Original Article

Early identification of high-risk individuals for monoclonal antibody therapy and prophylaxis is feasible by SARS-CoV-2 anti-spike antibody specific lateral flow assay

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ABSTRACT

Monoclonal antibody therapy has been approved for prophylaxis and treatment of severe COVID-19 infection. Greatest benefit appears limited to those yet to mount an effective immune response from natural infection or vaccination, but concern exists around ability to make timely assessment of immune status of community-based patients where laboratory-based serodiagnostics predominate. Participants were invited to undergo paired laboratory-based (Abbott Architect SARS-CoV-2 IgG Quant II chemiluminescent microparticle immunoassay) and lateral flow assays (LFA; a split SARS-CoV-2 IgM/IgG and total antibody test) able to detect SARS-CoV-2 anti-spike antibodies. LFA band strength was compared with CMIA titer by log-linear regression. Two hundred individuals (median age 43.5 years, IQR 30−59; 60.5% female) underwent testing, with a further 100 control sera tested. Both LFA band strengths correlated strongly with CMIA antibody titers ($P < 0.001$). LFAs have the potential to assist in early identification of seronegative patients who may demonstrate the greatest benefit from monoclonal antibody treatment.

1. Background

Since the onset of the Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) global pandemic, the collective medical community has sought novel therapeutic options to reduce associated disease morbidity and mortality. This has included several monoclonal antibodies which have been demonstrated to reduce morbidity when used either as treatment (e.g., casirivimab plus imdevimab, or sotrovimab) [1−3], or primary (tixagevimab plus cilgavimab) [4] and secondary (bamlanivimab plus etesevimab) [5] prophylaxis.

Where casirivimab plus imdevimab was used, particular evidence of reduced mortality and reduced hospital stays were shown to be most effective in those without notable serological response to either previous vaccination or infection [6]. Findings from the United States also suggested greater benefit in the outpatient setting for those with a high viral load yet to mount an immune response, with early treatment leading to fewer medical attendances [7]. Therefore, rapid

identification of this cohort of patients was essential to optimizing early management. It is likely that as further variants emerge, and as our understanding of all the various mAbs for treatment matures, we will see differential beneficial impact on those without effective B-cell response of their own. Separate to treatment, some areas of the world have now licensed a long-acting monoclonal antibody for primary prophylaxis [4]. Constrained access, and pharmaco-economics, however mean that its use must be optimized towards those who have failed to mount significant B-cell response to prior vaccination. Therefore easily deployable, low cost serodiagnostics must be considered to screen some segments of our population to aid optimal use of mAbs for this indication.

Confirming real-time antibody status via laboratory immunoassays faces inevitable logistical delay for community-based patients. Lateral flow assay (LFA) assessments prior to the vaccine rollout support their use at point-of-care as a potential alternative option to improve early screening, but anti-nucleocapsid LFAs utilized for delayed-case identification have had variable performance to date [8−10]. We have therefore undertaken an initial assessment of point-of-care LFA performance that identify antibodies developed to

the spike antigen in a post-vaccine population, comparing against a laboratory-based quantitative SARS-CoV-2 total IgG antibody immunoassay as current standard. We report the potential utility for their use in timely identification of patients most likely to benefit from mAb therapy at diagnosis.

2. Methods

Comparative testing was carried out in 2 steps, conducted via convenience sampling, across 300 paired tests. Firstly, health care workers at an acute London hospital were invited to attend for SARS-CoV-2 antibody testing. Inclusion was voluntary and no restrictions were placed on eligibility based on participant demographic or career employment group. Participants were consented for a single blood draw that was then tested concurrently on the quantitative Abbott Architect SARS-CoV-2 IgG Quant II chemiluminescent immunoassay (CMIA) and compared with results of a SARS-CoV-2 split IgM/IgG antibody and a total antibody LFA able to detect antibodies generated to epitopes of the SARS-CoV-2 spike antigen. Concurrently a cohort of sera from the SCALPEL (Sars-Cov-2 Antibody response in oLder PEopLe) study were also tested so that comparison could be made across a wider age spectrum.

LFA readers were blind to patient laboratory-based antibody results. Specificity testing was carried out using a cohort of pre-pandemic sera (antenatal) samples and a second real-time, post-pandemic cohort of sera that were seronegative following CMIA testing.

