Development of SARS-CoV-2 Rapid Antibody Detection Kit and Study on Dynamic Changes of Antibody in Infected Patients

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Abstract

OBJECTIVE: To develop a rapid detection kit for new coronavirus antibodies and use this reagent to study the dynamic changes of antibodies in clinical SARS-CoV-2 infected patients.

Methods: A (colloidal gold immunochromatography), B (fluorescence immunochromatography), and C (chemiluminescence) detection kits were developed. After clinical evaluation, the A kit was selected as a follow-up study. Serum SARS-CoV-2 IgM antibodies and IgG antibodies were tested in SARS-CoV-2 infected persons and non-SARS-CoV-2 infected persons, respectively. Positive sera of SARS-CoV-2 infected persons were further tested for antibody titers.

Results: The sensitivity of kit A was 50%, 70%, 92.5%, and 97.5% at 1-3 days, 4-6 days, 7-9 days, and> 9 days after admission, which were significantly higher than those of B and C kits. The specificity of A kit is 100%, but the specificity of A, B, and C kits is not

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statistically significant. Using the A kit as a follow-up study, the positive rates of SARS-CoV-2 IgM antibodies and IgG antibodies increased from 50% to 92.5% after 1-3 days, 4-6 days, and 7-9 days of admission, showing a clear upward trend. With the length of admission, the titers of SARS-CoV-2 IgM antibodies and IgG antibodies in positive specimens increased.

CONCLUSION: The positive rate and titer of SARS-CoV-2 antibody show a rapid increase with time. For patients who are negative for the first test, they should be tested again after 7 days. Patients who tested positive for the first time should have a titer test, and then test the titer again after 7 days to determine whether it is a SARS-CoV-2 infection based on the titer change. Other patients with respiratory infections had 2.5% false positives of IgM antibodies, and 0.5% false positives of IgM antibodies in pregnant women, which can be further confirmed by retesting after 1 week.

Background

SARS-CoV-2 is a pathogen that causes neo-coronary pneumonia. It belongs to the genus Coronavirus β and is the seventh coronavirus known to infect humans (1). Its genome is a linear single-stranded positive-stranded RNA with approximate 80% homology to the SARS-CoV gene (2). After SARS-CoV-2 infection, it causes respiratory tract inflammation, immune system disorders, and severe pneumonia. It can cause death in severe cases, and it is prone to hospital infection during treatment, posing a serious threat to people's life and health (3). The incubation period of the disease is generally 3-7 days, the shortest incubation period is 1 day, and the longest incubation period is 30 days, which is contagious during the incubation period. The disease is mainly transmitted from person to person through droplets and contact, and there may be a risk of aerosol transmission in closed, unventilated places (4,5). The aetiology basis of previous clinical diagnosis is either that the nucleic acid test result is positive or sequencing confirms that the virus gene sequence is highly homologous with SARS-CoV-2. However, a variety of factors have led to more false negative

results in nucleic acid testing at present (6), which has a huge impact on the diagnosis of SARS-CoV-2 infection and epidemic prevention and control. In order to avoid the lack of evidence for the diagnosis from a single pathology point of view, the National Health Commission's latest announcement on March 4, 2020, "New Coronavirus Infected Pneumonia Diagnosis and Treatment Guideline (Trial Version 7)" adds serological tests to confirm the diagnosed cases in addition to the original pathology evidence, that is, suspected cases plus "new coronavirus-specific IgM antibody and IgG antibody positive", or "new coronavirus-specific IgG antibody changed from negative to positive, or antibody level was 4 times higher during the recovery period than the acute phase can also be confirmed. At the same time, the exclusion of suspected cases needs to meet the condition: two consecutive tests of the novel coronavirus nucleic acid test are negative (sampling time interval of at least 24 hours), and the new coronavirus-specific antibodies IgM and IgG are still negative 7 days after the onset of illness.

Specific proteins of the new coronavirus, such as S protein or N protein, can stimulate the immune system of an infected person to initiate an immune response, producing virus-specific IgM and IgG antibodies. Detection of virus-specific IgM and IgG antibodies in the serum of suspected patients by using reagents produced by recombinant S protein or N protein antigens, can make up for the lack of pathogenic detection in the diagnosis and exclusion of suspected cases of new coronary pneumonia, and be effectively complementary to the pathogenic detection.

