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Practical Considerations for Implementation of SARS-CoV-2 Serological Testing in the Clinical Laboratory: Experience at an Academic Medical Center

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Abstract

Molecular techniques, especially reverse transcriptase polymerase chain reaction (RT-PCR), have been the gold standard for the diagnosis of acute severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Serological tests for SARS-CoV-2 have been widely used for serosurveys, epidemiology, and identification of potential convalescent plasma donors. However, the clinical role of serologic testing is still limited and evolving. In this report, we describe the experience of selecting, validating, and implementing SARS-CoV-2 serologic testing for clinical purposes at an academic medical center in a rural state. Successful implementation involved close collaboration between pathology, infectious diseases, and outpatient clinics. The most common clinician concerns were appropriateness/utility of testing, patient charges/insurance coverage, and assay specificity. In analyzing test utilization, serologic testing in the first month after go-live was almost entirely outpatient and appeared to be strongly driven by patient interest (including health care workers and others in high-risk occupations for exposure to SARS-CoV-2), with little evidence that the results impacted clinical decision-making. Test volumes for serology declined steadily through October 31, 2020, with inpatient ordering assuming a steadily higher percentage of the total. In a 5-month period, SARS-CoV-2 serology test volumes amounted to only 1.3% of that of reverse transcriptase polymerase chain reaction. Unlike reverse transcriptase polymerase chain reaction, supply chain challenges and reagent availability were not major issues for serology testing. We also discuss the most recent challenge of requirements for SARS-CoV-2 testing in international travel protocols. Overall, our experience at an academic medical center shows that SARS-CoV-2 serology testing assumed a limited clinical role.

Keywords

antibodies, immunoglobulin G, immunoglobulin M, SARS-CoV-2, serology, utilization

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Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was officially classified by the World Health Organization (WHO) as a pandemic on March 11, 2020.[1-3] As of this writing, an estimated 64 300 000 cases and 1 500 000 deaths have been attributed to SARS-CoV-2 throughout the world.[4,5] Diagnostic testing in patients who have symptoms consistent with

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COVID-19 or who have had close contact with infected persons has mainly relied on reverse transcriptase polymerase chain reaction (RT-PCR) on a variety of respiratory tract specimen types.[1,6] Antigen tests are an alternative with lower sensitivity, especially for asymptomatic disease, as well as lower specificity.[7]

Serologic assays that detect antibodies against SARS-CoV-2 represent an additional resource.[7,8] In contrast to the widespread use of serologic assays for the diagnosis of some other viral infections (e.g., hepatitis B and C), serologic assays have historically not played a major role in the clinical diagnosis of coronaviruses. The value of SARS-CoV-2 serological assays for clinical diagnosis and management is still evolving. Guidance from public health, infectious disease, and microbiology organizations has consistently proposed that antibody testing has the most established value for epidemiology and seroprevalence studies, selection of convalescent plasma donors, and evaluation of candidate vaccine efficacy.[9-12] The Infectious Disease Society of America (IDSA) published consensus guidelines for SARS-COV-2 serologic testing in May 2020.[13] In addition to use in research, proposed clinical uses for serologic testing include diagnosis of patients in the later course of suspected COVID-19, where upper respiratory RT-PCR may be negative or low positive or when a collection of a lower respiratory tract specimen is not feasible. Multiple publications have discussed the limitations of SARS-COV-2 serology assays.[14-17]

In the early phases of the COVID-19 pandemic, lack of commercially available serologic assays left laboratory-developed tests as the only option for clinical or research laboratories, often to support uses such as identification of convalescent plasma donors.[14,15] As commercial vendors entered the market, enzyme-linked immunosorbent assays (ELISAs) and point-of-care kits (many using lateral flow immunoassays) emerged. Ultimately, diagnostic vendors developed SARS-COV-2 serologic assays suitable for automated clinical immunoassay analyzers commonly used in hospital-based and reference clinical laboratories. The availability of automated assays makes large-scale serosurveys and epidemiology studies to assess the extent of the pandemic logistically easier.[18-20]

In the United States, SARS-CoV-2 serologic assays required Emergency Use Authorization (EUA) by the Food and Drug Administration (FDA) but originally were covered under section IV.D ("pathway D") of the FDA "Policy for Diagnostic Tests for Coronavirus Disease-2019," which minimally required commercial manufacturers to only notify the FDA of their validated product.[21] This led to the rapid marketing of nearly 200 SARS-CoV-2 serologic assays by May 1, 2020. On May 4, 2020, the FDA updated guidelines to require that manufacturers submit their validation data. Compared to the many vendors who originally marketed SARS-CoV-2 serological assays, the subsequent months have seen a smaller subset of vendors, including multinational diagnostic companies that market high-throughput automated clinical chemistry platforms, submit validation data to the FDA.[7,22] To supplement validation data reported in the assay package inserts, a number of clinical laboratories have published their detailed evaluation of marketed SARS-CoV-2 serologic assays, including studies that compared 3 or more assays.[18,23-28] The growing literature evaluating and comparing SARS-CoV-2 serologic assays thus provides a resource for laboratories interested in starting serologic testing or in moving from one assay to another. A more recent trend has been comparison of commercially marketed serologic assays with neutralizing antibodies, along with investigations of the time course and duration of antibody responses.[19,20,27,29-36]

In this report, we describe the process of selecting, validating, and performing SARS-CoV-2 serologic testing in an academic medical center that serves as a regional tertiary/quaternary care center. We discuss the challenges encountered and analyze utilization patterns for the serologic testing implemented. Finally, we compare and contrast the utilization of serology testing with COVID RT-PCR and other infectious disease assays.

