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## Short Review

# Protein-based lateral flow assays for COVID-19 detection

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## Abstract

To combat the enduring and dangerous spread of COVID-19, many innovations to rapid diagnostics have been developed based on proteinprotein interactions of the SARS-CoV-2 spike and nucleocapsid proteins to increase testing accessibility. These antigen tests have most prominently been developed using the lateral flow assay (LFA) test platform which has the benefit of administration at point-of-care, delivering quick results, lower cost, and does not require skilled personnel. However, they have gained criticism for an inferior sensitivity. In the last year, much attention has been given to creating a rapid LFA test for detection of COVID-19 antigens that can address its high limit of detection while retaining the advantages of rapid antibodyantigen interaction. In this review, a summary of these proteinprotein interactions as well as the challenges, benefits, and recent improvements to protein based LFA for detection of COVID-19 are discussed.

**Key words:** antibody test, antigen test, covid-19, lateral flow assay, diagnostics

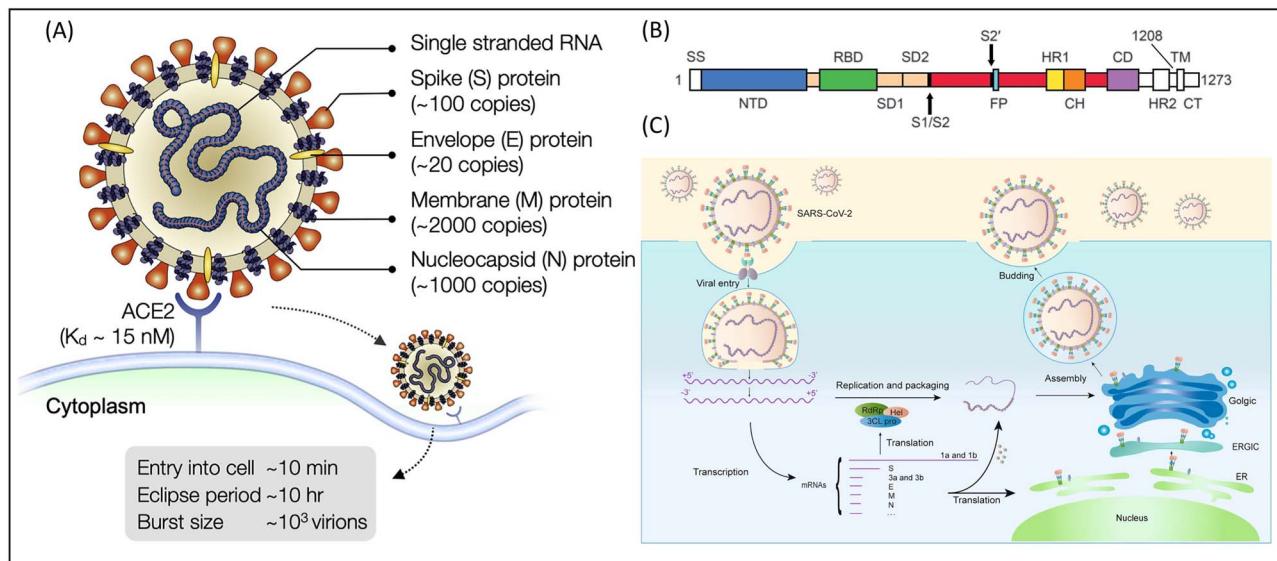
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## Introduction

Emergence of the COVID-19 pandemic has highlighted the importance of widespread testing to facilitate the management and control of disease during pandemics. Comprehensive early testing, followed by contact tracing and patient isolation, has proven effective in containing the spread of the SARS-CoV-2 in the first few months of the outbreak in several countries (Lee and Lee, 2020; Shim *et al.*, 2020). These efforts, however, have not curtailed the spread of the virus across the world. As an additional effort, countries such as the USA have sought rapid, sensitive and low-cost diagnostic methods, which remain a key challenge in controlling the outbreak in developed countries. Recent studies have highlighted the importance in researching affordable and reliable point-of-care (POC) testing (Pokhrel *et al.*, 2020). In this review, we aim to summarize the currently available POC diagnostic methods for SARS-CoV-2, with a focus on rapid POC lateral flow assay (LFA) testing methods. We briefly discuss the challenges in results readout, developing detection

protein candidates, and reported limits of detection (LoDs) for LFAs and describe some of the obstacles in employing antigen, serology and CRISPR (clustered regularly interspaced short palindromic repeats)-based diagnostic tests, as well as summarized list of currently available LFA tests for SARS-CoV-2.

COVID-19 is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is a single-stranded RNA virus belonging to the beta family of coronaviruses. The virus has a spherical lipid bilayer envelope with densely glycosylated spike (S) proteins located on its surface along with membrane (M) and envelope (E) proteins (Fig. 1A and B). A single strand of RNA and the nucleocapsid (N) protein are housed inside the envelope. The virus uses the S1 subunit of the spike protein to bind to the angiotensin-converting enzyme 2 (ACE2) receptor for entry into cells (Letko *et al.*, 2020; Wang *et al.*, 2020; Wrapp *et al.*, 2020). The cell entry is mediated by recognition of the peptidase domain of ACE2 by trimeric spike protein, cleavage and activation of spike protein by



**Fig. 1** SARS-CoV-2 virus and Spike (S) protein structure. **(A)** Schematic of SARS-CoV-2 virus and its proteins, bound to ACE2 receptor. Reproduced with permission (Kilic et al. (2020)). **(B)** Schematic of SARS-CoV-2 genome colored by domain. Domains consist of signal sequence (SS), N-terminal domain (NTD), receptor-binding domain (RBD), subdomain 1–2 (SD1–2), S2' protease cleavage site, fusion peptide (FP), heptad repeat 1–2 (HR1–2), central helix (CH), connector domain (CD), transmembrane domain (TM) and cytoplasmic tail (CT). Reproduced with permission (Wrapp et al. (2020)). **(C)** Lifecyle of coronavirus from binding to ACE2 and cell entry to synthesis and assembly of viral proteins. Reproduced with permission (Huang et al. 2020).

