CHIL COVID-19 ANTIGEN RAPID TEST KIT

Performance Evaluation Report

Product Name: CHIL COVID-19 ANTIGEN RAPID TEST

Packing specification: 40 Tests/Kit

Clinical evaluation category: Comparison with clinical PCR results

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Application company: CHILTıbbi Mal. San. Tic. Ltd. Sti.

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1.OVERVİEW

1.1 Abstract

Objective:

The aim of the test is to see whether CHIL Covid-19 antigen rapid test detection capabilityis comparable to clinical diagnostic standards when used in vitro for qualitative detection of SARS-CoV-2 antigen in human nasopharyngeal/oropharyngeal and nasal swab samples.

Methods:

Methodological comparison arrangement and synchronized blind test.

1.2 Abbreviation

Severe Acute Respiratory Syndrome Coronavirus 2：SARS-CoV-2

2.MAIN CONTENT

2.1 Basic Content

2.1.1 Introduction

The novel coronaviruses belong to the β-genus, a positive strand RNA virus. SARS- COV2 is an acute respiratory infectious disease which people are susceptible to infection. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be spread the virus. 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, loss of smell and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

2.1.2 Research purpose

To prove the capacity of detection of the CHIL Covid-19 antigen rapid test kit produced by CHIL Tıbbi Mal. San. Tic. Ltd. Sti. is equal when used for qualitative detection of antigen in nasopharyngeal/oropharyngeal and nasal samples in vitro to similar products on the market.

2.1.3 Testing Management

During the trial, the main investigator is in charge of the overall coordination and management, while the main participants are in charge of the actual trial work. Researcher monitors the testing laboratory's quality control. Any issues discovered during the test must be reported to the main researcher as soon as possible, and necessary steps must be taken. The person in charge of statistics analyzed the final test results statistically and confirmed by the main investigator. Finally, performance study report was prepared.

2.1.4 Research design

2.1.4.1 General Design

The synchronous blind test and methodological comparison design uses for this test.

This test uses a blind test to minimize the potential effect of individual prejudices and personal interests of researchers on test results during the clinical trial period. That is, the research staff in this test are unaware of the sample's specific details.

2.1.4.2 Measures to reduce and avoid bias

1)To avoid selection bias, subjects were screened solely according to the protocol's inclusion

2)After the trial is over, the data must be saved and sorted. When issues with the data are discovered, the researcher must double-check and confirm the information to prevent documenting errors.

2.1.4.3 Sample Selection

2.1.4.3.1 Inclusion criteria

The result of PCR was used as the classification basis in the consistency comparison of experimental reagent and reference group.

1)Inclusion criteria of sample: the sample should be a large enough sample including a well-documented source, with people of varying ages, and genders. Samples are collected and handled in compliance with the reagent specification or applicable regulations. Age, sex, sample collection date, and clinical diagnosis, such as confirmation or exclusion of SARS-CoV-2 infection, should all be included in the sample details.

2) Positive group with minimum 100 participants with symptoms (0-4 days sypmtom onset)

3) Negative group with minimum 100 asymptomatic participants

2.1.4.3.2 Exclusion criteria

1)There is no detail about the time of sample collection or the case.

2)The sample size is insufficient for the test to be completed.

3)Prior to the examination, it was discovered that the sample preservation process had been contaminated, resulting in turbidity.

4)According to the researchers, the sample does not meet the test criteria.

2.1.4.3.3 Rejection Criteria

1)Samples that fail to complete the test due to instrument or human error (sample contamination during operation).

2)The sample test results come from samples that were not processed and evaluated according to the experimental reagent's instructions.

2.1.4.4 Sample Distribution

The following are the specific criteria for clinical sample size:

1)There should be a minimum of 100 confirmed cases.

2)It is recommended that at least 100 cases be omitted.

2.1.4.5 Sample Collection, Storage and Transportation Methods

Collection of nasopharyngeal/oropharyngeal secretion: Insert the sterile swab into the place where the nasopharyngeal secretions are the most and rotate the swab close to the inner wall of the nasal cavity 3 times, remove the swab.

Collection of nasal secretion: Insert the sterile swab into the place where the nasal secretions are the most and rotate the swab against the inside of the nostril 5-6 times, remove the swab.

The collected nasopharyngeal/oropharyngeal and nasal samples are stable in 30 mins if they kept in the sample extraction solution provided with the kit. After the collection, samples must be tested as soon as possible.

