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IMMUNOASSAYS

Anti-SARS-CoV-2 Antibody Levels Measured by the AdviseDx SARS-CoV-2 Assay Are Concordant with Previously Available Serologic Assays but Are Not Fully Predictive of Sterilizing Immunity

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ABSTRACT With the availability of widespread SARS-CoV-2 vaccination, high-throughput quantitative anti-spike protein serological testing will likely become increasingly important. Here, we investigated the performance characteristics of the recently FDA-authorized semi-quantitative anti-spike protein AdviseDx SARS-CoV-2 IgG II assay compared to the FDA-authorized anti-nucleocapsid protein Abbott Architect SARS-CoV-2 IgG, Roche Elecsys anti-SARS-CoV-2-S, EuroImmun anti-SARS-CoV-2 enzyme-linked immunosorbent assay (ELISA), and GenScript surrogate virus neutralization assays and examined the humoral response associated with vaccination, natural protection, and vaccine breakthrough infection. The AdviseDx assay had a clinical sensitivity at 14 days after symptom onset or 10 days after PCR detection of 95.6% (65/68; 95% confidence interval [CI], 87.8 to 98.8%), with two discrepant individuals seroconverting shortly thereafter. The AdviseDx assay demonstrated 100% positive percent agreement with the four other assays examined using the same symptom onset or PCR detection cutoffs. Using a recently available WHO international standard for anti-SARS-CoV-2 antibody, we provide assay unit conversion factors to international units for each of the assays examined. We performed a longitudinal survey of healthy vaccinated individuals, finding that median AdviseDx immunoglobulin levels peaked 7 weeks after first vaccine dose at approximately 4,000 IU/ml. Intriguingly, among the five assays examined, there was no significant difference in antigen binding level or neutralizing activity between two seropositive patients protected against SARS-CoV-2 infection in a previously described fishing vessel outbreak and five health care workers who experienced vaccine breakthrough of SARS-CoV-2 infection, all with variants of concern. These findings suggest that protection against SARS-CoV-2 infection cannot currently be predicted exclusively using *in vitro* antibody assays against wild-type SARS-CoV-2 spike. Further work is required to establish protective correlates for SARS-CoV-2 infection.

KEYWORDS SARS-CoV-2, spike protein, spike IgG, serology, COVID-19, coronavirus, Abbott Architect, anti-SARS-CoV-2, correlates of protection, vaccination

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease 19 (COVID-19), is responsible for an ongoing global pandemic. In addition to infection control measures such as social distancing and masking, controlling the spread of the outbreak will require a global vaccination campaign. Currently, three vaccines have received FDA emergency use authorization, with other candidates in

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late-phase clinical trials (1). Common to all vaccine candidates is the inclusion of the receptor binding domain (RBD) or full-length spike (S) of SARS-CoV-2 (2).

For SARS-CoV-2 and related coronaviruses, antibodies to the RBD of the spike protein have demonstrated potent neutralizing activity at nanomolar concentrations (3, 4). In a recent meta-analysis of individuals with naturally acquired SARS-CoV-2 infection, neutralizing antibodies were first detectable between 7 and 15 days following symptom onset (5). Despite questions regarding the durability of the antibody response and documented cases of reinfection, longitudinal analysis of IgG levels and neutralizing potency suggest that immunity persists in most individuals for as long a time period as has been examinable to date (6, 7). However, some individuals, including those who are older or immunosuppressed, may be at risk for a suboptimal response to vaccination (8, 9).

The presence of neutralizing antibodies due to prior infection or vaccination has been shown to be a correlate of protection against SARS-CoV-2 infection (10–12). Although phase III vaccine trials demonstrated excellent efficacies among treated populations (12, 13), a number of subpopulations, including pregnant women and immunocompromised individuals, were excluded from these trials. Moreover, uncertainty exists over the durability of protection after vaccination (13). High-throughput, widely available laboratory measurements of protective correlates would be extremely helpful in these and other populations. The current gold standard test, known as the plaque reduction neutralization assay (PRNA), is resource intensive and requires biosafety level 3 (BSL-3) conditions for testing. Currently, one surrogate neutralization assay has received emergency use authorization (EUA) for clinical use. While this assay can be performed in BSL-2 laboratories and has shown excellent correlation to PRNAs, it also suffers from similar limitations of throughput and cost (14). The most widely used clinical platforms for monitoring immunity to vaccine-preventable diseases, including hepatitis B virus, measles virus, and varicella-zoster virus, are high-throughput, low-cost immunoassay analyzers, including the Roche cobas, Abbott Architect, and DiaSorin XL platforms, among others. Recently, the Abbott AdviseDx SARS-CoV-2 IgG II assay received emergency use authorization by the FDA. This chemiluminescent microparticle immunoassay (CIMA) for the Abbott Architect platform is designed for semiquantitative detection of IgG class antibodies to the RBD of the SARS-CoV-2 spike protein.

Our laboratory previously examined the clinical performance characteristics of the anti-N SARS-CoV-2 IgG assay for the Abbott Architect and found it to have adequate performance for determining prior SARS-CoV-2 infection in a hospitalized cohort (15). However, this assay is qualitative and designed to detect antibodies to the nucleocapsid, precluding the ability to monitor vaccine response. In this study, we examined the performance of the AdviseDx SARS-CoV-2 IgG II assay and correlated its performance to four other assays (Abbott Architect SARS-CoV-2 anti-nucleocapsid IgG, Roche Elecsys anti-SARS-CoV-2 S, EuroImmun anti-SARS-CoV-2 ELISA IgG, and the GenScript surrogate virus neutralization test). The WHO international standard was also run on each platform to evaluate analytical sensitivity using the manufacturer's cutoffs.

