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RBMO

REVIEW

Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays

BIOGRAPHY

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KEY MESSAGE

Molecular and serological assays for SARS-CoV-2 are being developed and implemented at an exponential rate. This suite of complementary tests will inform crucial decisions by healthcare providers and policy makers and understanding their strengths and limitations will be critical to their judicious application to treatment and public health strategies.

ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its associated coronavirus disease 2019 (COVID-19) pandemic has demanded rapid upscaling of in-vitro diagnostic assays to enable mass screening and testing of high-risk groups, and simultaneous ascertainment of robust data on past SARS-CoV-2 exposure at an individual and a population level. To meet the exponential demand in testing, there has been an accelerated development of both molecular and serological assays across a plethora of platforms. The present review discusses the current literature on these modalities, including nucleic acid amplification tests, direct viral antigen tests and the rapidly expanding laboratory-based and point of care serological tests. This suite of complementary tests will inform crucial decisions by healthcare providers and policy makers, and understanding their strengths and limitations will be critical to their judicious application for the development of algorithmic approaches to treatment and public health strategies.

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KEY WORDS
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INTRODUCTION

In December 2019, an outbreak of an unexplained pneumonia originated from the city of Wuhan, Hubei Province, China (*Guan et al., 2020*; *Huang et al., 2020*). After the initial outbreak, a novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) was quickly identified as the aetiological agent, and the associated disease defined as COVID-19 (an acronym from COrona VIrus Disease, where 19 stands for the year the virus was first detected). The exponential growth of affected individuals led the World Health Organization (WHO) to declare a global pandemic on 11 March 2020 (*Huang et al., 2020*), with 3,002,303 confirmed cases and 208,131 deaths worldwide as of 27 April 2020, and many more anticipated.

The use of direct molecular diagnostic testing based on sequencing of SARS-CoV-2 has been critical in identifying infected individuals. However, as lockdown measures have begun to bite, there has been a race to develop and approve tests with a different purpose, to assess not current viral infection but rather immunity to severe SARS-CoV-2 to facilitate a return to work. Despite this, antibody testing may also be relevant in the critical evaluation of the disease, including: (i) understanding the kinetics of the immune response to infection; (ii) understanding the immune response relative to disease severity and timeline; (iii) understanding whether cross-reactivity with other coronaviruses (CoV) leads to cross-protection; (iv) clarifying whether infection protects from future infection and how long immunity will last; and (v) determining the correlates of protection that can guide public health measures. In addition to these critical questions, immediate clinical applications include: (i) the diagnosis and triage of patients who seek medical attention in the later phases of the disease; (ii) contact tracing; (iii) stratifying workforces and patients if immunity is shown to be lasting; and (iv) sero-epidemiological studies to understand the extent of COVID-19 spread.

An understanding of the application and diagnostic performance of the different testing approaches for SARS-Cov-2 is essential in the fight against this pandemic. In the field of reproductive technology, these tests are believed by many to be one of the milestones for recommencing clinical activity. The recent European Society for Human Reproduction and Embryology (www.eshre.eu/Home/COVID19WG) position statement highlighted the current lack of understanding in the field of in-vitro diagnostic assays and in particular serological testing, and the American Society for Reproductive Medicine (www.asrm.org/news-and-publications/covid-19) has called for healthcare providers to be aware of the limitations of these tests. The purpose of this review is to provide an overview of current diagnostic approaches for SARS-CoV-2 and in particular highlight issues with serological testing, with the objective of providing a clear guide to clinicians on the assays currently available.

LITERATURE SEARCH

A literature search was carried out for studies that focused on the diagnostic and serological testing for SARS-CoV-2, using the keywords "coronavirus", "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" and "COVID-19". The PubMed, Google Scholar and EMBASE databases were searched without language restrictions from inception through to 16 April 2020 and updated on 15 May 2020. Given the rapidly developing field and rapid dissemination of scientific findings with respect to COVID-19, the preprint servers for both health sciences (medRxiv) and biology (bioRxiv) databases were also searched. Additional journal articles were identified from the bibliographies of included studies. For the main objective of this review, all original studies reporting on the sensitivity and/or specificity of antibodies against SARS-CoV-2 were included in the analysis. More than 20,000 articles have been published on SARS-CoV-2, of which 4182 articles are related to CoV and antibodies or serology. After screening the title and abstract, 887 full-text studies were retrieved, with 65 studies meeting the inclusion criteria and reporting data on test sensitivity and/or specificity, as summarized in TABLE 1.

CORONAVIRAL GENOME AND STRUCTURE

CoV belong to the subfamily *Coronavirinae*in the family of *Coronaviridae* of the order *Nidovirales*. In this subfamily four genera are included *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*. The genome of the virus is a single-stranded positive-sense RNA (+ssRNA) (around 30 kb in size) with a 5′-cap structure and 3′-poly-A tail. The genome and subgenomes of a typical CoV may present six, or even more, open reading frames (ORF). The first ORF (ORF1a/b) encompasses approximately 66% of the whole genome and encodes 16 non-structural proteins (nsp1–16), which are mainly involved in the replication of CoV. Other ORF encompassing one-third of the genome near the 3′-terminus encode the main structural proteins: spike (S), membrane (M), envelope (E) and nucleocapsid (N) proteins (*Chen et al., 2020a*).

The different CoV exhibit 54% identity of the whole RNA, with 58% exhibiting identity for the non-structural proteins coding region and 43% identity for the structural protein coding region. Sequence analysis shows that the new CoV incorporates the typical genome structure of CoV and belongs to the cluster of betac-CoV that includes bat-SARS-like (SL)-ZC45, bat-SL ZXC21, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). Based on the phylogenetic tree of CoV, 2019-nCoV is more closely related to bat-SL-CoV ZC45 and bat-SL-CoV ZXC21 and more distantly related to SARS-CoV (*Chen et al., 2020a*).

Four principal structural proteins are essential for assembly of the virion and its associated infective capacity. Homotrimers of S proteins make up the spikes on the viral surface, which are responsible for attachment to receptors on the host cells. The M protein has three transmembrane domains and shapes the virions, promotes membrane curvature and covers the nucleocapsid. The E protein participates in virus assembly and release, and is involved in viral pathogenesis. The N protein presents two domains, both of which can bind virus RNA genome via different mechanisms. The N protein binds to non-structural protein 3 (nsp3) protein to help tether the genome to the replication–transcription complex and package the encapsidated genome into virions. The N protein is also an antagonist of interferon and viral encoded repressor of RNA interference, which may be beneficial for viral replication.

