Immunologic Testing for SARS-CoV-2 Infection from the Antigen Perspective

Dandan Li,[a,b] Jinming Li[a,b,c]

[a]National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing, People's Republic of China
[b]Graduate School, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, People's Republic of China
[c]Beijing Engineering Research Center of Laboratory Medicine, Beijing, People's Republic of China

ABSTRACT Coronavirus disease 2019 (COVID-19), caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread globally as a severe pandemic. SARS-CoV-2 infection stimulates antigen-specific antibody responses. Multiple serologic tests have been developed for SARS-CoV-2. However, which antigens are most suitable for serological testing remains poorly understood. Specifically, which antigens have the highest sensitivity and specificity for serological testing and which have the least cross-reactivity with other coronaviruses are currently unknown. Previous studies have shown that the S1 domain of the spike (S) protein has very low cross-reactivity between epidemic coronaviruses and common human coronaviruses, whereas the S2 domain of the S protein and the nucleocapsid protein (N protein) show low-level cross-reactivity. Therefore, S1 is considered more specific than the native homotrimer of the S protein, and the receptor-binding domain as an antigen to test patient antibodies is more sensitive than the native N protein. In addition, an increasing number of studies have used multiantigen protein arrays to screen serum from convalescent patients with COVID-19. Antigen combinations demonstrated improved performance compared to each individual antigen. For rapid antigen detection, the sensitivity of the test is higher in the first week of onset of the disease with high viral loads. Highly sensitive and specific immunological diagnostic methods for antibodies or those that directly detect viral antigens in clinical samples would be beneficial for the rapid and accurate diagnosis of SARS-CoV-2 infection.

KEYWORDS COVID-19, SARS-CoV-2, antigen, cross-reaction, serology testing

Coronavirus disease 2019 (COVID-19), caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread globally as a severe pandemic (1). A key aspect of limiting this virus transmission is to ensure early and accurate diagnosis of the viral infection and appropriate quarantine measures for those infected.

There are four main methods currently available for the detection of SARS-CoV-2 infection. The first is virus isolation by inoculation of the patient's biological samples into cell cultures, such as Vero cell cultures, which requires biosafety level 3 laboratory facilities. The second is molecular techniques such as reverse transcription-PCR (RT-PCR), loop-mediated isothermal amplification, clustered regularly interspaced short palindromic repeats (CRISPR), and high-throughput sequencing, which is a powerful tool for the discovery of pathogens. The third is serological testing—antibody detection by enzyme-linked immunosorbent assay (ELISA), immunofiltration and immunochromatography tests, and chemiluminescent immunoassays (2). The last is antigen detection with specific monoclonal antibodies to the SARS-CoV-2 antigen.

RT-PCR has become the current standard diagnostic method for SARS-CoV-2. However, RT-PCR has high requirements for operators and laboratory conditions. The cost, complexity, and turnaround time limit its applications. Therefore, there is an urgent need for a rapid, simple, sensitive, and accurate test to quickly identify patients infected with SARS-CoV-2 at the initial stage, especially when RT-PCR results are difficult to obtain, prevent virus transmission, and ensure the timely treatment of patients (3).

The major structural proteins of SARS-CoV-2 are the spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins (4). The S protein is located on the surface of the viral particles and is, thus, potentially more accessible to the immune system. For coronavirus, N protein primarily encapsidates the viral genome and packages the viral genomic RNA to form the helical N and plays important roles in viral replication, virus particle assembly, release, and interference with the cell cycle processes of host cells. SARS-CoV-2 infection stimulates antigen-specific antibody responses. Serological tests are anticipated to function as a complementary approach for diagnosis. The N protein has high immunogenic activity and is abundantly expressed during infection in many coronaviruses. Both S and N proteins may be potential antigens for the serodiagnosis of COVID-19, as many diagnostic methods have been developed for diagnosing SARS-CoV-2 infection based on S and/or N proteins. Liu et al. found that the recombinant N and S proteins worked as antigens in the ELISA, which is an important screening method for COVID-19 diagnosis with high sensitivity, especially for the analysis of serum samples from patients after more than 10 days post-disease onset (d.p.o.) (5).

It has previously been reported that the seroprevalence of common human coronaviruses (HCoVs) increases throughout childhood to nearly 100% by adolescence (6). Antigens are important factors for immunological testing, and antigen choice is a significant element that can influence the performance of the assay, especially when detecting cross-reactive antibodies induced by previous infections with HCoVs. Any antibody cross-reactivity between common HCoVs and SARS-CoV-2 would result in false-positives, interfering with antibody-based testing and surveillance for SARS-CoV-2. Any serologic methodology for estimating the prevalence of SARS-CoV-2 needs to identify and reduce cross-reactivity with these common HCoVs. Therefore, it is important to choose the antigens for higher sensitivity and specificity. However, which antigens are most suitable for serological testing remains poorly understood. Specifically, which antigens have the highest sensitivity and specificity for serological testing, which have the least cross-reactivity with other coronaviruses, how the structural protein of SARS-CoV-2 is evaluated as an antigen, and whether the direct detection of viral antigens in clinical samples is a more sensitive immunological diagnostic method for the rapid and accurate diagnosis of COVID-19 are currently unknown. To investigate these areas, in this review, we summarize recent studies and provide a brief summary of the antigen performance of serologic assays, comment on which antigen is more suitable for detecting SARS-CoV-2 antibodies, and explain why antigen-based SARS-CoV-2 assays may have clinical significance.

ANTIGEN SELECTION

Among the four structural proteins (S, E, M, and N) of SARS-CoV-2, S and N proteins are the main immunogens, as described previously (7). Patients with COVID-19 usually produce antibodies against different antigens. The diagnostic performances of the corresponding antibody detection reagent methods are different.

Spike protein. S protein (a homotrimer) is a large (approximately 140 kDa) glycoprotein that consists of two subunits (S1 and S2) and forms a trimer on the viral membrane (8). Similar to SARS-CoV, the S glycoprotein on the surface of SARS-CoV-2 mediates membrane fusion and receptor recognition of the virus. The S protein is cleaved between the S1 and S2 subunits (S1/S2 cleavage site). The S1 subunit at the N-terminal region is responsible for virus attachment and contains the receptor-binding domain

TABLE 1 Percentage amino acid identity of coronavirus spike and nucleocapsid proteins with SARS-CoV-2 proteins

Virus	GenBank accession no.	Amino acid identity (%) for:				
		S	S1	RBD	S2	N
SARS-CoV	NC_004718.3	77	66	75	90	90
MERS-CoV	NC_019843.3	33	24	19	43	49
HCoV-OC43	NC_006213.1	33	25	21	42	34
HCoV-HKU1	NC_006577.2	32	25	19	40	34
HCoV-229E	NC_002645.1	30	24	19	35	28
HCoV-NL63	NC_005831.2	28	21	20	36	-

(RBD), which directly binds to the angiotensin converting enzyme 2 (ACE2) receptor on the host cell (1). S2 contains other basic elements required for membrane fusion.

