Flowflex™ SARS-CoV-2 Antigen Rapid Test

Evaluation Report

December 2020

Flow*flex* SARS-CoV-2 Antigen Rapid Test Evaluation Report

The Flow*flex* SARS-CoV-2 Antigen Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in nasal swab specimens directly from individuals who are suspected of COVID-19 by their healthcare provider within the first seven days of the onset of symptoms. The Flow*flex* SARS-CoV-2 Antigen Rapid Test does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the identification of SARS-CoV-2 nucleocapsid antigen. This antigen is generally detectable in upper respiratory samples during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.
Negative results from patients with more than seven days post symptom onset should be treated as presumptive and confirmed with a molecular assay, if necessary, for patient management. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

The Flow*flex* SARS-CoV-2 Antigen Rapid Test is intended for use by trained clinical laboratory personnel and individuals trained in point of care settings.

1. **Purpose:** To evaluate the performance of the Flow*flex* SARS-CoV-2 Antigen Rapid Test

2. **Study procedure and results**

2.1 Imprecision/reproducibility Study

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot#1:202009101, Lot#2:202009001, Lot#3:202009201
- Extraction Buffer, Lot1#:202008001, Lot2#:202008002, Lot3#:202008003
- SARS-CoV-2 Antigen Negative Sample Lot#: COVAG200904N
- SARS-CoV-2 Antigen Low Positive Sample P3 Lot#: COVAG200904P3
- SARS-CoV-2 Antigen Middle Positive Sample P2 Lot#: COVAG200904P2
- SARS-CoV-2 Antigen High Positive Sample P1 Lot#: COVAG200904P1

Procedure:

3 Lots of SARS-CoV-2 Antigen Rapid Test were tested according to the package insert by 3 operators. Each operator performed 2 tests on each control for 5 days in 2 sites in China. Total 180 tests were performed per each control: 2 replicates X 5 days X 3 lots X 3 operators X 2 sites = 180 tests.

Test results:

SARS-CoV-2 Samples	Lot 1	Lot 2	Lot 3
High Pos	+ / 60 replicates	+ / 60 replicates	+ / 60 replicates
Mid Pos	+ / 60 replicates	+ / 60 replicates	+ / 60 replicates
Low Pos	+ / 60 replicates	+ / 60 replicates	+ / 60 replicates
Neg	- / 60 replicates	- / 60 replicates	- / 60 replicates

Conclusions:

All three lots identified the samples 100% correctly as negative or positive.

2.2 Limit of Detection (LOD)

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot#1:202009101, Lot#2:202009001, Lot#3:202009201
- Extraction Buffer, Lot1#:202008001, Lot2#:202008002, Lot3#:202008003
- SARS-CoV-2 viral culture

Procedure:

1. Sample Application Method: Apply 4 drops of sample to the sample well on the test cassette, then start the timer, read the result at 15 minutes and 30 minutes.
2. Dilute the high concentration SARS-CoV-2 viral culture with the Extraction Buffer.
3. Use 3 lots of SARS-CoV-2 antigen rapid test to test the samples, and every sample is tested in 10 replicates. Calculate the detectable rate for each sample.
4. The minimum concentration with ≥95% detectable rate is defined as the minimum detectability (LOD).

Test results:

Culture sample:

Concentration	Lot	Test Result	Detectable rate
2.56×10^3 $TCID_{50}$/mL	**Lot 1**	+ / 10 replicates	100% (30/30)
	Lot 2	+ / 10 replicates	
	Lot 3	+ / 10 replicates	
1.28×10^3 $TCID_{50}$/mL	**Lot 1**	+ / 10 replicates	100% (30/30)
	Lot 2	+ / 10 replicates	
	Lot 3	+ / 10 replicates	
6.4×10^2 $TCID_{50}$/mL	**Lot 1**	+ / 10 replicates	100% (30/30)
	Lot 2	+ / 10 replicates	
	Lot 3	+ / 10 replicates	

3.2 x 10^2 $TCID_{50}$/mL	**Lot 1**	+ / 10 replicates	100% (30/30)
	Lot 2	+ / 10 replicates	
	Lot 3	+ / 10 replicates	
1.6 x 10^2 $TCID_{50}$/mL	**Lot 1**	+ / 10 replicates	96.7% (29/30)
	Lot 2	+ / 10 replicates	
	Lot 3	+ 9 replicates / - 1 replicate	
8 x 10 $TCID_{50}$/mL	**Lot 1**	- / 10 replicates	0% (0/30)
	Lot 2	- / 10 replicates	
	Lot 3	- / 10 replicates	

Conclusion:

According to the test result, the LOD is 1.6 x 10^2 $TCID_{50}$/mL.

2.3 **Clinical study – nasal swabs**

A multi-site clinical study was conducted to evaluate the performance of the SARS-CoV-2 Antigen Rapid Test, and the results are shown below.

