

Feasibility of SARS-CoV-2 Antibody Testing in Remote Outpatient Trials

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Background. During the coronavirus disease 2019 (COVID-19) pandemic, clinical trials necessitated rapid testing to be performed remotely. Dried blood spot (DBS) techniques have enabled remote HIV virologic testing globally, and more recently, antibody testing as well. We evaluated DBS testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody testing in outpatients to assess seropositivity.

Methods. In 2020, we conducted 3 internet-based randomized clinical trials and offered serologic testing via self-collected DBS as a voluntary substudy. COVID-19 diagnosis was based on the Centers for Disease Control and Prevention case definition with epidemiological link to cases. A minority reported polymerase chain reaction (PCR) testing at an outside facility. We tested for anti-SARS-CoV-2 immunoglobulin via antibody detection by agglutination-PCR (ADAP) and compared the results with enzyme-linked immunosorbent assay (ELISA).

Results. Of 2727 participants in the primary studies, 60% (1648/2727) consented for serology testing; 56% (931/1648) returned a usable DBS sample. Of those who were asymptomatic, 5% (33/707) had positive ADAP serology. Of participants with a positive PCR, 67% (36/54) had positive SARS-CoV-2 antibodies. None of those who were PCR-positive and asymptomatic were seropositive (0/7). Of 77 specimens tested for concordance via ELISA, 83% (64/77) were concordant. The challenges of completing a remote testing program during a pandemic included sourcing and assembling collection kits, delivery and return of the kits, and troubleshooting testing. Self-collection was successful for >95% of participants. Delays in US mail with possible sample degradation and timing of DBS collection complicated the analysis.

Conclusions. We found remote antibody testing during a global pandemic feasible although challenging. We identified an association between symptomatic COVID-19 and positive antibody results at a similar prevalence as other outpatient cohorts.

Keywords. antibody; COVID-19; humoral immunity; SARS-CoV-2; serology.

Early in the coronavirus disease 2019 (COVID-19) pandemic, the availability of polymerase chain reaction (PCR) testing (the gold standard for diagnosis) was limited in the United States. Supplies were scarce, and symptomatic persons with COVID-19 or high-risk exposures were unable to attend outpatient facilities due to infection control concerns restricting access. As a result, antibody testing was used to identify the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1].

Traditional serology is performed through venipuncture blood collection by trained personnel in a hospital or clinic setting. Thus, often only those with more severe disease are tested in medical settings. Alternatively, serology performed through a finger prick and dried blood spot (DBS) or similar technology with dried blood can be self-collected at home. This home-based approach allows for outpatients, often with less severe disease, to be tested. DBS serology is a well-established, rapid method used to identify infectious diseases and is the foundation for newborn screening [2–4]. DBS has been used to detect antibodies against SARS-CoV-2 antigens, and similar methods of at-home blood collection have been used by the National Health System in the United Kingdom for SARS-CoV-2 [5].

Serology tests for SARS-CoV-2 measure response to several proteins, including spike (S) and nucleocapsid (NP), the dominant antigens for host immune response [6, 7]. The S1 domain of the spike protein is the outermost domain and contains the receptor-binding domain (RBD) for the ACE2 receptor, which initiates cell entry [8]. Test validation of different SARS-CoV-2

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antibody tests has been controversial, particularly with respect to defining a gold standard [6].

Outpatients have been underrepresented in serology studies with disease correlation [9]. To assess the feasibility of using DBS for antibody testing during a pandemic and to better understand the serologic conversion rates in the outpatient population, we performed a serology substudy for persons with early COVID-19 and those with a high risk of infection enrolled in 3 COVID-19 clinical trials.

We hypothesized that participants with symptom-compatible COVID-19 infection would have detectable SARS-CoV-2 antibodies by DBS collection and antibody detection by agglutination-PCR (ADAP) analysis. We further hypothesized that participants with mild or asymptomatic illness would have lower seroconversion rates than participants with severe disease.

