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Full Length Article

Evaluation of the performance of a lateral flow device for quantitative detection of anti-SARS-CoV-2 IgG

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ABSTRACT

Introduction: The AbC-19™ lateral flow immunoassay (LFIA) performance was evaluated on plasma samples from a SARS-CoV-2 vaccination cohort, WHO international standards for anti-SARS-CoV-2 IgG (human), individuals ≥2 weeks from infection of RT-PCR confirmed SARS-CoV-2 genetic variants, as well as microorganism serology.

Methods: Pre-vaccination to three weeks post-booster samples were collected from a cohort of 111 patients (including clinically extremely vulnerable patients) from Northern Ireland. All patients received Oxford-AstraZeneca COVID-19 vaccination for the first and second dose, and Pfizer-BioNTech for the third (first booster). WHO international standards, 15 samples from 2 variants of concern (Delta and Omicron) and cross-reactivity with plasma samples from other microorganism infections were also assessed on AbC-19™.

Results: All 80 (100%) participants sampled post-booster had high positive IgG responses, compared to 38/95 (40%) participants at 6 months post-first vaccination. WHO standard results correlated with information from corresponding biological data sheets, and antibodies to all genetic variants were detected by LFIA. No cross-reactivity was found with exception of one (of five) Dengue virus samples.

Conclusion: These findings suggest BNT162b2 booster vaccination enhanced humoral immunity to SARS-CoV-2 from pre-booster levels, and that this antibody response was detectable by the LFIA. In combination with cross-reactivity, standards and genetic variant results would suggest LFIA may be a cost-effective measure to assess SARS-CoV-2 antibody status.

Introduction

The SARS-CoV-2 pandemic has been ongoing since 2019. The vaccination programme for the population of the United Kingdom commenced in December 2020. Since the start of July 2022, over 1400,000 first vaccinations, 1300,000 second vaccinations (approximately ≥8–12 weeks following the first dose) and 1100,000 third "booster" vaccinations (approximately 3 months following the second dose) have been administered in Northern Ireland [1]. However, the emergence of new variants, alongside the waning of antibody levels over time, warrants further investigation and monitoring of both the vaccine-induced immune response and the immune response from SARS-CoV-2 infection. SARS-CoV-2 IgG may be detected in saliva, sputum, bronchoalveolar lavage fluid or blood samples [2]. Our previous study [3] assessed plasma samples from participants for anti-SARS-CoV-2 spike protein IgG from pre-vaccination to six months after their first vaccination, as well as documented reports of SARS-CoV-2 infection from participants (i.e., the differences in antibody levels in participants that had been infected at some point within the vaccination process). The objective of this study is to semi-quantitatively assess the performance of the AbC-19™ lateral flow immunoassay on a selection of plasma samples which includes post booster vaccination, WHO international standards genetic variants and microorganism serology.

Methods

Study design

The eligibility criteria for the study required that participants be >18 years old with no contraindications to providing a blood sample, and that they were scheduled to receive the COVID-19 vaccine. All participants provided fully informed written consent prior to enrolment in

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Table 1
Timepoint description.

Timepoint (TP)	Description
1	Pre first vaccination
2	3 weeks post first vaccination
3	Pre second vaccination
4	3 weeks post second vaccination
5	6 months post first vaccination
6	9 months post first vaccination
7	Post booster vaccination

the study. The study was approved by the South Birmingham Research Ethics Service (REC 20/WM/0184, IRAS 286,041), and adhered to the Declaration of Helsinki.

Venous blood samples were collected from study participants in approximately 10 ml EDTA vacutainers and centrifuged at 3000 rpm for 15 min at 4 °C. The plasma was aliquoted and stored at −80 °C with a maximum freeze-thaw cycle of 1 upon testing on AbC-19™. The patients within the vaccine cohort received the Oxford-AstraZeneca COVID-19 vaccination for the first and second dose, and the Pfizer-BioNTech (BNT162b2) for the third dose (first booster). The sampling time points are described in Table 1. At each sampling time point participants received a questionnaire, and positive COVID-19 rapid antigen or PCR test results were recorded, as well as details of infection that include symptom severity (defined as mild, moderate or severe).

