

Clinical Study Report
of
COVID-19 Antigen Test Cassette

Testing Hospital: 安徽医科大学第一附属医院

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1. Purpose of the test

Through test on certain quantity of representative clinical specimens, and scientific and rational statistic analysis on the test result, evaluate the consistency of the result of COVID-19 Antigen Test Cassette produced by HangZhou Testsea Biotechnology and compare with RT-PCR product Shanghai ZJ Bio-Tech Co.,Ltd., and evaluate its clinical application capacity.

2. Overall design of the test

The test of parallel contrast is adopted, and RT-PCR similar product which has been launched is taken as comparison device. The COVID-19 Antigen Test Cassette produced by our company and the comparison device will parallel test on the same specimens, and the result will be recorded respectively.

3. Study method

A clinical study is received in which the estimation of the parameters of sensitivity, specificity of the test and Kappa index is observed. However, it is necessary to estimate all the complete parameters for the performance of the diagnostic test (sensitivity, specificity, positive predictive value and negative predictive value) and include the estimated Kappa index. The information should be presented in charts with its analysis and interpretation of the results

3.1 Specimens size and its confirmation basis

To investigate the function of product, a side-by-side comparison was conducted using The COVID-19 Antigen Test Cassette and a commercially available COVID-19 RT-PCR. Testing was performed on 291 clinical specimens previously collected from subjects present for COVID-19 IgG/IgM Testing. Allow the test, the Nasal Swab Specimen to reach room temperature (15-30℃) prior to testing.

3.2 Selection of specimen

The samples were selected from medical institution .To protect the privacy of the specimen suppliers, the providers' name shouldn't appear in the test record.

3.3 The selection of comparison device and confirmation method

RT-PCR product (Novel Coronavirus (SARS-CoV-2) Real Time Multiplex RT-PCR Kit) is selected as the comparison method, and the PCR product from Shanghai ZJ Bio-Tech Co.,Ltd.and the infection time information will be a supplementary explanation.

4. Clinical test result

4.1 Positive result

NO.	PCR	COVID-19 Antigen
Positive1# 10 days after infection	+	+
Positive2# 2 days after infection	+	-
Positive3# 7 days after infection	+	+
Positive4# 9 days after infection	+	+
Positive5# 11 days after infection	+	+
Positive6# 17 days after infection	+	+
Positive7# 16 days after infection	+	+
Positive8# 16 days after infection	+	+
Positive9# 15 days after infection	+	+
Positive10# 16 days after infection	+	+
Positive11# 13 days after infection	+	+
Positive12# 15 days after infection	+	+
Positive13# 16 days after infection	+	+
Positive14# 9 days after infection	+	-
Positive15# 7 days after infection	+	+
Positive16# 8 days after infection	+	+
Positive17# 14 days after infection	+	+
Positive18# 15 days after infection	+	+
Positive19# 17 days after infection	+	+
Positive20# 16 days after infection	+	+
Positive21# 10 days after infection	+	+
Positive22# 13 days after infection	+	+
Positive23# 15 days after infection	+	+

NO.	PCR	COVID-19 Antigen
Positive24# 16 days after infection	+	-
Positive25# 9 days after infection	+	+
Positive26# 14 days after infection	+	+
Positive27#17 days after infection	+	+
Positive28# 15 days after infection	+	+
Positive29# 11 days after infection	+	+
Positive30# 8 days after infection	+	-
Positive31# 2 days after infection	+	+
Positive32# 3 days after infection	+	+
Positive33# 11 days after infection	+	+
Positive34# 5 days after infection	+	-
Positive35# 4 days after infection	+	+
Positive36# 6 days after infection	+	+
Positive37# 13 days after infection	+	+
Positive38# 7 days after infection	+	+
Positive39# 9 days after infection	+	+
Positive40# 4 days after infection	+	+
Positive41# 1 day after infection	+	-
Positive42# 2 days after infection	+	+
Positive43# 3 days after infection	+	+
Positive44# 2 days after infection	+	+
Positive45# 5 days after infection	+	+
Positive46# 6 days after infection	+	+
Positive47# 13 days after infection	+	+

