

Performance of four automated SARS-CoV-2 serology assay platforms in a large cohort including susceptible COVID-19 negative and COVID-19 positive patients

Authors: Matthew D. Ward, Kristin E. Mullins, Elizabeth Pickett, VeRonika Merrill, Mark Ruiz, Heather Rebeck, Show-Hong Duh, and Robert H. Christenson

Department of Pathology, University of Maryland School of Medicine, Baltimore, MD

Running Title: SARS-CoV-2 serology assay discordance

Key words: serology, automated platforms, COVID-19, SARS-CoV-2

Abbreviations: US Food and Drug Administration, FDA; Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2; Real-time reverse transcriptase polymerase chain reaction, RT-PCR; Corona virus disease 2019, COVID-19; Nucleic acid amplification testing, NAAT; Enzyme-linked immunosorbant assay, ELISA; Immunoglobulin G, IgG; Immunoglobulin M, IgM; Immunoglobulin A, IgA; Spike, S; Nucleocapsid, N; Emergency Use Authorization, EUA.

Corresponding Author:
Robert Christenson, PhD, DABCC, FACB, FACC
University of Maryland School of Medicine
22 S. Greene Street
Baltimore, MD 21201
Telephone: (410) 328-8672
E-mail: rchristenson@umm.edu

Word limit 250 (currently 250)

ABSTRACT

Background. Anti-SARS-CoV-2 serological responses may have a vital role in controlling the spread of the disease. However, the comparative performance of automated serological assays has not been determined in susceptible patients with significant co-morbidities.

Methods. In this study, we used a large number of COVID-19 negative patient samples (n=2030) as well as COVID-19 positive patient samples (n=112) to compare the performance of four serological assay platforms; Siemens Healthineers Atellica IM Analyzer, Siemens Healthineers Dimension EXL Systems, Abbott ARCHITECT, and Roche cobas.

Results. All four serology assay platforms exhibited comparable negative percent agreement with negative COVID-19 status ranging from 99.2-99.7%, and positive percent agreement from 84.8-87.5% with positive real-time reverse transcriptase polymerase chain reaction (RT-PCR) results. Of the 2142 total samples, only 38 samples (1.8%) yielded discordant results on one or more platforms. However, only 1.1% (23/2030) of COVID-19 negative cohort results was discordant whereas discordance was 10-fold higher for the COVID-19 positive cohort at 11.3% (15/112). Of the total 38 discordant results, 34 were discordant on only one platform.

Conclusion. Serology assay performance was comparable across the four platforms assessed in a large population of COVID-19 negative patients with relevant comorbidities. The pattern of discordance shows that samples were discordant on a single

assay platform, and discordance rate was 10-fold higher in the COVID-19 positive population.

Impact statement. High negative percent agreement reinforces the reliability of serology testing especially in a cohort of at-risk patients. Serology platform discordance highlights the importance of a two-test strategy for properly identifying seroconverted patients.

Word limit 3,500 (*currently 2,244*)

INTRODUCTION

The COVID-19 pandemic has challenged the capacity and capabilities of national medical systems and has highlighted the importance of laboratory and diagnostic testing to control the spread of the disease through timely diagnosis and robust contact tracing. Nucleic acid amplification testing (NAAT) detecting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is central to identifying individuals with active infection and a COVID-19 diagnosis (1). To enhance surveillance efforts and control the virus spread, serological testing has emerged as an opportunity to identify patients who may have been exposed to the virus, who have recently recovered from an infection whether or not they were symptomatic, or to assess or identify the durability of immune responses (2).

The rapid emergence of serological assays has also challenged, and often out-paced, regulatory agencies as well as our understanding of COVID-19 serological responses.

The quality and performance of these assays was initially unknown, leading the US Food and Drug Administration (FDA) to generate a “removed” test list, for all assays considered for an emergency use authorization (EUA), but demonstrating poor clinical

performance (3). The overall utility of the assay, regardless of the manufacturer, has also been challenged, due to lack of supporting data for the proposed applications and the value of the test (4). These questions surrounding test utility and assay performance remain unresolved.

