

Verification and Validation Report

Product name: COVID-19 Antigen Test Cassette (Nasopharyngeal Swab)
Company: Hangzhou DIAN Biotechnology Co., Ltd.

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1 PERFORMANCE CHARACTERISTICS

1.1 Sample Correlation

The COVID-19 Antigen Test Cassette has been evaluated with specimens obtained from the patients. Novel Coronavirus(2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) (REF: S3102E from Sansure Biotech Inc.) is used as the reference method for the COVID-19 Antigen Test Cassette. Specimens were considered positive if RT-PCR indicated a positive result. Specimens were considered negative if RT-PCR indicated a negative result.

Table: Sample Correlation Results of Test Cassette

Swab Specimen

Days post Symptom onset	Number of samples	PCR Positive	COVID-19 Antigen Test Cassette	95% Confidence Interval
1	3	3	3 (100.0%)	39.8%-100%
2	9	9	9 (100.0%)	69.2%-100%
3	14	14	14 (100.0%)	78.2%-100%
4	17	17	17 (100.0%)	81.5%-100%
5	22	22	21 (95.4%)	78.1%-99.9%
6	26	26	25 (96.1%)	81.0%-99.9%
7	34	34	33 (97.0%)	85.1%-99.9%
Total	125	125	122 (97.6%)	94.9%-100%

The total sensitivity of COVID-19 Antigen is 97.6%; (94.9%-100%) The following data is provided for informational purposes:

The performance of COVID-19 Antigen Test Cassette with positive results stratified by the comparator method cycle threshold (Ct) counts were collected and assessed to better understand the correlation of assay performance to the cycle threshold, estimating the viral titer present in the clinical sample. As presented in the table below, the positive agreement of the COVID-19 Antigen Test Cassette is higher with samples of a Ct count <32.

COVID-19 Antigen Test Cassette	Comparator Method (RT-PCR from Sansure Biotech Inc) (POS by Ct Category)
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COVID-19 Antigen Test Cassette (Nasopharyngeal Swab)

	POS (Ct< 25)	POS (25< Ct<28)	POS (28<Ct<30)	POS (30<Ct<32)
Positive	48	27	30	17
Negative	0	0	0	3
Total	48	27	30	20
Positive agreement	100% 95% CI (92.0%-100%)	100% 95%CI (85.0%-100%)	100% 95%CI (89.2%-100%)	85% 95%CI (78.9%-91.1%)

Negative results:

Number of samples	PCR Negative result	COVID-19 Antigen Test Cassette
250	250	246/250=98.4%
Total	N/A	98.4%; 95%CI: (96.9%-99.9%)

The Clinical specificity is 98.4%;(96.9%-99.9%)

1.2 Detection Level Determination (LOD-Analytical sensitivity)

A recombinant COVID-19 protein was diluted with dilution to a low concentration to determine detection level. The assays were performed according to the package insert.

Note: "+" mean positive result, "-" mean negative result

Specimens concentration	YF20201101			YF20201102			YF20201103		
	15min			15min			15min		
100ng/ml	+	+	+	+	+	+	+	+	+
10ng/ml	+	+	+	+	+	+	+	+	+
1ng/ml	+	+	+	+	+	+	+	+	+
500pg/ml	+	+	+	+	+	+	+	+	+
200pg/ml	+	+	+	+	+	+	+	+	+
100pg/ml	+	+	+	+	+	+	+	+	+
50pg/ml	-	-	-	-	-	-	-	-	-

Gradient dilution of the COVID-19 virus dilution to a low concentration to determine detection level, the assays were performed according to the package insert.

