

Review of Current Advances in Serologic Testing for COVID-19

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ABSTRACT

Objectives: To examine and summarize the current literature on serologic methods for the detection of antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Methods: A literature review was performed using searches in databases including *PubMed*, *medRxiv*, and *bioRxiv*. Thirty-two peer-reviewed papers and 23 preprints were examined.

Results: The studies included lateral flow immunoassay, enzyme-linked immunosorbent assay, chemiluminescence immunoassay, and neutralizing antibody assays. The use of all major SARS-CoV-2 antigens was demonstrated to have diagnostic value. Assays measuring total antibody reactivity had the highest sensitivity. In addition, all the methods provided opportunities to characterize the humoral immune response by isotype. The combined use of IgM and IgG detection resulted in a higher sensitivity than that observed when detecting either isotype alone. Although IgA was rarely studied, it was also demonstrated to be a sensitive marker of infection, and levels correlated with disease severity and neutralizing activity.

Conclusions: The use of serologic testing, in conjunction with reverse transcription polymerase chain reaction testing, was demonstrated to significantly increase the sensitivity of detection of patients infected with SARS-CoV-2. There was conflicting evidence regarding whether antibody titers correlated with clinical severity. However, preliminary investigations indicated some immunoassays may be a surrogate for the prediction of neutralizing antibody titers and the selection of recovered patients for convalescent serum donation.

Key Points

- Current peer-reviewed and non-peer-reviewed studies of serologic methods have diverse information. Understanding the strengths and limitations of this literature is critical in the evolution of clinical applications of serologic testing.
- The use of total antibody or simultaneous IgG/IgM measurements (regardless of method) significantly adds sensitivity to reverse transcription polymerase chain reaction testing protocols early post onset of symptoms and becomes the most accurate diagnostic test at later time points.
- Additional studies are needed to determine if antibody titers correlate with disease severity and whether certain antigen-specific antibodies determined by routine serologic testing may be surrogate markers for the presence of neutralizing antibodies and long-term immunity.

The first cases of coronavirus disease 2019 (COVID-19) were reported in Wuhan, China, in December 2019.¹ As of June 2, 2020, 6,194,533 confirmed cases and 376,320 COVID-19-related deaths have been reported worldwide.² In the Americas, 2,905,432 cases and 163,248 related deaths were confirmed. The first case of COVID-19 in the United States was reported on January 19, 2020, in Washington.³ Five months later, the United States has become one of the most affected regions with 1,783,638 confirmed cases and 104,247 deaths.²

The rate of transmissibility, environmental stability of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the severity of disease in high-risk populations have all contributed to pandemic levels that challenge many health systems. Consequently, understanding and implementing effective evidence-based testing is the cornerstone to correctly identify cases, predict clinical outcomes, and develop treatment strategies. Reverse transcription polymerase chain reaction (RT-PCR) assays used to detect the presence of viral genetic material have become the gold standard of diagnosis. However, RNA extraction

techniques, the brief window of RT-PCR sensitivity post symptom onset, and variable levels of viral load have been demonstrated to lead to false-negative results.^{4,5} Therefore, the implementation of antibody immunoassays is an area of significant interest as an opportunity to increase the accuracy of diagnosis in different scenarios. The current literature describes over 200 immunoassays available worldwide.^{4,6-11} Understanding the strengths and pitfalls of these assays in different clinical and research scenarios is critical to move the utilization of serologic testing for SARS-CoV-2 forward.

Materials and Methods

Databases including PubMed, medRxiv, and bioRxiv were searched for published and preprint papers on COVID-19 and coronavirus serology. Search terminology included COVID-19 terms (COVID-19, SARS-CoV-2, novel corona, 2019-ncov) and serology, antibody, neutralizing antibody, seroconversion, rapid testing, IgG, IgM, IgA, LFD, ELISA, PRNT, chemiluminescence, and immunochromatography.

As of June 2, 2020, a total of 55 papers with performance data of serologic techniques were examined. The assays utilized in these studies included the neutralizing antibody assay, enzyme-linked immunosorbent assay (ELISA), automated chemiluminescence immunoassay (CLIA), and lateral flow immunoassay (LFIA). Thirty-two papers were peer-reviewed publications and 23 preprints were not peer-reviewed (medRxiv, 22; bioRxiv, 1).

The compiled information included the assay method with a focus on the detection of particular isotype(s) of antibody and selection of targeted antigen(s). In addition, the composition of cohorts of samples used in the validation studies was examined with focus on control groups. Details regarding the time of sample acquisition and characteristics of the clinical disease of confirmed SARS-CoV-2 patients were also recorded when available. Lastly, calculated test accuracy and information regarding comparison to other immunoassays and RT-PCR testing was noted. Two independent reviewers from the Department of Pathology and Laboratory Medicine and Department of Hematology at the University of Miami and Jackson Health System extracted the information.

