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A simple point-of-care assay accurately detects anti-spike antibodies after SARS-CoV-2 vaccination

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ABSTRACT

Objective: Lateral flow assays (LFA) are sensitive for detecting antibodies to SARS-CoV-2 proteins within weeks after infection. This study tested samples from immunocompetent adults, and those receiving treatments for chronic inflammatory diseases (CID), before and after mRNA SARS-CoV-2 vaccination.
Methods: We compared results obtained with the COVIBLOCK Covid-19 LFA to those obtained by anti-spike (S) ELISA.
Results: The LFA detected anti-S antibodies in 29 of 29 (100%) of the immunocompetent and 110 of 126 (87.3%) of the CID participants after vaccination. Semiquantitative LFA scores were statistically significantly lower in samples from immunosuppressed participants, and were significantly correlated with anti-S antibody levels measured by ELISA.
Conclusions: This simple LFA test is a practical alternative to laboratory-based assays for detecting anti-S antibodies after infection or vaccination. This type of test may be most useful for testing people in outpatient or resource-limited settings.

1. Background

Enormous efforts led to rapid advances in diagnostics following the emergence of the novel Coronavirus SARS-CoV-2 in 2019. While PCR and antigen-based diagnostics are the best tools for detecting active infection, antibody tests can be useful for detecting past infections, in individuals and in communities [13]. Another potential use for antibody testing is assessing anti-viral antibodies in immunocompromised individuals after infection or vaccination. Certain classes of immunosuppressed individuals are at increased risk for severe COVID-19 infection [7,11,12]. Some immunocompromised people also mount weaker antibody responses after vaccination [2,4,9,12]. Enzyme-linked immunosorbent assay (ELISA) can accurately and quantitatively measure antibodies to the SARS-CoV-2 spike (S) protein [1,4,14]. However, ELISAs can be costly and require clinical laboratory equipment and personnel. Several rapid antibody lateral flow assays (LFAs) detect antibodies to the SARS-CoV-2 S or nucleocapsid protein, or to specific domains within these proteins. Our group and others have evaluated these antibody-based LFAs in terms of sensitivity and specificity for SARS-CoV-2 infection [6,8]. Here, we use one of the better performing SARS-CoV-2 LFA to deter-

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mine whether the test detects anti-S antibodies in human serum before and after SARS-CoV-2 vaccination in immunocompetent and immunosuppressed people. Our results show that the LFA worked well for this purpose and that semi-quantitative results determined by LFA correlated with anti-S antibody ELISA results.

2. Methods

Ethics statement: All participants provided written informed consent. The trial was approved by the institutional review board at Washington University in St Louis and University of California at San Francisco.

Patient samples: Sera were collected from immunocompetent people in the WU368 study, and from immunosuppressed patients with chronic inflammatory diseases (CID) who were being treated with a variety of different immunosuppressive medications in the COVaRiPAD (COVID-19 Vaccine Response in Patients with Autoimmune Disease) study, as previously described [4]. Briefly, most sera were collected prior to vaccination and then again after the vaccination series was complete. All immunocompetent participants received the mRNA-based Pfizer-BioNtech vaccine. CID participants were vaccinated with two doses of mRNA-based vaccines: 101 (80%) with the Pfizer-BioNtech and 25 (20%) with the Moderna vaccine. 5 CID participants were excluded from this analysis as they were vaccinated with the adenovirus-vector based vaccine made by Johnson and Johnson.

For the immunocompetent study participants, samples for SARS-CoV-2 antibody LFA as well as ELISA were obtained 29–71 days (median 35) after the pre-vaccination blood draw. Two immunocompetent participants were excluded as post-vaccination sera were obtained 113 and 175 days after the pre-vaccination blood draw. For the CID participants, post-vaccination sera samples for ELISA were obtained 23–76 days (median 31 days) after the pre-vaccination sample. SARS-CoV-2 antibody LFA for this group were performed on samples obtained 54–105 days (median 66) after the pre-vaccination blood draw. This corresponded to 0–54 days (median 35) after the ELISA samples were collected.

