Towards effective diagnostic assays for COVID-19: a review

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ABSTRACT

Countries globally are affected by the COVID-19 pandemic, with nearly two million cases and 120 000 deaths occurring within 4 months of the discovery of the severe acute respiratory syndrome coronavirus-2 in December 2019 in China. Accurate diagnoses of cases is key in managing the pandemic by identification, isolation and treatment of patients and defining the epidemiology of the virus. By mid-January 2020, a scientist from China published the full genome of the virus, which facilitated the development of accurate molecular diagnostic assays. By the end of January 2020, the WHO, in collaboration with laboratories in Asia, Europe and the USA, published several real-time reverse transcriptase PCR (rtRT-PCR) protocols that allowed identification of cases and development of commercial assays. Clinical investigations facilitated development of accurate case definition and guidance for laboratories on the optimum specimens and procedures for diagnoses. Currently, laboratory-based rtRT-PCR is the recommended test for diagnoses of acute cases to ensure patients can be identified and isolated and to facilitate the public health response. However, due to delays in diagnoses, severe shortage of tests and laboratory capacity, point-of-care molecular or antigen tests are becoming more attractive. Although serological tests are not suitable for diagnoses of acute cases, they are important to define epidemiological questions, including attack rate in the population, and to identify immune individuals. This review aimed to summarise the current available information for diagnoses of cases and to aid laboratories and healthcare workers to select the best assays and procedures.

INTRODUCTION

Within 3 months of its emergence from Wuhan City, Hubei Province, China, a novel coronavirus,[1] now named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative agent of COVID-19,[2] has spread to all continents and has been declared a global pandemic.[3] By the end of March, the outbreak in China has started to decline; however, Europe and the USA has now exceeded the Western Pacific in new cases and deaths. To date, the least cases has been reported on the African continent, although most countries have now detected cases; the numbers are increasing daily; and several countries implemented emergency measures, including total lockdown to try to curb the spread of the virus. Accurate diagnosis is key in controlling the pandemic and in understanding the epidemiology of the disease. In this review, we aimed to summarise the available information for diagnoses of COVID-19 for laboratories as well as clinicians, including the recommendations from the WHO, available scientific data and references to available test options.

SARS-COV-2 VIROLOGY

The coronaviruses are enveloped positive-strand RNA viruses in the family Coronaviridae, suborder Cornidovirineae, order Nidovirales. the coronaviruses can be divided into four genera α-CoV/β-CoV/γ-CoV/δ-CoV with the α-CoV and β-CoV being able to infect mammals, while γ-CoV and δ-CoV tend to infect birds. The coronaviruses have similar gene organisations and genome expressions with 16 non-structural proteins (nsp1 through nsp16), encoded by open reading frame (ORF) 1 a/b at the 5′ end, followed by the structural proteins spike (S), envelope, membrane and nucleocapsid (N), encoded by ORFs at the 3′ end. The beta-CoV genus is divided into four lineages (A, B, C and D). Lineage A viruses also encode a smaller protein called haemagglutinin esterase, which is functionally similar to the S protein.[4] Based on phylogenetic analysis of the full genome, SARS-CoV-2 clusters within the genus *Betacoronavirus* subgenus *Sarbecovirus*, lineage B, and is closest to the severe acute respiratory syndrome (SARS)-related coronaviruses, which include human and bat isolates of SARS-CoV.[2 5] Ten genome sequences of SARS-CoV-2 from nine patients initially identified in China exhibited more than 99.98% sequence identity and 88% identity to two bat-derived SARS-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in Zhoushan, Eastern China in 2018. It was more distant to SARS virus (2002) (SARS-CoV) (~79%) (also lineage B) and Middle Eastern severe acute respiratory syndrome coronavirus (MERS-CoV) (~50%) (lineage C). Homology modelling suggested that SARS-CoV-2 had a receptor-binding domain structure similar to that of SARS-CoV, the ACE 2 receptor in humans. The authors postulated that, although bats might have been the original host of this virus, an intermediate host may have facilitated the emergence of the virus in humans.[5] The *Betacoronavirus* genus also contains several other coronaviruses of bat origin, as well as the human coronaviruses HKU1 and OC43 (lineage A). The other human coronaviruses NL63 and 229E cluster with the alphacoronaviruses. The coronaviruses have a moderate to high mutation rate with a large RNA genome that is prone to plasticity in genome modification by mutations and recombinations, increasing the probability for intraspecies variability and interspecies host jump, which allow novel CoVs to emerge under the right conditions.[4] The recognition of a several new coronaviruses

since the discovery of SARS-CoV has led to the development of several pancoronavirus PCRs, as well as specific PCRs for specific identification of the human viruses.[6 7] SARS-CoV-2 was initially identified using next-generation sequencing.[8 9] Availability of the whole genome sequence by January 2020 allowed for the development of initial specific diagnostic tests[5] for the new virus, as well as subsequent protocols by reference laboratories and commercial assays that will be discussed further.

