Clinical Validation report of Spring COVID-19 IgM/IgG Rapid Test Cassette

CONFIDENTIAL

Manufacturer: Spring Healthcare Services AG

Product name: Spring COVID-19 IgM/IgG Rapid Test Cassette

I. Clinical validation time

This clinical evaluation was conducted from February 2020 to March 4, 2020.

II. Background information for clinical evaluation

Since December 2019, Wuhan City, Hubei Province has successively discovered multiple cases of patients with new-type coronavirus pneumonia. With the spread of the epidemic, other cases in China and abroad have also been found. As an acute respiratory infectious disease, the disease has been included in the Class B infectious diseases stipulated in the Law of the People's Republic of China on the Prevention and Control of Infectious Diseases, and is managed as a Class A infectious disease. Based on the current epidemiological investigation, the incubation period is 1-14 days, mostly 3-7 days.

The main manifestations are fever, dry cough, and fatigue. A few patients have symptoms such as nasal congestion, runny nose, sore throat, myalgia and diarrhea. Severe patients usually have dyspnea and / or hypoxemia one week after the onset of symptoms, and severe patients can quickly progress to acute respiratory distress syndrome, septic shock, difficult to correct metabolic acidosis, coagulation dysfunction and multiple organ functional failure, etc. It is worth noting that in the course of severe and critically ill patients, there may be moderate to low fever, even without obvious fever.

Mild patients showed only low fever, mild fatigue, and no pneumonia. Judging from the current cases, most patients have a good prognosis, and a few patients are critically ill. The elderly and those with chronic underlying disease have a better prognosis. Symptoms in children are relatively mild.

The Spring COVID-19 IgM/IgG Rapid Test Cassette (Colloidal Gold) developed by our company can help diagnose whether patients are infected with the new coronavirus. It has further enriched the detection methods of new coronavirus, expanded the supply of detection reagents, and fully served the needs of epidemic prevention and control.

III. Test purposes

The Spring COVID-19 IgM/IgG Rapid Test Cassette produced by Spring Healthcare Services AG was used to verify the feasibility of clinical evaluation and the reliability of test results for Chinese subjects.

The purpose of research of the clinical test is: calculate the consistency percentage of negative/positive and the total consistency percentage and Kappa coefficient by making statistics of and analyzing test results through comparative experimental research.

IV. Test design

1. **Test plan selection and reasons**

   In vitro diagnostic reagents for testing and reference reagents were used to conduct comparative research tests on clinically suspected new-type coronavirus venous whole blood, serum, and plasma samples, and it was proved that the in vitro diagnostic reagents used in the test can achieve the expected assistance in infection of the new coronavirus.

2. **Sample volume required**

   The total number of clinical trials of this product is not less than 100 cases. The samples are classified into the positive group and the negative group as per the

test results of the reference product. Meanwhile, the samples shall be tested via the qualitative test strip tested and the swab specimen should be tested by reference product from the same patient and then the test results of the product tested and the reference product shall be compared, with statistical analysis being made.

3. **Sample collection, processing and storage**
Sample collection: Suitable for human serum, plasma or whole blood samples, including plasma or whole blood samples prepared from commonly used anticoagulants (EDTA, heparin, sodium citrate).

Sample processing: Before testing, slowly return the refrigerated or frozen samples to room temperature and mix them carefully. When clearly visible particulate matter is present in the sample, it should be centrifuged to remove sediment before testing. If the sample contains a large amount of lipid, hemolysis or turbidity, please do not use it, so as not to affect the result judgment.

Sample storage: The serum and plasma samples to be tested are stored at 2-8°C for 5 days. For long-term storage, store at -20°C.Avoid repeated freeze-thaw samples.
Anti-coagulated whole blood samples should not be stored for more than 72 hours atroom temperature; not more than 7 days at 2 to 8°C.

4. **In vitro diagnostic reagents and reference products for testing**

- **Test in vitro diagnostic reagents**

  Name: Spring COVID-19 IgM/IgG Rapid Test Cassette

  LOT: NO1G01T, NO1G02T, NO1G03T

  Expiry: August, 2020

  Storage Conditions: Store in a dry place at 2-30°C, protected from light. After opening the inner package, the test card will become invalid due to moisture absorption. Please use it within 1 hour.

  Source: Spring Healthcare Services AG

- **Reference products**

  Name: 2019-nCoV nucleic acid test kit (RT-PCR)

  Manufacturer: Shanghai ZJ Bio-Tech Co. Ltd.

  Storage Conditions: Store in a dry place at 2-8°C, protected from light.

