Appendix Ⅱ
Stability Report of the In Vitro Diagnostic Reagents

Product Name	COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
Packaging Specification	25 Tests/Kit
Test Site	3787#, East Yangguang Avenue, Dipu Street, Anji 313300,Huzhou,Zhejiang,China
Sponsor	Zhejiang Orient Gene Biotech Co., Ltd
Reporting Date	March 11, 2020

CONTENT

1. Accelerated Stability

Materials:

COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
Cassette Lot1: 2002155，Lot2:2002156，Lot3:2002157

Sample:

COVID-19(IgG/IgM) positive samples: M-P1, G-P1
COVID-19(IgG/IgM) negative sample: N2

Method:

Extrapolation of shelf life using accelerated stability data calculated with Arrhennius Equation

According to Arrhennius Equation which relates chemical reaction rate (k) to the absolute temperature (T):

d (In k)/dT = Δ Ea /RT2

Where: Ea is the activation energy; and R is the universal gas constant.

Assuming Ea = approx. 19.5 Kcal/mol, the following table, derived from the Arrhennius Equation, illustrates the estimated length of time at a particular storage temperature required for an Orient Gene product to achieve a one-year shelf life.

Storage Temperature °C	Days Required for 1 Year Stability
90.4	0.8
85.2	1.2
80.2	1.8
74.9	2.7
70.1	4.0
65.0	6.0
60.1	9.2
55.1	14.6
50.1	23.0
45.0	37.5
40.1	64.4
37.0	91.0
30.1	193.0
25.1	343.7
22.1	494.8
20.1	617.7
15.1	1145.3
12.0	1688.4

According to the above table, test kits must perform satisfactorily and meet preset stability criteria after storing at 25°C for 344 days, or 37°C for 91 days, or 45°C for 37.5 days or 55°C for 15 days in order to assign a one-year shelf life for the product.

In order to assign a two-year shelf life, product must be stored at a particular temperature for twice as long, perform satisfactorily and meet all stability criteria.

Part 1 Accelerated stability study at 55°C

Accelerated stability study at 55°C is to get a quick look at the predicted shelf life. For assigning final shelf life part 1 accelerated stability study needs to be done.

According to the Arrhenius equation, 14.6 days at 55°C is equivalent to 1 year at room temperature. To achieve a two-year life, product will be stressed for 35 days. Tests will be assayed according to the product test procedure at designated time points using two positive controls and one negative control. Run three replicates per control and read the result at the read time.

Following table illustrates the time points when the stability tests will be performed.

Storage Temperature	DAY 0*	DAY 7	DAY 14	DAY 21	DAY 28	DAY 30	DAY 35
55°C	X	X	X	X	X	X	X

* DAY 0: Run 10 test strips each with three controls.

Results:

Time	Lot	N2		M-P1		G-P1	
		IgG	IgM	IgG	IgM	IgG	IgM
0	Lot 1	10-	10-	10-	10+	10+	10-
	Lot 2	10-	10-	10-	10+	10+	10-
	Lot 3	10-	10-	10-	10+	10+	10-
7	Lot 1	3-	3-	3-	3+	3+	3-
	Lot 2	3-	3-	3-	3+	3+	3-
	Lot 3	3-	3-	3-	3+	3+	3-
14	Lot 1	3-	3-	3-	3+	3+	3-
	Lot 2	3-	3-	3-	3+	3+	3-
	Lot 3	3-	3-	3-	3+	3+	3-
21	Lot 1	3-	3-	3-	3+	3+	3-
	Lot 2	3-	3-	3-	3+	3+	3-
	Lot 3	3-	3-	3-	3+	3+	3-
28	Lot 1	3-	3-	3-	3+	3+	3-
	Lot 2	3-	3-	3-	3+	3+	3-
	Lot 3	3-	3-	3-	3+	3+	3-
30	Lot 1	3-	3-	3-	3+	3+	3-
	Lot 2	3-	3-	3-	3+	3+	3-
	Lot 3	3-	3-	3-	3+	3+	3-
35	Lot 1	3-	3-	3-	3+	3+	3-
	Lot 2	3-	3-	3-	3+	3+	3-
	Lot 3	3-	3-	3-	3+	3+	3-

Conclusion:

The accelerated stability study at 55°C for Orient Gene COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has been finished. The study showed that

COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) could meet the requirements at 24 months according to Arrhennius Equation.