Many serological tests have entered the healthcare market, most employing an immunoassay sandwich method using a SAR-CoV-2 envelope protein as an antigen ‘bait’ to detect immunoglobulins. The bait for these assays is often either recombinant-derived ectodomain of the spike (S) protein or the nucleocapsid (N), which is used as antigen to bind IgG, IgA, and/or IgM in a patient sample. Previous studies using commercially-available ELISA kits, have shown that both S and N proteins have near equivalent performance characteristics in detecting serological responses (5).

The present study was designed to compare head-to-head the performance characteristics of four automated analyzers, using a large and diverse population of patients. The study population was carefully selected to include a large number of COVID-19 positive patients (n=93; total samples, n=112) confirmed by reverse-transcriptase real-time polymerase chain reaction (RT-PCR), as well as a large group of COVID-19 negative patient samples (n=2030), collected in the United States (USA) prior to October 2019 and diagnosed with a respiratory or cardiovascular disorder that would otherwise increase risk for COVID-19 mortality (6).

METHODS

Study design and patient cohort. Study samples belonged to two general groups, a COVID-19 negative group (Table 1) collected in the USA prior to October 2019 (March 2009 to September 2019), the onset of the global pandemic in the USA, or a COVID-19

positive group (Table 2), diagnosed based on recent RT-PCR test. Both cohorts included remnant samples from patient plasma collected in lithium heparin collection containers. To compare assay agreement using a COVID-19 negative cohort, 1 mL frozen aliquots were withdrawn from a sample bank maintained by the study sponsor (Siemens). Samples were identified based on an associated diagnosis or condition causing dyspnea. To compare assay agreement using a COVID-19-positive cohort, remnant samples were collected from patients treated at the University of Maryland Medical Center with a diagnosis of COVID-19 and deidentified under IRB protocol HP-00092112. Samples were designated as positive based on a recent positive SARS-CoV-2 RT-PCR test. Due to logistical constraints including limited supply of reagents, several RT-PCR testing platforms and assays were used in the care and management of the COVID-19 positive patients (Supplemental Table 1). These platforms were validated, and shown to provide comparable results.

Analysis using four serology assay systems. Four testing platforms were used to compare serology assay performance including the Siemens Healthineers Atellica IM (SARS-CoV-2 Total (COV2T) Assay; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) (referred to as Siemens Atellica), Siemens Healthineers Dimension EXL (SARS-CoV-2 Total Antibody (CV2T) assay; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) (referred to as Siemens EXL), Abbott ARCHITECT (SARS-CoV-2 IgG assay; Abbott Laboratories, Abbott Park, IL, USA), and Roche cobas (Elecsys Anti-SARS-CoV2 assay; Roche Diagnostics GmbH, Mannheim, Germany) ((7–10) summarized in Table 3). All instruments were operated and maintained according to each respective manufacturer’s operations manual. Quality control and calibration materials

were prepared using the Instruction for Use document, and control results were within acceptable ranges prior to acceptance of study samples.

Sample handling. Specimens used in this study were frozen prior to use. Frozen specimens were thawed, mixed thoroughly, then centrifuged to remove particulates prior to testing. All specimens were assayed on the same freeze thaw cycle and assayed in parallel on each of the four platforms in singlicate.

Serology results interpretation. Serology results were reported as dichotomous outcomes, either ‘positive’ or ‘negative’ based on manufacturer determined threshold values. Results were reported as ‘positive’ if specimen values exceeded 1.0 index value (Siemens Atellica), 1000 qual units (Siemens EXL), 1.4 index value (ARCHITECT), and 1.0 index value (Cobas).

Statistical analysis. Assay performance was compared head-to-head using a paired Chi-squared analysis (McNemar’s test). Other statistical analysis, descriptive statistics and confidence intervals were generated using GraphPad Prism statistical software (San Diego, California USA).

Ethics statement. Sample collection and study design were approved by the University of Maryland Medical Center Institutional Review Board.

