COVID-19 Neutralizing Antibody Test Kit (Colloidal Gold）

Instruction for Use

REF: CG02Ab-01
REF: CG02Ab-25
REF: CG02Ab-50

【Product Name】

COVID-19 Neutralizing Antibody Test Kit (Colloidal Gold)

【Packing Specification】

1 Test/Kit, 25 Tests/Kit, 50 Tests/Kit

【Intended Use】

The AmonMed™ COVID-19 Neutralizing Antibody Test Kit (Colloidal Gold) is used for in vitro qualitative detection of neutralizing antibodies to SARS-CoV-2 in human serum, plasma and whole blood (venous and fingerstick). The test kit is for preliminary screening and for professional use only.

COVID-19 neutralizing antibodies are protective antibody produced by the human body after inoculation with novel coronavirus vaccine or infection with novel coronavirus. The kit is used to monitor the presence of neutralizing antibodies in subjects vaccinated with the novel coronavirus vaccine or in people who were infected with the novel coronavirus, it can be used to evaluate the immune effect after vaccination or whether neutralizing antibodies are produced in human body after infection with novel coronavirus.

【Test Principle】

This kit uses immunochromatography for qualitative detection of neutralizing antibodies to COVID-19 in human serum, plasma and whole blood (venous and fingerstick). The sample will move forward along the test card under capillary action. If the sample contains the COVID-19 neutralizing antibodies, the neutralizing antibodies will bind to the colloidal gold-labeled 2019-nCOV SRBD antigen. The immune complex will be captured by the other 2019-nCOV SRBD antigen which is pre-coated on membrane strip, then form a fuchsia line on the test line, it suggests that the result is positive; if the line does not show color, it suggests that the result is negative. The test card also contains a quality control line C which is pre-coated goat anti-mouse IgG antibody. No matter whether the sample contains neutralizing antibodies or not, the colloidal gold-labeled 2019-nCOV SRBD antigen will form a fuchsia line with goat anti-mouse IgG on the control line. A visible control line is required to indicate a test result is valid.

【Main Components】

Component	Description	Specification		
		1 Test/kit	25 Tests/kit	50 Tests/kit
Test card	Foil pouched test device containing one test strip which is encased in plastic device cassette.	1 bag/kit	25 bags/kit	50 bags/kit
Sample dilution	Used to dilute the sample(PH=8.0 Tris-Hcl)	0.3 mL×1 bottle	5 mL×1 bottle	5 mL×1 bottle
Blood lancet	Sterility, Sterilization by irradiation	1 piece/kit	25 pieces/kit	50 pieces/kit
Disposable pipette	Used for fingerstick blood collection	1 piece/kit	25 pieces/kit	50 pieces/kit
Alcohol prep pad	Used for fingertip disinfection	1 piece/kit	25 pieces/kit	50 pieces/kit
Instruction for use	Instruction for use	1 piece/kit	1 piece/kit	1 piece/kit

Note: The components of different lot numbers are not interchangeable.

【Materials Required but not Provided】

1. Timer
2. Any necessary personal protective equipment
3. Micropipette
4. Biohazard or sharps container
5. Centrifuge (for plasma and serum)

【Storage and Stability】

1. Store at 2 ~ 30℃ for 18 months.
2. After the aluminum foil bag is unsealed, the test card should be used as soon as possible within one hour (temperature 8℃~30℃, humidity 25% ~ 95%).
3. See manufacturing date and expiration date on label.

【Sample Requirements】

1. This kit is suitable for serum, plasma and whole blood (venous and fingerstick). Fresh samples are recommended.
2. Venous whole blood: venous whole blood should be tested within 8 hours after collection,
3. Fingerstick whole blood should be tested immediately after collection; severe hemolytic and lipemia samples shouldn't be used for testing.
4. Serum and plasma samples can be stored in a refrigerator at 2℃~8℃. After separation, serum and plasma samples should not be stored at 2℃~8 ℃ for more than 7 days. More than 7 days, they should be frozen at -20℃, which can be stored for 3 months. Frozen samples subjected to up to 3 freeze/thaw cycles have been evaluated. Be careful to return to room temperature before testing.

【Test Methods】

1. Place the test card, sample diluent and test sample on a flat surface and equilibrate to room temperature.
2. Open the aluminum foil pouch of the test card, and place the test card on a flat surface.
3. Write sample ID on the test card.
4. Fingerstick whole blood:

4.1 Rub the target finger to stimulate blood flow. Tear off the disposable alcohol pad, wipe your finger , and dry naturally.

