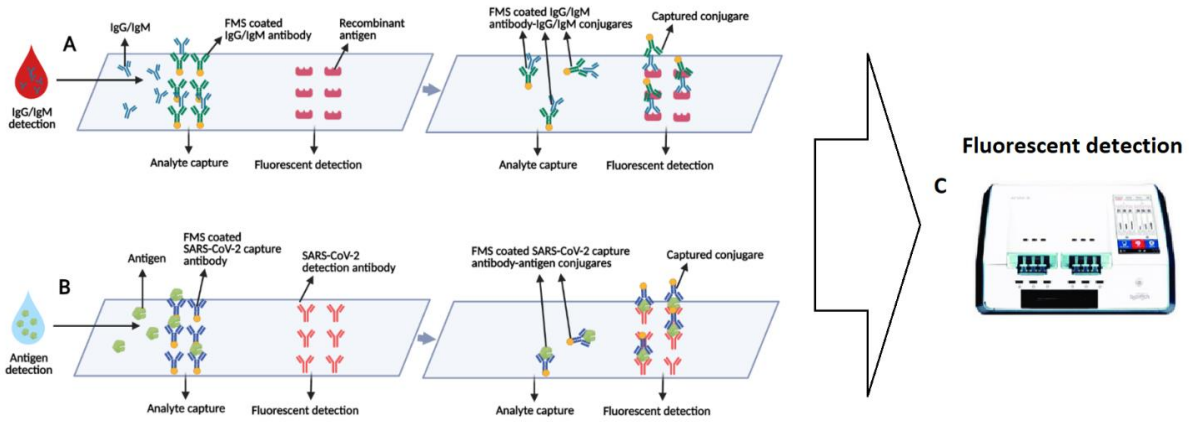

Figure 3. 3. Schematic of the microfluidic fluorescence immunoassay for simultaneous detection of the antibodies and antigens. (A) Schematics of IgG/IgM detection and (B) schematics of antigen detection of SARS-CoV-2 using microfluidic FIA. (C) The results of both of the tests are simultaneously reported by using a portable fluorescence detecting device.

LumiraDx SARS-CoV-2 Ag Test takes advantage of microfluidic FIA, antibodies specific to SARS-CoV-2 N protein are applied in the FIA and target viral N antigen in the collected specimens. The test has demonstrated no cross-reaction with other respiratory pathogens such as SARS-CoV-2 and reports the results in 12 min as one of the fastest antigenic tests. However, like the BD Veritor

System, the LumiraDx SARS-CoV-2 Ag Test does not present visual results and requires to be monitored using the LumiraDx Instrument. The sensitivity and specificity of the test are measured 97.6% and 96.6% with an LoD of 32 TCID50/mL [349]. BinaxNOW COVID-19 Ag Card developed by Abbott Diagnostics is an LFIA targeting virus N protein with high sensitivity of 97.1% and low LoD of 22.5 TCID50/mL. The obtained results can be uploaded in NAVICA, a smartphone application developed by Abbott company. By uploading the COVID-19 negative results to this application, NAVICA-enabled organizations such as banks or workplaces will easily have access to the COVID-19 status of the people [350, 351].

PCL Inc. has also introduced the new PCL COVID19 Ag Rapid FIA test. This test has received a certificate from different regulators including CE-IVD. This device is a POC rapid and cassette-based fluorescent immunoassay targeting SARS-CoV-2 N protein as an antigen is oropharyngeal, nasopharyngeal and sputum samples. The results are achieved in as fast as 10 min and FLA Analyzer is used as a fluorescent reader. Based on the manufacturing, the LoD of the test is 1000 PFU (active viruses), and its sensitivity and specificity are 100% and 97.78%, respectively [352]. Another research group has targeted the SARS-CoV-2 protein via Field-Effect Transistor-Based Biosensor using a specific IgG antibody against the SARS-CoV-2 spike protein [353].

The Simoa SARS-CoV-2 N Protein Antigen Test has recently been given FDA EUA for COVID-19 detection. Among all of the approved antigen tests, this test is the only high throughput kit with a higher TaT of about 80 min, although results are reported in 150 min for 96 tests. This test is a Paramagnetic Microbead-based Immunoassay requiring an analyzer for interpretation but does not differentiate SARS-CoV-2 and SARS-CoV from each other in the infected specimens [354]. The Clip COVID Rapid Antigen Test is the other authorized tool that is developed in a smartphone-based setting for the interpretation and measurement of the luminescence signal emitted from the luminescent nanomaterials [355].