RESULTS

Sample collection and patient population. The COVID-19-negative group included 2,030 unique patient samples (850 female, 1147 male, 33 not indicated; median age 69 years), including a range of pulmonary and cardiac diseases identified as conditions of breathlessness (Table 1). The COVID-19-positive group included 112 samples from 93

unique patients (41 female, 53 male; median age 55) (Table 2). Repeat samples from 18 patients were used in the COVID-19 positive group, however, these samples were drawn on different days following their first SARS-CoV-2 RT-PCR positive result.

Head-to-head comparison of serological assay performance. Each specimen was analyzed on each platform in parallel and their results were aggregated to include combined COVID-19 positive and COVID-19 negative groups (Table 4). The only statistically significant difference in assay performance by paired chi-squared analysis were the Abbott ARCHITECT and the Roche cobas platforms with a total of 22 discordant results; 5 negative serology results and 17 positive results on the Abbott ARCHITECT platform showed opposing results on the Roche cobas system. Although not statistically significant, the highest overall number of discordant results was observed when comparing the Abbott ARCHITECT and the Siemens EXL systems with a total of 31 discordant results out of 2142 samples, and the lowest discordance was observed when comparing the Siemens Atellica with the Roche cobas system with only 11 discordant results out of 2142 samples. Discordance was apparent between all platforms, including 22 samples for Abbott ARCHITECT vs Roche cobas, 25 samples for Abbott ARCHITECT vs Siemens Atellica, as compared to 13 samples for Siemens EXL vs Roche cobas, and 12 samples for Siemens EXL vs Siemens Atellica.

Negative and positive agreement and discordance of serology results. When compared to patient SARS-CoV-2 status, all platforms exhibited comparable agreement, with negative percent agreement from 99.2-99.7%, and positive percent agreement from 84.8-87.5% (Table 5). In the COVID-19 negative group, the Siemens Atellica and Roche cobas platforms had the highest percent negative agreement, and in the COVID-19

positive group, the Siemens Atellica had the highest percent positive agreement with SARS-CoV-2 positive RT-PCR test result.

The apparent pattern of discordance between the four platforms was perceptibly unique to each instrument (Table 6). Of the 38 discordant samples, 34 samples reported discordant results by only one platform with the other three platforms in agreement, without regard for SARS-CoV-2 status, either positive or negative for COVID-19. The four discordant samples reporting two positive results from the Siemens Atellica and EXL platforms and two negative results from the Roche cobas/Abbott ARCHITECT platforms were all from the COVID-19 positive group (see samples 1075, 1080, 1025-2, 1039). Notably, three discordant results (3/42; 7.1%) were near their respective cut-off values for the instruments; however, the accompanying concordant results were well above or below the imprecision of the instruments at the cut-off values.

It is important to note samples 1025-1 and 1025-2 were from the same patient, both reported discordant serology results, however the samples drawn 0-6 days post first positive RT-PCR test was only serology positive in the Siemens EXL, and a second positive serology result was only apparent in the Siemens Atellica from a sample drawn 7-14 days post-first positive RT-PCR test. The Abbott ARCHITECT and Roche cobas platforms were both negative at both time points.

DISCUSSION

In this study, we reported serology testing of the largest cohort of COVID-19 negative, high risk patients with significant co-morbidities causing dyspnea which has not been previously shown. This large study population demonstrated a high degree of negative agreement in serological responses across the four commercial platforms tested. Central

to this portion of the study was the inclusion of plasma samples collected prior to October 2019, intentionally including a population of COVID-19 negative patients with conditions of breathlessness, a group of co-morbidities that would otherwise have a higher mortality risk during the on-going pandemic (6). We have also shown a high degree of positive agreement across these serology platforms as compared to a recent COVID-19 diagnosis. Seroconversion and antibody responses to infection are of vital interest in vaccination efforts and understanding effectiveness and persistence of immunity. This work joins several studies in describing the performance characteristics of rapidly emerging SARS-CoV-2 serological assays (11,12,21,13–20).

