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Evaluation of a point of care lateral flow assay for antibody detection following SARS CoV-2 mRNA vaccine series

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ABSTRACT

Background: Breakthrough cases of SARS-CoV-2 infection correlate with decreased antibody immunity following mRNA vaccination. Measuring kinetics of vaccine efficacy using traditional laboratory approaches is more expensive and can be impractical. In this study, we evaluated the diagnostic performance of a validated COVID-19 point-of-care lateral flow assay (LFA) kit in detecting post-vaccination antibody response.

Methods: We conducted a prospective cohort study of whole blood and plasma samples to evaluate the performance of a LFA in detecting SARS-CoV-2-specific antibodies following mRNA vaccination compared to enzyme-linked immunosorbent assays (ELISAs). Health care workers at 2 tertiary centers who completed an initial BNT162b2 ($n = 103$) or mRNA-1273 ($n = 35$) vaccine series were enrolled between June and August of 2021. We performed an exploratory analysis to correlate band strength and antibody concentration of LFAs and ELISAs respectively.

Results: When compared to the ELISA, LFA results showed similar test positivity for plasma samples ($P = 0.55$), but not for whole blood samples ($P < 0.001$). For whole blood samples on the LFA, antibody detection differed between BNT162b2 (68.9%, 95% CI: 59.1%–77.7%) and mRNA-1273 (100%, 95% CI: 90.0%–100%, $P < 0.001$) vaccines. Higher plasma antibody concentrations correlated with greater LFA sensitivity. Samples with thick LFA bands had higher antibody concentrations compared to samples having faint LFA bands (81.8 arbitrary unit [AU]/mL vs. 57.1 AU/mL, $P < 0.01$).

Conclusions: The performance of a LFA in detecting SARS-CoV-2 antibodies was significantly better when plasma samples were used. The strength of label bands on the LFA may correlate with antibody concentration and could be a useful point-of-care monitoring tool for post-vaccine antibody status.

1. Introduction

Since the BNT162b2 (Pfizer-BioNTech) and the mRNA-1273 (Moderna) vaccines were approved using Emergency Use Authorization (EUA) in the United States, over 10 billion doses of SARS-CoV-2 vaccines have been distributed worldwide.(Ritchie et al., 2020) In clinical trials, both vaccines were shown to be efficacious against COVID-19 disease and current circulating SARS-CoV-2 variants/strains at any level of severity; results did not differ depending on age, sex, race, and ethnicity. (Baden et al., 2021; Polack et al., 2020) While completing the initial vaccine series elicited a neutralizing antibody response, antibody levels were shown to substantially decrease over time, leading to a growing risk of breakthrough infections.(Baden et al., 2021; Goldberg et al., 2021; Levin et al., 2021; Lumley et al., 2021; Shrotri et al., 2021).

Studies have suggested that neutralization titers correlate with immunity and that monitoring the level of neutralizing antibodies may be an important predictor of ongoing vaccine efficacy.(Corbett et al., 2021; Khoury et al., 2021) The formation and subsequent waning of an antibody response can vary depending on age, immunogenic response to vaccines, and other clinical factors.(Jeewandara et al., 2021; Levin et al., 2021; Pegu et al., 2021) While serial measurements of neutralizing antibodies can assist in predicting immunity level declines and guiding booster vaccination schedules, traditional or "gold standard" approaches to detect antibody levels can be cost prohibitive, and requires more complex laboratory testing, which limits large-scale application.

Immunochromatographic lateral flow assays (LFA) can be used as point-of-care (PoC) tools and can produce qualitative results in minutes. While numerous LFA PoC kits have been developed to detect

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convalescent antibodies, their ability to detect antibodies post-vaccination have not been tested. Furthermore, the threshold for qualitative serum antibody detection remains unknown. In this study, we critically evaluated the diagnostic performance of a COVID-19 PoC LFA kit in detecting post-vaccination antibody responses.