An important application of the rapid diagnostic tools is at home and near-patient testing. Fortunately, two rapid antigenic tests have recently been given FDA EUA to be applied as home tests with or without prescription, [259]. The Ellume COVID-19 Home Test respectively is the first FDA EUA authorized non-prescription fully at-home COVID-19 detecting test that can be completely performed at home with the patient to detect or follow up on the infection. This test employs fluorescent LFIA for the detection of N antigen of SARS-CoV-2 and requires a smartphone as the readout instrument to report the results [356]. The Clip COVID Rapid Antigen

Test is another FDA EUA approved tool for at-home testing which requires a prescription. Interestingly, the PPA and NPA of the Ellume COVID-19 Home Test are obtained 91% and 96%, respectively, for the asymptomatic cases, and 96% and 100%, respectively, for the symptomatic individuals which are very promising [357]. With such at-home tests, the self-isolation of the infected people and the disease follow up take place in a limited area minimizing the viral spread and assistance of the medical care system which not only significantly reduces the rate of the infection in the community but also provides critical information regarding the immune system during the quarantine days.

3.3 Other biosensors

Considering the increasing demand for rapid, cost-effective and accurate tests for COVID-19 detection, a wide range of technologies have been developed to come over the shortages in the testing area [195]. Researchers have recently reported a variety of biosensing strategies such as electrochemical, optical, electrical, mechanical and piezoelectric biosensors for the detection of pathogens. Among various biosensing technologies, field effective transistors have attracted scientists' attention due to the miniaturized size, fast and sensitive response and parallel sensing with the potential of being used in POC setting [197]. Seo and colleagues have introduced a graphene-based FET-based biosensor for SARS-CoV-2 rapid detection [353]. 2D graphene sheet has the advantage of high carrier mobility and electrode conductivity as well as large specific areas which make it a reliable material for sensing purposes [198]. In this COVID-19 FET sensor, the sensitive graphene layer on the device is coated with a commercially available IgG antibody against SARS-CoV-2 S spike protein. The performance of IgG antibodies was first validated using ELISA and then they were used as a receptor for SARS-CoV-2 detection. The immobilization of the IgG antibodies was completed using a probe linker, 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBASE), which is an efficient agent for interface coupling. The fabricated device observed a Real-time response and successfully detected SARS-CoV-2 S in both cultured SARS-CoV-2 virus and transport medium used for nasopharyngeal swabs antigens with high sensitivity and LoD of 1 fg/mL with the ability to differentiate it from MERS-CoV [353]. Apart from the considerable sensitivity and low LoD, the measuring set-up requires a costly and low-throughput semiconductor analyzer, also a high concentration of the antibody (250 µg/mL) is needed for the functionalization of the device.

COVID-19 has also been detected using field-deployable/portable plasmonic fibre-optic absorbance biosensors (P-FAB). This device is developed based on P-FAB with the LoD of detecting down to attomolar (10–18 M) protein concentrations. P-FAB technology monitors the changes in the intensity/absorbent or power loss in the light which is propagated in a multimode U-bent fibre-optic probe using a green LED and a photodetector. Two assays have been suggested for this aim, one using a labelled approach and another one is label-free. In the former, AuNPs are immobilized on the biosensor and then covalently conjugated with anti-N protein monoclonal antibodies (detector antibody) by thiol-PEG-NHS binding. Non-specific interactions are prevented by bovine serum albumin (BSA) treatment. These biofunctionalized probes will detect N protein of the SARS-CoV-2 in saliva samples in 15 min. On the other hand, the latter employs AuNP-labeled capture and detector antibodies in a sandwich immunoassay. The biosensor matrix is first coated with anti-N protein monoclonal antibodies on the U-bent fibre-optic probe and then treated with BSA. The sample should be mixed with anti-N protein antibodies-gold nanoparticles' conjugates and introduced to the sensing area. The results will be achieved in 5 min. Among these two introduced technologies, the label-free assay is more promising due to its one-step response with no need for reagents, but its drawback is poor specificity. In general, the sensitivity and low LoD of the devices over LFAs are considerable, and P-FAB technology has the potential to be developed as a COVID-19 diagnostic test to fight the pandemic [358].

