Early diagnosis · early isolation · early treatment

2019-nCoV Ab Test (Colloidal Gold)

• NMPA approved product under National Emergency Assessment •

Read results in 15 minutes

Characteristics of 2019-nCoV

Strong infectivity

Fast transmission

Long latent period

The latent period for 2019-nCoV is 1-14 days, with an average of 3-7 days. Mild patients only show symptoms such as low fever and mild fatigue without pneumonia. Some infected patients are asymptomatic but can also become a source of infection, which makes early diagnosis essential.

IgM and IgG Combined Detection

The clinical auxiliary diagnosis of 2019-nCoV requires simple, economical and feasible methods. The human immune system can produce specific IgM and IgG antibodies after virus infection. IgM is the earliest antibody that appears upon the first immune response. The detection of IgM antibody indicates a recent infection and can be used as auxiliary diagnosis of early infection. IgG is produced later and lasts long, which can be used as an indicator of previous or secondary infection.

The kit is intended for the qualitative detection of IgM and IgG antibodies against 2019 Novel Coronavirus (2019-nCoV) in human serum/plasma/venous whole blood specimen and for the auxiliary diagnosis of 2019-nCoV infection. The confirmation or exclusion of infection will be combined with the patient's clinical manifestations or further other methods.

2019-nCoV Ab Test
(Colloidal Gold)

Features

1. Venous Whole Blood/Serum/ Plasma
2. Result in 15 minutes
3. Equipment-free, suitable for POCT
4. Assisting confirmation of positive cases

Product Name

2019-nCoV Ab Test (Colloidal Gold)

Intended Use

The kit is intended for the qualitative detection of IgM and IgG antibodies against 2019 Novel Coronavirus (2019-nCoV) in human serum/plasma/venous whole blood specimen. It is only used as a supplementary detection indicator for suspected nucleic acid negative results or in conjunction with nucleic acid detection in the diagnosis of suspected cases. It cannot be used as a basis for the diagnosis and exclusion of COVID-19. It is not suitable for general screening.

A positive test result requires further confirmation. A negative test result does not rule out the possibility of infection.

This product is limited to clinical use and emergency reserve during the COVID-19 epidemic outbreak since December 2019, and cannot be used in the clinic as a conventional in vitro diagnostic reagent. The test results of this kit are for clinical reference only. It is recommended to conduct a comprehensive analysis of the condition based on the patient's clinical manifestations and other laboratory tests.

Laboratory testing of 2019-nCoV should meet the requirements of the "Technical Guidelines for Laboratory Testing of COVID-19 Infection" to do a better biosafety job.

Summary

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). 2019-nCoV is a new strain that has not been previously identified in humans.

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Standard recommendations to prevent infection spread include regular hand washing, covering mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs. Avoid close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing.

Current estimates of the incubation period range from 1-12.5 days with median estimates of 5-6 days. These estimates will be refined as more data becomes available. Based on information from other coronavirus diseases, such as MERS and SARS, the incubation period of 2019-nCoV could be up to 14 days. WHO recommends that the follow-up of contacts of confirmed cases is 14 days.

Principle

The kit detects 2019-nCoV IgM and IgG antibodies by immuno-capture method. The nitrocellulose membrane is coated by mouse-anti human monoclonal IgM antibodies, mouse-anti human monoclonal IgG antibodies, and goat-anti-mouse IgG antibodies. The recombinant 2019-nCoV antigen and mouse IgG antibodies are labeled with colloidal gold as a tracer. After addition of the specimens, if 2019-nCoV IgM antibodies are present, the antibodies will bind to colloidal gold-coated 2019-nCoV antigens to form compounds, which are further captured by pre-coated mouse-anti human IgM antibodies to form new compounds, and generate purple line (T). If 2019-nCoV IgG antibodies are present in specimen, the antibodies will bind to colloidal gold-labeled 2019-nCoV antigens to form compounds, and further form new

compounds by binding to pre-coated mouse-anti human monoclonal IgG antibodies, which give rise to purple line (T). The binding of colloidal gold-labeled mouse IgG antibodies with goat-anti-mouse IgG antibodies will present purple line, which is used as the control line(C).

