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Differentiation of Individuals Previously Infected with and Vaccinated for SARS-CoV-2 in an Inner-City Emergency Department

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ABSTRACT Emergency departments (EDs) can serve as surveillance sites for infectious diseases. The objective of this study was to determine the burden of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and to monitor the prevalence of vaccination against coronavirus disease 2019 (COVID-19) among patients attending an urban ED in Baltimore City. Using 1,914 samples of known exposure status, we developed an algorithm to differentiate previously infected, vaccinated, and unexposed individuals using a combination of antibody assays. We applied this testing algorithm to 4,360 samples from ED patients obtained in the spring of 2020 and 2021. Using multinomial logistic regression, we determined factors associated with infection and vaccination. For the algorithm, sensitivity and specificity for identifying vaccinated individuals were 100% and 99%, respectively, and 84% and 100% for previously infected individuals. Among the ED subjects, seroprevalence to SARS-CoV-2 increased from 2% to 24% between April 2020 and March 2021. Vaccination prevalence rose to 11% by mid-March 2021. Marked differences in burden of disease and vaccination coverage were seen by sex, race, and ethnicity. Hispanic patients, though accounting for 7% of the study population, had the highest relative burden of disease (17% of total infections) but with similar vaccination rates. Women and white individuals were more likely to be vaccinated than men or Black individuals. Individuals previously infected with SARS-CoV-2 can often be differentiated from vaccinated individuals using a serologic testing algorithm. The utility of this algorithm can aid in monitoring SARS-CoV-2 exposure and vaccination uptake frequencies and can potentially reflect gender, race, and ethnic health disparities.

KEYWORDS seroprevalence of SARS-CoV-2 antibody, emergency department, factors associated with SARS-CoV-2 infection, COVID-19 vaccination prevalence

As of November 2021, over 251 million cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which causes coronavirus disease 2019 (COVID-19), have been reported globally (1). The United States has recorded more than 750,000 deaths and documented infections in over 13% of the population. Within the U.S., Black and Latino individuals have experienced higher rates of infection and mortality than white individuals, reflecting the disproportionate effects of social determinants of health among U.S. racial and ethnic minority groups (2–5).

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TABLE 1 Description of cohorts[a]

Cohort name (IRB no.)	Purpose	No. of samples	Notes
NIH phase 1 vaccine trial (20-0003)	Positive control, known SARS-CoV-2 vaccinated	68	68 samples from vaccinated individuals with no prior infection
JHHS health care professionals (IRB00249350)	Positive control, known SARS-CoV-2 vaccinated and some previously infected	410	360 samples from vaccinated individuals with no prior infection; 28 samples from vaccinated individuals with prior infection; 18 samples from vaccinated individuals with suspected prior infection; 4 samples from unvaccinated individuals with prior infection
Potential convalescent plasma donors (IRB00250798)	Positive control, known SARS-CoV-2 infected	244	Known infected (PCR positive) and nonvaccinated
CCPSEI (IRB00247886 and IRB00091667)	Positive control, known SARS-CoV-2 infected	246	Known infected (PCR positive) and nonvaccinated
JHH ED 2016 (NA_00085477)	Prepandemic negative control	992	Prepandemic samples from the same survey site as pandemic surveillance site
JHH ED 2020: 16 March to 30 April (IRB00083646, CIR0016268)	Algorithm application	1,536	Population surveillance
JHH ED 2021: 11 January to 10 March (IRB00083646, CIR0016268)	Algorithm application	2,824	Population surveillance

[a]Abbreviations: CCPSEI, Clinical Characterization Protocol for Severe Infectious Diseases; ED, emergency department; JHHS, Johns Hopkins Healthcare System; JHH Johns Hopkins Hospital.

Currently, three vaccines for COVID-19 have been authorized by the U.S. Food and Drug Administration (6). The authorized vaccines from Pfizer, Moderna, and Johnson & Johnson each elicit an immune response against the spike protein of the SARS-CoV-2 virion (7–9). As of 1 January 2022, 80% of persons aged ≥5 years have received at least one dose of a COVID-19 vaccine in the United States (10). This uptake, however, has varied dramatically by race, socioeconomic status, and geographic location (11–13).

In contrast to vaccinated individuals, previously infected patients have antibodies to several parts of the virus, including the spike and nucleocapsid proteins (14). By comparing the results of serologic assays that detect antibodies to either spike (S1), the spike glycoprotein receptor-binding domain (RBD), or the nucleocapsid (N), it should be possible to distinguish between previously SARS-CoV-2-infected (either infected alone or infected and then vaccinated), vaccinated (with no evidence of prior infection), and uninfected individuals.

Emergency departments (EDs) have historically played a critical role in prior epidemics and pandemics (15–17). Although case reporting can provide an estimate of population-level seroprevalence, relying on case reporting alone may underestimate the burden of infection, emphasizing the need for accurate serologic assessment of seroprevalence (18). The goal of this study was to develop an algorithm to differentiate vaccinated, previously infected, and unexposed individuals using standard enzyme-linked immunosorbent assay (ELISA) and lateral flow assay (LFA) technology.