Methods

Institutional Setting

University of Iowa Hospitals and Clinics (UIHC) is an 845-bed tertiary/quaternary care medical center that includes pediatric and adult inpatient units (including multiple intensive care units), an emergency department with level I trauma capability, and outpatient services throughout the local region. UIHC receives many referrals, both in-state and out-of-state (primarily neighboring states such as Illinois). The state of Iowa is predominantly rural with few urban areas, and UIHC is currently the sole academic medical center in the state. Given limited intensive care unit beds in the state of Iowa and the bordering areas of adjacent states, UIHC represents a primary regional option for the management of patients with COVID-19 requiring inpatient care.

Patients

The present study had institutional review board approval as a retrospective study with a waiver of informed consent (protocols #202006433 and 202011042). The institutional electronic health record (EHR) is Epic Hyperspace (Epic, Inc., Verona, WI). The pathology laboratories use Epic Beaker Clinical Pathology as the laboratory information system for clinical laboratory testing.[37] Laboratory utilization data for SARS-CoV-2 serologic and RT-PCR testing were obtained using Epic Reporting Workbench as described previously.[38] In general, these data were extracted as discrete results (eg, negative, indeterminate, and positive) directly by Epic Reporting Workbench except for the first several weeks of RT-PCR testing, which required manual review to obtain the test results in scanned reports within the EHR. The first date of clinical RT-PCR testing in house at UIHC was March 7, 2020.

Clinical serologic testing at UIHC began on May 19, 2020. Chart review was performed using the EHR.

History of Serologic Testing at UIHC

The history of decisions and communications regarding SARS-CoV-2 serologic assays at UIHC was compiled by the laboratory director (MDK) who served as the primary pathology contact for inquiries regarding serologic testing. The laboratory director assembled and analyzed meeting notes, media transcripts, and emails to reconstruct the time course with the serologic assays. The associate director of clinical chemistry (AEM) oversaw the technical aspects of serologic assay validation. A system-wide broadcast on serology testing was issued throughout the University of Iowa Health Care system on May 18, 2020; this communication provided technical specifics on the testing (eg, specimen requirement and order codes) and indicated the following as recommended principles of testing based on evidence and guidelines available at the time: (1) The test should not be used to diagnose acute (within two weeks of symptoms onset) infection with SARS-CoV-2; (2) the test may have clinical value to identify individuals previously infected with SARS-CoV-2 who were more than 2 weeks from the onset of illness; and (3) the test does not determine whether a patient has developed protective immunity nor should the results of the test be used to guide personal protective equipment (PPE) use or adherence to physical distancing practices.

Results

Assessment of Need and Evaluation of Serologic Assays

The earliest application at our institution for serologic testing was to support a research protocol for convalescent plasma therapy. Testing to identify potential donors was developed and validated in an institutional research laboratory and is the subject of a separate publication. The first documented queries for clinical testing at our institution were on March 19, 2020, and were intermittent throughout the rest of March and April. During this phase, commercial reference laboratories were developing capacity for antibody testing, but availability was unknown as vendors were still ramping up production infrastructure.

As described in the Introduction, March through early May 2020 was a period of rapid growth for the marketing of SARS-CoV-2 serologic assays, many with limited or no validation data. The first assay considered for clinical use at our institution was an ELISA assay (EUROIMMUN), with separate assays for SARS-COV-2 immunoglobulin A (IgA) and immunoglobulin G (IgG) measurements. The UIHC clinical laboratories had instrumentation that could run this assay; however, the manual effort involved was a concern, given unknown test ordering volumes in the future and anticipated desire to support serosurvey research that may involve thousands of patients.

Ultimately, the decision was made to purchase a DiaSorin Liaison XL analyzer capable of running a SARS-CoV-2 IgG assay. This analyzer also had the capability of running additional assays of benefit to the laboratory, which factored into the purchasing decision. Approval to purchase this instrument was granted in mid-April 2020. The FDA approved a EUA for the DiaSorin assay on April 26, 2020. During this time frame, Roche Diagnostics, the vendor for the main clinical chemistry automated line in the UIHC core laboratory, also announced the development of a SARS-CoV-2 total antibodies assay, with a EUA for that assay approved on May 4, 2020. The UIHC core laboratory thus validated and compared both assays simultaneously, as we have published previously.[24] The validation study consisted of a total of 228 samples (n = 54 RT-PCR positive; n = 174 RT-PCR negative, including n = 139 pre-COVID samples) tested on Roche total antibodies and DiaSorin IgG assays.

At the time of decision-making for SARS-CoV-2 serology assays at UIHC, the overall seroprevalence data for Iowa were not available. In addition, RT-PCR testing at UIHC and other Iowa institutions in April and May 2020 was restricted, limited by supply chain, and influenced by triage steps such as telehealth screening prior to test approval or meeting established criteria for testing (eg, inpatient admission or preprocedural). From the limited published literature or publicly available information available in May 2020, a serosurvey of Boise, Idaho, conducted in early April 2020 reported a seroprevalence of approximately 2.0% in a state with roughly similar population density and likely similar viral spread patterns to Iowa in May 2020.[39] The 2.0% seroprevalence provided a reasonable initial estimate, with the expectation that seroprevalence would increase over time to 5% to 10% and possibly higher once more comprehensive data became available. Statewide seroprevalence data were not determined for Iowa until August 2020.[40] The seropositivity for August through October 2020 in Iowa averaged 8.0%. Interestingly, an 8.0% rate of RT-PCR positivity for samples tested at UIHC was also noted from May 19, 2020, to October 31, 2020.

Supplemental Table 1 summarizes estimates of negative and positive predictive values (NPV and PPV, respectively) for the Roche and DiaSorin assays performed either individually or in an orthogonal algorithm, with the Roche assay performed first and then reflexing to the DiaSorin IgG assay if the Roche test is positive. This uses package insert data for sensitivity and specificity and a calculator provided by the FDA.[41] The calculations presented in Supplemental Table 1 show results assuming seroprevalence of 2.0% or 8.0% and also sensitivity based on different days of onset from RT-PCR positivity. In a range of scenarios with these basic parameters, the 2-test orthogonal algorithm provides high NPV and PPV. An additional practical benefit was that the Roche assay could be run on an automated clinical chemistry line with redundant instrumentation as backup.

