Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)

Validation Report

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Validation Report

Validation Studies	Lot 1 NCP201001	Lot 2 NCP201002	Lot 3 NCP201003
1. Sample Flex Study	X	-	-
2. Read Time Flex Study	X	-	-
3. Temperature Flex Study	X	-	-
4. Sample Correlation Study	X	-	-
5. Cross Reactivity Study	X	X	X
6. Interference Study	X	X	X
7. Detection Level Determination and Dose Hook Study	X	X	X
8. Variability Study (Intra-assay, Inter-assay and Day-to-day)	X	X	X
9. Real Time Stability Study	X	X	X
10. Repeatability Study	X	X	X
11. Reproducibility Study	X	X	X
12. Mutation Strains Study	X	X	X

These studies will be performed to verify the performance of the transfer pilot lots.

Instruction for Validation Experiment

1. Evaluation/Validation Study Procedure:

All the Evaluation/Validation Study Procedures were made by

European Advisor: Careshield Gmbh

Asian Advisor: Zhejiang Quark Quality Test Room

European Representative: Wellkang Ltd.

Manufacturer: Zhejiang Quark Biotechnology Co., Ltd.

2. Negative Clinical Sample Data:

All the negative samples data used here were collected in China.

Shaoxing Central Hospital, Zhejiang Provincial People's Hospital, Zhejiang Hospital, Quark Quality Test Room.

3. Positive Standards:

The positive standards are the purified antigen from Zhejiang University of Chinese Medicine, College of Life Science.

4. Positive Clinical Samples Data:

All the positive samples data used here were collected in China.

Shaoxing Central Hospital, Zhejiang Provincial People's Hospital, Zhejiang Hospital, Quark Quality Test Room.

5. Operation methods

5.1 Remove the test device from the foil pouch and use it as soon as possible.

Allow the test device, specimen and buffer to equilibrate to room temperature (15-30℃) prior to testing. Best results will be obtained if the assay is performed within one hour.

5.2 Place the test device on a clean and level surface.

Allow the test device and specimens to equilibrate to temperature (15-30℃) prior to testing.

5.3 Sample Collection:

Collection method of Feces specimen:

Unscrew the sampling tube, use the sampling rod to pick up 50 mg of fresh Feces samples (equivalent to the size of a match head); or swab the Feces with a cotton swab. Put them into the tube and shake and mix completely.

Collection method of Saliva specimen:

Clean the hand and open Disposable paper cup, clean the throat, spit the saliva from the retro pharynx into the Disposable paper cup (repeat the action make the sample above 2 mL). Avoid the sampling tube contaminated by the saliva. The optimal sampling time is after waking up and before brushing teeth or drinking. Unscrew the sampling tube, use the dropper or a cotton swab to pick up 200-300 uL of fresh Saliva samples. Put them into the tube and shake and mix completely.

Collection method of Sputum specimen:

Clean the hand and open Disposable paper cup, clean the throat, spit the Sputum from the retro pharynx into the Disposable paper cup. Avoid the sampling tube contaminated by the sputum. Unscrew the sampling tube, use the dropper or a cotton swab to pick up 200-300 uL of fresh Sputum samples. Put them into the tube and shake and mix completely.

6. Interpretation standard and Gold Color Card

All the following experiments were performed according to above procedure except for special instruction.

1. *Sample Flex Study*

Purpose: The study is used to determine the sample size of the product and the amount of extraction reagent.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1

Samples:
SARS-CoV-2 S Positive Control: 2 ng/mL
Extraction Buffer: 300 uL, 500 uL, 800 uL

Methods:
Add the Positive Control into the Buffer of 300 uL, 500 uL, 800 uL. Homogenise well. Add 2, 3 and 4 drops of extracted sample and read the results at 15 minutes.

Results:

Control	4 drops			3 drops			2 drops		
	300 uL Buffer	500 uL Buffer	800 uL Buffer	300 uL Buffer	500 uL Buffer	800 uL Buffer	300 uL Buffer	500 uL Buffer	800 uL Buffer
Positive	G7	G6	G4	G8	G6	G4	G7	G6	G4
Negative	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Conclusions:
According to the results above, the excessive buffer will decrease the T-line signal, so 300 uL is the best one for the test device. Meanwhile, for the test device, the different volume of the extracted sample has the similar result but 3 drops of sample might overflow the sample well and the first drop of the buffer always gives less volume.
Therefore, the best sample volume for the test device is 300 uL extraction buffer and 3 drops of extracted sample.

2. *Read Time Flex Study*

Purpose: The study is used to determine the read time of the product.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1

Samples:
SARS-CoV-2 S Positive Control: 0.5 ng/mL, 2 ng/mL, 5 ng/mL
Extraction Buffer: 300 uL

Methods:
For the Positive Control (including Buffer), rate the results at 5, 10, 15, 20, 30 minutes and 1, 2, 8 and 24 hours.

