# Validity of self-testing at home with rapid SARS-CoV-2 antibody detection by lateral flow immunoassay

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**ABSTRACT**

**Background:** We explore SARS-CoV-2 antibody lateral flow immunoassay (LFIA) performance under field conditions compared to laboratory-based electrochemiluminescence immunoassay (ECLIA) and live virus neutralisation.

**Methods:** In July 2021, 3758 participants performed, at home, a self-administered Fortress LFIA on finger-prick blood, reported and submitted a photograph of the result, and provided a self-collected capillary blood sample for assessment of IgG antibodies using the Roche Elecsys® Anti-SARS-CoV-2 ECLIA. We compared the self-reported LFIA result to the quantitative ECLIA and checked the reading of the LFIA result with an automated image analysis (ALFA). In a subsample of 250 participants, we compared the results to live virus neutralisation.

**Results**: Almost all participants (3593/3758, 95.6%) had been vaccinated or reported prior infection. Overall, 2777/3758 (73.9%) were positive on self-reported LFIA, 2811/3457 (81.3%) positive by LFIA when ALFA-reported, and 3622/3758 (96.4%) positive on ECLIA (using the manufacturer reference standard threshold for positivity of 0.8 U $ml^{-1}$). Live virus neutralisation was detected in 169 of 250 randomly selected samples (67.6%); 133/169 were positive with self-reported LFIA (sensitivity 78.7%; 95% CI 71.8, 84.6), 142/155 (91.6%; 86.1, 95.5) with ALFA, and 169 (100%; 97.8, 100.0) with ECLIA. There were 81 samples with no detectable virus neutralisation; 47/81 were negative with self-reported LFIA (specificity 58.0%; 95% CI 46.5, 68.9), 34/75 (45.3%; 33.8, 57.3) with ALFA, and 0/81 (0%; 0.0, 4.5) with ECLIA.

**Conclusions:** Self-administered LFIA is less sensitive than a quantitative antibody test, but the positivity in LFIA correlates better than the quantitative ECLIA with virus neutralisation.

**Keywords:** SARS-CoV-2, COVID-19, lateral flow immunoassay, home-testing, antibodies

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## Introduction

In April 2020 the REal-time Assessment of Community Transmission-2 (REACT-2) study of at-home SARS-CoV-2 antibody testing using self-administered finger prick lateral flow immunoassays (LFIAs) was initiated to provide community prevalence estimates of antibodies to SARS-CoV-2 in England (1-4). As COVID-19 vaccination programmes are rolled out worldwide, large-scale LFIA antibody testing could have an important additional role in monitoring immune responses to vaccinations and informing policy regarding booster doses (5).

The REACT-2 programme conducted extensive clinical and laboratory evaluation of SARS-CoV-2 antibody LFIA performance (6-10), summarised in Supplementary Table S1. The LFIA selected (Fortress, Northern Ireland) was initially evaluated in a healthcare worker cohort known to have been infected with SARS-CoV-2, with a sensitivity 84.0% (95% confidence interval [CI] 70.5, 93.5) and specificity 98.6% (95% CI 97.1, 99.4) (6).

Prevalence studies based on self-administered LFIA have generally produced a lower estimate of population SARS-CoV-2 antibody positivity than those using quantitative laboratory assays, despite adjustment for test performance (11). As a threshold test, it is likely that the LFIA is predominantly missing people with low antibody titres. To investigate the utility of the Fortress LFIA under field conditions, we compare results of self-reported qualitative LFIA results against a quantitative laboratory-based electrochemiluminescence immunoassay (ECLIA) performed on simultaneously self-collected capillary blood. We also explore the relationship between LFIA results and antibody titres with viral neutralisation.

## Methods

### *Study design and sampling*

The study was conducted between 1st July 2021 and 10th August 2021.

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This study recruited participants from round 6 of the REACT-2 study of SARS-CoV-2 antibody prevalence in the community in England, UK. Methods for the REACT-2 study are published elsewhere (1, 12). Briefly, REACT-2 is a series of cross-sectional population surveys. At each round, we contacted a random sample of the population by sending a letter to named individuals aged 18 or over from the National Health Service (NHS) patient list (covering almost the whole population) and respondents were sent an LFIA self-testing kit to perform at home. The LFIA used (Fortress, Northern Ireland) detects antibody against the spike ("S") protein of the virus (contained in, or coded by, all UK licensed vaccines).

For this follow-up study, purposeful random sampling was carried out by re-contacting 7000 participants who had participated in round 6 of REACT-2 in May 2021, aiming to achieve a sample size of 4000. We invited equal numbers in each of the following categories based on results from round 6 – unvaccinated and LFIA negative, double vaccinated (>20 days previously) and LFIA negative, unvaccinated and LFIA positive, and double vaccinated and LFIA positive. This sampling frame was chosen to recruit sufficient people with positive and negative self-test results post-infection and post-vaccination, recognising that many people would have received further vaccination in the interim.

People were invited by post to register until approximately 4000 had signed up. Registration was undertaken online or by telephone. Those who registered were sent a further LFIA test kit to carry out at home, and asked to report the result online, upload a photograph of the result, and complete a short online questionnaire. In addition, participants were asked to take a 400 to 500μl capillary blood sample at the same time-point using an at-home self-collection blood device (Tasso-SST (13)) and return the sample for serological assessment of antibodies.

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***ALFA (Automated Lateral Flow Analysis): machine learning algorithm for automated analysis of LFIA images***

We have shown previously that participant reported LFIA interpretation is consistent with clinician interpreted results (9, 10). However, we developed a computational pipeline (ALFA) which used machine learning algorithms to analyse participant-submitted images of the Fortress LFIA from REACT-2 rounds 1 to 5. Methods for development of ALFA are published elsewhere (14). Automated analysis showed substantial agreement with human experts and performed consistently better than study participants, particularly for weak positive IgG results (14).