MATERIALS AND METHODS

Ethics statement. This study used blood samples from studies approved by The Johns Hopkins University (JHU) School of Medicine Institutional Review Board (IRB00245545, IRB00247886, IRB00091667, IRB00250798, IRB00249350, and NA_00085477). Samples from the Moderna vaccine trial were provided as part of the Division of Microbiology and Infectious Diseases protocol 20-0003. For those studies, all individuals provided written informed consent. The JHU School of Medicine Institutional Review Board (IRB00083646 and CIR00016268) approved the deidentified serosurvey performed on waste material. All studies were conducted according to the ethics standards of the Helsinki Declaration of the World Medical Association.

Samples for algorithm validation. Three sample sets from individuals with known previous infection with and vaccination against SARS-CoV-2 were used to validate the antibody testing algorithm (Table 1). Samples from individuals with known vaccination were drawn from a phase 1 trial (8) ($n = 68$) and vaccinated health care professionals (HCP, $n = 360$) (19, 20). The 494 samples from individuals known to have been infected by SARS-CoV-2 were drawn from three cohorts: convalescent plasma donors (CCP, $n = 244$) (18, 21), participants in the Clinical Characterization Protocol for Severe Infectious Diseases (CCPSEI, $n = 246$) (22), and HCP ($n = 4$) (23). All samples were from individuals with a known positive SARS-CoV-2 reverse transcription-PCR (RT-PCR) test result. The majority of the CCP donors had mild disease, with 9% of this cohort reporting hospitalization. Among the CCPSEI cohort, 14% received oxygen therapy, 33% received ventilation, and 13% died. All HCP had mild disease. Additionally, there were 46 HCP who were infected and subsequently vaccinated, 28 with a confirmed PCR date and 18

who were suspected as having prior infection (PCR negative, but symptomatology indicative of an infection). The specificity of the testing algorithm was assessed using 992 samples from prepandemic remnant complete blood count (CBC) samples collected from Johns Hopkins Hospital Emergency Department (JHH ED) patients between December 2015 and January 2016 (24).

Samples for algorithm application. The testing algorithm was subsequently applied to two serosurveys conducted among patients attending the JHH ED from 16 March to 30 April 2020 and from 11 January to 10 March 2021. As previously described in an identity-unlinked seroprevalence study (25), remnant CBC blood samples from ED patients aged >17 years were collected during the study period. Each sample was assigned a unique study code, processed, and stored at −80°C. Basic patient demographic characteristics (age, sex, race, and ethnicity) were abstracted from the ED administrative database, and all identifiers and protected health information were removed from the data set. Data regarding COVID-19 vaccination status were not available. Laboratory testing was performed on stored specimens after delinking the demographic data set.

Laboratory methods. The testing algorithm required three serologic assays that could differentiate serologic reactivity to SARS-CoV-2 S1, RBD, and nucleocapsid. These assays were limited to standard ELISA and point-of-care assays, as chemiluminescent detection equipment is expensive and not readily available to most laboratories. We selected ELISA-based technologies for the initial high-throughput screening. Confirmatory testing was done with point-of-care assays. Additional information on the assays is available in Table S1 in the supplemental material.

Plasma and serum samples were analyzed using three commercially available serologic assays: the Euroimmun anti-SARS-CoV-2 ELISA (Mountain Lakes, NJ), the CoronaCHEK COVID-19 IgG/IgM rapid test cassette (Hangzhou Biotest Biotech Co. Ltd.), and the Bio-Rad Platelia SARS-CoV-2 total-antibody ELISA (Marnes-la-Coquette, France). Each assay was selected for speed of testing (using an ELISA as an initial screen as the throughput is many times faster than using point-of-care testing), previously determined performance (18, 26), ease-of-use characteristics (standard ELISA technology, no large pieces of equipment necessary) and availability. The Euroimmun ELISA measures IgG responses to the S1 protein of SARS-CoV-2, whereas the Bio-Rad ELISA measures total antibodies to nucleocapsid. Both ELISAs generate a ratio of the optical density of the sample to that of the control (referred to as a signal-to-cutoff ratio [S/C]). For the Euroimmun and Bio-Rad ELISAs, an S/C of ≥0.8 was considered a positive result. The CoronaCHEK lateral flow assay (LFA) tests for the presence of both IgM and IgG antibodies to the receptor-binding domain (RBD) of the spike protein. Any visible band was considered a positive result. Each assay was performed according to the manufacturer's instructions.

An algorithm composed of the Euroimmun, Bio-Rad, and CoronaCHEK assays was used to differentiate samples into three groups: previously infected (who may or may not subsequently be vaccinated), vaccinated (who were never infected), and unexposed (Fig. 1). All samples were first tested using the Euroimmun ELISA (S1). Next, all positive and indeterminate samples were subsequently tested on CoronaCHEK (RBD). Samples that tested positive on Euroimmun and negative on CoronaCHEK were assumed to be false positives and classified as not previously infected or vaccinated (unexposed). Samples that tested positive on CoronaCHEK were tested with the Bio-Rad Total Ab assay (N). Samples which were reactive by Euroimmun and CoronaCHEK but negative by the Bio-Rad assay were considered vaccinated. Samples with a positive or indeterminate result on the Bio-Rad assay were considered previous infections.

