Covid-19 Rapid IgM/IgG Combined Antibody Assay Pre-Screening Kit Package Insert

Model Number: SP-GR 525

For the qualitative assessment of new coronavirus (2019-nCOV) IgM/IgG in human serum/plasma/whole blood.

INTENDED USE

The Covid-19 Rapid IgM/IgG Combined Antibody Assay Pre-Screening Kit is a rapid chromatographic immunoassay for the qualitative detection of IgM & IgG antibody of WUHAN New Coronavirus in human whole blood, serum, or plasma from anticoagulated blood (Li+ heparin, K2EDTA and sodium citrate).The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection.

SUMMARY

Coronavirus (CoV) belongs to the genus Nestovirus, Coronaviridae and is divided into three genera: α, β, and γ.
The genus α and β are only pathogenic to mammals. The genus mainly causes bird infections CoV is mainly transmitted through direct contact with secretions or through aerosols and droplets. There is also evidence that it can be transmitted through the fecal-oral route. So far, there are 7 types of human coronavirus (HCoV) that causes human respiratory diseases: HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HCoV-HKU1,
MERS-Co and new coronaviruses (2019), Is an important pathogen of human respiratory infections. Among them, the new coronavirus (2019) was discovered due to Wuhan virus pneumonia cases in 2019. The clinical manifestations are systemic symptoms such as fever and fatigue accompanied by dry cough and dyspnea, etc., which can rapidly develop into severe pneumonia, respiratory failure, and acute breathing. Distress syndrome, septic shock, multiple organ failure, severe acid-base metabolism disorders, etc. are even life-threatening.

PRINCIPLE

This kit uses immunochromatography. The test card contains: 1) colloidal gold-labeled recombinant new coronavirus antigen and quality control antibody gold markers; 2) two detection lines (G and M lines) and one quality Control line (C line) of nitrocellulose membrane. The M line is immobilized with a monoclonal anti-human IgM antibody for detecting a new coronavirus IgM antibody; the G line is immobilized with a reagent for detecting a new coronavirus IgG antibody; and the C line is immobilized with a quality control antibody.
When an appropriate amount of the test sample is added to the sample hole of the test card, the sample will move forward along the test card under the action of the capillary. If the sample contains an IgM antibody, the antibody will bind to the colloidal gold-labeled new coronavirus antigen. The immune complex will be captured by the anti-human IgM antibody immobilized on the membrane to form a purple-red M line showing that the new coronavirus IgM antibody is positive. If the sample contains an IgG antibody, the antibody will bind to the colloidal gold-labeled new coronavirus antigen and the immune complex will be captured by the reagent immobilized on the membrane to form a purple-red G line, indicating that the new coronavirus IgG antibody is positive. If the test lines G and M are not colored a negative result is displayed. The test card also contains a quality control line C. The fuchsia quality control line C should appear regardless of whether a test line appears. The quality control line is a color band of the quality control antibody immune complex. If the quality control line C does not appear, the test result is invalid, and the sample needs to be tested again with another test card.

REAGENTS

The test contains 2019-nCOV virus envelope protein particles and anti-human IgG anti-human IgM antibody conjugated gold particles coated on the membrane.

WARNING AND PRECAUTIONS

1. For professional in vitro diagnostic use only. Do not use the kit beyond the expiration date.
2. Do not eat, drink or smoke in the area where the specimens or kits are handled.
3. Do not use the test if the pouch is damaged.
4. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
5. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
6. Negative results do not rule out 2019-nCOV/COVID-19 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
7. Result from antibody testing should not be used as the sole basis to diagnose or exclude 2019-nCOV/COVID-19 infection or to inform infection status.
8. Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
9. Temperature and humidity can harm the accuracy of the results (especially with an RH over 80%). Testing must be conducted within 60 minutes of opening the pouch.
10. Please ensure the package insert is read completely before conducting the test. Failure to do so will result in inaccurate test results.
11. Not to be used if the pouch/tube is damaged or broken.
12. Test must only be used once. It must never be re-used.
13. All samples must be handled as if they contain infectious agents. Comply with established precautions against microbiological hazards throughout testing process and abide by the standard procedures for correct disposal of samples.