4.2 Twist the lancet cap for over 90° and remove it.

4.3 Place the blood lancet firmly on side of finger (avoid callus) to trigger it, gently press around the site of puncture to obtain a drop of blood (avoid excessive bleeding);

4.4 Wipe away the first drop of blood with the alcohol prep pad.

4.5 Allow a new drop of blood to form and collect with provided disposable pipette.

4.6 Use disposable pipette to deliver 1 drop of whole blood(about 30μl) to the sample well of test card,

4.7 Add two drops of sample diluent（about 60μl) to the sample well of test card.

4.8 Start the timer and read the test results at 15 minutes. Do not read results after 20 minutes.

5. Venous whole blood sample:

5.1 Use a micropipette to remove 30μl of whole blood into the test sample well of the test card

5.2 Add two drops of sample dilution (about 60μl) to the sample well of the test card.

5.3 Start the timer and read the test results at 15 minutes. Do not read results after 20 minutes.

6. Serum and plasma samples:

6.1 Use a micropipette to remove 20μl serum/plasma sample into the test sample well of the test card.

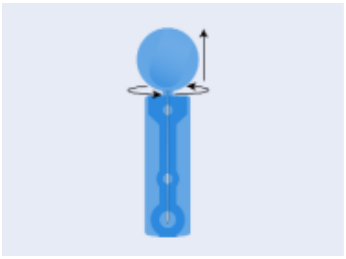
6.2 Add two drops of sample dilution (about 60μl) to the sample well.

6.3 Start the timer and read the test results at 15 minutes. Do not read results after 20 minutes.

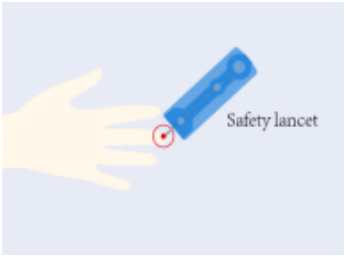
Note: Take care not to produce obvious bubbles when drawing and adding sample.

For fingerstick whole blood

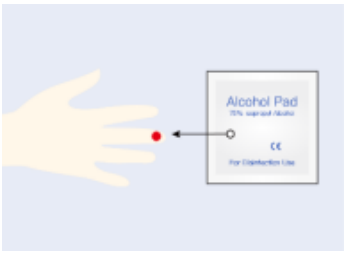
4.1 Disinfect the fingertip with an alcohol prep pad



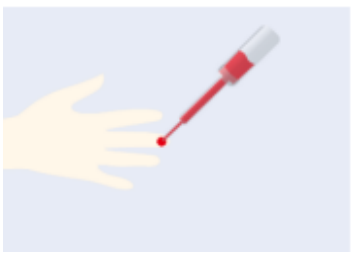
4.2 Twist the lancet cap for over 90° and remove it.



4.3 Place the lancet firmly on side of finger (avoid callus) to trigger it.



4.4 Gently press the bleeding point. Wipe away the first drop of blood.



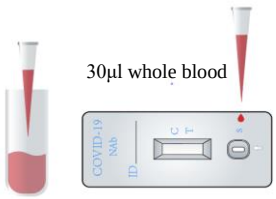
4.5 Use disposable pipette to collect specimen.

4.6 Use disposable pipette to deliver 1 drop of whole blood (about 30μl) to the sample well of test card.

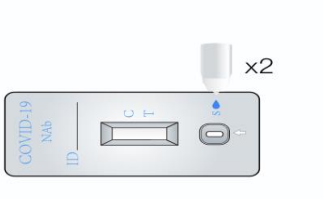
4.7 Add two drops of sample dilution (about 60μl) to the sample well,take care not to produce obvious bubbles when drawing and adding sample.

4.8 Start the timer and read the test results at 15 minutes. Do not read results after 20 minutes.

For Venous whole blood



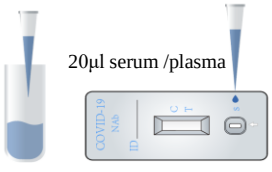
5.1 Use a micropipette to remove 30μl of whole blood into the test sample well.



5.2 Add two drops of sample dilution (about 60μl) to the sample well,take care not to produce obvious bubbles when drawing and adding sample.

5.3 Start the timer and read the test results at 15 minutes. Do not read results after 20 minutes.

For serum/plasma



6.1 Use a micropipette to remove 20μl of serum/plasma into the test sample well.

6.2 Add two drops of sample dilution (about 60μl) to the sample well,take care not to produce obvious bubbles when drawing and adding sample.

