Antibody Profiles According to Mild or Severe SARS-CoV-2 Infection, Atlanta, Georgia, USA, 2020

William T. Hu, J. Christina Howell, Tugba Ozturk, Karima Benameur, Leda C. Bassit, Richard Ramonell, Kevin S. Cashman, Shama Pirmohammed, John D. Roback, Vincent C. Marconi, Irene Yang, Valerie V. Mac, Daniel Smith, Ignacio Sanz, Whitney Wharton, F. Eun-Hyung Lee, Raymond F. Schinazi

Among patients with coronavirus disease (COVID-19), IgM levels increased early after symptom onset for those with mild and severe disease, but IgG levels increased early only in those with severe disease. A similar pattern was observed in a separate serosurveillance cohort. Mild COVID-19 should be investigated separately from severe COVID-19.

Coronavirus disease (COVID-19) emerged in December 2019 (*1*,*2*), and by June 2020, ≈10 million persons worldwide had acquired the disease. The confirmatory test for severe acute respiratory syndrome virus 2 (SARS-CoV-2) infection remains real-time reverse transcription PCR, but this test poses challenges in terms of sensitivity (*3*), reagent or equipment availability, and specialized personnel training. Serologic assays can be readily performed in most clinical laboratories, with faster turnaround times, but their association with COVID-19 has largely been reported for hospitalized patients with severe disease (*4*; E. Adams et al., unpub. data, https://www.medrxiv.org/content/10.1101/2020.04.15.20066407v1.full.pdf). Whether mild and severe COVID-19 represent 2 interlinked stages on a severity continuum or 2 distinct phenotypes of an infectious process (*5*) remains incompletely understood; detailed cross-sectional characterization of IgM and IgG reactive against SARS-CoV-2 antigens may provide insight into the temporal evolution of antibodies. Detection of cross-reactive antibodies from a pre-2020 cohort can also indicate whether past exposure to other coronaviruses is associated with cross-reactive protection against SARS-CoV-2.

In addition to IgG targeting the receptor-binding domain (RBD) of the spike protein subunit S1

(*6*), we developed and validated an IgM assay targeting the full-length S1 protein. We further developed and validated an IgM assay targeting the small full-length envelope (E) protein, which is highly shared between SARS-CoV and SARS-CoV-2 (*2*), is accessible on the surface, and increases during virus replication (*7*). Using these assays, we characterized the IgM and IgG profiles of participants with COVID-19, pre-2020 control participants, and a community cohort of 116 persons who had recovered from self-limited illness during March and April 2020 in Atlanta, Georgia, USA.

The Study

We recruited 28 participants hospitalized for severe COVID-19 (20 requiring artificial ventilation; samples collected during hospitalization a median of 15.5 days after symptom onset) and 15 participants who had recently recovered from mild COVID-19 (samples collected a median of 15 days after symptom onset; Table 1). Compared with hospitalized participants, participants with mild illness were less likely to be African American (*8*) and more likely to be younger and to have nasal congestion or anosmia.

Compared with control participants, hospitalized participants had higher levels of IgG against S1-RBD ($\log_{10}$ transformed because of nonnormal distribution; Student *t* [56.7] = 12.183; p<0.0001; Figure 1, panel A), IgM against S1 (Student *t* [33.29] = 3.713; p<0.001; Figure 1, panel B), and IgM against E (*t* [129] = 2.279; p = 0.024; Figure 1, panel C). The same was true among participants with mild illness for IgG against S1-RBD (Student *t* [116] = 4.246; p<0.0001; Figure 1, panel A), IgM against S1 (Student *t* [116] = 6.764; p<0.0001; Figure 1, panel B), and IgM against E (Student *t* [116] = 3.398; p = 0.001; Figure 1, panel C). However, an IgG diagnostic

Table 1. Demographic and other information for persons with known coronavirus disease, pre-2020 controls, and persons with influenza-like illness but negative for SARS-CoV-2, Atlanta, Georgia, USA, 2020*