Results

Overview of Immunoassays

The SARS-CoV-2 virus is genetically related to the severe acute respiratory syndrome coronavirus

(SARS-CoV), which emerged in 2002 to 2003 and the Middle East respiratory syndrome coronavirus (MERS-CoV), which was identified in 2012.¹ The genomic sequence of SARS-CoV-2 has approximately 82% homology to SARS-CoV and 89% homology with bat SARS-like-CoVZXC21.¹

SARS-CoV-2 is a large (50-200 nm) positive-sense single-stranded RNA virus with 4 major structural proteins: nucleocapsid protein (NP) holding the viral RNA and 3 envelope structural proteins including the spike protein (SP), envelope protein (EP), and membrane protein (MP).¹² The NP is the most abundant viral protein made and shed during infection. The SP consists of 2 subunits referred to as S1 and S2. S1 contains the receptor-binding domain (RBD) needed for binding to the host angiotensin-converting enzyme 2 (ACE2) receptor.¹² S2 contains elements needed for membrane fusion. MP is the most abundant protein on the virion, and EP is the smallest protein and involved in the assembly and release of virions. The SP, RBD, and NP proteins appear to be the main targets of the humoral immune response in coronavirus infections including SARS-CoV-2 and were the antigens used in the majority of the serologic assays examined in this literature review.¹²⁻¹⁴

Studies varied widely in the detection of the different isotypes of antibody: IgM, IgA, and IgG. Many immunoassays, referred to as total antibody assays, were constructed to detect levels of all isotypes simultaneously. While a few studies presented quantitative data, the large volume of current literature described mostly qualitative and semiquantitative immunoassays.

In the analysis of these studies, it is essential to examine the details of sample size and the patient population(s).^{15,16} The design of a comprehensive validation study to address assay specificity requires the assessment of 3 groups of samples: confirmed SARS-CoV-2 infected patients, confirmed healthy negative controls, and secondary sets of controls from patients with other viral infections and diseases. The latter group should include samples from patients with other human coronavirus infections. For sensitivity data, consideration of the timing of sample acquisition related to the onset of symptoms is crucial. Samples acquired at very early times may represent a period where antibody levels are not present or are below the level of detection. Finally, the performance of the assays will be affected by the technical component of the assays themselves. For LFIA, reactivity is determined by visual inspection of bands on the immunochromatography paper present in single use devices. In the case of the semiautomated ELISA and automated CLIA methods, the quantitation is provided by spectrometry and luminescence detection, respectively.

It is important to note that many non-peer-reviewed manuscripts were included in this review; the reader is advised to search for final refereed versions that may have updated data and discussion points.

Lateral Flow Immunoassay (LFIA)

The variable sensitivity and specificity of LFIA have been the focus of the media and numerous studies due

to the growing number of commercially available devices.^{6,17} Several investigations reported high sensitivity and specificity of these assays although many lacked information regarding the target antigens and well-defined negative control groups (ie, human coronavirus controls).

Table 1 summarizes the review of studies using LFIA methods.^{6,7,18-29}

The experimental design and data of 7 studies were judged to provide insight into LFIA method

Table 1
Studies Reporting the Use of Lateral Flow Immunoassay Methods in the Detection of Antibody to SARS-CoV-2

Antigen	No. of Positive Patient Samples ^a	No. of Negative Patient Samples ^b	No. of Other Samples ^c	Sensitivity, Specificity (%)	Comments	Reagent Manufacturer	Reference
RBD	397	128	ND	89, 91	IgM, IgG	BioMedomics	Li et al ⁷
Unk	30	Unk	Unk	83-93, 100	Variable performance, 32 samples used in specificity were not defined	Acro Biotech, Arton Labs, Autobio, CTK Biotech, Dynamiker, Hangzhou Alltest Biotech	Lassaunière et al ¹⁸
Unk	91	35	ND	82, 100	IgM, IgG	Zhuhai Livzon Diagnostics	Xiang et al ¹⁹
NP	109 ^d	60	14	77, 100	IgM	Zhuhai Livzon Diagnostics	Xiang et al ²⁰
NP	109 ^d	60	14	83, 95	IgG	Zhuhai Livzon Diagnostics	Xiang et al ³⁷
Unk	191 ^d	ND	ND	30, 89	IgM, IgG; PCR+ vs PCR-	Viva-Chek	Paradiso et al ²¹
NP	80	209	ND	86, 100	IgG	Wantai	Lou et al ²²
RBD	80	209	ND	89, 98	IgM	Wantai	
RBD	80	209	ND	98, 95	Total antibody	Wantai	
Unk	90	ND	64 ^e	85,91	IgM, IgG; timeline, severity	Unknown	Liu et al ²³
Unk	86	ND	ND	Sensitivity only: 11 (<7 d), >90 after day 8	IgM, IgG; timeline	Zhuhai Livzon Diagnostics	Pan et al ²⁴
Unk	76	ND	ND	Sensitivity only: IgM 50%, IgG 92%	Examined serology vs PCR in timeline post symptom onset	Beijing Innovita Biological Technology	Yong et al ²⁵
SP + NP	93	ND	9-44	87-89, 81-100	IgM, 2 devices examined	Cellex, Orient Gene	Geurtsvankessel et al ²⁶
SP + NP	93	ND	9-44	84-92, 85-100	IgG, 2 devices examined	Cellex, Orient Gene	
NP	93	ND	64	88, 74	IgM	Intec	
NP	93	ND	64	95,77	IgG	Intec	
Unk	128	72	ND	69-72, 96-100	IgM, IgG, 3 devices	LabOn Time, Avioq, QuickZen	Montesinos et al ²⁷
Multiple	77	60	40	Specificity 98-100	IgM, IgG timeline for sensitivity	Wantai, Hangzhou All Biotech	Traugott et al ²⁸
Multiple	167	ND	103	Specificity 97-100	IgM, IgG timeline for sensitivity	Clungene, OrientGene, VivaDiag, StrongStep, Dynamiker, Multi-G, Prim	Van Elslande et al ²⁹
Multiple	130	108	52	81-100, 84-100	IgM, IgG timeline	BioMedomics, Bioperfectus, DecomBio, DeepBlue, Innovita, Premier, Sure, UCP, VivaChek, Wondfo	Whitman et al ⁶

ND, not done; NP, nucleocapsid protein; RBD, receptor-binding domain; RT-PCR, reverse transcription polymerase chain reaction; SP, spike protein; Unk, unknown.

