todylaboratories®

Rapid Tests

CE IVD REF C12RT1029

Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Colloidal Gold Immunochromatographic Assay, Anti-Spike Protein, Whole Blood/Serum/Plasma) · Instructions For Use

For *In-Vitro* diagnostic and professional use only. Store at (2-30°C).

INTENDED USE

The Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of neutralizing antibodies to SARS-CoV-2 in human whole blood, serum, or plasma.

INTRODUCTION

COVID-19 is an interspecies infectious disease that is caused by a novel coronavirus with a nucleocapsid of helical symmetry that envelopes a positive single stranded RNA genome. Infection by this virus is zoonotic; which means it can be transmitted between species. Symptoms, however, vary drastically. In humans, the novel coronavirus causes acute respiratory tract complications that can range from mild to lethal. Symptoms most commonly include; fever, dry cough, fatigue, sputum production, sore throat, and headache. Infected individuals usually show signs of infection within 2 to 14 days of exposure, and will recover without the need for special treatment. Elderly, diabetic, immunocompromised, and those patients with cardiovascular conditions, for instance, are at higher risk of developing severe responses to the virus, such as pneumonia, kidney failure, and even death.

The 1960s witnessed the discovery of human coronaviruses. Other members of the family have emerged since, including SARS-CoV in 2003, HCoV NL63 in 2004, HKU1 in 2005, MERS-CoV in 2012, and the novel SARS-CoV-2 in 2019.

According to the European Centre for Disease Prevention and Control, the COVID-19 virus spreads primarily through common substances of human origin that include body fluids, such as mucus and saliva.

PRINCIPLE

In this test, gold particles coated with SARS-CoV-2 spike protein antigens are immobilized on the conjugate pad, and a capture reagent is, in turn, immobilized on the test line. This allows the formation of a mixture between the sample and the spike protein. This mixture migrates chromatographically along the test strip. If neutralizing antibodies are found in the sample, capturing occurs at the test line and a colored line develops indicating a positive result.

Otherwise, in the case of negative samples, no test line appears. To serve as a procedural control, a colored line will always change from blue to red in the control line region.

MATERIALS

Material Provided

• Test cassette • Buffer • Micro-pipette • Lancet • Alcohol pad • Package insert.

Materials required but not provided

• Specimen collection container • Timer • Centrifuge.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after the expiration date indicated on the package.
- Do not use the test if the foil pouch is damaged. Do not reuse tests.
- Never smoke, drink, or eat in the assay laboratory.
- The test cassette should remain in the sealed pouches until use.
- Dispose of all specimens and materials used to perform the test as biohazardous waste.
- Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
- Handle the specimens as though they contain infectious agents
- This package insert must be read completely before performing the test.
- Do not interchange the buffer and test cassettes of different lots.
- The used tests, specimens and potentially contaminated materials should be discarded according to the local regulations.
- Humidity and temperature can adversely affect results.
- Do not use the pouch if it is unprinted and it was not mentioned the product expiry date.

STORAGE AND STABILITY

- The tests are packaged in the sealed pouch at room temperature or refrigerated 2-30°C.
- The test cassette is stable through the expiration date printed on the sealed pouch.
- Do not freeze.
- The test cassette must remain in the sealed pouch until use.
- Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

1. The Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test can be performed using whole blood, serum or plasma.
2. Only clear, non-hemolyzed specimens are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
3. Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days. For long term storage, specimens should be kept at -20°C.
4. Allow sample to reach room temperature before proceeding. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
5. If specimens are to be shipped, they should be packed in compliance with local regulations.
6. Whole blood or plasma could be collected with tube containing Heparin or Citrate.

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (18-30°C) before use.

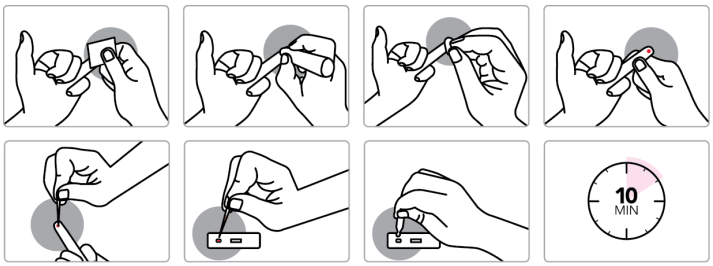
1. Take the test cassette from the sealed pouch.
2. Place the test cassette on a clean and level surface.

For serum or plasma specimen:

3. Hold the micro-pipette vertically, draw the specimen up to the fill line **(approximately 10 µL)** and transfer specimen to the specimen well (**S**) of the test cassette, then add **2 drops of buffer (approximately 80 µL)** and start the timer. Avoid trapping air bubbles in the specimen well. See the illustration below.
4. Wait for the colored line(s) to appear. Read the result after 10 minutes. Do not interpret results after 20 minutes.