Quantitative assessments of antibodies to SARS-CoV-2 spike protein: Quantification of anti-spike IgG was conducted by enzyme-linked immunosorbent assay (ELISA), as previously described [4].

Detection of anti-S antibodies by LFA. Antibody responses were measured using the COVIBLOCK Covid-19 rapid test cassette (Clarity, Boca Raton, Fl, USA). This product currently has emergency use authorization from the Federal Drug Administration for identification of anti-SARS-CoV-2 antibodies, and was donated by a distributor. The test was performed according to manufacturer's instructions to assess the presence or absence of IgM and IgG antibodies reactive to the receptor binding domain of the SARS-CoV-2 Spike protein as previously described [6]. Sera were de-identified and barcoded so that investigators performing the rapid tests were unaware of the immune and vaccination status of each study participant and sample. LFA test results were independently assessed by two readers. If there was a discrepancy between the results, a third reader served as a tie breaker. We also used a simple visual scoring system to provide a semi-quantitative assessment of the LFA. Negative tests were scored as 0, (no visible test line); samples that produced a test line that was less intense than the procedural control line were scored as 1; samples that produced a test line that was as intense as the control line were scored as 2; samples that produced a test line that was more intense than the control line were scored as 3.

Data Analysis: Data were entered into an Excel database (Microsoft, Redmond, Washington, USA). Data were analyzed with Excel or Prism 9 (GraphPad Software, San Diego, Ca, USA) to calculate median, geometric mean, and 95% confidence intervals (CI).

3. Results

The WU368 study enrolled 53 immunocompetent participants and the COVaRiPAD study enrolled 136 participants with CID, as previously described [4]. These participants were enrolled between 12/2020 and 3/2021. They were assessed before and after the full series of SARS-CoV-2 mRNA vaccination. One immunocompetent participant later reported a diagnosis of rheumatoid arthritis after enrolling in the study. For this analysis, that individual was included in the CID cohort. Of this cohort, we had data from 29 immunocompetent and 126 CID participants to analyze.

Participant demographics are shown in Table 1. The mean age of immunocompetent participants was lower than that of the CID participants, and there were more female participants in the CID cohort. The CID diagnoses and medications are shown in Supplemental Table 1. Other co-morbidities in both the immunocompetent and CID cohort are also listed in Supplemental Table 1. The most common diagnoses in the CID group were inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Most of the CID patients were receiving immunosuppressive treatments at the time of vaccination. These included glucocorticoids, hydroxychloroquine, disease modifying antirheumatic drugs (DMARDs) (methotrexate, mycophenolate, and azathioprine), B cell depleting therapies (rituximab and ocrelizumab), anti-tumor necrosis factor (TNF) therapies (adalimumab, certolizumab pegol, etanercept, golimumab and infliximab), anti-IL12/23 therapy (ustekinumab), and targeted synthetic DMARDs (tsDMARDs) (Janus kinase inhibitor (JAKi) tofacitinib).

We tested samples from 29 immunocompetent participants and 126 CID participants by SARS-CoV-2 LFA. Table 2 demonstrates the number of samples with detectible anti-S IgM and IgG by LFA before and after vaccination.

The LFA detected anti-S IgG in 149 of 170 (87.6%) individuals with anti-S IgG detected by ELISA. This included 7 of 8 (87.5%) pre-vaccination samples and 27 of 27 (100%) post vaccination samples from immunocompetent participants, and 7 of 23 (30%) pre-vaccination samples and 108 of 112 (96.4%) post-vaccination samples from CID participants. Table 3 demonstrates the high level of agreement between LFA and ELISA test results for samples from the CID cohort after vaccination, where 95.5% of the LFA tests were concordant with ELISA results (Cohen's kappa score of $k = 0.77$).

