bioPerfectus technologies

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Novel Corona Virus (SARS-cov-2) Ag Rapid Test Kit

Stability Evaluation Report

1 Purpose

This is to set the protocol for stability evaluation of Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit and applicable specimens.

2 Reference standards and regulations (EU harmonized standards, ISO, and FDA standards)

(1) EN ISO 23640:2015 In vitro diagnostic medical devices - Evaluation of stability of in vitro diagnostic reagents (ISO 23640:2011)

(2) CLSI EP25-A: Evaluation of Stability of in Vitro Diagnostic Reagents; Approved Guideline.

(3) CLSI EP05-A3: Evaluation of Precision of Quantitation Measurement Procedures.

(4) CLSI EP15-A3: User Verification of Precision and Estimation of Bias.

(5) CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

3 Product name, batch and size

Product name	Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit
Batch No.	20200213-1，20200214-1，20200215-1
Specification：	20 Tests

4 Reference panel

Reference Name	Code	Component	Characteristic
Positive	P1	SARS-CoV-2 virus	Inactivated virus
	P2	SARS-CoV-2 virus	Inactivated virus
	P3	SARS-CoV-2 Recombinant protein	Recombinant antigen
	P4	SARS-CoV-2 Recombinant protein	Recombinant antigen
Negative	N1	Influenza A virus	Inactivated virus
	N2	Influenza B virus	Inactivated virus
	N3	Adenovirus	Inactivated virus
	N4	Respiratory syncytial virus	Inactivated virus
	N5	Rhinovirus	Inactivated virus
	N6	Group A streptococcus	Inactivated bacteria
Sensitivity	S1	SARS-CoV-2 virus	Inactivated virus
	S2	SARS-CoV-2 virus	Inactivated virus
	S3	SARS-CoV-2 virus	Inactivated virus
	S4	Sample extraction buffer	Sample extraction buffer
Repeatability	J	SARS-CoV-2 Recombinant protein	Recombinant antigen

5 Evaluation methods

5.1 Shelf life stability test protocol

5.1.1 Stability under recommended storage conditions (4℃～30℃ storage test)

Products of three batches (20200213-1, 20200214-1 and 20200215-1) were stored at room temperature avoid sunlight (2 and 32℃, respectively). Randomly select product from storage on the 0th day, the 3th, 6th, 9th, 12th, 18th, 24th and 27th month to evaluate the performance of the kit with the reference panel, including Positive coincidence rate reference, Negative coincidence rate reference, Limit of detection reference and Precision reference. Use a different randomized order to process the test samples each day. Visually inspect the product before use, and record signs of potential stability related changes.

5.1.2 Stability after high temperature transportation simulation studies

Kits from 3 batch were stored at 45℃ for 4 weeks. Then the kits were stored at room temperature avoid sunlight (25℃). Randomly select product from storage on the 0th day, the 3th, 6th, 9th, 12th, 18th, 24th and 27th month to evaluate the performance of the kit with the reference panel, including Positive coincidence rate reference, Negative coincidence rate reference, Limit of detection reference and Precision reference. Use a different randomized order to process the test samples each day. Visually inspect the product before use, and record signs of potential stability related changes.

5.1.3 Stability after freeze/thaw simulation studies

Kits from 3 batches were stored at -40℃ for 3 days and then placed at 45℃ for 3days and repeat the process for 3 times. Then the kits were stored at room temperature avoid sunlight (25℃). Randomly select product from storage on the 0th day, the 3th, 6th, 9th, 12th, 18th, 24th and 27th month to evaluate the performance of the kit with the reference panel, including Positive coincidence rate reference, Negative coincidence rate reference, Limit of detection reference and Precision reference. Use a different randomized order to process the test samples each day. Visually inspect the product before use, and record signs of potential stability related changes.

5.1.4 Acceptance criteria

(1) LOD: Test the gradient diluted limit of detection (LOD) references. S1-S2 are tested as positive; S3 is positive or possibly negative; S4 must be detected as negative.

(2) Consistency for positive reference: Four positive references (P1-P4) are tested. Four positive references are detected as positive and the consistency (+/+) is 4/4.

(3) Consistency for negative reference: Six negative references (N1-N6) are tested. All 6 negative references are tested as negative with consistency (-/-) of 6/6.

(4) Repeatability: Repeatability reference J is tested for 10 times as positive with consistent color intensity.

5.2 High temperature accelerated stability test protocol

5.2.1 45℃ accelerated stability test

According to 'Chinese Requirements for Biological Products (supplemented in 2002)', detection kit stability can be tested by high temperature accelerated destructive test. Products of three batches (20200213-1, 20200214-1 and 20200215-1) are kept at 45℃. They are tested for the consistency of positive reference, consistency of negative reference and that of LOD reference as well as the repeatability after 0 day, 3 days, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks and 9 weeks storage.

5.2.2 60℃ accelerated stability test

According to 'Chinese Requirements for Biological Products (supplemented in 2002)', detection kit stability can be tested by high temperature accelerated destructive test. Products of three batches (20200213-1, 20200214-1 and 20200215-1) are kept at 60℃. They are tested for the consistency of positive reference, consistency of negative

reference and that of LOD reference as well as the repeatability after 0 day, 2 days, 4 days, 6 days, 8 days, 10 days.

5.2.3 Acceptance criteria

(1) LOD: Test the gradient diluted limit of detection (LOD) references. S1-S2 are tested as positive; S3 is positive or possibly negative; S4 must be detected as negative.

