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RESEARCH ARTICLE

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Clinical sensitivity and interpretation of PCR and serological COVID-19 diagnostics for patients presenting to the hospital

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Abstract

The diagnosis of COVID-19 requires integration of clinical and laboratory data. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostic assays play a central role in diagnosis and have fixed technical performance metrics. Interpretation becomes challenging because the clinical sensitivity changes as the virus clears and the immune response emerges. Our goal was to examine the clinical sensitivity of two most common SARS-CoV-2 diagnostic test modalities, polymerase chain reaction (PCR) and serology, over the disease course to provide insight into their clinical interpretation in patients presenting to the hospital. We conducted a single-center, retrospective study. To derive clinical sensitivity of PCR, we identified 209 PCR-positive SARS-CoV-2 patients with multiple PCR test results (624 total PCR tests) and calculated daily sensitivity from date of symptom onset or first positive test. Clinical sensitivity of PCR decreased with days post symptom onset with >90% clinical sensitivity during the first 5 days after symptom onset, 70%-71% from Days 9 to 11, and 30% at Day 21. To calculate daily clinical sensitivity by serology, we utilized 157 PCR-positive patients with a total of 197 specimens tested by enzyme-linked immunosorbent assay for IgM, IgG, and IgA anti-SARS-CoV-2 antibodies. In contrast to PCR, serological sensitivity increased with days post symptom onset with >50% of patients seropositive by at least one antibody isotype after Day 7, >80% after Day 12, and 100% by Day 21. Taken together, PCR and serology are complimentary modalities that require time-dependent interpretation.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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Superimposition of sensitivities over time indicate that serology can function as a reliable diagnostic aid indicating recent or prior infection.

KEYWORDS
biomarker, COVID, qPCR, SARS-CoV-2

1 | INTRODUCTION

While many measures to mitigate the multifactorial impact of COVID-19 are being implemented, one critical component of this strategy is the widespread testing and identification of individuals currently or previously infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The delivery of effective care and mitigation of infection depend on the performance of SARS-CoV-2 diagnostic testing and the clinical interpretation of results. The lack of a full understanding of the natural history and immunopathogenesis of COVID-19 infection creates unique challenges in the implementation of diagnostic testing strategies. SARS-CoV-2 diagnostic assays have fixed technical performance metrics (eg, sensitivity and specificity). Clinical sensitivity depends on more than technical performance and is also a function of pre-analytical variables and the disease state of the patient. Interpretation becomes challenging because the clinical sensitivity changes as the virus clears and the immune response emerges.

The goal of this study is to examine the clinical sensitivity and provide insights into the interpretation of the two most common SARS-CoV-2 diagnostic test modalities: polymerase chain reaction (PCR) and serology. Laboratory-based diagnosis of active SARS-CoV-2 infection relies on the direct detection of virus-specific nucleic acids, most commonly obtained from the nasopharynx of infected patients. Indirect markers of infection include the detection of SARS-CoV-2 specific antibodies, generated as part of the human immune response to the virus. Serologic testing holds promise as a blood-based diagnostic aid, as a marker of viral exposure, and potentially as an indicator of protective immunity. Understanding the presence of these biomarker in relationship to one another over the natural course of infection is required to effectively utilize these available diagnostic tests in clinical practice.[1-3]

Here, we share our experience of SARS-CoV-2 PCR sensitivity and separately obtained IgM, IgA, and IgG sensitivity of an in-house enzyme-linked immunosorbent assay (ELISA) during the natural course of disease in a cohort of patients presenting to the hospital.

2 | METHODS

2.1 | Setting and design

The project was conducted within the clinical laboratories of the Massachusetts General Hospital (MGH), a Clinical Laboratory Improvement Amendments-certified laboratory. The study was designed as a single-center, retrospective review of PCR results and serology data. PCR results were obtained between 3 March 2020 and 15 April 2020 and we superimposed serology data obtained from confirmed COVID-19 positive patients as part of ongoing clinical validation studies of an ELISA for regulatory approval. The study was conducted with approval from the Mass General Brigham Institutional Review Board. We also used previously published data as a comparison dataset (Wölfel et al[4]).

