

Clinical Validation Report of Coronavirus (Sars-Cov-2) Antigen Rapid Test Device (Saliva)

Product name: Coronavirus (Sars-Cov-2) Antigen Rapid Test Device (Saliva)

Manufacturer: Innovation Biotech (Beijing) Co., Ltd

1. Background information for clinical evaluation

The SARS-CoV-2 belong to the genus Nestovirus, Coronaviridae, and is divided into three genera: α , β , and γ . The genus α and β are only pathogenic to mammals. COVID-19 is an acute respiratory infectious disease infected with SARS-CoV-2. 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.

The Coronavirus (Sars-Cov-2) Antigen Rapid Test Device (Saliva) Test Device developed by Innovation Biotech (Beijing) Co., Ltd is a rapid chromatographic immunoassay for the qualitative detection of specific antigens to SARS-CoV-2 present in human saliva. The test provides more convenient technical support for timely blocking the spread of the virus.

2. Test purposes

To verify the accuracy and the reliability of the Coronavirus (Sars-Cov-2) Antigen Rapid Test Device (Saliva) produced by Innovation Biotech (Beijing) Co., Ltd. using the clinical specimen.

3. Test design

3.1 Test plan

Coronavirus (Sars-Cov-2) Antigen Rapid Test Device (Saliva) are used to conduct comparative research tests on clinically suspected COVID-19, and to prove that the Coronavirus (Sars-Cov-2) Antigen Rapid Test Device (Saliva) can be used to detect antigen in the patient with the SAR-Cov-2 infection with symptom onset of 3-7 days. The test results will be compared to reference PCR (BGI Genomics Co. Ltd.) results.

3.2 Testing Site

3.2.1 Jiangsu Provincial Center for Disease Control and Prevention

Director: Zhu Fengcai, Operators: Tan zhongmin, Bao changjun

3.2.2 Kangwon National University Hospital (Korea)

Director: Dr. Lee, Operators: Sue Kim

samples are classified into the potential positive group (symptomatic and asymptomatic) and the negative group (asymptomatic) as per the test results of the reference PCR. All sample were retrospectively collected either from hospitalized COVID-19 patients or during routine hospital visits.

Enrollment criteria: Samples must be greater than 100uL. If either is the case, sample volume less than 100uL, the sample will be excluded.

The test results of both the Antigen test strip and the reference PCR method shall be compared with statistical analysis.

The reference method is PCR for SARS-CoV-2 nucleic acids in nasal swabs and/or bronchoalveolar lavage fluid (BALF). The name of the PCR test: Real-Time Fluorescent RT-PCR Kit for Detecting SARS-nCoV-2.

The manufacture information of PCR test: BGI Genomics Co.,Ltd.

LOD:100copies/mL

3.3 Sample collection, processing and storage

Sample collection: human saliva has been demonstrated suitable for COVID-19 antigen test strip in the bench study. Collect saliva sample following the procedure described in the instruction for use (IFU).

Sample processing: Before testing, slowly return the refrigerated or frozen samples to room temperature and mix them carefully.

Sample storage: For the samples to be tested short term, storage was at 2-8°C for max 7days. For long-term storage, store at -20°C. Avoid repeated freeze-thaw samples.

4. In vitro diagnostic reagents and reference products for testing

4.1 Test in vitro diagnostic reagents

Name: Coronavirus (Sars-Cov-2)

Antigen Rapid Test Device (Saliva)

4.2 Cat#: CO-03, (20 tests/kit)

LOT: 20201011

Expiry: Oct, 2022

Storage Conditions: Store in a dry place at 2-30°C, protected from light. Once the cassette pouch is open, it should be used within 30 minutes. The cassette will become invalid due to moisture absorption.

4.3 Reference reagents

The reference method is PCR, SARS-CoV-2 nucleic acids in nasal swabs from swab samples. The name of the PCR test: Real-Time Fluorescent RT-PCR Kit for Detecting SARS-nCoV-2.

The manufacture: BGI Genomics Co., Ltd. LOD:100copies/mL

5. Experiment method

5.1 Following product instruction, A total of 335 samples including 200 negatives and 135 positives from patients hospitalized or routine visit with symptom onset within 7 days, who were confirmed with PCR reference method.