In this study we also assess the comorbidities and the medications of the clinically extremely vulnerable (CEV) participants. CEV patients would now be defined as high risk for death or serious illness from COVID, however, at the point of recruitment patients were categorized as CEV or non-CEV. The 10 genetic variant samples (5 samples per variant of concern to include Delta (mutations; G142D, E156-F157, R158G, L452R, T4278K) and Omicron (B.1.1.529) were sourced and tested on the AbC-19™ device by Abingdon Health (York, UK). The WHO international standards were obtained from NIBSC (National Institute for Biological Standards, Herts, UK). The 101 microorganism serology samples, as listed in Table S2., were obtained from AbBaltis (Kent, UK), Trina (Trina Bioreactives AG, Switzerland) and NIBSC.

The AbC-19™ Rapid Test lateral flow immunoassay was used in accordance with manufacturer's instructions to test the plasma samples for anti-SARS-CoV-2 IgG (including neutralising antibodies to the trimeric spike protein). To allow for comparison of the immune response post-booster vaccination, the semi-quantitative approach, as documented within our earlier study [3] was implemented as follows;

Negative= AbC-19™ score of 0
Low positive= AbC-19™ score of 1–2
High positive= AbC-19™ score of 4–10

Statistical analysis

All data analysis was carried out using SPSS for Windows version 27 (property of IBM). Continuous data was described using the mean ± standard error of the mean and 95% confidence intervals. Differences were tested using the Mann Whitney U test. Categorical data was described by number and percentages. One-way analysis of variance (ANOVA) was used to compare the differences between groups and the Games-Howell post hoc test was applied.

Results

Vaccinated sample demographics

From our initial cohort of 111 participants, as documented by Robertson et al. [3], we were able to follow-up on 24 participants at TP6 (all except for 1 participant were followed up at TP7) and 80 participants for TP7. CEV participants accounted for 53.2% of the total cohort (59/111). Of the 59 CEV participants, 19 (32.2%) were hypertensive, 10 had cardiovascular disease (16.9%), 8 were diabetic (13.6%), 15 were asthmatic (25.4%) and 10 had cancer (17.0%). Additionally, 20 of the CEV participants had more than one medical condition. The majority of CEV participants were on medications (84.7%), with the most common medication within this cohort being statins (37.3%).

Immune response post-booster vaccination (TP7)

The results of the anti-SARS-CoV-2 IgG AbC-19™ test shown in Fig. 1 demonstrate high positive IgG antibody levels for all 80 participants sampled at TP7. There was a significant mean increase in AbC-19™ score of 5.73 between TP5 and TP7 as shown in Fig. S2 ($p<0.0001$). TP5 AbC-19™ scores ranged from 0 to 10, whilst TP7 AbC-19™ scores ranged from 5 to 10.

Furthermore, the one participant previously reported to be SARS-CoV-2 IgG negative within the cohort following second vaccination (TP4), returned a high positive (5) AbC-19™ score following booster vaccination (TP7) for. The participant was CEV and reported having no medical history of SARS-CoV-2 infection. A further 6 participants who tested IgG negative at TP5, and an additional 3 participants who tested negative at TP6 all elicited an immune response following their booster vaccination. The AbC-19™ scores of the 6 negative individuals at TP5 increased by 6, 8, 9, 6, 7 and 5. The AbC-19™ scores of the 3 negative individuals at TP6 increased by 10, 8 and 9. These individuals were all low positives at TP4.

Effect of previous SARS-CoV-2 infection on response to booster vaccination

SARS-CoV-2 infection confirmed by rapid antigen or PCR test was recorded. The AbC-19™ score of the 17 participants who had previously been infected with SARS-CoV-2 was compared with the scores of those who reported no infection (Fig. 2). The scores of those who reported previous infection were significantly higher, increased by a mean AbC-19™ score of 0.8 ($p = 0.049$). Multiple comparisons analysis is presented in Table S1 for infection status and grade. However, there is no difference in the scores when stratified for symptomatic or non-symptomatic infection, as shown in Fig. 3. Furthermore, the mean increase in AbC-19™ score was significantly less for the infected individuals at 5.80 compared to those not previously infected at 2.43, as shown in Figure S3 ($p = 0.001$). Therefore, this difference is likely to be due to infected individuals having already increased antibody levels.