Results:

SARS-CoV-2 S Positive Control	Rate Time (minutes)								
	5	**10**	**15**	**20**	**30**	**1*60**	**2*60**	**8*60**	**24*60**
Background	Purple	Purple	Good	Good	Good	Good	Good	Yellow	Yellow
5 ng/mL	G6	G7	G9	G9	G9	G10	G10	G10	G10
2 ng/mL	G5	G5	G7	G6	G6	G7	G7	G7	G8
0.5 ng/mL	G3	G3	G4	G4	G5	G5	G6	G6	G6
Buffer	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Conclusions:
According to the results above, the results after 15 minutes will have clear background and strong T-line intensity. Meanwhile, the negative results can maintain for a long time (at least 2 hours). Consider the suitable read time for customer, the read time is from 15 minutes to 20 minutes.

3. *Temperature Flex Study*

Purpose: The study is conducted to confirm the effect of ambient temperature on the use of the product.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1

Samples:
SARS-CoV-2 S positive control: 0.5 ng/mL, 2 ng/mL, 5 ng/mL
Extraction Buffer: 300 uL

Methods:
Place the test devices and Positive Control (including Buffer) at 2~8℃, 30℃ and 37℃ for 30 minutes to equilibrate. Test them in duplicate and read results at 15 minutes.

Results:

SARS-CoV-2 S Positive Control	**Testing temperature**		
	2~8℃	**30**℃	**37**℃
5 ng/mL	G8	G9	G9
2 ng/mL	G6	G7	G7
0.5 ng/mL	G4	G4	G4
Buffer	Neg.	Neg.	Neg.

Conclusions:
According to the results above, the products can be run under 2~8℃ to 37℃, so we can list the temperature range from 2 to 30℃.

4. Sample Correlation Study

Purpose: This study is to determine the performance of the product compared with the competitors to obtain the clinical research analysis.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1
Competitor: Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit
(Produced by Shanghai ZJ Bio-Tech Co., Ltd., a commercial SARS-CoV-2 kit approved by CFDA, is used as the "gold standard" reagent.)

Samples:
Feces/Saliva/Sputum specimen of the tester.
Extraction Buffer: 300 uL

Methods:
117 samples of the Novel Coronavirus and 305 samples of healthy people were tested with test reagents. Compared with the Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR kit (as RT-PCR Reagent), and the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum) (as Test Reagent)

Clinical Research Results:

Feces		**RT-PCR Reagent**		**Total**
		Positive	**Negative**	
Test Reagent	**Positive**	111	2	113
	Negative	6	303	309
Total		117	305	422

Saliva	**RT-PCR Reagent**		**Total**
	Positive	**Negative**	

Test Reagent	**Positive**	106	2	108
	Negative	11	303	314
Total		117	305	422

Sputum		**RT-PCR Reagent**		**Total**
		Positive	**Negative**	
Test Reagent	**Positive**	109	2	111
	Negative	8	303	311
Total		117	305	422

Statistics Analysis:

Feces Analysis	**Statistics**	**95% Confidence Interval**
Clinical Sensitivity	111/117 = 94.87%	(89.17% ~ 98.09%)
Clinical Specificity	303/305 = 99.34%	(97.65% ~ 99.92%)
Positive Predict Value	111/113 = 98.23%	(93.75% ~ 99.79%)
Negative Predict Value	303/309 = 98.06%	(95.82% ~ 99.28%)
Total agreement rate	414/422 = 98.10%	(96.30% ~ 99.18%)

Saliva Analysis	**Statistics**	**95% Confidence Interval**
Clinical Sensitivity	106/117 = 90.60%	(83.80% ~ 95.21%)
Clinical Specificity	303/305 = 99.34%	(97.65% ~ 99.92%)
Positive Predict Value	106/108 = 98.15%	(93.47% ~ 99.78%)
Negative Predict Value	303/314 = 96.50%	(93.82% ~ 98.24%)
Total agreement rate	409/422 = 96.92%	(94.79% ~ 98.35%)

Sputum Analysis	**Statistics**	**95% Confidence Interval**
Clinical Sensitivity	109/117 = 93.16%	(86.97% ~ 97.00%)
Clinical Specificity	303/305 = 99.34%	(97.65% ~ 99.92%)
Positive Predict Value	109/111 = 98.20%	(93.64% ~ 99.78%)

Negative Predict Value	303/311 = 97.43%	(94.99% ~ 98.88%)
Total agreement rate	412/422 = 97.63%	(95.69% ~ 98.86%)

Clinical Research Records:

No	Tester	Test Reagent			RT-PCR Reagent	
		Feces	Saliva	Sputum	Throat Swab	Ct Value
1	Confirmed for 4 Day	+	+	+	+	18.08
2	Confirmed for 3 Day	+	+	+	+	26.30
3	Confirmed for 2 Day	+	+	+	+	25.05
4	Confirmed for 6 Day	+	+	+	+	18.01
5	Confirmed for 8 Day	+	+	+	+	21.56
6	Confirmed for 2 Day	+	+	+	+	23.24
7	Confirmed for 5 Day	+	+	+	+	23.24
8	Confirmed for 5 Day	+	+	+	+	22.05
9	Confirmed for 7 Day	+	+	+	+	18.36
10	Confirmed for 1 Day	+	+	+	+	27.24
11	Confirmed for 2 Day	+	+	+	+	27.24
12	Confirmed for 2 Day	+	+	+	+	25.02
13	Confirmed for 5 Day	+	+	+	+	22.04
14	Confirmed for 7 Day	+	+	+	+	21.06
15	Confirmed for 9 Day	+	-	+	+	26.12
16	Confirmed for 3 Day	+	+	+	+	21.12
17	Confirmed for 3 Day	+	+	+	+	25.42
18	Confirmed for 5 Day	+	+	+	+	25.48
19	Confirmed for 3 Day	+	+	+	+	19.16
20	Confirmed for 3 Day	+	+	+	+	18.12
21	Confirmed for 1 Day	+	+	+	+	23.06
22	Confirmed for 3 Day	+	+	+	+	25.10
23	Confirmed for 5 Day	+	+	+	+	24.18
24	Confirmed for 4 Day	+	+	+	+	26.48
25	Confirmed for 4 Day	+	+	+	+	27.21
26	Confirmed for 5 Day	+	+	+	+	27.12
27	Confirmed for 3 Day	+	+	+	+	19.04
28	Confirmed for 5 Day	+	+	+	+	23.01
29	Confirmed for 7 Day	+	+	+	+	26.32
30	Confirmed for 9 Day	+	+	+	+	23.56

31	Confirmed for 6 Day	+	+	+	+	21.40
32	Confirmed for 3 Day	+	+	+	+	20.09
33	Confirmed for 6 Day	+	+	+	+	26.08
34	Confirmed for 6 Day	+	+	+	+	23.16
35	Confirmed for 9 Day	+	+	+	+	26.21
36	Confirmed for 1 Day	+	+	+	+	24.48
37	Confirmed for 7 Day	+	+	+	+	22.07
38	Confirmed for 5 Day	+	+	+	+	20.02
39	Confirmed for 7 Day	+	+	+	+	20.18
40	Confirmed for 9 Day	+	+	+	+	18.04
41	Confirmed for 1 Day	+	+	+	+	26.20
42	Confirmed for 3 Day	+	+	+	+	18.06
43	Confirmed for 5 Day	+	+	+	+	26.18
44	Confirmed for 8 Day	-	-	-	+	27.10
45	Confirmed for 9 Day	+	+	+	+	20.06
46	Confirmed for 1 Day	+	+	+	+	21.08
47	Confirmed for 9 Day	+	-	-	+	26.14
48	Confirmed for 5 Day	+	+	+	+	27.04
49	Confirmed for 7 Day	+	+	+	+	21.24
50	Confirmed for 9 Day	+	+	+	+	19.35
51	Confirmed for 1 Day	+	+	+	+	24.12
52	Confirmed for 10 Day	+	+	+	+	21.24
53	Confirmed for 5 Day	+	+	+	+	24.12
54	Confirmed for 7 Day	+	+	+	+	18.21
55	Confirmed for 10 Day	+	+	+	+	22.12
56	Confirmed for 11 Day	+	+	+	+	23.08
57	Confirmed for 3 Day	+	+	+	+	20.08
58	Confirmed for 5 Day	+	+	+	+	24.64
59	Confirmed for 7 Day	+	+	+	+	21.10
60	Confirmed for 1 Day	+	+	+	+	25.25
61	Confirmed for 1 Day	+	+	+	+	21.06
62	Confirmed for 3 Day	+	+	+	+	22.10
63	Confirmed for 5 Day	+	+	+	+	21.01
64	Confirmed for 7 Day	+	+	+	+	19.28
65	Confirmed for 9 Day	-	-	-	+	25.28
66	Confirmed for 3 Day	+	+	+	+	23.12
67	Confirmed for 3 Day	+	+	+	+	20.07
68	Confirmed for 5 Day	+	+	+	+	22.24