^aPositive RT-PCR results for SARS-CoV-2.

^bHealthy controls.

^cInclusion of banked samples from patients with other viral infections including other human coronaviruses.

^dCohort included some samples from probable COVID-19 patients based on clinical presentation and other test results.

^eInclusion of samples from patients with autoimmune diseases.

applications. Li et al⁷ evaluated a combined IgM/IgG LFIA using the RBD antigen in a sample set obtained from 397 positive patients defined by positive RT-PCR and COVID-19 symptoms and 128 negative patients. The assay sensitivity was 89% and the specificity was 91%. Notably, 64% of the samples from the positive patient group demonstrated both IgM and IgG reactivity. Lou et al²² reported the use of 3 different LFIA: IgM and total antibody using RBD antigen and IgG detection using NP antigen. Using a large group of COVID-19–negative patient samples (n = 209) but not a control group of samples from patients with other viral infections or diseases, an excellent specificity ranging from 95% to 100% was reported for all 3 devices. Notably, the sensitivity of the total antibody LFIA had the highest performance at 98%. Also, the performance of all the assays compared well with similarly constructed and validated ELISA in the same study. Using 147 control samples from patients with various viral infections and 93 samples from COVID-19 patients, Geurtsvankessel et al²⁶ reviewed 3 LFIA including the Cellex. This device uses a combination of SP and NP antigens. The sensitivity and specificity were calculated as 87% and 81% for IgM detection and 84% and 85% for IgG detection, respectively. Interestingly, the Orient LFIA, also constructed with the same 2 antigens, showed a sensitivity of 89% for IgM detection and 92% for IgG detection. The assay specificity for each isotype was 100%, but it should be noted that the latter study tested a very small sample set of negative controls. Another study utilized samples from both negative patients and patients with other viral infections and examined 6 LFIA devices.¹⁸ Although no target antigen information was presented, the patient sample sets were robust. While evaluation of 2 of the LFIA demonstrated a poor performance, the remaining devices demonstrated 83% to 93% sensitivity and 100% specificity values. Lastly, Traugott et al²⁸ presented data regarding 2 LFIA with specificity ranging from 98%–100%, but sensitivity was reported as poor at less than 5 days post onset of symptoms (13%–20%), poor to moderate at days 6 to 10 (20%–80%), and excellent at or after day 11 (100%).

One study examined the accuracy of 10 different LFIA and 2 ELISA immunoassays using samples obtained from COVID-19 patients at different time points of disease.⁶ Specificity was assessed using 108 samples from healthy subjects and 52 samples from patients with other respiratory illnesses (not specified). The highest detection rate observed using LFIA devices was obtained with combined IgM and IgG detection. Accuracy peaked at days 16 to 20 post onset of symptoms with sensitivity results greater than 80%

and specificity results greater than 95%. Notably, this study reported several false-positive results obtained from banked specimens collected before the pandemic. These false-positive results suggest nonspecific binding by plasma proteins or cross-reactivity with antibodies produced during other viral infections. In a separate study of 7 LFIA devices, Van Elslande et al²⁹ also used a secondary set of 103 control samples to assess assay performance and reported a range of 97% to 100% specificity with the combination of positive IgM and IgG results but an 85% to 98% range of specificity when only IgM or IgG positive results were used. In their study, sensitivity was also reported to vary with the timing of sample acquisition post onset of symptoms.

LFIA is an appealing platform for SARS-CoV-2 testing with a relatively low cost per test and the advantage of potential use for point-of-care testing.³⁰ LFIA described in the literature thus far provided results for both IgM and IgG. The majority of the reports demonstrated a higher sensitivity and specificity with the detection of both isotypes, and a higher specificity was observed with the detection of the IgG isotype over the detection of the IgM isotype alone. Antigens frequently used in LFIA include NP, RBD, and combined use of NP and SP. A superior test accuracy based on the use of specific target antigen(s) was not evident at this time. Studies utilizing samples obtained at various time points demonstrated the accuracy of LFIA was optimal at approximately 2 weeks post onset of symptoms. Overall, the understanding of the limitations or advantages for the implementation of LFIA-based assays is severely impacted by the presence of only a few properly designed studies in the current literature (Table 1).

Enzyme-Linked Immunosorbent Assay (ELISA)

Publications that described ELISA method validation often sought to address which antigens and antibody isotypes provided the best sensitivity and specificity (Table 2).^{6,18,19,22,26-28,31-40} In many of these studies, these calculations were reported solely to compare the performance of different antigens or the detection of different isotypes and not necessarily to propose the use of the assay for clinical implementation. Additionally, as noted in Table 2, many investigations utilized the ELISA method to study the timeline of antibody expression in SARS-CoV-2 patients.