Comparison with other studies.

By comparison, most similar studies have used a considerably smaller COVID-19 negative cohort from a collection of historic patient samples simply termed pre-COVID-19 or pre-pandemic samples without regard for co-morbidities (12,15–17,21), or from healthy individuals (19). The benefit of using pre-pandemic samples allows for definitive identification of any apparent false positives, since the virus was not known to exist prior to the year 2019. Other studies have collected contemporaneous patient samples during the pandemic with an associated negative RT-PCR test (18,20). We believe the use of COVID-19 negative patient samples with specific high-risk co-morbidities is most representative of patients likely to receive a COVID-19 associated test, including SARS-CoV-2 RT-PCR or SARS-CoV-2 serological test, during the pandemic. Without regard for cohort selection criteria, the majority of the platforms compared in these studies demonstrate sensitivities (positive percent agreement) and specificities (negative percent

agreement) greater than 90%, using their select populations of COVID positive and COVID negative study participants.

Another important consideration in selection of COVID-19 negative samples is cross-reactivity from similar viral respiratory infections or other coronaviruses. Several studies have previously shown minimal or no cross-reactivity with these other infectious diseases (15,17,19,21). *That said, these issues will be studied systematically when the manufacturers seek FDA clearance or approval for their assays.*

Considerations and limitations. We avoided comments on specificity and sensitivity of the assay performance since a “gold standard” for SARS-CoV-2 serology has not been established. Therefore, discordant results were not considered false positive or false negatives for the purposes of this study, even though the FDA has applied a standard in which positive percent agreement is used as a surrogate for sensitivity and negative percent agreement for specificity (22). It is important to note that RT-PCR positive COVID-19 patients with an undetectable serological response may always result as a ‘false negative’ using this scheme(12). For this reason, the Center for Disease Control (CDC) does not recommend using serology testing to diagnose previous SARS-CoV-2 infection (23).

The cause of disagreement between serological testing and RT-PCR is summarized in CDC guidance for the use and interpretation of serological testing (23). A negative serology test may not preclude a previous infection, as some infected patients may never develop antibodies. And, a positive serology test may not indicate a previous or current infection, because these antibodies may reflect an infection with a different virus from the same family of viruses.

Similar studies have analyzed how serological sensitivity changes over time using serial blood draws from the same patients (11–13). One study has shown higher positive agreement between assays >14 days post first-positive RT-PCR test (13). One head-to-head comparison study employed two assays both targeting the SARS-CoV-2 nucleocapsid (N) antibodies, displaying similar degrees of assay platform discordance with serial samples from the same patients (12). Future studies investigating the complete serological response using multiple platforms and serial blood draws will identify if there is, or is not, a time point of convergence for positive agreement in these assays.

Implications. These results suggest that a two-test strategy to serology testing using two systems will improve overall agreement with RT-PCR results, as suggested previously (20). Using this two-test strategy, a single positive result would indicate a positive result, increasing positive percent agreement with a modest decrement in negative percent agreement.

In this study, the detected immunoglobulin sub-type (IgG, IgM, IgA) and the antigen employed as bait, spike (S) or nucleocapsid (N), do not seem to be important factors based on the pattern of discordance observed in this study. Elucidating the explicit cause of discordance among the serology assays is complicated and was not part of this study's design. Because the virus was unknown to exist prior to 2019, the false positive results are thought to be the result of non-specific or cross-reactivity in antibody binding, perhaps with other related viruses. However, it is also possible that other unspecified interferences to each assay's individual reagents, measurement strategy and principles may have contributed to the observed discordance. It is noteworthy that all of the assays included here were available by EUA, and have not yet undergone the full rigor of FDA

clearance or approval that, in part, includes thorough investigation of cross-reactivity and interferences.”