(i) The use of S protein to establish an antibody detection method and cross-reaction with other human coronaviruses. As HCoV-OC43, -229E, -NL63, and HKU1 are responsible for a large proportion of common cold infections each year, cross-reactivity between SARS-CoV-2 and these seasonal coronaviruses is of particular importance and warrants further investigation.

Table 1 shows the percentage of amino acid identity of the coronavirus S and N proteins with SARS-CoV-2 proteins. The SARS-CoV-2 S protein has the highest amino acid homology with the SARS-CoV S protein. The amino acid homology between SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV), HCoV-OC43, -HKU1, -229E, and -NL63 is 33%, 33%, 32%, 30%, and 28%, respectively (9). Seo et al. verified the performance of the SARS-CoV-2 spike antibody by ELISA, and the results revealed that the antibody binds to the SARS-CoV-2 S protein but not the MERS-CoV S protein or bovine serum albumin, which confirms that the antibody is specific for the SARS-CoV-2 S protein and, thus, suitable for detecting SARS-CoV-2 (10). However, in the study of Hicks et al. (11), they found that there existed potential cross-reactivity of SARS-CoV-2 IgG antibodies with MERS, SARS-CoV, OC43, and HKU1 S protein, as well as for the sequence and structural homology of SARS-CoV-2 and SARS-CoV S protein. There also exists cross-reactivity between SARS-CoV to other viruses. A previous study analyzed the cross-reactivity of SARS-CoV and human coronaviruses. Of 20 patients with confirmed SARS CoV infection, the positive serum antibody of 12 patients reacted with OC43 and 229E (12). Collectively, the SARS-CoV-2 S protein antibody may have cross-reaction with other human coronaviruses.

Okba et al. analyzed three patients diagnosed with SARS-CoV-2 infection; positive serum was collected from 13 to 21 days after the onset of the disease. SARS-CoV-2, SARS-CoV, and MERS-CoV recombinant S and S1 proteins were coated as antigens for the ELISA. The results showed that there was cross-reactivity with the SARS-CoV S and S1 proteins, and to a lower extent with MERS-CoV S protein, but not with MERS-CoV S1 protein (9). Compared with the amino acid homology of the SARS-CoV-2 S1 subunit with the other six human-pathogenic coronaviruses, the SARS-CoV-2 S2 subunit has higher amino acid homology with the other six human-pathogenic coronaviruses. A previous study showed that the antibody profiles of local residents include high immunoglobulin G (IgG) reactivity to common cold coronaviruses with low-level cross-reactivity with S2 domains from SARS-CoV-2 and other epidemic coronaviruses, which is not surprising given the high degree of sequence homology among the S2 domains of SARS-CoV, MERS, and common cold coronaviruses HKU1 and OC43. The results showed that the lowest specificity was observed for SARS-CoV-2 S2 for IgG detection (13). Khan et al. used a coronavirus antigen microarray measured by mean fluorescence intensity to analyze the cross-reactivity between common HCoVs and SARS-CoV-2 and found that the S1 domain of the S protein, including the RBD, demonstrates very low cross-reactivity between epidemic coronaviruses and common HCoVs, whereas the S2 domains of the S protein show low-level cross-reactivity between these coronavirus

subtypes (14). Overall, the S2 subunit is more conserved and, thus, plays a role in the cross-reactivity seen when the whole S protein is used as an antigen.

The RBDs of SARS-CoVs, which bind to the ACE2 receptor on the host cells, are also major targets of human antibodies. The RBD amino acid sequence identities between SARS-CoV-2 and SARS-CoV, MERS, OC43, HCoV-HKU1, -229E, and -NL63 are 75%, 19%, 21%, 19%, 19%, and 20%, respectively. As the RBD is a common target of human antibodies and is poorly conserved between SARS-CoVs and other pathogenic HCoVs, this domain is a promising candidate for use in antibody-based diagnostic assays.

The recombinant SARS-CoV-2 RBD antigen is highly specific for the detection of antibodies induced by SARS-CoVs. To evaluate the specificity of SARS-CoV-2 RBD for serology, Premkumar et al. collected sera from 20 healthy American adults before the SARS-CoV-2 pandemic. The sera had high levels of antibodies to the recombinant RBDs of HCoV-NL63 patients ($n = 19$) and HCoV-HKU1 patients ($n = 16$), but not to those of patients with SARS-CoV and SARS-CoV-2. To further assess the specificity of the SARS-CoV-2 RBD for serology, the authors obtained two HCoV-NL63, one HCoV-43, and two HCoV-HKU1 sera from patients who had recently recovered from a laboratory-confirmed common cold HCoV infection. None of the immune sera from those exposed to recent HCoV infections cross-react with the recombinant RBD of SARS-CoVs (15).

Nucleocapsid protein. The N protein (molecular weight [mol. wt.], ~40 kDa) is composed of 413 amino acids and can be intertwined with the viral genome RNA to form a viral N protein. It participates in viral genome replication and particle assembly and plays an important role in the synthesis of viral RNA. It is the most abundant viral phosphoprotein produced, is shed during infection, and has strong immunogenicity. Early infection can induce the body to produce a high level of immune response. N protein exhibits high immunogenicity and can be detected in either serum or urine samples during the first 2 weeks of infection, with peak viral shedding approximately 10 days after infection (16).

(i) Use of N protein to establish an antibody detection method and cross-reaction with other human coronaviruses. N proteins of known coronaviruses are relatively conserved. As shown in Table 1, SARS-CoV-2 N protein has the highest amino acid homology with SARS-CoV N protein. The amino acid homology between SARS-CoV-2 and MERS-CoV, HCoV-OC43, -HKU1, and -229E is 49%, 34%, 34%, and 28%, respectively. Approximately, 90.0% of amino acid sequence homology was observed with SARS-CoV, which may explain the cross-reactivity between the two viruses.

