Novel Coronavirus (2019-nCoV) IgG/IgM Antibody Testing Kit (Colloidal Gold)

Intended use

The cassette is used for the qualitative detection of new coronavirus IgM and IgG antibodies in human whole blood, serum or plasma.

Background

Currently there are seven types of human coronaviruses (HCoV), which are important pathogen of human respiratory infections that can cause human respiratory diseases, HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HcoV-HKU1, MERS-CoV, and new coronaviruses (2019-nCoV). Among them, the new coronavirus (2019-nCoV) was discovered due to the virus pneumonia cases in Wuhan, 2019. The clinical manifestations are systemic symptoms such as fever and fatigue, accompanied by dry cough and dyspnea. It can quickly develop into severe pneumonia, respiratory failure, Acute respiratory distress syndrome, septic shock, multiple organ failure, severe acid-base metabolism disorders, etc., are even life-threatening.

Test principle

The test method is immunochromatographic， the membrane is pre-coated with recombinant anti-human IgG, IgM antibodies on the test line region of the cassette. During testing, the whole blood, serum or plasma specimen reacts with recombinant anti-human IgG, IgM antibodies conjugated colloid gold. The sample migrates upward on the membrane chromatographically by capillary action to react with recombinant anti-human IgG, IgM antibodies on the membrane.

If contains IgM antibodies, the M line will be generate a colored line. If contains IgG antibodies, the G line will be generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred. If the control line does not appear, indicating the test result is meaningless, this sample must be re-tested.

Reagents and Materials

Reagents and Materials Provided

Components	Kit Size (#of Tests)	25	40
	Test Cassette(#)	25	40
	Sample Diluent (# of Bottles)	1	1
	Transfer pipette	26	41
	IFU Leaflet	1	1

Transfer pipette is packaged inside the test cassette pouch.

The kit for 25 people filled with 3 ml of dilution liquid, the kit for 40 people with 4.5 ml of dilution.

Composition

Conjugate Pad	Recombined coronavirus antigen and chicken IgY antibody
G Line	Anti-human IgG
M Line	Anti-human IgM
C Line	Sheep-anti- chicken IgG
Sample Buffer	Sodium chloride, Sodium phosphate, Sodium casein salt

Other Material Required But Not Provided

Centrifuge tube, Pipette, Disposable tips, Timer , Alcohol sanitizing cotton tablet, Blood sampling needle

Storage and stability

Sealed: The kit must be stored at 4-30℃, valid for 18 months.

Opened: The cartridge must be used within 1 hour once its foil pouch is opened.

Specimen collection and preparation

1. The Cassette can be performed using whole blood, serum or plasma. Includes plasma samples prepared with commonly used anticoagulants (EDTA, heparin, sodium citrate).
2. Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
3. Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens must be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.
4. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
5. If the sample contains a large amount of lipid, hemolysis or turbidity, please do not use it.

Test Procedure

Step 1：Allow test cassette, specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing. After thawing, mix samples thoroughly before testing.

Step 2：Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour. Place the test cassette on a clean and level surface.

Step 3: Mark the sample Number in the cassette.

Step 4: Add the sample. Avoid trapping air bubbles in the specimen well. See the illustration below.

Serum/Plasma Sample

Hold the dropper vertically and transfer 1 drop of serum or plasma (approximately 20 μL) to the specimen well of the test cassette, then add 3 drops of buffer (approximately 80 μL) and start the timer.

Whole Blood Sample

Hold the dropper vertically and transfer 1 drop of whole blood (approximately 20 μL) to the specimen well of the test cassette, then add 3 drops of buffer (approximately 80 μL) and start the timer.

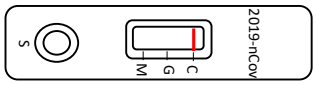
Fingertip Blood Sample

Hold the dropper vertically and transfer 1 drop of fingertip blood (approximately 20 μL) to the specimen well of the test cassette, then add 3 drops of buffer (approximately 80 μL) and start the timer.

Step 5: Wait for the colored line(s) to appear. The test result should be read in 15 minutes. Do not interpret the result after 15 minutes. After observing and recording the results, please discard the test card to avoid confusing the result judgment. For long-term storage, take photos of the results.

