Diagnostic Kit for Antibody IgM/IgG to Novel Coronavirus SARS-CoV-2

(Colloidal Gold Immunochromatography Assay)

Clinical Verification Report

30th April 2020

Product name:

Diagnostic Kit for Antibody IgM/IgG of SARS-CoV-2 by Colloidal Gold Immunochromatography Assay

Objective:

Evaluation of clinical performance of test product

Test category:

Clinical verification

Company name:

REDACTED

Contents

1. Introduction

1.1 Background

1.1.1 Disease research background

The novel coronavirus SARS-CoV-2 which causes COVID-19 is a member of the Coronavirus category of viruses, which causes a mostly mild disease in humans but can lead to a severe pneumonia, multiple organ failure and death. COVID-19 has currently reached pandemic proportions globally however in many countries a measure of control has been achieved. As an acute respiratory infectious disease, the disease has been classified as a Class B infectious disease as stipulated in "*the Law of the People's Republic of China on the Prevention and Control of Infectious Diseases"*, but has been managed as a Class A infectious disease in view of it's infectivity and potential severity.

SARS-CoV-2 has significantly different genetic characteristics from SARSr-CoV and MERSr-CoV and belongs to the new coronavirus of the genus β in which the viruses are enveloped, round or oval in shape (often polymorphic), and 60-140 nm in diameter. Current evidence shows that SARS-CoV-2 has more than 85% homology with a bat SARS-like coronavirus (bat-SL-CoVZC45). Using in vitro isolation and culture techniques, SARS-CoV-2 can be found in human respiratory epithelial cells in about 96 hours and it takes about 6 days to isolate and culture in Vero E6 and Huh-7 cell lines. The SARS-CoV-2 virus is sensitive to UV light and can be inactivated effectively by heating at 56 ℃ for 30 minutes.

Ether, 75% alcohol, chlorine-containing disinfectant, peracetic acid and chloroform can effectively inactivate the virus, however chlorhexidine is ineffective. Coronavirus Spike protein (S protein), the largest and most complex structural protein of the virus, is a type I transmembrane glycoprotein that is responsible for virus-target cell binding, membrane fusion, and entry into target cells. Nucleocapsid protein (N protein), expressed in large quantities during viral infection, is another important structural protein of the virus that affects viral RNA replication and transcription. Therefore, specific antibodies against N and S proteins in serum can serve as important detection targets

1.1.2 Disease diagnosis background

According to the diagnostic criteria of the "*Diagnosis and Treatment of Pneumonia of New Coronavirus Infection (Trial Version 6)*" issued on February 3, 2020 by the General Office of the National Health and Health Commission and the Office of the State Administration of Traditional Chinese Medicine, the clinical standards currently adopted for this indication and the laboratory diagnosis method are a comprehensive analysis that combines epidemiological history, clinical manifestations and laboratory testing. Suspected cases will have one of the following criteria of evidence of COVID-19 infection: 1) tested positive in Real-time fluorescent RT-PCR of respiratory specimens or blood specimens for detection of new coronavirus nucleic acid; and 2) sequencing results are highly similar to those of known new coronavirus source (a confirmed case) in the viral gene sequencing of respiratory specimens or blood specimens.

1.2 Detection principle

The [redacted] Diagnostic Kit uses colloidal gold immunochromatography ('lateral flow' technology) to detect SARS-CoV-2 antigen-specific IgM and IgG antibodies in serum or plasma. This kit is made of anti-human IgM, IgG antibody solid-phase nitrocellulose membrane, colloidal gold-labeled SARS-CoV-2 S/N protein and other reagents. During testing, the gold-labeled antigen forms a complex with specific antibodies in the testing sample. Specifically, if SARS-CoV-2 antigen-specific IgM or IgG antibodies are present in the sample, the antibody will form a complex with the gold-labeled antigen, which, due to chromatography, moves forward along the test strip and is captured by the solid-phase anti-human IgM or anti-human IgG antibody in the detection line, forming a complex that condenses and develops colour. If it is a negative specimen, even if a complex can be formed, it does not develop color because it cannot capture the specific antigen component in the detection area. Whether there is a SARS-CoV-2 antibody in the sample or not, a colored band will appear in the quality control area to serve as the internal control standard of whether the chromatography process is normal and whether the reagent is invalid or not.

2. Test purposes

The purpose of this study is to evaluate the clinical performance of the Diagnostic Kit for Antibody IgM/IgG to Novel Coronavirus SARS-CoV-2 by Colloidal Gold Immunochromatography Assay against clinical reference standards by testing of clinical samples using the Diagnostic Kit.

3. Experiment method

3.1 Preparation of reagents

Leave all reagents and samples to equilibrate to room temperature for 30 minutes.