***Laboratory Methods***

Serological assessment was performed in a commercial laboratory on the Roche Elecsys® Anti-SARS-CoV-2 ECLIA which reports a quantitative anti-Spike (anti-S) antibody titre. This assay has been previously validated by Public Health England who reported a specificity of 100% (95% CI 99.1, 100), and a sensitivity of 98.5% (95% CI 96.9, 99.4) in samples 21 days post-onset in people with PCR-confirmed infection (15). In addition, the Roche ECLIA demonstrates prolonged antibody detection compared to many other SARS-CoV-2 laboratory-based assays (16, 17). The threshold value for antibody positivity for the Roche ECLIA is 0.8 U $ml^{-1}$ based on manufacturer instructions (15). The lower limit of quantification is 0.4 U $ml^{-1}$ (18). Measurements below this value were truncated at 0.4 U $ml^{-1}$. The assay was analysed in its original scale (U $ml^{-1}$). WHO international standard units are BAU $ml^{-1}$ for anti-spike IgG to allow comparison across studies and platforms (19). The conversion factor for U $ml^{-1}$ to BAU $ml^{-1}$ for the Roche Elecsys® Anti-SARS-CoV-2 assay:

$$\text{BAU } ml^{-1} = \text{U } ml^{-1} / 0.972 \quad (18)$$

In addition, we selected 250 serum samples at random for assessment on a live virus neutralisation assay. Serum samples were heat-inactivated and a 2-fold dilution series was performed in 96-well plates. Serum dilutions were incubated with 100 $TCID_{50}$ SARS-CoV-2 (WT D614G) for 1 hour at 37°C.

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Vero E6 cells modified to overexpress ACE2 and TMPRSS2 (VAT cells) were then added to the wells and incubated at 37°C for 72 hours before assessing the cells for the presence or absence of virus-induced cytopathic effect (CPE). The neutralisation titre of a serum sample was defined as the reciprocal of the highest serum dilution at which CPE was not observed, demonstrating antibody-mediated protection from virus, e.g. protection of cells at a 1:20 dilution of serum gives a neutralisation titre value of 20. Serum samples were titrated 2-fold in duplicate with a starting dilution of 1:10 meaning if 1 of the 2 replicate wells were protected at this first dilution, the titre was expressed as 7.1, halfway to the 1:10 dilution on a log2 scale. Serum samples for which CPE was observed in all wells were therefore defined as having neutralisation titre of <7.1. Using a calculated conversion factor of 2.6 BAU per neutralisation titre unit, the lower limit of detection of 7.1 equates to 18.5 BAU $ml^{-1}$ (20) (Supplementary Figure S1).

***Data analysis***

We report on positivity based on three results for each participant: self-administered and reported LFIA (hereafter self-LFIA), self-administered and machine-read LFIA (hereafter ALFA) and Roche Elecsys® platform (hereafter ECLIA) using the manufacturer recommended threshold ≥0.8 U $ml^{-1}$. As the manufacturer's threshold for antibody positivity for the ECLIA is likely too low to correlate with moderate-to-high levels of protection from infection based on recent studies in the UK population (21, 22), we also report positivity at different thresholds of ≥100 U $ml^{-1}$, ≥350 U $ml^{-1}$ and ≥1000 U $ml^{-1}$ – equivalent to ≥103 BAU $ml^{-1}$, ≥360 BAU $ml^{-1}$ and ≥1029 BAU $ml^{-1}$, respectively. In addition, we report the distribution of quantitative ECLIA results for self-LFIA and ALFA positive and negative results.

We assessed the association between self-LFIA, ALFA, ECLIA and live virus neutralisation titres, with the threshold of neutralisation detection defined as a titre of ≥7.1 (equivalent to 18.5 BAU $ml^{-1}$). We then used this as a standard to determine sensitivity and specificity of self-LFIA, ALFA and ECLIA at

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different thresholds as a measure of neutralisation. The Mann-Whitney test was performed to compare neutralisation titres according to whether positive or negative by self-LFIA, and to compare IgG antibody titres according to whether positive or negative by self-LFIA. The threshold for statistical significance was $p < 0.05$.

As a supplementary analysis, we used multiple linear regression to quantify associations between demographic characteristics, history of COVID-19, vaccination status and time since double vaccinated (two doses) and log10-transformed antibody titres. Methods and results are described in Supplementary Table S3.

Data analysed using statistical packages STATA version 15.0 and GraphPad Prism 9.0.0.

**Ethics**

Ethical approval from South Central–Berkshire B Research Ethics Committee (20/SC/0206; IRAS 283805).

**Results**

Overall, 71.0% (4972/7000) of invited individuals agreed to take part in the study, of whom, 1214 (24.4%) were excluded from the analysis due to either a missing or invalid self-LFIA result (n=327) or a missing or void ECLIA result (n=887). The reasons for the large number of missing or void ECLIA results include insufficient and incorrectly labelled samples and laboratory error, but the distribution of these was not provided by the commercial laboratory performing the tests. A total of 3758 participants had paired self-LFIA and ECLIA results, 96.6% (3457/3578) of whom also uploaded a photograph of their self-LFIA test which enabled analysis using ALFA. Participant characteristics are shown in Table 1. Most participants had received one (862, 22.9%) or two (2430, 64.7%) COVID-19

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vaccine doses, and 27.4% reported suspected or confirmed past COVID-19 (Table 1), meaning that almost all participants (3593/3758, 95.6%) reported either vaccine or prior infection.

***IgG anti-S positivity and antibody titres***

Self-LFIA positivity was 73.9% (2777/3758, 95% CI 72.5, 75.3) (Table 1); ALFA positivity was 81.3% (2811/3457, 95% CI 80.0, 82.6), and ECLIA positivity was 96.4% (3622/3758, 95% CI 95.7, 97.0) using the manufacturer's threshold of ≥0.8 U $ml^{-1}$. ECLIA positivity decreased to 83.1% (95% CI 81.9, 84.3), 62.7% (95% CI 61.1, 64.2) and 47.0% (95% CI 45.4, 48.6) by increasing the ECLIA threshold to ≥100 U $ml^{-1}$, ≥350 U $ml^{-1}$ and ≥1000 U $ml^{-1}$, respectively.