**Table 1.** Comparison of widely used methods for COVID-19 diagnostics

Type	Target	Target details	Biomarker	Platform	Assay time	POC (Y/N)	Sample
xPCR	Viral RNA	S, N, E, ORF1ab	Nucleic acid	Plate	2–8 h	N	Swab, saliva
RT-LAMP	Viral RNA	ORF1ab, N	Nucleic acid	Kit, Cartridge	30–45 m	Y	Swab, saliva
CRISPR	Viral RNA	Nucleic acid	Nucleic acid	Kit	<2 h	Y	Swab, serum
NGS	Viral RNA	Nucleic acid	Nucleic acid	Kit	Days	N	Swab, saliva
IFM	Antigen, IgG/IgM, viral RNA	Nucleic acid	Protein	Manual	3 h	N	Swab, saliva, serum
ELISA	Antigen, IgG/IgM, viral RNA	S, N, S-RBD, S1	Protein	Plate	2–4 h	N	Swab, saliva, serum
CLIA	Antigen, IgG/IgM	S, N, S-RBD, S1	Protein	Cartridge	30–45 m	N	Swab, saliva, serum
LFA	Antigen, IgG/IgM, viral RNA	S, N, S-RBD, S1	Protein	Cartridge	10–20 m	Y	Swab, saliva, serum
MESIA	Antigen, IgG/IgM, viral RNA	S, N, C	Protein	Plate, Manual	10–20 m	N	Swab, saliva, serum

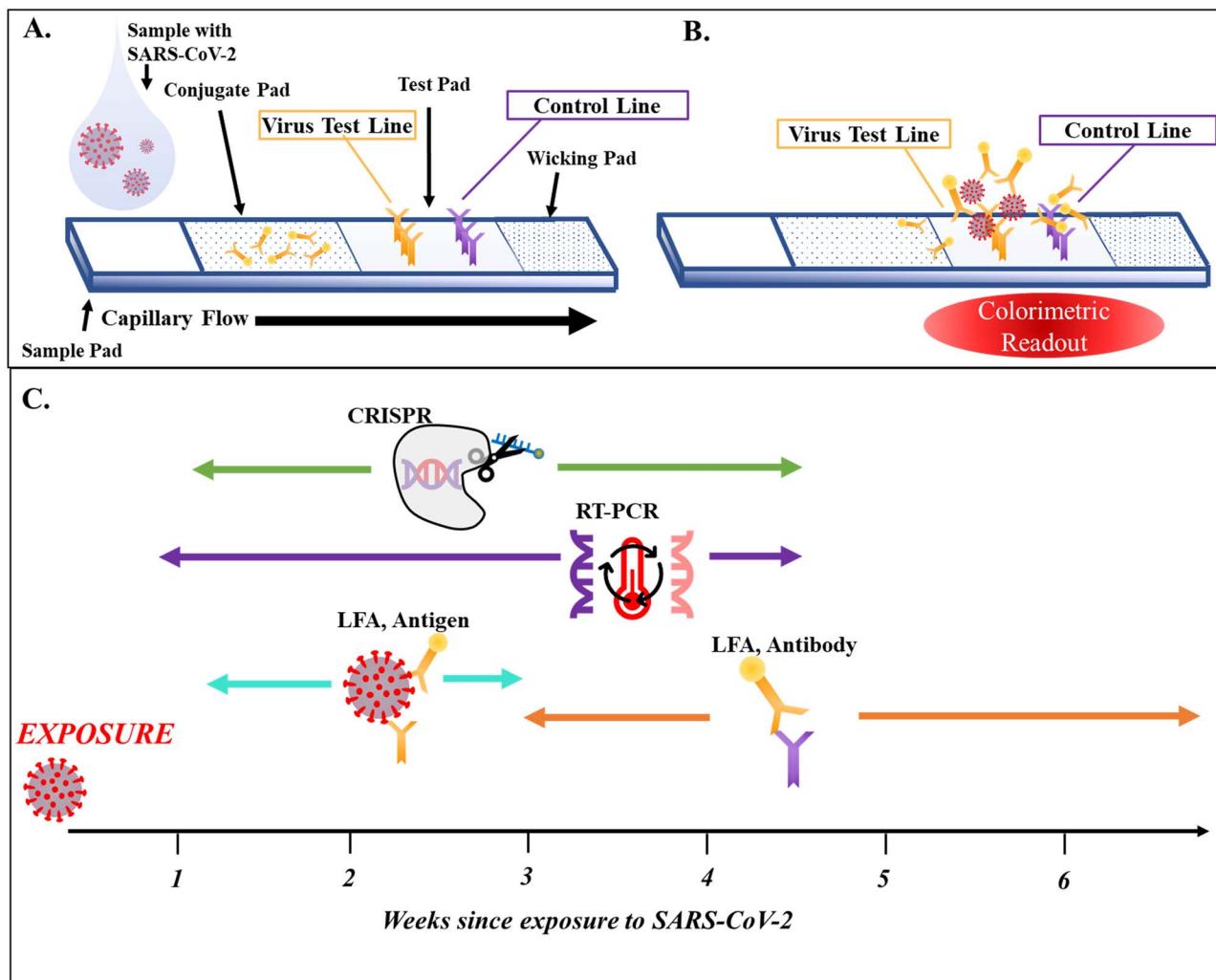
MESIA, magnetic force-assisted electrochemical sandwich immunoassay; NGS, next-generation sequencing.

transmembrane protease serine 2 (TMPRSS2), followed by viral and cell membrane fusion through S2 subunit (Fig. 1C). After entering the host cell, the next steps in the lifecycle of the coronavirus include release, translation, replication and transcription of the viral RNA and eventually synthesis, assembly and release of viral proteins by the cell (Fehr and Perlman, 2015).