Statistical methods. To evaluate the diagnostic accuracy of the testing algorithm for a particular state (vaccinated or previously infected), the sensitivity for each state was determined from samples with that known status (the training sample sets) (Table 1). Calculation of specificity included all samples from unexposed individuals and the samples from individuals of the other state. Since sample collection had occurred prior to the availability of the COVID-19 vaccines, both previously infected and prepandemic samples were considered negative samples to calculate the sensitivity and specificity of the algorithm for the detection of vaccinated samples. To calculate algorithm sensitivity and specificity for previously infected samples, samples from the vaccinated (known to be uninfected) and prepandemic cohorts were considered negative samples. The 48 samples from individuals known or suspected to be infected and subsequently vaccinated were not included in determining the performance of the algorithm. Statistically significant differences in the ELISA S/C values between vaccinated and previously infected individuals were determined using a *t* test. Chi-squared and Fisher's exact tests was used to examine the differences in population demographics between the 2020 and 2021 serosurveys. For the JHH ED serosurvey sample sets, factors associated with previous infection or vaccination were assessed with logistic regression.

RESULTS

Samples from vaccinated individuals without prior SARS-CoV-2 infection (n = 428), unvaccinated individuals with PCR-confirmed infection (n = 494), and those seen in the ED prepandemic (n = 992) were tested on all three assays (Fig. 2). The Euroimmun S1 ELISA was positive for 100% (95% confidence interval [CI], 99.1 to 100.0%), 89% (95% CI, 86.2 to 91.9%), and 3.2% (95% CI, 2.2 to 4.5%) of samples from vaccinated, previously infected, and prepandemic cohorts, respectively. Similarly, for the Bio-Rad N ELISA, 0% (95% CI, 0.0 to 0.7%), 91% (95% CI, 88.2 to 93.5%), and 1.4% (95% CI, 0.7 to 2.4%) were positive for vaccinated, previously infected, and prepandemic cohorts. For the CoronaCHEK RBD assay, 100% (95% CI, 99.1 to 100.0%), 91% (95% CI, 87.8 to 93.1%), and 0.5% (95% CI, 0.2 to 1.2%) had any reactive band for samples from vaccinated, previously infected, and prepandemic cohorts. For samples from

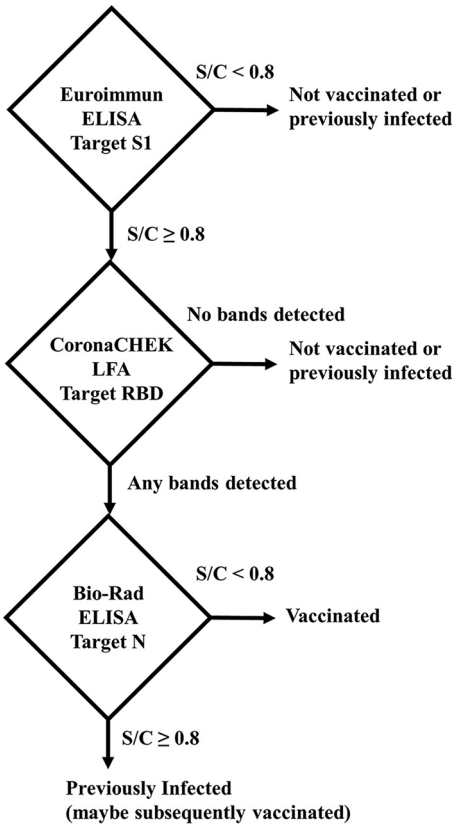

FIG 1 Antibody testing algorithm. An S/C of ≥0.8 on the Euroimmun anti-SARS-CoV-2 ELISA (S1) and a positive result on the CoronaCHEK COVID-19 IgG/IgM rapid test cassette (RBD) were considered positive for the SARS-CoV-2 spike protein. An S/C of ≥0.8 on Bio-Rad Platelia SARS-CoV-2 total-antibody ELISA (nucleocapsid) was considered a previous infection, whereas an S/C of <0.8 in combination with a positive result for spike/RBD indicated vaccination. Samples with negative tests by either Euroimmun or CoronaCHEK were considered unexposed to either SARS-CoV-2 infection or COVID-19 vaccination.

vaccinated individuals, algorithm sensitivity and specificity were 100% (95% CI, 99.1 to 100.0%) and 98.9% (95 CI, 98.2 to 99.3%), respectively (Table 2; Fig. S1a). For samples from previously infected individuals, algorithm sensitivity and specificity were 84.4% (95% CI, 80.9 to 87.5%) and 100% (95% CI, 99.7 to 100.0%), respectively (Table 3; Fig. S1b).

