2019-nCoV IgG / IgM Detection Kit (Colloidal Gold-Based)
Instruction for Use (Version 3.0)

PRODUCT NAME
2019-nCoV IgG / IgM Detection Kit (Colloidal Gold-Based).

CATALOG NUMBER & SIZE
C6603C : 50 tests / kit.

INTENDED USE
This product is intended for the detection of 2019-Novel Coronavirus (2019-nCoV). It is suitable for qualitative detection of IgG / IgM antibodies in human serum, plasma, and whole blood.

2019-Novel Coronavirus belongs to the new coronavirus of the genus β, which has an envelope, the particles are round or oval, often polymorphic, and the diameter is 60-140nm. Its genetic characteristics are significantly different from SARSr-CoV and MERSr-CoV. Current research shows that it has more than 85% homology with bat SARS-like coronavirus (bat-SL-CoVZC45). After infection with 2019-nCoV, the common symptoms are fever, fatigue, dry cough, dyspnea etc. Some severe patients appear the symptoms including acute respiratory distress syndrome, septic shock, metabolic acidosis that is difficult to correct, and coagulation disorders. Some patients have mild symptoms and no fever. Most of patients have a good prognosis, while a few are in critical condition or even die.

Both IgG and IgM are immunoglobulin which are produced by the immune system to provide protection against the 2019-nCoV. The level of IgM antibody begins to rise within 1 week and achieves the peak at 2-3 weeks after the initial infection. While the IgG appears later than IgM (usually in 14 days after infection) and achieves the peak at 5 weeks, lasting for 6 months or even several years.

PRINCIPLE OF DETECTION
This product is based on capture and solid-phase immunochromatography methods for determination. The specimen (whole blood / serum / plasma) flows from the blood separator through to the conjugate release pad (which occurs the conjugation reaction between IgG / IgM antibody in the specimen and the antigen colloidal gold of 2019-nCoV to form an immune complex of IgG / IgM antibody and colloidal gold-labeled antigen) due to capillary action. Then migrate to a capture zone of nitrocellulose membrane-immobilized antibody (mouse-anti-human IgM antibody) to form an immune complex of colloidal gold-labeled antigen, IgM antibody and mouse-anti-human IgM antibody, thereby generating a IgM red line. The unreacted immune complex continues to flow upward, will be captured by the mouse-anti-human IgG antibodies to form an immune complex of colloidal gold-labeled antigen, IgG antibody and mouse-anti-human IgG antibody, thereby generating a IgG red line. The remaining uncaptured immune complex moves upward, combining with C line (quality control line) to indicate the completion of this reaction.

Components

Components	Ingredients
Test Cassette	Aluminum foil pouch, desiccant, test strip and plastic card. Test strip composing blotting paper, nitrocellulose membrane, specimen separator, colloidal gold-labeled pad and PVC. IgM line (Test line) coating 1.0 mg/mL mouse-anti-human IgM antibody. IgG line (Test line) coating 1.0 mg/mL mouse-anti-human IgG antibody. C line (quality control line) coating 1.0 mg/mL actin protein C. Conjugate release pad containing 40 OD 2019-nCoV antigen-colloidal gold conjugate complex.
Specimen Dilution	HEPES Buffer containing casein (0.1 M), 5 mL/bottle.
Dropper	According to different packing specifications: 10 droppers /pack, 50 droppers/pack.

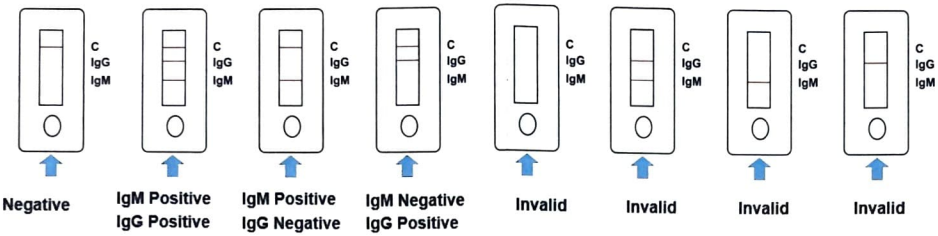
Note: Do not interchange the components from different batches.

STORAGE & SHELF LIFE
This kit should be stored at 4°C~30°C for 18 months in a sealed condition. Once the inner packaging of strip is opened (4°C~30°C, humidity < 65%), it must be used in 1 hour. The opening specimen dilution buffer should be stored at 4°C, it is valid within 1 month. It is recommended to mark the opening date of the specimen dilution buffer.