COVID-19: clinical presentation and risk groups

Although the initial cases of COVID-19 were linked to probable zoonotic transmission at a seafood market in Wuhan,[10] expansion of the outbreak despite the closure of the seafood market suggested that the subsequent outbreak was due to human-to-human transmission with animals playing limited to no further role in the global spread. Analysis of 44 672 cases diagnosed based on positive viral nucleic acid test result on throat swab samples from China helped to define the presentation of the disease.[11] According to this, most cases were mild (81%; ie, non-pneumonia and mild pneumonia); 14% were severe (presenting with dyspnoea, respiratory frequency ≥30 breaths/min, blood oxygen saturation≤93%, partial pressure of arterial oxygen to fraction of inspired oxygen ratio<300 and/or lung infiltrates>50% within 24–48 hours); and 5% were critical (presenting with respiratory failure, septic shock, and/or multiple organ dysfunction or failure). Asymptomatic infection was also identified in 889 people (1%). The case fatality rate was 2.3% but differed significantly between age groups. No deaths occurred in the group aged 9 years and younger, while among patients aged ≥80 years, 14.8% died, and in patients aged 70–79 years, 8.0% died. The case fatality rate was also elevated among those with pre-existing comorbid conditions, in particular cardiovascular disease, diabetes, chronic respiratory disease, hypertension and cancer. By April 2020 the global mortality ratio is 5.9% according to WHO-reported cases but differs significantly between countries, depending on the criteria used for and the amount of testing done.[12 13] Recently, a study of hospitalised cases in New York also suggested obesity to be a high risk for severe disease.[14]

WHO case definition

In order to decide if a patient should be tested, WHO published case definitions for surveillance but encouraged countries to adapt this depending on their local epidemiological situation and other factors.[15] According to this, a *suspect case* is (1) a patient with acute respiratory illness (fever and at least one sign/symptom of respiratory disease, eg, cough, shortness of breath) and a history of travel to or residence in a location reporting community transmission of COVID-19 disease during the 14 days prior to symptom onset; or (2) a patient with any acute respiratory illness and having been in contact with a confirmed or probable COVID-19 case in the last 14 days prior to symptom onset; or (3) a patient with severe acute respiratory illness (fever and at least one sign/symptom of respiratory disease, eg, cough, shortness of breath, and requiring hospitalisation) and in the absence of an alternative diagnosis that fully explains the clinical presentation.

Probable case

A probable case is a suspect case for whom testing for the COVID-19 virus is inconclusive (1) or a suspect case for whom testing could not be performed for any reason (2).

Confirmed case

A confirmed case is a person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technicalguidance/laboratory-guidance).

Contact

A contact is a person who experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case:

1. Face-to-face contact with a probable or confirmed case within 1 m and for more than 15 min.
2. Direct physical contact with a probable or confirmed case.
3. Direct care for a patient with probable or confirmed COVID-19 disease without using proper personal protective equipment.

2 or 4. For confirmed asymptomatic cases, the period of contact is measured as the 2 days before through the 14 days after the date on which the sample was taken, which led to confirmation.

Diagnostic testing guidelines

Accurate diagnostic tests are key in identification of cases but also of contacts who need to be quarantined and guided on epidemiological questions around the virus. Selection of the relevant specimen and knowledge of the incubation period, viraemia and shedding period are important in diagnosing individual cases and defining transmissibility to inform policies on isolation periods for patients.

The WHO has published testing guidelines that are being updated as necessary in two documents that are of importance,[16 17] which can be found online (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance).

According to these guidelines, clinical and epidemiological factors linked to an assessment of the likelihood of infection should guide the decision to test but should be adapted to the local situation and national policy for testing. Asymptomatic or mildly symptomatic contacts can be considered for individuals who have had contact with a COVID-19 case. Nucleic acid testing is recommended for diagnosis of acute cases. Serological assays have an important role in epidemiological questions, including determining the attack rate and establishing immunity of individuals who have recovered, but are not relevant for diagnoses of acute cases.

