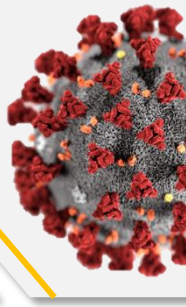
SEROLOGY TEST FOR COVID-19

Based on available evidence up to 15 May 2020



INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) began in Wuhan, China, in December 2019, and rapidly spread to neighbouring Asian and Western countries that on March 12, the World Health Organisation (WHO) declared COVID-19 to be a pandemic.[1] The WHO Strategic Preparedness and Response Plan towards COVID-19 stated that the overarching goal is for all countries to control the pandemic by slowing down the transmission and reducing mortality associated with COVID-19. This is achieved by rapidly finding and isolating all cases, providing them with appropriate care, and tracing, quarantining, and supporting all contacts.[2] Thus far, the molecular testing of respiratory tract samples using reverse transcriptase Polymerase Chain Reaction (RT-PCR) test is the recommended method for the identification and laboratory confirmation of COVID-19 cases.[3]

The main limitations with RT-PCR test is that it requires well-equipped laboratory facilities, highly skilled technologists and multiple reagents.[4] Currently, infrastructure limitations and global supply shortages of laboratory RT-PCR capacity and reagents are limiting testing capacity below the growing demand for COVID-19 diagnostics. In response to this, other types of diagnostic tests are being developed, based either on, the detection of proteins from the COVID-19 virus in respiratory samples (*antigen test*) or detection of human antibodies or immunoglobulins (Ig) in blood or serum (*serology test or antibody test*).[4] Table 1 shows comparison between different types of tests for COVID-19. Serology testing will be further discussed in this rapid review.

Table 1: What tests are available in the context of the COVID-19 outbreak?

	Molecular diagnostic tests	**Serology tests**
Test objective	Detection of virus in the organism	Detection of immune response to the virus

Technique	RT-PCR	Direct SARS-CoV-2 antigen detection (still under development)	ELISA tests	Immunochromatographic assays (rapid tests)
What does it look for?	Looks for presence of viral genetic material (RNA)	Looks for the presence of viral antigens	Look for the presence of an immune response (antibodies) against virus in patients' blood	
What does a positive test mean?	The virus is present in a patient		Patient has been exposed to the virus and is either recovering or has already recovered	
What is the test used for?	To know whether a patient is currently infected by SARS-CoV-2		To know whether a patient has been exposed to SARS-CoV-2 and is therefore protected against new infections (and may not spread the disease anymore)	
Pros	● If done properly, RT-PCR is very sensitive and specific ● Rapid RT-PCR can be used at point of care	● Simple ● Rapid ● Could be used at point of care	● More precise than immunochromatographic assays ● Provides a quantitative information(concentration of antibodies)	● Less resource intensive than ELISA tests ● Could be performed at point of care once the technique is fully validated (could potentially be sold to the public) ● Rapid results (10-30 minutes)
Cons	● Labour intensive ● Need to be processed in a lab ● Not all labs can process RT-PCR (need the right device	● Complex to develop	● Possible false negative (if performed too early in the infection process as antibodies have not yet been produced ● Possible false positives (interaction with other diseases) ● Needs to be performed in a lab ● Resource intensive	● Provides only a qualitative information (presence or not

	and a special authorisation to handle hazardous material) ● Risk of false negative (mainly due to poor sampling technique) ● Possible shortages of swabs and reagents		(1 to 5 hours) ● Possible shortages of reagents ● Kits being produced not tested yet	of antibodies) ● Kits being produced not tested yet

Adapted from: OECD. Tackling Coronavirus (COVID-19):Contributing To A Global effort. Testing for COVID-19: A way to lift confinement restrictions. 4 May 2020[14]

In general, the immune system typically produces immunoglobulin M (IgM) soon after infection as the first line of defense during viral infections, and immunoglobulin G (IgG) is generated later and persists in the body longer than IgM. This contributes to long term immunity and immunological memory. [5] IgA is another type of antibody, typically found in mucous membranes, that can be produced in high quantities during infections.

These immunoglobulins are detected through three types of serology assays; rapid diagnostic test (RDT), enzyme-linked immunosorbent assay (ELISA) and neutralization assay/ plaque reduction neutralization test (PRNT).[6] In the context of SARS-CoV-2, rapid diagnostic tests (RDTs) and enzyme-linked immunosorbent assays (ELISAs) have been designed to detect antibodies specific to the spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins of the SARS-CoV-2 virus. The RDT is typically a qualitative (positive or negative) lateral flow assay that is simple, portable, and can be used at point of care (POC) to detect the presence or absence of antibodies against the virus present in patient serum. ELISA is generally a lab-based test (either qualitative or quantitative), to detect antibody-viral protein complexes, using whole blood, plasma, or serum samples from patients. Neutralization assay provides quantitative information on the ability of patient antibodies to confer protective immunity. It is the most time-consuming and skill-based of the three tests described. Lab-based methods is utilised to detect the presence of active antibodies in patient serum that are able to inhibit virus growth ex vivo, in a cell culture system.[6]

