

Clinical Validation Report

Product Name: COVID-19 antigen rapid test kit (Short Nose)

Manufacturer: BEIJING BEIER BIOENGINEERING CO.,LTD

Testing agency: Tianjin Haihe Hospital



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Introduction

A novel coronavirus (2019-nCoV) was identified in December 2019, which has resulted in hundreds of thousands of confirmed human infections 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, shortness of breath. The SARS-CoV-2 virus has four structural proteins known as S (spike), E (envelope), M (membrane) and N (nucleocapsid) proteins; the N protein holds the RNA genome and the S, E and M proteins together form the viral envelope.

Currently, the research on the Company's Covid-19 Antigen Rapid Test Kit (Short Nose) has been completed. In order to validate its clinical suitability and accuracy, we are prepared to carry out clinical validation. Entrusted by Beijing Beier Bioengineering Co., Ltd, Tianjin Haihe Hospital undertook the clinical trial on the Covid-19 Antigen Rapid Test Kit (Short Nose) produced hereby in the clinical study.

1. The protocol for clinical experiment

1.1 Basic information for clinical experiment

1.1.1 The purpose for clinical experiment

To evaluate the diagnosis Performance of COVID-19 Antigen Rapid Test kit (Short nose) manufactured by BEIJING BEIER BIOENGINEERING CO., LTD, take the kit of Novel Coronavirus(2019-nCoV) Real Time RT-PCR Kit (Real-time Fluorescent PCR method) Manufactured by Shanghai ZJ Bio-Tech Co., Ltd. as comparator method. Test the samples with the kit manufactured by BEIJING BEIER BIOENGINEERING CO., LTD and the comparator method in parallel, and then evaluate the Percent Positive Agreement and Negative Percent Agreement of the kit to be evaluated.

1.1.2 The requirement for Principal Investigator

The Principal Investigator shall be profession clinical Inspector, and trained prior to clinical implementation.

They are at least two operators in each clinical experiment sit, one operator to number the sample, another to test the sample, and record the result.

1.1.3 The planned time for clinical experiment

2020.10~ 2021.1

1.2 The design of the clinical experiment

1.2.1 Clinical experiment site

The clinical trail is conducted by Tianjin Haihe Hospital. As the applicant, Beijing Beier Bioengineering Co.,Ltd was responsible for communication and contact during the clinical trail.

1.2.2 The information of the kit to be evaluated

Product name: COVID-19 Antigen Rapid Test kit(Short nose)

Manufacturer: BEIJING BEIER BIOENGINEERING CO., LTD

1.2.3 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: 1×10^3 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.

1.2.4 The 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.

1.3 Requirement for the sample collection

- 1) Method for the sample collection
Nasal Swab Specimen Collection

The nasal swab specimen collection method : Insert the swab tip up to 2.5cm(1 inch) from edge of the nostril. Roll the swab along the mucosa inside the nostril 5 times for both nasal cavities. Withdraw the swab from the nasal cavity.

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.

1.4 Inclusion/exclusion criteria of sample

- 1) Inclusion criteria
- a) specimens from patients suspected 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 nasal swabs.

2)Exclusion criteria:

- a.)specimen contaminated by microorganism;
- b.) the specimen's storage condition not conform to the requirement state 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 state in section 4.4.
- f.)any specimen that the Principal Investigator think it not 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.

1.5 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.

1.6 Sample confirmation method:

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

1.7 The rule of the sample randomization and the blind method

The experiment is planned to conduct in method of single blind experiment. The sample numbered randomly by one operator, test and record by another operator.

