Clinical Evaluation Report

Rapid SARS-CoV-2 Antigen Test (Colloidal Gold)

A lateral flow colloidal gold chromatographic immunoassay

for the detection of SARS-CoV-2 antigens in swab specimens

Manufacturer: InTec PRODUCTS, INC.

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Product Name

Rapid SARS-CoV-2 Antigen Test (Colloidal Gold)

Manufacturer

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Introduction

COVID-19 is a SARS-CoV-2 (also known as 2019-nCoV) associated pneumonia. A few patients have developed severe pneumonia, pulmonary oedema, ARDS, or multiple organ failure and have died. The Rapid SARS-CoV-2 Antigen Test is based on immunochromatography for detection of SARS-CoV-2 antigen in the specimen collected by the nasopharyngeal and nasal swab. It is simple, visual qualitative and presents the result within 20 minutes.

The novel coronaviruses belong to the β genus.COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

Results are for the identification of SARS-CoV-2 nucleocapsid antigen. The antigen is generally detectable in upper respiratory samples or lower respiratory samples during the acute phase of infection. The positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. The positive results do not rule out bacterial infection or co-infection with other viruses. The antigen detected may not be the definite cause of disease. The negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. The negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with SARS-CoV-2 and confirmed with a molecular assay, if necessary for patient management.

Test principle

Gold conjugated mouse anti-SARS-CoV-2 N-protein IgG and gold conjugated rabbit nonspecific IgG are pre-coated on the sample pad. SARS-CoV-2 antigen (N protein) can react with the gold conjugated mouse SARS-CoV-2 specific IgG and form an immune complex. The specimen will move forward along the test strip.

If the specimen contains SARS-CoV-2 antigen (N protein) and the concentration is above the minimum detection limit, the complex will be captured by the mouse anti-SARS-CoV-2 N-protein IgG pre-coated at the test band region, and form a purplish red band. If the specimen does not contain SARS-CoV-2 antigen or the concentration is below the minimum detection limit, there will be no purplish red band shown at the test band region.

Regardless of whether the analyte exist in the specimen, the gold conjugated rabbit nonspecific IgG will be captured by the goat anti-rabbit IgG. A purplish red band will appear at the control band

region.
Only when the control band appears, the correlated result is valid.

Purpose

InTec PRODUCTS,INC. intends to introduce Rapid COVID-19 Antigen Test(Colloidal Gold) into the market. The objective of this study was designed to evaluate the user performance of Rapid SARS-CoV-2 Antigen Test. We expected to determine the sensitivity and specificity of the Rapid SARS-CoV-2 Antigen Test when testing intended use populations who are suspected of COVID-19 by healthcare professionals and trained healthcare workers as an aid in the diagnosis of SARS-CoV-2 infection.
The test was performed in hospitals, clinic and same testing laboratories. The test results of samples collected from clinical cases were compared with PCR results of cases to verify the clinical performance of the test reagent.

Design

Sample population and size

The clinical evaluation will be conducted at the actual user site and the study population will be participants and patients. To support the test performance, clinical specimens will be tested with the goal of testing a minimum of 200 positive specimens and 500 negative specimens, the positive specimens were collected by targeted and planed fashion, the negative specimens in a randomized, blinded fashion. Overall sample size's plan are summarized in the following table (Tab1) ：

Tab1 Number of clinical specimens

Group	PCR result (Nasopharyngeal swab)	Sample type	Number
1	Positive	Nasopharyngeal swab	At least 200 cases
		Nasal swab	
2	Negative	Nasopharyngeal swab	At least 500 cases
		Nasal swab	

The testing to be conducted will include the following:
A. Enroll more than 200 subjects known to be positive for COVID-19 by a RT-PCR assay within 14 days. These would be the patients that are already under the PI's care.
B. Enroll more than 500 subjects where the healthcare provider suspects or may have COVID-19 infection based on the CDC description of COVID-19 symptoms. Also the individual volunteers from the enterprise.
C. All the subjects will agree to be simultaneously sampled for a COVID-19 RT-PCR test and sampled for an antigen test at the clinical site.
D. Each subject has been collected the Nasopharyngeal and Nasal Swab specimen at the same time.
E. If a subject has a known RT-PCR result less than 14 days ago, the RT-PCR test can be waived.