This clinical workflow, with the Roche assay as the first test and reflexing to the DiaSorin IgG assay if the Roche test was positive, went live on May 19, 2020. We elected to result cases

Table 1. SARS-CoV-2 Serology Assay Considerations.

Factor	Variables	Comments
Type of assay	Lateral flow immunoassay	Common in point-of-care assays
	ELISA	Labor-intensive
	Automated immunoassay	High throughput
Verification/validation studies	In-house studies	Required for moderate- and high-complexity assays; scale is at the discretion of the medical director
	Package insert data	Wide variability in early marketed assays Steadily grew starting in June 2020
	Publications	Uncommon as a standalone assay
Antibody type	IgA	
	IgG	Many assays target IgG
	IgM	Uncommon as standalone assay*
	Total	Detection of IgM may aid early detection
Antigenic target of assay	Spike (S)	Common target of most vaccines
	Nucleocapsid (N)	
Testing protocol†	One stage	Use of single assay has higher false-positive rate
	Two stage	Lowers false-positive rate but reduces sensitivity

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; ELISA, enzyme linked immunosorbent assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.
*Exception is some international travel screening protocols that require SARS-CoV-2 IgM testing.
†An added variable is the specific combination of assays chosen, for example, targeting the same or different antigenic target, IgG vs total antibodies.

where the Roche total antibodies assay was positive but the DiaSorin IgG negative as "Indeterminate," providing an interpretive comment that such results could represent true positives on the Roche (perhaps due to detection of IgM or IgA antibodies not detected on the DiaSorin assay) or false positives. Both the Roche and the DiaSorin assays have also been used for multiple research studies at our institution.

Table 1 summarizes some of the key considerations for selecting a SARS-CoV-2 serology assay. In our case, testing demand made point-of-care or ELISA assays infeasible relative to assays that could run on automated clinical immunology analyzers. Both of the assays selected for clinical workflow at UIHC had fairly extensive data in the package insert that was supplemented with in-house studies and, later, corroborated by research publications.[18,26,42,43] In addition to differing by type of antibodies (IgG only vs total antibodies), the Roche and DiaSorin assays differ by antigenic target, which may have implications as vaccines that target different antigens enter clinical use. Most vaccines in the current pipeline target the spike protein.[44] The clinical importance of assay antigenic target is an area of ongoing research. For example, the correlation of the assays with neutralizing antibodies was unknown at the time of selection although recent publications have examined this issue.[27,29,34,35]

Identification of Concerns and Development of Guidelines and Practices for SARS-COV-2 Serologic Testing

In preparation for offering the tests for clinical ordering, clinical laboratory leadership sought the input of UIHC infectious disease, epidemiology, and institutional clinical leadership. The most common clinical concerns related to test characteristics were assay specificity and clinical utility. Concerns on assay specificity were allayed by the consistency between package insert and internal validation data related to the performance of the Roche assay. This has subsequently been further confirmed by published studies.[18,24,26,42,43] Recommended guidelines for clinical utility were drafted into communications to health care staff and EHR order entry prompts (see Methods section).

A system-wide broadcast on the availability of serologic testing and the recommended guidelines went out prior to go-live with the laboratory director as the contact person. Calls and emails to the laboratory director were mainly related to insurance coverage, concerns over direct request for testing by patients (including the possibility of providers being "flooded" by patient requests), and suggestions for restrictions on testing (eg, requiring approval from infectious disease consult) (Table 2). Unlike RT-PCR testing, reagent availability has not been a limiting factor for serologic testing at our institution. Therefore, restrictions designed to conserve reagent and specimen supplies, as used by our institution throughout the pandemic for RT-PCR testing, have not been needed.

Multidisciplinary discussions led to educational materials for dissemination, decisions on recommended language in fielding patient and provider queries related to serologic testing, and guidance for questions related to insurance coverage and potential patient financial liability (which was essentially unknown at that time except for the patient charge for the testing if not covered by insurance). Calls and emails to the laboratory director were heavily concentrated on the day of the system-wide broadcast, the subsequent day of assay go-live, and the following day. After this, queries tended to be related to the logistics of the testing, with the majority wondering whether serologic testing had any restrictions

Table 2. Number of Communications to Laboratory Director Regarding SARS-CoV-2 Serology Assay.

Category	Before assay go-live*	After assay go-live*
Test availability†	28	21
Restrictions/guidelines on testing	10	31
Convalescent plasma	17	11
Vendor query	42	23
Test characteristics (eg, sensitivity, specificity)	17	27
Test charges/insurance coverage	7	19
Research testing using the assay	23	10
Informatics and regulatory issues	15	9
Media query on serology testing	3	6

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.
*Assay went live on May 19, 2020. Numbers indicate queries from unique individuals and do not reflect any subsequent back-and-forth communications on the original query.
†Includes both whether test is available and how to order.

similar to those put in place for SARS-CoV-2 RT-PCR. These were handled by verbiage that the testing only required a licensed provider order similar to most routine laboratory tests, but that insurance coverage was not certain. Chart review of patient testing showed that this verbiage was utilized widely by clinical providers in documentation in the EHR and those fielding calls. One question that came up intermittently from patients was whether they could obtain serologic testing by donating blood, even if not related to a convalescent plasma study or protocol. This question seemed to mainly arise from the practice of some larger commercial blood collection centers (eg, American Red Cross) to offer SARS-CoV-2 serology testing free of charge to blood donors.[45]

The laboratory director did a total of 6 interviews with local media including newspaper, radio, and television. These were all between May 5, 2020, and June 17, 2020, with the exception of 1 follow-up interview on July 20, 2020. Some of the radio and television interviews included audience questions, examples of which were use of testing for identifying convalescent plasma donors, concerns over the quality of serologic assays, and whether positive testing indicated immunity to the disease.

