Registration File of

2019-nCoV IgG/IgM Rapid Test Cassette Single Use Kit

(Fingerstick Whole Blood)

INCP-402S

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1 BACKGROUND

Early January 2020, a novel coronavirus (2019-nCoV) was identified as the infectious agent causing an outbreak of viral pneumonia in Wuhan, China, where the first cases had their symptom onset in December 2019.[1]

Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds and that cause respiratory, enteric, hepatic, and neurologic diseases.[2] Six coronavirus species are known to cause human disease.[3] Four viruses — 229E, OC43, NL63, and HKU1 — are prevalent and typically cause common cold symptoms in immunocompetent individuals.[3] The two other strains — severe acute respiratory syndrome coronavirus (SARS-COV) and Middle East respiratory syndrome coronavirus (MERS-COV) — are zoonotic in origin and have been linked to sometimes fatal illness.[4]

Coronaviruses are zoonotic, meaning they are transmitted between animals and people.

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.[5]

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. [5]

1.1 Test Principle

The 2019-nCoV IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) is a qualitative membrane-based immunoassay for the detection of IgG and IgM antibodies to 2019-nCoV in whole blood, serum or plasma specimen. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in IgG test line region. During testing, the specimen reacts with 2019-nCoV antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region, if the specimen contains IgG antibodies to 2019-nCoV. A colored line will appear in IgG test line region as a result of this. Similarly, anti-human IgM is coated in IgM test line region and if specimen contains IgM antibodies to 2019-nCoV, the conjugate-specimen complex reacts with anti-human IgM. A colored line appears in IgM test line region as a result.

Therefore, if the specimen contains 2019-nCoV IgG antibodies, a colored line will appear in IgG test line region. If the specimen contains 2019-nCoV IgM antibodies, a colored line will appear in IgM test line region. If the specimen does not contain 2019-nCoV 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 appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

1.2 Illustrations

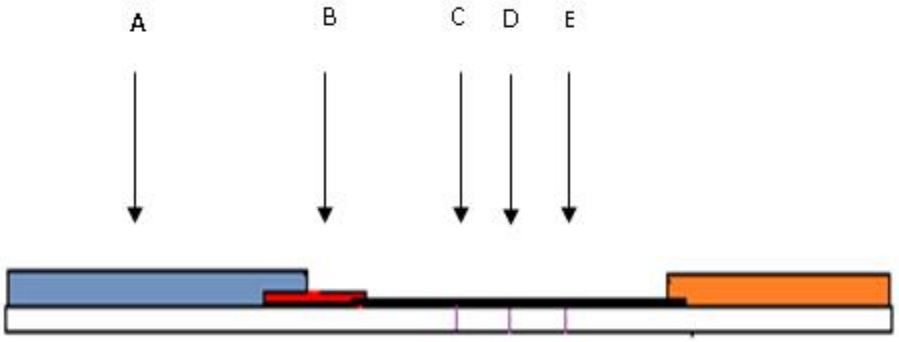

Figure 1: Test Principle

As shown in illustration above, the specimen (A) migrates via capillary action along the membrane to react with the gold conjugate (B). 2019-nCoV IgG or/and IgM present in the specimen binds to the conjugate, forming a colored Novel coronavirus antibody-antigen complex. The mouse anti-human IgG and mouse anti-human IgM immobilized in the test zone of the membrane captures the test region (C) and test region (D). The formation of a visible colored line in the test region indicates a positive result (C) or (D). The absence of a colored line in the test zones suggests a negative result. In the control zone of the membrane, immobilized reagents capture colored conjugate regardless of test specimen composition. The resulting visible colored band (E) confirms control line.

1.3 Precautions

- For professional in vitro diagnostic use only. Do not use after the expiration date.
- Do not eat, drink or smoke in the area where the specimen or kits are handled.
- Do not use test if pouch is damaged.
- Handle all the 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.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Please ensure that an appropriate amount of samples are used for testing. Too much or too little sample size may lead to deviation of results.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

1.4 Storage

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

1.5 Stability

The 2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) is stable for 24 months from the date of production when stored properly in unopened aluminum foil pouches with desiccant.

1.6 Directions for Use

Bring tests, specimens, buffer, and/or controls to room temperature (15-30°C) before use.