Figure 1 shows the distribution of ECLIA titres for samples that were positive and negative on self-reported LFIA. The self-LFIA positive samples had a median anti-S titre of 1702.0 U $ml^{-1}$ (IQR 357.9 to 7416.0) and a range of 0.40 U $ml^{-1}$ to 25000.0 U $ml^{-1}$. The self-LFIA negative samples had a median anti-S titre of 142.6 U $ml^{-1}$ (IQR 46.6 to 384.0). There were 859 discrepant results with a negative self-LFIA and a positive ECLIA; for these samples the median anti-S titre was 197.6 U $ml^{-1}$ (IQR 78.9 to 443.7) indicating that these were weaker positives on average. Of the self-LFIA positive samples with a negative ECLIA (n=14), the median anti-S titre was 0.4 U $ml^{-1}$; anti-S titre ranged from 0.4 U $ml^{-1}$ to 0.75 U $ml^{-1}$ indicating false positives (Table 2).

Table 2 also shows the comparison using the machine-read (ALFA) LFIA results; for samples with a negative ALFA and positive ECLIA, the median anti-S titre was lower than self-LFIA at 131.67 (IQR 63.3-267.3) suggesting that ALFA was better at detecting weaker positives.

Supplementary Table S2 shows the same results calibrated with anti-S thresholds of ≥100 U $ml^{-1}$, ≥350 U $ml^{-1}$ and ≥1000 U $ml^{-1}$.

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***Live Virus Neutralisation***

Neutralisation assays were performed on 250 randomly selected serum samples, including 167 self-reported positive and 83 self-reported negative LFIA participants.

Live virus neutralisation was detected in 169 of 250 samples. The self-LFIA had an estimated sensitivity of 78.7% (133/169; 95% CI 71.8, 84.6) and specificity of 58.0% (47/81; 95% CI 46.5, 68.9) using detectable neutralisation (equivalent to at least 18.5 BAU $ml^{-1}$) as the comparator (Table 3). The ALFA-LFIA had an estimated sensitivity of 92.3% (142/155; 95% CI 86.9, 95.9) and specificity of 45.3% (34/75; 95% CI 33.8, 57.3) (Table 3). The ECLIA had a sensitivity of 100% (95% CI 97.8, 100.0) and specificity of 0% (95% CI 0.0, 4.5) as all neutralisation titres <7.1 threshold were positive on the ECLIA (Table 3). All 250 samples remained positive by ECLIA when the anti-S titre threshold was increased to 1000 U $ml^{-1}$.

Figure 2 shows the distribution of live virus neutralisation titres against anti-S titres, with points labelled for LFIA positive and negative. Neutralisation titres were higher in participants with positive compared to negative LFIA results ($p<0.0001$). A similar association was observed for anti-S titres and LFIA result ($p<0.0001$).

The conversion of neutralisation titres to BAU $ml^{-1}$ following titration of a WHO antibody reference standard showed that 34.9% (59/169) of the neutralisation positive samples had a titre of ≥100 BAU $ml^{-1}$ (Supplementary Figure 1).

**Discussion**

The self-administered LFIA offers a validated qualitative tool that provides a means for obtaining community-wide SARS-CoV-2 antibody positivity prevalence estimates rapidly and at scale, at reasonable cost by adjusting the results for known test performance. The threshold for positivity of

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the LFIA is higher than that of laboratory-based quantitative assays, producing lower estimates of population antibody prevalence.

Although the LFIA has a threshold that means it does not detect a proportion of positive anti-spike IgG registered on the ECLIA, that threshold is close to the level at which neutralising antibody can be reliably measured. Indeed, we demonstrated that the estimated specificity of the self-administered self-reported Fortress LFIA against positive neutralisation titres was substantially higher than that of the Roche ECLIA with manufacturer's threshold of 0.8 U $ml^{-1}$ (58.0% vs. 0%). There is evidence that the presence of neutralising antibodies in sera is highly predictive of protection from symptomatic disease following SARS-CoV-2 infection and that declining levels of neutralising antibody titres correlate with increased risk of symptomatic infection and severe disease (23).

We question the clinical and epidemiological significance of detectable but low antibody titres (post-infection or post-vaccine) picked up by the low thresholds for positivity used for quantitative laboratory assays and suggest that these cut-offs may need to be recalibrated (upwards) to be a useful marker of protection from infection and/or severe disease. The LFIA is predominantly missing people with low antibody titres. The implications of a higher threshold for IgG detection on LFIA testing are not yet well understood and may represent an important marker of protection. Wei at al. recently explored the association between anti-spike IgG levels and protection from SARS-CoV-2 infection with majority Delta (B.1.617.2) variant in a large representative sample of households with longitudinal follow-up (22). They showed that protection against infection rose sharply as antibody levels increased in unvaccinated participants with prior infection, with 67% protection at 33 BAU $ml^{-1}$ using the OmniPATH 384 Combi SARS-CoV-2 IgG ELISA (Thermo Fisher Scientific) assay. Higher antibody levels were required to reach the same level of protection after vaccination, with 67% protection at 107 BAU $ml^{-1}$ or 94 BAU $ml^{-1}$ with ChAdOx1 (Oxford-AstraZeneca) or BNT162b2 (Pfizer), respectively (22). The threshold for determining IgG positivity for the assay used was ≥23

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BAU $ml^{-1}$ (22). Similarly, Fent et al. showed a vaccine efficacy of 80% against symptomatic infection with majority Alpha (B.1.1.7) variant was achieved with 264 BAU $ml^{-1}$ (21).