Fig. 1 shows that LFA semi-quantitative antibody scores were significantly correlated with ELISA half-maximal titers. (Spearman correlation of $r = 0.92$, $p < 0.0001$). The medians at the half-maximal dilution for the LFA scores were significantly different by Kruskal-Wallis ($p < 0.0001$). The indicated LFA scores were also significantly different by the Mann-Whitney U test with a Bonferroni correction ($p < 0.0001$). There was some overlap in ELISA results for each LFA score. The geometric mean half-maximal dilution for an LFA score of 0 was 33.8, (95% CI: 31.5–36.3), for an LFA score of 1 was 185.1 (95% CI: 108.8–314.9), for an LFA score of 2 was 520.1 (95% CI:174.4–1551), and for an LFA score of 3 was 4697 (95% CI: 3783–5831).

A small number of samples with negative LFA tests were positive for anti-S antibodies by ELISA. These included 1 immunocompetent participant before vaccination, 15 CID participants before vaccination and 4 CID participants after vaccination. The half-maximal dilution for those who were ELISA positive but LFA negative ranged from 1:35–1:584. Most of these individuals had low half-maximal titers; 15 of 20 (75%) had ELISA titers < 1:100.

We then compared the LFA scores and ELISA half-maximal dilution titers according to the participants' clinical and vaccination status (Fig. 2). The median half-maximal dilution for LFA scores were significantly different (Kruskal-Wallis $p < 0.0001$). The indicated LFA scores were also significantly different by the Mann-Whitney U test with a Bonferroni correction ($p < 0.0001$). Results in Fig. 2 show that CID participants had lower LFA scores than immunocompetent participants following vaccination. All immunocompetent participants had LFA scores of 3 and 24 of 27 (88%) had half-maximal dilution values > 1:1000 after vaccination. In contrast, only 93 of 126 (74%) and 91 of 126 (72%) of post-vaccination CID samples had LFA scores of 3 or ELISA half-maximal dilution titers >1:1000, respectively. However, there was not a signifi-

Table 1
Participant demographics.

	Immunocompetent	CID
Age, mean in yrs (range)	42.3 (28–73)	47.2 (22–82)
Female, n (%)	12 (41)	100 (73.5)
Male, n (%)	17 (59)	36 (26.5)
LFA Seropositive after vaccination, n (%)[a]	29 (100)	110 (87.3)
ELISA Seropositive after vaccination, n (%)[b]	27 (100)	112 (88.8)

[a] LFAs were performed for 29 immunocompetent and 126 CID participants.
[b] ELISAs were performed for 27 immunocompetent and 126 CID participants.

Table 2
Lateral flow antibody test results.

	Immunocompetent		CID	
	Pre-vaccine	**Post-vaccine**	**Pre-vaccine**	**Post-vaccine**
IgM (-) IgG (-)	20	0	117	16
IgM (+) IgG (-)	0	0	2	0
IgM (+) IgG (+)	7	28	4	71
IgM (-) IgG (+)	2	1	3	39

Table 3
Lateral flow assay and ELISA antibody test results for samples from participants with chronic inflammatory disease after vaccination.

	ELISA IgG positive	ELISA IgG negative
LFA IgG positive	108 (86%)	2 (1.5%)
LFA IgG negative	4 (3%)	12 (9.5%)