NO.	PCR	COVID-19 Antigen
Positive48# 6 days after infection	+	+
Positive49# 3 days after infection	+	+
Positive50# 10 days after infection	+	+
Positive51 # 5 days after infection	+	+
Positive52 # 4 days after infection	+	+
Positive53 # 2 days after infection	+	+
Positive54 # 9 days after infection	+	+
Positive55# 5 days after infection	+	+
Positive56# 11 days after infection	+	+
Positive 57# 2 days after infection	+	+
Positive58# 6 days after infection	+	+
Positive59# 13 days after infection	+	+
Positive60 # 12 days after infection	+	+
Positive61 # 7 days after infection	+	+
Positive62 # 11 days after infection	+	+
Positive63 # 9 days after infection	+	+
Positive64 # 5 days after infection	+	+
Positive65 # 6 days after infection	+	+
Positive66 # 4 days after infection	+	+
Positive 67# 7 days after infection	+	+
Positive 68 # 10 days after infection	+	+
Positive 69 # 4 days after infection	+	+
Positive 70 # 5 days after infection	+	+

NO.	PCR	COVID-19 Antigen
Positive 71 # 7 days after infection	+	+
Positive 72 # 5 days after infection	+	+
Positive 73 # 6 days after infection	+	+
Positive 74 # 4 days after infection	+	+
Positive 75 # 4 days after infection	+	+
Positive 76 # 5 days after infection	+	+

4.2 Negative result

NO.	PCR	COVID-19 Antigen
Negative#1	-	-
Negative#2	-	-
Negative#3	-	-
Negative#4	-	-
Negative#5	-	-
Negative#6	-	-
Negative#7	-	-
Negative#8	-	-
Negative#9	-	-
Negative#10	-	-
Negative#11	-	-
Negative#12	-	-
Negative#13	-	-
Negative#14	-	-
Negative#15	-	-
Negative#16	-	-
Negative#17	-	-
Negative#18	-	-
Negative#19	-	-
Negative#20	-	-
Negative#21	-	-
Negative#22	-	-
Negative#23	-	-
Negative#24	-	-

NO.	PCR	COVID-19 Antigen
Negative#25	-	-
Negative#26	-	-
Negative#27	-	-
Negative#28	-	-
Negative#29	-	-
Negative#30	-	-
Negative#31	-	-
Negative#32	-	-
Negative#33	-	-
Negative#34	-	-
Negative#35	-	-
Negative#36	-	-
Negative#37	-	-
Negative#38	-	-
Negative#39	-	-
Negative#40	-	-
Negative#41	-	-
Negative#42	-	-
Negative#43	-	-
Negative#44	-	-
Negative#45	-	-
Negative#46	-	-
Negative#47	-	-
Negative#48	-	-

NO.	PCR	COVID-19 Antigen
Negative#49	-	-
Negative#50	-	-
Negative#51	-	-
Negative#52	-	-
Negative#53	-	+
Negative#54	-	-
Negative#55	-	-
Negative#56	-	-
Negative#57	-	-
Negative#58	-	-
Negative#59	-	-
Negative#60	-	-
Negative#61	-	-
Negative#62	-	-
Negative#63	-	-
Negative#64	-	-
Negative#65	-	-
Negative#66	-	-
Negative#67	-	-
Negative#68	-	-
Negative#69	-	-
Negative#70	-	-
Negative#71	-	-
Negative#72	-	-

NO.	PCR	COVID-19 Antigen
Negative#73	-	-
Negative#74	-	-
Negative#75	-	-
Negative#76	-	-
Negative#77	-	-
Negative#78	-	-
Negative#79	-	-
Negative#80	-	-
Negative#81	-	-
Negative#82	-	-
Negative#83	-	-
Negative#84	-	-
Negative#85	-	-
Negative#86	-	+
Negative#87	-	-
Negative#88	-	-
Negative#89	-	-
Negative#90	-	-
Negative#91	-	-
Negative#92	-	-
Negative#93	-	-
Negative#94	-	-
Negative#95	-	-
Negative#96	-	-

NO.	PCR	COVID-19 Antigen
Negative#97	-	-
Negative#98	-	-
Negative#99	-	-
Negative#100	-	-
Negative#101	-	-
Negative#102	-	-
Negative#103	-	-
Negative#104	-	-
Negative#105	-	-
Negative#106	-	-
Negative#107	-	-
Negative#108	-	-
Negative#109	-	-
Negative#110	-	-
Negative#111	-	-
Negative#112	-	-
Negative#113	-	-
Negative#114	-	-
Negative#115	-	-
Negative#116	-	-
Negative#117	-	-
Negative#118	-	-
Negative#119	-	-
Negative#120	-	-