2.3.1 Study in China

Clinical site:

Sample collection and testing site	Responsible person/Qualification	Coordinator/Qualification
Shenzhen CDC No. 8 Longyuan Road, Nanshan District, Shenzhen, P.R. China	Renli Zhang, MD	Fangli Tong, Technologist
Adicon No.208 Zhenzhong Road, West Lake District, Hangzhou, Zhejiang, P.R. China	Cheng Zeng, Technologist	

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot# 202009001
- Jiangsu Changfeng Medical nasal swabs
- Comparison method: RT-PCR, Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing), manufactured by Sansure BioTech Inc.
- Extraction Buffer, Lot1#:202008001

- Nasal swab samples from infected patients and non-infected patients

Procedure:

1. Study was conducted in China
    - 452 clinical nasal swabs were collected from patients who were suspected of COVID-19. All the samples were confirmed with RT-PCR.
    - 70 positive clinical nasal swabs collected from patients. 63 samples with Ct counts <33, 7 samples with Ct counts ≥33.

2. Following product package insert, performed the test and read the result at reading time.

Test results:

Candidate method		RT-PCR method		
		Negative	Positive	Total
Flow*flex* Test Results	Negative	381	2*	383
	Positive	1	68	69
	Total	382	70	452

***2 samples with PCR CT value 34-35**

2.3.2 Clinical Study in USA

Clinical sites:

- Sample collection sites in USA:

Patient sample collection site	Responsible person/Qualification	Coordinator/Qualification
Boca Raton 6877 SW 18th Street Boca Raton, FL 33433	Dr. Peter Miller, MD	David Cantor, CRO
COVID CLINIC Westminster (WM) 2109 Westminster Mall Westminster, CA 92683	Dr. Matthew Abinante, DO, MPH	
COVID CLINIC La Mesa (LM) 5601 Grossmont Center Drive La Mesa, CA 91942		
COVID CLINIC Downtown San Diego 1350 Third Avenue San Diego, CA 92101		

- Testing sites in USA:

Testing sites	Operator name/Qualification	Coordinator /Qualification
7200 Parkway Drive, Suite 117 La Mesa, CA 91942	Dr. Shannyn Fowl, MD	David Cantor, CRO
COVID CLINIC Westminster (WM) 2109 Westminster Mall Westminster, CA 92683	Dr. Matthew Abinante, DO, MPH	
COVID CLINIC La Mesa (LM) 5601 Grossmont Center Drive La Mesa, CA 91942		
COVID CLINIC Downtown San Diego 1350 Third Avenue San Diego, CA 92101		

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot# 202009001
- Puritan Medical Products nasal swabs (#25-1506 1PF 100), and Jiangsu Changfeng Medical nasal swabs
- Comparison method: TaqPath COVID-19 Combo Kit, FDA authorized RT-PCR test for emergency use, manufactured by Thermo Fisher Scientific, Inc.
- Nasal swab samples from infected patients and non-infected patients

Procedure:

1. Study is being conducted in multiple U.S. sites in California and Florida, and it is ongoing. So far, 125 clinical nasal swabs were collected from patients who were suspected of COVID-19. All the samples were confirmed with RT-PCR method.
2. Following product package insert, performed the test and read the result at reading time.

Test results:

Candidate method		RT-PCR method		
		Negative	Positive	Total
Flow*flex* Test Results	Negative	32	3*	35
	Positive	1	89	90
	Total	33	92	125

***3 samples with PCR CT value 32.9-33**

2.3.3 Summary of combined clinical studies at all sites:

Candidate method		RT-PCR method		
		Negative	Positive	Total
Flow*flex* Test Results	Negative	413	5	418
	Positive	2	157	159
	Total	415	162	577

2.3.4 Conclusions:

The sensitivity, specificity, and accuracy are meeting MHRA acceptable requirement, which has sensitivity greater than 80% and specificity greater than 95%.

	Performance	95% CI
Sensitivity	96.9% (157/162)	92.8%-98.9%
Specificity	99.5% (413/415)	98.1%- 99.9%
Accuracy	98.8% (570/577)	97.5% -99.5%

2.4 Cross Reactivity (Analytical Specificity)

To demonstrate the related pathogens and microorganisms that are reasonably likely to be present in the nasal cavity do not interfere with test performance of Flow*flex* SARS-Cov-2 Antigen Test.

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot#202009001
- Extraction Buffer, Lot#102820
- Pooled human negative matrix

Procedure: Cross-Reactivity Wet Testing

Samples were prepared by spiking each stock microorganism into the pooled human negative matrix. Each microorganism was tested in triplicate with Flow*flex* SARS-CoV-2 Antigen Rapid Test.

Test Results:

No cross-reactivity was observed with the following bacteria and viruses when tested at the concentration presented in the table below.

