Comparison of serological assays for the detection of SARS-CoV-2 antibodies

Technical White Paper

SureScreen Diagnostics

Introduction

As of the 1st June 2020, there have been over 6 million confirmed cases of COVID-19 worldwide, with almost 400,000 deaths (https://covid19.who.int/). The WHO, as well as many governments, have emphasised the necessity of increased testing as a means of screening the disease, understanding its epidemiology, and potentially assessing the safety of the public to return to work. Real time (RT) PCR (or antigen testing) is currently the most common method of detecting the virus, however, there are drawbacks to this method of testing.

In order to assess the usefulness of antibody tests, seven LFIAs (including SureScreen's device), one chemiluminescent assay and two commercial ELISA tests were rigorously tested at London's Guy's and St. Thomas' Hospital. Evaluation was conducted using samples from patients with and without COVID-19 infection. Additionally, King's College London developed a highly sensitive ELISA method for the detection of the antibodies, allowing the performance of these LFIA tests to be compared directly against the laboratory method. This White Paper discusses this independent evaluation study.

Antigen and Antibody Testing

Whilst PCR antigen testing is currently the main screening tool adopted in the UK, the research conducted at Guy's and St. Thomas's noted that there is a clear requirement for accurate serology testing as a companion diagnostic to PCR-based testing. Using devices such as the SureScreen test alongside PCR will lead to increased accuracy in testing, allowing a wider detection window (period of time from infection when a positive result can be found). This kind of testing would provide solutions to the delayed-onset syndromes such as paediatric inflammatory multiorgan syndrome (PIMS), that are increasingly being reported post-peak pandemic.

Monitoring population seroprevalence (level of antibodies in the population) will also be central to future public health planning based on disease susceptibility and herd immunity. For this to be meaningful, it is imperative that antibody detection methods are affordable, reliable, and readily accessible.

SureScreen Diagnostics have developed a rapid lateral flow immunoassay (LFIA) test for the qualitative detection of two antibodies specific to SARS-CoV-2, namely Immunoglobulin M and G (IgM and IgG). Advantages of this test are low sample volume, multiple sample types, speed of test, ease of use, cost-effectiveness and easy to read results.

Study Outcomes

The study allowed many different aspects of the testing kits and individuals' antibody response to be analysed:

- Sensitivity, specificity and accuracy of the antibody test(s), as functions of time and disease severity
- Antibody levels over time post infection
- Comparison of tests from different manufacturers

Methods

Patient and sample collection
110 serum samples were collected from 87 patients diagnosed with COVID-19 at Guy's and St. Thomas' hospital between 4th March and 21st April 2020. Cases were diagnosed using RT-PCT from nasopharyngeal samples (antigen positive). Samples ranged from 1 to 30 days after onset of self-reported symptoms. For the longitudinal study (antibody levels over time), 17 serum samples (days 6 to 24 days post symptoms) were obtained from 5 patients (3-4 samples each). Two patients overlapped between the two studies, meaning in total there were 90 unique infected patients.

Pre-pandemic (negative) samples were also used; 50 serum samples from 50 patients at the same hospital in March 2019 and plasma samples taken from patients 7 days after H1N1 vaccination (as part of a trial).

Development of in-house ELISA test
As a positive control for antibody testing, King's College London prepared a highly specific and sensitive laboratory ELISA method for the detection of SARS-CoV-2 specific antibodies. This was achieved by isolating three virus specific proteins. Using these proteins as the basis of the ELISA method ensured that only antibodies associated with SARS-CoV-2 were detected.

Assessment of test kits
To determine the specificity and sensitivity of the test kits, the same clinical samples were run across all tests. Firstly, they were run through the ELISA, then through each of the LFIA devices and commercial ELISA test kits. All results were noted (IgM and IgG). The results were then compared across all platforms.

All tests were performed in line with their instructions for use.

Results

Antibody levels over time
In general, there was an increase in both IgM and IgG levels in the samples over time from onset of symptoms. Although IgM was detected in some patients using ELISA from day 1 post symptoms, it was not detected in all of the patients until day 9. IgG was also detected in some patients at day 1 but was not detected in 100% of patients until day 16. This gives an indication of the timing of when the two antibodies are produced in response to the infection and illustrates that the production of antibodies is different for each person.

During the initial period after symptoms, there is only very small concentrations of antibodies present in the blood, at levels which are too low to be detected. The levels of antibodies rapidly increased however after this period, and between days 20 to 30 post symptoms, both antibodies were detected in all samples, except one.