2.2 | PCR

Nucleic acid testing was performed as part of clinical care at MGH using three real-time PCR assays, each of which received EUA by the FDA. Our laboratory-developed real-time PCR assay uses the Centers for Disease Control and Prevention (CDC) primers targeting regions of the N gene of SARS-CoV-2, the cobas SARS-CoV-2 Test performed on the cobas 6800 (Roche) targets regions of the ORF1a and E genes, and the Xpert Xpress SARS-CoV-2 assay run on the GeneXpert Infinity (Cepheid) targets regions of the N and E genes. Choice of which testing platform to use was determined by access to reagents available at the time of clinical testing provided for patient care. Our laboratory-developed assay was validated to detect SARS-CoV-2 at or above 5 copies/μL with 100% technical sensitivity and specificity. For commercial assays, we internally validated the assays and found 100% technical sensitivity and specificity. Within our validation cohort of known positive patients, we found 100% concordance between all three platforms. Despite excellent (technical) performance characteristics, pre-analytical factors may decrease the performance of viral detection. These factors may include timing during the course of infection, improper sampling, specimen handling and others.

2.3 | Serology

An in-house ELISA developed by Massachusetts General Hospital (Boston, MA) and the Ragon Institute of MGH, MIT, and Harvard (Cambridge, MA), was used to measure IgG, IgA, and IgM antibodies that target the SARS-CoV-2 receptor binding domain (RBD) within the spike protein. The optical density (OD) was read at 450 and 570 nm on a plate reader. OD values were adjusted by subtracting the

TABLE 1 Demographics of cohorts used in analysis

	PCR total	PCR-positive	PCR multiple tests	Serology
	n (%)	n (%)	n (%)	n (%)
Patients	11 698	3163 (27)	209	157
Age				
Median	46	47	46*	57*
Average	47.0	48.0	48.6	57
Range	0 to 102	0 to 102	21 to 93	22 to 98
Gender				
Female	6411 (55)	1584 (25)	110 (53)**	55 (35)**
Male	5270 (45)	1576 (30)	99 (47)	102 (65)
Other	22 (0.2)	3 (14)	0	0

Note: PCR-positive is the SARS-CoV-2 PCR-positive subset of the all tested patients (PCR Total). PCR Multiple Tests is a cohort of patients with multiple PCR test results (and at least one positive result) used for clinical sensitivity analysis shown in Figure 1. Serology is an independent subset of PCR-positive patients.

* $P < .01$ from student t test;