Table 2 summarizes communication to the laboratory director regarding SARS-CoV-2 serology testing, dividing queries into before and after assay go-live on the morning of May 19, 2020.

Overall Testing Statistics for Serology and RT-PCR Testing

From May 19, 2020, through October 31, 2020, a total of 1466 SARS-CoV-2 serology tests were performed on 1440 unique patients at UIHC, with 119 (8.1%) positives, 16 indeterminates (Roche total antibodies positive, DiaSorin IgG negative; 1.1%), and 1331 (90.8%) negatives. In the same time frame, a total of 102 708 RT-PCR tests were performed on a total of 65 178 unique patients, with 7047 (6.9%) positives, 109 (0.1%) indeterminates, and 95 552 negatives. During this time frame, RT-PCR testing had a range of restrictions and protocols for test ordering.

Clinical Utilization of the Testing: Those Testing Negative in the First Month

To gain insight into the clinical utilization of SARS-CoV-2 serologic testing after the order was first available, we performed a detailed chart review on all orders for the first month investigating the presence of symptoms commonly associated with COVID-19 infection. We did not examine the grouping of symptoms. We will first describe the cohort that tested negative (n = 411 unique patients; 163 males and 248 females). This group was predominantly outpatients (97.1%), and 107 (26.0%) were health care workers (Table 3). Teachers (n = 37, 9.0%), students (n = 52, 12.7%), and retirees (43, 10.5%) were also common. A total of 283 (68.9%) people had documentation in the EHR of at least 1 symptom known to be associated with COVID-19 infection. Fever (n = 147, 51.9%), cough (n = 170, 60.1%), shortness of breath/difficulty breathing (n = 83, 29.1%), and fatigue (n = 82, 29.0%) were the most common presenting symptoms in those testing negative (Table 4). For those in which timing of symptoms could be ascertained from the EHR documentation, 227 (97.0%) of 234 had the first appearance of symptoms 2 weeks or longer prior to testing.

Loss of taste and smell has received attention as symptoms relatively unique to COVID-19 infection compared to other similarly presenting respiratory infections.[46-48] Among individuals with negative serology who reported a new loss of taste and/or smell (n = 21), 8 patients had previous documented RT-PCR testing prior to serology testing, with all 8 being RT-PCR negative. In 5 of the 21 patients, loss of taste and/or smell was the main chief complaint documented in the chart. In 8 of the 21 patients, the clinical history was the onset of suspected COVID symptoms more than 1 month prior to serology.

One or more comorbidities associated with more severe COVID-19 clinical course was documented in the EHR in 175 (42.6%) of those who tested negative, with obesity being the most common (Table 5). SARS-CoV-2 PCR was performed in only 93 of the 411 who tested negative in the first month (22.6%), with only 1 positive. Testing for other infectious diseases including Epstein-Barr virus (EBV), cytomegalovirus (CMV), influenza A, influenza B, and parvovirus B-19 was uncommon; none of these diseases were tested in more than 5 (1.2%) patients negative for SARS-CoV-2 serology in the first month (Table 6; note that RT-PCR reporting follows EUA for the Thermo-Fisher RT-PCR assay[49] used; see second footnote of Table 6).

A majority of those testing negative in the first month had no documentation in the EHR (n = 301, 73.2%) on the reason for testing. For those who did have some documentation, "want to know" (n = 38, 9.2%) and explanation of previous symptoms (n = 57, 13.9%) were the most common reasons cited (Table 7). While it was rare for change in clinical management due to serology to be documented in the EHR, we identified a

Table 3. Demographics and Characteristics of Patients Tested for COVID Serology.

	Negative serology* (413 tests in 411 patients)	Indeterminate/positive* serology (61 tests in 58 patients)
Number of unique patients (male/female/total)[†]	163/248/411	28/30/58
Age in years (mean/median/range)	46.9/48 (0.6-89 years)	40.3/38.5 (5-79 years)
Patients with more than 1 serology test	2	1
Total number of serology tests	413	61
Location of sample collection[‡]		
Inpatient	11 (2.7%)	14 (23.0%)
Outpatient	401 (97.1%)	45 (73.8%)
Emergency Department	1 (0.2%)	2 (3.3%)
Employment status/profession[§]		
Agriculture	4 (1.0%)	0 (0.0%)
Childcare	3 (0.7%)	1 (1.7%)
Construction	4 (1.0%)	2 (3.4%)
Disabled/medical leave	10 (2.4%)	0 (0.0%)
Food processing/food services	7 (1.7%)	7 (12.1%)
Health care	107 (26.0%)	7 (12.1%)
Teaching (postsecondary)	25 (6.1%)	3 (5.2%)
Teaching (K–12 education)	12 (2.9%)	1 (1.7%)
None	23 (5.6%)	1 (1.7%)
Other	75 (18.2%)	14 (24.1%)
Retired	43 (10.5%)	5 (8.6%)
Student	52 (12.7%)	10 (17.2%)
Unknown	46 (11.2%)	7 (12.1%)

*Includes data from May 19, 2020, to June 19, 2020, for the negative cohort and May 19, 2020, to August 12, 2020, for the positive cohort. "Indeterminate" serology was positive by Roche Diagnostics total antibodies assay but negative by the DiaSorin IgG assay.
[†]Includes one transgender female in the negative result cohort.
[‡]Includes repeated tests (n = 413 for negative results; n = 61 for positive or indeterminate results).
[§]Includes unique patients (n = 411 for negative results; n = 58 for positive or indeterminate results).

Table 4. Symptoms of Patients Tested for COVID Serology.