Important questions remain as to what binding-antibody levels may be considered protective against SARS-CoV-2 infection. We explored this question using the following approach. First, we calculated median immunoglobulin values following SARS-CoV-2 vaccination over 11 weeks in a group of healthy volunteers. Second, we compared the antibody response between a pair of seropositive patients who were protected from SARS-CoV-2 infection during a previously described outbreak on a fishery vessel and a group of five fully vaccinated healthy individuals who subsequently experienced breakthrough SARS-CoV-2 infection. This study not only demonstrates the acceptable analytical clinical performance of the Abbott AdviseDx assay but also provides context for how these values may be interpreted in vaccinated individuals.

MATERIALS AND METHODS

Study population and specimen collection. A total of 128 residual plasma specimens from 91 patients with a history of PCR-confirmed SARS-CoV-2 infection or anti-N IgG antibodies were included in this study (the raw data are presented in Data Set S1 in the supplemental material). Residual plasma samples included EDTA-treated and heparinized plasma. Residual serum from 104 individuals collected

between June and August of 2019 for anti-HSV Western blot analysis was used as the negative control. One hundred fifty-five samples from 27 vaccinated asymptomatic ambulatory adult health care workers were collected to assess the longitudinal response (raw data are available in Data Set S2). These individuals received either the Moderna mRNA-1273 or Pfizer/BioNTech BNT162b2 vaccine. Samples were obtained from two seropositive individuals who previously demonstrated protection against SARS-CoV-2 infection during a previously described fishery vessel outbreak (10). Five samples were obtained from fully vaccinated health care workers with PCR-confirmed SARS-CoV-2 infection and mild COVID-19 symptoms. Diagnostic SARS-CoV-2 PCR tests included a Washington State authorized CDC-based laboratory developed assay or FDA-authorized Roche cobas SARS-CoV-2, Abbott Alinity m SARS-CoV-2, or Hologic Panther Fusion SARS-CoV-2 assays (16). SARS-CoV-2 whole-genome sequencing was performed using the Swift Biosciences v2 or Illumina COVID-Seq amplicon tiling assays (17). This study was approved by the University of Washington Institutional Review Board.

Anti-N Abbott Architect SARS-CoV-2 IgG and anti-S AdviseDx SARS-CoV-2 IgG II assays. The emergency use-authorized anti-N Architect SARS-CoV-2 IgG and anti-S AdviseDx SARS-CoV-2 IgG II assays (Abbott, Chicago, IL) are chemiluminescent microparticle immunoassays (CIMA) designed to measure IgG antibodies binding the N protein and S protein, respectively, and were performed on an Architect i2000SR analyzer. Results from the anti-N SARS-CoV-2 IgG assay are reported as index values. Index values of 1.40 or greater were classified as positive per the manufacturer's recommendation for the anti-N Abbott Architect SARS-CoV-2 IgG assay. Results from the anti-S AdviseDx SARS-CoV-2 IgG II assay are reported as arbitrary units (AU) per milliliter. The manufacturer's suggested positive cutoff of 50 AU/ml was used. Interday and interassay studies were performed over 3 days by two different operators.

Roche Elecsys anti-SARS-CoV-2 S. The Elecsys anti-SARS-CoV-2 S assay (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) is an electrochemiluminescence immunoassay which uses a double-antigen sandwich design for the detection of immunoglobulins (predominantly IgG, but also IgA and IgM) to the RBD of the S protein. Samples were prepared according to the manufacturer's instructions and analyzed on the Roche cobas e 411 platform. Using the manufacturer's guidelines, sample values of $\geq$0.8 AU/ml were classified as positive for anti-SARS-CoV-2 antibodies. Dilutions were performed on specimens with values greater than 250 AU/ml according to the manufacturer's guidelines.

EuroImmun anti-SARS-CoV-2 ELISA. The EuroImmun anti-SARS-CoV-2 IgG assay is a semiquantitative enzyme-linked immunosorbent assay (ELISA) detecting antibodies which bind the S1 subunit of the spike protein. Samples are loaded into reagent wells coated with the spike protein, washed, and then incubated with enzyme-conjugated anti-human IgG generating a colorimetric signal. Results are provided as a semiquantitative measurement of the signal of the experimental sample divided by the signal of the calibrator (optical density [OD] ratio). Per the manufacturer's insert, values of $<$0.8 are considered negative, values of $\geq$0.8 to $<$1.0 are borderline, and values of $\geq$1.1 are positive. For this study, we classified borderline results as positive.

GenScript surrogate virus neutralization test. The GenScript surrogate virus neutralization test (GenScript, Piscataway, NJ, USA) assay was performed according to the manufacturer's instructions. The test examines the ability of sera to block binding of SARS-CoV-2 spike RBD to the human ACE2 receptor. Absorbance was read at 450 nm on a Victor Nivo (PerkinElmer, Waltham, MA, USA) reader. Values are reported as percent neutralization relative to a negative-control sample provided by the manufacturer. Samples demonstrating $\geq$30% inhibition of ACE2 binding were classified as positive, as recommended by the manufacturer.

SARS-CoV-2 spike pseudotyped lentivirus neutralization assay. 293T-ACE2 cells (1.25×10^4) were seeded in 96-well plates and incubated in Dulbecco's modified Eagle medium (DMEM) plus 10% fetal bovine serum (FBS) for 16 to 18 h. The following day, 1.0×10^7 relative light units (RLU)/well SARS-CoV-2 D614G spike pseudotyped lentivirus was diluted 1:10 in complete medium consisting of DMEM with 10% FBS (18). Serum was diluted 1:20 in DMEM with 10% FBS, and seven 3-fold serial dilutions were prepared. Equal parts diluted serum and pseudovirus were combined and incubated for 1 h at 37°C. The mixture was added to the cells and incubated for 52 h at 37°C. Following incubation, the medium was removed, and 30 μl of luciferase substrate (Promega, Madison, WI, USA) was added. After 2 min of incubation, luminescence was measured on the Victor Nivo, and the 50% inhibitory concentration (IC_{50}) was calculated from a standard curve using the CV30 monoclonal antibody (Absolute Antibody, Oxford, UK).