METHODS

Study Design

We conducted 3 randomized, double-blind, placebo-controlled trials investigating the use of hydroxychloroquine as prophylaxis or treatment for early COVID-19 disease [10–12]. Two trials, 1 investigating hydroxychloroquine as postexposure prophylaxis and 1 investigating hydroxychloroquine as early pre-emptive treatment, enrolled participants from March 17, 2020, to May 6, 2020; follow-up concluded on May 20, 2020. The third trial investigating the efficacy of hydroxychloroquine as pre-exposure prophylaxis enrolled participants from April 6, 2020, to May 26, 2020; follow-up concluded on July 13, 2020. Details regarding the study protocols and analysis of the primary end points can be found on ClinicalTrials.gov (identifiers NCT04308668 and NCT04328467, respectively).

Inclusion criteria for the postexposure prophylaxis trial required persons to have a known exposure (within the previous 4 days) to a lab-confirmed SARS-CoV-2 case either as a health care worker, a first responder, or a household contact. Individuals were enrolled into the preemptive early treatment trial with COVID-19 symptoms (≤ 4 days duration) and either lab-confirmed SARS-CoV-2 or high-risk exposure to a known case within 14 days of symptom onset. The pre-exposure prophylaxis trial inclusion criteria required persons to be high-risk health care workers or first responders with ongoing occupational exposure to COVID-19 patients.

Study Participants

Participants were screened and enrolled in the trials if they met the respective inclusion criteria for each study via an internet-based survey using Research Electronic Data Capture (REDCap) [13]. While overall trial participation included persons living in the United States or select Canadian provinces, only participants in the United States were eligible for participation in the serology substudy. Full details regarding inclusion and exclusion criteria have been published elsewhere [10–12].

Enrollment and follow-up data were collected via self-report through REDCap. Participants were asked to report COVID-19 exposure and lab-confirmed SARS-CoV-2 results (if available) on follow-up surveys to assess ongoing risk and status. PCR and outside serology test results were self-reported and were not verified by the study. Only a minority of individuals had PCR testing given the limitations of COVID-19 testing in the United States during March–June of 2020. The majority of participants were health care workers.

Reported symptoms were adjudicated as probable or possible COVID-19 disease by 3 independent physicians using the Council of State and Territorial Epidemiologists reporting guidelines [14]. Per this US case definition, probable COVID-19 cases had (A) at least 2 of the following symptoms: fever (measured or subjective), chills, rigors, myalgia, headache, sore throat, or new olfactory and taste disorder(s); or (B) at least 1 of the following symptoms: cough, shortness of breath, or difficulty breathing. Possible cases per our case definition in this and the other parent studies included at least 1 of the following symptoms: fever (measured or subjective), chills, rigors, myalgia, headache, sore throat, loss of smell and taste disorder(s), and epidemiologic linkage of exposure to a PCR-positive case [10–12].

Patient Consent

Before study enrollment, participant comprehension of clinical trial design, purpose, and drug randomization was assessed via an online survey. Informed consent was obtained through electronic signature capture. All institutional review board (IRB) approvals were obtained through the University of Minnesota.

Collection of Dried Blood Spots

Participants were invited via email to enroll in the serology substudy to investigate the prevalence of SARS-CoV-2 antibodies, irrespective of clinical trial or study arm randomization. A separate consent was delivered and signed via REDCap. Upon enrollment into the substudy, participants were mailed DBS collection kits that included 2 lancets, a pre-labeled Whatman Proteinsaver card, 2 bandages, alcohol wipes, a zippered plastic bag for storage, a mailing envelope with return postage, and detailed instructions on how to prepare the sample (Supplementary Figure 1). The instructions on DBS preparation were adapted from commercial instructions and used with permission from Great Plains Laboratory, Inc. These instructions were adapted by the study team and IRB approved. Kits for DBS self-collection were sent to participants in May and June of 2020. Samples were collected by participants and returned between May and July 2020. Upon receipt, returned samples were refrigerated at 4°C and subsequently shipped for testing.

