Clinical Validation Report

Product Name: COVID-19 antigen rapid test kit

Manufacturer: BEIJING BEIER BIOENGINEERING CO., LTD.

Testing Agency: Beijing Bohao Yuntian Medical Technology Co., Ltd

Content

1. Background

A novel coronavirus(2019-nCoV) was identified in December 2019, which has resulted in over 180 millions of confirmed human infectious worldwide. On February 11, 2020 the International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2. The symptoms of COVID-19 are similar to other viral respiratory diseases and include fever, cough and shortness of breath.
Currently, the research on the Company's Covid-19 Antigen Rapid Test Kit has been completed. In order to validate its clinical suitability and accuracy, we are prepared to carry out clinical validation. Entrusted by Beijing Bohao Yuntian Medical Technology Co., Ltd. undertook the clinical trial on the Covid-19 Antigen Rapid Test Kit produced hereby in the clinical study.

2. Clinical purpose

The purpose of this research is to compare the COVID-19 Antigen Rapid Test Kit (hereinafter candidate kit) manufactured by Beijing Beier Bioengineering Co., Ltd with Novel Coronavirus (2019-nCoV) Real Time RT-PCR Kit (Real-time Fluorescent PCR method) Manufactured by Shanghai ZJ Bio-Tech Co., Ltd., to evaluate the clinical effectiveness of candidate kit.

3. Protocol

3.1 The requirement for Principal Investigator

The principal Investigator shall be professional clinical inspector and trained prior to clinical implementation.

3.2 The planned time for clinical experiment

2020.08-2020.10

3.3 The design of the clinical experiment

3.3.1 Clinical experiment site

The clinical experiment has been implemented in three hospitals (Peking Union Medical College Hospital, Shijiazhuang Fifth Hospital, and Tianjin Haihe Hospital). All the samples are collected and tested in the three hospitals listed above.

3.3.2 The information of the kit to be evaluated

Product name：COVID-19 Antigen Rapid Test Kit

Manufacturer: BEIJING BEIER BIOENGINEERING CO., LTD

3.3.3 Information of the comparator method

Product Name: Novel Coronavirus (2019-nCoV) Real Time RT-PCR Kit (Real-time Fluorescent PCR method).

Approved by NMPA（Registration Number: GXZZ 20203400057）

Approved CE marked

Manufacturer: Shanghai ZJ Bio-Tech Co., Ltd.

The Limit of Detection (LoD) of the comparator is：$1x10^3$ copies / mL The analysis specificity of the comparator is:

The comparator has no cross-reaction with influenza A (H1N1), influenza B (Yamagata), Respiratory syncytial virus (type A), adenovirus (type 2), Parainfluenza virus (type 1), Mycoplasma pneumoniae, Chlamydia pneumoniae, Bordetella pertussis, Streptococcus pneumoniae, rhinovirus (type A), Legionella pneumophila, Middle East Respiratory Syndrome coronavirus (MERSr COV), human coronavirus HCoV-299E, human coronavirus HCoV-HKU1, human coronavirus HCoV-NL63, human coronavirus HCoV-OC43, influenza A virus (H3N2), influenza A virus (H3N2),influenza A virus (H5N1), influenza A virus (H7N9), influenza B virus (Victoria), Human Metapneumovirus, EB virus, measles virus, human cytomegalovirus, rotavirus, norovirus, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Streptococcus pyogenes.

3.3.4 Instrument required for the comparator method

Product Name: MIC qPCR Cycler

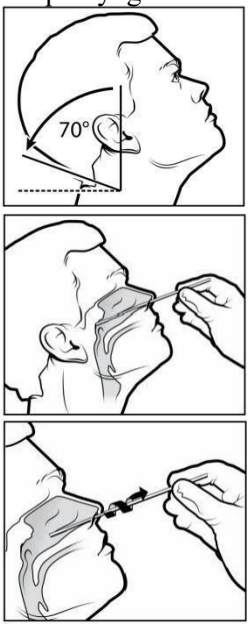
Approved by NMPA（Registration Number GXZZ 20203220013）

Manufacturer: Shanghai ZJ Bio-Tech Co., Ltd.