(2) Consistency for positive reference: Four positive references (P1-P4) are tested. Four positive references are detected as positive and the consistency (+/+) is 4/4.

(3) Consistency for negative reference: Six negative references (N1-N6) are tested. All 6 negative references are tested as negative with consistency (-/-) of 6/6.

(4) Repeatability: Repeatability reference J is tested for 10 times as positive with consistent color intensity.

5.3 Transportation stability test protocol

5.3.1 Simulated high temperature transportation stability test

Products of one batch (20200213-1) are kept at 45℃ for 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks. Kits undergo different storage time were tested with the reference panel and kits from shelf-life studies were tested and set as control. Each sample was tested in duplicate.

5.3.2 Simulated low temperature transportation stability test

Products of one batch (20200213-1) are at -40℃ for 3 days and then placed at 45℃ for 3days. The whole freeze/thaw process was repeated for 1 time, 2 times, 3 times and 4 times. Kits undergo different freeze/thaw times were tested with the reference panel and kits from shelf-life studies were tested and set as control. Each sample was tested in duplicate.

5.3.3 Humidity study

Kits from 1 batch were placed in the 30%, 60%, 85% ±5% relative humidity environment for 3 days, 1week and 2 weeks. Kits undergo different RH environment were tested with the reference panel.

5.3.4 Real transport process simulation study

Kits from 1 batch were stored at varied environment for different time as list below. Kits undergo different conditions were tested with the reference panel. Randomly select product from storage on the 0th day, the 1th, 6th, 12th, 18th, 24th, and 30th month to evaluate the performance of the kit.

Anticipated Conditions	Time in hours	Temperature in℃±2℃	Humidity in %	Simulation Process
Extreme Cold, Uncontrolled RH	24	-40	Uncontrolled RH	Ari Transport
Controlled Conditions	72	25	50%RH±5%	Temporary Storage
Extreme Cold, Uncontrolled RH	24	-40	Uncontrolled RH	Ari Transport
Controlled Conditions	72	25	50%RH±5%	Temporary Storage
Hot, Humid	72	45	85%RH±5%	Land Transport
Extreme Heat, Dry	72	60	20%RH±5%	Complex Environment
Severe Cold, Uncontrolled RH	72	-20	Uncontrolled RH	Land Transport

5.3.5 Physical conditions simulation

a) Impact simulation

Well packaged Kits from 1 batch were placed in corrugated box in a 4x3x5 (X,Y,Z) mode. Then the box was lifted to 1.2 m and dropped to the ground. Repeat the lift/drop process for 3 times. Repeat the whole process on each face of the box.

b) Stacking simulation

Well packaged Kits from 1 batch were placed in corrugated box in a 4x3x5 (X,Y,Z) mode. Then place the corrugated boxes on a pallet in a 2x3x3 (X,Y,Z) mode.

5.3.6 Performance Acceptance criteria

(1) LOD: Test the gradient diluted limit of detection (LOD) references. S1-S2 are tested as positive; S3 is positive or possibly negative; S4 must be detected as negative.

(2) Consistency for positive reference: Four positive references (P1-P4) are tested. Four positive references are detected as positive and the consistency (+/+) is 4/4.

(3) Consistency for negative reference: Six negative references (N1-N6) are tested. All 6 negative references are tested as negative with consistency (-/-) of 6/6.

(4) Repeatability: Repeatability reference J is tested for 10 times as positive with consistent color intensity.

5.3.7 Appearance acceptance criterion:

95% of the appearance of the corrugated boxes are complete without damage.

5.4 Open vial stability test protocol

5.4.1 Open vial stability of test cards

Unwrap the foil pouch of the cassette at the 0th，1th hour，2th hours，4th hours，8th hours，24th hours and 25th hours since opening at 20~30℃with humidity of 80%, respectively. Test the cassette with the reference panel, including Positive coincidence rate reference, Negative coincidence rate reference, Limit of detection reference and Precision reference to evaluate the performance of cassettes after unwrapped for different time period. Visually inspect the product before use, and record signs of potential stability related changes.

5.4.2 Open vial stability of sample extraction buffer

Unwrap the sample extraction buffer from 1 batch and then tightly capped the cover. Store the sample extraction buffer at room temperature avoid sunlight (4-30℃) for 0 day, the 2, 4, 8, 12, 16, 20, 24 and 25 weeks to evaluate the performance of the sample extraction buffer with the reference panel, including Positive coincidence rate reference, Negative coincidence rate reference, Limit of detection reference and Precision reference after unwrapped for different time period. Visually inspect the product before use, and record signs of potential stability related changes.

5.4.3 Acceptance criteria

(1) LOD: Test the gradient diluted limit of detection (LOD) references. S1-S2 are tested as positive; S3 is positive or possibly negative; S4 must be detected as negative.

(2) Consistency for positive reference: Four positive references (P1-P4) are tested. Four positive references are detected as positive and the consistency (+/+) is 4/4.

(3) Consistency for negative reference: Six negative references (N1-N6) are tested. All 6 negative references are tested as negative with consistency (-/-) of 6/6.

(4) Repeatability: Repeatability reference J is tested for 10 times as positive with consistent color intensity.

5.5 Specimen refrigeration stability test protocol

5.5.1 Stability test for specimens refrigerated at 2°C~8°C

Heat-inactivated SARS-CoV-2 virus was spiked into the pooled human nasal swab matrix obtained from multiple healthy volunteers who were confirmed as SARS-CoV-2 negative by RT-PCR to prepare positive samples. 50 μL samples with different tilers were added to swabs to form positive samples A1, A2 and A3. Stored the swabs in clean, dry transport tubes at 2°C-8°C. Detection kit of 20200213-1 batch is used to test the samples for positive consistency after storage for 0 day, 1 day, 3 days, 5 days, 7 days. Each sample were test for 5 times.