Currently, biosafety level (BSL)-2 conditions are recommended for handling of specimens for molecular testing, while attempts to culture the virus requires BSL-3 facilities at a minimum. Guidance for the use of PPE and infection prevention and control is provided by the WHO.[18]

Type of specimen and sampling period

The recommended specimens for diagnoses of acute cases for reverse transcriptase PCR (rtRT-PCR) are upper or lower respiratory track specimens: nasopharyngeal (NP) and oropharyngeal (OP) swabs or wash in ambulatory patients and/or sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease or lung tissue postmortem. Healthcare workers should take respiratory precautions when performing aerosol-generating procedures. Specimens for nucleic acid testing should be collected on presentation. Testing of specimens from multiple sites (eg, upper and lower respiratory tracts) may improve the sensitivity of the RT-PCR and reduce false-negative test results, especially

in the second week of illness.[3] Repeated sampling may be useful to monitor clearance but is not essential for all deisolation protocols.[19]

All specimens should preferably be collected in virus transport media (if available) and transported on ice to reach the lab as soon as possible. Specimens may be frozen at −20°C or ideally −80°C and shipped on dry ice if there is going to be a delay in reaching the lab, but repeat freeze thawing should be avoided. The WHO documents the summary of the optimum sample collection procedures, which is similar to those for influenza. Dacron or polyester flocked swabs are recommended for OP/NP swabs and sterile sample collection containers for washes or bronchoalveolar lavage fluid (BAL) specimens, urine or stool. For whole blood, EDTA tubes should be used, and for serum, separator tubes should be used.[16]

A recent study described the findings of 1070 clinical samples collected from 205 patients with COVID-19 in China. The study group had a mean age of 44 years (range 5–67 years) and mostly presented with fever, dry cough and fatigue, while 19% of patients had severe illness. BAL specimens showed the highest positive rates (14/15, 93%), followed by sputum (72/104, 72%), nasal swabs (5/8, 63%), fibrobronchoscope brush biopsy (6/13, 46%), pharyngeal swabs (126/398, 32%), faeces (44/153, 29%) and blood (3/307, 1%), and 0/72 urine specimens tested positive. NP swabs had a lower cycle threshold (Ct=24) relative to OP swabs (Ct>30). This suggests that combined OP/NP swabs may help to improve the positivity rate but should be guided by the availability of swabs.[20]

A study investigating the viral load in posterior OP saliva or other respiratory specimens suggested a viral load of 5.2 log10 copies per mL (IQR 4.1–7.0) at presentation and correlated well, but 3/30 patients who tested positive on initial respiratory samples did not test positive on OP saliva. Salivary viral load was highest during the first week after symptom onset. In one patient, viral RNA could still be detected 25 days after symptom onset. Higher viral loads were detected in older patients. The viral load did not correlate with disease severity. Viral RNA was detected in rectal specimens of only 4/30 and in blood of 5/30 but not in urine samples.[21]

A study in three clusters of COVID-19 in Singapore suggests that the median incubation period of SARS-CoV-2 was 4 days (IQR 3–6). The serial interval between transmission pairs ranged between 3 and 8 days.[22] Current WHO recommendation for surveillance of patients with COVID-19 or exposed individuals is 14 days,[15] although prolonged RNA shedding may occur up to day 37 in some patients.[23] According to a guidance document by the European Centre for Disease Prevention and Control (ECDC), viral RNA shedding of SARS-CoV-2 does not equate with infectivity, unless there is proof that the virus can be isolated and cultured from the particular samples.[19] The availability of diagnostic test versus the ability to do virus isolation may influence the decision to end isolation; however, the correlation between RNA shedding and infectivity requires further investigation.

Since saliva/NP or OP swabs may miss early infection, repeat sampling may be needed. Repeat sampling or a lower respiratory tract sample may facilitate the diagnoses of more severe cases and may be important if a patient has a clinical picture of viral pneumonia, and/or radiographical findings (chest CT or MRI scan) consistent with COVID-19 pneumonia or a potential exposure history. Consideration should also be given to the optimal specimen to exclude other respiratory pathogens.[24]

Since SARS-CoV-2 and most other respiratory viruses are RNA-based, care should be taken to select extraction and inactivation protocols that will not damage RNA, although for differential diagnoses that include DNA viruses such as adenovirus, total nucleic extraction protocols should be used. A recent study suggests that heat inactivation at 56°C for 30 min may result in false negatives for samples with low viral load, while guanidinium-based lysis for preservation of these specimens resulted in fewer false negatives (2 of 15 samples, 13.3%) and significantly less increase in Ct values than heat inactivation.[25]

PROTOCOLS FOR MOLECULAR DIAGNOSES OF COVID-19

Following the identification of COVID-19, WHO published a list of protocols used by public health and specialist research labs for identification of SARS-CoV-2 (summarised in table 1A from https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance).