Composition

1. Sealed foil pouches each containing:
   a. One cassette device
   b. One desiccant
2. Specimen diluent
3. Instructions for use

Storage and Stability

1. Store at 4℃~ 30℃ (39.2℉~ 86℉) .
2. Use the test within 1 hour after opening the pouch under 60% humidity.
3. See production date and expiration date on label.

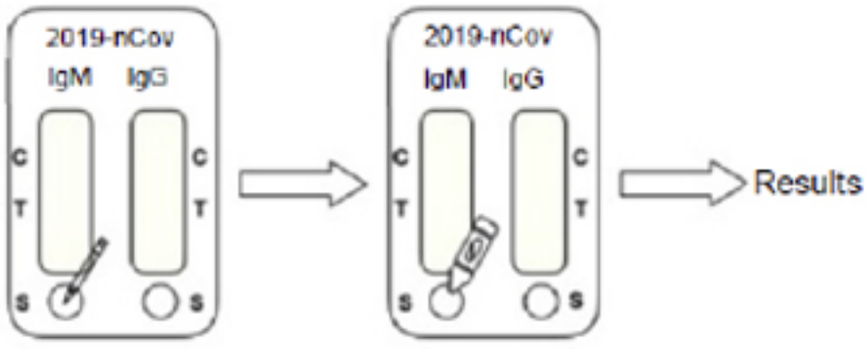
Specimen Collection and Handling

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

1. The kit is intended for test only in serum/plasma/venous whole blood specimens.
2. Specimens should be collected by standard protocol.
3. The venous whole blood specimens could be stored at 2℃~ 8℃ (36℉~46℉) for up to 3 days, and it couldn't be frozen. Venous whole blood specimens can be anti-coagulated with routine dosage of heparin (9.8-28IU/mL), sodium citrate (3.8%, equivalent to 129mmol/L), ethylenediaminetetraacetic acid (EDTA) (4.55mmoL / mL ± 0.85 mmol/mL).
4. The serum or plasma specimen s could be stored at 2℃~ 8℃ (36℉~46℉) for up to 7 days, and could be frozen at -20℃ (-4℉) for 6 months. The specimens are repeatedly frozen and thawed no more than 8 times; it should be the best to test the sample after collection immediately.
5. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Test Procedure

1. Allow the test, specimen diluent and/or controls to reach room temperature 10℃~30℃ (50℉~86℉) prior to testing.
2. Remove the test device from the sealed pouch and use it as soon as possible.
3. Place the test device on a clean and level surface.
4. **FROM THE TOP OF THE SPECIMEN WELL:** Add 20μL venous whole blood or 10μL serum/plasma specimen into each specimen well.
5. **FROM THE BOTTOM OF THE SPECIMEN WELL:** Add 80μL or 2 drops of specimen diluent into each specimen well.
6. Wait for the colored line(s) to appear. Read results within 15 minutes. Do not read the result after 15 minutes.

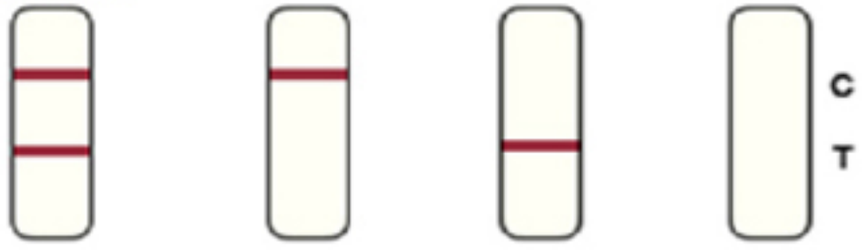


Add 20 μL venous whole blood or 10 μL serum/plasma

Add 80 μL(or 2 drops) of specimen diluent

Results Interpretation

1. **IgM Positive**: The presence of two purple bands (T and C) within the IgM result window indicates positive for 2019-nCoV IgM antibody.
2. **IgG Positive**: The presence of two purple bands (T and C) within the IgG result window indicates positive for 2019-nCoV IgG antibody.
3. **Negative**: Only one purple band appearing at the control line (C) indicates negative result.
4. **Invalid**: If control line (C) fails to appear, no matter whether the T line is visible or not, the test is invalid. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, you should immediately stop using the kit with the same LOT No. and contact your local distributor.