5.5.2 Freeze-thaw stability

Heat-inactivated SARS-CoV-2 virus was spiked into the pooled human nasal swab matrix obtained from multiple healthy volunteers who were confirmed as SARS-CoV-2 negative by RT-PCR to prepare positive samples. 50 μL samples with different tilers were added to swabs to form positive samples A1, A2 and A3. Then the swabs were treated with sample extraction buffer. The eluate were stored in a refrigerator at -20±5℃, and then freeze-thaw one time every other day for 0 time (control group), 2 times, 3 times, 4 times, 5 times and 6 times, respectively. The samples with different times of freeze-thaw process were tested and the positive rates were calculated and analyzed.

5.5.3 Transport stability

Swabs were placed in clean, dry transport tubes and then placed in a cold closet or placed with ice bag in a foam box, then sealed and placed at room temperature. The transport process was simulated for 0 days (control group), 1 day, 2 days, 3 days, 4 days and 5days. The samples were tested every day, and the positive rates were calculated and analyzed.

5.5.4 Acceptance criteria

(1) Negative consistency

All negative samples show negative results.

(2) Positive consistency

Detection results for positive reference samples meet corresponding requirements.

6 Evaluation steps

（1） Carry out the experiments according to the above-mentioned method.

（2） Operate the tests according to the instruction for use.

（3） Record and statistically analyze the data.

7 Operator/Test Report review

Responsibilities	Signature	Remarks
Operator:		
Report reviewer:		

8 Relevant records form

8.1 4°C~30°C storage stability test for the kit

Products of three batches (20200213-1, 20200214-1 and 20200215-1) were kept at room temperature. They were tested for the consistency of positive reference, consistency of negative reference and that of LOD reference as well as the repeatability after 0 day, 4 months, 8 months, 12 months, 18 months and 24 months storage.

8.1.1 Consistency of positive reference

Table 1-1. 2°Cstorage stability test for the kit

Batch	Storage time (months)	P1	P2	P3	P4
20200213-1	0	+	+	+	+

	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				
20200214-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				
20200215-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				

Table 1-2. 32°Cstorage stability test for the kit

Batch	Storage time (months)	P1	P2	P3	P4
20200213-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				
20200214-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				
20200215-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				

Table 1-3. Stability after high temperature transportation simulation studies

Batch	Storage time (months)	P1	P2	P3	P4

20200213-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				
20200214-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				
20200215-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				

Table 1-4. Stability after freeze/thaw simulation studies

Batch	Storage time (months)	P1	P2	P3	P4
20200213-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				
20200214-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				
20200215-1	0	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	12				
	18				
	24				

8.1.2 Consistency of negative reference

Table 2-1. 2°Cstorage stability test for the kit							
Batch	Storage time (months)	N1	N2	N3	N4	N5	N6
20200213-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						
20200214-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						
20200215-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						

Table 2-2. 32°Cstorage stability test for the kit							
Batch	Storage time (months)	N1	N2	N3	N4	N5	N6
20200213-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						
20200214-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						
20200215-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						

	24						

Table 2-3. Stability after high temperature transportation simulation studies

Batch	Storage time (months)	N1	N2	N3	N4	N5	N6
20200213-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						
20200214-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						
20200215-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						

Table 2-4. Stability after freeze/thaw simulation studies

Batch	Storage time (months)	N1	N2	N3	N4	N5	N6
20200213-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						
20200214-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						
	18						
	24						
20200215-1	0	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	12						

	18						
	24						

8.1.3 LOD

Table 3-1. 2°Cstorage stability test for the kit					
Batch	Storage time (months)	S1	S2	S3	S4
20200213-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				
20200214-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				
20200215-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				

Table 3-2. 32°Cstorage stability test for the kit					
Batch	Storage time (months)	S1	S2	S3	S4
20200213-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				
20200214-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				
20200215-1	0	+	+	-	-
	4	+	+	-	-

	8	+	+	-	-
	12				
	18				
	24				

Table 3-3. Stability after high temperature transportation simulation studies					
Batch	Storage time (months)	S1	S2	S3	S4
20200213-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				
20200214-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				
20200215-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				

Table 3-4. Stability after freeze/thaw simulation studies					
Batch	Storage time (months)	S1	S2	S3	S4
20200213-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				
20200214-1	0	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				
20200215-1	0	+	+	-	-

	4	+	+	-	-
	8	+	+	-	-
	12				
	18				
	24				

8.1.4 Repeatability

Table 4-1. 2°Cstorage stability test for the kit		
Batch	Storage time (months)	J
20200213-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	
20200214-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	
20200215-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	

Table 4-2. 32°Cstorage stability test for the kit		
Batch	Storage time (months)	J
20200213-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	
20200214-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	
20200215-1	0	10/10(100%)

	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	

Table 4-3. Stability after high temperature transportation simulation studies		
Batch	Storage time (months)	J
20200213-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	
20200214-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	
20200215-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	

Table 4-4. Stability after freeze/thaw simulation studies		
Batch	Storage time (months)	J
20200213-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	
20200214-1	0	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	12	
	18	
	24	
20200215-1	0	10/10(100%)
	4	10/10(100%)

	8	10/10(100%)
	12	
	18	
	24	

8.2 45°C accelerated destructive test

According to 'Chinese Requirements for Biological Products (supplemented in 2002)', detection kit stability can be tested by high temperature accelerated destructive test. Products of one batch (20200213-1) are kept at 45°C. They are tested for the consistency of positive reference, consistency of negative reference and that of LOD reference as well as the repeatability after 0 day, 3 days, 1 week, 2 weeks, 4 weeks, 6 weeks and 8 weeks storage.