5.2 Each sample was blind labeled and tested in random order.

5.3 The testing procedure follows below. For details, please refer to the product instruction:

Step 1: If the sample is stored refrigerated or frozen, let the samples to equilibrate to room temperature (15-30°C). After thawing, mix the samples thoroughly before testing.

Step 2: When prepared for the test, open the aluminum foil pouch and remove the Cassette. Place the Cassette flat on a horizontal table.

Step 3: Label the sample number on the Cassette.

Step 5: Using a timer wait 10 minutes, then read the results. Do not read after 15 minutes.

Note: The test steps need to be completed under protection against infection.

6. Statistical methods of statistical analysis of clinical research data

6.1 Methods evaluating clinical performance

Whether various indexes can reach the standards of clinical evaluation shall be judged by calculating the consistency percentage of negative/positive and the total consistency percentage in the test results of the product tested and the reference product, to validate the accuracy and applicability of the product in clinical applications. The product tested shall be subject to tests through the sample of different types, with statistics on the results. Meanwhile, different types of sample

of the subjects shall be subject to determination by the product tested synchronously, and then the determination results of both shall be compared. The test results recorded shall be subject to statistical analysis upon completion of determination of all clinical samples, to calculate the consistency percentage of negative/positive and the total consistency percentage. Afterwards, equivalence of both shall be evaluated as per these statistical indexes

6.2 Statistical method

The products launched on the market shall be subject to comparative study and evaluation. Kappa inspection: each sample shall be tested with the product and the reference PCR product respectively, and then the consistency in statistical results of these two inspection methods shall be compared through Kappa inspection.

The data shall be subjected to Kappa inspection and analysis and the Kappa coefficient shall be calculated. Favorable consistency can be proven if Kappa is > 0.8. The consistency in test results of the product and the reference PCR product are evaluated as per the evaluation standards.

7. Criteria of clinical evaluation

The coincidence rate shall be calculated by comparing with the reference product. The product performance shall meet the following requirements.

Coincidence rate of negative: More than 90% of the samples tested should indicate negative results for both the product and the reference PCR product.

Coincidence rate of positive: More than 90% of the samples tested should indicate positive results for both the product and the reference PCR product.

8. Provisions for amendments to clinical validation

In general, the clinical validation should not be changed. Any modification to the project during the test must be documented and explained. The time, reason for the change, process of the change, and whether there is a record of the change need to be explained in detail and its impact on the entire research result explained.

9. Results and Analysis of Clinical Tests

In total, 200 normal negative and 135 potential positive symptomatic and asymptomatic patient candidates saliva and nasal swab samples were collected. The test results are summarized in the table below:

Table 1. The result for accuracy

		Coronavirus (SARS-Cov-2) Antigen rapid test device (saliva)		Total Result
		+	-	
PCR	+	129	6	135
	-	0	200	200
Total Results		129	206	335

As summarized in the above table, all 200 negative specimens gave negative results; out of 135 positive specimens, 130 shown antigen positive, The result shows:

Relative sensitivity: $129/135 = 95.6\%$ (95%CI 93.79%~98.66%)

Relative specificity: $200/200 > 99\%$ (95%CI 97.12%~100%)

INVBIO agreement: $(129+200)/(129+0+6+200)*100\% = 98.21\%$ (95%CI 96.55%~99.36%)

CI: Confidence Interval

The samples include different gender and age. Most positive sample tested are in early stage of infection which is listed below.

10. Analysis on consistency in Test Results

According to the test result, there are 329 samples showing the consistency results for rapid test and RT-PCR. For qualitative rapid test, the result will show positive, negative and invalid, for RT-PCR detection, the Ct/Cq value will indicate the result, Ct/Cq value > 40 means the detection result is negative, in our validation test, the amount of both RdRP and N gene Ct/Cq value are all below 40 is 135. The median of RdRP gene Ct/Cq value is 22.9, while the RdRP gene Ct/Cq value is 23.09.

Table 2. The consistency in Test Result

Consistency result number	RdRP gene Ct/Cq value			N gene Ct/Cq value		
	Maximum	Minimum	Median	Maximum	Minimum	Median

124	34.86	14.01	22.9	35.46	14.01	23.09
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11. Analysis on Inconsistency in Test Results

6 confirmed by PCR positive samples, showed Antigen negative. This may due to the insufficient of sample collected.

