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RESEARCH ARTICLE

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Development, performance evaluation, and clinical application of a Rapid SARS-CoV-2 IgM and IgG Test Kit based on automated fluorescence immunoassay

Keren Kang[1,2] | Lingfang Huang[1,2] | Chaolin Ouyang[1,2] | Jiaming Du[1,2] | Bin Yang[1,2] | Yaqin Chi[1,2] | Shuyu He[1,2] | Le Ying[1,2] | Guoling Chen[1,2] | Jihua Wang[1,2]

[1]Guangdong Medical Laboratory Technology (Rapid Diagnostic) Engineering Technology Research Center, Guangzhou, China

[2]Guangzhou Wondfo Biotech Co., Ltd., Guangzhou, China

Correspondence
Keren Kang, Guangdong Medical Laboratory Technology (Rapid Diagnostic) Engineering Technology Research Center, Guangzhou 5l0663, China.
Email: keren_kang@wondfo.com.cn

Abstract

The ongoing coronavirus disease 2019 (COVID-19) epidemic has made a huge impact on health, economies, and societies all over the world. Although reverse transcription-polymerase chain reaction (RT-PCR)-based nucleic acid detection has been primarily used in the diagnosis of COVID-19, it is time-consuming with limited application scenarios and must be operated by qualified personnel. Antibody test, particularly point-of-care antibody testing, is a suitable complement to nucleic acid test as it provides rapid, portable, and cost-effective detection of infections. In this study, a Rapid Antibody Test Kit was developed based on fluorescence immunochromatography for the sensitive, accurate, and automated detection of immunoglobulin M (IgM) and IgG antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human serum, plasma, and whole blood samples within 10 min. The sensitivity, specificity, precision, and stability of the test kit were of good performance. No cross-activity and no interference was observed. In the multiple-center parallel study, 223 samples from hospitalized patients were used to evaluate the clinical specificity of the test. Both SARS-CoV-2 IgM and IgG achieved a clinical specificity of 98.21%. The clinical sensitivities of SARS-CoV-2 IgM and IgG were 79.54% and 87.45%, respectively, among 733 reverse transcription-polymerase chain reaction (RT-PCR) confirmed SARS-CoV-2 samples. For the combined IgM and IgG assays, the sensitivity and specificity were 89.22% and 96.86%, respectively. Our results demonstrate that the combined use of IgM and IgG could serve as a more suitable alternative detection method for patients with COVID-19, and the developed kit is of great public health significance for the prevention and control of the COVID-19 pandemic.

KEYWORDS

automated detection, COVID-19, fluorescence immunochromatography, IgM and IgG, rapid antibody test, SARS-CoV-2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a novel infectious disease, which has plunged the whole world into a state of emergency and declared as a global pandemic by the World Health Organization. The outbreak and prevalence of COVID-19 have brought severe challenges to the healthcare system and made a huge impact on the world economy and society. As of August 5, 2020, more than 200 countries and territories have officially reported 18,354,342 confirmed cases and 696,147 deaths,[1] the pandemic remains a public health emergency of international concern.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of COVID-19. It belongs to the genus β-coronavirus, with a single-stranded and positive-sense RNA viral genome of ~30 kilobases.[2] SARS-CoV-2 encodes four structural proteins, including the spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid (N) protein, which are involved in transcription, assembly, budding, envelope formation, and the pathogenesis of the virus.[2,3]

The accurate, sensitive, and rapid detection of SARS-CoV-2 is of great significance for the prevention and control of the COVID-19 pandemic. In the past few months, various commercially available COVID-19 test kits have been developed to meet the urgent need of several countries. These detection techniques fall into two main categories: nucleic acid detection and immunological detection. The nucleic acid test detects SARS-CoV-2 viral RNA based on reverse transcription-polymerase chain reaction (RT-PCR), isothermal amplification, genome sequencing, or other methods. The target of immunological detection is SARS-CoV-2 viral protein antigens or antibodies produced in response to the infection. Immunological detection methods mainly include enzyme-linked immunosorbent assay, lateral flow immunoassay (LFIA), chemiluminescence immunoassay (CLIA).[4]

Although RT-PCR-based nucleic acid detection has been primarily used as the gold standard in the diagnosis of COVID-19, it is time-consuming with limited application scenarios and must be operated by qualified personnel. Antibody testing, particularly the point-of-care antibody testing method, is a suitable complement to the nucleic acid detection method as it provides a rapid, portable, and cost-effective method of detection. The LFIA for SARS-CoV-2 is a typical point-of-care approach for clinical auxiliary diagnosis of infection. Traditional colloidal gold-based LFIA for SARS-CoV-2 immunoglobulin M (IgM) or immunoglobulin G (IgG) detection has been developed,[5] it takes 15 min to get results with the naked eye. However, the test process is carried out on a manual basis and it is difficult to avoid the error caused by visual inspection. Fluorescence-based LFIA has higher sensitivity and can achieve automatic/semi-automatic operation by a small portable device. A lanthanide-doped nanoparticles-based LFIA method has been reported, it provides semiquantitative results of anti-SARS-CoV-2 IgG in human serum.[6] So far the National Medical Products Administration in China and other administrations have approved just a few well-evaluated rapid fluorescence-based LFIA immunoassays.

