

Early data on the performance of a combined SARS-CoV-2 spike-nucleocapsid antibody lateral flow device compared to a nucleocapsid-only device

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Abstract

Background: There is a critical need for reliable antibody detection methods in order to study and evaluate the public health and clinical response to the ongoing COVID-19 pandemic. Lateral flow immunoassay (LFIA) devices offer the prospect of rapid point-of-care testing (POCT), but the performance of these devices must be evaluated for robustness before they can be adopted for routine clinical and public health use.

Methods: Plasma and serum specimens from SARS-CoV-2 RNA-positive ($n = 131$) and RNA-negative ($n = 16$) patients were taken from various time points with respect to the onset of symptoms. All 147 anonymised specimens were tested for IgM and IgG using the Hangzhou AllTest 2019-nCoV IgG/IgM Rapid Test Cassette and the Abbexa COVID-19 IgG/IgM Rapid Test Kit.

Results: IgM sensitivity ranged from 13% to 68%, depending on the date of symptom onset and the device. Regarding IgG, the Abbexa device outperformed the Hangzhou device at all cumulative timeline brackets, with sensitivity of 97·87% (Abbexa) versus 68·09% (Hangzhou) for samples beyond 21 days from symptom onset. Day 21 was therefore chosen as the cut-off for ascertaining test performance characteristics, beyond which the specificity was 100% for both devices and negative predictive value was 0·94 (Abbexa) versus 0·50 (Hangzhou).

Discussion: Based on this limited dataset, the performance characteristics of the Abbexa LFIA device were substantially better than those of the Hangzhou device. Applying a 21-day cut-off for the Abbexa device meets the minimum (98%) sensitivity and specificity thresholds set by the UK Medicine & Healthcare products Regulatory Agency. The Abbexa device captures antibodies against both SARS-CoV-2 spike and nucleocapsid proteins, as opposed to Hangzhou that targets only the nucleocapsid protein. We therefore propose that spike glycoprotein antibodies be considered as part of the standard diagnostic approach towards SARS-CoV-2 antibody profiling to improve clinical sensitivity and potentially specificity, pending follow-up studies to confirm this approach.

Keywords: COVID-19, SARS-CoV-2, pandemic, serology, IgG, IgM, antibodies, lateral flow immunoassay, epidemiology, public health

Introduction

Understanding the antibody response is an essential element for evaluating the nature of any new emergent infectious pathogen and the same applies for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is the cause of the current coronavirus disease 2019 (COVID-19) pandemic. Currently, the most widely employed tool for laboratory diagnosis of COVID-19 is identification of present infection with SARS-CoV-2 by detection of virus-specific RNA from nasopharyngeal swab specimens using real-time reverse transcriptase PCR (rRT-PCR, i.e. PCR).¹ This requires specialist equipment, staff and reagents, which creates a significant delay between testing and diagnosis. Furthermore, PCR cannot be used to determine past exposure or immunity, whereas tests that detect antibodies against SARS-CoV-2 could potentially be used to identify both present infection and past exposure, depending on detection of the immunoglobulin (Ig) classes IgM, IgG, or both.² Lateral flow immunoassay (LFIA) devices, also referred to as rapid diagnostic test (RDT) devices, offer the prospect of rapid point-of-care testing (POCT), which involves a capillary blood draw by fingerstick and immediate application of that blood to an LFIA device. POCT with LFIA devices might be particularly advantageous in the outpatient, GP and community settings, as it can be performed near the bedside. However, the accuracy and robustness of sample collection methods must be evaluated before LFIA devices can be adopted for routine clinical use.³

Current UK national regulations dictate further evaluation of LFIA devices is necessary before they can be used widely.⁴ We sought to evaluate two LFIA devices to start with by comparing their performance against a well characterised residual seraset at the following timeline brackets: day 4, 7, 14, 21, 28, and >28 from onset of symptoms. We chose two LFIA devices (Figure 1): the Hangzhou AllTest 2019-nCoV IgG/IgM Rapid Test Cassette (Hangzhou AllTest Biotech Co Ltd, Hangzhou, China), as it was amongst the first on the market,⁵ and the Abbexa COVID-19 IgG/IgM Rapid Test Kit (Abbexa Ltd, Cambridge, UK), based on stated kit performance characteristics.⁶ The samples were all venous blood samples, providing an early insight into LFIA performance whilst further data on capillary blood samples evolves.