We investigated whether a two-step algorithm could be used, such as an initial screen with the CoronaCHEK RBD assay followed by the Bio-Rad N ELISA. This two-set algorithm had performance characteristics similar to those of the three-step algorithm described above but increased the time to testing by a factor of 5, as the initial screening with an ELISA is much more efficient than a point-of-care test. For samples from vaccinated individuals, the two-step algorithm sensitivity and specificity were 100% (95% CI, 99.1 to 100.0%) and 98.5% (95 CI, 97.8 to 99.1%), respectively (Fig. S2a). For previously infected individuals, algorithm sensitivity and specificity were 87.3% (95% CI, 84.0 to 90.1%) and 100% (95% CI, 99.7 to 100.0%), respectively (Fig. S2b).

There were significant differences between SARS-CoV-2 serostatus and the level of antibody reactivity to spike and nucleocapsid among the cohorts used for algorithm validation (Fig. 3). For vaccinated individuals, the median S/C value for antibody reactivity against spike was 8.9 (interquartile range [IQR] = 83, 95) compared to 5.2 (IQR = 2.3, 7.6) for infected persons ($P < 0.001$). Among the vaccinated persons without previous infection, no individuals had an S/C value for antibody reactivity against nucleocapsid greater than 0.8, the threshold for a positive result, whereas previously infected patients with no history of vaccination had a median S/C of 4.3 (IQR = 3.9, 4.5) ($P < 0.001$). Among HCP, there were 28 samples from individuals with a known SARS-CoV-2 PCR-positive date who were vaccinated 7 to 103 days later. These individuals had spike antibody S/C values similar to those of vaccinated

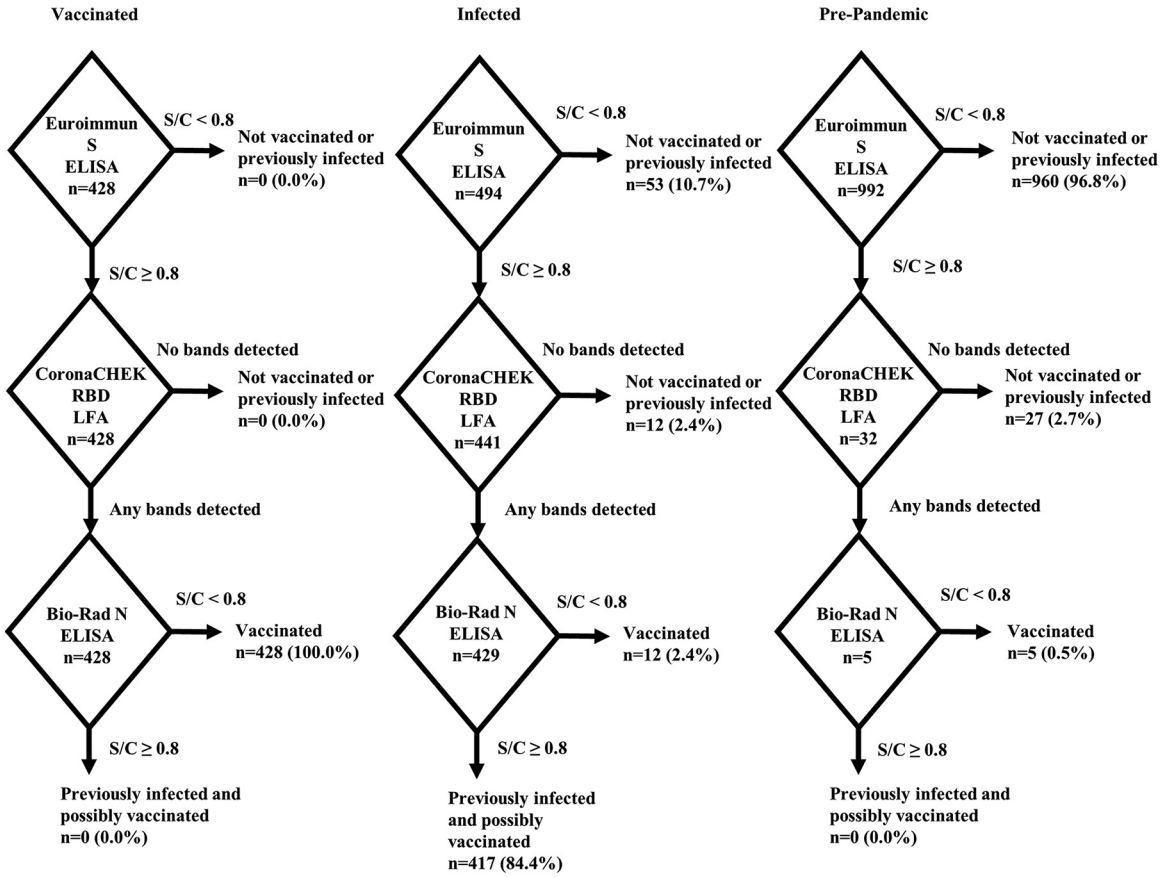

FIG 2 Testing algorithm results on samples from known vaccinated, previously infected, and prepandemic samples.

individuals (median = 9.5, IQR = 9.1, 10.0) and nucleocapsid antibody S/C values similar to those of previously infected individuals (median = 3.4, IQR = 1.4, 4.4). Additionally, 18 HCP who were SARS-CoV-2 PCR negative but had suspected infection had values similar to those of individuals with known infection followed by vaccination, with spike antibody levels (median = 10.0, IQR = 9.0, 10.1), and nucleocapsid antibody S/C values (median = 3.3, IQR = 2.2, 4.1). Because of the timing of sample collection relative to vaccination, it is very unlikely that these 18 samples represented breakthrough infections. It is more likely that these infections were unconfirmed infections that occurred prior to vaccination. There was little reactivity for samples from prepandemic samples.