To date, few investigations have focused on immunoassays utilizing the full SP antigen.^{31,34} The SP antigen includes the RBD antigen as well as other essential peptides that may be the target of the humoral immune response. Amanat et al³¹ generated 2 different versions of the

Table 2

Studies Reporting the Use of Enzyme-Linked Immunosorbent Assay Methods in the Detection of Antibody to SARS-CoV-2

Antigen	No. of Positive Patient Samples ^a	No. of Negative Patient Samples ^b	No. of Other Samples ^c	Sensitivity, Specificity (%)	Comments	Reagent Manufacturer	Reference
SP, RBD	16	0	50	ND	Also isotype expression	Lab-developed test	Amanat et al ³¹
RBD	30	10	72	93, 100	Total antibody	Wantai	Lassaunière et al ¹⁸
S1	30	10	72	67, 96	IgG	Euroimmun	
S1	30	10	72	93, 93	IgA	Euroimmun	
NP	208 ^d	150	140	ND	IgM, IgG, IgA timeline	Lab-developed test	Guo et al ³²
RBD	161	213	ND	93, 99	Total antibody timeline	Lab-developed test	Zhao et al ³³
RBD	143	213	ND	83, 99	IgM timeline	Lab-developed test	
NP	112	197	ND	65, 99	IgG timeline	Lab-developed test	
SP, S1, NP, RBD	41	76	192	ND ^e	IgG and IgA, PRNT	Euroimmun and lab-developed tests	Okba et al ³⁴
NP	214	100	ND	80, 100	IgM, IgG timeline	Lizhu	Liu et al ³⁵
RBD	214	100	ND	82, 100	IgM, IgG timeline	Hotgen	
NP	80	100	ND	89, 100	IgG	Wantai	Lou et al ²²
RBD	80	300	ND	98, 100	Total antibody	Wantai	
RBD	80	300	ND	93, 100	IgM	Wantai	
NP	238	120	ND	82, 94	vs PCR results, timeline	Lizhu	Liu et al ³⁶
NP + RBD	12	6	ND	ND	Also quantitative titers	Lab-developed test	Ni et al ³⁷
NP	16	ND	ND	Sensitivity: 94 IgG, 88 IgM	IgM, IgG	Lab-developed test	To et al ³⁸
RBD	16	ND	ND	Sensitivity: 100 IgG, 94 IgM		Lab-developed test	
Unknown	63	35	ND	87, 100	Compared to LFIA	Zhuhai Livzon Diagnostics	Xiang et al ¹⁹
RBD	76	ND	150	99, 99	Total antibody	Wantai	Geurtsvankessel et al ²⁶
RBD	76	ND	150	89, 99	IgM	Wantai	
S1	43	ND	161	82, 99	IgG	Euroimmun	
S1	76	ND	161	97, 94	IgA	Euroimmun	
S1, RBD	77	60	40	Specificity 83 (IgA), 97-98 (others)	IgA, IgG, IgM, total antibody; sensitivity presented by timeline	Euroimmun, Wantai	Traugott et al ²⁸
RBD	130	108	52	>80, >95	IgM/IgG 16 d after symptoms	Epitope Diagnostics, lab-developed test	Whitman et al ⁶
S1	128	10	72	84, 88	IgG, IgA, timeline	Euroimmun	Montesinos et al ²⁷
S1	69	412	ND	97, 98	Also asymptomatic study	Lab-developed test	Zhao et al ³⁹
SP, NP	130	16	ND	ND	IgG and prognosis	Lab-developed test	Sun et al ⁴⁰

LFIA, lateral flow immunoassay; ND, not done; NP, nucleocapsid protein; PRNT, plaque reduction neutralization test; RBD, receptor-binding domain; RT-PCR, reverse transcription polymerase chain reaction; SP, spike protein.

^aPositive RT-PCR results for SARS CoV-2.

^bHealthy controls.

^cInclusion of banked samples from patient with other viral infections including other human coronaviruses.

^dCohort included some samples from probable COVID-19 patients based on clinical presentation and other test results.

^eAddressed in study but no formal calculations.

SARS-CoV-2 spike protein. One construct expressed the full length of SP and a second construct presented only the RBD. Evaluating a small number of samples from RT-PCR–positive patients, reactivity to both antigens was excellent as demonstrated by optical density values. However, SP reactivity was significantly higher than that observed for RBD. IgG3, IgM, and IgA were the dominant isotypes observed in these patient samples. The investigators further reported that reactivity to a sample

bank of sera obtained from patients with other human coronavirus infections was negligible.

Okba et al³⁴ produced an array of different ELISA to examine antibody reactivity to SP, S1, NP, and RBD antigens. A large cohort of samples was obtained from patients with other viral respiratory infections and was inclusive of 116 banked samples from patients infected with various human coronaviruses. Only samples from some SARS-CoV and MERS-CoV patients cross-reacted with

the SARS-CoV-2 antigens. The RBD and NP ELISA were the most effective in the detection of antibodies in patients with mild infection. These 2 antigens were examined in a second study using samples obtained from a cohort of 16 patients obtained 14 days or longer after symptom onset.³⁸ The RBD ELISA showed higher sensitivity (100% for IgG and 94% for IgM) compared to the NP ELISA (94% for IgG and 88% for IgM).

Okba et al³⁴ also examined commercial ELISA using the S1 antigen for the detection of IgA and IgG antibodies. Both ELISA showed some cross-reactivity with samples obtained from other human coronavirus positive patients. Overall, the IgA ELISA displayed a higher sensitivity and the IgG ELISA displayed a higher specificity.