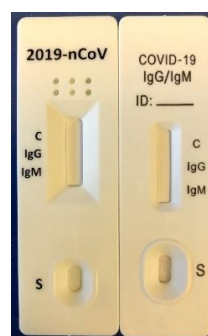


Figure 1: Hangzhou COMBRA COVID-19 IgM/IgG Rapid Test Cassette (left) and Abbexa COVID-19 IgG/IgM Rapid Test Kit (right) LFIA devices.

Common targets detected by these LFIA devices include antibodies against the SARS-CoV-2 spike (S) glycoprotein and the nucleocapsid (N) protein. The S glycoprotein is comprised of two subunits; S₁ mediates viral attachment and entry through the host cell ACE2 receptor and S₂ the fusion of viral and cellular membranes.⁷ The N protein is the SARS-CoV-2 structural protein that binds to the viral genomic RNA.⁸ The Abbexa device is designed to detect antibodies against both S and N,⁶ whilst the Hangzhou device is designed to detect antibodies against N only.⁹ We sought to compare the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each device against PCR.

Materials and methods

Samples

According to the UK Medicine & Healthcare products Regulatory Agency (MHRA), capillary whole blood, serum, and plasma are the desired sample types for LFIA testing.¹⁰ In the present study, residual plasma and serum samples from SARS-CoV-2 PCR-positive ($n = 131$) and PCR-negative ($n = 16$) patients, a total of 147 specimens, were identified from various timeline brackets with respect to the onset of symptoms as follows: RNA-positive at 4 days post symptom onset ($n = 13$), RNA-positive at day 7 ($n = 20$), RNA-positive at day 10 ($n = 20$), RNA-positive at day 14 ($n = 31$), RNA-positive at day 21 ($n = 25$), RNA-positive at day 28 ($n = 12$), and RNA-positive beyond day 30 ($n = 10$). Also included in this study were 4 specimens from patients who were PCR-negative, 7 specimens that were collected prior to the pandemic, and 5 specimens from patients who were RNA-positive for non-SARS-CoV-2 seasonal coronavirus species OC43. Date of symptom onset (D_0) was defined as the date on which the patient first reported or was found to have either fever or respiratory signs or symptoms, as reported in their hospital records.

Testing protocol

All 147 anonymised specimens were tested on both devices. All tests were performed exactly as per the instructions included in the respective package inserts. For the Hangzhou device, 10 μ L of plasma or serum was added to the sample well, followed by two drops of the proprietary buffer and incubation at room temperature for ten minutes. For the Abbexa device, 20 μ L of plasma or serum was added to the sample well, followed by one drop of the proprietary buffer and incubation at

room temperature for ten minutes. Test results were read synchronously from both devices within 20 minutes of initiation.

Results interpretation

The result of each test was indicated by pink bands, where a positive result is indicated by the appearance of test and control bands, a negative result is indicated by the presence of the control band only. The absence of the control band or a band in the wrong place indicated an invalid test result.

Statistical analysis

Analysis was conducted using Microsoft Excel. For each device, sensitivity, specificity, PPV, and NPV were calculated for each group.

Results

Analysis of the performance characteristics of the Hangzhou and Abbexa LFIA devices identified differences in test sensitivity throughout the timeline of convalescence. Both devices had capabilities for distinguishing IgM and IgG with distinct reading lines for each, allowing observation of differences in antibody class characteristics over the timeline of convalescence.

Evolution of IgM and IgG responses

There were great disparities in the responses to IgM and IgG between the two devices, as exemplified in Figure 2. Day 4 following onset of symptoms was the earliest timeline bracket investigated, which showed sensitivity to be low (IgM 30.77% and IgG 38.46%) for both devices (Figure 3A). For the Hangzhou device, the sensitivity for IgM was poor, peaking at 7 days with a sensitivity of 45%. By 30 days post symptom onset, IgM could no longer be detected using the Hangzhou device. Conversely, in the Abbexa device, IgM was detected in specimens from most time points tested, peaking with a sensitivity of 68% at day 21 post symptom onset.

Similar to IgM, IgG could be detected from day 4 following symptom onset (Figure 3B). For both devices, as expected, sensitivity for IgG detection improved over time from symptom onset. Sensitivity for IgG in the Abbexa device was substantially higher compared to the Hangzhou device, at both day 21 (96% versus 68%) and beyond day 30 (100% versus 70%).

