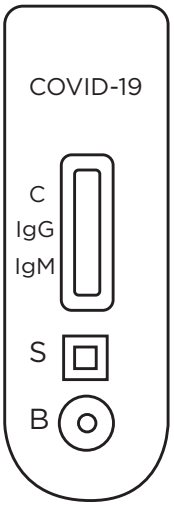
Coronavirus

Technical White Paper (3)

SureScreen Diagnostics

Clinical Assessments of COVID-19 Rapid Immunoassay Test



Study Details:
902 samples from patients in Hubei, China. 446 tested for IgG and 456 tested for IgM

Procedure:
Each sample analysed using test cassette. Simultaneously, SARS-CoV-2/IgG/IgM presence confirming using lab test

Results:
For both IgG and IgM
Sensitivity > 91%
Specificity > 99%
Accuracy > 97%

Background

Recently, a new strain of coronavirus (SARS-CoV-2) has been identified as causing human illness, which has been named COVID-19 (coronavirus disease 2019). Originating in Wuhan, Hubei, China in December 2019, it has now spread to over 50 countries. To date, there have been over 300,000 confirmed cases and 13,000 deaths[1], and cases continue to rise, prompting the WHO to declare it a pandemic. Symptoms typically take 2-14 days to appear and display as fever, dry cough, sore throat, headache and muscle pains[2]. Most cases are mild, and patients recover quickly. However, a small number of cases are severe and require medical attention. The current fatality rate is 1-2%[2].

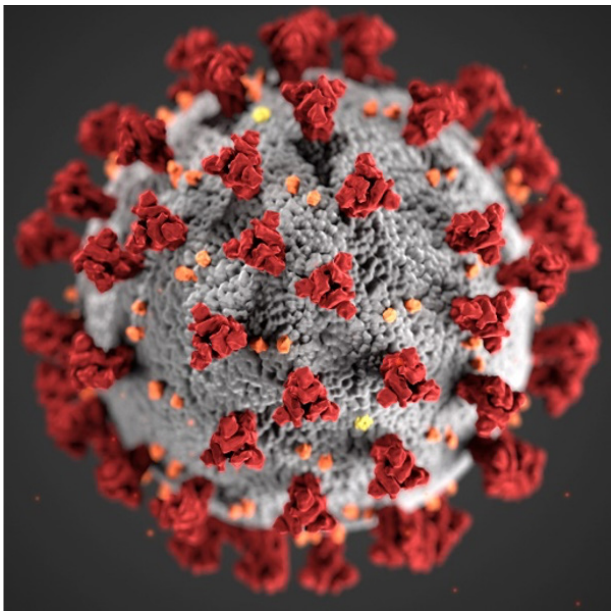


Fig. 1. An illustration of coronavirus, showing the surface proteins/antigens in red[3].

Current diagnosis of COVID-19 involves measuring body temperature, CT scans and Real-Time PCR (RT-PCR), which sequences the viral genome, allowing identification of SARS-CoV-2. However, RT-PCR test kits have some limitations; they take at least 2 hours to obtain a result, they require certified laboratories and personnel and they are known to give several false positives[4]. Hence, there is a need for rapid diagnostics, which are easy to use and can give fast results from the lowest volume of sample possible.

IgG and IgM

Immunoglobulins are immune glycoproteins, also generally known as antibodies. When a person is infected by a pathogen (such as SARS-CoV-2), immunoglobulins are produced by B-cells (a type of white blood cell) in response to it. The immunoglobulins bind to specific antigens on the surface of the pathogen, which 'flags' the pathogen as foreign matter. This triggers a secondary immune response, including the complement system[5], which attacks and clears the pathogen.

Immunoglobulin M (IgM) is the largest immunoglobulin in humans and is the first antibody to be produced upon infection. Since it has multiple antigen binding sites, it can be thought of as a 'general' antibody and is compatible with many pathogen species/types. In the case of COVID-19, IgM levels in the blood rise to a detectable level 3-7 days after the onset of infection.

Immunoglobulin G (IgG) is a smaller structure and is produced in response to a specific antigen on the pathogen. Its levels rise later than IgM (>7 days) and indicates exposure to the SARS-CoV-2 sometime previously[6].