The immune response elicited by the booster vaccination in the CEV was compared to those not CEV. Although the mean response to the booster vaccine was stronger in non-CEV participants, the 0.23 increase in the mean AbC-19™ score for the 32 non-CEV participants compared to the 48 CEV participants was not statistically significant ($p = 0.49$) (Fig. 4). Similarly, the 0.41 increase in the mean AbC-19™ score found at TP5 for 35 non-CEV compared to 54 CEV participants was not statistically significant ($p = 0.522$).

WHO international standards and genetic variants

The MDCG (2021) guidelines advocate the testing of the WHO International SARS-CoV-2 IgG NIBSC standards, genetic variants, and potentially interfering/cross-reacting serology [4]. The WHO international SARS-CoV-2 IgG NIBSC standards were developed for the purposes of standardization and calibration of SARS-CoV-2 IgG antibody tests [5]. This analysis was performed to assess if the AbC-19™ could correctly rank the standards in accordance with the NIBSC ranking, as well as to investigate if the AbC-19™ was able to detect antibodies to a selection of genetic variants. The WHO international standards performed as expected in accordance with the ranking of the NIBSC standards (*S*=spike protein, *N*=nucleocapsid); NIBSC 20/150 (high) scored 6, 20/148 (mid) scored 3–4, 20/144 (low S, high N) scored 1–2, 20/140 (low) scored 1,