Any of the proteins involved in the S1-ACE2 interactions are critical in the SARS-CoV-2 infection and provide potential targets for drug therapies (Morse et al., 2020). For diagnostic purposes, since SARS-CoV-2 is an RNA virus, all previously known RNA

detection methods can potentially be utilized to detect the virus. These tests mainly fall into molecular tests, e.g. reverse transcription polymerase chain reaction (RT-PCR), isothermal amplification and CRISPR methods, which detect viral sequence in N, E, S or Rd-RP genes of SARS-CoV-2 (Kilic et al., 2020), and protein tests or immunoassays, e.g. serological and antigen tests, which detect various proteins of the virus. Table 1 summarizes the testing methods currently available for COVID-19.

Currently, the most prominent testing method for SARS-CoV-2 is RT-PCR that detects the amplified DNA complements transcribed



**Fig. 2** (A, B) Schematic representation of LFA testing for SARS-CoV-2 at sample application and at test results. (C) Timeline of expected use of various testing methods for SARS-CoV-2 for accurate detection after exposure to SARS-CoV-2.

from the viral genome. The assay was mostly developed during the SARS-CoV and MERS-CoV outbreaks (Fehr and Perlman, 2015). The high throughput, analytical sensitivity and low LoD have made PCR the preferred method for COVID-19 detection in clinical circumstances (Chan *et al.*, 2020; Liu *et al.*, 2020; Suo *et al.*, 2020). However, the need for expensive instrumentation, skilled personnel, consumables and relatively long assay time has diminished its accessibility. As a result, more cost-effective, easy-to-use and rapid diagnostic tools such as LFA tests are suitable for global management of the current pandemic.

LFAs consist of a sample pad, conjugate pad (usually cellulose) loaded with a labeled detector protein, a test pad (usually nitrocellulose) loaded with immobilized antibodies to the sample protein or detector protein and a wicking pad to absorb excess sample and induce flow. Sample is loaded onto a sample pad that allows the analyte to conjugate to the primary antibodies labeled with a colorimetric marker on the conjugate pad. Analyte-antibody conjugates then flow to a test pad that has been striped with the immobilized primary antibodies to the analyte (analyte test line) and secondary antibodies (control line sensitive to the analyte primary antibodies). Schematic of a LFA for SARS-CoV-2 is visualized in Fig. 2A and B. Generally, LFA assays developed for viral and serology detection

of COVID-19 have lower sensitivity compared to molecular tests (Cantera *et al.*, 2020), but recent studies point out the importance of frequency, short turnaround time and POC setup of testing on effective surveillance and curbing the spread of the current pandemic (Alexandersen *et al.*, 2020; He *et al.*, 2020; Larremore *et al.*, 2020).

While the development of PCR testing was a reasonable first response to the COVID-19 pandemic, the development of LFA immunoassays that can be rapidly produced, administered at POC, require little to no clinical guidance and deliver results in just a few minutes is the next step in the fight against SARS-CoV-2. Here, we review the recent LFA antigen and antibody tests for COVID-19 with an emphasis on the ones that received emergency use authorization (EUA) from Federal Drug Administration (FDA) by November 2020. A complete list of FDA-cleared commercial tests is available at <https://www.fda.gov>.

### In Vitro Antigen LFA Tests for COVID-19

The most sought-after use of SARS-CoV-2 protein-protein interactions has thus far been through antigen detection of the S1 receptor-binding domain and the nucleocapsid N protein. As discussed, detection of the SARS-CoV-2 antigen has largely been inspired by the

pathogenesis and immunogenicity of the virion in the human body. Notably, most antigen tests have utilized highly sensitive antibodies in their LFA design. Most of these antibodies have been to the N protein since studies have shown that concentration of N protein can be higher than S (~25 per virion) protein in samples prepared for LFAs (Burbelo et al., 2020; Ke et al., 2020). A list of FDA-cleared antigen tests as of November 2020 can be viewed in Table 2. Efficacy of these proteins are largely derived by the high binding affinity proven through preliminary studies of SARS-CoV2-S1 in ELISA (enzyme-linked immunosorbent assay) and BLI (Biolayer Interferometry), which have shown results in the range of 9.6–16.1 nM and 0.745–10.8 nM, respectively (Wang et al., 2020).

Alternatively, the virion pathogenesis in the body has inspired the use of another protein for antigen detection in LFAs: the human angiotensin converting enzyme-2 (hACE2), which is the cell entry receptor of SARS-CoV-2 (Letko et al., 2020). hACE2 has similarly demonstrated a high binding and strong specificity to the SARS-CoV-2 S protein with affinities in the range of 1.2–44.2 nM as reported by surface plasmon resonance, ELISA and BLI (Walls et al., 2020; Yi et al., 2020; Xu et al., 2020a,b). Harnessing a similar level of protein-protein affinity and specificity has provided increasingly faster and more reliable POC tests; however, there still exists several barriers to using protein-based diagnostics as a ubiquitous testing platform.

Enabling detection of COVID-19 in a reliable and user-friendly format has become the foremost problem facing wide integration of antigen tests into the market. FDA EUA antigen tests include LFAs and immunoassays. These antigen tests can be further characterized by visual reads and instrument reads defining their usability as an at-home or healthcare setting test. However, in June 2020, antigen-based testing reported inferior sensitivity in comparison with PCR testing (Mak et al., 2020). The inherent lack of an amplification step and machine readability, like those in PCR, are among the underlying causes (Guglielmi, 2020). Improving the sensitivity thus represents the main barrier to entry for these rapid antigen tests that have, so far, negated its ability to surveil asymptomatic or otherwise healthy patients with low viral loads (Rubin, 2020). Additionally, recent studies pointed out the importance of testing frequency and turnaround time over sensitivity in order to effectively control the current pandemic (Alexandersen et al., 2020; Larremore et al., 2020). Despite a lower sensitivity, studies reveal that antigen tests exhibit promising clinical diagnosis in the first week of showing symptoms, which has been shown to identify when a person is infectious (He et al., 2020).

