Performance Evaluation studies

Coronavirus-19 (COVID-19) Antibody (IgM/IgG) Rapid Test Kit (Colloidal gold immunochromatography)

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Content

Introduction

COVID-19 IgG/IgM Rapid Test kit(Colloidal gold immunochromatography method), developed by UN science, is used for the the qualitative detection of IgG and IgM antibodies to COVID-19 in human whole blood, serum or plasma as an aid in the diagnosis of primary and secondary COVID-19 infections.

According to the regulation of In vitro diagnostic reagent registration application and instructions, we designed the experiments to do the product performance evaluation. We evaluate every parameter analytical performance by aquiring related target experiment data and doing the statistical analysis.

1. General information

1.1 Specification

According to the number of coronavirus (COVID-19) antibody (IgM / IgG) detection kits in each box, our company has one packaging specifications: 40 tests / kit. The difference in packaging specifications is the number of novel coronavirus (COVID-19) antibody (IgM / IgG) detection kits assembled in the box. See Table 1:

Table 1：Volume

Packages	Test Cassette	Sample diluent
40T/kit	40tests	7mL/bottle

Each kit consists of a test cassette and a desiccant. The test cassette consists of a test strip and a plastic card shell. The test strip consists of a sample pad and a chromatographic membrane (the detection area is coated with a mouse anti-human IgM monoclonal antibody and a mouse anti-human IgG monoclonal antibody and goat anti-mouse IgG antibody), colloidal gold binding pad (coated with colloidal gold-labeled recombinant novel coronavirus (COVID-19) antigen and mouse lgG antibody), liner and absorbent pad. Each kit is packed for 1 test, so the difference in packaging specifications has no effect on the performance of the product. Therefore, the following experiment uses only one packaging specification, and the product information is as follows:

Table 2：Product

Lot. NO.	20200226	20200228	20200302
Date of production	Feb. 26, 2020	Feb. 28, 2020	Mar. 2, 2020
Specification	40T/kit	40T/kit	40T/kit

1.2 Operation Procedure

Please make sure to read the instruction manual and the applicable instrument instruction completely before using the kit. Please make sure the kit is recovered into

room temperature and perform the kit under the room temperature(15℃~30℃).

1. Open the aluminum foil bag of the test cassette and take out of the test card.

2. Take 20μL sample of the serum or plasma, or 20μL sample of the whole blood, add them to the sample well, and add 1drop(for serum or plasma)/3 drops(for whole blood) of the sample dilution solution, and keep it at room temperature for 10 minutes to observe the results.

2. Physical Character Evaluation

2.1 Experimental Requirement

A. Appearance: smooth and clean, no damage, no pollution, all components are complete, the position is correct, the paste is firm without falling off.

B. Composition: the number of individual test bags inside the kit is correct; Aluminum foil bag is printed with production batch number, clear and correct valid period; Contents inside the bag are correct; The manual is complete.

C. The membrane strip width shall be within 3.0mm±0.10mm.

D. Liquid migration speed: the liquid migration speed shall not be less than 10 mm/min.

2.2 Experimental methods

A. Appearance

Visual examination with corrected vision under natural light.

B. Composition

Random check 2 kits and check the corresponding parameters.

C. Membrane strip width

Three reagent strips were randomly selected from the kit and the width of the strip was measured with a vernier caliper. Record the measurement data.

D. Liquid migration speed

Three tests in the same batch of kit were randomly selected, the shell was opened respectively, the strip of test paper was taken out, the time was started after the sample diluent was added to the sample end of the test paper, and the time was recorded as T when the liquid reached the boundary between zone C and zone D as shown in the figure. The length of the liquid moving distance (zone A + zone B + zone C) was measured with a calibrated general or special measuring tool, denoted as L, and the moving speed was calculated according to L/T.

