Comparison of Serological Assays For The Detection of **SARS-CoV-2** Antibodies

TECHNICAL WHITE PAPER

SPRING HEALTHCARE

INTRODUCTION

As of the 4th of October 2020, over 34 million cases of SARS-CoV-2 have been confirmed worldwide, accounting for more than 1 million deaths (https://covid19.who.int/). Although treatment and vaccines are important to cure and prevent a disease, screening of an infection is the first and the most important step toward cure. Screening may help to understand the magnitude and behavior of the epidemic to ascertain the methods of prevention and cure.

The most common screening test available for COVID-19 yet is RT-PCR that has its own shortcomings. There is a dire need of a fast, accurate and reliable test. In this regard, antibody screening tests are promising. Spring Healthcare Services AG has developed a highly sensitive but reliable rapid test. The present white paper presents a rigorous evaluation of this test by using samples from patients with and without COVID-19.

RATIONALE

The RT-PCR are highly sensitive and reliable tests but slow as being laboratory method need to send samples to the specialized laboratories. These tests are also useful for an early detection of the infection. However, viral load gets decline with the time lapsed. Therefore, the RT-PCR can give false negative if samples are taken after onset of symptoms. This suggests need of an accurate and reliable test that along with RT-PCR would increase the screening capacity and avoid false negatives. Antibodies screening would also help to understand the epidemiology for the control, management and treatment options.

Spring Healthcare Services AG have developed a SARS-CoV-2 IgM/IgG Combined Ab Assay that is an Immunochromatographic rapid test intended for qualitative detection of IgM & IgG antibodies specific to SARS-CoV-2 in samples from human. The SARS-CoV-2 IgM/IgG Combined Ab Assay is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. This test produces valid and reliable results by using a minute sample volume and various types of samples. It also does not need any technical expert and gives results in a very short time.

STUDY OUTCOMES

This evaluation presented different salient features of the SARS-CoV-2 Ab rapid test in terms of sensitivity, specificity and accuracy of the antibody test(s) in comparison to the nucleic acid (RT-PCR) test.

METHOD

a) Samples

In this trial, 1300 clinical samples were selected. There were 300 positive samples and 1000 negative samples. The SARS-CoV-2 IgM Ab rapid test and the SARS-CoV-2 PCR test were detected simultaneously, and the positive coincidence rate, negative coincidence rate, and total coincidence rate were calculated.

b) Development of test

The test uses anti-human IgM antibody (test line IgM) , anti-human IgG (test line IgG) and rabbit IgG (control line C) immobilized on a nitrocellulose strip. The burgundy colored conjugate pad contains colloidal gold conjugated to recombinant COVID-19 antigens (SARS-CoV-2 Spike S1 antigen) conjugated with colloid gold (COVID-19 conjugates). These antibodies detect IgM and IgG antibodies only specific to SARS-CoV-2.

c) Test performance

The blood was drawn with a sterilized needle. The test cassette, specimen, buffer and controls were allowed to equilibrate to room temperature (15-30˚C) prior to testing. The test cassettes were then removed from the sealed foil pouch and were used instantly. The device was placed on a clean and level surface. By using a 5 µL mini plastic dropper in vertical manner to transfer 1 drop of whole blood (about 10 µL) to the specimen well (S) of the test device, then added 2 drops (about 80 µL) of sample buffer to the buffer well (B) immediately.

d) Reading

The colored line in the control line region (C) changed from blue to red, and 2 colored lines appeared in test line regions M and G. The test results indicated the presence of IgM and IgG anti-SARS-CoV-2 antibodies.

RESULT

a) IgM

Table 1. presents the result of screening of COVID-19 specific IgM antibodies in patients that were confirmed by using RT-PCR. Out of total 300 cases of positive samples confirmed by RT-PCR when tested with SARS-CoV-2 IgM Ab rapid test gave 276 cases positive, and 24 cases as negative. Further, a total of 1000 cases that were confirmed negative by RT-PCR when tested with SARS-CoV-2 IgM Ab rapid test all 1000 were negative for IgM.

b) IgG

Table 2. presents the result of screening of COVID-19 specific IgG antibodies in patients that were confirmed by using RT-PCR. Out of total 300 cases of positive samples confirmed by PCR Test when tested with SARS-CoV-2 IgG Ab rapid test gave 288 cases positive, and 12 cases as negative. Further, a total of 1000 cases that were confirmed negative by PCR Test when tested with SARS-CoV-2 IgG Ab rapid test, 1000 cases were negative, 0 cases were positive.

c) Specificity, Sensitivity and Accuracy

Table 3. presents the values of specificity, sensitivity and accuracy of the SARS-CoV-2 IgM and IgG Ab rapid test. The sensitivity, specificity and accuracy for IgM were 92%, 100% and 96%, respectively. The values of sensitivity, specificity and accuracy for IgG were 96%, 100% and 98%, respectively

d) Cross Reactivity Study:

Table 4. presents the results of cross reactivities with various other pathogens. Specimens which tested positive with following various agents from patients were investigated with SARS-CoV-2 IgM and IgG Ab Rapid Test (Lateral Flow Method). The results showed no cross reactivity.

d) Interferences Study:

Table 5. presents the results of interference assay. The test result of SARS-CoV-2 IgM and IgG Ab Rapid Test (Lateral Flow Method) does not interfere with the substance at the following concentration:

Table 1. Comparison of results of rapid test for IgM antibodies and PCR test.