TABLE 1 SUMMARY OF THE ORIGINAL ARTICLES REPORTING ON SARS-COV-2 ANTIBODY TESTING

Author, Year	Design of the study	N	Population	Country of the test population	Antibody used	Methodology	Main findings and/or conclusions	Sensitivity (%)	Specificity (%)	PPV	NPV
PubMed articles											
Li Z et al., 2020	Retrospective	525	397 RNA-positive patients, 128 controls	China	Commercial assay	Jiangsu Medomics Medical Technologies, LFIA	The test time was from day 8 to day 33 after symptoms appeared. The IgM–IgG combined assay has better utility and sensitivity than a single IgM or IgG test. Results demonstrate that the IgG–IgM combined antibody test kit can be used as a POC test	88.66	90.63	NA	NA
Xiao D et al., 2020	Prospective	34	SARS-CoV-2 confirmed patients	China	Commercial assay	Shenzhen Yahuilong Biotechnology, chemiluminescence assay	After 2 weeks from the onset of symptom, all but two subjects had positive results from the test. From the 5th to 7th weeks IgM became negative, while all had high levels of IgG	94.1	NA	NA	NA
Zhao J et al., 2020	Prospective	535 samples from 173 subjects	173 RNA-positive patients	China	Commercial assay	Beijing Wantai Biological Pharmacy Enterprise, ELISA	The seroconversion rates for Ab, IgM and IgG were 93.1%, 82.7% and 64.7%. The cumulative seroconversion curve showed that the rates for antibody and IgM reached 100% around 1 month of illness	100 (>15 days)	NA	NA	NA
Du Z et al., 2020	Retrospective	60	Convalescent patients (6–7 weeks from onset)	China	Commercial assay	ELISA	All patients tested positive for IgG against the virus, while 13 patients tested negative for IgM	78 IgM 100 IgG	NA	NA	NA
Cassaniti I et al., 2020	Prospective	110	30 RNA-positive patients, 50 patients with respiratory symptoms, 30 controls	China	Commercial assay	Rapid VivaDiag IgM /IgG immunoassay	The rapid test is not recommended for triage of patients with suspected COVID-19 in the emergency room	18.4	91.7	87.5	26.2
Guo L et al., 2020	Prospective	208 samples from 140 subjects	82 confirmed and 58 probable cases	China	In-house assay	ELISA for IgA, IgM and IgG	IgA, IgM and IgG were detected in 92.7%, 85.4% and 77.9% of samples from a median time of 5 days from the onset of symptoms	75.6 (IgM in confirmed cases) 93.1 (IgM in probable cases)	NA	NA	NA
Jin Y et al., 2020	Retrospective	76	43 RNA-positive patients, 33 probable cases	China	Commercial assay	Shenzhen YHLO Biotech, chemiluminescence assay	Viral serological testing is an effective means of diagnosing SARS-CoV-2 infection. The positive rate and titre variance of IgG are higher than those of IgM	48.1 IgM 88.9 IgG	100 IgM 90.9 IgG	NA	NA
Pan Y et al., 2020	Retrospective	105	105 patients	China	In-house assay	Immunochromatography	The positive rates of Ig in the early stage are relatively low and gradually increase during disease progression. The IgM-positive rate rose from 11.1% in early-stage to 74.2% in late-stage disease. The IgG-positive rate in confirmed patients was 3.6% in early-stage and 96.8% in late-stage disease	68.6	NA	NA	NA
Padoan A et al., 2020	Retrospective	87 samples from 37 subjects	37 patients	Italy	Commercial assay	Snibe, MAGLUM 2000 Plus 2019-nCov IgM and IgG assays	After the 11th day from the onset of symptoms, all patients were found to be positive for IgG (100%), while the higher positivity of IgM (88%) was achieved only after the 13th day. The imprecision and repeatability of the test were acceptable	88 IgM 100 IgG	NA	NA	NA
Zhong L et al., 2020	Cross-sectional	347	47 RNA-positive patients, 300 controls	China	Commercial assay	ELISA and chemiluminescence detection assay	The ELISA and chemiluminescence methods were consistent in detecting IgG and IgM antibodies by the recombinant N and S proteins of SARS-CoV-2	97.9 IgM 95.7 IgG	99.7 IgM 85.7 IgG	NA	NA

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Table 1 – (continued)

Author, Year	Design of the study	N	Population	Country of the test population	Antibody used	Methodology	Main findings and/or conclusions	Sensitivity (%)	Specificity (%)	PPV	NPV
Infantino M et al., 2020	Cross-sectional	125	61 RNA-positive patients and 64 controls	China	Commercial assay	iFlash CLIA, Chemiluminescence assay	The ROC AUC were 0.918 and 0.980 for IgM and IgG anti-SARS CoV-2 antibodies	73.3 (IgM) 76.7 (IgG)	92.2 100	81.5 NA	88.1 90.1
Xiang F et al., 2020	Retrospective	216 samples from 109 subjects	85 confirmed and 24 suspected cases	China	Commercial assays	Zhu Hai LivZon Diagnostics, ELISA	The seropositive rate of IgM increased gradually and notably. IgG was increased sharply on the 12th day after onset. Diagnostic performance calculated from samples obtained after 13 days from the onset	77.3 IgM 83.3 IgG	100 95	100 94.8	80 83.8
Lee YL, et al., 2020	Retrospective	33 samples from 14 subjects, 28 samples from 28 controls	14 RNA-positive patients and 28 controls	China	Commercial assay	Alltest, Rapid Test	Antibody response varied with different clinical manifestations and disease severity. Patients with symptoms and development of anti-SARSCoV-2 IgM antibodies had a shorter duration of a positive rRT-PCR result and no worsening clinical conditions compared with those without the presence of anti-SARS-CoV-2 IgM antibodies	78.6	100	NA	NA
Long QX et al., 2020	Cross-sectional	285 patients	285 RNA-positive patients	China	Commercial assay	Chemiluminescence Bioscience assay	The positive rate of IgG reached 100% around 17–19 days after symptom onset, while the IgM seroconversion rate reached its peak of 94.1% around 20–22 days after symptom onset	94 (IgM) 100 (IgG)	NA	NA	NA
Perera R et al., 2020	Retrospective	51 samples from 24 patients	24 RNA-positive patients	China	In-house assay	ELISA	IgG and IgM were reliably positive after 29 days from illness onset with no detectable cross-reactivity in age-stratified controls	74	100	NA	NA
Qu J et al., 2020	Retrospective	347 samples from 41 patients and 38 samples from controls	41 RNA-positive patients and 38 controls	China	Commercial assay	YHLO Biotech, chemiluminescence assay	The majority of the patients developed robust antibody responses between 17 and 23 days after illness onset	87.8 (IgM) 97.6 (IgG)	NA	NA	NA
Shen B et al., 2020	Prospective	150 patients	150 suspected cases, of whom 97 were RNA positive	China	Commercial assay	Shanghai Outdo Biotech, rapid immunochromatography test	The colloidal gold immunochromatography assay for SARS-Cov-2-specific IgM/IgG antibody shows the potential for a useful rapid diagnosis test for COVID-19	71	96	97	64
Zhao R et al., 2020	Retrospective	481	69 affected subjects and 412 controls	China	In-house assay	ELISA	The overall accuracy of the ELISA test was 97.3%	97.5	97.5	NA	NA
Cai X et al., 2020	Retrospective	276 samples from 276 subjects, 200 samples from 200 controls	276 RNA-positive patients, and 200 healthy controls	China	In-house assay	Chemiluminescence assay	Combining immunoassay with real-time RT-PCR might enhance the diagnostic accuracy of COVID-19	57.2 (IgM) 71.4 (IgG)	NA	NA	NA
Dohla M et al., 2020	Prospective	Samples from 49 symptomatic patients	22 RNA-positive and 27 RNA-negative patients	Germany	Commercial assay	Rapid test	The rapid test was substantially inferior to the RT-qPCR testing and should therefore be used for neither individual risk assessment nor decisions on public health measures	36.4	88.9	72.7	63.1

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Table 1 – (continued)