It is recommended that antibody detection be performed using a validated assay meeting acceptable and documented performance standards. The accuracy of a serological test can be directly related to the mechanism of the test itself, or it can be influenced by epidemiologic conditions, such as expected or known disease prevalence in the population.[7] The sensitivity of a serological test is the ability of the test to correctly produce a positive result for a sample that has the antibodies in question. Specificity refers to the ability of the test to correctly return a negative result for a sample that does not have the antibodies. If serology testing is going to be used to make policy decisions or to guide individual actions around patient health and safety, it is critical that both sensitivity and specificity are as high as possible to avoid false-positive and false-negative results. Positive predictive value (PPV) and negative predictive value (NPV) provide insight into how accurate the positive and negative test results are expected to be in a given population, by factoring in both test accuracy and the prevalence of the disease in the population.[7]

As of April 20, the Food and Drug Administration (FDA) who is responsible for the approval and regulation of serology tests available in the United States, has authorized four emergency use authorization (EUA).[8] These include some rapid tests to indicate the presence of antiviral antibodies, such as those developed by Cellex and Chembio Diagnostic Systems as well as COVID-19 ELISA developed at Mount Sinai Laboratory, that can measure antibody levels in an individual's blood.[8]

Guidelines and Technical Reports Recommendations on Serology Testing

1. In the latest technical brief, WHO recognises the role of serological assays in research and surveillance and it supports testing for SARS-CoV-2 antibodies at the population level or in specific groups (such as health workers, close contacts of known cases, or within household antibodies) as they are critical for understanding the extent of and risk factors associated with infection. WHO however does not recommend serology testing for COVID-19 case diagnosis and detection. As there is currently no evidence that people who have recovered from COVID-19 and have antibodies are protected from a second infection, the accuracy of an "immunity passport" or "risk-free certificate" that would enable individuals to travel or to return to work can not be guaranteed.[9]

2. The FDA highlights that serology tests should not be used as the sole test to diagnose or exclude active SARS-CoV-2 infection.[8]

3. The Chinese Center for Disease Control and Prevention's Laboratory Testing for COVID-19 guideline states that serum antibody tests (colloidal gold, magnetic particle chemiluminescence, ELISA) are used as supplementary test for the following conditions;[10]
   a. cases that are negative for 2019-nCoV nucleic acid tests and used in addition to nucleic acid tests in diagnosing suspected cases; and
   b. serological and past exposure surveys of concerned population groups

   Laboratory confirmed positive cases need to meet one of the following two conditions:
   a. Serum IgM antibodies and/or IgG antibodies are positive
   b. Serum IgG antibodies turn from negative to positive or the IgG antibody titers of recovery period are 4 times or more higher than that of acute phase.[10]

   If a serology test which is conducted in the acute phase within 7 days after the onset of disease detects IgM and IgG is negative, repeat collection for testing within 10 days after the onset of disease is recommended.[10]

4. According to the guidelines by Korean Society for Laboratory Medicine and the Korea Centers for Disease Prevention and Control, serology tests are not recommended for diagnosing COVID-19 in clinical laboratories in Korea, other than RT-PCR and may be considered for public health purposes.[11]

5. The Johns Hopkins Center for Health Security in their report "Developing a National Strategy for Serology (Antibody Testing) in the United States" stated that the test may be used to identify whether people were previously infected by SARS-CoV-2 and the priority lies in ensuring accuracy, validity, and comparability of available serology tests.[12] If the serology tests can be determined to correlate with immunity to the disease, they should then be made available to:
   (a) public health authorities, to conduct surveillance and to estimate the prevalence of disease;
   (b) essential workers, with priority for healthcare workers and those who interact with vulnerable populations (eg, nursing home residents); and
   (c) individuals who may use them to assess their personal risk of becoming infected with SARS-CoV-2 (COVID-19 disease).

   Currently however, there are significant and urgent areas of uncertainty particularly in the validation of serology tests that will need to be addressed.[12]

6. The European Centre for Disease Prevention and Control (ECDC) states that SARS-CoV-2 antibody detection tests have limited usefulness in the early diagnosis of COVID-19 because it may take 10 days or more after onset of symptoms for patients to become positive.[13]

7. An OECD report on testing for COVID-19 states that validated serology tests can be used for two purposes;
   (a) to allow people who have acquired immunity to return to work safely, and
   (b) to provide intelligence on the evolution of the epidemic across the population.
   Nevertheless, rapid serology test kits need to be developed and their clinical performance needs to be demonstrated before implementation at large scale can happen.[14]

8. Public Health England does not recommend the use of rapid test kits for the diagnosis of COVID-19 infection in community settings. The UK government has launched a nationwide surveillance study to track the prevalence of infection with SARS-CoV-2 in the general population. The study includes swab testing and serology testing to look at both the current rates of infection and how many people are likely to have developed antibodies to the virus.[15]

9. The Public Health Laboratory Network of Australia does not recommend point-of-care serology as first line tests in diagnosing acute viral infection due to significant limitations.[16] Validated tests have some utility in determining past infection or screening purposes if used properly by trained healthcare professionals.