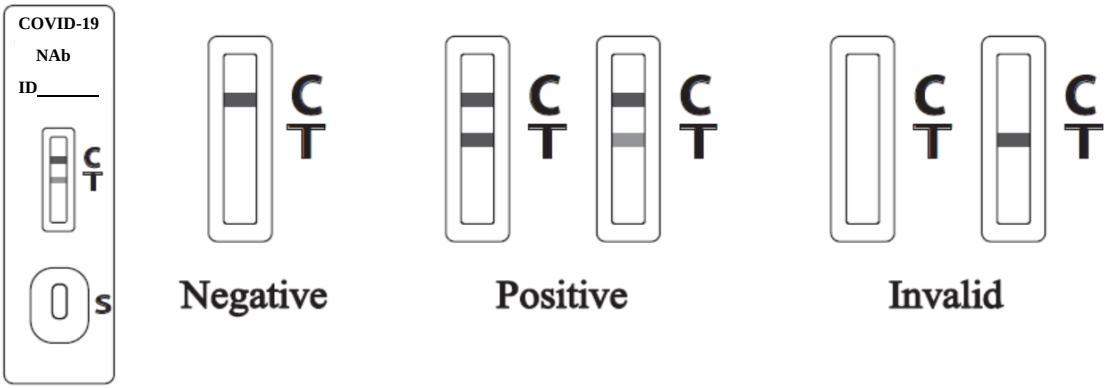
6.3 Start the timer and read the test results at 15 minutes. Do not read results after 20 minutes.

【Interpretation of Test Results】

Negative result: If there is only a quality control line (C), the detection line is colorless, indicating that COVID-19 neutralizing antibody has not been detected or lower than limit of detection so that the result is negative.

Positive result: If both the quality control line (C) and the detection line (T) appear, COVID-19 neutralizing antibody has been detected and the result is positive.

Invalid result: If the quality control line (C) is not observed, it will be invalid regardless of whether there is detection line (as shown in the figure below), and the test shall be conducted again.



【Performance Characteristics】

1. Limit of Detection (LoD)

The LoD for the COVID-19 Neutralizing Antibody Test Kit (Colloidal Gold) is 0.065 μg/mL.

2. Cross Reactivity

Cross-reactivity of the AmonMed™ COVID-19 Neutralizing Antibody Test Kit (Colloidal Gold) was designed to evaluate potential cross reactants. The results are listed in the following table:

Category	Number of samples	Cross Reactivity (Yes/NO)	Category	Number of samples	Cross Reactivity（Yes/NO）
Human Coronavirus antibodies (HKU1, OC43, NL63, 229E)	16	NO	Respiratory syncytial virus antibodies	10	NO
Influenza A virus antibodies	20	NO	CMV antibodies	10	NO
Influenza B virus antibodies	14	NO	EB virus antibodies	10	NO
Human immunodeficiency virus antibodies	8	NO	M.Pneumonia antibodies	6	NO
Hepatitis C virus antibodies	10	NO	Adenovirus antibodies	10	NO
Hepatitis B virus antibodies	10	NO	ANA	5	NO

3. Interfering Substances

A study was performed demonstrate that 36 potentially interfering substances that may be found in the serum do not cross-react or interfere with the negative and positive test results of the reagent, and there is no cross-reaction. The final test

concentrations of the interfering substances are documented in the table below.

Substance	Concentration	Substance	Concentration	Substance	Concentration
Bilirubin	40 mg/dL	Ribavirin	90 mg/dL	Mucin	250 mg/dL
Triglycerides	1000 mg/dL	Ceftriaxone sodium	80 mg/dL	RF	1500 IU/mL
Hemoglobin	2000 mg/dL	Mometasone	2.5 mg/dL	Albumin	6 g/dL
HAMA	30 ng/mL	Budesonide	3.0 mg/dL	Anti-Mitochondrial	1:64 (titer)
Fluticasone propionate	2.5 mg/dL	Total IgG	1600 mg/dL	Interferon α	1500 U/mL
Levofloxacin	1.55 mg/dL	Total IgM	280 mg/dL	Phenylephrine hydrochloride	1 mg/dL
Azithromycin	1.2 mg/dL	Total IgA	500 mg/dL	Meropenem	80 mg/dL
Tobramycin	2.5 mg/dL	Oseltamivir	1.2 mg/dL	Dexamethasone	18 mg/dL
Oxymetazoline	2.5 mg/dL	Sodium chloride	45 mg/dL	Beclomethasone	2.5 mg/dL
Triamcinolone acetonide	5.5 mg/dL	Biotin	5 mg/dL	Peramivir	60 mg/dL
Lopinavir	45 mg/dL	Ritonavir	120 mg/dL	Arbidol	36 mg/dL
Flunisolide	2.5 mg/dL	Zanamivir	1.2 mg/dL	Histamine dihydrochloride	4.5 mg/dL