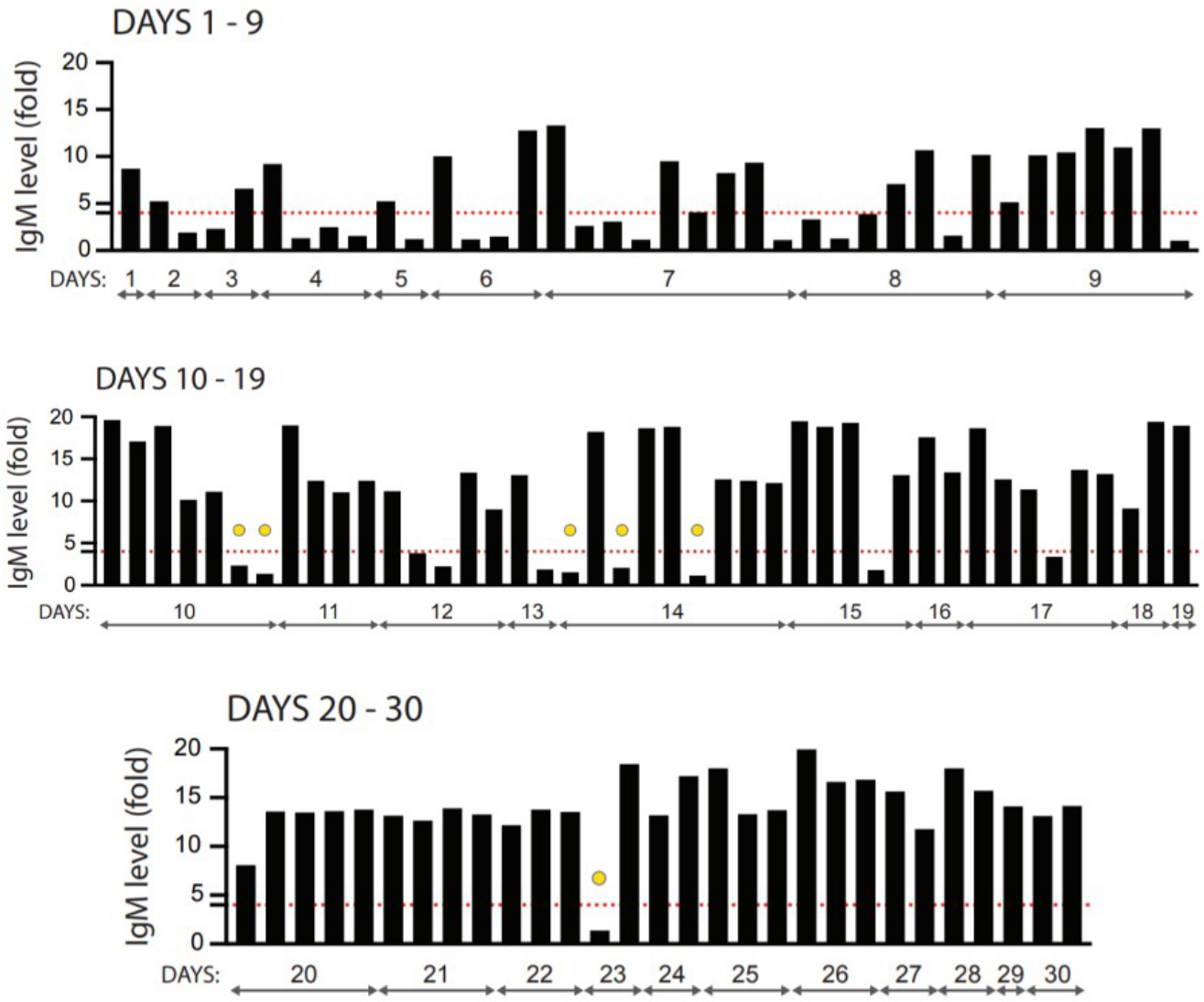

Fig. 1. IgM antibody levels over time post onset of symptoms, as determined by ELISA. Each bar represents an individual result. The red dashed line represents the cut-off level, above which a true positive is confirmed. Yellow circled represent an antibody negative result. Antibody levels can be seen to increase over time.