1. Materials and Methods

1.1 Choice of antigen

The SARS-CoV-2 N antigen obtained from two expression systems of PET28 vector + BL21 (DE3) strain, and pCMVp-NEO-BAN vector + HEK293 cell line. These are verified by the serum of SARS-CoV-2 infected persons and non-SARS-CoV-2 infected persons. The SARS-CoV-2 N antigen obtained from the expression system of pCMVp-NEO-BAN vector + HEK293 cell line showed better IgM antibodies and IgG antibodies performance than those expressed by PET28 vector + BL21 (DE3) strain in

testing the serum.

1.2 Development of kits

The research team developed three detection kits A (colloidal gold immunochromatography), B (fluorescence immunochromatography), and C (chemiluminescence), and evaluated the sensitivity and specificity of the three kits.

1.3 Test principle

Principle of colloidal gold immunochromatography: SARS-CoV-2 IgM / IgG was detected using SARS-CoV-2 recombinant antigen and mouse anti-human IgM / IgG antibody. The SARS-CoV-2 IgM / IgG reacted with colloidal gold-bound SARS-CoV-2 recombinant antigen in the sample. The complex is chromatographed along a membrane and reaches a detection line (T) with a murine anti-human IgM antibody and a murine anti-human IgG antibody. When the result is positive, the colloidal gold SARS-CoV-2 recombinant antigen-antibody complex is bound to the IgM or IgG detection line (T) and is purple-red. When the result is negative, the sample does not contain any SARS-CoV-2 recombinant antigen-antibody complex that can bind to the IgM / IgG detection line (T), so the color is not visible.

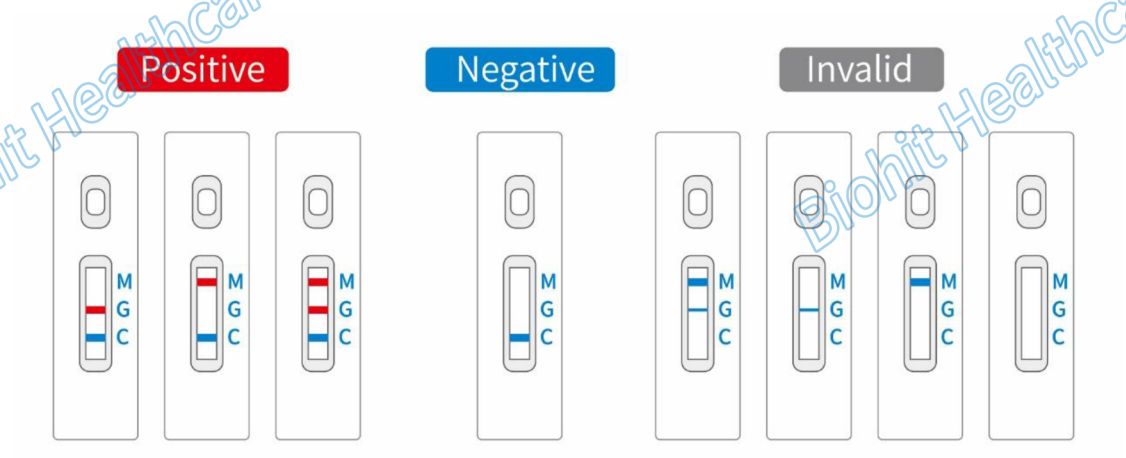

Figure 1 Colloidal gold immunochromatographic test results

Principle of fluorescent immunochromatography: SARS-CoV-2 recombinant antigen and mouse anti-human IgM / IgG antibody are used to detect SARS-CoV-2 IgM / IgG.

In the sample, the SARS-CoV-2 IgM / IgG reacted with the microsphere-associated SARS-CoV-2 recombinant antigen. The complex is chromatographed along a membrane and reaches a detection line (T) with a murine anti-human IgM antibody and a murine anti-human IgG antibody. When the result is positive, the microsphere SARS-CoV-2 recombinant antigen-antibody complex is bound to the IgM or IgG detection line (T); when the result is negative, there is no microsphere SARS-CoV-2 recombinant antigen-antibody complex and IgM. Or IgG detection line (T) binding. The more analytes in the sample, the stronger the fluorescence signal of the T line, and the fluorescence signal intensity can be detected and analyzed by the supporting fluorescence reader produced by Biohit Healthcare (Hefei) Co., Ltd.