Author, Year	Design of the study	N	Population	Country of the test population	Antibody used	Methodology	Main findings and/or conclusions	Sensitivity (%)	Specificity (%)	PPV	NPV
Hoffman T et al., 2020	Cross-sectional	Samples from 153 subjects	29 RNA-positive patients and 124 controls	China	Commercial assay	Zhejiang Orient Gene Biotech, rapid COVID test	The test is suitable for assessing previous virus exposure, although negative results may be unreliable during the first weeks after infection	69 (IgM) 93 (IgG)	100 (IgM) 99.2 (IgG)	100 (IgM) 96.4 (IgG)	93.2 (IgM) 98.4 (IgG)
Hou H et al., 2020	Retrospective	338 subjects	338 RNA-positive patients	China	Commercial Assay	YHLO, ELISA	Quantitative detection of IgM and IgG antibodies against SARS-CoV-2 quantitatively has potential significance for evaluating the severity and prognosis of COVID-19	82.7 (IgM) 88 (IgG)	NA	NA	NA
Imai K et al., 2020	Retrospective	139 samples from 112 patients and 48 controls	112 RNA-positive patients and 48 controls	Artron, Canada	Commercial assay	Artton, One Step IgM/IgG Rapid Test	Immunoassay had low sensitivity during the early phase of infection, and thus immunoassay alone is not recommended for initial diagnostic testing for COVID-19	40	NA	NA	NA
Lippi G et al., 2020	Prospective	48 patients	48 RNA-positive patients	Snibe Cehmiluminescence Maglumi, Italy Euroimmun, Italy	Commercial assays	Snibe, Chemiluminescence MAGLUMI; EUROIMMUN, ELISA	The results of MAGLUMI are well aligned with those of the EUROIMMUN test	10 (<5days) 100 (>10 days)	NA	NA	NA
Pan Y et al., 2020b	Retrospective	86 samples from 67 cases	67 RNA-positive patients	China	Commercial assay	Zhuhai Livzon Diagnostic, rapid lateral flow assays	Serology may be considered a supplementary approach in clinical diagnosis	11 (<7 days) 92 (7–14 days) 96 (>14 days)	NA	NA	NA
Spicuzza et al., 2020	Cross-sectional	41 subjects	27 RNA-positive patients, 7 symptomatic RNA-negative patients and 7 controls	China	Commercial assay	Beijing Diagreat Biotechnologies, rapid lateral flow assay	Antibody test is quite reliable and useful as it has the advantage of being a POC test that gives a response within minutes	83	93	NA	NA
Sun B et al., 2020	Cross-sectional	130 samples from 38 patients, 16 samples from 16 controls	38 RNA-positive patients and 16 controls	China	In-house assay	ELISA	IgM and IgG increased gradually after symptom onset and can be used for detection of SARS-CoV-2 infection. Analysis of the dynamics of IgG may help to predict prognosis	75 (after 1 week) 94.7 (after 2 weeks) 100 (after 3 weeks)	NA	NA	NA
To K et al., 2020	Cross-sectional	16 patients	16 RNA-positive patients	China	In-house assay	ELISA	Serological assay can complement RT-qPCR for diagnosis	88 (IgM) 94 (IgG)	NA	NA	NA
Xie J et al., 2020	Prospective	56 patients	56 symptomatic patients	China	Commercial assay	YHLO Biological Technology, chemiluminescence assay	A combination of nucleic acid and Ig testing is a more accurate approach for diagnosing COVID-19	93.7 (IgM) 100 (IgG)	NA	NA	NA
Yonh G et al., 2020	Retrospective	76 samples from 38 patients	38 symptomatic patients	China	Commercial assay	Rapid assay GICA kit	Antibody detection could be used as an effective indicator of the virus in the absence of viral RNA	50 (IgM) 92.1 (IgG)	NA	NA	NA
Bryan A et al., 2020	Cross-sectional	6001 subjects	1020 controls and 125 patients. 4856 subjects from the general population	USA	Commercial assay	Abbott, chemiluminescence SARS-CoV-2 IgG test	This study demonstrates excellent analytical performance of the Abbott SARS-CoV2 test as well as the limited circulation of the virus in the western USA	53.1 (day 7) 82.4 (day 10) 96.9 (day 14) 100 (day 17)	99.9	NA	NA

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Table 1 – (continued)

Author, Year	Design of the study	N	Population	Country of the test population	Antibody used	Methodology	Main findings and/or conclusions	Sensitivity (%)	Specificity (%)	PPV	NPV
Demey B et al., 2020	Prospective	21 subjects	21 RNA-positive patients	France	Commercial assays	Four rapid lateral flow assays	Immunochromatographic tests for detection of the virus may have a role in the diagnosis of COVID-19	9-24 (day 5) 67–82 (day 10) 100 (day 15)	99.8	NA	NA
Jaaskeilainen A et al., 2020	Retrospective	77 subjects	40 RNA-positive patients and 37 controls	Germany	Commercial assay	EUROIMMUN, ELISA	The median time after onset of symptoms was 12 days (13 patients, range 5–20 days) for detection of IgG and 11days (24 patients, range 5–20 days) for detection of IgA	NA	91.9 (IgG) 73 (IgA)	NA	NA
Montesinos J et al., 2020	Retrospective	400 subjects	272 controls and 128 RNA-positive patients	Germany	Commercial assays	MAGLUMI, chemiluminescence; EUROIMMUN, ELISA; rapid assay	The sensitivity of the tests increased with time from onset of symptoms	64.3 (MAGLUMI) 84.4 (EUROIMMUN) 70 (rapid assay)	99 100	NA	NA
Tang MS et al., 2020	Retrospective	201 subjects	48 patients and 153 controls	Abbott USA Euroimmun USA	Commercial assays	Abbott, chemiluminescence assay; EUROIMMUN, ELISA	Both assays have poor sensitivity during the first days of the disease. Abbott tests generally performed better than the EUROIMMUN test	Abbott: 0 (<3 days) 30 (3–7 days) 47.8 (8–13 days) 93.8 (>14 days) EUROIMMUN: 0 (<3 days) 25 (3–7 days) 56.5 (8–13 days) 85.4 (>14 days)	99.4 (Abbott)	NA	NA
MedRxiv articles											
Wang X et al., 2020	Prospective study with longitudinal follow-up	117 samples in 70 subjects	Inpatients and convalescent patients	China	In-house assay	Modified cytopathogenic assay	The seropositivity rate reached up to 100.0% within 20 days after onset. Patients with a worse clinical classification had a higher antibody titre	100	NA	NA	NA
Garcia PF et al., 2020	Prospective	163	55 RNA-positive patients, 63 RNA-negative patients, 45 controls	China	Commercial Assay	AllTest, COV 19 IgG IgM immunoassay	The sensitivity of the test was 73.9% after 2 weeks from the onset of symptoms	73.9	100	NA	NA
Lassauniere R et al., 2020	Cross-sectional	111	30 SARS-CoV-2 patients, 10 healthy controls, 71 patients with respiratory diseases other than SARS-CoV-2	Denmark	Commercial assays	3 ELISA tests and 6 POC lateral flow tests	The diagnostic performance of the commercial assays analysed may vary	65–90 (ELISA) 83–93 (POC)	96–100 (ELISA) 80–100 (POC)	82–100 (ELISA) 100 (POC)	89–98 (ELISA) 74–92 (POC)
Yangchun F, 2020	Cross-sectional	294	186 RNA-positive patients, 98 RNA-negative patients	China	Commercial assay	ELISA	Antibody testing has a very good diagnostic performance in identifying positive subjects	96.1 (IgG)	92.4 (IgG)	96.09 (IgG)	90.1 (IgG)

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Table 1 – (continued)