STORAGE AND STABILITY

1. The original packaging should be stored at 4~ 30 °C to avoid light, keep dry.
2. The test device is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. DO NOT FREEZE.
3. Do not use beyond the expiration date, especially at temperatures above 30 °C or under high humidity conditions,should be used immediately once it is opened.

SPECIMEN COLLECTION AND PREPARATION

1. The Spring COVID-19 IgM/IgG Rapid Test Cassette can be performed using with venous whole blood, serum or plasma.
2. The COVID-19 IgM/IgG Rapid Test Cassette (WholeBlood/Serum/Plasma) test has not been evaluated with fingerstick specimens. Use of this test must not be with fingerstick blood.
3. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
4. Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage,specimens should be kept below -20°C for up to one month.Whole blood specimens must be stored at 2-8˚C if not tested immediately and tested within 24 hours of collection. Do not freeze whole blood specimens.
5. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens cannot be frozen and thawed more than 3 times.
6. If specimens are to be shipped, they should be packed in compliance with federal regulations for transportation of etiologic agents.

MATERIALS

Materials provided

- Test Devices – 10 Cassettes
- Disposable plastic pipette - 10
- Alcohol pads - 10
- Buffer – 1 Package
- Insert - 1

Materials required but not provided

- Specimen collection containers
- Micropipette Centrifuge (for plasma only)
- Timer

DIRECTIONS FOR USE

Allow the test device, specimen, buffer, and/or controls to reach room temperature (15-30°C) prior to testing.
1. Bring the pouch to room temperature before opening Remove the test device from the sealed pouch and use itas soon as possible.
2. Place the test device on a clean and level surface.

a. For Serum or Plasma Specimens:

With a 5 µL mini plastic dropper provided, draw serum/plasma specimen to exceed the specimen line as showed in the following image and then transfer drawn serum/plasma specimen into the sample well (S). Then add 2 drops (about 80 µL) of sample buffer to the buffer well (B) immediately. Avoid air bubbles.

Note: *Practice a few times prior to testing if you are not familiar with the mini dropper. For better precision, transfer specimen by pipette capable to deliver 5 µL of volume.*

b. For Venous whole blood Specimens:

Hold the 5 µL mini plastic dropper vertically and transfer 1 drop of whole blood (about 10 µL) to the specimen well (S) of the test device, then add 2 drops (about 80 µL) of sample buffer to the buffer well (B) immediately. Avoid air bubbles.

3. Wait for the colored line(s) to appear. After 2 minutes, if the red color has not moved across the test window or if blood is still present in the specimen well (S), add 1 additional drop of the sample buffer to the buffer well(B).
4. The result should be read in 10 minutes. Positive results may be visible as soon as 2 minutes. Do not interpret the result after 15 minutes.

INTERPRETATION OF RESULTS

IgG POSITIVE: The colored line in the control line region (C) appears and a colored line appears in test line region IgG. The result is positive for 2019-nCOV-IgG antibodies.
IgM POSITIVE: The colored line in the control line region (C) appears and a colored line appears in test line region IgM.
IgG AND IgM POSITIVE: The colored line in the control line region (C) appears and two colored lines should appear in test line regions IgG and IgM. The color intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies.
NOTE: *The intensity of the color in the test line region(s) IgG and/or IgM will vary depending on the concentration of 2019- nCOV antibodies in the specimen. Therefore, any shade of color in the test line region(s) IgG and/or IgM should be considered positive.*
NEGATIVE:
The colored line in the control line region (C) appears. No line appears in test line regions IgG or IgM.
INVALID:
There is no line appear in the c region. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in the test. A color line appearing in the control region (C) is an internal positive procedural control It confirms sufficient specimen volume and correct procedural technique.
Control standards are not supplied with this kit however it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