Principle of chemiluminescence method: SARS-CoV-2 recombinant antigen and mouse anti-human IgM / IgG antibody are used to detect SARS-CoV-2 IgM / IgG. The SARS-CoV-2 IgM / IgG in the sample reacts with the SARS-CoV-2 recombinant antigen bound to magnetic particles to form a complex. Under the action of a magnetic field, magnetic particles are adsorbed to the reaction tube wall, and unbound materials are washed away by a cleaning solution. A mouse anti-human IgM / mouse anti-human IgG antibody HRP marker was added to form an antigen-antibody-secondary antibody complex. Finally, an enzymatic luminescent substrate is added and the resulting chemiluminescence reaction is measured, expressed as relative light intensity (RLU).

1.4 Verification of three kits

Verification of sensitivity and specificity of three kits using serum samples from 40 clinically confirmed COVID-19 patients and 94 non-COVID-19 populations.

1.5 Research object

40 patients with new coronavirus infection，age between 21～71，median is 46 years； 281 patients with other respiratory infections (including Mycoplasma pneumoniae, parainfluenza virus, adenovirus, and influenza B virus), age between 2

to 99 years, with a median of 51 years; 252 non-respiratory patients (including 30 cases of rheumatic immune system diseases and 20 cases of severe liver disease), aged 1 to 90 years, with a median of 50 years; 416 pregnant women, aged 18 to 34 years, with a median of 27 years; 112 cases of normal physical examination population, aged 23 to 72 years, median number is 50 years old. The age distribution of each group of people is shown in Figure 1.

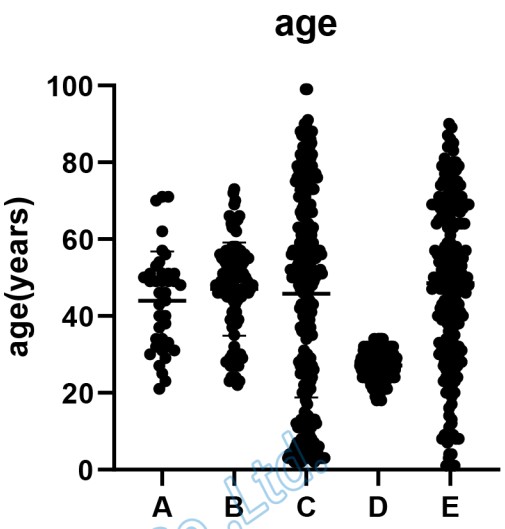

Figure 2 age distribution in five groups. A: patients with COVID-19, B: Physical examination, C: patients with other respiratory infectious diseases, D: pregnant women, E: patients with other system diseases

1.6 Specimen collection and processing

The type of sample used in this study was serum samples. Blood samples were collected from patients with SARS-CoV-2 infection 1-3 days, 4-6 days, 7-9 days, and 9 days after admission. Blood samples were collected and stored at room temperature for 30 minutes, centrifuged at 3500 rpm for 5 minutes, serum was collected, and SARS-CoV-2 IgM antibodies and IgG antibodies were tested.

Non-SARS-CoV-2 serum are from remaining samples from clinical testing.

1.7 Antibody titer testing

The remaining serum samples of patients with non-respiratory infections were

collected, mixed and tested with a new coronavirus IgM / IgG antibody detection A kit to confirm as SARS-CoV-2 IgM and SARS-CoV-2 IgG negative. COVID-19 patients' serum samples were diluted with the negative serum collected above and then tested with the new coronavirus IgM / IgG antibody detection A kit until the diluted samples were tested to be SARS-CoV-2 IgM and SARS-CoV-2 IgG negative, determine the highest dilution factor.

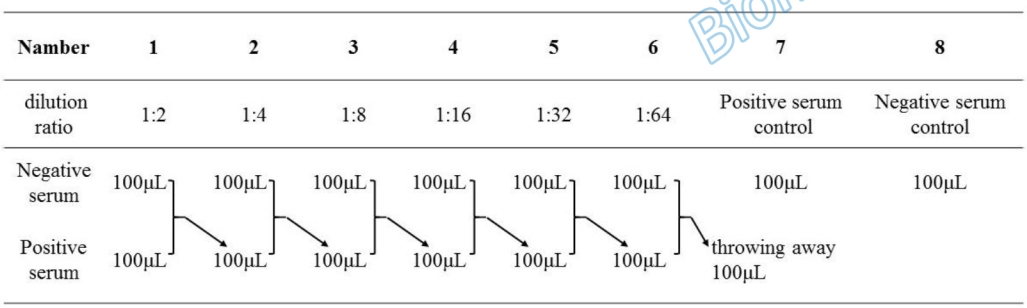

Figure 3 dilution of sample

1.8 Statistical methods

x^2 inspection was used for comparison（chi-square test）。

2. Results

2.1 Comparison of A, B, and C kits

2.1.1 Sensitivity of three kits A, B, and C to detect SARS-CoV-2 IgM antibodies and IgG antibodies

A, B, and C kits were used to detect IgM antibodies and IgG antibodies in serum samples from 40 clinical COVID-19 patients, and the sensitivity of the three kits was calculated. The sensitivity of IgM antibody and IgG antibody detected by A kit was higher than that of B and C kits (the results are shown in Table 1 and Table 2).