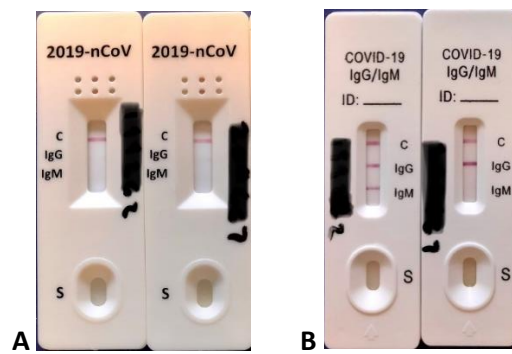


Figure 2: Example of performance discrepancies of Hangzhou (A) versus Abbexa (B) LFIA devices at day 21 from symptom onset. A and B represent the same two specimens tested on the Hangzhou and Abbexa devices, respectively. Discrepancies were observed in both specimens between the two devices. The Hangzhou device failed to detect IgM or IgG in either specimen, whereas the Abbexa device identified antibodies in both specimens.

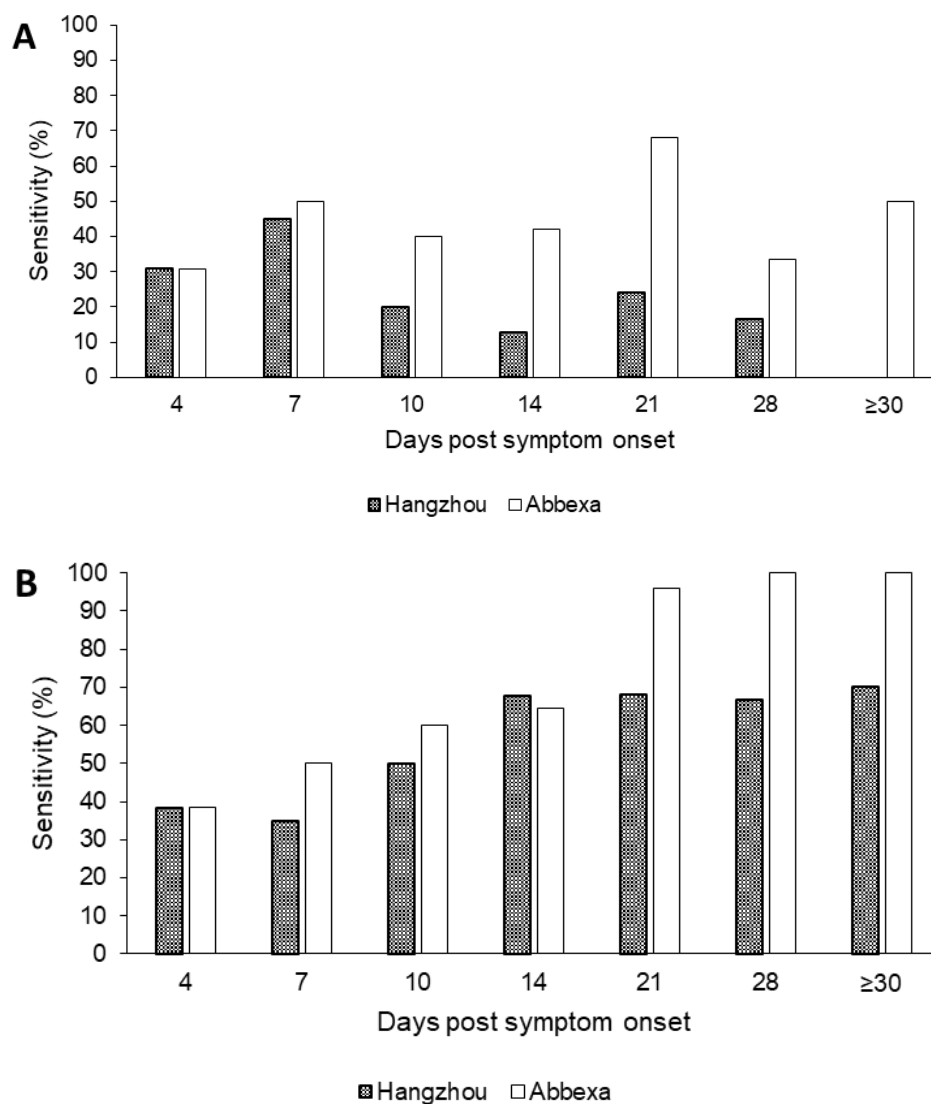


Figure 3: Development of IgM (A) and IgG (B) over the convalescent timeline using the Hangzhou and Abbexa LFIA devices.