If samples must be transported using vNAT solution, only a small amount of dilution is recommended, as dilution can decrease test sensitivity. To prevent unnecessary dilution of the patient sample, use 1 mL or less wherever possible. Nasopharyngeal/oropharyngeal in vNAT will last up to 48 hours at 2°C-8°C.

2.1.4.6 Reagents and Instruments for Clinical Research

1)Table 1 shows the evaluation reagent information:

Table 1: Evaluation Reagent Information

Name of Reagent	CHIL Covıd-19 Antigen Rapid Test
Specification	40 Tests/Kit
Company	CHILTıbbi Mal. San. Tic. Ltd. Sti.

Lot Number	CCOV-201.22CRD.01
Preservation Condition	2℃ ~ 30℃

2) Table 2 shows the information of reference reagent:

Table 2: Information of Reference Reagent

Name of Reagent	Covid-19 Coronavirus Real Time PCR kit
Specification	1 Test/Kit
Company	BioPerfectus Technologies Co., Ltd.
Preservation Condition	-25℃ ~ -15℃

2.1.4.7 Quality Control

1) Definition:

The operation of techniques and activities, such as monitoring, under the quality assurance system to verify that the study quality meets the specifications is known as quality control. To ensure that all data is trustworthy and properly located, quality control must be applied at everypoint of data processing.

2) Monitoring of Study:

Authorized and qualified inspectors will perform routine remote primary data checks in accordance with the monitoring plan to verify compliance with protocols and regulations and assist investigators during the outbreak.

3) Quality Control of Laboratory

A unified test index, standard operating procedures, and quality management procedures mustbe developed by the testing laboratory.

4) Testing Process of Reagent Quality Control

The quality control line in each test must have a red stripe (qualified quality control). If the quality control line does not have a red strip (unqualified quality control), the cause must be determined and the quality control result must be retested before the quality control result isqualified, ensuring the system's reliability and stability.

5) Researcher's Qualification

Researchers taking part in the clinical trial must meet the clinical trial's specialization, qualification, and skill requirements, as well as pass the qualification test. Personnel specifications should be fairly consistent.

2.1.4.8 Statistical analysis method of clinical trial data

For statistical analysis, use statistical software or the formula below.

Table 3: Data analysis method of clinical trial data

Experimental Group	Reference Group		Sum
	Positive	Negative	
Positive	a	b	a + b
Negative	c	d	c + d
Sum	a + c	b + d	a + b + c + d
Sensitivity	a / (a + c) d / (b + d)		
Specificity			

2.2 Clinical Trial Results and Analysis

2.2.1 Overall Distribution of Samples

A total of 490 samples nasopharyngeal/oropharyngeal and 200 samples nasal, which known as Omicron variant, were enrolled in this study for the accuracy comparison of nasopharyngeal/ oropharyngeal detection results by experimental reagent vs nasopharyngeal/oropharyngeal detection results by RT-PCR result, and nasal detection results by experimental reagent vs nasopharyngeal/oropharyngeal detection results by RT-PCR result, 0 duplicate samples were omitted, leaving a total of 690 samples for statistical analysis. There were 490 positive samples and 200 negative samples of RT-PCR results among them.

Table 4: Clinical trials proportion and concentration distribution

Sample Type	**Number of total cases**	**Positive samples**		**Negative samples**	
		Number of cases	**Ratio**	**Number of cases**	**Ratio**
Nasopharyngeal/ Oropharyngeal	490	390	79,60%	100	20,40%
Nasal	200	100	66,66%	100	33,33%

2.2.2 Consistency analysis of test result

2.2.2.1 Consistency comparison of the detection results of nasopharyngeal/oropharyngeal samples byexperimental reagent and the clinical diagnostic criteria

A total of 490 samples were used in this study to compare the accuracy of nasopharyngeal/oropharyngeal sample detection results by experimental reagent and PCR results. Among them 390 samples were detested as positive and 7 of them detected negative.

The test kit's sensitivity is 98.20% and the specificity is 100%. The accuracy of the test kit calculated as 98.57%.

Table 5: Analytical Results with correlation of Ct-values of the samples

Ct Value	Number of Samples	Number of true positive rapid test samples	Number of false negative rapid test samples	Sensitivity of CHIL™ SARS- CoV-2 Rapid Antigen Test Kit
<25	143	143	0	100%
25<x<30	116	115	1	99.14%
30<x<35	131	125	6	95.41%
35<	3	2	1	66.66%

As the detection limit of the rapid test kits is max CT35, the results for >35CT is excluded from final calculations.

