Clinic Evaluation Report

Product name: SARS-CoV-2 Antigen Rapid Test（COLLOIDAL GOLD）

Company name:

Nantong Egens Biotechnology Co., Ltd.

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

Clinic Evaluation Report

1 Product Review

Product Name: SARS-CoV-2 Antigen Rapid Test (Colloidal Gold)

Specification：25 tests/kit.

Manufacturer: Nantong Egens Biotechnology Co., Ltd.

2 Intended Use

This kit is a double-antibody sandwich method for the rapid, qualitative and differential detection of SARS-COV-2 nucleocapsid protein antigen in nasopharyngeal swab or oropharyngeal swab samples.

Testing is limited to specialized laboratories.

3 Technical Principles

The reagent was pre-packaged with a colloidal gold labeled novel Coronavirus monoclonal antibody and mouse IgG on the binding pad, and then wrapped with a novel Coronavirus monoclonal antibody and a Goat anti-mouse polyclonal antibody respectively on the nitric acid membrane detection line and the quality control line. Then the novel Coronavirus antigen in a human sample was qualitatively detected by applying the principle of colloidal gold immunochromatography and double antibody sandwich method.

When the samples were tested positive, the novel Coronavirus antigen and the colloid gold labeled Novel Coronavirus monoclonal antibody in the samples were combined to form a complex. As the chromatography complex moved forward along the strip, it was combined with the pre-packaged Novel Coronavirus monoclonal antibody to form a sandwich after passing the test line. Colloidal gold labeled mouse IgG was combined with goat anti-mouse polyclonal antibody at the quality control line.

4 Background of Clinical Evaluation

Since December 2019, the novel coronavirus infection cases of acute respiratory tract infection have been confirmed by a number of cases of unknown pneumonia

The novel coronavirus which caused the epidemic of pneumonia in Wuhan was officially named “2019 coronavirus” (SARS-CoV-2) in January 12,2020.

Typical symptoms: fever, fatigue and dry cough are the main manifestations, and dyspnea may occur in severe cases.

Common symptoms: The incubation period of this disease is generally 3 ~ 7 days, and the longest is not more than 14 days. Fever, fatigue and dry cough are the main manifestations. A few patients have nasal obstruction, runny nose, diarrhea and other symptoms. Severe cases usually have dyspnea after 1 week. Severe cases rapidly progress to acute respiratory distress syndrome, septic shock, metabolic acidosis which is difficult to correct, and coagulation dysfunction.

Other symptoms: Some patients showed only low fever, slight fatigue, etc., no pneumonia, and recovered after 1 week.

At present, the pneumonia epidemic caused by 2019-nCoV has seen explosive growth in many provinces and cities and regions in China and worldwide.

The novel coronaviruses belong to the β genus.SARS-CoV-2 is an acute respiratory infectious disease. Currently, the patients infected by 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. The main manifestation include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.Coronavirus are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds and that cause respiratory, enteric, hepatic, and neurologic diseases. Seven coronavirus species are known to cause human disease. Four viruses - 229E, OC43, NL63, and HKU1 - are prevalent and typically cause common cold symptoms in immunocompetent individuals. The three other strains - severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and 2019 Novel Coronavirus (SARS-CoV-2) - are zoonotic in origin and have been linked to sometimes fatal illness. The SARS-CoV-2 Antigen Rapid Test (COLLOIDAL GOLD) can detect pathogen antigens directly from nasopharyngeal swab or oropharyngeal swab specimens.

5 Introduction of Clinical trials

5.1 To prove the kits' safety and effectiveness, clinical trials are necessary.

5.2 Clinical trials were conducted in 2020 March in Guangzhou Medical College.

5.3 Enrollment criteria: patients who were tested positive for SARS-COV-2 based on clinical and epidemiological criteria, normal persons who were tested negative for SARS-COV-2.

5.4 No IRB approval because it was for Emergency Use.

5.5 Proposed comparator: Real-time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCOV by BGI Genomics Co. Ltd.

5.6 Specimen Collection: No special patient's preparation required. Collect the nasopharyngeal swab or oropharyngeal swab specimens in accordance with the normal laboratory practice.