Conclusion. We have completed the largest serology platform comparison to-date including 93 unique COVID-19 positive patients and 2030 COVID-19 negative patients, with samples run in parallel on four different platforms. We have shown a unique pattern of discordance across these platforms, which have implications in assay selection and suggests a two-testing strategy will improve overall agreement and reduce apparent false negative results.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

K.E. Mullins, administrative support, provision of study material or patients; S.-H. Duh, statistical analysis; R.H. Christenson, financial support, administrative support, provision of study material or patients.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: H. Rebeck, University of Maryland Baltimore; R.H. Christenson, *The Journal of Applied Laboratory Medicine*, AACC.

Consultant or Advisory Role: R.H. Christenson, Roche Diagnostics, Quidel, Becton Dickinson, Beckman Coulter, Pixell Medical, Spingotech.

Stock Ownership: None declared.

Honoraria: R.H. Christenson, Roche Diagnostics, Quidel, Becton Dickinson, Beckman Coulter, Pixell Medical, Spingotech.

Research Funding: H. Rebeck, University of Maryland Baltimore; R.H. Christenson, Roche Diagnostics, Quidel, Becton Dickinson, Beckman Coulter, Pixell Medical, Spingotech, Siemens Healthineers.

Expert Testimony: None declared.

Patents: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

References:

1. World Health Organization. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. Vol. 2019, WHO - Interim guidance. 2020.
2. Mayne ES, Scott HL, Semete B, Julsing A, Jugwanth S, Mampeule N, et al. The role of serological testing in the SARS-CoV-2 outbreak. *South African Med J* 2020;110:842–5.
3. FDA. Certain COVID-19 Serology/Antibody Tests Should Not Be Used - Letter to Clinical Laboratory Staff and Health Care Providers | FDA. 2020;2–5. Available from: <https://www.fda.gov/medical-devices/letters-health-care-providers/certain-covid-19-serologyantibody-tests-should-not-be-used-letter-clinical-laboratory-staff-and>
4. Theel ES, Slev P, Wheeler S, Couturier MR, Wong SJ, Kadkhoda K. The role of antibody testing for sars-cov-2: Is there one? *J Clin Microbiol* 2020;58:1–7.
5. Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, et al. Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2. *J Clin Microbiol* 2020;58:1–7.
6. Jordan RE, Adab P, Cheng KK. Covid-19: Risk factors for severe disease and death. *BMJ [Internet]*. 2020;368(March):1–2. Available from: <http://dx.doi.org/doi:10.1136/bmj.m1198>
7. Siemens. SARS - CoV - 2 Total Antibody assay (COV2T). 2020;1–20.
8. Siemens. SARS-CoV-2 Total (COV2T) Assay for the Detection of Total Antibodies to SARS-CoV-2. 2020;Atellica I:1–26. Available from: <https://www.fda.gov/media/138442/download#:~:text=The Atellica® IM SARS,the Atellica® IM Analyzer>.
9. Abbott Laboratories. Abbott ARCHITECT IgG SARS-CoV-2 assay. 2020 [Internet]. 2020;(June):12. Available from: <https://www.fda.gov/media/137383/download>
10. Roche. Elecsys Anti-SARS-CoV-2 assay. Cobas:1–5.
11. Tang MS, Hock KG, Logsdon NM, Hayes JE, Gronowski AM, Anderson NW, et al. Clinical performance of two SARS-CoV-2 serologic assays. *Clin Chem* 2020;66:1055–62.
12. Hubbard JA, Geno KA, Khan J, Szczepiorkowski ZM, de Gijzel D, Ovalle AA, et al. Comparison of two automated immunoassays for the detection of SARS-CoV-2 nucleocapsid antibodies. *J Appl Lab Med* 2021;6:429-40.
13. Favresse J, Eucher C, Elsen M, Tré-Hardy M, Dogné J-M, Douxfils J. Clinical performance of the elecsys electrochemiluminescent immunoassay for the detection of SARS-CoV-2 total antibodies. *Clin Chem* 2020;66:1104–6.
14. GeurtsvanKessel CH, Okba NMA, Igloi Z, Bogers S, Embregts CWE, Laksono BM, et al. An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. *Nat Commun* 2020;11:1–5.
15. Merrill AE, Jackson JB, Ehlers A, Voss D, Krasowski MD. Head-to-Head Comparison of Two SARS-CoV-2 Serology Assays. *J Appl Lab Med*. 2020;1–7.
16. Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. Multi-platform comparison of SARS-CoV-2 serology assays for the detection of COVID-19. *J Appl Lab Med* 2020;5:1324–1336.