3.2 Detection steps

Pipette a 10μL sample of plasma into the sample application area with a micropipette, add 100uL of the diluent, and then start timing. Wait for the fuchsia-coloured band to appear, and the results should be interpreted within 10-20 minutes. Interpretation of a result more than 20 minutes after adding diluent is ruled invalid.

4. Statistical analysis of data

4.1 Analysis Method

The positive reference samples were banked samples from patients confirmed to have COVID-19 infection by clinical and radiological evidence and RT-PCR diagnosis methods. The negative reference samples were taken from subjects with no confirmed evidence of SARS-CoV nucleic acid presence as determined by RT-PCR. The Diagnostic Kit for Antibody IgM/IgG of Novel Coronavirus SARS-CoV-2 by Colloidal Gold Immunochromatography Assay was the product assessed and the clinical samples were taken as the reference standards and tested on the Diagnostic Kit. The analysis method for determining the performance characteristics of the Diagnostic Kit as against the clinical samples is summarized in Table 4-1 below:

Table 4-1: Comparison of the test kit results with clinical reference standards

Test Kit results	Clinical Reference Standard Results		Total
	Positive Samples	Negative Samples	
Positive Samples	A	B	A+B
Negative Samples	C	D	C+D
Total	A+C	B+D	A+B+C+D

4.2 Evaluation method

The results of the Diagnostic Kit for Antibody IgM/IgG of Novel Coronavirus SARS-CoV-2 by Colloidal Gold Immunochromatography Assay were therefore compared with the clinical sample results to determine sensitivity and specificity performance using the following methodology:

Sensitivity: A/（A+C）×100%;

Specificity: D/（B+D）×100%;

Total coincidence rate: = (A+D) ／ (A+B+C+D) ×100%.

5. Number of clinical trial samples and enrollment

A total of 1,000 samples were tested, comprising 450 COVID-19 positive patient samples (150 patients at 1-7 days after onset of symptoms, 150 at 8-14 days after onset of symptoms and 150 patients >14 days after onset of symptoms) and 550 COVID-19 negative samples. The tests were carried out in three hospitals in China specializing in Infectious Disease. The study was conducted during March/April 2020 according to a Protocol as specified by the Chinese Health Authority, the NMPA. The laboratory tests were conducted according to the [redacted] Technical Laboratory Manual and to the Protocol specified by NMPA.

6. Clinical research results and statistical analysis

The experimental results of the clinical performance verification study are shown below in Table 6-1:

Table 6-1: Comparison of test results with clinical reference standards

Test Kit results	Clinical Reference Standard Results		Total
	Positive Samples	Negative Samples	
Positive Samples	405	1	406
Negative Samples	45	549	594
Total	450	550	1,000

Sensitivity: A / (A+C) ×100%; 405/ (405+45) ×100％ = 90.22%

Specificity: D / (B+D) ×100％; 549/ (549+1） ×100％ = 99.82%

Total coincidence rate: = (A+D) / (A+B+C+D) ×100%

(405+549)/ (405+45+1+549) = 95.40%

6.1 Sensitivity results by time from onset of symptoms

An analysis was conducted on the sensitivity performance of the Diagnostic Kit based on 450 positive reference samples collected from patients at different time intervals from the onset of symptoms. The three time periods after onset of symptoms were 1~7 days, 8-14 days and greater than 14 days. The results are as follows:

Time from symptoms onset	**Positivity Rate**	**Sensitivity**
1 – 7 days	122/150	81.33%
8 – 14 days	136/150	90.67%
>14 days	148/150	98.66%

7. Conclusions

The [redacted] Diagnostic Kit for Antibody IgM/IgG of Novel Coronavirus SARS-CoV-2 by Colloidal Gold Immunochromatography Assay was evaluated in a total of 1,000 samples comprised of 450 positive samples and 550 negative samples.

The sensitivity of the kit was 90.22% when performance measures were averaged across the 450 positive reference samples stratified by sample collection time number of days after onset of symptoms. The specificity of the kit was 99.82% in the 550 negative samples.

A further analysis was made on kit performance depending on the sample collection time number of days after onset of symptoms in the 450 positive samples, because it is known that antibody titers of IgM and IgG vary over time. The sensitivity of the kit increased from 81.33% at 1 – 7 days, to 90.67% sensitivity at 8 – 14 days, and to 98.66% sensitivity at >14 days after onset of symptoms in patients.

The results indicate that the [redacted] Diagnostic test performed well overall and that from > 14 days after patients' onset of symptoms, the kit performs with 98.66% sensitivity and 99.82% specificity.

With its test results being highly consistent with the confirmed clinical reference standards, the [redacted] Diagnostic Kit has a strong clinical utility in the diagnosis of COVID-19 infection.

References

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