V. Experiment method

Get the remaining serum specimens from patients with positive and negative persons. Each serum specimen needs to be tested in random order using in vitro diagnostic reagents for the test.

The operation steps of the in vitro diagnostic reagents for the test are as follows. For details, please refer to the product instruction manual:

Step 1: If the sample is stored refrigerated or frozen, remove the test sample and

required reagents from the storage conditions and equilibrate to room temperature (15-30°C). After thawing, mix the samples thoroughly before testing.

Step 2: When preparing for testing, open the aluminum foil bag from the tear. Remove the test card and lay it flat on a horizontal table.

Step 3: Label the sample number on the test card.

Step 4: Whole blood sample: Use a sample gun or a dropper to draw a whole blood sample from the sample tube and add 1 drop (about 20μl) to the sample hole on the test card, and immediately add 1 Drops (about 35～50μL) of sample dilution, and ensure that no air bubbles are generated during the operation.

Step 5: Time counting and interpret the results within 10 minutes.

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

VI. Statistical methods of statistical analysis of clinical research data

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.

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 tested and the reference product respectively, and then the consistency in statistical results of these two inspection methods shall be compared through Kappa inspection.

The data shall be subject 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 tested and the reference product is evaluated as per the evaluation standard.

VII. Standards of clinical evaluation

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

1. Coincidence rate of negative: the sample whose test results are negative for both the product tested and the reference product and the proportion in the sample whose test results are negative for the reference product shall be more than 90%.

2. Coincidence rate of positive: the sample whose test results are positive for both the product tested and the reference product and the proportion in the sample whose test results are positive for the reference product shall be more than 90%.

3. Total coincidence rate: the sample whose test results are the same for the product tested and the reference product and its proportion in the total number of samples shall be more than 90%.

Method (For IgG)		2019-nCoV nucleic acid test kit (RT-PCR)		Total Results
Spring COVID-19 IgM/IgG Rapid Test Cassette	Results	Positive	Negative	
		A	B	A+B
		C	D	C+D
Total Results		A+C	B+D	A+B+C+D

Clinical sensitivity =A/(A+C)*100%
Clinical specificity = D/(B+D)*100%
Accuracy: (A+D)/(A+B+C+D)*100%

Method (For IgM)		2019-nCoV nucleic acid test kit (RT-PCR)		Total Results
Spring COVID-19 IgM/IgG Rapid Test Cassette	Results	Positive	Negative	
		A	B	A+B
		C	D	C+D
Total Results		A+C	B+D	A+B+C+D

Clinical sensitivity =A/(A+C)*100%
Clinical specificity = D/(B+D)*100%
Accuracy: (A+D)/(A+B+C+D)*100%

If the coincidence rate of positive/negative can meet clinical requirements, two methods or Products are considered as equivalent; if the coincidence rate of positive/negative is greatly different, the clinical scheme should be re-designed.

4. Kappa consistency analysis shall be adopted for statistical analysis of reference reagents.
   The results of the product tested are statistical materials and can be per the table below:

Method (For IgG)		2019-nCoV nucleic acid test kit (RT-PCR)		Total Results
Spring COVID-19 IgM/IgG Rapid Test Cassette	Results	Positive	Negative	
		A	B	A+B
		C	D	C+D
Total Results		A+C	B+D	A+B+C+D

P0= (A+D)/(A+B+C+D)*100%
Pe=((A+B)(A+C)+(A+B)(B+D)) /$(A+B+C+D)^2$
Kappa: (P0 - Pe)/(1-pe)

Method (For IgM)		2019-nCoV nucleic acid test kit (RT-PCR)		Total Results
Spring COVID-19 IgM/IgG Rapid Test Cassette	Results	Positive	Negative	
		A	B	A+B
		C	D	C+D
Total Results		A+C	B+D	A+B+C+D

P0= (A+D)/(A+B+C+D)*100%
Pe=((A+B)(A+C) +(A+B)(B+D)) /$(A+B+C+D)^2$
Kappa: (P0 - Pe)/(1-pe)

If conducting Kappa consistency analysis for the base data above, high consistency can be judged if the Kappa coefficient is >0.8, and both systems are considered as equivalent. Consistency is considered if 0.4<Kappa coefficient <0.8, and the coincidence rate of positive/negative shall be compared, with statistical analysis being made. Two such systems are considered as inconsistent and not equivalent if the Kappa coefficient is <0.4.