Note: "+" mean positive result, "-" mean negative result

COVID-19 Antigen Test Cassette (Nasopharyngeal Swab)

The virus concentration	YF20201101			YF20201102			YF20201103		
	15min			15min			15min		
100TCID ₅₀ /ml	+	+	+	+	+	+	+	+	+
50TCID ₅₀ /ml	+	+	+	+	+	+	+	+	+
25TCID ₅₀ /ml	-	-	-	-	-	-	-	-	-

Conclusion: Minimal detection limits of COVID-19 Antigen Test Cassette is 100pg/ml for recombinant COVID-19 protein. Minimal detection limits of COVID-19 Antigen Test Cassette is 50TCID₅₀/ml for COVID-19 virus

1.3 Interfering Substances

The interfering substances below were spiked with negative and SARS-COV-2 Antigen weak positive. Test them according to the package insert in triplicate. Read the result at 15 minutes. Results were present in table below.

Note: "+" mean positive result, "-" mean negative result.

Table: Interfering Substances

Analytes	Cone.	YF20201101					
		Negative			SARS-COV-2 Antigen weak Positive		
Whole Blood	20 µl/ml	-	-	-	+	+	+
Mucin	50pg/ml	-	-	-	+	+	+
Budesonide Nasal Spray	200 µl/ml	-	-	-	+	+	+
Dexamethasone	0.8mg/ml	-	-	-	+	+	+
Flunisolide	6.8ng/ml	-	-	-	+	+	+
Mupirocin	12mg/ml	-	-	-	+	+	+
Oxymetazoline	0.6mg/ml	-	-	-	+	+	+
Phenylephrine	12mg/ml	-	-	-	+	+	+
Rebetol	4.5pg/ml	-	-	-	+	+	+
Relenza	282ng/ml	-	-	-	+	+	+
Tamiflu	1.1 pg/ml	-	-	-	+	+	+
Tobryamycin	2.43mg/ml	-	-	-	+	+	+
Analytes	Cone.	YF20201102					
		Negative			SARS-COV-2 Antigen weak Positive		
Whole Blood	20 µl/ml	-	-	-	+	+	+
Mucin	50pg/ml	-	-	-	+	+	+
Budesonide Nasal Spray	200 µl/ml	-	-	-	+	+	+
Dexamethasone	0.8mg/ml	-	-	-	+	+	+

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Flunisolide	6.8ng/ml	—	—	—	+	+	+
Mupirocin	12mg/ml	—	—	—	+	+	+
Oxymetazoline	0.6mg/ml	—	—	—	+	+	+
Phenylephrine	12mg/ml	—	—	—	+	+	+
Rebetol	4.5pg/ml	—	—	—	+	+	+
Relenza	282ng/ml	—	—	—	+	+	+
Tamiflu	1.1 pg/ml	—	—	—	+	+	+
Tobryamycin	2.43mg/ml	—	—	—	+	+	+
Analytes	Cone.	YF20201103					
		Negative			SARS-COV-2 Antigen		
Whole Blood	20 µl/ml	—	—	—	+	+	+
Mucin	50pg/ml	—	—	—	+	+	+
Budesonide Nasal Spray	200 µl/ml	—	—	—	+	+	+
Dexamethasone	0.8mg/ml	—	—	—	+	+	+
Flunisolide	6.8ng/ml	—	—	—	+	+	+
Mupirocin	12mg/ml	—	—	—	+	+	+
Oxymetazoline	0.6mg/ml	—	—	—	+	+	+
Phenylephrine	12mg/ml	—	—	—	+	+	+
Rebetol	4.5pg/ml	—	—	—	+	+	+
Relenza	282ng/ml	—	—	—	+	+	+
Tamiflu	1.1 pg/ml	—	—	—	+	+	+
Tobryamycin	2.43mg/ml	—	—	—	+	+	+

Conclusion: No substances showed any interference with the test. There were no obvious differences among the 3 lots of products.

1.4 Cross Reactivity

1x10⁸org/ml following samples were spiked onto swabs and tested according to the package insert in triplicate. Read the results at 15 minutes. Results were presented in table below.