By far, the most prominently developed solution to more rapid testing using antigens is LFAs. Lateral flow immunoassays are qualitative POC tests that use antibodies to a protein of interest in any of a variety of bodily fluid samples including blood and saliva (Carter et al., 2020). These assays have the benefit of being portable, fast and relatively inexpensive. However, they tend to have low analytical performance compared to other antigen-based immunoassays especially for detecting early stages of the disease when the antigen level is low in the body (Qin et al., 2012; Carter et al., 2020; Rubin, 2020). This can be largely attributed to the nature of protein assembly used for detection of COVID-19 both before and after assembly on LFA components.

LFAs present significant limitations to these protein detectors due to the physical and chemical challenges to optimizing the interaction kinetics of the protein complex as well as low sensitivity of the visual readout or signaling methods (Tang et al., 2017; Bishop et al., 2019). The readout method of the LFA is usually a colorimetric marker such

as gold nanoparticles that can be observed visually by the human eye. Alternatives such as fluorescent markers, thermal contrast agents or laser excited markers can improve the sensitivity of LFAs by introducing a more robust signaling method, but their use requires specialized equipment and personnel (Qin et al., 2012; Koczula and Gallotta, 2016; Ye et al., 2020). Other FDA-EUA-approved antigen tests include magnetic force-assisted electrochemical sandwich immunoassay and microfluidic immunofluorescence assay.

In addition to optimizing protein interactions, labeling and readability obstacles, the most challenging aspect of developing an efficacious COVID-19 antigen test is designing a protein that is sensitive to SARS-CoV-2 antigen and is capable of adopting the aforementioned properties. One of the main challenges is that *de novo* design of binding proteins takes considerable time and effort (Bishop et al., 2019). Although there have been quite a few *de novo* protein designs for COVID-19 diagnostic tests, computational modeling and process optimization for each produces a barrier to entrance in comparison with their non-synthetic counterparts. To add to this, with the exception of some nanobodies or recombinant *Escherichia coli*-based proteins, production of a human-like protein requires extensive study to reduce post-translational misfolding, immunogenicity and non-human glycosylation (Frenzel et al., 2013). With this in mind, creating more sensitive proteins has been a prominent strategy in overcoming the low LoDs in LFAs (Pan et al., 2018). Thus, currently available LFAs for virion detection are mostly based on antibodies to their respective antigen, predominantly monoclonal antibodies (mAB) derived from mouse hybridomas, which confer specific antibodies in large quantities (Azzi et al., 2004, 2020; Koczula and Gallotta, 2016; Ariffin et al., 2020).

Monoclonal antibodies are highly preferred because they bind to their targets with relatively high specificity and affinity (Hristov et al., 2019). However, for use as a diagnostic agent, antibodies are often engineered to have improved biochemical characteristics. These include binding affinity or specificity to the antigen, solubility, stability, glycosylation, isoelectric point and labeling abilities (Hristov et al., 2019). Biochemical modifications of mABs are often made as either targeted or random changes that involve altering complementarity-determining regions of the antibody or exposed hydrophobic residues to affect aggregation and solubility (Ducancel and Muller, 2012; Arslan et al., 2019). Further modifications of mABs can be achieved by elimination of non-binding regions of the antibody and preservation of the antigen binding fragment (Fab), single-chain variable fragment (scFv) or nanobodies (camelid heavy chain only antibodies) (Pinto Torres et al., 2018). These fragments provide the advantage of ease of manufacturing and integration into an LFA and ability to be incorporated into multivalent or tagged constructs (Nelson, 2010; Vincke et al., 2012; Pinto Torres et al., 2018). Currently, mABs are used in LFAs to target the spike and nucleocapsid protein of the SARS-CoV-2 virion (Table 1). At the time of writing this manuscript (March 2021), 15 antigen tests have received FDA-EUA, out of which 10 are LFA-based, 12 are cleared for patient care settings under Clinical Laboratory Improvement Amendments (CLIA) certificate waiver and 3 are cleared for at-home use. Table 1 provides a summary of available antigen tests with FDA-EUA.

It is noticeable that most tests report very high sensitivity values, comparable to that of PCR tests. However, special attention should be drawn to these values due to the nature of their reported LODs. Rather than being reported in viral RNA copies/ml, antigen tests report LoDs as TCID50 or median tissue culture infectious dose: the virus titer required to infect 50% of cultured cells (Smithers et al., 2013). The TCID50 is not standardized, nor are virion copies

**Table 2.** Summary of available antigen and antibody tests for SARS-CoV-2 with FDA-EUA as of March 2021

AO14	Test	Company	Detection protein	Target protein	Assay time (m)	Sensitivity (%)	Specificity (%)	LoD if specified (TCID50/ml)
Antigen tests								
	BinaxNOW COVID-19 Ag Card <sup>b</sup>	Abbot	NA	NA	15	97.7	98.5	22.5
	BinaxNOW COVID-19 Ag Card Home Test <sup>b</sup>	Abbot	NA	NA	15	84.6	98.5	140.6
	CareStart COVID-19 Antigen Test <sup>b</sup>	Access Bio	NA	NA	10	88.4	100	800
	BD Veritor System	BD	NA	NA	15	84	100	140
	Sampinute COVID-19 Antigen MIA	Celltrion USA, Inc	NA	S	10	94.4	100	30
	LumiraDx SARS-CoV-2 Ag Test	LumiraDx UK Ltd.	NA	NA	12	97.6	96.6	32
	Sofia 2 SARS Antigen FIA <sup>b</sup>	Quidel	NA	NA	15	80	100	113
	Sofia 2 Flu + SARS Antigen FIA <sup>b</sup>	Quidel	NA	NA	15	95.2	100	91.7
	QuickVue SARS Antigen Test <sup>b</sup>	Quidel	NA	NA	10	96.6	99.3	7570
	QuickVue At-Home COVID-19 Test <sup>b</sup>	Ortho Clinical Diagnostics	NA	NA	10	NA	NA	NA
	VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack	Ortho Clinical Diagnostics	NA	NA	60	92.3	NA	NA
	Simoa SARS-CoV-2 N Protein Antigen Test	Quanterix	NA	NA	35	97.7	100	310
	Ellume COVID-19 Home Test <sup>b</sup>	Ellume Limited	NA	NA	15	91	96	6300
	Status COVID-19/Flu <sup>b</sup>	Princeton BioMeditech Corp.	NA	NA	15	93.9	100	2700
	Clip COVID Rapid Antigen Test <sup>b</sup>	Luminostics	NA	NA	35	96.6	100	88