Lassaunière et al¹⁸ evaluated ELISA constructed with RBD and S1 antigens using a control group of samples from patients with other non-SARS-CoV-2 viral infections. The performance of a sandwich ELISA for the detection of total antibody reactivity to RBD was superior to that observed for the commercial IgG and IgA ELISA using the S1 antigen. The ELISA for total antibody detection provided the highest sensitivity and specificity of 93% and 100%, respectively. The specificity of the IgG and IgA assays were similar, but the IgA ELISA had a superior sensitivity of 93% vs 67% for IgG. The authors reported a similar cross-reactivity of the commercial S1 ELISA to other human coronaviruses as observed by Okba et al.³⁴

Zhao et al³³ also utilized a sandwich ELISA to measure total antibody reactivity to RBD antigen as well as a RBD targeted ELISA to measure IgM reactivity alone and a NP targeted ELISA to detect IgG reactivity. Examining a cohort of over 300 patient samples, the total antibody assay had superior performance vs the other assays with a sensitivity and specificity of 93% and 99%, respectively. While the specificity of the IgM and IgG ELISA was 99%, the sensitivity of the IgG ELISA (NP) was 65% compared to the IgM ELISA (RBD) at 83%.

Whitman et al⁶ evaluated 2 different ELISA and 10 different LFIA. In their study, the sensitivity for the ELISA assays using samples obtained after day 16 post onset of symptoms was greater than 80% and the specificity was greater than 95%. The agreement of the ELISA with different LFIA ranged from 75 to 95%.

Overall, ELISA assays with RBD and NP antigens were the most frequently used in these early studies. Although the sensitivity of the assays was affected by the timing of sample acquisition, a higher overall sensitivity was consistently observed with the use of total antibody detection. The ELISA technique is labor intensive and unsuitable for point-of-care testing; however, it may offer the advantage of determining antibody titers

and selective isotype detection. The importance of these options as clinical applications is unknown at this time. Overall, while several ELISA-based studies were robust in experimental design, many reports did not include examination of important control samples to best evaluate method specificity (Table 2).

Direct Chemiluminescence Immunoassay (CLIA)

The use of CLIA in the detection of SARS-CoV-2 antibody is of particular interest, as this method has excellent sensitivity with a high signal-to-noise ratio in the detection of other viral infections. CLIA uses recombinant antigen coated magnetic beads and a lumigen substrate with analysis on automated platforms. In current reports, NP, combined NP and SP, and RBD antigens were most commonly used **Table 3**.^{8,22,26,27,41-46}

Many studies utilized CLIA to address differences in patient populations and time of onset of symptoms vs serologic results. Long et al⁴² reported the use of the NP and SP antigen combination in a study of 363 samples from confirmed and suspect COVID-19 patients. In their report, IgG-positive serostatus approached 100% at 20 days after onset of symptoms, and the median seroconversion for IgM and IgG was 13 days. Using a similar NP and SP assay but a smaller cohort of positive and negative patients, Jin et al⁸ reported a 48% sensitivity and 100% specificity for IgM detection. However, for IgG, the reported sensitivity was 89% and specificity was 91%. Accuracy was higher for combined IgM and IgG testing with a posterior probability of greater than 99%; this was followed by IgG alone (90%) and IgM alone (86%) and was corroborated in a separate study.⁴⁷

Five studies warranted a closer examination, as each included control samples from patients with non-SARS-CoV-2 infections. Lin et al⁴³ used NP antigen to detect IgM and IgG reactivity. Sensitivity for both IgM and IgG assays was reported as 82%, and the specificity was 81% for IgM detection and 98% for IgG detection. Ma et al⁴⁴ compared RBD and NP antigen targeted CLIA and reported a higher sensitivity and specificity when using the RBD antigen. A superior sensitivity and specificity were also demonstrated with the detection of IgA over IgM or IgG detection alone. In addition, the use of IgA in tandem with the detection of either IgM or IgG increased assay sensitivity and specificity. Geurtsvankessel et al²⁶ examined a total antibody assay for S1/S2 reactivity and reported a sensitivity of 73% and a specificity of 98%. Bryan et al⁴⁶ validated an IgG antibody assay using NP antigen with a specificity of 100% based on the analysis of banked samples originally submitted for HSV testing. This study also

Table 3

Studies Reporting the Use of Chemiluminescence Immunoassay Methods in the Detection of Antibody to SARS-CoV-2

Antigen	No. of Positive Patient Samples ^a	No. of Negative Patient Samples ^b	No. of Other Samples ^c	Sensitivity, Specificity (%)	Comments	Reagent Manufacturer	Reference
NP + SP	228	225	222	ND	IgM and IgG	Shenzhen YHLO Biotech	Zhang et al ⁴¹
NP + SP	363 ^d	ND	ND	ND	IgM and IgG	Bioscience	Long et al ⁴²
NP	79	29	51	82, 81	IgM	Lab-developed test	Lin et al ⁴³
NP	79	29	51	82, 97	IgG	Lab-developed test	
NP + SP	43	33	ND	48, 100	IgM	Shenzhen YHLO Biotech	Jin et al ⁸
NP + SP	43	33	ND	89, 91	IgG	Shenzhen YHLO Biotech	
RBD	80	300	ND	96, 99	Total antibody	Xiamen InnoDx Biotech	Lou et al ²²
RBD	80	300	ND	86, 99	IgM	Xiamen InnoDx Biotech	
RBD	216	330	153	97, 100	IgG	Lab-developed test	Ma et al ⁴⁴
NP	216	ND	20	96, 100	IgG	Lab-developed test	
RBD	216	330	153	97, 92	IgM	Lab-developed test	
NP	216	ND	20	78, 95	IgM	Lab-developed test	
RBD	216	330	153	99, 98	IgA	Lab-developed test	
NP	216	ND	20	90, 85	IgA	Lab-developed test	
S1/S2	53	ND	69	74, 99	Total antibody	DiaSorin	Geurtsvankessel et al ²⁶
NP + SP	54	180	55	100, 99	IgM and IgG at day 15	Diazyme	Suhandynata et al ⁴⁵
Unknown	122	10	72	64, 100	IgM and IgG	Maglumi	Montesinos et al ²⁷
NP	689	ND	1,020	53-100, 100	IgG	Abbott, sensitivity examined over time	Bryan et al ⁴⁶