2. Methods

2.1. Study design and population

We conducted a prospective cohort study evaluating the performance of a LFA kit (Humasis®, Anyang, South Korea) for its ability to detect SARS-CoV-2-specific antibodies following the BNT162b2 or mRNA-1273 initial 2-dose vaccine series. We compared the LFA performance to that of Enzyme-Linked Immunosorbent Assays (ELISA), a traditional laboratory method for detecting antibody presence. We used 2 commercially available EUA designated ELISA kits: Genscript® (NJ, USA) and Kantaro® (NY, USA), which detect antibodies against the receptor binding domain (RBD) of SARS-CoV-2. The Genscript® ELISA is designed to detect neutralizing antibodies to SARS-CoV-2. Both the BNT162b2 and mRNA-1273 vaccines generated antibodies against the critical neutralizing domain within the RBD of spike protein.(Min and Sun, 2021) We also used a third ELISA kit (Bio-Rad® Platelia [CA, USA]) that detects regions of the nucleocapsid gene to distinguish the presence of neutralizing antibodies generated by vaccination alone from a mixed state of vaccination and prior COVID-19 exposure.

We enrolled 138 health care workers at the University of California San Francisco (UCSF) and Zuckerberg San Francisco General Hospital (ZSFG) who had completed either their BNT162b2 or mRNA-1273 initial 2-dose vaccine series. Samples were collected between June and August of 2021 when the Delta variant was most prevalent.(CA.Gov, 2022) The eligibility criteria required participants to be 18-years-old or older and at least 14 days from their second dose vaccination date. Those who received 1 dose of an mRNA vaccine and 1 non-mRNA vaccine (mix-and-match vaccination) were not considered for the study. Study participants were asked to document any side effects of vaccination. All study participants gave explicit written informed consent. The study protocol was approved by the Institutional Review Board (#21–33,856).

2.2. Specimen collection and assay protocol

To determine whether the source of a sample can affect LFA performance, capillary whole blood and plasma samples were tested separately. Per manufacturer guidelines, 10 μL of capillary whole blood samples were drawn using finger pricks. A separate 3-mL sample of venous whole blood was drawn into an ethylenediaminetetraacetic acid-coated microtainers. The tubes were centrifuged for 10 min at 3000 *G*. The plasma fraction was collected and stored in a − 80 °C freezer. The samples were later thawed at room temperature immediately prior to laboratory assay analyses.

From an initial finger prick, 10 μL of whole blood or plasma was deposited onto a test kit, followed by a buffer supplied by the manufacturer. Test kits were read 15 min after the buffer was placed. Each test kit was numerically labeled to match the sample number. For ELISA assays, we followed the description and methodologic protocols outlined by each manufacturer.

2.3. Exploratory analysis

We assessed whether neutralizing plasma antibody concentrations influenced LFA performance. Both ELISA kits for RBD-specific antibody detection can be used semi-quantitatively. While LFAs are designed to be qualitative, the strength of lateral flow bands can vary by sample. Using the control band strength as a baseline, the strengths of the test bands were designated as "none," "faint" (band strength <30% of the control), "moderate" (>30% band strength, < 70% of control band), or "full/thick" (> 70% of control band). We then evaluated whether the strength of the test bands correlated with plasma antibody concentration. Examples of band strength provided in Supplement A.

2.4. Statistical analysis

The mean (standard deviation [SD]) and median (inter-quartile range [IQR]) were used to describe the normal and non-normal distributed quantitative variables. The Kruskal-Wallis test was used to compare different non-normal distributed variables. Chi-squared and the equality of proportion tests were used to analyze the difference between categorical variables. Univariate and multivariate logistic regression models were developed to identify significant variables that affected the presence of neutralizing antibodies and the thickness of LFA band strength. The 95% 2-sided confidence intervals (CIs) were used for significance analysis. Data were aggregated and analyzed using Visual Studio Code v.1.62 (Microsoft®, Redmond, USA) and STATA v15.1 (College Station, USA).

3. Results

3.1. Study population

The characteristics of the study population are shown in Table 1. Between June and August of 2021, 138 volunteers participated in the study; 103 received the BNT162b2 vaccine and 35 received the mRNA-1273 vaccine. The Delta variant was the most predominant variant during this period. The mean age was 43.7 years and 61.6% of participants identified as female. For age and sex, there were no significant differences between the BNT162b2 vaccine or the mRNA-1273 vaccine. The most commonly reported vaccine side effects were pain, swelling, fever, chills, headache, and myalgia; 7 participants (5.1%) reported no side effects. Within the study cohort, only 3 participants tested positive for anti-nucleocapsid antibodies, which suggests prior or recent infection with SARS-CoV-2; 2 out of the 3 received the BNT-162b2 vaccine.