17. Harb R, Remaley AT, Sacks DB. Evaluation of three commercial automated assays for the detection of anti-SARS-CoV-2 antibodies. *Clin Chem* 2020;66:1351–3.
18. Plebani M, Padoan A, Negrini D, Carpinteri B, Sciacovelli L. Diagnostic performances and thresholds: The key to harmonization in serological SARS-CoV-2 assays? *Clin Chim Acta* [Internet]. 2020;509(May):1–7. Available from: <https://doi.org/10.1016/j.cca.2020.05.050>
19. Grzelak L, Temmam S, Planchais C, Demeret C, Tondeur L, Huon C, et al. A comparison of four serological assays for detecting anti-SARS-CoV-2 antibodies in human serum samples from different populations. *Sci Transl Med* 2020;12(559).
20. Krüttgen A, Cornelissen CG, Dreher M, Hornef M, Imöhl M, Kleines M. Comparison of four new commercial serologic assays for determination of SARS-CoV-2 IgG. *J Clin Virol* [Internet]. 2020;128(April):104394. Available from: <https://doi.org/10.1016/j.jcv.2020.104394>
21. Tan SS, Saw S, Chew KL, Huak CY, Khoo C, Pajarillaga A, et al. Head-to-head evaluation on diagnostic accuracies of six SARS-CoV-2 serological assays. *Pathology* 2020;(September).
22. Food and Drug Administration U. EUA Authorized Serology Test Performance [Internet]. 2020 [cited 2020 Oct 29]. Available from: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance>
23. CDC. Test for Past Infection | CDC. Centers Dis Control Prev [Internet]. 2020; Available from: <https://www.cdc.gov/coronavirus/2019-ncov/testing/serology-overview.html>

Table 1. Patient demographics and characteristics; COVID-19 negative group.

	n (%)
All patients	2030
Sex, n (%)	
Women	850 (41.8%)
Men	1147 (56.4%)
Not indicated	33 (1.6%)
Age, n (%)	
0-10	6 (0.3%)
11-20	45 (2.2%)
21-30	158 (7.8%)
31-40	142 (7.0%)
41-50	157 (7.7%)
51-60	214 (10.5%)
61-70	344 (16.9%)
71-80	400 (19.7%)
81-90	409 (20.1%)
91-100	103 (5.1%)
Not indicated	52 (2.6%)
Associated diagnosis	
Conditions of breathlessness	
Unspecified, elevated BNP	1266 (62.2%)
COPD	212 (10.4%)
CHF	89 (4.4%)
Pneumonia	60 (3.0%)
SOB	58 (2.9%)
Bronchitis	30 (1.5%)
Cystic Fibrosis	19 (0.9%)
Respiratory failure	19 (0.9%)
Dilated cardiomyopathy	16 (0.8%)
Asthma	4 (0.2%)
Lung cancer	2 (0.1%)
Pulmonary edema	2 (0.1%)
Cough congestion	1 (0.05%)
Lung injury	1 (0.05%)
MI	1 (0.05%)
Pulmonary Congestion	1 (0.05%)
Respiratory distress	1 (0.05%)
Multiple diagnoses; Pneumonia, MI, CHF, and/or COPD	12 (0.6%)
Other Conditions	
Normal	199 (9.8%)
Colorectal cancer	37 (1.9%)

Abbreviations: BNP, B-type natriuretic peptide; COPD, Chronic obstructive pulmonary disease; CHF, Congestive heart failure; SOB, Shortness of breath; MI, myocardial infarction

Table 2. Patient, sample characteristics; COVID-19 positive group.