Criteria for Participant

Inclusion Criteria

1. Age: no restriction, the neonatal excluded. Gender : Male or female.
2. Has symptoms that lead the healthcare provider to suspect the individual of possibly having SARS-CoV-2 infection.
3. Was exposed to a COVID-19 patient within 14 days that leads the healthcare provider to suspect the individual of possibly having SARS-CoV-2 infection
4. Has an immediate need to determine COVID-19 status for occupational purposes.
5. Must be willing to provide a sample for COVID-19 RT-PCR testing if the subject has not been previous tested for COVID-19 RT-PCR within 14 days.
6. Must be willing to provide a sample for additional tests required by the study site.(antigen test or RT-PCR).
7. Must be able to sign a consent form.
8. Must be able to provide swab samples.

Exclusion Criteria

1. Is receiving treatment with infusion of convalescent plasma or other antibody therapy related to SARS-CoV-2 infections.
2. Is participating in a SARS-CoV-2 vaccine study.
3. Tested positive for COVID-19 positive more than 14 days ago.

Test Interval

October, 2020 to February, 2021

Materials and Equipment

(1) Candidate Test
Rapid SARS-CoV-2 Antigen Test (Colloidal Gold)
Lot：S20200910
Manufacturer：InTec PRODUCTS,INC.
Disposables including tips for the pipette；timer

(2) Comparator Test & Equipment
Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV
Lot：S1572054 Manufacturer：BGI Genormics Co,Ltd.

Real time fluorescence quantitative PCR
Type：ABI 7500 Manufacturer：Applied Biosystems

The comparator tests included high sensitivity Authorized RT-PCR tests used at each testing site as the routine testing method for COVID-19 diagnostics. The RT-PCR tests use a chemically step followed by solid phase extraction of nucleic acid. The patient specimens were all prospective

collected and immediately tested with candidate test by operators. Multiple RT-PCR tests were used as the comparator assay because Manufacturer had no control of which assay the test site used for patient testing. RT-PCR test because the subject was already sampled twice (once for the clinical testing and once for the investigational testing).

Evaluation Sites & Main Researcher and Investigator

The evaluation sites were chosen by high sensitivity Authorized RT-PCR tests or designated laboratory. All the clinical specimens were collected or tested by the following sites:

(1) POC in Guangzhou,China
Primary Researcher
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(2) POC in Quanzhou,China
Primary Researcher
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Co-Investigator
Zheng Youxian
Title: Chief Technical Engineer of clinical medical laboratory, Masters Degree
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(3) POC in Beijing,China

Primary Researcher

Wang Yibo

Title: Technical Engineer in charge of Virus Research Laboratory, Doctor Degree

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Co-Investigator

Dai Wei, Li Yong

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(4) POC in Wuhan,China

Primary Researcher

Cai Kun

Title: Deputy senior engineer in charge of clinical medical laboratory，Doctor Degree

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Co-Investigator

Li jing，Liu Yue，Zhou Kangping

Title: Deputy senior engineer，Doctor Degree

Zhu Yuexian

Title: Deputy senior engineer，Masters Degree

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(5) POC in Xi’an,China

Primary Researcher

Che Hui

Title: Technical Engineer in charge of clinical medical laboratory，Doctor Degree

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Co-Investigator

Zhao Miaomiao, Zhu Jingbo

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The above POC are Authorized Centre of Disease Control for COVID-19 testing, and each POC is operated by 3-5 professionals.

Clinical specimens storage

(1) Applicable clinical specimens type: Nasopharyngeal swab specimen, Nasal Swab specimen
(2) Storage: Samples should be tested as soon as possible after collection. Rapid tests and Multiple RT-PCR tests were tested within 24 hours. Processed samples (add Extraction Solution) are stable for up to 24-hours at room temperature or 2° to 8°C and cannot be frozen.
(3) The specimens must be balanced to room temperature before testing.

Test Procedure

(1) Specimens collection and information record
The main researchers of the clinical institutions designate special personnel to select the eligible cases according to the enrollment criteria, and collect the clinical information of the enrolled specimens, including: age, gender, clinical symptoms, clinical classification(mild or moderate), sample collection time and other information. The specimens are numbered according to the sequence before and after grouping, i.e. Specimens number.

(2) Specimens blinding
The main researchers of the clinical institution designated the person to randomly number the specimens in the group with the random number generating tool, record the random number of the specimens and the corresponding specimens number, and the person arranged the specimens according to the sequence of the random number, and handed them to the test operator for testing according to this sequence, noting that the person and the test operator cannot be the same person.

(3) Testing
Perform the Test according to the Instructions for Use (IFU)package insert.The test operator shall test the specimens and operate according to the instructions. PCR test is used for in vitro qualitative detection of novel coronavirus (2019-nCoV) ORF1ab, N gene and E gene in nasopharyngeal swab, oropharyngeal swab,sputum, and alveolar lavage fluid samples.
The test device and swab is provided with the test kit. The fresh specimens were tested immediately, and no transport media was used for shipping the samples to a different location for testing.

