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: 1 approved]

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Abstract
Background: Lateral flow immunoassays (LFIs) are able to achieve affordable, large scale antibody testing and provide rapid results without the support of central laboratories. As part of the development of the REACT programme extensive evaluation of LFIA performance was undertaken with individuals following natural infection. Here we assess the performance of the selected LFIA to detect antibody responses in individuals who have received at least one dose of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine.

Methods: This was a prospective diagnostic accuracy study. Sampling was carried out at renal outpatient clinic and healthcare worker testing sites at Imperial College London NHS Trust. Two cohorts of patients were recruited; the first was a cohort of 108 renal transplant

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Any reports and responses or comments on the article can be found at the end of the article.
patients attending clinic following two doses of SARS-CoV-2 vaccine, the second cohort comprised 40 healthcare workers attending for first SARS-CoV-2 vaccination and subsequent follow up. During the participants visit, finger-prick blood samples were analysed on LFIA device, while paired venous sampling was sent for serological assessment of antibodies to the spike protein (anti-S) antibodies. Anti-S IgG was detected using the Abbott Architect SARS-CoV-2 IgG Quant II CMIA. A total of 186 paired samples were collected. The accuracy of Fortress LFIA in detecting IgG antibodies to SARS-CoV-2 compared to anti-spike protein detection on Abbott Assay

**Results:** The LFIA had an estimated sensitivity of 92.0% (114/124; 95% confidence interval [CI] 85.7% to 96.1%) and specificity of 93.6% (58/62; 95% CI 84.3% to 98.2%) using the Abbott assay as reference standard (using the threshold for positivity of 7.10 BAU/ml)

**Conclusions:** Fortress LFIA performs well in the detection of antibody responses for intended purpose of population level surveillance but does not meet criteria for individual testing.

**Keywords**
SARS-CoV-2, Covid-19, Lateral flow, LFIA, Antibodies, Neutralisation, Seroprevalence

This article is included in the Coronavirus (COVID-19) collection.
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Introduction
As vaccination programmes for coronavirus disease 2019 (COVID-19) are rolled out worldwide, population antibody testing is useful in monitoring immune responses to vaccinations, informing discussion and decisions about booster doses, and assessing levels of potential protective immunity in the population.\(^1\)

Lateral flow immunoassays (LFIAs) have the potential to deliver affordable, large-scale testing of individuals and provide rapid results without the support of central laboratories. Antigen lateral flow testing is already being used widely. This approach, using antibody lateral flow devices, has been used across England in the REACT2 (REal time Assessment of Community Transmission)\(^3\) study to estimate the number of infections during the first wave of the COVID-19 pandemic,\(^4\) monitor the decline in antibody positivity over time, and assess population antibody prevalence following vaccine roll-out, most recently in Round 5 of the study published in February 2021.\(^5\)

Prior to the scale up of antibody testing for surveillance, extensive clinical and laboratory evaluation of diagnostic accuracy following natural infection was performed on a range of LFIA antibody tests\(^6,7\), identifying one for subsequent use. The test selected (Fortress, Northern Ireland) detects antibody against the spike protein of the virus (contained in all licensed vaccines) and would therefore be expected to detect vaccine induced antibody responses. This study examined the accuracy of the Fortress LFIA device in detecting antibodies in two cohorts of vaccinated individuals and explored the relationship between LFIA results and viral neutralisation.

Methods
This was a prospective diagnostic accuracy study conducted between 20th December 2020 and 26th May 2021. Samples were collected from two groups: renal transplant patients (cohort 1) and healthcare workers (cohort 2).

Bias
Every attempt was made to address potential sources of bias. All eligible participants were offered enrolment where practical and every effort was made to ensure understanding of the participant information sheet (PIS) and study procedure, using translation services where necessary. Potential participants were given time to consider participation and trained research staff were able to answer questions relating to the study.

Eligibility criteria
Eligibility for both cohorts was defined as:

1) Adult (\(\geq\)18 years old)
2) Able to understand and consent to study
3) Received either one or two doses of any UK approved vaccine for COVID-19
4) Able to comply with study procedure/ study not thought to be risk to patient

Sample size
Sample size was computed based on an expected sensitivity of 90% and specificity of 95%, with a minimal acceptable lower confidence limit of -10% for both estimates. Under power 1 - \(\beta\) = 0.85 and \(\alpha\) = 0.15, the minimum sample size required is 106 cases and 76 controls. Patients were pragmatically enrolled to ensure minimum sample size achieved.