2. Specimen Storage Stability

Purpose

To evaluate the expiration date of specimen (whole blood. serum, plasma) after collection and the influence of freeze/thaw cycle (serum, plasma) on testing results

Method

1.Specimen Storage: store the collected whole blood specimens under 2～8℃,Test the specimens under different time point. Store the collected serum/plasma specimens under 2～8℃ and -20℃ respectively, Test the specimens under different time point. At different time point, each specimen should be tested once by three lots respectively

2.Freeze/thaw cycle: divide the collected serum/plasma into two tubes, one tube is stored under 2～8℃ as the baseline, the other tube undergoes three freeze/thaw cycles (Note: One cycle: store the specimen under -20℃ for 24 hours and then take out and allow the specimen to equilibrate to room temperature),the specimen should be tested once by three lots respectively after every freeze/thaw cycle.

Test material

Three Lots of COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
Cassette Lot1: 2002155，Lot2:2002156，Lot3:2002157

Procedure

1) Specimen preparation
Whole blood: Positive specimens, negative specimens
Serum/Plasma: Positive specimens, negative specimens
2) Allow test cassette and samples reference to equilibrate to room temperature (20-30°C) prior to testing.
3) Remove the test cassette from the sealed foil pouch and use it as soon as possible.
4) Place the test device on a clean and level surface.
For Serum or Plasma Specimens:
With a dropper provided, draw 5μl specimen in the sample well (S). Then add vertically 2 drops of sample buffer to the buffer well (B) immediately. And start the time.
For Whole blood Specimens:

With a dropper provided, draw 10μl specimen in the sample well (S). Then add vertically 2 drops of sample buffer to the buffer well (B) immediately. And start the time.

5) The result should be read in 10 to 15minutes.

Results

Note: "3+"=3 positive results, "3-" =3 negative results

Results of 2～8℃ :

Specimens		2～8℃				
		Day 1	2 Days	3 Days	7 Days	15 Days
Whole blood	Positive	3+	3+（hemolysis）	3+（hemolysis）	3+（hemolysis）	3+(hemolysis）
	Negative	3-	3-（hemolysis）	3-（hemolysis）	3-（hemolysis）	3-（hemolysis）
Serum	Positive	3+	3+	3+	3+	3+
	Negative	3-	3-	3-	3-	3-
Plasma	Positive	3+	3+	3+	3+	3+
	Negative	3-	3-	3-	3-	3-

Results of -20℃:

Specimen		-20℃			
		Day 1	7 Days	15 Days	1 month
Serum	Positive	3+	3+	3+	3+
	Negative	3-	3-	3-	3-
Plasma	Positive	3+	3+	3+	3+
	Negative	3-	3-	3-	3-

Results of three Freeze/thaw cycles：

Specimens		Baseline (stored under 2～8℃)	Freeze/thaw cycles		
			1 cycles	2 cycles	3 cycles
Serum	Positive	3+	3+	3+	3+
	Negative	3-	3-	3-	3-
Plasma	Positive	3+	3+	3+	3+
	Negative	3-	3-	3-	3-

Conclusion

We conclude from the study above,

1) Venipuncture Whole Blood specimen stored under 2-8 ℃ for 1 days does not impact test performance; Over 2 days, hemolysis appears in whole blood specimen and increases with time. It impacts results reading due to the red background. It is recommended that venipuncture Whole Blood Specimen shall be used within 24 hours to avoid hemolysis.

2) Serum/plasma specimen stored under 2-8 ℃ for 15 days does not impact test performance. Serum/plasma specimen stored under -20℃ for 30 days does not impact

the test performance. Clinical test recommendation: clinical serum/plasma specimen could be stored under 2-8℃ for 7 days, -20℃ for longer preservation.

3) Serum/plasma specimen underwent three freeze/thaw cycles does not impact test performance. It is recommended to avoid repeated freeze/thaw cycles and over three freeze/thaw cycles in consideration of antibody protein damage resulted from repeated freeze/thaw cycles.