In June 2020, the only assay validated for DBS was the ADAP method [15]. The validation was done using samples from PCR-positive individuals with symptomatic COVID-19 who had not

been hospitalized (J Tsai, Personal Communication, 2021). Our specimens were sent by courier to Enable Biosciences in San Francisco in 2 batches in July and August 2020. SARS-CoV-2 serologic testing was later validated for DBS via enzyme-linked immunosorbent assay (ELISA) [16].

Antibody Testing of Dried Blood Spots by ADAP

Dried blood spots were processed to detect antibodies against SARS-CoV-2 spike protein, S1, as previously described [15]. Briefly, 3-mm discs were isolated from the dried blood spots, incubated in an elution buffer at 37°C for 90 minutes, and subsequently run through a molecular weight column. The recovered sample was then used in an ADAP test to detect the presence of and quantify immunoglobulin antibodies against SARS-CoV-2 spike protein. The ADAP system constitutes an antigen-DNA conjugate which, when exposed to its cognate antibody, leads to immune complex formation [17]. A ligation solution containing DNA ligase and the bridging oligonucleotide was added to the newly formed immune complexes and subsequently mixed with PCR master mix. The final product was analyzed by quantitative PCR, producing a cycle threshold value; high DNA quantity equated to high antibody levels, while low DNA quantity equated to low antibody levels. S1 spike protein amino acid 1-674 with Fc tag served as the target antigen and was conjugated to a custom oligonucleotide sequence from Integrated DNA Technologies [15]. Samples were run in batches without technical replicates. Each batch contained an internal positive control consisting of a dried blood spot sample with S1 antibodies as well as a negative control consisting of a dried blood spot sample without S1 antibodies present.

Hydroxychloroquine and Seropositivity

To evaluate whether hydroxychloroquine affected seropositivity, we compared seropositivity by treatment arm and using the chi-square test.

Timing of Seropositivity

We calculated the number of days between the development of COVID-compatible symptoms and DBS collection. If a DBS arrived without a date, we imputed the date as 2 weeks after the date of informed consent. Similarly, we calculated the number of days between the PCR collection and DBS collection.

Seropositivity by COVID-19-Specific Symptoms

To evaluate the correlation between seropositivity, we selected individuals with COVID-compatible illness and compared seropositivity by number of symptoms. We grouped the number of symptoms into 1, 2–3, 4–5, and 6–7 among those with cough, shortness of breath, fever, myalgia, headache, sore throat, and loss of smell.

Antibody Testing of Dried Blood Spots by ELISA

ELISA for detecting immunoglobulin G (IgG) antibodies against SARS-CoV-2 spike protein S1 domain was performed on a subset of DBS to externally validate ADAP results. The ELISA kit was purchased from Euroimmun (EI 2606-9601-G), which received Emergency Use Authorization (EUA) from the Food and Drug Administration (FDA) on May 5, 2020 [16]. This ELISA assay was validated using stored residual serum and plasma samples submitted to the University of Chicago Medicine Clinical Laboratories.

Per the manufacturer's instructions, DBS were eluted with sample buffer, transferred to a pre-coated ELISA plate, incubated, and subsequently treated with enzyme conjugate and substrate solution. The plate was read at a wavelength of 450 nm. As this was a qualitative analysis, cutoffs were set at positive ≥ 1.1 , borderline ≥ 0.8 – < 1.1 , and negative < 0.8 , as established by Euroimmun [16]. Samples were run in singles, and 10% of samples were randomly selected to be run in duplicate. As evaluated by calculating the coefficient of variance, experimental precision ranged from 0.55% and 17.45%. A raw readout of the calculated ELISA ratio was compared with raw ADAP results and analyzed by simple linear regression with Pearson's correlation coefficient (r), and P values were reported. We also analyzed the ELISA and PCR qualitative results by McNemar test for concordance.