The sensitivity was calculated using IgM antibody + IgG antibody (the specimen SARS-CoV-2 antibody is positive when IgM antibody, IgG antibody alone or both are positive), the sensitivity of the A kit is also higher than the B and C kits (the results are shown in Table 3).

Table1 Sensitivity of A、B、C three kits to detect SARS-CoV-2 IgM antibodies

Kit	1-3day	4-6day	7-9day	>9day

A kit	50%	70%	92.5%	97.5%
B kit	27.5%[a]	50%[a]	75%[a]	77.5%[a]
C kit	20%[b]	60%	75%	80%
Chi-square	8.889	3.333	5.271	3.720
p	0.003	0.06	0.016	0.04

Note： a: A kit vs B kit p<0.05
b: A kit vs C kit p<0.05

Table2 Sensitivity of A、B、C three kits to detect SARS-CoV-2 IgG antibodies

Kit	1-3day	4-6day	7-9day	>9day
A kit	50%	70%	92.5%	97.5%
B kit	35%	60%	80%	85%
C kit	30%[a,b]	65%	87.5%	92.5%
Chi-square	9.018	0.879	2.740	4.145
p	0.018	0.083	0.105	0.116

Note： a: A kit vs C kit p<0.05， b: B kit vs C kit p<0.05

Table3 Total sensitivity of A、B、C three kits for SARS-CoV-2 IgM antibody + IgG antibody

Kit	1-3day	4-6day	7-9day	>9day
A kit	55%	75%	95%	97.5%
B kit	45%	65%	85%	90%
C kit	40%	70%	75%[a]	80%[a]
Chi-square	1.875	0.952	6.275	6.384
p	0.08	0.086	0.005	0.006

Note： a: A kit vs C kit p<0.05

2.1.2 The specificity of A, B, C three kits to detect SARS-CoV-2 IgM antibodies and IgG antibodies

A, B, and C kits were used to detect IgM antibodies and IgG antibodies in 94

non-COVID-19 serum samples. The specificity of I, M, and IgG antibodies and IgM antibodies + IgG antibodies was calculated for three, A, B, and C kits. The specificity of the A kit is 100%, and the specificity of the B and C kits are less than 100%, but the difference in specificity is not statistically significant (see Table 4 for results).

Table4 The specificity of A, B, C three kits to detect SARS-CoV-2 IgM antibodies and IgG antibodies

Kit	IgM antibody	IgG antibody	IgM+IgG antibody
A kit	100%	100%	100%
B kit	98.9%	98.9%	97.9%
C kit	98.9%	100%	98.9%
Chi-square	0.000	0.000	0.339
p	0.503	0.503	0.377

Based on the overall consideration of detection sensitivity, specificity, ease of operation, and whether or not an instrument is needed, the sensitivity of A kit is better than that of B and C kits, and no instrument is required. We choose A kit (colloidal gold immunochromatography) as the final kit to continue for subsequent research.

2.2 Study on Kinetics of Antibody from SARS-CoV-2 Infected Population

2.2.1 Changes in IgM antibody and IgG antibody positive rates at different times of SARS-CoV-2 infection

From 1-3 days to 7-9 days after admission, the antibody-positive rate increased from 50% to 92.5%, and more than 9 days after admission, the positive rate increased to 97.5% (see Figure 4 for the results). But one patient was still negative for IgM antibodies and IgG antibodies on day 22 after admission.