Using the testing algorithm, 1,536 JHH ED 2020 samples and 2,824 JHH ED 2021 samples were evaluated. During the two collection periods, combined seroprevalence of antibodies to SARS-CoV-2 from 1.6% (95% CI, 1.1 to 2.5%) to 23.8% (95% CI, 22.2 to 25.4%) (Fig. 4). During the 7 weeks of the second survey, the prevalence of vaccination significantly increased from 2.8% (95% CI, 0.9 to 6.3%) in mid-January to 11% (95% CI, 8.6 to 13.7%) by mid-March 2021. The age, sex, and race/ethnic demographics of the two survey periods were similar (Table 4). For both surveys, approximately 27% of participants were ≥60 years of age, 52% were female, 60% were Black, 26% were white, and 7% were Hispanic. The prev-

TABLE 2 Classification as vaccinated by testing algorithm

	No. of samples	
Classification by algorithm	**Known vaccinated**	**Not vaccinated**
Vaccinated (Euroimmun and CoronaCHEK positive, Bio-Rad negative)	428	17
Not vaccinated (any other result)	0	1,469

TABLE 3 Classification as previously infected by testing algorithm

Classification by algorithm	No. of samples	
	Infected	Not infected
Infected (Euroimmun, CoronaCHEK, and Bio-Rad positive)	417	0
Not infected (any other result)	77	1,420

alence of infection in the spring of 2020 did not vary significantly by age, ethnicity, race, and sex. In contrast, by the spring of 2021, significant differences in infection by age, ethnicity, race, and sex were observed.

The prevalence of antibodies to SARS-CoV-2 indicating previous infection or vaccination is presented in Table 4. White women and men had the lowest prevalence of infection both in 2020 and 2021. In the 2021 survey, white women accounted for 9% of all infections in 2021 but 27% of all vaccinations. For all other groups, the prevalence of exposure to SARS-CoV-2 was higher than the frequency of vaccination. By the spring of 2021, Hispanic patients had the highest evidence of prior SARS-CoV-2 infection within any ethnic group, at 38%.

In the 2021 survey, there were no statistically different rates of infection between age groups (Table 5). In contrast, 45- to 59-year-olds were less likely to be vaccinated than the youngest individuals (adjusted odds ratio [aOR] = 0.71; 95% CI, 0.52 to 0.98). Compared to Black women, white women were less likely to be previously infected (aOR, 0.46; 95% CI,