Preparation of the international standard. The WHO first international standard for SARS-CoV-2 antibody was prepared according to the manufacturer's instructions (19). The lyophilized sample was provided at 250 IU/ampoule and resuspended in 250 μl of deionized water to create a 1,000-IU/ml stock solution. The stock was diluted 1:10 to prepare a working solution with sufficient volume for analysis across the various platforms.

Statistical analysis. Correlation studies were performed using Spearman's coefficient. Assay performance, linear regression, and curve fitting calculations were performed using Prism 9 (GraphPad Software, LLC, San Diego, CA, USA).

RESULTS

The AdviseDx SARS-CoV-2 IgG II assay is 95.6% sensitive and 100% specific in individuals at least 14 days after symptom onset or 10 days after first positive PCR result. To assess the sensitivity and specificity of the AdviseDx SARS-CoV-2 IgG II assay, a total of 172 patient samples (68 positive, 104 negative) were tested. Positive patients were classified as individuals with a PCR-confirmed diagnosis of SARS-CoV-2 infection who were at least 14 days beyond symptom onset or 10 days past the first positive PCR result. Negative sera were collected during July and August 2019, prior to when SARS-CoV-2 was

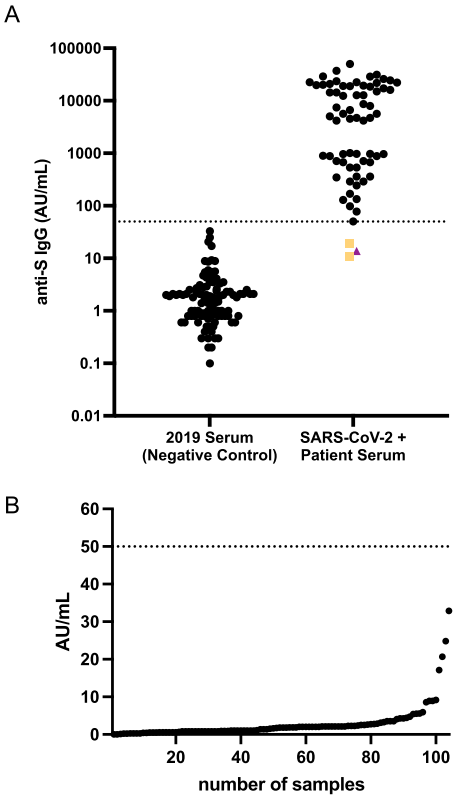

FIG 1 AdviseDx SARS-CoV-2 IgG II values for patients with a PCR-confirmed SARS-CoV-2 infection who were >14 days beyond symptom onset or >10 days beyond the first positive PCR result and control serum collection prior to the SARS-CoV-2 pandemic (A). The dotted line represents the assay positive cutoff (50 AU/ml) set by the manufacturer. Gold squares indicate patients who seroconverted at 13 and 18 days following their first positive PCR result. The purple triangle represents a severely ill COVID-19 patient who died 17 days after symptom onset. One hundred four serum samples obtained prior to the emergence of SARS-CoV-2 were analyzed for assay specificity (B). The median value of these negative controls was 1.8 AU/ml (range, 0 to 32.9). The dotted line represents the positive threshold.

thought to be circulating in the western Washington area (20). Using these criteria, the assay had a sensitivity of 95.6% (65/68 samples; 95% confidence interval [CI], 87.8 to 98.8%) (Fig. 1A; Table S1). Importantly, two of the three cases that initially tested negative were in clinically asymptomatic individuals detected by preadmission SARS-CoV-2 PCR screening. Based on the manufacturer's recommended cutoff of 50 AU/ml, these two individuals tested antibody negative on days 11 (13.8 AU/ml) and 13 (10.9 AU/ml) and seroconverted on days 13 (77.3 AU/ml) and 18 (168.3 AU/ml) post-PCR, respectively. The third individual was borderline anti-S seronegative (19.5 AU/ml) at day 15 after symptom onset and died due to COVID-19 pneumonia on day 17 after symptom onset. Specificity was calculated to be 100% (104/104 samples; 95% CI, 96.4 to 100%) (Table 1B). The median AU/ml of these specimens was 1.8 AU/ml (range, 0 to 32.9 AU/ml), and the clinical cutoff, calculated as the mean of negative samples plus 3 standard deviations (SD), was 17.3 AU/ml, well below the manufacturer's recommended cutoff of 50 AU/ml.

The AdviseDx SARS-CoV-2 IgG II assay is linear over the analytic measurement interval, with a coefficient of variation <5% near the positive cutoff. Per the manufacturer's insert the AdviseDx SARS-CoV-2 IgG II has a limit of quantitation of 22.0 AU/ml and an upper limit of quantitation of 25,000 AU/ml. To assess the linearity of the assay, we performed 1:2 serial dilutions of a high-positive sample from 37,256 AU/ml to ~12 AU/ml. Each sample dilution was measured in triplicate. The concentration of the 37,256 AU/ml neat sample was calculated from a 1:10 dilution. Results demonstrated excellent linearity beyond the manufacturer's analytical measurement interval (R^2 = 0.9989) (Fig. S1). To assess for assay reproducibility, the coefficient of variation (CV) was

TABLE 1 Agreement between the AdviseDx SARS-CoV-2 IgG II assays and the Abbott SARS-CoV-2 IgG, EuroImmun, and Roche Elecsys anti-SARS-CoV-2 S enzyme immunoassays and the GenScript surrogate virus neutralization assay[a]

A

	Abbott SARS-CoV-2 IgG (anti-N) +	Abbott SARS-CoV-2 IgG (anti-N) -
AdviseDx +	54	0
AdviseDx -	0	3

PPA: 100% (93.4 to 100%)

B

	EuroImmun +	EuroImmun -
AdviseDx +	44	2
AdviseDx -	0	3

PPA: 100% (92.0 to 100%)

C

	Roche anti-S +	Roche anti-S -
AdviseDx +	46	0
AdviseDx -	0	3

PPA: 100% (92.3 to 100%)

D

	GenScript +	GenScript -
AdviseDx +	89	9
AdviseDx -	0	4

PPA: 100% (95% CI: 95.9-100%)

