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REVIEW ARTICLE

Diagnostic performance of COVID-19 serology assays

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Abstract

Introduction: The World Health Organization (WHO) declared COVID-19 outbreak as a world pandemic on 12th March 2020. Diagnosis of suspected cases is confirmed by nucleic acid assays with real-time PCR, using respiratory samples. Serology tests are comparatively easier to perform, but their utility may be limited by the performance and the fact that antibodies appear later during the disease course. We aimed to describe the performance data on serological assays for COVID-19. *Materials and Methods:* A review of multiple reports and kit inserts on the diagnostic performance of rapid tests from various manufacturers that are commercially available were performed. Only preliminary data are available currently. *Results:* From a total of nine rapid detection test (RDT) kits, three kits offer total antibody detection, while six kits offer combination SARS-CoV-2 IgM and IgG detection in two separate test lines. All kits are based on colloidal gold-labeled immunochromatography principle and one-step method with results obtained within 15 minutes, using whole blood, serum or plasma samples. The sensitivity for both IgM and IgG tests ranges between 72.7% and 100%, while specificity ranges between 98.7% to 100%. Two immunochromatography using nasopharyngeal or throat swab for detection of COVID-19 specific antigen are also reviewed. *Conclusions:* There is much to determine regarding the value of serological testing in COVID-19 diagnosis and monitoring. More comprehensive evaluations of their performance are rapidly underway. The use of serology methods requires appropriate interpretations of the results and understanding the strengths and limitations of such tests.

Keywords: antibody, antigen, COVID-19, performance, SARS-CoV-2, rapid test, serology

INTRODUCTION

A cluster of pneumonia cases infected with a novel coronavirus was reported in Wuhan, a city in Hubei province of China in December 2019.[1,2] Chinese authorities officially announced a novel coronavirus as the aetiological agent on 7 January 2020. The acute respiratory disease is known as coronavirus disease 2019 or COVID-19.[3] The virus that caused this disease is designated severe acute respiratory syndrome coronavirus 2 or SARS-CoV-2. The World Health Organization (WHO) declared this outbreak a public health emergency of international concern (PHEIC) on 30 January 2020. On 12th of March, WHO declared COVID-19 as a world pandemic.[4]

Workflows for COVID-19 laboratory diagnosis by screening and confirmation steps by molecular method has been designed and validated, with the use of synthetic nucleic acid technology.[5] This was followed by development of numerous nucleic acid assays.[6,7,8] Diagnosis of suspected cases is confirmed by RNA assays with real-time PCR, using respiratory samples.[9,10] RNA-based molecular tests require facilities set up and instruments, with appropriate biosafety measures, skilled laboratory technicians at significant cost. Most centres face shortage and limitation of testing capacity by molecular method, either in the manpower or the resources such as limited equipment and reagents for testing. Other issues that need to be addressed are the safety of healthcare personnel collecting, storing and transporting the samples and laboratory personnel handling and processing the potentially infectious samples.[11,12,13] According to WHO, full personal protective equipment (PPE) which includes gloves, medical masks, goggles or a face shield, and gowns, as well as

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for specific procedures, respirators (i.e., N95 or FFP2 standard or equivalent) and aprons need to be donned to minimize the risk of transmission.[13]

Serology tests are comparatively easier to perform, requiring less technical expertise and equipment compared to nucleic acid detection. Samples are blood that is collected in tubes, which pose less potential risk to the staff handling the samples. It can be performed in a basic clinical laboratory and smaller community settings, therefore reaching a wider application. However, their utility may be limited by the performance issues of rapid tests in general, and the fact that antibodies appear later during the disease course. In this review we aimed to describe the currently available performance data on rapid serological assays for COVID-19 and their potential in diagnosis and management of disease.

MATERIALS AND METHODS

A review of multiple reports and kit inserts on rapid tests from various manufacturers that are commercially available were performed. Only preliminary evaluation data on the diagnostic performance of the kits are available currently, and for some kits external evaluations have been done and reported.

RESULTS

There was a total of nine rapid detection tests (RDTs) available and the kit inserts and evaluation reports were reviewed. A range of serological assays and their performance is shown in Table 1. Three kits offer total antibody detection, while six kits offer combination IgM and IgG detection. All kits are based on the principle of colloidal gold-labeled immunochromatography (ICT) and use capture reaction to detect SARS-CoV-2 IgM/IgG or total antibody in the samples. For combination IgM and IgG kit, the test card or cassette has two test lines (M and G lines) and a quality control line (C line). The M line is fixed with a monoclonal anti-human IgM antibody for detecting SARS-CoV-2 antibody; the G line is fixed with a reagent for detecting SARS-CoV-2 antibody; C line is fixed with a quality control antibody. All kits offer a one-step method with results obtained within 15 minutes. Samples that can be used are whole blood, serum or plasma samples.