PERFORMANCE CHARACTERISTICS

1) Assay Clinical Performance

Study 1: Spring Healthcare Agreement Validation

The clinical performance of the COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood) was evaluated by testing a total of 1300 clinical sample—300 positive samples and 1000 negative samples) from individual patients exhibiting pneumonia, respiratory symptoms and fever etc. Testing was performed from January to mid-March 2020. COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) results for IgM and IgG detection were compared to the results of RT-PCR assays for SARS-CoV-2 from oropharyngeal swabs. The time from RT-PCR result to collection of specimens (plasma) ranged from 15-45 days and 0-38 days (Site #2). The time from collection of specimens (plasma) from each individual to testing ranged from 12-23 days (Site #1) and 3-29 days (Site #2). Overall study results are shown in below (Table 1).

Table 1: Assay Clinical Study Results

SARS-CoV2 IgM Ab	PCR Test		
Rapid Test	**Positive**	**Negative**	**Total**
Positive	276	0	276
Negative	24	1000	1024
Total	300	1000	1300

Analysis of coincidence rate of SARS-CoV-2 IgM Ab rapid test and PCR test in serum samples

Positive coincidence rate= 276 / (276+24) x 100%=92% (Sensitivity)
Negative coincidence rate= 1000/ (0+1000) x 100%=100% (Selectivity)
Total coincidence rate= (276+1000)/ (276+24+0+1000 ×100%=96% (Accuracy)

Conclusion: SARS-CoV-2 IgM Ab rapid test and PCR test positive coincidence rate (Sensitivity) of 92%, negative coincidence rate (Selectivity) of 100%, total coincidence rate (Accuracy) of 96%.

Table 2: Result Statistics Table

SARS-CoV2 IgG Ab	PCR Test		
Rapid Test	**Positive**	**Negative**	**Total**
Positive	288	0	304
Negative	12	1000	1012
Total	300	1000	1300

Analysis of coincidence rate of SARS-CoV-2 IgG Ab rapid test and PCR test in serum samples

Positive coincidence rate= 288/（288+12）×100%=96% (Sensitivity)
Negative coincidence rate= 1000/（0+1000）×100%=100% (Specificity)
Total coincidence rate= (288+1000）/（288+12+0+1000）×100%=98% (Accuracy)

Conclusion: SARS-CoV-2 IgG Ab rapid test and PCR test positive coincidence rate (Sensitivity) of 96%, negative coincidence rate (Specificity) of 100%, total coincidence rate of 98% (Accuracy).

Study 2: Dr. Wegene Borena, Institute for Virology, Medical University of Innsbruck

1. Methods

Covid-19 Rapid IgM/IgG Combined Antibody Assay is an immunochromatographic rapid test for the qualitative detection of SARS-CoV-2-specific IgG and IgM antibodies.

Sample material
40 Plasma samples of people tested positive with SARS- CoV-2 PCR
74 Plasma samples of blood donors from the previous year (2019)

IgG Sensitivity
In order to validate the sensitivity of IgG antibodies samples of 40 people were used which had been tested positive for SARS- CoV-2 beforehand by means of PCR. These sera had to be positive in at least one of the following serological processes to be included in the evaluation as a positive standard.

1)Euroimmun, anti-SARS-CoV-2 -IgG ELISA (anti-S1evidence, Euroimmun, Lübeck, Germany)
2)Abbott, SARS-CoV-2 IgG immunoassay (anti-Nevidence, Abbott, Illinois, USA)

IgM Sensitivity
In order to validate the sensitivity of IgG antibodies samples of 40 people were used which had been tested positive for SARS- CoV-2 beforehand by means of PCR. The time between the PCR test and blood sampling was also taken into account.

IgG specifity of IgG and IgM
In order to assess the specifity 74 plasma samples of blood donors from 2019 were defined as negative standard and used for the calculation - as it was assumed that they could not have developed antibodies (IgM, IgA or IgG) against the new Corona virus SARS-CoV-2.