Study participants who received the BNT162b2 vaccine had lower concentrations of plasma antibodies against SARS-CoV-2 spike proteins compared to those who received the mRNA-1273 vaccine (52.5 vs. 74.7 arbitrary units [AU]/mL, $P < 0.001$, Fig. 1). However, the BNT162b2 cohort had a longer elapsed time after receiving their second vaccine dose (165 days vs. 140.0 days, $P < 0.001$). For both vaccines, the plasma antibody concentration trended downward the further removed they were from completing their vaccine series. Even after controlling for the time after last vaccine dose, participants who received BNT162b2 had lower concentrations of plasma antibodies ($P < 0.05$).

3.2. Primary outcome

With the LFA, 76.8% (95% CI, 68.9%–83.6%) of the whole blood samples tested positive. The antibody positivity on the LFAs was

Table 1
Characteristics of study participants.

	BNT162b2 ($n = 103$)	mRNA-1273 ($n = 35$)	P – Value
Sex			
Female	60 (58.3%)	25 (71.4%)	0.169
Age (Mean)	43	45	0.283
Symptoms			
Pain/Swelling	35 (34.0%)	16 (45.7%)	
Fever/Chills	30 (29.1%)	25 (71.4%)	
Fatigue	61 (59.2%)	29 (82.7%)	
Myalgia	40 (38.8%)	23 (65.7%)	
Nausea/Vomiting	9 (8.7%)	3 (8.6%)	
Headache	79 (76.7%)	22 (62.9%)	
Any symptoms	96 (93.2%)	35 (100%)	0.115
Number of Days from			
Second Dose	165 days	139 days	< 0.001