Although IgG detection on LFIA or quantitative laboratory-based assays is not designed to document the presence of neutralising antibodies, these findings suggest that antibody positivity on the LFIA could be useful to measure waning of vaccine induced immunity in the population. This approach would indeed be more useful than quantitative assays with low thresholds for positivity: these could result in false reassurance, as the lower thresholds are not as well associated with positive neutralisation titres. Given the strong evidence of a protective role for neutralising serum antibodies (23, 24), and evidence for correlation between SARS-CoV-2 IgG antibody values and neutralisation titres (21), calibrated to the appropriate positivity threshold for protection, rapid antibody testing by LFIA may prove a valuable tool for monitoring the distribution of protective serological antibody responses in the population to inform policy for subsequent vaccination programmes, including the targeting of booster vaccines, and could be useful as a screening tool for identifying individuals in the community with below threshold antibody levels who may benefit from further vaccination or other prevention measures or treatment, including anti-viral therapy, as laboratory-based methods may cause a delay in initiating treatment. However, a cost-effectiveness analysis comparing the use of LFIAs to other options for targeting prevention and treatment programmes would be required to inform future policy.

***Strengths and Limitations***

Unlike previous evaluations of the Fortress LFIA, this study replicates the 'real-world' application of LFIAs in large-scale population antibody prevalence studies where users are self-administering the test in their own homes following detailed instructions. Therefore, the study authentically explores the accuracy of the Fortress LFIA under the field conditions in which it is most likely to be deployed for surveillance.

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Our purposeful sampling strategy of selecting approximately equal numbers of unvaccinated and LFIA negative, double vaccinated and LFIA negative, unvaccinated and LFIA positive, and double vaccinated and LFIA positive may have introduced biases. By purposive selection of vaccinated LFIA negative individuals there is the possibility that we enriched our sample for low level antibody titres that might be less common at population level, thus overall figures on sensitivity cannot be extrapolated to real world use in a random population sample.

We used data from 1st July 2021 to 10th August 2021- that is, while the Delta (B.1.617.2) variant accounted for nearly all cases (25). Our neutralisation assays used a first wave isolate as target, with antigenicity the same as the Wuhan strain. In settings in which Delta is not the dominant variant causing disease, or where neutralisation assays use different strains of the virus, the relationships between IgG antibody positivity by LFIA or quantitative anti-S assays and neutralisation titres shown here may not apply. Indeed, Wall et al. demonstrated neutralising antibody titres were 5.8-fold lower against Delta relative to the Wuhan variant after two doses of BNT162b2 (26). Neutralising antibody titres against Omicron (B.1.1.529) have been shown to be eight-fold lower than with Delta after two BNT162b2 vaccinations (27). As such, emerging viral variants might need higher antibody levels for the same level of neutralising activity (23). In the case where relationships between antibody levels and levels of protection do not change with other variants and assuming that neutralisation is a major mechanism of protection (or that the mechanism of protection remains correlated with neutralisation over time), future LFIAs could be calibrated to the appropriate antibody positivity threshold for protection.

**Conclusion**

At-home self-testing and reporting with LFIAs provide a rapid and cost-effective means to assess population antibody prevalence of SARS-CoV-2. In the future, calibrating the threshold for antibody

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positivity of LFIAs to binding or neutralising antibody levels correlated with protection from infection and/or severe disease, could provide a valuable role for home-testing by LFIA to inform vaccination and treatment strategies going forward. As a first step it would be important to understand the extent to which a positive LFIA result is predictive of protection against infection, illness and hospitalisation.

## NOTES


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### Author contributions

HW, CJA and GSC conceptualized and designed the study and drafted the manuscript. CJA, HW, MW, MM, JCB, NCKW, AAB and WSB undertook data collection and data analysis. DA provided statistical advice. HW, GSC, WSB, PE, CAD, SR and AD provided study oversight. AD and PE obtained funding. SR, RAM, AAB, DA, WSB, CAD, AD and PE critically reviewed the manuscript. All authors read and approved the final version of the manuscript. HW is the guarantor for this paper. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted, had full access to all the data in the study, and had final responsibility for the decision to submit for publication.

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## Data Availability Statement

All data underlying the results are available as part of the article and no additional source data are required.


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## Declaration of interests

We declare no competing interests.

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## References


1. Riley S, Atchison C, Ashby D, Donnelly CA, Barclay W, Cooke GS, et al. REal-time Assessment of Community Transmission (REACT) of SARS-CoV-2 virus: Study protocol. Wellcome Open Res. 2020;5:200.

2. Ward H, Atchison C, Whitaker M, Ainslie KEC, Elliott J, Okell L, et al. SARS-CoV-2 antibody prevalence in England following the first peak of the pandemic. Nature Communications. 2021;12(1):905.

3. Ward H, Cooke GS, Atchison C, Whitaker M, Elliott J, Moshe M, et al. Prevalence of antibody positivity to SARS-CoV-2 following the first peak of infection in England: Serial cross-sectional studies of 365,000 adults. Lancet Reg Health Eur. 2021;4:100098.

4. Ward H, Cooke G, Whitaker M, Redd R, Eales O, Brown JC, et al. REACT-2 Round 5: increasing prevalence of SARS-CoV-2 antibodies demonstrate impact of the second wave and of vaccine roll-out in England. medRxiv. 2021:2021.02.26.21252512.

5. Maple PAC. Population (Antibody) Testing for COVID-19—Technical Challenges, Application and Relevance, an English Perspective. Vaccines. 2021;9(6).

6. Flower B, Brown JC, Simmons B, Moshe M, Frise R, Penn R, et al. Clinical and laboratory evaluation of SARS-CoV-2 lateral flow assays for use in a national COVID-19 seroprevalence survey. Thorax. 2020:thoraxjnl-2020-215732.