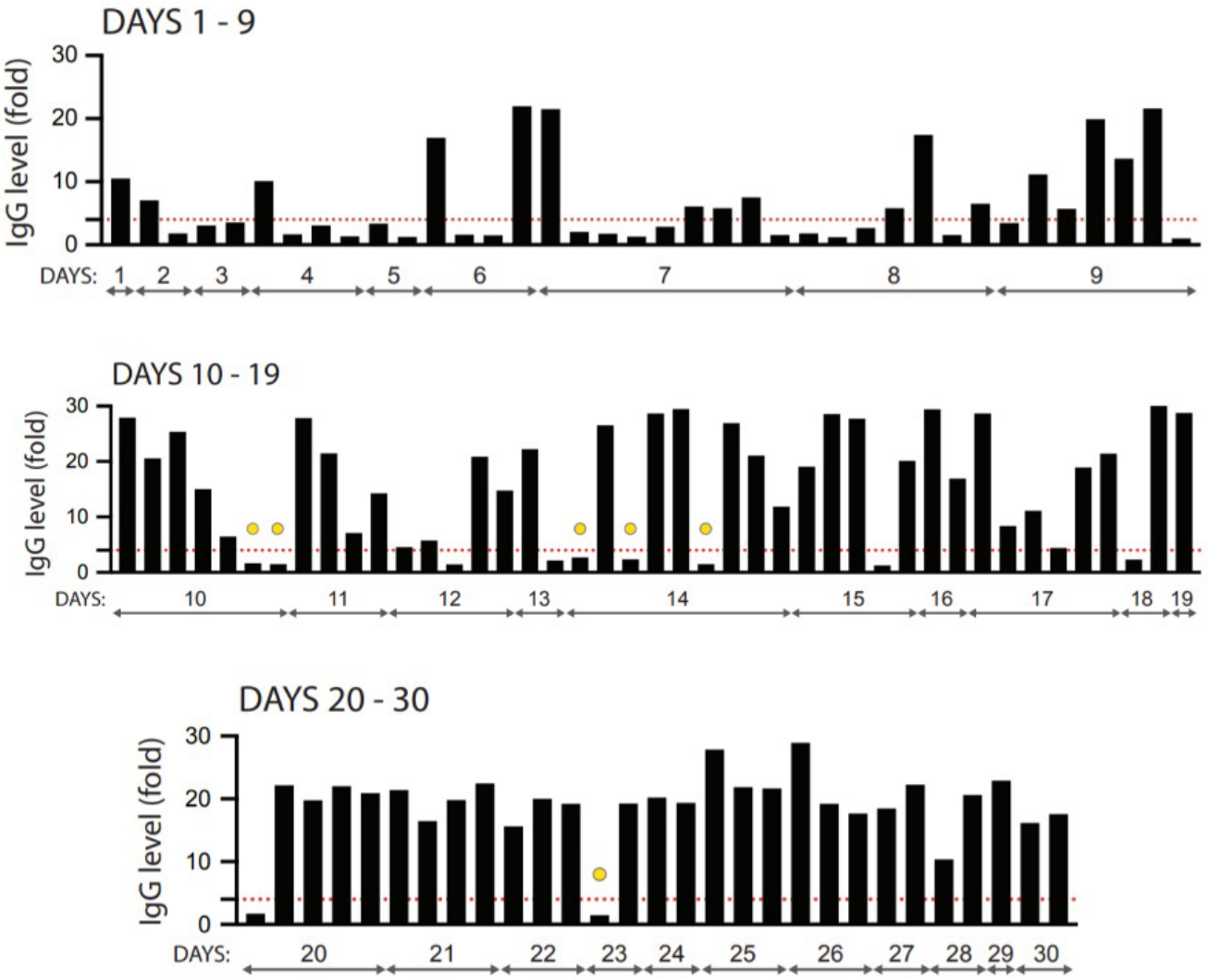

Fig. 2. IgG antibody levels over time post onset of symptoms, as determined by ELISA. Each bar represents an individual result. The red dashed line represents the cut-off level, above which a true positive is confirmed. Yellow circled represent an antibody negative result. Antibody levels can be seen to increase over time.

Performance of tests compared

Specificity
There was a wide variation in the performance of the different tests, with specificity (accurately diagnosing negative results) ranging from 82% to 100%.

Specificity was calculated to be 100% on the SureScreen test (50 out of 50 negative results reported correctly), with a 95% confidence interval of 92.87 – 100%. The SureScreen device had joint highest specificity across all tests trialled.