2. Results

IgG Sensitivity
Of the 40 blood samples which came from PCR-confirmed cases 39 were recognized as IgG-positive using the Covid-19 Rapid IgM/IgG Combined Antibody Assay. So, the sensitivity is 98.5%. The analysis of the data resulted in a comparable sensitivity taking the time between the PCR test and the blood sampling into account.

Table 1. Sensitivity (IgG) of Covid-19 Rapid IgM/IgG Combined Antibody Assay (n=40)

Days post-PCR(n)	positive	negative	total	Sensitivity
<21	12	1	12	100%
≥21	27	0	28	96.4%

IgM Sensitivity
Of the 40 blood samples which came from PCR-confirmed cases 38 were recognized as IgG-positive using the Covid-19 Rapid IgM/IgG Combined Antibody Assay. Therefore, the sensitivity for proving an existing or recently occurred SARS- CoV-2 infection is 97.5%. The analysis of the data shows that the sensitivity of the IgM evidence does not change significantly taking the time between the PCR test and the blood sampling into account (Table 2).

Table 2. Sensitivity (IgM) of Covid-19 Rapid IgM/IgG Combined Antibody Assay (n=40)

Days post-PCR(n)	positive	negative	total	Sensitivity
<21	12	0	12	100%
≥21	26	2	28	92.8%

IgG Specifity
All 74 blood donors from the time before SARS-Cov-2 were recognized as negative using the Covid-19 Rapid IgM/IgG Combined Antibody Assay. Therefore, the specifity of the test is 100%.

IgM Specifity
Of the 74 samples of the blood donors from the previous year, which should normally be negative, 2 samples were recognized as IgM-positive using the Covid-19 Rapid IgM/IgG Combined Antibody Assay. Therefore, the specifity of the IgM evidence is 97.3%.

3.Conclusions

The evaluation of the Covid-19 Rapid IgM/IgG Combined AntibodyAssay using the PCR and the antibody positive standard samplesresulted in a sensitivity of 98.5% for IgG and 97.5% for IgM. Thespecifity is 100% for IgG and 97.3% for IgM.
The test seems to be suitable to be used in serum prevalence studies. In order to analyze populations with a low serum prevalence (<5%) and for individual immunity tests the high specifity would have to be confirmed with at least another 200 negative samples taken in the last year.

Study 3: PASEO DE LA UNIVERSIDAD #300 Y JUAN DÍAZ – URB. IÑAQUITO ALTO

1. Method

In this trial, 347 clinical samples were selected. There were 77 PCR-positive and 270 PCR-negative samples that were tested from lot20200418 with expiration date 04/2022. The SARS-CoV-2 IgG Acrapid test and SARS-CoV-2 PCR were detected by means ofimmunochromatography and the positive and negative match rates were calculated.

2. Statistical Calculation

Data	Values
Accuracy, meanning (d) (0-1)	0.05
Population size	10000
Hypothetical proportion of measurement occurrence in the population of COVID-19(p)(0-1)	0.9
Z(T Student)	1.96

Sample size 136

3. IgM Ac and IgG Ac results

1. 73 cases of positive samples confirmed by the nucleic acidtest (RT-PCR): tested with the SARS-CoV-2 IgM Ac rapid test;67 cases were positive, and 6 cases were negative.
2. 270 cases negative samples confirmed by nucleic acid test(RT-PCR): tested by the SARS-CoV-2 IgM Ac rapid test; 265cases were negative, and 5 cases were positive
3. 75 cases positive samples confirmed by nucleic acid test(RT-PCR): tested by the SARS-CoV-2 IgG Ac rapid test; 69cases were positive, and 6 cases were negative.
4. 270 cases of negative samples confirmed by nucleic acidtest (RT-PCR): tested by SARS-CoV-2 IgG Ac rapid test; 268cases were negative, and 2 cases were positive.