Continued

**Table 2.** Continued

	Test	Company	Detection protein	Target protein	Assay time (m)	Sensitivity (%)	Specificity (%)	LoD if specified (TCID <sub>50</sub> /ml)
CRISPR LFA tests <sup>a</sup>	STOPCovid.v2 SARS-CoV-2 DETECTR LFA	Sherlock BioSciences Mammoth Biosciences	Cas13 Cas12	S (RNA) N (RNA) E (RNA) IgG, IgM	15–45 40	93.1 95	98.5 100	33 gRNA copies/ml 10 gRNA copies/ml
Antibody LFA tests	Cellex qSARS-CoV-2 IgG/IgM Rapid Test Wantai SARS-CoV-2 Ab Rapid Test	Cellex Inc Beijing Wantai Biological Pharmacy Enterprise	S and N protein spike-RBD	IgG, IgM	15–20	93.8	96	NA
	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test Cassette	Sienna-Clarity Salofa Oy	spike-RBD	IgG, IgM	10	93.3	98.8	NA
	Rapid COVID-19 IgM/IgG Combo Test Kit	Magna Health	N protein	IgG, IgM	15	100	95	NA
	LYTHER Novel Coronavirus (2019-nCoV) IgM/IgG Antibody Combo Test Kit	Hangzhou Laihe Biotech	S1 subunit	IgG, IgM	10	100	98.8	NA
	RightSign COVID-19 IgG/IgM Rapid Test Cassette	Hangzhou Biotech Biotech Co., Ltd.	spike-RBD	IgG, IgM	10	100	100	NA
	SGTi-flex COVID-19 IgG Assure COVID-19 IgG/IgM Rapid Test Device	Sugentech, Inc. Assure Tech. (Hangzhou Co.)	S-RBD and N protein S1 and N protein	IgG IgG, IgM	10 15	96.7 100	100 98.8	NA NA
	MidaSpot COVID-19 Antibody Combo Detection Kit	Nirmidas Biotech, Inc.	S-RBD	IgG, IgM	18	100	96.2	NA
	Nirmidas COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit	Nirmidas Biotech, Inc.	S1 and spike-RBD	IgG, IgM	10–15	96.6	97.9	NA
	Biohit SARS-CoV-2 IgM/IgG Antibody Test Kit	Biohit Healthcare (Hefei) Co. Ltd.	N protein	IgG, IgM	15–20	96.7	95	NA
	CareStart COVID-19 IgM/IgG	Access Bio, Inc.	S1 and N protein	IgG, IgM	10	98.4	98.9	NA

Continued

**Table 2.** Continued

Test	Company	Detection protein	Target protein	Assay time (m)	Sensitivity (%)	Specificity (%)	LoD if specified (TCID50/ml)
BIOTIME SARS-CoV2 IgG/IgM Rapid Qualitative Test	Xiamen Biotime Biotechnology Co., Ltd.	S protein	IgG, IgM	15	100	96.2	NA
Orwell IgM/IgG Rapid Test	Jiangsu Well Biotech Co., Ltd.	spike-RBD	IgG, IgM	10	100	94.8	NA
Innovita 2019-nCoV Ab Test (Colloidal Gold)	Innovita (Tangshan) Biological Technology Co., Ltd.	S and N protein	IgG, IgM	10	98.8	93.3	NA
ACON SARS-CoV-2 Ag/G/IgM Rapid Test	ACON Laboratories	NA	IgG, IgM	15	99.1	98.2	NA
Tell Me Fast Novel Coronavirus (COVID-19) IgG/IgM Antibody Test	Biocan Diagnostics Inc.	S and N protein	IgG, IgM	15-20	93.3	96.2	NA
TBG SARS-CoV2 IgG/IgM Rapid Test Kit	TBG Biotechnology Corp.	S and N protein	IgG, IgM	15	93.3	95	NA
COVID-19 Test	Adwate	N Protein	IgG	15	90	95.2	NA
COVID-19 IgG/IgM Rapid Test Cassette	Healgen	S1 subunit	IgG, IgM	15	100	97.5	NA

<sup>a</sup>Data for CRISPR LFAs are taken from their respective companies which do not have FDA clearance (Broughton *et al.*, 2020; Joung *et al.*, 2020).

<sup>b</sup>LFA antigen test.

the same as genome copies, and may refer to viral copy numbers in different ways depending on the viral preparation and units of copies/volume (Arnaout *et al.*, 2020). Translationally, approximately 1 of 10 000 genome copies may be associated with a tissue culture infectious viral particle; for example, the Sofia2 SARS Antigen Test's sensitivity has been calculated to have an estimated clinical sensitivity of only 31% (rather than its reported 96.7%), when translating the LoD from TCID50 to genomic copies/ml (Arnaout *et al.*, 2020). This has manifested in academic studies reporting lower clinical sensitivities or higher LoDs than reported in the tests' FDA-EUAs (Corman *et al.*, 2020; Mak *et al.*, 2020; Scohy *et al.*, 2020).

Although the translational differences of RNA (PCR) and virion (antigen-based) based tests cause disagreement in the diagnosis of a SARS-CoV-2 positive or SARS-CoV-2 negative person, rapid antigen testing may present a potential diagnostic advantage. While viral load, as measured by viral RNA copies/ml, is the highest during the infectious period, ranging from 2 days after exposure to 12 days after showing symptoms, it only indicates evidence of SARS-CoV-2 and not necessarily an individual that is capable of transmitting SARS-CoV-2 to others (Manabe *et al.*, 2020). Virus culture, on the other hand, has shown to correspond with transmission of SARS-CoV-2 in animal models (Manabe *et al.*, 2020; Sia *et al.*, 2020).