Characteristic	Hospitalized, n = 28	Mild disease, n = 15	Pre-2020 control, n = 103	p value
Sex, %				0.273
F	14 (50)	7 (47)	65 (63)	
M	14 (50)	8 (53)	38 (37)	
Median age, y (range)	61.5 (29–85)†	32 (26–81)†	62.5 (24–87)	<0.0001
Race, no. (%)				<0.0001
Asian	3 (11)	0	2 (2)	
African American	18 (64)†	1 (7)†	15 (14)	
Non-Hispanic Caucasian	6 (21)	12 (80)	82 (80)	
Hispanic	1 (4)	1 (7)	0	
Other	0	1 (7)	4 (4)	
Clinical features				
Inpatient/outpatient	28/0	1/14	NA	<0.0001
Respiratory failure requiring intubation, %	20 (71)†	0†	NA	<0.0001
Median days since symptom onset (range)	15.5 (4–42)	15 (9–33)	NA	0.427
Clinical signs/symptoms				
Cough	22 (79)	10 (67)	NA	0.473
Fever/chills	22 (79)	9 (64)	NA	0.287
Shortness of breath	20 (71)	5 (33)	NA	0.024
Myalgia	7 (25)	9 (60)	NA	0.045
Headaches	7 (25)	7 (47)	NA	0.184
Sore throat	5 (18)	6 (40)	NA	0.150
Nasal congestion/rhinorrhea	2 (7)	8 (53)	NA	0.001
Diarrhea	5 (18)	3 (20)	NA	1.000
Anosmia	1 (4)	7 (47)	NA	0.001
Fatigue	3 (11)	3 (20)	NA	0.647
Vomiting	0	1 (7)	NA	0.349
Never symptomatic	0	0	NA	0.012
Laboratory features				
SARS-CoV-2 detected by rRT-PCR	28/28	10/10	NA	<0.0001
Mean anti-S1-RBD IgG (± SD), OD	1.72 (0.72)†	0.71 (0.60)†	0.26 (0.36)	<0.0001
Mean anti-S1 IgM (± SD), OD	1.76 (0.74)	2.12 (0.53)	1.21 (0.48)	<0.0001
Mean anti-E IgM (± SD), OD	1.85 (0.90)	2.16 (0.72)	1.48 (0.71)	0.001

*E, envelope protein; NA, not applicable; OD, optical density, RBD, receptor-binding domain; rRT-PCR, real-time reverse transcription PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S1, spike protein subunit S1.
†Different between patients who were hospitalized and who had mild disease at p<0.005.

threshold of 0.82 optical density (OD) (Appendix, https://wwwnc.cdc.gov/EID/article/26/12/20-3334-App1.pdf) from the hospitalized participants identified only 4 (26.7%) of 15 participants with mild disease because of the lower IgG levels early after symptom onset in the group with mild disease. Elevated IgG only weeks after symptom onset among participants with mild COVID-19 is consistent with prior reports (9; E. Adams et al., unpub. data, https://www.medrxiv.org/content/10.1101/2020.04.15.20066407v1.full.pdf), and linear regression analysis projected that their IgG would reach the threshold of hospitalized participants an average of 29 days after symptom onset.

Conversely, IgM negatively correlated with time since symptom onset for hospitalized participants but not for those with mild disease. An anti-S1 IgM level of 1.60 OD from hospitalized patients during the first 21 days—before significant IgM decline—and 50-fold randomly selected control participants showed sensitivity of 81.0% and median specificity of 80.4% (range 76%–85.5%). The threshold of 1.60

OD was in range with values derived from pre-adsorption experiments that used S1 antigen (1.75 OD; Appendix) and identified participants with mild disease with sensitivity of 80.0% and median specificity of 80.5% (range 80%–86.7%). Anti-E IgM levels showed similar associations with time from symptom onset and severity but did not increase identification of COVID-19 participants.

Because many persons with mild influenza-like (ILI) symptoms in the metropolitan Atlanta area did not or could not access SARS-CoV-2 testing during early 2020, we also analyzed antibody levels in 116 adults who had recovered from self-limited ILI symptoms (Table 2). Compared with participants with mild COVID-19, this cohort was less likely to have anosmia (11% vs. 47%; p = 0.002) or fatigue (4% vs. 20%; p = 0.048) but was otherwise similar in terms of sex, race, age, and signs/symptoms. Of 31 participants with symptom onset 7–29 days before blood collection, 1 (3%) had elevated IgG, and 11 (12.9%) of 85 with symptom onset 30–60 days before participation had elevated IgG. None of the clinical signs/symptoms

strongly predicted antibody levels. A liberal threshold of anti-S1 IgM $\geq$1.60 OD identified 18/31 (58%) and 57/85 (67%) participants, and a more stringent threshold of 2.00 OD to reduce false positives identified 7/31(22%) and 41/85 (48%) participants.