Author, Year	Design of the study	N	Population	Country of the test population	Antibody used	Methodology	Main findings and/or conclusions	Sensitivity (%)	Specificity (%)	PPV	NPV
Liu R et al., 2020	Retrospective	133	Samples from patients	China	Commercial Assay	YHLO, IG detection kit	In symptomatic patients, IgM was superior to RT-PCR in detecting affected subjects. The positive rate for IgM was 79.55% in moderate cases, 82.69% 156 in severe cases and 72.97% in critical cases. The IgG antibody test positive rate was 93.18% in moderate cases, 100.00% in severe cases and 97.30% in critical cases	78.95 (IgM) 93.18 (IgG)	NA	NA	NA
Liu Y et al., 2020	Retrospective	179	Patients, RNA positive (n = 90) and RNA negative (n = 89)	China	Commercial assay	Rapid immunoassay	The accuracy of the antibody testing increased over time (from 40% in the first week from onset of symptoms to 93.9% 2 weeks later)	85.6	91	95.1	82.7
Yong G et al., 2020	Retrospective	38	Patients	China	Commercial assay	Rapid assay GICA IgG IgM detection kit	The accuracy of the test 8 days after the onset of symptoms	50	92.1	NA	NA
Lin D et al., 2020	Retrospective	149	79 RNA-positive patients	China	Commercial assay	Darui Biotech, ELISA	The sensitivity of the test increased with time from onset of the disease	82.2	97.5	NA	NA
Lou B et al., 2020	Cross-sectional	380	80 RNA-positive patients. 300 healthy controls	China	Commercial assay	ELISA and lateral flow assay	The overall seroconversion rate was 98.8% at a median of 9 days from the onset of disease	98.8	94.3	NA	NA
Liu L et al., 2020	Cross-sectional	238	238 patients, 153 of them RNA-positive. 120 controls	China	Commercial assay	Lizhu, ELISA	Antibody detection should be used as a major viral diagnostic test for patients with symptoms for more than 10 days. The combination of ELISA and RT-PCR assays will greatly improve detection efficacy, even in the early stage of infection	81.5	NA	NA	NA
Bendavid E et al., 2020	Cross-sectional	3300	3300 subjects from the general population	China	Commercial	Premier Biotech, LFIA	The population prevalence of COVID-19 in Santa Clara, CA, ranged from 2.49% to 4.16%, 50- to 85-fold more than reported cases	80.3	99.5	NA	NA
Paradiso AV et al., 2020a	Prospective	191	191 symptomatic patients	China	Commercial	Rapid VivaDiag IgM /IgG immunoassay	The performance of the test at the onset of symptoms was low. Sensitivity was 66.7% 15 days later	30	89	NA	NA
Jia X et al., 2020	Retrospective	59	59 suspected patients, 24 of whom were RNA positive	China	Commercial assay	Diagreat, immunofluorescence assay	IgM and IgG may provide a quick, simple and accurate detection method for suspected COVID-19 patients	87.5	NA	NA	NA
Zhang J et al., 2020	Retrospective	736	228 suspected cases, 3 positive. 508 controls	China	Commercial assay	Shenzhen Yahuilong Biotechnology, chemiluminescence assay	Detection of specific antibodies in patients with fever can be a good complement to nucleic acid diagnosis for early diagnosis of suspected cases	100	97	75	100
Xiang J et al., 2020	Retrospective	189	154 patients, 35 controls	China	Commercial assays	Zhu Hai Liv Zon Diagnostics, ELISA and GICA assays	There is no difference between the sensitivity of ELISA and GICA assay; both are simple and fast, and the results can be used for clinical reference	87.3 (ELISA) 82.4 (GICA)	100 (ELISA) 100 (GICA)	NA	NA
Hu Q et al., 2020	Prospective	993 samples from 221 subjects	221 hospitalized patients	China	Commercial assay	BioScience, chemiluminescence assay	IgG and IgM antibodies examined every 3 days revealed increasing antibody levels that peaked on day 19–21. SARS-CoV-2 IgG and IgM antibody testing should be combined with RT-PCR as an early diagnosis method	73.6 IgM 97.8 IgG (day 13–18 after the onset)	NA	NA	NA

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Table 1 – (continued)

Author, Year	Design of the study	N	Population	Country of the test population	Antibody used	Methodology	Main findings and/or conclusions	Sensitivity (%)	Specificity (%)	PPV	NPV
Ma H et al., 2020	Cross-sectional	216 samples from 87 subjects	87 RNA-positive patients	China	In-house assay	Chemiluminescence	Measuring SARS-CoV-2 specific antibodies IgA, IgM and IgG in serum provides better serological testing with improved sensitivity and specificity	98.6 IgA 96.8 IgM 96.8 IgG	98.1 IgA 92.3 IgM 99.8 IgG	NA	NA
Qian C et al., 2020	Prospective, multicentric	2061 subjects from 10 hospitals	972 non-COVID patients, 586 controls, 503 RNA-positive patients	China	Commercial assay	Shenzhen YHLO Biotech, chemiluminescence assay	The assay showed a coefficient of variation of less than 5%. SARS-CoV-2 IgM and IgG showed clinical specificity of over 97% and 86.54% for suspected cases	85.8 IgM 96.6 IgG	99 IgM 99 IgG	NA	NA
National COVID testing Scientific Advisory Board, 2020	Cross-sectional	182	40 RNA-positive patients, 142 controls	UK	Commercial assays	Elisa and 9 commercial LFIA	The performance of current LFIA devices is inadequate for most individual patient applications. ELISA can be calibrated to be specific for detecting and quantifying SARSCoV-2 IgM and IgG and is highly sensitive for IgG from 10 days following symptom onset	85 (ELISA) 55-70 (LFIA versus RT-PCR)	100 (ELISA) 65–85 (LFIA versus ELISA)	NA	NA
Burbelo PD et al., 2020	Cross-sectional	100	68 patients, 32 controls	USA	In-house assay	Luciferase 44 immunoprecipitation assay systems to the nucleocapsid (NP) and spike proteins (SP)	Antibody to the nucleocapsid protein of SARS-CoV-2 is more sensitive than 56 spike protein antibody for detecting early infection	100 (anti-NP) 91 (anti-SP)	100 (anti-NP) 100 (anti-SP)	NA	NA
Adams ER et al., 2020	Retrospective	834 samples	270 positive samples, 564 negative samples	UK	Commercial assay	Mologic, ELISA	The ELISA tested had good diagnostic performance	88	97	NA	NA
Meyer B et al., 2020	Retrospective	357 subjects	176 controls, 181 RNA-positive patients	Germany	Commercial assay	EUROIMMUN, ELISA	The assay displays an optima diagnostic accuracy using IgG, with no obvious gain from IgA serology	82	100	100	46
Norman M et al., 2020	Retrospective	81 subjects	81 subjects	USA	In-house assay	Single Molecular Array (SIMOA)	The SIMOA serological platform provides a powerful analytical tool	86	100	NA	NA
Tuaillon E et al., 2020	Prospective	58	38 RNA-positive patients and 20 controls	Euroimmun, Germany IdVet, France	Commercial assay	Elisa by EUROIMMUN and IdVet and 5 rapid lateral flow tests	The second week of COVID-19 seems to be the best period for assessing the sensitivity of commercial serological assays	86.7 (ELISA) 80–93.3 (rapid tests)	80–85 (ELISA) 65–100 (rapid tests)	NA	NA
Wajnberg A et al., 2020	Prospective	1343 subjects	1343 symptomatic subjects, of whom 624 were RNA-positive	Roche, USA	Commercial assay	Roche, chemiluminescence assay	The vast majority of confirmed COVID-19 patients seroconvert, potentially providing immunity to reinfection	82	NA	NA	NA
Wan Y et al., 2020	Retrospective	180	50 RNA-positive patients and 130 controls	China	Commercial assay	Four chemiluminescence assay systems	Systems for CoVID-2019 IgM/IgG antibody test may perform differently	26–92	78–99	NA	NA

(continued on next page)

Table 1 – (continued)

Author, Year	Design of the study	N	Population	Country of the test population	Antibody used	Methodology	Main findings and/or conclusions	Sensitivity (%)	Specificity (%)	PPV	NPV
Xiao T et al., 2020	Retrospective	56 subjects	56 RNA-positive patients (33 symptomatic and 23 asymptomatic)	China	Commercial assay	Chemiluminescence microparticle immunoassay	Asymptomatic carriers were found to have a lower initial viral load, undetectable IgM and moderate levels of IgG	90.9 95.5 90.9 63.2	NA	NA	NA
Zhou Q et al., 2020	Retrospective	419 subjects	19 RNA-positive patients and 400 controls	China	Commercial assay	Chemiluminescence	Viral serological testing is an effective means of detecting SARS-CoV-2 infection	91.6	NA	NA	NA
Ozturk T et al., 2020	Cross-sectional	148 subjects	32 RNA-positive patients, 116 controls	USA	Commercial assay	GenScript, ELISA	There is a complex relationship between antibody levels, disease severity and time since symptom onset, so caution is needed in using serological assay to inform public policies	88.9	92.3	NA	NA
Rosado J et al., 2020	Retrospective	594	259 RNA-positive patients, 335 controls	France	In-house assay	Multiplex serological assay using a serological signature of IgG to four antigens	Serological signatures based on antibody responses to multiple antigens can provide more accurate and robust serological classification of individuals with previous SARS-CoV-2 infection	96.1	99.1	NA	NA

Searched up to 15 May 15. Case reports and review articles have not been included.

AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; GICA, gold immunochromatographic assay; Ig; LFIA, lateral flow immunoassay; NA, not available; NPV, negative predictive value; PPV, positive predictive value; qPCR, quantitative PRC; POC, point of care; ROC, receiver operating characteristic; RT-PCR, reverse transcription-PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

DIAGNOSTIC TESTS FOR THE SARS-COV-2

On 22 May 2019, the database held by the Foundation for Innovative New Diagnostics, which is the WHO Collaborating Centre for Laboratory Strengthening and Diagnostic Technology Evaluation, contained 560 SARS-CoV-2 laboratory tests for the diagnosis of COVID-19. These comprised 273 molecular assays and 287 immunoassays. Excluding those intended for research use only, 152 of these are molecular assays and 211 immunoassays are CE-marked for in-vitro diagnostic devices. There are principally two types of tests available for COVID-19: viral tests and antibody tests. The viral tests are direct tests as they are designed to detect the virus and therefore reflect current infection. In contrast, the antibody tests are indirect tests, as they do not detect the virus, but rather ascertain established seroconversion to previous infection, or early seroconversion to ongoing infection.

Direct tests

The recommended test for diagnosis of SARS-CoV-2 infection involves detection of viral RNA using nucleic acid amplification tests (NAAT), such as reverse transcription (RT)-PCR (www.ecdc.europa.eu). In areas with widespread community transmission of SARS-CoV-2 and when laboratory resources are limited, detection by RT-PCR of a single discriminatory target is considered sufficient. There are still, however, specific technical considerations for laboratory testing, including specimen collection (variable collection methods), which samples to collect (upper or lower respiratory tract biospecimens, or other samples), time of collection in relation to the course of disease, and the availability of different laboratory test methods and kits (not all of which may be standardized or approved by authorities such as the US Food and Drug Administration). Then there are infrastructure considerations: are the approved laboratory facilities and trained manpower available, can the methodology be rapidly scaled up, and how are test results interpreted and false negatives excluded?

These issues have been faced by the whole scientific community, with a collective response to develop guidance. The currently used protocol was developed and optimized for the detection of the novel CoV at the Charité University Hospital, Geneva, Switzerland in collaboration with several other laboratories in Germany, the Netherlands, China, France, the UK and Belgium (*Corman et al., 2020*). Additionally, the existing protocol was further optimized by the Centers for Disease Control (CDC) in the USA through the comprehensive comparison and validation of alternative kits available for nucleic acid extraction and the use of alternative probe and primer sets for efficient SARS-CoV-2 detection in clinical samples (www.cdc.gov/coronavirus). Similar approaches are being undertaken by other national authorities as they continue to scale up provision for laboratories not using CE-marked assays (www.england.nhs.uk/coronavirus/).

The importance and variability of specimen collection was initially highlighted from comparison of positive rates from pharyngeal, nasal, blood, sputum, faeces, urine and bronchoalveolar lavage fluid specimens and fibrobronchoscope brush biopsy of patients with confirmed COVID-19 (*Zou L et al., 2020*). At present the CDC recommend collecting and testing an upper respiratory specimen, with a nasopharyngeal specimen being the preferred choice for swab-based SARS-CoV-2 testing. When collection of a nasopharyngeal swab is not possible, the following are acceptable alternatives: an oropharyngeal specimen, a nasal mid-turbinate specimen (using a flocked tapered swab), an anterior nares (nasal swab) specimen (using a flocked or spun polyester swab) or a nasopharyngeal wash/aspirate or nasal aspirate specimen. For individuals having invasive procedures, lower respiratory tract specimens are also recommended if available. Although the virus can be detected in other specimens, such as blood and stools, these have been generally less reliable than respiratory specimens.

At present it is recommended that specimens should be collected as soon as possible once a decision has been made to pursue SARS-CoV-2 testing, regardless of the time of symptom onset. The viral load in throat swabs is greatest at the time of viral onset and decreases monotonically thereafter (*To et al., 2020*; *Zou L et al., 2020*). Analysis of these temporal dynamics suggests that viral shedding may begin 2–3 days before the appearance of the first symptoms, facilitating pre-symptomatic or asymptomatic transmission (*He et al., 2020*). CoV have a number of molecular targets within their +ssRNA genome that can be used for RT-PCR assays. The WHO has provided primers for the genes that encode the structural proteins of the viral envelope (E) and the nucleocapsid (N), and for the RNA-dependent RNA polymerase (RdRp), which is a key part of the virus's replication machinery that makes copies of its RNA genome (*Corman et al., 2020*). However, there has been no demonstration that any one of these three sequences (E, N or RdRP) might offer an advantage for clinical diagnostic testing, with different targets being preferred by different authorities. For example, the Public Health England assay employs two probes against RdRp; one is a Pan Sarbeco-probe that will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoV, while the second probe is specific to 2019-NCoV. Continued refinement of these NAAT assays is ongoing to facilitate their upscaling, while maintaining laboratory safety, a low cost and a high sensitivity (*Won et al., 2020*).

Detection of isolated viral antigens

Great efforts have been made in order to develop tests for the rapid detection of SARS-CoV-2 antigens. Antigen detection tests are designed to directly detect viral particles in biological samples such as nasopharyngeal secretions. Several rapid antigen tests have been proposed (*Diao et al., 2020*); however, the principal concerns are the false-negative rate due to either a low or variable viral load, and the variability in sampling, the latter having the potential to further compound the problem in cases with low viral titres, thereby increasing the false-negative rate (*Tang YW et al., 2020*).

Diao and colleagues (*Diao et al., 2020*) have reported preliminary results from the use of a fluorescence immunochromatographic assay for detecting the N protein of SARS-CoV-2 in both nasopharyngeal swab samples and urine from 239 participants, with comparison to NAAT testing where the intersection of the amplification curve and diagnostic threshold line (cycle threshold [Ct] value) was set at either ≤30 or ≤40 (*Diao et al., 2020*). With a higher viral load in the sample, the prespecified Ct value may be lower, as fewer replication cycles are required to achieve a detectable signal; however, with a low viral load, a greater number of replication cycles (higher Ct value) will be required for a detectable signal to be

attained. For this assay with a prevalence of 87%, although the positive predictive value (PPV) was 100%, the negative predictive value (NPV) was 32% for a Ct ≤40, increasing to 97% for patients with a higher viral load as demonstrated by a Ct ≤30. This would suggest that, at present, this assay would only be useful in excluding those with high viral loads. Whether alternative approaches as previously suggested for influenza viruses in children including the use of colloidal gold-labelled immunoglobulin (Ig) G as the detection reagent (*Li et al., 2020*), to increase the sensitivity of rapid antigen tests for respiratory viruses, are feasible is still under consideration, with monoclonal antibodies specifically against SARS-CoV-2 under development. Further validation of this technique and similar approaches in larger populations including asymptomatic cases is warranted. Consideration of approaches to try to concentrate antigen and amplify the detection phase are, however, likely to be needed for these methods to have any clinical utility (*Loeffelholz et al., 2020*).