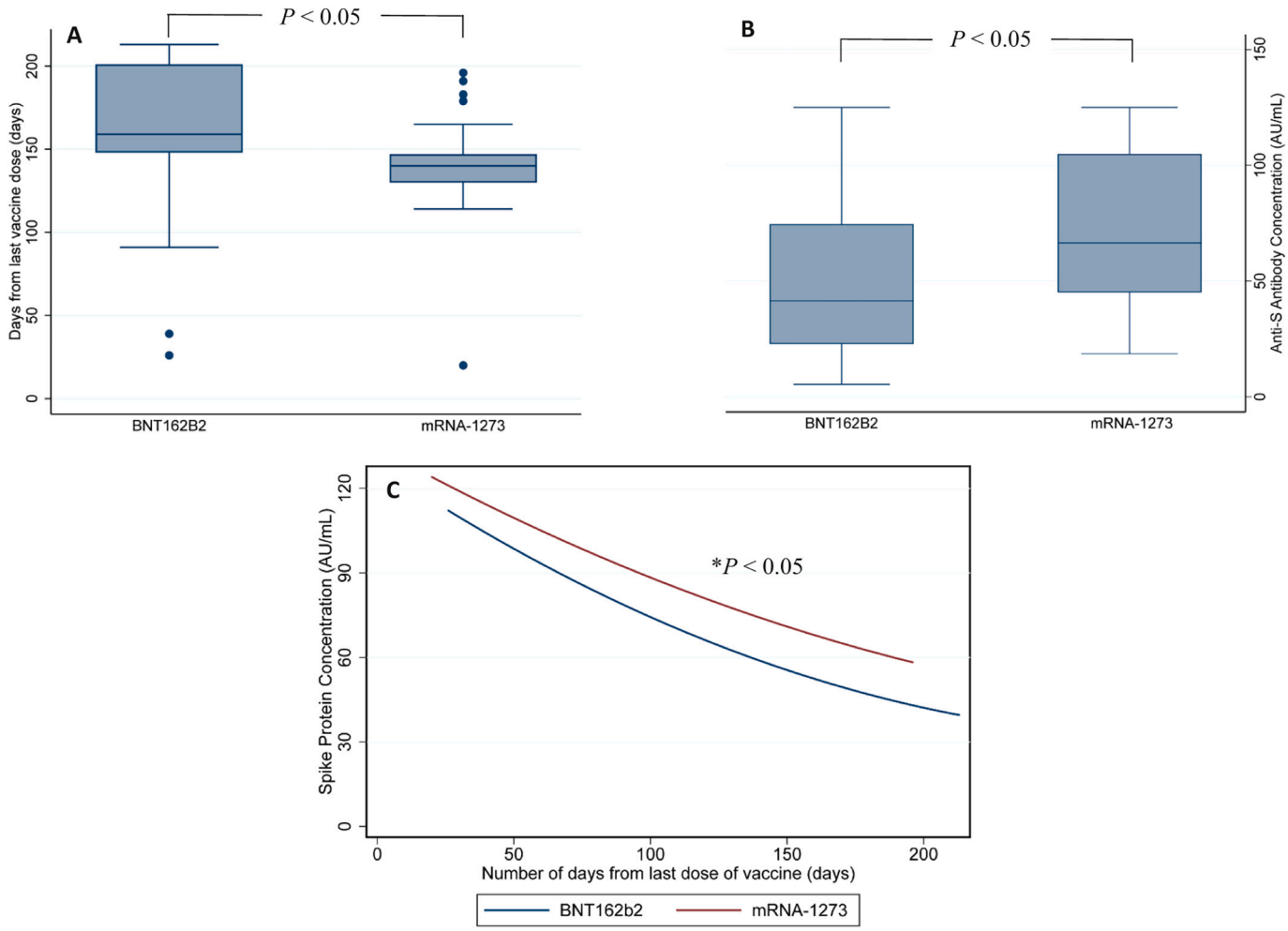

Fig. 1. Relationship Between SARS-CoV-2 mRNA Vaccination and Anti-Spike Antibody Concentration. ELISA kits assessed the vaccine-induced antibody responses using plasma samples from study participants who received either the BNT162b2 ($n = 103$) or the mRNA-1273 ($n = 35$) SARS-CoV-2 vaccines, including (A) the average number of days since study participants completed initial vaccine series for either BNT162b2 or mRNA-1273, (B) the average plasma Anti-S (Spike Protein) antibodies measured by vaccine type, and (C) the relationship between plasma Anti-S antibody concentration and the number of days since completion of initial vaccine series. Lines indicate the cross-sectional average using quadratic fit model for each group and colored accordingly. In A-B, the box plots show the median in the 25th and 75th percentiles and the minimum and maximum whiskers; dots beyond the whiskers represent outliers.

significantly higher with plasma samples than whole blood samples (94.2%, 95% CI, 88.9%–97.5%, $P < 0.001$). All samples tested positive for plasma antibodies using the Genscript ELISA kit; only 2 samples tested negative using the Kantaro ELISA kit. These two samples also tested negative on the LFA for both WB and plasma. There was a significant difference in antibody positivity on the LFA based on vaccine type. All whole blood and plasma samples from study participants who received the mRNA-1273 vaccine were positive, but only 68.9% (95% CI, 59.1%–77.7%, $P < 0.001$) of whole blood samples and 92.2% (95% CI, 85.3%–96.6%, $P = 0.09$) of plasma samples from participants who received the BNT162b2 vaccine tested positive (Fig. 2).

Fig. 3 shows a subgroup analysis that correlates plasma antibody concentration with LFA sensitivity. Using whole blood samples, the LFA was able to detect antibodies in plasma antibody concentration in 87.6% of samples with 30 AU/mL or more ($n = 105$, 95% CI, 79.8%–93.2%, $P < 0.05$) and in 93.0% of samples with 60 AU/mL or more ($n = 57$, 95% CI, 83.0%–98.1%, $P < 0.01$). For participants who completed the BNT162b2 vaccine series, those with plasma antibody concentrations >30 AU/mL (82.0%, 95% CI, 71.1%–90.0%, $P < 0.05$) were also more likely to test positive on the LFA than those with <30 AU/mL. For the LFA plasma samples, higher plasma antibody concentration correlated with higher detection, but this was not statistically significant.

3.3. Exploratory analysis

We performed an exploratory analysis that correlated the strength of the LFA bands with plasma antibody concentrations. Using a "faint" band as a reference for whole blood samples, the presence of any band strength correlated with higher antibody concentration (57.1 AU/mL vs. 33.4 AU/mL, $P < 0.01$) and higher antibody activity (90.6% vs. 81.4% inhibition, $P < 0.001$) than samples with no band (Supplement A). "Thick/full" band samples were likely to have significantly higher antibody concentration (81.8 AU/mL vs. 57.1 AU/mL, $P < 0.01$) and antibody neutralizing capacity (97.9% v. 90.6% inhibition, $P < 0.01$) when compared to "faint" band samples. There was no statistically significant difference between samples with "faint" or "moderate" band strength. Plasma samples with "thick/full" band strength correlated with significantly higher antibody concentration (73.1 AU/mL vs. 38.2 AU/mL, $P < 0.001$) and greater antibody neutralizing capacity (96.7% vs. 80.5% inhibition, $P < 0.001$) than samples with "faint" band strength. Unlike whole blood samples, plasma samples had no statistically significant differences between no band strength and "faint" band strength (Table 2).