Potential Cross -Reactant		Test Concentration	Cross-Reactivity (in the absence of SARS-CoV-2 virus)
Virus	Adenovirus	1.14×10^6 $TCID_{50}$/mL	No 3/3 negative
	Enterovirus	9.50×10^5 $TCID_{50}$/mL	No 3/3 negative
	Human coronavirus 229E	1.04×10^5 $TCID_{50}$/mL	No 3/3 negative
	Human coronavirus OC43	2.63×10^5 $TCID_{50}$/mL	No 3/3 negative
	Human coronavirus NL63	1.0×10^5 $TCID_{50}$/mL	No 3/3 negative
	Human Metapneumovirus	1.25×10^5 $TCID_{50}$/mL	No 3/3 negative
	MERS-coronavirus	7.90×10^5 $TCID_{50}$/mL	No 3/3 negative
	Influenza A	1.04×10^5 $TCID_{50}$/mL	No 3/3 negative
	Influenza B	1.04×10^5 $TCID_{50}$/mL	No 3/3 negative
	Parainfluenza virus 1	1.25×10^5 $TCID_{50}$/mL	No 3/3 negative
	Parainfluenza virus 2	3.78×10^5 $TCID_{50}$/mL	No 3/3 negative
	Parainfluenza virus 3	1.0×10^5 $TCID_{50}$/mL	No 3/3 negative
	Parainfluenza virus 4	2.88×10^6 $TCID_{50}$/mL	No 3/3 negative
	Respiratory syncytial virus	3.15×10^5 $TCID_{50}$/mL	No 3/3 negative
	Rhinovirus	3.15×10^5 $TCID_{50}$/mL	No 3/3 negative
Bacteria	Bordetella pertussis	2.83×10^9 CFU/mL	No 3/3 negative
	Chlamydia trachomatis	3.13×10^8 CFU/mL	No 3/3 negative
	Haemophilus influenzae	1.36×10^8 CFU/mL	No 3/3 negative
	Legionella pneumophila	4.08×10^9 CFU/mL	No 3/3 negative
	Mycobacterium tuberculosis	1.72×10^7 CFU/mL	No 3/3 negative
	Mycoplasma pneumoniae	7.90×10^7 CFU/mL	No 3/3 negative
	Staphylococcus aureus	1.38×10^7 CFU/mL	No 3/3 negative

	Staphylococcus epidermidis	2.32 x 10^9 CFU/mL	No 3/3 negative
	Streptococcus pneumoniae	1.04 x 10^8 CFU/mL	No 3/3 negative
	Streptococcus pyogenes	4.10 x 10^6 CFU/mL	No 3/3 negative
	Pneumocystis jirovecii-S. cerevisiae	8.63 x 10^7 CFU/mL	No 3/3 negative
	Pseudomonas aeruginosa	1.87 x 10^8 CFU/mL	No 3/3 negative
Yeast	Candida albicans	1.57 x 10^8 CFU/mL	No 3/3 negative

Flow*flex* SARS-CoV-2 Antigen Rapid Test does not differentiate between SARS-CoV and SARS-CoV-2.

2.5 Microbial Interference Studies

To demonstrate that false negatives will not occur with Flow*flex* SARS-Cov-2 Antigen Test when SARS-CoV-2 is present in a specimen with other microorganisms.

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot# 202009001
- Heat inactivated SARS-CoV-2 virus: Isolate USA-WA1/2020, Cat# 0810587CFHI, Lot#324615
- Extraction Buffer, Lot#102820
- Pooled human negative matrix

Procedure:

The samples were prepared by spiking each microorganism and the heat inactivated SARS-CoV-2 virus into the pooled human negative matrix. Each microorganism in the presence of low concentration of the heat inactivated SARS-CoV-2 virus was tested in triplicate with Flow*flex* SARS-CoV-2 Antigen Rapid Test.

Test Results:

No interference was observed in the presence of heat inactivated SARS-CoV-2 virus with the following bacteria and viruses when tested at the concentration presented in the table below.

Potential Cross -Reactant		Test Concentration	Interference (in the presence of SARS-CoV-2 virus)
Virus	Adenovirus	1.14×10^6 $TCID_{50}$/mL	No 3/3 positive
	Enterovirus	9.50×10^5 $TCID_{50}$/mL	No 3/3 positive
	Human coronavirus 229E	1.04×10^5 $TCID_{50}$/mL	No 3/3 positive
	Human coronavirus OC43	2.63×10^5 $TCID_{50}$/mL	No 3/3 positive
	Human coronavirus NL63	1.0×10^5 $TCID_{50}$/mL	No 3/3 positive
	Human Metapneumovirus	1.25×10^5 $TCID_{50}$/mL	No 3/3 positive
	MERS-coronavirus	7.90×10^5 $TCID_{50}$/mL	No 3/3 positive
	Influenza A	1.04×10^5 $TCID_{50}$/mL	No 3/3 positive
	Influenza B	1.04×10^5 $TCID_{50}$/mL	No 3/3 positive
	Parainfluenza virus 1	1.25×10^5 $TCID_{50}$/mL	No 3/3 positive
	Parainfluenza virus 2	3.78×10^5 $TCID_{50}$/mL	No 3/3 positive
	Parainfluenza virus 3	1.0×10^5 $TCID_{50}$/mL	No 3/3 positive
	Parainfluenza virus 4	2.88×10^6 $TCID_{50}$/mL	No 3/3 positive
	Respiratory syncytial virus	3.15×10^5 $TCID_{50}$/mL	No 3/3 positive
	Rhinovirus	3.15×10^5 $TCID_{50}$/mL	No 3/3 positive
Bacteria	Bordetella pertussis	2.83×10^9 CFU/mL	No 3/3 positive
	Chlamydia trachomatis	3.13×10^8 CFU/mL	No 3/3 positive
	Haemophilus influenzae	1.36×10^8 CFU/mL	No 3/3 positive
	Legionella pneumophila	4.08×10^9 CFU/mL	No 3/3 positive
	Mycobacterium tuberculosis	1.72×10^7 CFU/mL	No 3/3 positive
	Mycoplasma pneumoniae	7.90×10^7 CFU/mL	No 3/3 positive
	Staphylococcus aureus	1.38×10^7 CFU/mL	No 3/3 positive