2.3 Experimental results

Table 3 Performance evaluation -- Physical character detection results

Test item	Test result		
	Lot No：20200226	Lot No：20200228	Lot No：20200302
Appearance	Smooth and clean, no damage, no pollution, all components are complete, the position is correct, the paste is firm without falling off	Smooth and clean, no damage, no pollution, all components are complete, the position is correct, the paste is firm without falling off	Smooth and clean, no damage, no pollution, all components are complete, the position is correct, the paste is firm without falling off
Membrane strip width（mm）	3.02mm	3.01mm	3.04mm
Liquid migration speed	15.2mm/min	15.3mm/min	15.9mm/min

2.4 Interpretation of Result

According to the test data in the above table, the physical characters of the novel coronavirus (COVID-19) antibody (IgM/IgG) detection kit (colloidal gold immunochromatography) are intact, and the test results of reagent width meet the

requirements of film width within the range of 3.0mm±0.10mm, and the liquid migration speed is no less than 10mm/min, which meets the technical requirements.

3. SAMPLE DILUENT EVALUATION

3.1 Experimental Requirement

a. Appearance: The appearance of the liquid is a colorless, clear and transparent liquid without insoluble substance such as impurities and precipitates.

b. Filling volume: The actual filling volume of the sample diluent for the kit is 7mL ± 0.1mL / vial.

c. PH value: The pH value of the sample diluent of the kit is 7.4 ± 0.2.

3.2 Experimental Method

a. Appearance: Randomly take a bottle of sample diluent, and visually check the appearance of the liquid under natural light with corrected vision.

b. Loading volume: The loading volume of sample diluent A should be measured with a calibrated measuring instrument. Repeat the measurement 3 times with the loading volume 7mL ± 0.1mL / vial.

c. PH value: 2 bottles of sample diluent A were randomly sampled and mixed, and measure the pH value according to the requirements of the Chinese Pharmacopoeia 2015 Edition Appendix VI pH measurement method. To record the test results.

3.3 Experimental Result

Table 4 : Performance evaluation—Test results of sample diluent

Test item	Test results		
	Lot number: 20200226	Lot number: 20200228	Lot number: 20200302

Appearance	Liquid appearance is colorless, clear and transparent liquid, no insoluble matter such as impurity precipitation.	Liquid appearance is colorless, clear and transparent liquid, no insoluble matter such as impurity precipitation.	Liquid appearance is colorless, clear and transparent liquid, no insoluble matter such as impurity precipitation.
Loading volume	7mL	7mL	7mL
pH value	7.26	7.25	7.26

3.4 Interpretation of Result

According to the data in the table above, the verification results of the novel coronavirus (COVID-19) antibody (IgM/IgG) detection kit (colloidal gold immunochromatography) meet the appearance of a colorless clear transparent liquid without precipitation. The loading volume is 7mL ± 0.1mL / vial, and the design value between pH 7.4 ± 0.2 meets the actual requirements.

4. COMPLIANCE RATE EVALUATION OF NEGATIVE/ POSITIVE RFERENCE

4.1 Experimental Method

Positive reference compliance rate：Repeat the test 5 times using the enterprise positive reference, and the results should all be positive.

Negative reference compliance rate：Repeat the test 10 times using the enterprise negative reference, and the results should all be negative.

4.2 Experimental Result

The results and statistics of the positive / negative reference of three Lot numbers of kits are as follows:

Table 5: COVID-19 Positive reference product compliance test results

Lot Number	Results of 5 measurements (IgM / IgG)				
20200226	+/-	+/+	+/-	-/+	-/+
20200228	Results of 5 measurements (IgM / IgG)				
	+/-	+/+	+/-	-/+	-/+
20200302	Results of 5 measurements (IgM / IgG)				
	+/-	+/+	+/-	-/+	-/+

Table 6: COVID-19 Negative reference product compliance rate test results

Lot Number	Results of 10 measurements (IgM / IgG)				
20200226	-/-	-/-	-/-	-/-	-/-
	-/-	-/-	-/-	-/-	-/-
20200228	Results of 10 measurements (IgM / IgG)				
	-/-	-/-	-/-	-/-	-/-
	-/-	-/-	-/-	-/-	-/-
20200302	Results of 10 measurements (IgM / IgG)				
	-/-	-/-	-/-	-/-	-/-
	-/-	-/-	-/-	-/-	-/-

4.3 Interpretation of Result

Three batches of kits meet the requirements for positive / negative reference.