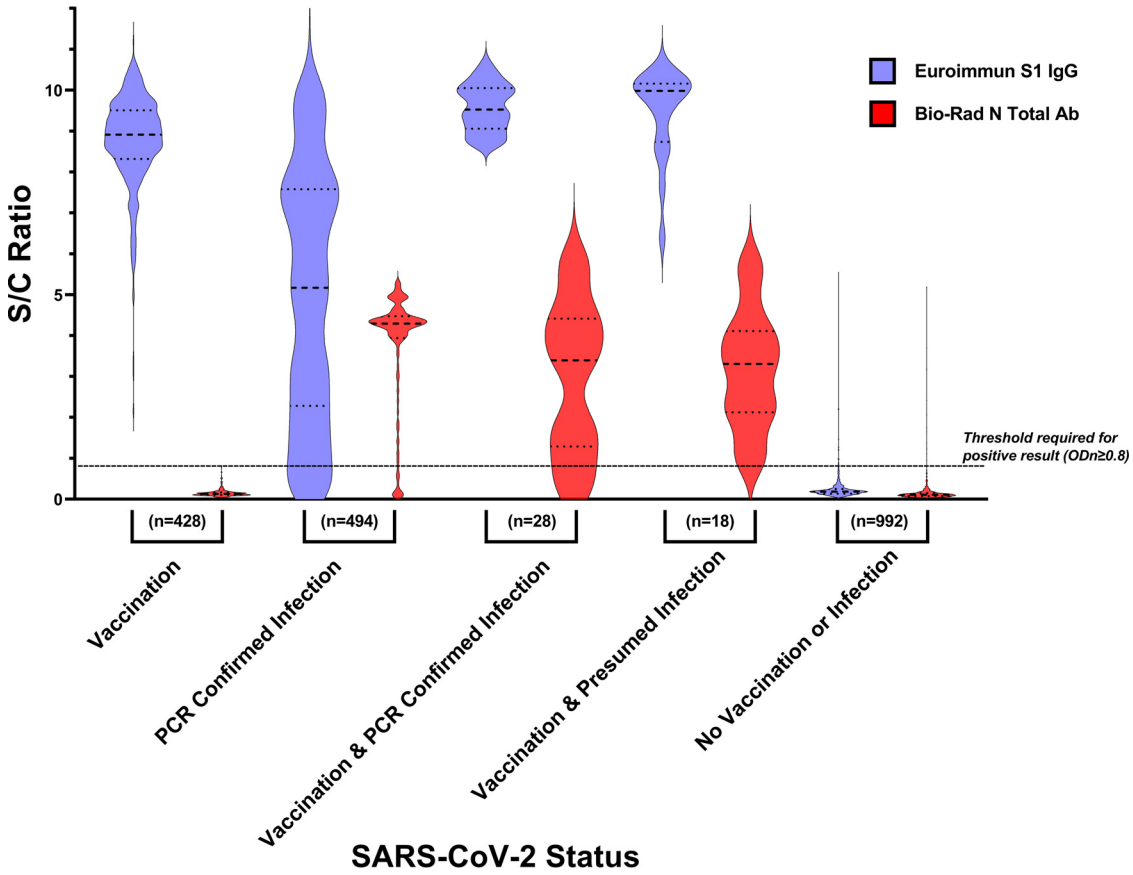

FIG 3 Comparison of ELISA values between vaccinated and previously infected individuals. Samples with known serostatus from the algorithm validation cohorts were tested on both the Euroimmun anti-SARS-CoV-2 IgG ELISA (spike) and on Bio-Rad Platelia SARS-CoV-2 total-antibody ELISA (nucleocapsid). Each ELISA generates a ratio of the optical density of the sample to that of a manufacturer-provided calibrator. The *y* axis is given as a signal-to-cutoff ratio (S/C). Medians and interquartile ranges are displayed for each violin plot. The vaccinated group comprised individuals with documented vaccination and no previous positive PCR or serological result. SARS-CoV-2 infections were confirmed by a positive PCR result. The vaccination-and-confirmed-infection group was composed of individuals with both documented vaccination and PCR-positive infection. Presumed infections were characterized by a lack of PCR-positive result but a positive result for nucleocapsid on the Bio-Rad assay. Samples in the not-vaccinated-or-infected category were obtained from the JHH ED in 2016, prior to the advent of the COVID-19 pandemic.

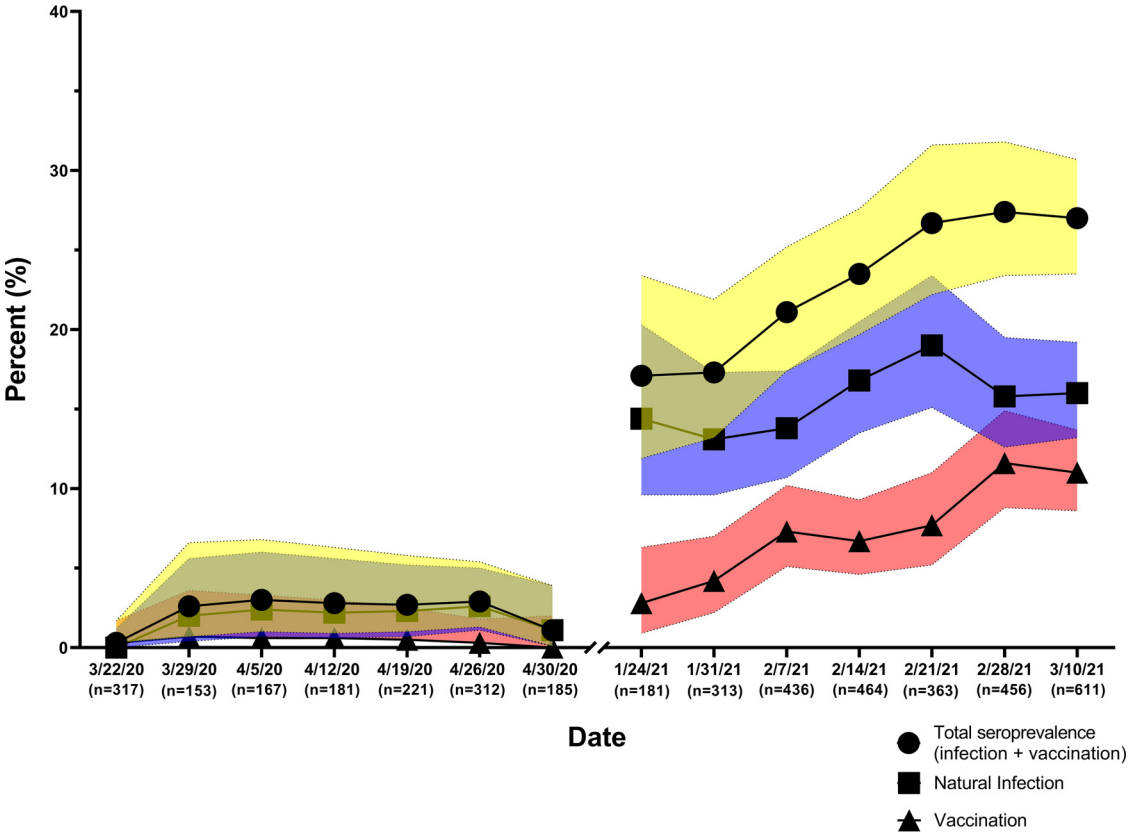

FIG 4 Seroprevalence of antibodies of SARS-CoV-2 2020 to 2021. JHH ED samples from 2020 and 2021 were tested on the previously mentioned algorithm and categorized according to the date on which the sample was drawn.