7. Moshe M, Daunt A, Flower B, Simmons B, Brown JC, Frise R, et al. SARS-CoV-2 lateral flow assays for possible use in national covid-19 seroprevalence surveys (React 2): diagnostic accuracy study. BMJ. 2021;372:n423.

8. Cann A, Clarke C, Brown J, Thomson T, Prendecki M, Moshe M, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody lateral flow assay for antibody prevalence studies following vaccination: a diagnostic accuracy study [version 1; peer review: awaiting peer review]. Wellcome Open Res. 2021;6(358).


ACCEPTED MANUSCRIPT

9. Davies B, Araghi M, Moshe M, Gao H, Bennet K, Jenkins J, et al. Acceptability, Usability, and Performance of Lateral Flow Immunoassay Tests for Severe Acute Respiratory Syndrome Coronavirus 2 Antibodies: REACT-2 Study of Self-Testing in Nonhealthcare Key Workers. Open Forum Infectious Diseases. 2021;8(11):ofab496.

10. Atchison C, Pristerà P, Cooper E, Papageorgiou V, Redd R, Piggin M, et al. Usability and Acceptability of Home-based Self-testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antibodies for Population Surveillance. Clinical Infectious Diseases. 2021;72(9):e384-e93.

11. UK Government. Coronavirus (COVID-19) latest insights: Antibodies 2022 [Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/articles/coronaviruscovid19latestinsights/antibodies.

12. Ward H, Whitaker M, Tang SN, Atchison C, Darzi A, Donnelly CA, et al. Vaccine uptake and SARS-CoV-2 antibody prevalence among 207,337 adults during May 2021 in England: REACT-2 study. medRxiv. 2021:2021.07.14.21260497.

13. Hendelman T, Chaudhary A, LeClair A, van Leuven K, J C, SL F. Self-collection of capillary blood using Tasso-SST devices for Anti-SARS-CoV-2 IgG antibody testing. PLoS ONE. 2021;16(9):e0255841.

14. Wong NCK, Meshkinfamfard S, Turbé V, Whitaker M, Moshe M, Bardanzellu A, et al. Machine learning to support visual auditing of home-based lateral flow immunoassay self-test results for SARS-CoV-2 antibodies. Communications medicine. 2022;2:78.

15. Public Health England. Evaluation of Roche Elecsys Anti SARS-CoV-2 S serology assay for the detection of anti-SARS-CoV-2 S antibodies 2021 [Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/989460/Evaluation_of_Roche_Elecsys_anti_SARS_CoV_2_S_assay_PHE.pdf.

ACCEPTED MANUSCRIPT

16. Theel ES, Johnson PW, Kunze KL, Wu L, Gorsh AP, Granger D, et al. SARS-CoV-2 Serologic Assays Dependent on Dual-Antigen Binding Demonstrate Diverging Kinetics Relative to Other Antibody Detection Methods. J Clin Microbiol. 2021;59(9):e0123121.

17. Nakagama Y, Nitahara Y, Kaku N, Tshibangu-Kabamba E, Kido Y. A Dual-Antigen SARS-CoV-2 Serological Assay Reflects Antibody Avidity. J Clin Microbiol. 2022;60(2):e0226221.

18. Lukaszuk K, Kiewisz J, Rozanska K, Podolak A, Jakiel G, Woclawek-Potocka I, et al. Is WHO International Standard for Anti-SARS-CoV-2 Immunoglobulin Clinically Useful? medRxiv. 2021:2021.04.29.21256246.

19. Infantino M, Pieri M, Nuccetelli M, Grossi V, Lari B, Tomassetti F, et al. The WHO International Standard for COVID-19 serological tests: towards harmonization of anti-spike assays. International Immunopharmacology. 2021;100:108095.

20. Mattiuzzo G, Bentley EM, Hassall M, Routley S, Richardson S, Bernasconi V, et al. WHO/BS.2020.2403 Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody2020; (20 April 2022). Available from: https://www.nibsc.org/documents/ifu/20-268.pdf.

21. Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nature Medicine. 2021;27(11):2032-40.

22. Wei J, Pouwels KB, Stoesser N, Matthews PC, Diamond I, Studley R, et al. Antibody responses and correlates of protection in the general population after two doses of the ChAdOx1 or BNT162b2 vaccines. Nature Medicine. 2022.

23. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nature Medicine. 2021;27(7):1205-11.

24. McMahan K, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature. 2021;590(7847):630-4.

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25. UK Health Security Agency. SARS-CoV-2 variants of concern and variants under investigation in England. 2021 [Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1025827/Technical_Briefing_25.pdf.

26. Wall EC, Wu M, Harvey R, Kelly G, Warchal S, Sawyer C, et al. Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination. Lancet. 2021;397(10292):2331-3.

27. Nemet I, Kliker L, Lustig Y, Zuckerman N, Erster O, Cohen C, et al. Third BNT162b2 Vaccination Neutralization of SARS-CoV-2 Omicron Infection. New England Journal of Medicine. 2021;386(5):492-4.

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**Table 1: Demographic and clinical characteristics of the study participants by antibody status for self-LFIA, ALFA and ECLIA at 0.8U ml$^{-1}$**