RESULTS

Of 2727 participants in the primary clinical trials, 931 participants (34.1%) enrolled in the serology substudy and returned a DBS for testing. Of these 931 participants, 266 enrolled in the postexposure prophylaxis trial, 123 enrolled in the preemptive early treatment trial, and 542 enrolled in the pre-exposure prophylaxis trial (Table 1). The median age of participants in the serology substudy was 43 years. Of those returning a DBS, 54% were women, 84% self-identified as White, and 88% identified as health care workers (Supplementary Table 1).

We found that the highest rate of seropositivity was among those in the early treatment trial (29%, 36/123), all with mild COVID-19. Antibody seropositivity was 11% (29/266) in participants receiving postexposure prophylaxis and 4% (19/542) in participants receiving pre-exposure prophylaxis.

COVID-19 Symptoms and Seropositivity

Among participants with symptoms consistent with probable COVID-19 [14], 29% (51/178) had detectable antibodies. Among participants with possible COVID-19 with only 1 COVID-compatible symptom [14], 0% (0/46) tested positive for antibodies.

Among participants without symptoms of COVID-19 from the prophylaxis trials, 5% (33/707) had detectable SARS-CoV-2 antibodies. This may reflect asymptomatic

Table 1. Serology Results Summary by Study, PCR Confirmation, and Symptoms

	Postexposure Prophylaxis	Early Treatment	Pre-exposure Prophylaxis	All Studies Combined
	n = 266	n = 123	n = 542	n = 931
	% [No.]	% [No.]	% [No.]	% [No.]
Overall results				
Positive antibody	11 [29/266]	29 [36/123]	4 [19/542]	9 [84/931]
Probable COVID-19	32 [9/28]	32 [36/114]	17 [6/36]	28 [51/178]
Possible COVID-19	0 [0/8]	0 [0/9]	0 [0/29]	0 [0/46]
Without COVID-19 symptoms	9 [20/230]	0 [0/0]	3 [13/477]	5 [33/707]
PCR positivity*				
	% Antibody Positive [No.]	% Antibody Positive [No.]	% Antibody Positive [No.]	% Antibody Positive [No.]
Positive PCR	83 [5/6]	66 [23/35]	62 [8/13]	67 [36/54]

Symptom designations per Council of State and Territorial Epidemiologists [14]: probable symptoms are >1 of cough, fever, or shortness of breath or >2 of fever, myalgia, headache, sore throat, or loss of taste and an epidemiologic link to a COVID-19 case.

Abbreviations: COVID-19, coronavirus disease 2019; PCR, polymerase chain reaction.

*Total with PCR tests at any time was 121 (13%) out of 931 enrolled.

seroconversion or false positives. One of these individuals self-reported an external antibody test performed at enrollment that was also positive. Of the other 32 cases without COVID-19 symptoms and positive antibody results, we evaluated if they had reported trial medication side effects, which could have been reported as a COVID-19 symptom. We found that 63% (20/32) had no reported medication side effects or COVID-19 symptoms.

SARS-CoV-2 seropositivity increased with the number of COVID-19-specific symptoms. Nine percent of participants reporting 1 COVID-19-specific symptom seroconverted compared with 50% of those with 6–7 COVID-19-specific symptoms (n = 224) (Figure 1).

Seropositivity and PCR Positivity

Of 931 participants with antibody testing, 121 (13%) reported PCR testing. Of the 54 participants with positive PCR results, 67% (n = 36) had detectable SARS-CoV-2 antibodies. Given the possibility that persons with more recent illness seroconverted after DBS collection, we performed a secondary analysis of those with DBS collection >21 days from PCR-positive testing. We found the seropositivity to be similar (64% [28/44] antibody positivity) in those >21 days from PCR testing compared with the whole cohort.