	Negative serology* (413 tests in 411 patients)	Positive serology* (61 tests in 58 patients)
Specific symptoms mentioned in EMR		
At least 1 specific symptom	283 (68.9%)	39 (67.2%)
Fever	147 (51.9%)	24 (61.5%)
Cough	170 (60.1%)	22 (56.4%)
Fatigue/tired	82 (29.0%)	17 (43.6%)
Aches/pains	64 (22.6%)	19 (48.7%)
Sore throat	51 (18.0%)	7 (17.9%)
Diarrhea	23 (8.1%)	9 (23.1%)
Conjunctivitis	5 (1.8%)	0 (0.0%)
Headache	45 (15.9%)	16 (41.0%)
New loss of taste and/or smell	21 (7.4%)	12 (30.8%)
Rash on skin and/or discolorations of fingers/toes	10 (3.5%)	1 (2.6%)
Shortness of breath/difficulty breathing	83 (29.3%)	18 (46.2%)
Persistent chest pain and/or pressure	23 (8.1%)	8 (20.5%)
Neurologic changes	2 (0.8%)	0 (0.0%)
Bluish lips or face (cyanosis)	0 (0.0%)	0 (0.0%)
Timing of symptoms (if present) to testing		
First appearance of symptoms < 3 weeks	7 (3.0%)	4 (10.5%)
First appearance of symptoms ≥3 weeks	227 (97.0%)	34 (89.5%)
Range of first appearance of symptoms	7-196 days	7-156 days

Abbreviation: EMR, electronic medical record.
*Includes data from May 19, 2020, to June 19, 2020, for the negative cohort and May 19, 2020, to August 12, 2020, for the positive cohort. "Indeterminate" serology was positive by Roche Diagnostics total antibodies assay but negative by the DiaSorin IgG assay.

Table 5. Comorbidities of Patients Tested for COVID Serology.

	Negative Serology* (413 tests in 411 patients)	Positive Serology* (61 tests in 58 patients)
Comorbidities		
One or more comorbidities	175 (42.6%)	29 (50.0%)
Asthma	21 (5.1%)	7 (12.1%)
Chronic obstructive pulmonary disease	6 (1.5%)	1 (1.7%)
Atrial fibrillation	5 (1.2%)	2 (3.4%)
Hypertension	64 (15.5%)	7 (12.1%)
Congestive heart failure	4 (1.0%)	2 (3.4%)
Coronary artery disease	7 (1.7%)	1 (1.7%)
Obstructive sleep apnea	9 (2.2%)	5 (8.6%)
Dyslipidemia	5 (1.2%)	1 (1.7%)
Diabetes mellitus	20 (4.9%)	4 (6.9%)
Obesity	150 (36.5%)	18 (31.0%)
HIV	12 (2.9%)	1 (1.7%)
Malignancy	22 (5.4%)	3 (5.2%)
Immunosuppressant medication	5 (1.2%)	1 (1.7%)
Pregnant in 2020	4 (1.0%)	5 (8.6%)
Transplant recipient	3 (0.7%)	2 (3.4%)

*Includes data from May 19, 2020, to June 19, 2020, for the negative cohort and May 19, 2020, to August 12, 2020, for the positive cohort. "Indeterminate" serology was positive by Roche Diagnostics total antibodies assay but negative by the DiaSorin IgG assay.

Table 6. SARS-CoV-2 RT-PCR and Other Infectious Disease Testing.

	Negative serology* (413 tests in 411 patients)	Positive Serology* (61 tests in 58 patients)
COVID RT-PCR Testing		
COVID RT-PCR performed	93	29
RT-PCR positive†	1 (1.1%)	23 (79.3%)
RT-PCR negative†	92 (98.9%)	5 (17.2%)
RT-PCR indeterminate†	0 (0.0%)	1 (3.4%)
Other infectious disease testing		
EBV	5 (1.2%)	3 (5.2%)
CMV	2 (0.5%)	1 (1.7%)
HSV-1	1 (0.2%)	0 (0.0%)
HSV-2	2 (1.0%)	0 (0.0%)
Influenza A	4 (1.0%)	0 (0.0%)
Influenza B	3 (0.7%)	1 (1.7%)
Parvovirus B-19	2 (0.5%)	0 (0.0%)
Hepatitis A	5 (1.2%)	1 (1.7%)
Hepatitis B core antibodies	2 (0.5%)	0 (0.0%)

Abbreviations: RT-PCR, reverse transcriptase-polymerase chain reaction; EBV, Epstein-Barr Virus; CMV, cytomegalovirus; HSV, herpes simplex virus.
*Includes data from May 19, 2020, to June 19, 2020, for the negative cohort and May 19, 2020, to August 12, 2020, for the positive cohort. "Indeterminate" serology was positive by Roche Diagnostics total antibodies assay but negative by the DiaSorin IgG assay.
†Positive, negative, and indeterminate rate calculated out of those tested by RT-PCR. Positive RT-PCR results had a cycle threshold $\leq$37. Indeterminate results had cycle thresholds for any target between 37 and 40, and this low-positive result was confirmed by repeating the PCR before reporting.

case where a negative serology result ruled out a previous COVID infection in a patient under quarantine following an exposure; the quarantine was continued in this patient. We identified positive serology results used to justify proceeding with a procedure (n = 2), to transfer a patient to a different unit (n = 1), to return to work (n = 1), to justify not treating a patient for active COVID infection (n = 1), and to rule out an active infection during a workup (n = 1). Involvement of infectious disease (n = 11, 2.7%) and documentation of any clinical management change other than informing the patient of the results (n = 1, 0.2%) were uncommon. Patients in obstetric/gynecology clinics accounted for 13 (32.5%) of 40 orders on first 2 days of testing; however, testing volumes from these clinics amounted to only 77 (5.3%) of 1466 of all orders through October 31, 2020.