10. The Philippine College of Physician and Society For Microbiology and Infectious Diseases in their Position Statement on Rapid Antibody Tests do not recommend it for clinical use and triaging in the first 14 days of illness. Disease surveillance using serology tests during the peak of pandemic is not recommended. However, validated serology tests are recommended for seroprevalence surveys during the downward trend of the pandemic. [17]

Some governments including Chile, Germany, Italy, the UK, and the USA are looking at the use of immunity “passports”; digital or physical documents that certify an individual has been infected and is purportedly immune to SARS-CoV-2, but research is still ongoing.[18]

EVIDENCE ON EFFECTIVENESS AND SAFETY

There were six articles retrieved from the scientific databases (Medline, EMBASE, PubMed) and from the general search engines [Google Scholar and US Food and Drug Administration (US FDA)] on diagnostic performance of serology tests for COVID-19 (Table 1). Four studies were conducted in China while two were conducted in Europe; The Netherlands and Sweden. There was no evidence on safety of the serology test retrieved from the scientific databases.

1. Okba et al. validated and tested various antigens in different platforms developed in-house, as well as a commercial platform using a well-characterized cohort of serum samples from PCR-confirmed SARS-CoV-2 and patients PCR-confirmed to be infected with seasonal coronaviruses and other respiratory pathogens.19 Serum samples (n = 10) were collected from three PCR-confirmed patients: two with mild COVID-19 and one with severe COVID-19 from France and serum samples (n = 31) were collected from nine patients with PCR-confirmed cases of COVID-19 cases from Germany. Plaque reduction neutralization test (PRNT) was used as a reference coronavirus serologic assay for this study, and anti-SARS-CoV-2 S1 IgG and IgA ELISAs were performed using β-versions of two commercial kits and in-house ELISAs.[19]

After infection, all three patients from France seroconverted between days 13 and 21 after onset of disease and antibodies were elicited against the SARS-CoV-2 S, S1 subunit, and RBD, but only 2/3 patients had detectable antibodies to the N-terminal (S1A) domain. Because the N protein of SARS-CoV-2 is 90% similar to that of SARS-CoV, the authors used SARS-CoV N protein as an antigen to test for SARS-CoV-2 N protein–directed antibodies in an ELISA format and found that antibodies were elicited against the N protein in all three patients. When tested in a PRNT, serum samples from all three patients neutralized SARS-CoV-2 infection.

The author observed cross-reactivity with the SARS-CoV S and S1 proteins, and to a lower extent with MERS-CoV S protein, but not with the MERS-CoV S1 protein. Further analysis showed that the S2 subunit is more conserved and thus plays a role in the cross-reactivity seen when the whole S was used as antigen. Thus, S1 is more specific than S as an antigen for SARS-CoV-2 serologic diagnosis. The authors further assessed the specificity of the S1 assay by using validation cohorts A–E which were composed of serum samples from healthy blood donors (A), PCR-confirmed acute respiratory non-CoV infections (B), acute-phase and convalescent-phase PCR-confirmed α- and β-HCoV infections (C), PCR-confirmed MERS-CoV infections (D), and PCR-confirmed SARS-CoV infections (E). None of the serum samples from specificity cohorts A–D were reactive using in-house S1 ELISA at the set cutoff value, indicating 100% specificity. The

authors also validated the sensitivity and specificity of two commercial ELISA kits for detecting S1-specific IgG and IgA and all 3 COVID-19 patients had reactive antibodies detected by the IgG (6/10 serum samples) and IgA (7/10 serum samples) ELISAs. However, serum samples from the validation cohorts A–D showed reactivity in 11/203 for IgA and 8/203 for IgG ELISAs and serum samples from 2 patients infected with HCoV-OC43 (a betacoronavirus) were reactive in both IgG and IgA ELISA kits.[19]

Further validation of IgG and IgA ELISA was performed using 31 serum samples collected from nine COVID-19 patients in Germany that were previously confirmed to seroconvert at days 6–15 after onset of disease by use of a recombinant immunofluorescence test and PRNT. A total of 8/9 seroconverted patients showed reactivity above the implemented cutoff values in the IgG and IgA ELISA. A serum sample from one patient had an antibody level slightly below the cutoff value, which might be explained by an overall reduced antibody response of this patient (PRNT90 = 10).[19]

2. Li et al. reported and developed a rapid and simple point-of-care lateral flow immunoassay (LFIA) test product, which can detect IgM and IgG simultaneously in human blood within 15 minutes and can detect patients at different infection stages.[20] They assumed the antibody generation process is similar to MERS and SARS outbreak since COVID-19 belongs to the same large family of viruses. Therefore, the detection of the IgG and IgM antibody against SARS-CoV-2 will be an indication of infection.