** $P < .01$ from Fisher's exact test.

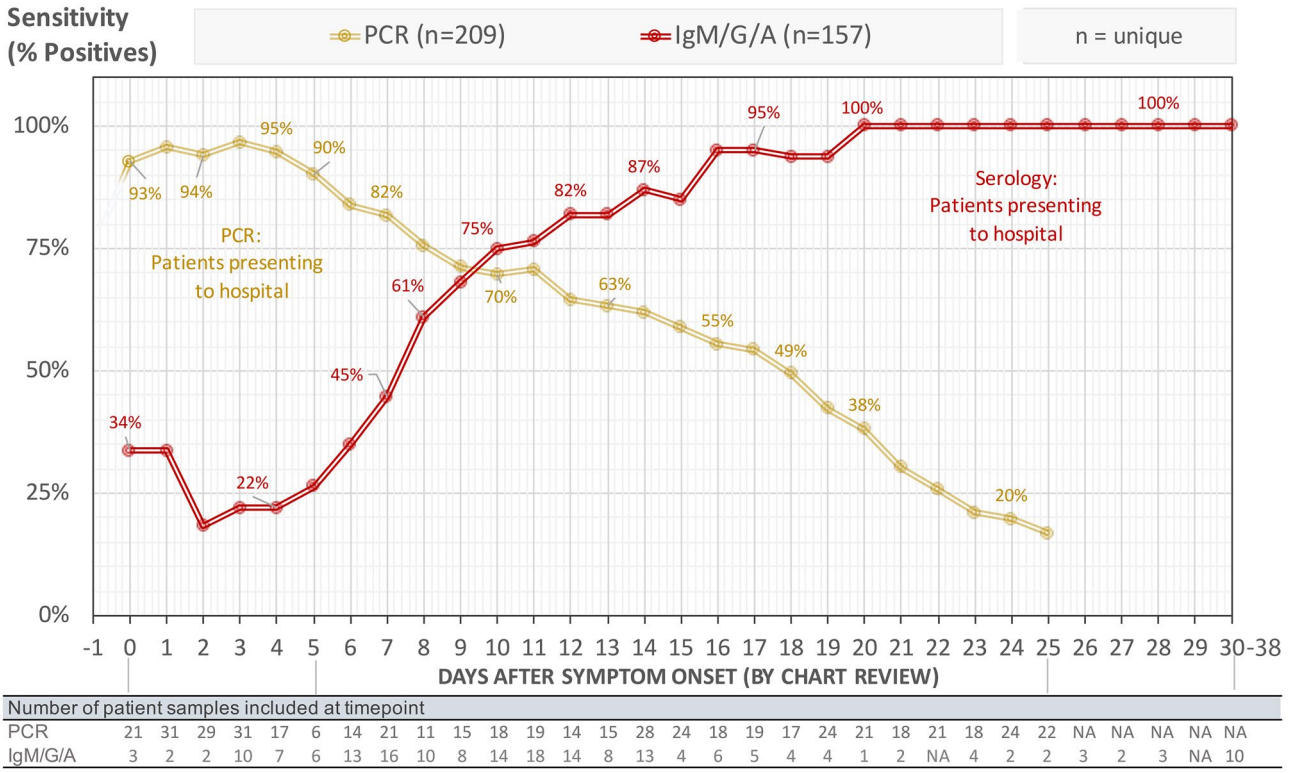

FIGURE 1 Sensitivity by assay modality over time. Blood-based serologic sensitivity in 157 patients superimposed onto NP swab PCR data from 209 patients. Results for all patient samples from initial symptom onset are plotted: PCR—516, Serology—588 (196 samples × 3 isotypes). Serology sensitivity is based on detection of IgM, IgG, or IgA. Sample results prior to Day 0 were excluded. PCR and serology samples were obtained in largely different patient populations; therefore, sensitivities are not additive. Data is plotted as 5-day moving average against the days since symptom onset. NA, none assessed

570 nm OD from the 450 nm OD. To estimate antibody titers, we generated isotype-specific standard curves using anti-SARS-CoV-1/2 monoclonal IgG, IgA, and IgM antibodies. We used this standard curve to calculate the concentration of anti-RBD IgG, IgA, and IgM expressed in U/mL. Positive specimens were identified as those that had an U/mL three standard deviations above the mean of negative control specimens. (data not shown). The overall specificity was 98.6% for IgM, 99.0% for IgA, and 99.5% for IgG in 207 samples obtained before the pandemic (1 March 2019-14

January 2020) when no detectable antibody responses would be expected.

2.4 | Determination of date of symptom onset

The date of symptom onset was determined by review of the electronic medical record by physician investigators. The onset date was determined in by one of two ways from the medical record: (a) as explicitly defined in the chart from an MD-written note as "COVID-19+ date of symptom onset" or (b) determined from MD and non-MD notes that stated date of symptom onset for any COVID-19–related symptom that developed acutely and was new from baseline (fever, chills, loss of smell or taste, body aches, fatigue, runny nose, congestion, sore throat, cough, and shortness of breath). Cases for which the date of symptom onset could not be determine were excluded from analysis (21/359, or 5.8%, of all PCR and serology cases).

2.5 | Patient cohorts and statistical analysis

Clinical laboratory test results are stored in a laboratory information management system connected to the electronic medical record. We performed two data queries with different end-dates: an initial PCR-query (3 March 2020 to 15 April 2020) and a second PCR-query (3 March 2020 to 4 May 2020).

The initial PCR-query was performed to delineate clinical sensitivity over time, and analysis was restricted to patients with multiple PCR test results and at least one positive (ie, the most informative subset). These patients were considered confirmed COVID-19 positive and taken as true positives. All PCR test results regardless of specimen type were used to confirm a patient as SARS-CoV-2 positive; however, only PCR test results from a NP-swab specimen were used for sensitivity calculations. The resulting dataset consists of 624 PCR results from 209 unique subjects. In this subset, 83% were inpatients, 13% were patients from the ED, and 4% were outpatients. For each specimen, we manually mapped date of symptom onset and all test results on a daily scale and calculated: (a) the time (in days) from the date of symptom onset to the date of specimen collection, (b) the duration from the first positive PCR test result to any subsequent positive PCR test result, and (c) clinical detection rates (PCR-positive over total tests per day) at each day in relation to symptom onset or first PCR-positive, respectively. We modeled a linear daily regression trend after first positive PCR test, to estimate the time when PCR sensitivity reaches zero (foot-point analysis).