1.8 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: $a / (a+c) \times 100 \%$

The 95% confidence interval of the Positive Percent Agreement: $Q1=2 \times a + 1.96^2$,

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

$Q3=2 \times (a+c+1.96^2)$

The lower limit of the 95% confidence interval of the Positive Percent Agreement:
 $(Q1-Q2) / Q3 \times 100\%$

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

2)Negative Percent Agreement: $d / (b+d) \times 100 \%$

The 95% confidence interval of the Negative Percent Agreement:

$$Q1=2 \times d + 1.96^2,$$

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

$$Q3 = 2 \times (d + b + 1.96^2)$$

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

Agreement: $(Q1 - Q2) / Q3 \times 100\%$

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

$$(Q1 + Q2) / Q3 \times 100\%$$

3) Total Percent Agreement: $(a + d) / (a + b + c + d)$

$\times 100\%$ The 95% confidence interval of the total Percent Agreement:

$$Q1 = 2 \times (a + d) + 1.96^2,$$

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

$$Q3 = 2 \times (n + 1.96^2)$$

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

$$(Q1 - Q2) / Q3 \times 100\%$$

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

$$(Q1 + Q2) / Q3 \times 100\%$$

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

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

1.9 Acceptable criteria

Positive Percent Agreement should $\geq 80\%$;

Negative Percent Agreement should $\geq 98\%$;

The total Percent Agreement should $\geq 90\%$; Kappa should ≥ 0.9 ;

1.10 The operation method

1.10.1 The operation method of the kit to be evaluated

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

- 1) Bring the Test cassette to room temperature before opening the foil pouch. The test cassette should be used within 1 hour after take out from the sealed pouch.
- 2) Place the test cassette on a clean and level surface.
- 3) Take the test according to instruction strictly.
- 4) Wait for the colored line(s) to appear. Read the results in 15 -20 minutes. Do not interpret the result exceed 20 minutes.

1.10.2 The operation method of the comparator method

1) RNA-Extraction

Take Different brand RNA extraction kits are available. You may use your own extraction systems 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-0012 1	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 1µl/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 following protocol in the instrument MIC POC Dx48:

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

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; otherwise the sample results are invalid.

Control Channel	Ct value			
	FAM(Gene ORF1ab)	HEX/VIC/JOE (Gene N)	Cal Red 610 (Gene E)	Cy5
Negative control	UNDET	UNDET	UNDET	25-40
Positive control	≤35	≤35	≤35	≤35

1.11 The train for clinical experiment

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

1.12 Quality control method the clinical experiment

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

- 1)whether the clinical trial is carried out according to the clinical experiment protocol;
- 2)whether record of the date is consistent with the test result;
- 3)whether the record of the date is integrated

1.13 Notes on ethical matters

The specimens involved in this clinical trial are all the specimens for the clinical institution's test, has nothing 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 did 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. so we apply to the Ethics Committee for exemption from informed consent.

2.Clinical Performance study report

2.1 The implemented time of the clinical experiment

2020.10.6~ 2021.1.22

2.2 The information of the reagent and the instrument

2.2.1 The information of the kit to be evaluated

Product name: COVID-19 antigen rapid test kit(Short Nose)
Manufacturer: BEIJING BEIER BIOENGINEERING CO.,LTD
Model: 20Tests / Kit
Lot:20200801
Date of expiry: 04/30/2022

2.2.2 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

2.3 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. Each of clinical swab samples by nasal swab, and confirmed by RT-PCR The information about sample is listed in table below.

Table : State of sample

State of sample	Nasal swab
Positive	171
Negative	303

2.4 The Statistical analysis results

2.4.1 The results of evaluated antigen kit and comparator nucleic acid kit of positive sample

Evaluated antigen kit		Comparator nucleic acid kit	
Positive	negative	CT value	number
76	0	<25	76
65	0	25 <CT < 28	65
65	0	25 <CT < 28	65
23	3	28 <CT < 31	26
1	3	31 <CT < 34	4
165	6		171

2.4.2 The results of evaluated antigen kit and comparator nucleic acid kit of negative sample

evaluated antigen kit		comparator nucleic acid kit	
Positive	negative	CT value	Number
1	302	Negative	303

2.4.3 Coincidence rate between evaluated antigen kit and comparator nucleic acid kit

Reagents		Clinical diagnosis (nucleic acid Reagents)		Total
		Positive	Negative	
	Positive	165	1	166

Beier Reagents	Negative	6	302	308
Total		171	303	474

Results analysis:

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%)

2.5 Conclusion

Compared with the Test Kit and Novel Coronavirus(2019-nCoV) Real Time RT-PCR Kit (Real-time Fluorescent PCR method) Manufactured by Shanghai ZJ Bio-Tech Co,Ltd. The test results show that the diagnostic specificity, diagnostic sensitivity, total coincidence rate percentage of the Test Kit are relatively high, meet the requirement of the test kit.