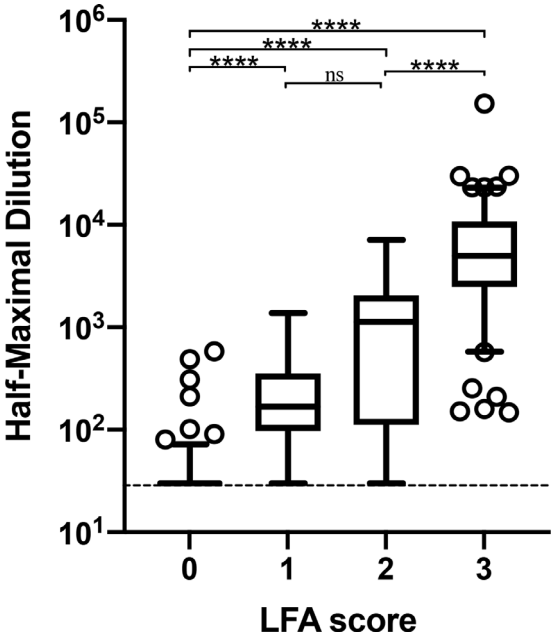

Fig. 1. Lateral flow assay scores correlate with antibody titers assessed by ELISA. SARS-CoV-2 IgG LFA scores for both immunocompetent and CID participants before and after vaccination, as correlated with half maximal dilution titer by ELISA. The half-maximal titer threshold for positivity was 1:30 (shown with a dotted line). The box spans the 25th to 75th percentile, black line indicates the median, the whiskers span the 5th to 95th percentile, and open circles indicate outliers. ****Represents a significant difference between the indicated comparison ($p < 0.0001$, Mann-Whitney U test with Bonferroni correction), while insignificant differences are labeled "ns".

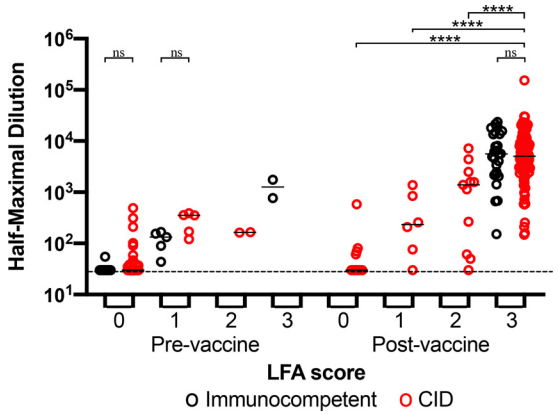

Fig. 2. Lateral flow assay scores for immunocompetent and CID participants before and after vaccination. SARS-CoV-2 IgG LFA results, before and after vaccination for immunocompetent and CID participants, plotted against anti-S half-maximal dilution titers by ELISA. The half-maximal titer threshold for positivity was 1:30 (shown with a dotted line). The median half-maximal dilution is shown with a black line. **** Represents a significant difference between the indicated comparisons ($p < 0.0001$, Mann-Whitney U test with Bonferroni correction), while insignificant differences are labeled "ns".

cant difference in the half-maximal dilution values for the immunocompetent and CID participants who had an LFA score of 3.

As seen in Fig. 2, some of the immunocompetent and CID participants in the study had anti-S antibodies before vaccination. Some of these participants had known prior COVID-19. Others probably had undiagnosed SARS-CoV-2 infections, although we cannot exclude false positive tests results that might have resulted from prior infection with a different Coronavirus. Post-vaccination data were very similar to those in Fig. 2 when results from persons with anti-S antibodies prior to vaccination were excluded (data not shown).

Prior studies have shown that people receiving immunosuppressive treatments are less likely to mount strong immune responses after SARS-CoV-2 vaccination. This is especially true for patients receiving glucocorticoids or B cell depleting therapies [4,5]. We found similar results with the SARS-CoV-2 antibody LFA. Our CID cohort included 10 participants taking B cell depleting therapies: 6 had no anti-S antibodies by LFA or ELISA, 2 had negative LFA and ELISA antibody dilutions < 1:100 and 2 had detectible anti-S antibodies by LFA and ELISA. Our CID cohort also included 11 participants taking prednisone: 4 had no anti-S antibodies by LFA or ELISA and 7 had detectible anti-S antibodies by both LFA and ELISA.

4. Discussion

We tested sera from immunocompetent people and those with CID to assess the ability of a simple SARS-CoV-2 LFA to detect anti-S antibodies before and after SARS-CoV-2 vaccination. The LFA detected anti-S IgG in 149 of 170 (87.6%) individuals with detectible anti-S IgG

by ELISA. However, the LFA identified anti-S IgG in 135 of 139 (97%) post-vaccination sera samples with anti-S IgG detected by ELISA. This included 27 of 27 (100%) post-vaccination samples from immunocompetent participants and 108 of 112 (97%) post-vaccination samples from CID participants with anti-S antibodies by ELISA. The higher rate of agreement between the LFA and ELISA after vaccination is likely due to the higher anti-S antibody titers after vaccination compared with those in pre-vaccination samples. The majority of anti-S antibodies missed by LFA were in samples with very low anti-S antibody titers; 15 of 20 (75%) of these samples had ELISA half-maximal dilutions < 1:100. While we did not specifically measure the limit of detection for the LFA, the fact that 75% of those samples where LFA failed to detect anti-S antibody had ELISA titers < 1:100 suggests that the lower limit of detection for the LFA is approximately 3 times higher than that of the ELISA.