	n (%)
Number of unique samples, total	112
<i>Days since positive SARS-CoV-2 PCR assay</i>	
0-6 days	62 (55.3%)
7-14 days	16 (14.2%)
15-62 days	34 (30.4%)
Number of unique patients	93
Patients with 2 serial blood draws	17
Patients with 3 serial blood draws	1
Sex,	
Female	41 (43.6%)
Male	53 (56.4%)
Age, years	
0-10	0
11-20	3 (3.2%)
21-30	8 (8.6%)
31-40	16 (17.2%)
41-50	13 (14.0%)
51-60	22 (23.7%)
61-70	17 (18.3%)
71-80	10 (10.8%)
81-90	4 (4.3%)
91-100	0

Table 3. Serology platforms and assays used in comparison study.

Manufacturer	Platform	Assay	Principle of test	Antigen	Ig
Siemens	Atellica	SARS-CoV-2 Total (COV2T)	sandwich chemiluminescent immunoassay (acridinium ester)	Spike (S); S1 receptor binding domain (RBD)	Total Ig (IgG and IgM)
Siemens	EXL	SARS-CoV-2 Total Antibody (CV2T)	sandwich chemiluminescent immunoassay (fluorescein-isothiocyanate; LOCI-reagents)	Spike (S); S1 receptor binding domain (RBD)	Total Ig (IgG and IgM)
Abbott	ARCHITECT	SARS-CoV-2 IgG assay	chemiluminescent microparticle immunoassay (CMIA; acridinium ester)	Nucleocapsid (N)	IgG
Roche	cobas	Elecsys Anti-SARS-CoV2	sandwich chemiluminescent immunoassay (ruthenium complex)	Nucleocapsid (N)	Total Ig (IgG, IgA, IgM)

Table 4. Head-to-head comparison of serological assay performance.

		Method									
		Siemens Atellica		Siemens EXL		Abbott ARCHITECT		Roche Cobas Elecsys			
		+	-	+	-	+	-	+	-		
Method	Siemens Atellica	+	104								
		-		2038							
	Siemens EXL	+	99	7	106						
		-	5	2031		2036					
		<i>p = 0.77283</i>									
	Abbott ARCHITECT	+	96	17	94	19	113				
		-	8	2021	12	2017		2029			
		<i>p = 0.1096</i>		<i>p = 0.2812</i>							
	Roche Cobas Elecsys	+	97	4	95	6	96	5	99		
		-	7	2034	11	2030	17	2024		2043	
		<i>p = 0.54649</i>		<i>p = 0.33198</i>		<i>* p = 0.01902</i>					

Table 5. Negative and positive percent agreement (NPA/PPA) relative to positive/negative SARS-CoV-2 status.

	NPA (CI)	PPA (CI)
Siemens Atellica	99.7% (99.4-99.9%) n = 2024 / 2030	87.5% (80.1-92.4%) n = 98 / 112
Siemens EXL	99.6% (99.2-99.8%) n = 2021 / 2030	86.6% (79.1-91.7%) n = 97 / 112
Abbott ARCHITECT	99.2% (98.7-99.5%) n = 2014 / 2030	86.6% (79.1-91.7%) n = 97 / 112
Roche cobas	99.7% (99.4-99.9%) n = 2024 / 2030	84.8% (77.0-90.3%) n = 95 / 112
Combined agreement for all platforms and SARS-CoV-2 status	98.7% (98.1-99.1%) n = 2004 / 2030	79.5% (79.4-92.4%) n = 89 / 112

Abbreviations: CI, 95% Confidence Interval; NPA, negative percent agreement; PPA, positive percent agreement

Note: NPA = (Neg serology / Neg SARS-CoV-2); PPA = (Pos serology / Pos SARS-CoV-2)

Table 6. Discordant SARS-CoV-2 serology specimens.