We further explored seropositivity by the duration between PCR and DBS collection and by symptoms. None of the 7 participants with asymptomatic, PCR-confirmed SARS-CoV-2

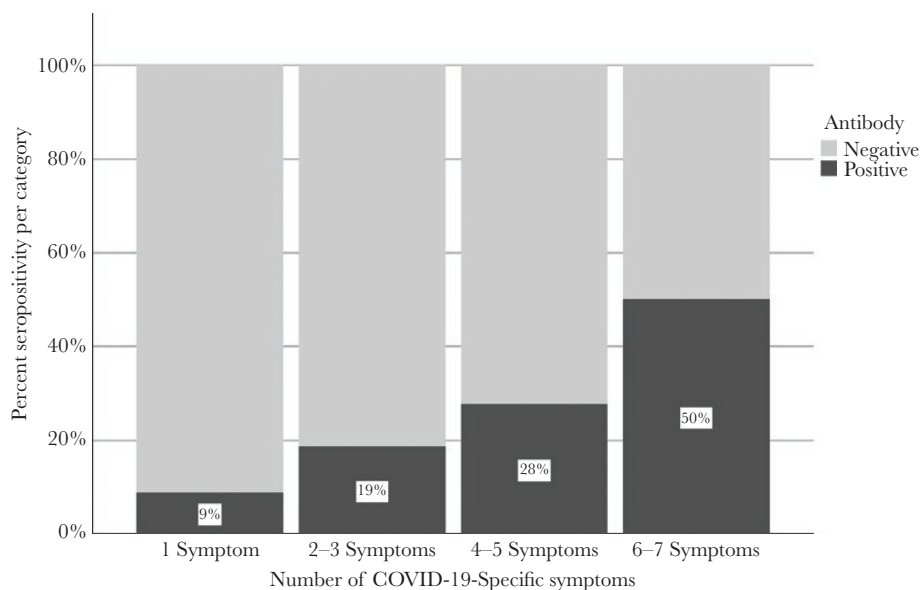


Figure 1. SARS-CoV-2 antibody positivity by number of COVID-19-specific symptoms among those with COVID-19-compatible illness. Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

infection had a positive antibody via DBS. Thus, among persons who were symptomatic with COVID-19 and PCR-positive, seropositivity was 72% (34/47) (Table 2).

Hydroxychloroquine and Seropositivity

We evaluated the effect of hydroxychloroquine on seropositivity. We compared seropositivity by study as well as combined among all study cohorts. We found no significant association between the use of hydroxychloroquine and SARS-CoV-2 seropositivity (5% [27/497] of those randomized to hydroxychloroquine vs 5% [21/428] randomized to placebo; $P = .72$).

Validation Study

Of the 77 participants who had DBS tested via ELISA, 64 (83%) had consistent qualitative results (positive or negative) by the ADAP assay. ELISA has a borderline category, while ADAP is only positive or negative. By ELISA, 4 samples were borderline positive, 3 of which were positive by ADAP (Figure 2). Of the 27 people with positive ELISA results, 23 (85%) were positive by ADAP. Of the 45 participants who were negative by ELISA, 38 (84%) were negative by ADAP. The correlation between ADAP and ELISA was moderate (Pearson $r = 0.68$; $P < .001$).

When the borderline ELISAs were classified as positive for qualitative concordance, the McNemar statistic was 0.29 ($P = .77$). When the borderline ELISAs were classified as negative, the McNemar statistic was 1.34 ($P = .19$). Thus, classification of borderline ELISA as positive improved concordance with ADAP testing.

Remote Lab-Based Testing Program During a Pandemic

The challenges of completing a remote testing program during a pandemic were related to supply chain challenges, delivery via US mail, and securing an appropriate testing method. Given the supply chain issues in 2020 [18], sourcing supplies, especially lancets, was difficult. Also, troubleshooting lancet size was more difficult than initially anticipated: The smallest

lancet possible is preferred for participant comfort, but this must be balanced against obtaining enough blood for the sample to be usable. Given limited funding, the investigators assembled the DBS collection kits. We created a set of instructions for self-collection. Some individuals continued to have challenges collecting DBS. As an additional reference tool, we created an instructional video that was distributed online to participants (available in the Supplementary Data). Over 95% of individuals who tried to submit a sample were successful. We used the US Postal Service in order to limit costs of collection. Unfortunately, there were extensive delays in the postal service during 2020 [19]. These delays in mail resulted in lost kits and samples, which were returned after the July cutoff. The longest time frame for a misdelivered kit with return to sender was 9 months. These delays in mail delivery may have contributed to sample degradation in several instances. One of the biggest challenges was ensuring appropriate testing. We initially attempted to run assays internally; however, we determined the ADAP method to be more appropriate, requiring outsourcing of the testing. Further transport time may have also affected results.