These protocols were used during the initial identification of the virus by public health and reference labs internationally. The tests are a combination of specific and pancoronavirus PCR tests but are all based on more than one target gene using real-time RT-PCR protocols for confirmation (table 1A). New specific assays were subsequently developed and control material was made available through the European Virus Archive-Global[26] by the end of January 2020.[26] The viral genes targeted include

Table 1A Summary table of in-house protocols published by public health and research labs at the time of discovery of COVID-19 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance)

Country	Institute	Gene targets	Reference
China	China CDC	ORF1ab and N	http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html
Germany	Charité	RdRP, E, N	https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2
Hong Kong SAR	HKU	ORF1b-nsp14, N	https://www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf?sfvrsn=af1aac73_4
Japan	National Institute of Infectious Diseases, Department of Virology III	Pancorona and multiple targets, spike protein	https://www.who.int/docs/default-source/coronaviruse/method-niid-20200123-2.pdf?sfvrsn=fbf75320_7
Thailand	National Institutes of Health	N	https://www.who.int/docs/default-source/coronaviruse/conventional-rt-pcr-followed-by-sequencing-for-detection-of-ncov-rirl-nat-inst-health-t.pdf?sfvrsn=42271c6d_4
USA*	US CDC	Three targets in N gene	https://www.fda.gov/media/134922/download
France	Institut Pasteur, Paris	Two targets in RdRP	https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2

*CDC update effective from 15 March 2020.
CDC, Centers for Disease Control and Prevention; ORF, open reading frame.

the N, E, S and RdRP genes. The Centers for Disease Control and Prevention (CDC) had also developed kits that are available to public health laboratories. The updated CDC protocol is listed in table 1A. These early protocols guided development of commercial assays, which are now available for wider testing. Although wisely used, limited studies exist to compare the efficiency of the early protocols. A reprint has recently appeared that compares the individual primer and probe pairs, suggesting that, although all will detect the virus, there are some differences in sensitivity and specificity. The authors suggested that the most sensitive primer-probe sets were the E-Sarbeco (Charité), HKU-ORF1 (HKU), HKU-N (HKU) and 2019-nCoV_N1 (US CDC), but that the RdRp-SARSr (Charité) primer-probe set had the lowest sensitivity, possibly stemming from a mismatch in the reverse primer. They also identified some background cross-reactivity for the China CDC and US CDC assays on samples prior to the pandemic.[27] Since all of the assays currently consist of a confirmatory assay, these issues may not affect the accurate diagnoses of SAR-CoV-2; nevertheless, this should be kept in mind when selecting an assay and requires further investigation. The WHO has recently listed the first two diagnostic tests for emergency use during the COVID-19 pandemic. This gives countries access to quality-assured, accurate tests for the disease, which can be supplied by the United Nations and other procurement agencies supporting the COVID-19 response. The Emergency Use Listing procedure was established to expedite the availability of diagnostics needed in public health emergency situations (https://www.who.int/news-room/detail/07-04-2020-who-lists-two-covid-19-tests-for-emergency-use).

These are the *genesig Real-Time PCR Coronavirus (COVID-19)* and *cobas SARS-CoV-2 Qualitative Assay for Use on the cobas 6800/8800 Systems* (https://www.who.int/diagnostics_laboratory/200407_eul_sars_cov2_product_list.pdf?ua=1, table 1B).

In order to overcome limitations on availability of tests for domestic and global needs, the Food and Drug Administration (FDA) approved commercial assays under emergency use approval (EUA) for use in the USA that also provides some quality insurance for wider use (table 2) (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd). This approval requires that the companies define the limit of detection–analytical sensitivity of their SARS-CoV-2 assay and demonstrate 100% detection of published SARS-CoV-2 sequences with the assay's primers and probes. The approved tests are mostly real-time RT-PCR assays, as well as manual and automated commercial platforms, with the exception of the Cellex qSARS-CoV-2 IgG/IgM Rapid Test, which is discussed under rapid tests in the following section.

Abbott has recently released a rapid real-time molecular assay that can produce a positive result within 5 min and a negative result within 13 min. This is a molecular point-of-care (POC) test for acute cases that can be carried out in the doctor's office. The test has also received EUA from the FDA. The Abbott ID NOW COVID-19 test will run on the ID NOW platform, which is a portable instrument already available in a wide range of healthcare settings but is only available in the USA at this stage. This is currently the only rapid molecular assay with EUA approval (table 2).

OTHER MOLECULAR TESTS WITH A WIDELY AVAILABLE PLATFORM

The FIND Foundation has catalogued an extensive list of tests that are currently commercially available, as well as those in development. This includes 137 manual molecular assays and 47 automated test at the time of this review. An updated list can be found online (https://www.finddx.org/covid-19/sarscov2-eval-molecular/).

However, these tests have not yet received FDA or WHO approval and should be evaluated individually for sensitivity and specificity. The FIND Foundation currently has an initiative to independently evaluate molecular assays if applicants meet a set of minimum standards, including ability to upscale and availability. The findings of the first round of evaluations will be published on their website.