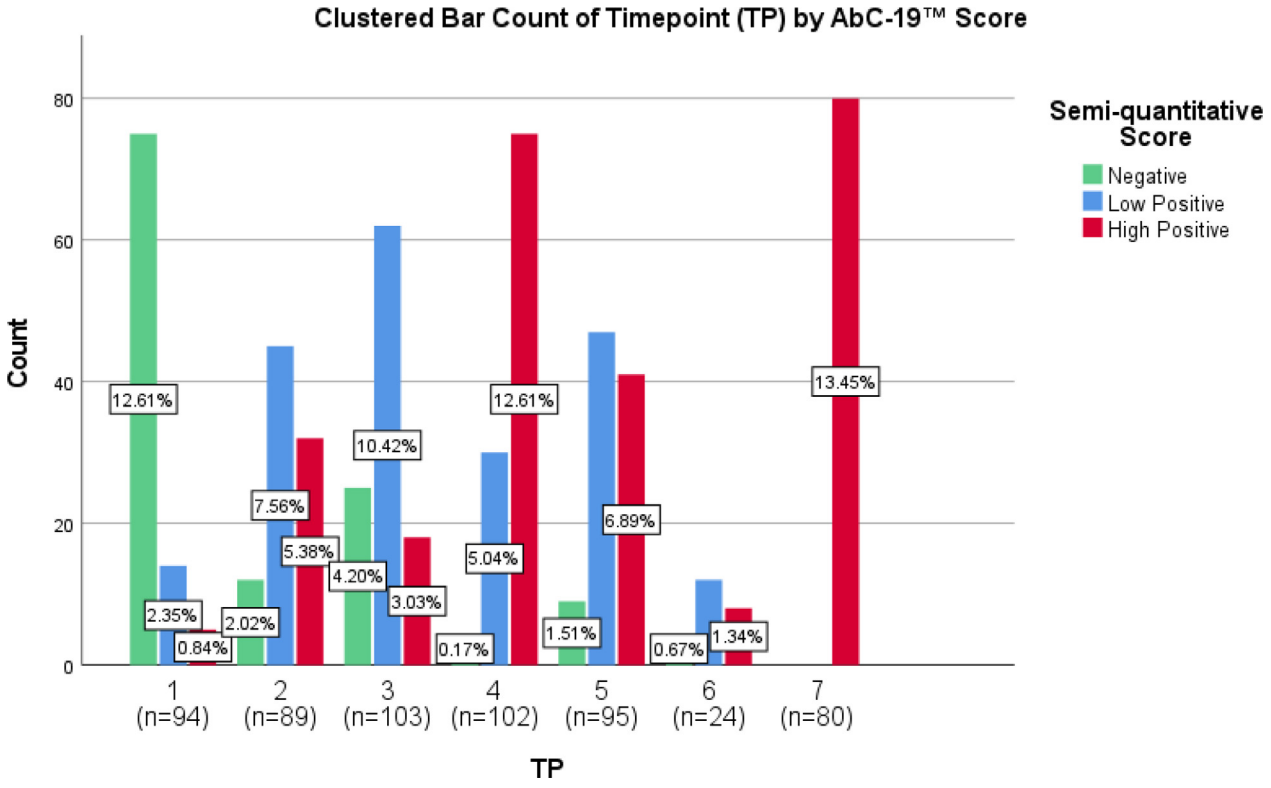

Fig. 1. Semi-quantitative scoring of AbC-19™ result for participants at seven time points.

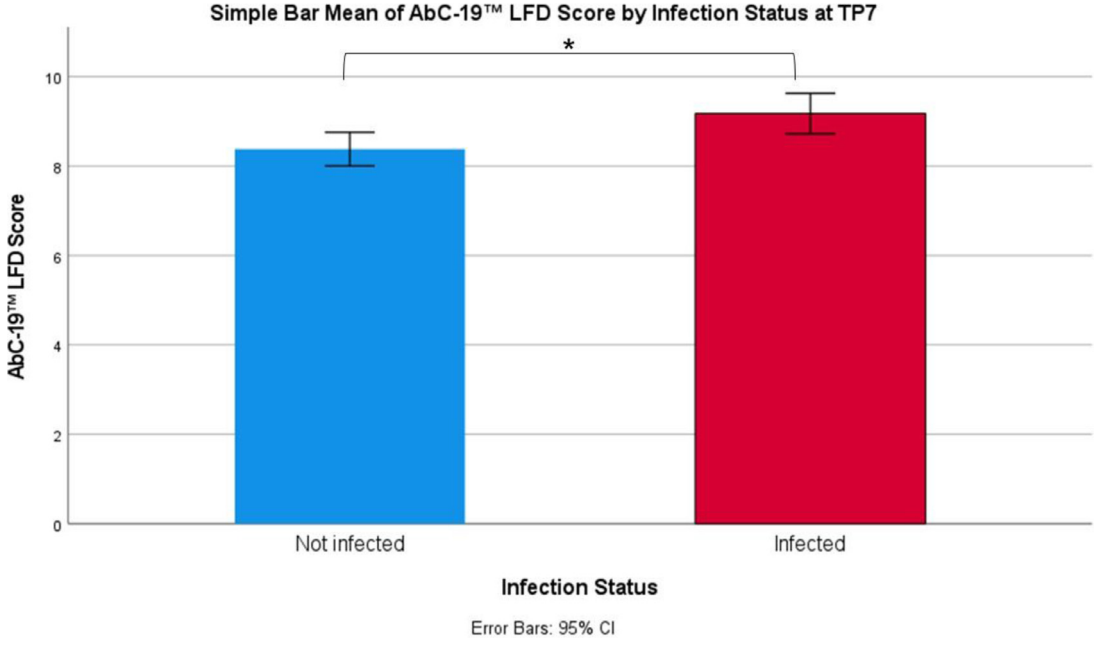

Fig. 2. AbC-19™ score by infection status for TP7.
Not infected n = 63 vs Infected n = 17 (Pre-Vaccination n = 7, TP1-TP7 n = 10). LFD= Lateral Flow Device.

20/142 (negative) scored 0 and 21/234 (high) scored 6. The results of the genetic variant samples are presented in Table 2.

Cross-reactivity

This cross-reactivity analysis was carried out to evaluate the cross-reactivity to antibodies elicited by other microbial infections. There were 101 microorganism serology samples tested on AbC-19™ as documented in Table S2. No cross-reactivity was detected in any sample with the exception of a marginal cross-reactivity that was observed in one of five pre-pandemic dengue serology samples with a score of 1 on AbC-19™, and as confirmed by repeat testing and multiple observers.