4. Analysis of IgM Ac and IgG Ac

	PCR		
IgM	POSITIVE	NEGATIVE	TOTAL
POSITIVE	67	5	**72**
NEGATIVE	6	265	**271**
TOTAL	**73**	**270**	**343**

SENSITIVITY	**92%**
SPECIFICITY	**98%**

NOTE: 4 PCR positive patients are positive for IgG negative for IgM (73+4=77)

	PCR		
IgG	POSITIVE	NEGATIVE	TOTAL
POSITIVE	69	2	**71**
NEGATIVE	6	268	**274**
TOTAL	**75**	**270**	**345**

SENSITIVITY	**92%**
SPECIFICITY	**99%**

NOTE: 2 PCR positive patents are positive for IgM and negative for IgG (75+2=77).

5. Conclusion

1. The sample size was calculated at 136 patients for a total of10,000 tests and it was performed successfully on 235patients, exceeding the minimum number required.
2. The SARS-CoV-2 IgM Ac rapid test and PCR test had apositive match rate (sensitivity) of 92%, and a negativematch rate (effectiveness) of 98%.
3. The SARS-CoV-2 IgG Ac rapid test and PCR test positivematch rate (sensitivity) of 92%, and a negative match rate(effectiveness) of 99%.

5. Recommendations

1. Sensitivity and specificity analysis should be extended toother lots to confirm these percentages.
2. If possible, the serum sample should be used because it hasa higher serum avidity and reading speed.

2) Cross Reactivity Study

Specimens which tested positive with following various agents from patients were investigated with SARS-CoV-2 IgM Ab Rapid Test (Lateral Flow Method). The results showed no cross reactivity.

Parainfluenza virus antibody
Influenza A antibody
Influenza B antibody
Chlamydia pneumonia antibody
Mycoplasma pneumoniae antibody
Adenovirus antibody
Respiratory syncytial virus antibody
Hepatitis B surface antibody
Hepatitis C virus antibody

Treponema pallidum antibody
HIV antibody
EB virus antibody
Measles virus antibody
Cytomegalovirus antibody
Enterovirus type 71 antibody
Mumps antibody
Varicella-zoster virus positive sample

3) Interferences study

The test result of SARS-CoV-2 IgM Ab Rapid Test (Lateral Flow Method) does not interfere with the substance at the following concentration:

Substance	Concentration
Bilirubin	250 umol/L
Hemoglobin	9 g/L
Triglyceride	15 mmol/L
Rheumatoid factors	80 IU/mL
Antinuclear antibody (ANA) titer	1:240
Anti-mitochondrial antibody	80 U/mL
Mouse IgG	1000 ug/ml

LIMITATION OF USE

1. The accuracy of the test depends on the sample collection process. Improper sample collection, improper storage of samples, unfresh samples, or repeated freeze-thaw cycles of samples will affect the test results.
2. The test cassette only provides qualitative detection of the COVID-19 antibody in the sample. If you need to detect the specific content of an indicator, please use the relevant professional instruments.
3. The test result of this kit is for clinical reference only and should not be used for clinical diagnosis and treatment. The clinical management of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests, and treatment responses.
4. It is recommended to review the suspicious negative results by using nucleic acid detection or virus culture identification methods.
5. Analysis of the possibility of false negative results：

① Unreasonable sample collection, transportation and processing may lead to false negative results.
② Genetic variations of virus can cause changes in antibody determinants, which can lead to false negative results.
③ The optimal sample type and sampling time after infection have not been verified, so collecting samples at different times on the same patient may avoid false negative results.

Symbol

Symbol	Meaning	Symbol	Meaning
IVD	In vitro diagnostic medical device	2°C 30°C	Storage temperature limit
	Manufacturer	EC REP	Authorized representative in the European Community
	Date of Manufacture		Use by date
	Do not reuse		Consult instruction foe use
LOT	Batch code	CE	Meet the requirements of EC Directive 98/79/EC
REF	Catalogue number	Σ	The number of test

Spring Healthcare Services Sp zoo
Ul. Bartycka, Nr. 22B/21A
00-716 Warsaw, Poland

Number: 1101311601
Version: 1.0
Effective Date: 2020-04-05