Rapid diagnostic assays

The WHO currently recommend COVID-19 diagnosis to be carried out by laboratories using molecular tests targeting SARS-CoV-2 virus RNA. This ensures laboratory results can be traced and patients identified for isolation and treatment and for tracking the pandemic. However, due to current infrastructure limitations and supply shortages which limit testing capacity access to reliable rapid diagnostic tests, in particular, rapid antigen tests are being investigated to expand laboratories' testing capacity to meet the most urgent medical and public health needs. The ECDC has recently published a review of the status of such tests.[28] According to their definition, rapid tests are qualitative or semiquantitative in vitro diagnostics involving small or single quantities, non-automated procedures and have been designed to give results within 10–20 min rather than hours as is the case with molecular assays and can be performed either in the lab or POC. These are usually direct antigen tests or indirect antibody tests. The ECDC is working in close cooperation with the European Commission, member state authorities, FIND (https://www.finddx.org/) and WHO on validating rapid tests and will make the results available as soon as possible on the FIND website.

At the time of this review 5 out of 17 antigen-detection RDTs and 27 out of 53 antibody-detection (serological) RDTs were under evaluation (table 4), (https://www.finddx.org/covid-19/sarscov2-eval-immuno/). Not all of these are CE approved or widely available, and of the rapid antibody tests, only the Cellex IgG/IgM rapid test is also on the FDA EUA list. There is some concern that PCR tests may miss some patients following day 7 of infection, and a suggestion that a combination of rtRT-PCR and IgM/IgG test may help to detect these patients or patients

Table 1B WHO-endorsed commercial assays for emergency use

Test	Details	Web link
Genesig Real-Time PCR Coronavirus (COVID-19) (Primerdesign, UK)	Open system suitable for laboratories with moderate sample testing capacity	https://www.who.int/diagnostics_laboratory/eul_0489_185_00_path_covid19_ce_ivd_ifu_issue_2.0.pdf?ua=1
Cobas SARS-CoV-2 for use on the cobas 6800/8800 Systems (Roche,USA)	Closed system assay for larger laboratories	https://www.who.int/diagnostics_laboratory/eul_0504-046-00_cobas_sars_cov2_qualitative_assay_ifu.pdf?ua=1

Table 2 Commercial molecular diagnostic tests that received EUA from the Food and Drug Administration of the USA as listed on their website at the time of this review. The website should be checked regularly for updates. (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd)

Date EUA was issued	Manufacturer	Diagnostic (letter of authorisation)	Fact sheet for healthcare providers	Fact sheet for patients	Manufacturer instructions/package Insert	Other documents
2 April 2020	Becton, Dickinson & Company	BioGX SARS-CoV-2 Reagents for BD MAX System	Healthcare providers	Patients	IFU	None
1 April 2020	Ipsum Diagnostics, LLC	COV-19 IDx Assay	Healthcare providers	Patients	EUA summary	None
1 April 2020	Cellex*	qSARS-CoV-2 IgG/IgM Rapid Test	Healthcare providers	Patients	IFU	None
30 March 2020	QIAGEN GmbH	QIAstat-Dx Respiratory SARS-CoV-2 Panel	Healthcare providers	Patients	IFU	None
30 March 2020	NeuMoDx Molecular	NeuMoDx SARS-CoV-2 Assay	Healthcare providers	Patients	IFU	None
27 March 2020	Luminex Molecular Diagnostics	NxTAG CoV Extended Panel Assay	Healthcare providers	Patients	IFU	None
27 March 2020	Abbott Diagnostics Scarborough	ID NOW COVID-19	Healthcare providers	Patients	IFU	None
26 March 2020	BGI Genomics Co	Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV	Healthcare providers	Patients	IFU	None
25 March 2020	Avellino Lab USA	AvellinoCoV2 test	Healthcare providers	Patients	EUA summary	None
24 March 2020	PerkinElmer	PerkinElmer New Coronavirus Nucleic Acid Detection Kit	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (1 April 2020)
23 March 2020	Mesa Biotech	Accula SARS-Cov-2 Test	Healthcare providers	Patients	IFU	None
23 March 2020	BioFire Defense, LLC	BioFire COVID-19 Test	Healthcare providers	Patients	IFU	None
20 March 2020	Cepheid	Xpert Xpress SARS-CoV-2 Test	Healthcare providers	Patients	IFU for labs IFU for point of care	None
20 March 2020	Primerdesign	Primerdesign Ltd COVID-19 genesig Real-Time PCR Assay	Healthcare providers	Patients	IFU	None
19 March 2020	GenMark Diagnostics	ePlex SARS-CoV-2 Test	Healthcare providers	Patients	IFU	None
19 March 2020	DiaSorin Molecular LLC	Simplexa COVID-19 Direct Assay	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (26 March 2020)
18 March 2020	Abbott Molecular	Abbott RealTime SARS-CoV-2 Assay	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (1 April 2020)
17 March 2020	Quest Diagnostics Infectious Disease	Quest SARS-CoV-2 rRT-PCR	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (26 March 2020)
17 March 2020	Quidel Corporation	Lyra SARS-CoV-2 Assay	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (23 March 2020)
16 March /2020	Laboratory Corporation of America	COVID-19 RT-PCR Test	Healthcare providers	Patients	EUA summary	None
16 March 2020	Hologic	Panther Fusion SARS-CoV-2	Healthcare providers	Patients	IFU	None
13 March 2020	Thermo Fisher Scientific	TaqPath COVID-19 Combo Kit	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (24 March 2020)
12 March 2020	Roche Molecular Systems	cobas SARS-CoV-2	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (31 March 2020)
29 February 2020	Wadsworth Centre, New York State Department of Public Health's (CDC)	New York SARS-CoV-2 Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (15 March 2020)
4 February 2020	CDC	CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)	Healthcare providers	Patients	IFU	Letter granting EUA amendment(s) (30 March 2020)