In this study, we report the development of a rapid automated detection method based on fluorescence immunochromatography for the determination of SARS-CoV-2 IgG and IgM antibodies in human serum, plasma, and whole blood.

2 | MATERIALS AND METHODS

2.1 | Materials and reagents used in the kit production

SARS-CoV-2 recombinant antigen was purchased from Sino Biological Inc. The recombinant antigen is the receptor-binding domain of the surface unit, S1, of the S protein. Mouse anti-human IgM (μ chain) antibody and mouse anti-human IgG antibody were purchased from Fapon Biotech Inc. Polyclonal goat anti-chicken IgY antibody and chicken IgY antibody were purchased from Hangzhou Clongene Biotech Co. Fluorescent latex was purchased from Merck. Nitrocellulose membranes were purchased from Sartorius. Glass fiber was obtained from Shanghai Shangzhuo Filtration Co., Ltd. The contact spray film machine was obtained from Imagene Technology Company.

2.2 | COVID-19 and non-COVID-19 sample collection

From January 11, 2020 to March 11, 2020, a total of 733 samples of patients with COVID-19 were collected from nine hospitals in five different provinces in China. Patients diagnosed with SARS-CoV-2 infection were confirmed by an RT-PCR nucleic acid test, following the guideline of diagnosis and treatment of COVID-19.[7] The average age of this cohort was 51 years (4–92 years) and 52.0% were males. Clinical samples from 223 hospitalized patients diagnosed with non-COVID-19 diseases were used as controls. The average age of this cohort was 43 years (1–86 years) and 50.6% were males. Blood samples from COVID-19 patients and controls, including serum/plasma or whole blood, were tested for the presence of SARS-CoV-2 IgM and IgG. All patients were informed of the use of their blood samples for immunodiagnostic study and all consented to participate in the study. Venous blood samples were collected into anticoagulant tubes, and then the serum and plasma were separated by centrifugations (3400 rpm, 10 min) for immediate testing or stored at 2–8°C for use within 7 days.

2.3 | Preparation of SARS-CoV-2 IgM and IgG Rapid Test Kits

The test strip is composed of the sample pad, nitrocellulose membrane, conjugate pad, absorbent pad, and backing card. SARS-CoV-2 recombinant antigen conjugate and chicken IgY antibody were labeled in the fluorescent latex solution, then evenly sprayed on glass

fiber to prepare a conjugate pad. The mouse anti-human IgM (μ chain) antibody, mouse anti-human IgG antibody, and polyclonal goat anti-chicken IgY antibody were coated on nitrocellulose membranes to prepare test lines (T1 line and T2 line) and control line (C line), respectively. The sample pad was made of glass fiber and pretreated with polyethylene glycol 6000 (2%, wt/vol), bovine serum albumin (1%, wt/vol), and Tween-20 (2%, wt/vol). Finally, the nitrocellulose membrane, absorbent pad, conjugate pad, and sample pad were laminated onto a backing card, cut into 4-mm-wide strips, and then assembled into a test cassette for subsequent use in the screening of clinical samples.

2.4 | Cut-off value determination of the rapid antibody test

About 120 SARS-CoV-2 negative serum samples were detected with Finecare™ FIA Meter and analyzed using the percentile method. The signal ratio of the test line to control line (T/C) gives the detection value of each specimen, and then the mean value ($\bar{X}$) and the standard deviation (*SD*) were calculated. Regarding the technical specifications of in vitro diagnostic kit products, the mean value plus three times the *SD* was regarded as the detection threshold value; hence, the formula $\bar{X}$+3*SD* was used as the cut-off (CO) value for the test kit. The value of the (T/C)/CO indicates the ratio of the detected value to the CO value. Samples with (T/C)/CO < 1.0 were considered negative, (T/C)/CO ≥ 1.0 were considered positive.

2.5 | Sensitivity and specificity evaluation of the rapid antibody test

For investigating the sensitivity of the assay, three positive samples determined by commercial SARS-CoV-2 antibody test kits were diluted with negative serum at different concentrations. Three replicates were prepared for each dilution and each sample was tested 20 times. The limit of detection was determined as the antibody level with a positive rate greater than 95%. Two negative reference samples and four weakly positive reference samples with different concentration gradients of interfering substances were evaluated to investigate interference. These interfering substances include bilirubin, hemoglobin, triglyceride, rheumatoid factor, antinuclear antibody, antimitochondrial antibody, human anti-mouse antibody, histamine dihydrochloride, α-interferon, zanamivir, ribavirin, oseltamivir, peramivir, lopinavir, ritonavir, abidor, levofloxacin, azithromycin, ceftriaxone, meropenem, and tobramycin. Moreover, clinically diagnosed viral and bacterial samples of coronavirus (HKU1, OC43, NL63, and 229E), parainfluenza virus, influenza A (H1N1) virus, influenza A (H3) virus, avian influenza A (H7N9) virus, influenza B virus, *Chlamydia pneumonia*, *Mycoplasma pneumonia*, respiratory syncytial virus, hepatitis B virus, hepatitis C virus, *Treponema pallidum*, human immunodeficiency virus, Epstein–Barr virus, measles virus, cytomegalovirus, mumps virus, varicella-zoster virus, enterovirus, rhinovirus, adenovirus type 3 virus, adenovirus type 4 virus, adenovirus type 7 virus, and adenovirus type 55 virus were also evaluated to investigate the cross-reactivity of the assay.