VIII. Provisions for amendments to clinical validation

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

IX. Results and Analysis of Clinical Tests

In total, 100 test samples (60 for male and 40 for female) are included for the unit and all test samples included are tested. Statistics on test results and those of the product tested are as follows:

For IgG:

Method (For IgG)		2019-nCoV nucleic acid test kit （RT-PCR）		Total Results
Spring COVID-19 IgM/IgG Rapid Test Cassette		Positive	Negative	
	Positive	48	0	48
	Negative	2	50	52
Total Results		50	50	100

Clinical sensitivity = 48/50*100%=96%
Clinical specificity = 100/100*100%= 100%
Accuracy: (48+50)/(48+0+2+50)*100%=98%

P_0 = (48+50)/(48+0+2+50)*100% = 0.98
P_e = ((48*50)+(48*50))/(100*100)=0.48
Kappa: (P_0 - P_e)/(1-P_e) =0.96

According to the above table, 50 are proven negative of 50 negative specimens, 48 are proven positive of 100 positive specimens. The sensitivity and accuracy are more than 95%, indicating favorable consistency with the reference product. The Kappa=0.96＞ 0.8, indicating favorable and high consistency of two methods and equivalence of two such systems.

For IgM:

Method (For IgM)		2019-nCoV nucleic acid test kit （RT-PCR）		Total Results
Spring COVID-19 IgM/IgG Rapid Test Cassette		Positive	Negative	
	Positive	46	0	46
	Negative	4	50	54
Total Results		50	50	100

Clinical sensitivity = 46/50*100%=92%
Clinical specificity = 50/50*100%= 100%
Accuracy: (46+50)/(46+0+4+50)*100%=96%

P_0 = (46+50)/(46+0+4+50)*100% = 0.96
P_e = (46*50+46*50)/(100*100)=0.46
Kappa: (P_0 - P_e)/(1-P_e) =0.93

According to the above table, 50 are proven negative of 50 negative specimens, 46 are proven positive of 50 positive specimens. The sensitivity and accuracy are more than 95%, indicating favorable consistency with the reference product. The Kappa=0.93 ＞ 0.8, indicating favorable and high consistency of two methods and equivalence of two such systems.

X. Analysis on Inconsistency in Test Results

NO.	Gender	Age	Spring COVID-19 IgM/IgG Rapid Test Cassette		2019-nCoV nucleic acid test kit （RT-PCR）	Clinical Diagnosis
			IgG	IgM	N/A	
23	F	45	NEG	NEG	POS	Subsequent visit of pneumonia triggered by COVID-19
24	F	66	POS	NEG	POS	Cured
52	F	76	NEG	NEG	POS	Non-pneumonia triggered by COVID-19
90	F	32	POS	NEG	POS	Cured

For those subjected to subsequent visit, IgM in the blood may be degraded and IgG definite diagnosis is more effective.

XI. Discussion and Conclusions

Discussion

1. Results of comparative analysis of the product tested and the reference product: Test results of the serum sample of the product tested and the reference result: Both the coincidence rate of negative/positive and the total coincidence rate are larger than 90%, indicating favorable consistency with the reference product. In the analysis results of Kappa inspection, Kappa was proven >0.8, indicating favorable and high consistency of both methods. Both systems were proven equivalent.

2. Statistical analysis results of the product tested for different types of clinical sample: While testing the SARS-CoV-2 antibody through the product tested for different types of clinical sample, the consistency percentages of negative/positive are 100.0% and the total consistency percentage is 100.0%. The Kappa coefficient = 1.00 (>0.8) in the results of Kappa inspection and analysis, indicating favorable and complete consistency of two methods and equivalence of two such systems.

Test Conclusions

By analyzing the test results of the product tested and the reference product, the consistency percentage of negative/positive and the total consistency percentage are proved to be 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. Meanwhile, the test results of the product tested for the serum and plasma sample of the same patient are completely identical. Therefore, such product is applicable to qualitative clinical analysis on the SARS-CoV-2 antibody in the serum and plasma sample of humans, and can be used for auxiliary diagnosis of those suffering from pneumonia triggered by COVID-19.

XII. Quality control methods

On-site quality control

During the course of this study, clinical implementers appointed clinical inspectors to conduct regular on-site supervision visits to the research hospital. Through monitoring

visits, it was found that all the contents of the research plan were strictly observed, and the correctness of the research data was also guaranteed. Participating researchers have undergone unified training, unified recording methods and judgment standards. The entire clinical trial process 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.

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 for each trial to ensure the quality of clinical trials.