Clinical Utilization of the Testing: Those Testing Positive in the First 3 Months

The first month of SARS-CoV-2 serology testing at our institution yielded only 14 positive results (Roche and DiaSorin both positive) and 1 indeterminate result (Roche positive,

Table 7. Rationale for Testing and Impact on Clinical Care.

	Negative serology* (413 tests in 411 patients)	Positive serology* (61 tests in 58 patients)
Documented reason for testing		
"Want to know"	38 (9.2%)	7 (12.1%)
Diagnostic	13 (3.2%)	9 (15.5%)
Exposure to confirmed COVID case	6 (1.5%)	9 (15.5%)
Exposure to suspected COVID case	13 (3.2%)	2 (3.4%)
Unknown/other	301 (73.2%)	17 (29.3%)
Explain previous symptoms	57 (13.9%)	23 (39.7%)
Convalescent plasma	1 (0.2%)	2 (3.4%)
Involvement of infectious disease service	11 (2.7%)	10 (17.2%)
Documented change in clinical management	1 (0.2%)†	6 (10.2%)‡

*Includes data from May 19, 2020, to June 19, 2020, for the negative cohort and May 19, 2020, to August 12, 2020, for the positive cohort.
†One patient with negative testing had documentation in chart that this was used in decision-making for quarantine related to possible recent COVID exposure.
‡For 6 patients with positive serology testing, documented changes in clinical management included the following: allowed surgical procedure to proceed (n = 2), transfer to COVID unit (n = 1), return to work (n = 1), decision not to treat for active COVID infection (n = 1), and rule out of infection in infectious disease workup (n = 1).

DiaSorin negative) out of testing on 426 unique patients (3.3% positive and 0.2% indeterminate). The patient with the 1 indeterminate result ended up being retested 1 month later and was positive for both assays. To obtain a larger cohort to assess clinical utilization, we expanded a detailed chart review of patients with positive and indeterminate results through August 12, 2020.

The cohort testing positive or indeterminate was mostly outpatients (n = 45, 73.8%); in terms of occupation, health care workers (n = 7, 12.1%) and employment in food processing/food services (n = 7, 12.1%) together accounted for approximately one-quarter of patients (Table 3). For presenting symptoms, fever (n = 24, 61.5%), cough (n = 22, 56.4%), headache (n = 16, 41.0%), new loss of taste and/or smell (n = 12, 30.8%), shortness of breath/difficulty breathing (n = 18, 46.2%), and persistent chest pain and/or pressure (n = 8, 20.5%) were common in those testing positive (Table 4). Fever and cough were the most common presenting symptoms in cohorts testing negative and positive, illustrating the diagnostic challenge in differentiating COVID-19 from other viral illnesses. Comorbidities for the cohort testing positive or indeterminate are shown in Table 5.

Testing for SARS-CoV-2 RT-PCR was performed in 50% of the cohort testing positive for serology, with 23 (79.3%) of 29 patients testing positive by RT-PCR (Table 6). We identified a single case of a patient who had a negative serology result roughly 1 month after testing positive by RT-PCR; this patient was undergoing chemotherapy including rituximab for primary mediastinal (thymic) large B-cell lymphoma. We speculate impaired humoral immune response prevented production of antibodies in response to infection with SARS-CoV-2. In contrast, other infectious disease testing was not commonly performed in the cohort testing positive for SARS-CoV-2 serology (Table 6). The most common documented reasons for testing were exposure to confirmed COVID-19 case (n = 9, 15.5%), establish diagnosis of active SARS-CoV-2 infection (n = 9, 15.5%), and explain previous symptoms consistent with infection (n = 23, 39.7%; Table 7). Infectious disease service was involved in 10 cases (17.2%).

As mentioned earlier, 1 patient tested indeterminate in the first month of serology testing. Overall, there were only 16 unique patients total testing indeterminate through October 31, 2020. Repeat serology testing was only performed in 1 patient.

Comparison of SARS-CoV-2 Serology Testing With RT-PCR Testing

Figure 1 shows ordering volumes for SARS-CoV-2 RT-PCR and serology testing, revealing opposite trends in order volumes over time. RT-PCR testing steadily increased from March 2020 and reached an average of more than 700 tests/day in September and October 2020. In contrast, the highest average daily ordering for serology occurred in May 2020, with slightly more than 14 tests/day. In October 2020, approximately 56 positive RT-PCR tests were seen daily (Figure 1A) compared to slightly more than 1 positive serology test per day (Figure 1B).

Increased RT-PCR testing at our institution resulted from multiple factors including routine testing of inpatient admissions (including Labor and Delivery), increased emergency department testing, expansion of influenza-like illness clinic, drive-through testing, and increased preprocedural testing. This is reflected in steady increases in outpatient, emergency department, and inpatient order volumes for RT-PCR (Figure 1C). The most notable trends for serology testing have been a steady decline in outpatient testing and a 4-fold increase in inpatient testing from May to October 2020 (Figure 1D).

Ordering Volumes of SARS-CoV-2 Serology Compared to Other Infectious Disease Serologies

Finally, we also compared ordering volumes of SARS-CoV-2 serology with other infectious disease serologies performed in the core clinical laboratories. In the retrospective period of