[a]High levels of categorical agreement between the AdviseDx SARS-CoV-2 IgG II assays and Abbott SARS-CoV-2 IgG (A), EuroImmun (B), and Roche Elecsys anti-SARS-CoV-2 S (C) enzyme immunoassays were seen. Positive percent agreement was calculated for samples collected more than 14 days from symptom onset or 10 days after the first positive PCR result. Positive percent agreement between the AdviseDx SARS-CoV-2 IgG II and GenScript surrogate virus neutralization assays was calculated irrespective of collection time (D). In discrepant cases (AdviseDx positive but GenScript negative), the AdviseDx result ranged from 50.1 to 290.7 AU/ml, above the assay positive threshold of 50 AU/ml.

measured in four samples near the manufacturer's suggested positive cutoff over 4 days. The assay demonstrated a CV less than 5% at all dilutions above the positive threshold (550, 140, and 70 AU/ml) during intraday and interday measurements. For the sample below the positive threshold (40 AU/ml), the CV was 5% and 7.7% on intraday and interday measurements, respectively (Table S2).

The AdviseDx SARS-CoV-2 IgG II has 100% positive agreement with three other EUA immunoassays. Results of the AdviseDx assay were compared to those of three serologic binding assays with prior EUAs, Abbott Architect SARS-CoV-2 IgG (nucleocapsid), EuroImmun anti-SARS-CoV-2 ELISA (spike S1 subunit), and Roche Elecsys anti-SARS-CoV-2 S (spike RBD). Using a cutoff of 14 days after symptom onset or 10 days after first positive PCR result, the positive percent agreement (PPA) of the AdviseDx with the three other assays was 100% (Table 1A to C). When patient samples were examined regardless of collection time, the AdviseDx PPA agreements for the Abbott Architect, EuroImmun, and Roche assays were 98.3% (114/116), 100% (95/95), and 100% (102/102), respectively (Table S3).

A total of 13 samples with at least one qualitative test result discrepancy were observed, which were mostly driven by negative results on the EuroImmun test (Table S4). Discrepant cases occurred near the time of seroconversion in patients with available clinical data. Of the discrepant cases, two were considered to be false positives by the Abbott Architect anti-N assay based on negative results on the other three platforms. Both of these cases had Abbott Architect anti-N index values close to the cutoff at 1.53 and 1.44. Of the remaining cases, 11/11 were AdviseDx positive, 10/11 were Abbott Architect anti-N positive, 8/11 were Roche positive, and 0/11 were EuroImmun positive. Quantitative results of the AdviseDx assay were highly correlated with the three other platforms as measured by Spearman's coefficient: Abbott Architect anti-N ($r = 0.89$), EuroImmun anti-S1 ($r = 0.95$), Roche anti-RBD ($r = 0.83$) ($P < 0.001$ for all comparisons). AdviseDx values

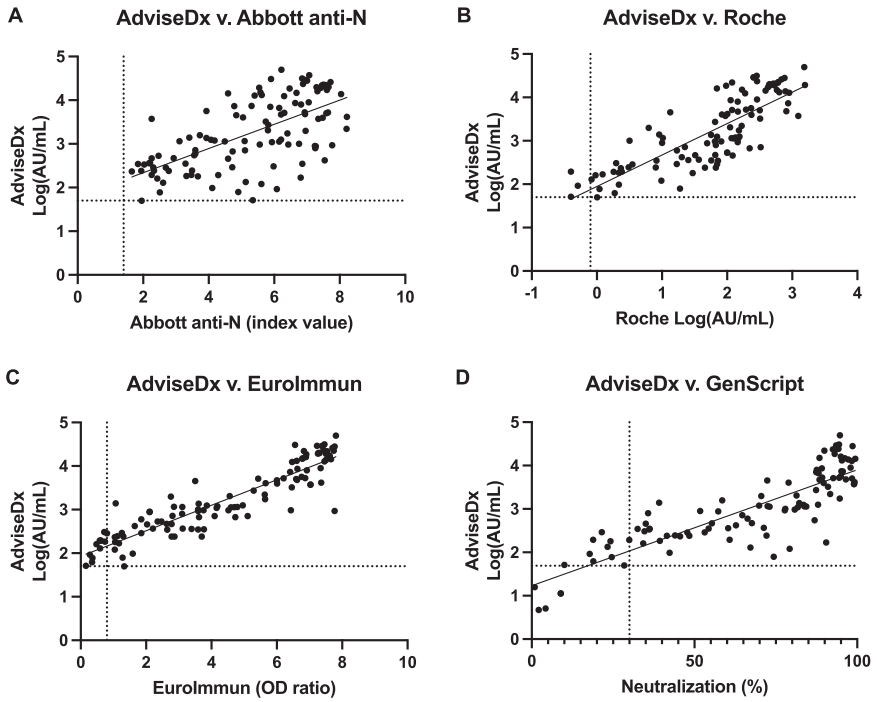

FIG 2 Correlation of AdviseDx SARS-CoV-2 IgG II assays results to the Abbott Architect anti-N (A), Roche anti-S (B), EuroImmun anti-S (C), and GenScript (D) assays. The same specimens were run on all five platforms. AdviseDx and Roche results were log-transformed before performing linear regression. Results demonstrated strong agreement, and goodness of fit (R^2) was measured as 0.42, 0.66, 0.73, and 0.74, respectively. Dotted lines represent the manufacturer's positive cutoff values.

were compared to the other platforms by linear regression and goodness of fit. The values for goodness of fit (R^2) of the log-transformed AdviseDx values to the quantitative results of the Abbott Architect anti-N, log-transformed Roche anti-S/RBD assays, and EuroImmun anti-S1 were 0.42, 0.66, and 0.73, respectively (Fig. 2A to C), which was chiefly affected by the limited reportable range of these assays. Categorical agreement and assessment of linearity between the Abbott Architect anti-N, EuroImmun, Roche, and GenScript assays are available in Fig. S2 and Table S5.