1. Remove the test cassette from the foil pouch and use it within one hour. Best results will be obtained if the test is performed immediately after opening the foil pouch.
2. Place the cassette on a clean and level surface.
3. Use the provided alcohol swab to clean the fingertip of the middle finger or ring finger as the puncture site.
4. Carefully rotate and pull off the sterile lancet cap. Push the sterile lancet firmly into the fingertip of the middle finger. Do not use the first drop of blood. To increase blood flow, use the thumb and forefinger to gently apply pressure around the puncture site.
5. Hold the dropper vertically, draw the blood to 1cm above the fill line and transfer 1 full drop of whole blood (approximately 20 μL) to the specimen well (S), then add 2 drops of buffer (approximately 80 μL), and start the timer. See illustration below.
6. Wait for the colored line(s) to appear. Read results at 10 minutes. Do not interpret the result after 20 minutes.
7. Place the used tests into the plastic zip lock bags provided and seal, discard according to local regulations.

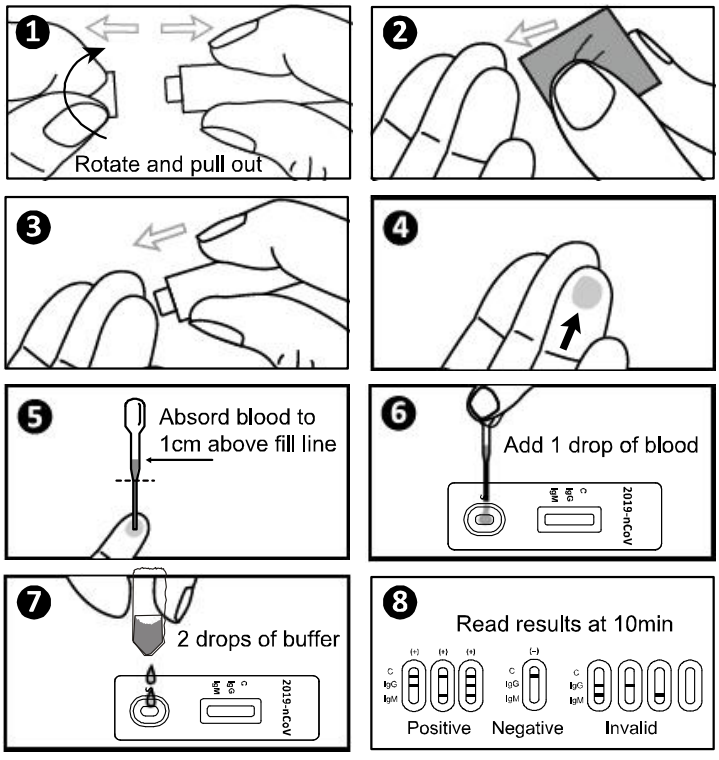

Figure 2: Interpretation of Results

1.7 Interpretation of Result

IgG POSITIVE:* Two colored lines appear. One colored line should always appear in the control line region (C) and another line should be in the IgG line region.
IgM POSITIVE:* Two colored lines appear. One colored line should always appear in the control line region (C) and another line should be in the IgM line region.
IgG and IgM POSITIVE:* Three colored lines appear. One colored line should always appear in the control line region (C) and two test lines should be in the IgG line region and IgM line region.
***NOTE:** The intensity of the color in the test line regions may vary depending on the concentration of 2019-nCoV antibodies present in the specimen. Therefore, any shade of color in the test line region should be considered positive.
NEGATIVE: **One colored line appears in the control line region (C)**. No line appears in the IgG region and IgM region.
INVALID: **Control line fails to appear**. 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. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

1.8 Quality Control

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking 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.

1.9 Limitation

1. The 2019-nCoV IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) is for in vitro diagnostic use only. This test should be used for detection of IgG and IgM antibody to 2019-nCoV in Fingerstick Whole Blood specimens. Neither the quantitative value nor the rate of increase in the concentration of IgG or IgM antibodies to 2019-nCoV can be determined by this qualitative test.
2. The 2019-nCoV IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) will only indicate the presence of IgG and IgM antibodies to 2019-nCoV in the specimen and should not be used as the sole criteria for the diagnosis of 2019-nCoV infections.
3. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of 2019-nCoV infection.

5. The test will show negative results under the following conditions: The titer of the novel coronavirus antibodies in the sample is lower than the minimum detection limit of the test, or the novel coronavirus antibody has not appeared at the time of sample collection (Asymptomatic stage).

1.10 Description of Test Methods

1.10.1 General remarks

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

1.10.2 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.

1.11 Composition of Product

A) Mouse anti-human IgG (Capture)
B) Mouse anti-human IgM (Capture)
C) Mouse IgG
D) Goat anti-mouse IgG
E)2019-nCov antigen (Detection)
F) Adhesive plastic backing
G) Buffer
H) Label pad
I) Absorbant pad
J) Sample pad
K) NC membrane
L) Desiccant (in pouch)
M) Pouch
N) Sample dropper
O) Lancets
P) Alcohol pads

1.12 Manufacturing Procedure

- Coat the gold conjugated recombine 2019-nCov antigen and mouse IgG on the label pad.
- Use the sprayer to dispense goat anti-mouse IgG, mouse anti-human IgG and mouse anti-human IgM onto the membrane respectively.
- Assemble the membrane, label pad, absorbent pad and sample pad on the plastic backing.
- Use the cutter to cut the plastic backing into strips of selected size.
- Test the strips according to the QC procedure and release the finished product.