3.4 Requirement for the sample collection

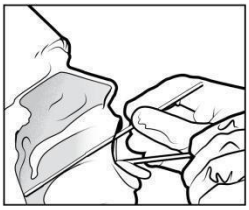
1) Method for the sample collection

Nasopharyngeal Swab Specimen Collection



1. Tilt patient's head back 70 degrees
2. Insert swab into nostril. (Swab should reach depth equal to distance from nostrils to outer opening of the ear.) Leave swab in place for several seconds to absorb secretions.
3. Slowly remove swab while rotating it. (Swab both nostrils with same swab.)

Oropharyngeal Swab Specimen Collection



1. For oropharyngeal swab, take a second dry polyester swab, insert into mouth, and swab the posterior pharynx and tonsillar areas. (Avoid the tongue.)

Specimen Transport and Storage:

Freshly collected specimen should be processed as soon as possible.
Collected swabs can be stored at -70°C for long time.

3. 5 Inclusion/exclusion criteria of sample

1) Inclusion criteria

a) specimens from patients suspected of infection of SARS-CoV-2 or other respiratory viruses.

b) Specimens positive in SARS-CoV-2 confirmed by nucleic acid assay.

c) Specimens negative in SARS-CoV-2 confirmed by nucleic acid assay from different symptoms.

d) Specimens from the patient with the symptoms of fever, cough, shortness of breath, and dyspnea.

e) the information of specimens is integrated.

f) the specimens are not limited in sex, age, territory, race.

g) the specimen types are oropharyngeal or nasopharyngeal swabs.

2) Exclusion criteria:

a) specimen contaminated by micro organism;

b) the specimen's storage condition not conform to the requirement stated in section 4.4.

c) the specimen volume is insufficient for the test.

d) the information of the specimen is not integrated e) the specimen type is not what is stated in section 4.4.

f) any specimen that the Principal Investigator considers not to be selected into the experiment.

3) Rejection criteria

a) the information of specimen is incorrect.

b) there is an error in the sample test, but the residual sample volume is not enough to conduct

the test again.

3.6 The requirements about the sample number

The samples number positive in SARS-CoV-2 should not less than 100. The samples negative in SARS-CoV-2 should not less than 200.

3.7 Sample confirmation method:

The samples infection status is confirmed by clinical symptoms (fever and / or respiratory symptoms) and RT-PCR test results.

3.8 The rule of the sample randomization and the blind method

The experiment is planned to conduct in method of blind experiment.

3.9 Statistical method

The results of the kit to be evaluated and the comparator method were expressed by table listed below (table 1). The Percent Positive Agreement, Negative Percent Agreement, total Percent Agreement, confidence interval and kappa value (K) for the kit to be evaluated to the comparator method were calculated.

Table 1 Test results of kit to be evaluated and comparator method

Kit to be evaluated	Comparator method		Total
	Positive(+)	Negative(-)	
Positive(+)	a	b	a+b
Negative(-)	c	d	c+d
Total	a+c	b+d	a+b+c+d

1) Positive Percent Agreement：a/（a＋c）×100%

The 95% confidence interval of the Positive Percent Agreement:

Q1=2×a+1.96^2,

Q3=2×（a+c+1.96^2）

$$Q2 = 1.96 \times \sqrt{1.96^2 + \frac{4 \times a \times c}{(a + c)}}$$

The lower limit of the 95% confidence interval of the Positive Percent Agreement：

(Q1-Q2）/Q3×100%

The upper limit of the 95% confidence interval of the Positive Percent Agreement:
(Q1+Q2)/Q3×100%

2) Negative Percent Agreement：d/（b＋d）×100％

The 95% confidence interval of the Negative Percent Agreement:

Q1=2×d+1.96^2,

$$Q2 = 1.96 \times \sqrt{1.96^2 + \frac{4 \times d \times b}{(d + b)}}$$

Q3=2×（d+b+1.96^2）

The lower limit of the 95% confidence interval of the Negative Percent

Agreement：（Q1-Q2）/Q3×100%

The upper limit of the 95% confidence interval of the Negative Percent

Agreement：（Q1+Q2）/Q3×100%.