| Characteristic | All participants | Self-LFIA | | ALFA | | ECLIA (0.8 U ml$^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | N (% of total) | no. positive/ total | positivity %[b], (95% CI) | no. positive/ total | positivity %[b], (95% CI) | no. positive/ total | positivity %[a], (95% CI) |
| **All participants** | 3758 | 2777/3758 | 73.9 (72.5, 75.3) | 2811 /3457 | 81.3 (80.0, 82.6) | 3622/3758 | 96.4 (95.7, 97.0) |
| **Sex** | | | | | | | |
| **Female** | 2275 (60.5) | 1760/2275 | 77.4 (75.6, 79.0) | 1766/2095 | 84.3 (82.7, 85.8) | 2192/2275 | 96.4 (95.5, 97.0) |
| **Male** | 1483 (39.5) | 1017/1483 | 68.6 (66.2, 70.9) | 1045/1362 | 76.7 (74.4, 78.9) | 1430/1483 | 96.4 (95.3, 97.3) |
| **Age group (years)** | | | | | | | |
| **18-24** | 385 (10.2) | 343/385 | 89.1 (85.5, 91.8) | 341/372 | 91.7 (88.4, 94.1) | 369/385 | 95.8 (93.3, 97.4) |
| **25-34** | 704 (18.7) | 624/704 | 88.6 (86.1, 90.8) | 625/688 | 90.8 (88.4, 92.8) | 669/704 | 95.0 (93.1, 96.4) |
| **35-44** | 430 (11.4) | 386/430 | 89.8 (86.5, 92.3) | 381/481 | 91.2 (88.0, 93.5) | 416/430 | 96.7 (94.6, 98.1) |
| **45-54** | 163 (4.3) | 128/163 | 78.5 (71.5, 84.2) | 126/157 | 80.3 (73.2, 85.8) | 152/163 | 93.3 (88.2, 96.2) |
| **55-64** | 628 (16.7) | 449/628 | 71.5 (67.8, 74.9) | 459/574 | 80.0 (76.5, 83.0) | 592/628 | 94.3 (92.1, 95.8) |
| **65-74** | 1292 (34.4) | 756/1292 | 58.5 (55.8, 61.2) | 795/1125 | 70.7 (67.9, 73.3) | 1270/1292 | 98.3 (97.4, 98.9) |
| **75+** | 156 (4.2) | 91/156 | 58.3 (50.4, 65.9) | 84/123 | 68.3 (59.4, 76.0) | 154/156 | 98.7 (94.9, 100.0) |
| **Ethnicity** | | | | | | | |
| **White** | 3420 (91.6) | 2502/3420 | 73.2 (71.6, 74.6) | 2533/3136 | 80.8 (79.4, 82.1) | 3298/3420 | 96.4 (95.8, 97.0) |
| **Mixed** | 59 (1.6) | 49/59 | 83.1 (70.9, 90.8) | 52/59 | 88.1 (76.7, 94.4) | 56/59 | 94.9 (84.9, 98.4) |
| **Asian** | 152 (4.1) | 124/152 | 81.6 (74.5, 87.0) | 126/146 | 86.3 (79.6, 91.0) | 147/152 | 96.7 (92.3, 98.6) |
| **Black** | 69 (1.9) | 59/69 | 85.5 (74.8, 92.1) | 57/63 | 90.5 (80.0, 95.8) | 66/69 | 95.7 (87.0, 98.6) |
| **Other** | 35 (0.9) | 25/35 | 71.4 (53.6, 84.4) | 24/31 | 77.4 (58.4, 89.3) | 32/35 | 91.4 (75.4, 97.4) |
| **History of COVID-19** | | | | | | | |
| **Positive PCR test** | 489 (13.0) | 468/489 | 95.7 (93.5, 97.2) | 459/470 | 97.7 (95.8, 98.7) | 488/489 | 99.8 (98.6, 100.0) |
| **Suspected by doctor** | 54 (1.4) | 48/54 | 88.9 (76.9, 95.1) | 49/53 | 92.5 (81.0, 97.2) | 52/54 | 96.3 (85.8, 99.1) |
| **Suspected by self** | 487 (13.0) | 421/487 | 86.5 (83.1, 89.2) | 417/469 | 88.9 (85.7, 91.5) | 455/487 | 93.4 (90.8, 95.3) |
| **No** | 2728 (72.6) | 1840/2728 | 67.5 (65.7, 69.2) | 1886/2465 | 76.5 (74.8, 78.1) | 2627/2728 | 96.3 (95.5, 96.9) |
| **No. of pre-existing health conditions[b]** | | | | | | | |
| **>1** | 701 (18.7) | 433/701 | 61.8 (58.1, 65.3) | 443/621 | 71.3 (67.6, 74.8) | 668/701 | 95.3 (93.4, 96.6) |
| **1** | 881 (23.4) | 606/881 | 68.8 (65.6, 71.8) | 626/800 | 78.3 (75.2, 81.0) | 855/881 | 97.0 (95.7, 98.0) |
| **0** | 2176 (57.9) | 1738/2176 | 79.9 (78.1, 81.5) | 1742/2036 | 85.6 (84.0, 87.0) | 2099/2176 | 96.5 (95.6, 97.2) |
| **Vaccine status** | | | | | | | |
| **0** | 466 (12.4) | 335/466 | 71.9 (67.6, 75.8) | 329/444 | 74.1 (69.8, 78.0) | 363/466 | 77.9 (73.9, 81.4) |
| **1** | 862 (22.9) | 793/862 | 92.0 (90.0, 93.6) | 789/837 | 94.3 (92.5, 95.7) | 856/862 | 99.3 (98.5, 99.7) |
| **2** | 2430 (64.7) | 1649/2430 | 67.9 (66.0, 69.7) | 1693/2176 | 77.8 (76.0, 79.5) | 2403/2430 | 98.9 (98.4, 99.2) |
| **Vaccine type** | | | | | | | |
| **Pfizer-BioNTech** | 1965 (59.8) | 1733/1965 | 88.2 (86.7, 89.5) | 1704/1836 | 92.8 (91.5, 93.9) | 1948/1965 | 99.1 (98.6, 99.5) |
| **AstraZeneca** | 1210 (36.8) | 599/1210 | 49.5 (46.7, 52.3) | 671/1066 | 63.0 (60.0, 65.8) | 1195/1210 | 98.8 (98.0, 99.3) |
| **Moderna** | 110 (3.4) | 105/110 | 95.5 (89.4, 98.1) | 102/104 | 98.1 (92.5, 99.5) | 109/110 | 99.1 (93.7, 99.9) |
| **Time since second vaccination (N=2396) (weeks)** | | | | | | | |
| **0-3** | 326 (13.6) | 312/326 | 95.7 (92.9, 97.4) | 306/317 | 96.5 (93.8, 98.1) | 326/326 | 100 (98.9, 100) |
| **4-12** | 268 (11.2) | 175/268 | 65.5 (59.4, 70.8) | 178/232 | 76.7 (70.8, 81.8) | 268/268 | 100 (98.6, 100) |
| **13-23** | 1766 (73.7) | 1122/1766 | 63.5 (61.3, 65.7) | 1171/1571 | 74.5 (72.3, 76.6) | 1739/1766 | 98.5 (97.8, 98.9) |
| **24+** | 36 (1.5) | 21/36 | 58.3 (41.1, 73.7) | 23/31 | 74.2 (55.1, 87.1) | 36/36 | 100 (90.3, 100) |