(4) Record
At the end of the test, according to the corresponding relationship between random number and specimens number, record the test results.

(5) Result determination
All clinical specimens tested in this submission were tested and evaluated in accordance with the

proposed diagnostic algorithm. The test results should be statistically analyzed with clinical diagnosis to evaluate the clinical application performance of the product.

Control method

(1) Before the start of clinical research, the enterprise shall train the researchers to make them familiar with and master the operation method and technical performance of the product, so as to minimize the test error.
(2) Researchers should strictly follow the product's operation specifications and related requirements for testing to ensure that the testing error can be minimized.
(3) The supervisors shall check the relevant activities and documents of the clinical trial, whether the trial is conducted in accordance with the test scheme, standard operating procedures and relevant regulations, and whether the test data is recorded in a timely, clear, accurate and complete manner.

Data management

(1) Traceability of data, filling and transfer of case report form
Ensure the traceability of clinical trial data. According to the original observation records of the subjects, the researchers recorded the data in the case report form in a timely, complete, accurate and clear manner. The supervisor shall monitor whether the trial is carried out in accordance with the plan, confirm that the case report form is filled in correctly and completely, and is consistent with the original data. In case of any mistake or omission, the researcher shall be required to correct it in time. The original record shall be kept clear and visible during modification, and the correction shall be signed and dated by the researcher.

(2) Data entry and modification
In order to ensure the accuracy of data, two data entry personnel are responsible for independent entry and proofreading. During the data analysis, for the questions in the case report form, the researcher should answer and return them as soon as possible, and the statistician should modify, confirm and input them according to the researcher's answers.

(3) Lock of database
At the end of the test, after data entry, the researcher and the sponsor check the data, and lock the data after confirming that the data is correct, and then lock the data for statistical analysis.

Data record

According the test Test Procedure, the operator personnel was recorded the sample information and the test results. After data entry, the researcher and the sponsor check the data. At last, the test results were finished in a total sheet. The detail sample information and test results were recorded in the Annex sheet.

Results and Statistical Analysis

Each subject has been collected the Nasopharyngeal and Nasal Swab specimen at the same time.

For Nasopharyngeal and Nasal Swab respectively two kinds of samples, the test results were statistical analyzed with the proposed diagnostic algorithm. We got the clinical performance analysis for Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) in different specimen type.

(1) Results for Nasopharyngeal swab specimen

Clinical Performance of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) was evaluated by being involved in 3 sites within china where patients were enrolled and tested. Testing was performed by Healthcare Workers that were not familiar with the testing procedure. A total of 755 fresh Nasopharyngeal swab samples was collected and tested, which includes 233 positive samples and 522 negative samples with Rapid SARS-CoV-2 Antigen Test (Colloidal Gold). Among these nasopharyngeal swab specimens, only 9 samples results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) were negative and disagree with RT-PCR assays. The other results of two methods are consistent. Overall study results are shown in the following table (Tab2)：

Tab2 Analysis table of clinical specimens results (Nasopharyngeal Swab)

		RT-PCR result		
		Positive	Negative	Total
Rapid SARS-CoV-2	Positive	233(a)	0(b)	233(a+b)
Antigen Test (Colloidal	Negative	9(c)	513(d)	522(c+d)
Gold) result	Total	242(a+c)	513(b+d)	755(a+b+c+d)

For the 755 test results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) (Nasopharyngeal Swab) and RT-PCR assays, the Clinical sensitivity (Positive Coincidence rate) calculation with statistical analysis is 96.3%, and 95% confidence interval is about 93.1%~98.0%, the Clinical specificity (Negative Coincidence rate) calculation with statistical analysis is 100%, and 95% confidence interval is about 99.3%~100%. Overall study results are shown in the following table (Tab3)：

Tab3 Coincidence rate and 95% confidence interval

	Coincidence rate	95% confidence interval
Clinical sensitivity	96.3%	93.1%~98.0%
Clinical specificity	100%	99.3%~100%
Total coincidence rate	98.8%	97.8%~99.4%

(2) Results for Nasal Swab specimen

Clinical Performance of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) was evaluated by being involved in 3 sites within china where patients were enrolled and tested. Testing was performed by Healthcare Workers that were not familiar with the testing procedure. A total of 755 fresh Nasal Swab samples was collected and tested, which includes 233 positive samples and 522 negative samples with Rapid SARS-CoV-2 Antigen Test (Colloidal Gold). Among these Nasal Swab specimens, only 2 samples results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) were

positive and disagree with RT-PCR assays. About 11 samples results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) were negative and disagree with RT-PCR assays. The other results of two methods are consistent. Overall study results are shown in the following table (Tab4)：