Comparison of IgM and IgG performance characteristics for Abbexa versus Hangzhou

The manufacturers' stated performance characteristics are as follows: for IgM, Hangzhou claims a sensitivity of 85% and specificity of 96%. For IgG, Hangzhou claims a sensitivity of >99.9% and specificity of 97%. Abbexa, meanwhile, only claims combined performance characteristics of 98.58% sensitivity and 97.94% specificity for IgM and IgG together.

Table 1 highlights the differential cumulative performance characteristics for IgM and IgG between the two devices, categorised as timeline since symptom onset. For the Abbexa device, IgM positivity rate initially increases across the seraset timeline, with the greatest sensitivity observed in specimens collected at least 21 days following symptom onset (55.32%). As expected, the sensitivity of IgM then drops in samples collected at least 28 days after symptom onset. Conversely, the Hangzhou device IgM sensitivity decreased across the seraset timeline from 22.14% at symptom onset to 9.09% by day 28.

The IgG positivity rates of both devices increased across the seraset timeline but with quite different profiles. The sensitivity of the Abbexa device increased incrementally (70.99% to 100%), whilst the Hangzhou device plateaued by 14 days following symptom onset (57.25% to 68.18%). As early as day 21, the sensitivity of the Abbexa device for IgG was very close to the manufacturer's reported figures: 97.87% against 98.6%, and 100% by day 28. A maximum sensitivity of 68.18% was calculated against the manufacturer's claimed 99.9% for the Hangzhou device at day 28. Neither device met the manufacturer's claim for IgM sensitivity. In light of the significant improvement in sensitivity of the Abbexa device in detecting IgG against SARS-CoV-2 between days 14 and 21 post symptom onset, day 21 was chosen as the cut-off for ascertaining test performance for IgG, below which a negative result could not be relied upon.

Limited cross-reactivity was identified with only a single specimen collected prior to the COVID-19 pandemic testing positive for IgM, resulting in an IgM specificity of 93.75% for both devices, compared to the 98% specificity reported by each manufacturer. The IgG specificity was 100% for both devices. The NPV performance in Abbexa was superior to Hangzhou across all timeline brackets, with Abbexa reaching an NPV of 0.94 by day 21 and beyond for IgG, versus 0.50 for Hangzhou by the same timeline bracket.

Performance characteristics	IgM		IgG	
	Hangzhou	Abbexa	Hangzhou	Abbexa
From D ₀ of symptom onset (<i>n</i> = 147)				
Sensitivity (%)	22·14	46·56	57·25	70·99
Specificity (%)	93·75	93·75	100·00	100·00
PPV	0·97	0·98	1·00	1·00
NPV	0·13	0·18	0·21	0·28
≥7 days from symptom onset (<i>n</i> = 134)				
Sensitivity (%)	21·19	48·31	59·32	74·58
Specificity (%)	93·75	93·75	100·00	100·00
PPV	0·96	0·98	1·00	1·00
NPV	0·14	0·20	0·24	0·33
≥14 days from symptom onset (<i>n</i> = 94)				
Sensitivity (%)	15·38	50·00	67·95	84·62
Specificity (%)	93·75	93·75	100·00	100·00
PPV	0·92	0·98	1·00	1·00
NPV	0·19	0·28	0·38	0·56
≥21 days from symptom onset (<i>n</i> = 63)				
Sensitivity (%)	17·02	55·32	68·09	97·87
Specificity (%)	93·75	93·75	100·00	100·00
PPV	0·89	0·96	1·00	1·00
NPV	0·28	0·42	0·50	0·94
≥28 days from symptom onset (<i>n</i> = 38)				
Sensitivity (%)	9·09	40·91	68·18	100·00
Specificity (%)	93·75	93·75	100·00	100·00
PPV	0·67	0·90	1·00	1·00
NPV	0·43	0·54	0·68	1·00

Table 1: Comparative performance characteristics of Hangzhou and Abbexa LFIA devices for detecting SARS-CoV-2 IgM and IgG in PCR-positive patients at varying time points following symptom onset.

Discussion

There is a critical need for reliable antibody detection methods in order to study and evaluate clinical and public health knowledge regarding the ongoing COVID-19 pandemic, which could then instruct clinical and public health interventions.