Positive **Negative** **Invalid** **Invalid**

Performance Characteristics

1. Use the national or enterprise reference controls for testing, and the results meet the detection requirements of national or enterprise reference controls.
2. Test the samples with a titer of 1:320 at the original concentrations with the 2019-nCoV IgM antibody and 2019-nCoV IgG antibody. No hook effect was observed.
3. The clinical trial of this product is based on the clear diagnosis / exclusion criteria of the disease identified in the "COVID-19 Diagnosis and Treatment Program". Clinical research was conducted in 5 institutions and the total cases were 447. Using this kit, 110 cases out of 126 clinically confirmed cases are positive, with the sensitivity of 87.3% (95% CI: 80.40% to 92.0%); 62 cases of clinically excluded cases are totally negative with the specificity of 100% (95% CI: 94.20% to 100%).
4. Avoid using special samples: red background may appear in the hyperlipemia (triglyceride concentration higher than 25mg/ml), icteric samples (Bilirubin concentration higher than 0.2mg/mL) and hemolytic specimen (hemoglobin concentration more than 5.0mg/mL). which may affect the test result.
5. The 2019-nCoV IgM test was also evaluated with samples that are IgM positive for other diseases as listed in the following table. No cross reactivity was observed.

Coronavirus HKU1-IgM	Coronavirus OC43-IgM
Coronavirus NL63-IgM	Coronavirus 229E-IgM
Influenza A virus H1N1 (new type influenza A virus H1N1 2009, seasonal influenza virus H1N1) IgM	H3N2-IgM
H5N1-IgM	H7N9-IgM
Influenza B virus IgM	Respiratory Syncytial Virus IgM
Adenovirus IgM	Rhinovirus IgM
Enterovirus A-IgM	EB virus IgM
Measles virus IgM	Cytomegalovirus IgM
Rotavirus IgM	Mumps IgM
Varicella-zoster virus IgM	Parainfluenza virus IgM
Mycoplasma pneumoniae IgM	Chlamydia pneumoniae IgM
Coxsackievirus group B IgM	

6. The 2019-nCoV IgG test was also evaluated with samples that are IgG positive for other diseases as listed in the following table. No cross reactivity was observed.

Coronavirus HKU1-IgG	Coronavirus OC43-IgG
Coronavirus NL63-IgG	Coronavirus 229E-IgG
IInfluenza A virus H1N1 (new type influenza A virus H1N1 2009, seasonal influenza virus H1N1) IgG	H3N2-IgG
H5N1-IgG	H7N9-IgG
Influenza B virus IgG	Respiratory Syncytial Virus IgG
Adenovirus IgG	Rhinovirus IgG
Enterovirus A-IgG	EB virus IgG
Measles virus IgG	Cytomegalovirus IgG
Rotavirus IgG	Mumps IgG
Varicella-zoster virus IgG	Parainfluenza virus IgG
Mycoplasma pneumoniae IgG	Chlamydia pneumoniae IgG
Coxsackievirus group B IgG	