Guo et al. evaluated the potential cross-reactivity of N proteins between SARS-CoV-2 and other HCoVs. The results showed that there was no cross-reactivity of SARS-CoV-2 recombinant N protein with human plasma positive for IgG antibodies against HCoV-NL63, -229E, -OC43, and -HKU1. However, strong cross-reactivity was observed between SARS-CoV-positive human plasma and SARS-CoV-2 recombinant N protein by Western blot analysis and ELISA (35). Okba et al. used SARS-CoV-2 N protein as the coated antigen and two serum samples from patients with SARS and seven serum samples from patients with MERS for ELISA detection. The results showed that two serum samples from the patients with SARS and one from a patient with MERS reacted with the SARS-CoV-2 N protein (9). The serum positive for SARS- and MERS-CoV could react with the SARS-CoV-2 N protein.

Since the SARS-CoV-2 N protein has over 90% homology to the SARS-CoV N protein, the conserved residues at the N-terminal domain of the N protein show a high degree of similarity. A highly conserved motif (FYYLGTGP) occurs in the N-terminal for half of all coronavirus N proteins, and other conserved residues have been reported to occur near this highly conserved motif. Chen et al. found that the COOH terminus, which is an important region of the SARS-CoV N protein, has a higher immunogenicity than the NH$_2$ terminus (17). To reduce the cross-reaction of the whole N protein in SARS-CoV with other coronaviruses and high rates of false positivity while testing healthy donor sera, a truncated SARS-CoV N protein (ΔN-NP)-based ELISA system using recombinant

techniques was used. The ΔN-NP is devoid of homogenous conserved residues at the N-terminal region.

Yu et al. analyzed serum samples collected from 175 healthy volunteers in Vietnam before the SARS outbreak. When the N protein (amino acids 5 to 422) was used as the coating antigen, 38 of the 175 serum samples showed titers higher than 100, ranging from 100 to 3,200. In contrast, when the SARS-CoV ΔN-NP (amino acids 122 to 422, NΔ121 protein) was used, the highly conserved motif (FYYLGTGP) was deleted. Only 11 out of 175 samples showed weak reactions, with titers ranging from 100 to 260. The results showed that ΔN-NP is more specific than the whole-length N protein. The false-positive results may be due to cross-reactivity of SARS CoV with existing antibodies to other circulating coronaviruses (18). In addition, the recombinant NΔ121 protein-based ELISA is more sensitive and can detect seroconversion earlier than the virus-infected cell lysate-based ELISA system. Another study by the same team investigated patients using recombinant truncated SARS-CoV N protein as the antigen; the median seroconversion time using the newly developed immunoglobulin M (IgM) capture-ELISA was 3 days earlier, and the seroconversion rate by the second week after the illness for IgM was significantly higher than with the IgG assay (19).

Similarly, the ΔN-NP showed better performance in SARS-CoV-2 antibody tests. Yamaoka et al. stated that ΔN-NP is more suitable than whole-length N protein for developing high-sensitivity diagnostic assays for COVID-19. They collected samples from 70 healthy donors before the outbreak of COVID-19, and whole-length N protein showed higher false positivity than ΔN-NP. In the time course analysis, the ΔN-NP ELISA detected lower levels of antibodies that began to increase from the second week onward, whereas whole-length N protein ELISA at the same cutoff would have falsely reported these as negative (20).

In summary, cross-reactivity of the SARS- and MERS-CoV serum was positive for SARS-CoV-2 N protein. Caution should be used while interpreting assay results when the full-length recombinant N protein of SARS-CoV-2, whole-virus antigen extracts, or virus-infected cells are used as reagents for the diagnosis of SARS-CoV-2 infections in humans. The SARS-CoV-2 ΔN-NP protein-based IgG ELISA is a more reliable, specific, and sensitive test for the diagnosis of SARS-CoV-2 infection even though there is still some low-level cross-reactivity.

Comparison of performance of S and N proteins as immunogens for detecting antibodies. It is important to select an appropriate antigen that can improve the sensitivity and specificity of antibody detection. Compared to S, M, and E proteins, antibodies against N proteins are longer-lived and occur in greater abundance than antibodies against other viral components. Previous studies analyzed the sera from 148 healthy blood donors as controls and 95 patients with SARS-CoV pneumonia to detect IgM and IgG by ELISA. The sensitivities were 58.9% and 74.7%, respectively. The sensitivity of the N proteins IgG ELISA (94.7%) was significantly higher than that of the S protein IgG ELISA. The difference is speculated to be due to the relatively late IgG response to the S protein compared to the IgG response to the N protein in patients with SARS (21).

Similar conclusions were drawn in the study of SARS-CoV-2. Several studies conducted for the comparison of sensitivity and specificity of the S and N proteins are shown in Table 2. Burbelo et al. analyzed PCR-positive patient samples collected >14 days after the onset of symptoms. Seropositive N protein antibodies were detected in all samples, yielding both a sensitivity and specificity of 100%. The S protein antibody showed a slightly lower sensitivity of 91% (32/35) with 100% specificity, but this was not statistically different from that of the N antibody. The authors also evaluated the samples collected <14 days after symptom onset, and the sensitivity was reduced, but specificity was maintained. The sensitivity for antibodies to the N and S proteins at this time point was 51% (33/65) and 43% (28/65), respectively. Among five immunocompromised patients with COVID-19, two were negative for the S protein antibody but positive for the N protein antibody. The antibody to the N protein of SARS-CoV-2 is more sensitive than the S protein antibody for detecting early infection (22).