69	Confirmed for 1 Day	+	+	+	+	20.15
70	Confirmed for 9 Day	+	+	+	+	22.21
71	Confirmed for 1 Day	+	+	+	+	19.14
72	Confirmed for 3 Day	+	+	+	+	22.16
73	Confirmed for 4 Day	+	+	+	+	27.06
74	Confirmed for 4 Day	+	+	+	+	24.06
75	Confirmed for 4 Day	+	+	+	+	22.14
76	Confirmed for 5 Day	+	+	+	+	21.08
77	Confirmed for 5 Day	-	-	-	+	26.08
78	Confirmed for 5 Day	+	+	+	+	22.08
79	Confirmed for 7 Day	+	+	+	+	19.56
80	Confirmed for 9 Day	+	+	+	+	20.32
81	Confirmed for 11 Day	+	-	-	+	23.21
82	Confirmed for 3 Day	+	+	+	+	23.12
83	Confirmed for 5 Day	+	+	+	+	24.24
84	Confirmed for 6 Day	-	-	-	+	26.20
85	Confirmed for 6 Day	+	+	+	+	19.15
86	Confirmed for 1 Day	+	+	+	+	24.08
87	Confirmed for 3 Day	+	+	+	+	23.08
88	Confirmed for 5 Day	-	-	-	+	25.32
89	Confirmed for 1 Day	+	+	+	+	18.56
90	Confirmed for 1 Day	-	-	-	+	26.14
91	Confirmed for 8 Day	+	+	+	+	27.10
92	Confirmed for 3 Day	+	+	+	+	21.02
93	Confirmed for 5 Day	+	+	+	+	23.15
94	Confirmed for 7 Day	+	+	+	+	18.12
95	Confirmed for 8 Day	+	+	+	+	18.21
96	Confirmed for 9 Day	+	+	+	+	20.08
97	Confirmed for 3 Day	+	-	+	+	26.14
98	Confirmed for 9 Day	+	+	+	+	24.06
99	Confirmed for 9 Day	+	+	+	+	20.18
100	Confirmed for 9 Day	+	+	+	+	19.24
101	Confirmed for 2 Day	+	+	+	+	23.42
102	Confirmed for 2 Day	+	+	+	+	22.12
103	Confirmed for 2 Day	+	+	+	+	24.12
104	Confirmed for 2 Day	+	+	+	+	18.20
105	Confirmed for 2 Day	+	-	+	+	26.08
106	Confirmed for 1 Day	+	+	+	+	25.15

107	Confirmed for 1 Day	+	+	+	+	25.14
108	Confirmed for 1 Day	+	+	+	+	23.04
109	Confirmed for 1 Day	+	+	+	+	22.42
110	Confirmed for 2 Day	+	+	+	+	20.32
111	Confirmed for 2 Day	+	+	+	+	21.15
112	Confirmed for 3 Day	+	+	+	+	26.09
113	Confirmed for 5 Day	+	+	+	+	20.30
114	Confirmed for 2 Day	+	+	+	+	26.02
115	Confirmed for 2 Day	+	+	+	+	22.35
116	Confirmed for 2 Day	+	+	+	+	18.56
117	Confirmed for 3 Day	+	+	+	+	25.40
118	Healthy	+	+	+	-	43.33
119	Healthy	+	+	+	-	42.56
120~422	Healthy	-	-	-	-	>41

5. *Cross Reactivity Study*

Purpose: The study is to confirm that the pathogens and organisms do not interfere with the performance of the product in certain concentration.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1, Lot 2, Lot 3

Samples and Method:
Some positive specimens of other pathogens and organisms were combined into the Novel Coronavirus positive and negative specimens and tested separately by each batch of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Results:

Pathogens/ Organisms	Specimen Negative	Specimen Positive	Cross-Reactivity (Yes/No)
SARS-CoV	- (30/30)	+ (30/30)	No
MERS-CoV	- (30/30)	+ (30/30)	No
HCoV-NL63	- (30/30)	+ (30/30)	No
HCoV-HKU1	- (30/30)	+ (30/30)	No
HCoV-229E	- (30/30)	+ (30/30)	No
HCoV-OC43	- (30/30)	+ (30/30)	No
Influenza A H1N1	- (30/30)	+ (30/30)	No
Influenza B	- (30/30)	+ (30/30)	No
Human RSV(B1)	- (30/30)	+ (30/30)	No
Adenovirus	- (30/30)	+ (30/30)	No
M. Pneumonia	- (30/30)	+ (30/30)	No
Measles virus	- (30/30)	+ (30/30)	No
Streptococcus Pneumoniae	- (30/30)	+ (30/30)	No
Staphylococcus Aureus	- (30/30)	+ (30/30)	No
EBV	- (30/30)	+ (30/30)	No
Coxsachie virus CA16	- (30/30)	+ (30/30)	No

