2019-nCoV Neutralizing Antibody Test Kit (Colloidal Gold)

[Intended Use]

For The 2019-nCoV RBD antibodies are protective antibody produced by the human body after inoculation with 2019-nCoV vaccine or infection with 2019-nCov.
The test is intended as an aid to assess the adaptive humoral immune response to the 2019-nCoV.
For in vitro diagnostic use only. For professional use only.

[SUMMARY]

The 2019-nCoV belongs to the B genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible.
The current research has found that the 2019-nCoV binds to their host cell receptor, angiotensin-converting enzyme 2 (ACE2) through RBD from the spike protain (S protein), and undergoes a process of cell membrane fusion. Then the virus enters the cell through endocytosis, replicates and infects the body.
Antibodies against 2019-nCoV with strong neutralizing capacity, especially potent if directed against the RBD, have been idantified. Numerous vaccines for COVID-19 are in development, many of which focus on eliciting an immune response to the RBD.

[PRINCIPLE]

2019-nCoV Neutralizing Antibody Test Kit (Colloidal Gold) is based on the principle of double antigen sandwich immunoassay for determination of 2019-nCoV RBD antibodies in human whole blood, serum and plasma specimen.
Add specimens into the sample well to initiate a test.
When the 2019-nCoV RBD antibodies level in the specimen is at or above the detection limit of the test, the RBD antibodies bound to the colloidal gold-labeled 2019-nCoV recombinant antigen and form immune complexes which are captured by RBD Immobilized in the Test Region (T) of the nitrocellulose membrane, and this produces a colored test band that indicates a positive result.
When the 2019-nCoV RBD antibodies level is zero or below the detection limit, there is no visible colored band in the Test Region (T) of the device. This indicates a negative result.
To serve as a procedure control, a colored line will appear at the Control Region (C), if the test has been performed properly.

[Material Required but Not Provided]

1. Specimen Collection Containers
2.Centrifuge (for serum/plasma specimen)
3.Timer
4.Personal protective equipment, such as medical gloves, medical mask, goggles and lab coat.
5.Appropriate biohazard waste containers and disinfectants.

[STORAGE AND STABILITY]

1. Store at 2-30℃ in the sealed pouch up to the expiration dale printed on the package. Do not freeze.
2.The test cassette should be used within 1 hour after taking out from the foil envelope. Buffer solution should be re-capped in time after use.
3. Keep away from sunlight, moisture and heat.
4. Kit contents are stable until the expiration date printed on the outerbox.
5.The production date is printed on the outer box.

[SPECIMEN COLLECTION AND PREPARATION]

The test can be performed with fingerstick whole Blood, venous whole blood, serum and plasma.
For finger stick Whole Blood:
1.Clean the area to be lanced with an aloohol pad. Allow the finger to dry thoroughly.
2.Use a sterile lancet, puncture the skin just off the center of the finger pad. Apply gentle pressure beside the point of the puncture. Wipe away the first drop of blood. Allow a new drop of blood to form. If blood flow is Inadequate, the subject's finger may have to be gently

massaged at the finger base to produce a droplet of sufficient volume.
3.Draw 40ul of finger blood with the disposable dropper. Whole blood specimen collected by- fingerstick should be tested Immediately.
For Venous Whole Blood
1.According to standard venous blood collection procedure, collect a venipuncture whole blood specimen using a blood collection tube with suitable anticoagulant (containing EDTA or Heparin). Other anticoagulants have not been validated and may give incorrect result.
2.1t is recommended that whole blood specimen is tested at the time of specimen collection. If the specimens are not tested immediately, they may be stored at 2-8°C for up to 3 days. Prior to testing, mix the blood by gentle inversion several times, do not freeze or heat whole blood specimens.

[PRECAUTION]

1.This kit is for in vitro diagnostic use only.
which is for storage purposes only, and is not used in test procedure.
3.All specimens should be trealed as capable of transmitting diseases. Use appropriate precautions in the collection, handling, storage and disposal of testing samples and used kit contents. And follow biosafety level 2 or higher guidelines.
4.Wear appropriate personal protective equioment (a.g. medical gloves, medical mask, goggles and lab coat) when handling the contents of this kit.
5.Proper specimen collection, storage and transport are citical to the performance of this test. Discard after first use. The lest cannot be used more than once.
6. Avoid excessively high temperature in the lesting environment. Test Cassettes and Detection Buffer stored at low temperature need to be returned to room temperature before opening to avoid moisture absorption.
7.Do not touch the reaction area of test strip.
8.Do not use test kit beyond the expiration date.
9. The Test Cassette should remain in its original sealed pouch until use. Do not use the kit if the pouch is punctured or not well sealed.
10. Do not use damaged or stained detection buffer tube.
11. Testing should be applied by professional trained staff working in certified laboratories or clinics at which the specimen(s) is taken by qualified medical personnel.