Another protein-based SARS-CoV-2 test that emerged early in this pandemic are CRISPR/Cas-based protein tests. Widely used as a gene editing tool since 2013, CRISPR first found a new application as a diagnostic agent for ZIKA and Dengue (Koonin and Makarova, 2013; Wu *et al.*, 2013; Abudayyeh *et al.*, 2016; Chen *et al.*, 2018; Harrington *et al.*, 2018). Those used for diagnostic purposes, including Cas12, Cas13 and Cas14, had promiscuous cleavage activities and shared similar reactive components: gRNA, probes, nucleotide activators and buffers (Ai *et al.*, 2019). These Cas proteins have one enzymatic domain that binds to a nucleotide activator and another enzymatic domain to cleave small nucleotide probes after the protein is activated. Cas12 recognizes double-stranded DNA and cleaves single-strand DNA (ssDNA). Cas13 recognizes and cleaves ssRNA. Cas 14 recognizes and cleaves ssDNA (Abudayyeh *et al.*, 2016; Chen *et al.*, 2018; Harrington *et al.*, 2018).

Rapid tests for SARS-CoV-2 have thus far utilized Cas12 and Cas13. These proteins bind specifically to the Orf1ab, N or E gene of the SARS-CoV-2 gRNA and produce a fluorescent signal from cleaved probes due to collateral nuclease activity of Cas proteins (Broughton *et al.*, 2020; Hou *et al.*, 2020). This in turn signals the presence of SARS-CoV-2 via the fluorescent signal. As a POC test, CRISPR-based tests have the advantage of taking as little as 40 min and can even be integrated into an LFA—two of which have gained FDA-EUA (Broughton *et al.*, 2020). These tests have shown to have potentially greater sensitivity than that of PCR (Broughton *et al.*, 2020; Hou *et al.*, 2020).

## In Vitro Antibody Tests for COVID-19

Molecular tests are ideal for diagnosing viral infections during the first weeks of a disease, whereas serological tests detect the presence of host response proteins, such as immunoglobulin G (IgG), IgM, IgA and other host components in whole blood, plasma or serum. As discussed before, in order to manage and control the COVID-19 pandemic, there is an urgent need to develop rapid, accessible, cost-effective, POC viral and serological diagnostic tests (Xu *et al.*, 2020a). Antibody tests are used mostly to complement molecular and antigen tests in the COVID-19 diagnosis as they can provide

information on past infections and the dynamics of the individual's infection response by detecting the presence of IgG and IgM. Studies show that both SARS-CoV-2-[IgG and IgM] levels rise gradually after symptom onset with IgM being detectable as early as 5 days post-illness onset. However, the accuracy of detection of antibodies reaches its peak after 10–14 days after infection (Long *et al.*, 2020; Xiang *et al.*, 2020) as illustrated in Fig. 2C.

Serology diagnostic tests are developed on various platforms such as ELISA, EIA (Enzyme ImmunoAssay), CLIA or LFA with turnaround times ranging between a few hours and as little as 10 min (Weissleder *et al.*, 2020). Accuracy of each test is strongly dependent on the viral antigen or recombinant proteins used to capture the host antibodies. These tests employ recombinant viral proteins or other proteins expressed in *E. coli* or HEK 293 cells as antibody capture proteins to S and N proteins, the main immunogenic proteins of SARS-CoV-2 (Okba *et al.*, 2020). Although high analytical sensitivity and specificity (comparable to ELISA and CLIA methods after 14 days of symptom onset) have been observed in serological LFAs, variations in sample collection, test administration, varying performance among different brands and their inability to detect acute infections in the early days of the disease hinder them from becoming a standalone diagnostic method for COVID-19 (Liu *et al.*, 2020; Long *et al.*, 2020; Nicol *et al.*, 2020; Weissleder *et al.*, 2020; Xiang *et al.*, 2020). However, they are suitable and needed for contact tracing, epidemiologic and vaccine evaluation studies.

At the time of writing this manuscript (March 2021), 72 serology tests have received FDA-EUA, out of which 20 are LFA-based and 5 are cleared for patient care settings under CLIA certificate waiver, whereas none are cleared for at-home use. Table 2 provides a summary of available LFA serology tests with FDA-EUA.

## Conclusion and Future Directions

Curbing the COVID-19 outbreak likely requires the use of combination of different diagnostic assays. While RNA-based assays provide an impressive launchpad for diagnosis and contact tracing at the start of the COVID-19 pandemic, rapid POC testing can provide a route towards immediate and convenient knowledge of COVID-19 infection and response. Some helpful advancements have been recently published that have pushed LFA technology to compete with the performance of PCR-based testing. The Chilkoti research group has made advancements on their D4 assay technology, which introduces an inkjet-printed assay using a non-fouling polymer brush to increase the signal-to-noise ratio of the assay (Joh *et al.*, 2017). They are currently applying this work to test for COVID-19 antibodies. Zhang *et al.* (2020) have recently reported improvement of the LoD up to 100-fold by test-zone pre-enrichment using human chorionic gonadotropin and Aflatoxin B1 as model targets. Salminen *et al.* (2019) have improved the sensitivity of a LFA to free prostate-specific antigen by 100-fold using a fluorescent europium (III)-doped nanoparticle (Eu-np) reporter instead of gold.

Given the need for an improvement in sensitivity of rapid antigen testing, it may be best used as a screening platform accompanied by a molecular test to confirm diagnosis. With recent advancements in our understanding of SARS-CoV-2 pathology and epidemiology, rapid diagnostic tests are achieving higher sensitivity and specificity every day. These improvements pave the way for broad and repeated testing

that is required for timely identification and treatment of infectious individuals (Studdert and Hall, 2020). Among the diagnostic tools, LFAs are the most promising method for mass testing due to their portability and expense. Current LFAs developed for COVID-19 antigen and antibody diagnosis are highly specific, but their variable sensitivity prevents their use as a primary detection method. At the moment, they remain most useful as seroprevalence surveys for detection of past infections and immunity to the diseases.

## Conflict of interest

The authors declare no competing financial interest.