TABLE 2 Selected comparison of published sensitivities and specificities of the S and N proteins used in antibody assays[a]

Protein	Method	d.p.o.[b]	Sensitivity	Specificity (%)	Manufacturer and reference
S	ELISA	0–55	77.1 (165/214) for IgM 74.3 (159/214) for IgG		Lizhu, Zhuhai, China; 5
N	ELISA	0–55	68.2 (146/214) for IgM 70.1 (150/214) for IgG		Lizhu, Zhuhai, China; 5
S	Luciferase immunoprecipitation systems	≤14	43 (28/65)	100	22
N	Luciferase immunoprecipitation systems	≤14	51 (33/65)	100	22
S	Luciferase immunoprecipitation systems	≥15	91 (32/35)	100	22
N	Luciferase immunoprecipitation systems	≥15	100 (35/35)	100	22
RBD	ELISA	1–7	38.3 (36/94) for total antibody 28.7 (27/94) for IgM	99.1 (211/213) for total antibody 98.6 (210/213) for IgM	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 23
N	ELISA	1–7	19.1 (18/94) for IgG	99.0 (195/197) for IgG	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 23
RBD	ELISA	8–14	89.6 (121/135) for total antibody 73.3 (99/135) for IgM	99.1 (211/213) for total antibody 98.6 (210/213) for IgM	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 23
N	ELISA	8–14	54.1 (77/135) for IgG	99.0 (195/197) for IgG	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 23
RBD	ELISA	15–39	100.0 (90/90) for total antibody 94.3 (83/90) for IgM	99.1 (211/213) for total antibody 98.6 (210/213) for IgM	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 23
N	ELISA	15–39	79.8 (71/90) for IgG	99.0 (195/197) for IgG	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 23
RBD	ELISA	0–7	64.1 (25/39) for total antibody 33.3 (13/39) for IgM	100.0 (300/300) for total antibody 100.0 (300/300) for IgM	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 3
N	ELISA	0–7	33.3 (13/39) for IgG	100.0 (300/300) for IgG	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 3
RBD	ELISA	8–14	98.7 (74/75) for total antibody 86.7 (65/75) for IgM	100.0 (300/300) for total antibody 100.0 (300/300) for IgM	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 3
N	ELISA	8–14	76.0 (57/75) for IgG	100.0 (300/300) for IgG	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 3
RBD	ELISA	15–29	100.0 (60/60) for total antibody 94.3 (58/60) for IgM	100.0 (300/300) for total antibody 100.0 (300/300) for IgM	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 3
N	ELISA	15–29	93.3 (56/60) for IgG	100.0 (300/300) for IgG	Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.; 3
RBD	EIA	≥14	100 (16/16) for IgG 94 (15/16) for IgM		24
N	EIA	≥14	94 (15/16) for IgG 88 (14/16) for IgM		24

[a]The enrolled positive cases meet the following criteria: fever and/or respiratory symptoms, abnormal lung imaging findings, and confirmed to be infected with SARS-CoV-2 through real-time RT-PCR from oropharyngeal or nasopharyngeal swabs. All the controls had not reported close contact with any confirmed COVID-19 patient and had negative real-time RT-PCR results.
[b]d.p.o., post-disease onset.

Zhao et al. tested total antibody, IgM, and IgG antibodies against SARS-CoV-2 in plasma samples using ELISA kits. The ELISA for total antibody and IgM detection was developed using the RBD of the S protein of SARS-CoV-2. IgG antibodies were tested using a recombinant N protein. The sensitivity of total antibody and IgM using the RBD antigen was higher than that of IgG using the N protein at 1 to 7, 8 to 14, and 15 to 39 d.p.o. The specificities of the assays for total antibody, IgM, and IgG were found to be 99.1% (211/213), 98.6% (210/213), and 99.0% (195/197), respectively, by testing the samples collected from healthy individuals before the outbreak of SARS-CoV-2 (23). Similarly, using the RBD antigen for total antibody and IgM with N protein as an antigen for IgG, the sensitivity of total antibody and IgM was higher than that of IgG at 0

to 7, 8 to 14, and 15 to 29 d.p.o. The specificities were overall comparable, and all of them were 100%. Total antibody detection was based on double-antigen sandwich methodology, which can detect any type of antibody, including IgM, IgG, and IgA. In addition, the two Fab arms of the same antibody molecule need to bind to the coated and enzyme-conjugated antigens, which guarantees the specificity of the test and allows a high concentration of antigens to be used for coating and second binding to increase the sensitivity of the assay. Therefore, it is not unexpected that the sensitivity of total antibody detection is superior to that of IgM and IgG detection (3).

Another study revealed that at least 10 days after symptom onset, more patients had earlier seropositivity for anti-RBD than anti-N protein for both IgG and IgM. For 16 patients with serum specimens available for 14 days or longer after symptom onset, the rates of seropositivity were 94% for anti-N protein IgG, 88% for anti-N protein IgM, 100% for anti-RBD IgG, and 94% for anti-RBD IgM (24). The data are shown in Table 1. It was concluded that for the RBD antigen for IgG and IgM detection, the sensitivity was higher than that of N protein as an antigen.

In summary, the antibody to the N protein of SARS-CoV-2 is more sensitive than an S protein antibody for detecting early infection. The RBD as an antigen to test the patient antibody is more sensitive than the N protein, and the specificities are overall comparable.

Evaluation of multiple testing and combination-antigen tests. With the development of diagnostic methods, microfluidics-based diagnostic systems have been extensively developed and applied, which integrate sample preparation, reaction, and detection steps into a miniaturized chip. Lin et al. described a point-of-care microfluidic platform integrating a homemade fluorescence detection analyzer that detects three biomarkers (IgG, IgM, and antigens) of SARS-CoV-2. The combination of multiple biomarker detection enhances the accuracy and sensitivity of detection. The multiple-testing platform was demonstrated to be easy-to-use, portable, and highly sensitive for point-of-care detection of SARS-CoV-2 within 15 min. It was approved by the Center for Medical Device Evaluation (CMDE) in China and obtained European CE certification (25).

Furthermore, an increasing number of studies have used multiantigen protein arrays, allowing for high-throughput, multiplexed screening of numerous parameters within a single experiment (13, 14) to screen the serum from convalescent patients with COVID-19 against multiple SARS-CoV-2 antigens simultaneously and rapidly. A coronavirus antigen microarray was constructed containing 65 antigens. The viral antigens printed on this array included SARS-CoV-2, SARS-CoV, MERS-CoV, common cold coronaviruses (HKU1, OC43, NL63, and 229E), and multiple subtypes of the virus. The SARS-CoV-2 antigens on this array included the S protein, RBD, S1 and S2 domains, and N protein. Among the high-performing antigens, four were ranked as high performing for both IgG and IgA—SARS-CoV-2 N protein, SARS-CoV N protein, SARS-CoV-2 S1+S2, and SARS-CoV-2 S2. The receiver operating characteristic curve with area under the curve (AUC), sensitivity, and specificity was calculated for each combination. For IgG, the best discrimination was achieved with the two-antigen combination of SARS-CoV-2 S2 and SARS-CoV N protein (AUC, 0.994; sensitivity, 0.944; specificity, 1). For IgA, the highest performance was achieved with the two-antigen combination of SARS-CoV-2 S1 plus S2 and SARS-CoV N protein (AUC, 0.969; sensitivity, 0.944; specificity, 0.895) (13).