Sample ID	Age	Gender	COVID-19 Ag Test	PCR	
				RdRP	N gene
NS224	45	F	-	34.17	35.46
NS257	34	F	-	>40	34.28
NS262	39	F	-	>40	34.15
NS304	32	M	-	>40	32.29
NS314	22	M	-	>40	34.22
NS329	42	M	-	>40	34.21

12. Discussion and Conclusions

12.1 Discussion

Results of comparative analysis of the product tested and the reference product:

Test results of the antigen test and the reference PCR product: both the coincidence rate of negative/positive are larger than 90%, indicating favorable consistency with the reference PCR product. In the analysis results of Kappa inspection, Kappa was proven >0.8, indicating favorable and high consistency of both methods. Both systems were considered equivalent.

12.2 Test conclusions

By analyzing the test results of the product tested and the PCR reference product, the consistency percentage of negative/positive is proven high. Moreover, according to the results of statistical analysis, there is no remarkable difference in test results of both, indicating favorable consistency in diagnosis and equivalence of two such systems. Therefore, such product is applicable to qualitative clinical analysis on the SARS-CoV-2 antigen in the human saliva, and can be used for auxiliary diagnosis of those suffering from pneumonia triggered by COVID-19.

13. Quality control methods

13.1 On-site quality control

The entire clinical evaluation is conducted under strict operation, and the test content is complete and authentic. All observations and findings in the clinical trials

have been verified and the data are reliable. The conclusions in the clinical trials are derived from the original data.

13.2 Quality control of clinical experiment process

During the evaluation, quality control was performed daily to ensure that the product was under control. Strict quality control is performed to ensure the quality of the clinical testing.

14. Prediction of adverse events

The is an in vitro diagnostic test and the test results are only used for comparative studies. It involves personal privacy, does not serve as a basis for auxiliary diagnosis, does not bring any risk to the subject.

15. References

1. The "Technical Review Points for the Registration of New Coronavirus Antigen / Antibody Detection Reagents in 2019 (Trial)" issued by the State Drug Administration Medical Device Technical Evaluation Center on February 25, 2020,
2. "Pneumonitis Diagnosis and Treatment Program for New Coronavirus Infection (Trial Version 6)" issued by the National Health Committee on February 19, 2020.

Annex : The Data of Clinical Sample Tests

Sample ID	Age	Gender	COVID-19 Ag Test	PCR		Sample ID	Age	Gender	COVID-19 Ag Test	PCR	
				RdRP	N gene					RdRP	N gene
NS1	42	F	-	>40	>40	NS101	28	F	-	>40	>40
NS2	39	M	-	>40	>40	NS102	29	M	-	>40	>40
NS3	40	M	-	>40	>40	NS103	48	F	-	>40	>40
NS4	17	F	-	>40	>40	NS104	44	M	-	>40	>40
NS5	26	M	-	>40	>40	NS105	61	F	-	>40	>40
NS6	23	F	-	>40	>40	NS106	29	M	-	>40	>40
NS7	16	M	-	>40	>40	NS107	57	F	-	>40	>40
NS8	43	F	-	>40	>40	NS108	32	M	-	>40	>40
NS9	51	M	-	>40	>40	NS109	54	M	-	>40	>40
NS10	22	M	-	>40	>40	NS110	71	F	-	>40	>40
NS11	45	F	-	>40	>40	NS111	28	F	-	>40	>40
NS12	34	F	-	>40	>40	NS112	29	M	-	>40	>40
NS13	61	F	-	>40	>40	NS113	48	F	-	>40	>40
NS14	54	M	-	>40	>40	NS114	44	M	-	>40	>40
NS15	34	F	-	>40	>40	NS115	61	F	-	>40	>40
NS16	23	M	-	>40	>40	NS116	29	M	-	>40	>40
NS17	22	M	-	>40	>40	NS117	57	F	-	>40	>40
NS18	20	F	-	>40	>40	NS118	32	M	-	>40	>40
NS19	18	M	-	>40	>40	NS119	42	F	-	>40	>40
NS20	16	F	-	>40	>40	NS120	45	F	-	>40	>40