2.6 | Precision and stability evaluation of the rapid antibody test

Repeatability, within-lab precision, and site-to-site reproducibility were evaluated according to the Clinical and Laboratory Standards Institute document EP05-A3—Evaluation of Precision of Quantitative Measurement Procedures. Each batch was tested continuously for 5 days, and each sample was repeated five times a day. The mean, *SD*, and coefficient of variation (CV) were then calculated. CV less than 10% was considered acceptable. (CV = *SD*/mean × 100%).

Furthermore, the accelerated test was used to assess the stability of the kit. The test strips were incubated at 50°C and tested after 7 days and 28 days, respectively, using reference panels from Wondfo Biotech.

2.7 | Quality control panel verification

For quality control, use the national reference panel for the SARS-CoV-2 Antibody Detection Kit from the National Institute for Food and Drug Control. The IgM antibody test national reference panel was composed of 38 samples, consisting of 10 positive (P1–P10), 25 negative (N1–N25), 1 limit of detection (S), 1 precision reference (R), and 1 substrate plasma (S0). S0 was used to make nine serial dilutions of S, then the original S and the nine diluted samples were labeled from L1 to L10. The composition of the IgG antibody test national reference panel was the same as IgM. Test kit performance was verified by the national reference panels. The IgM and IgG national reference panels were tested with our newly developed kits for IgM and IgG, respectively. The positive compliance rate, negative compliance rate, the limit of detection, and repeatability were evaluated to determine whether these indexes met the required criteria.

2.8 | Clinical sensitivity and specificity evaluation of the rapid antibody test

The specificity of the assay was accessed by measuring hospitalized patients with non-COVID-19 diseases (223 samples). Clinical sensitivity was evaluated on samples from patients diagnosed with SARS-CoV-2 infection by an RT-PCR nucleic acid test (733 Samples).

2.9 | Statistical analysis

The specificity and sensitivity of the Rapid Test Kits were computed as follows: specificity (%) = 100 × [True Negative/(True Negative + False Positive)], and sensitivity (%) = 100 × [True Positive (True

Positive + False Negative)]. Ninety-five percent confidence interval = $p \pm 1.96 \times [p(1-p)/n]^{1/2}$ ("p" is the consistent rate and "n" is the number of samples), if $p > .9$, then use the Wilson score method for correction.

3 | RESULTS

3.1 | Principle and procedure of SARS-CoV-2 IgM and IgG Antibody Rapid Test Kit

The test kit was based on the fluorescence immunoassay technology for the detection of IgM/IgG antibodies against SARS-CoV-2 in human whole blood, serum, and plasma. As shown in Figure 1, the fluorescence-labeled detector SARS-CoV-2 antigen binds to SARS-CoV-2 IgM/IgG antibodies in blood specimens and forms immune complexes. As the complexes migrate on the nitrocellulose matrix by capillary action, the SARS-CoV-2 IgM antibody can be captured by anti-human IgM antibodies that have been immobilized on the M line. The SARS-CoV-2 IgG antibody can be captured by anti-human IgG antigens that have been immobilized on the G line. Thus, the higher the level of the SARS-CoV-2 IgM/IgG antibodies in the specimen, the higher the signal value scanned by Finecare FIA Meters, and the stronger the positive degree of the specimen. Thus, negative and positive samples could be easily differentiated.

3.2 | The CO value of SARS-CoV-2 IgM and IgG Antibody Test Kit

About 120 negative serum specimens were for both IgM and IgG. For the IgM antibody test, the average value was 0.69 ± 0.03, and the CO value was equal to 0.77. For the IgG antibody test, the average value was 0.67 ± 0.04, and the CO value was equal to 0.8. In the detection process, (T/C)/CO < 1.0 indicated "negative" result; (T/C)/CO ≥ 1.0 indicated "positive" result. The greater the (T/C)/CO value, the higher the SARS-CoV-2 concentration.