As an individual antigen, S2 demonstrates cross-reactivity with negative-control sera, which leads to low specificity. However, this antigen adds predictive power when combined with the more specific N protein antigen. The coronavirus antigen microarray containing a panel of antigens from SARS-CoV-2 in addition to other HCoVs was able to reliably distinguish convalescent plasma of PCR-positive COVID-19 cases from the negative-control sera collected prior to the pandemic. It is important to inform the selection and design of antigens for population surveillance, clinical diagnostic assays, and vaccine development. Antigen combinations, including both S proteins and N

protein, demonstrated improved performance compared to each individual antigen. The analysis of antibody reactivity to multiple SARS-CoV-2 and related antigens will provide broad insight into the humoral immune response to SARS-CoV-2.

ANTIGEN SYNTHESIS

Antigens and antibodies are two important factors for immunological testing. Obtaining antigens that can be recognized by antibodies is the basis for accurate detection of specific antibodies. SARS-CoV-2 antigens used for antibody detection are usually artificial and mainly prepared using genetic engineering technology. Generally, the gene encoding the amino acid sequence of the antigen is amplified and connected with an appropriate vector by molecular biology technology and then transferred to the recipient cell for expression. The recombinant protein can be used as an antigen after purification. The new coronavirus isolation and culture activities must be carried out in a biosafety level 3 (BSL-3) laboratory. Viral RNA extraction, gene amplification, plasmid construction, cell transfection, and antigen purification can be performed in a BSL-2 laboratory.

Spike protein. Due to the global COVID-19 pandemic, the SARS-CoV-2 S protein has become an important target for clinical investigations, and future studies will require efficient and streamlined production of this protein. Usually, two different versions of the SARS-CoV-2 S protein are developed. One is a full-length trimeric and stabilized version of the S protein, and the other is the much smaller RBD. Stadlbauer et al. stated that the expression levels of the RBD are high (>20 mg/liter culture), whereas the expression levels of the full-length S protein (OptSpike2) are lower (approximately 4 to 5 mg/liter) (26). It was also necessary to validate whether the recombinant S protein and RBD could be utilized in a serum ELISA screen for serology testing at different clinical sites. The basic process of S protein purification is shown in Fig. S1 in the supplemental material.

S protein and RBD are preferentially expressed in mammalian cells. The antigen produced in mammalian cells often retains posttranslational modifications of the antigen, unlike bacterial recombinant proteins or peptide-based ELISAs (22). The natural S protein gene needs to be translated into the endoplasmic reticulum and transported to the Golgi apparatus for processing and modification to form the S protein with a natural conformation. The S protein, in its natural conformation, can fully exert its immunogenicity. S proteins must be synthesized in eukaryotic cells containing organelles such as the endoplasmic reticulum and Golgi apparatus.

N protein and ΔN-NP. The SARS-CoV-2 strain isolated from a patient was propagated in a Vero E6 cell line and, to construct the recombinant plasmids, the gene for the SARS-CoV-2 N protein was amplified by RT-PCR. PCR amplification was carried out using primers to generate a gene for the whole-length N protein or the ΔN-NP. The recombinant SARS-CoV-2 N proteins were expressed by inserting recombinant plasmids containing the whole-length N protein or the ΔN-NP sequences into *Escherichia coli* (BL21, XL1-Blue, JM105, etc.). The basic process of N protein purification is shown in Fig. S1.

N protein and ΔN-NP were expressed in *E. coli*. The natural N protein gene can usually undertake replication, transcription, and protein synthesis in the host cytoplasm. Prokaryotic cells have only ribosomes, and N protein can be synthesized directly into these. The recombinant product can be obtained within 1 week after cloning, and the expression and purification procedures are simple and easy to perform with low costs (19), which would be especially useful in developing countries and in areas undertaking large-scale epidemiological investigations.

ANTIGEN TEST

In addition to antibody detection, an increasing number of studies are focusing on SARS-CoV-2 antigen detection. The principle of antigen detection is based on the immune response of antibodies against the structural proteins of SARS-CoV-2 in the
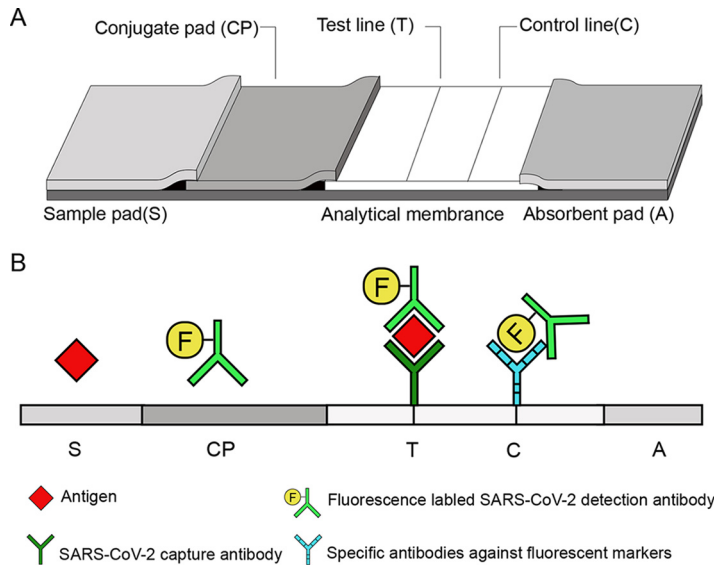
FIG 1 (A) Components of the lateral flow assay strip. (B) Detection principle of a lateral flow assay test. The red square indicates the antigen to be tested, the yellow circle with F indicates the fluorescence, the light green antibody indicates the SARS-CoV-2 capture antibody, the dark green antibody indicates the SARS-CoV-2 detection antibody, and the blue antibody indicates the specific antibodies against fluorescent markers.

specimen. The SARS-CoV-2 antigen detection kits reported thus far primarily detect N protein. Being a large protein, it can be detected via a sandwich immunoassay. To date, only a small number of antigen-detecting lateral flow assays (LFAs) have been developed. A typical LFA test strip is composed of several overlapping membranes. These usually include a sample pad, a conjugate pad, an analytical membrane (typically nitrocellulose), and an absorption or wicking pad (as shown in Fig. 1A). The detection principle of LFA (as shown in Fig. 1B) comprises a clinical sample containing the detected antigen that is applied to the sample pad. The sample, sometimes assisted by a running buffer, travels through the conjugate pad and conjugates with the fluorescence-labeled SARS-CoV-2 detection antibody or the detector-antibody gold conjugate, flowing through an analytical membrane striped with a capture antibody (the test line). Most lateral flow tests include an additional control line downstream of the test line to validate proper fluid flow through the test as well as the activity of the assay reagents (27). The SARS-CoV-2 antigen detection results are shown in Table 3.