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## References

Abudayyeh, O.O., Gootenberg, J.S., Konermann, S. *et al.* (2016) *Science*, **353**, aaf5573-1 to aaf5573-9.

Ai, J.-W., Zhou, X., Xu, T. *et al.* (2019) *Emerg. Microbes Infect.*, **8**, 1361–1369.

Alexandersen, S., Chamings, A., Bhatta, T.R. (2020) *medRxiv*.

Ariffin, N., Yusof, N.A., Abdullah, J., Rahman, S.F.A., Raston, N.H.A., Kusnini, N., Suraiya, S. (2020) *J. Sensors*, **2020**, 1365983.

Arnaout, R., Lee, R.A., Lee, G.R., Callahan, C., Yen, C.F., Smith, K.P., Arora, R., Kirby, J.E. (2020) *bioRxiv*.

Arslan, M., Karadağ, D., Kalyoncu, S. (2019) *Turk. J. Biol.*, **43**, 1–12.

Azzi, A., Manaresi, E., Zakrzewska, K., DeSantis, R., Musiani, M., Zerbini, M. (2004) *J. Med. Virol.*, **72**, 679–682.

Azzi, L., Baj, A., Alberio, T., Lualdi, M., Veronesi, G., Carcano, G., Ageno, W. *et al.* (2020) *J. Infect.*, **81**, e75–e78.

Bishop, J.D., Hsieh, H.V., Gasperino, D.J., Weigl, B.H. (2019) *Lab Chip*, **19**, 2486–2499.

Broughton, J.P., Deng, X., Yu, G., Fasching, C.L., Servellita, V., Singh, J., Miao, X. *et al.* (2020) *Nat. Biotechnol.*, **38**, 870–874.

Burbelo, P.D., Riedo, F.X., Morishima, C., Rawlings, S., Smith, D., Sanchita Das, J.R., Strich, D.S., Chertow, D.R.T.Jr., Cohen, J.I. (2020) *J. Infect. Dis.*, **222**, 206–213.

Cantera, J., Cate, D., Golden, A., Peck, R., Lillis, L., Domingo, G., Murphy, E. *et al.* (2020) *Chemrxiv*.

Carter, L.J., Garner, L.V., Smoot, J.W., Li, Y., Zhou, Q., Saveson, C.J., Sasso, J.M. *et al.* (2020) *ACS Central Sci.*, **6**, 591–605.

Chan, J.F.-W., Yip, C.C.-Y., To, K.K.-W., Tang, T.H.-C., Wong, S.C.-Y., Leung, K.-H., Fung, A.Y.-F. *et al.* (2020) *J. Clin. Microbiol.*, **58**, e00310–e00320.

Chen, J.S., Ma, E., Harrington, L.B., Da Costa, Tian, X., Palefsky, J.M., Doudna, J.A. (2018) *Science*, **360**, 436–439.

Corman, V.M., Haage, V.C., Bleicker, T., Schmidt, M.L., Mühlmann, B., Zuchowski, M., Lei, W.K.J. *et al.* (2020) *medRxiv*.

Ducancel, F. and Muller, B.H. (2012) *MABS*, **4**, 445–457.

Fehr, A.R. and Perlman, S. (2015) *Methods Mol. Biol.*, **1282**, 1–23.

Frenzel, A., Hust, M., Schirrmann, T. (2013) *Front. Immunol.*, **4**, 217.

Guglielmi, G. (2020) *Nature*, **583**, 506–509.

Harrington, L.B., Burstein, D., Chen, J.S., Paez-Espino, D., Ma, E., Witte, I.P., Cofsky, J.C., Kyriides, N.C., Banfield, J.F., Doudna, J.A. (2018) *Science*, **362**, 839–842.

He, X., Lau, E.H.Y., Wu, P., Deng, X., Wang, J., Hao, X., Lau, Y.C. *et al.* (2020) *Nat. Med.*, **26**, 672–675.

Hou, T., Zeng, W., Yang, M., Chen, W., Ren, L., Ai, J., Wu, J. *et al.* (2020) *PLoS Pathog.*, **16**, e1008705.

Hristov, D.R., Rodriguez-Quijada, C., Gomez-Marquez, J., Hamad-Schifferli, K. (2019) *Sensors (Basel)*, **19**, 554.

Huang, Y. *et al.* (2020) Structural and functional properties of sars-cov-2 spike protein: potential antivirus drug development for covid-19. *Acta Pharmacologica Sinica*, **41**(9), 1141–1149.

Joh, D.Y., Hucknall, A.M., Wei, Q., Mason, K.A., Lund, M.L., Fontes, C.M., Hill, R.T. *et al.* (2017) *Proc. Natl. Acad. Sci.*, **114**, E7054–E7062.

Joung, J., Ladha, A., Saito, M., Nam-Gyun Kim, A.E., Woolley, M.S., Barreto, R.P.J. *et al.* (2020) *N. Engl. J. Med.*, **383**, 1492–1494.

Ke, Z., Oton, J., Kun, Q., Cortese, M., Zila, V., McKeane, L., Nakane, T. *et al.* (2020) *Nature*.

Kilic, T., Weissleder, R., Lee, H. (2020) *iScience*, **23**, 101406.

Koczula, K.M. and Gallotta, A. (2016) *Essays Biochem.*, **60**, 111–120.

Koonin, E.V. and Makarova, K.S. (2013) *RNA Biol.*, **10**, 679–686.

Larremore, D.B., Wilder, B., Lester, E., Soraya Shehata, J.M., Burke, J.A., Hay, M.T., Mina, M.J., Parker, R. (2020) *medRxiv*.

Lee, D. and Lee, J. (2020) *Transport. Res. Interdiscip. Perspect.*, **5**, 100111.

Letko, M., Marzi, A., Munster, V. (2020) *Nat. Microbiol.*, **5**, 562–569.

Liu, Y., Liu, Y., Diao, B., Ren, F., Wang, Y., Ding, J., Huang, Q. (2020) *medRxiv*.