[a] Percentages are calculated from non-missing values; [b] A pre-existing health condition is any physical or mental illness or health condition that existed at the time of study.

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**Table 2: Comparison of results from paired self-LFIA and ALFA, and ECLIA (using the manufacturer's threshold of ≥0.8 U ml$^{-1}$), N=3758**

| Self-LFIA | ECLIA positive<br>N<br>(median (IQR) titre) | ECLIA negative<br>N<br>(median (IQR) titre) | Total<br>N<br>(median (IQR) titre) |
|---|---|---|---|
| **Positive** | 2763<br>(1715.0; 368.9-7489.0) | 14<br>(0.4; 0.4-0.4) | 2777<br>(1702.0; 357.9-7416.0) |
| **Negative** | 859<br>(197.6; 78.9-443.7) | 122<br>(0.4; 0.4-0.4) | 981<br>(142.6; 46.6-384.0) |
| **Total** | 3622<br>(925.4; 207.5-4655.0) | 136<br>(0.4; 0.4-0.4) | 3758<br>(824.1; 168.5-4286.0) |
| **ALFA** | | | |
| **Positive** | 2798<br>(1566.5; 313.0-7119.0) | 13<br>(0.4; 0.4-0.4) | 2811<br>(1541.0; 306.2-7079.0) |
| **Negative** | 531<br>(131.6; 63.3-267.3) | 115<br>(0.4; 0.4-0.4) | 646<br>(102.7; 24.7-235.7) |
| **Total** | 3329<br>(947.4; 201.4-4990.0) | 128<br>(0.4; 0.4-0.4) | 3457<br>(831.5; 165.1-4668.0) |

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**Table 3: Comparison of results from self-LFIA and ALFA, ECLIA and SARS-CoV-2 neutralisation titre (NT) (neutralisation titres of 7.1 have been assigned an arbitrary threshold of 0.1), N=250 (Self-LFIA) and N=230 (ALFA).**

| Self- LFIA | NT positive (median (IQR) titre) | NT negative (median (IQR) titre) | Total (median (IQR) titre) | Performance (95% CI) |
|---|---|---|---|---|
| **Positive** | 133 (20.0; 10.0-113.1) | 34 (0.1; 0.1-0.1) | 167 (14.1; 7.1-80.0) | Sensitivity: 78.7 (71.8-84.6) |
| **Negative** | 36 (10.0;7.1-14.1) | 47 (0.1; 0.1-0.1) | 83 (0.1; 0.1-10.0) | Specificity: 58.0 (46.5-68.9) |
| **Total** | 169 (20.0;10.0-80.0) | 81 (0.1; 0.1-0.1) | 250 (10.0; 0.1-28.3) | |
| **ALFA** | | | | |
| **Positive** | 142 (20.0; 10.0-104.8) | 41 (0.1; 0.1-0.1) | 183 (14.1; 7.1-56.6) | Sensitivity: 91.6 (86.1-95.5) |
| **Negative** | 13 (10.0;7 .1-14.1) | 34 (0.1; 0.1-0.1) | 47 (0.1; 0.1-7.1) | Specificity: 45.3 (33.8-57.3) |
| **Total** | 155 (20.0; 10.0-80.0) | 75 (0.1; 0.1-0.1) | 230 (10.0; 0.1-28.3) | |
| **ECLIA (≥0.8 U ml$^{-1}$)** | | | | |
| **Positive** | 169 (20; 10-80) | 81 (0.1; 0.1-0.1) | 250 (10; 0.1-28.3) | Sensitivity: 100% (97.8-100) |
| **Negative** | 0 - | 0 - | 0 - | Specificity: 0% (0-4.5) |
| **Total** | 169 (20; 10-80) | 81 (0.1; 0.1-0.1) | 250 (10; 0.1-28.3) | |

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## Figure Legends

**Figure 1: Box plot (median and quartiles) illustrating the distribution of quantitative ECLIA antibody titres by self-LFIA result (N=3758)**

**Figure 2: Relationship between SARS-CoV-2 live virus neutralisation titre and ECLIA by self-LFIA.**

FOOTNOTE: Positive self-LFIA results are represented in blue and negative LFIA results are represented in red. The threshold of SARS-CoV-2 neutralisation detection is defined as ≥7.1, equivalent to 18.5 BAU $ml^{-1}$, as denoted by the vertical black dotted line and samples below this are marked as not detected (n.d.) Both axes use a Log 10 scale. ECLIA anti-Spike antibody thresholds of ≥100 U $ml^{-1}$, ≥350 U $ml^{-1}$ and ≥1000 U $ml^{-1}$ are denoted by horizontal dotted lines.

Statistical significance is reported by performing a non-parametric Mann-Whitney test for neutralisation titres by self-LFIA positive and negative results (p= 0.0001), and for ECLIA anti-Spike antibody titres by self-LFIA positive and negative results (p=0.0001).