2 PERFORMANCE CHARACTERISTICS

2.1 Sample Correlation

Method

The 2019-nCoV IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) was compared with a leading commercial PCR; the results show that 2019-nCoV IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) has a high sensitivity and specificity.

Table: Sample Correction Result

Performance of IgG Specimen

Method		**PCR**		**Total Result**
2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood)	Results	Positive	Negative	
	Positive	20	1	21
	Negative	0	49	49
Total Result		20	50	70

Relative sensitivity: 100% (95%CI*: 86.0%~100%);
Relative specificity: 98.0% (95%CI*: 89.4%~99.9%);
Accuracy: 98.6% (95%CI*: 92.3%~99.96%). *Confidence Intervals

Performance of IgM Specimen

Method		**PCR**		**Total Result**
2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood)	Results	Positive	Negative	
	Positive	17	2	19
	Negative	3	48	51
Total Result		20	50	70

Relative sensitivity: 85.0% (95%CI*: 62.1%~96.8%);
Relative specificity: 96.0% (95%CI*: 86.3%~99.5%);
Accuracy: 92.9% (95%CI*: 84.1%~97.6%). *Confidence Intervals

Conclusion

The 2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) products have been compared with a commercial PCR using clinical specimen. The results show that the relative sensitivity of the 2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) is 98.6% and the relative specificity is 92.7%.

2.2 Interfering Substances

Method

Analytes were spiked into negative specimen at the following concentrations listed. The specimens were tested in triplicate with visual interpretations occurring at 10 minutes after specimen application. Results are presented in table below.

Table: Interfering Substance

Substance	Conc.	Lot : COV20010002-R		
		Negative		
Triglyceride	50mg/dl	-	-	-
Hemoglobin	1000mg/dl	-	-	-
Ascorbic Acid	20mg/dl	-	-	-
Total cholesterol	6mmol/l	-	-	-
Bilirubin	60mg/dl	-	-	-
Substance	Conc.	Lot : COV20010002-R		
		IgG positive		
Triglyceride	50mg/dl	+	+	+
Hemoglobin	1000mg/dl	+	+	+
Ascorbic Acid	20mg/dl	+	+	+
Total cholesterol	6mmol/l	+	+	+
Bilirubin	60mg/dl	+	+	+
Substance	Conc.	Lot : COV20010002-R		
		IgM positive		
Triglyceride	50mg/dl	+	+	+
Hemoglobin	1000mg/dl	+	+	+
Ascorbic Acid	20mg/dl	+	+	+
Total cholesterol	6mmol/l	+	+	+
Bilirubin	60mg/dl	+	+	+

Note: "-" mean negative result, "+"mean positive result

Conclusion

No substances showed any interference with the test. There were no differences observed between the results at 10 minutes.

2.3 Cross Reactivity

Method

Anti-influenza A virus, anti-influenza B virus, anti-RSV, anti-Adenovirus, HBsAg, anti-Syphilis, anti-H. Pylori, anti-HIV and anti-HCV positive specimens as confirmed by ELISA or other method were tested with the 2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) occurred at 10 minutes and 20 minutes after specimen application**.** Results were presented in Table below.

Table: Cross Reactivity

Specimen \ INCP-402		COV20010002-R	
		10min	20min
3 Anti-influenza A virus positive samples	1	-	-
	2	-	-
	3	-	-
3 Anti-influenza B virus positive samples	1	-	-
	2	-	-
	3	-	-
3 anti-HIV Positive Samples	1	-	-
	2	-	-
	3	-	-
3 anti-HCV Positive Samples	1	-	-
	2	-	-
	3	-	-
3 anti-Syphilis Positive Samples	1	-	-
	2	-	-
	3	-	-
3 HBsAg Positive Samples	1	-	-
	2	-	-
	3	-	-
3 Anti-RSV Positive Samples	1	-	-
	2	-	-
	3	-	-
3 Anti-Adenovirus Positive Samples	1	-	-
	2	-	-

	3	-	-
3 Anti-H.pylori Positive Samples	1	-	-
	2	-	-
	3	-	-

Note: "-" mean negative result

Conclusion

There was no cross-reaction with anti-influenza A virus, anti-influenza B virus, anti-RSV, anti-Adenovirus, HBsAg, anti-Syphilis, anti-H. Pylori, anti-HIV and anti-HCV positive specimens at 10 and 20 minutes.