3) Total Percent Agreement：（a＋d）/（a＋b＋c＋d）×100%

The 95% confidence interval of the total Percent Agreement:

Q1=2×（a+d）+1.96^2,

$$Q2 = 1.96 \times \sqrt{1.96^2 + \frac{4 \times (a + d) \times (b + c)}{n}}$$

Q3=2×(n+1.96^2）

The lower limit of the 95% confidence interval of the total Percent Agreement：

（Q1-Q2) /Q3×100%

The upper limit of the 95% confidence interval of the total Percent Agreement：

（Q1+Q2) /Q3×100%。

4) kappa value (K) is calculated with the formula:

$$\text{Kappa(K)} = \frac{2(ad - bc)}{(a+b)(b+d) + (a+c)(c+d)}$$

3.10 Acceptable criteria

Positive Percent Agreement should be ≥80%;

Negative Percent Agreement should be ≥98%;

The total Percent Agreement should be ≥90%;

Kappa should be ≥0.9;

3.11 The operation method

3.11.1 The operation method of the kit to be evaluated

Allow the Test cassette, Sample lysis and specimens to equilibrate to temperature (15-30℃ or 59-86°F) before testing.

1) Bring the Test cassette to room temperature before opening the foil pouch.

2) Place the test cassette on a clean and level surface.

3) take the test strictly according to the instructions.

4) Wait for the colored line(s) to appear. Read the results in 15 -20 minutes. Do not interpret the result after exceeding 20 minutes.

3.11.2 The operation method of the comparator method

1) RNA-Extraction

Take Different brand of RNA extraction kits that are available. You may use your own extraction system or the commercial kits based on the yield. For the RNA extraction, please follow the manufacturer's instructions. The recommended extraction kits are as follows:

Nucleic Acid Isolation Kit	Cat. Number	Manufacturer

RNA Isolation Kit	ME-0010/ME-00I2	ZJ Biotech

It is noted that the negative control in this kit should be nucleic acid extracted with the same protocol for specimens. The positive control doesn't need to be nucleic acid extracted.

2) Internal Control

The internal control in this kit should be added into the extraction mixture with lμl/rxn to monitor the whole process.

3) RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:

The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and samples prepared. Molecular Grade Water is used as the negative control. For reasons of imprecise pipetting, always add an extra virtual sample. Mix completely and then spin down briefly with a centrifuge.

Pipet 20μl Master Mix with micropipettes of sterile filter tips to each of the Real Time PCR reaction well. add 5μl sample (nucleic acid extracted from negative control and specimen, positive control with no extraction) to different well respectively. close the plates/tubes with the cap immediately. to avoid contamination.

Spin down briefly to collect the Master Mix in the bottom of the reaction tubes.

4) Perform the fallowing protocol in the instrument MIC POC Dx48：

Selection of Fluorescence Channels	
FAM	ORF1ab
HEX/VIC/JOE	Gene N
Cal Red 610/ROX/TEXAS RED	Gene E
Cy5	IC

45°C for 10min	1 cycle
95°C for 90sec	1 cycle
95°C for 3sec, 58°C for 20sec (Fluorescence measured at 58°C)	45cycles

5) Threshold Setting: Just above the maximum level of molecular grade water.

6) Quality Control: Negative Control and Positive Control must be performed correctly. Failing which the sample results will be invalid.

Control Channel	Ct Value			
	FAM	HEX/VIC/JOE	Cal Red 610	Cy5

	(Gene ORF1ab)	(Gene N)	（Gene E）	
Negative control	UNDET	UNDET	UNDET	25-40
Positive control	≤35	≤35	≤35	≤35

3.12 The training for clinical experiment

To ensure that the clinical experiment can be implemented correctly, the operation training was provided by BEIJING BEIER BIOENGINEERING CO., LTD., prior to the clinical trial.

3.13 Quality control method the clinical experiment

The CRC will inspect the clinical trials periodically. The main work of the CRC includes:

(1) if the clinical trial is carried out according to the clinical experiment protocol;

(2) if record of the date is consistent with the test result;

(3) if the record of the date is integrated.