Discussion

Vaccination and infection

In this study we assessed both samples from infected individuals and samples from vaccinated individuals. Assessment of SARS-CoV-2 antibodies provides a better understanding of the immune response to both

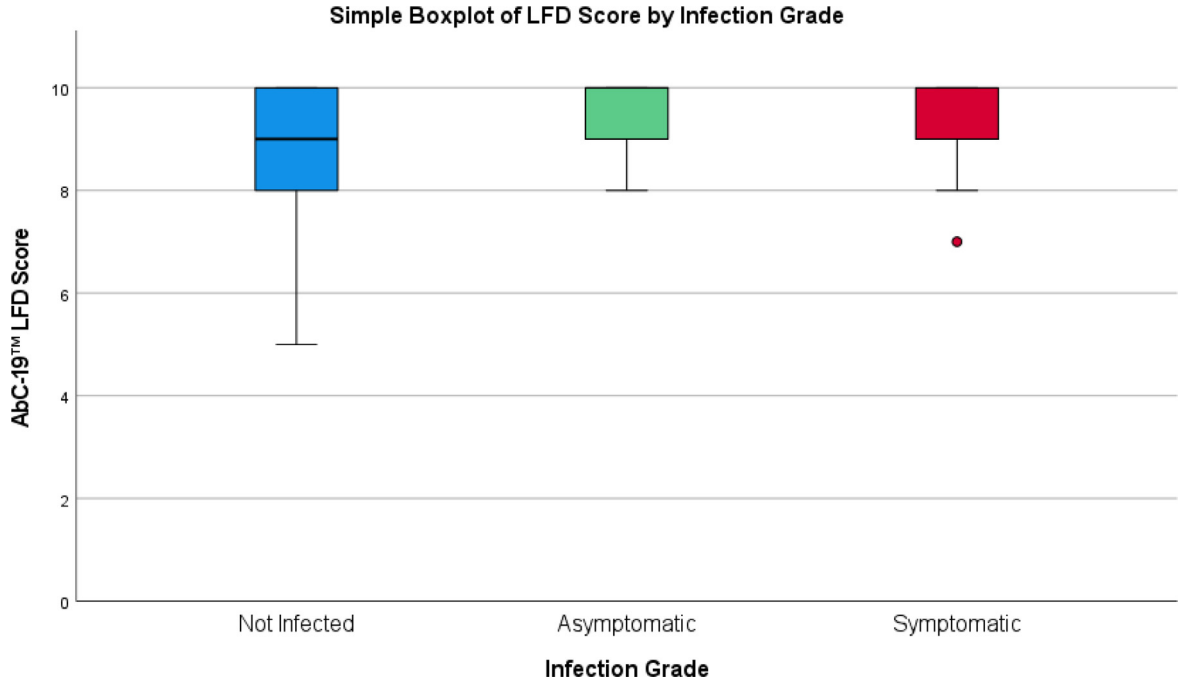

Fig. 3. AbC-19™ score by infection grade for TP7.
Not infected $n = 63$, Asymptomatic $n = 7$ (Non-CEV $n = 3$, CEV $n = 4$), Symptomatic $n = 10$ (Mild $n = 4$ (Non-CEV $n = 2$, CEV $n = 2$), Moderate $n = 3$ (Non-CEV $n = 2$, CEV $n = 1$), Severe $n = 3$ (Non-CEV $n = 0$, CEV $n = 3$)).