8.2.1 Consistency of positive reference

Table 5-1. 45°C accelerated destruction test for the kit

Batch	Storage time	P1	P2	P3	P4
20200213-1	0 days	+	+	+	+
	3 days	+	+	+	+
	1 week	+	+	+	+
	2 weeks	+	+	+	+
	4 weeks	+	+	+	+
	6 weeks	+	+	+	+
	8 weeks	+	+	+	+
20200214-1	0 days	+	+	+	+
	3 days	+	+	+	+
	1 week	+	+	+	+
	2 weeks	+	+	+	+
	4 weeks	+	+	+	+
	6 weeks	+	+	+	+
	8 weeks	+	+	+	+
20200215-1	0 days	+	+	+	+
	3 days	+	+	+	+
	1 week	+	+	+	+
	2 weeks	+	+	+	+
	4 weeks	+	+	+	+
	6 weeks	+	+	+	+
	8 weeks	+	+	+	+

8.2.2 Consistency of negative reference

Table 5-2. 45°C accelerated destruction test for the kit

Batch	Storage time	N1	N2	N3	N4	N5	N6
20200213-1	0 days	-	-	-	-	-	-
	3 days	-	-	-	-	-	-
	1 week	-	-	-	-	-	-
	2 weeks	-	-	-	-	-	-

	4 weeks	-	-	-	-	-	-
	6 weeks	-	-	-	-	-	-
	8 weeks	-	-	-	-	-	-
20200214-1	0 days	-	-	-	-	-	-
	3 days	-	-	-	-	-	-
	1 week	-	-	-	-	-	-
	2 weeks	-	-	-	-	-	-
	4 weeks	-	-	-	-	-	-
	6 weeks	-	-	-	-	-	-
	8 weeks	-	-	-	-	-	-
20200215-1	0 days	-	-	-	-	-	-
	3 days	-	-	-	-	-	-
	1 week	-	-	-	-	-	-
	2 weeks	-	-	-	-	-	-
	4 weeks	-	-	-	-	-	-
	6 weeks	-	-	-	-	-	-
	8 weeks	-	-	-	-	-	-

8.2.3 LOD

Table 5-3. 45°C accelerated destruction test for the kit

Batch	Storage time	S1	S2	S3	S4
20200213-1	0 days	+	+	-	-
	3 days	+	+	-	-
	1 week	+	+	-	-
	2 weeks	+	+	-	-
	4 weeks	+	+	-	-
	6 weeks	+	+	-	-
	8 weeks	+	+	-	-
20200214-1	0 days	+	+	-	-
	3 days	+	+	-	-
	1 week	+	+	-	-
	2 weeks	+	+	-	-
	4 weeks	+	+	-	-
	6 weeks	+	+	-	-
	8 weeks	+	+	-	-
20200215-1	0 days	+	+	-	-
	3 days	+	+	-	-
	1 week	+	+	-	-
	2 weeks	+	+	-	-
	4 weeks	+	+	-	-
	6 weeks	+	+	-	-

	8 weeks	+	+	-	-

8.2.4 Repeatability

Table 5-4. 45°C accelerated destruction test for kit		
Batch	Storage time	J
20200213-1	0 days	10/10(100%)
	3 days	10/10(100%)
	1 week	10/10(100%)
	2 weeks	10/10(100%)
	4 weeks	10/10(100%)
	6 weeks	10/10(100%)
	8 weeks	10/10(100%)
20200214-1	0 days	10/10(100%)
	3 days	10/10(100%)
	1 week	10/10(100%)
	2 weeks	10/10(100%)
	4 weeks	10/10(100%)
	6 weeks	10/10(100%)
	8 weeks	10/10(100%)
20200215-1	0 days	10/10(100%)
	3 days	10/10(100%)
	1 week	10/10(100%)
	2 weeks	10/10(100%)
	4 weeks	10/10(100%)
	6 weeks	10/10(100%)
	8 weeks	10/10(100%)

8.3 60°C accelerated destructive test

According to 'Chinese Requirements for Biological Products (supplemented in 2002)', detection kit stability can be tested by high temperature accelerated destructive test. Products of one batch (20200213-1) are kept at 60°C. They are tested for the consistency of positive reference, consistency of negative reference and that of LOD reference as well as the repeatability after 0 day, 2 days, 4 days, 6 days, 8 days, 10 days' storage.

8.3.1 Consistency of positive reference

Table 6-1. 60°C accelerated destruction test for the kit					
Batch	Storage time	P1	P2	P3	P4
20200213-1	0 days	+	+	+	+
	2 days	+	+	+	+
	4 days	+	+	+	+
	6 days	+	+	+	+
	8 days	+	+	+	+
	10 days	+	+	+	+
20200214-1	0 days	+	+	+	+
	2 days	+	+	+	+

	4 days	+	+	+	+
	6 days	+	+	+	+
	8 days	+	+	+	+
	10 days	+	+	+	+
20200215-1	0 days	+	+	+	+
	2 days	+	+	+	+
	4 days	+	+	+	+
	6 days	+	+	+	+
	8 days	+	+	+	+
	10 days	+	+	+	+