	Staphylococcus epidermidis	2.32 x 10^9 CFU/mL	No 3/3 positive
	Streptococcus pneumoniae	1.04 x 10^8 CFU/mL	No 3/3 positive
	Streptococcus pyogenes	4.10 x 10^6 CFU/mL	No 3/3 positive
	Pneumocystis jirovecii-S. cerevisiae	8.63 x 10^7 CFU/mL	No 3/3 positive
	Pseudomonas aeruginosa	1.87 x 10^8 CFU/mL	No 3/3 positive
Yeast	Candida albicans	1.57 x 10^8 CFU/mL	No 3/3 positive

Conclusion:

Based on the data generated by this study, the microorganisms tested do not cross-react or interfere with Flow*flex* SARS-CoV-2 Antigen Rapid Test.

2.6 Endogenous Interfering Substances

To determine if the substances that naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity interfere with Flow*flex* SARS-CoV-2 Antigen Rapid Test.

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot# 202009001
- Heat inactivated SARS-CoV-2 virus: Isolate USA-WA1/2020, Cat# 0810587CFHI, Lot#324615
- Extraction Buffer, Lot# 102820
- Pooled human negative matrix

Procedure 1: Test the endogenous substances in the absence of heat inactivated SARS-Cov-2 virus.

The samples were prepared by spiking each substance into the human negative matrix to the test concentration listed in the table below. Each sample was tested in triplicate with Flow*flex* SARS-CoV-2 Antigen Rapid Test according to the package insert.

Test Results:

No cross-reactivity was observed with the endogenous interfering substances when tested at the concentration presented in the table below.

Procedure 2: Test the endogenous substances in the presence of heat inactivated SARS-CoV-2 virus.

The samples were prepared by spiking each substance and heat inactivated SARS-Cov-2 virus into the human negative matrix to the test concentration in the presence of low concentration of heat inactivated SARS-CoV-2 virus. Each sample was tested in triplicate according to the package insert.

Test Results:

No interference was observed.

Endogenous Interference Substances Study Results

Interfering Substance	Active Ingredient	Concentration	Test Results (in the absence of SARS-CoV-2 virus)	Test Results (in the presence of SARS-CoV-2 virus)
Endogenous	Biotin	2.4 mg/mL	3/3 negative	3/3 positive
	Mucin	0.5% w/v	3/3 negative	3/3 positive
	Whole Blood	4% v/v	3/3 negative	3/3 positive
Afrin Original Nasal Spray	Oxymetazoline	15% v/v	3/3 negative	3/3 positive
ALKALOL Allergy Relief Nasal Spray	Homeopathic	1:10 Dilution	3/3 negative	3/3 positive
Chloraseptic Max Sore Throat Lozenges	Menthol, Benzocaine	1.5 mg/mL	3/3 negative	3/3 positive
CVS Health Fluticasone Propionate Nasal Spray	Fluticasone propionate	5% v/v	3/3 negative	3/3 positive
Equate Fast-Acting Nasal Spray	Phenylephrine	15% v/v	3/3 negative	3/3 positive
Equate Sore Throat Phenol Oral Anesthetic Spray	Phenol	15% v/v	3/3 negative	3/3 positive
Original Extra Strong Menthol Cough Lozenges	Menthol	1.5 mg/mL	3/3 negative	3/3 positive
NasalCrom Nasal Spray	Cromolyn	15% v/v	3/3 negative	3/3 positive
NeilMed NasoGel for Dry Noses	Sodium Hyaluronate	5% v/v	3/3 negative	3/3 positive
Throat Lozenge	Dyclonine Hydrochloride	1.5mg/mL	3/3 negative	3/3 positive
Zicam Cold Remedy	Galphimia glauca, Luffa operculata, Sabadilla	5% v/v	3/3 negative	3/3 positive
Antibiotic	Mupirocin	10 mg/mL	3/3 negative	3/3 positive
Tamiflu	Oseltamivir Phosphate	5 mg/mL	3/3 negative	3/3 positive
Antibiotic	Tobramycin	4 µg/mL	3/3 negative	3/3 positive

Conclusion:

Based on the data generated by this study, the endogenous interfering substances tested do not cross-react or interfere with Flow*flex* SARS-CoV-2 Antigen Rapid Test.