Bioelectric recognition assay has been used in the development of a novel rapid and portable cell-based biosensor for COVID-19 detection. This method is based on Molecular Identification through Membrane Engineering, in which human chimeric spike S1-RBD specific antibody, recombinant human IgG1, was inserted into mammalian Vero cells via electroinserting. By the presence of viral S proteins is positive samples, they attach to their specific antibody on the surface of the cells and make a unique difference in biorecognition elements' electric properties. This hyperpolarization was measured and recorded by a cell-biosensor, a customized multichannel potentiometer with a polydimethylsiloxane (PDMS) layer containing eight holes on its electrode's polyester part. After the application, for the readout section, the potentiometer was connected to a tablet and recorded the measurements. The test did not require any sample-preparation steps, and the LoD of the test was 1 fg/mL and showed no cross-reaction with SARS-CoV-2 N protein. The range of responses was semi-linear from 10 fg to 1 µg/mL [359].

PathSensors Inc. is another company developing its sensor, CANARY biosensor, for novel coronavirus detection using a cell-based technology in 5 min. The test is based on CANARY ™ technology. In summary, the test is made by genetically engineering B lymphocytes with bioluminescence from jellyfish and specific antibodies developed in mice. The engineered cells are designed to emit light when they are exposed and attached to a secondary pathogen [360]. When SARS-CoV-2 is present, it binds to the specific antibodies on the surface of the engineered cells, and the biosensors detect the viruses and report their presence by emitting light. The presence of the target pathogen is confirmed by measuring light output from the cell [361].

Convat project is one of the funded projects by the H2020 European Union Framework program with the main goal of developing a POC nanophotonic sensor based on silicon photonics interferometric technology and microfluidics lab-on-chip integration. For this aim, three distinct assays are in development, the first one for viral genomic analysis and two others for direct virus detection and serological testing. For direct detection of the virus, SARS-CoV-2 specific monoclonal antibodies and nanobodies are produced, linked to the chip for capturing the complete virus and evaluated using deactivated SARS-CoV-2 virus and real samples from COVID-19 positive patients. The test quantifies the viral load in the sample. For direct RNA detection, WHO recommended sequences for SARS-CoV-2 PCR detection were evaluated and three highly specific candidate sequences were selected as targets with a similarity of 100% to SARS-CoV-2 and 0% to other genomes (E, N1 and N gene) with no need for PCR amplification. Different complementary probes are immobilized on the chip to hybridize with the target viral-specific sequences. The device is evaluated with synthetic RNA targets, viral SARS-CoV-2 RNA and RNA of other coronaviruses and the target sequence in the E gene and N1 gene have an LoD of 1 nM and 3 nM, respectively. Direct virus detection takes place by immobilizing anti-S1 antibodies on the surface of the chip and evaluating of its affinity and specificity. The LoD is measured at 19 ng/mL for this test. For the serological test, the targets are viral N, S1 and RBD antigens, and the intact virus is detected. The device is evaluated using S serum samples and COVID-19 positive samples. The LoD value of this chip is equal to 446 FFU/mL. The tests are performed in 30 min [362].

Förster or fluorescence resonance energy transfer (FRET) is another method achieving high resolutions of detection in the range of 1–10 nm compared with optical techniques. Through FRET signals, protein–protein interactions, protein conformation changes and proteolytic cleaves can be studied even in living cells. For viral protein detection, FRET is capable of sensing protein–protein

interactions such as antibody–antigen [363]. FRET technology relies on the energy transfer between a pair of donors and acceptor molecules in a distance-dependent manner. The interaction between two fluorescent-labelled proteins emitting specific colours while distancing, with an overlap in fluorescence emission spectrums, produces a novel third fluorescent colour that differs from the two initial emissions [364]. A useful technology for SARS-CoV-2 study and detection can be FRET-based biosensors. For this aim, viral proteins such as S can be fused to FRET pair-proteins. It also can be utilized to investigate the enzymatic reactions in human cells during COVID-19 infection [365]. SPR-based optical biosensors are other tools which have previously been developed for SARS-CoV detection and can be considered as the other potential tools to be developed for the accurate detection of the SARS-CoV-2 [366].

3.4 Discussion

In this chapter, we discussed the current and potential protein-based strategies which are developed for COVID-19 detection and have the potential to be adapted for the detection of other pathogens in future pandemics. Having many unsuspected asymptomatic COVID-19 carriers interacting with other people in the society increases the risk of infecting healthy people and makes the virus spread much more quickly. This situation may lead to problems such as overloaded clinics and hospitals. This fact has raised concern regarding not only new coronavirus but also to avoid such probable outbreaks in the future. Early detection and isolation of the positive cases is pivotal for controlling any outbreak. For this aim, the development of rapid and adaptable diagnostic tools plays a critical role to limit the spread of the virus in the earliest stages in the future to avoid such pandemics. Although vaccination has the potential of improving the immune state of our bodies, the challenge is that, after the initiation of each outbreak, at least a few months are required to develop a safe and effective vaccine and large-scale vaccination itself required a long time. More importantly, natural mutations in virus RNA may immunize the virus against the vaccine. For these reasons, mass production of sensitive and low-cost POC diagnostic tools is critical to saving thousands of lives during the first months of the upcoming outbreaks.