For Serum and Plasma

1.According to standard venous blood collection procedure, collect a venipuncture whole blood specimen using a blood collection tube. If collecting plasma use a blod collection tube containing suitable anticoagulant (containing EDTA or Heparin). Other anticoagulants have not been validated and may give incorrect result.
2.Separate the serum/plasma from blood as soon as possible to avoid hemolysis.
3. Test should be performed within 8 hours after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods.
4.If the specimens are not tested within 8 hours, they should be kept at 2-8°C for up to 7 days. For storage more than 7 days, specimens should be kept below -20°C for long time.
Note: Bring specimens to room temperature before testing Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly. Severe hemolytic or heat-inactivated specimens are not recommended

[TEST PROCEDURE]

Please read the instruction for use carefully before performing the test.
1.Allow the device, buffer and specimen to equilibrate to room temperature prior to testing.
2. Romove a test cassette from the sealed pouch by tearing at the notch and place it on a level surface.
3. Add 20 ul serum/plasma or 40 ul. (1 drop) fingerstick/venous whole blood specimen into the sample well (small well) with the disposable dropper, and then add 80 uL (2-4 drops) detection buffer (in a dropper bottle or capsule) into the buffer well (large well) as the below picture shows.

4. As the test begins to work, you will see purple color move across the result window in the center of the test device. Wait for 15 minutes and read the results.
Note: Results after more than 30 minutes may be not accurate and should not be read.
5. The test result should be interpret by the physician along with clinical findings and other laboratory test results.
6. Disposal of the diagnostic: All secimens and the used kit has the infectious risk. The process of disposing the diagnostic must follow the local infectious disposal law or laboratory regulation.
7. If you have questions or suggestions during the use of this reagent, please contact the manufacturer.

[MATERIAL]

Components	Test Cassette	1
	Detection Buffer	1
	Disposable Droppers	1
	IFU	1
	Sterile Lancet	1
	Alcohol Pad	1

Each sealed pouches containing: 1 Test Cassette and 1 Desiccant Pouch
There are Three types of Detection Buffer: buffer in capsule (100ul/capsule) and buffer in a dropper bottle (2.5 mL/bottle) or (5.0 mL bottle)

Reactive ingredients of main components
The test cassette consists of test strip and plastic cassette. The test strip includes: nitrocellulose membrane, sample pad, conjugated pad, absorbent paper and PVC board. Nitrocellulose membrane is coated with RBD, goat anti-rat lgG polyclonal antibodies; Conjugate pad contains 2019-nCov recombinant antigen and rat IgG.
Note: To ensure the accuracy of test results, components in different lots cannot be mixed-used.

[RESULT INTERPRETATION]

Positive Result
Colored band appear at both test line (T) and control line (C). It indicates that the concentration of the 2019-nCoV RBD antibodies at or above the detection limit of the test.
Negative Result
Colored band appears only at control line (C). It indicates that the concentration of the 2019-nCoV RBD antibodies is zero or below the detection limit of the test.
Invalid Result
No visible colored band appear at control line after performing the test. The directions may not have been followed correctly. Pay particular attention to whether the sample volume is sufficient. It is recommended that the specimen be re-tested.

[QUALITY CONTROL]

A procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient liquid volume, adequate membrane wicking and correct procedural technique.
Good laboratory practice recommends the use of the control materials.

[LIMITATIONS OF PROCEDURE]

1.This reagent is designed to detect RBD antibodies against 2019-nCov in human whole blood, serum or plasma specimen.
2. The accuracy of the test depends on the specimen collection process. Improper specimen collection, improper specimen storage, or repeated freezing and thawing of the specimen will affect the test result.
3. This reagent is a qualitative assay. It is not designed to determine the quantitative concentration of 2019-nCoV RBD anibodies. If you need to test the quantitative concentration, please use the relevant professional instruments.
4. A positive result cannot completely rule out the risk of infection with the coronaviruses (2019-nCov).
5. Limited by the method of antibody test reagents, for negative test results, it is

recommended to use other methods for review and confirmation.
6. A negative result of this reagent can be caused by:
1) improper specimen collection, improper specimen transfer or handling, the antibodies titer in the specimen is too low
2) variations in viral genes may cause changes in antibodies determinants.