Last, we performed plaque-reduction neutralization assays (PRNT; Appendix) for a subgroup of participants with confirmed or probable COVID-19 and pre-2020 control participants (75% with elevated antibody levels; Figure 2, panel A). All 6 hospitalized participants and 5 participants with mild

disease (2 weak neutralizing results $\leq$1:40) demonstrated >90% plaque reduction in Vero cells compared with 2 of 15 control participants who also showed weak neutralization. Using positive PRNT at >1:40 as a specific threshold, we found simultaneously elevated IgM and IgG most predictive of positive PRNT (p = 0.008 compared with IgM alone, p = 0.07 compared with IgG alone; Appendix), although plasma from 1 hospitalized participant with neutralizing plasma had reference IgM and IgG levels. PRNT for community participants
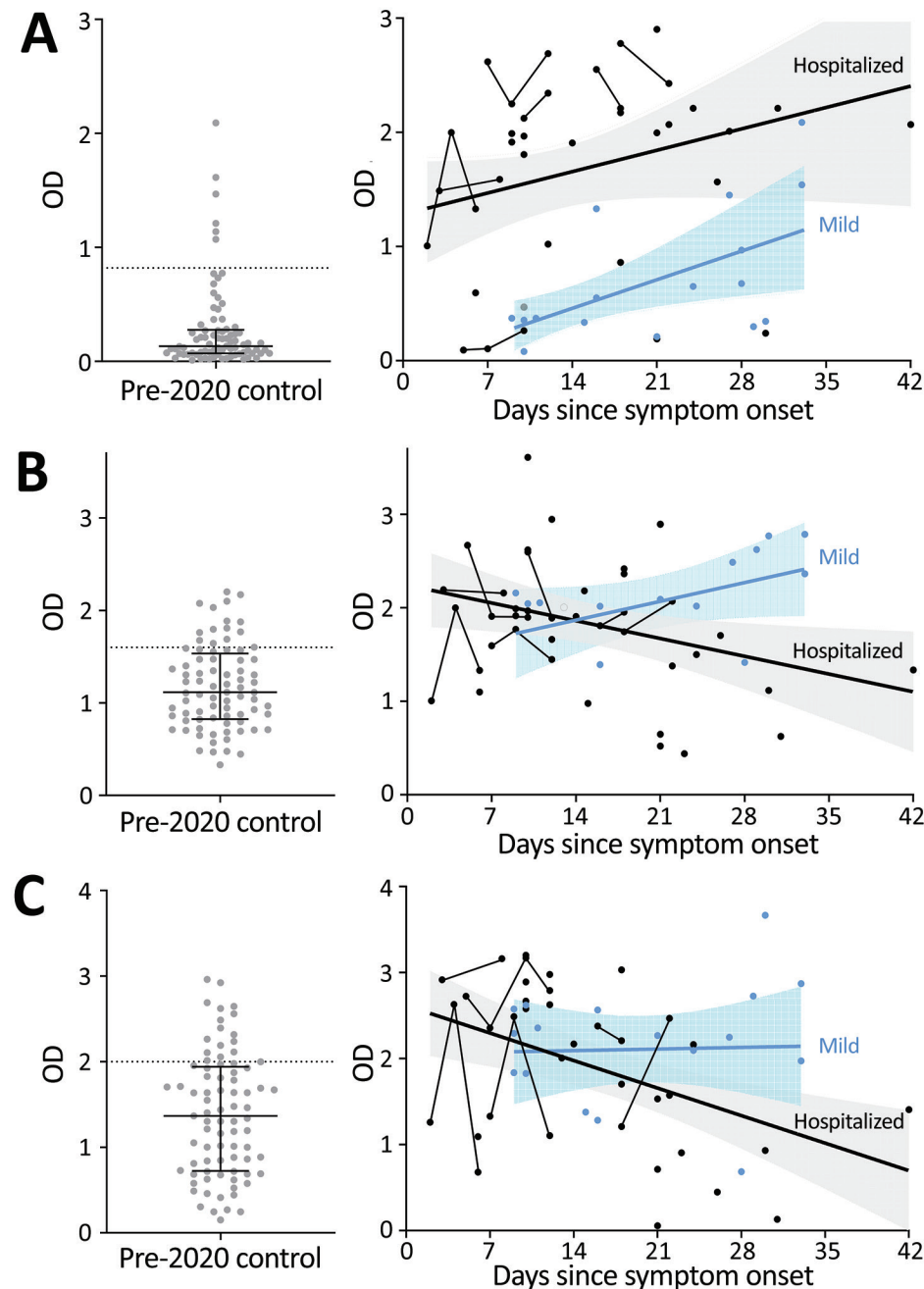

Figure 1. Serologic assay results for study participants with coronavirus disease (COVID-19), Atlanta, Georgia, USA, 2020. Levels of IgG against the receptor-binding domain (RBD) of the spike protein subunit S1 (A), IgM against S1 (B), and IgM against envelope protein (C) were analyzed for hospitalized patients with severe COVID-19 (black circles) and patients who had recovered from mild COVID-19 (blue circles) according to time from symptom onset. Levels in pre-2020 HC participants (gray circles) are shown for comparison; dotted lines represent optimal threshold levels for receiver operating characteristic curve analysis. Best fit lines for relationships between time since symptom onset and antibody levels were calculated separately for hospitalized participants and participants with mild COVID-19. OD, optical density.