5. LOD EVALUATION

5.1 Experimental Method

The determination reference: S1 is negative, S2(S2A, S2B)、S3 are positive.

5.2 Experimental Result

The results statistics of the three batches' minimum detection limit are as follows:

Table 7: COVID-19 minimum detection limit test results（IgM/IgG）

Lot No.	S1 Three times' detection results			S2A Three times' detection results			S2B Three times' detection results			S3 Three times' detection results		
20200226	-/-	-/-	-/-	-/+	-/+	-/+	+/-	+/-	+/-	+/+	+/+	+/+
20200228	-/-	-/-	-/-	-/+	-/+	-/+	+/-	+/-	+/-	+/+	+/+	+/+
20200302	-/-	-/-	-/-	-/+	-/+	-/+	+/-	+/-	+/-	+/+	+/+	+/+

5.3 Interpretation of Result

According to the above three batches of experimental results, the test values of reference material S1 of this kit are all negative, and the test values of S2(S2A, S2B) and S3 are positive.

6. Reproducibility

6.1 Experimental Method

The enterprise reference samples S2A, S2B and S3 were repeatedly determined for 10 times, and the results should be positive.

6.2 Experimental Result

The repeatability test results of the three batches of kits are as follows:

Table.8 Results of Covid-19 repeatability test (IgM/IgG)

Reagent batches	S2A, the results of ten repeats		S2B, the results of ten repeats		S3, the results of ten repeats	
20200226	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
20200228	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+

	-/+	-/+	+/-	+/-	+/+	+/+
20200302	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+
	-/+	-/+	+/-	+/-	+/+	+/+

6.3 Interpretation of Result

In the repeatability tests of three batches of novel coronavirus (COVID-19) antibody (IgM/IgG) detection kit (colloidal gold immunochromatography), the ten repeatability test results of enterprise reference samples S2A, S2B and S3 were positive.

7. Evaluation of Specifity Analysis

7.1 Experimental Method

Cross-reactivity: Determination of parainfluenza virus antibodies, influenza A virus antibodies, influenza B virus antibodies, Chlamydia pneumoniae antibodies, Mycoplasma pneumoniae antibodies, adenovirus antibodies, respiratory syncytial virus antibodies, hepatitis B surface antibodies, hepatitis C virus Antibodies, Treponema pallidum antibodies, human immunodeficiency virus antibodies, EB virus antibodies, measles virus antibodies, cytomegalovirus antibodies, enterovirus 71 antibodies, mumps antibodies, chicken pox-zoster virus antibodies, HKU1 virus antibodies, OC43 virus antibodies, NL63 virus antibodies, 229E virus antibodies, the test results should be negative.

Interfering substances: test the samples separately under the condition that the bilirubin concentration in the samples is ≤250 μmol / L, the hemoglobin content is ≤9g / L, the triglyceride content is ≤15 mmol / L, the rheumatoid factor content is ≤ 80IU / mL, and antinuclear antibody (ANA) titer is ≤ 1: 240, the anti-mitochondrial antibody (AMA) is ≤80 U / mL, and the mouse IgG content is

≤1000 μg / mL, the test result should be negative. Test samples separately, and add histamine hydrochloride, alpha-interferon, zanamivir, ribavirin, oseltamivir, paramivir, lopinavir, ritonavir, abidol, levofloxacin , Azithromycin, Ceftriaxone, Meropenem, Tobramycin. The test results should be negative.

7.2 Experimental Result

Form 9 COVID-19 Assay result for specific analysis（IgM/IgG）

Sample	Lot number：20200226 Result for specific analysis			Lot number：20200228 Result for specific analysis			Lot number：20200302 Result for specific analysis		
Parainfluenza virus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Influenza A virus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Influenza B virus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Chlamydia pneumoniae antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Mycoplasma pneumoniae antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Adenovirus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Respiratory syncytial virus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Hepatitis B surface antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Hepatitis C virus Antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Treponema pallidum antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Human immunodeficiency virus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
EB virus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Measles virus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Cytomegalovirus antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