DISCUSSION

Here we present antibody results from 3 internet-based, outpatient randomized trials of individuals with early COVID-19 or those at high risk of SARS-CoV-2 infection. We found a seropositivity rate of 28% among those with probable COVID-19 symptoms, 0% among those with possible COVID-19 symptoms, and 5% among those without COVID-19-specific symptoms. Seropositivity was not detected among those with asymptomatic PCR positivity or with very mild disease (defined as only 1 COVID-compatible symptom plus epidemiologic linkage to a PCR-positive COVID case).

The seroprevalence of participants with COVID-19-compatible symptoms and SARS-CoV-2 PCR-confirmed disease was similar to other outpatient studies. Mitja et al. reported 27% (17/64) IgM or IgG seropositivity among PCR-positive persons with symptomatic COVID-19 and 21% (13/61)

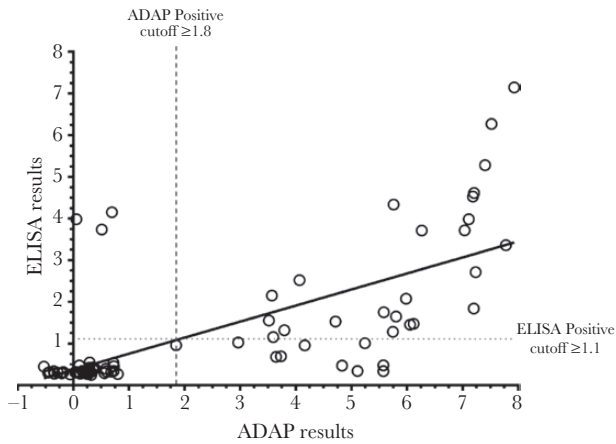
Table 2. SARS-CoV-2 Antibody Positivity by Days From Positive PCR to Dried Blood Spot Collection Shows Seropositivity Is Similar by Collection Timing

Days After PCR+	Total Samples		PCR+ COVID-19-Specific Probable Symptoms ^a		PCR+ Without COVID-19-Specific Symptoms	
	Samples Tested, No.	Positive Samples, % (No.)	Samples Tested, No.	Positive Samples, % (No.)	Samples Tested, No.	Positive Samples, % (No.)
0–14	5	60 (3/5)	3	100 (3/3)	2	0 (0/2)
15–30	4	75 (3/4)	3	100 (3/3)	1	0 (0/1)
31–75	45	62 (28/45)	41	68 (28/41)	4	0 (0/4)
Total	54	63 (34/54)	47	72 (34/47)	7	0 (0/7)

Designations per Council of State and Territorial Epidemiologists [14]: probable symptoms are >1 of cough, fever, or shortness of breath or >2 of fever, myalgia, headache, sore throat, or loss of taste and an epidemiologic link to a COVID-19 case.

Abbreviations: COVID-19, coronavirus disease 2019; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aNo participant with COVID-19-specific possible symptoms had a positive PCR or SARS-CoV-2 antibody positivity.



Concordance for SARS-CoV-2 ADAP and ELISA

	Total	Positive by ADAP	Negative by ADAP
Total		34	43
Positive by ELISA	27	24	3
Borderline by ELISA	4	3	1
Negative by ELISA	46	7	39

Correlation between ADAP and ELISA results was assessed by simple linear regression. Best fit line followed the equation $y = 0.3853x + 0.3709$. The r value was 0.6819, with a P value $< .001$.