Stain	Concentration
Candida albicans	1x10 ⁸ org/ml
Staphylococcus aureus subspaureus	1x10 ⁸ org/ml
Staphylococcus epidermidis	1x10 ⁸ org/ml
Corynebacterium	1x10 ⁸ org/ml
Streptococcus pneumoniae	1x10 ⁸ org/ml
Escherichia coli	1x10 ⁸ org/ml
Streptococcus pyogenes	1x10 ⁸ org/ml
Moraxella catarrhalis	1x10 ⁸ org/ml
Streptococcus salivarius	1x10 ⁸ org/ml
Neisseria lactamica	1x10 ⁸ org/ml
Streptococcus sp group F	1x10 ⁸ org/ml

Neisseria subflava	1x10 ⁸ org/ml
Pseudomonas aeruginosa	1x10 ⁸ org/ml
Arcanobacterium	1x10 ⁸ org/ml

Table: Cross Reactivity Result

Treatment	YF20201101			YF20201102			YF20201103		
	15min			15min			15min		
Candida albicans	-	-	-	-	-	-	-	-	-
Staphylococcus aureus subspaureus	-	-	-	-	-	-	-	-	-
Corynebacterium	-	-	-	-	-	-	-	-	-
Streptococcus pneumoniae	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	-	-	-
Streptococcus pyogenes	-	-	-	-	-	-	-	-	-
Moraxella catarrhalis	-	-	-	-	-	-	-	-	-
Streptococcus salivarius	-	-	-	-	-	-	-	-	-
Neisseria lactamica	-	-	-	-	-	-	-	-	-
Streptococcus sp group F	-	-	-	-	-	-	-	-	-
Neisseria subflava	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-
Arcanobacterium	-	-	-	-	-	-	-	-	-

Note: “-” mean negative result, “+”mean positive result

Conclusion: There was no cross-reaction with the substances above at 15 minutes.

1.5 Specificity Testing with Strains

Following strains at specified concentrations were spiked onto swabs and tested according to the package insert. Read the results at 15 minutes. Results were presented in table below.

Table: Specificity Testing with Strains

Description	Concentration	Result		
		YF20201101	YF20201102	YF20201103
Human coronavirus 229E	1 x10 ⁶ TCID ₅₀ /ml	-	-	-
Human coronavirus QC43	2.45 x 10 ⁶ LD ₅₀ /ml	-	-	-
Coronavirus NL63	1 x 10 ^{5.07} u/ml	-	-	-
MERS	1.5 x10 ⁶ TCID ₅₀ /ml	-	-	-
Influenza A H1N1	3.16 x 10 ⁵ TCID ₅₀ /ml	-	-	-
Influenza A H3N2	1 x10 ⁵ TCID ₅₀ /ml	-	-	-
Influenza B	3.16 x 10 ⁶ TCID ₅₀ /ml	-	-	-
Human Rhinovirus 2	2.81 x 10 ⁴ TCID ₅₀ /ml	-	-	-

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Human Rhinovirus 14	1.58 x 10 ⁶ TCID ₅₀ /ml	-	-	-
Human Rhinovirus 16	8.89 x 10 ⁶ TCID ₅₀ /m	-	-	-
Measles	1.58 x 10 ⁴ TCID ₅₀ /ml	-	-	-
Mumps	1.58 x 10 ⁴ TCID ₅₀ /ml	-	-	-
Parainfluenza virus 2	1.58 x 10 ⁷ TCID ₅₀ /ml	-	-	-
Parainfluenza virus 3	1.58 x 10 ⁸ TCID ₅₀ /ml	-	-	-
Respiratory syncytial virus	8.89 x 10 ⁴ TCID ₅₀ /ml	-	-	-

Note: “-” mean negative result, “+” mean positive result

TCID₅₀ = Tissue Culture Infectious Dose is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated. LD₅₀ = Lethal Dose is the dilution of virus that under the conditions of the assay can be expected to kill 50% of the sucklingmice inoculated.

Conclusion: All the results showed negative when tested with different strains at theconcentration above.