Specificity			
Total N	**Negative**	**%**	**95% CI**
50	50	100.00	92.87 - 100

Table 1. Sensitivity result for SureScreen device, CI = 95% confidence interval. Please see the appendix for full results table.

Sensitivity
Sensitivity (accurately diagnosing positive results) also varied considerably across the devices.

The sensitivity of SureScreen's test increased over time from the onset of symptoms; as expected in line with the patients developing antibodies in their system to a detectable level over time. Throughout each time period the SureScreen test achieved high sensitivity compared to the other tests in the trial. By day 20, the SureScreen test achieved 100% sensitivity. Results are tabulated below.

Sensitivity			
<10 days	**≥10 days**	**≥14 days**	**≥20 days**
71.05% (55.24 - 83.00%)	86.11% (76.29 - 92.28%)	90.74% (80.09 - 95.98%)	100% (87.94 - 100%)

Table 2. Sensitivity values for SureScreen's test against RT-PCR confirmed samples at different time intervals post onset of symptoms. Results are shown as the result and the 95% confidence interval in parentheses. For full sensitivity results, please see the appendix.

Overall
Across all samples tested, the SureScreen test achieved the highest sensitivity of any test trialled with 100% specificity. This included both LFIA devices and ELISA testing kits.

Discussion

Recommendations from the Study
Head-to-head comparisons such as the study done at Guy's and St. Thomas's, using identical clinical samples on a number of different test kits provide a robust assessment of individual testing kits performance. Seroprevalence (level of antibodies in the population) varies considerably depending on a number of factors such as time since onset of infection, and the severity of infection. As such, only considering manufacturer's validation data can make it difficult to compare the accuracy of tests on the market; since the kits have been validated with samples from different conditions and time points.

In this study, cross-comparison of overall specificities and sensitivities across all tests led to the study shortlisting the SureScreen test as "*one of the best performing,*" demonstrating the highest sensitivity across all devices tested with a specificity of 100%.

This study endorsed the SureScreen test for use, and pointed out that early reports in publications and the media around LFIAs having insufficient sensitivity may have been due to the type of samples tested; for example, samples sourced from people during very early infection before antibodies had been developed by the body, so absent from the samples being tested.

Although LFIAs lack the semi-quantitative information provided by an ELISA tests, they have a clear utility advantage in practice over ELISA or chemiluminescence-based technologies for antibody testing, such as portability, stability, and ease of use, cost-effectiveness among others.

The report noted: *"The results demonstrate that LFIAs may have utility in a hospital setting as of now, particularly if deployed where a rapid result could aide a clinical pathway or decision in real time, such as ward location or prioritisation of further diagnostics and follow up. The ease of use and affordability of the LFIAs weighs heavily in their favour, especially for potential in resource-poor settings or as point-of-care solutions in hospitals."*

Screening Timelines
As previously reported, IgM is the first antibody to be produced after incubation of the SARS-CoV-2 virus, and within this study it was shown to be at a completely detectable level in all patients 9 days post symptoms. IgG is produced later, and it was shown to be completely detectable level in all patients 16 days after symptoms.

As the diagnostic sensitivity of the LFIA tests is dependent on antibody concentration (and therefore time), it is important that the devices are used at the right timepoint post infection. Antibody screening with a LFIA device in the very early stages of symptoms may lead to a negative result as the individual's body has not had chance to produce the antibodies yet, so not present in the blood to allow detection by the test device.

The study also confirmed that individuals with a more severe infection produced antibodies much more readily. This should also be taken into consideration in a screening protocol. If the correct timelines and protocols are followed, it has been shown by this study that the SureScreen device can be used as a robust antibody screening test.

Conclusion

Overall, SureScreen's antibody tests have been shown to perform excellently in practice. The test has been formally endorsed by Guy's and St. Thomas Hospital and King's College London and is now being used as standard protocol for patients within the hospital.

The test has been shown to add significant benefit to screening protocols in a hospital setting alongside PCR based testing, and has been suggested that it's use for wider screening to understand infection rates in the community is central to future public health planning. Given the results of this study found specificity and sensitivity results of 100% at the post 20-day post infection point, the SureScreen device also has an important part to play in a wider setting in monitoring population infection rates and seroprevalence.

Acknowledgements

We thank the researchers and clinicians from King's College London and Guy's and St. Thomas' hospital and anyone helping or supporting this work.