2.7 Hook effect

To evaluate if the false negative result can be observed when test very high levels of heat inactivated SARS-CoV-2 virus with Flow*flex* SARS-Cov-2 Antigen Rapid Test.

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot# 202009001
- Heat inactivated SARS-CoV-2 virus: Isolate USA-WA1/2020, Cat# 0810587CFHI, Lot#324615
- Extraction Buffer, Lot#102820
- Pooled human negative clinical matrix

Procedure:

The nasal swabs from healthy donors were collected and eluted with PBS buffer. The swab eluates were combined and mixed thoroughly to create a negative clinical matrix pool. The heat-inactivated SARS-CoV-2 virus was diluted in the negative clinical matrix pool to generate a positive sample.

For each test, 50 µL of the positive sample was added to a nasal swab. The spiked swab was processed in the extraction buffer tube and tested on the Flow*flex* SARS CoV-2 Antigen Rapid Test according to the package insert. The testing concentration for the heat-inactivated SARS-CoV-2 virus was 1.43×10^5 $TCID_{50}$/mL.

Conclusion:

No high dose hook effect was observed when tested with up to a concentration of 1.43×10^5 $TCID_{50}$/mL of heat inactivated SARS-CoV-2 virus with Flow*flex* SARS-CoV-2 Antigen Rapid Test.

2.8 Read Time Flex

To demonstrate that the test result is stable when read within the recommended time window.

Material:

SARS-CoV-2 Antigen Rapid Test, Lot# COV0110005
Buffer, Lot#: TDE20110009
SARS-CoV-2 Antigen Negative Sample Lot#: 20201104
SARS-CoV-2 Antigen Low Positive Control Lot#: COVAG200930L
SARS-CoV-2 Antigen Middle Positive Control Lot#: COVAG200930M
ACON Rapid Flow Test Color Card, Lot#20200112

Procedure:

SARS-CoV-2 Antigen negative, high, middle and low positive sample are tested with SARS-CoV-2 Antigen Rapid Test according to package insert. Each test was performed in triplicate. The test results were recorded at 5, 10, 15, 20 and 30 mins.

Test results:

SARS-CoV-2 Samples	5 min	10 min	15 min	20 min	30 min
Neg	- / 3 replicates	- / 3 replicates	- / 3 replicates	- / 3 replicates	- / 3 replicates
Low Pos	- / 3 replicates	+/ 3 replicates	+ / 3 replicates	+ / 3 replicates	+ / 3 replicates
Mid Pos	+ / 3 replicates	+ / 3 replicates	+ / 3 replicates	+ / 3 replicates	+ / 3 replicates
High Pos	+ / 3 replicates	+ / 3 replicates	+ / 3 replicates	+ / 3 replicates	+ / 3 replicates

Conclusion:

The results are stable when read between 10 minutes to 30 minutes.

2.9 Stability Study

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot#1:202009101, Lot#2:202009001, Lot#3:202009201
- Extraction Buffer, Lot1#:202008001, Lot2#:202008002, Lot3#:202008003
- SARS-CoV-2 Antigen Negative Sample Lot#: COVAG200904N
- SARS-CoV-2 Antigen Low Positive Sample P3 Lot#: COVAG200904P3
- SARS-CoV-2 Antigen Middle Positive Sample P2 Lot#: COVAG200904P2
- SARS-CoV-2 Antigen High Positive Sample P1 Lot#: COVAG200904P1
- SARS-CoV-2 Antigen positive control swab, Lot#1: 202009003P-1, Lot#2: 202009003P-2, Lot#3: 202009003P-3
- SARS-CoV-2 Antigen negative control swab, Lot#1: 202009003N-1, Lot#2: 202009003N-2, Lot#3: 202009003N-3

2.9.1 Accelerated stability

Estimate the shelf life for SARS-CoV-2 Antigen Rapid Test, Extraction Buffer and Control Swabs basing on the accelerate stability study.

Procedure:

Accelerated stability study for three lots (including tests in individual pouches, control swabs in individual pouches, extraction buffer in tube) will be stored at 55°C/65°C to estimate product stability. Tests will be assayed according to package insert at designated time points. For each device lot, run 3 replicates per sample at each time points. Read the results according to package insert.

