Novel Coronavirus 2019-nCoV Antigen Test

(Colloidal Gold)

Clinical Study Report

Subject Product: Novel Coronavirus 2019-nCoV Antigen Test

(Colloidal Gold)

Test start time: October 9, 2020

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Model specifications: 40T/kit

Submitted by: Beijing Hotgen Biotech Co., Ltd.

Contact: 5.1.2e

Tel: 5.1.2e

Beijing Hotgen Biotech Co., Ltd.

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1. Background of the Clinical Study

Coronaviruses are positive-sense single-stranded RNA viruses, with four genera of α, β, γ, and δ. The novel coronavirus is a new type of coronavirus discovered in Wuhan viral pneumonia cases in 2019. On January 12, 2020, the World Health Organization named the virus as COVID-19, which belongs to the β genus. The S protein of 2019-nCoV is located on the viral surface to form a rod-like structure, and it is one of the main antigen proteins of the virus. The S gene is also the main gene for coronavirus typing. The 2019-nCoV can cause viral pneumonia, with main clinical manifestations of fever, fatigue, and respiratory symptoms such as dry cough. Some patients gradually develop breathing difficulties, and in severe cases, acute respiratory distress syndrome, septic shock, irreversible metabolic acidosis, and coagulopathy may occur.

2. Intended Clinical Use and Principle of Subject Product

This kit is used for in vitro qualitative determination of novel coronavirus antigen in human anterior nasal swab samples. It is used as rapid investigation for suspected cases of novel coronavirus, can also be used as a reconfirmation method for nucleic acid detection in discharged cases.

This kit is based on the colloidal gold immunochromatographic technology, and uses double antibody sandwich method to detect the novel coronavirus antigen in human anterior nasal swab samples. The detection line (T line) of the novel coronavirus antigen test cassette was coated with novel coronavirus antibody, and the quality control line (C line) was coated with sheep anti-mouse. During the test, the sample is dropped into the test cassette and the liquid is chromatographed upward under the capillary effect. The novel coronavirus antigen in the sample first binds to the colloidal gold-labelled novel coronavirus antibody to form a solid phase novel coronavirus antibody-novel coronavirus antigen-labelled novel coronavirus antibody-colloidal gold complex at the T line position, and form a solid phase sheep anti-mouse-labelled novel coronavirus antibody- colloidal gold complex was formed at the C line position. After the test is completed, observe the colloidal gold color reaction of T line and C line to determine results of novel coronavirus antigen in human anterior nasal swab samples.

3. Purpose of the Clinical Study

The purpose of this clinical study was to investigate the clinical performance of "Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold)" produced by Beijing Hotgen Biotech Co., Ltd. to detect novel coronavirus (2019-nCoV) antigen in human anterior nasal swab samples.

4. Overall Study protocol

4.1 Establishment of clinical trial protocol

The clinical trial protocol was formulated by the applicant in consultation with the clinical trial institution before the clinical trial, and according to the clinical trial protocol, the responsibilities of the applicant, the researcher, and the person in charge of statistics were clearly defined. The applicant organization organizes participation in the trial. All researchers were to be trained in clinical trial protocols and use of in vitro diagnostic reagents for testing.

4.2 Study method introduction

The clinical specimens used in the trial were prospectively taken from the valid specimens (human anterior nasal swab samples) from clinical trial institutions. Patients were sequentially enrolled and tested blindly. All collected specimens can be traced back to the corresponding clinical information, including case number, age, gender, type of specimens, collection time, confirmation or exclusion of the novel coronavirus infection, and the nucleic acid test results for disease diagnosis (include the name of nucleic acid detection kit).

The subject product of this study is "Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold)" (*hereinafter referred to as "Antigen Test"*) produced by Beijing Hotgen Biotech Co., Ltd. The product selected for the comparison is RT-PCR Kit.

Results of the Antigen Test and Nucleic Acid Test are compared to evaluate the consistency between the Antigen Test and Nucleic Acid Test. Cases with different test results were comprehensively analyzed by combining the patients' epidemiological background, clinical symptoms, disease outcome, and other information. In this way, the clinical performance of the Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) (produced by Beijing Hotgen Biotech Co., Ltd) to detect the novel coronavirus (2019-nCoV) antigen in human anterior nasal swab specimens was evaluated.

4.3 Investigators

The investigators participating in the clinic study were 3 principal investigators, 3 investigators in charge of statistics, 6 operators used to run the assay and several participants used to assist the study.

4.4 Quality control

(1) Select and manage samples strictly in accordance with the requirements of research samples, and samples that do not meet the requirements during the experiment should be excluded;

(2) The clinical trial testing process was strictly performed in accordance with the requirements of the kit instructions;

(3) truthfully, detailed, timely, and carefully record all content to ensure that the content of the test record form is complete, true, reliable, and traceable;

(4) Invalid results due to the kit or other reasons should be re-tested;

(5) When the retesting of the sample occurs due to human error operation, instrument failure, and sample addition failure, the retesting result shall prevail, and the reason for retesting shall be indicated.