Patients were recruited who conform to the diagnostic criteria of suspected cases of COVID-19 according to guideline of diagnosis and treatment of COVID-1915 including typical epidemiological history and clinical characteristics. These samples were collected from various hospitals (total number of hospitals of eight) and CDC testing laboratories at six different provinces in China. The respiratory tract specimen, including pharyngeal swab and sputum, was used to confirm COVID-19 cases, and the blood, including serum and plasma, was used to test the IgM and IgG antibody.[20]

Blood samples were collected from COVID-19 patients in order to test the detection sensitivity and specificity of SARS-CoV-2 IgG-IgM combined antibody test. A total of 525 cases (N=525) were tested consist of 397 (positive) clinically confirmed (including PCR test) SARS-CoV-2-infected patients and 128 non-SARS-CoV-2-infected patients (128 negative) (n positive=397; n negative= 128). Of the 397 blood samples from SARS-CoV-2-infected patients, 352 tested positive, resulting in a sensitivity of 88.66% (352/397). Twelve of the blood samples from the 128 nonSARS-CoV-2

infection patients tested positive, generating a specificity of 90.63%. (116/128). It was also found that 64.48% (256/397) of positive patients had both IgM and IgG antibodies.[20]

3. Zhang et al. conducted and developed IgM and IgG detection methods using a cross-reactive nucleocapsid protein (NP) from another SARSr-CoV Rp3, which is 92% identical to 2019-nCoV NP.[21] Using these serological tools, they demonstrate viral antibody titres increase in patients infected with 2019-nCoV.

They collected human samples, including oral swabs, anal swabs and blood samples of all consented patients from Wuhan pulmonary hospital. They divided the investigation into two groups of selected patients. In the first group, they collected samples from 39 patients, seven of which were in severe conditions. In the second group, they collected samples from 139 patients, yet their clinical records were not available. In a serological test, in-house anti-SARSr-CoV IgG and IgM ELISA kits were developed using SARSr-CoV Rp3 NP as antigen, which shared above 90% amino acid identity to all SARSr-CoVs.[21]

The target patients (those who received around 10 days of medical treatments upon admission) were tested for both viral antibody and viral nucleotide levels by previously established method. The results showed that both IgM and IgG titres were relatively low or undetectable on day 0 (the day of first sampling). On day 5, an increase of viral antibodies can be seen in nearly all patients, which was normally considered as a transition from earlier to later period of infection. IgM positive rate increased from 50% (8/16) to 81% (13/16), whereas IgG positive rate increased from 81% (13/16) to 100% (16/16). This is in contrast to a relatively low detection positive rate from the first part of investigation using the molecular test. Both molecular and serological tests are needed to definitively confirm a virus carrier.[21]

4. Pan et al. conducted a study using the colloidal gold-based immunochromatographic (ICG) strip targeting viral IgM or IgG antibody and compared it with real-time Polymerase Chain Reaction (RT-PCR) for patients hospitalised in Zhongnan Hospital of Wuhan University, China.[22] A total of 134 samples from 105 patients (48 male vs. 57 female), with a median age of 58 years (range from 20 to 96 years old) were enrolled in the study. The blood samples were collected, and blood serum, plasma or whole blood were subjected to ICG assay in accordant with the manufacturer's protocol (Zhuhai Livzon Diagnostic Inc.). In comparison, throat swab samples were collected and tested for SARS-CoV2 with the Chinese Center for Disease Control and Prevention (CDC) recommended Kit (BioGerm, Shanghai, China). All samples were processed simultaneously at the Department of Laboratory Medicine of Zhongnan Hospital of Wuhan University. All patients were

tested for SARSCoV-2 on samples from the respiratory tract as real-time reverse-transcription PCR (real-time RT-PCR).[22]

Results showed that the positive rates of IgM or IgG in the early stage are relatively low, and gradually increase during the disease progression. The IgM positive rate increased from 11.1% of early stage to 78.6 and 74.2% in intermediate and late stage, respectively. The IgG positive rate in the confirmed patients is 3.6% in early, 57.1% in intermediate and 96.8% in late stage, respectively. Additionally, by combining the result of IgM and IgG, i.e. patients with either IgM or IgG positive, would significantly increase the sensitivity of ICG assay, especially at the intermediate stage. While IgM and IgG positive rates at the intermediate stage are 78.6% and 57.1%, respectively, combining both parameters would bring a positive rate to 92.9%.[22]