Lambert-Niclot et al. analyzed 94 nasopharyngeal samples confirmed by RT-PCR. Among the 94 RT-PCR-positive samples, the rapid antigen test only detected 47 specimens, resulting in a sensitivity of 50.0% (95% confidence interval [CI], 39.5 to 60.5). The specificity of the test was 100% (95% CI, 91.8 to 100) (28). In another study, among the 106 positive samples, the COVID-19 Ag Respi-Strip detected 32 samples. For samples with a cycle threshold (C_T) of <25 ($n = 10$), <30 ($n = 34$), and <35 ($n = 64$), there were 1.8×10, 9.4×10^3, and 494.8 copies/ml, respectively. The COVID-19 Ag Respi-Strip had sensitivities of 100%, 70.6%, and 46.9%, respectively. However, the overall sensitivity was 30.2% (95% CI, 21.7 to 39.9) (29). Similarly, another study involved 75 swabs from patients tested positive by SARS-CoV-2 PCR and 75 swabs negative by SARS-CoV-2 PCR. The assay's sensitivity with samples with a cycle threshold of <25, 25 to 29, 30 to 34, and ≥35 was 100%, 95%, 44.8% and 22.2%, respectively. The overall sensitivity is 70.7% and the specificity is 96% (30). Of 127 samples from patients with suspected SARS-CoV-2, 82 were RT-PCR positive for SARS-CoV-2, and the overall sensitivity and specificity of the evaluated antigen detection was 93.9% (95% CI, 86.5 to 97.4) and 100% (95% CI, 92.1 to 100), respectively (31); from this study, the evaluated antigen detection showed high sensitivity (>90%) in samples mainly obtained during the first

TABLE 3 Sensitivity and specificity of SARS-CoV-2 antigen (N protein) detection[a]

Country and reference	Test and manufacturer	Sample type	Method	Sensitivity and specificity (%) (95% CI)
France; 28	COVID-19 Ag Respi-Strip Coris (BioConcept, Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	Sensitivity, 50.0 (39.5–60.5) Specificity, 100 (91.8–100)
Belgium; 29	COVID-19 Ag Respi-Strip (Coris BioConcept, Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	Sensitivity, 30.2 (21.7–39.9) Specificity, 100
Germany; 30	SARS-CoV-2 rapid antigen test (Roche, Switzerland)	Nasopharyngeal swabs	Lateral flow assay	Sensitivity, 70.7 (59.0–81.0) Specificity, 96 (89.0–99.0)
Chile; 31	SARS-CoV-2 antigen test (Bioeasy Biotechnology Co., Shenzhen, China)	Nasopharyngeal, oropharyngeal swabs	Fluorescence immunochromatographic	Sensitivity, 93.9 (86.5–97.4) Specificity, 100 (92.1–100)
Belgium; 32	COVID-19 Ag Respi-Strip assay (Coris Bioconcept, Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	Sensitivity, 30 (16.7–47.9) Specificity, 100
USA; 34	Sofia 2 SARS antigen FIA (Quidel Corporation)	Nasopharyngeal swabs	Lateral flow immunofluorescent sandwich assay	Sensitivity, 80 (68–88)

[a]The enrolled positive cases were confirmed to be infected with SARS-CoV-2 through real-time RT-PCR from oropharyngeal or nasopharyngeal swabs. All the controls had negative real-time RT-PCR results.

week of symptom onset with high viral loads. The forest plots for the sensitivity and specificity of SARS-CoV-2 antigen detection are shown in Fig. S2. However, among the 714 antigenic-negative strips, there were 159 positive PCR samples. This shows that there is a certain false-negative rate (approximately 22.3%) in the antigen detection of SARS-CoV-2 (32).

Compared with molecular technology, the rapid antigen detection tests were less sensitive than RT-PCR (33). Only when the samples have high viral loads, for example, in the first week of a patient's illness or the samples with a C_T of <25, the sensitivity and specificity of antigen detection may be high (>90%) (29–31). Collectively, the sensitivity of antigen detection is relatively low. And the low prevalence of high viral load samples further limits the use of rapid antigen detection in clinical settings. Additionally, the negative results from this rapid antigen detection method cannot exclude SARS-CoV-2 virus infection confidently, and thus, results should be verified by further RT-PCR testing. The rapid antigen test serves only as an adjunct to the RT-PCR test because of the potential for false-negative results. The rapid antigen test alone is not recommended for initial COVID-19 diagnosis because of its low sensitivity. However, antigen detection (LFAs) has the advantages of simple operation, rapidity, low cost, and no need for specialist equipment. LFA antigen tests may be a scalable solution to population-scale diagnosis of SARS-CoV-2. Antigen detection kits that have undergone rigorous performance evaluation and meet the requirements may be used as preliminary tests to quickly identify affected patients.

Recently, most antigen tests have been approved by the United States Food and Drug Administration (FDA) under emergency use authorization (EUA) and are Conformité Européenne (CE) (https://www.finddx.org/covid-19/pipeline), as shown in Table S1. This is an excellent start, and further research is needed to validate the sensitivity of the antigen assays. In view of the limited data currently available, although the World Health Organization encourages research on the performance and potential diagnostic utility of antigen rapid detection tests, they are not currently recommended for the diagnosis and treatment of patients infected with SARS-CoV-2.