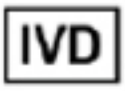
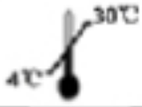
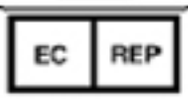
7. RF, ANA and AMA don't exhibit cross reactivity with the test.
8. Common antivirals such like Epistine hydrochloride (≤4mg/L), Ribavirin (≤40mg/L), Interferon (≤200mg/L), Oseltamivir (≤30mg/L), Abidol (≤40mg/L), Levofloxacin (≤200mg/L), Azithromycin (≤100mg/L), Ceftriaxone (≤400mg/L), Meropenem (≤200mg/L) have no interference effect on the detection of this kit.
9. Systemic lupus erythematosus has no interference effect on the detection of this kit.
10. Non-specific IgM antibody (≤0.8mg/mL) and non-specific IgG antibody (≤4mg/mL) have no interference effect on the detection of this kit.
11. Heparin, sodium citrate, EDTA and other anticoagulants have no interference effect on the detection of this kit.
12. The precision experiments were carried out by different experimenters, at different times and at different places, and the results met the product performance requirements.
13. After the specific IgM positive sample was destroyed by β-mercaptoethanol, the IgM test result was negative.
14. After preliminary evaluation, it is basically confirmed that the clinical performance of the product can meet the emergency needs of the epidemic. The product will further collect clinical data to confirm the clinical performance of the product after it is marketed.

Limitations

1. The kit is for qualitative detection and auxiliary diagnosis use only.
2. In the early phase of infection, no IgM or IgG antibody will be produced, or the titer will be very low, thus, negative result will occur. Re-testing will be conducted in 7-14 days, and the sample that is collected last time will be detected in parallel during re-testing to confirm whether the serology turns positive or the titer increases significantly.
3. The reference value of serological antibody detection is limited for the immune-compromised patients or patients who receive immunosuppressive therapy.
4. IgM antibody positive will occur not only in primary infection, but also in secondary infection.
5. IgG antibody positive indicates previous infection or secondary infection.
6. The confirmation or exclusion of infection will be combined with the patient's clinical manifestations or other further methods.

Precaution

1. Use fresh specimens whenever possible.
2. Results after 15 minutes are considered invalid.
3. The product should be used as soon as possible once the foil pouch is open, in case of long-term exposure to environment.
4. Follow standard biosafety guidelines for handling and disposal of potential infective material.

	Do not reuse		For in vitro diagnostic use only
	Stored between 4-30℃		Consult instruction for use
	Caution		Lot number
	Use by		Contains sufficient for <n> tests
	Keep away from sunlight		Keep dry
	Manufacturer		Do not use if package is damaged
	Authorized Representative in the European Community		
	CE Mark		

COVID-19 Diagnosis and Treatment Program (7th Edition)