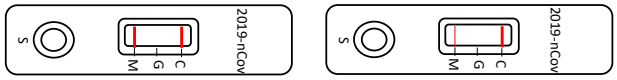
Explanation of results

1. **Negative Results:** One color line appears in the control region (C). No apparent red or pink line appears in the test region (G or M). As Below:

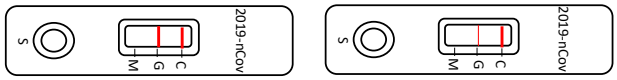


2. **Positive Results**

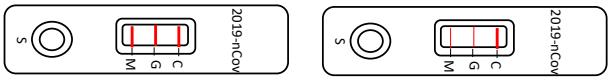
2.1 Two distinct colored lines appear. One color line should be in the control region (C) and another color line should be in the test region (M), means IgM antibody positive（As Below）



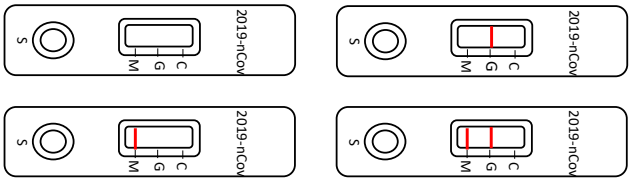
2.2 Two distinct colored lines appear. One color line should be in the control region (C) and another color line should be in the test region (G), means IgG antibody positive（As Below）



2.3 Three colored lines appear. One color line should be in the control region (C) and another color line should be in the test region (G and M), means IgG and IgM antibody positive（As Below）



3. **Invalid Result:** If the QC Line C is not observed, the detection should be re-detected regardless of whether or not the detection line is displayed (see below).



Performance characteristics

1. Study of: Testing of RT-PCR positive clinical specimens

To study the performance of the Norman nCov IgG/IgM on fresh whole blood (with EDTA) specimens.

The nCOV IGG/IGM Ab Test produced by Norman is used to screen 92 nCOV PCR positive patient Samples and 200 nCOV PCR negative patient Samples. Whole blood (with EDTA) specimens collected according to proper procedure.

		RT-PCR Testing result		
		Pos	Neg	Total
Norman nCov	IgM+/IgG-	9		9
	IgM-/IgG+	0		0
	IgM-/IgG-	4		4
	IgM+/IgG+	79		79
	Neg		200	200
	Total	92	200	292

IgG: Relative Sensitivity: 85.87%
Relative Specificity: 100%
Accuracy: 95.55%
* 95% Confidence Interval
IgM: Relative Sensitivity: 95.65%
Relative Specificity: 100%
Accuracy: 98.63%
* 95% Confidence Interval

2. Assay Cross Reactivity

In order to further evaluate the performance of Novel Coronavirus IgG / IgM Antibody Testing Kit, clinical cooperation units were commissioned to collect the samples of several common infectious diseases or common endogenous interference factors. RF positive autoantibody samples were purchased from Bio-Heme, USA, and ANA autoantibody positive samples were purchased from Keystone Biologicals, USA.

Sample	Result of interference factor testing	Result of report reagent	Conclusion
Positive Hepatitis A antibody sample 1	S/co 15.2	Negative	No cross
Positive Hepatitis A antibody sample 2	S/co 18.9	Negative	No cross
Positive Hepatitis A antibody sample 3	S/co 13.3	Negative	No cross
Positive Hepatitis A antibody sample 4	S/co 10.4	Negative	No cross
Positive Hepatitis A antibody sample 5	S/co 9.5	Negative	No cross
Positive HIV antibody samples 1	S/co 12.8	Negative	No cross
Positive HIV antibody samples 2	S/co 15.3	Negative	No cross
Positive HIV antibody samples 3	S/co 18.6	Negative	No cross
Positive HIV antibody samples 4	S/co 9.4	Negative	No cross
Positive HIV antibody samples 5	S/co 17.3	Negative	No cross
Hepatitis B surface antigen sample 1	S/co 14.5	Negative	No cross
Hepatitis B surface antigen sample 2	S/co 16.3	Negative	No cross
Hepatitis B surface antigen sample 3	S/co 9.6	Negative	No cross
Hepatitis B surface antigen sample 4	S/co 7.9	Negative	No cross
Hepatitis B surface antigen sample 5	S/co 7.5	Negative	No cross
Positive HCV antibody sample 1	S/co 15.4	Negative	No cross
Positive HCV antibody sample 2	S/co 16.2	Negative	No cross
Positive HCV antibody sample 3	S/co 9.6	Negative	No cross
Positive HCV antibody sample 4	S/co 18.5	Negative	No cross
Positive HCV antibody sample 5	S/co 16.4	Negative	No cross
Positive syphilis antibody sample 1	(++)	Negative	No cross
Positive syphilis antibody sample 2	(++)	Negative	No cross
Positive syphilis antibody sample 3	(++)	Negative	No cross
Positive syphilis antibody sample 4	(++)	Negative	No cross
Positive syphilis antibody sample 5	(++)	Negative	No cross
Positive Hepatitis E antibody sample 1	S/co 15.4	Negative	No cross
Positive Hepatitis E antibody sample 2	S/co 16.2	Negative	No cross
Positive Hepatitis E antibody sample 3	S/co 9.6	Negative	No cross
Positive Hepatitis E antibody sample 4	S/co 18.5	Negative	No cross
Positive Hepatitis E antibody sample 5	S/co 16.4	Negative	No cross
RF sample 1	24 IU/ml	Negative	No cross
RF sample 2	260 IU/ml	Negative	No cross
RF sample 3	2960 IU/ml	Negative	No cross
ANA (+)	1:360	Negative	No cross
ANA (+)	1:120	Negative	No cross
ANA (+)	1:240	Negative	No cross
ANA (+)	1:300	Negative	No cross
ANA (+)	1:420	Negative	No cross
ANA (+)	1:180	Negative	No cross
ANA (+)	1:240	Negative	No cross
ANA (+)	1:360	Negative	No cross