0.31 to 0.67), while Hispanic women and men were three times as likely to be previously infected (aOR, 3.11 [95% CI, 1.98 to 4.86] and 2.92 [1.86 to 4.58], respectively). White women and men and Hispanic men were all significantly more likely than Black women to have evidence of vaccination, with aOR of 2.42 (95% CI, 1.64 to 3.56), 1.59 (1.02 to 2.47), and 2.04 (1.02 to 4.08), respectively.

TABLE 4 Demographic characteristics and seroprevalence of infection and vaccination in emergency department patients in the spring of 2020 and 2021

		No. of patients (%)				
		16 March to 30 April 2020		11 January to 10 March 2021		
Characteristic	Category[a]	Total (n = 1,536)	Previously infected (n = 26)	Total (n = 2,824)	Previously infected (n = 444)	Vaccinated (n = 229)
Age (yrs)	18–29	285	3 (1.1)	578	103 (23.2)	51 (8.8)
	30–44	411	2 (0.5)	763	128 (28.8)	70 (9.2)
	45–59	434	9 (2.1)	664	100 (22.5)	31 (4.7)
	60–74	318	9 (2.8)	599	80 (18.0)	58 (9.7)
	≥75	87	3 (3.4)	215	32 (14.9)	19 (8.8)
	Missing	1	0 (0.0)	5	1 (20.0)	0 (0.0)
Ethnicity, race, sex	NH Black female	490	4 (0.8)	880	150 (17.0)	55 (6.3)
	NH white female	201	0 (0.0)	442	38 (8.6)	61 (13.8)
	Hispanic female	54	2 (3.7)	95	37 (38.9)	8 (8.4)
	Other female	47	3 (6.4)	108	17 (15.7)	15 (13.9)
	NH Black male	436	7 (2.3)	738	101 (13.7)	33 (4.4)
	NH white male	197	3 (1.7)	382	51 (13.4)	37 (9.7)
	Hispanic male	61	2 (3.3)	96	36 (37.5)	11 (11.5)
	Other male	50	2 (4.0)	83	14 (16.9)	9 (10.8)

[a]NH, non-Hispanic.

TABLE 5 Factors associated with positivity for SARS-CoV-2 antibodies among individuals attending the JHH ED between 11 January and 10 March 2021[a]

		Previous infection		Vaccination	
Characteristic	Category	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
Age (yrs)	18–29	1	1	1	1
	30–44	0.93 (0.70–1.24)	0.86 (0.64–1.16)	1.04 (0.72–1.52)	0.99 (0.67–1.46)
	45–59	0.82 (0.61–1.11)	0.84 (0.62–1.14)	**0.51 (0.32–0.80)**	**0.51 (0.32–0.82)**
	60–74	**0.71 (0.52–0.98)**	0.76 (0.55–1.06)	1.11 (0.75–1.64)	1.13 (0.75–1.69)
	≥75	0.81 (0.52–1.24)	0.94 (0.60–1.46)	1.00 (0.58–1.74)	0.88 (0.50–1.55)
	Missing	NE	NE	NE	NE
Ethnicity, race, sex	NH Black female	1	1	1	1
	NH white female	**0.46 (0.31–0.67)**	**0.46 (0.32–0.67)**	**2.40 (1.64–3.53)**	**2.42 (1.64–3.56)**
	Hispanic female	**3.11 (1.98–4.86)**	**3.08 (1.96–4.84)**	1.38 (0.64–2.99)	1.36 (0.62–2.97)
	Other female	0.91 (0.53–1.57)	0.92 (0.53–1.60)	**2.42 (1.32–4.45)**	**2.47 (1.34–4.57)**
	NH white male	0.75 (0.53–1.06)	0.77 (0.55–1.09)	**1.61 (1.04–2.49)**	**1.59 (1.02–2.47)**
	NH Black male	0.77 (0.59–1.02)	0.79 (0.60–1.04)	0.70 (0.45–1.09)	0.72 (0.46–1.13)
	Hispanic male	**2.92 (1.86–4.58)**	**3.01 (1.90–4.77)**	1.94 (0.98–3.85)	**2.04 (1.02–4.08)**
	Other male	0.99 (0.54–1.80)	0.99 (0.54–1.81)	1.82 (0.87–3.84)	1.87 (0.89–3.94)

[a]ORs with *P* values of <0.05 are shown in boldface type. NH, non-Hispanic; NE, not examined.