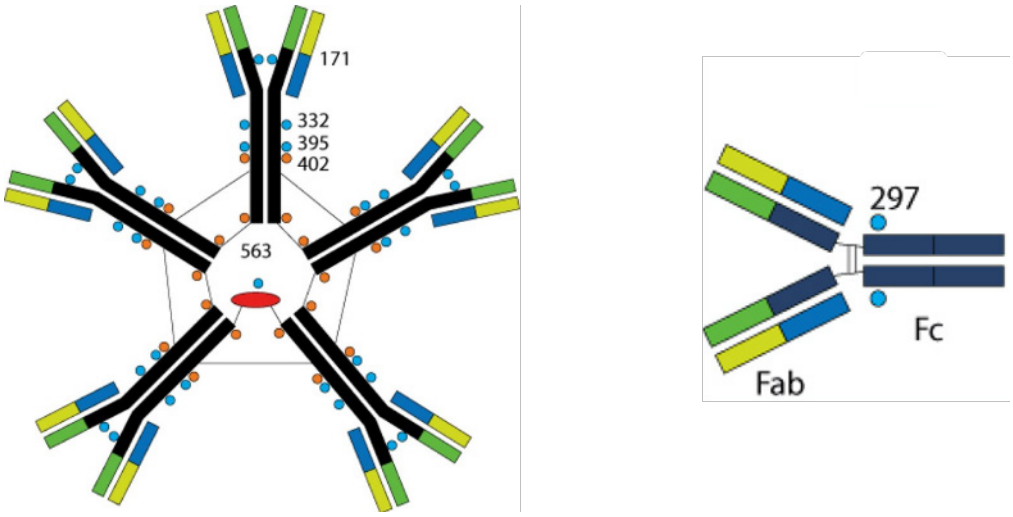


Fig. 2. The structures of IgM (L) and IgG (R)[5].

IgG also plays a role in long term immunity and immunological memory[7]. How long IgG remains in the circulation after the infection has cleared is highly variable. IgG has a circulation half-life of 7-30 days[8], and it may be detected after many half lives. This may allow immunity/immunological memory to be confirmed in patients who contract COVID-19.

The combination of IgG and IgM can be used to detect early and late stage COVID-19, as well as long-term immunity after recovery. In this context, it is important that the IgG is specific to SARS-CoV-2. Recently, IgG specific for the SARS-CoV-2 virus has been isolated[9-11] from the serum of patients who had contracted COVID-19. This specific IgG and the genralised IgM can be used to assemble a simple lateral flow immunoassay diagnostic test for the novel coronavirus.

Rapid Immunoassay Test Cassette

SureScreen Diagnostics has developed a rapid immunoassay test, which detects the presence of the SARS-CoV-2 specific IgG and general IgM for the qualitative diagnosis of early and late stage COVID-19. As mentioned, IgG is specific to SARS-CoV-2 and confirms secondary illness (usually >7 days), whereas IgM confirms primary illness (3-7 days). Both are elevated in the secondary illness and can be used to confirm diagnosis. Hypothetically, detection of these biomarkers should rule out other viral infections and false diagnosis, allowing confirmation of COVID-19.

To assemble the cassette, anti-human IgG and IgM are mobilised onto a nitrocellulose membrane to generate the two test lines and other antibodies are used to generate the control line. The conjugation pad contains gold nanoparticles conjugated to either COVID-19 specific antigen (for test) or the other antibodies (for control). All components are assembled onto an adhesive card base and enclosed in a plastic casing.

For testing, the sample (whole blood, serum or plasma) is placed at the inlet, before the addition of a buffer solution. Any IgG/IgM present in the sample will bind to the gold-COVID-19 antigen conjugate and flow to the two test lines, which will capture any IgG/IgM in the sample. The control line will then capture the other gold-antibody conjugate, confirming the test is valid. By 10 minutes, test lines will appear for both IgG and IgM for a positive result, and a control line for quality control. Only one test line (IgG or IgM) is required for a positive diagnosis. This test has the advantages of being portable, rapid and low cost.