Appendix A-Information For samples of Clinical Trace

Serial number	Sample Type Antigen	Beier Kit Result	COVID-19 Diagnostic	Name of RT-PCR	RT-PCR Result
1	nasal	+	+	MIC-qPCR	+
2	nasal	+	+	MIC-qPCR	+
3	nasal	+	+	MIC-qPCR	+
4	nasal	+	+	MIC-qPCR	+
5	nasal	+	+	MIC-qPCR	+
6	nasal	+	+	MIC-qPCR	+
7	nasal	+	+	MIC-qPCR	+
8	nasal	+	+	MIC-qPCR	+
9	nasal	+	+	MIC-qPCR	+
10	nasal	+	+	MIC-qPCR	+
11	nasal	+	+	MIC-qPCR	+
12	nasal	+	+	MIC-qPCR	+
13	nasal	+	+	MIC-qPCR	+
14	nasal	+	+	MIC-qPCR	+
15	nasal	+	+	MIC-qPCR	+
16	nasal	+	+	MIC-qPCR	+
17	nasal	+	+	MIC-qPCR	+
18	nasal	+	+	MIC-qPCR	+
19	nasal	+	+	MIC-qPCR	+
20	nasal	+	+	MIC-qPCR	+
21	nasal	-	+	MIC-qPCR	+
22	nasal	+	+	MIC-qPCR	+
23	nasal	+	+	MIC-qPCR	+
24	nasal	+	+	MIC-qPCR	+
25	nasal	+	+	MIC-qPCR	+
26	nasal	+	+	MIC-qPCR	+
27	nasal	+	+	MIC-qPCR	+
28	nasal	+	+	MIC-qPCR	+
29	nasal	+	+	MIC-qPCR	+
30	nasal	+	+	MIC-qPCR	+
31	nasal	+	+	MIC-qPCR	+
32	nasal	+	+	MIC-qPCR	+
33	nasal	+	+	MIC-qPCR	+

34	nasal	+	+	MIC-qPCR	+
35	nasal	+	+	MIC-qPCR	+
36	nasal	+	+	MIC-qPCR	+
37	nasal	+	+	MIC-qPCR	+
38	nasal	+	+	MIC-qPCR	+
39	nasal	+	+	MIC-qPCR	+
40	nasal	+	+	MIC-qPCR	+
41	nasal	+	+	MIC-qPCR	+
42	nasal	+	+	MIC-qPCR	+
43	nasal	+	+	MIC-qPCR	+
44	nasal	+	+	MIC-qPCR	+
45	nasal	+	+	MIC-qPCR	+
46	nasal	+	+	MIC-qPCR	+
47	nasal	+	+	MIC-qPCR	+
48	nasal	+	+	MIC-qPCR	+
49	nasal	+	+	MIC-qPCR	+
50	nasal	+	+	MIC-qPCR	+
51	nasal	+	+	MIC-qPCR	+
52	nasal	-	+	MIC-qPCR	+
53	nasal	+	+	MIC-qPCR	+
54	nasal	+	+	MIC-qPCR	+
55	nasal	+	+	MIC-qPCR	+
56	nasal	+	+	MIC-qPCR	+
57	nasal	+	+	MIC-qPCR	+
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64	nasal	+	+	MIC-qPCR	+
65	nasal	+	+	MIC-qPCR	+
66	nasal	+	+	MIC-qPCR	+
67	nasal	+	+	MIC-qPCR	+
68	nasal	+	+	MIC-qPCR	+
69	nasal	+	+	MIC-qPCR	+
70	nasal	-	+	MIC-qPCR	+
71	nasal	+	+	MIC-qPCR	+