8.3.2 Consistency of negative reference

Table 6-2. 60°C accelerated destruction test for the kit

Batch	Storage time	N1	N2	N3	N4	N5	N6
20200213-1	0 days	-	-	-	-	-	-
	2 days	-	-	-	-	-	-
	4 days	-	-	-	-	-	-
	6 days	-	-	-	-	-	-
	8 days	-	-	-	-	-	-
	10 days	-	-	-	-	-	-
20200214-1	0 days	-	-	-	-	-	-
	2 days	-	-	-	-	-	-
	4 days	-	-	-	-	-	-
	6 days	-	-	-	-	-	-
	8 days	-	-	-	-	-	-
	10 days	-	-	-	-	-	-
20200215-1	0 days	-	-	-	-	-	-
	2 days	-	-	-	-	-	-
	4 days	-	-	-	-	-	-
	6 days	-	-	-	-	-	-
	8 days	-	-	-	-	-	-
	10 days	-	-	-	-	-	-

8.3.3 LOD

Table 6-3. 45°C accelerated destruction test of the kit

Batch	Storage time	S1	S2	S3	S4
20200213-1	0 days	+	+	-	-
	2 days	+	+	-	-
	4 days	+	+	-	-
	6 days	+	+	-	-
	8 days	+	+	-	-

	10 days	+	+	-	-
20200214-1	0 days	+	+	-	-
	2 days	+	+	-	-
	4 days	+	+	-	-
	6 days	+	+	-	-
	8 days	+	+	-	-
	10 days	+	+	-	-
20200215-1	0 days	+	+	-	-
	2 days	+	+	-	-
	4 days	+	+	-	-
	6 days	+	+	-	-
	8 days	+	+	-	-
	10 days	+	+	-	-

8.3.4 Repeatability

Table 6-4. 45°C accelerated destruction test for kit

Batch	Storage time	J
20200213-1	0 days	10/10(100%)
	2 days	10/10(100%)
	4 days	10/10(100%)
	6 days	10/10(100%)
	8 days	10/10(100%)
	10 days	10/10(100%)
20200214-1	0 days	10/10(100%)
	2 days	10/10(100%)
	4 days	10/10(100%)
	6 days	10/10(100%)
	8 days	10/10(100%)
	10 days	10/10(100%)
20200215-1	0 days	10/10(100%)
	2 days	10/10(100%)
	4 days	10/10(100%)
	6 days	10/10(100%)
	8 days	10/10(100%)
	10 days	10/10(100%)

8.4 Transportation stability test protocol

8.4.1 Simulated high temperature transportation stability test

Products of one batch (20200213-1) are kept at 45℃ for 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks. Kits undergo different storage time were tested with the reference panel and kits from shelf-life studies were tested and set as control. Each sample was tested in duplicate.

8.4.1.1 Consistency of positive reference

Table7-1. High temperature transport stability test for the kit

Batch	Storage time	P1	P2	P3	P4
20200213-1	0 day	+	+	+	+
	1 week	+	+	+	+
	2 weeks	+	+	+	+
	3 weeks	+	+	+	+
	4 weeks	+	+	+	+
	5 weeks	+	+	+	+

8.4.1.2 Negative reference compliance rate

Table 7-2. High temperature transport stability test for kit

Batch	Storage time	N1	N2	N3	N4	N5	N6
20200213-1	0 day	-	-	-	-	-	-
	1 week	-	-	-	-	-	-
	2 weeks	-	-	-	-	-	-
	3 weeks	-	-	-	-	-	-
	4 weeks	-	-	-	-	-	-
	5 weeks	-	-	-	-	-	-

8.4.1.3 LOD

Table 7-3. High temperature transport stability test for kit

Batch	Storage time	S1	S2	S3	S4
20200213-1	0 day	+	+	-	-
	1 week	+	+	-	-
	2 weeks	+	+	-	-
	3 weeks	+	+	-	-
	4 weeks	+	+	-	-
	5 weeks	+	+	-	-

8.4.1.4 Repeatability

Table 7-4. High temperature transport stability test for kit

Batch	Storage time	J
20200213-1	0 day	10/10(100%)
	1 week	10/10(100%)
	2 weeks	10/10(100%)
	3 weeks	10/10(100%)
	4 weeks	10/10(100%)
	5 weeks	10/10(100%)

8.4.2 Simulated low temperature transportation stability test

Products of one batch (20200213-1) are at -40℃ for 3 days and then placed at 45℃ for 3days. The whole freeze/thaw process was repeated for 1 time, 2 times, 3 times and 4 times. Kits undergo different freeze/thaw times were tested with the reference panel and kits from shelf-life studies were tested and set as control. Each sample was tested in duplicate.

8.4.2.1 Consistency of positive reference

Table 8-1. Low temperature transport stability test for kit					
Batch	Freeze/thaw time	P1	P2	P3	P4
20200213-1	0 time	+	+	+	+
	1 time	+	+	+	+
	2 times	+	+	+	+
	3 times	+	+	+	+
	4 times	+	+	+	+

8.4.2.2 Consistency of negative reference

Table 8-2. Low temperature transport stability test for kit							
Batch	Freeze/thaw time	N1	N2	N3	N4	N5	N6
20200213-1	0 time	-	-	-	-	-	-
	1 time	-	-	-	-	-	-
	2 times	-	-	-	-	-	-
	3 times	-	-	-	-	-	-
	4 times	-	-	-	-	-	-

8.4.2.3 LOD

Table 8-3. Low temperature transport stability test for kit					
Batch	Freeze/thaw time	S1	S2	S3	S4
20200213-1	0 time	+	+	-	-
	1 time	+	+	-	-
	2 times	+	+	-	-
	3 times	+	+	-	-
	4 times	+	+	-	-