5.8 The total numbers of samples tested: 240 positive samples and 500 negative samples.

6 Performance Results

6.1 Summary of Clinical Study

To evaluate the clinical sensitivity, we sent our reagents to clinical partners for evaluation. Totally 240 nasopharyngeal swab samples in viral transport medium (VTM) from patients clinical confirmed with Coronavirus (SARS-CoV-2) infection were collected. Antigen detection in the samples of SARS-CoV-2 patients has a high consistency with nucleic acid detection in swab. The positive rate is 95.8% (230/240). Through the evaluation of normal population and interfering samples, it is confirmed that the Company's SARS-CoV-2 Antigen Rapid Test meet the testing

requirements in specificity (99.8%, 499/500) and sensitivity (95.8%, 230/240). It is recommended that the Company's SARS-CoV-2 Kit for detecting antigen of coronavirus (SARS-CoV-2) can be used in clinical assisting examination.

6.2 Results

Part 1: Clinical sensitivity: test samples collected from positive clinical patients with corona virus (SARS-CoV-2) infection.

To evaluate the clinical sensitivity, we sent our reagents to clinical partners for evaluation. Totally 240 nasopharyngeal swab VTM samples from patients clinical confirmed with Coronavirus (SARS-CoV-2) infection were collected and tested. The results were as follows:

Table 1: Test results for 240positive samples by RT-PCR

No.	Result	No.	Result	No.	Result	No.	Result
1	+	31	+	61	+	91	+
2	+	32	+	62	+	92	+
3	+	33	+	63	−	93	+
4	+	34	+	64	+	94	+
5	+	35	+	65	+	95	+
6	+	36	+	66	+	96	+
7	+	37	+	67	+	97	+
8	+	38	+	68	+	98	−
9	+	39	+	69	+	99	+
10	+	40	+	70	+	100	+
11	+	41	+	71	−	101	+
12	+	42	−	72	+	102	+
13	+	43	+	73	+	103	+
14	+	44	+	74	+	104	−
15	+	45	+	75	+	105	+
16	+	46	+	76	+	106	+
17	+	47	+	77	+	107	+
18	+	48	+	78	+	108	+
19	+	49	+	79	+	109	+
20	+	50	+	80	+	110	+
21	+	51	+	81	+	111	+
22	+	52	+	82	+	112	+
23	+	53	+	83	+	113	+
24	+	54	+	84	+	114	+
25	+	55	+	85	+	115	+
26	+	56	+	86	+	116	+
27	+	57	+	87	+	117	+
28	+	58	+	88	+	118	+
29	+	59	+	89	+	119	+
30	+	60	+	90	+	120	+

121	+	151	+	181	+	211	+
122	+	152	+	182	+	212	+
123	+	152	+	183	−	213	+
124	+	154	+	184	+	214	+
125	+	155	+	185	+	215	+
126	+	156	+	186	+	216	+
127	+	157	+	187	+	217	+
128	+	158	+	188	+	218	−
129	+	159	+	189	+	219	+
130	+	160	+	190	+	220	+
131	+	161	+	191	−	221	+
132	+	162	−	192	+	222	+
133	+	163	+	193	+	223	+
134	+	164	+	194	+	224	−
135	+	165	+	195	+	225	+
136	+	166	+	196	+	226	+
137	+	167	+	197	+	227	+
138	+	168	+	198	+	228	+
139	+	169	+	199	+	229	+
140	+	170	+	200	+	230	+
141	+	171	+	201	+	231	+
142	+	172	+	202	+	232	+
143	+	173	+	203	+	233	+
144	+	174	+	204	+	234	+
145	+	175	+	205	+	235	+
146	+	176	+	206	+	236	+
147	+	177	+	207	+	237	+
148	+	178	+	208	+	238	+
149	+	179	+	209	+	239	+
150	+	180	+	210	+	240	+

According to table 1, the quantity of o Antigen positive cases were 230. The positive rate is 95.8% （230/240）.

Conclusions: Antigen detection in the nasopharyngeal swab VTM samples of COVID-19 patients has a high consistency with nucleic acid detection. The positive rate is 95.8%, 95% confidence interval (CI) for sensitivity is 90.62%-98.21%.