Total Ig antibody detection kits

Three kits offer a total Ig antibody test in which the test card has only one test line (T line) and a quality control line (C line). This kit does not differentiate IgM or IgG, thus results are interpreted as positive or negative without suggesting which SARS-CoV-2 antibody is detected. Sensitivity were between 86.4% and 90.6% and specificity were reported to be more than 99%.

Combination IgM and IgG antibodies kit

Six of the rapid test kits provide separate IgM and IgG lines to be visualised on the same test kit. The sensitivity for both IgM and IgG tests ranges between 72.7% and 100%, while specificity ranges between 98.7% to 100%. Some kits reported the sensitivity of individual IgM and IgG tests according to the number of days of illnesses or from initial PCR samples taken. Standard Q IgM/IgG Duo Test (by SD Biosensor) reported that their kits showed sensitivity for IgM and IgG of 91.7% and 79.2% respectively in samples taken 7 days after the PCR samples. Their sensitivity for IgM and IgG increased to 100% in samples nine days and 12 days after initial PCR confirmation, respectively. From this review, all test kits compared their results with PCR results, except PureChek IgG/IgM Test Kit which compared their results with another IgM/IgG results but the details of the compared test kit was not stated in the report. Positive coincidence rate of all kits were between 37.5% and 97.1% and negative coincidence rate were between 96% and 100%.

Antigen detection tests by RDT using respiratory samples

Two antigen detection tests by RDT using respiratory samples are commercially available. Standard Q COVID-19 Ag Test by SD Biosensor is an immunochromatography RDT, using nasopharyngeal or throat swab for detection of COVID-19 specific antigen.[14] Based on 7 samples tested, the test showed sensitivity of 80% and specificity of 100%.[15] Another product by the same manufacturer is Standard F Ag Test Fluorescent Immunoassay (FIA), also using the same sample types and giving results in 30 minutes using an analyser.[16] Based on 7 samples tested, the test showed sensitivity of 100% and specificity of 100%.[17] A positive result using these methods provides initial screening information, requiring retesting with a more specific method for confirmation of infection.

TABLE 1: Serological assays for SARS-CoV-2 and preliminary evaluation data

No.	Manufacturer	Kit name	Antibody detected	Evaluation Study/ Report	Results	Performances Summary
1	Biologix Corporation	SARS-CoV-2 Antibody test	Total Ig	596 clinical case (361 confirmed case; 235 excluded cases by PCR)	Sensitivity: 312/361 = 86.43% Specificity: 234/235 = 99.57%	Sensitivity: 86.43% (95% CI: 82.41% ~ 89.58%) Specificity: 99.57% (95% CI: 97.63% ~ 99.92%) Total consistent = 91.61% (95% CI: 89.10% ~ 93.58%)
2	Guangzhou Wondfo Biotech	WONDFO® One Step COVID-19 (SARS-CoV-2 Antibody) Test	Total Ig	Guangdong. 38 PCR-positive; 10 PCR-negative samples	Positive coincidence rate: 39/38 = 100% Negative coincidence rate: 9/10 = 90% Total coincidence rate: (38+9)/(38+10) = 97.92%	Positive coincidence rate = 100% Negative coincidence rate = 90% Total coincidence rate = 97.92% Kappa value = 0.9344 (95%CI 0.807~1.0613)
3	Zhuhai Livzon Diagnostic Inc.	Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2)	Total Ig	644 samples (286 clinically diagnosed samples; 358 clinically excluded samples)	Sensitivity: 259 / 286 = 90.6% Specificity: 355 / 358 = 99.2% Total accordance rate: (259+355) / 644 = 95.3%	Sensitivity: 90.6% Specificity: 99.2% Total accordance rate: 95.3%
4	Shanghai Eugene Biotech	Eugene® SARS-CoV2 (COVID-19) IgG/IgM Rapid Test	Combo IgM/IgG	A total of 208 subjects; 55 PCR-positive and 153 PCR-negative	Sensitivity: 53/55 = 96.4% Specificity: 151/153 = 98.7%	Relative sensitivity: 96.4% (95% CI:*90.3%-98.6%) Relative specificity: 98.7% (95% CI:*94.5%-99.6%) Accuracy: 98.1% (95% CI:*93.8%-99.3%)