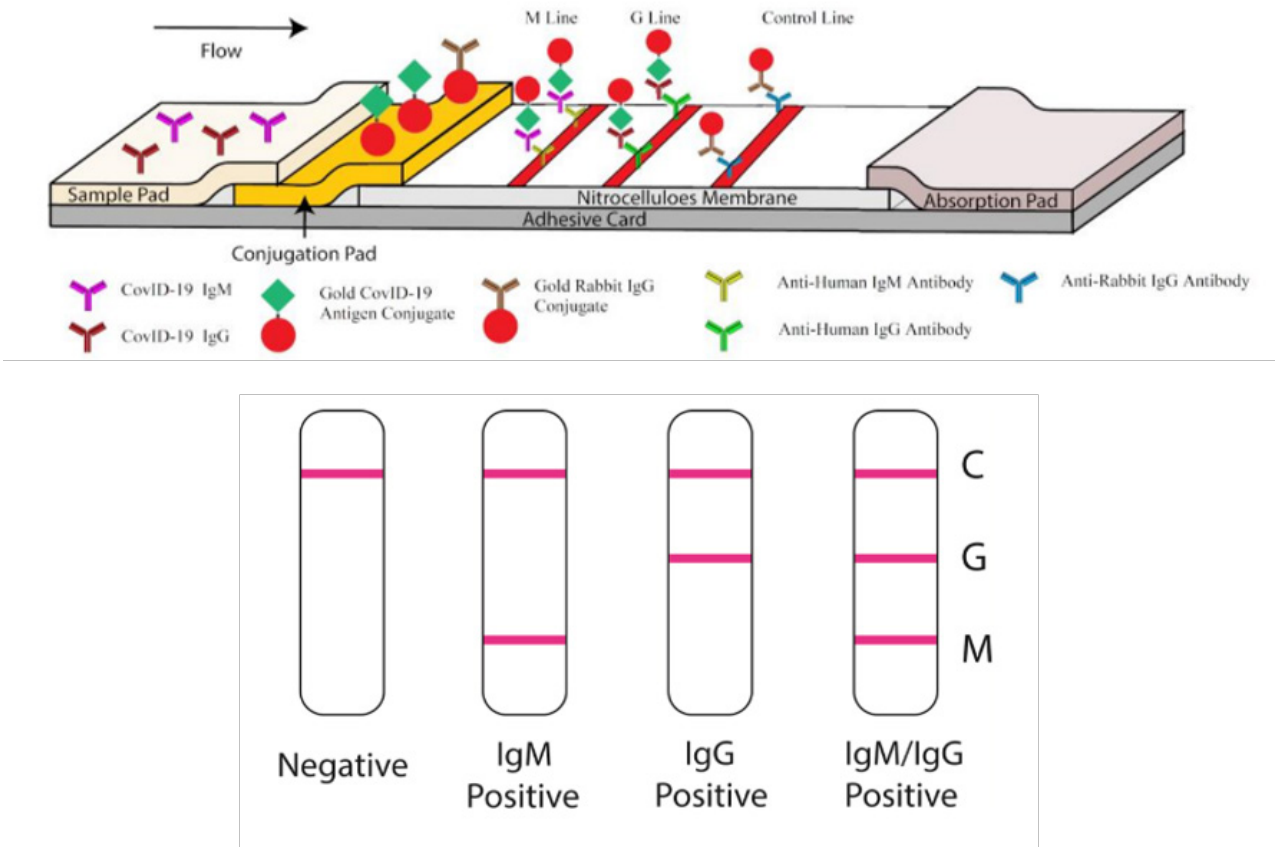


Fig. 2. A schematic of the SARS-CoV-2 lateral flow immunoassay test.

To test the sensitivity, specificity and accuracy of the test cassette, tests were sent to the epicenter of the outbreak, Hubei, China. Results were compared for the same samples between the test cassette and the standard laboratory method(s) of detecting the immunoglobulins, mainly ELISA.

Study Details

446 samples were tested for IgG and 456 samples for IgM. Samples were obtained from patients from Hubei and all analysed using the same clinical protocol. Blood samples were taken and centrifuged to obtain plasma, which was tested using the cassette and compared with the clinical diagnostic procedure (IgG and IgM analysis).

Results

*Comparison between clinical diagnosis and COVID-19 rapid test cassette – **IgG results***		Clinical Diagnosis (Confirmed)		Total Results
		Positive	Negative	
Rapid Test Cassette	Positive	75	2	77
	Negative	0	369	369
Total Results		75	371	446

*Comparison between clinical diagnosis and COVID-19 rapid test cassette – **IgM results***		Clinical Diagnosis (Confirmed)		Total Results
		Positive	Negative	
Rapid Test Cassette	Positive	78	3	81
	Negative	7	368	375
Total Results		85	371	456

In summary:

IgG sensitivity	100% (75/75)
IgG specificity	99.5% (369/371)
IgG Accuracy	99.6% (444/446)
IgM sensitivity	91.8% (78/85)
IgM specificity	99.2% (368/371)
IgM Accuracy	97.8% (446/456)

Conclusion

Overall, the SARS-CoV-2 rapid immunoassay test is suitable for both clinical diagnosis and screening of COVID-19. Providing it is used correctly, it has a high sensitivity, specificity and accuracy against two key biomarkers of COVID-19, IgG and IgM. Furthermore, a variety of sample types can be used (whole blood, plasma, serum) at low volumes (single droplets). The test cassette is therefore a useful tool in the diagnosis of COVID-19 and can act as a first line screening test prior to clinical assessment. Additionally, patients who recovered from the illness continued to test positive for IgG for at least 33 days after displaying symptoms, indicating that the test could be used to assess longer term immunity. Moreover, the test cassette does not react with Rheumatoid Factor and will therefore not have the common cross-reactivity issues associated with other immunoassay tests.

References

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