Table 2. Demographic and other information for a prospective cohort who recovered from an influenza-like illness, Atlanta, Georgia, USA, 2020

Characteristic	IgG <0.82, IgM <2.00, n = 60	IgG <0.82, IgM ≥2.00, n = 44	IgG ≥0.82, IgM <2.00, n = 8	IgG ≥0.82, IgM ≥2.00, n = 4	p value
Symptom onset, no. (%)					0.029
7–29 d earlier	23 (38)	7 (16)	1 (12)	0	
30–60 d earlier	37 (62)	37 (84)	7 (88)	4 (100)	
Sex, no. (%)					0.042
F	29 (48)	33 (75)	4 (50)	3 (75)	
M	31 (52)	11 (25)	4 (50)	1 (25)	
Median age, y (range)	45.5 (19.4–73.7)	34.9 (25.9–73.3)	43.6 (31.7–62.3)	37.3 (33.5–48.2)	0.113
Non-Hispanic Caucasian	47 (78)	36 (82)	6 (75)	4 (100)	0.715
Healthcare worker	35 (58)	27 (61)	4 (50)	2 (50)	0.918
Potential exposure to coronavirus disease	37 (62)	22 (50)	5 (62)	2 (50)	0.662
Never smoker	51 (85)	38 (86)	7 (88)	2 (50)	0.119
Clinical signs/symptoms					
Cough	38 (63)	37 (84)	2 (25)	4 (100)	0.002
Fever/chills	21 (35)	21 (48)	2 (25)	3 (75)	0.214
Shortness of breath	21 (35)	13 (29)	1 (12)	3 (75)	0.166
Myalgia	34 (57)	21 (48)	4 (50)	4 (100)	0.228
Headaches	38 (63)	21 (48)	3 (37)	3 (75)	0.238
Sore throat	27 (45)	25 (57)	3 (37)	4 (100)	0.117
Nasal congestion/rhinorrhea	37 (62)	30 (68)	3 (37)	0	0.029
Diarrhea	11 (18)	13 (29)	2 (25)	2 (50)	0.352
Anosmia	6 (10)	5 (11)	0	2 (50)	0.067
Fatigue	1 (2)	4 (9)	0	0	0.262
Vomiting	2 (3)	3 (7)	0	0	0.717

with the 10 most elevated IgG levels showed a similar trend (Figure 2, panel B).

Conclusions

IgM reactive toward S1 and E proteins increased early regardless of disease severity, but IgG increased early only in hospitalized participants with severe COVID-19. This pattern was observed in a separate cohort of community participants who had recovered from self-limited ILI. Positive PRNT—a surrogate for antibody-mediated immune protection—may be better associated with elevated IgM and IgG than either antibody alone.

A diagnostic algorithm of IgG from hospitalized participants performed poorly for detection of mild COVID-19. Similarly, other studies found delayed or low-to-medium neutralizing antibody titers in persons who recovered from mild COVID-19 (E. Adams et al., unpub. data, https://www.medrxiv.org/content/10.