Table 6: PCR results statistical analysis

SARS-CoV-2 Rapid Antigen Test Kit	RT-PCR comparative test result		
	Positive (+)	Negative (-)	Total
Positive	383	0	383
Negative	7	100	107
Total	390	100	490
Sensitivity: 383/390 98.20%			
Specificity: 100/100 100%			
Accuracy:483/490x100% =98.57%			

2.2.2.2 Consistency comparison of the detection results of nasal samples by experimental reagent and the clinical diagnostic criteria

A total of 202 samples were used in this study to compare the accuracy of nasal sample detection results by experimental reagent and nasopharyngeal/oropharyngeal detection results by RT-PCR results. Among them 100 samples were detected as positive and 2 of them detected negative.

The test kit's sensitivity is 98% and the specificity is 100%. The accuracy of the test kit calculated as 99%.

Table 7: Analytical Results with correlation of Ct-values of the samples

Ct Value	Number of Samples	Number of true positive rapid test samples	Number of false negative rapid test samples	Sensitivity of CHIL™ SARS- CoV-2 Rapid Antigen Test Kit
<25	39	39	0	100%
25<x<30	27	26	1	96,29%
30<x<35	34	33	1	97,05%
35<	2	2	0	100%

As the detection limit of the rapid test kits is max CT35, the results for >35CT is excluded from final calculations.

Table 8: PCR results statistical analysis

SARS-CoV-2 Rapid Antigen Test Kit	RT-PCR comparative test result		
	Positive (+)	Negative (-)	Total
Positive	98	0	98
Negative	2	100	102
Total	100	100	200
Sensitivity: 98/100 98%			
Specificity: 100/100 100%			
Accuracy:198/200x100% 99%			

2.3 Test Reliability

1)All test sample collection and preservation methods are accurate.

2)During the evaluation phase, the operators received special training to ensure the test results' reliability.

2.4 Cross reaction testing

This crossreaction study is performed to verify the influence of common respiratory pathogens on the detection performance of CHIL COVID-19 Antigen Test. The following respiratory pathogens are selected for cross-reactivity tests: Influenza A virus H1N1, influenza B virus, Mycoplasma pneumoniae, Rhinovirus A, Rotavirus, Large intestine Escherichia, respiratory syncytial virus, adenovirus, etc.

The concentration of bacterial specimens is set to 106 cfu/mL or higher, and the concentration of virus specimens is set to 105 pfu/mL or higher. The test results are shown in the table 9 below.

Table 9. Cross reaction (cfu: colony-forming unit, pfu: plaque-forming unit)

Pathogen	Concentration	CHIL COVID-19 Antigen Rapid Test Results
HKU1	105 pfu/mL	Negative
OC43	105 pfu/mL	Negative
NL63	105 pfu/mL	Negative
229E	105 pfu/mL	Negative
MERS-coronavirus	105 pfu/mL	Negative
Human Metapneumovirus	105 pfu/mL	Negative
Influenza A virus H1N1	105 pfu/mL	Negative
Influenza A virus H3N2	105 pfu/mL	Negative
Influenza A virus H5N1	105 pfu/mL	Negative
Influenza A virus H7N9	105 pfu/mL	Negative
Influenza B virus	105 pfu/mL	Negative
Mycoplasma pneumoniae	106 cfu/mL	Negative
Rhinovirus A	105 pfu/mL	Negative
Rhinovirus B	105 pfu/mL	Negative
Rhinovirus C	106 pfu/mL	Negative
Adenovirus 1	105 pfu/mL	Negative
Adenovirus 2	105 pfu/mL	Negative
Adenovirus 3	105 pfu/mL	Negative
Adenovirus 4	105 pfu/mL	Negative
Adenovirus 5	105 pfu/mL	Negative
Adenovirus 7	105 pfu/mL	Negative
Adenovirus 55	105 pfu/mL	Negative
Enterovirus A	105 pfu/mL	Negative
Enterovirus B	105 pfu/mL	Negative
Enterovirus C	105 pfu/mL	Negative
Enterovirus D	105 pfu/mL	Negative
EB Virus	105 pfu/mL	Negative
Measles virus	105 pfu/mL	Negative
Human cytomegalovirus	105 pfu/mL	Negative
Rotavirus	105 pfu/mL	Negative
Norovirus	105 pfu/mL	Negative

Mumps virus	105 pfu/mL	Negative
Varicella-zoster virus	105 pfu/mL	Negative
Respiratory syncytial virus	105 pfu/mL	Negative
Mycoplasma pneumoniae	106 cfu/mL	Negative
Escherichia Coli	106 cfu/mL	Negative

Experiment results on twenty COVID-19 Antigen negative specimens of healthy people have confirmed as all negative for the concentration of microorganisms or viruses mentioned above. These tested pathogens have no cross reaction effect on the detection performance of the COVID-19 Antigen Rapid Test.