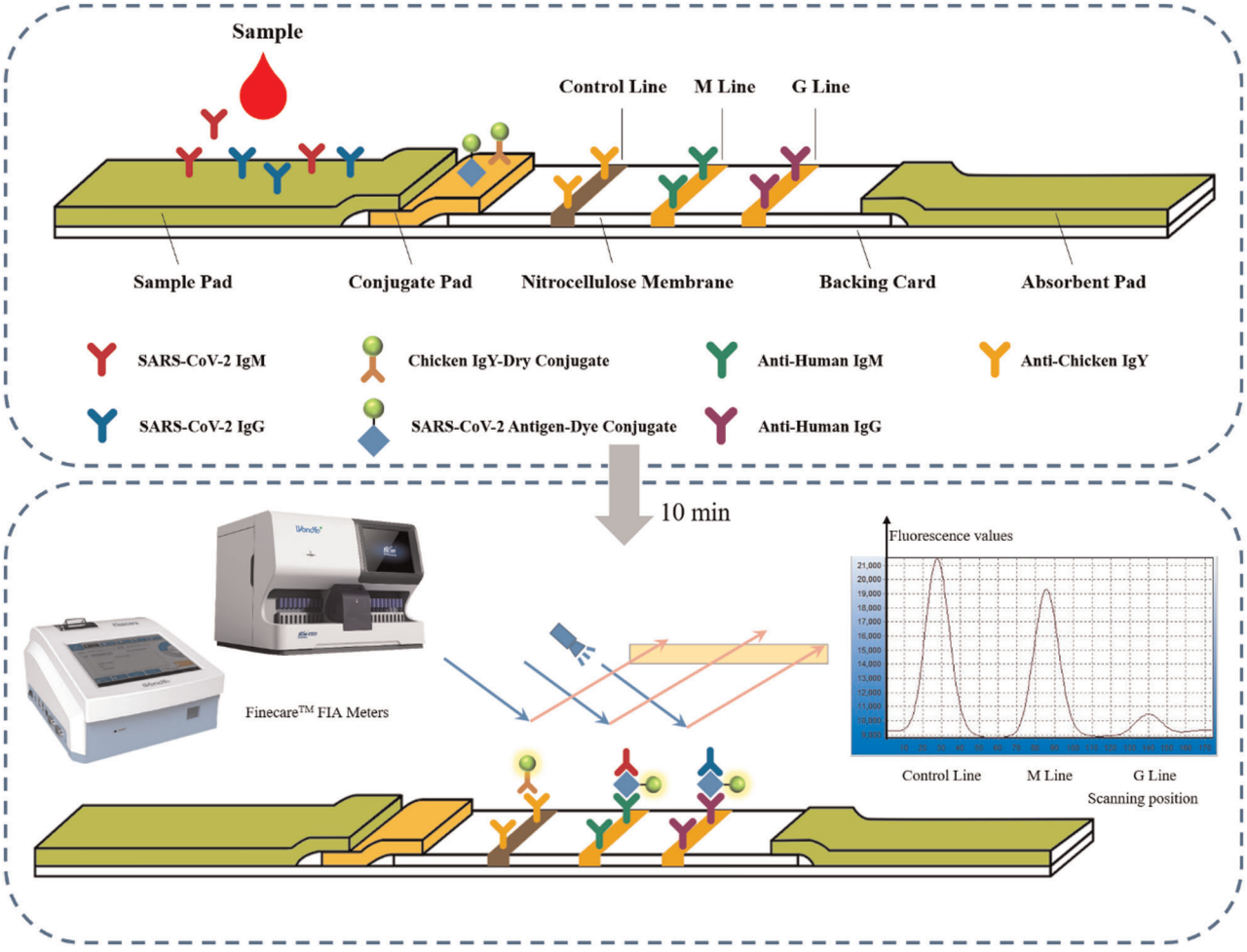

FIGURE 1 Principle and procedure of SARS-CoV-2 IgM and IgG Antibody Rapid Test Kit. (A) Schematic diagram of SARS-CoV-2 IgM and IgG Antibody Rapid Test Kit. (B) Scanning and analysis process, using auto-immunofluorescence analyzer (multichannel) or immunofluorescence analyzer (single channel). IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

3.3 | Determination of the sensitivity and specificity of the Antibody Test Kit

The limit of detection was conducted by detecting serial dilutions of samples of IgM and IgG antibodies. As shown in Figure 2, the IgM positivity rate was ≥95% for dilution ratio, ≥1:20 for low-level IgM samples, ≥1:50 for medium-level IgM samples, and ≥1:100 for high-level IgM samples. The IgG positivity rate was ≥95% for dilution ratio, ≥1:20 for low-level IgG samples, ≥1:60 for medium-level IgG samples, and ≥1:120 for high-level IgG samples.

The specificity of our assay was determined by evaluating its reactivity with common interfering factors. The negative and weakly positive reference samples with high concentrations of common interfering substances were tested and our data showed that these common interfering substances did not influence the test results (Figure 3). Also, cross-reactivity tests were performed on other clinical samples obtained from individuals with other infections other than SARS-CoV-2. Taken together, these results showed that our newly developed assay was highly specific for SARS-CoV-2 antibody detection and showed no cross-reactivity with other substances.

3.4 | Precision and stability of the Antibody Test Kit

The precision of the assay was determined using IgM/IgG negative serum or whole blood samples, IgM positive/IgG weak positive serum or whole blood samples, IgM weak positive/IgG negative serum or whole blood samples, and IgM negative/IgG positive serum or whole blood samples. The CV of repeatability ranged from 3.22% to 4.75%, while the CV of within-lab precision ranged from 3.24% to 4.89%, and the CV of reproducibility ranged from 3.25% to 4.89%. All the results were lower than 10%, indicating a high degree of precision and acceptability of our test kit.

Moreover, since the validity period of the Rapid Detection Kit plays a very important role in their clinical application, we studied the stability of our test kit by an accelerated test using reference panels from the Wondfo Biotech Co., Ltd. The results showed that the test strips remained stable after 28 days at 50°C accelerated test, and the positive compliance rate, negative compliance rate, limit of detection, and repeatability all conformed to the standard requirement of test kits. Taken together, these results suggest that our newly developed test kits are of an excellent standard and could serve as an alternative auxiliary diagnostic tool for the detection of SARS-Cov-2 antibody.

3.5 | Quality control panel verification

The test strips were tested using the national reference panels. The positive and negative compliance rates, the limit of detection, and repeatability all met the required criteria (Table 1).