5. Clinical Trial Procedures

5.1 Case screening and enrollment

The case enrollment was based on the clinical diagnostic information provided by the clinical trial institutions/centers. All enrolled cases meet the trial requirements for clinical information and specimens.

5.2 Testing of specimens

Specimens of enrolled cases were blindly tested using the Antigen Test and Nucleic Acid Test according to kit instructions, and the results were recorded.

5.3 Statistical analysis of test results

Generate a data table with the information of the case specimen, the corresponding test results of the Antigen Test and Nucleic Acid Test results of the same case at the same period, the confirmation/exclusion results of COVID-19, the disease processes, and the clinical severity of the disease, etc. After verification, the clinical trial database of the project is established.

6. Clinical Trial Specimens

6.1 Specimen types

There is a sample type in this trial: human anterior nasal swab specimens.

6.2 Collection, processing and storage of specimens

Specimens collection, processing, storage should meet the Instructions for Use of the subject product and the comparator method.

Here are the requirements for subject products.

6.2.1 Collection and treatment of specimens

Gently insert the entire soft tip of the swab into one nostril for 1.5cm until you feel a bit of resistance. Using medium pressure, rub the swab slowly in a circular motion around the inside wall of your nostril 4 times for a total time of 15 seconds to ensure that as many cells and mucus are collected. Repeat the same process with the same swab in the other nostril. After the collection is complete, put the swab with the sample into the sample extraction buffer for processing.

6.2.2 Storage of specimens

The sample should be used as soon as possible after collection, and cannot be stored for a long time at room temperature. If it cannot be sent for inspection in time, anterior nasal swab samples can be stored for 24 hours at 2°C~8°C.

6.3 Entry Criteria of Specimens

6.3.1 Inclusion criteria

(1) The total number of enrolled cases is no less than 100, of which no less than 30 positive COVID-19 cases and no less than 30 negative cases.

(2) The enrolled cases should cover a certain number of recovered cases, suspected cases and try to cover patients with various respiratory infectious diseases. The enrolled cases should cover patients with different clinical severity (such as mild, moderate, severe, and critical patients), as well as patients with different disease stages (such as early, middle, and mid-late stage patients).

(3) The specimen meets the requirements for specimen collection, processing and storage.

(4) The relevant information of the specimen is complete, including the case number, age, gender, type of species, collection time, the confirmation or exclusion of the novel coronavirus infection, etc., and the nucleic acid testing results used for the diagnosis of the disease (including nucleic acid test kit name).

6.3.2 Exclusion criteria of specimens

Cases that do not meet the inclusion criteria, such as

(1) Specimens type does not meet the test requirements;

(2) Does not meet the requirements for collection, processing and storage;

(3) Cases with incomplete clinical information;

(4) Specimens whose quantity does not meet the requirements for testing.

6.3.3 Removal criteria of specimens

(1) Specimens deteriorated;

(2) Specimens that do not meet the entry criteria, or meet the criteria for exclusion but are still tested.

(3) Re-tested specimens due to operational error, instrument failure, and/or sample addition failure. If a retest occurs, remove the earlier result and record the retest result (reasons for a retest should be indicated).

7. Blind Design

The study was designed as a blind study. In the trial, one special researcher de-identifies the test specimens and then hand them over to another test operator for testing, so that the testing operator is completely unclear about the background information of the specimens. After loading the samples and the testing finished, a special data collator merges the background information of the specimens with the test results of the investigational kit to ensure the objectivity of the trial and reduce the bias caused by the expectations of the researchers who are clear about the background of the specimens.

8. Statistical and Analytical Plans

8.1 Data collection

1) Establish a database in Excel, and enter the traceable information of all specimens, background clinical diagnosis, epidemiological data, onset/visit time, sampling time, and diagnosis/exclusion results, etc.

2) Check the data. In principle, no data shall be deleted. Any dropouts shall be explained and recorded. The final statistical data shall be locked and backed up.

8.2 Data statistics

Summarize and compare the Antigen Test and Nucleic Acid Test results in a crosstab (Table 1.), and evaluate the positive consistency rate (sensitivity), negative consistency rate (specificity), and other indicators of Antigen Test and Nucleic Acid Test results. All inconsistent results shall be fully analyzed based on the confirmation/exclusion results, patient's epidemiological background, clinical symptoms, disease outcome and other information.

Table 1. Statistics of Antigen Test and Nucleic Acid Test Results

		Nucleic Acid Test results		Total
		Positive (+)	Negative (-)	
Antigen testing	Positive (+)	A	B	A+B
	Negative (-)	C	D	C+D
Total		A+C	B+D	A+B+C+D

Notes: If there are specimens results of the same case in different periods in the above evaluation, any positive Antigen Test result should be taken into analysis. The same analysis method should apply to the statistics of Nucleic Acid Test results.