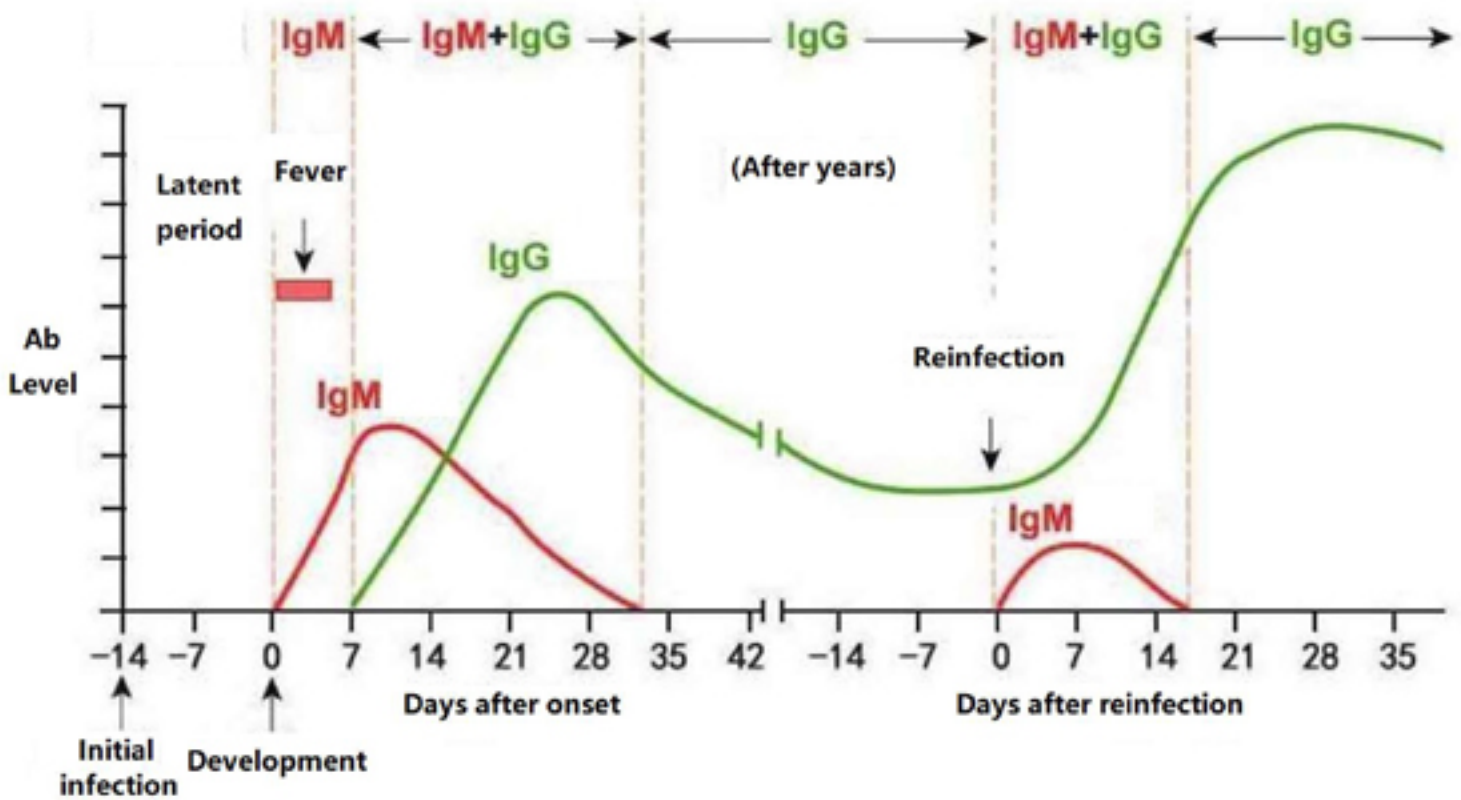
Section 5. Diagnosis Criteria

II. Confirmed Cases

Suspected cases having either one of the following etiology or serological evidences:

1. Nucleic acid positive by real-time fluorescence RT-PCR;
2. Highly homologous to known SARS-COV-2 by gene sequencing;
3. Specific IgM and IgG antibodies both positive; or
   Specific IgG antibody changed from negative to positive; or
   Specific IgG antibody increased by at least 4 times in recovery phase compared to acute phase.

Significance of IgM & IgG



Test Result Reference

Nucleic acid	IgM	IgG	Nucleic acid and antibody test results reference
Positive	-	-	Probably in "window period" of infection.
	+	-	Probably in the early stage of infection, but no IgG is produced or the IgG concentration does not reach the lowest limit of detection.
	-	+	Probably in the advanced stage of infection or recurrent infection.
	+	+	Active phase of infection. But the human body has developed immunity with persistent IgG antibody being produced.
Negative	+	-	Highly probably in the acute stage of infection. At this time, it is necessary to consider cases where the nucleic acid test result is suspect or the patient has other diseases. Rheumatoid factor has been found to result in weak positive or positive in IgM.
	-	+	Probably previous infection. But the body has recovered or the virus has been cleared. IgG produced by the immune response maintains for a long time and is still detectable in blood.
	±	-	Initial infection with very low virus load at early stage; When the viral load is lower than the lowest detection limit of nucleic acid, a small amount of IgM was produced, and no IgG was produced; Or rheumatoid factor positive of the patient resulted in IgM false positive.
	+	+	The patient has recently been infected and is in the recovery phase. The virus in the body has been cleared and IgM has not been reduced to the lowest limit of detection; Or the nucleic acid test result is false negative and the patient is in active phase of infection.