Test results:

Result of SARS-CoV-2 Antigen Rapid Test

55°C

SARS-CoV-2 Samples	0 day	7 days	14 days
Neg	- / 3 tests x 3 lots	- / 3 tests x 3 lots	- / 3 tests x 3 lots
Low Pos	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots
Mid Pos	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots
High Pos	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots

65°C

SARS-CoV-2 Samples	0 day	7 days	14 days
Neg	- / 3 tests x 3 lots	- / 3 tests x 3 lots	- / 3 tests x 3 lots
Low Pos	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots
Mid Pos	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots
High Pos	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots

Result of SARS-CoV-2 Antigen Control swab:

55°C

Samples	0 day	7 days	14 days
Positive Control Swab	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots
Negative Control Swab	- / 3 tests x 3 lots	- / 3 tests x 3 lots	- / 3 tests x 3 lots

65°C

Samples	0 day	7 days	14 days
Positive Control Swab	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots
Negative Control Swab	- / 3 tests x 3 lots	- / 3 tests x 3 lots	- / 3 tests x 3 lots

Conclusion:

SARS-CoV-2 Antigen Rapid Test, extraction buffer and SARS-CoV-2 Antigen Control Swabs are stable at 65°C for 14 days, so the shelf life can be estimated at least 24 months.

2.9.2 Real time stability

Estimate the shelf life for SARS-CoV-2 Antigen Rapid Test, Extraction Buffer and Control Swabs basing on the real time stability study.

Procedure:

Real time stability study for three lots (including tests in individual pouches, control swabs in individual pouches, extraction buffer in tube) will be stored at 2-8°C/30°C to estimate product stability. Tests will be assayed according to package insert at designated time points every 3 months until the timepoints that performance does not meet the acceptance criteria. For each device lot, negative and different levels of positive samples will be tested, run 3 replicates per sample at each time points. Read the results according to package insert.

Acceptance criteria:

Negative sample will generate negative result

Low positive, medium positive and high positive sample will generate positive results

Test results:

Result of SARS-CoV-2 Antigen Rapid Test:

2-8°C

SARS-CoV-2 Samples	Neg	Low Pos	Mid Pos	High Pos
0 day	- / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots
3 months				
6 months				
9 months				
12 months				

30°C

SARS-CoV-2 Samples	Neg	Low Pos	Mid Pos	High Pos
0 day	- / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots	+ / 3 tests x 3 lots
3 months				
6 months				
9 months				
12 months				

Result of SARS-CoV-2 Antigen Control swab:

2-8°C

SARS-CoV-2 Samples	Neg control swab	Pos control swab
0 day	- / 3 tests x 3 lots	+ / 3 tests x 3 lots
3 months		
6 months		
9 months		
12 months		

30°C

SARS-CoV-2 Samples	Neg control swab	Pos control swab
0 day	- / 3 tests x 3 lots	+ / 3 tests x 3 lots
3 months		
6 months		
9 months		
12 months		

Conclusion:

The real time stability of SARS-CoV-2 Antigen Rapid Test, extraction buffer and SARS-CoV-2 Antigen Control Swab are still in process. It is scheduled to finish in December 2022.

4.0 Mimicking Shipping Study

To evaluate the performance of Flow*flex* SARS-CoV-2 Antigen Rapid Test by mimicking shipping conditions.

Materials:

	SARS-CoV-2 Antigen Rapid Test, Lot1	SARS-CoV-2 Antigen Rapid Test, Lot2	SARS-CoV-2 Antigen Rapid Test, Lot3
Test lot number	Lot 202009101	Lot 202009001	Lot 202009201
Negative control swab	Lot 202009003N-1	Lot 202009003N-2	Lot 202009003N-3
Positive control swab	Lot 202009003P-1	Lot 202009003P-2	Lot 202009003P-3

Heat-inactivated SARS-CoV-2 virus: ZeptoMetrix Corporation, Lot#324615

Dry ovens

Refrigerator, -20℃

Method:

1) Study at 3XFT/25°C:

SARS-CoV-2 Antigen Rapid Tests were stored at -20°C for 24 hours and then stored at RT for 24 hours. 3 freeze/thaw cycles were repeated to mimic harsh shipping conditions. At the last thaw, the products were stored at 65°C for a certain period. Performed the tests with control swabs, negative and positive samples in 5 replicates at designated timepoints as below:

Temperature	Day 0	Day 7	Day 14
65°C	X	X	X

The nasal swabs from healthy volunteers were collected and eluted with PBS buffer. The swab eluates were combined and mixed thoroughly to create a negative clinical matrix pool. The heat-inactivated SARS CoV-2 virus was spiked in the negative clinical matrix pool to generate a positive sample.

50 ul of negative clinical matrix pool and spiked positive sample were applied to each swab, respectively. The swab was inserted to the extraction buffer tube, processed and tested with SARS CoV-2 Antigen Rapid Test following package insert at different time point and different mimic shipping condition. Each sample was tested in 5 replicates.

2) Shipping under condition of 55°C for two days.

Accelerated stability study at 55°C was performed for 35 days in a separated study report, which supports that product still maintain good stability after 55°C/2 days shipping condition.

Accepted Criteria:

Negative control swab and negative sample should generate negative results.

Positive control swab and positive sample should generate positive results.