Our results are consistent with a recent meta-analysis that reported 86% of patients with inflammatory rheumatic diseases produced SARS-CoV-2 IgG antibodies after vaccination [12]. Thus, the LFA is a useful surrogate for ELISA results, although it was slightly less sensitive. It is possible that different LFAs have somewhat different sensitivity. The LFA used in this study detects antibodies to the receptor binding domain of the S protein while the ELISA detects antibodies to the entire S protein, which might impact sensitivity. As some of the CID samples tested by LFA were obtained longer after vaccination than the samples tested by ELISA, this may explain some of the discrepant results.

In this study, our semi-quantitative LFA scores were statistically correlated with quantitative titers by ELISA. While usually designed to provide binary results (positive or negative), other studies have shown the value of scoring LFAs semi-quantitatively [3,10,15]. This study confirms the utility of the semi-quantitative LFA scoring in assessing anti-S antibody levels. Although it is possible to increase the sensitivity of LFAs by increasing the amount of antigen used, we think our results demonstrate that the LFA test we evaluated is clinically useful and sensitive enough to use as a surrogate for quantitative assays.

Prior studies have shown that people receiving immunosuppressive therapies are less likely to mount antibody responses after SARS-CoV-2 vaccination, especially if they are receiving glucocorticoids or B cell depleting therapies [4,5,12]. However, some CID participants were able to produce robust antibody responses, and there were not significant differences between the quantitative titers for immunocompetent and CID participants who had the same LFA score.

As the SARS-CoV-2 pandemic continues with successive waves related to new variants, we believe that antibody testing will gain increased prominence as a tool for testing individual patients and for monitoring antibody prevalence in populations. LFAs are especially attractive for antibody testing in outpatient or low resource settings. These LFAs currently cost $10–15 per test, and they do not require specialized laboratory equipment or personnel. Many different COVID vaccines are used around the world, and their efficacies vary in different patient populations. LFAs that provide an indication of prior infection or effective vaccination may be useful for testing individual patients and for seroprevalence surveys. Additional studies will be needed to determine whether LFA test results or scores correlate with protection from infection or the risk of severe disease when infections occur. LFA test results may also be useful for following antibody responses to guide decisions regarding the timing of revaccination.

Conferences

This work was presented at internal university meetings in 2022 (Washington University in St. Louis Infectious Disease Annual Research Symposium and the St. Louis Children's Hospital Pediatric Research Retreat) but not at national conferences.

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Ethics statement

All participants provided written informed consent. The trial was approved by the institutional review board at Washington University in St Louis and University of California at San Francisco.

Declaration of Competing Interest

MAP receives research support from Lilly paid to the institution and received consultant fees from AbbVie, JK Market Research, and Priovant Therapeutics. **PD:** Consultant or on an advisory board for Janssen, Pfizer, Prometheus Biosciences, Boehringer Ingelheim, AbbVie, Arena Pharmaceuticals, Boehringer Ingelheim, CorEvitas LLC and Scipher Medicine Corporation. He has also received funding under a sponsored research agreement unrelated to the data in the paper from Takeda Pharmaceutical, Arena Pharmaceuticals, Bristol Myers Squibb-Celgene, and Boehringer Ingelheim. **SPJW** has filed a patent with Washington University for VSV-SARS-CoV-2 mutants to characterize antibody panels (PCT/Us2021/027275). SPJW has received unrelated funding support in sponsored research agreements with Vir Biotechnology.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2023.100135.

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