No.	Manufacturer	Kit name	Antibody detected	Evaluation Study/Report	Results	Performances Summary
5	Healgen Scientific	COVID-19 IgG/IgM Rapid Test	Combo IgM/IgG	113 samples (with pneumonia/respiratory symptoms); tested singly - IgM compared to PCR results (99 PCR-positive and 14 PCR-negative). 36 samples during convalescent period tested for IgG.	IgM test: Sensitivity: 87/99 = 87.9% Specificity: 14/14 = 100% IgG test: Sensitivity: 35/36 = 97.2%	IgM: Sensitivity: 87.9% Specificity: 100% IgG: Sensitivity: 97.2% (during convalescent)
				An evaluation in IMR, Malaysia; 22 PCR-positive samples (within 5-8 days of onset) - using combo IgM/IgG cassette	The sensitivity of COVID-19 IgG/IgM Rapid test cassette for IgM against COVID-19 is 72.7% while the sensitivity of IgG against COVID-19 is 50.0% and as this is a combo IgG/IgM, the overall sensitivity is 72.7%	Sensitivity for IgM: 72.7% Sensitivity for IgG: 50.0% Overall sensitivity: 72.7%
6	Qi ngdao Hightop Biotech Co., Ltd	COVID-19 IgM/IgG Ab Rapid test	Combo IgM/IgG	1300 samples - 300 positive, 1000 negative NAT	<u>FOR 300 POSITIVE SAMPLES</u>: IgM Ab rapid test: 246 cases were positive, 54 cases were negative. IgG Ab rapid test: 279 cases were positive. 21 cases were negative. <u>FOR 1000 NEGATIVE SAMPLES</u>: IgM Ab rapid test, 960 cases were negative, 40 cases were positive. IgG Ab rapid test, 975 cases were negative, 25 cases were positive.	Positive coincidence rate: For IgM: 246/(246+54) = 82% For IgG: 279/(279+21) = 93% Negative coincidence rate: For IgM: 960/(40+960) = 96% For IgG: 975/(25+975) = 97.5% Total coincidence rate: For IgM: 92.8% For IgG: 96.5%

No.	Manufacturer	Kit name	Antibody detected	Evaluation Study/Report	Results	Performances Summary
7	VivaChek Laboratories	VivaDiag™ COVID-19 IgM/IgG Rapid test	Combo IgM/IgG	80 positive cases	Positive coincidence rate: For IgM: 65/80 = 81.25% For IgG: 30/80 = 37.5% Total positive coincidence rate = 81.25%	Positive coincidence rate: IgM: 81.25% IgG: 37.5%
				70 positive cases	Positive coincidence rate For IgM: 68/70 = 97.1% For IgG: 67/70 = 95.7% Total positive coincidence rate = 97.1%	Positive coincidence rate: IgM: 97.1% IgG: 95.7%
				50 negative cases	Negative coincidence rate For IgM: 100% For IgG: 100%	Negative coincidence rate: IgM: 100% IgG: 100%
8	SD Biosensor	Standard™ Q COVID-19 IgM/IgG Duo Test	Combo IgM/IgG	40 samples (24 PCR-pos; 16 PCR-neg);	Specificity: 100% (16/16)	Specificity = 100%
				After 7 days of initial PCR	Sensitivity: For IgM: 22/24 (91.7%) For IgG: 19/24 (79.2%)	Sensitivity: IgM = 91.7% IgG = 79.2%
				After 9 days of initial PCR (for IgM) & 12 days after initial PCR (for IgG)	Sensitivity: For IgM: 24/24 (100%) For IgG: 24/24 (100%)	Sensitivity: IgM = 100% IgG = 100%

No.	Manufacturer	Kit name	Antibody detected	Evaluation Study/Report	Results	Performances Summary
9	PureChek	COVID-19 IgG/IgM Rapid Test	Combo IgM/IgG	IgM-positive specimens (n=112), patients who have been diagnosed and are in acute development. Analysis results were compared with other IgG/IgM test	For IgM Positive coincidence rate 33/34=97.06% Negative coincidence rate 76/78=97.44% Total coincidence rate 109/112 = 97.32% Kappa value 0.9372 (highly consistent)	For IgM (acute samples): Positive predictive value: 94.29% Negative predictive value: 98.70%
				IgG-positive specimens (n=106), patients who have been diagnosed and are recovering	For IgG Positive coincidence rate 33/34=97.06% Negative coincidence rate 71/72=98.61% Total coincidence rate 104/106 = 98.11% Kappa value 0.9567 (highly consistent)	For IgG (recovering samples) Positive predictive value: 97.06% Negative predictive value: 98.61%