Figure 2. Severe acute respiratory syndrome coronavirus 2 virus neutralization measures according to anti-S1-RBD IgG and anti-S1 IgM levels, Atlanta, Georgia, USA, 2020. Open circles represent negative plaque-reduction neutralization test (PRNT) result, and solid circles represent positive PRNT result (sizes of filled circles are proportional to maximal dilution with positive PRNT result). Dotted lines indicate threshold values. A) Among participants with coronavirus disease (COVID-19) (mild disease and hospitalized), pre-2020 controls with elevated antibody levels, and pre-2020 controls with normal antibody levels, positive PRNT results were most associated with simultaneously elevated IgM and IgG levels (Appendix, https://wwwnc.cdc.gov/EID/article/26/12/20-3334-App1.pdf). B) Analysis of a group of 116 persons who reported recovery from self-limited illness 7–60 days prior showed a similar trend. ND, not done; RBD, receptor-binding domain; S1, spike protein subunit 1.
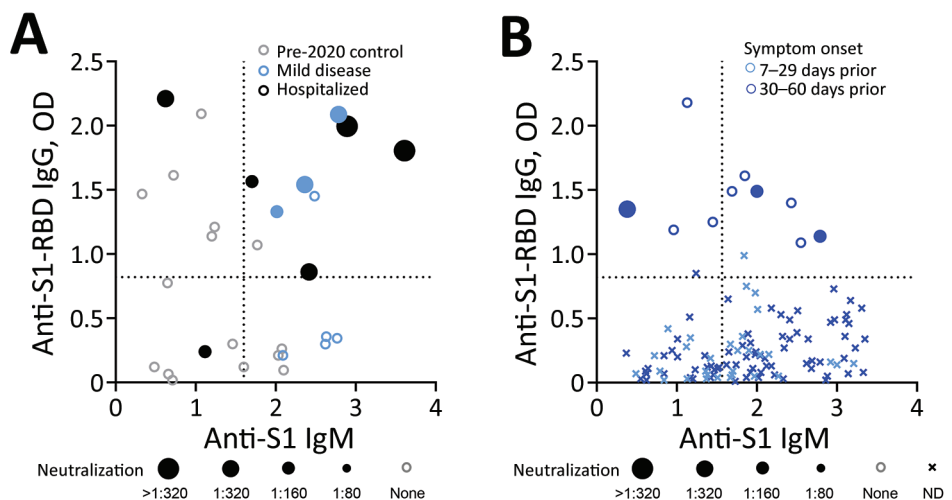
1101/2020.04.15.20066407v1.full.pdf; F. Wu et al., unpub. data, https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2). The delayed increase in IgG and neutralizing antibodies in persons with mild COVID-19 also suggests that mild cases do not necessarily represent an intermediate stage between severe and asymptomatic COVID-19. A corollary of slow IgG increases in persons with mild COVID-19 may be longer persistence of IgM, but more definitive characterization of IgM+ memory B cells (*10*) and long-term decay of antibody levels (*11*) is needed.

Our study has limitations. Our small cross-sectional cohort of patients with well-characterized and laboratory-confirmed COVID-19 limits generalization. The overrepresentation of African Americans in the more severely ill cohort may mediate some differences in antibody profiles (*8*), and we did not measure IgA levels or antibodies targeting other SARS-CoV-2 gene products (currently under development and validation). We also did not measure antibody levels in historic SARS or MERS case-patients, and cross-reactive antibody response against homologous regions cannot be ruled out.

We did confirm a complex relationship between antibody levels, disease severity, and time since symptom onset. Examining IgM and IgG against multiple SARS-CoV-2–related antigens may thus better inform natural history and vaccine studies than any one antibody.

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About the Author

Dr. Hu is a physician-scientist at Emory University in Atlanta, GA. His research interests involve reliable fluid biomarkers for human diseases related to inflammation.

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Address for correspondence: William T. Hu, Neurology, Emory University, 615 Michael St, 505F, Atlanta, GA 30322; email: wthu@emory.edu

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Appendix

Materials and Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by Emory University Institutional Review Board. Written consents were obtained from all participants or their legally authorized representatives (when appropriate). Sample size was calculated based on one previous study (*1*) when the current study began using a more conservative effect size (0.8 vs. >1), with an estimated disease prevalence of 5%-20%. Plasma was collected from 15 hospitalized participants, ten mild participants, and all pre-2020 HC and those with negative rRT-PCR, while serum was collected from the remaining 13 hospitalized participants and five mild participants.

Study Participants

All hospitalized participants had rRT-PCR confirmation. Mild participants were recruited up until 35 days (inclusive) following symptom onset. Among the mild participants, ten had rRT-PCR confirmation, four developed influenza-like illness (ILI) following direct contact with confirmed COVID-19 cases but were not eligible for rRT-PCR, and one developed ILI following direct contact with confirmed COVID-19 cases but did not seek rRT-PCR. Mild participants did not differ in antibody levels or time since symptom onset according to rRT-PCR result availability, and excluding them from the cross-sectional analysis of antibody level vs. time did not change the time-associated trends. Furthermore, two of four without rRT-PCR confirmation (vs. one of four with rRT-PCR confirmation) had positive neutralizing antibodies (see below and results). Therefore, the five participants without rRT-PCR confirmation were thus included in analysis. Pre-2020 HC participants (n=103) were recruited through inflammation studies targeting the young (PI: WTH),(*2*) middle-aged (PI: WW),(*3*) or older (PI: WTH) healthy adults.