Clinical Evaluation and Application of Detection for SARS-CoV-2 IgM and IgG Antibodies with Colloidal Gold Immunochromatography Assay

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Abstract: The pandemic of the novel coronavirus (SARS-CoV-2) infection has seriously threated global public health, a rapid and easy operated method for coronavirus disease (COVID-19) diagnosis is needed. To evaluate the clinical application efficacy of the colloidal gold rapid test kit for detection of the IgM/IgG antibodies to SARS-CoV-2, a total of 304 clinical diagnosed case, 138 health donor (of which 114 showed SARS-CoV-2 viral RNA negative and 64 other fever patients with respiratory symptoms were selected for the study and the plasma or serum samples were tested for both IgM and IgG with the kit. The comparison of the detection coincidence of the samples from whole blood and plasma or serum were also performed; Furthermore, the time distribution of SARS-CoV-2 viral RNA and IgM/IgG antibodies detections were analyzed. The results showed that, of the 304 clinical diagnosed cases, 105 cases were positive for viral RNA detection, among which the detection sensitivity of IgM and IgG antibodies to SARS-CoV-2 by colloidal gold rapid assay was 76.2% (80/105) and 86.6% (91/105), respectively, and the overall coincidence rate of IgM/IgG antibody was 96.1% (101/105); and 73 cases were negative for both nucleic acid and antibody detection. Of the remaining 126 clinical diagnosed cases, the positive rate of IgM and IgG was 69.2% (87/126) and 98.3% (125/126), respectively, and the overall coincidence rate of IgM/IgG antibody was 100% (126/126). In detections for healthy and other fever patients, the specificity of IgM and IgG was 99% (200/202) and 98% (198/202), respectively, and the total coincidence rate of antibody detection results of homologous whole blood and plasma samples was 99%, indicating a high degree of consistency. In this study, the detection assay of SARS-CoV-2 antibodies using colloidal gold method showed satisfactory detection effect, and it could be used for clinical auxiliary diagnosis and epidemiological investigation, which could be applied in a wide range of scenarios and play a valuable role in the prevention and control of SARS-CoV-2 pandemic. [1]

Key words: SARS-CoV-2; Antibody detection; Colloidal gold; IgM antibody; IgG antibody