Figure 2. Linear regression comparing SARS-CoV-2 ADAP and ELISA. Abbreviations: ADAP, antibody detection by agglutination–polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

seropositivity among asymptomatic PCR-positive persons after exposure [20]. Mitja et al. collected specimens ~16–20 days after exposure [20]. Our prevalence of COVID-19 antibodies among PCR-positive participants was 67%. This seroprevalence is below previously published surveys of hospitalized patients (80%–100% in China) [21, 22]. However, several recent studies have shown that antibody production varies significantly by disease severity; outpatients and those with asymptomatic disease have been shown to have lower antibody conversion and decreased duration of detectable antibodies [22–25]. Additionally, some data suggest that persons with asymptomatic carriage and positive SARS-CoV-2 PCR have low seroconversion rates [26].

The seroprevalence of our sample of mostly health care workers is similar to other surveys of high-risk health care workers. A sample of health care workers in New York City from April 20, 2020, to June 23, 2020 (during the height of their local incidence), demonstrated a seroprevalence of 14% [27]. A multicenter study reporting data from 13 centers across the United States showed an overall prevalence of 6% [28], which is similar to our rate among high-risk health care workers of 4%.

The lack of a clear “gold standard” for evaluating serologic testing in COVID-19 remains challenging. Some studies recommend using PCR as the gold standard [6]. However, as previously discussed, PCR correlation with antibody seroprevalence is highly dependent on disease severity and timing of measurement. PCR results were only available from a subset of our participants, given the limited availability of PCR in

March to June of 2020, further limiting the utility of this comparison. ELISA assays have been in general use longer than the ADAP assay; however, both were validated using samples from participants with PCR-confirmed COVID-19 with high sensitivity and specificity [16, 17]. Of note, the ADAP assay validation used outpatient samples [15], while the ELISA used waste samples from a hospital system [16]. The ADAP and ELISA techniques have not otherwise been compared directly, and neither is known to be superior. Further work is needed to establish a clear diagnostic gold standard, and the current lack thereof remains a limitation in interpreting our results.

DBS may alter the sensitivity of the COVID-19 antibody test as there is a large amount of debris that can interfere with the signal, although DBS-based methods have been validated for similar use cases [15]. Other studies using DBS samples showed a high seroprevalence of COVID-19 [29], and our seropositivity is similar to other studies among high-risk health care workers [27, 28], which suggests that the difference is not from assay variations resulting from using DBS. Our lower prevalence is more likely because of low disease severity or asymptomatic carriage, variable time from disease, and DBS collection issues [26].

Finally, we found that that a remote lab-based testing program with DBS was feasible, although challenging. Pragmatic needs like rapid testing across the country must be weighed against quality control issues. While the kits utilized in our study were suitable, Mitra Specimen Collection Kits have been used for some pharmacokinetic studies; although more expensive, they require minimal labor to assemble and may be a more appropriate option when considering future remote serologic testing.

Limitations

We enrolled the parent studies early in the pandemic via internet-based randomized controlled trials. As such, our testing was performed using self-collected DBS, and there was no validation with fresh plasma samples given the constraints of the pandemic at the time and the nature of our trials. The ADAP assay is a relatively new technology, and the assay only targeted the spike protein, which may underrepresent the seroprevalence. However, unlike other tests, the developers of the ADAP assay in outpatients. At the time, the FDA had no regulation regarding antibody tests. All symptoms and PCR results were self-reported, and while we have confidence in our sample of mostly health care workers, we cannot independently verify their results.

CONCLUSIONS

We tested COVID-19 antibodies using DBS from 931 clinical trial participants with PCR-confirmed COVID-19 or COVID-19-compatible symptoms, in addition to individuals at high risk for

SARS-CoV-2 infection, during the first wave of the pandemic. We found that SARS-CoV-2 seropositivity is correlated with symptom severity, with increasing seropositivity with increasing number and severity of symptoms. The percent seropositivity is lower than prior inpatient studies but consistent with previous outpatient studies.

DBS sampling is potentially a feasible method to perform remote lab testing in internet-based clinical trials. However, further work is needed in the serologic testing field to establish a gold standard and test outpatient samples. New assays specific to outpatients or those with asymptomatic carriage would be helpful for future work.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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