Cohort 1: Renal transplant cohort
Participants. Participants were recruited between 1st February 2021 and 26th May 2021. Those recruited were 108 renal transplant recipients who were attending clinic at Hammersmith Hospital 28 days (allowing range from 21 to 42 days) following a second dose of a SARS-CoV-2 vaccine, (either BNT162b2, Pfizer/BioNTech or ChAdOx1, Oxford/AstraZeneca). Participants were recruited directly from clinic by trained medical and nursing staff who explained the study and provided with PIS and informed consent form (ICF). Participants underwent a finger-prick capillary blood draw for immediate testing on the LFIA device and, at the same time, gave a venous blood samples for later serology testing. Clinical characteristics were obtained from electronic medical records (including basic demographic data, past medical history, vaccination status; see Table 1 for full details). All patients provided written informed consent.

Lateral flow immunoassay testing. Participants were supplied with an LFIA testing kit as used in the REACT home testing programme.\(^8\) The LFIA (Fortress, NI) detects IgG and IgM to the S1 subunit of the spike protein. Participants were also provided with verbal instructions on how to use the test by a member of the research team, prior to performing self-testing, with support provided where necessary. The LFIA result was assessed independently by two observers. The results were reported by the colour intensity of the IgG band, and documented as either a positive or negative result.

Cohort 2: Healthcare worker cohort
Participants. Participants were recruited between 20th December 2020 and 31st January 2021. Overall, 39 healthcare workers were consented when attending for first vaccination with BNT162b2 Pfizer-BioNTech. Participants were approached by trained members of the research team at the vaccination centre and provided with a PIS and ICF with explanation of the study. Of these participants, 38 had repeat samples taken at 21 days post vaccination and one further participant had samples taken at 21 days who had not had day 0 samples. In total there were 40 participants. Data was collected on age and gender. Medical records of participants were not accessed for this cohort.

Lateral flow immunoassay testing. Participants were supported in capillary blood draw for use with the Fortress LFIA devices as described above. Results were reviewed and recorded by the study team.

Serological testing. Serological assessment was performed on the Abbott Architect SARS-CoV-2 IgG Quant II CMIA which
reports a quantitative anti-Spike antibody titre. The threshold value for positivity of 7.10 binding antibody units (BAU)/ml. At the time of the study in the healthcare worker cohort (cohort 2), quantitative antibody titres were reported in AU/ml. To allow combination with cohort 1 data, these were converted to BAU/ml by multiplying by 0.142. Double antigen binding assay (DABA) testing for discordant results (positive LFIA with negative serological) was performed on available stored samples from cohort 1. Detailed methodology of DABA has been described previously. Briefly, the Imperial Hybrid DABA is a sequential two step double binding assay for the detection and measurement of antibody directed to the receptor binding domain of SARS-CoV-2. The proteins employed were expressed and gifted by the Crick Institute, London. In order to evaluate specificity the Hybrid DABA was tested on stored plasma and serum samples predating the SARS-CoV-2 outbreak (n=825) in which 0 samples tested positive, giving a specificity of 100%.