Part 2: Clinical specificity: test samples collected from normal human.

To evaluate the specificity of the reagent, 500 nasopharyngeal swab samples were collected from normal human. The test results illustrated as follows:

Table2: Test results for normal human

No.	Result	No.	Result	No.	Result	No.	Result	No.	Result

1	—	101	—	201	—	301	—	401	—
2	—	102	—	202	—	302	—	402	—
3	—	103	—	203	—	303	—	403	—
4	—	104	—	204	—	304	—	404	—
5	—	105	—	205	—	305	—	405	—
6	—	106	—	206	—	306	—	406	—
7	—	107	—	207	—	307	—	407	—
8	—	108	—	208	—	308	—	408	—
9	—	109	—	209	—	309	—	409	—
10	—	110	—	210	—	310	—	410	—
11	—	111	—	211	—	311	—	411	—
12	—	112	—	212	—	312	—	412	—
13	—	113	—	213	—	313	—	413	—
14	—	114	—	214	—	314	—	414	—
15	—	115	—	215	—	315	—	415	—
16	—	116	—	216	—	316	—	416	—
17	—	117	—	217	—	317	—	417	—
18	—	118	—	218	—	318	—	418	—
19	—	119	—	219	—	319	—	419	—
20	—	120	—	220	—	320	—	420	—
21	—	121	—	221	—	321	—	421	—
22	—	122	—	222	—	322	—	422	—
23	—	123	—	223	—	323	—	423	—
24	—	124	—	224	—	324	—	424	—
25	—	125	—	225	—	325	—	425	—
26	—	126	—	226	—	326	—	426	—
27	—	127	—	227	—	327	—	427	—
28	—	128	—	228	+	328	—	428	—
29	—	129	—	229	—	329	—	429	—
30	—	130	—	230	—	330	—	430	—
31	—	131	—	231	—	331	—	431	—
32	—	132	—	232	—	332	—	432	—
33	—	133	—	233	—	333	—	433	—
34	—	134	—	234	—	334	—	434	—
35	—	135	—	235	—	335	—	435	—
36	—	136	—	236	—	336	—	436	—
37	—	137	—	237	—	337	—	437	—
38	—	138	—	238	—	338	—	438	—
39	—	139	—	239	—	339	—	439	—
40	—	140	—	240	—	340	—	440	—
41	—	141	—	241	—	341	—	441	—
42	—	142	—	242	—	342	—	442	—
43	—	143	—	243	—	343	—	443	—

44	—	144	—	244	—	344	—	444	—
45	—	145	—	245	—	345	—	445	—
46	—	146	—	246	—	346	—	446	—
47	—	147	—	247	—	347	—	447	—
48	—	148	—	248	—	348	—	448	—
49	—	149	—	249	—	349	—	449	—
50	—	150	—	250	—	350	—	450	—
51	—	151	—	251	—	351	—	451	—
52	—	152	—	252	—	352	—	452	—
53	—	153	—	253	—	353	—	453	—
54	—	154	—	254	—	354	—	454	—
55	—	155	—	255	—	355	—	455	—
56	—	156	—	256	—	356	—	456	—
57	—	157	—	257	—	357	—	457	—
58	—	158	—	258	—	358	—	458	—
59	—	159	—	259	—	359	—	459	—
60	—	160	—	260	—	360	—	460	—
61	—	161	—	261	—	361	—	461	—
62	—	162	—	262	—	362	—	462	—
63	—	163	—	263	—	363	—	463	—
64	—	164	—	264	—	364	—	464	—
65	—	165	—	265	—	365	—	465	—
66	—	166	—	266	—	366	—	466	—
67	—	167	—	267	—	367	—	467	—
68	—	168	—	268	—	368	—	468	—
69	—	169	—	269	—	369	—	469	—
70	—	170	—	270	—	370	—	470	—
71	—	171	—	271	—	371	—	471	—
72	—	172	—	272	—	372	—	472	—
73	—	173	—	273	—	373	—	473	—
74	—	174	—	274	—	374	—	474	—
75	—	175	—	275	—	375	—	475	—
76	—	176	—	276	—	376	—	476	—
77	—	177	—	277	—	377	—	477	—
78	—	178	—	278	—	378	—	478	—
79	—	179	—	279	—	379	—	479	—
80	—	180	—	280	—	380	—	480	—
81	—	181	—	281	—	381	—	481	—
82	—	182	—	282	—	382	—	482	—
83	—	183	—	283	—	383	—	483	—
84	—	184	—	284	—	384	—	484	—
85	—	185	—	285	—	385	—	485	—
86	—	186	—	286	—	386	—	486	—