The second PCR query was performed to capture hospital-wide testing metrics, and to assess whether the above subset analysis of SARS-CoV-2 patients with multiple PCR results is representative of the entire tested population in our setting. We extracted admission date, encounter, discharge date (when applicable), age, gender, and collection types and times, reporting dates and times along with results from all SARS-CoV-2 PCR tests. By clinical encounter, 55.5% of orders originated in the outpatient setting, 12.1% originated in the emergency department (ED), and 32.2% of orders originated from the inpatient setting. The overall PCR positivity rate was 27.0% (n = 3163/11 703) in unique individuals and 28.3% of all tests performed (n = 4320/15 251; Table 1). All test results were used for calculations of test number over time, positivity rate and age as well as gender calculations (Figures S1 and S2).

To compare our data of mainly hospitalized patients to a population with mild disease, we used data derived from Wölfel et al,[4] in which patients with a known exposure were instructed to present to the clinic at the first sign of symptoms. A positive PCR was necessary for inclusion into the study. We applied the validated limit of detection of our laboratory-developed assay (5 copies/μL) to the data derived from Wölfel et al,[4] and used the same calculations for time-dependent clinical sensitivity for PCR.

Serologic analysis of IgM, IgA and IgG status was performed in a subset of the above SARS-CoV-2 PCR-positive patients for which we had excess material in the MGH core laboratories for clinical validation studies. For each sample, we determined the days post symptom onset at the collection date and calculated daily sensitivity for each antibody isotype as well as detection rate of any isotype. Although serology is currently considered a diagnostic aid (as opposed to a primary diagnostic test), we calculated sensitivity as the number of seropositive samples over the total number of tested samples from patients with the disease (ie, who tested positive by PCR).

We plotted the sensitivity for both test modalities (PCR and serology) as percentages per overlapping 5-day leading intervals against the days since symptom onset. Statistical analysis consisted of Fisher's exact test (association of SARS-CoV-2 status with dichotomous factors), χ^2 with Yates correction, or *t* test (comparison of means).

3 | RESULTS

3.1 | PCR sensitivity for SARS-CoV-2 nucleic acid decreased with days post symptom onset

SARS-CoV-2 viral RNA levels decline over the course of infection.[4] This decline of RNA levels clearly impacts the clinical sensitivity of PCR testing. It is not possible to determine the false negative rate of the PCR test from patients with a single PCR result. Therefore, we identified patients

with multiple PCR tests who had at least one positive test result (considered true positives). The resulting dataset is composed of 624 test results from 209 patients (6.6% of all PCR-positive patients, Table 1). We compared this subset to all tested patients (Figure S1, Table S1) and contingency analysis of the multi-PCR vs. all single PCR-positive patient subset showed no significant differences in age, gender, and test type (Table S1, Figure S2). Thus, we consider the multi-PCR subset demographically representative of our tested patient population.

To derive clinical sensitivity over disease course, we needed to define an anchor point. We chose two different anchor points: symptom onset (subjective) and first positive test (objective). First, we examined the time course by anchoring test results to the day of symptom onset (Figures 1 and S3). Clinical sensitivity remains above 90% for the first 5 days after symptom onset. In our cohort of patients presenting to the hospital, patients were admitted to the hospital a median of 8 days after symptom onset (interquartile range: 4-16 days; mean: 10 days), and between Days 6 and 8, the clinical sensitivity of PCR ranged from 84% to 76%. On subsequent days the sensitivity decreases, and at Day 18 the sensitivity decreases below 50%. We also compared sensitivity data from an earlier study of mildly symptomatic patients[4] and noted a steeper PCR sensitivity decline, consistent with viral levels dropping more quickly in this population (Figure S4). This data provide sensitivity estimates at the time of presentation (eg, a patient presents at Day 10 after symptom onset). Second, we also modeled how PCR sensitivity decreases over time after the first positive PCR test (Figures S3 and S5). Regression modeling and extension of PCR positivity decay (foot-point analysis) revealed that in our cohort, NP-swab specimens could stay PCR-positive beyond 20 and up to 40 days (Figure S5).