3. Open-pouch Stability

Purpose

To evaluate the open pouch stability of COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Method

Place open-pouch products under different Relative Humidity (RH) condition: (RH) <40%, 40-60%, 60-80%, >80%. Then take out and use the open-pouch products at 0.5h, 1h and 2h to test internal negative standards, positive standard and limit of detection standard (threshold standard).

Test Material

Three Lots of COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Cassette Lot1: 2002155，Lot2:2002156，Lot3:2002157

Internal Standards

Procedure

1) Allow test cassette, specimen and buffer to equilibrate to room temperature (20-30°C) prior to testing.

2) Remove the test cassette from the sealed foil pouch and use it as soon as possible.

3) Place the test device on a clean and level surface.

With a dropper provided, draw 5μl specimen in the sample well (S). Then add vertically 2 drops of sample buffer to the buffer well (B) immediately. And start the time.

4) The result should be read in 10 to 15minutes.

Test Results

Results of (RH)<40% and 40-60%

Internal Standard	(RH)and Time Point	
	<40%	40-60%

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	0.5h	1h	2h	0.5h	1h	2h
10 negative standards(-/-)	10/10	10/10	10/10	10/10	10/10	10/10
3IgG positive standards(+/+)	3/3	3/3	3/3	3/3	3/3	3/3
3IgM positive standards(+/+)	3/3	3/3	3/3	3/3	3/3	3/3
Limit of detection standard G-L1	-	-	-	-	-	-
Limit of detection standard G-L2	+	+	+	+	+	+
Limit of detection standard G-L3	+	+	+	+	+	+
Precision IgG-J	+	+	+	+	+	+
Limit of detection standard M-L1	-	-	-	-	-	-
Limit of detection standard M-L2	+	+	+	+	+	+
Limit of detection standard M-L3	+	+	+	+	+	+
Precision IgM-J	+	+	+	+	+	+

Results of (RH)60-80% and >80%

Internal Standard	(RH)and Time Point					
	60%-80%			>80%		
	0.5h	1h	2h	0.5h	1h	2h
10 negative standards(-/-)	10/10	10/10	10/10	10/10	10/10	10/10
3IgG positive standards(+/+)	3/3	3/3	3/3	3/3	3/3	3/3
3IgM positive standards(+/+)	3/3	3/3	3/3	3/3	3/3	3/3
Limit of detection standard G-L1	-	-	-	-	-	-
Limit of detection standard G-L2	+	+	+	+	+	-
Limit of detection standard G-L3	+	+	+	+	+	-
Precision IgG-J	+	+	+	+	+	+
Limit of detection standard M-L1	-	-	-	-	-	-
Limit of detection standard M-L2	+	+	+	+	+	-
Limit of detection standard M-L3	+	+	+	+	+	-
Precision IgM-J	+	+	+	+	+	+

Conclusion

From the study above, we can conclude that relative humidity will influence the test result, especially over (RH) 80%. The sensitivity of the test becomes weak after 2 hours' opening. That is the density of T line become weak. It is recommended that perform the test within one hour after opening and use it as soon as possible in clinical use.

4. Transport Simulation

Purpose

Appendix II

Product transportation is an essential part before use, and transportation stability is one of the important indicators to ensure the safety and effectiveness of products. Some extreme conditions may be encountered in the transportation of products, such as temperature change, humidity change and violent turbulence. This study is to confirm whether the performance of products can still meet the requirements after these extreme conditions.

Test Method

Considering the foil pouch is good sealing and buffer bottle is solid enough, humidity changes outside will not have an effect on the product with good package. If not tearing violently, foil pouch will not be damaged. The study is to observe and record the influences of severe turbulence and temperature changes between high temperature, room temperature and low temperature.

1) Let the product packed with box fall freely from the height of about 1.5m, repeat for three times, and observe whether the outer package and inner package of the kit are damaged.
2) To store the product at oven at 45±3℃for two days. Then take out and store at room temperature for one day.
3) Then store the product at refrigerator at -20±10℃for two days. Then take out and store at room temperature for one day and to test them for five replicates.

Material

Three Lots of COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Cassette Lot1: 2002155，Lot2:2002156，Lot3:2002157

Internal specimen standard:

2Limit of detection standard (M-L3, G-L3)

2positive sample standard (M-P1, G-P1)

1negative sample standard (N1)

Test Procedure

1) Allow test cassette and samples reference to equilibrate to room temperature (20-30°C) prior to testing.