Authorization Documents include the Healthcare Provider (HCP) and Patient Fact Sheets and either the Manufacture Instructions/Package Insert (abbreviated to IFU)
*Antibody rapid test.
CDC, Centers for Disease Control and Prevention; EUA, emergency use approval.

who seroconverted. However, rapid antibody tests are not indicated for diagnoses of clinical cases by themselves.

While antigen tests may detect virus early in infection, they may have limitations on sensitivity relative to nucleic acid amplification tests and potential to cross-react with other coronaviruses. Indirect antibody assays have the potential to cross-react with other coronaviruses and are not of value for early diagnoses of clinical cases but may be valuable to determine immunity of individuals or answering epidemiological questions as discussed further.

Serological assays

Serological assays are not useful for diagnoses of acute cases in the first week of illness since IgM and IgG antibody responses are only detectable after approximately 6–15 days postdisease onset.[19] In two studies by Guo *et al*[29] and Zhao *et al*,[23] it was suggested that the combination of IgM ELISA and/or total antibodies versus SARS-CoV-2 plus rtRT-PCR can increase the sensitivity of diagnosis in the second week of illness, as the sensitivity of rtRT-PCR, especially on upper respiratory tract specimens, declines significantly during the immunological phase of illness. IgG against SARS-CoV-2 will have an important role to determine if someone is immune against SARS-COV-2. This could help to identify healthcare workers who can safely treat patients, identify serum donors and determine the true infection fatality rate of the COVID-19 pandemic. It will also be useful as part of vaccine trials. Early clinical studies help to define the kinetics of the immune response and appropriate target proteins for serological test development.

Table 4 Antigen rapid tests and serological assays under evaluation for sensitivity and specificity at the time of this review by FIND

Antigen-based Rapid Detection Tests to be included in the first-round evaluation

Company	Assay	Country of manufacturer	Interpretation	Regulatory status
Coris BioConcept	COVID-19 Ag Respi-Strip	Belgium	Visual	CE-IVD
RapiGEN	BIOCREDIT COVID-19 Ag	South Korea	Visual	RUO
SD BIOSENSOR	STANDARD F COVID-19 Ag FIA	South Korea	Reader	CE-IVD
SD BIOSENSOR	STANDARD Q COVID-19 Ag Test	South Korea	Visual	CE-IVD
Shenzhen Bioeasy Biotechnology Co*	BIOEASY 2019-nCoV Ag Fluorescence Rapid Test Kit (time-resolved fluorescence)	China	Reader	CE-IVD