Enterovirus 71 antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Mumps antibodies	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
HKU1 virus antibody	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
OC43 virus antibody	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
NL63 virus antibody	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
229E virus antibody	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Chicken pox-zoster virus antibody	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Bilirubin 250μmol/L	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Hemoglobin 9g/L	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Triglyceride 15mmol/L	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Rheumatoid factor 80IU/mL	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Antinuclear antibody（ANA）1:240	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Anti-mitochondrial antibody（AMA）80U/mL	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Mouse IgG1000μg/mL	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Histamine hydrochloride	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Alpha-interferon	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Zanamivir	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ribavirin	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Oseltamivir	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Paramivir	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Lopinavir	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ritonavir	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Abidol	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Levofloxacin	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Azithromycin	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Ceftriaxone	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

Meropenem	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Tobramycin	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

7.3 Interpretation of Result

According to the above experimental results, there is no cross-reaction and interference to the above substances.

8. Evaluation of the influence of Disrupted COVID-19 IgM antibody

8.1 Experimental Method

Add 10μl/mL mercaptoethanol in serum sample taken from patient infected COVID-19, compared whether it interfere the detection result of COVID-19 IgM antibody.

8.2 Experimental Result

Table 10: Detection result of (IgM/IgG) with interference of disrupted COVID-19 IgM antibody.

（IgM/IgG）

NO	Result	Detection result (Lot#20200226) with interference	Detection result (Lot#20200228) with interference	Detection result (Lot#20200302) with interference
Sample 1	+/-	-/-	-/-	-/-
Sample2	+/+	-/+	-/+	-/+
Sample 3	+/+	-/+	-/+	-/+
Sample 4	+/-	-/-	-/-	-/-
Sample 5	+/-	-/-	-/-	-/-
Sample 6	+/-	-/-	-/-	-/-
Sample 7	+/+	-/+	-/+	-/+
Sample 8	+/+	-/+	-/+	-/+
Sample 9	+/-	-/-	-/-	-/-
Sample 10	+/+	-/+	-/+	-/+

8.3 Interpretation of Result

Based on above experimental results, it can be confirmed that the detection of the kit is not interfered by the disrupted COVID-19 IgM antibody.

9. Conclusion of Product performance Evaluation

Through the analysis of performance evaluation experiment of novel coronavirus(COVID-19)antibody (IgM/IgG) detection kit (colloidal gold immunochromatography) from three batches produced by Wuhan UNscience, the product analysis performance is summarized as follows:

1. Positive reference compliance rate：The enterprise compliance rate of positive reference products is 5/5.

2. Negative reference compliance rate: The enterprise compliance rate of negative reference products is 10/10.

3. Minimum detection limit: The enterprise minimum detection limit of reference S1 is negative, S2A, S2B and S3 are positive.

4. Repeatability: Three samples of enterprise repeatable references were tested, and each test was repeated for 10 times, all were positive.

5. Analytical specificity

5.1 Cross reaction: this product has no cross-reaction with Parainluenza virus antibody, Influenza A virus antibody, Influenza b virus antibody, Chlamydiapneumoniae antibody , M.Pneumonia antibody, Adenovirus antibody , Respiratory syncytial virus antibody, Anti-HBs, HCV-Ab, TP-Ab, HIV antibody, EB virus antibody, Measles virus antibody, CMV antibody, Human enterovirus 71 antibody, Mumps virus antibody, HKU1 virus antibodies, OC43 virus antibodies, NL63 virus antibodies, 229E virus antibodies and varicella-zoster virus antibodies positive samples .

5.2 Interfering substances: when bilirubin concentration ≤250 μmol/L, hemoglobin content ≤9g/L, triglyceride content ≤15mmol/L, rheumatoid factor content ≤

80IU/mL, ANA titer ≤1:240, anti-mitochondrial antibody (AMA) ≤80U/mL, and mouse IgG content ≤1000μg/mL, there will be no interference with the detection results of this product.2) histamine hydrochloride, beta-interferon, zanamivir, ribavirin, oseltamivir, peramivir, lopinavir, ritonavir, abidor, levofloxacin, azithromycin, ceftriaxone, meropenem and tobramycin have no effect on the test results of this product.

6. The test results of this product are not affected by the destroyed novel coronavirus (COVID-19) specific antibody（IgM）.