For detecting antibodies in nucleic acid-negative “clinically diagnosed” patients, a total of 39 samples from 37 clinically diagnosed patients were included. Among these samples, nine (23.1%) of them were positive to IgM and 15 (38.5%) of them were positive to IgG; when combined the IgG and IgM results in total 17 (43.6%) of samples were positive from 39 nucleic acid negative cases. Twenty-two samples from clinical diagnosis patients with disease duration information were included. Nine cases were at early stage, six cases at the intermediate stage and seven cases at late stage. The positive percentages of IgM at early, intermediate and late stages were 22.2%, 33.3% and 57.1%, respectively; the positive rate of IgG at early, intermediate and late stages were 44.4%, 66.7% and 71.4%, respectively. When it came to IgM and IgG combination, the positive rate boosted to 83.3% in cases at intermediate stage. Even by comparing with confirmed cases, the IgG and IgM positive rates were similar, albeit relatively lower.[6] From this study, they provided a sensitive and consistent serology diagnostic approach in complementary to the current clinically used real-time RT-PCR testing in diagnosis with SARS-CoV-2 infected COVID-19 patients.[22]

5. Hoffman et al. evaluated a commercial rapid test cassette (Zhejiang Orient Gene Biotech Co Ltd, Huzhou, Zhejiang, China) for detection of SARS-CoV-2-specific IgM and IgG.[23] Twenty nine capillary blood samples or serum from COVID-19 cases from Uppsala Biobank, obtained during disease or convalescence and previously confirmed by PCR, were used as ‘true positives’ while 124 samples from healthy volunteers from Uppsala Academic Hospital, without any known history of SARS-CoV-2 infection were used as negative controls. The results revealed a sensitivity of 69% and 93.1% for IgM and IgG, respectively. The assay specificities were shown to be 100% for IgM and 99.2% for IgG. None of the healthy volunteers tested positive for IgM while 1 tested positive for IgG. Using PCR-positive cases as true positives, the accuracy of the test was 94.1% and 98.0% for IgM and IgG, respectively. The positive and negative predictive values for IgM were

100% and 93.2%, respectively. For IgG, the corresponding values were 96.4% and 98.4%. Detectable IgM and IgG were recorded in some patients at day 9, while in other patients the seroconversion seems to occur later (last detected at Day 29).This indicates that the test is suitable for assessing previous virus exposure, although negative results may be unreliable during the first weeks after infection. In contrast to Li et al. (2020), this study found less indications for using this test for clinical diagnosis. Nevertheless, it might contribute to detecting potential asymptomatic infections and the magnitude of the spread of the infection. The high negative predictive value indicates that the rapid test could be useful for detecting past infections and possible immunity, which may be crucial for restoring social functions after lockdown. Due to the small sample size, the study results should be interpreted with caution.[23]

6. Jin et al. evaluated a SARS-CoV-2 IgM and IgG chemiluminescence immunoassay (CLIA) kits (by Shenzhen YHLO Biotech Co., Ltd, China) in which its magnetic beads were coated with two antigens of SARS- CoV-2 (nucleocapsid protein or N protein, spike protein or S protein).[24] Forty-three patients from Xixi Hospital of Hangzhou, China with a laboratory-confirmed infection and at least one viral serological test performed in the hospital were enrolled in this study. Thirty-three patients with suspected SARS- CoV-2 infection, in whom the disease was eventually excluded in the hospital and who quarantined at home, were included as a control group. Laboratory confirmation of the virus was based on the result of real-time RT-PCR. In the COVID-19 group, 27 patients were tested for viral antibody before becoming virus-negative (including oral swabs, anal swabs, or sputum).

The results revealed that the median duration from first symptoms to serological testing in these 27 patients was 16 days (IQR 9–20 days). Among these people, 13 were IgM-positive (48.1%) and 24 were IgG-positive (88.9%). IgM and IgG were also both found to be positive in one case before laboratory confirmation for the first time. Therefore, COVID-19 should be considered when serum IgM or IgG is positive. Three IgG-negative patients were also IgM-negative. The duration from symptom onset to this serological test in these three patients was 0 days, 5 days, and 8 days, respectively. It was observed that serum viral antibodies increased only slightly in the early stage of the disease. These antibodies may not have been produced yet and could be undetectable. Thus, COVID-19 cannot be excluded at an early stage when viral serological testing is negative. In the control group, the IgM and IgG positive rates were 0% and 9.1% (3 cases; all weak positive IgG), respectively. Looking at the dynamic variance of the antibodies, the IgM-positive rate increased slightly at first and then decreased as the number of days from laboratory confirmation to serological detection increased; in contrast, the IgG-positive rate increased to 100% and was higher than IgM at all times. This was in accordance with findings by Zhang et al (2020).[24]

In terms of diagnostic performance, compared to RT-PCR, the sensitivities of serum IgM and IgG antibodies to diagnose COVID-19 were 48.1% and 88.9%, and the specificities were 100% and 90.9%, respectively. The positive predictive values (PPVs) of IgM and IgG antibodies were 100% and 88.9%, respectively, while the negative predictive values (NPVs) were 70.2% and 90.9%, respectively. The study results should be interpreted with caution as the sample size was small.[24]