ND, not done; NP, nucleocapsid protein; RBD, receptor-binding domain; SP, spike protein.

^aPositive RT-PCR results for SARS CoV-2.

^bHealthy controls.

^cInclusion of banked samples from patient with other viral infections including other human coronaviruses.

^dCohort included some samples from probable COVID-19 patients based on clinical presentation and other test results.

included 689 samples from confirmed COVID-19 patients. The assay sensitivity increased from 53% at day 7 to 100% at day 17. Lastly, Zhang et al⁴¹ used a large cohort of samples and showed an area under the curve of 0.99 and 1.00 for IgM and IgG, respectively, using a combined NP and SP CLIA.

The CLIA assay is traditionally considered a very sensitive method with the capability to detect low levels of antibodies. CLIA assays are automated and allow for a high throughput of samples. In the current literature review, RBD and NP were the most commonly targeted antigens, and the studies detected IgM and IgG isotypes as well as total antibody reactivity. The use of RBD antigen and IgG detection produced the highest accuracy data among studies. It is important to consider that the variation in performance among different platforms may have been related to sample acquisition timing, which was not consistently reported in the studies.

Practical Implementation of Serologic Testing

Diagnosis in Symptomatic Patients

RT-PCR testing for viral detection is a frontline tool to detect patients with suspected SARS-CoV-2 infection. However, several studies showed that the sensitivity of RT-PCR testing decreased over time post onset of

symptoms and that this change was observed to be concurrent with the increasing sensitivity of antibody detection methods.

Guo et al³² evaluated the use of a NP targeted ELISA using IgM, IgA, and IgG detection in a sample set of 82 RT-PCR confirmed cases and 58 suspected cases with negative RT-PCR. Early in disease (5 days post onset of symptoms), IgM detection was positive in 93% of suspected cases with negative RT-PCR results and 76% of the RT-PCR confirmed cases. Overall, the sensitivity of RT-PCR testing alone was 52%; however, when using RT-PCR in combination with the ELISA, the total sensitivity increased to 99%. Similar results were observed in another study where combined detection of IgG and IgM identified over 70% of suspected cases that were negative by RT-PCR testing.³⁸

As the course of the disease progresses, the utility of serology increases as well. Zhao et al³³ evaluated the sensitivity of a total antibody ELISA to the RBD antigen. In the early (1-7) days post onset of symptoms, the sensitivity was 67% for RT-PCR; this increased to 79% when RT-PCR was combined with ELISA testing. The sensitivity for RT-PCR decreased to 54% at days 8 to 14 while the ELISA sensitivity increased to 90%. At this time point, the use of both methods resulted in a sensitivity of 97%. Lastly, on days 15 to 39, RT-PCR sensitivity was reported as 46%, and the

ELISA sensitivity was 100%. Similar trends were also observed with an IgM RBD ELISA and IgG NP ELISA.

Serology Use in Monitoring Disease Course

Numerous studies indicated that antibody responses may vary according to disease severity, and some reports proposed that monitoring titers may be applied in clinical practice to guide earlier aggressive treatment. Traditionally, the hallmarks of a humoral immune response include the early expression of IgM isotype, which then matures into IgG isotype expression. Notably, many reports of SARS-CoV-2 patients indicated that IgM expression was observed concurrently with IgG expression. Long et al⁴² conducted a large multicenter study using an NP and SP targeted CLIA. The median seroconversion of both isotypes was recorded at day 13. In addition, a different NP and SP targeted CLIA studied by Suhandyanata et al⁴⁵ showed a median seroconversion on days 4 to 5 for IgM and IgG.

The goal of several ELISA studies was to define the period of seroconversion, and all demonstrated the higher sensitivity of the assays by the second week post onset of symptoms.^{20,28,32,33} Zhao et al³³ reported that the median seroconversion time of IgM and IgG to RBD antigen was days 12 and 14, respectively. Guo et al³² reported the median appearance of IgM and IgA at day 5 and IgG at day 14 post symptom onset. Their study used serial samples obtained from the same patients and an ELISA for antibody detection to the NP antigen. Xiang et al²⁰ also used a NP targeted ELISA and reported the median appearance of antibody at day 4 post onset of symptoms. Overall, while these results are similar to that reported in a review of serologic testing for MERS-CoV and SARS-CoV, they may have been affected by choice of target antigens, the various immunoassay kits, and the level of detail of case history used to categorize the time of sample acquisition post onset of symptoms.¹⁴