In addition, for cohort 2, individuals provided blood for assessment of neutralisation assays. The ability of sera to neutralise the SARS-CoV-2 virus was assessed by neutralisation assay on Vero cells. Sera were serially diluted in OptiPRO SFM (Life Technologies) and incubated for 1h at room temperature with 100 TCID50/well of SARS-CoV-2/England/IC19/2020 and transferred to 96-well plates pre-seeded with Vero-E6 cells. Serum dilutions were performed in duplicate. Plates were incubated at 37°C, 5% CO₂ for 42 h before fixing cells in 4% PFA (paraformaldehyde). Cells were treated with methanol 0.6% H₂O₂ and stained for 1h with a 1:3000 dilution of 40143-R019 rabbit mAb to SARS-CoV-2 nucleocapsid protein (Sino Biological). A 1:3000 dilution of sheep anti-rabbit HRP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients N=108 (%)</th>
<th>Anti-S Seronegative* N= 36 (%)</th>
<th>Anti-S Seropositive* N= 72 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>74 (68.5)</td>
<td>25 (69.4)</td>
<td>49 (68.1)</td>
</tr>
<tr>
<td>Female</td>
<td>34 (31.5)</td>
<td>11 (30.6)</td>
<td>23 (31.9)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years (Range)</td>
<td>54 (41–65)</td>
<td>44 (38–74)</td>
<td>56 (44–64)</td>
</tr>
<tr>
<td>Ethnicity</td>
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<tr>
<td>White</td>
<td>52 (48.1)</td>
<td>17 (47.2)</td>
<td>35 (48.6)</td>
</tr>
<tr>
<td>Black</td>
<td>8 (7.4)</td>
<td>2 (5.6)</td>
<td>6 (8.3)</td>
</tr>
<tr>
<td>Asian</td>
<td>34 (31.5)</td>
<td>11 (30.6)</td>
<td>23 (31.9)</td>
</tr>
<tr>
<td>Other</td>
<td>14 (13.0)</td>
<td>6 (16.7)</td>
<td>8 (11.1)</td>
</tr>
<tr>
<td>Cause of End Stage Kidney Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>9 (8.3)</td>
<td>4 (11.1)</td>
<td>5 (6.9)</td>
</tr>
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<td>Glomerulonephritis</td>
<td>41 (38.0)</td>
<td>12 (33.3)</td>
<td>29 (40.3)</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>18 (16.7)</td>
<td>7 (19.4)</td>
<td>11 (15.3)</td>
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<tr>
<td>Urological</td>
<td>7 (6.5)</td>
<td>2 (5.6)</td>
<td>5 (6.9)</td>
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<td>Unknown</td>
<td>26 (24.1)</td>
<td>8 (22.2)</td>
<td>18 (25.0)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (6.5)</td>
<td>3 (8.3)</td>
<td>4 (5.6)</td>
</tr>
<tr>
<td>Vaccinated ≤1 year post transplant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (5.6)</td>
<td>1 (2.8)</td>
<td>5 (6.9)</td>
</tr>
<tr>
<td>No</td>
<td>102 (94.4)</td>
<td>35 (97.2)</td>
<td>67 (93.1)</td>
</tr>
<tr>
<td>Time vaccinated post-transplant</td>
<td>Years (Median)</td>
<td>5.7 (2.8–11.7)</td>
<td>6.5 (2.9–12.0)</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>75 (69.4)</td>
<td>27 (75.0)</td>
<td>48 (66.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>33 (30.6)</td>
<td>9 (25.0)</td>
<td>24 (33.3)</td>
</tr>
<tr>
<td>Vaccine type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNT162b2</td>
<td>51 (47.2)</td>
<td>12 (33.3)</td>
<td>39 (54.2)</td>
</tr>
<tr>
<td>ChAdOx1</td>
<td>57 (52.8)</td>
<td>24 (66.7)</td>
<td>33 (45.8)</td>
</tr>
<tr>
<td>Time between vaccinations</td>
<td>Days (median)</td>
<td>77 (73–80)</td>
<td>77 (74–80)</td>
</tr>
<tr>
<td>Time of serological test post-boost</td>
<td>Days (median)</td>
<td>34 (29–38)</td>
<td>34 (30–38)</td>
</tr>
<tr>
<td>Prior COVID-19 exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>89 (82.4)</td>
<td>36 (100.0)</td>
<td>53 (73.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>19 (17.6)</td>
<td>-</td>
<td>19 (26.4)</td>
</tr>
</tbody>
</table>
(horseradish peroxidase) conjugate (Sigma) was then added for 1 h. TMB (3,3',5,5'-Tetramethylbenzidine) substrate (Europa Bioproducts) was added and developed for 20 mins before stopping the reaction with 1M hydrogen chloride (HCl). Plates were read at 450nm and 620nm and the concentration of serum needed to reduce virus signal by 50% was calculated to give NT50 values.

**Performance analysis**
The primary outcome of the study was sensitivity and specificity of the LFIA device in detecting SARS-CoV-2 IgG antibodies identified by the Abbott platform.

A secondary analysis was conducted using reference standard as either Abbott or, for discordant results (positive LFIA negative serology) in cohort 1 using in house DABA as reference standard for serological positivity.