A large cohort of people who recovered from self-limited ILI but did not seek rRT-PCR confirmation were prospectively recruited as part of a larger baseline serological surveillance study conducted at Emory University during April and May 2020. Participants were screened for temperature and COVID-19-related symptoms to ensure they were asymptomatic at time of blood collection, and asked to recall if they had a standardized list of any influenza- or COVID-19-like symptoms in the prior four months. Dates of symptom onset and resolution were also recorded, along with information on if they were healthcare workers and if they were exposed to someone with confirmed COVID-19. Participants who recorded any symptoms as well as onset within the past 7-60 days (116/369, 31%) were included in the current study.

Specimen Collection

For subjects with plasma (n=244), blood was collected in K_2EDTA tubes and placed on ice immediately. Blood samples were centrifuged at 4°C and 2500 g for 10 minutes, and plasma was removed, aliquoted, labeled, and frozen immediately at -80°C until analysis. For subjects with serum (n=28), blood was collected in silicone-coated tubes and allowed to clot in the upright position at room temperature for 30 minutes. After centrifugation at 4°C and 2500 g for 10 minutes, serum was removed, aliquoted, labeled, and frozen immediately at -80°C until analysis

IgG Serological Assay

A commercial anti-S1 receptor binding domain (RBD) IgG indirect ELISA assay (GenScript, Piscataway, NJ) was purchased and performed per manufacturer's protocol, except two plasma dilutions (1:16 and 1:64) were selected from a range of 1:8 – 1:256 performed in a subgroup of COVID-19 and pre-2020 HC participants. To derive an empirical threshold for COVID-19, receiver operating characteristics (ROC) curve analysis using 18 hospitalized COVID-19 participants (all with rRT-PCR confirmation) and 75 pre-2020 HC participant showed area under the curve (AUC) of 0.941 (95% CI 0.891-0.990). None of the pre-2020 HC participants were tested for IgG against SARS-CoV or other coronaviruses.

Development and Validation of IgM Serological Assays

To detect IgM targeting S1 and E proteins, we developed two novel assays based on our prior experience in modifying ELISA and multiplex immunoassays to measure targets of low abundance. Synthetic SARS-CoV-2 S1 (230-01101-100, produced from *E. coli*) and SARS-

related E (228-11400-2, produced from *E. coli*) peptides were purchased from RayBiotech (Peachtree Corners, GA). 100 µL of 2.5 µg/mL antigen in PBS with 2% non-fat dried milk (nfdm) and 0.1% Tween was applied to standard 96-well plate at 4°C overnight. Alternate diluents (including sodium bicarbonate buffer, TBS, PBS with 1% nfdm, PBS with 2% albumin) and concentrations (1 µg/mL, 4 µµ/mL) resulted in insufficient sensitivity or greater non-specific binding. During assay development, multiple plasma dilutions (1:2, 1:8, 1:32…1:1,024), blocking conditions (PBS with 0.1%, 1%, 5% albumin, 8% casein, or 4% nfdm, or PBS with 4% nfdm and 0.1% Tween), and detecting antibody mix (donkey- vs. goat-derived; PBS, PBS with 1-3% nfdm with or without Tween) were tested to derive the final protocol. Following overnight coating, ELISA plates were washed three times with PBS and blocked with PBS with 4% nfdm before 50 µL of 1:20,000 affinity-purified goat anti-human IgM fc (109-035-043, Jackson ImmunoResearch Laboratories, West Grove, PA; in PBS) was added to each dilution condition for 30 min. Wells were then washed again, treated with strepavidin-HRP (1:200, 50 µL per well) for 20 min in the dark, washed, incubated with substrate mix for 20 min in the dark, and treated with reaction stop solution. Plates were then read at 450 nm (Molecular Devices, SpectraMax-M2) followed by background (570 nm) subtraction to derive relative units (in optical density [O.D.]). We did not include an IgG-removal (from plasma) process because the affinity-purified anti-human IgM Fc antibody does not react to human IgG in our hands (using humanized monoclonal antibody standards).