CI = Confidence Intervals; Ig = Immunoglobulin; NAT = nucleic acid testing; IMR = The Institute for Medical Research, Kuala Lumpur

Note:

1. Biologix Corporation. SARS-CoV-2 Antibody Test (Lateral Flow Method) Catalog No: W195 (kit insert). Shandong, China.
2. i) Guangzhou Wondfo Biotech. One Step COVID-19 (SARS-CoV-2 Antibody) Test (Immunochromatography Assay) Catalog No: W195 (kit insert). Guangzhou, China. ii) Chunrong Huang & Ruimao Lin. Clinical Report of Guangdong No.2 People's Hospital, 1 Feb 2020.
3. i) Zhuhai Livzon Diagnostic Inc. Livzon Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Lateral Flow). Guangdong, China. ii) Zhuhai Livzon Diagnostic Inc. FAQ for 2019-nCoV IgM/IgG Antibody Detection Kit.
4. Shanghai Eugene Biotech. Eugene® SARS-CoV-2 (COVID-19) IgG/IgM Rapid Test (REF RINCOV2) (kit insert). Shanghai, China.
5. i) Healgen Scientific. Product brochure for Healgen COVID-19 IgG/IgM Rapid Test Cassette. ii) Healgen Scientific. Package Insert for Healgen COVID-19 IgG/IgM Rapid Test Cassette. iii) Clinical Samples Study Report COVID-19 IgG/IgM Rapid Test Cassette. Houston, Texas, USA. iv) Report on the brief evaluation of a COVID 19 IgG/IgM Rapid Test Cassette, 28 Feb 2020. The Institute for Medical Research, Kuala Lumpur, Malaysia.
6. i) Qingdao Hightop Biotech Co., Ltd. COVID-19 IgM/IgG Ab Rapid test (Immunochromatography) (kit insert) Qingdao, Shandong, China. ii) Comparative Test Report Novel Coronavirus (COVID-19) IgM Ab Rapid Test (Immunochromatographic Method) Version 3.0.
7. VivaChek Laboratories, Inc. VivaDiag™ COVID-19 IgM/IgG Rapid test (Ref VID35-08-011). Wilmington, USA.
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9. PureChek. Clinical Performance Evaluation Report Novel Coronavirus (2019-nCoV) IgG/IgM Test Kit (Colloidal Gold), 10 Mar 2020.

DISCUSSION

The decision to test is based on clinical and epidemiological factors, such as exposure to confirmed positive cases or history of travel to or residing in affected countries within the last 14 days before the onset of illness.[10] PCR is used in the diagnosis of COVID-19 in those with respiratory illness, as well as screening of contacts. SARS-CoV-2 virus can initially be detected 1 to 2 days prior to onset of symptoms in the upper respiratory samples and can persist for 7 to 12 days in moderate cases and up to 2 weeks in severe cases.[18] Molecular tests which detect viral RNA may have false negative results. Even though analytical sensitivity is generally known to be very high, detection is dependent on several crucial factors such as sampling timing related to the day of illness, sample types,[19] correct sampling technique, sample quality, transport and storage conditions, detection kits and gene target sequence mutations. A study in China using PCR detection showed that bronchoalveolar lavage samples had the highest positive rates (93%), followed by sputum (72%), nasal swabs (63%) and pharyngeal swabs (32%).[19] PCR method may not detect the virus in the very early stage of infection or late stage when the viral load is very low. The value of antigen detection RDTs using respiratory samples is also subject to further evaluation, as they would also be dependent on the same sample factors, with the advantage of a simplified laboratory procedure.

Serological assay detects presence of IgG, IgM or both. A positive interpretation has been defined as a positive lgM, or convalescent sera with an increased lgG titer more than 4 times than that in the acute phase. COVID-19 IgG and IgM are detected using whole blood, serum or plasma. Antibodies rise late in the course of illness, where the median duration of COVID-19 IgM antibody detection was found to be 5 days, while IgG detection around 14 days after symptom onset.[20] Antibody dynamics was found to be similar to an acute viral infection, where IgG levels increase as IgM levels start to decrease.[3] Unlike respiratory samples which may suffer from false negative results due to sampling factors, the presence of antibodies in blood can be uniformly detected.[21] Samples are easier to obtain compared to respiratory samples, involving less risk to the operator. The test is easy to operate, rapid, requires no instrumentation and can provide results in just 15 minutes. With shortage of PCR tests, availability of serological tests could also provide a crucial diagnostic method, with a different role. It offers a good alternative especially for community clinics and smaller community hospital labs that do not have access to the equipment and expertise needed for molecular testing. The advantage of cheap, rapid tests for healthcare workers for example, would allow them to be cleared and return to work. Furthermore, the availability and use of automated ELISA platforms in future has the potential of high testing capacity compared to PCR assays.