SAMPLING & HANDLING
1. Suitable specimen type: serum, plasma, and whole blood.
2. Sediment and suspended matter in the specimen may affect the test result. It should be removed by centrifugation at 3000 g for 10 minutes.
3. Severe hematolytic, lipemic and turbid specimens should not be used.
4. Whole blood/plasma specimens can be treated with heparin sodium or EDTA anticoagulant. After specimen collection, the test should be completed within the same day. If not, please store it as the following protocol:
For whole blood specimens, store at 2℃ ~8℃ for 3 days.
For Serum/plasma specimens, store at 2℃ ~8℃ for 7 days, or at < -20℃ for 12 months.
5. Specimens must be fully restored to room temperature (18℃ -28℃) before testing. Freeze-preserved specimens should be completely melted, reheated and mixed thoroughly before use.

PROTOCOL
Read the instructions carefully before operating.
1. Fully incubate the test strips at room temperature before use. The test must be operated at room temperature.
2. Remove the test strips from the aluminum foil pouch and place it on a horizontal and dry table.
3. Dilute the serum, plasma and whole blood specimens, using provided dropper sampling to add 1 drop (about 20 μL) to the sample loading position, then open the black cap of the drop bottle, add 3 drops of dilution buffer(about 60 μL)to the sample loading position. Start timing.
4. Observe the test card after 10 minutes and analyze the results.

INTERPRETING TEST RESULTS



The test results are analyzed as follows:

1. Negative result: Only one red quality control line (C line) appears in the detection area.
2. IgM positive, IgG positive result: Three clear red lines appear in the detection area, one is quality control line (C line), one is IgM detection line, and the other is IgG detection line.
3. IgM positive, IgG negative result: Two clear red line appear in the detection area, one is quality control line (C line) and another is IgM detection line.
4. IgM negative, IgG positive result: Two clear red line appear in the detection area, one is quality control line (C line) and another is IgG detection line.
5. Invalid result: No red quality control line (C line) appears in the detection area (e.g. without any red lines or only test lines (IgM, IgG line), indicating that the test error or the test result is invalid, and the test should be retested.

LIMITATIONS OF TEST METHODS

1. The test results of this product are only for clinical reference and should not be used as the only basis for clinical diagnosis and treatment.
The clinical management of patients should be considered in combination with their symptoms / signs, medical history, treatment reactions and epidemiology and other laboratory tests. It is recommended to repeat the test for suspicious samples at intervals.
2. The accuracy of detection is affected by the sample collection process. Improper sample collection and storage process will affect the test results and should avoid high temperature and direct sunlight.
3. This production provides a qualitative test for the novel coronavirus IgM antibody and IgG antibody in the sample, but not quantified detection.
4. Due to the limitation of the testing methodologies, it cannot rule out the possibility of the novel coronavirus infection based on negative results. It is recommended to combine other test results and clinical symptom to make an accurate diagnosis.

PRODUCT PERFORMANCE INDICATOR

1.Lowest limit of detection
Test with the in-house LOD references. S1 and S2 are positive for novel coronavirus IgG antibody, negative for IgM antibody; S3 is negative for novel coronavirus IgG/IgM antibodies; S4 and S5 are positive for novel coronavirus IgM antibody, negative for IgG antibody; and S6 is negative for novel coronavirus IgG/IgM antibodies.
2.Negative coincidence rate
Test with the in-house negative references, and the results are all negative for novel coronavirus IgG/IgM antibodies, with a coincidence rate of 100%.
3.Positive coincidence rate
Test with the in-house positive references. PC01-PC05 are all positive for novel coronavirus IgG/IgM antibodies, with a coincidence of 100%; PC06-PC10 are all negative for novel coronavirus IgG antibody, and all positive for IgM antibody, with a coincidence rate of 100%; PC11-PC15 are all negative for novel coronavirus IgM antibody, and all positive for IgG antibody, with a coincidence rate of 100%.
4.Precision
Intra-batch difference: Test with the in-house repetitive references. CV1 and CV2 are positive for novel coronavirus IgG antibody and negative for IgM antibody; CV3 and CV4 are negative for novel coronavirus IgG antibody and positive for IgM antibody, with uniform color development.
Inter-batch difference: Test with the in-house repetitive references. The results of the kit of three batch numbers are shown as follows: CV1 and CV2 are positive for novel coronavirus IgG antibody and negative for IgM antibody; CV3 and CV4 are negative for novel coronavirus IgG antibody and positive for IgM antibody, with uniform color development.
5.Analytical specificity:
5.1 Cross reaction
This product will not cross react with positive samples of human coronavirus HKU1, OC43, 229E, influenza A H1N1 virus, seasonal H1N1 influenza virus, H3N2, H5N1, H7N9, influenza B Yamagata, Victoria, respiratory syncytial virus, parainfluenza virus, rhinovirus species A, B and C, adenovirus types 1, 2, 3, 4, 5, 7 and 55, coxsackievirus (enterovirus species B), enterovirus 71 (enterovirus species A), enterovirus 68 (EV-D68) (enterovirus species D), EB virus, measles virus, human cytomegalovirus, rotavirus, norovirus, mumps virus, varicella-zoster virus, mycoplasma pneumoniae, chlamydia pneumoniae IgG/IgM antibodies.
5.2 Interferents
When bilirubin $\leqslant$0.2 g/L, triglyceride $\leqslant$10 g/L, hemoglobin $\leqslant$5 g/L, rheumatoid factor $\leqslant$500 IU/mL, antinuclear antibody titer $\leqslant$1:240, anti-mitochondrial antibody titer $\leqslant$1:160, HAMA $\leqslant$20 ng/mL, total IgG $\leqslant$50 mg/L and total IgM $\leqslant$5 mg/L, they will not interfere with the test results.
Oseltamivir, levofloxacin, ceftriaxone, zanamivir, interferon alpha (IFN-α), ribavirin, peramivir, lopinavir, ritonavir, arbidol, azithromycin, meropenem, tobramycin, histamine hydrochloride, phenylephrine, oxymetazoline, sodium chloride, beclomethasone, dexamethasone, flunisolide, triamcinolone acetonide, budesonide, mometasone and fluticasone have no effect on the test results.
6.Hook effect
Hook effect will occur at the concentration levels that exceed the lowest limit of detection of IgG antibody of this product by more than 1280 times and the lowest limit of detection of IgM antibody by more than 640 times. If novel coronavirus pneumonia is highly suspected but the antibody test result is negative, the sample should be re-tested after dilution.
7.After the specific IgM positive sample is destroyed, the IgM antibody test result is negative, and the IgG antibody test is not affected.
8.Heparin sodium and EDTA anticoagulants have no effect on the detection of this kit.
9.The precision test is conducted by different test personnel at different time with this kit, and the results comply with the requirements of product performance.
10.For virus infection samples from different regions, the lowest limit of detection and detection repeatability of the reagent comply with the requirements.
11.Clinical study
The clinical trial of this product was carried out in 5 sites based on the criteria for disease confirmation/exclusion specified in the Diagnosis and

