Information for:

nCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum)

CONFIDENTIAL

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1 BACKGROUND

NCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum) is a rapid test to qualitatively detect the NCoV-19 antibody. The test utilizes colloid gold conjugate to NCoV-19 IgG/IgM in whole blood.

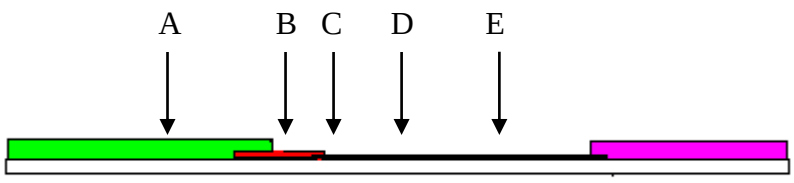
1.1 Test Principle

The NCoV-19 IgG/IgM Rapid Test is a qualitative membrane-based immunoassay for the detection of NCoV-19 antibodies in whole blood, serum, or plasma. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in test line region 1 of the test. During testing, the specimen reacts with NCoV-19 antigen-coated particles in the test strip. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in test line region 1. If the specimen contains IgG antibodies to NCoV-19, a colored line will appear in test line region 1. In the IgM component, anti-ligand is coated in test line region 2 of the test. During testing, the specimen reacts with ligand anti-human IgM. NCoV-19 IgM antibodies, if present in the specimen, reacts with the ligand anti-human IgM and the NCoV-19 antigen-coated particles in the test strip, and this complex is captured by the anti-ligand, forming a colored line in test line region 2.

Therefore, if the specimen contains NCoV-19 IgG antibodies, a colored line will appear in test line region 1. If the specimen contains NCoV-19 IgM antibodies, a colored line will appear in test line region 2. If the specimen does not contain NCoV-19 antibodies, no colored line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a colored line will always change appeared in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

1.2 Illustrations

Figure 1: Test Principle



As shown in Figure 1 above, the specimen (A) migrates via capillary action along the membrane to react with the colored conjugate (B). NCoV-19 antibody or antigen present in the specimen bind to the conjugate, forming a colored antibody-antigen complex (C). NCoV-19 antigen or antibody immobilized in the test line region of the membrane captures the complex. The formation of a visible red line indicates a positive result (D). The absence of a red line formation in the test line region indicates a negative result.

In the control line region of the membrane, immobilized reagents capture colored conjugate regardless of the test specimen composition. The resulting visible red line (E) confirms that the assay is functioning correctly.

1.3 Storage

Store the test at 2-30°C. Freezing must be avoided.

1.4 Stability

NCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum) is stable for 24 months from the date of production when stored properly in unopened aluminum foil pouches with desiccant.

1.5 Description of Test Methods

1.5.1 GENERAL REMARKS

The Quality Control department performs testing according to written procedures. Testing equipment is checked prior to use and calibrated at scheduled intervals.

1.5.2 RECEIVING INSPECTION AND CONTROL OF RAW MATERIALS

A sample batch of each raw material (chemicals, packaging and labeling) is inspected/tested (where applicable) for suitability and functionality. Primary packaging is inspected for correct dimensions, cleanliness and suitability. Only QC approved raw material is employed for production.

1.6 Composition of Product

A) NCoV-19 Antigen	B) Goat anti-Chicken IgY
C) Monuse anti-human IgM	D) Mouse anti-human IgG
E) Chicken IgY	Gold conjugate

F) Membrane
G) Adhesive plastic backing
H) Sample pad
I) Label pad
J) Absorbant pad
K) Desiccant (in pouch)
L) Plastic device
M) Buffer
N) Pouch

1.7 Manufacturing Procedure

a) Precoat the colored NCoV-19 antigen and Chicken IgY Gold conjugate on the label pad.

b) Use the sprayer to dispense Mouse anti-human IgG/IgM and Goat anti- Chicken IgY onto the membrane.

c) Assemble the membrane, label pad, absorbant pad and sample pad on the plastic backing.

d) Use the cutter to cut the plastic backing into strips of selected size.

e) Lay the strip into the plastic device and pack the device and a desiccant packet.

f) Test the device according QC procedure and release the finished product.

1.8 Quality Control

1.8.1 Internal Quality Control

Internal procedural controls are included in the test. A red line appearing in the control region (C) is an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.

1.8.2 External Quality Control

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.

1.8.3 Procedure for External Quality Control

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: For whole blood samples:

For whole blood sample:

Fill the dropper with the specimen then add 1 drop (about 40 μL) of specimen into the sample well. Making sure that there are no air bubbles. Then add 1 drop (about 40 μL) of Sample Diluent immediately into the sample well.

For Plasma/ Serum sample:

Fill the dropper with the specimen then add 1 drop (about 40 μL) of specimen into the sample well. The volume is around 80μL. Making sure that there are no air bubbles. Then add 1 drop (about 40 μL) of Sample Diluent immediately into the sample well.