NS21	14	M	-	>40	>40	NS121	36	M	-	>40	>40
NS22	19	F	-	>40	>40	NS122	15	F	-	>40	>40
NS23	20	M	-	>40	>40	NS123	17	M	-	>40	>40
NS24	27	M	-	>40	>40	NS124	42	F	-	>40	>40
NS25	26	F	-	>40	>40	NS125	45	F	-	>40	>40
NS26	25	F	-	>40	>40	NS126	36	M	-	>40	>40
NS27	28	F	-	>40	>40	NS127	15	F	-	>40	>40
NS28	29	M	-	>40	>40	NS128	17	M	-	>40	>40
NS29	48	F	-	>40	>40	NS129	16	F	-	>40	>40
NS30	44	M	-	>40	>40	NS130	54	M	-	>40	>40
NS31	61	F	-	>40	>40	NS131	43	F	-	>40	>40
NS32	29	M	-	>40	>40	NS132	72	M	-	>40	>40
NS33	57	F	-	>40	>40	NS133	49	F	-	>40	>40
NS34	32	M	-	>40	>40	NS134	42	F	-	>40	>40
NS35	54	M	-	>40	>40	NS135	45	F	-	>40	>40
NS36	71	F	-	>40	>40	NS136	42	F	-	>40	>40
NS37	34	M	-	>40	>40	NS137	45	F	-	>40	>40
NS38	42	F	-	>40	>40	NS138	36	M	-	>40	>40
NS39	45	F	-	>40	>40	NS139	55	F	-	>40	>40
NS40	36	M	-	>40	>40	NS140	17	M	-	>40	>40
NS41	65	F	-	>40	>40	NS141	16	F	-	>40	>40
NS42	37	M	-	>40	>40	NS142	64	M	-	>40	>40
NS43	56	F	-	>40	>40	NS143	53	F	-	>40	>40

NS44	44	M	-	>40	>40	NS144	12	M	-	>40	>40
NS45	53	F	-	>40	>40	NS145	42	F	-	>40	>40
NS46	22	M	-	>40	>40	NS146	45	F	-	>40	>40
NS47	19	F	-	>40	>40	NS147	36	M	-	>40	>40
NS48	28	F	-	>40	>40	NS148	42	F	-	>40	>40
NS49	29	M	-	>40	>40	NS149	45	F	-	>40	>40
NS50	48	F	-	>40	>40	NS150	36	M	-	>40	>40
NS51	44	M	-	>40	>40	NS151	15	F	-	>40	>40
NS52	61	F	-	>40	>40	NS152	17	M	-	>40	>40
NS53	29	M	-	>40	>40	NS153	16	F	-	>40	>40
NS54	57	F	-	>40	>40	NS154	14	M	-	>40	>40
NS55	32	M	-	>40	>40	NS155	13	F	-	>40	>40
NS56	54	M	-	>40	>40	NS156	12	M	-	>40	>40
NS57	71	F	-	>40	>40	NS157	42	F	-	>40	>40
NS58	42	F	-	>40	>40	NS158	42	F	-	>40	>40
NS59	45	F	-	>40	>40	NS159	46	F	-	>40	>40
NS60	36	M	-	>40	>40	NS160	36	M	-	>40	>40
NS61	15	F	-	>40	>40	NS161	54	F	-	>40	>40
NS62	17	M	-	>40	>40	NS162	23	M	-	>40	>40
NS63	63	F	-	>40	>40	NS163	16	F	-	>40	>40
NS64	34	M	-	>40	>40	NS164	34	M	-	>40	>40
NS65	23	F	-	>40	>40	NS165	53	F	-	>40	>40
NS66	22	M	-	>40	>40	NS166	34	M	-	>40	>40