1.5 Dose Hook Study

A recombine COVID-19 protein was diluted with dilution to 100ug/ml, 10ug/ml, 1ug/ml, 100ng/ml, 10ng/ml, 1ng/ml, 100pg/ml, 50pg/ml and 1pg/ml and tested according to the package insert in replicates of three. The results were rated at 15 minutes. Results were presented in Tablebelow.

Table: Dose Hook Study Result

Specimens	YF20201101			YF20201102			YF20201103		
	15min			15min			15min		
100ug/ml	+	+	+	+	+	+	+	+	+
10ug/ml	+	+	+	+	+	+	+	+	+
1ug/ml	+	+	+	+	+	+	+	+	+
100ng/ml	+	+	+	+	+	+	+	+	+
10ng/ml	+	+	+	+	+	+	+	+	+
1ng/ml	+	+	+	+	+	+	+	+	+
100pg/ml	+	+	+	+	+	+	+	+	+
50pg/ml	-	-	-	+	-	-	-	-	-
1pg/ml	-	-	-	-	-	-	-	-	-
Dilution	-	-	-	-	-	-	-	-	-

Note: “-” mean negative result, “+” mean positive result

Conclusion: Test results showed that there was no dose hook effect of the 3 lots of COVID-19 products.

1.6 Variability (Inter/Intra/Day to day assay)

Negative, SARS-COV-2 Antigen Weak Positive, SARS-COV-2 Antigen Strong Positive weretested according to the package inset. Ten replicates of each level were tested each day for 3 consecutive days using all the 3 lots. Read the results at 15 minutes. Results were

presented in Table below.

Table: Variability Results

Negative (Dilution)

Day	Lot#:	1	2	3	4	5	6	7	8	9	10
Day 1	YF20201101	-	-	-	-	-	-	-	-	-	-
	YF20201102	-	-	-	-	-	-	-	-	-	-
	YF20201103	-	-	-	-	-	-	-	-	-	-
Day 2	YF20201101	-	-	-	-	-	-	-	-	-	-
	YF20201102	-	-	-	-	-	-	-	-	-	-
	YF20201103	-	-	-	-	-	-	-	-	-	-
Day 3	YF20201101	-	-	-	-	-	-	-	-	-	-
	YF20201102	-	-	-	-	-	-	-	-	-	-
	YF20201103	-	-	-	-	-	-	-	-	-	-

SARS-COV-2 Antigen Weak Positive

Day	Lot#:	1	2	3	4	5	6	7	8	9	10
Day 1	YF20201101	+	+	+	+	+	+	+	+	+	+
	YF20201102	+	+	+	+	+	+	+	+	+	+
	YF20201103	+	+	+	+	+	+	+	+	+	+
Day 2	YF20201101	+	+	+	+	+	+	+	+	+	+
	YF20201102	+	+	+	+	+	+	+	+	+	+
	YF20201103	+	+	+	+	+	+	+	+	+	+
Day 3	YF20201101	+	+	+	+	+	+	+	+	+	+
	YF20201102	+	+	+	+	+	+	+	+	+	+
	YF20201103	+	+	+	+	+	+	+	+	+	+

SARS-COV-2 Antigen Strong Positive

Day	Lot#:	1	2	3	4	5	6	7	8	9	10
Day 1	YF20201101	+	+	+	+	+	+	+	+	+	+
	YF20201102	+	+	+	+	+	+	+	+	+	+
	YF20201103	+	+	+	+	+	+	+	+	+	+
Day 2	YF20201101	+	+	+	+	+	+	+	+	+	+
	YF20201102	+	+	+	+	+	+	+	+	+	+
	YF20201103	+	+	+	+	+	+	+	+	+	+
Day 3	YF20201101	+	+	+	+	+	+	+	+	+	+
	YF20201102	+	+	+	+	+	+	+	+	+	+
	YF20201103	+	+	+	+	+	+	+	+	+	+

Note: “-” mean negative result, “+” mean positive result

Conclusion: Test results were consistent between the 3 lots of test cassette.