Treatment Protocol for Novel Coronavirus Pneumonia. The enrolled cases were suspected cases of novel coronavirus infection, including 201 confirmed cases and 369 excluded cases, with 51 early cases in confirmed cases. Clinical sensitivity of this product: 91.54% (95% CI: 86.87%, 94.65%) and specificity: 97.02% (95% CI: 94.74%, 98.33%). The sample types for clinical evaluation were serum, plasma and whole blood After preliminary evaluation, it is basically confirmed that the clinical performance of the product can meet the emergency needs of the epidemic. The clinical data for the product after marketing will be further collected to confirm the clinical performance of the product.

NOTE

1. This kit is only for in vitro diagnosis.
2. It should be operated by professionally trained inspectors, read the product manual carefully before operation, and conduct the test operation strictly in accordance with the kit instructions.
3. Protective measures against infectious diseases should be took. Thorough sterilization must be done after operation of handling reagents and specimens.
4. Keep it clean and treat the pollutants as wastes. The waste treatment should be performed in accordance with WS / T249-2005 "Clinical Laboratory Waste Disposal Principles" for the safe disposal of waste and the safe disposal of infectious waste. Please handle with care.

REFERENCES

1. Hui DS, et al. (2020). The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health-The latest 2019 novel coronavirus outbreak in Wuhan, China. International Journal of Infectious Diseases, 91, 264–266.
2. Templeton KE, et al. (2004). Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza A and influenza B viruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3 and 4. Journal of clinical microbiology 42(4): 1564-1569.
3. Smith AB, et al. (2003). Rapid detection of influenza A and B viruses in clinical specimens by Light Cycler real time RT-PCR. Journal of Clinical Virology 28(1): 51-58.

CONTACT

Manufacturer: Nanjing Vazyme Medical Technology Co., LTD.
Address: Building C1-2, Red Maple Park of Technological Industry, Nanjing, China. Tel: +86 25 8436 5701
Customer Service Provider: Nanjing Vazyme Medical Technology Co., LTD. Tel: +86 25 8436 5701
Address: Building C1-2, Red Maple Park of Technological Industry, Nanjing, China.

DATE OF APPROVAL AND MODIFICATION OF INSTRUCTION

February 27th, 2020

DATE OF MANUFACTURE AND EXPIRATION

See packaging.

Symbols

EC REP	Authorized Representative
IVD	For in vitro diagnostic Use only
30°C 4°C	Store between 4-30°C

Σ	Tests per Kit
REF	Catalog #
LOT	Lot Number

	Manufacturer
	Expiry Date
	Consult instructions for use

Nanjing Vazyme Medical Technology Co., LTD.
Building C1-2, Red Maple Park of Technological Industry, Nanjing, China
www.vazymemedical.com

EC REP
Polgen Sp. z o.o. Sp.K.
92-516 Lodz, Puszkina Str. 80, Poland