SARS-CoV-2 IgM Ab Rapid Test	PCR Test		Total
	Positive	Negative	
Positive	276	0	276
Negative	24	1000	1024
Total	300	1000	1300

Table 2. Comparison of results of rapid test for IgG antibodies and PCR test.

SARS-CoV-2 IgG Ab Rapid Test	PCR Test		Total
	Positive	Negative	
Positive	288	0	304
Negative	12	1000	1012
Total	300	1000	1300

Table 2. Comparison of results of rapid test for IgG antibodies and PCR test.

Antibody	Measure	Calculations	Estimates
IgM	Sensitivity (Positive coincidence rate)	276/(276+24)x100%	92%
	Specificity (Negative coincidence rate)	1000/(0+1000)×100%	100%
	Accuracy (Total coincidence rate)	(276+1000)/(276+24+0+1000×100%	96%
IgG	Sensitivity (Positive coincidence rate)	288/(288+12)×100%	96%
	Specificity (Negative coincidence rate)	1000/(0+1000)×100%	100%
	Accuracy (Total coincidence rate)	288+1000/(288+12+0+1000×100%	98%

Table 4. Assay cross reactivity results

Pathogens	Cross reactivity
Parainfluenza virus antibody	0
Influenza A antibody	0
Influenza B antibody	0
Chlamydia pneumonia antibody	0
Mycoplasma pneumoniae antibody	0
Adenovirus antibody	0
Respiratory syncytial virus antibody	0
Hepatitis B surface antibody	0
Hepatitis B surface antibody	0
Hepatitis C virus antibody	0
Treponema pallidum antibody	0
HIV antibody	0
EB virus antibody	0
Measles virus antibody	0
Cytomegalovirus antibody	0
Enterovirus type 71 antibody	0
Mumps antibody	0
Varicella-zoster virus positive sample	0

Table 5. Assay interfering substance results

Substance	Concentration
Bilirubin	250 umol/L
Hemoglobin	9 g/L
Triglyceride	15 mmol/L
Rheumatoid factors	80 IU/mL
Antinuclear antibody (ANA) titer	1:240
Anti-mitochondrial antibody (AMA)	80 U/mL
Mouse IgG	1000 ug/ml

RESULT

Recommendations

The comparisons of Spring Healthcare Services AG SARS-CoV-2 IgM and IgG Ab Rapid Test with RT-PCR by using identical clinical samples provided a robust assessment of the rapid test. In this study, cross-comparison of overall specificities, sensitivities and accuracies confirms validity and reliability of the Spring Healthcare Services AG rapid test. Therefore, the present study endorsed the Spring Healthcare Services AG rapid test for use, and suspected that previous publications showing insufficient sensitivity of rapid tests were due to bad quality of samples, non-specific antibodies used for the development of tests, and mistakes in performance of test. This test replicated all the results same to those produced by RT-PCR. This test has advantage over other test methods due to ease of use, option of using multiple types of samples, cost-effectiveness, fast and reliable results. This test could be adopted in laboratory and non-laboratory setting due to the above-mentioned advantages. In hospital or laboratory setting it can be used as a preliminary test that may help to do advanced decision rapidly, such as isolation, or recommendation of further tests and follow up. In non-laboratory setting it can help to understand epidemiology of the infection and decision about isolation.

Screening Timelines

As already known that IgM appears first post-incubation of a pathogen and IgG appears later. This rapid test is sensitive to the concentration of antibodies; therefore, the device should be used at the right timepoint post infection. Screening by using this test at early stages may give a negative result as the patient's blood lacks antibodies because body could not produce the antibodies yet. Therefore, if used by following the screening protocol at the correct timelines the Spring Healthcare Services AG rapid test can be promising to screen antibodies specific to COVID-19 in a valid and reliable way.

RESULT

The Spring Healthcare Services AG SARS-CoV-2 IgM and IgG Ab Rapid Test showed excellent results overall. The test assures a complementary testing method to RT-PCR test. The high values of specificity, sensitivity and accuracy of the test recommend to use this test confidently to understand dynamics of epidemics in populations for prevention, control and management decision well in time.

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For the full study please visit: https://www.medrxiv.org/content/10.1101/2020.06.02.20120345v1.full.pdf