Discordant samples in COVID-19 negative group							
Patient ID	Sex	Age	Medical diagnosis / condition of breathlessness	Siemens Atellica	Siemens EXL	Abbott ARCHITECT	Roche cobas Elecsys
PM412180	F	21-30	COPD	Pos	Pos	Neg	Pos
12227238	M	81-90	Unspecified, elevated BNP	Pos	Neg	Neg	Neg
12236951	M	31-40	Unspecified, elevated BNP	Pos	Neg	Neg	Neg
20918128	F	31-40	Normal	Neg	Pos	Neg	Neg
3911P302	M	81-90	Unspecified, elevated BNP	Neg	Pos	Neg	Neg
3911P361	M	51-60	Unspecified, elevated BNP	Neg	Pos	Neg	Neg
12301252	M	71-80	Unspecified, elevated BNP	Neg	Pos	Neg	Neg
PM412119	F	31-40	COPD	Neg	Pos	Neg	Neg
PM412226	M	41-50	COPD	Neg	Neg	Pos	Neg
5530262987	M	51-60	Normal	Neg	Neg	Pos	Neg
5530263144	F	21-30	Normal	Neg	Neg	Pos	Neg
DLS0087086	M	71-80	Unspecified, elevated BNP	Neg	Neg	Pos	Neg
CPTR01-00113	M	51-60	MI & CHF	Neg	Neg	Pos	Neg
895100342	M	81-90	Pneumonia	Neg	Neg	Pos	Neg
38P9059	M	81-90	Unspecified, elevated BNP	Neg	Neg	Pos	Neg
38P9064	M	81-90	Unspecified, elevated BNP	Neg	Neg	Pos	Neg
12229935	F	81-90	Unspecified, elevated BNP	Neg	Neg	Pos	Neg
12233081	M	51-60	Unspecified, elevated BNP	Neg	Neg	Pos	Neg
12237007	M	61-70	Unspecified, elevated BNP	Neg	Neg	Pos	Neg
12330737	M	71-80	Unspecified, elevated BNP	Neg	Neg	Pos	Neg
PM412246	M	51-60	COPD	Neg	Neg	Pos	Neg
12330387	F	81-90	Unspecified, elevated BNP	Neg	Neg	Neg	Pos

PM412190	F	71-80	COPD	Neg	Neg	Neg	Pos
----------	---	-------	------	-----	-----	-----	-----

Discordant samples in COVID-19 positive group							
Patient ID	Sex	Age	Days since 1st PCR, diagnosis	Siemens Atellica	Siemens EXL	Abbott ARCHITECT	Roche cobas Elecsys
1070	F	41-50	0-6 days, COVID-19 positive	Neg	Neg	Neg	Pos
1063	F	61-70	0-6 days, COVID-19 positive	Neg	Neg	Pos	Neg
1099	M	51-50	0-6 days, COVID-19 positive	Neg	Neg	Pos	Neg
1093	F	51-60	0-6 days, COVID-19 positive	Neg	Neg	Pos	Neg
1025-1	M	51-60	0-6 days, COVID-19 positive	Neg	Pos	Neg	Neg
1089	F	61-70	7-14 days, COVID-19 positive	Neg	Pos	Pos	Pos
1069	M	51-60	0-6 days, COVID-19 positive	Pos	Neg	Pos	Pos
1096	M	31-40	0-6 days, COVID-19 positive	Pos	Neg	Pos	Pos
1074	F	51-60	0-6 days, COVID-19 positive	Pos	Neg	Pos	Pos
1075	F	31-40	7-14 days, COVID-19 positive	Pos	Pos	Neg	Neg
1080	F	11-20	0-6 days, COVID-19 positive	Pos	Pos	Neg	Neg
1025-2	M	51-60	7-14 days, COVID-19 positive	Pos	Pos	Neg	Neg
1039	F	31-40	0-6 days, COVID-19 positive	Pos	Pos	Neg	Neg
1036	M	21-30	>14 days, COVID-19 positive	Pos	Pos	Neg	Pos
1020	F	31-40	0-6 days, COVID-19 positive	Pos	Pos	Pos	Neg

0