XIII. Prediction of adverse events

Because the Spring COVID-19 IgM/IgG Rapid Test Cassette (Colloidal Gold) is an in vitro diagnostic reagent product, no direct contact with patients is required in clinical trials, no test report is provided to patients, 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, and does not cause adverse events.

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: Data of Clinical Tests

NO.	Gender	Age	Spring COVID-19 IgM/IgG Rapid Test Cassette		2019-nCoV nucleic acid test kit (RT-PCR)
			IgG	IgM	
1	F	23	NEG	NEG	NEG
2	M	13	NEG	NEG	NEG
3	F	32	NEG	NEG	NEG
4	M	32	NEG	NEG	NEG
5	F	56	NEG	NEG	NEG
6	F	45	NEG	NEG	NEG
7	M	32	NEG	NEG	NEG
8	M	43	NEG	NEG	NEG
9	F	21	NEG	NEG	NEG
10	M	65	NEG	NEG	NEG
11	M	4	NEG	NEG	NEG
12	M	14	NEG	NEG	NEG
13	F	34	NEG	NEG	NEG
14	M	98	NEG	NEG	NEG
15	M	87	NEG	NEG	NEG
16	F	32	NEG	NEG	NEG

17	M	23	NEG	NEG	NEG
18	M	33	NEG	NEG	NEG
19	M	25	NEG	NEG	NEG
20	F	76	NEG	NEG	NEG
21	F	54	POS	POS	POS
22	F	65	POS	POS	POS
23	F	45	NEG	NEG	POS
24	F	66	POS	NEG	POS
25	M	65	POS	POS	POS
26	M	43	POS	POS	POS
27	M	56	NEG	NEG	NEG
28	F	64	POS	POS	POS
29	F	33	POS	POS	POS
30	F	33	POS	POS	POS
31	F	87	POS	POS	POS
32	M	32	POS	POS	POS
33	M	45	POS	POS	POS
34	F	54	POS	POS	POS
35	M	22	POS	POS	POS
36	F	25	NEG	NEG	NEG
37	F	45	NEG	NEG	NEG
38	M	33	POS	POS	POS
39	M	44	POS	POS	POS
40	M	33	POS	POS	POS
41	M	24	POS	POS	POS
42	F	15	POS	POS	POS
43	F	89	POS	POS	POS
44	M	54	NEG	NEG	NEG
45	M	33	NEG	NEG	NEG
46	M	76	NEG	NEG	NEG
47	M	47	NEG	NEG	NEG
48	F	98	NEG	NEG	NEG
49	M	45	POS	POS	POS
50	F	34	POS	POS	POS
51	F	56	POS	POS	POS
52	F	76	NEG	NEG	POS
53	M	75	POS	POS	POS
54	M	33	POS	POS	POS
55	M	56	POS	POS	POS
56	M	43	POS	POS	POS
57	M	65	NEG	NEG	NEG
58	M	54	NEG	NEG	NEG
59	M	87	NEG	NEG	NEG

60	M	46	NEG	NEG	NEG
61	M	86	NEG	NEG	NEG
62	M	54	NEG	NEG	NEG
63	M	46	NEG	NEG	NEG
64	F	32	POS	POS	POS
65	M	17	POS	POS	POS
66	F	98	POS	POS	POS
67	F	34	POS	POS	POS
68	F	23	POS	POS	POS
69	M	45	POS	POS	POS
70	M	76	POS	POS	POS
71	M	77	NEG	NEG	NEG
72	F	22	POS	POS	POS
73	F	35	POS	POS	POS
74	M	23	POS	POS	POS
75	M	36	NEG	NEG	NEG
76	M	76	POS	POS	POS
77	M	34	POS	POS	POS
78	M	98	POS	POS	POS
79	M	56	POS	POS	POS
80	M	79	NEG	NEG	NEG
81	F	21	POS	POS	POS
82	M	45	POS	POS	POS
83	M	75	NEG	NEG	NEG
84	M	63	POS	POS	POS
85	M	66	POS	POS	POS
86	F	23	POS	POS	POS
87	F	67	POS	POS	POS
88	M	56	NEG	NEG	NEG
89	M	15	POS	POS	POS
90	F	32	POS	NEG	POS
91	F	89	NEG	NEG	NEG
92	F	32	NEG	NEG	NEG
93	M	54	NEG	NEG	NEG
94	F	24	NEG	NEG	NEG
95	F	24	NEG	NEG	NEG
96	M	73	NEG	NEG	NEG
97	M	43	NEG	NEG	NEG
98	F	75	NEG	NEG	NEG
99	M	24	NEG	NEG	NEG
100	M	34	NEG	NEG	NEG