CONCLUSION

Serological testing is conventionally defined as a diagnostic procedure used to identify the presence of an immune response against an infectious agent. Serology testing for SARS-CoV-2 can be defined as the analysis of plasma, serum, or whole blood for the detection of antibodies, which are specific for SARS-CoV-2 antigens, including the S glycoprotein and N protein. The S2 subunit is more conserved and plays a role in the cross-reactivity seen when the whole S protein is used as an antigen. S1 is more specific than the S protein as an antigen for SARS-CoV-2 serologic diagnosis. Antigen combinations, including both S protein and N protein, have demonstrated improved

performance compared to each individual antigen through multiantigen protein arrays. In addition to antibody detection, SARS-CoV-2 antigen detection is also important, and SARS-CoV-2 antigen detection primarily detects N protein by LFAs. The sensitivity of the test is higher when the virus content of the specimen is high. More work needs to be done to understand the utility and performance of antigen-based detection for SARS-CoV-2.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

REFERENCES

1. Cao Y, Su B, Guo X, Sun W, Deng Y, Bao L, Zhu Q, Zhang X, Zheng Y, Geng C, Chai X, He R, Li X, Lv Q, Zhu H, Deng W, Xu Y, Wang Y, Qiao L, Tan Y, Song L, Wang G, Du X, Gao N, Liu J, Xiao J, Su XD, Du Z, Feng Y, Qin C, Qin C, Jin R, Xie XS. 2020. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. Cell 182:73–84.e16. https://doi.org/10.1016/j.cell.2020.05.025.

2. He Q, Manopo I, Lu L, Leung BP, Chng HH, Ling AE, Chee LL, Chan SW, Ooi EE, Sin YL, Ang B, Kwang J. 2005. Novel immunofluorescence assay using recombinant nucleocapsid-spike fusion protein as antigen to detect antibodies against severe acute respiratory syndrome coronavirus. Clin Diagn Lab Immunol 12:321–328. https://doi.org/10.1128/CDLI.12.2.321-328.2005.

3. Lou B, Li TD, Zheng SF, Su YY, Li ZY, Liu W, Yu F, Ge SX, Zou QD, Yuan Q, Lin S, Hong CM, Yao XY, Zhang XJ, Wu DH, Zhou GL, Hou WH, Li TT, Zhang YL, Zhang SY, Fan J, Zhang J, Xia NS, Chen Y. 2020. Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. Eur Respir J 56:2000763. https://doi.org/10.1183/13993003.00763-2020.

4. Kandeel M, Ibrahim AA, Fayez M, Al-Nazawi M. 2020. From SARS and MERS CoVs to SARS-CoV-2: moving toward more biased codon usage in viral structural and nonstructural genes. J Med Virol 92:660–666. https://doi.org/10.1002/jmv.25754.

5. Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, Wang Q, Tan L, Wu W, Tang S, Xiong Z, Zheng S. 2020. Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2. J Clin Microbiol 58:e00461-20. https://doi.org/10.1128/JCM.00461-20.

6. Zhou W, Wang W, Wang H, Lu R, Tan W. 2013. First infection by all four non-severe acute respiratory syndrome human coronaviruses takes place during childhood. BMC Infect Dis 13:433. https://doi.org/10.1186/1471-2334-13-433.

7. Meyer B, Drosten C, Müller MA. 2014. Serological assays for emerging coronaviruses: challenges and pitfalls. Virus Res 194:175–183. https://doi.org/10.1016/j.virusres.2014.03.018.

8. Millet JK, Whittaker GR. 2018. Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells. Virology 517:3–8. https://doi.org/10.1016/j.virol.2017.12.015.

9. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, Lamers MM, Sikkema RS, de Bruin E, Chandler FD, Yazdanpanah Y, Le Hingrat Q, Descamps D, Houhou-Fidouh N, Reusken C, Bosch BJ, Drosten C, Koopmans MPG, Haagmans BL. 2020. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease 2019 patients. Emerg Infect Dis 26:1478–1488. https://doi.org/10.3201/eid2607.200841.

10. Seo G, Lee G, Kim MJ, Baek SH, Choi M, Ku KB, Lee CS, Jun S, Park D, Kim HG, Kim SJ, Lee JO, Kim BT, Park EC, Kim SI. 2020. Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS Nano 14:5135–5142. https://doi.org/10.1021/acsnano.0c02823.

11. Hicks J, Klumpp-Thomas C, Kalish H, Shunmugavel A, Mehalko J, Denson JP, Snead K, Drew M, Corbett K, Graham B, Hall MD, Esposito D, Sadtler K. 2020. Serologic cross-reactivity of SARS-CoV-2 with endemic and seasonal Betacoronaviruses. medRxiv https://doi.org/10.1101/2020.06.22.20137695.

12. Chan KH, Cheng VC, Woo PC, Lau SK, Poon LL, Guan Y, Seto WH, Yuen KY, Peiris JS. 2005. Serological responses in patients with severe acute respiratory syndrome coronavirus infection and cross-reactivity with human coronaviruses 229E, OC43, and NL63. Clin Diagn Lab Immunol 12:1317–1321. https://doi.org/10.1128/CDLI.12.11.1317-1321.2005.

13. de Assis RR, Jain A, Nakajima R, Jasinskas A, Felgner J, Obiero JM, Adenaiye O, Tai S, Hong F, Norris P, Stone M, Simmons G, Bagri A, Schreiber M, Buser A, Holbro A, Battegay M, Milton DK, Davies H, Corash LM, Busch MP, Felgner PL, Khan S. 2020. Analysis of SARS-CoV-2 antibodies in COVID-19 convalescent plasma using a coronavirus antigen microarray. bioRxiv https://doi.org/10.1101/2020.04.15.043364.

14. Khan S, Nakajima R, Jain A, de Assis RR, Jasinskas A, Obiero JM, Adenaiye O, Tai S, Hong F, Milton DK, Davies H, Felgner PL. 2020. Analysis of serologic cross-reactivity between common human coronaviruses and SARS-CoV-2 using coronavirus antigen microarray. bioRxiv https://doi.org/10.1101/2020.03.24.006544.

15. Premkumar L, Segovia-Chumbez B, Jadi R, Martinez DR, Raut R, Markmann A, Cornaby C, Bartelt L, Weiss S, Park Y, Edwards CE, Weimer E, Scherer EM, Rouphael N, Edupuganti S, Weiskopf D, Tse LV, Hou YJ, Margolis D, Sette A, Collins MH, Schmitz J, Baric RS, de Silva AM. 2020. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. Sci Immunol 5:eabc8413. https://doi.org/10.1126/sciimmunol.abc8413.

16. Paules CI, Marston HD, Fauci AS. 2020. Coronavirus infections: more than just the common cold. JAMA 323:707. https://doi.org/10.1001/jama.2020.0757.