72	nasal	+	+	MIC-qPCR	+
73	nasal	+	+	MIC-qPCR	+
74	nasal	+	+	MIC-qPCR	+
75	nasal	+	+	MIC-qPCR	+
76	nasal	+	+	MIC-qPCR	+
77	nasal	+	+	MIC-qPCR	+
78	nasal	+	+	MIC-qPCR	+
79	nasal	+	+	MIC-qPCR	+
80	nasal	+	+	MIC-qPCR	+
81	nasal	+	+	MIC-qPCR	+
82	nasal	+	+	MIC-qPCR	+
83	nasal	+	+	MIC-qPCR	+
84	nasal	+	+	MIC-qPCR	+
85	nasal	-	+	MIC-qPCR	+
86	nasal	+	+	MIC-qPCR	+
87	nasal	+	+	MIC-qPCR	+
88	nasal	+	+	MIC-qPCR	+
89	nasal	+	+	MIC-qPCR	+
90	nasal	+	+	MIC-qPCR	+
91	nasal	+	+	MIC-qPCR	+
92	nasal	+	+	MIC-qPCR	+
93	nasal	+	+	MIC-qPCR	+
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96	nasal	+	+	MIC-qPCR	+
97	nasal	+	+	MIC-qPCR	+
98	nasal	+	+	MIC-qPCR	+
99	nasal	+	+	MIC-qPCR	+
100	nasal	+	+	MIC-qPCR	+
101	nasal	-	+	MIC-qPCR	+
102	nasal	+	+	MIC-qPCR	+
103	nasal	+	+	MIC-qPCR	+
104	nasal	+	+	MIC-qPCR	+
105	nasal	+	+	MIC-qPCR	+
106	nasal	+	+	MIC-qPCR	+
107	nasal	+	+	MIC-qPCR	+
108	nasal	+	+	MIC-qPCR	+
109	nasal	+	+	MIC-qPCR	+
110	nasal	+	+	MIC-qPCR	+

111	nasal	+	+	MIC-qPCR	+
112	nasal	+	+	MIC-qPCR	+
113	nasal	+	+	MIC-qPCR	+
114	nasal	+	+	MIC-qPCR	+
115	nasal	+	+	MIC-qPCR	+
116	nasal	+	+	MIC-qPCR	+
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123	nasal	+	+	MIC-qPCR	+
124	nasal	+	+	MIC-qPCR	+
125	nasal	+	+	MIC-qPCR	+
126	nasal	+	+	MIC-qPCR	+
127	nasal	+	+	MIC-qPCR	+
128	nasal	+	+	MIC-qPCR	+
129	nasal	+	+	MIC-qPCR	+
130	nasal	+	+	MIC-qPCR	+
131	nasal	+	+	MIC-qPCR	+
132	nasal	+	+	MIC-qPCR	+
133	nasal	-	+	MIC-qPCR	+
134	nasal	+	+	MIC-qPCR	+
135	nasal	+	+	MIC-qPCR	+
136	nasal	+	+	MIC-qPCR	+
137	nasal	+	+	MIC-qPCR	+
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144	nasal	+	+	MIC-qPCR	+
145	nasal	+	+	MIC-qPCR	+
146	nasal	+	+	MIC-qPCR	+
147	nasal	+	+	MIC-qPCR	+
148	nasal	+	+	MIC-qPCR	+
149	nasal	+	+	MIC-qPCR	+

150	nasal	+	+	MIC-qPCR	+
151	nasal	+	+	MIC-qPCR	+
152	nasal	+	+	MIC-qPCR	+
153	nasal	+	+	MIC-qPCR	+
154	nasal	+	+	MIC-qPCR	+
155	nasal	+	+	MIC-qPCR	+
156	nasal	+	+	MIC-qPCR	+
157	nasal	+	+	MIC-qPCR	+
158	nasal	+	+	MIC-qPCR	+
159	nasal	+	+	MIC-qPCR	+
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163	nasal	+	+	MIC-qPCR	+
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165	nasal	+	+	MIC-qPCR	+
166	nasal	+	+	MIC-qPCR	+
167	nasal	+	+	MIC-qPCR	+
168	nasal	+	+	MIC-qPCR	+
169	nasal	+	+	MIC-qPCR	+
170	nasal	+	+	MIC-qPCR	+
171	nasal	+	+	MIC-qPCR	+