The AdviseDx SARS-CoV-2 IgG II assay demonstrates 100% positive agreement with FDA emergency use-authorized surrogate virus neutralization test. As the GenScript surrogate virus neutralization test is the only currently available neutralization-based assay that has been authorized by the FDA, we compared its results to the AdviseDx SARS-CoV-2 IgG II results. Categorical evaluation demonstrated a positive precent agreement of 100% (89/89 samples; 95% CI, 95.9 to 100%) in patients with a documented history of SARS-CoV-2 infection (Table 1D). AdviseDx values positively correlated with increasing percent neutralization by Spearman's analysis ($r = 0.86$; $P < 0.001$). Following log-transformation of the AdviseDx values, a linear regression model was fitted to the data (Fig. 2D). Neutralization values of 30% (positive cutoff), 50%, and 80% on the GenScript assay corresponded to 107, 369, and 2,340 AU/ml in the Abbott IgG II assay, respectively. Similarly, a 18% neutralization value correlated with 50 AU/ml, the positive cutoff, for the AdviseDx assay.

Increases in anti-S binding antibodies and neutralization activity are observed in individuals following SARS-CoV-2 infection and vaccination. The kinetics of the GenScript and AdviseDx assays were measured over 10 to 15 days in four patients hospitalized for COVID-19 and over 44 to 59 days in four individuals who received mRNA vaccines to SARS-CoV-2. Both anti-S immunoglobulins and neutralization values increased over time with high neutralizing levels achieved in all patients (Fig. 3). Patients 1 and 2 had positive AdviseDx results on days 8 to 9, with a positive GenScript 1 to 2 days later. Patient 4 tested positive by both assays 10 days after symptom onset. For patient 3, clinical samples were available starting on day 5 after symptom onset, and both were found to be positive at

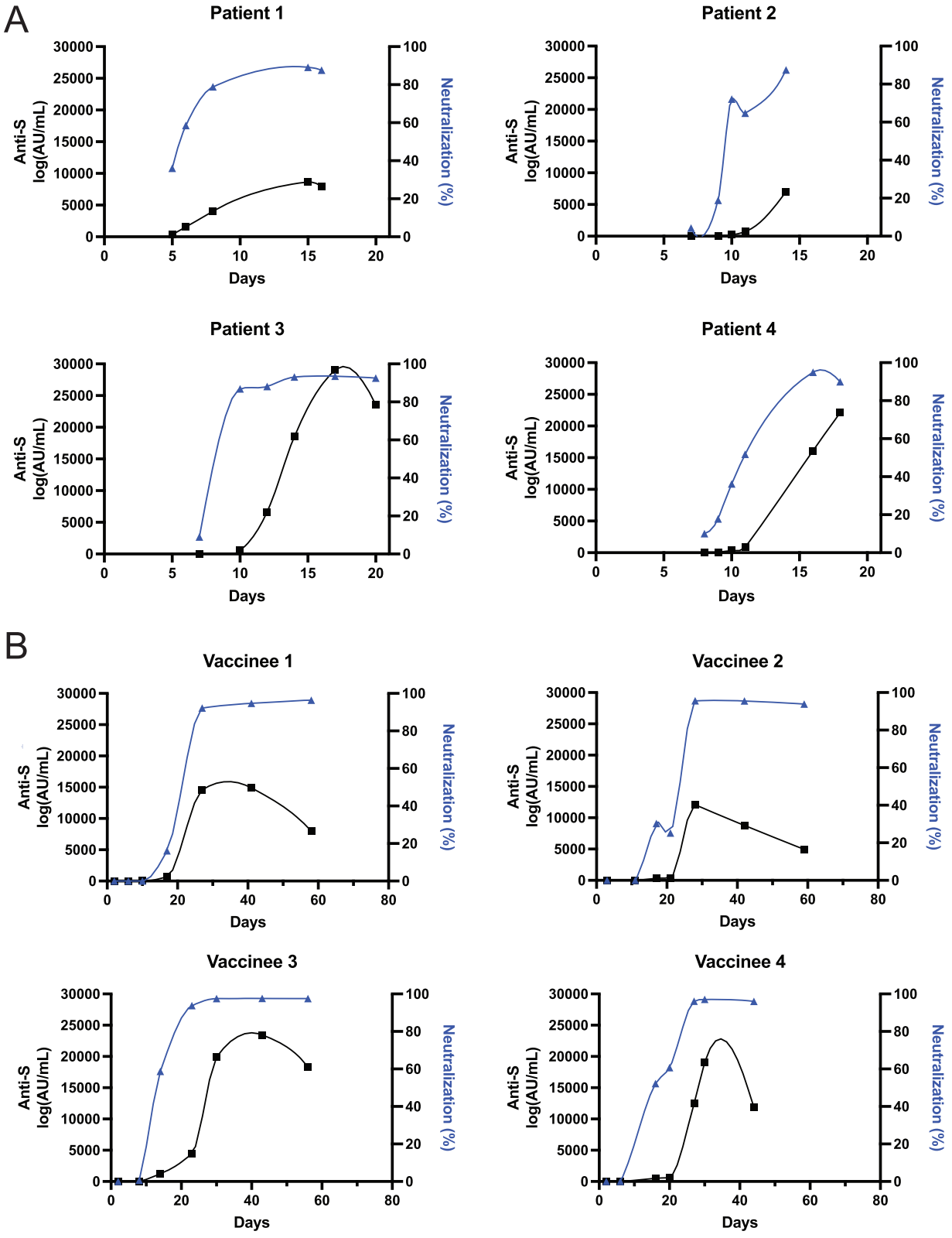

FIG 3 Serial measurements of anti-S IgG levels and surrogate neutralization results in four patients with acute SARS-CoV-2 infection (A) and four patients who received the mRNA-1273 or BNT162b2 SARS-CoV-2 vaccine (B). Increasing anti-S levels and neutralization were observed in all patients as time from exposure increased. Black squares represent anti-S AdviseDx SARS-CoV-2 results, and blue triangles show percent neutralization calculated from the GenScript neutralization assay. At later time points, vaccinated patients had lower anti-S levels with sustained neutralization activity. Curves connected data points were plotted in Prism 9 using the Akima spline.