At the time of writing (25 April 2020), the non-governmental organization Foundation for Innovative New Diagnostics (https://www.finddx.org/) has listed four CE-marked rapid SARS-CoV-2 antigen detection tests, which are primarily lateral flow immunochromatographic assays based on the presence of a colloid gold conjugate pad and a membrane strip pre-coated with antibodies specific to SARS-CoV-2 antigens on a test line. If SARS-CoV-2 antigens are present in the specimen withdrawn from a nasopharyngeal swab, a visible band appears on the test line as antibody–antigen–antibody gold conjugate complex forms. The evaluation of these diagnostic tests has, however, been limited, and their CE mark means that the manufacturers state that they conform with the relevant EU legislation, but they may still not be available to purchase. According to European Union Directive 98/79/EC for in-vitro diagnostic devices, in order to affix the CE mark to COVID-19 diagnostic devices to be used by health professionals, the manufacturer has to specify device performance characteristics and self-declare conformity with the safety and performance requirements listed in the Directive. In addition, self-tests intended to be used by patients themselves must also be assessed by a third-party body (a notified body), which for these tests has yet to happen.

Although direct antigen tests are being registered by several health authorities, the sensitivity of these tests is lower than that of RT-PCR, with previous antigen-detecting enzyme-linked immunosorbent assays (ELISA) developed for SARS-CoV having limits of detection of 50 pg/ml (*Che et al., 2004*; *Di et al., 2005*). Furthermore, clarification of their specificity for SARS-CoV-2 is awaited, given the potential for cross-reaction with other human CoV. Despite these limitations, the chief advantages of antigen tests, including their rapidity (10–30 min compared with hours for NAAT testing), ease of interpretation and limited technical skill and infrastructure required compared with NAAT-based testing, continue to make them worth pursuing. However, experience with influenza antigen testing invites caution as these tests may have low sensitivity and specificity; moreover, as noted, false–negatives rate will be critical (*Tang YW et al., 2020*). Their greatest utility if they come to fruition may be in symptomatic patients, when the viral load will be at its greatest, to enable accurate triage.

Building an indirect test for SARS-CoV-2: serological testing

In contrast to NAAT-based testing, where as soon as the sequence is known, a diagnostic test can be built, the diagnostic technology and methodology underlying the development of serological tests is quite different, with a substantially longer timeline to obtain a robust product that is suitable for routine deployment. The principal difference is that antibody tests require identification of distinct proteins that form the viral coat, with elucidation of which proteins are most divergent from previous CoV proteins, then identification of specific antibodies to these proteins that are part of the acquired immune response to viral exposure, and finally testing to ensure that there is limited cross-reactivity with antibodies developed to other historical CoV.

With the previous two CoV, a variety of assays encompassing different methodologies were developed, including ELISA, chemiluminescence, western blotting, protein microarray and immunofluorescence platforms. However, only ELISA and chemiluminescence were deemed suitable for clinical application because of costs, time-to-results, relative simplicity and ability to scale to very large throughput. It is these platforms that are once again being examined for detection of antibodies to SARS-CoV-2.

Appraisal of test performance

Appropriate thresholds for sensitivity and specificity of an antibody test depend on its purpose and must be considered prior to implementation. For diagnosis in symptomatic patients, high sensitivity is required (generally ≥90%). In this context, a slight reduction in specificity may be acceptable as some false-positive results may be tolerated, provided other potential diagnoses are considered and there is acceptance that overdiagnosis may result in unnecessary interventions that, for SARS-CoV-2, may include quarantining. However, if antibody tests were deployed as an individual-level approach to inform release from social isolation and return to normal activities, high specificity would be essential, as false-positive results would return non-immune individuals to risk of exposure. It is with these purposes in mind that the UK Medicines and Healthcare products Regulatory Agency set a minimum 98% specificity threshold for flow immunoassays (LFIA). This is particularly challenging, particularly given the scale of validation study required for a suitable candidate LFIA, because to demonstrate a high specificity if the true underlying value were 98%, 1000 negative controls would be required to estimate the specificity of an assay to ±1% with approximately 90% power.

As part of the evaluation of test performance, the influence of population prevalence also needs to be considered, acknowledging that this is at present rapidly changing (*Brenner and Gefeller, 1997*). This can be considered as the proportion of all positive tests that are wrong, as well as the number of incorrect positive tests per 1000 people tested. For example, for a point of care (POC) test with 70% sensitivity and 98% specificity, the proportion of positive tests that are wrong is 35% at 5% population seroprevalence (19 false positives/1000 tested), 13% at 20% seroprevalence (16 false positives/1000 tested) and 3% at 50% seroprevalence (10 false positives/1000 tested).

According to available data, the prevalence of seropositivity is still low. The prevalence of antibodies to SARS-CoV-2 among a high risk category such as healthcare personnel is 5.9% in Utah,

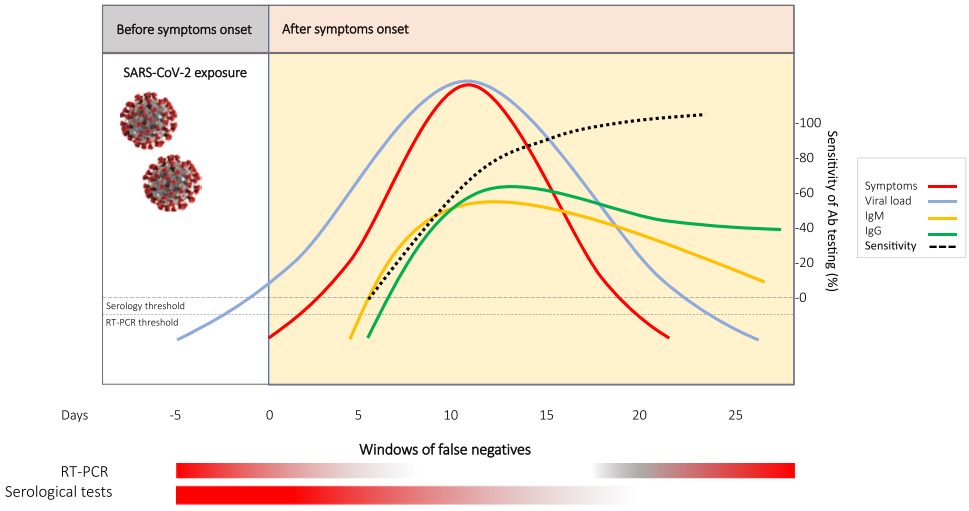

FIGURE 1 The time relationship between viral load, symptoms and positivity on diagnostic tests. The onset of symptoms (day 0) is usually 5 days after infection (day –5). At this early stage corresponding to the window or asymptomatic period, the viral load could be below the RT-PCR threshold and the test may give false-negative results. The same is true at the end of the disease, when the patient is recovering. Seroconversion may usually be detectable between 5–7 days and 14 days after the onset of symptoms; therefore, in the first phase of the disease, the serological tests are more likely to give false-negative results. The dotted black line in the graph illustrates the sensitivity of the chemiluminescent assay as derived from the data sheet of a commercial test (Abbott Diagnostics, USA). Ig, immunoglobulin; RT-PCR, reverse transcription-PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

USA (*Masden et al., 2020*), 5.4% in Lyon, France (*Solodky et al., 2020*), 17.3% in Trieste (*Comar et al., 2020*), 5.25% in Padua (*Tosato et al. 2020*) and 1.5% in Bari, Italy (*Paradiso et al., 2020a*), 1.6% in Germany (*Korth et al., 2020*) and 2.6% in Barcelona, Spain (*Tuaillon et al., 2020*). In the general population it has been reported as being 0.13% in Rio Grand do Sul, Brazil (*Silveira et al., 2020*), 1.5% in Santa Clara, California (*Benavid et al. 2020*), 1.79 % in Idaho (*Bryan et al., 2020*) and 7.1% in Atlanta, USA (*Zou J et al., 2020*), 1.2% in Edinburgh, Scotland (*Thompson et al., 2020*), 3% in Paris, France (*Grzelak et al., 2020*), 1.7% in Denmark (*Erikstrup et al., 2020*), 3.3% in Kobe, Japan (*Doi et al., 2020*), 9.6% in Wuhan, China (*Wu et al., 2020*) and 21% in Guilan, Iran (*Shakiba et al., 2020*). Large-scale seroprevalence studies are ongoing, but understanding the background rate is essential for accurate interpretation of diagnostic tests.