Outcomes are presented with the corresponding binomial exact 95% confidence interval (95% CI). Statistical analyses (specificity and sensitivity) were performed with open access online website MedCalc diagnostic test evaluation calculator (version 20.015). Graphical analyses was performed using GraphPad Prism 9.1.2 software. An open-source alternative is R.

**Ethics approval**
Ethics approvals were sought for each cohort prior to commencement of the study.

The renal cohort ethics were obtained from Health Research Authority, Research Ethics Committee (Reference: 20/W A/0123 - The Impact of COVID-19 on Patients with Renal disease and Immunosuppressed Patient).

For the healthcare worker cohort, we got ethics from the South Central-Berkshire B Research Ethics Committee (IRAS ID: 283805), and Medicines and Healthcare products Regulatory Agency approval for use of the LFIA for research purposes only.

### Table 2. Clinical characteristics of healthcare worker cohort.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients N=40 (%)</th>
<th>Anti-S Seronegative</th>
<th>Anti-S Seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D0 N=26 (%)</td>
<td>D21 N=0 (%)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13 (32.5)</td>
<td>10 (38.5)</td>
<td>3 (23.0)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (67.5)</td>
<td>16 (61.5)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years average (range)</td>
<td>43 (23–71)</td>
<td>46</td>
<td>38</td>
</tr>
<tr>
<td><strong>Vaccine type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNT162b2</td>
<td>40 (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Time between vaccinations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days average (range)</td>
<td>65 (57–92)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Results**

**Cohort characteristics**
The characteristics of the participants are described in Table 1 and Table 2. In total, 186 samples were tested using both LFIA and serological testing.

**LFIA IgG positivity and antibody titres in serum**
The combined results describe both cohort 1 and cohort 2 (n=186 samples, Figure 1). Of those samples which scored positive on LFIA (n=118), 4 had undetectable laboratory anti-S levels using Abbott Architect assay. Three of these samples (from the renal transplant cohort) were subsequently re-tested using an in-house DABA which detected antibodies in 1 sample and confirmed negativity in 2. The remaining 114 samples had a median anti-S titre of 229.5 BAU/ml and mean of 229.5 BAU/ml; anti-S titre ranged from 9.7 BAU/ml to 5680 BAU/ml. Of those which scored negative on LFIA (n=68), anti-S antibodies were detected in 10 samples, of which 7 had anti-S titre levels <10 BAU/ml (7.8, 8.0, 8.5, 8.8, 9.2, 9.4, 9.7). The other 58 negative LFIA tests had undetectable anti-S levels (<7.1 BAU/ml).

**Test sensitivity and specificity**

**Primary analysis (Abbott as reference standard).** Using the threshold value for positivity on serological testing of ≥7.10 BAU/ml, the LFIA had an estimated sensitivity of 92.0% (114/124; 95% CI 85.7% to 96.1%) and specificity of 93.6% (58/62; 95% CI 84.3% to 98.2%) using the Abbott assay as reference standard.

**Secondary analysis (Abbott or DABA as reference standard).** Using the threshold vale for positivity on serological testing of ≥7.10 BAU/ml (n=183) on Abbott Architect Assay and confirmatory DABA result for available discordant samples (n=3) as the reference standard, the overall performance of the test in these combined cohorts produce an estimate of sensitivity...
of 92.0% (115/125; 95% CI 85.8% to 96.1%) and specificity of 95.1% (58/61; 95% CI 86.3% to 99.0%). Results were similar when analysing cohort 1 and cohort 2 individually (see Figure 2).

Live virus neutralisation
Neutralisation titres were available for 64/78 samples in the healthcare worker cohort. Neutralisation titres (NT50) were significantly higher in those with positive LFIA compared to those without (Figure 3a). Only one LFIA-negative sample had detectable neutralisation assay using a threshold for positivity of (NT50 of 15 with an anti-S antibody titre of 7.8 BAU/ml). For individuals with detectable IgG on LFIA only 2/34 did not have significant evidence of viral neutralisation.