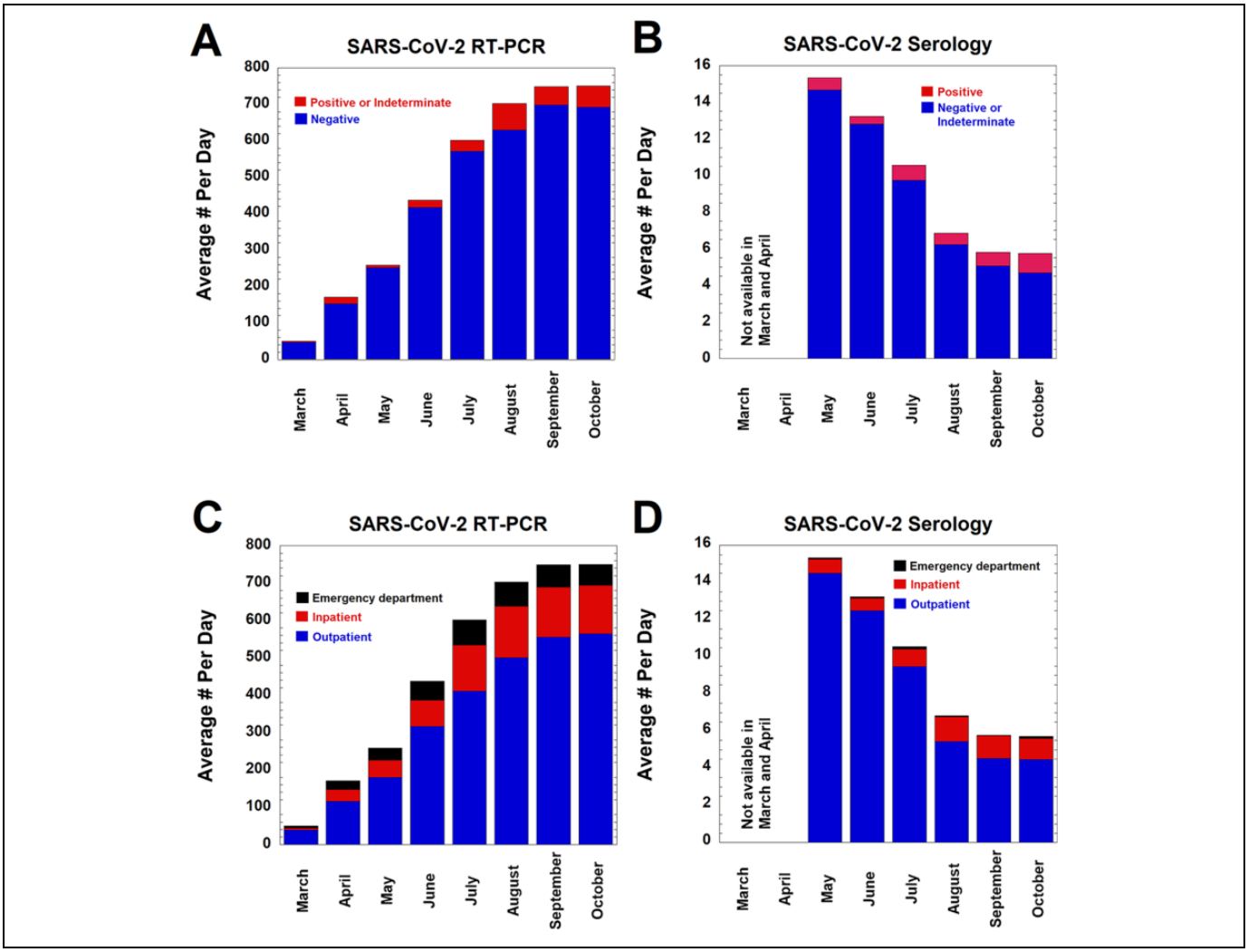

Figure 1. Ordering volumes for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) reverse transcriptase polymerase chain reaction (RT-PCR)and serology broken down by results (A, B) and location of order (C, D). For serology, cases where the Roche total antibodies assay was positive, but the DiaSorin IgG negative were classified as "indeterminate."

analysis (May 19, 2020, through October 31, 2020), SARS-CoV-2 serology was ordered a total of 1466 times. This is lower than hepatitis C antibody (5199 orders), hepatitis B surface antigen (5087 orders), HIV antigen/antibody combo (5063 orders), syphilis antibodies (2852 orders), hepatitis B surface antibody (3222 orders), and hepatitis B core total antibodies (2265 orders). SARS-CoV-2 serology was ordered more frequently than varicella-zoster IgG antibodies (1006 orders), measles IgG antibodies (541 orders), and mumps IgG antibodies (479 orders). In the same time period, orders of SARS-CoV-2 serology for research studies were similar in volume to clinical ordering volumes.

Introduction of SARS-CoV-2 IgM Testing for International Travel Protocols

Our clinical laboratory introduced a test for SARS-CoV-2 IgM antibodies on November 20, 2020. This test was introduced solely to meet requirements issued on October 29, 2020, by the Chinese government that dictated travelers must have negative SARS-CoV-2 RT-PCR and IgM serology results along with a subsequent green health code within 48 hours of boarding direct flights to China.[50] After discussion with clinical leadership at our institution, we offered this test with clear warning prompts to indicate that the purpose was to satisfy international travel requirements, and current published data did not support clinical use in other contexts. The main challenge with this testing is the risk of false positives, with no simple way to adjudicate a possible false positive other than performing another IgM test. The requirement specifically dictates IgM and not another alternative such as serology testing of total antibodies. Assay manufacturers may not make any specific claims toward detection of IgM in the package insert (as in the case for the Roche total antibodies used in the present study), and published research may not specifically address detection of IgM alone.[18,26,42,43]

Discussion

In this report, we summarize experience with SARS-CoV-2 serology testing at an academic medical center. While serology testing has an established role in seroprevalence and epidemiology studies, evidence for clinical utility remains limited.[7,8,22] We will in turn discuss what our results indicate in terms of clinical impact/benefit, clinician perspective on test utility, and patient perceptions.