TABLE 2 Comparison of WHO international standard results across multiple platforms demonstrates various levels of sensitivity[a]

	Result with assay									
	Abbott Architect anti-N (index value)		AdviseDx anti-S (AU/ml)		GenScript (% neutralization)		Roche Elecsys anti-S (AU/ml)		EuroImmun (OD ratio)	
Level (IU/ml)	Mean (% CV)	Categorical	Mean (% CV)	Categorical	Mean (% CV)	Categorical	Mean (% CV)	Categorical	Mean (% CV)	Categorical
100	2.5 (1.2)	POS	618.9 (0.9)	POS	48.3 (3.4)	POS	88.2 (1.0)	POS	2.30 (2.5)	POS
50	1.27 (1.2)	NEG	301.6 (0.6)	POS	36.6 (13.6)	POS	40.3 (0.1)	POS	1.29 (3.1)	POS
10	0.2 (0)	NEG	59.5 (4.6)	POS	3.9 (78.5)	NEG	6.2 (0.1)	POS	0.46 (14.6)	NEG
5	0.09 (0)	NEG	29.9 (1.0)	NEG	3.5 (108)	NEG	2.4 (0.03)	POS	0.2 (22.9)	NEG
1	0.01 (43)	NEG	6.1 (5.7)	NEG	0.4 (1032.8)	NEG	0.4 (0)	NEG	0.08 (12.5)	NEG

[a]The positive cutoffs for the Abbott Architect anti-N, AdviseDx, Roche, and EuroImmun assays were calculated as 56.2 IU/ml, 8.4 IU/ml, 3.2 IU/ml, and 30.2 IU/ml, respectively. Each sample was analyzed in triplicate. Manufacturer's positive cutoffs are as follows: Abbott Architect anti-N, 1.4; AdviseDx, 50 AU/ml; GenScript, 30%; Roche, 0.8 AU/ml; EuroImmun, 0.8.

that time. In the vaccinated patients, AdviseDx results began to wane around day 40, but the surrogate neutralizing results remained elevated.

Implementation of the WHO international standard demonstrates variable sensitivity among serologic assays for SARS-CoV-2 using the manufacturer's recommended cutoffs. To determine the positivity threshold for each assay in standardized international units, a dilution series of the WHO international standard for SARS-CoV-2 antibody was prepared and run in triplicate on all platforms (Table 2). The positive cutoff, in international units per milliliter, was calculated by linear regression for each assay. In increasing order, the manufacturers' recommended assay cutoffs were determined to be as follows: Roche, 3.2 IU/ml; AdviseDx, 8.4 IU/ml; EuroImmun, 30.2 IU/ml; GenScript, 10 to 50 IU/ml; and Abbott Architect anti-N, 56.2 IU/ml. All assays demonstrated an acceptable coefficient of variation (less than 20%) at values near their positive cutoffs.

Longitudinal measurement of anti-SARS-CoV-2 immunoglobulins following vaccination. To understand how anti-S levels change in healthy individuals during vaccination, we examined a total of 155 weekly samples obtained from 27 volunteers (age range, 20 to 72) over up to 11 weeks after vaccination with either the mRNA-1273 or BNT162b2 SARS-CoV-2 vaccine. Sera were analyzed by the AdviseDx, EuroImmun, and Roche anti-S assays, and median values along with the 10th to 90th percentiles were calculated for the assay results (Table 3). Sera were universally positive by all assays by week 3 after the first vaccine dose. Median AdviseDx anti-spike IgG levels increased by more than 100-fold 2 weeks after the first dose and by more than 10-fold after administration of the second dose. Peak antibody levels were observed 7 weeks after first vaccine dose at 23,881 AU/ml (7,304 to >25,000 AU/ml, 10th to 90th percentiles).

SARS-CoV-2 anti-S immunoglobulin levels and neutralization results do not significantly differ between individuals protected after exposure and vaccine breakthrough cases. Prior to the widespread emergence of SARS-CoV-2 variants, we described an outbreak of SARS-CoV-2 aboard a fishing vessel in which three individuals with preexisting SARS-CoV-2 antibodies demonstrated immunity to reinfection (10). These individuals most closely represent the results of a human infection challenge model where an individual with known immunity experiences multiple known exposures to a pathogen. Residual samples were available for two of these individuals with AdviseDx values of 5,303 AU/ml and 1,240 AU/ml. These samples were also tested using across the other available platforms in our study; however, the sample for patient 2 was exhausted during this process. Antibody levels and neutralizing activity were well above the positive cutoff as measured on all platforms, including anti-N (Abbott Architect) and anti-S (AdviseDx, EuroImmun, Roche) antibody responses. When results were standardized to international units, protection could be observed at levels as low as 81 IU/ml (Table 4).

A second group of samples were collected from five health care workers who experienced mild SARS-CoV-2 infection more than 4 weeks following their second vaccine dose. All individuals endorsed some form of upper respiratory symptoms after receiving the BNT162b2 vaccine and had surprisingly strong viral loads, with an average cycle threshold (C_T) of 18.4 (range,

TABLE 3 Longitudinal antibody response in a group of healthy volunteers following vaccination with mRNA-1273 or BNT162b2 SARS-CoV-2 as measured by three different anti-S assays[a]