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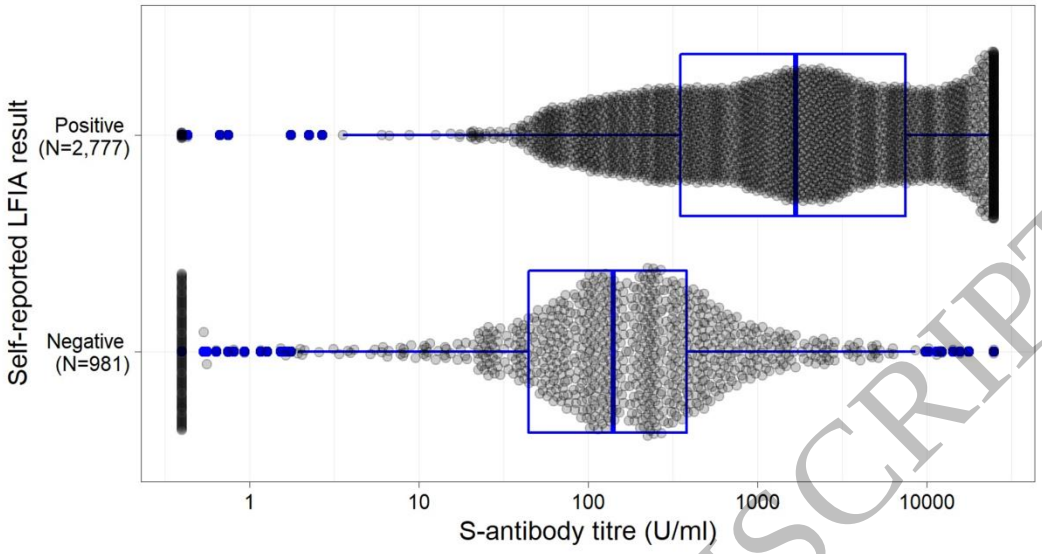


**Figure 1**
**339x190 mm (.74 x  DPI)**

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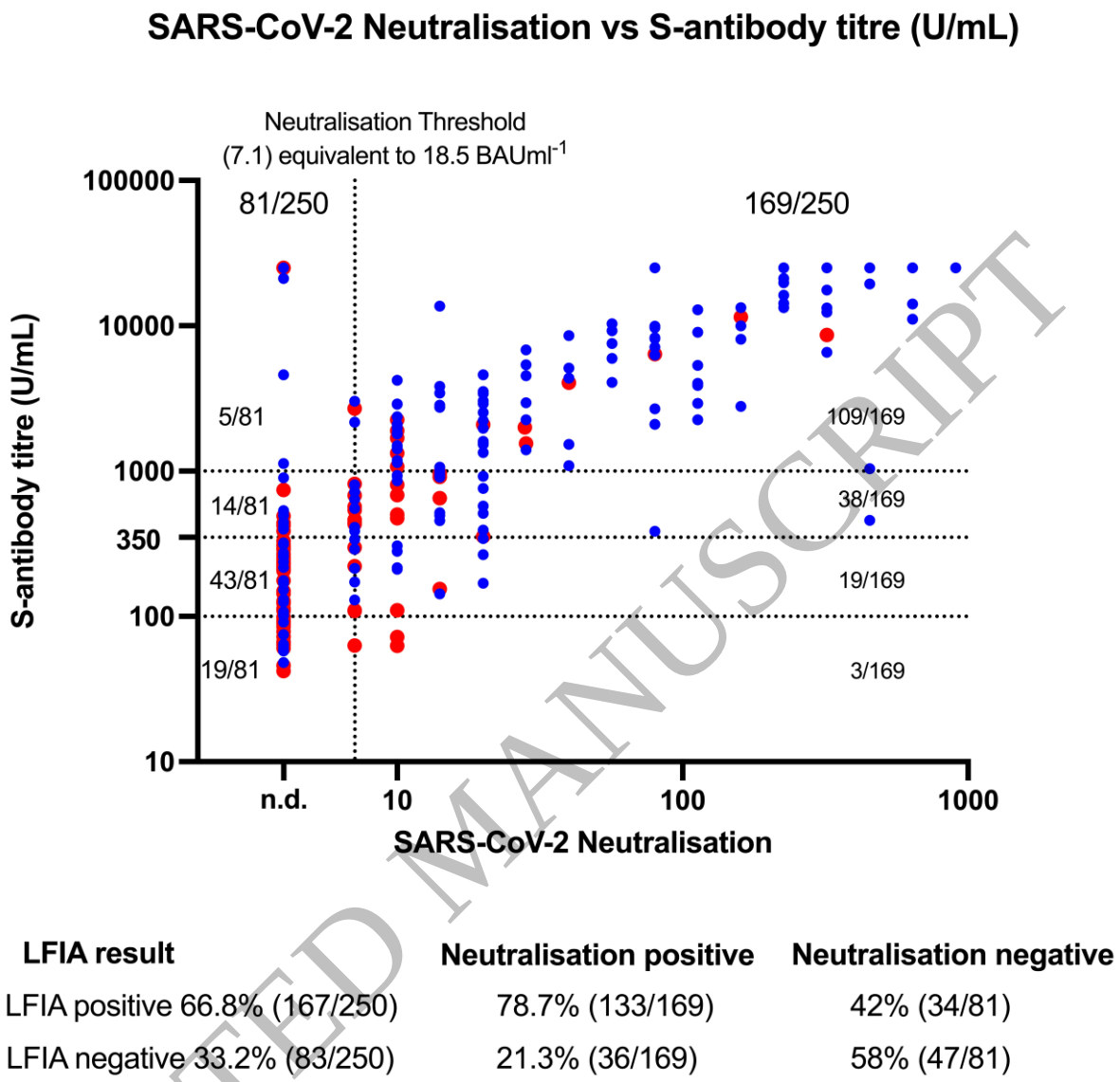


**Figure 2**
**167x153 mm (.74 x DPI)**

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