4. Clinical Performance

The AmonMed™ COVID-19 Neutralizing Antibody Test Kit (Colloidal Gold) has been evaluated to study product performance. The study included 321 samples (101 was determined by testing 101 samples confirmed SARS-CoV-2 VNT $50 \geq 20$, and 220 samples from subjects neither SARS-CoV-2 infection nor vaccination).

Method		cVNT		Sub-total
COVID-19 Neutralizing Antibody Test Kit(Colloidal Gold)	Results	Positive	Negative	
	Positive	95	2	97
	Negative	6	218	224
Sub-total		101	220	321
Sensitivity	95/101	94.06% (95% CI*: 87.64%～97.25%)		
Specificity	218/220	99.09% (95% CI*: 96.75%～99.75%)		
Accuracy	313/321	97.51% (95% CI*: 95.16%～98.73%)		

*** 95% Confidence Interval**

5. Hook Effect

The highest concentration of the samples (2×10^2 μg/mL) was tested. There was no hook effect detected.

【Limitations of Test Method】

1. This test kit is only suitable for qualitative in vitro diagnosis.
2. This reagent only detects human serum, plasma and whole blood (venous and fingerstick) samples. The results of testing with other samples or solutions may be incorrect.
3. Operation procedures must be strictly followed to obtain the correct results, any changes to the operating procedures may affect the results.
4. False negative results may be caused by improper sample collection, transport and handling, and too low concentration of measured substance in the samples.
5. This reagent is only used for preliminary screening and cannot be used as a diagnostic result.

6. Bacterial contamination of the samples may affect the test results.
7. The test results of this kit can only be used to determine the production of neutralizing antibodies, and can be used to evaluate the immune effect of vaccination or the evaluation of the presence of neutralizing antibodies in human body after infection with novel coronavirus, however, it is not applicable to the evaluation of protective ability after vaccination or after infection with novel coronavirus. It is unknown at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
8. Results should be used in conjunction with patient's medical history, clinical examination and other findings.
9. If the COVID-19 neutralizing antibody results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
10. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassay. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed.

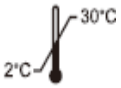
【Warnings and Precautions】

1. Test results are meant to be visually determined.
2. To avoid erroneous results, samples must be processed as indicated in the assay procedure sections.
3. Do not eat, drink, or smoke in the area where the samples and kit contents are handled.
4. Use appropriate precautions in the collection, handling, storage and disposal of patient samples and used kit contents.
5. If the sample dilution contacts the skin or eye, flush with copious amounts of water immediately.
6. Handle all samples as though they contain infectious agents.
7. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of samples.
8. Do not interchange kit contents from different lots.
9. Inadequate or inappropriate sample collection, storage and transport can result in incorrect results.
10. Specific training or guidance is recommended if operators are not experienced with sample collection and handling procedures. Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when samples are collected and evaluated. Pathogenic microorganisms, including hepatitis viruses and Human immunodeficiency virus, may be present in clinical samples. Standard precautions and institutional guidelines should always be followed in handling, storing, and disposing of all samples and all items contaminated with blood or other body fluids.
11. For additional information on hazard symbols, safety, handling and disposal of the components within this kit.
12. Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
13. Do not store the test kit in direct sunlight.

【Approval Date and Revision Date of the Instruction for use】

Approval Date: 2021.05.21
Revision Date: 2021.05.21
Date of Issue: 2021.05.21
Version: V2.0

【Index of Symbols】

	For In Vitro Diagnostic Use		Please don’t reuse it
	Expiry date		Please read the instruction book carefully before using
	Warning, please refer to the instruction in the annex		Manufacturer
	Temperature scope within which the product is reserved		Batch number
	European union authorization representative		Keep dry
	Avoid overexposure to the sun		Don’t use the product when the package is damaged
	Date of manufacture		Catalog Number
	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC		

【Basic Information】

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