(1) Calculation of positive consistency rate (sensitivity), negative consistency rate (specificity) and overall consistency rate (accuracy)

Positive consistency rate (sensitivity) = A/(A+C)×100.00% (95% confidence interval)

Negative consistency rate (specificity) = D/(B+D)×100.00% (95% confidence interval)

Overall consistency rate (accuracy) = (A+D)/(A+B+C+D)×100.00% (95% confidence interval)

The 95% confidence interval is directly calculated using statistical software MedCalc v19.0.7.

(2) Kappa agreement analysis

Calculate the Kappa value of the Antigen Test and Nucleic Acid Test results by the following formula, compare the Kappa value grading in Table 2. to evaluate the consistency of the Antigen Test and Nucleic Acid Test results.

Kappa (K)=[N(A+D) — (R1C1+R2C2)] /[N^2 — (R1C1+R2C2)]

Table 2. Consistency Judgment

No.	Kappa Value	Consistency Grading
1	<0	Very poor
2	0~0.2	Poor
3	0.21~0.40	Fair
4	0.41~0.60	Good
5	0.61~0.80	Very good
6	0.81~1.00	Excellent

9. Clinical Trial Results and Analysis

This clinical trial was led by Beijing Hotgen Biotech Co., Ltd., with specimens from 3 clinical trial institutions. The confirmed patient specimens from each clinical trial institution have traceable disease onset dates and Nucleic Acid Test results.

9.1 Composition and number of trial specimens

This clinical trial enrolled a total of 346 clinical cases, including 139 nucleic acid positive cases and 207 nucleic acid negative cases. A total of 346 human anterior nasal swab specimens were tested in this trial. The distribution of enrolled cases and numbers in each clinical trial institution are as follows:

Table 4. The distribution of enrolled cases

Sample Collection and Testing sites	PCR Negative	PCR Positive	Total
CPL	67	52	119
SPH	60	50	110
AMM	80	37	117
Total	207	139	346

In addition, the enrolled cases cover recovered cases, suspected cases, and multiple respiratory infections, as well as patients with different severity of disease (such as mild, common, severe, and critical COVID-19 patients), as well as patients with different disease processes (such as early, middle, mid-late stage patients).

The enrolled population covers children, adults, and the elderly, and cover males and females evenly.

9.2 Statistical analysis of test results

This trial enrolled a total of 346 human anterior nasal swab specimens, of which 139 were positives for Nucleic Acid Test, 207 negatives for Nucleic Acid Test; As for data collection of the corresponding Nucleic Acid Test results.

Summarize the Antigen Test and Nucleic Acid Test results (see Table 5.), and evaluate the positive consistency rate, negative consistency rate, and overall consistency rate of Antigen Test and Nucleic Acid Test.

Table 5. Statistics of Antigen Test and Nucleic Acid Test Results (human anterior nasal swab specimens)

		Nucleic Acid Test results		Total
		Positive (+)	Negative (-)	
Antigen Test	Positive (+)	136	1	137
	Negative (-)	3	206	209
Total		139	207	346

The sensitivity, specificity, overall consistency rate, and Kappa value are calculated as follows:

Statistics	Ratio	Percentage (95% confidence interval)
Positive consistency rate (sensitivity)	136/139	97.84% (93.82%~99.55%)
Negative consistency rate (specificity)	206/207	99.52% (97.34%~99.99%)
Overall consistency rate (accuracy)	342/346	98.84% (97.07%~99.68%)
Kappa value	0.976, excellent agreement	

The above statistical results show that results between Antigen Test and Nucleic Acid Test (human anterior nasal swab specimens) are highly consistent. 3 human anterior nasal swab specimens that were positive for the Nucleic Acid Test were

negative for the Antigen Test. The disagreement may because that viral load was below the lower detection limit of the Antigen Test and resulted in a false negative.

10. Discussion and Conclusions

10.1 Clinical trial implementation centers

This clinical trial was conducted by Beijing Hotgen Biotech Co., Ltd., with specimens from 3 clinical trial institutions.

10.2 Amounts of specimens in the trial

346 human anterior nasal swab specimens were tested in this trial, 139 nucleic positive and 207 negatives for nucleic acid test. The enrollment cases cover discharged cases, suspected cases, and cases with other respiratory infections. The enrollment cases cover different severities of disease (i.e. mild, moderate, severe, and critical), different disease stages (i.e. early, middle, mid-late stages), and also cover different ages (children, adults, and elders).

10.3 Analysis of test results

Statistical analysis of the results of the Antigen Test of human anterior nasal swab specimens and the results of Nucleic Acid Test, positive consistency rate (sensitivity), negative consistency rate (specificity), overall consistency rate (accuracy), Kappa value were: 97.84%, 99.52%, and 98.84%; Kappa(K)= 0.976.

In summary, using the Antigen Test kit, the Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) produced by Beijing Hotgen Biotech Co., Ltd. to detect human anterior nasal swab specimens, the results showed excellent agreement with clinical diagnosis results and the Nucleic Acid Test results. The comparison test results of human anterior nasal swab specimens are highly consistent. Therefore, the Antigen Test kit has good clinical performance.