NO.	PCR	COVID-19 Antigen
Negative#121	-	-
Negative#123	-	-
Negative#124	-	-
Negative#125	-	-
Negative#126	-	-
Negative#127	-	-
Negative#128	-	-
Negative#129	-	-
Negative#130	-	-
Negative#131	-	-
Negative#132	-	-
Negative#133	-	-
Negative#134	-	-
Negative#135	-	-
Negative#136	-	-
Negative#137	-	-
Negative#138	-	-
Negative#139	-	-
Negative#140	-	-
Negative#141	-	-
Negative#142	-	-
Negative#143	-	-
Negative#144	-	-
Negative#145	-	-

NO.	PCR	COVID-19 Antigen
Negative#146	-	-
Negative#147	-	-
Negative#148	-	-
Negative#149	-	-
Negative#150	-	-
Negative#151	-	-
Negative#152	-	-
Negative#153	-	-
Negative#154	-	-
Negative#155	-	+
Negative#156	-	-
Negative#157	-	-
Negative#158	-	-
Negative#159	-	-
Negative#160	-	-
Negative#161	-	-
Negative#162	-	-
Negative#163	-	-
Negative#164	-	-
Negative#164	-	-
Negative#165	-	-
Negative#166	-	-
Negative#167	-	-
Negative#168	-	-

NO.	PCR	COVID-19 Antigen
Negative#169	-	-
Negative#170	-	-
Negative#171	-	-
Negative#172	-	-
Negative#173	-	-
Negative#174	-	-
Negative#175	-	-
Negative#176	-	-
Negative#177	-	+
Negative#178	-	-
Negative#179	-	-
Negative#180	-	-
Negative#181	-	-
Negative#182	-	-
Negative#183	-	-
Negative#184	-	-
Negative#185	-	-
Negative#186	-	-
Negative#187	-	-
Negative#188	-	-
Negative#189	-	-
Negative#190	-	-
Negative#191	-	-
Negative#192	-	-

NO.	PCR	COVID-19 Antigen
Negative#193	-	-
Negative#194	-	+
Negative#195	-	-
Negative#196	-	-
Negative#197	-	-
Negative#198	-	-
Negative#199	-	-
Negative#200	-	-
Negative#201	-	-
Negative#202	-	-
Negative#203	-	-
Negative#204	-	-
Negative#205	-	-
Negative#206	-	-
Negative#207	-	-
Negative#208	-	-
Negative#209	-	-
Negative#210	-	-
Negative#211	-	-
Negative#212	-	-
Negative#213	-	-
Negative#214	-	-
Negative#215	-	-

4.3 Statistic analysis method of test result

This test will adopt statistic analysis on pair enumeration data, and will record analysis in the form of fourfold table, see below:

Table 1 fourfold table for evaluating diagnostic test

		Comparison		Total
		positive	negative	
Test device	positive	a	b	γ_1
	negative	c	d	γ_2
total		C_1	C_2	N

Positive conformity rate= $[a/(a+c)] \times 100\%$

Negative conformity rate= $[d/(b+d)] \times 100\%$

Total conformity rate= $[(a+d)/(a+b+c+d)] \times 100\%$

We'll do Kappa consistency test on the result, and when the Kappa value is between 0-1, it means better consistency between the test result of the two devices. It is normally considered consistent when the Kappa value bigger than 0.75.

$$\text{Kappa} = \frac{N^*(a+b) - (\gamma_1 C_1 + \gamma_2 C_2)}{N^2 - (\gamma_1 C_1 + \gamma_2 C_2)}$$

4.4 Discussion and conclusion

4.4.1 positive predictive value and predictive value

Table 2 fourfold table for COVID-19 IgG result

		PCR		total
		positive	negative	
COVID-19 Antigen Test Cassette	positive	70	4	74
	negative	6	211	217
Total		76	215	291
% Agreement		≥92.1%	>98.1%	>93.8%

Positive Agreement= $70/76 \times 100\% = 92.1\%$ (95%CI* (87.7%~93.9%))

Negative Agreement= $211/215 \times 100\% = 98.1\%$ (95%CI*:95.3%~99.5%)

TotalAgreement= $(70+211)/(76+215) \times 100\% = 96.5\%$ (95%CI*:92.9%~99.7%)

Kappa=0.91

4.4.2 COVID-19 Antigen Positive Results

Days post symptom onset	Number of samples	# PCR positive	COVID-19 IgG/IgM Test Cassette positive
≤3	9	9	7
4~7	33	33	32
8~14	25	25	23
>14	9	9	8

4.4.3 COVID-19 Antigen Positive Results by time from symptom onset

Table 3 COVID-19 Antigen PPA

Days post symptom onset	Number of samples	PCR positive	COVID-19 Antigen Test Cassette
≤3	9	9	7/9=77.8%
4~7	33	33	32/33=97.0%
8~14	25	25	23/25=92%
>14	9	9	8/9=88.9%
Total	76	76	70/76=92.1% 95% CI: (87.7%~93.9%)

Note: the nasal swab samples were collected from the same patients for serology testing between 1 day and > 30 days after PCR sample collection.