The potential risk of a test providing false reassurance and release from being sheltered for non-immune individuals can therefore be widely based on the underlying seroprevalence, and this still assumes antibody positivity as a correlate of protective immunity, which may be incorrect.

Dynamics of seroconversion

Understanding viral and host interactions during the acute and convalescent phases is critical to be able to understand both the timing of initial seroconversion after exposure to SARS-CoV-2 and the subsequent duration of antibodies. However, at present the studies regarding seroconversion are being developed in parallel to the assays, limiting some conclusions. The data do suggest that seroconversion after exposure to SARS-CoV-2 is very similar to that after other acute viral infections, with IgG concentration beginning to rise as IgM concentrations reach a plateau (FIGURE 1). However, observations have shown that IgM and IgA growth is relatively slow related to other respiratory viruses, which has been suggested to contribute to the heterogeneous pathogenicity of SARS-CoV-2 in COVID-19 patients (*Zhao J et al., 2020*).

The most comprehensive study to date of seroconversion assessed 173 patients affected by COVID-19 using an assay developed to detect antibodies against the receptor binding domain of the S protein of SARS-CoV-2 (*Zhao J et al., 2020*). The median seroconversion times of total antibody, IgM and IgG were 11, 12 and 14 days (*Zhao J et al., 2020*). The respective seroconversion rates for total antibody, IgM and IgG were 93.1%, 82.7% and 64.7% (*Zhao J et al., 2020*), with the cumulative seroconversion curve suggesting that the rate for total antibody and IgM reached 100% 30 days after the onset. These studies have also highlighted the temporal nature of testing as, despite all patients subsequently being confirmed as COVID-19 positive, in the early phase of illness (within 7 days since the onset) the NAAT test only exhibited 66.7% sensitivity, with the antibody assays having an even lower positive rate of 38.3% (*Zhao J et al., 2020*). However, the sensitivity of antibody overtook that of RNA testing from day 8 after symptom onset and reached over 90% across day 12 after onset. Among samples from patients in the later phase (day 15–39 after onset), the sensitivities for total antibody, IgM and IgG were 100.0%, 94.3% and 79.8%, respectively. In contrast, RNA was detectable in only 45.5% of samples from days 15–39. In a separate small series of nine cases, seroconversion occurred after 7 days in 50% of patients (after 14 days in all) but was not followed by a rapid decline in viral load (*Wolfel et al., 2020*). An analysis of 285 patients further supports IgG seroconversion within 19 days after symptom onset (*Long et al., 2020*). Collectively, these data suggest that there is a role for both tests depending on where the patient is on their infection journey, with the combined use of NAAT and antibody tests markedly improving the sensitivity of a pathogenic diagnosis for COVID-19 patients in different phases of the illness.

With respect to antibody titres and disease severity, critically ill hospitalized patients have been reported to exhibit

significantly higher antibody titre values than non-critical patients in some (*Long et al., 2020*; *Zhao J et al., 2020*) but not all studies. In the previous epidemics of SARS-CoV and MERS-CoV, antibody titres were positively associated with disease severity (*Choe et al., 2017*; *Okba et al., 2019*). In a limited case series (n = 57 confirmed SARS-CoV-2 cases), six patients with detectable viral RNA in the blood were at increased risk of severe disease progression compared with those with low titres, but unfortunately the authors did not measure antibody titres (*Chen W et al., 2020b*). Clarification is awaited of whether even in previously healthy individuals a high viral titre and/or high antibody titre can predict disease severity and likely progression.

Diagnostic performance of the immunoassays

Our extensive search identified 24 peer-reviewed articles (*Xiao DAT et al., 2020*; *Zhao J et al., 2020*; *Du Z et al., 2020*; *Guo L et al., 2020*; *Jin Y et al., 2020*; *Pan Y et al., 2020*; *Padoan A et al., 2020*; *Zhong L et al., 2020*; *Infantino M et al., 2020*; *Xiang F et al., 2020*; *Long QX et al., 2020*; *Perera R et al., 2020*; *Qu J et al., 2020*; *Zhao R et al., 2020*; *Cai X et al., 2020*; *Hou H et al., 2020*; *Lippi G et al., 2020*; *Sun B et al., 2020*; *To K et al., 2020*; *Xie J et al., 2020*; *Bryan A et al., 2020*; *Jaaskeilanen A et al., 2020*; *Montesinos I et al., 2020*; *Tang MS et al., 2020*) and 25 pre-print studies (*Wang X et al., 2020*; *Lassauniere R et al., 2020*; *Yangchun F, 2020*; *Liu R et al., 2020*; *Lin D et al., 2020*; *Lou B et al., 2020*; *Liu L et al., 2020*; *Jia X et al., 2020*; *Zhang J et al., 2020*; *Xiang J et al., 2020*; *Hu Q et al., 2020*; *Ma H et al., 2020*; *Qian C et al., 2020*; *National COVID Testing Scientific Advisory Panel, 2020*; *Burbelo PD et al., 2020*; *Adams ER et al., 2020*; *Meyer B et al., 2020*; *Norman M et al., 2020*; *Tuaillon E et al., 2020*; *Wajnberg A et al., 2020*; *Wan Y et al., 2020*; *Xiao T et al., 2020*; *Zhou Q et al., 2020*; *Ozturk T et al., 2020*; *Rosado J et al., 2020*) reporting on the sensitivity and specificity of immunoassays for COVID-19, with a sample size ranging from 16 to 6001 subjects (**TABLE 1**). Most studies were conducted in China, with only a few coming from western countries.

Overall sensitivity ranged from 0% to 100% and specificity from 78% to 100%, with performance highly time-sensitive, reflecting the dynamics of seroconversion. In general, most assays performed better shortly after initial symptom resolution, accepting the very limited time frames evaluated for all studies to date. In an evaluation of nine commercially available SARS-CoV-2 immunoassays, the sensitivities varied with the duration of disease: early phase, 7–13 days after the onset of disease symptoms (sensitivities 40–86%); middle phase, 14–20 days after the onset of disease symptoms (sensitivities 67–100%); and late phase, ≥21 days after the onset of disease symptoms (sensitivities 78–89%) (*Lassauniere et al., 2020*).

The range of assays being released is extensive, with apparently very limited validation. Gonzalez and colleagues reviewed four web databases for SARS-CoV-2 immunoassay, and by 4 April 2020, 226 immunoassays from 20 different countries had already been listed. The technical data sheet was available online for only 22% of the tests, and despite 23 claiming regulatory certification only four had PubMed-listed papers (*González JM et al., 2020*). Despite wide claims for sensitivity and specificity, practically at present it is almost impossible to conclude which antibody test would be the one to use. A pragmatic choice would be to use an automated immunoassay that is scalable, from a well-known established manufacturer, with a complete and clear technical data sheet, which has received regulatory certification issued by the health authority and been independently validated.