8.4.2.4 Repeatability

Table 8-4. Low temperature transport stability test for kit		
Batch	Freeze/thaw time	J
20200213-1	0 time	10/10(100%)
	1 time	10/10(100%)
	2 times	10/10(100%)
	3 times	10/10(100%)
	4 times	10/10(100%)

8.4.3 Humidity study

8.4.3.1 Consistency of positive reference

Table 9-1. Humidity study of the kit					
Humidity	Storage time	P1	P2	P3	P4
30% RH±5%	0 day	+	+	+	+
	3 days	+	+	+	+
	1 week	+	+	+	+

	2 weeks	+	+	+	+
60% RH±5%	0 day	+	+	+	+
	3 days	+	+	+	+
	1 week	+	+	+	+
	2 weeks	+	+	+	+
85% RH±5%	0 day	+	+	+	+
	3 days	+	+	+	+
	1 week	+	+	+	+
	2 weeks	+	+	+	+

8.4.3.2 Consistency of negative reference

Table 9-1. Humidity study of the kit

Humidity	Storage time	N1	N2	N3	N4	N5	N6
30% RH±5%	0 day	-	-	-	-	-	-
	3 days	-	-	-	-	-	-
	1 week	-	-	-	-	-	-
	2 weeks	-	-	-	-	-	-
60% RH±5%	0 day	-	-	-	-	-	-
	3 days	-	-	-	-	-	-
	1 week	-	-	-	-	-	-
	2 weeks	-	-	-	-	-	-
85% RH±5%	0 day	-	-	-	-	-	-
	3 days	-	-	-	-	-	-
	1 week	-	-	-	-	-	-
	2 weeks	-	-	-	-	-	-

8.4.3.3 LOD

Table 9-3. Humidity study of the kit

Humidity	Storage time	S1	S2	S3	S4
30% RH±5%	0 day	+	+	-	-
	3 days	+	+	-	-
	1 week	+	+	-	-
	2 weeks	+	+	-	-
60% RH±5%	0 day	+	+	-	-
	3 days	+	+	-	-
	1 week	+	+	-	-
	2 weeks	+	+	-	-
85% RH±5%	0 day	+	+	-	-
	3 days	+	+	-	-
	1 week	+	+	-	-
	2 weeks	+	+	-	-

8.4.3.4 Repeatability

Table 9-4. Humidity study of the kit

Humidity	Storage time	J
30% RH±5%	0 day	10/10(100%)
	3 days	10/10(100%)
	1 week	10/10(100%)
	2 weeks	10/10(100%)
60% RH±5%	0 day	10/10(100%)
	3 days	10/10(100%)
	1 week	10/10(100%)
	2 weeks	10/10(100%)
85% RH±5%	0 day	10/10(100%)
	3 days	10/10(100%)
	1 week	10/10(100%)
	2 weeks	10/10(100%)

8.4.4 Real transport process simulation study

8.4.4.1 Consistency of positive reference

Table 10-1. Real transport process simulation study of the kit

Storage time	P1	P2	P3	P4
0 days	+	+	+	+
1 month	+	+	+	+
6 months	+	+	+	+
12 months				
18 months				
24 months				
30 months				

8.4.4.2 Consistency of negative reference

Table 10-2. Real transport process simulation study of the kit

Storage time	N1	N2	N3	N4	N5	N6
0 days	-	-	-	-	-	-
1 month	-	-	-	-	-	-
6 months	-	-	-	-	-	-
12 months						
18 months						
24 months						
30 months						

8.4.4.3 LOD

Table 10-3. Real transport process simulation study of the kit

Storage time	S1	S2	S3	S4
0 days	+	+	-	-
1 month	+	+	-	-

6 months	+	+	-	-
12 months				
18 months				
24 months				

8.4.4.4 Repeatability

Table 10-4. Real transport process simulation study of the kit	
Storage time	J
0 days	10/10(100%)
1 month	10/10(100%)
6 months	10/10(100%)
12 months	
18 months	
24 months	
30 months	

8.4.5 Physical conditions simulation

a) Impact simulation

48 boxes of Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit were placed in a corrugated box. After the impact simulation, 2 boxes of the kit at the corner were different degrees of damaged. That indicate the package are capable to protect the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit from damage.

b) Stacking simulation

All the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit were flawless and perfect after staked for 2 weeks. That indicate the package are capable to protect the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit from damage caused by stacking.

8.5 Open-vial stability of test cards

Products of one batch (20200213-1) are kept open in 80% or higher humidity condition. They are tested for the consistency of positive reference, consistency of negative reference and that of LOD reference as well as the repeatability at the 0th，1th hour，2th hours，4th hours，8th hours，24th hours and 25th hours since opening.