The total sensitivity of COVID-19 Antigen is 92.1%; 95% CI:(87.7% - 93.9%)

4.4.4 specificity of COVID-19 Antigen Test Cassette

Table 4 COVID-19 Antigen NPA

Number of samples	PCR Negative result	COVID-19 Antigen Test Cassette
215	215	211/215=98.3%
Total	N/A	98.3% 95%CI: (95.3%-99.5%)

Total specificity from the above table is 98.3%; 95%CI: (94.2%-100%)

4.5 Conclusion

Clinical study has been conducted on altogether 291 specimens. Include 76 positive specimens and 215 Negative specimens . Testsea tests were parallel comparison studied with comparison device,the total positive conformity rate of the test result of Testsea test and of PCR product for comparison is $\geq 90\%$.the total Negative conformity rate of the test result of Testsea test and of PCR product for comparison is $\geq 90\%$;

The total sensitivity of COVID-19 Antigen is 92.1%;Total specificity from the above table is 98.3%,The Positive predictive value is 92.1%;The Negative predictive value is 98.1%.

Attachment 1

Detailed description of the population

NO	Sex	Age	Sampling distance Symptom time(days)	Race	Country
1	Man	50	10	Han	China
2	Woman	24	2	Han	China
3	Woman	26	7	Han	China
4	Woman	54	9	Han	China
5	Man	50	11	Han	China
6	Woman	34	17	Han	China
7	Man	33	16	Han	China
8	Man	35	16	Han	China
9	Man	60	15	Han	China
10	Man	46	16	Han	China
11	Man	38	13	Han	China
12	Woman	35	15	Han	China
13	Man	44	16	Han	China
14	Man	22	9	Han	China
15	Woman	34	7	Han	China
16	Woman	54	8	Han	China
17	Woman	56	14	Han	China
18	Man	45	15	Han	China
19	Woman	50	17	Han	China
20	Man	46	16	Han	China
21	Woman	57	10	Han	China
22	Man	51	13	Han	China
23	Woman	34	15	Han	China
24	Woman	29	16	Han	China
25	Woman	55	9	Han	China

NO	Sex	Age	Sampling distance Symptom time(days)	Race	Country
26	Man	79	14	Han	China
27	Man	41	17	Han	China
28	Woman	46	15	Han	China
29	Man	78	11	Han	China
30	Woman	76	8	Han	China
31	Woman	67	2	Han	China
32	Man	66	3	Han	China
33	Man	65	11	Han	China
34	Man	64	5	Han	China
35	Woman	64	4	Han	China
36	Man	45	6	Han	China
37	Man	34	13	Han	China
38	Woman	29	7	Han	China
39	Man	57	9	Han	China
40	Woman	68	4	Han	China
41	Man	69	1	Han	China
42	Man	27	2	Han	China
43	Man	36	3	Han	China
44	Woman	45	2	Han	China
45	Man	20	5	Han	China
46	Man	21	6	Han	China
47	Woman	65	13	Han	China
48	Woman	67	6	Han	China
49	Man	46	3	Han	China
50	Woman	28	10	Han	China
51	Man	21	5	Han	China
52	Woman	77	4	Han	China

NO	Sex	Age	Sampling distance Symptom time(days)	Race	Country
53	Woman	35	2	Han	China
54	Man	66	9	Han	China
55	Woman	46	5	Han	China
56	Man	66	11	Han	China
57	Woman	54	2	Han	China
58	Woman	64	6	Han	China
59	Man	53	13	Han	China
60	Woman	32	12	Han	China
61	Woman	40	7	Han	China
62	Man	50	11	Han	China
63	Woman	35	9	Han	China
64	Man	31	5	Han	China
65	Woman	41	6	Han	China
66	Woman	38	4	Han	China
67	Man	34	7	Han	China
68	Woman	21	10	Han	China
69	Man	27	4	Han	China
70	Man	78	5	Han	China
71	Woman	67	7	Han	China
72	Man	71	5	Han	China
73	Man	39	6	Han	China
74	Man	32	4	Han	China
75	Man	34	4	Han	China
76	Man	65	5	Han	China