87	—	187	—	287	—	387	—	487	—
88	—	188	—	288	—	388	—	488	—
89	—	189	—	289	—	389	—	489	—
90	—	190	—	290	—	390	—	490	—
91	—	191	—	291	—	391	—	491	—
92	—	192	—	292	—	392	—	492	—
93	—	193	—	293	—	393	—	493	—
94	—	194	—	294	—	394	—	494	—
95	—	195	—	295	—	395	—	495	—
96	—	196	—	296	—	396	—	496	—
97	—	197	—	297	—	397	—	497	—
98	—	198	—	298	—	398	—	498	—
99	—	199	—	299	—	399	—	499	—
100	—	200	—	300	—	400	—	500	—

Conclusions: The specificity in normal population is 99.8% (499/500), 95% confidence interval (CI) for specificity is 98.9%-100.0%.

Part 3: Cross reactivity test

Many types of pneumonia are companied by fever, cough and other respiratory symptoms. In order to eliminate similar clinical symptoms of other types of pneumonia effects, the positive swab samples of pneumonia mycoplasma, influenza A, influenza B, pneumococcus etc. were detected for the specific assessment.

Table 3: specificity verification with pneumonia mycoplasma positive

No.	MP Ag	SARS-CoV-2 Ag	No.	MP Ag	SARS-CoV-2 Ag
1	+	—	6	+	—
2	+	—	7	+	—
3	+	—	8	+	—
4	+	—	9	+	—
5	+	—	10	+	—

The 10 cases of pneumonia mycoplasma infection patient swab samples, the Company's SARS-CoV-2 Antigen Rapid Test Antigen negative.

Table 4: specificity verification with influenza A positive

No.	FLU A Ag	SARS-CoV-2 Ag	No.	FLU A Ag	SARS-CoV-2 Ag
1	+	—	6	+	—
2	+	—	7	+	—
3	+	—	8	+	—
4	+	—	9	+	—
5	+	—	10	+	—

The 10 cases of influenza A infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 5: specificity verification with influenza B positive

No.	FLU B Ag	SARS-CoV-2 Ag	No.	FLU B Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of influenza B infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 6: specificity verification with adenovirus antigen-positive

No.	Adenovirus Ag	SARS-CoV-2 Ag	No.	Adenovirus Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of Adenovirus infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 7: specificity verification with hMPV antigen-positive

No.	hMPV Ag	SARS-CoV-2 Ag	No.	hMPV Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of hMPV infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 8: specificity verification with parainfluenza virus antigen-positive

No.	Parainfluenza Ag	SARS-CoV-2 Ag	No.	Parainfluenza Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of Parainfluenza infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 9: specificity verification with Enterovirus 71 antigen-positive

No.	Enterovirus 71 Ag	SARS-CoV-2 Ag	No.	Enterovirus 71 Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of enterovirus 71 infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 10: specificity verification with respiratory syncytial virus antigen-positive

No.	SPN Ag	SARS-CoV-2 Ag	No.	SPN Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of respiratory syncytial virus infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 11: specificity verification with Rhinovirus antigen-positive

No.	Rhinovirus Ag	SARS-CoV-2 Ag	No.	Rhinovirus Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−

5	+	−	10	+	−

The 10 cases of rhinovirus infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 12: specificity verification with chlamydia pneumoniae antigen-positive

No.	chlamydia pneumoniae Ag	SARS-CoV-2 Ag	No.	chlamydia pneumoniae Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of chlamydia pneumoniae infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 13: specificity verification with streptococcus pneumoniae antigen-positive