	Result with assay								
	Abbott AdviseDx (anti-S) (AU/ml)			Roche Elecsys (anti-S) (AU/ml)			EuroImmun (anti-S) (index value)		
Wk	Median	10th percentile	90th percentile	Median	10th percentile	90th percentile	Median	10th percentile	90th percentile
1 ($n = 35$)	2.2	0.68	4.7	0.4	0.4	0.4	0.183	0.103	0.312
2 ($n = 22$)	5.15	1.36	448.29	1.8325	0.36	147.77	0.29	0.157	4.216
3 ($n = 24$)	794.3	303.16	3,823.89	40.84	8.856	197.52	4.355	2.767	9.092
4 ($n = 22$)	2,109.85	508.75	23,958.06	457.2	18.09	3,569	7	4.43	9.702
5 ($n = 16$)	2,1962.1	6,283.65	>25,000	1,992	293.592	7,991	9.525	5.755	10.455
6 ($n = 13$)	16,919.1	5,200.66	>25,000	1,886.5	410.35	6,429.5	10.53	9.272	11.73
7 ($n = 8$)	23,881.35	7,304.41	>25,000	2,419.5	1,156.23	5,208	10.61	10.039	11.379
8 ($n = 3$)	14,648.2	7,439.64	22,929.64	1,436	606.42	3,306.2	9.76	8.912	10.384
9 ($n = 8$)	8,747.15	4,049.91	>25,000	691.2	315.06	3,215.4	9.41	7.802	11.379
10-11 ($n = 4$)	21,770.25	15,256.8	>25,000	2,625	2,180.2	3,722.6	11.625	11.239	12.396

[a]Initial detectable positive values were identified at week 3, with values peaking at week 7, consistent with booster response. Values are reported as the median antibody levels along with the 10th to 90th percentiles.

16.0 to 20.8) (Table S6). Serum specimens were collected 1 to 4 days following symptom onset, tested across all platforms, and standardized to international units per milliliter when applicable (Table 4). Consistent with a history of prior vaccination and early infection, detectable anti-S and undetectable anti-N antibody responses were observed. In four of five patients, antibody levels and surrogate neutralization results were equal to or greater than those in samples from the fishing vessel cohort. One patient (HCW_02) demonstrated low positive surrogate neutralization (35.6%; positive cutoff, 30%) and low AdviseDx values compared to the other members of the cohort. However, this patient's anti-S antibody binding activity as measured on the EuroImmun and Roche platforms was greater than that of at least one of the protective fishing vessel samples. Pseudovirus neutralization results from this cohort demonstrated a 50% neutralizing dilution (ND) ranging between 188 and 788. Whole-genome sequencing of the health care worker isolates identified the CAL.20C variant of concern (4 20C/B.1.429 isolates and 1 20C/B.1.427 isolate). Patient demographics, symptoms, time to first positive PCR result, C_T value, and time to draw for serology for the health care worker cohort are provided in Table S6.

DISCUSSION

Widespread vaccination campaigns are under way, and they present an opportunity to elucidate correlates of protection from SARS-CoV-2 infection. While many methodologies

TABLE 4 Comparison of SARS-CoV-2 IgG results among three individuals with demonstrated resistance to multiple SARS-CoV-2 challenges and five individuals >6 weeks following vaccination who subsequently developed SARS-CoV-2 infection[a]

	Result with assay										
	AdviseDx		EuroImmun		Roche		Abbott Architect	GenScript	Pseudovirus neutralization		
Individual	AU/ml	IU/ml	OD ratio	IU/ml	AU/ml	IU/ml	(index value)	(% neutralization)	μg/ml	IC_{50}	Strain
Fisheries 1	5,303	858.0	6.87	306.4	NA	N/A	6.93	89.2	ND	ND	ND
Fisheries 2	1,240	200.9	3.86	169.4	70.16	81.0	4.07	83.6	ND	ND	ND
Fisheries 3	ND	ND	ND	ND	ND	ND	4.72	93.1	ND	ND	ND
HCW_1	1,639	265.4	7	312.3	483.1	428.8	0.01	89.6	0.005311	1:188	20C/B.1.429
HCW_2	1,059	171.5	5.2	230.4	397.3	352.3	0.04	35.6	0.006564	1:152	20C/B.1.429
HCW_3	7,100	1,148.6	9.5	426.0	1,265	1,126.3	0.06	96.5	0.001729	1:577	20C/B.1.429
HCW_4	3,267	528.7	9.7	435.1	627.3	557.5	0.01	89.9	0.002727	1:366	20C/B.1.427
HCW_5	10,857	1,756.2	10.5	471.5	1,384	1,232.4	0.02	97.5	0.001282	1:788	20C/B.1.429

[a]Individuals with resistance to multiple infectious challenge (Fisheries) and individuals with vaccine breakthrough infections (HCW) demonstrated similar levels of anti-S titers and activity. In only one of five vaccine-breakthrough cases were AdviseDx levels and GenScript neutralization values lower than in fishing vessel individuals. International units per milliliter were calculated for the AdviseDx, EuroImmun, and Roche assays. As expected, Abbott anti-nucleocapsid IgG levels were negative among vaccinated health care workers because they were tested prior to seroconversion to their breakthrough infection, indicating the anti-S levels measured are due to vaccination.

exist to assess and monitor humoral immunity, the most tractable for clinical laboratory workflows are enzyme immunoassays, which can be run at high throughput with relatively low cost. We found the AdviseDx SARS-CoV-2 IgG II assay to have acceptable performance characteristics and strong agreement with four other serologic tests previously granted emergency use authorization. Compared to the manufacturer's reported sensitivity and specificity of 98.1% and 99.6%, the clinical sensitivity calculated in our study may have been lower due to strict case definitions for infected individuals (14 days after symptom onset or 10 days following first positive SARS-CoV-2 PCR result) and the inclusion of acutely ill patients (21, 22). Of the three COVID-19 patients who tested negative using this cutoff for the AdviseDx assay, two seroconverted over the following 8 days and one died from COVID-19 pneumonia. Our estimated clinical cutoff of 17.3 AU/ml compares favorably to the manufacturer's recommended cutoff of 50 AU/ml, suggesting that negative samples are rarely near the positive threshold and unlikely to cause a false-positive result due to analytic variation. In contrast to the Abbott Architect anti-N assay, which has demonstrated poor linearity at index values above 3, the AdviseDx demonstrated excellent linearity even when values outside the manufacturer's analytical measurement range were tested (23).