3.6 | Application of the IgM and IgG Antibody Test Kit in the detection of clinical samples

A total of 223 samples from hospitalized patients were used to assess the clinical specificity of the assay. SARS-CoV-2 IgM and IgG both showed a specificity of 98.21%. Next, we tested 733 RT-PCR confirmed SARS-Cov-2 samples to evaluate the sensitivity of the assays in the detection of SARS-Cov-2 antibodies. The sensitivities of SARS-CoV-2 IgM and IgG test kits were 79.54% and 87.45%, respectively. For the combined IgM and IgG test, the sensitivity and specificity were 89.22% and 96.86%, respectively (Table 2).

3.7 | The detectability of antibodies in patients at different time points of infection

Next, we analyzed the detectability of antibodies according to the time course since the onset of illness in the cohort. As shown in Figure 4, during the early phase of illness (within 7 days since onset), IgM and IgG assays only showed a positivity rate of 45.53% and 47.97%, respectively. However, the sensitivity increased dramatically to 82.14% for IgM and 85.71% for IgG from the second week. The positivity rate of IgM peaked in the third week after onset, then decreased after 6 weeks postonset of illness. The positivity rate of IgG also showed 98.75% within the third week and remained high even after 7 weeks postonset of illness. The sensitivity of combined IgM and IgG (IgM or IgG positive) was high at all stages of disease development as compared to IgM and IgG only.

Furthermore, to compare the detectability of antibodies in patients with different clinical types, we classified patients as moderate/severe cases or mild cases. As shown in Table 3, the positive rate of IgM was lower in the mild cases but there was no significant difference in IgG or combined IgM/IgG between the mild cases and moderate/severe cases in all disease stages.

3.8 | Correlation between SARS-CoV-2 IgM and IgG antibody tests in whole blood and plasma/serum samples

Although antibodies are more stable in serum/plasma samples, the whole blood samples are more convenient to use. Therefore, we assessed the performance of the newly developed kits using whole blood samples. About 101 COVID-19 patients and 68 patients with other diseases were enrolled in this study. Whole blood and serum/plasma from the same patient were tested. Among the COVID-19 whole blood samples, 89 were IgM positive and 97 were IgG positive, and all non-COVID-19 control samples tested negative. All of the positive and negative test results matched with high consistency between whole blood and plasma/serum samples, which indicated that both SARS-CoV-2 IgG and IgM Antibody Test Kits can be performed using whole blood samples from suspected patients.