1.7 Reading Time Flex Study

Negative, SARS-COV-2 Antigen weak positive, and SARS-COV-2 Antigen strong positive standards have been tested according to the directions for use in replicates of three. The test was rated as positive or negative at 3, 5, 10, 15, 20, 30 minutes, 1 hour and 2 hours.

Table: Reading Time Flex Study Result

Time	Lot: YF20201101
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COVID-19 Antigen Test Cassette (Nasopharyngeal Swab)

	Negative			SARS-COV-2 Antigen weak positive			SARS-COV-2 Antigen strong positive		
3min	-	-	-	-	-	-	+	+	+
5min	-	-	-	+	+	+	+	+	+
10min	-	-	-	+	+	+	+	+	+
15min	-	-	-	+	+	+	+	+	+
20min	-	-	-	+	+	+	+	+	+
30min	-	-	-	+	+	+	+	+	+
1 h	-	-	-	+	+	+	+	+	+
2hs	-	-	-	+	+	+	+	+	+
	Lot: YF20201102								
Time	Negative			SARS-COV-2 Antigen weak positive			SARS-COV-2 Antigen strong positive		
3min	-	-	-	-	-	-	+	+	+
5min	-	-	-	+	+	+	+	+	+
10min	-	-	-	+	+	+	+	+	+
15min	-	-	-	+	+	+	+	+	+
20min	-	-	-	+	+	+	+	+	+
30min	-	-	-	+	+	+	+	+	+
1 h	-	-	-	+	+	+	+	+	+
2hs	-	-	-	+	+	+	+	+	+
	Lot: YF20201103								
Time	Negative			SARS-COV-2 Antigen weak positive			SARS-COV-2 Antigen strong positive		
3min	-	-	-	-	-	-	+	+	+
5min	-	-	-	+	+	+	+	+	+
10min	-	-	-	+	+	+	+	+	+
15min	-	-	-	+	+	+	+	+	+
20min	-	-	-	+	+	+	+	+	+
30min	-	-	-	+	+	+	+	+	+
1 h	-	-	-	+	+	+	+	+	+
2hs	-	-	-	+	+	+	+	+	+

Note: “-” mean negative result, “+” mean positive result

Conclusion: This study demonstrated the ability of the assay to give correct results with the prescribed read time of 15 minutes. For the samples tested, the result remained consistent within a 1hr period.

1.8 Specimen Volume Flex Study

Negative, SARS-COV-2 Antigen weak positive spiked extraction reagent were added into the sample well of COVID-19 Antigen Test Cassette with the following operation method, read the results at 15 minutes, a suitable specimen volume should be validated in this study.

Method A-2 drops specimen

Method B-3 drops specimen

Method C-4 drops specimen

Method D-5 drops specimen

Table: Specimen Volume Flex Study Result

Lot	Method	A		B		C		D	
		15min	20min	15min	20min	15min	20min	15min	20min
YF20201101	Specimen	15min	20min	15min	20min	15min	20min	15min	20min
	Negative	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
	SARS-COV-2 Antigen weak positive	+	+	+	+	+	+	+	+
		+	+	+	+	+	+	+	+
+		+	+	+	+	+	+	+	
YF20201102	Specimen	15min	20min	15min	20min	15min	20min	15min	20min
	Negative	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
	SARS-COV-2 Antigen weak positive	+	+	+	+	+	+	+	+
		+	+	+	+	+	+	+	+
+		+	+	+	+	+	+	+	
YF20201103	Specimen	15min	20min	15min	20min	15min	20min	15min	20min
	Negative	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
	SARS-COV-2 Antigen weak positive	+	+	+	+	+	+	+	+
		+	+	+	+	+	+	+	+
+		+	+	+	+	+	+	+	

Note: “-” mean negative result, “+” mean positive result

Conclusion: This study demonstrated the ability of the assay to give correct results with 3 drops specimen volume.