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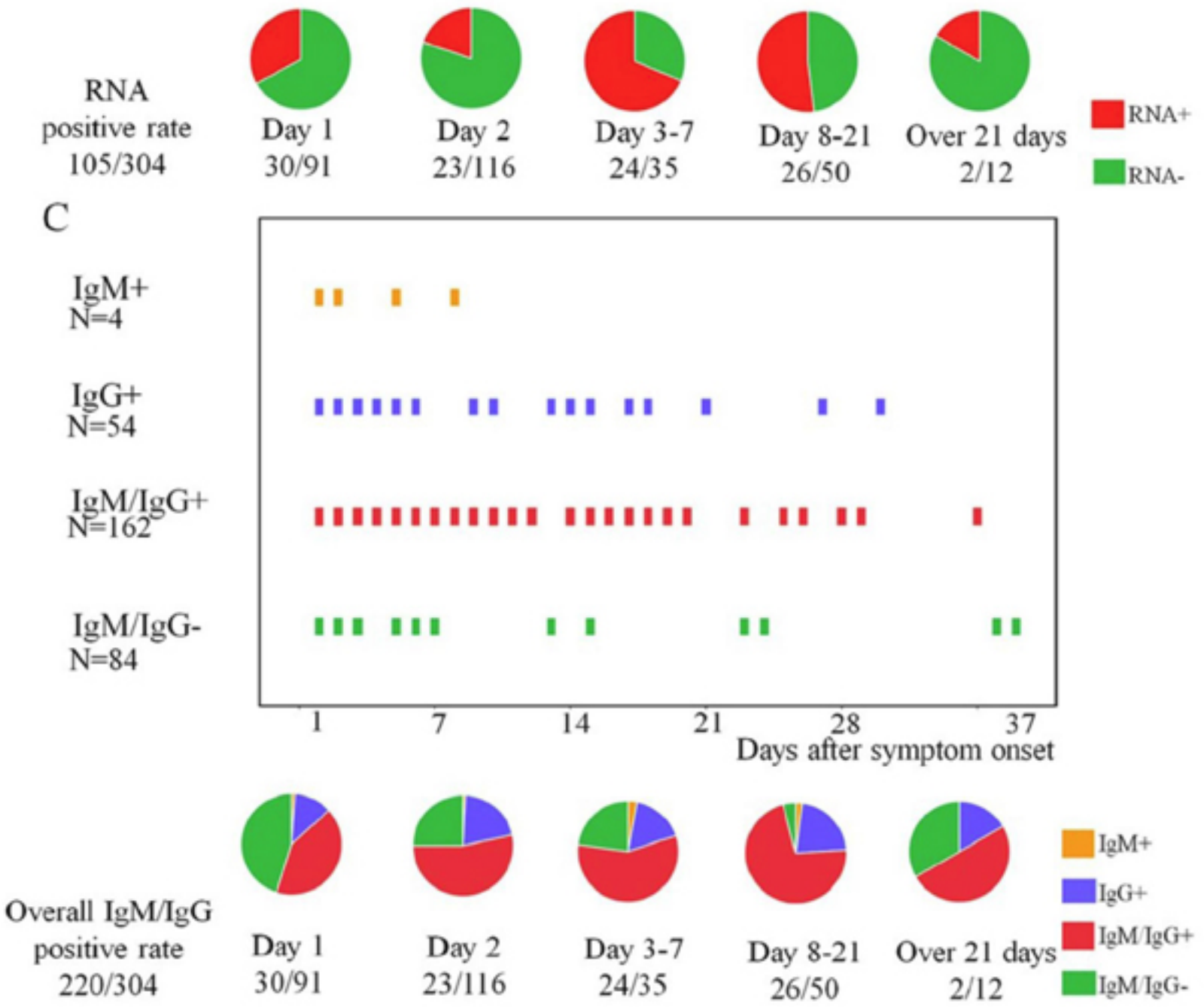
Funding: This study was supported by the Ministry of Science and Technology Project, title: Development of Novel Coronavirus (2019-nCoV) Rapid Detection Product


RNA
positive rate
105/304
Day 1
30/91
Day 2
23/116
Day 3-7
24/35
Day 8-21
26/50
Over 21 days
2/12
RNA+
RNA-
C
IgM+
N=4
IgG+
N=54
IgM/IgG+
N=162
IgM/IgG-
N=84
1
7
14
21
28
37
Days after symptom onset
Overall IgM/IgG
positive rate
220/304
Day 1
30/91
Day 2
23/116
Day 3-7
24/35
Day 8-21
26/50
Over 21 days
2/12
IgM+
IgG+
IgM/IgG+
IgM/IgG-

References

Hospitals using our 2019-n-CoV Ab test

Peking University Third Hospital	Hubei Provincial Hospital of Integrated Traditional Chinese and Western Medicine
Beijing Friendship Hospital	Wuhan Xiehe Hospital
Beijing Youan Hospital	Hubei Provincial Hospital of Traditional Chinese Medicine
Beijing Chaoyang Hospital	Wuhan Children's Hospital
Beijing Armed Police General Hospital	Wuhan Fangcai Hospital
Children's Hospital of Fudan University	Beijing Children's Hospital
Shanghai Xinhua Hospital	Capital Institute of Pediatrics
Shanghai Children's Medical Center	Shanghai Xinhua Hospital
Shanghai Children's Hospital	Affiliated Children's Hospital of Fudan University
Henan Provincial Center for Disease Control	Shanghai Children's Hospital
Guangdong Provincial Center for Disease Control	Suzhou Children's Hospital
Sichuan Provincial Center for Disease Control	Xuzhou Children's Hospital
Beijing Centers for Disease Control	Tianjin Children's Hospital
Disease Control Center of Shanxi Province	Shenyang Children's Hospital
Wuhan Tongji Hospital	Children's Hospital of Hebei Province