Band strength was subjectively assessed as either (1) not present, (2) faint, (3) moderate, or (4) thick/full by comparing the label band to the control band thickness. Genscript ELISA was used to detect antibody

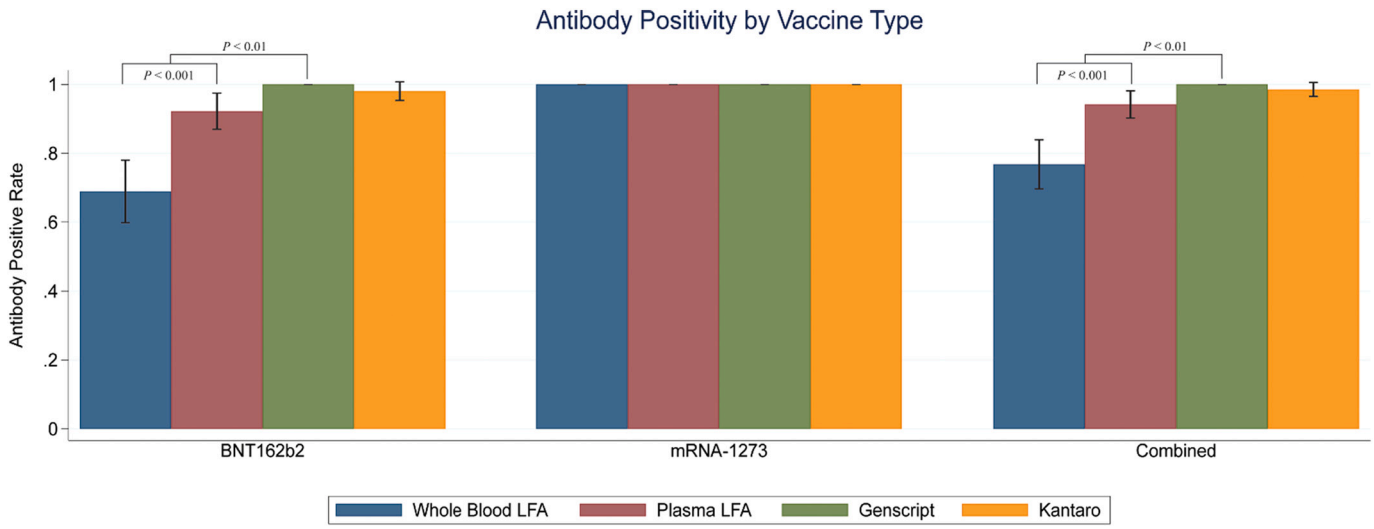

Fig. 2. Rate of antibody positivity seen on either Lateral Flow Assay (LFA) or Enzyme-Linked Immunosorbent Assays (ELISA) from study participants who completed BNT162b2 ($n = 103$) or mRNA-1273 ($n = 35$) vaccine series. The LFA assays were conducted with either whole blood from finger pricks or plasma samples. For each LFA test, 10 μL of samples were drawn per manufacturer recommendations. Two ELISA devices (Genscript and Kantaro) were tested using plasma samples. The combined category represents all study participants; the error bars represent 95% CI.