3.2 | Serological assay sensitivity increases with days post symptom onset

Seroconversion is also a dynamic response to the virus and assay sensitivity changes over time. To assess the sensitivity of our serology assay over time, we tested for IgM, IgG, and IgA antibodies against the RBD of SARS-CoV-2 spike protein in 157 SARS-CoV-2 PCR-positive patients using an in-house ELISA (Table 1). For some patients, we were able to assess serology at multiple time points (n = 591 total isotype tests on 197 total specimens). Anchoring our serologic results to days after symptom onset shows that seroconversion starts as early as symptom onset, is detectable in 50% of subjects after Day 7, and continues to increase with >80% of patients showing seropositivity after Day 12 (Tables 2 and S2; Figures 1 and S4). In the subset of patients with multiple serological tests we saw isotype switching occur as short as 1-4 days, consistent with recent reports.[5] Of note, we detected IgA prior to IgM or IgG in a number of individuals, with two subjects having only detectable IgA within 3 days of symptom onset. We also documented cases of IgG positivity prior to IgM or IgA, highlighting the utility of measuring seroconversion using all three isotypes (Table S3, Figure S6). The superimposition of serologic sensitivities with PCR sensitivities shows that, after Day 7, seroconversion is a reliable diagnostic aid indicating recent or prior infection (Figure 1).

4 | DISCUSSION

We present the dynamic clinical sensitivity of SARS-CoV-2 PCR and our serology platform in patients presenting to the hospital. We performed this single-center, retrospective analysis to share real-world evidence that can inform interpretation of PCR and serologic testing. We focused on assessing the clinical utility of both modalities in conjunction by direct superimposition of both sensitivity time courses. The direct superimposition shows that serology can function as a reliable diagnostic aid indicating recent or prior infection—in particular at times when PCR sensitivity is lower than 70%. Our findings emphasize that understanding the specific sensitivity kinetics of both modalities is paramount for interpretation and effective utilization of SARS-CoV-2 diagnostics.

There is considerable interest in moving SARS-CoV-2 diagnostics to evidence-based principles.[6-9] While clinicians await formal guidance from large, prospective, multi-center studies—which will be challenging during the ongoing pandemic—there is considerable uncertainty surrounding SARS-CoV-2 diagnostics in clinical practice.[3,10-13] Using available published data[3-7,12,14] and data presented here from our hospital, we offer the following five diagnostic principles for consideration:

1. **In symptomatic patients, all interpretations are anchored on days post symptom onset.** Understanding performance characteristics of SARS-CoV-2 diagnostics over the course of the infection is key to interpretation of results. We provide two distinct approaches to anchor interpretation over the course of infection: subjective (date of symptom onset) and objective (first PCR-positive result). Both approaches are valid and have limitations. For example, the quality of patient histories is variable and in many cases the day of symptom onset is unknown or cannot easily be reconstructed. As a practical suggestion, to make this data easily obtainable and searchable, we recommend placing the date of symptom onset (when available) in the front page of the (electronic) medical record of patients diagnosed with COVID-19.
2. **PCR is the diagnostic gold standard during acute infection.** PCR testing using consensus primers has an estimated

TABLE 2 Sensitivity of anti-SARS-CoV-2 serology by isotype, age, gender, and days post symptom onset