2.1. Laboratory-based immunoassays

Serum was tested for SARS-CoV-2 total IgG antibodies with the qualitative and quantitative Abbott Architect SARS-CoV-2 IgG Quant II CMIA as per the manufacturer's instructions. A threshold value for positive results was established by the manufacturer at 7.1 BAU/ml. The Abbott Architect IgG Quant II CMIA is a 2-step automated immunoassay that detects total IgG antibodies including toward the S1 subunit of the spike protein. The Abbott units (AU/ml) are multiplied by a factor of 0.142, giving a binding antibody unit (BAU)/ml result, reflecting a comparative result with the World Health Organization international standard for SARS-CoV-2 IgG [11]. Following assessment by plaque reduction assay on the SARS-CoV-2 reference strain, the manufacturer guidelines report antibody titers measured by this assay are highly likely to correlate with neutralizing antibody titers.

2.2. Lateral flow assays

The performance of 2 antibody LFAs were compared; the Dixion SARS-CoV-2 split antibody IgM/IgG Duo (Duo Ab LFA) test and the Dixion SARS-CoV-2 total antibody anti-receptor binding domain (total anti-RBD LFA) test. Both LFAs were completed concurrently as per manufacturer's instructions. The authors have previously described a scoring system for LFA based on colorimetric band intensity and results were assessed accordingly [8]. In brief, this involved negative results scored as 0 and positive results scored as 1 to 4 (very weak, weak, moderate and strong respectively) based on increasing band intensity. Each LFA was independently scored by 2 individuals and scores collated.

2.3. Statistical analysis

A pre-established statistical plan was utilized. Demographics, vaccine and prior infection status, and assay performance characteristics were assessed using descriptive statistics. Positive and negative predictive values were calculated for 2 separate community groups based on current UK Office of National Statistics data (i) vaccine uptake within the local area [12] and (ii) national average seroprevalence estimate data in order for results to be reflective across populations [13]. Concordance between paired tests (Abbott SARS-CoV-2 total IgG CMIA with both LFA separately) were assessed using log-linear regression. All data was included in the final analysis with no adjustments required for any missing data. Statistical evidence for an association was quantified with p values derived from likelihood ratio tests. Analysis of results was carried out in R (Version 4.1.1).

3. Results

Two hundred participant samples underwent paired testing (median age 43.5 years, IQR 30−59; 60.5% female). Results were available for Abbot SARS-CoV-2 total IgG CMIA and both LFAs for all participants. A further 50 pre-pandemic samples and 50 post-pandemic known CMIA IgG negative control samples underwent testing. 185/200 (92.5%) of participants had detectable Abbott SARS-CoV-2 total IgG CMIA results (>7.1 BAU/ml) (Fig. 1).

Of those with positive Abbott SARS-CoV-2 total IgG CMIA IgG CMIA results, 184 of 185 had a positive anti-RBD LFA result
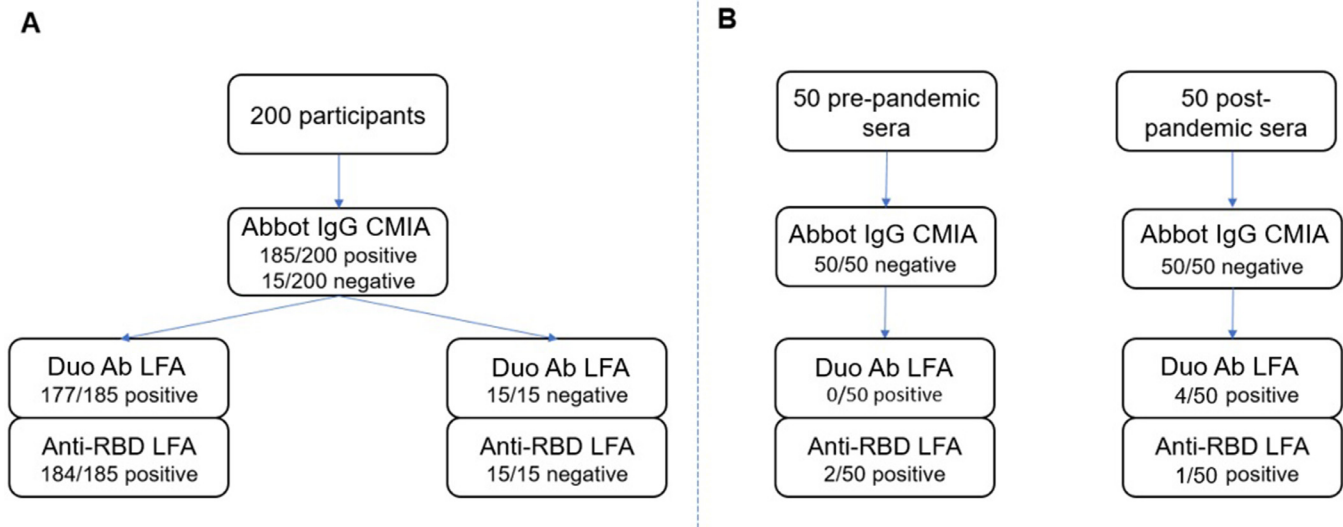
Fig. 1. Results of the Duo Ab LFA and anti-RBD LFA against quantitative Abbott SARS-CoV-2 total IgG CMIA CMIA results, London, UK, July 2021. (A) 200 paired participant samples (B) 100 control samples of which 50 were derived from pre-pandemic sera and 50 from post-pandemic samples that were found to be seronegative following testing with the Abbott Architect SARS-CoV-2 IgG Quant II CMIA. LFA = lateral flow assay; CMIA = chemiluminescent microparticle assay; Duo Ab = SARS-CoV-2 split IgM/IgG Duo antibody test; RBD = receptor binding domain.