Table 1: Diagnostic performance of serology tests

Study /author	Population (n)		Intervention	Comparator/ Reference	Sensitivity (%)		Specificity (%)		Day of sero-conversion	Comments
	Cases	Control			IgM	IgG	IgM	IgG		
Okba et al (Netherlands)	France samples 3 PCR-confirmed cases; 2 mild COVID-19 and 1 severe COVID-19 (serum samples=10) Berlin samples 31 samples from PCR-confirmed cases	45 healthy blood donors	ß version of 2 commercial kit (EUROIMMUN Medizinische Labordiagnostika AG) anti-SARS-CoV-2 S1 IgG and IgA ELISA	Assay validation PCR-diagnosed infections with human coronaviruses or other respiratory viruses,cytomegalovirus, Epstein-Barr virus, or Mycoplasma pneumoniae Accuracy Plaque reduction neutralization test (PRNT)	IgA 70.0	60.0	S1 assay: 100		Day 13-21	**Cross-reactivity** Observed with; -SARS-CoV S and S1 proteins -MERS-CoV S protein
Li et al (China)	8 hospitals & CDC testing laboratories at six different provinces in China (N=525) Positive cases & clinically confirmed (including PCR test: 397	128 non-SARS-CoV-2-infected patients	Rapid and simple point-of-care lateral flow immunoassay (LFIA) test	RT-PCR	88.7		90.3		Day 8-33 -in 1 subset of patients	
Zhang et al (China)	From Wuhan pulmonary hospital n=39 which 7 in severe conditions	n= 139 patients	cross-reactive nucleocapsid protein (NP) from another SARSr-CoV Rp3	RT-PCR	Positivity rate: IgM : 50 - 81 IgG: 81 - 100				Day 5 -10	

Pan 2020 (China)	105 hospitalised patients,Wuhan (134 samples)		Colloidal gold-based immunochroma-tographic IgG & IgM	RT-PCR	Positivity rate: IgM: early-late 11.1-74.2 IgG: early-late 3.6-96.8				NA	
Hoffman et al 2020 (Sweden)	COVID-19 cases (n=29) from Uppsala Biobank, Sweden	Healthy volunteers (n=124) from Uppsala Academic Hospital	Rapid test cassette of SARS-CoV-2 IgG & IgM (Zhejiang Orient Gene Biotech Co Ltd, China)	RT-PCR	69.0	93.1	100	99.2	Day 9-29	**PPV** IgM 100% IgG 93.2% **NPV** IgM 96.4% IgG 98.4% **Accuracy** IgM 94.1% IgG 98%
Jin et al 2020 (China)	43 cases from Xixi Hospital of Hangzhou, China	33 excluded cases in hospital or, home-quarantined	SARS-CoV-2 IgM & IgG chemilumines-cence immunoassay (CLIA) kits	RT-PCR	48.1	88.9	100	90.9	NA	**PPV** IgM 100% IgG 88.9% **NPV** IgM 70.2% IgG 90.9%

***NA= Not available, PPV= Positive Predictive Value, NPV= Negative Predictive Value**

CONCLUSION

There were 10 guidelines and technical reports retrieved on serology testing from various countries and six articles retrieved from the scientific databases on diagnostic performance of serology tests for COVID-19.

The serology tests could help identify people who have been exposed to the virus by being able to detect antibodies to SARS-CoV-2 virus.