Total specimens tested	197		Male	Female	Age < 70	Age ≥ 70
Days post symptom onset	n	%	(% male)	(% female)	(% <70)	(% ≥70)
<8 days (total)	**60**		**39**	**21**	**34**	**26**
Positive for IgG	9	15	7 (18)	2 (10)	6 (18)	3 (12)
Positive for IgG or IgM	12	20	9 (23)	3 (14)	9 (26)	3 (12)
Positive for IgG or IgM or IgA	15	25	12 (31)	3 (14)	10 (28)	5 (12)
8 to 14 days (total)	**85**		**58**	**27**	**70**	**15**
Positive for IgG	45	53	33 (57)	12 (44)	40 (57)	5 (33)
Positive for IgG or IgM	53	62	38 (66)	15 (56)	45 (64)	8 (53)
Positive for IgG or IgM or IgA	58	68	40 (69)	18 (67)	50 (71)	8 (53)
>14 days (total)	**52**		**35**	**17**	**47**	**5**
Positive for IgG	46	88	30 (86)	16 (94)	42 (89)	4 (80)
Positive for IgG or IgM	47	90	31 (89)	16 (94)	43 (91)	4 (80)
Positive for IgG or IgM or IgA	49	94	33 (94)	16 (94)	45 (96)	4 (80)

specificity of >99%.[15] Based on early reports from Wuhan[16] the overall clinical sensitivity is reported around 70%. We found a clinical sensitivity around 95% in the first 5 days after symptom onset and although PCR is an imperfect standard, concurrent IgM/IgA/IgG antibody assessment in the first 5 days post symptom onset does not significantly aid in rendering a current diagnosis; at no point during active infection should serology replace PCR for diagnosis.

3. **Clinical sensitivity of PCR decreases with days post symptom onset.** In clinical practice many symptomatic patients present to medical care after Day 1 of symptom onset. Our data show PCR sensitivity decreases with days post symptom onset (Figure 1) and with days post first PCR-positive test result (Figure S5). Notably, some patients may have an initial PCR-negative result at presentation (Figure S3). Our data also indicate that severely ill patients (many patients in our cohort) remain PCR-positive for a longer period than mildly ill patients (patients in the Wölfel et al[4] cohort Figure S4). Both time since symptom onset and disease severity may be key elements for the interpretation of PCR results.
4. **Serological assay sensitivity increases with days post symptom onset.** By positivity alone, in our cohort, seropositivity surpasses PCR positivity after Days 8-10 post symptom onset. Remarkably, we find seroconversion does not follow the typical kinetics of IgM antibodies followed by class-switched IgG and IgA antibodies. Rather, all appear simultaneously at a cohort level, with IgG or IgA seropositivity preceding IgM responses in some cases. Supporting this are other studies that report overall low IgM responses to SARS-CoV-2 that are often preceded by IgG.[14,17] These data highlight the benefit of measuring all three anti-SARS-CoV-2 antibody isotypes to maximize sensitivity. In the case of SARS-CoV-2, it is unknown for how long IgM, IgA, or IgG antibodies remain detectable after infection. It is important to note that a positive serologic result for IgM, IgA, and/or IgG does not conclusively indicate that a patient's presenting symptoms are due to a current SARS-CoV-2 infection. Distinguishing a prior versus an acute infection by serology will require isotype-specific interpretation, serial serologic testing to demonstrate seroconversion, and integration with clinical data.
5. **Negative results do not completely preclude SARS-CoV-2 infection.** Ruling out SARS-CoV-2 infection remains challenging. Supplementing PCR results with serologic assessment can increase sensitivity (serology as a diagnostic aid). However, our data clearly show that there is a window period (Day 6-12 from symptom onset) when clinical sensitivity of PCR and serological assays are below 90%. In a symptomatic patient, if multiple PCR tests are negative and serological results after 8-12 days are also negative, we believe the likelihood of active SARS-CoV-2 infection to be low. Clinical judgment is needed in this situation as there are rare scenarios where PCR negativity may be due to disease at a different anatomic site and/or serologic negativity may be due to an immunocompromised state.