Table 1
Performance characteristics of the Duo Ab LFA and anti-RBD LFA against quantitative Abbott SARS-CoV-2 total IgG CMIA results, London, July 2021.

	Duo Ab LFA positive	Duo Ab LFA negative	Anti-RBD LFA positive	Anti-RBD LFA negative
Abbott SARS-CoV-2 total IgG CMIA Detected	177	8	184	1
Abbott SARS-CoV-2 total IgG CMIA Not detected	4	111	3	112
Sensitivity		95.7%, 95% CI 91.7−98.1		99.5%, 95% CI 97.0−100.0
Specificity		96.5%, 95% CI 91.3−99.0		97.4%, 95%CI 92.6−99.5
Overall test concordance		96.0%, 95 CI 93.1−97.9		98.7%, 95% CI 96.6−99.6

Results of paired SARS-CoV-2 IgG serological testing of health care workers and long-term care facility residents with the Abbott Architect SARS-CoV-2 IgG Quant II chemilumines-cent microparticle immunoassay and 2lateral flow assays; the Dixion split IgM/IgG antibody Duo test and the Dixion total antibody anti-receptor binding domain test.
CMIA = chemiluminescent immunoassay; LFA = lateral flow assay; Duo Ab = SARS-CoV-2 split IgM/IgG Duo antibody test; RBD = receptor binding domain.

(sensitivity 99.5%, 95% CI 97.0−100.0). Of all those with negative Abbott SARS-CoV-2 total IgG CMIA results, 3 of 115 had a positive Abbott SARS-CoV-2 total IgG CMIA IgG CMIA (2 pre-pandemic, 1 post-pandemic controls, specificity 97.4%, 95% CI 92.6−99.5). 177/185 (95.7%) participants had a positive Duo Ab LFA result (sensitivity 95.7%, 95% CI 91.7-98.1) while 4 of 115 negative CMIA results had a positive Duo Ab LFA (0 pre pandemic, 4 post-pandemic controls, specificity 96.5% 95%CI 91.3−99.0) (Table 1).

The band strength of the anti-RBD was strongly associated to the Abbott SARS-CoV-2 total IgG CMIA value (log-linear regression LRT $P < 0.001$). Compared to patients with no visible band, patients with a very weak band have on average 6 times the amount of IgG (2−17); 34 times (11−100) for weak, 58 times (30−110) for moderate,

105 times (60−182) for strong. (Fig. 2). The band strength of the Duo Ab LFA was also strongly associated to the CMIA IgG value (log-linear regression LRT $P < 0.001$). Compared to patients with no visible band, patients with a very weak band have on average 14 times the amount of IgG (8−25); 28 times (16−49) for weak, 46 times (29−72) for mod, 100 times (65−152) for strong (Fig. 2). Of note, the Duo Ab LFA has a relatively even distribution of band strengths, whilst the anti-RBD has a large majority (69%) of strong bands with only 6% that are weak or very weak (Fig. 2).