To demonstrate assay specificity in the finalized protocol, we pre-adsorbed plasma from RT-PCR confirmed COVID-19 and pre-2020 HC subjects using soluble S1 or E protein (corresponding to each assay). In pre-adsorption experiments, antigen-specific antibodies present in the plasma would be bound to soluble antigens of markedly greater concentration, and remaining antibodies which bind to the plated antigens then bind either 1) non-specifically, 2) preferentially to post-translationally modified native protein, or 3) to antigen in a non-soluble conformational state. For each tested sample, we calculated O.D. difference between O.D. with and without antigen pre-adsorption (Appendix Figure 1). This showed linear relationships between antibody levels associated with plated antigen (X-axis) and soluble antigen (Y-axis) when the former exceeds 1.75 O.D. in S1 (R^2=0.789) and 2.01 O.D. (R^2=0.443) in E. Because antigen (S1 or E) binding to ELISA plates requires a hydrophobic reaction between *synthetic protein* and the plastic surface (sometimes facilitated by the use of sodium bicarbonate coating

buffer) which may alter the target antigen's conformation, we do not consider detected antibody levels below these two thresholds as *exclusively* non-specific. For the purpose of this descriptive study, we therefore elected to report the total measured antibody levels. We did not have access to stored blood samples from patients with SARS-CoV, MERS, or other coronavirus infections to determine if they have elevated IgM levels beyond the empirically determined thresholds. While IgM levels normalized within weeks to months after infection in the 2003 SARS outbreak (*4,5*) and no pre-2020 participant from our studies had reported prior MERS infection, we do not exclude the possibility that even low degree homology between SARS-CoV-2 and other coronaviruses could have contributed to the artificially high O.D. from pre-2020 HC participants.

Viral Neutralization Assays

Viral neutralization was determined by plaque reduction neutralization test (PRNT) using enzyme-linked immunospot as a read out. Serially diluted (1:20 – 1:2560) plasma was incubated with SARS-CoV-2 USA-WA1 ($\approx$70 foci/well) for 1 hour at 37°C, along with negative and positive (convalescent serum, BEI Resources, Manassas, VA) control samples. Confluent Vero cells were subsequently incubated with the virus-plasma mix for 1 hr, followed by addition of overlay media (Opti-MEM, 2% FBS, 2.5 μg/mL amphotericin B, 20 μg/mL Ciprofloxacin, 2% methylcellulose) and incubation for three days. PRNT was then performed using monoclonal anti-SARS recombinant IgG1 (NR-52392, BEI) as a positive control, with >90% reduction considered as a positive response in this study. All assays were performed by experienced scientists blinded to diagnosis.

Statistical Analyses

All statistical analyses were performed using SPSS 26 (IBM SPSS, Armonk, NY) except curve-fitting was performed in Prism 8.4.3 (GraphPad Software, San Diego, CA). Chi-squared or Fisher's exact test was used to analyze differences in categorical variables and Student's T-tests were used to analyze differences in continuous variables between hospitalized and mild COVID-19 participants. Only non-HC participants were analyzed for differences in clinical symptoms. Anti-S1-RBD IgG levels were $\log_{10}$-transformed before analysis due to its non-normal distribution. Given the expected effect sizes, Bonferroni correction was used to adjust for multiple comparisons.

For each antibody, linear regression between days after symptom onset and antibody levels was compared against other higher order models (second- or third-order polynomial, and exponential growth for anti-S1-SBD IgG in recovered cases) based on Akaike Information Criteria. Linear functions provided better fit than more complex models for all three antibodies.

Receiver-operating characteristic (ROC) curve analysis for IgM was trained on hospitalized COVID-19 participants up to 21 days after symptom onset and 75 randomly selected pre-2020 HC participants 50 times to derive antibody thresholds. These thresholds were then tested in mild COVID-19 participants and the remaining pre-2020 HC participants to report median sensitivity and specificity. One example is shown in Appendix Figure 2.

To determine the best predictors of positive PRNT results, we analyze elevated anti-S1 IgM, elevated anti-S1-RBD IgG, and elevated levels of both relative to PRNT outcomes in eight participants with mild COVID-19, six participants hospitalized for severe COVID-19, and 16 pre-2020 HC participants (11 with elevated anti-S1 IgM or anti-S1-RBD IgG). Sensitivity and specificity improvement was determined by McNemar's χ^2 Test using positive (for sensitivity) or negative (specificity) PRNT cases (Appendix Table) (6).

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Appendix Table. Relationship between anti-S1 IgM, anti-S1-RBD IgG, and PRNT results in mild and hospitalized COVID-19 participants (greater than 90% plaque reduction at >1:40 dilution was considered a true positive response)

Variable	Elevated anti-S1 IgM (> 1.60 O.D.)	Elevated Anti-S1-RBD-IgG (> 0.82 O.D.)	Elevated anti-S1 IgM & anti-S1-RBD IgG
(+)PRNT	7/9	7/9	7/9
(-) PRNT	11/21	7/21	2/21
Sensitivity for (+)PRNT	77.8%	77.8%	77.8%
Specificity for (+)PRNT	47.6%	66.7%	90.5%*
Accuracy for PRNT	56.7%	70.0%	86.7%