Step 5: Set up a timer. Read the result at 15 minutes. Don't read result after 30 minutes. To avoid confusion, discard the test device after interpreting the result.

2 PERFORMANCE CHARACTERISTICS

2.1 Specimen Correlation

NCoV-19 IgG/IgM Rapid Test:

The specimens obtained from a population of symptomatic and asymptomatic individuals. Results were confirmed by the PCR test.

1. Clinical Performance For IgM Test

A total of 146 patient samples from susceptible subjects were tested by the 2019-nCoV IgG/IgM Rapid Test and by a reference PCR. Comparison for all subjects is showed in the following table:

	2019-nCoV IgM Rapid Test		
PCR Results	Positive	Negative	Total
Positive	42	2	44
Negative	0	102	102
Total	42	104	146

95%CI
Relative Sensitivity: 95.5% (89.3%~100%)
Relative Specificity: 100% (99.8%~100%)
Overall Agreement: 98.6% (96.7%~100%)

2. Clinical Performance For IgG Test

A total of 156 patient samples from susceptible subjects were tested by the 2019-nCoV IgG/IgM Rapid Test and by a reference PCR. Comparison for all subjects is showed in the following table:

	2019-nCoV IgG/IgM Rapid Test		
PCR Results	Positive	Negative	Total
Positive	54	0	54
Negative	2	100	102
Total	56	100	156

95%CI
Relative Sensitivity: 100% (99.7%~100%)
Relative Specificity: 98.0% (95.4%~100%)
Overall Agreement: 98.7% (97.0%~100%)

2.2 Interfering Substances

Analytes were spiked into negative plasma/serum and low positive specimens of IgG/IgM at the concentrations listed. The specimens were tested in triplicate with 3 lots of test devices. Visual interpretations were made at 15 minutes after specimen application. The results are presented in Table 1 and Table 3 and below.

Table 1: Interfering Substances of NCoV-19 IgG

Analytes	Concentration	202002001		202002002		202002003	
		Neg.	Low positive	Neg.	Low positive	Neg.	Low positive
Ascoribic acid	20mg/mL	-	+	-	+	-	+
Hemoglobin	1000mg/dL	-	+	-	+	-	+
Gentistic acid	20mg/dL	-	+	-	+	-	+
Oxalic acid	60mg/dL	-	+	-	+	-	+
Bilirubin	1000mg/dL	-	+	-	+	-	+
Uric acid	20mg/mL	-	+	-	+	-	+
Acetoaminophen	20mg/dL	-	+	-	+	-	+
Aspirin	20mg/dL	-	+	-	+	-	+
Methanol	10%	-	+	-	+	-	+
Creatine	200mg/dL	-	+	-	+	-	+
Albumin	2000mg/dL	-	+	-	+	-	+
Caffeine	20mg/dL	-	+	-	+	-	+

Table 2: Interfering Substances of NCoV-19 IgM

Analytes	Concentration	202002001		202002002		202002003	
		Neg.	Low positive	Neg.	Low positive	Neg.	Low positive
Ascoribic acid	20mg/mL	-	+	-	+	-	+
Hemoglobin	1000mg/dL	-	+	-	+	-	+
Gentistic acid	20mg/dL	-	+	-	+	-	+
Oxalic acid	60mg/dL	-	+	-	+	-	+
Bilirubin	1000mg/dL	-	+	-	+	-	+
Uric acid	20mg/mL	-	+	-	+	-	+
Acetoaminophen	20mg/dL	-	+	-	+	-	+
Aspirin	20mg/dL	-	+	-	+	-	+
Methanol	10%	-	+	-	+	-	+
Creatine	200mg/dL	-	+	-	+	-	+
Albumin	2000mg/dL	-	+	-	+	-	+
Caffeine	20mg/dL	-	+	-	+	-	+

Conclusion:

No substances showed any interference with the test. There were no differences observed between the results at 30 minutes.

2.3 Cross-Reactivity

HBV+, HAV+, HCV+, Rf+, HIV+, and heterophilic specimens were tested in triplicate with 3 lots of test devices. Visual interpretations were made at 15 and 30 minutes after specimen application. The results are presented in the Table 4.

Table 4: Non Cross-Reacting Compounds

Treatment	Lot					
	202002001		**202002002**		**202002003**	
	15min	30min	15min	30min	15min	30min
HAV+	-	-	-	-	-	-
HCV+	-	-	-	-	-	-
Rheumatoid Factor +	-	-	-	-	-	-
HIV+	-	-	-	-	-	-
Heterophilic	-	-	-	-	-	-
hCG urine	-	-	-	-	-	-
Cancer	-	-	-	-	-	-
HBsAg	-	-	-	-	-	-
HBsAb	-	-	-	-	-	-
HBeAg	-	-	-	-	-	-
HBeAb	-	-	-	-	-	-
HBcAb	-	-	-	-	-	-

Conclusion:

There was no cross-reaction with above diseases above in 30 minutes.