2) Remove the test cassette from the sealed foil pouch and use it as soon as possible.

3) Place the test device on a clean and level surface.

With a dropper provided, draw 5 μL specimen in the sample well (S). Then add vertically 2 drops of sample buffer to the buffer well (B) immediately. And start the time.

4) The result should be read in 10 to 15minutes.

Result

Lot	References

	N1	M-L3	G-L3	M-P1	G-P1
2002155	5-	5+	5+	5+	5+
2002156	5-	5+	5+	5+	5+
2002157	5-	5+	5+	5+	5+

Note: “5+”= 5positive results, “5-”= 5negative results

Conclusion:

Orient Gene COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) can be used after transport simulation and we can conclude that Orient Gene COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is stable under normal transportation conditions.

5. Real Time Stability

Purpose

To verify product stability of Orient Gene COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) over length of storage time

Test Method

Place three lots of Orient Gene COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) at 2~8℃and 30±3℃ respectively, dry and avoid light. To test references by Orient Gene COVID-19 IgM/IgG Rapid Test over length of storage time as table below.

Material

Three Lots of COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Cassette Lot1: 2002155，Lot2:2002156，Lot3:2002157

Internal specimen standard:

2Limit of detection standard (M-L3, G-L3)

2positive standard (M-P1, G-P1)

1negative standard (N1)

Test Procedure

1) Place COVID-19 IgM/IgG Rapid Tests at 2~8℃and 30±3℃ respectively.

2) Take out tests over length of storage time at the table

Storage Temp	**DAY**	**Month**	**Month**	**Month**	**Month**	**Month**	**Month**	**Month**	**Month**
	0	**1**	**3**	**6**	**9**	**12**	**18**	**24**	**27**
2~8℃	X	X	X	X	X	X	X	X	X
30±3℃	X	X	X	X	X	X	X	X	X

3) Run five replicates per control and read at the product read time.

4) Allow test cassette and samples reference to equilibrate to room temperature (20-30°C) prior to testing.

5) Remove the test cassette from the sealed foil pouch and use it as soon as possible.

6) Place the test device on a clean and level surface.

With a dropper provided, draw 5μl specimen in the sample well (S). Then add vertically 2 drops of sample buffer to the buffer well (B) immediately. And start the time.

7) The result should be read in 10 to 15minutes.

Result:

The results of tests stored at 2~8℃

Lot1	Reference	replicates	months								
			0	1	3	6	9	12	18	24	27
2002155	N1	5	5-	5-							
	M-L3	5	5+	5+							
	G-L3	5	5+	5+							
	M-P1	5	5+	5+							
	G-P1	5	5+	5+							
Lot2	Reference	replicates	months								
			0	1	3	6	9	12	18	24	27
2002156	N1	5	5-	5-							
	M-L3	5	5+	5+							
	G-L3	5	5+	5+							
	M-P1	5	5+	5+							
	G-P1	5	5+	5+							
Lot3	Reference	replicates	months								
			0	1							
2002157	N1	5	5-	5-							
	M-L3	5	5+	5+							
	G-L3	5	5+	5+							
	M-P1	5	5+	5+							
	G-P1	5	5+	5+							

The results of tests stored at 30±3℃

Lot1	Reference	replicates	months								
			0	1	3	6	9	12	18	24	27
2002155	N1	5	5-	5-							
	M-L3	5	5+	5+							

	G-L3	5	5+	5+							
	M-P1	5	5+	5+							
	G-P1	5	5+	5+							
Lot2	Reference	replicates	months								
			0	1	3	6	9	12	18	24	27
2002156	N1	5	5-	5-							
	M-L3	5	5+	5+							
	G-L3	5	5+	5+							
	M-P1	5	5+	5+							
	G-P1	5	5+	5+							
Lot3	Reference	replicates	months								
			0	1							
2002157	N1	5	5-	5-							
	M-L3	5	5+	5+							
	G-L3	5	5+	5+							
	M-P1	5	5+	5+							
	G-P1	5	5+	5+							

Note: “5+”= 5positive results, “5-”= 5negative results

Conclusion:

From the results, Orient Gene COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) can be used after stored at 2~8℃ and 30±3℃ for a month. And the real time stability study is still to be finished.