Limitations in our study include relatively small numbers, a retrospective design, and selection bias due to the specific setting and testing practice. We evaluated symptomatic, mostly hospitalized patients and we cannot derive recommendations for asymptomatic or mildly symptomatic patients from our data. Due to limited availability of tests and time constraints during an ongoing pandemic, we did not perform daily sampling. Date of symptom onset is not consistently available, subjective, and affected by recall biases—yet, it represents a useful anchor point for disease time course in symptomatic patients. Notably, the eclipse period ranges from 2 to 14 days[18-21] and some patients already mounted a serologic response at the time of presentation, which can be taken as an argument for the early y-axis deviation from zero in the serology curve (Figure 1) and a confirmation of date of symptom onset as an imperfect marker. We caution that the presented serology data are specific to our ELISA, and we cannot extrapolate to anti-SARS-CoV-2 antibody responses in general. Nonetheless, other publications indicate that the time courses are comparable.[14,17,22] Our serological studies measured antibodies to the RBD of SARS-CoV-2. We chose this viral antigen because of its specificity to SARS-CoV-2, and because anti-RBD antibodies are typically neutralizing. Plaque reduction neutralization tests are the gold standard for assessing neutralizing ability,[23-26] and ongoing studies are in progress to confirm anti-RBD antibodies are neutralizing in SARS-CoV-2 infection.[27] Some commercially available assays measure the more abundant nucleocapsid protein, which may increase sensitivity to detect a serologic response early in the course of infection, therefore, shifting the seroconversion curve to the left. However, antibodies to nucleocapsid protein are unlikely to provide protective immunity as nucleocapsid protein is inaccessible to antibodies in an intact virus. Therefore, serology results also depend on the specific antigen employed in testing. Finally, our multi-PCR cohort and serologic patient cohort are largely non-overlapping PCR-positive patients (n = 20/209). To enable additive sensitivity calculations from combined PCR and serology assays, prospective and systematically obtained repeated parallel PCR and quantitative serologic data will be necessary.

Our real-world data outline the strengths and weaknesses of two SARS-CoV-2 test modalities over the natural course of infection. We hope these data, in conjunction with the five diagnostic principles for consideration, will contribute to effective utilization and interpretation of COVID-19–related laboratory data for patient care.

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CONFLICT OF INTEREST

Dr Gelfand reports personal fees from Henry Schein Inc, outside the submitted work; Dr Turbett reports grants from Centers for Disease Control and Prevention, outside the submitted work; Dr Anahtar reports personal fees and other from Day Zero Diagnostics, outside the submitted work; Dr Ryan reports grants from CDC, during the conduct of the study. Dr. Branda reports grants from Zeus Scientific, grants from bioMerieux, grants from Immunetics, personal fees from T2 Biosystems, personal fees from DiaSorin, personal fees from Roche Diagnostics, grants from Bay Area Lyme Foundation, grants from Lyme Disease Biobank Foundation, outside the submitted work.

AUTHOR CONTRIBUTIONS

T.E. Miller, W.F. Garcia Beltran, A.Z. Bard, T. Gogakos, E.S. Rosenberg, and J.K. Lennerz conceived and designed the study. T.E. Miller, W.F. Garcia Beltran, A.Z. Bard, T. Gogakos, M.N. Anahtar, M.G. Astudillo, D. Yang, J. Thierauf, A.S. Fisch, G.K. Mahowald, M.J. Fitzpatrick, V. Nardi, A. Dighe, R.C. Charles, E.T. Ryan, J.A. Branda, V.M. Pierce, M.R. Murali, A.J. Iafrate, E.S. Rosenberg, and J.K. Lennerz collected and assembled the data. T.E. Miller, W.F. Garcia Beltran, A.Z. Bard, T. Gogakos, M.N. Anahtar, V.M. Pierce, M.R. Murali, A.J. Iafrate, E.S. Rosenberg, and J.K. Lennerz analyzed and interpreted the data. T.E. Miller, W.F. Garcia Beltran, and J.K. Lennerz drafted the article. T.E. Miller, W.F. Garcia Beltran, A.Z. Bard, T. Gogakos, M.N. Anahtar, J.A. Gelfand, M.C. Poznansky, B.E. Bernstein, D.N. Louis, A. Dighe, R.C. Charles, E.T. Ryan, V.M. Pierce, M.R. Murali, A.J. Iafrate, E.S. Rosenberg, and J.K. Lennerz provided critical revisions and important intellectual content. All authors provided final approval of the article. J. Feldman, B.M. Hauser, T.M. Caradonna, G. Alter, A.G. Schmidt, J.B. Harris, R.C. Charles, and E.T. Ryan provided study materials or patients. J. Thierauf, H.D. Marble, L.L. Ritterhouse, S.E. Turbett, J. Batten, N.Z. Georgantas, D.N. Louis, and A. Dighe provided administrative, technical, or logistic support.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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