2.5 High-dose Hook Effect:

Three batches of CCOV-201.20.C1, CCOV-201.20.C2 and CCOV-201.20.C3 CHIL® COVID-19 Antigen Rapid Test were used to test COVID-19 antigen strong positive sample obtained from NIBSC company with a high concentration of 9.73x106TCID50/mL.. Each gradient was tested in parallel for five times with CHIL® COVID-19 Antigen Rapid Test and test results are shown in the table 10.

Table 10: High-dose Hook Effect Study

Batch Number		**COVID-19 Antigen Concentration**				**CHIL COVID-19 Antigen Rapid Test Results on Positive Specimens**	
1		2		3	4		5
CCOV-201.20.C1	9.73x10$_6$TCID50/mL	+	+	+	+	+	
CCOV-201.20.C2	9.73x10$_6$TCID50/mL	+	+	+	+	+	
CCOV-201.20.C3	9.73x10$_6$TCID50/mL	+	+	+	+	+	

The CHIL® COVID-19 Antigen Rapid Test detected the positive samples of the COVID- 19 antigen without obvious up to 9.73x106TCID50/mL hook effect.

2.6 Interference testing

Interference testing is performed to evaluate CHIL COVID-19 Antigen Rapid Test performance with common substances. The study is designed as to add the following substances to negative and weakly positive specimens to evaluate the interference effect on the COVID-19 Antigen Rapid Test results (see the table 11).

Table 11: Interference testing

Substances	Concentratios	CHIL COVID-19 Antigen Rapid Test Results on Negative specimens	CHIL COVID-19 Antigen Rapid Test Results on Positive specimens
Mucin	200 mg/ml	Negative	Positive
Hemoglobin	10 mg/ml	Negative	Positive
Histamine Hydrochloride	4.0mg/L	Negative	Positive
Human albumin	60 mg/ml	Negative	Positive
α- interferon	2 ng/ml	Negative	Positive
Lopinavir	2 µg/ml	Negative	Positive
Tobramycin	10 mg/L	Negative	Positive
Ribavirin	40 mg/L	Negative	Positive
Tramadol	12 µg/ml	Negative	Positive
Azithromycin	5 µg/ml	Negative	Positive
Meropenem	10 mg/ml	Negative	Positive
Oseltamivir	1000 ng/ml	Negative	Positive
Benzocaine	1.5 mg/ml	Negative	Positive
Peramivir	20 µg/ml	Negative	Positive

The test results show that the above mentioned common substances have no interference effect on the detection performance of the COVID-19 Antigen Rapid Test. However, high concentrations of Hemoglobin may affect the test performance. In accordance with this possibility, hemolyzed specimens should not be used for avoiding to have false test results.

2.7 Discussion and Conclusion

2.7.1 Nasopharyngeal/Oropharyngeal Swab

In this clinical trial with fresh samples, 490 samples were taken from RT-PCR positive or negative patients. Among them,390 cases of "positive group" Nasopharyngeal/Oropharyngeal samples and 100 Nasopharyngeal/Oropharyngeal samples of "negative group" were determined by nucleic acid detection (RT-PCR). Among them 390 positive Nasopharyngeal/Oropharyngeal samples and 100 negative Nasopharyngeal/Oropharyngeal samples were detected by the assessment reagent. The positive coincidencerate of the assessment reagent was 98.20 % and the negative coincidence rate was 100%.

2.7.2 Nasal Swab

In this clinical trial with fresh samples, 200 samples were taken from RT-PCR positive or negative patients. Among them,100 cases of "positive group" Nasopharyngeal/Oropharyngeal samples and 100 Nasopharyngeal/Oropharyngeal samples of "negative group" were determined by nucleic acid detection (RT-PCR). Among them 100 positive nasal samples and 100 negative nasal samples were detected by the assessment reagent. The positive coincidencerate o ssment reagent was 98 % and the negative coincidence rate was 100%.

Conclusion:

In summary, the detection results of CHIL Covid-19 Antigen Rapid Test Kit developed by CHILTıbbi Mal. San. Tic. Ltd. Sti and the nucleic acid detection (RT-PCR) results are in good agreement, and the SARS-CoV-2 antigen detection function can meet the needs of clinical application.

SIGNATURE