2.4 Precision

2.4.1 INTRA-ASSAY

Samples were spiked with low, medium and high positive samples and tested according to the package insert. Fifteen replicates of each level were tested using the same lot. The devices were interpreted at the prescribed read time. The results show that the nCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum) performs as expected. Results are presented in Table 5.

Table 5: Intra-Assay

Specimen	Test Results of 202002001	
	Negative	Positive
Negative	15	0
IgG Low positive	0	15
IgG Medium positive	0	15
IgG High positive	0	15
IgM Low positive	0	15
IgM Medium positive	0	15
IgM High positive	0	15

Conclusion:

100% of actual results were consistent with expected results. No distinct difference was detected on the ten replicates of the same lot.

2.4.2 INTER-ASSAY

Samples were spiked with low, medium and high positive samples and tested according to the package insert. Fifteen replicates of each level were tested using the three separate lots of test devices. The devices were interpreted at the prescribed read time. The results show that the nCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum) performs as expected. Results are presented in Table 6.

Table 6: Inter-Assay

Lot	Negative	Low Positive	Medium Positive	High Positive
202002001	15-	15+	15+	15+
202002002	15-	15+	15+	15+
202002003	15-	15+	15+	15+

Note:

15- indicates negative test results with 15 replicates.

15+ indicates positive test results with 15 replicates.

Conclusion:

No distinct difference was detected in three pilot lots at 5 minutes after sample application.

2.5 Real Time Stability

Real Time Stability of the NCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum) was evaluated using samples from three different lots. These samples were placed in an incubator with the temperature calibrated at 2-8℃ and 30±3℃ with relative humidity (RH) calibrated at 60%. A series of stability tests were performed at 0, 3, 6, 9, 12, 15, 18, 21, 24 and 27 months. Test devices were assayed using negative, IgG middle positive and IgM middle positive specimens. Testing at each specific time interval consisted of 3 replicates for each specimen. The tests were performed according to the package insert. The results are presented in Table 7.

Table 7: Real Time Stability Summary

Month	Specimen	Lot					
		202002001		202002002		202002003	
		2-8℃	30℃	2-8℃	30℃	2-8℃	30℃
0	Negative	3-	3-	3-	3-	3-	3-
	IgG Positive	3+	3+	3+	3+	3+	3+
	IgM Positive	3+	3+	3+	3+	3+	3+
3	Negative						
	IgG Positive						
	IgM Positive						
6	Negative						
	IgG Positive						
	IgM Positive						
9	Negative						
	IgG Positive						
	IgM Positive						
12	Negative						
	IgG Positive						
	IgM Positive						
15	Negative						
	IgG Positive						
	IgM Positive						
18	Negative						
	IgG Positive						
	IgM Positive						
21	Negative						
	IgG Positive						
	IgM Positive						
24	Negative						
	IgG Positive						
	IgM Positive						
27	Negative						
	IgG Positive						
	IgM Positive						

Note:

3- indicates negative test results with 3 replicates

3+ indicates positive test results with 3 replicates

Conclusion:

The NCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum) is just developed and the stability is under going, it should be finished by the year 2022, May.

2.6 Accelerated Stability

Accelerated Stability of the NCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum) was evaluated using samples from three different lots. These samples were placed in an incubator with the temperature calibrated at 45℃ and relative humidity (RH) calibrated at 60%. A series of stability tests were performed at 0, 7, 14, 21, 28, 35, 42, 56 and 77days. Test devices were assayed using negative, IgG and IgM Middle Positive specimens. Testing at each specific time interval consisted of 3 replicates for each specimen. The tests were performed according to the package insert. The results are presented in Table 8.

Table 8: Accelerated Stability Summary

Day	Specimen	Lot		
		202002001	202002002	202002003
0	Negative	3-	3-	3-
	IgG Positive	3+	3+	3+
	IgM Positive	3+	3+	3+
7	Negative	3-	3-	3-
	IgG Positive	3+	3+	3+
	IgM Positive	3+	3+	3+
14	Negative	3-	3-	3-
	IgG Positive	3+	3+	3+
	IgM Positive	3+	3+	3+
21	Negative	3-	3-	3-
	IgG Positive	3+	3+	3+
	IgM Positive	3+	3+	3+
28	Negative	3-	3-	3-
	IgG Positive	3+	3+	3+
	IgM Positive	3+	3+	3+
35	Negative			
	IgG Positive			
	IgM Positive			
42	Negative			
	IgG Positive			
	IgM Positive			
56	Negative			
	IgG Positive			
	IgM Positive			
77	Negative			
	IgG Positive			
	IgM Positive			

Note:
3- indicates negative test results with 3 replicates
3+ indicates positive test results with 3 replicates

Conclusion:

The NCoV-19 IgG/IgM Rapid Test Device (Whole Blood/Plasma/Serum) is stable at 45℃ for 28 days till now and the accelerated stability study will be go on for other 49 days under 45℃. These data were plotted on an Arrhenius Plot and the shelf life of this product was determined to be at least 9 months from the date of manufacture. The shelf life would be updated when the study is going on.

3 BIBLIOGRAPHY

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