In accordance with this, the most recent novel assays use fully automated chemiluminescence immunoassays implemented on high-throughput laboratory instrumentation. These systems include the MAGLUMI 2000 Plus 2019-nCov IgM and IgG assays (Snibe, China), which has been independently validated in accordance with the Clinical and Laboratory Standards Institute EP15-A3 guideline (*Padoan et al. 2020*) and the CE-marked Euroimmun, Italy Anti-SARS-CoV-2 IgA and IgG assays, with others, including from Beckman Coulter, Italy for their Access platform and Roche Diagnostics, Italy for their Elecsys platform, under development. However, in independent validation the EUROIMMUN assay exhibited some cross-reactivity in both ELISA with serum samples from the two seasonal CoV patients (HCoV-OC43) who had previously cross-reacted with the MERS-CoV S1 IgG ELISA (*Okba et al., 2019*). On comparison of their respective performances for 131 known cases, there was concordance for the IgG assays of only 88% (kappa statistic 0.47; 95% confidence interval [CI] 0.26–0.68). Despite involving different immunoglobulin classes, an analogous analysis between MAGLUMI 2019-nCoV IgM positive/negative and EUROIMMUN Anti-SARS-CoV-2 IgA positive/negative results yielded an overall concordance of 90% (kappa statistic 0.39; 95% CI 0.14–0.65). The IgG assays also exhibited different concordance during the early phases of symptom onset, with concordance improving 10–21 days after symptom onset. Further studies with longer timelines and known cases with a range of symptoms will help confirm the alignment of these assays. Inevitably, it is anticipated that there will be an enormous number of studies comparing the available assays, with the advantages and disadvantages of the respective assays being discussed at length.

Rapid serological tests

POC immunoassays have also been developed for the rapid detection of SARS-CoV-2 antibodies (IgG and IgM). The primary advantage of these tests, as with a home pregnancy test, is being able to obtain a diagnosis without sending samples to centralized laboratories. This enables communities without the necessary laboratory infrastructure to detect SARS-CoV-2-exposed subjects using only finger prick testing rather than formal blood draws, thereby reducing training requirements and allowing clinicians to have a validated test at the bedside. As these devices are cheap to manufacture, store and distribute, and provided that a positive antibody test were confirmed to be an accurate surrogate for immunity to infection, they would also be able inform decision making. This would particularly be the case as secure confirmation of antibody status would reduce anxiety, provide confidence to allow individuals to relax social distancing measures, and guide policy makers in the staged release of population lockdown, potentially in tandem with digital approaches to contact tracing.

The rapid POC immunoassays are generally LFIA (*Li et al., 2020*). In lateral flow assays, a membrane strip is coated with two lines: gold nanoparticle–antibody conjugates are located on one line and bind antibodies on the other. The blood

sample from the patient is put on the membrane, and the proteins are drawn through the membrane strip by capillary action. As it passes the first line, the antigen binds to the gold nanoparticle–antibody conjugate, and the complex flows across the membrane. Generally, rapid assays have a low diagnostic performance compared with ELISA assays, which is explained not only by the well-known technical differences between the two methodologies, but also by possible low antibody concentrations that may further contribute to the false-negative results observed with the rapid tests.

At present, 12 peer-reviewed articles (*Li Z et al., 2020*; *Cassaniti I et al., 2020*; *Lee YL, et al., 2020*; *Shen B et al., 2020*; *Dohla M et al., 2020*; *Hoffman T et al., 2020*; *Imai K et al., 2020*; *Pan Y et al., 2020b*; *Spicuzza et al., 2020*; *Yong G et al., 2020*; *Demey B et al., 2020*; *Montesinos I et al., 2020*) and 9 pre-print studies (*Garcia FP et al., 2020*; *Lassauniere R et al., 2020*; *Liu Y et al., 2020*; *Yong G et al., 2020*; *Lou B et al., 2020*; *Bendavid E et al., 2020*; *Paradiso AV et al., 2020a*; National COVID testing Scientific Advisory Board, 2020; *Tuaillon E et al., 2020*) have reported on the diagnostic performance of rapid assays (summarized in TABLE 1).

In the published studies, sensitivity and specificity ranged from 9% to 88.6% and from 88.9% to 91.7%, respectively (TABLE 1), while in the pre-print articles sensitivity and specificity ranged from 30% to 98.8% and from 89% to 100%, respectively. Of note the sensitivity of these tests performed in countries other than China were substantially lower than those reported for studies conducted in China. Extensive evaluation of manufacturers' claims related to the performance of these tests and optimal timing will be required before they are suitable for widespread routine clinical use. For example, the performance of the VivaDiag, VivaCheck Biotech, China COVID-19 IgM/IgG Rapid Test was evaluated in 30 cases 7 days after confirmed NAAT testing, and despite this five (16.7%) cases were negative for both IgG and IgM (*Cassaniti et al., 2020*). Furthermore, when evaluating 50 patients with acute illness presenting in the emergency room, of whom 38 were positive on RT-PCR, the sensitivity of the VivaDiag COVID-19 IgM/IgG Rapid Test was only 18.4%, its specificity was 91.7%, while the NPV was 26.2% and the PPV was 87.5% (*Cassaniti et al., 2020*). The same VivaDiag test was evaluated in 525 healthcare workers in Italy, with only six testing positive; none was positive by NAAT testing or symptomatic, and only three had a confirmed positive result on the MAGLUMI chemiluminescence IgG assay (*Paradiso et al., 2020b*). Evaluation of six POC tests in a mix of 111 patients with COVID-19, other CoV or other viruses and negative controls revealed sensitivities ranging from 83% to 93% and NPV of 74–92% (*Lassauniere et al., 2020*). In keeping with other studies, the diagnostic performance of these tests reflected the duration of the illness, with the worst performance observed in the first 2 weeks after symptom onset (*Lassauniere et al., 2020*). Finally, formal evaluation of nine commercially available LFIA in a case-control mix of 182 samples revealed sensitives of 55–70% (*National COVID Testing Scientific Advisory Panel, 2020*).

For all studies to date, sample size has been limited, and further testing across a large diverse population from a range of geographical locations and ethnic groups is required, with inclusion of children and individuals with autoimmune disease and immunosuppression. With extensive evaluation, it is likely that technical performance may deteriorate. At present, evaluation of the current LFIA devices suggest that although they may provide some information for population-level surveys, their performance is inadequate for most individual patient applications.

Clinical interpretation of the COVID-19 tests

The interpretation of a test for SARS-CoV-2 will depend on a combination of the accuracy of the test and the estimated risk of COVID-19 prior to performing the test (*Watson et al., 2020*). A positive direct antigen test and specifically NAAT is strongly suggestive of current infection due to its high specificity but moderate sensitivity, and the patient can be reassured that the clinician is confident that they have COVID-19 and should be managed in accordance with local policies regarding positive cases. In contrast, negative tests need to be interpreted with caution, and a single negative SARS-CoV-2 test in a patient with strongly suggestive symptoms should not be relied upon to exclude COVID-19. In this situation, it would still be safer for the patient to be treated as a positive case, and local policies regarding re-testing and isolation to be followed. For the serological tests, the clinical implication of seroconversion with respect to future immunity continues to be elucidated, but similar principles for evaluating the test result in the clinical context and history of previous infection or exposure is critical, particularly as a false-positive result could lead to false reassurance and inappropriate behaviour that might enhance community disease transmission.

CONCLUSIONS

At present, NAAT based methodologies remain the cornerstone of in-vitro diagnostic assays for SARS-CoV-2. There is an urgent need for the development of serological assays with high sensitivity for screening and adequate specificity to avoid unnecessary interventions, and confirmation that seropositivity equates to immunity. At present, none of the POC diagnostic tests for SARS-CoV-2 appears suitable for wide-scale deployment, and large prospective studies are urgently needed to clarify their utility. Evaluation of the performance of the potentially scalable high-throughput immunoassays is ongoing, but extensive validation across different populations will be required before they can be routinely used to inform critical decision making for clinicians, the public health community and policy makers.

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DECLARATION

ALM has participated in Advisory Boards and received speaker's and consultancy fees from Beckman Coulter, Gedeon Richeter, Ferring, IBSA, Merck, MSD, Roche Diagnostics and Theramex. SMN has participated in Advisory Boards and received speaker's and consultancy fees from Access Fertility, Beckman Coulter, Ferring, Finox, Merck, MSD, Roche Diagnostics and The Fertility Partnership. The other authors report no financial or commercial conflicts of interest.

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