*p=0.008 compared to IgM only and p=0.07 compared to IgG only by McNemar's χ^2 test.
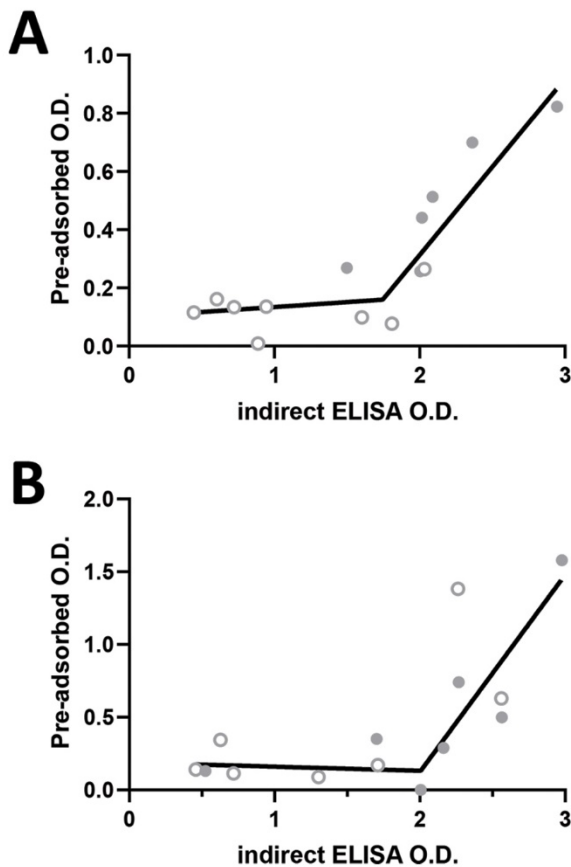
Appendix Figure 1. Correlation between directly measured antibody levels and calculated pre-adsorbed antibody levels using soluble S1 and E proteins.
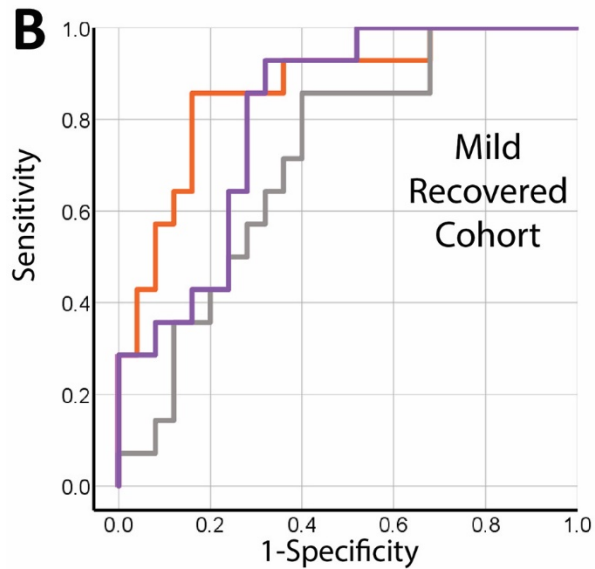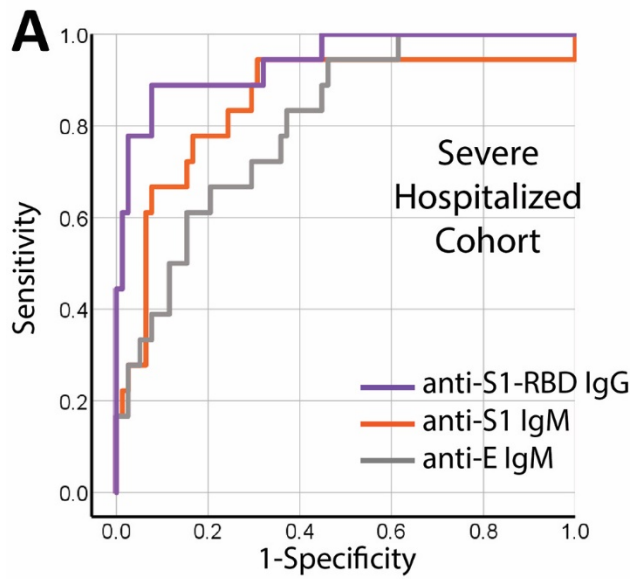
Appendix Figure 2. Example of ROC curve analysis using the three serological assays to distinguish severe hospitalized (A) and mild recovered (B) COVID-19 participants from randomly selected HC participants. This process was repeated 50 times to generate median O.D. for anti-S1 IgM and specificity.