Novel Coronavirus 2019-nCoV Antigen Test

(Colloidal Gold)

Self-test Performance Study

Subject Product: Novel Coronavirus 2019-nCoV Antigen Test

(Colloidal Gold)

Test start time: Oct.10 th, 2020

Test completion time: Feb. 03th, 2021

Model specifications: 40T/kit

Submitted by: Beijing Hotgen Biotech Co., Ltd.

Beijing Hotgen Biotech Co., Ltd.

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1. Background of the 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 2019-nCoV, 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 Use and Principle of subject product

This kit is used for in vitro qualitative determination of novel coronavirus antigen in human anterior nasal swab. 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 test is also for self-test in non-healthcare settings by individuals and results are for the detection of 2019-nCoV antigen.Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for 2019-nCoV by a healthcare provider is necessary.

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. 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.

3 Purpose of the Study

The purpose of this study was to investigate the self-test 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 specimens.

4 Overall Study protocol

4.1 Establishment of the study protocol

The study protocol was formulated by the applicant in consultation with the clinical institution before the study, and according to the study 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 trained in the study protocol.

4.2 Study method introduction

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 RT-PCR Test are compared to evaluate the consistency between the Antigen Test and RT-PCR 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 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.

The specimens collection and testing for antigen test were conducted by individuals in non-healthcare settings while the collection and testing of the specimens for RT-PCT were accomplished by the investigators.

The anterior nasal swab specimens used for antigen test were prospectively collected. Patients were sequentially and randomly enrolled .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 RT-PCR Test results for disease diagnosis.

4.3 Investigators

The investigators participating in the study were 3 principal investigators, 3 investigators in charge of statistics, 6 operators used to run the RT-PCR 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 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 Study Trial Procedures

5.1 Case screening and enrollment

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

5.2 Specimens collection and testing

Antigen test: The specimens collection and testing were conducted by individuals in non-healthcare settings accroding to the instruction of use. and the results were recorded.

RT-PCR test: Specimens of all enrolled cases were tested using the RT-PCR 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 RT-PCR 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 trial database of the project is established.

6. 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.

6.2.1 Collection and treatment of specimens

Gently insert the entire soft tip of the swab into one nostril for 2.5-3cm 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 4-6 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 of treated should be tested within 1h. Specimens that can't be detected within 24 hours should be kept at -70°C or below. Repeated freezing and thawing should be avoided during specimen transportation.

6.3 Entry Criteria of Specimens

6.3.1 Inclusion criteria

(1) The total number of enrolled cases is no less than 200, of which no less than 100 positive COVID-19 cases and no less than 100 negative cases.

(2) Enrolled cases included patients from clinic institutions with definitely clinic information and no less than 30 healthy subjects from non-clinic institutions(eg.staffs in supermarket, students in school).

(3) 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 middle-late stage patients).

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

(5) 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 RT-PCR Testing results used for the diagnosis of the disease .

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 Statistical and Analytical Plans

7.1 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.

7.2 Data statistics

Summarize and compare the Antigen Test and RT-PCR 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 RT-PCR 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 RT-PCR Test Results

		RT-PCR Test		Total
		Positive (+)	Negative (-)	
Antigen Test	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 RT-PCR 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 RT-PCR Test results by the following formula, compare the Kappa value grading in Table 2. to evaluate the consistency of the Antigen Test and RT-PCR 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

8.Trial Results and Analysis

The confirmed patient specimens from each clinical institution have traceable disease onset dates and all enrolled cases were with RT-PCR Test results to ensure that the collected specimens tested are compatible with a true positive/negative status of the subject.

8.1 Composition and number of trial specimens

This study enrolled a total of 223 clinical cases, including 108 RT-PCR positive cases and 115 RT-PCR negative cases. A total of 223 human anterior nasal swab spec imens were tested in this trial.

In addition, the enrolled cases cover 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, middle-late stage patients).

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

8.2 Statistical analysis of test results

This study enrolled a total of 223 human anterior nasal swab specimens, of which 108 were positives for RT-PCR Test, 115 negatives for RT-PCR Test; As for data collection of the corresponding RT-PCR Test results.

Summarize the Antigen Test and RT-PCR Test results (see Table 3.), and evaluate the positive consistency rate, negative consistency rate, and overall consistency rate of Antigen Test and RT-PCR Test.

Table 3. Statistics of Antigen Test and RT-PCR Test Results
(human anterior nasal swab specimens)

		RT-PCR Test		Total
		Positive (+)	Negative (-)	
Antigen Test	Positive (+)	103	1	104
	Negative (-)	5	114	119
Total		108	115	223

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

Statistics	Ratio	Percentage (95% confidence interval)
Positive consistency rate (sensitivity)	103/108	95.37% (89.62%~98.01%)
Negative consistency rate (specificity)	114/115	99.13% (95.24%~99.85%)
Overall consistency rate (accuracy)	217/223	97.31% (94.26%~98.76%)
Kappa value	0.9461, excellent agreement	

The above statistical results show that results between Antigen Test and RT-PCR Test (human anterior nasal swab specimens) are highly consistent. 4 specimens that were positive for the RT-PCR 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. In addition, 1 specimen that were negative for the RT-PCR Test were positive for the Antigen Test, and the disagreement may be caused by individual differences or medication.

9.Discussion and Conclusions

9.1 Trial implementation centers

This Antigen Tests were conducted by individuals themselves in clinic institutions and several non-clinic institutions. The RT-PCR Tests were conducted by investigator in professional laboratory.

9.2 Amounts of specimens in the trial

223 human anterior nasal swab specimens were tested in this trial, 108 RT-PCR positive and 115 negatives for RT-PCR Test. The enrollment cases cover 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, middle-late stages), and also cover different ages (children, adults, and elders).

9.3 Analysis of test results

Statistical analysis of the results of the Antigen Test of human anterior nasal swab specimens and the results of RT-PCR Test, positive consistency rate (sensitivity), negative consistency rate (specificity): ,overall consistency rate (accuracy),Kappa value were:95.37%, 99.13%, and 97.31%; Kappa（K）=0.9461.

In summary, individuals self-test in non-healthcare settings by 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 the RT-PCR Test results. The comparison test results of human anterior nasal swab specimens are highly consistent. Therefore, the Antigen Test kit has a good self-test performance.

10. Appendices

See the appendix "Clinical Trial Specimens Information and Test Results Record Sheet" for the original information and test results of the trial specimens.