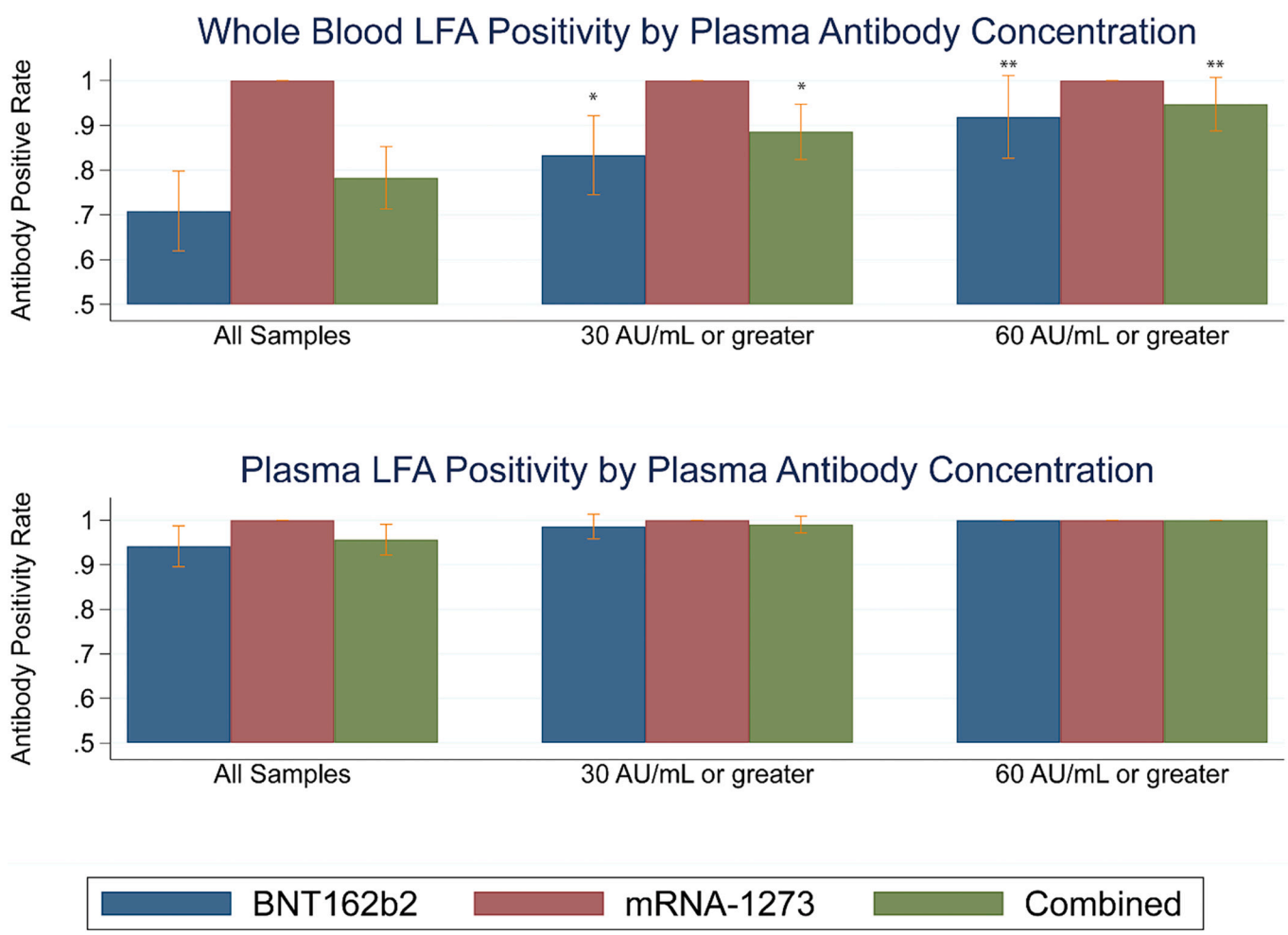

Fig. 3. A Correlation of SARS-CoV-2 Antibody Concentration and LFA Detection Level. Antibodies were detected using (a) whole blood samples or (b) plasma samples for both vaccine types. Samples were categorized by antibody concentration (30 AU/mL or greater and 60 AU/mL or greater). The combined category represents all study participants; error bars represent 95% CI. * denotes $P < 0.05$, ** denotes $P < 0.01$.

Table 2
Exploratory analysis correlating the strength of LFA bands with SARS-CoV-2 specific antibody concentration.

	LFA Band Strength	Number of Samples (%)	Antibody Inhibition (%)	*P*-Value	Mean Plasma Antibody Concentration (AU/mL)	*P*-Value
Whole Blood	Not Present	32 (23.2%)	81.4	< 0.001	33.4	< 0.01
	Faint	52 (37.7%)	90.6	Ref	57.1	Ref
	Moderate	29 (21.0%)	95.0	0.08	66.8	0.20
	Thick/Full	25 (18.1%)	97.9	< 0.01	81.8	< 0.01
Plasma	Not Present	8 (5.8%)	73.7	0.09	30.1	0.54
	Faint	25 (18.1%)	80.5	Ref	38.2	Ref
	Moderate	38 (27.5%)	90.5	< 0.001	50.7	0.14
	Thick/Full	67 (48.6%)	96.7	< 0.001	73.1	< 0.001

inhibition, whereas Kantaro ELISA was used to quantify plasma antibody concentration. A "faint" line was used as a reference (ref). Differences were considered statistically significant if $P < 0.05$.