3.14 Notes on ethical matters

The specimens involved in this clinical trial are all the specimens for the clinical institution's test and has no risk to the subjects, and the test results of this trial will not be reported to the subjects. Therefore, the subjects will not face any risk of false negative or false positive results. The subjects also did not need to do additional examination and do not need to pay additional fees. In the clinical trial, we only analyze the data of the enrolled specimens. We will check the original data only to confirm the authenticity of the specimen source, code all the enrolled specimens, hide the name, medical record number and other information of the subject and fully guarantee the privacy of the subject. Therefore, we apply to the Ethics Committee for exemption from informed consent.

4. Clinical Performance Study Report

4.1 The implemented Time of the Clinical Experiment

2020.08.1~ 2020.10.05

4.2 The information of the reagent and the instrument

4.2.1 The information of the kit to be evaluated

Product name：COVID-19 antigen rapid test kit

Manufacturer：BEIJING BEIER BIOENGINEERING CO., LTD

Model: 20Tests / Kit Lot: 20200301

Date of expiry: 09/11/2021

4.2.2 Information of the comparator method

Product Name: Novel Coronavirus(2019-nCoV) Real Time RT-PCR Kit (Real-time Fluorescent PCR method).

Manufacturer: Shanghai ZJ Bio-Tech Co., Ltd. Registration No: GXZZ 20203400057

Model: 25Tests / kit

Lot: P20200801

Date of expiry: 01/31/2021

The reagent required but not provide for the comparator method

Product Name: Autrax automatic nucleic acid detection system

Registration No：HXZZ 20172410039

Manufacturer：Shanghai ZJ Bio-Tech Co., Ltd. Model: Autrax192

The instrument required for the comparator method

Product Name: MIC qPCR Cycler

Approved by NMPA（Registration No:GXZZ 20203220013）

Manufacturer: Shanghai ZJ Bio-Tech Co., Ltd.

4.3 The distribution of the enrolled samples

There are 303 negative clinical samples and 171 positive clinical samples collected from patients with signs and symptoms of an upper respiratory infection. The information about sample is listed in table below

Table : State of sample

State of sample	Sample Type		Total
	NP	OP	
Positive	109	62	171
Negative	182	121	303

Total	291	183	474

4.4 The Statistical analysis results

4.4.1 The results of evaluated antigen kit and comparator PCR kit of positive sample

Candidate test kit		Comparator test kit	
Positive	Negative	CT value	Sample Size
76	0	< 25	76
65	0	25 < CT < 28	65
23	3	28 < CT < 31	26
1	3	31 < CT < 34	4
		> 34	0
165	6		171

4.4.2 Coincidence rate between evaluated antigen kit and PCR kit

1) For the Nasopharyngeal specimen

		PCR Kit		Total
		Positive	Negative	
Candidate kit	Positive	106	1	107
	Negative	3	181	184
Total		109	182	291

Results and analysis:

Sensitivity=97.2%(95% CI:94.6%-99.7%)

Specificity=99.5%(95% CI: 98.9%-99.9%)

Positive predictive values= 99.1% (95% CI: 97.7%-99.9%)

Negative predictive values= 98.4% (95% CI: 95.3%-99.2%)

Total consistent=98.6%(95% CI: 96.7%-99.5%)

2) For the Oropharyngeal specimen

		PCR Kit		Total
		Positive	Negative	
Candidate kit	Positive	59	0	59
	Negative	3	121	124
Total		62	121	183

Sensitivity=95.2%(95% CI: 92.5%-97.6%)

Specificity=100%(95% CI: 99.0%-100%)

Positive predictive values= 100% (95% CI: 99.1% -100%)

Negative predictive values=97.6%(95% CI:93.7%-98.6%)

Total consistent=98.4%(95% CI: 96.2%-99.5%)

3) For the Nasopharyngeal specimen and oropharyngeal specimen together:

		PCR Kit		Total
		Positive	Negative	
Candidate kit	Positive	165	1	166
	Negative	6	302	308
Total		171	303	474

Sensitivity=96.5%(95% CI: 93.7-99.3%)

Specificity=99.7%(95% CI: 99.0%-100%)

Positive predictive values= 99.4% (95% CI: 98.2% -100%)

Negative predictive values= 98.0% (95% CI: 96.5%-99.6%)

Total consistent=98.5%(95% CI:97.4%-99.6%)

5. Conclusion

In the present study, the Covid-19 Antigen Rapid Test Kit manufactured by Beijing Beier Bioengineering Co.,Ltd has shown high sensitivity, specificity and total accuracy on

nasopharyngeal samples and oropharyngeal samples.