Few studies have examined the differences in titers by case severity classification, and there was no consensus among the findings. Tan et al⁴⁹ reported that the IgG and IgM detection occurred earlier for severe cases compared to nonsevere cases ($P < .05$). Higher titers were also observed in the former group. Wang et al⁵⁰ evaluated the titers of IgM and IgG in 116 confirmed cases of which 101 of the cases had mild to moderate disease and 15 patients died. Levels of IgM were significantly higher in deceased patients ($P = .019$). However, no significant correlation was observed between case outcome and IgG titers. In contrast, a large study of 338 confirmed COVID-19 patients reported elevated IgM titers but lower IgG levels in critical cases.⁵¹ Ma et al⁴⁴ evaluated IgA titers in a cohort

of 216 patients and observed a significant correlation with disease severity ($P < .0001$) and peak levels of IgA 16 to 20 days after symptom onset. Sun et al⁴⁰ reported significantly higher IgG titers to SP antigen in non-ICU patients, whereas IgG titers to NP antigen were elevated in ICU patients. Lastly, To et al,³⁸ using NP and RBD targeted ELISA observed no correlation between titers and severity.

Previously, quantification performed by ELISA and neutralizing antibody assays showed that individuals over 60 years of age had higher antibody titers than young healthy adults with human coronavirus (non-SARS-CoV-2) infection.⁵² Notably, in a study of patients recovered from COVID-19, samples obtained from elderly and middle-aged individuals had higher titers of SP reactive antibody compared to young adults.⁵³ Thus, while increased age is often associated with severe SARS-CoV-2 disease and poor outcomes, high levels of antibody production do not appear to be detrimental given these preliminary reports.

Serology Use for Screening Asymptomatic Patients

There were limited reports of testing approaches for the detection of asymptomatic individuals. Much of the current literature was weakened given the limitations in the experimental design of studies used to validate the serologic methods. For example, Paradiso et al⁵⁴ used an LFIA for IgM and IgG detection to screen 525 health care workers. Previously, this group reported an overall 30% sensitivity and 89% specificity obtained during the validation of this LFIA. It was acknowledged that the reduced sensitivity was related to the selection of validation samples from patients early after the onset of symptoms.²¹ In the health care worker screening study, only 1.1% of the cases were seropositive; these cases tested negative by RT-PCR. All the cases were positive for IgM, and 1 case was positive for both IgM and IgG. Another study from the same investigation group examined asymptomatic cases presenting to the emergency department.²¹ Twenty nine percent of the cases were positive by RT-PCR testing and 21% of the cases were positive by LFIA testing. As the LFIA was not fully validated, it is problematic to apply these results to develop screening strategies for asymptomatic patients.

Bendavid et al⁵⁵ used LFIA in a study of 3,330 individuals from Santa Clara County, CA, and found an unadjusted antibody prevalence of 1.5% (95% confidence interval [CI], 1.11%-1.97%). The weighted population prevalence was 2.8% (95% CI, 2.24%-3.37%).⁵⁵ The kit was validated using samples from confirmed negative and positive SARS-CoV-2 patients with a combined IgM/IgG

sensitivity of 80% and specificity of 99%. No secondary controls with non-SARS-CoV-2 viral infections were included in the validation study. As a result, this study likely overestimated the specificity of this LFIA.

Zhao et al³⁹ used a lab-developed ELISA for the detection of IgG antibody to S1 antigen with reported excellent sensitivity and specificity, as determined by the use of samples from symptomatic hospitalized patients and healthy individuals. In a subsequent study, the same ELISA was examined in 276 asymptomatic health care workers. Ten percent of health care workers had seropositive results. Samples from a small cohort of close contact individuals were also examined. All subjects tested negative by RT-PCR, but 1 individual was seropositive. As with the study by Bendavid et al,⁵⁵ the specificity of the ELISA assay was not fully assessed; this complicates the interpretation of these results.

A report of serologic testing of a family cluster of 6 individuals provided interesting data regarding the complexity of test sensitivity.⁵⁶ Two family members were hospitalized with SARS-CoV-2 symptoms. One individual tested positive by RT-PCR on presentation, and the second patient tested negative twice before obtaining a positive result on day 5. Both individuals were positive for IgM reactivity by ELISA. The 4 remaining family members remained asymptomatic. However, 3 of 4 tested positive for IgM reactivity, and 2 of 3 were positive by RT-PCR testing at later dates. The fourth individual remained clinically asymptomatic and was negative on all testing.

Implementation of serology testing to screen the general population and asymptomatic health care workers is currently of significant interest. Nonetheless, the available evidence is limited to support its use in these scenarios. With unclear population prevalence and the use of immunoassays that are not fully validated, the limitations of test sensitivity and specificity in the evaluation of asymptomatic individuals may be difficult to overcome.⁵⁷

Utility for Possible Convalescent Serum Donors

Many studies focused on the detection of neutralizing antibodies for the potential use as a predictor of clinical outcome and in the identification of convalescent serum for use in a treatment strategy. Several of these studies evaluated the correlation of data from ELISA using RBD, S1, S2, and NP antigens to the presence of neutralizing antibodies. Wu et al⁵³ studied neutralizing antibodies using a pseudotyped-lentiviral-vector-based assay using plasmids for SARS-CoV and SARS-CoV-2 SP protein. Titers of neutralizing antibodies were reported as ID50 (highest dilution resulting in 50% reduction of