Results:

Test Result of 3XFT/25°C:

1) Accelerated stability study results with lot 1:

Results with quality control swabs:

65°C stability with Lot 1	Day 0	Day 7	Day 14
Negative control swab	- (5/5)	- (5/5)	- (5/5)
Positive control swab	+ (5/5)	+ (5/5)	+ (5/5)

The results at 15min were the same as at 30min

Results with contrived samples:

65°C stability with Lot 1	Day 0	Day 7	Day 14
Negative specimen	- (5/5)	- (5/5)	- (5/5)
Low positive specimen	+ (5/5)	+ (5/5)	+ (5/5)

The results at 15min were the same as at 30min

2) Accelerated stability study results with lot 2:

Results with quality control swabs:

65°C stability with Lot 2	Day 0	Day 7	Day 14
Negative control swab	- (5/5)	- (5/5)	- (5/5)
Positive control swab	+ (5/5)	+ (5/5)	+ (5/5)

The results at 15min were the same as at 30min

Results with contrived samples:

65°C stability with Lot 2	Day 0	Day 7	Day 14
Negative specimen	- (5/5)	- (5/5)	- (5/5)
Low positive specimen	+ (5/5)	+ (5/5)	+ (5/5)

The results at 15min were the same as at 30min

3) Accelerated stability study results with lot 3:

Results with quality control swabs:

65°C stability with Lot 3	Day 0	Day 7	Day 14
Negative control swab	- (5/5)	- (5/5)	- (5/5)
Positive control swab	+ (5/5)	+ (5/5)	+ (5/5)

The results at 15min were the same as at 30min

Results with contrived samples:

65°C stability with Lot 3	Day 0	Day 7	Day 14
Negative specimen	- (5/5)	- (5/5)	- (5/5)
Low positive specimen	+ (5/5)	+ (5/5)	+ (5/5)

The results at 15min were the same as at 30min

4) Study with storage temperature at 55°C:

Product performance met the acceptable criteria under the shipping condition of 55°C for two days (detailed results are available in **3.9.1** Accelerated stability study).

6. Conclusion:

The study results of mimicking shipping condition support that the shelf life of SARS-CoV-2 Antigen Rapid Test is over two years under mimic harsh shipping conditions.

AZURE
INSTITUTE

Clinical Study Report
for
Flowflex SARS-CoV-2 Antigen
Rapid Test

I. Intend for Use

The Flowflex SARS-CoV-2 Antigen Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in nasal swab specimens directly from individuals who are suspected of COVID-19 by their healthcare provider. The Flowflex SARS-CoV-2 Antigen Rapid Test does not differentiate between SARS-CoV and SARS-CoV-2.

II. Objective

A multi-site clinical study was conducted in China and USA to evaluate the performance of the Flowflex SARS-CoV-2 Antigen Rapid Test when compared to RT-PCR method.

III. Clinical Study Site and Study Period

Clinical Study Sites in USA:

Sample collection sites in USA	Testing sites in USA
Site 1: Boca Raton 6877 SW 18th Street Boca Raton, FL 33433	Site 1: Dr. Fowl 7200 Parkway drive, Suite 117, La Mesa, CA91942
Site 2: COVID CLINIC Westminster (WM) 2109 Westminster Mall Westminster, CA 92683	Site 2: COVID CLINIC Westminster (WM) 2109 Westminster Mall Westminster, CA 92683
Site 3: COVID CLINIC La Mesa (LM) 5601 Grossmont Center Drive La Mesa, CA 91942	Site 3: COVID CLINIC La Mesa (LM) 5601 Grossmont Center Drive La Mesa, CA 91942
Site 4: COVID CLINIC Down Town San Diego (DTSD) 1350 Third Avenue San Diego - San Diego County	Site 4: COVID CLINIC Down Town San Diego (DTSD) 1350 Third Avenue San Diego - San Diego County

Clinical Study Sites in China:

Sample collection sites in China	Testing sites in China
Site 1: Shenzhen CDC No. 8 Longyuan Road, Nanshan District, Shenzhen, P.R. China	Site 1: Shenzhen CDC No. 8 Longyuan Road, Nanshan District, Shenzhen, P.R. China
Site 2: Adicon No.208 Zhenzhong Road, West Lake District, Hangzhou, Zhejiang, P.R. China	Site 2: Adicon No.208 Zhenzhong Road, West Lake District, Hangzhou, Zhejiang, P.R. China

Study Period

Study Initiation Date: September, 2020

Study Completion Date: December, 2020

IV. Study acceptance criteria

Total Sensitivity: ≥85%

Total Specificity: ≥98%

V. Study Procedure:

The clinical performance of the Flowflex SARS-CoV-2 Antigen Rapid Test was evaluated at four (4) investigational sites in U.S and two (2) investigational sites in China using a total of 605 nasal swab specimens collected from the patients at multiple sites in U.S and China.

5.1 Clinical Study in USA

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot# 202009001
- Comparison method:

TaqPath COVID-19 Combo Kit, FDA authorized RT-PCR test for emergency use, manufactured by Thermo Fisher Scientific, Inc.
CDC 2019-nCoV RT-PCR, ABI 7500DX, FDA authorized RT-PCR test for emergency use

- Nasal swab samples from infected patients and non-infected patients

Procedure:

A total of 153 nasal swab specimens were collected from the patients at multiple sites in U.S. The patients presenting the COVID-19 like symptoms within 14 days of symptom onset at the collection sites are enrolled.