Tab4 Analysis table of clinical specimens results (Nasal Swab)

		RT-PCR result		
		Positive	Negative	Total
Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) result	Positive	231(a)	2(b)	233(a+b)
	Negative	11(c)	511(d)	522(c+d)
	Total	242(a+c)	513(b+d)	755(a+b+c+d)

For the 755 test results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) (Nasal Swab) and RT-PCR assays, the Clinical sensitivity (Positive Coincidence rate) calculation with statistical analysis is 95.5%, and 95% confidence interval is about 92.0%~97.4%, the Clinical specificity (Negative Coincidence rate) calculation with statistical analysis is 100%, and 95% confidence interval is about 98.6%~99.9%. Overall study results are shown in the following table (Tab5)：

Tab5 Coincidence rate and 95% confidence interval

	Coincidence rate	95% confidence interval
Clinical sensitivity	95.5%	92.0%~97.4%
Clinical specificity	99.6%	98.6%~99.9%
Total coincidence rate	98.3%	97.1%~99.0%

(3) Results for Total specimen (Nasopharyngeal and Nasal Swab)

At last, all the test results of different sample (Nasopharyngeal and Nasal Swab) were statistics analyzed. A total of 1510 fresh Nasopharyngeal and Nasal Swab samples were collected and calculated, which includes 466 positive samples and 1044 negative samples with Rapid SARS-CoV-2 Antigen Test (Colloidal Gold). Among these nasopharyngeal swab specimens, only 2 samples results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) were positive and disagree with RT-PCR assays. About 20 samples results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) were negative and disagree with RT-PCR assays. The other results of two methods are consistent. Overall study results are shown in the following table (Tab6)：

Tab6 Analysis table of clinical specimens results

		RT-PCR result		
		Positive	Negative	Total
Rapid COVID-19 Antigen Test(Colloidal Gold) result	Positive	464(a)	2(b)	466(a+b)
	Negative	20(c)	1024(d)	1044(c+d)
	Total	484(a+c)	1026(b+d)	1510(a+b+c+d)

For the 755 test results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) (Nasopharyngeal and Nasal Swab) and RT-PCR assays, the Clinical sensitivity (Positive Coincidence rate) calculation with statistical analysis is 95.9%, and 95% confidence interval is about 93.7%~97.3%, the Clinical specificity (Negative Coincidence rate) calculation with statistical analysis is 99.8%, and 95% confidence interval is about 99.3%~99.9%. Overall study results are shown in the following table (Tab7)：

Tab7 Coincidence rate and 95% confidence interval

	Coincidence rate	95% confidence interval
Clinical sensitivity	95.9%	93.7%~97.3%
Clinical specificity	99.8%	99.3%~99.9%
Total coincidence rate	98.5%	97.8%~99.0%

For the total 1510 test results of Rapid SARS-CoV-2 Antigen Test (Colloidal Gold) and RT-PCR assays, The total coincidence rate calculation with statistical analysis is 98.5%, the Kappa value(K) calculation with statistical analysis is 0.966, K=0.966>0.75, which indicates that the high consistency of two methods and equivalence of two such systems.

Conclusion

(1) 1510 samples were tested in this clinical trial, included 755 nasopharyngeal swabs, and 755 Nasal Swab.
(2) Among them, there were 1488cases with product test results consistent with PCR result in this clinical trial, included 464 cases were positive, 1024 cases were negative.
(3) There were 22 cases of product test results inconsistent with PCR result in this clinical trial.

PCR result	Product result	Number
-	N(+)	2
+	NP(-),N(-)	9
+	N(-)	2
* Nasopharyngeal swab:NP, Nasal Swab: N		

More detail information refers to Annex sheet.

(4) The sensitivity of product testing and PCR result are 96.3%(for Nasopharyngeal swab) and 95.5%(for Nasal Swab) respectively. The total sensitivity of product testing and PCR result is 95.9%(for Nasopharyngeal swab and Nasal Swab). The specificity of product testing and PCR result are 100%(for Nasopharyngeal swab) and 99.6%(for Nasal Swab) respectively. The total specificity of product testing and PCR result is 99.8%(for Nasopharyngeal and Nasal Swab).
(5) Among 1510 samples clinical trial, K=0.966>0.75 indicates that the high consistency of two methods and equivalence of two such systems. The total coincidence rate of product testing and PCR result is 98.5%.
(6) The clinical valuation study were designed and executed with the criteria of EN13612:2002 and EC/98/79 directives. The performance of InTec Rapid SARS-COV-S Antigen Test are met with the relevant criteria.

Annex I

The data record sheet for Rapid SARS-CoV-2 Antigen Test (Colloidal Gold)