8.5.1 Consistency of positive reference

Table 11-1. Open-vial stability test of kit					
Batch	Time for staying open (hours)	P1	P2	P3	P4
20200213-1	0	+	+	+	+
	1	+	+	+	+
	2	+	+	+	+
	4	+	+	+	+
	8	+	+	+	+
	24	+	+	+	+
	25	+	+	+	+

8.5.2 Consistency of negative reference

Table 11-2. Open-vial stability test of kit							
Batch	Time for staying open (hours)	N1	N2	N3	N4	N5	N6
20200213-1	0	-	-	-	-	-	-

	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	4	-	-	-	-	-	-
	8	-	-	-	-	-	-
	24	-	-	-	-	-	-
	25	-	-	-	-	-	-

8.5.3 LOD

Table 11-3. Open-vial stability test of kit					
Batch	Time for staying open (hours)	S1	S2	S3	S4
20200213-1	0	+	+	-	-
	1	+	+	-	-
	2	+	+	-	-
	4	+	+	-	-
	8	+	+	-	-
	24	+	+	-	-
	25	+	+	-	-

8.5.4 Repeatability

Table 11-4. Open-vial stability test of kit		
Batch	Time for staying open (hours)	J
20200213-1	0	10/10(100%)
	1	10/10(100%)
	2	10/10(100%)
	4	10/10(100%)
	8	10/10(100%)
	24	10/10(100%)
	25	10/10(100%)

8.5.5 Open vial stability of sample extraction buffer

Unwrap the sample extraction buffer from 1 batch and then tightly capped the cover. Store the sample extraction buffer at room temperature avoid sunlight (4-30℃) for 0 day, the 2, 4, 8, 12, 16, 20, 24 and 25 weeks to evaluate the performance of the sample extraction buffer with the reference panel, including Positive coincidence rate reference, Negative coincidence rate reference, Limit of detection reference and Precision reference after unwrapped for different time period. Visually inspect the product before use, and record signs of potential stability related changes.

8.5.6 Consistency of positive reference

Table 12-1. Open-vial stability test of kit					
Batch	Time	P1	P2	P3	P4
20200213-1	0day	+	+	+	+
	2 weeks	+	+	+	+
	4 weeks	+	+	+	+
	8 weeks	+	+	+	+

	16 weeks	+	+	+	+
	20 weeks	+	+	+	+
	24 weeks	+	+	+	+
	25 weeks	+	+	+	+

8.5.7 Consistency of negative reference

Table 12-2. Open-vial stability test of kit

Batch	Time for staying open (hours)	N1	N2	N3	N4	N5	N6
20200213-1	0day	-	-	-	-	-	-
	2 weeks	-	-	-	-	-	-
	4 weeks	-	-	-	-	-	-
	8 weeks	-	-	-	-	-	-
	16 weeks	-	-	-	-	-	-
	20 weeks	-	-	-	-	-	-
	24 weeks	-	-	-	-	-	-
	25 weeks	-	-	-	-	-	-

8.5.8 LOD

Table 12-3. Open-vial stability test of kit

Batch	Time for staying open (hours)	S1	S2	S3	S4
20200213-1	0day	+	+	-	-
	2 weeks	+	+	-	-
	4 weeks	+	+	-	-
	8 weeks	+	+	-	-
	16 weeks	+	+	-	-
	20 weeks	+	+	-	-
	24 weeks	+	+	-	-
	25 weeks	+	+	-	-

8.5.9 Repeatability

Table 12-4. Open-vial stability test of kit

Batch	Time for staying open (hours)	J
20200213-1	0day	10/10(100%)
	2 weeks	10/10(100%)
	4 weeks	10/10(100%)
	8 weeks	10/10(100%)
	16 weeks	10/10(100%)
	20 weeks	10/10(100%)
	24 weeks	10/10(100%)
	25 weeks	10/10(100%)

8.6 Sample stability test protocol

8.6.1 Stability test for samples refrigerated at 2℃~8℃

Heat-inactivated SARS-CoV-2 virus was spiked into the pooled human nasal swab matrix obtained from multiple healthy volunteers who were confirmed as SARS-CoV-2 negative by RT-PCR to prepare positive samples. 50 μL samples with different tilers were added to swabs to form positive samples A1, A2 and A3. Stored the swabs in clean, dry transport tubes at 2°C-8°C. Detection kit of 20200213-1 batch is used to test the samples for positive consistency after storage for 0 day, 1 day, 3 days, 5 days, 7 days. Each sample were test for 5 times.

Table 13. Stability test for samples store at 2℃~8℃

Batch	storage time before test	A1	A2	A3
20200213-1	Instant test	+	+	+
	1 day	+	+	+
	2 days	+	+	+
	3 days	+	+	+
	4 days	+	+	-
	5 days	+	+	-
	6 days	+	+	-
	7 days	+	-	-

8.6.1 Freeze-thaw stability

Heat-inactivated SARS-CoV-2 virus was spiked into the pooled human nasal swab matrix obtained from multiple healthy volunteers who were confirmed as SARS-CoV-2 negative by RT-PCR to prepare positive samples. 50 μL samples with different tilers were added to swabs to form positive samples A1, A2 and A3. Then the swabs were treated with sample extraction buffer. The eluate were stored in a refrigerator at -20±5°C, and then freeze-thaw one time every other day for 0 time (control group), 2 times, 3 times, 4 times, 5 times and 6 times, respectively. The samples with different times of freeze-thaw process were tested and the positive rates were calculated and analyzed.

Table 14. Stability test for samples with different freeze-thaw cycles

Batch	Frozen storage time before test	A1	A2	A3
20200213-1	0 time	+	+	+
	1 time	+	+	+
	2 times	+	+	+
	3 times	+	+	-
	4 times	+	+	-
	5 times	+	-	-

8.6.2 Transport stability

Swabs were placed in clean, dry transport tubes and then placed in a cold closet or placed with ice bag in a foam box, then sealed and placed at room temperature. The transport process was simulated for 0 days (control group), 1 day, 2 days, 3 days, 4 days and 5days. The samples were tested every day, and the positive rates were calculated and analyzed.