The nasal swabs were randomized and blinded tested by operators following product package insert.

A companion nasopharyngeal (NP) swab was also collected from the same patient and confirmed as positive or negative and validated with Ct counts by the FDA EUA RT-PCR as a comparator method.

Test results:

Candidate method		RT-PCR method		
		Negative	Positive	Total
Flow*flex* Test Results	Negative	52	3*	55
	Positive	1	97	98
	Total	53	100	153

***3 samples with PCR CT value 32.9-33**
Relative Sensitivity: 97.0% (95% CI: 91.2%-99.4%)
Relative Specificity: 98.1% (95% CI: 89.1%-99.9%)
Accuracy: 97.4% (95% CI: 93.2%-99.2%)

5.2 Clinical Study in China

Material:

- SARS-CoV-2 Antigen Rapid Test, Lot# 202009001
- RT-PCR, Novel Coronavirus (2019-nCoV) Nucleia Acid Diagnostic Kit (PCR-Fluorescence Probing), FDA authorized RT-PCR test for emergency use, manufactured by Sansure BioTech Inc.
- Nasal swab samples from infected patients and non-infected patients

Procedure:
A total of 452 nasal swab specimens were collected from the patients at multiple sites in China. The patients presenting the COVID-19 like symptoms within 14 days of symptom onset at the collection sites are enrolled.

The nasal swabs were randomized and blinded tested by operators following product package insert.

A companion nasopharyngeal (NP) swab was also collected from the same patient and confirmed as positive or negative and validated with Ct counts by the FDA EUA RT-PCR as a comparator method.

Also the RT-PCR test results were confirmed by the clinical diagnostic result. RT-PCR positive specimens were all from diagnosis of COVID-19 patients

and RT-PCR negative specimens were all from non COVID-19 patients.

Test results:

Candidate method		RT-PCR method		
		Negative	Positive	Total
Flow*flex* Test Results	Negative	381	2*	383
	Positive	1	68	69
	Total	382	70	452

***2 samples with PCR CT value 34-35**

Relative Sensitivity: 97.1% (95% CI: 89.6%-99.8%)
Relative Specificity: 99.7% (95% CI: 98.4%-99.9%)
Accuracy: 99.3% (95% CI: 98.0%-99.9%)

5.3 Summary of combined clinical studies at all sites:

Candidate method		RT-PCR method		
		Negative	Positive	Total
Flow*flex* Test Results	Negative	433	5	438
	Positive	2	165	167
	Total	435	170	605

Relative Sensitivity: 97.1% (95% CI: 93.1%-98.9%)
Relative Specificity: 99.5% (95% CI: 98.2%-99.9%)
Accuracy: 98.8% (95% CI: 97.6%-99.5%)

5.4 Positive results to be reported by different Ct value range

Ct value	RT-PCR Positive (+)	Proportion	Flowflex SARS-CoV-2 Antigen Rapid Test Positive (+)	PPA
≤27	86	50.6%	86	100%
27-30	38	22.4%	38	100%
>30-33	29	17.1%	27	93.1%
>33	9	5.3%	6	66.7%

Note: There are eight samples only have the PCR result of positive and no Ct value available.

Comparing with RT-PCR, the positive percent agreement (PPA) of the Flowflex SARS-CoV-2 Antigen Rapid Test is 100% for samples with Ct value ≤30, 93.1% for samples with Ct value from >30 to 33. For samples with Ct value >33, the PPA is 66.7%.

5.5 Positive results to be reported by days since symptom onset

Days Since Symptom Onset	RT-PCR Positive (+)	Proportion	Flowflex SARS-CoV-2 Antigen Rapid Test Positive (+)	PPA
0-3	81	46.3%	80	98.8%
4-7	62	37.0%	60	96.8%
>7	19	11.7%	17	89.5%

Note: There are four patients is asymptomatic individuals. And there are four patients lack "Days Since Symptom Onset" information.

Nasal swab specimens obtained early (≤7 days) after symptom onset may contain higher viral concentration.

5.6 Patient Demographics

Age Group	Total	RT-PCR Positive (+)	Flowflex SARS-CoV-2 Antigen Rapid Test Positive (+)	PPA
Children (Age < 18)	13	12	11	91.7%
Adult (Age 18 to 60)	565	132	128	97.0%
Elderly (Age ≥ 60)	23	22	22	100%

Note: There are four patients lack age information.

VI. Conclusions:

Using a total of 605 specimens tested at multiple sites in U.S and China, the Flowflex SARS-CoV-2 Antigen Rapid Test has sensitivity of 97.1%, specificity of 99.5%, and accuracy of 98.8% when comparing with FDA EUA RT-PCR.

*Clinical data was collected in USA and China. Data analysis was performed by Azure Institute.

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