PPV based on local vaccine uptake (63.5% with at least 1 vaccination) [12] for the anti-RBD LFA was calculated at 98.5% (95% CI 95.6−99.5) and the NPV at 99.0% (95% CI 93.6−99.9). For the Duo Ab LFA the PPV was 98.0% (95% CI 94.8−99.2), NPV92.8 (95% CI
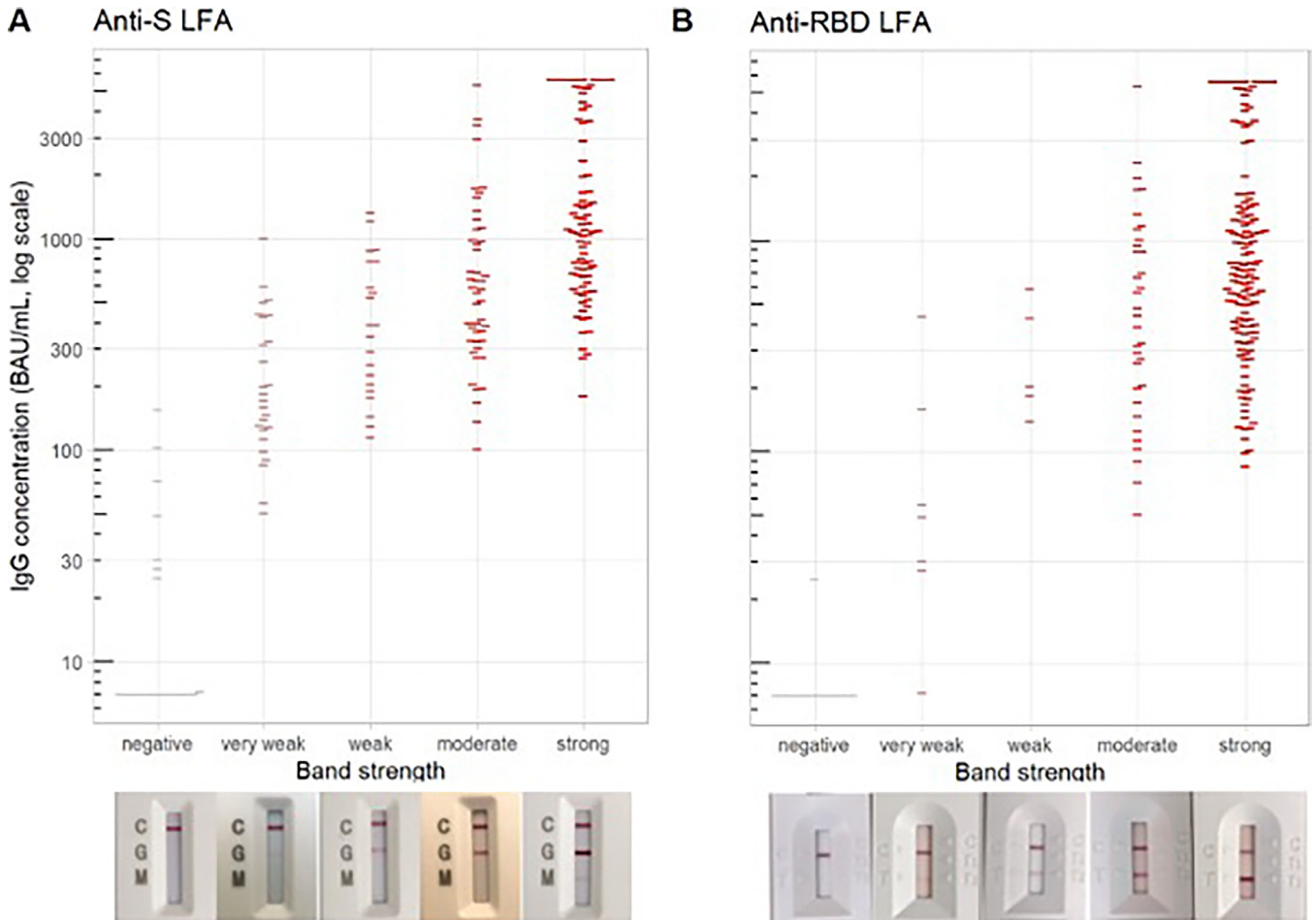
Fig. 2. Comparison of visual band strength with quantified Abbott SARS-CoV-2 total IgG CMIA values, London, July 2021. Qualitative comparison of LFA band strength as previously described. [(Pallett et al., 2020)] Reported band strength is correlated against quantitative Abbott SARS-CoV-2 total IgG CMIA titre demonstrating a positive relationship of visual band strength with rising titre by log-linear regression for (A) the SARS-CoV-2 split IgM/IgG Duo antibody LFA ($P < 0.001$) and (B) the SARS-CoV-2 total antibody anti-RBD LFA ($P < 0.001$). LFA = lateral flow assay; CMIA = chemiluminescent microparticle assay; Duo Ab = SARS-CoV-2 split IgM/IgG Duo antibody test; RBD = receptor binding domain.