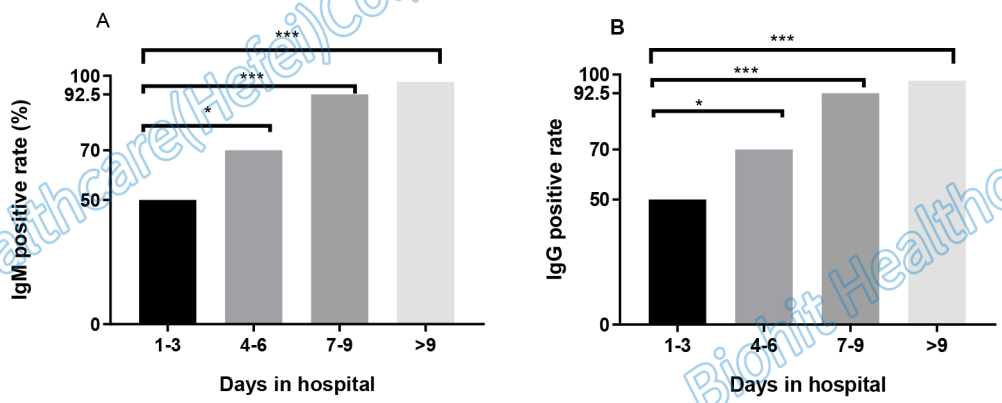

Figure 4 Changes in IgM and IgG antibody positive rates in patients with SARS-CoV-2 infection at different days of admission

2.2.2 Changes in antibody titers of SARS-CoV-2 infected patients after admission

The titers of IgM antibody-positive patients were mainly 1-2 times on 1-3 days after admission. Two patients had IgM antibody titers of more than 4 times. IgM antibody titers were mainly 2 to 8 times at 4-6 days, 2 patients had titers of more than 8 times, 7 to 9 days were mainly 2 to 16 times, and 4 patients had titers of more than 16 times, exceeding 9 days the IgM antibody titer was mainly 4-32 times, and 4 cases reached 64 times. No patient had an IgM antibody titer exceeding 64 times.（See Figure5A）

IgG antibody titers showed a similar trend to IgM antibody titers.（See Figure5B）

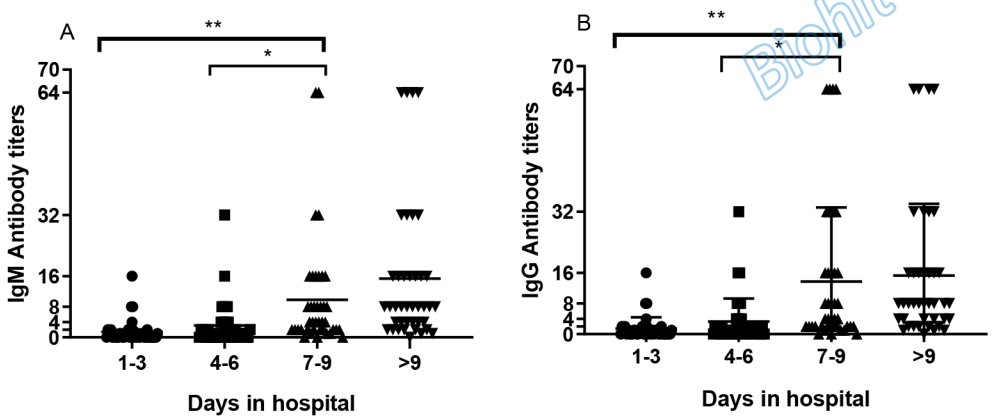

Figure 5 IgM antibody and IgG antibody titers in patients with SARS-CoV-2 infection on different days of admission

2.3 Analysis of false positive rates of SARS-CoV-2 IgM antibodies and IgG antibodies in non-SARS-CoV-2 infected people

Of the 1061 non-SARS-CoV-2 infected people, IgM test results were positive in 6 samples from other respiratory infection patients, 2 positive samples from pregnant women, and all 8 IgM positive samples were weak positive. After 1 week, the results were negative.（See results in Table5）

Table5 Analysis of IgM test results in 1061 non-SARS-CoV-2 infected people

S/N	Sample type	Sample amount	IgM(+)	IgM(-)	False positive rate
1	Samples of patients with other respiratory infections	281	6	275	2.1%
2	Pregnant woman sample	416	2	414	0.5%
3	Non-respiratory infection patient samples	252	0	252	0
4	Physical examination sample	112	0	112	0
5	Total	1061	8	1053	0.75%

There were no false positive results of IgG in 1061 non-SARS-CoV-2 infected people. The results are shown in Table 6.