Saliva	- (30/30)	+ (30/30)	No
Negative serum/plasma	- (30/30)	+ (30/30)	No

Conclusions:

According to the results above, there were no cross reactions with the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

6. *Interference Study*

Purpose: The study is to confirm that the substances do not interfere with the performance of the product in certain concentration.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1, Lot 2, Lot 3

Samples and Method:
Some positive specimens of the common Biological Analytes and Endogenous/Exogenous potential interfering substances were combined into the Novel Coronavirus positive and negative specimens and tested separately by each batch of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Results:

Endogenous Substances	Concentration	Specimen Negative	Specimen Positive	Interference (Yes/No)
Albumin	20 mg/mL	- (30/30)	+ (30/30)	No
Bilirubin	20 ug/mL	- (30/30)	+ (30/30)	No
Hemoglobin	15 mg/mL	- (30/30)	+ (30/30)	No
Glucose	20 mg/mL	- (30/30)	+ (30/30)	No
Uric Acid	200 ug/mL	- (30/30)	+ (30/30)	No
Lipids	20 mg/mL	- (30/30)	+ (30/30)	No

Exogenous Substances/ Biological Analytes	Concentration	Specimen Negative	Specimen Positive	Interference (Yes/No)
Acetaminophen	200 ug/mL	- (30/30)	+ (30/30)	No
Acetoacetic Acid	200 ug/mL	- (30/30)	+ (30/30)	No
Acetylsalicylic Acid	200 ug/mL	- (30/30)	+ (30/30)	No
Benzoylecgonine	100 ug/mL	- (30/30)	+ (30/30)	No
Caffeine	200 ug/mL	- (30/30)	+ (30/30)	No

EDTA	800 ug/mL	- (30/30)	+ (30/30)	No
Ethanol	1.0%	- (30/30)	+ (30/30)	No
Gentisic Acid	200 ug/mL	- (30/30)	+ (30/30)	No
P- Hydroxybutyrate	200,000 ug/mL	- (30/30)	+ (30/30)	No
Methanol	10.0%	- (30/30)	+ (30/30)	No
Phenothiazine	200 ug/mL	- (30/30)	+ (30/30)	No
Phenylpropanolamine	200 ug/mL	- (30/30)	+ (30/30)	No
Salicylic Acid	200 ug/mL	- (30/30)	+ (30/30)	No

Conclusions:

According to the results above, there were no interference reactions with the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

7. *Detection Level Determination and Dose Hook Study:*

Purpose: This study is to find out the LOD of the product using SARS-CoV-2 S protein expressed in vitro and National Standard Reference sample.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1, Lot 2, Lot 3

Samples and Method:
SARS-CoV-2 S protein:
Spike a healthy Feces sample, a healthy Saliva sample and a healthy Sputum sample into saline water, respectively. Prepare the supernatant for subsequent use.
Spiked a group of serially diluted SARS-CoV-2 S protein (50 ng/mL, 20 ng/mL, 5 ng/mL, 2 ng/mL, 0.5 ng/mL, 0.2 ng/mL 0 ng/mL,) in supernatant described above, respectively. Each supernatant is tested 6 times.

SARS-CoV-2:
Spike a healthy Feces sample, a healthy Saliva sample and a healthy Sputum sample into saline water, respectively. Prepare the supernatant for subsequent use.
Spiked a group of serially diluted National Standard Reference sample for SARS-CoV-2 (1×10^5 $TCID_{50}$/mL, 1×10^4 $TCID_{50}$/mL, 1×10^3 $TCID_{50}$/mL, 1×10^2 $TCID_{50}$/mL, 10^1 $TCID_{50}$/mL, 0 $TCID_{50}$/mL) in supernatant described above, respectively. Each supernatant is tested 6 times.

Results:

SARS-CoV-2 S protein	Feces Sample			Saliva Sample			Sputum Sample		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
50 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
20 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)

2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.2 ng/mL	+ (3/6)	+ (2/6)	+ (2/6)	+ (1/6)	+ (1/6)	+ (2/6)	+ (3/6)	+ (3/6)	+ (4/6)
0 ng/mL	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)

SARS-CoV-2	**Feces Sample**			**Saliva Sample**			**Sputum Sample**		
	Lot 1	**Lot 2**	**Lot 3**	**Lot 1**	**Lot 2**	**Lot 3**	**Lot 1**	**Lot 2**	**Lot 3**
1×10^5 $TCID_{50}$/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
1×10^4 $TCID_{50}$/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
1×10^3 $TCID_{50}$/mL	+ (5/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (4/6)	+ (6/6)	+ (6/6)	+ (5/6)	+ (5/6)
1×10^2 $TCID_{50}$/mL	+ (2/6)	+ (4/6)	+ (2/6)	+ (2/6)	+ (1/6)	+ (1/6)	+ (3/6)	+ (2/6)	+ (2/6)
1×10^1 $TCID_{50}$/mL	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)
0 $TCID_{50}$/mL	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)

Conclusion:

The LOD of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum) meets the requirements in product specification, which supports its feasibility in clinical applications.