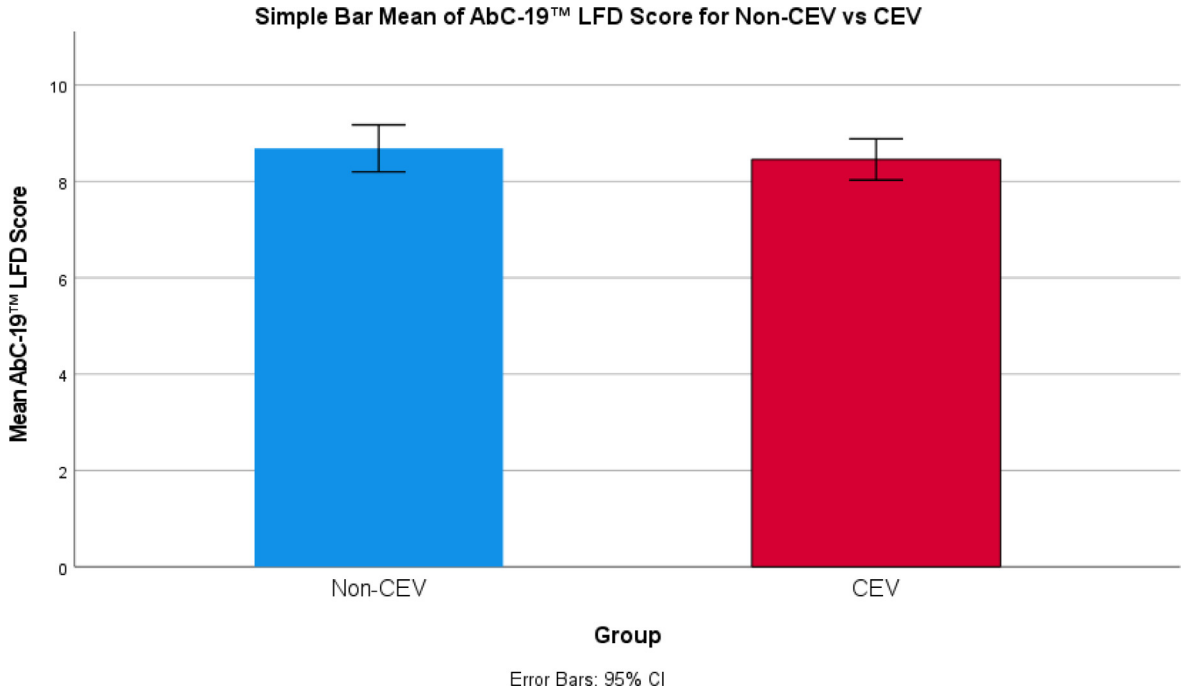

Fig. 4. AbC-19™ score for Non-CEV vs CEV at TP7.
Non-CEV (n = 32) vs *CEV (n = 48)*.

vaccination, as well as infection. For example, in a recent study the odds of infection by Omicron were shown to be higher than that of Delta [6]. Additionally, Beaney et al. [7] also found significant variation over time for hospitalization and mortality risk from SARS-CoV-2 infection. These findings may suggest continual monitoring of infection and immune response is crucial with the emergence of new variants. In the same study by Chaguza et al., there was shown to be a lower PCR test positivity rate after three mRNA vaccine doses, highlighting the importance of booster vaccinations.

There are few studies assessing the SARS-CoV-2 immune response of samples from vaccinated individuals compared to those of infected individuals [8], The conclusions of the studies by Salvagno et al. [9] and Chuguza et al., recognized the need for booster vaccinations following a decline in antibody levels after 6 months. This is particularly important given the indication of protection from COVID-19 associated with [10], as well as reports of reduction in risk of symptomatic infection in correlation with higher levels of immune markers to include anti-spike, anti-receptor binding domain and neutralising antibodies [11].

Table 2
Genetic variants.

Sample	Operator 1						Operator 2						Average T-Line	Mode T-Line	Max	Min
	Rep 1		Rep 2		Rep 3		Rep 1		Rep 2		Rep 3					
	C1	T1	C2	T2	C3	T3	C1	T1	C2	T2	C3	T3				
1. Delta	10	1	10	1	10	1	9	1	9	1	9	1	1.00	1	1	1
2. Delta	10	3	10	3	10	2	9	3	9	3	9	2	2.67	3	3	2
3. Delta	10	2	10	2	10	2	9	2	9	3	9	2	2.17	2	3	2
4. Delta	10	2	10	2	10	2	9	2	9	1	9	1	1.67	2	2	1
5. Delta	10	4	10	3	10	3	9	4	9	3	9	3	3.33	3	4	3
6. Omicron	10	7	10	7	10	6	9	7	9	7	9	7	6.83	7	7	6
7. Omicron	10	6	10	6	10	6	9	6	9	6	9	6	6.00	6	6	6
8. Omicron	10	4	10	5	10	5	9	4	9	4	9	4	4.33	4	5	4
9. Omicron	10	7	10	5	10	6	9	6	9	6	9	6	6.00	6	7	5
10. Omicron	10	7	10	7	10	6	9	7	9	7	9	7	6.83	7	7	6
Buffer alone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Rep= Repeat, C= Control-Line and *T* = T-Line.