Table6 Analysis of IgG test results in 1061 non-SARS-CoV-2 infected people

S/N	Sample type	Sample amount	IgG（+）	IgG（-）	False positive rate
1	Samples of patients with other respiratory infections	281	0	282	0
2	Pregnant woman sample	416	0	416	0
3	Non-respiratory infection	252	0	252	0

	patient samples				
4	Physical examination sample	112	0	112	0
5	Total	1061	0	1061	0

3. Discussion

In this study, the SARS-CoV-2 N antigen obtained from two expression systems of PET28 vector + BL21 (DE3) strain, pCMVp-NEO-BAN vector + HEK293 cell line was verified. and was expressed. The SARS-CoV-2 N antigen obtained by pCMVp-NEO-BAN vector + HEK293 cell line in the detection of IgM antibodies and IgG antibodies in serum was better than PET28 carrier + BL21 (DE3) strain. It may be that prokaryotic expression system can not form the correct formation of protein spatial structure. Therefore, pCMVp-NEO-BAN vector + HEK293 cell line was selected to express the antigen for subsequent research.

This project first developed SARS-CoV-2 IgM antibody and IgG antibody kits in three methodologies: colloidal gold immunochromatography, fluorescence immunochromatography, and chemiluminescence. After verification with SARS-CoV-2 infected persons and non-SARS -CoV-2 infected patients' serum, colloidal gold immunochromatography kit has high sensitivity (see Table 1 and Table 2), specificity reaches 100% (see Table 4). It is simple to operate, no instrument is required, through comprehensive consideration, we chose the colloidal gold immunochromatography kit as the finalized kit. The sensitivity of the chemiluminescence method kit is poor, which may be caused by the immature development of the kit, and further technical research is needed on magnetic bead labeling, enzyme-labeled reagent diluent formulation, and stability.

A medical team from Guangzhou established a rapid IgM-IgG antibody detection method and used the kit for clinical research verification. The clinical sensitivity and specificity of the test were determined using blood samples from 397 COVID-19 patients and 128 negative patients confirmed by PCR from 8 different hospitals. The detection sensitivity was 88.66%, and the specificity was 90.63%. This study

indicates that the combined IgM-IgG test has better practicality and sensitivity than a single IgM or IgG test (7). Our results also show that the combined detection of IgM and IgG can increase the detection rate of infected patients (see Table 3). Guo L et al.'S report showed that the positive rates of IgM antibodies and IgG antibodies in SARS-CoV-2 infected patients increased significantly after 7-14 days of symptoms (8).

Considering the uncertainty about the time when patients recall the symptoms, this study used the number of days of admission as the basis for grouping. The results showed that IgM antibodies and IgG were 1-3 days, 4-6 days, 7-9 days, and more than 9 days after admission. Antibody positive rates were 50%, 70%, 92.5%, and 97.5% (see Figure 4), suggesting that the antibody positive rate increased rapidly in the early stages of infection, and antibody detection can be used as a interpretation indicator for SARS-CoV-2 infection. However, one infected person was negative for IgM antibody and IgG antibody at the third week. Checking the medical records, this infected person only had a history of exposure and did not show clinical symptoms. The nucleic acid test continued to be positive, suggesting that there was a delay in antibody production in the individual and attention should be paid to. At the same time, we found an interesting phenomenon, the positive rate of IgM antibody and IgG antibody showed a parallel rise, and further research is needed.

With the length of hospital stay, serum antibody titers of COVID-19 patients will increase, but there are individual differences. For more than 9 days of hospitalization, antibody titers of 70% of patients can rise to more than 4 times, and some patients can rise to 32 ~ 64 times (see Figure 5).

The test results of 1061 non-SARS-CoV-2 infected people showed that SARS-CoV-2 IgM showed weak positive results in 8 cases, 6 cases of other respiratory infections, 2 cases of pregnant women, and the results were reviewed after 1 week. The results were negative. It is suggested that there are factors that interfere with the detection of SARS-CoV-2 IgM in other respiratory infections and pregnant women, but this can be confirmed by a retest after 1 week. Negative health checkups and patients with other systemic diseases, including rheumatic immune system disease

and severe liver disease, did not affect the test results.

4. Conclusion

4.1 The positive rate and titer of SARS-CoV-2 antibody showed a rapid increase with time. For patients who were negative for the first test, they should be tested again after 7 days. Patients who tested positive for the first time should have a titer test, and then test the titer again after 7 days to determine whether it is a SARS-CoV-2 infection based on the titer change.

4.2 Other patients with respiratory infections had 2.5% false positives of IgM antibodies, and 0.5% false positives of IgM antibodies in pregnant women, which can be identified by retesting after 1 week.

Disclaimer: The antigen screening, methodological selection and reagent production of this study were completed in the research and development department of Biohit Healthcare (Hefei) Co., Ltd. Methodological evaluation and performance verification were completed in the First Affiliated Hospital of Anhui Medical University. All authors have no conflicts of interest.

Ethics: This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

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