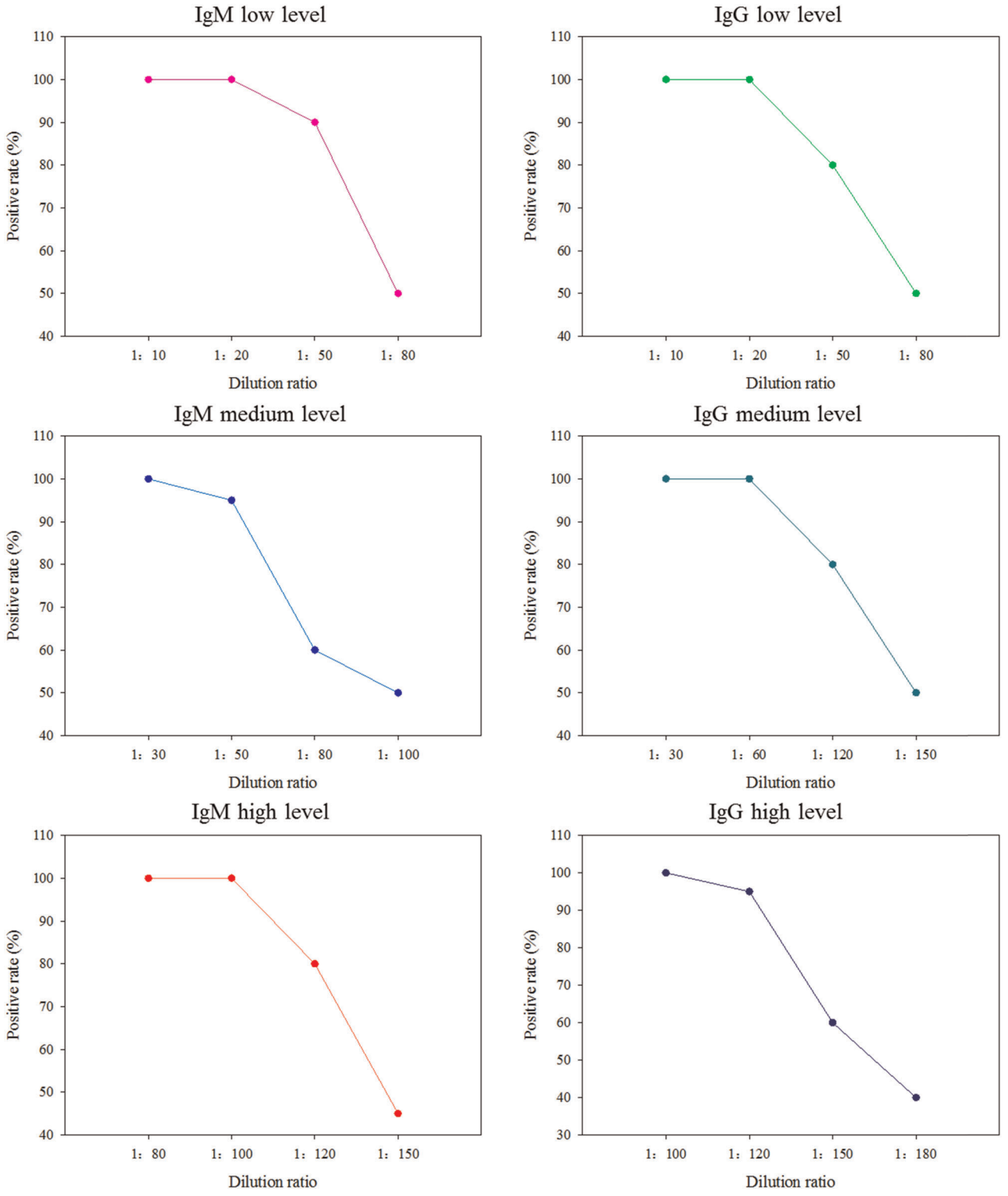

FIGURE 2 The limit of detection of the developed Rapid SARS-CoV-2 IgM and IgG Antibody Test Kit. IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

4 | DISCUSSION

SARS-CoV-2 is the third highly pathogenic and transmissible coronavirus, after the SARS-CoV and Middle East respiratory syndrome coronavirus in humans.[8] Accurate identification of SARS-CoV-2 infection is essential for an effective diagnosis of COVID-19, and this is important not only for the patients but also for public health.

The positive result of viral nucleic acid testing is the key and direct evidence of SARS-CoV-2 infection, and it is considered the gold standard for the diagnosis of COVID-19. However, it is difficult

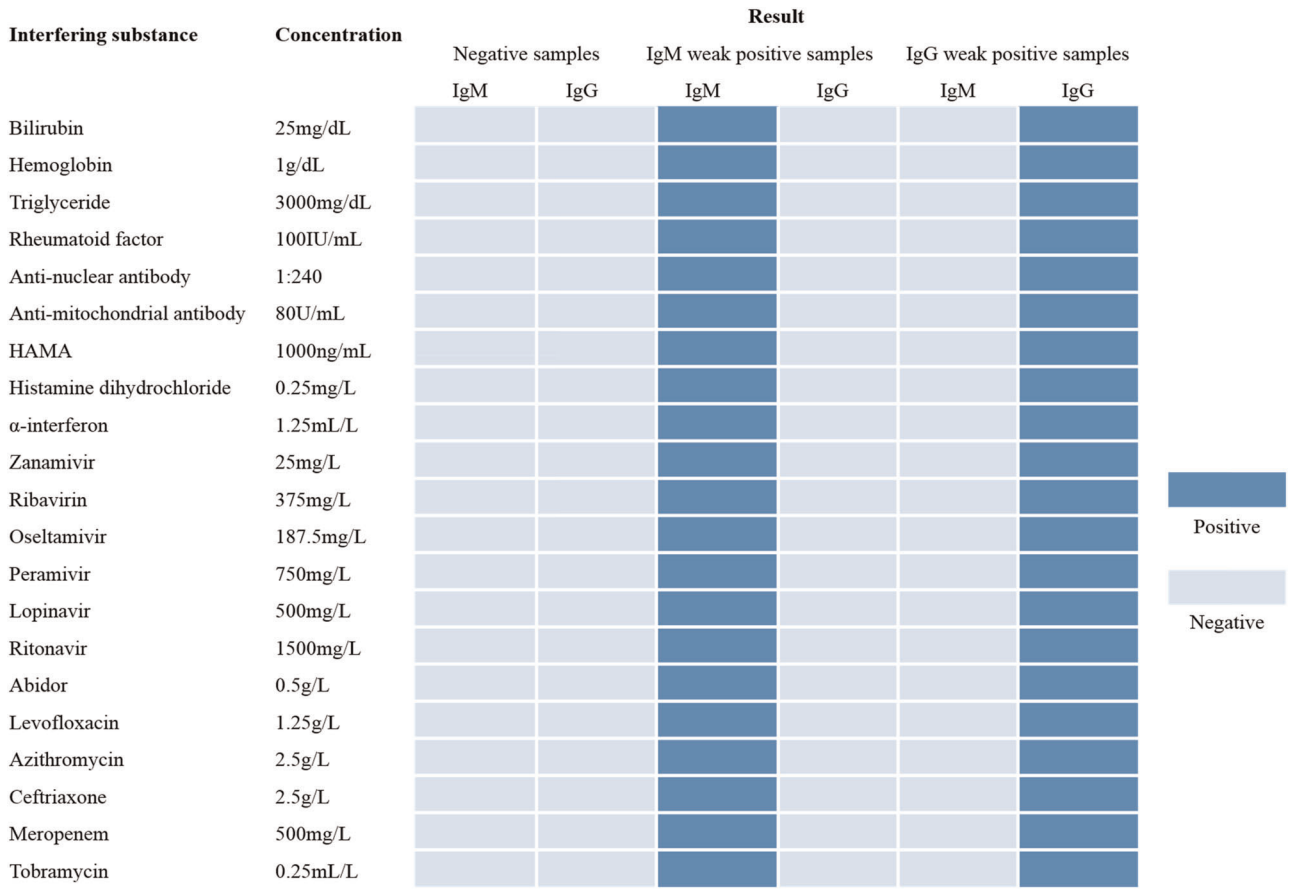

FIGURE 3 Cross-reactivity and specificity assays of the newly developed Rapid SARS-CoV-2 IgM/IgG Antibody Test Kit. HAMA, human anti-mouse antibody; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