NS67	19	F	-	>40	>40	NS167	42	F	-	>40	>40
NS68	42	F	-	>40	>40	NS168	42	F	-	>40	>40
NS69	45	F	-	>40	>40	NS169	45	F	-	>40	>40
NS70	36	M	-	>40	>40	NS170	36	M	-	>40	>40
NS71	15	F	-	>40	>40	NS171	35	F	-	>40	>40
NS72	23	M	-	>40	>40	NS172	67	M	-	>40	>40
NS73	32	F	-	>40	>40	NS173	66	F	-	>40	>40
NS74	54	M	-	>40	>40	NS174	44	M	-	>40	>40
NS75	53	F	-	>40	>40	NS175	63	F	-	>40	>40
NS76	32	M	-	>40	>40	NS176	22	M	-	>40	>40
NS77	19	F	-	>40	>40	NS177	42	F	-	>40	>40
NS78	42	F	-	>40	>40	NS178	46	F	-	>40	>40
NS79	45	F	-	>40	>40	NS179	37	M	-	>40	>40
NS80	36	M	-	>40	>40	NS180	18	F	-	>40	>40
NS81	15	F	-	>40	>40	NS181	25	M	-	>40	>40
NS82	65	M	-	>40	>40	NS182	16	F	-	>40	>40
NS83	46	F	-	>40	>40	NS183	45	M	-	>40	>40
NS84	34	M	-	>40	>40	NS184	43	F	-	>40	>40
NS85	53	F	-	>40	>40	NS185	65	M	-	>40	>40
NS86	72	M	-	>40	>40	NS186	42	F	-	>40	>40
NS87	19	F	-	>40	>40	NS187	45	F	-	>40	>40
NS88	42	F	-	>40	>40	NS188	36	M	-	>40	>40
NS89	45	F	-	>40	>40	NS189	15	F	-	>40	>40

NS90	37	M	-	>40	>40	NS190	17	M	-	>40	>40
NS91	25	F	-	>40	>40	NS191	46	F	-	>40	>40
NS92	57	M	-	>40	>40	NS192	14	M	-	>40	>40
NS93	36	F	-	>40	>40	NS193	63	F	-	>40	>40
NS94	56	M	-	>40	>40	NS194	29	M	-	>40	>40
NS95	53	F	-	>40	>40	NS195	42	F	-	>40	>40
NS96	32	M	-	>40	>40	NS196	45	F	-	>40	>40
NS97	19	F	-	>40	>40	NS197	36	M	-	>40	>40
NS98	42	F	-	>40	>40	NS198	35	F	-	>40	>40
NS99	45	F	-	>40	>40	NS199	57	M	-	>40	>40
NS100	36	M	-	>40	>40	NS200	26	F	-	>40	>40
NS201	47	M	+	16.29	15.96	NS219	57	F	+	26.2	25.94
NS202	46	F	+	14.42	14.01	NS220	32	M	+	30.84	30.22
NS203	34	M	+	27.24	27.78	NS221	54	M	+	27.18	27.65
NS204	23	F	+	31.24	30.93	NS222	71	F	+	20.14	21.32
NS205	52	M	+	14.65	14.73	NS223	42	F	+	25.84	26.12
NS206	28	F	+	31.76	31.44	NS224	45	F	-	34.17	35.46
NS207	42	F	+	22.90	23.45	NS225	36	M	+	25.68	25.68
NS208	45	F	+	22.65	23.10	NS226	35	F	+	20.14	19.74
NS209	36	M	+	22.94	22.71	NS227	17	M	+	35.12	34.21
NS210	17	M	+	31.85	31.55	NS228	56	F	+	27.18	27.45
NS211	57	F	+	34.86	33.76	NS229	77	M	+	23.49	23.42
NS212	32	M	+	14.25	14.39	NS230	16	F	+	22.87	23.09