17. Chen Z, Pei D, Jiang L, Song Y, Wang J, Wang H, Zhou D, Zhai J, Du Z, Li B, Qiu M, Han Y, Guo Z, Yang R. 2004. Antigenicity analysis of different regions of the severe acute respiratory syndrome coronavirus nucleocapsid protein. Clin Chem 50:988–995. https://doi.org/10.1373/clinchem.2004.031096.

18. Yu F, Le MQ, Inoue S, Thai HT, Hasebe F, Del Carmen Parquet M, Morita K. 2005. Evaluation of inapparent nosocomial severe acute respiratory syndrome coronavirus infection in Vietnam by use of highly specific recombinant truncated nucleocapsid protein-based enzyme-linked immunosorbent assay. Clin Diagn Lab Immunol 12:848–854. https://doi.org/10.1128/CDLI.12.7.848-854.2005.

19. Yu F, Le MQ, Inoue S, Hasebe F, del Carmen Parquet M, Morikawa S, Morita K. 2007. Recombinant truncated nucleocapsid protein as antigen in a novel immunoglobulin M capture enzyme-linked immunosorbent assay for diagnosis of severe acute respiratory syndrome coronavirus

infection. Clin Vaccine Immunol 14:146–149. https://doi.org/10.1128/CVI.00360-06.

20. Yamaoka Y, Jeremiah SS, Miyakawa K, Saji R, Nishii M, Takeuchi I, Ryo A. 2020. Whole nucleocapsid protein of SARS-CoV-2 may cause false positive results in serological assays. Clin Infect Dis https://doi.org/10.1093/cid/ciaa637.

21. Woo PC, Lau SK, Wong BH, Tsoi HW, Fung AM, Kao RY, Chan KH, Peiris JS, Yuen KY. 2005. Differential sensitivities of severe acute respiratory syndrome (SARS) coronavirus spike polypeptide enzyme-linked immunosorbent assay (ELISA) and SARS coronavirus nucleocapsid protein ELISA for serodiagnosis of SARS coronavirus pneumonia. J Clin Microbiol 43:3054–3058. https://doi.org/10.1128/JCM.43.7.3054-3058.2005.

22. Burbelo PD, Riedo FX, Morishima C, Rawlings S, Smith D, Das S, Strich JR, Chertow DS, Davey RT, Cohen JI. 2020. Sensitivity in detection of antibodies to nucleocapsid and spike proteins of severe acute respiratory syndrome coronavirus 2 in patients with coronavirus disease 2019. J Infect Dis 222:206–213. https://doi.org/10.1093/infdis/jiaa273.

23. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, Wang X, Yuan J, Li T, Li J, Qian S, Hong C, Wang F, Liu Y, Wang Z, He Q, Li Z, He B, Zhang T, Fu Y, Ge S, Liu L, Zhang J, Xia N, Zhang Z. 2020. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clin Infect Dis 71:2027–2034. https://doi.org/10.1093/cid/ciaa344.

24. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, Yip CC, Cai JP, Chan JM, Chik TS, Lau DP, Choi CY, Chen LL, Chan WM, Chan KH, Ip JD, Ng AC, Poon RW, Luo CT, Cheng VC, Chan JF, Hung IF, Chen Z, Chen H, Yuen KY. 2020. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 20:565–574. https://doi.org/10.1016/S1473-3099(20)30196-1.

25. Lin Q, Wen D, Wu J, Liu L, Wu W, Fang X, Kong J. 2020. Microfluidic immunoassays for sensitive and simultaneous detection of IgG/IgM/antigen of SARS-CoV-2 within 15 min. Anal Chem 92:9454–9458. https://doi.org/10.1021/acs.analchem.0c01635.

26. Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmeier S, Arunkumar GA, Tan J, Bhavsar D, Capuano C, Kirkpatrick E, Meade P, Brito RN, Teo C, McMahon M, Simon V, Krammer F. 2020. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. Curr Protoc Microbiol 57:e100. https://doi.org/10.1002/cpmc.100.

27. Hsieh HV, Dantzler JL, Weigl BH. 2017. Analytical tools to improve optimization procedures for lateral flow assays. Diagnostics (Basel) 7:29. https://doi.org/10.3390/diagnostics7020029.

28. Lambert-Niclot S, Cuffel A, Le Pape S, Vauloup-Fellous C, Morand-Joubert L, Roque-Afonso AM, Le Goff J, Delaugerre C. 2020. Evaluation of a rapid diagnostic assay for detection of SARS CoV-2 antigen in nasopharyngeal swab. J Clin Microbiol 58:e00977-20. https://doi.org/10.1128/JCM.00977-20.

29. Scohy A, Anantharajah A, Bodéus M, Kabamba-Mukadi B, Verroken A, Rodriguez-Villalobos H. 2020. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. J Clin Virol 129:104455. https://doi.org/10.1016/j.jcv.2020.104455.

30. Krüttgen A, Cornelissen CG, Dreher M, Hornef MW, Imöhl M, Kleines M. 2020. Comparison of the SARS-CoV-2 rapid antigen test to the Real Star Sars-CoV-2 RT PCR kit. J Virol Methods 288:114024. https://doi.org/10.1016/j.jviromet.2020.114024.

31. Porte L, Legarraga P, Vollrath V, Aguilera X, Munita JM, Araos R, Pizarro G, Vial P, Iruretagoyena M, Dittrich S, Weitzel T. 2020. Evaluation of novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. Int J Infect Dis 99:328–333. https://doi.org/10.1016/j.ijid.2020.05.098.

32. Blairon L, Wilmet A, Beukinga I, Tré-Hardy M. 2020. Implementation of rapid SARS-CoV-2 antigenic testing in a laboratory without access to molecular methods: experiences of a general hospital. J Clin Virol 129:104472. https://doi.org/10.1016/j.jcv.2020.104472.

33. Mak GC, Cheng PK, Lau SS, Wong KK, Lau CS, Lam ET, Chan RC, Tsang DN. 2020. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. J Clin Virol 129:104500. https://doi.org/10.1016/j.jcv.2020.104500.

34. FDA. 2020. Individual EUAs for antigen diagnostic tests for SARS-CoV-2. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individual-antigen.

35. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, Dela Cruz CS, Wang Y, Wu C, Xiao Y, Zhang L, Han L, Dang S, Xu Y, Yang Q, Xu S, Zhu H, Xu Y, Jin Q, Sharma L, Wang L, Wang J. 2020. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis 71:778–785. https://doi.org/10.1093/cid/ciaa310.