Although a large number of the COVID-19 tests have been developed over the last year, it is still difficult to recommend the advantage of each technology over the other ones due to the lack of sufficient clinical reports for most of these tests. However, the main goal of this review is to open the discussion and stimulate the questions for the Bioengineering community and also the designers

who are interested in developing and contributing in the diagnostic technologies. For this reason, in each section, we have provided the performance, advantages and disadvantages of each strategy, and we hope this review opens new discussions in the development of the novel assays such as combining the current technologies with electronic biosensors or developing fully integrated devices. As an illustration, in the future, we expect to see the fully integrated lower-cost technologies adoptable for the detection of pathogens in the early stages of the spread to avoid any pandemics. Generally, the protein-based tests have been demonstrated to be very useful during the previous SARS and MERS pandemics, and, in the meantime, they are widely employed for COVID-19 diagnostics as attractive strategies especially for large-scale screening purposes. In conclusion, novel, reliable, accurate, prompt and adaptable protein-based strategies are urgently needed to assist us in the current pandemic and future hazards.

Chapter 4

Conclusion and future works

This chapter is partially taken from our review paper published in Micromachines, entitled "Towards Fully Integrated Portable Sensing Devices for COVID-19 and Future Global Hazards: Recent Advances, Challenges, and Prospects" by Tina Shaffaf, Saghi Forouhi and Ebrahim Ghafar-Zadeh [367]. The COVID-19 pandemic caused by the new coronavirus 2019 has affected the countries and territories around the world and created a medical and socio-economic crisis [368]. Diagnostic tests play a pivotal role in response to all unexpected outbreaks including COVID-19. Since the onset of this pandemic, FDA has been issuing EUA for the diagnostic assays to be performed in the authorized laboratories in the outbreak situation to protect public health [369]. The pandemic situation reveals some shortcomings of laboratory-based assays like requiring costly materials and specific instruments limiting many laboratories worldwide, even in the high-income countries, not to be able to purchase the instrument themselves. Furthermore, the time required for collecting and analysing the sample to obtain an actionable result is sometimes so long that patients might lose their opportunity for treatment. Moreover, breaking quarantine for getting COVID-19 test at health centres can increase the risk of being infected, especially for high-risk people. Implementing PoC self-assessment tools could control the spread of the viruses by reducing the time required to achieve an actionable result, enhancement of the level of social distancing, and the early identification of the disease.

Due to the fast expansion of the infection, the diagnostic tests are preferred to have short hands-on and turnaround time without the need for any sample preparation to be capable of being performed at home or in a PoC setting to accelerate reporting the results and decrease the risk of infection by avoiding involvement of several laboratory members [370]. Such limitations have inspired the researchers and manufacturers to take advantage of different strategies to cover the current shortcomings and conjugate the current tests with other novel strategies. Despite the commercialization of many PoC devices for COVID-19 detection, some challenges such as high-throughput measurement, short turnaround time, high precision, reliability, and low cost of the PoC platforms are still under investigation. Moreover, the possibility of sample collection and sending the report directly from patients to centralized clinicians, especially in the time of effective social distancing measures, is still an open subject to study which can help to control and fight against such pandemics.

Based on the literature [371-374], electrochemical and optical techniques have attracted much attention for COVID-19 applications. A CMOS biosensor including the transducers and the readout circuits on a single chip which is incorporated in a microfluidic platform. Fully integrated CMOS-based technologies are potential to act as as an alternative solution to address the aforementioned challenges of the existing PoC devices. Standard CMOS technology by offering the striking features of reliability, accessibility, considerably low cost, low power consumption, and most importantly scalability and the rapid design-to-product cycle is the best alternative technology to develop PoC devices during an urgent pandemic situation such as COVID-19. CMOS-based devices are categorized into three groups including optical, electrochemical (e.g., impedimetric, capacitive, voltammetry, amperometry, potentiometry), and magnetic techniques. This technology allows for the monolithic integration of a large number of high-speed biosensors and actuators on a single chip and consequently gives the opportunity of high-throughput measurements in a short time. Considering the recent advances in the development of low-noise, high-speed, or high-frequency electrical circuits using CMOS technology and the huge investment in CMOS foundries, this cutting-edge technology is a promising candidate for the new generation of PoC platforms with the capability of wireless data transferring and the possibility of low-cost batch fabrication in urgent situations so that they would be affordable for the end-users.