In summary, the Covid-19 Antigen Rapid Test Kit has shown satisfying sensitivity, specificity, and total accuracy in the present evaluation. It can be used as a rapid tool to assist the early diagnosis of Covid-19 cases.

Appendix : Clinical Trace Data

No.	Specimen type	Ag results	Specimen type for RT-PCR	RT-PCR name	RT-PCR results
1	NP	Positive	NP	MIC-qPCR	Positive
2	NP	Positive	NP	MIC-qPCR	Positive
3	NP	Positive	NP	MIC-qPCR	Positive
4	NP	Positive	NP	MIC-qPCR	Positive
5	NP	Positive	NP	MIC-qPCR	Positive
6	NP	Positive	NP	MIC-qPCR	Positive
7	NP	Positive	NP	MIC-qPCR	Positive
8	NP	Positive	NP	MIC-qPCR	Positive
9	NP	Positive	NP	MIC-qPCR	Positive
10	NP	Positive	NP	MIC-qPCR	Positive
11	NP	Positive	NP	MIC-qPCR	Positive
12	NP	Positive	NP	MIC-qPCR	Positive
13	NP	Positive	NP	MIC-qPCR	Positive
14	NP	Positive	NP	MIC-qPCR	Positive
15	NP	Positive	NP	MIC-qPCR	Positive
16	NP	Positive	NP	MIC-qPCR	Positive
17	NP	Positive	NP	MIC-qPCR	Positive
18	NP	Positive	NP	MIC-qPCR	Positive
19	NP	Positive	NP	MIC-qPCR	Positive
20	NP	Positive	NP	MIC-qPCR	Positive

21	OP	Negative	NP	MIC-qPCR	Positive
22	OP	Positive	NP	MIC-qPCR	Positive
23	OP	Positive	NP	MIC-qPCR	Positive
24	OP	Positive	NP	MIC-qPCR	Positive
25	OP	Positive	NP	MIC-qPCR	Positive
26	OP	Positive	NP	MIC-qPCR	Positive
27	OP	Positive	NP	MIC-qPCR	Positive
28	OP	Positive	NP	MIC-qPCR	Positive
29	OP	Positive	NP	MIC-qPCR	Positive
30	OP	Positive	NP	MIC-qPCR	Positive
31	OP	Positive	NP	MIC-qPCR	Positive
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37	OP	Positive	NP	MIC-qPCR	Positive
38	OP	Positive	NP	MIC-qPCR	Positive
39	OP	Positive	NP	MIC-qPCR	Positive
40	OP	Positive	NP	MIC-qPCR	Positive
41	NP	Positive	NP	MIC-qPCR	Positive
42	NP	Positive	NP	MIC-qPCR	Positive
43	NP	Positive	NP	MIC-qPCR	Positive
44	NP	Positive	NP	MIC-qPCR	Positive
45	NP	Positive	NP	MIC-qPCR	Positive

46	NP	Positive	NP	MIC-qPCR	Positive
47	NP	Positive	NP	MIC-qPCR	Positive
48	NP	Positive	NP	MIC-qPCR	Positive
49	NP	Positive	NP	MIC-qPCR	Positive
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66	NP	Positive	NP	MIC-qPCR	Positive
67	NP	Positive	NP	MIC-qPCR	Positive
68	NP	Positive	NP	MIC-qPCR	Positive
69	NP	Positive	NP	MIC-qPCR	Positive
70	OP	Negative	NP	MIC-qPCR	Positive

71	NP	Positive	NP	MIC-qPCR	Positive
72	NP	Positive	NP	MIC-qPCR	Positive
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75	OP	Positive	NP	MIC-qPCR	Positive
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138	NP	Positive	NP	MIC-qPCR	Positive
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141	OP	Positive	NP	MIC-qPCR	Positive
142	OP	Positive	NP	MIC-qPCR	Positive
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171	NP	Positive	NP	MIC-qPCR	Positive