Multiple lines of evidence in our study indicate that serology testing overall had a minimal clinical impact. First, order volumes of the serology testing are much smaller than that for RT-PCR, with total serology orders through October 31, 2020, amounting to only 1.3% of the testing volume of RT-PCR. Second, we found scant documentation of changes in clinical management resulting from serology testing other than simply notifying the patient of the results. Also, in contrast to RT-PCR testing, the serology testing on its own did not influence protocol-based decisions on patient isolation or use of PPE or other protective measures by health care staff. Third, documented reasons for ordering serology testing often did not follow evidence-based guidelines or were not provided at all. Fourth, only a small percentage of patients for whom SARS-CoV-2 serology was ordered had additional infectious disease testing performed and/or infectious disease consult requested. This would be consistent with a desire to determine SARS-CoV-2 antibody status as the sole goal as opposed to using the testing to resolve a differential diagnosis that includes other infectious diseases or medical conditions. These results parallel another study of SARS-CoV-2 serology ordering at an academic medical center, which analyzed in detail all orders within 1 month of go-live.[51] That study concluded that a high percentage of serology testing appeared to be driven mainly by patient request and clinical curiosity. This is perhaps not surprising, given all of the media and public health attention to COVID-19.

In the preparation and subsequent rollout of SARS-CoV-2 serology testing at our institution, clinician input was valuable in formulating recommendations for testing and dissemination of educational material. Concerns regarding the appropriateness of testing and test characteristics (eg, sensitivity and specificity) help to guide system-wide broadcast communications, Question and Answer education documents on the medical center intranet, and prompts in the EHR order entry system. It is noteworthy that questions about clinical serology testing to the laboratory director declined substantially after the first week following go-live. Clinician feedback also factored into the decision to use two-step testing (discussed below).

Our experience indicates that patient interest in being tested played a role in order volumes. This was evident from direct patient queries on the testing, including interest in convalescent plasma studies and obtaining serology testing with regular blood donation. The ordering patterns of SARS-CoV-2 serology testing at our institution would be consistent with an initial phase of ordering heavily driven by patient interest, especially patients from obstetric/gynecology clinics and those who were health care workers and others with a higher risk of occupational exposure. This type of ordering declined over a few months. In this process, outpatient ordering decreased, while inpatient orders increased, becoming a higher percentage of the total. Inpatient ordering was scattered throughout the various adult and pediatric units and did not appear to be the result of any systematic change in clinical practice. To our knowledge, no approved order sets or protocols incorporated SARS-CoV-2 serology testing into inpatient management during the retrospective time frame.

In terms of operational factors, the availability of the assay on the main automated chemistry line meant minimal impact in terms of labor to run the testing. The major effort occurred upfront in terms of validating the assay and installing the DiaSorin Liaison analyzer which ran the secondary assay in our testing algorithm for SARS-CoV-2 antibodies. Unlike SARS-CoV-2 RT-PCR, supply chain issues with the serology reagents were minimal.

One area of ongoing debate is the algorithm for performing SARS-CoV-2 antibody testing.[19,29,52] There are multiple variables to consider including assay antigenic target, type of antibody assay, and assay performance characteristics. We implemented an orthogonal testing approach, with a total antibodies assay performed first and then reflexing to an IgG-specific assay if the total antibodies assay is positive. This approach enhances specificity (addressing a common concern among clinicians) but does lead to a small percentage of patients with total antibodies positive and IgG-specific antibodies negative. Our initial estimates of seroprevalence in May 2020 were between 2.0%, with an expectation that seroprevalence would increase beyond 5% by autumn 2020 (as described in Results and Supplemental Table 1). In this range of prevalence, the orthogonal testing algorithm yields high NPV and PPV for a high percentage of patients, given the known sensitivity and specificity of the serology assays. Only 1.1% of patients had indeterminate results with total antibodies positive and IgG-specific antibodies negative.

The planned introduction of vaccines also adds another variable. Most vaccines in the current pipeline target the spike antigen; thus, assays targeting the spike antigen would be logical choices to assess post-antibody.[44] On the other hand, an assay targeting a different antigen (eg, the Roche total antibodies used in the present study that targets the nucleocapsid antigen) may provide information on natural infection versus immunization. There are also serology assays that will provide quantitative antibody values. To this end, Roche Diagnostics recently received a EUA from FDA for a quantitative assay targeting the SARS-CoV-2 spike protein,[53] and other vendors have similar assays in development. It is currently unclear what clinical value may be provided by quantitative SARS-CoV-2 assays.

As of publication time, we have stayed with the current orthogonal testing strategy but with a plan to evaluate and likely transition to quantitative assays. Although there have been scattered inquiries from clinicians at our institution about using serologic testing targeting the spike protein to test vaccination efficacy, these have not yet translated to changes in

testing. Discussions with infectious disease have reinforced that there is value in having an assay that targets the spike protein (detect vaccination and natural infection) and another that targets another protein such as nucleocapsid (to detect natural infection).

As a final serology-related issue, we also recently encountered the challenge of new travel requirements from China that require both SARS-CoV-2 RT-PCR and IgM serology testing prior to boarding of direct flights.[50] Our medical center is part of a large university that includes many international students and staff, including foreign nationals who are visiting scholars. This requirement was issued without prior warning and impacted travelers who had already set flights to China before encountering this barrier. There are several main challenges with the IgM testing requirement. First, IgM serology assays for viruses in general are notorious for risk of false positives.[54] Second, SARS-CoV-2 IgM assays have not been widely used in the United States and other countries. Thus, prior clinical experience was not available. Third, while detection of IgM theoretically can detect active SARS-COV-2 earlier than IgG antibodies, this difference is likely only several days on average for SARS-CoV-2.[55-57] Finally, the performance characteristics of SARS-CoV-2 IgM assays have generally been inferior to IgG or total antibodies assays.[55,57]

Limitations of our study include data from a single academic center with an overall low prevalence of COVID-19 in spring and summer of 2020. The test volume and patient population were affected by the employee population within the medical center and broader university community. Finally, the choice of assays was influenced by existing testing platforms within our clinical laboratories.

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Supplemental Material

Supplemental material for this article is available online.

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