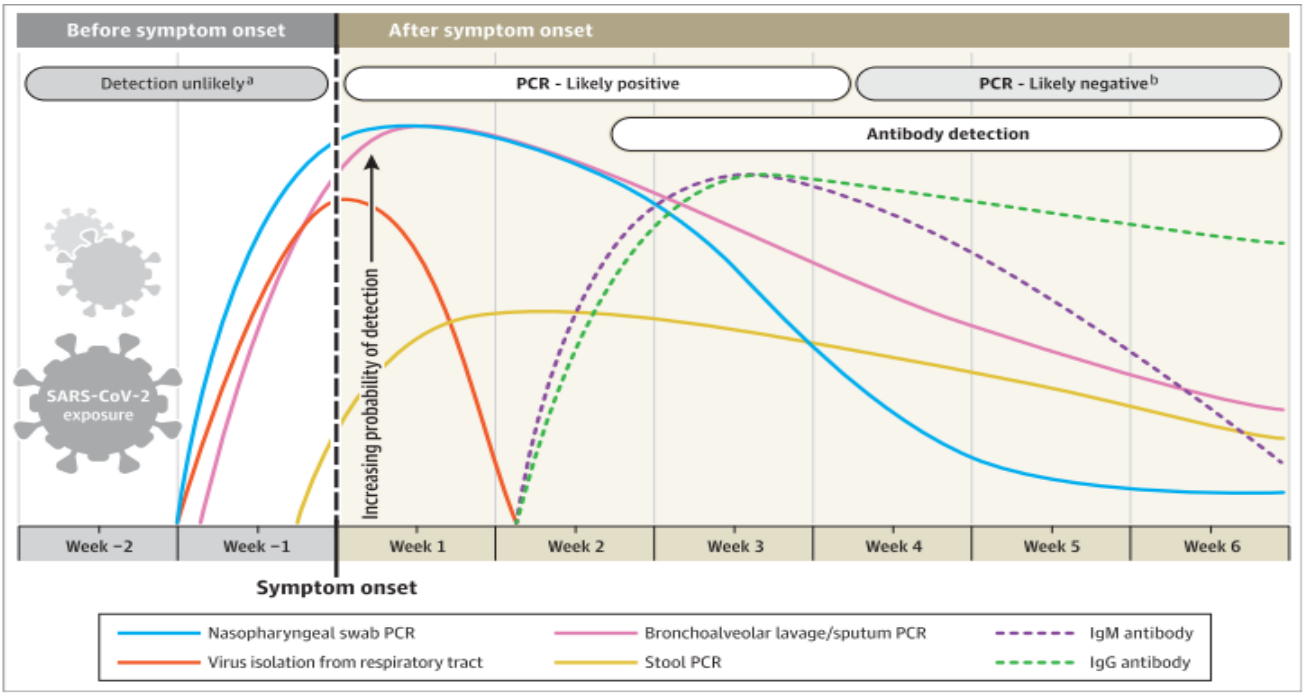
These tests however, are of limited value in the early diagnosis of a patient with COVID-19 infection, given antibody responses to infection take days to weeks to be detectable. Retrieved studies suggested that most of the patients seroconverted in 7–11 days after exposure to the virus, although some patients may develop antibodies sooner or later (up to 29 days of infection). Therefore, antibody testing is not useful in the setting of an acute illness.

As the IgM and IgG were found to increase only slightly in the early stage of the disease, COVID-19 cannot be excluded at an early stage when viral serological testing is negative. Figure 1 illustrated clinically useful timeline of diagnostic markers for detection of COVID-19 (as adapted from Senthuraman et al).[25]

Cross-reactivity with other coronavirus proteins SARS-CoV and MERS-CoV was found to occur in some studies and this can lead to false positive results.

In terms of diagnostic accuracy of the tests, the sensitivity and specificity varies greatly depending on the timing of the test relative to the duration of illness, highlighting the need for more independent validation studies of serology tests.The tests perform poorly during the early phase of the disease (less than 8 days from onset of symptoms). Low sensitivity and specificity leads to high false positive and false negative rates. High false negative leads to false reassurance, ignoring public health measures and inadvertent exposure. This will pose threat to health workers, the patients themselves as well as the family and communities.

Figure 1: Estimated variation over time in diagnostic tests for detection of SARS-CoV-2 infection relative to symptom onset [25]



SARS-CoV-2=severe acute respiratory syndrome Coronavirus 2, PCR=Polymerase Chain Reaction
[a]Detection only occurs if patients are followed up proactively from the time of exposure
[b]More likely to register a negative than a positive result by PCR of a nasopharyngeal swab
*Estimated time intervals and rates of viral detection are based on data from several published reports. Because of variability in values among studies, estimated time intervals should be considered approximations and the probability of detection of SARS-CoV-2 infection is presented quantitatively.

In conclusion, current evidence does not support the routine use of serology tests for definitive diagnosis of COVID-19.

In the acute phase of illness - serology testing is not recommended for triage, clinical use and diagnosis in the first 14 days of illness because of high false positive and high false negative rates.

During the downward trend of pandemic - appropriately validated serology testing is recommended for seroprevalence surveys in determining the true prevalence of COVID-19 (by identifying people who were not diagnosed by PCR or who may have had asymptomatic or

subclinical infection), monitor the emergence of herd immunity and can potentially help the medical community better understand how the immune response against the SARS-CoV-2 virus develops in patients over time.

Testing for the purpose of assessing immunity is still being explored as there is no study to date that evaluated whether the presence of antibodies to SARS-CoV-2 confers immunity to subsequent infection. In addition to this, it is unclear on what level of response is necessary for immunity, or how long such protection might last. Research is still in progress on “immunity passports” or “risk-free certificates” that certify antibody protection and allow people to return to public spaces.