1.9 Open Pouch Stability Study

Negative, SARS-COV-2 Antigen weak positive and SARS-COV-2 Antigen strong positive specimen were run in triplicate using the COVID-19 Antigen Test Cassette which were opened pouch after 10 minutes, 20 minutes, 30 minutes, 1 hour, 1.5 hours and 2 hours at following condition. All results were read as positive or negative at 15 and 20 minutes after sample application.

Condition 1: Room temperature and high humidity (>90%)

Condition 2: 45°C and normal humidity (≤60%)

Condition 3: 45°C and high humidity (>90%)

Condition 4: Room temperature and normal humidity (≤60%)

Table: Open Pouch Stability Study Results

Sample	Time after Open Pouch	Results of Condition 1								
		YF20201101			YF20201102			YF20201103		
Negative	10min	-	-	-	-	-	-	-	-	-
	20min	-	-	-	-	-	-	-	-	-
	30min	-	-	-	-	-	-	-	-	-
	60min	-	-	-	-	-	-	-	-	-
	90min	-	-	-	-	-	-	-	-	-
	120min	-	-	-	-	-	-	-	-	-
SARS-COV-2 Antigen weak positive	10min	+	+	+	+	+	+	+	+	+
	20min	+	+	+	+	+	+	+	+	+
	30min	+	+	+	+	+	+	+	+	+
	60min	+	+	+	+	+	+	+	+	+
	90min	+	+	+	+	+	+	+	+	+
	120min	+	+	+	+	+	+	+	+	+
SARS-COV-2 Antigen strong positive	10min	+	+	+	+	+	+	+	+	+
	20min	+	+	+	+	+	+	+	+	+
	30min	+	+	+	+	+	+	+	+	+
	60min	+	+	+	+	+	+	+	+	+
	90min	+	+	+	+	+	+	+	+	+
	120min	+	+	+	+	+	+	+	+	+
Sample	Time after Open Pouch	Results of Condition 2								
		YF20201101			YF20201102			YF20201103		
Negative	10min	-	-	-	-	-	-	-	-	-
	20min	-	-	-	-	-	-	-	-	-
	30min	-	-	-	-	-	-	-	-	-

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	60min	-	-	-	-	-	-	-	-	-
	90min	-	-	-	-	-	-	-	-	-
	120min	-	-	-	-	-	-	-	-	-
SARS-COV-2 Antigen weak positive	10min	+	+	+	+	+	+	+	+	+
	20min	+	+	+	+	+	+	+	+	+
	30min	+	+	+	+	+	+	+	+	+
	60min	+	+	+	+	+	+	+	+	+
	90min	+	+	+	+	+	+	+	+	+
	120min	+	+	+	+	+	+	+	+	+
SARS-COV-2 Antigen strong positive	10min	+	+	+	+	+	+	+	+	+
	20min	+	+	+	+	+	+	+	+	+
	30min	+	+	+	+	+	+	+	+	+
	60min	+	+	+	+	+	+	+	+	+
	90min	+	+	+	+	+	+	+	+	+
	120min	+	+	+	+	+	+	+	+	+
Sample	Time after Open Pouch	Results of Condition 3								
		YF20201101			YF20201102			YF20201103		
Negative	10min	-	-	-	-	-	-	-	-	-
	20min	-	-	-	-	-	-	-	-	-
	30min	-	-	-	-	-	-	-	-	-
	60min	-	-	-	-	-	-	-	-	-
	90min	-	-	-	-	-	-	-	-	-
	120min	-	-	-	-	-	-	-	-	-
SARS-COV-2 Antigen weak positive	10min	+	+	+	+	+	+	+	+	+
	20min	+	+	+	+	+	+	+	+	+
	30min	+	+	+	+	+	+	+	+	+
	60min	+	+	+	+	+	+	+	+	+
	90min	+	+	+	+	+	+	+	+	+
	120min	+	+	+	+	+	+	+	+	+
SARS-COV-2 Antigen strong positive	10min	+	+	+	+	+	+	+	+	+
	20min	+	+	+	+	+	+	+	+	+
	30min	+	+	+	+	+	+	+	+	+
	60min	+	+	+	+	+	+	+	+	+
	90min	+	+	+	+	+	+	+	+	+
	120min	+	+	+	+	+	+	+	+	+
Sample	Time after Open Pouch	Results of Condition 4								
		YF20201101			YF20201102			YF20201103		