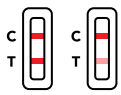
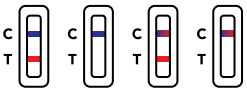
For Fingerstick whole blood specimen:

5. Wash the patient's hand with soap and warm water or clean with an alcohol pad. Allow to dry.
6. Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
7. Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
8. Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
9. Add the Fingerstick Whole Blood specimen to the test cassette by using a provided micro-pipette.
10. Hold the micro-pipette vertically, draw the specimen up to the fill line **(approximately 10 µL)** and transfer and specimen to the specimen well (**S**) of the test cassette, then add **2 drops of buffer (approximately 80 µL)** and start the timer. Avoid trapping air bubbles in the specimen well. See the illustration below.
11. Wait for the colored line(s) to appear. Read the result after 10 minutes. Do not interpret results after 20 minutes.



INTERPRETATION OF RESULTS

(PLEASE REFER TO THE ILLUSTRATIONS BELOW)

	**POSITIVE: Two Lines appear.** The colored line in the control line region (C) changes from **Blue to Red**, and other colored line should appear in the test line region (T). ***NOTE**: The intensity of the color in the test line region will vary depending on the concentration of neutralizing antibodies to SARS-COV-2 antibodies in the specimen. Therefore, any shade of color in the test line region should be considered positive.
	**NEGATIVE:** The colored line in the control line region (C) changes from **Blue to Red**. No line appears in the line region (T).
	**INVALID:** Control line (C) is still completely or partially blue and fails to completely change from **Blue to Red**. 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 cassette. 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 colored line appearing in the control region (C) is an internal valid procedural control, it confirms adequate membrane wicking.

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.

LIMITATIONS

- The Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole Blood/Serum/Plasma) is for *in vitro* diagnostic use only. The test should be used for the detection of neutralizing antibody in whole blood, serum or plasma specimens only.
- The best time to detect the presence of neutralizing antibodies is 10 days from the second vaccination day in case of vaccination with vaccines containing inactivated viral particles, and 15 days from the second vaccination day in case of mRNA vaccines.

NOTE: COVID-19 Vaccinations are given on two days usually with a 28-days gap in-between. Here, the number of days is to be calculated from the second vaccination day.

- Results from the Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole Blood/Serum/Plasma) should not be used as the sole basis to diagnose or exclude the presence of neutralizing antibodies to SARS-CoV-2.
- The continued presence or absence of neutralizing antibodies cannot be used to determine the success or failure of therapy or vaccination.
- Results from immunosuppressed patients should be interpreted with caution.
- As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
- This test should not be used for blood donor screening.

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PERFORMANCE CHARACTERISTICS

Generally, the higher the titer of neutralizing antibody, the better the individual protection. Microneutralization Assay (MNA_{50}) is an established method of assessing the humoral response. Positive results obtained with a 1:10 dilution in MNA_{50} assay indicate that individuals are already protected.
Positive result obtained with a 1:20 dilution indicate that individuals have strong protection. Therefore, MNA_{50} assay at 1:10 dilution was selected as the comparison method to evaluate the efficacy of the Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole Blood/Serum/Plasma).

1. The Cutoff of MNA_{50} is maintained at 1:10 dilution and the clinical characteristics are shown below:
In order to evaluate the clinical performance of Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole Blood/Serum/Plasma), the comparison was established against Microneutralization Assay (MNA) and SARS-CoV-2 Neutralizing Antibody Elisa Kits.

Value Result (Dilution Titer)	Result	Test Result interpretation
≥1:10	Positive	SARS-CoV-2 Neutralizing antibodies are detected at 50% viral neutralization.
<1:10	Negative	SARS-CoV-2 Neutralizing antibodies are not detected at 50% viral neutralization

Part 1: Clinical Performance using MNA_{50} titer as the comparator method with samples from convalescent patients, or healthy unvaccinated individuals
A total of 48 samples were retrospectively collected from convalescent patients, or healthy unvaccinated individuals (30 MNA_{50} positive and 18 MNA_{50} negative) and were evaluated with the Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test.

Item		Microneutralization Assay (MNA_{50})		Total Result
Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole blood/Serum/Plasma)	Result	Positive	Negative	
	Positive	30	0	30
	Negative	0	18	18
Total Result		30	18	48

Sensitivity: 100% (95%CI*: 90.5%~100%)
Specificity: 100% (95%CI*: 84.7%~100%)
Accuracy: 100% (95%CI*: 93.9%~100%)
* Confidence Interval

Part 2: Clinical Performance using MNA_{50} titer as the comparator method with samples from vaccinated individuals, or healthy unvaccinated individual.
A total of 47 samples were collected from vaccinated individuals (Inactivated SARS-CoV-2 Vaccine), or healthy unvaccinated individuals (29 MNA_{50} positive and 18 MNA_{50} negative) and were evaluated with the Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test.