luciferase luminescence). Parallel ELISA for SARS-CoV and SARS-CoV-2 RBD, S1, and S2 were conducted. The study samples were obtained from 175 patients with mild to moderate symptoms who recovered from COVID-19. Levels of neutralizing antibodies were low (ID50, <200) before day 10 and appeared at 10 to 15 days after the onset of symptoms. Titers persisted at similar levels on repeat testing 2 weeks later. The levels of neutralizing antibodies in the patients were categorized as follows: low to mid in 17% (ID50, 500-999), mid to high 39% (ID50, 1,000-2,500), and high in 14% (ID50, >2,500). Samples from 30% of patients had very low levels (ID50, <500) and included samples from 10 patients who had reactivity below the level of detection (ID50, <40). Results obtained from ELISA using RBD, S1, and S2 antigens correlated moderately with neutralizing antibody titers. Correlation coefficients ranged from 0.42 to 0.51 ($P < .0001$). Ni et al³⁷ examined RBD and NP ELISA in tandem with the pseudovirus neutralization test in cohorts of 6 patients tested at the time of discharge and 2 weeks later. Significant levels of IgM and IgG were present at both time points as determined by ELISA methods. Notably, while neutralizing antibody was detected at the time of discharge, levels were lower in 5 of 6 patients 2 weeks later.

Okba et al³⁴ compared the results of IgA and IgG ELISA for RBD, S1, and NP to data obtained from a plaque reduction neutralization assay (using Vero E6 cells) and reported a significant correlation ($r = 0.88$, $P < .01$) with all ELISA. The strongest correlation was observed with IgA reactivity ($r = 0.93$, $P < .001$). It is important to note that various ELISA in this study were validated with a large secondary sample set obtained from patients with other respiratory viral infections (Table 2).

The identification of novel antigenic epitopes that may be important in the humoral immune response was the focus of a limited number of studies. Poh et al¹³ evaluated the reactivity of 25 convalescent serum samples. Six samples showed significant neutralization activity (ID50, >600) and were selected for further analysis. Additional experiments to characterize antigen targets showed that the S14 and S21 peptides (within the SP) provided the strongest reactivity by ELISA methods, and these results correlated with neutralizing activity. Jiang et al⁵⁸ developed a microarray of 28 SARS-CoV-2 proteins to profile IgG/IgM responses of 29 convalescent sera. All patients had combined IgM/IgG responses to NP and S1 antigens but not to S2 antigen. Furthermore, a significant number of samples were positive for anti-ORF9b and anti-NSP5 antibodies. These peptides may represent good predictors for immunity and possible therapeutic targets. Overall, understanding the utility of routine serologic methods (ie, ELISA, CLIA) in the prediction of convalescence is

complex. Further comprehensive studies in this area are warranted.

Discussion

Numerous immunoassays for the detection of antibodies to SARS-CoV-2 are rapidly emerging. These immunoassays have the potential to improve the diagnosis and monitoring of infection in different scenarios.^{17,59-61} Published and non-peer-reviewed studies varied dramatically in the definition of patient groups, time of sample acquisition, sample size, and inclusion of relevant control sera from patients with non-SARS-CoV-2 respiratory infections. These are all critical variables that will affect the sensitivity and specificity of the different immunoassays and should be a fundamental part of the review of any validated assay.¹⁵ Determining the final roles of LFIA and ELISA immunoassays in SARS-CoV-2 testing and research is difficult at this time, as this is limited by the pitfalls in the experimental design of many of these foundational studies. Further well-validated assays determined through studies with rigorous experimental design are needed.

In the current literature, the study methods were heterogeneous regarding the specific antigens used and the different isotypes of antibodies measured; few studies compared these variables simultaneously. The most common antigens used in the assays included RBD, S1, and NP. There was preliminary evidence that the use of particular target antigens may provide value to increase the sensitivity of antibody detection methods. However, additional comprehensive studies need to be conducted to reproduce this early reported data. In addition, antigenic epitopes within the SP appeared to be important in the immune response and thus, this region remains of interest for future detailed studies.

There is compelling evidence that using total antibody or combined IgG/IgM detection offered the highest sensitivity of detection. Data from preliminary studies indicated that additional investigations should examine the clinical correlation of different isotypes and titers to disease severity. It is also clear that the timing of sample acquisition is a crucial determinant of test accuracy, although this important information was not always clearly presented in the current literature.¹⁶ The earliest positive results were reported by day 5 post onset of symptoms, and accuracy peaked by the second week of symptoms. Early in the course of the disease, when RT-PCR sensitivity was reported as 50% to 60%, the concomitant use of serologic tests

significantly added sensitivity with consistently reported values over 90%. Moreover, after 10 to 14 days post onset of symptoms, the sensitivity of RT-PCR dropped significantly while serology testing reached its peak. As fully validated methods become commercially available, serology methods may be utilized as an adjunct tool to RT-PCR testing protocols in patients with suspected infection.

Nearly all of the current literature focused on the results obtained using serologic testing in symptomatic patients. It will be essential to define antibody responses in individuals with subclinical and mild disease before immunoassays can be used in screening and seroprevalence studies. At the time of this writing, while the potential importance of quantitative titers was raised in the literature, testing platforms available to provide this information are largely absent from clinical laboratories. Lastly, defining convalescence and the presence of long-lasting immunity to SARS-CoV-2 after infection or future vaccination is important. Additional studies are needed to determine if ease-of-use assays of antibody detection and quantitation will compare well with traditional neutralizing antibody assays. While serologic testing continues to hold promise for various applications, there are still knowledge gaps that must be clarified to give meaningful recommendations for its use in different clinical scenarios.

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