TABLE 1 Performance of test kit on national reference panels

Reference panel		Positive compliance rate	Negative compliance rate	Limit of detection	Repeatability
IgM antibody test National reference panel	Criterion	10/10+/+	24/25−/−	≥2/10+/+, L1+, L2+	10/10+/+
	Results	10/10+/+	24/25−/−	3/10+/+, L1+, L2+, L3+	10/10+/+
IgG antibody test National reference panel	Criterion	≥9/10+/+	24/25−/−	≥1/10+/+, L1+	10/10+/+
	Results	9/10+/+	25/25−/−	1/10+/+, L1+	10/10+/+

for some countries or regions to build up the ability to cope with a large number of patients in a short period under the epidemic because of the high need for sophisticated equipment and specialized personnel to carry out nucleic acid testing. Besides, many factors could contribute to false-negative results,[9] such as the different disease courses, specimen collection sites, nonstandard operation, or even drugs. Therefore, it is a critical need to develop a supplementary detection method to compensate for these limitations.

TABLE 2 The clinical specificity and sensitivity of IgM and IgG Antibody Test Kit

	Patients with COVID-19			Controls		
	Positive	Negative	Sensitivity (%)	Positive	Negative	Specificity (%)
IgM	583	150	79.54	4	219	98.21
IgG	641	92	87.45	4	219	98.21
IgM or IgG	654	79	89.22	7	216	96.86

Abbreviations: COVID-19, coronavirus disease 2019; IgG, immunoglobulin G.

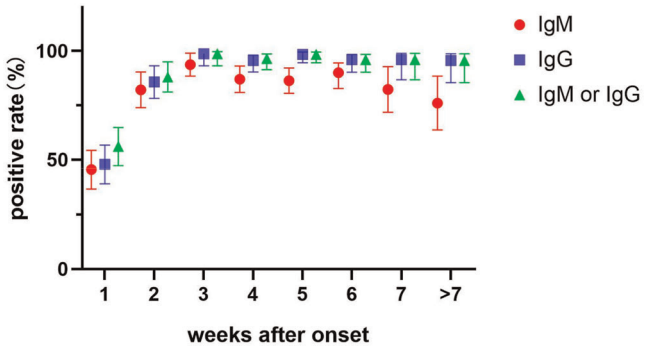

FIGURE 4 The positivity rate of immunoglobulin M (IgM) and IgG during the different stage after onset

In China, according to the guideline of diagnosis and treatment of COVID-19 (version 8),[10] either etiological or serological evidence can be used as the diagnostic criteria for suspected cases. Besides real-time fluorescence quantitative RT-PCR and gene sequencing, SARS-CoV-2 specific IgM and IgG antibody detection can also be used as an important tool to confirm suspected patients.

Antibodies could be produced by the immune system after pathogen infection and could be tested by immunological detection methods. Since the samples used for antibody testing is blood, compared to nasopharyngeal swabs, sample collection is easier to control and less affected by collection location and operational errors, ensuring repeatability of test results. To some extent, it can also reduce biosecurity risks for the test operators. Nevertheless, antibody assays may give false-positive results in clinical practice because of interfering substances in patients and cross-reaction with other viruses. Hence, the SARS-CoV-2 Antibody Test Kit needs to be fully evaluated to avoid false-positive results but with excellent sensitivity and specificity in COVID-19 detection.

In this study, we developed SARS-CoV-2 IgM and IgG immunoassays for the rapid test of COVID-19. The assays were based on fluorescence immunochromatography and operated on an autoimmunofluorescence analyzer. The specific IgM and IgG antibodies could be automatically measured and the whole detection process takes about 10 min as compared to 3–4 h of performing a nucleic acid test. Although some commercial immunoassays have been produced based on CLIA and colloidal gold method, our assay is cheaper and easier to build capacity to detect SARS-CoV-2 antibodies than CLIA and is especially suitable for developing countries and areas with limited laboratory conditions. This assay is more stable and provides quantitative detection results as compared to the colloidal gold method that must be observed with the naked eye. Besides, our newly developed kits are suitable for the different types of Finecare FIA Meters and immunofluorescence analyzers with single channel can also be selected according to actual need. Since many factors such as other types of coronavirus infections and autoimmune diseases could play a role in the determination of the specificity of SARS-CoV-2 IgM and IgG immunoassays,[11–13] we verified the interference and cross-reactivity of our assays. Interestingly, both assays showed excellent and reliable test results comparable to the nucleic acid test in the detection of the SARS-CoV-2 virus. Also, we performed multicenter large sample trials across nine hospitals in China. As a result, the specificity of SARS-CoV-2 IgM and IgG assays was both 98.21%, and the clinical sensitivities of SARS-CoV-2 IgM and IgG were 79.54% and 87.45%, respectively.