No.	streptococcus pneumoniae Ag	SARS-CoV-2 Ag	No.	streptococcus pneumoniae Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of streptococcus pneumoniae infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 14: specificity verification with mycobacterium tuberculosis antigen-positive

No.	mycobacterium tuberculosis Ag	SARS-CoV-2 Ag	No.	mycobacterium tuberculosis Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of mycobacterium tuberculosis infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Table 15: specificity verification with EBV antigen-positive

No.	EBV Ag	SARS-CoV-2 Ag	No.	EBV Ag	SARS-CoV-2 Ag
1	+	−	6	+	−
2	+	−	7	+	−
3	+	−	8	+	−
4	+	−	9	+	−
5	+	−	10	+	−

The 10 cases of EBV virus infection patient samples, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive.

Cross Reactivity Test Conclusions: The 10 cases of pneumonia mycoplasma, 10 cases of influenza A, 10 cases of influenza B, 10 cases of adenovirus, 10 cases of hMPV, 10 cases of parainfluenza, 10 cases of enterovirus 71, 10 cases of respiratory syncytial virus, 10 cases of rhinovirus, 10 cases of Chlamydia pneumonia, 10 cases of streptococcus pneumonia and 10 cases of mycobacterium tuberculosis infection patient samples were tested, the Company's SARS-CoV-2 Antigen Rapid test no Antigen positive, no cross reactivity were found.

Part 4: Sample Matrix

Three types of samples such as direct nasopharyngeal swab, nasal or throat swabs specimens and oropharyngeal samples in VTM from the same ten patients and ten normal persons were tested in order to evaluate if the sample kind and matrix can give different results.

Table 20: sensitivity and specificity by sample kind and matrix

No.	nasopharyngeal swab	nasal swab	throat swab	oropharyngeal swab in VTM
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+
11	−	−	−	−
12	−	−	−	−
13	−	−	−	−
14	−	−	−	−
15	−	−	−	−
16	−	−	−	−
17	−	−	−	−

18	—	—	—	—
19	—	—	—	—
20	—	—	—	—

The 10 cases of patient samples and 10 cases of normal samples were tested, different kinds of samples can give the same results.

7 Clinical Performance Conclusions:

Through the evaluation of normal population and interfering samples, it is confirmed that the Company's SARS-CoV-2 kit meet the testing requirements in specificity (99.8%, 499/500) and sensitivity (95.8%, 230/240). It is recommended that the Company's SARS-CoV-2 kit for detecting antigen of coronavirus (SARS-CoV-2) can be used in clinical assisting examination.

7.1 A total of 240 positive samples and 500 negative samples were detected in this clinical trial, and each of them was evaluated by the evaluation product and contrast product.

		PCR test result	
		positive	negative
Result	positive	230 true positive (a)	1 false positive (b)
	negative	10 false negative (c)	499 true negative (d)

7.2 Coincidence rate analysis:

Positive coincidence rate=a/(a+c)*100%=95.8%

Negative coincidence rate=d/(b+d)*100%=99.8%

Accuracy=(a+d)/(a+b+c+d)*100%=98.5%

Sensitivity=a/(a+c)*100%=95.8%

Specificity=d/(d+b)*100%=99.8%

7.3 Conclusions

The kit which its working principle is clear, the design is finalized, the process is mature, the clinical application is extensive, and no serious adverse event records and/or product defects have been found. The performance indicators of the product's safety and effectiveness have been established in the test report and have been fully verified without passing the clinical trial. At the same time, the product is of the same clinical use and the same operating object with the same kind of registered and marketed products, and is substantially equivalent in terms of basic principle, structure composition, product manufacturing materials, main performance indicators, application scope, use method and so on.

Therefore, compared to other similar products, SARS-CoV-2 Antigen Rapid Test does not reduce the clinical effectiveness, nor increase the clinical safety risk. The production and application technology of this product is mature, and its functional principle, expected clinical use effect have been fully affirmed in the relevant clinical application field.

In conclusion, SARS-CoV-2 Antigen Rapid Test Kit can meet the expected use, and can ensure the safety and effectiveness of its clinical use.