4. Discussions

This study evaluated the performance of a Lateral Flow Assay (LFA) in detecting antibodies following a SARS-CoV-2 two-dose mRNA vaccination series. When whole blood samples were used, the LFA was significantly less likely to detect the presence of SARS-CoV-2 specific antibodies compared to ELISA assays. When plasma samples were used, the LFA was able to detect antibodies at similar levels as the ELISA assays (Fig. 3). The difference in the LFA's ability to detect antibodies based on the sample source may be due to a threshold effect. Although we used equivalent volumes for each sample, plasma contained larger concentrations of antibodies because of the removal of cell volume, which could explain the significant increase in the LFA antibody detection for samples with high plasma antibody concentration. Blood cells in samples may also affect the flow through the nitrocellulose membrane, affecting the sensitivity of the assay. (Kasetsirikul et al., 2020).

When testing whole blood samples, we found significant differences in LFA antibody positivity rate between BNT162b2 and mRNA-1273 vaccine recipients. The quantity of mRNA in the vaccine (30 μg for BNT162b2 and 100 μg for mRNA-1273) and the nanoparticles used for packaging the mRNA may explain the significant difference in antibody levels.(Dickerman et al., 2022) With the emergence of more infectious variants, the mRNA-1273 vaccine may have higher efficacy against both infection and hospitalization than the BNT162b2 vaccine, perhaps because of the higher antibody immunity induced by the mRNA-1273 vaccine.(Britton et al., 2022; Grannis et al., 2021; Tang et al., 2021; Wang et al., 2022) In one study, heterologous boosting after the primary BNT162b2 series was associated with lower SARS-CoV-2 incidence rates than homologous boosting.(Tan et al., 2022) Nevertheless, the mRNA-1273 cohort had a significantly higher antibody positivity rate, even after controlling for plasma antibody concentration. The data suggest that the LFA can better detect higher concentrations of antibody present in patient serum and whole blood.

In our exploratory analysis, the strength of the label band on the LFA correlated with antibody concentration; for example, samples with "thick/full" bands had significantly higher antibody activity than samples with "faint" or no bands. While visual evaluations of colorimetric assays are prone to human bias and misinterpretation,(Dungchai et al., 2010) longitudinally trending band strength may provide early warning of waning immunity and guide targeted booster vaccination schedules. The presence of SARS-CoV-2 specific antibodies is associated with reduced risk of infection and reinfection following prior exposure.(Lucas et al., 2021; Tartof et al., 2021) As antibody levels naturally wane, the risk of breakthrough infection may increase.

Understanding the kinetics of vaccine efficacy and optimal timing for booster vaccination may be crucial in continuing control of the pandemic. Large scale monitoring of immunity can provide key information, especially in a setting with emerging infectious variants. (Accorsi et al., 2022; Chivu-Economescu et al., 2022; Favresse et al., 2021; Sun et al., 2020) For community-wide epidemiologic assessment, LFAs may be useful PoC diagnostic devices that can be deployed as an alternative for expensive and labor intensive laboratory-based assessments, especially in resource poor or restricted settings.(Kasetsirikul et al., 2020; Morbioli et al., 2017; Yetisen et al., 2013) The availability of a low-cost hand centrifuge may allow collection of plasma samples to achieve highest accuracy in these settings.(Bhamla et al., 2017).

This study has several limitations. The generalizability of this study is limited because the participants were health care workers at 2 large urban academic centers located in the same city. We studied an LFA device from a single manufacturer, and the quality of devices from other manufacturers may vary significantly.(Cassaniti et al., 2020) We only included study participants who received mRNA-based vaccines; the LFA performance for those who received vector-based or other types of SARS-CoV-2 vaccines remains unknown. Finally, the effects of variant-specific vaccines in development could affect the LFA performance in detecting vaccine-mediated antibody specific immunity.

In conclusion, a LFA can be a useful tool to qualitatively detect the presence of antibody specific immunity after receiving mRNA-based SARS-CoV-2 vaccines, especially when plasma samples are tested. Further studies could help determine whether LFAs deployed for surveillance can judiciously guide booster vaccination schedules.

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Author's contributions

Won Lee: This author contributed to conception, design, conduct, analysis, and interpretation of the study as well as drafting the manuscript as the lead author.

Philip Kurien: This author contributed to conception, design, analysis, and interpretation of the study, and provided critical input in revision of the manuscript.

Declaration of Competing Interest

We declare no competing interest for all authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2022.113410.

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