On the basis of previous studies of SARS-CoV, the median serum conversion time of IgM and IgG was at least 7–10 days after onset, and IgM and IgG could last for 2–3 years.[14,15] Although several studies on SARS-CoV-2 antibody detection have been published, the longitudinal change of SARS-CoV-2 antibodies is still debated. To verify the suitability of our test kit at different stages of the disease, we tested the sensitivity of the assay in patients at different infection time points. We observed that IgM and IgG were detectable as early as Day 1 after onset in some patients. However, similar to some other studies on SARS-CoV-2 antibody,[16,17] the sensitivity was low for either IgM or IgG within the first week, and the positivity rate showed little difference between IgM and IgG, suggesting that IgG could appear immediately after IgM or at the same time. The positivity rate of IgM and IgG rapidly increased to more than 80% in the second-week postonset. IgM peaked in the third week, and then kept decreasing but the positive rate remained higher than 75% at 49 days after onset. While IgG positive rate remained high after the first-week postonset. Combined IgM/IgG tests performed best in almost all stages of the onset of COVID-19. In addition, we found that the positive rate of SARS-CoV-2 IgM was lower in the mild cases than in the moderate/severe cases within 5 weeks after onset, and there were statistical differences at 4 and 5 weeks after onset. The same trend was not shown for more than 5 weeks, possibly because of the small sample size. For IgG, there was no significant difference between the two groups. Since specific IgG appears nearly IgM in patients with COVID-19, combined IgM/IgG performed best in either mild or moderate/severe cases. Therefore, we recommend the combined use of IgM and IgG rather than individual testing of IgM or IgG for all patients. Taken together, our newly developed test kit provides suitable point-of-care testing of patients with COVID-19 after the first week of illness.

In this COVID-19 pandemic, the need for antibody testing among asymptomatic and symptomatic individuals and their close contacts would be critical to combating the pandemic. In addition to its role in complementing nucleic acid detection, antibody tests may assist community epidemiological investigation, and evaluate SARS-CoV-2 infection and immune response in the population to manage the return to normal activities. Nevertheless, it is important to note that the results of antibody assays may be false-negative when antibody levels are below the limit of detection. Also, differences in the individual immune responses to infection may contribute to false-negative results in suspected patients.[5,18] Hence, the use of antibody detection should be done in combination with other detection methods such as RT-PCR, epidemiological history, and clinical symptoms.

Despite all the advantages provided by our newly developed SARS-CoV-2 IgM/IgG assays, there are few limitations to our study

TABLE 3 Presence of antibodies against SARS-CoV-2 in patients with different clinical types at different stage after onset

Days after onset	Moderate/severe cases									Mild Cases								
	IgM			IgG			IgM or IgG			IgM			IgG			IgM or IgG		
	Positive	Negative	Sensitivity (%)	Positive	Negative	Sensitivity (%)	Positive	Negative	Sensitivity (%)	Positive	Negative	Sensitivity (%)	Positive	Negative	Sensitivity (%)	Positive	Negative	Sensitivity (%)
1–7	52	62	45.61	54	60	47.37	64	50	56.14	4	5	44.44	5	4	55.56	5	4	55.56
8–14	60	9	86.96	61	8	88.41	63	6	91.30	9	6	60.00	11	4	73.33	11	4	73.33
15–21	68	4	94.44	71	1	98.61	71	1	98.61	7	1	87.50	8	0	100.00	8	0	100.00
22–28	73	6	92.41	77	2	97.47	77	2	97.47	28	9	75.68*	34	3	91.89	35	2	94.59
29–35	89	9	90.82	98	0	100.00	98	0	100.00	25	9	73.53*	32	2	94.12	32	2	94.12
36–42	57	7	89.06	61	3	95.31	61	3	95.31	34	3	91.89	36	1	97.30	36	1	97.30
>42	69	19	78.41	85	3	96.59	85	3	96.59	8	1	88.89	8	1	88.89	8	1	88.89

Abbreviations: IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

*$p < .05$ (mild cases vs. moderate/severe cases).

that remained to be addressed. First, we did not conduct a virus neutralization test, so the neutralizing activities of the detected IgG antibodies are unknown. The relationship between our test and neutralizing antibodies need to be explored. Second, the monitoring time for patients with COVID-19 was not long enough to meet the disappearance of specific antibodies, which might last for years according to reports on SARS.[14,15] A long-term follow-up study for patients after SARS-CoV-2 virus infection is needed. Also, we did not evaluate the accuracy of RT-PCR and the combined IgM–IgG antibody test among suspected cases. These limitations need to be further investigated in the future.

5 | CONCLUSION

A sensitive, rapid, and accurate fluorescence immunochromatography method has been developed to detect SARS-CoV-2 in serum, plasma, or whole blood samples. The assay was an improved automated immunoassay, less time consuming, and with higher sensitivity. The rapid development of this assay is of great public health significance in the management and control of the COVID-19 pandemic and would serve as an important tool in response to the outbreak.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: Keren Kang, Lingfang Huang, Bin Yang, and Jihua Wang. *Performed the experiments*: Chaolin Ouyang, Jiaming Du, Yaqin Chi, and Guoling Chen. *Analyzed the data*: Chaolin Ouyang, Shuyu He, Le Ying, and Guoling Chen. *Wrote the paper*: Keren Kang, Shuyu He, and Le Ying.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Le Ying http://orcid.org/0000-0002-3620-9637

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