We disaggregated the data by sex, race, and ethnicity. In terms of previous infection (Table S2a), individuals between the ages of 45 to 74 were 22% less likely to have evidence of prior infection than the 18- to 29-year-old JHH ED patients. White individuals were 30% less likely to have been infected than Black individuals. Hispanic individuals had more than three times the burden of infection compared to non-Hispanic individuals, with an aOR of 3.31 (95% CI, 2.16 to 5.07). After adjusting for age and race/ethnicity, women had an increased odds for vaccination compared to men, with an aOR of 1.35 (95% CI, 1.02 to 1.80) (Table S2b). In comparison to patients aged 18 to 29, patients aged 45 to 59 years were less likely to be vaccinated, with an aOR of 0.50 (95% CI, 0.31 to 0.80). Furthermore, white patients had more than twice the odds of vaccination compared to Black patients, with an aOR of 2.26 (95% CI, 1.67 to 3.07).

DISCUSSION

This study describes a method for distinguishing between SARS-CoV-2-vaccinated, previously infected, and uninfected individuals using commercially available serologic assays when no vaccination or infection history is available. The algorithm utilized in this study indicates a 10-fold increase in seropositivity to SARS-CoV-2 infection in the Baltimore metropolitan area from April 2020 to March 2021. Furthermore, this study highlights disparities based on sex and race/ethnicity in SARS-CoV-2 prevalence and vaccine distribution within metropolitan Baltimore during the spring of 2021.

This study followed the work of Suhandynata et al. (27) in the ability to differentiate vaccinated from infected individuals based on antibody responses to the S1 and N proteins of SARS-CoV-2. In contrast to the Suhandynata et al. study, which utilized chemiluminescent assays (Roche Elecsys Anti-SARS-CoV-2 S and N antibody) (27), we applied more commonly available ELISA and LFA methods. Other chemiluminescent assays, such as the Meso Scale Diagnostics assay, provide testing where multiple antigens (S1, RBD, and N) can be evaluated in one well. This assay relies on chemiluminescent technology, which requires expensive technology not affordable to many laboratories worldwide. We further expanded the study by Suhandynata et al. by using assays that do not need expensive chemiluminescent technology, incorporating a larger validation cohort, and applying the algorithm to population-level surveillance.

This study confirms previous reports of high burden of COVID-19 among the Baltimore Hispanic population (28). Additionally, the discrepancies in vaccine uptake among racial and ethnic minority groups are clearly demonstrated. While recent data suggest that racial and ethnic gaps in vaccination have narrowed (10), our data from early 2021 suggest that disparities in vaccination were present in the initial stages of the vaccine rollout. Surprisingly, despite prioritizing older Americans during the vaccine rollout (29), patients older than 60 were as likely to be vaccinated as those between the ages of 18 to 44.

This method demonstrated 100% sensitivity in identifying individuals who were fully vaccinated with both the Pfizer and Moderna vaccines. One critical potential limitation to the use of antispike and antinucleocapsid antibody testing to differentiate previously infected from vaccinated individuals is differential loss of antibody reactivity to these two targets. In a cohort of 3,276 health care workers in the United Kingdom, Lumley et al. estimated that anti-nucleocapsid IgG antibodies exhibit a half-life of 85 days from the maximum titer (95% CI, 81 to 90) (30). In contrast, the half-life of antispike IgG antibodies could not be measured, as 94% of health care workers did not exhibit significant loss during follow-up (30). Additionally, antinucleocapsid antibody decline was more rapid in younger patients and those with milder symptoms. Thus, in the proposed algorithm, a proportion of previously infected individuals will be misclassified as vaccinated, as antinucleocapsid antibodies wane with time. This effect will be differential by age and initial symptomology. The time to seroreversion of spike and nucleocapsid antibodies in SARS-CoV-2-infected patients is significantly affected by both disease severity and assay platform. The effect of these variations should be considered when interpreting the results of serosurveillance studies.

This study has several additional limitations. We did not test any individuals with known breakthrough infection (vaccinated then infected), nor could we distinguish between previously infected individuals who were and were not subsequently vaccinated. Furthermore, a lack of seroreactivity occurred in a minority of previously infected individuals. The lack of seroconversion in infected individuals has been observed in other studies and occurs most frequently in individuals with asymptomatic infection (31–33). Using this testing algorithm, 16% of individuals with a previous positive RT-PCR test were seronegative by the algorithm. Similarly, Self et al. found that in a convenience sample of 156 mildly infected frontline health care personnel, 93.6% experienced a decline in antibody response and 28.2% seroreverted within 60 days (34). These studies illustrate the difficulty of identifying infected persons several months after infection, especially in cases of mild infection. It should be noted that antibody reactivity is also dependent on the assay used, especially at 6 months after SARS-CoV-2 infection (35).

Although the correlates of antibody protection for previously infected individuals are not well established (36), we demonstrated that a serosurvey can be performed to differentiate vaccinated, previously infected, and at-risk unexposed individuals in a population when vaccination or infection history is not available. This information provides evidence for targeted public health intervention in preparation for the continued spread of endemic SARS-CoV-2 infections.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.

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