Discussion
This study demonstrates that the Fortress LFIA device performs well in detecting IgG antibodies in vaccinated individuals when comparing against a serological assay widely used in routine practice. LFIA has been a helpful tool the assessment of population antibody prevalence of SARS-CoV-2, and can play a role in informing vaccination strategy going forwards. The Fortress LFIA has been assessed previously for its performance following natural infection, though did not meet Medicines and Healthcare products Regulatory Agency (MHRA) criteria for individual use which recommend antibody tests should have a sensitivity of >98% (95% CI 96% to 100%) and specificity of >98% on a minimum of 200 known negative controls. The test has undergone extensive evaluation for home self-testing and has since been used widely in community studies of antibody prevalence in England.

The performance of the LFIA in the cohorts of vaccinated individuals here demonstrates slightly higher sensitivity than previously reported for natural infection, though this difference is not significant. This is likely to reflect higher background titres of antibody following vaccination, particularly after second doses, when compared to natural infection in the community, at least in the healthcare worker cohort. The LFIA device does not detect very low levels of antibody which may still correlate with protection from severe disease and/or hospitalisation. However, in the general population, the number of such individuals with low titres following two vaccinations will be low (in contrast to the renal transplant cohort studied here).

A small number of LFIA tests appear to produce false positive results (n=4) with undetectable antibodies in the commercial laboratory assay. To understand whether these were genuine false positives, these four samples were tested with a second sensitive assay (DABA). Only one if these discordant samples tested positive.

There is growing evidence that the presence of neutralising antibodies in sera is highly predictive of protection from symptomatic COVID-19 disease. Although the LFIA studied has a threshold below which it can’t detect Spike specific antibody that is present, that threshold is close to the level at which neutralising antibody can be reliably measured (Figure 3a). This suggests that antibody positivity on the LFIA may give some indication of protection from symptomatic disease and thus could be useful to measure any waning of vaccine induced immunity in different populations.

In terms of study limitations, it is important to note that, although the LFIA tests were self-tested in the clinic or vaccination centre, participants had access to support from trained healthcare professionals when required. This does not fully replicate the ‘real-world’ application of LFIA where users will be following a detailed guide in their own homes. Furthermore, the patient cohort includes healthcare workers and as such may have greater understanding and/or experience of self-testing than members of the general population. However, the primary purpose of this study was to evaluate the diagnostics accuracy of the test.

The performance of the LFIA evaluated is sufficiently good that it can continue to play a helpful role in the assessment of population antibody responses resulting from widespread infection and high levels vaccination coverage, particularly given the correlation of LFIA results with the functional measure of live virus neutralisation. Over time, antibody titres will begin to wane and ongoing population surveillance can play a helpful role in informing decisions on policy for subsequent vaccination programmes, the targeting of booster vaccines. Rapid antibody testing may prove useful in initial screening of patients to receive monoclonal antibody therapy as lab methods may cause a delay in therapy to potentially eligible patients.
**Figure 2.** Flowchart detailing testing results for Cohort 1 and Cohort 2 and Combined Cohort. LFIA=Fortress lateral flow immuno-assay.

LFIAs are lateral flow immunoassays used for rapid detection of antigens or antibodies in bodily fluids. They are commonly used in point-of-care diagnostics for their ease of use and speed. The flowchart illustrates the testing process and the results obtained from each cohort. The sensitivity and specificity of the LFIA tests are calculated based on the true positive (TP), true negative (TN), false positive (FP), and false negative (FN) results.
Data availability

Underlying data

This project contains the following underlying data:
- Anti Spike Protein LFIA For HCW Cohort.tab
- Neutralisation Titres for HCW Cohort.tab
- DABA testing of LFIA Positive Abbott Negative Renal Transplant Samples.tab

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

References


10. MHRA: Target product profile antibody tests to help determine if people have immunity to SARS-CoV-2. [Accessed 21.6.21]. Reference Source


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Ankur Gupta-Wright
Institute for Global Health, University College London, London, UK

This study assesses the diagnostic accuracy of the Fortress LFIA for SARS-CoV-2 anti-S Ab compared to a lab-based serological assay reference standard. The study is appropriately conducted, based on two different cohorts but giving similar results. The authors have attempted to understand if FP results are due to FN results from the reference standard by using an additional assay for discordant results.

Comments that need addressing:

Discussion

- The small sample size leads to wide CIs - this needs to be emphasised in the discussion/limitations section.

- Please state what the previously reported diagnostic accuracy for natural infection was for this assay.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes
Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Diagnostics, Infectious Diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.