Surface modifications could be used and implemented with the sensing devices which have not been employed for COVID-19 detection yet. By changing the capture molecules employed on the

surface of the device and employing the suitable recognition elements specific for the target of interest, the device would be easily adaptable for detecting a large group of viruses. During the last two decades, the modification of the surface of different sensors has significantly advanced and many promising materials have been developed to be immobilized on top of the surfaces. The presence of more reliable deposition technologies and chemical materials has improved the process of commercializing advanced tools with more effective BREs and such strategies will be used for developing new surface chemistries based on the new demands. Although they have been widely beneficial for capturing the desired protein structures, surface modification strategies are associated with some challenges such as the immobilization of the capture molecules, their specificity and orientation, the stability of the coating layer, as an illustration, the COVID-19 diagnostic devices are probably not to distinguish between SARS-CoV and SARS-CoV-2 viruses due to low specificity of the device including the immobilized capture molecules. Hence, in the future, specific and promising chemical structures need to be developed for more accurate detection of the desired targets.

As depicted in Figure 4.1, a fully integrated PoC device includes a disposable electronic biosensor (cartridge) and a handheld reader. This biosensor is incorporated in a microfluidic structure to prepare the sample, extract the target biological cells or molecules (e.g. viruses or antibodies), and direct them towards a sensing system. This system features a sensor and an interface circuit. A biorecognition element (BRE) is coated on the top of the sensor to selectively detect the target biomarker. The required custom-made integrated sensors and circuits for PoC testing devices can be developed using CMOS technology.

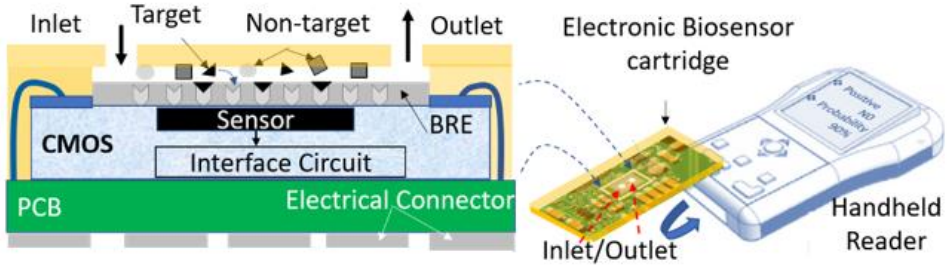

Figure 4. 1. Schematic view of fully integrated CMOS-based PoC system including an electronic disposable cartridge and a reader. CMOS biosensor features a CMOS sensor and circuit, BRE layer, and microfluidic with inlet/outlet.

Recent decades have witnessed unprecedented advances in the CMOS sensors using optical [375], electrochemical [216, 220, 221], and magnetic [376, 377] techniques alike for a variety of applications such as detection of different viruses (like human respiratory viruses [378, 379], Zika virus [221], and dengue virus [376]), as well as monitoring various bacteria (e.g., Streptococcus pneumoniae [380], bacillus globigii [381], Staphylococcus epidermidis [375], and Escherichia coli [220]) and detecting parasites (such as Plasmodium falciparum malaria diagnosis [216]). There are other opportunities for reconfiguring these devices for the diagnosis of similar diseases. The electronic parts of these sensors can be used for similar applications but sensor calibration and normalization of the design metrics such as dynamic range, resolution, the LoD, SNR, and alike would be different. If the current materials and methods reported for COVID-19 detection are CMOS-compatible meaning that they can be fabricated and implemented by CMOS technology, this technology can open a new avenue to develop more efficient PoC platforms for detecting this virus or similar ones. Thus, in this paper, after reviewing the protein-based techniques and the materials reported for COVID-19 detection (which are more practical than nucleic acid-based techniques), the potential of CMOS biosensors to be adapted to this application is discussed. Antibody and antigen immune-sensing devices have the potential of being batch produced and distributed as accurate, cost-effective, portable and adaptable PoC and at-home devices for disease detection purposes. So, in the future, more studies should concentrate on simplifying all user steps at a minimal cost.

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