Negative	10min	-	-	-	-	-	-	-	-	-
	20min	-	-	-	-	-	-	-	-	-
	30min	-	-	-	-	-	-	-	-	-
	60min	-	-	-	-	-	-	-	-	-
	90min	-	-	-	-	-	-	-	-	-
	120min	-	-	-	-	-	-	-	-	-
SARS-COV-2 Antigen weak positive	10min	+	+	+	+	+	+	+	+	+
	20min	+	+	+	+	+	+	+	+	+
	30min	+	+	+	+	+	+	+	+	+
	60min	+	+	+	+	+	+	+	+	+
	90min	+	+	+	+	+	+	+	+	+
	120min	+	+	+	+	+	+	+	+	+
SARS-COV-2 Antigen strong positive	10min	+	+	+	+	+	+	+	+	+
	20min	+	+	+	+	+	+	+	+	+
	30min	+	+	+	+	+	+	+	+	+
	60min	+	+	+	+	+	+	+	+	+
	90min	+	+	+	+	+	+	+	+	+
	120min	+	+	+	+	+	+	+	+	+

Note: "+"mean positive result, "-"mean negative result.

Conclusion: Results above indicated best results of COVID-19 Antigen Test Cassette will be got within 1 hr after opening the pouch.

1.10 Accelerated Stability Study

Accelerated Stability of the COVID-19 Antigen Test Cassette (Swab) was evaluated using samples from three different batches. These were placed in an incubator with the temperature calibrated at 45°C and 55°C. Relative humidity (RH) calibrated at about 60%. A series of stability tests were performed at 0, 7, 14, 21, 28, 35, 42, 56, 77, 84 days for 45°C.

About 55°C, some performance study would be tested at 0, 7, 14, 21, 28, 35, 42, 56, 77, 84 days according to Arrhenius Plot. See Table in below. Test cassettes were assayed using negative, SARS-COV-2 Antigen weak positive and SARS-COV-2 Antigen strong positive specimens. Testing at each specific time interval consisted of 3 replicates for each specimen. The tests were performed according to the package insert. Results are presented in Table below.

Arrhenius Formula:

In K=-Ea/RT + In A

"K" mean Rate constant; "A" mean Arrhenius constant; "Ea" mean Activation energy

"R" mean Gas constant; "T" mean Temperature in Kelvin

Table: Time Line for Accelerated Stability Study

Day Temperature	0 day	7 days	14 days	21 days	28 days	35 days	42 days	56 days	77 days	84 days
45°C	√	√	√	√	√	√	√	√	√	√
55°C	√	√	√	√	√	√	√	√	√	√

Table: 45°C Accelerated Stability Summary

Day	Specimen	YF20201101			YF20201102			YF20201103		
0	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
7	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
14	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
21	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
28	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
35	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
42	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
56	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
77	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
84	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+

Table: 55°C Accelerated Stability Summary

Day	Specimen	YF20201101			YF20201102			YF20201103		
0	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
7	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
14	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
21	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
28	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
35	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
42	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
56	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
77	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+
84	Negative	-	-	-	-	-	-	-	-	-
	Weak positive	+	+	+	+	+	+	+	+	+
	Strong positive	+	+	+	+	+	+	+	+	+

Note: "-" mean negative result, "+" mean positive result

Conclusion: The COVID-19 Antigen Test Cassette was stable at 45°C for 84 days and at 55°C for 84 days. These data were plotted on an Arrhenius Plot and the shelf life of this product was determined to be at least 24 months from the date of manufacture.