[PERFORMANCE CHARACTERISTICS]

A. Sensitivity and Specificity/ Duyarlılık ve Özgüllük
A total of 238 samples retrospectively collected from 2019-nCoV PCR positive and negative individuals (65 positive and 173 negative) were tested with the 2019-nCoV Neutralizing Antibody Test Kit (Colloidal Gold). The sampling date of positive samples were both more than 7 days after diagnosis with PCR The performance of the kit was compared to the results of a nasopharyngeal or Oropharyngeal swab tested with a commercialized molecular assay.

Method		RT-qPCR		Total
2019-nCoV Neutralizing Antibody Test Kit (Colloidal Gold)	Result	Positive	Negative	
	(+)	62	1	63
	(-)	3	172	175
Total		65	173	238

Sensitivity: 95.38% (95%Cl 87.29%-98.42%)
Specificity: 99.42% (95%Cl 96.8% - 99.90%)
Overall agreement 98.32% (95%Cl: 95.76%-99.34%)

B. Correlation of test results to serum neutralization capacity/
The 2019-nCoV Neutralizing Antibody Test Kit (Colloidal Gold) was compared to The Pseudovirus Neutralization Assay. The following tables show the correlation between $NtAb_{50}$" of Pseudovirus Neutralization Assay and results of the 2019-nCoV Neutralizing Antibody Test Kit (Colloidal gold)

Method		Pseudovirus Neutralization Assay			Total
			< 1:20	1:20 – 1.160	
2019-nCoV Neutralizing Antibody Test Kit (Colloidal Gold)	(-)	20	1	0	21
	(+)	1	6	24	31
Total		21	7	24	52

*NtAb50: Antibody titers resulting in 50% virus neutralization

Dilution Titer	Result	Test Result Interpretation
<1:20	Negative	Neutrolizing antibodies for 2019-nCoV are not detected at 50% virus neutralization
1.20 – 1:160	Low titer	Low neutrolizing antibodies for 2019-nCoV are not detected at 50% virus neutralization
>1:160	High titer	High neutrolizing antibodies for 2019-nCoV are not detected at 50% virus neutralization

C. Cross reactivity/Çapraz reaktivite
Cross reaktivity of the 2019-nCoV Neutralizing Antibody Test Kit (Colloidal Gold) was evaluated using specimens containing the antibodies listed below. The results showed no cross reactivity with the following:

Parainfluenza virus antibodies	Treponema pallidum antibodies
Influenza A antibodies	HIV antibodies
Influenza B antibodies	EB virus antibodies
Chlamydia pneumonia antibodies	Meales virus antibodies
Mycoplasma pneumoniae antibodies	Cytomegalovirus antibodies
Adenovirus antibodies	Enterovirus type 71 antibodies
Respiratory syncytial virus antibodies	Mumps antibodies
Hepatit B surfece antibodies	Varicella-zoster virus antibodies
Hepatit C virus antibodies	

D. Interference
The test result of 2019-nCoV Neutralizing Antibody Test Kit (Colloidal Gold) were not be interfered with the substance at the following concentration:

Substance	Concentration
Bilirubin	250 μmol/L
Hemoglobin	9 g/L
Triglyceride	15 mmol/L
Rheumatoid factors	80 IU/mL
Antinuclear antibodies (ANA) titer	1:240
Anti-mitochondrial antibodies (AMA)	80 U/mL
HAMA	1000 μG/mL

E. Hook effect/

Within the titer range of clinically positive specimens of 2019-nCoV RBD antibodies, there was no hook effect in the test results of this product.

F. Precision/

1.Within-run Precision was determined by testing separately two positive specimens in 10 times. The agreement rate was 100%.

2. Between-run Precision was determined by testing three different specimens including positive and negative in 3 different lots of test devices. The negative agreement rate and the positive agreement rate were 100%.

[Explanation of Symbols on Label and Package]

	Consult instructions for use		Use-by date		Authorized representative in the European Community
	In vitro diagnostic medical device		Batch code		Do not re-ıse

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