REFERENCE

1. World Health Organization (WHO). Coronavirus disease (COVID-19) pandemic. https://www.who.int/emergencies/diseases/novel-coronavirus-2019. (Accessed on 23 April 2020)
2. World Health Organization (WHO). COVID-19 Strategy Update. 14 April 2020. Available from: https://www.who.int/docs/default-source/coronaviruse/covid-strategy-update-14april2020.pdf?sfvrsn=29da3ba0_19 (Accessed on 23 April 2020)
3. World Health Organization (WHO). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. 2020. Available from: https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117 (Accessed on 23 April 2020)
4. World Health Organization (WHO). Advice on the use of point-of-care immunodiagnostic tests for COVID-19. Updated 8 April 2020. Available at: https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19 (Accessed on 17 April 2020)
5. Li Z, Yi Y, Luo X, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. J Med Virol. 2020;0–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32104917
6. Global Progress on COVID-19 Serology-Based Testing. Johns Hopkins Bloomberg School of Public Health. Available at: https://www.centerforhealthsecurity.org/resources/COVID-19/serology/Serology-based-tests-for-COVID-19.html (Accessed on 23 April 2020)
7. Gordis L. Epidemiology. 5th ed. Philadelphia, PA: Elsevier/Saunders; 2014
8. Food and Drug Administration (FDA). Coronavirus (COVID-19) Update: Emergency Use Authorizations. https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#code 19 dvd (Accessed on 23 April 2020)

9. World Health Organization (WHO). “Immunity passports” in the context of COVID-19. 24 April 2020. Available from: WHO/2019-nCoV/Sci_Brief/Immunity_passport/2020.1(Accessed on 24 April 2020)

10. Chinese Center for Disease Control, Prevention. Technical Guidelines for COVID-19 Laboratory Testing. China CDC Wkly. 2020;2(19):1–6. Available from:http://weekly.chinacdc.cn//article/id/0f6dc581-08f4-4828-97f0 9cc805969bbb

11. Hong KH, Lee SW, Kim TS, et al. Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea. Ann Lab Med. 2020;40(5):351–60

12. Gronvall G, Connel N, Kobokovich A, et al. Developing a National Strategy for Serology (Antibody Testing) in the United States. Johns Hopkins Bloomberg School of Public Health. 2020

13. European Center for Disease Prevention and Control. Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK– ninth update. Rapid Risk Assess. 23 April 2020

14. OECD. Tackling Coronavirus (COVID-19):Contributing To A Global effort. Testing for COVID-19: A way to lift confinement restrictions. 4 May 2020. Available from: www.oecd.org/coronavirus

15. Rough E. Coronavirus: Testing for Covid-19. 2020. Available from: www.parliament.uk/commons-library

16. Communicable Diseases Network Australia. PHLN guidance on laboratory testing for SARS-CoV-2. Public Health Laboratory Network. 2020. Available from: https://www1.health.gov.au/internet/main/publishing.nsf/Content/2FCDB8DA4EB40BA9CA257BF000211F2A/$File/PHLN-Lab-Test-Guide-SARS-CoV-2-v1.9.pdf

17. Philippines Society for Microbiology and Infectious Diseases. PSP and PSMID position statement on rapid antibody tests for COVID-19. 11 April 2020. Available at:https://www.psmid.org/pcp-and-psmid-position-statement-on-rapid-antibody-tests-for-covid-19/

18. Bartlett J. Chile’s “immunity passport” will allow recovered coronavirus patients to break free from lockdown, get back to work. Washington Post, April 20, 2020. https://www.washingtonpost.com/world/the_americas/chile-coronavirus-immunity-passport-antibody-testing-card/2020/04/20/8daef326-826d-11ea-81a3-9690c9881111_story.html (Accessed April 27, 2020)

19. Okba NMA, Müller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2−specific antibody responses in coronavirus disease 2019 patients. Emerg Infect Dis. 2020 Jul. https://doi.org/10.3201/eid2607.200841

20. Li Z, Yi Y, Luo X, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. J Med Virol. 2020;0–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32104917

21. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect. 17 Feb 2020;9(1):386–389.

22. Pan Y, Li X, Yang G, et al. Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. J Infect. 2020;S0163-4453(20)30175-4. Available at: https://doi:10.1016/j.jinf.2020.03.051

23. Hoffman T, Nissen K, Krambrich J, et al. (2020) Evaluation of a COVID-19 IgM and IgG rapid test; an efficient tool for assessment of past exposure to SARS- CoV-2, Infection Ecology & Epidemiology, 10:1, 1754538 Available at: https://doi.org/10.1080/20008686.2020.1754538 (Accessed on 21 April 2020)

24. Jin Y, Wang M, Zuo Z, et al. Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. Int J Infect Dis. 2020;94:49–52. Available from: https://doi.org/10.1016/j.ijid.2020.03.065 (Accessed on 21 April 2020)

25. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. Jama. 6 May 2020;2019:2019–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32374370

Based on available evidence up to 15 May 2020.

Disclosure: The authors of this report has no competing interest in this subject and the preparation of this report is totally funded by the Ministry of Health, Malaysia.

Malaysian Health Technology Assessment Section (MaHTAS),
Medical Development Division,
Ministry of Health, Malaysia.