NS213	54	M	+	31.76	31.44	NS231	34	M	+	20.14	19.74
NS214	71	F	+	24.09	24.57	NS232	33	F	+	19.34	19.34
NS215	42	F	+	27.34	26.79	NS233	42	M	+	30.21	30.42
NS216	45	F	+	19.24	19.43	NS234	19	F	+	24.64	24.15
NS217	36	M	+	27.18	26.91	NS235	42	F	+	18.43	18.25
NS218	15	F	+	19.39	19.51	NS236	33	F	+	19.47	19.29
NS237	54	M	+	31.76	31.44	NS254	55	M	+	20.14	19.74
NS238	71	F	+	24.09	24.57	NS255	53	F	+	19.34	19.34
NS239	42	F	+	27.34	26.79	NS256	24	M	+	33.01	33.21
NS240	45	F	+	19.24	19.43	NS257	34	F	-	>40	34.28
NS241	36	M	+	27.18	26.91	NS258	45	F	+	18.43	18.25
NS242	54	M	+	31.76	31.44	NS259	64	M	+	20.14	19.74
NS243	71	F	+	24.09	24.57	NS260	43	F	+	19.34	19.34
NS244	42	M	+	27.34	26.79	NS261	52	M	+	23.21	23.16
NS245	45	F	+	19.24	19.43	NS262	39	F	-	>40	34.15
NS246	36	M	+	27.18	26.91	NS263	62	F	+	18.43	18.25
NS247	54	M	+	31.76	31.44	NS264	74	M	+	20.14	19.74
NS248	71	M	+	24.09	24.57	NS265	56	F	+	19.34	19.34
NS249	42	F	+	27.34	26.79	NS266	76	M	+	23.91	24.21
NS250	45	F	+	19.24	19.43	NS267	59	F	+	24.64	24.15
NS251	36	M	+	27.18	26.91	NS268	46	F	+	18.43	18.25
NS252	54	M	+	31.76	31.44	NS269	64	M	+	20.14	19.74
NS253	71	F	+	24.09	24.57	NS270	63	F	+	19.34	19.34

NS271	42	F	+	27.34	26.79	NS294	22	M	+	35.31	34.25
NS272	45	F	+	19.24	19.43	NS295	18	F	+	24.64	24.15
NS273	36	M	+	27.18	26.91	NS296	43	F	+	18.43	18.25
NS274	54	M	+	31.76	31.44	NS297	24	M	+	20.14	19.74
NS275	72	F	+	24.09	24.57	NS298	43	F	+	19.34	19.34
NS276	43	F	+	27.34	26.79	NS299	22	M	+	18.23	18.25
NS277	45	M	+	19.24	19.43	NS300	59	M	+	24.64	24.15
NS278	46	M	+	27.18	26.91	NS301	42	F	+	18.43	18.25
NS279	54	M	+	31.76	31.44	NS302	54	M	+	20.14	19.74
NS280	71	F	+	24.09	24.57	NS303	53	F	+	19.34	19.34
NS281	45	M	+	27.34	26.79	NS304	32	M	-	>40	32.29
NS282	43	F	+	19.24	19.43	NS305	69	F	+	24.64	24.15
NS283	36	M	+	27.18	26.91	NS306	42	F	+	18.43	18.25
NS284	54	M	+	31.76	31.44	NS307	64	M	+	20.14	19.74
NS285	54	F	+	24.09	24.57	NS308	43	F	+	19.34	19.34
NS286	40	F	+	27.34	26.79	NS309	32	M	+	23.42	22.21
NS287	42	F	+	19.24	19.43	NS310	59	F	+	24.64	24.15
NS288	36	M	+	27.18	26.91	NS311	42	F	+	18.43	18.25
NS289	54	M	+	31.76	31.44	NS312	24	M	+	20.14	19.74
NS290	71	M	+	24.09	24.57	NS313	53	F	+	19.34	19.34
NS291	42	F	+	27.34	26.79	NS314	22	M	-	>40	34.22
NS292	45	F	+	19.24	19.43	NS315	49	F	+	24.64	24.15
NS293	36	M	+	27.18	26.91	NS316	45	F	+	18.43	18.25

NS317	54	M	+	31.76	31.44	NS327	64	M	+	20.14	19.74
NS318	71	F	+	24.09	24.57	NS328	63	F	+	19.34	19.34
NS319	42	F	+	27.34	26.79	NS329	42	M	-	>40	34.21
NS320	45	F	+	19.24	19.43	NS330	29	F	+	24.64	24.15
NS321	36	M	+	27.18	26.91	NS331	46	M	+	18.43	18.25
NS322	15	F	+	19.39	19.51	NS332	72	F	+	24.09	24.57
NS323	71	F	+	24.09	24.57	NS333	46	F	+	27.34	26.79
NS324	42	F	+	27.34	26.79	NS334	44	F	+	19.24	19.43
NS325	45	F	+	19.24	19.43	NS335	33	M	+	27.18	26.91
NS326	36	M	+	27.18	26.91	/	/	/	/	/	/