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

Appendix

	Specificity			
	Total N	Negative	%	95% CI
Deep Blue	50	41	82.00	69.20 - 90.23
Accu-Tell	50	49	98.00	89.50 - 99.90
GenBody	50	50	100.00	92.87 - 100.0
SureScreen	50	50	100.00	92.87 - 100.0
Spring	50	49	98.00	89.50 - 99.90
Biohit	50	47	94.00	83.78 - 98.36
Medomics	97	93	95.88	89.97 - 98.38
Watmind	50	41	82.00	69.20 - 90.23
EUROIMMUN IgA	50	50	100.00	92.87 - 100.0
EUROIMMUN IgG	50	50	100.00	92.87 - 100.0
In-house IgM S	105	104	99.05	94.80 - 99.95
In-house IgG S	105	105	100.00	96.47 - 100.0

Table A.1 Sensitivity results from all assays tested, seven LFIAs, the Watmind clinical antibody test, the chemiluminescent EUROIMMUN tests and the in-house ELISA.

	OVERALL				<10 days				≥10 days				≥14 days				≥20 days			
	Total N	Positive	%	95% CI	Total N	Positive	%	95% CI	Total N	Positive	%	95% CI	Total N	Positive	%	95% CI	Total N	Positive	%	95% CI
Deep Blue	110	96	87.27	79.76 - 92.27	38	30	78.95	63.65 - 88.93	72	60	91.67	82.99 - 96.12	54	51	78.95	84.49 - 98.49	28	28	100.00	87.94 - 100.0
Accu-Tell	110	93	84.55	76.64 - 90.12	38	29	76.32	60.79 - 87.01	72	64	88.89	79.58 - 94.26	54	49	76.32	80.09 - 95.98	28	27	96.43	82.29 - 99.82
GenBody	110	69	62.73	53.41 - 71.19	38	13	34.21	21.21 - 50.11	72	56	77.78	66.91 - 85.83	54	43	34.21	67.10 - 88.23	28	25	89.29	72.80 - 96.29
SureScreen	110	89	80.91	72.57 - 87.16	38	27	71.05	55.24 - 83.00	72	62	86.11	76.29 - 92.28	54	49	71.05	80.09 - 95.98	28	28	100.00	87.94 - 100.0
Spring	110	93	84.55	76.64 - 90.12	38	29	76.32	60.79 - 87.01	72	64	88.89	79.58 - 94.26	54	50	76.32	82.45 - 97.08	28	28	100.00	87.94 - 100.0
Biohit	110	83	75.45	66.64 - 82.55	38	22	57.89	42.19 - 72.15	72	61	84.72	74.68 - 91.25	54	47	57.89	75.58 - 93.58	28	27	96.43	82.29 - 99.82
Medomics	110	81	73.64	64.71 - 80.97	38	20	52.63	37.26 - 67.52	72	61	84.72	74.68 - 91.25	54	48	52.63	77.81 - 94.81	28	27	96.43	82.29 - 99.82
Watmind	110	67	60.91	51.57 - 69.51	38	14	36.84	23.38 - 52.72	72	53	73.61	62.42 - 82.41	54	42	36.84	65.06 - 86.80	28	24	85.71	68.51 - 94.30
EUROIMMUN IgA	110	87	79.09	70.57 - 85.64	38	25	65.79	49.89 - 78.79	72	62	86.11	76.29 - 92.28	54	48	65.79	77.81 - 94.81	28	28	100.00	87.94 - 100.0
EUROIMMUN IgG	110	66	60.00	50.66 - 68.67	38	10	26.32	14.97 - 42.01	72	56	77.78	66.91 - 85.83	54	46	26.32	73.40 - 92.30	28	27	96.43	82.29 - 99.82
In-house IgM S	110	82	74.55	65.67 - 81.76	38	21	55.26	39.71 - 69.85	72	61	84.72	74.68 - 91.25	54	48	55.26	77.81 - 94.81	28	28	100.00	87.94 - 100.0
In-house IgG S	110	81	70.91	61.83 - 78.58	38	20	52.63	37.26 - 67.52	72	61	84.72	74.68 - 91.25	54	47	52.63	75.58 - 93.58	28	27	96.43	82.29 - 99.82

Table A.2 Sensitivity results for all assays tested against RT-PCR, at different time intervals post symptoms. Results are shown as the result and the 95% confidence interval in parentheses.

info@surescreen.com
+44 (0)1332 365318
surescreen.com
SureScreen Diagnostics, 1 Prime Parkway, Prime Enterprise Park, Derby, DE1 3QB, United Kingdom

SureScreen Diagnostics