86.7−96.2). In high vaccine uptake situations (UK overall data 85.9% eligible individuals to receive first dose), high seroprevalence (modelled seroprevalence of those vaccinated of 93.6%) [13] the PPV for the anti-RBD LFA was 99.4% (95% CI 98.0−99.8) and NPV was 97.8% (86.4−99.7). For the Duo Ab LFA the PPV was 99.1 (95% CI 97.7−99.7), NPV 84.8 (95% CI 73.9−91.7).

4. Discussion

The findings of this study suggest a potential utility in the use of anti-spike LFAs for (i) rapid assessment of suitability of patients who may have had suboptimal adaptive immune response to previous vaccination or infection, and (ii) for screening for those segments of the population who have failed to mount a vibrant SARS-CoV-2 IgG response, and who may be candidates for primary prophylaxis with mAbs in the community. High concordance of LFA and CMIA results allowed for identification of all seronegative patients within our cohort at point-of-care, reflecting their capacity to positively impact targeted distribution of mAbs.

Given logistic challenges and cost pressures currently associated with SARS-CoV-2 mAbs, current laboratory serodiagnosis is suboptimal due to comparatively lengthy laboratory test turnaround time, need for formal venipuncture, high-cost, and limited availability. In contrast, near-patient lateral flow devices offer minimal turn-around time, can be conducted via finger prick, are relatively cheap, and are not constrained by platform capacity issues. The highest risk in following a LFA strategy would be the misidentification of seronegative individuals as seropositive, and so adversely influencing clinical decision making around mAb treatment and prophylaxis. Test specificity is thus integral to justifying any benefit incurred by LFAs in improving the turnaround time for serological assessment. Our results suggest that the anti-RBD LFA would allow for early identification of seronegative individuals in approximately 24 of 25 cases, affording an opportunity for earlier access to treatment or appropriate referral for long acting mAb prophylaxis in the vast majority of cases. Pathways would however need to consider follow up testing, e.g.,with serum on the CMIA, for those testing seropositive as the current model to ensure the 1 of 25 individuals are not disadvantaged.

Sensitivity for the Duo Ab and anti-RBD LFAs were both high and both LFA had a strong correlation of band strength with quantified Abbott SARS-CoV-2 total IgG CMIA titer (Fig. 2). Where LFA were unable to detect present antibody, this was exclusively in samples with very low Abbott SARS-CoV-2 total IgG CMIA results (mean 78.3 BAU/ml). Early data suggests that the greatest benefit conferred by mAbs are seen in seronegative patients [6-7,14]. While it remains unclear if this also holds true for those with an attenuated response, it seems reasonable to consider some benefit may be provided for those with results around the threshold index. There may therefore be a potential benefit in such patients infected with a high viral load, but further assessment from clinical trials will be required to better understand this relationship.

PPV and NPV have been considered for both the cohort setting, where the vaccine uptake is particularly low in comparison to the national average and also for a high seroprevalence setting based off of national antibody surveillance data. While the NPV was higher in our low vaccination setting, it remained reasonable as seroprevalence increased providing greater confidence for use across the current immune landscape. The use of an LFA as part of a strategy to assist identification of those most likely to benefit from mAb therapy in the community based on these findings therefore appears practical.

The chief limitation of this study is in assessment of only 2 of the commercially available LFAs, and while our findings support a proof of concept for use of LFA at point-of-care to screen for anti-spike antibody serostatus, variation seen between the 2 assays preclude automatically extrapolating findings to other LFAs. Based on observed LFA specificity, any strategy looking to employ LFA in this manner would need to consider follow up testing of seropositive individuals with a laboratory-based assay to offset the small risk of false positive LFA results impacting decision making on eligibility for mAb therapy.

5. Conclusions

Anti-spike antibody LFAs have the potential to support laboratory-based testing for the early identification of patients most likely to benefit from mAb therapy and as a potential screening mechanism to identify vaccine non-responders who may benefit from long acting mAb primary prophylaxis. The LFA were able to correctly identify the vast majority of seronegative patients at point-of-care and so confer a potential to significantly reduce time to therapy. Concordance between LFA results and quantified SARS-CoV-2 total IgG CMIA values was high. A very small number of false positive results, more so with the Duo Ab LFA than the anti-RBD LFA, would necessitate follow up testing through the traditional laboratory route for serology.

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Authors' contributions

SJCP, MR, GWD and LSPM designed the study. SJCP, MR, JH and GWD carried out LFA testing. PR carried out CMIA testing. AM conducted statistical analysis. SJCP, MR and LSPM drafted the initial manuscript. All authors reviewed the manuscript and contributed comments to its development. All authors agreed with the final draft for submission.

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