The LOD of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum) ranges between 0.2 ng/mL to 0.5 ng/mL of the SARS-CoV-2 N protein.

The LOD of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum) ranges between 1×10^2 $TCID_{50}$/mL to 1×10^3 $TCID_{50}$/mL of the SARS-CoV-2.

There is no Hook-Effect observed in the test.

8. Variability Study (Intra-assay, Inter-assay & Day-to-day)

Purpose: The study determines the variability of the product.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1, Lot 2, Lot 3

Samples:
SARS-CoV-2 S Positive Control: 0.5 ng/mL, 2 ng/mL
Extraction Buffer: 300 uL

Methods:
Run all the samples in each time point and get the results at 15 minutes.

Results:

Lot 1						
Day	**Positive Control**	**Run**				
		1	**2**	**3**	**4**	**5**
Day 1 Morning	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 1 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 2 Morning	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 2 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 3 Morning	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4

	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 3 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.

Lot 2						
Day	**Positive Control**	**Run**				
		1	**2**	**3**	**4**	**5**
Day 1 Morning	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 1 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 2 Morning	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 2 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 3 Morning	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 3 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.

Lot 3						
Day	**Positive Control**	**Run**				
		1	**2**	**3**	**4**	**5**
Day 1 Morning	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 1 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 2	2 ng/mL	G7	G7	G7	G7	G7

Morning	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 2 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 3 Morning	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.
Day 3 Afternoon	2 ng/mL	G7	G7	G7	G7	G7
	0.5 ng/mL	G4	G4	G4	G4	G4
	Buffer	Neg.	Neg.	Neg.	Neg.	Neg.

Conclusion:

According to the results above, there's no significant variability among the in Intra-assay, Inter-assay & Day-to-day test of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

9. Real Time Stability Study

Purpose: The study is used to determine the stability of the product.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1, Lot 2, Lot 3

Samples:
Negative Control
SARS-CoV-2 S Positive Control: 2 ng/mL
Extraction Buffer: 300 uL

Methods:
The Shelf-Life Stability: The test devices will be run at 0 Day, 20℃ to estimate product stability. Test devices will be assayed using Negative Control and Positive Control. Run sample and read at 15 minutes.

The Accelerated Stability: The test devices will be run at 20℃, 37℃ and 45℃ to estimate product stability. Test devices will be assayed using Negative Control and Positive Control. Run sample and read at 15 minutes.

The Freeze-Thaw Stability: The test devices will be subjected to 3 freeze-thaw cycles and analyzed to determine product robustness under possible harsh shipping conditions (Simulated transport stability). Test devices will be assayed using Negative Control and Positive Control. Run sample and read at 15 minutes.

Worst-Case Stability: An additional set of test devices will also be stored at 55℃ for two days prior to testing. Test devices will be assayed using Negative Control and Positive Control. Run sample and read at 15 minutes.

The Real-Time Stability: All above Tests will be continued on-going until 39th Month.

Temperature	Days										Months									
	0*	7	14	21	28	35	42	56	77	84	3	4	5	6	7	8	9	10	11	12
20℃	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
37℃	X	X	X		X			X	X	X	X	X	X	X	X	X	X	X	X	X
45℃	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
3 Freeze Thaw /25℃		X			X			X						X**						
2 Days @ 55℃ /25℃		X			X			X						X**						

* Day 0: Run 10 test devices each with 6 controls.

** Continue testing every 3 months thereafter until the 39th Month.

The In-Use/Open Stability: The test devices will be run at 15℃ to 30℃ (Room temperature) to estimate product stability, after open product at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours and 6 hours. Test devices will be assayed using Negative Control and Positive Control. Run sample and read at 15 minutes.

Temperature	15 mins	30 mins	1 hour	2 hours	3 hours	6 hours
15℃ to 30℃	X	X	X	X	X	X

The Transport Stability will be run from China to Germany by Air Transport (about 7 days, temperature is 2~30℃) to estimate product stability. Test devices will be assayed using Negative Control and Positive Control. Run sample and read at 15 minutes.

The test is said to be stable when the testing levels produce the correct results. When the test results do not meet above criteria for stability, the previous data point can be used to extrapolate the estimated shelf life of the test. If only one test does not meet above criteria, repeat 10 tests. The stability study can be continued if all of the repeat tests meet criteria, otherwise, the date of the previous test data can be used to extrapolate the estimated shelf-life of the test.