Long, Q.-x., Deng, H.-j., Chen, J., Hu, J.-L., Liu, B.-z., Liao, P., Lin, Y. *et al.* (2020) *medRxiv*.

Mak, G.C.K., Cheng, P.K.C., Lau, S.S.Y., Wong, K.K.Y., Lau, C.S., Lam, E.T.K., Chan, R.C.W., Tsang, D.N.C. (2020) *J. Clin. Virol.*, **129**, 104500.

Manabe, Y.C., Sharfstein, J.S., Armstrong, K. (2020) *JAMA*.

Morse, J.S., Lalonde, T., Xu, S., Liu, W.R. (2020) *Chem. Bio Chem.*, **21**, 730–738.

Nelson, A.L. (2010) *MABS*, **2**, 77–83.

Nicol, T., Lefevre, C., Serri, O., Pivert, A., Joubaud, F., Dubée, V., Kouatchet, A., Ducancelle, A., Lunel-Fabiani, F., Le Guillou-Guillemette (2020) *J. Clin. Virol.*, **129**, 104511.

Okba, N.M.A., Muller, M.A., Li, W., Wang, C., Kessel, C.H.G., Corman, V.M., Lamers, M.M., Sikkema, R.S., Bruin, Chandler, F.D. (2020) *Emerg. Infect. Dis.*, **26**, 1478–1488.

Pan, RUILI, YUJUN Jiang, LUHONG Sun, RUI Wang, KEJIN Zhuang, YUEMING Zhao, Hui Wang, MR ASLAM Ali, HONGHUA Xu, and CHAOXIN Man. 2018. “”. *J. Dairy Sci.* **101** (): 3835–43.

Pinto Torres, J.E., Goossens, J., Ding, J., Li, Z., Lu, S., Vertommen, D., Naniima, P. *et al.* (2018) *Sci. Rep.*, **8**, 9019–9019.

Pokhrel, P., Hu, C., Mao, H. (2020) *ACS Sensors*, **5**, 2283–2296.

Qin, Z., Chan, W.C.W., Boulware, D.R., Akkin, T., Butler, E.K., Bischof, J.C. (2012) *Angew. Chem. Int. Ed.*, **51**, 4358–4361.

Rubin, R. (2020) *JAMA*, **324**, 1813–1815.

Salminen, T., Juntunen, E., Talha, S.M., Pettersson, K. (2019) *J. Immunol. Methods*, **465**, 39–44.

Scohy, A., Anantharajah, A., Bodéus, M., Kabamba-Mukadi, B., Verroken, A., Rodriguez-Villalobos, H. (2020) *J. Clin. Virol.*, **129**, 104455–104455.

Shim, E., Tariq, A., Choi, W., Lee, Y., Chowell, G. (2020) *Int. J. Infect. Dis.*, **93**, 339–344.

Sia, S.F., Yan, L.M., Chin, A.W.H., Fung, K., Choy, K.T., Wong, A.Y.L., Kaewpreedee, P. *et al.* (2020) *Nature*, **583**, 834–838.

Smithier, S.J., Lear-Rooney, C., Biggins, J., Pettitt, J., Lever, M.S., Jr.Olinger, G.G. (2013) *J. Virol. Methods*, **193**, 565–571.

Studdert, D.M. and Hall, M.A. (2020) *N. Engl. J. Med.*.

Suo, T., Liu, X., Feng, J., Guo, M., Hu, W., Guo, D., Ullah, H. *et al.* (2020) *Emerg Microbes Infect*, **9**, 1259–1268.

Tang, R., Yang, H., Gong, Y., Liu, Z., Li, X., Wen, T., Qu, Z., Zhang, S., Mei, Q., Xu, F. (2017) *Sci. Rep.*, **7**, 1360.

Vincke, C., Gutiérrez, C., Wernery, U., Devoogdt, N., Hassanzadeh-Ghassabeh, G., and Muyldermans, S. 2012. Generation of single domain antibody fragments derived from camelids and generation of manifold constructs. *In Antibody Engineering: Methods and Protocols*, 2nd edn, 145–76. Totowa, NJ: Humana Press.

Walls, A.C., Park, Y.-J., Tortorici, M.A., Wall, A., McGuire, A.T., Veesler, D. (2020) *Cell*, **181**, 281, e6–292.

Wang, C., Li, W., Drabek, D., Okba, N.M.A., van Haperen, Osterhaus, A.D.M.E., van Kuppeveld, Haagmans, B.L., Grosveld, F., Bosch, B.-J. (2020) *Nat. Commun.*, **11**, 2251.

Weissleder, R., Lee, H., Ko, J., Pittet, M.J. (2020) *Sci. Transl. Med.*, **12**, eabc1931.

Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.-L., Abiona, O., Graham, B.S., McLellan, J.S. (2020) *Science*, **367**, 1260.

Wu, Y., Liang, D., Wang, Y., Bai, M., Tang, W., Bao, S., Yan, Z., Li, D., Li, J. (2013) *Cell Stem Cell*, **13**, 659–662.

Xiang, F., Wang, X., He, X., Peng, Z., Yang, B., Zhang, J., Zhou, Q. *et al.* (2020) *Clin. Infect. Dis.*, **71**, 1930–1934.

Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., Zhong, W., Hao, P. (2020a) *Sci. China Life Sci.*, **63**, 457–460.

Xu, Y., Xiao, M., Liu, X., Xu, S., Tiekuang, D., Xu, J., Yang, Q., Xu, Y., Yang, H., Li, T. (2020b) *Emerg Microbes Infect*, **9**, 924–927.

Ye, H., Liu, Y., Zhan, L., Liu, Y., Qin, Z. (2020) *Theranostics*, **10**, 4359–4373.

Yi, C., Sun, X., Ye, J., Ding, L., Liu, M., Yang, Z., Lu, X. *et al.* (2020) *Cell. Mol. Immunol.*, **17**, 621–630.

Zhang, Y., Liu, X., Wang, L., Yang, H., Zhang, X., Zhu, C., Wang, W., Yan, L., Li, B. (2020) *Sci. Rep.*, **10**, 1–9.