2.4 Between Day Reproducibility

Method

2019-nCov negative specimen were run individually on 3 separate days using the same lot of 2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood). Results were rated visually as negative or positive at 10 minutes and 20 minutes after specimen application. Results are presented in table below.

Table: Between Day Results

Day 1 Results						
Lot	Negative					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	-	-	-	-	-	-
Day 2 Results						
Lot	Negative					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	-	-	-	-	-	-
Day 3 Results						
Lot	Negative					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	-	-	-	-	-	-
Day 1 Results						
Lot	IgG positive					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	+	+	+	+	+	+
Day 2 Results						
Lot	IgG positive					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	+	+	+	+	+	+
Day 3 Results						
Lot	IgG positive					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	+	+	+	+	+	+

Day 1 Results						
Lot	IgM positive					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	+	+	+	+	+	+
Day 2 Results						
Lot	IgM positive					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	+	+	+	+	+	+
Day 3 Results						
Lot	IgM positive					
COV20010002-R	1		2		3	
	10 min	20 min	10 min	20 min	10 min	20 min
	+	+	+	+	+	+

Note: “-” mean negative result, “+” mean positive result

Conclusion

100% of actual results were consistent with expected results. No distinct difference was detected in intra lots.

2.5 Accelerated Stability

Method

Accelerated Stability of the 2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) was evaluated using samples from 1 batch. These were placed in an incubator with the temperature calibrated at 45℃ and 55℃. Relative humidity (RH) calibrated at about 60%. A series of stability tests were performed at 0, 7 and 14 days for 45℃. About 55℃, some performance study would be tested at 0, 7, 14 days according to Arrhenius Plot. See Table in below. Test cassettes were assayed using negative specimen. Testing at each specific time interval consisted of 3 replicates for each specimen. The tests were performed according to the package insert. Results are presented in Table below.

Arrhenius Formula:

In K=-Ea/RT + In A

"K" mean Rate constant

"A" mean Arrhenius constant

"Ea" mean Activation energy

"R" mean Gas constant

"T" mean Temperature in Kelvin

Table: Time line for Accelerate Stability Study

Day / Temp.	0day	7days	14 days	21 days	28 days	35 days	42 days	56 days	77 days	84 days
45℃	×	×	×	×	×	×	×	×	×	×
55℃	×	×	×	×	×	×	×			

Table: 45℃Accelerated Stability Study Result

Day	Specimen	2019-nCov IgG/IgM Rapid Test Cassette		
		COV20010002-R		
0	Negative	-	-	-
	IgG positive	+	+	+
	IgM positive	+	+	+
7	Negative	-	-	-
	IgG positive	+	+	+
	IgM positive	+	+	+
14	Negative	-	-	-
	IgG positive	+	+	+
	IgM positive	+	+	+
21	Negative	-	-	-

	IgG positive	+	+	+
	IgM positive	+	+	+
28	Negative	-	-	-
	IgG positive	+	+	+
	IgM positive	+	+	+
35	Negative			
	IgG positive			
	IgM positive			
42	Negative			
	IgG positive			
	IgM positive			
56	Negative			
	IgG positive			
	IgM positive			
77	Negative			
	IgG positive			
	IgM positive			
84	Negative			
	IgG positive			
	IgM positive			

Note: "-" mean negative result, "+" mean positive result

Table: 55℃ Accelerated Stability Study Result

Day	Specimen	2019-nCov IgG/IgM Rapid Test Cassette		
		COV20010002-R		
0	Negative	-	-	-
	IgG positive	+	+	+
	IgM positive	+	+	+
7	Negative	-	-	-
	IgG positive	+	+	+
	IgM positive	+	+	+
14	Negative	-	-	-
	IgG positive	+	+	+
	IgM positive	+	+	+
21	Negative	-	-	-
	IgG positive	+	+	+
	IgM positive	+	+	+
28	Negative	-	-	-

	IgG positive	+	+	+
	IgM positive	+	+	+
35	Negative			
	IgG positive			
	IgM positive			
42	Negative			
	IgG positive			
	IgM positive			

Note: "-" mean negative result, "+" mean positive result

Conclusion

2019-nCov IgG/IgM Rapid Test Cassette (Fingerstick Whole Blood) is stable at 45℃ for 28 days and at 55℃ for 28 days, the whole study will be finished at about Middle of April 2020.

3 BIBLIOGRAPHY

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Document History Summary

Version No.	Date	Description	Remark
01	2020.02.19	Initial version	N/A