Results:

The Shelf-Life Stability 0 Day, 20℃						
Run Times	Lot 1		Lot 2		Lot 3	
	Positive	Negative	Positive	Negative	Positive	Negative
1	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)

2	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
3	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
4	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
5	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
6	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
7	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
8	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
9	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
10	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)

The Accelerated Stability 20 ℃						
Time	**Lot 1**		**Lot 2**		**Lot 3**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
0 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
7 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
14 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
21 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
28 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
35 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
42 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
56 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
77 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
84 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
3 Months	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
4 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
5 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
6 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
7 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
8 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
9 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
10 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add

The Accelerated Stability 37 ℃						
Time	**Lot 1**		**Lot 2**		**Lot 3**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
0 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)

7 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
14 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
28 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
56 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
77 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
84 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
3 Months	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
4 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
5 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
6 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
7 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
8 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
9 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
10 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
11 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
12 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add

The Accelerated Stability 45 ℃						
Time	**Lot 1**		**Lot 2**		**Lot 3**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
0 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
7 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
14 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
21 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
28 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
35 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
42 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
56 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
77 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
84 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
3 Months	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
4 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
5 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
6 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
7 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
8 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
9 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add

10 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add

The Freeze-Thaw Stability **3 Freeze Thaw /25℃**						
Time	**Lot 1**		**Lot 2**		**Lot 3**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
7 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
28 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
56 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
6 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
9 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
12 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
15 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
18 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
21 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
24 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
27 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
30 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
33 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
36 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
39 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add

Worst-Case Stability **2 Days @55 ℃ /25 ℃**						
Time	**Lot 1**		**Lot 2**		**Lot 3**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
7 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
28 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
56 Days	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
6 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
9 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
12 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
15 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
18 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
21 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
24 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
27 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add

30 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
33 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
36 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add
39 Months	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add	To Be Add

The In-Use/Open Stability **15℃ to 30℃**						
Time	**Lot 1**		**Lot 2**		**Lot 3**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
15 mins	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
30 mins	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
1 hour	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
2 hour	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
3 hour	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)
6 hour	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)	+ (6/6)	- (6/6)

The Transport Stability **Air Transport, 2~30℃**						
Time	**Lot 1**		**Lot 2**		**Lot 3**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
China - Germany 7 Days	+ (10/10)	- (10/10)	+ (10/10)	- (10/10)	+ (10/10)	- (10/10)

Conclusion:

The Shelf-Life and Accelerate Stability result showed that the product has the estimated shelf life at 24 months at a temperature of 2~30℃.

The In-Use/Open Stability is recommended that the product be used within 1 hour after opening the package.

The Transport Stability result meet the requirements and can be used as a reference for global transportation.

10.Repeatability Study

Purpose: This study is to validate the repeatability of the product.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1, Lot 2, Lot 3

Samples:
SARS-CoV-2 S Positive Control: 2 ng/mL
Extraction Buffer: 300 uL

Methods:
Each Lot should be tested by the above test samples for 10 times.
If the test results of the batches (Lots) are positive, and the color degree of the test results is uniform, it indicates that the **Within-Batch Repeatability** is good.
If the test results from the different batches (Lots) are positive, and the color degree of the test results is uniform, it indicates that the **Between-Run Repeatability** is good.

Results:

SARS-CoV-2 S Positive Control			
Rum Time	**Lot 1**	**Lot 2**	**Lot 3**
1	+ (6/6)	+ (6/6)	+ (6/6)
2	+ (6/6)	+ (6/6)	+ (6/6)
3	+ (6/6)	+ (6/6)	+ (6/6)
4	+ (6/6)	+ (6/6)	+ (6/6)
5	+ (6/6)	+ (6/6)	+ (6/6)
6	+ (6/6)	+ (6/6)	+ (6/6)
7	+ (6/6)	+ (6/6)	+ (6/6)
8	+ (6/6)	+ (6/6)	+ (6/6)
9	+ (6/6)	+ (6/6)	+ (6/6)
10	+ (6/6)	+ (6/6)	+ (6/6)

Conclusion:

All of the test results were positive, and the color degree of the test results was uniform. It indicates that the Within-Batch Repeatability and Between-Run Repeatability of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum) are both good.

11.Reproducibility Study

Purpose: This study is to validate the reproducibility of the product.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1, Lot 2, Lot 3

Samples:
SARS-CoV-2 S Positive Control: 0.5 ng/mL, 2 ng/mL
Extraction Buffer: 300 uL

Methods:
Reproducibility studies were performed for Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum) at three physician office laboratories (POL). Each specimen was run for 3 days at each POL.
The **Intra-assay Agreement** should not be less than 99% at each site, and the **Inter-site Agreement** should not be less than 99% as well.