If shown to be robust enough, antibody testing with LFIA devices could play a critical role in the epidemiologic management of the ongoing COVID-19 pandemic by expanding testing well beyond the laboratory. There are several such devices emerging on the market, and as with any diagnostic technology, their performance needs to be evaluated against manufacturers' claims before they can be adopted for widespread, routine clinical use. High clinical and analytical specificity and sensitivity are required for antibody testing if the results are to be used for clinical management decisions such as de-isolating individuals from quarantine.¹¹

Our seraset comparison was framed along the lines of the Royal College of Pathologists (RCPATH) guidance on verification of SARS-CoV-2 serology assays.¹² Overall, the data presented in this study support the use of IgG as a biomarker of exposure to SARS-CoV-2 in samples collected at least 21 days from onset of COVID-19 symptoms where the assay performance is considered acceptable. The poor performance of IgM detection for both devices across all timeline brackets highlights the transient nature of IgM and its limited clinical utility. Detection of IgM by either of these two LFIA devices was not sufficiently sensitive to replace PCR as the gold standard for diagnosing active infection. However, since both IgM and IgG become detectable at the same time,¹³ the absence of both could rule out active infection.

It was quite evident in our study that the Abbexa device outperformed the Hangzhou device consistently across all timeline brackets for the various statistical parameters, i.e. sensitivity, specificity, NPV, for both IgG and IgM, reaching the MHRA standards that the manufacturer claims as achieved by day 21. The IgG positivity reaching close to 85% by day 14 in the Abbexa device is also quite reassuring. Furthermore, specificity was maintained throughout the convalescent time period in the Abbexa device, at levels mandated by MHRA (98%),¹⁰ which was also similar for the Hangzhou device. The comparatively poor NPV of the Hangzhou device raises questions about its utility in informing clinical and public health practice.

In our study, the Abbexa device significantly outperformed the Hangzhou device. This is of interest because there is currently a major point of debate in health circles as to the clinical and public health relevance of SARS-CoV-2 antibodies in terms of performance characteristics evidencing exposure,

infection, or immunity and protection from severe disease. The single major difference in the technical configuration between the two devices is the additional capacity of the Abbexa LFIA device to detect antibodies against the S glycoprotein. There is a further need to understand the functional elements of the spike glycoprotein subunits, S₁ and S₂. There are enzyme-linked immunosorbent assays (ELISAs) currently available, such as the COVID-19 IgG Confirmation assay (Dia.Pro Diagnostic Bioprobes, Sesto San Giovanni, Italy), which could be evaluated to provide further insight into the frequency and magnitude of specific antibody responses against S₁ and S₂ and their discriminatory diagnostic and clinical value. There may be value in combining spike and nucleocapsid antigen targets to ascertain antibody responses in individuals following exposure to SARS-CoV-2, rather than attempting to compare antibody response against one protein moiety in contrast to the other.

Limitations

The prevalence of infection in our study sample is not reflective of the general population. This study presents comparison data from mostly known-positive cases with only a small proportion of samples from RNA-negatives, non-COVID OC43 coronavirus-positive, and pre-COVID sera to cover for analytical and clinical specificity. Therefore, calculations of false positivity rates (FPR) are limited, and PPV cannot be inferred. Larger sample data sets with proportional representation from true negative samples matching current population prevalence are required for those characteristics to be derived.

A second limitation is that only ten specimens were collected after 30 days from symptom onset, up to a maximum of 40 days. It is therefore not possible to draw any conclusions regarding the longevity of IgG past this time. Further analysis of convalescent sera collected across a greater timeline is therefore required to draw more general conclusions and to improve the clinical utility of these tests.

Conclusion

Based on this limited dataset, the Abbexa LFIA device was found to have superior performance characteristics over the Hangzhou device. Future studies are needed to test large numbers of PCR-negative and pre-COVID-era specimens in order to calculate FPR, PPV, and further confirmation of the sensitivity, specificity, and NPV data presented in this study. Consideration should be given to include the S glycoprotein, cognisant of S₁ and S₂ subunit inclusion, as a standard target in LFIA devices to ascertain the clinical sensitivity and specificity of immune response to SARS-CoV-2.

Contributors

SM designed and drafted the experiment plan and supervised the study. CAL conducted the experiments. FR analysed the data and presented the results. CAL and FR co-authored the manuscript, which was edited and finalised by CAL, FR, and SM.

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Declaration of interests

We have no competing interests to declare.

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