Item		Microneutralization Assay (MNA_{50})		Total Result
Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole blood/Serum/Plasma)	Result	Positive	Negative	
	Positive	29	0	29
	Negative	0	18	18
Total Result		29	18	47

Sensitivity: 100% (95%CI*: 90.2%~100%)
Specificity: 100% (95%CI*: 84.7%~100%)
Accuracy: 100% (95%CI*: 93.8%~100%)
* Confidence Interval

2. The cutoff of MNA_{50} is maintained at 1:20 (dilution titer) and the clinical characteristics are showed below:
In order to evaluate the clinical performance of Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole Blood/Serum/Plasma), the comparison was established against Microneutralization Assay (MNA) and SARS-CoV-2 Neutralizing Antibody Elisa Kits.

Value Result (Dilution Titer)	Result	Test Result interpretation
≥1:20	Positive	SARS-CoV-2 Neutralizing antibodies are detected at 50% viral neutralization.
<1:20	Negative	SARS-CoV-2 Neutralizing antibodies are not detected at 50% viral neutralization

Part 1: Clinical Performance using MNA_{50} titer as the comparator method with samples from convalescent patients, or healthy unvaccinated individuals
A total of 48 samples were retrospectively collected from convalescent patients, or healthy unvaccinated individuals (30 MNA_{50} positive and 18 MNA_{50} negative) and were evaluated with the Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test.

Item		Microneutralization Assay (MNA_{50})		Total Result
Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole blood/Serum/Plasma)	Result	Positive	Negative	
	Positive	30	0	30
	Negative	0	18	18
Total Result		30	18	48

Sensitivity: 100% (95%CI*: 90.5%~100%)
Specificity: 100% (95%CI*: 84.7%~100%)
Accuracy: 100% (95%CI*: 93.9%~100%)
* Confidence Interval

Part 2: Clinical Performance using MNA_{50} titer as the comparator method with samples from vaccinated individuals, or healthy unvaccinated individual
A total of 47 samples were collected from vaccinated individuals (Inactivated SARS-CoV-2 Vaccine), or healthy unvaccinated individuals (26 MNA_{50} positive and 21 MNA_{50} negative) and were evaluated with the Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test.

Item		Microneutralization Assay (MNA_{50})		Total Result
Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole blood/Serum/Plasma)	Result	Positive	Negative	
	Positive	26	3	29
	Negative	0	18	18
Total Result		26	21	47

Sensitivity: 100% (95%CI*: 89.1%~100%)
Specificity: 85.7% (95%CI*: 63.7%~97%)
Accuracy: 93.6% (95%CI*: 82.5%~98.7%)
* Confidence Interval

3. Clinical Performance using Elisa kit as the comparator method with samples from vaccinated individuals, or healthy unvaccinated individual
A total of 60 samples were collected from vaccinated individuals (Inactivated SARS-CoV-2 Vaccine), or healthy unvaccinated individuals (30 Elisa positive and 30 Elisa negative) and were evaluated with the Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test.

Item		Microneutralization Assay (MNA_{50})		Total Result
Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole blood/Serum/Plasma)	Result	Positive	Negative	
	Positive	30	0	30
	Negative	0	30	30
Total Result		30	30	60

Sensitivity: 100% (95%CI*: 90.5%~100%)
Specificity: 100% (95%CI*: 96.3%~100%)
Accuracy: 100% (95%CI*: 95.1%~100%)
* Confidence Interval

4. Cross-reactivity
The Coronavirus (SARS-CoV-2) Neutralizing Antibody Rapid Test (Whole Blood/Serum/Plasma) has been verified against anti-influenza A virus, anti influenza B virus, anti-RSV, anti-Adenovirus, HBsAb, anti-Syphilis, anti-H.Pylori, anti-HIV, anti-HCV. No cross-reactivity was observed.

5. Interfering Substances
The following potentially interfering substances were added to SARS-CoV-2 neutralizing antibody negative and spiked positive specimens.

SUBSTANCE	CONCENTRATION	RESULT	
		Negative Specimen	Spiked with Positive Specimen
Acetaminophen	20 mg/dL	Negative	Positive
Caffeine	20 mg/dL	Negative	Positive
Albumin	2 g/dL	Negative	Positive
Acetylsalicylic Acid	20 mg/dL	Negative	Positive
Gentisic Acid	20 mg/dL	Negative	Positive
Ethanol	1%	Negative	Positive
Ascorbic Acid	2 g/dL	Negative	Positive
Creatine	200 mg/dL	Negative	Positive
Bilirubin	1 g/dL	Negative	Positive
Hemoglobin	1000 mg/dL	Negative	Positive
Oxalic Acid	60mg/dL	Negative	Positive
Uric Acid	20 mg/ml	Negative	Positive

None of the substances at the concentrations tested interfered with the assay.

INDEX OF SYMBOLS

	Manufacturer
IVD	For *in vitro* diagnostic use only
LOT	Lot number
REF	Product code
	Use by
2°C 30°C	Store between 2-30°C
CE	European Conformity
	Consult Instructions For Use
Σ	Tests per kit
	Do not use if package is damaged
	Do not reuse
	Keep away from sunlight
	Keep dry

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