The study presented here obtained from the vaccinated cohort aimed to address this matter further.

The high positive anti-SARS-CoV-2 IgG response to the booster vaccine was found to be significantly increased for those who were previously infected, reflecting the findings from our previous study [3]. Although all participants scored high positive AbC-19^{TM} results at TP7. Contrastingly, results from the study by Reynolds et al. [12] suggest that after the third vaccine dose the anti-spike protein S1 receptor binding domain antibody response increased to similar levels in all 3 groups assessed (Wuhan variant infected, Alpha variant infected and uninfected). However, this study used lab-based immunoassays such as ROCHE Elecsys electrochemiluminescence immunoassay (ECLIA).

Genetic variants and who international standards

The MDCG (2021) guidelines propose acceptable performance metrics of a SARS-CoV-2 IgG immunoassay to be ≥90% diagnostic sensitivity for samples taken >21 days post symptom onset (for ≥400 samples), and a high specificity value of >99% (for ≥400 samples from non-vaccinated and non-infected individuals) should also be met. However, MHRA (2022) guidelines suggest a minimum clinical sensitivity and specificity of >98% [13]. In the evaluation of analytical sensitivity, the AbC-19^{TM} LFIA successfully profiled the WHO International standard results by correctly identifying those samples with negative, low, mid and high antibody levels. The results of this study also show successful detection of a neutralising humoral antibody response for the 2 variants (Delta and Omicron). In our earlier collaborative study, we reported cross-reactivity of spike glycoprotein induced antibody against the Delta and Omicron variants pre and post booster vaccination in a range of non-CEV and CEV individuals [14]. Therefore, SARS-CoV-2 immunoassays may prove to be a valuable tool in the monitoring and response to new variants with the possibility of immune escape [15]. However, the performance of the existing LFD test should be measured for each new variant to ensure that the test performs to the required standard.

Cross-reactivity

Cross-reactivity of samples from dengue serology as well as malaria serology samples have previously been reported to present false positive results on SARS-CoV-2 assays [16]. Therefore, whilst only 1 out of the 5 Dengue samples tested demonstrated this weak cross-reactivity on AbC-19^{TM}, this should still be taken into consideration to prevent overestimating SARS-CoV-2 seroprevalence in regions where these infections are widespread. However, it is likely that this positive result was due to non-specific binding of the anti-dengue IgG in this one sample [17]. Furthermore, the MHRA (2022) guidelines desired criteria are met as no cross-reactivity with any other coronaviruses or respiratory pathogen antibody positive serology was detected, and Dengue is not presented in the list for analytical specificity analysis.

Limitations

This study examines only the humoral antibody response and not the cellular antibody response. Additionally, given the longitudinal nature of the study it was anticipated that vaccinated participants may not attend all sampling timepoints. Lastly, we acknowledge this study is limited as it only reports on the results from one immunoassay and Salvagno et al. [18] suggest that assessment of pre and post booster humoral response is dependant on the immunoassay used.

In conclusion, the AbC-19^{TM} immunoassay detected high positive IgG responses post-booster for all 80 participants sampled. Furthermore, the AbC-19^{TM} test also successfully classified all WHO international standards and detected IgG responses with all genetic variants tested. Cross-reactivity was only observed with one dengue serology sample. Therefore, the AbC-19^{TM} immunoassay would be a cost-effective tool to indicate antibody status.

Authorship statement

all authors meet the ICMJE authorship criteria.

ICMJE statement

TM and JADM conceived the study. LJR, GC, JSM and TM performed all laboratory analyses. JSM, LJR and GC analysed data. JSM performed all statistical analyses/interpretations and produced figures. TM, RKP, MAN, JF and AB coordinated participant recruitment, consent and sampling. LJR, JSM and GC performed sample collection and processing. JSM, LJR, MAN and TM wrote the manuscript. All authors reviewed and approved the final manuscript.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Professor Tara Moore acted as a consultant for Abingdon Health during the final period of sampling. At time of conception and commencement of this study, none of the authors received payment from Abingdon Health.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.clicom.2022.09.001.

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