The AdviseDx assay demonstrated performance similar to that of three other EUA serological binding assays (Abbott Architect SARS-CoV-2 IgG, EuroImmun anti-SARS-CoV-2 ELISA, and Roche Elecsys anti-SARS-CoV-2 S) measuring SARS-CoV-2 immunoglobulins. Of note, a comparison of the anti-N Abbott Architect SARS-CoV-2 IgG assay with three anti-spike protein assays on (DiaSorin Liaison, Ortho Vitros, and Euroimmun) also found a high degree of concordance (24). Interestingly, we found the EuroImmun assay to be less clinically sensitive than the Abbott Architect anti-N assay but more analytically sensitive based on the WHO international standard. The discrepancy may reflect a higher relative presence of anti-S antibodies compared to anti-N immunoglobulins in the international standard. Other studies have suggested that the EuroImmun assay may be more sensitive than the Abbott Architect anti-N (25, 26). It also worth noting that assay sensitivity is heavily dependent on the nonstandardized placement of a given manufacturer's chosen cutoff (27, 28).

We also found a high degree of correlation between the AdviseDx and the only FDA authorized surrogate neutralization assay, which has demonstrated excellent correlation with the gold-standard PRNAs (14). Our results suggest that most discrepant results between these assays would be AdviseDx positive and GenScript negative. Of note, five of our discrepant GenScript-negative results fell between 20% and 30% inhibition, arising from the modification made by GenScript in their positive cutoff from 20% inhibition (in research-use-only [RUO] documents) to 30% inhibition in their EUA application (29). While the functional data provided by the GenScript assay (i.e., inhibition of ACE2 binding) may better assess protective antibodies, four of five patients with SARS-CoV-2 infection following vaccination were found to have inhibition levels of $>$89%. These results encourage the cautious interpretation of surrogate inhibition assays as markers of sterilizing immunity, especially in the context of emerging variants.

Patients with innate or acquired immunosuppressive conditions have demonstrated poor antibody responses following vaccination against other viral pathogens (8, 9). Similar trends are being documented following SARS-CoV-2 vaccination (30). Given the severe impact COVID-19 can have on these individuals, longitudinal monitoring of antibody levels may impact decisions related to administering booster vaccinations and altering immunosuppressive regimens. Our results from otherwise healthy vaccinated individuals presented in Table 3 can help guide physician and laboratory interpretation on what may be considered a typical antibody response on several different platforms. These results are mainly limited by their relatively short follow-up. Certainly, more work is needed on longitudinal monitoring of antibody levels following vaccination and their functional correlates.

Our study is limited by the relatively small number of patients sampled. While we examined over 100 specimens to determine assay specificity, a larger number should be examined if the AdviseDx assay were to be employed for use in serosurveys to improve the confidence interval of specificity. The samples used for determining test

sensitivity were primarily obtained from patients who were hospitalized with COVID-19. While seroconversion may begin as early as 6 days following infection, three patients in our cohort were classified as seronegative when a cutoff of 14 days after symptom onset or 10 days after the first PCR-positive result in asymptomatic cases was used. Two of the patients who failed to seroconvert within this time frame were asymptomatic and eventually seroconverted 13 and 18 days following their first positive SARS-CoV-2 PCR result, suggesting that anti-S levels are likely associated with the degree of symptoms or infection (22). The third patient who failed to seroconvert died from COVID-19 pneumonia at 17 days after symptom onset. Several published reports support the view that a cutoff of 14 days after symptom onset may be too conservative when calculating the sensitivity of SARS-CoV-2 serologic assay. In a series of SARS-CoV-2-infected patients who did not require ICU admission, the 95% CI for seroconversion extended to 25 days after symptom onset (22). Similarly, delayed antibody response at the time of hospital admission for COVID-19 may be associated with poor outcomes (31). These data suggest that a longer duration from symptom onset may be appropriate when assessing sensitivity in previously hospitalized or severely ill patients. Additionally, our cohort included plasma samples collected in EDTA and heparin blood tubes. There is evidence to suggest the that anticoagulant in these tubes may also affect neutralization and antibody binding results (32, 33).

During the early phase of the pandemic, serologic assays were used to diagnose patients with prior SARS-CoV-2 infection and to perform seroprevalence studies. However, with the availability of FDA-authorized vaccines and more candidates entering late-phase trials, the ability to quantitatively measure the immune response to SARS-CoV-2 may be useful as a biomarker for protection. Such an approach would be similar to the current practice used in hepatitis B serologic monitoring, where anti-HBs IgG levels above 10 mIU/ml are considered protective (34). To accomplish this task for SARS-CoV-2, studies must be designed to assess the protective threshold. Intriguingly, our early attempt here to investigate protective thresholds found no difference in the antibody levels between individuals protected from infection and those who suffered vaccine breakthrough infection. Three of the individuals with breakthrough infection had AdviseDx values greater than 3,000 AU/ml. It is worth noting that the fishing vessel samples were collected prior to the emergence of clinically significant variants, while in the vaccine breakthrough group, all individuals were infected with the 20.C B.1.427/B.1.429 variant of concern lineage (35). Compared to the wild-type strain, the B.1.427/B.1.429 variants demonstrate an L452R mutation in the RBD and may be associated with increased transmissibility (36). When serum from vaccinated individuals was tested against the B.1.427/B.1.429 variant, it demonstrated a 2- to 3-fold reduction in neutralization compared to the wild type (36, 37). The emergence of novel variants highlights several challenges for diagnostic assays, including the difficulty in establishing protective thresholds and impact on the design of capture antigens for enzyme immunoassays (EIAs). Whether standardized adjustments based on cross-neutralization of different variants can be made to the quantitative anti-spike binding assays profiled here to establish a correlate of protect remains to be seen. Though it is exceedingly difficult to profile strain-specific immunity in the context of high-throughput FDA-authorized assays, given the rapid pace of viral evolution, further work in this space is required to realize more perfect measurements of immune protection.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.

SUPPLEMENTAL FILE 3, XLSX file, 0.01 MB.

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