Positive infections samples of HIV, Syphilis, Tuberculosis, Hepatitis A, Hepatitis B, Hepatitis E, and Hepatitis C, as well as serum of patients with autoantibody (RF and ANA), will be not cross.
In addition, we commissioned a clinical partner to conduct cross-reaction testing on samples of non- 2019-nCoV strains， such as HKU1, NL63, OC43 and 229E. The testing cards of batch number F0216D2 were randomly selected, and the samples were tested according to the instructions. The results are as follows:

Types of Samples	Number of Samples	Results					
		IgM			IgG		
		Pos	Neg	%CR	Pos	Neg	%CR
HKU1	5	0	5	100	0	5	100
NL63	5	0	5	100	0	5	100
OC43	5	0	5	100	0	5	100
229E	5	0	5	100	0	5	100

The results show that the testing of HKU1, NL63, OC43 and 229E samples were conducted by using Novel Coronavirus IgG/IgM Antibody Testing kit from Nanjing Norman Biotechnology Co., Ltd. And it did not produce cross-reactions.
3. Interference test
Some clinical substances commonly used (such as: antipyretic analgesics) may affect the performance of product. The following substances were added to four kinds of positive samples with negative, weak positive, medium positive and strong positive levels. No false positivity or false negativity was found with the following:

Substances	Concentration
Albumin	2000 mg/dl
Caffeine	1000 mg/dl
Glucose	2000 mg/dl
Heme	1000 mg/dl
Human IgG	1000mg/dl
EDTA	0.5mM
Lecithin	2000 mg/dl
Penicillin G	1000 mg/dl
Ketone	2000 mg/dl

Warnings
For *in vitro* diagnostic use.
The package insert must be carefully followed. Reliability of the test results cannot be guaranteed if there are any deviations from the package insert.
Safety precautions
CAUTION: This product requires the handling of human samples. It is recommended that all human sourced materials are considered potentially infectious and be handled in accordance with the OSHA Standard on Blood borne Pathogens. Biosafety Level 211 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
Appropriate protective measures should be taken in the collection, processing, storage, mixing of the sample and testing process; once the sample and the reagent contact skin, wash with plenty of water; if skin irritation or rash occurs, get medical advice/attention.
Samples, used cartridge and disposable tips may be potentially infectious. Proper handling and disposal methods should be established by the laboratory in accordance with local regulations.

Handling precautions
Do not use the kit beyond the expiration date. The production date and expiration date are on the label.
Different batches of sample diluent and cartridges cannot be mixed.
The cartridge is disposable and cannot be reused.
Please do not use obviously damaged kit or cartridge.
Please avoid high temperature in the lab.
To avoid contamination, wear clean gloves when operating with kits and samples.

Limitation of the Procedure
1. This reagent is for in vitro diagnostics only.
2. This reagent is only used to detect human serum, plasma and whole blood samples, and the results of other sample tests may be incorrect.
3. This reagent is only used for qualitative testing and does not indicate the number of the new coronavirus antibody in the sample.
4. This reagent is only a clinical auxiliary diagnostic tool. If the results are positive, it is recommended to use other methods for further examination and to follow the doctor's diagnosis.
5. The test results of this product are for clinical reference only and should not be used as the sole basis for clinical diagnosis and treatment. The clinical management of patients should be combined with their symptoms, signs, medical history, other laboratory examinations, treatment response and epidemiology.

References
[1]. The situation and prevention and control of the new coronavirus infection outbreak.
[2]. Overview of the new coronavirus.

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