Serological, Ab-based RDTs to be included in the first round evaluation

Company	Assay	Target	Country of manufacturer	Interpretation	Regulatory status
Beijing Diagreat Biotechnologies Co	2019-nCoV IgG/IgM Antibody Determination Kit	IgM/IgG	China	Reader required	CE-IVD
Beijing Tigsun Diagnostics Co	Tigsun COVID-19 Combo IgM/IgG Rapid Test (lateral flow)	IgM/IgG	China	Visual	CE-IVD, India
Beijing Wantai Biological Pharmacy Enterprise Co	Wantai SARS-CoV-2 Ab Rapid Test	Total Ab	China	Visual	Australia
BioMedomics	COVID-19 IgM-IgG Combined Antibody Rapid Test	IgM/IgG	China	Visual	CE-IVD, India
Boditech	AFIAS/iChroma COVID-19 Ab	IgM/IgG	Korea	Reader required	RUO
BTNX	Rapid Response COVID-19 IgG/IgM Test Cassette (whole blood/serum/plasma)	IgM/IgG	China	Visual	RUO
Cellex	Cellex qSARS-CoV-2 IgG/IgM Cassette Rapid Test	IgM/IgG	USA	Visual	CE-IVD
Changsha Sinocare	SARS-CoV-2 Antibody Test Strip	IgM/IgG	China	Visual	CE-IVD
		IgM/IgG	China	Visual	CE-IVD
Dynamiker Biotechnology Co	2019-nCOV IgG/IgM Rapid Test	IgG/IgM	China	Visual	CE-IVD
GenBody	GenBody COVID-19 IgM/IgG	IgM/IgG	Republic of Korea	Reader optional	CE-IVD
Guangzhou Wondfo Biotech Co	Wondfo SARS-CoV-2 Antibody Test (lateral flow method)	IgM/IgG	China	Visual	China, Australia, India; CE-IVD
Hangzhou AllTest Biotech Co	2019 nCoV IgG/IgM Rapid Test Cassette (whole blood, serum, plasma)	IgM/IgG	China	Visual	Australia, CE-IVD
Hangzhou Biotest Biotech Co	COVID-19 IgG/IgM Rapid Test Cassette (whole blood, serum, plasma)	IgM/IgG	China	Visual	CE-IVD
Innovita (Tangshan) Biological Technology Co	2019-nCoV Ab Test (colloidal gold)	IgM/IgG	China	Visual	China, CE-IVD
InTec Products	Rapid SARS-CoV-2 Antibody Test	IgM/IgG	China	Visual	RUO
InTec Products	Rapid SARS-CoV-2 Antibody (IgM/IgG) Test	IgM/IgG	China	Visual	RUO
Jiangsu Bioperfectus Technology Co	PerfectPOC Novel Corona Virus (SARS-CoV-2) IgM/IgG Rapid Test Kit	IgM/IgG	China	Visual	CE-IVD
Qingdao Hightop Biotech Co	HIGHTOP COVID-19 IgM/IgG Ab Rapid Test Kit	IgM/IgG	China	Visual	RUO
RapiGEN	BIOCREDIT COVID-19 IgG+IgM Duo	IgM/IgG	Republic of Korea	Visual	RUO
SD BIOSENSOR	STANDARDTM Q COVID-19 IgM/IgG Duo Test	IgM/IgG	Republic of Korea	Visual	CE-IVD, Brazil
Shanghai Kehua Bio-engineering Co	DIAGNOSTIC KIT FOR SARS-CoV-2 IgM/IgG ANTIBODY (colloidal gold)	IgM/IgG	China	Visual	CE-IVD
Shenzhen Bioeasy Biotechnology Co†	BIOEASY 2019-nCoV Ab (IgG/IgM) GICA Rapid Test Kit (gold colloidal immunoassay)	IgM/IgG	China	Visual	CE-IVD
Shenzhen Bioeasy Biotechnology Co†	BIOEASY 2019-nCov Total Ab GICA Rapid Test Kit (gold colloidal immunoassay)	Total Ab	China	Visual	CE-IVD
VivaChek Biotech (Hangzhou) Co	VivaDiagTM COVID-19 IgM/IgG Rapid Test	IgM/IgG	China	Visual	CE-IVD; Singapore, India, Australia
Zhuhai Livzon Diagnostics	Diagnostic Kit for IgG Antibody to Corona Virus (nCoV-2019) (colloidal gold)	IgG	China	Visual	CE-IVD, China
Zhuhai Livzon Diagnostics	Diagnostic Kit for IgM Antibody to Corona Virus (nCoV-2019) (colloidal gold)	IgM	China	Visual	CE-IVD, China

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*This fluorescence-based test is different from the colloidal gold Ag test that has now been withdrawn by the company.
†These colloidal gold Ab tests are different from the colloidal gold Ag test that has now been withdrawn by the company.
Ab, antibody; CE-IVD, Conformité Européenne (EU Certification) In Vitro Diagnostics; RUO, research use only.