Table 15. Stability test for samples transport at 2℃~8℃

Batch	storage time before test	A1	A2	A3
20200213-1	Instant test	+	+	+
	1 day	+	+	+
	2 days	+	+	+
	3 days	+	+	+

	4 days	+	+	-
	5 days	+	+	-

9 Conclusions and discussion

9.1 Conclusion of shelf life stability experiment

This study employs 4℃~30℃ storage experiment to test the shelf-life stability of the kit and recommended storage conditions and after transportation simulation by the indexes of the consistency of positive reference and negative reference as well as LOD and repeatability. Those four indicators are tested in different storage times. The results are shown in Table 1~4. The stability is evaluated based on the variations of these indicators. In this study, the kits of three batches are tested in parallel.

The experiment is still in progress, and the result on the 8th month are available at present. The products remain stable for 8 months after release.

9.2 Conclusion of stability in high temperature accelerated destructive test

This test includes kit products stored at 45℃ for at most 60 days and at 60 ℃ for 10 days to check the consistency of positive reference, consistency of negative reference, LOD and repeatability. The results are shown in Table 5~6. Stability is evaluated based on the variations of those four indicators tested for kits stored for different duration. In this study, three batches of kits are tested.

The results of this study show that after being kept at 45℃ for 8 weeks or at 60 ℃ for 4 days, the kit shows unchanged consistency of positive reference and negative reference, LOD and repeatability.

9.3 Conclusion of the transportation stability experiment

Products of one batch (20200213-1) are kept at 45℃ for 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks to monitor transportation at tropical areas.

Products of one batch (20200213-1) are kept at -40℃ for 3 days and then placed at 45℃ for 3days to monitor air transportation or transportation at cold areas. The whole freeze/thaw process was repeated for 1 time, 2 times, 3 times and 4 times.

Products of one batch (20200213-1) were placed in the 30%, 60%, 85% ±5% relative humidity environment for 3 days, 1week and 2 weeks. Kits undergo different RH environment were tested with the reference panel.

Products of one batch (20200213-1) were stored at varied environment for different time as list below. Kits undergo different conditions were tested with the reference panel. Randomly select product from storage on the 0th day, the 1th, 6th, 12th, 18th, 24th, and 30th month to evaluate the performance of the kit.

Well packaged Kits from 1 batch were placed in corrugated box in a 4x3x5 (X,Y,Z) mode. Then the box was lifted to 1.2 m and dropped to the ground. Repeat the lift/drop process for 3 times. Repeat the whole process on each face of the box.

Well packaged Kits from 1 batch were placed in corrugated box in a 4x3x5 (X,Y,Z) mode. Then place the corrugated boxes on a pallet in a 2x3x3 (X,Y,Z) mode.

The results shows that the products are stable after 4 weeks' high temperature simulated transportation or at least 3 times' freeze/thaw process.

Kits undergo 30%, 60%, 85% ±5% relative humidity environment for 3 days, 1week and 2 weeks are remain stable.

The Bioperfectus Technologies Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit are remain stable for at least 2 weeks at dry conditions or wet conditions.

Kits undergo simulated transport conditions in varied environment for different time are still stable. The experiment is still in progress.

Impact simulation:48 boxes of Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit were placed in a corrugated

box. After the impact simulation, 2 boxes of the kit at the corner were different degrees of damaged. That indicate the package are capable to protect the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit from damage.

Stacking simulation:All the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit were flawless and perfect after staked for 2 weeks. That indicate the package are capable to protect the Novel Corona Virus (SARS-CoV-2) Ag Rapid Test Kit from damage caused by stacking.

9.4 Conclusion of Open-vial stability test study

The test kit kept open under humidity greater than 80% for different time were evaluated in the study. The variation of consistency of positive reference and negative reference, LOD and repeatability duration the progress of open-vial storage is used to test the open-vial stability of this kit. The kit from one batch were tested in this study.

The stability of sample extraction buffer after open vial was also investigated. The results are shown in Table 11~12.

The results of this study show when kept open under conditions of more than 80% humidity the consistency of positive reference and negative reference, LOD and repeatability of the kit remain unchanged within 25 hours. In order to ensure the product performance, it is recommended that this test be finished within 60 minutes of opening.

The sample extraction buffer showed consistent performance after 25 weeks upon open vial.

9.5 Conclusion of refrigeration stability of swab samples

The collected negative and positive blood samples were stored at 2~8°C for different time. The consistency of positive samples was recorded, and the variation of these two indicators were used to evaluate the corresponding stability. The results are shown in Table 13.

The results of this study showed that swab samples kept for 3 days at 2~8°C have unchanged consistency of positive samples and negative samples. Therefore, it can be determined that the swab samples could be kept in clean, dry tubes for at most 3 days at 2~8°C. However, we still insist to test soon after sample collection.

9.6 Conclusion of Freeze-thaw stability of sample eluate

After different times of repeated freeze-thaw cycles of positive samples treated with sample extraction, the stability of the samples is tested based on the positive consistency. The results are shown in Table 14. According to the results of this study, the stability of sample eluate decreases gradually with the increase of freeze-thaw times, so the repeated freeze-thaw should be avoided in the process of sample preservation, and the freeze-thaw times should be controlled within 2 times. However, we still insist to test soon after sample collection.

9.7 Conclusion of Transportation stability of swab samples

After different simulated transport time period of positive swab samples, the stability of the samples is tested based on the positive consistency. The results are shown in Table 15.

The results show that the positive rates of samples transported under the simulated transport process for 0 day (control group), 1 days, 2 days and 3 days are100%. That is, the samples can be transported for at most 3 days in a cold closet or in a foam box with ice bags. However, we still insist to test soon after sample collection.