However, serology results alone cannot confirm or exclude diagnosis or inform infection status. Serology-based tests are not currently recommended by health organisations for diagnosis. Interpretations of results require an understanding of the strengths and limitations of serological assays, and further testing which may be required. Reporting test results may include information that a negative result does not rule out SARS-CoV-2 infection, particularly in those who have been in close contact with confirmed positive cases. Follow-up testing with a molecular diagnostic test should be considered to rule out infection in individuals with risk factors or history of exposure. SARS-CoV-2 belongs to betacoronavirus, in the same family as SARS-CoV and MERS-CoV. Thus there is possibility of cross-reactivity with other coronaviruses occurring.[21] Positive results may also be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E. Cross-reactivity with these other coronaviruses and other bat-related SARS coronaviruses remains to be clearly determined.

Most evaluation reports of these rapid test kits compared the performance against PCR as the gold standard. Evaluation of serology tests are therefore not quite straightforward, since PCR detects viral nucleic acid while serology tests detect antibodies or host response to the infection. The virus can be detected 1–2 days prior to symptom onset in upper respiratory tract samples, and can persist for 7–12 days in moderate cases and up to 2 weeks in severe cases.[18] The comparison has to include evaluation of antibody profiles and timeline during disease, early stage vs late stage of infection or convalescence period. Sensitivity of detection for the different modalities would depend largely on the time of testing or day of illness. To date, only one serology test has been validated by the US Food and Drug Administration (FDA) in the Emergency Use Authorisation (EUA) category,[22]

which is the Cellex qSARS-CoV-2 IgG/IgM Rapid Test. More than 70 other test developers have since notified the FDA of the availability of their serological tests.

Serology method is incorporated into the current local guideline for testing asymptomatic contacts of positive cases, at day 13 of home surveillance.[10] Negative IgM at this point would allow discharge of the case from quarantine. A similar approach is used in Italy, where viral clearance is indicated by negative PCR accompanied by specific IgG detection.[23] Apart from the potential use of serology assays in contact screening, detection of antibodies has been shown to improve diagnosis of positive cases.[20,24] Antibody kinetics data from China showed that positive detection rate increased significantly, when PCR is used in combination with IgM ELISA assay (98.6%) compared to PCR alone (51.9%), and using antibody detection can improve diagnosis of COVID-19 including subclinical cases.[20] Compared to PCR, the IgM detection rate was reported to be lower in the first 5 days post symptom onset (100% for PCR vs.71.4% for IgM), but was higher afterwards (44.3% for PCR vs 87.9% for IgM).[20]

Incorporation of serology assays in diagnostic algorithms and discharge criteria may ease the burden or divert the workload from nucleic acid detection, which is applicable for some clinical situations.[21] Serology assays may be a tool in studying the seroepidemiology of COVID-19. Testing for antibodies may enable assessment of the true scope of COVID-19 infections, among asymptomatic or subclinical infection, as well as symptomatic cases. Seroepidemiology studies enable estimation of the proportion or extent of the population which has already been infected in the community, and the epidemiology of the infection across different demographics retrospectively. Availability of tests with good performance will give a more accurate picture of the overall spread of COVID-19.

Further data need to be explored such as description of antibody profiles in COVID-19 infections, presence of antibodies and correlates of protective immunity, and duration of protection among other things. These assessments will guide the use of serology tests in diagnosis, management of disease and assessment of epidemiology of infection. Improved performance of serological testing may provide information for public and healthcare workers assessment and monitoring. Assessment of immunity in population, particularly in areas identified as hotspots may inform future response and strategies in future waves of the pandemic.

CONCLUSION

There is much to determine regarding the value of serological testing in COVID-19 diagnosis and monitoring. More comprehensive evaluations of the performance of serology tests are rapidly underway. Considerations for the use of serology methods for COVID-19 require the correct and appropriate interpretations of the results and understanding the strengths and limitations of such tests.

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