In a study in Finland, the index COVID-19 case was followed up for 3–23 days from developing symptoms using immunofluorescence antibody tests and serum neutralisation assays. The authors demonstrated neutralising antibody response appeared within 9 days, along with specific IgM and IgG response, targeting particularly N and S proteins.[30] In a large study in China, enzyme immune assays, western blots and serum neutralisation assays were used to investigate immune responses to recombinant SARS-CoV-2 nucleoprotein (NP) and S protein receptor-binding domain (RBD) in 23 patients. An increase was noted in IgG or IgM antibody levels against NP or RBD for most patients at 10 days or later after symptom onset. More patients had earlier seropositivity for anti-RBD than anti-NP for both IgG and IgM. More patients had earlier seroconversion for IgG than IgM for anti-NP and anti-RBD. In 16 patients who had serial serum samples available for 14 days or longer,

the rates of seropositivity were 94% for anti-NP IgG (n=15), 88% for anti-NP IgM (n=14), 100% for anti-RBD IgG (n=16) and 94% for anti-RBD IgM (n=15). Anti-SARS-CoV-2-NP or anti-SARS-CoV-2-RBD IgG levels correlated with virus neutralisation titre (R^2>0.9).[21] A reprint has recently become available that describes the development of an ELISA that may be easily reproduced by research laboratories.[31] In this study, the authors describe a test based on recombinant antigens derived from the S protein of SARS-CoV-2. They used negative control samples representing pre-COVID-19 background immunity in the general population and samples from patients with COVID-19 to evaluate the assay. The S protein is a target for the neutralising antibody response and reflects protective immunity. The authors describes production of the subunit antigen and evaluation of the ELISA against 59 negative sera collected prior to the COVID-19 era and three patients with COVID-19 sera that were serially diluted. They showed no cross reaction with the negative sera and could demonstrate IgG3, IgM and IgA in the positive patients. Although this is a small study and has yet to be peer-reviewed, it provides a useful protocol for researchers to assess.[31]

Neutralisation assays to the virus may also be used to evaluate the immune response to SARS-CoV-2 and to evaluate cross reactions but requires a BSL-3 lab.

Currently,ere are no serological assays that have undergone extensive external validation, but an extensive list of >250 serological assays that are either already available or in development can be found under the FIND foundation website (https://www.finddx.org/covid-19/pipeline/?section=immunoassays#diag_tabhttps://www.finddx.org/covid-19/pipeline/?section=immunoassays#diag_tab). Several claims to have the CE certification mark that indicates conformity with health, safety and environmental protection standards for products sold within the European Economic Area. The FDA EUA list should also be monitored for updated information on serological assays. Due to the potential cross reaction between SARS-CoV-2 and other human coronaviruses, care should be taken when selecting an assay.

In a recent reprint published by investigators of the Department of Virus and Microbiological Special Diagnostics, Statens Serum Institut, Denmark, the sensitivity and specificity of nine commercially available serological tests were evaluated, including three ELISAs and six POC lateral flow tests. The investigators validated the assays against serum samples from SARS-CoV-2 PCR-positive patients with a known day of onset of disease, archived sera from healthy individuals prior to the emergence of SARS-CoV-2 in China, and sera from patients with acute viral respiratory tract infections associated with other coronaviruses or unrelated viruses. The Wantai SARS-CoV-2 Total Antibody ELISA had 100% specificity, while the Euroimmun IgA ELISA had 93% specificity and the Euroimmun IgG ELISA had 96% specificity, while sensitivities of 90%, 90% and 65%, respectively, were determined. The performance of the POC tests was more variable and ranked in the order of AutoBio Diagnostics>Dynamiker Biotechnology=CTK Biotech>Artron Laboratories>Acro Biotech≥Hangzhou Alltest Biotech. Sensitivities of 93% for AutoBio Diagnostics, 90% for Dynamiker Biotechnology and CTK Biotech, and 83% for Artron Laboratories were measured. The POC tests had a positive predictive value of 100%, while the negative predictive values ranged at 91%, 89%, 89% and 74%, respectively. Both ELISA and POC test varied according to the stage of disease with 70%–80% positivity at day 7 and 15% and 100% positivity by day 21. The authors also concluded that in ELISA format, the sensitivity of SARS-CoV-2 RBD-specific antibodies was superior to assays detecting spike-specific IgA or IgG only.[32]

TO CONCLUDE

Within a month of the emergence of SARS-CoV-2, rapid development and availability of molecular diagnostic assays have allowed countries globally to identify local cases and to describe the progression of the pandemic. High numbers of cases and testing requirements to track the virus globally have increased the demand for specific but cost-effective molecular diagnostic assays with an increased demand for POC molecular or antigen rapid tests. Serological assays are now required to define the attack rate and immunity in communities, but limited validation reports are so far available. Evaluation of sensitivity and specificity of both molecular and serological, as well as rapid assays remains a high priority to bring the pandemic under control.

Take home messages

- Accurate diagnostic tests are key in controlling the COVID-19 pandemic through identification of acute cases, describing the clinical presentation, isolation of infectious individuals and understanding the epidemiology of the virus.
- The WHO has facilitated an early public health response by publishing available PCR protocols for testing by mid-January 2020, defining the case definition using clinical data from China and advising on control measures.
- Continued validation of molecular, point-of-care and serological assays is required for rapid diagnosis and for defining measures of immunity.

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