Results:

Lot 1				
Day	**Positive Control**	**Feces**	**Feces**	**Sputum**
Day 1	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)
Day 2	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)
Day 3	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)

Intra-assay Agreement	100%	100%	100%
Inter-site Agreement	100%		

Lot 2				
Day	**Positive Control**	**Feces**	**Feces**	**Sputum**
Day 1	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)
Day 2	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)
Day 3	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)
Intra-assay Agreement		100%	100%	100%
Inter-site Agreement		100%		

Lot 3				
Day	**Positive Control**	**Feces**	**Feces**	**Sputum**
Day 1	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)
Day 2	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)
Day 3	2 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	0.5 ng/mL	+ (20/20)	+ (20/20)	+ (20/20)
	Buffer	- (20/20)	- (20/20)	- (20/20)
Intra-assay Agreement		100%	100%	100%
Inter-site Agreement		100%		

Conclusion:

The intra-assay agreements were 100%. The inter-site agreement was 100 %.

It indicates that the reproducibility of Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum) is good.

12.Mutation Strains Study

Purpose: This study is to find out the effectiveness of the product using the mutant strains of the SARS-CoV-2 spike glycoprotein expressed.

Reference: The study was performed according to the technology specification of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum).

Device: Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum)
Lot: Lot 1, Lot 2, Lot 3

Samples:

1. SARS-CoV-2 spike RBD - His Recombinant Protein (Sino Biological, Cat:40592-V08H)
2. SARS-CoV-2 spike RBD (N501Y) - His Recombinant Protein (Sino Biological, Cat:40592-V08H82) British mutant B.1.1.7
3. SARS-CoV-2 spike RBD (D614G) - His Recombinant Protein (Sino Biological, Cat:40591-V08H3) British mutant B.1.1.7
4. SARS-CoV-2 spike RBD (Y453F) - His Recombinant Protein (Sino Biological, Cat: 40592-V08H80) Minks mutant
5. SARS-CoV-2 spike RBD (K417N) - His Recombinant Protein (Sino Biological, Cat: 40592-V08H59) South Africa mutant B.1.351.

Methods:

SARS-CoV-2 RBD concentration:
Spike a healthy Feces sample, a healthy Saliva sample and a healthy Sputum sample into saline water, respectively. Prepare the supernatant for subsequent use.
Spiking a group of serially diluted SARS-CoV-2 spike glycoprotein with above mutation strains RBD (5 ng/mL, 2 ng/mL, 1 ng/mL, 0.5 ng/mL, 0.2 ng/mL and 0.1 ng/mL) in above supernatant, respectively.
Each supernatant is tested 6 times with 3 sequential Lots.

Result:

SARS-CoV-2 Spike RBD	Feces			Saliva			Sputum		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
1 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.1 ng/mL	+ (2/6)	+ (4/6)	+ (3/6)	+ (1/6)	+ (2/6)	+ (2/6)	+ (2/6)	+ (3/6)	+ (3/6)
blank	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)

SARS-CoV-2 Spike RBD (N501Y)	Feces			Saliva			Sputum		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
1 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.1 ng/mL	+ (3/6)	+ (4/6)	+ (4/6)	+ (3/6)	+ (2/6)	+ (2/6)	+ (1/6)	+ (4/6)	+ (3/6)
blank	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)

SARS-CoV-2 Spike RBD (D614G)	Feces			Saliva			Sputum		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
1 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.1 ng/mL	+ (3/6)	+ (4/6)	+ (2/6)	+ (1/6)	+ (1/6)	+ (3/6)	+ (4/6)	+ (4/6)	+ (2/6)
blank	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)

SARS-CoV-2 Spike RBD (Y453F)	Feces			Saliva			Sputum		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
1 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.1 ng/mL	+ (3/6)	+ (3/6)	+ (2/6)	+ (3/6)	+ (2/6)	+ (3/6)	+ (3/6)	+ (1/6)	+ (2/6)

blank	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)

SARS-CoV-2 Spike RBD (K417N)	Feces			Saliva			Sputum		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
1 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.5 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.2 ng/mL	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)	+ (6/6)
0.1 ng/mL	+ (3/6)	+ (3/6)	+ (4/6)	+ (2/6)	+ (4/6)	+ (3/6)	+ (3/6)	+ (3/6)	+ (2/6)
blank	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)

Conclusion:

Test results presented above shows that the sensitivity of the Novel Coronavirus Ag Rapid Test Device (Feces/Saliva/Sputum) ranges between 0.1 ng/mL to 0.2 ng/mL to the mutant strains of the SARS-CoV-2 spike glycoprotein.