Performance Analysis Evaluation Data
SARS-CoV-2 Antigen Saliva
Rapid Test Kit
(Immunochromatography)

Labnovation Technologies, Inc.

Preface

This report complies with the standard of “EN13612:2002 Performance evaluation of in vitro diagnostic medical devices”.

As the below tests show, the SARS-CoV-2 Antigen Saliva Rapid Test Kit meets the claimed specifications and practical clinical applications. The performance evaluation of the SARS-CoV-2 Antigen Saliva Rapid Test Kit demonstrates that the device has proved itself to be as effective and safe enough for routine use in clinical laboratories as the legally marketed predicate devices.

Refer to the detailed information in R&D original document.

Contents

1. Abstract

SARS-CoV-2 Antigen Saliva Rapid Test Kit (Immunochromatography) (hereinafter referred to as the kit). It was designed and manufactured by LABNOVATION TECHNOLGEIS INC. The purpose of the study was to evaluate the performance of the kit in several routine assays. The evaluation consisted of the kit's positive coincidence rate, negative coincidence rate, repeatability, limit of detection, and cross-reactivity, and compared it with PCR test to assess whether it meets the requirements of research design. The evaluation plan was designed according to the guidelines of CLSI, and conforms to the criterion of EN 13612:2002.

2. Evaluation Plan

2.1Performance Evaluating Center and Participants

The performance evaluation center and participants are described in the table1.

Table 1 Performance evaluation center and participants

Performance Evaluation Center	RUMAH SAKIT UMUM DR.R.M. PATOMO BAGANSIAPIAPI	
Coordinator	Jiming Zhang（Clinical Application manager）	
Investigator	**Position**	**Investigator**
Shan Bowen	Engineer	Tan Hui

Note:

The performance evaluation supervisor takes a total charge of the evaluation and ensures the evaluation can proceed normally.

The testers are assigned by the evaluation center, and must possess related specialties, qualification and capacities and be familiar with the materials and documents related to the evaluation, which can be conducted successfully.

2.2 Time Table

		Date
1	Internal validation	30 January 2021
2	External validation	RUMAH SAKIT UMUM DR.R.M. PATOMO BAGANSIAPIAPI December 27, 2020 - January 30, 2021
3	Evaluation report (performance evaluation)	30 January 2021

2.3 Product and General Information

2.3.1 Product under test

SARS-CoV-2 Antigen Saliva Rapid Test Kit is provided by LABNOVATION TECHNOLGEIS, INC.

2.3.2 Kit batch number and specifications

The kit specifications for analytical performance evaluation are 1Test/Kit and 20 Tests/Kit, which is individually packaged in aluminum foil bags for single servings.

The batch numbers of the kits are: 20200513, 20200519, 20200526.

2.3.3 Reference Materials

National reference: The National Reference Panel for SARS-Cov-2 Antigen Detection Kit from the National Institutes for Food and Drug Control.

Coincidence Rate of negative reference products: National negative reference materials (N1-N20).

Coincidence Rate of positive reference products: tested with national positive reference products (P1-P8).

Repetitive Reference: National repetitive reference material (R).

For the determination of Limit of Detection reference material (L): SARS-CoV-2 inactivated viral sample will be purchased from Biocome Co,Ltd.

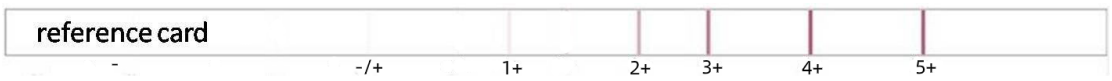
All the potential cross-reacting organism for analytical specificity will be purchased from Genetimes Technology Inc.

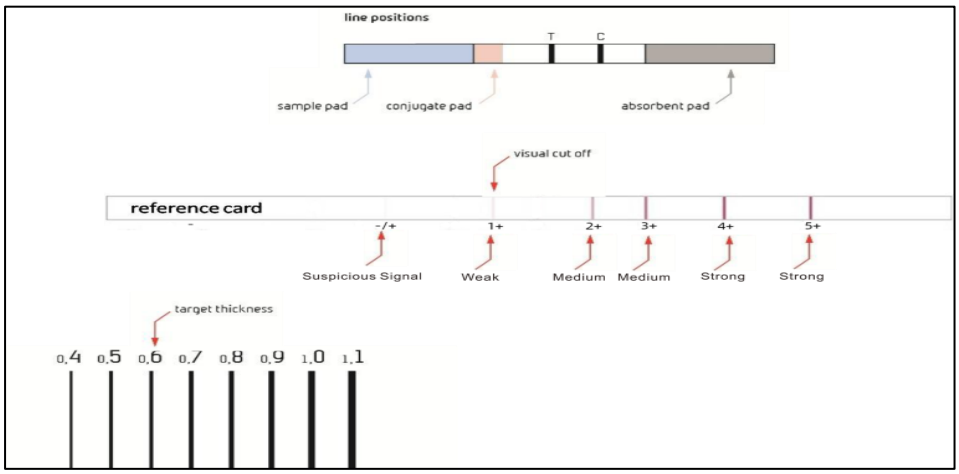
2.4 Instrument

The main instruments used for analytical performance evaluation are the instruments of equipment required by ordinary laboratories, micro-samplers, stopwatches, thermometers and hygrometers.

2.5 Reference card

The positive result was represented by '+', and the negative result was represented by '-', the quantity of + are shown the degree of color.




line positions
T
C
sample pad
conjugate pad
absorbent pad
visual cut off
reference card
-
-/+
1+
2+
3+
4+
5+
Suspicious Signal
Weak
Medium
Medium
Strong
Strong
target thickness
0,4
0,5
0,6
0,7
0,8
0,9
1,0
1,1

3. Performance Evaluation

3.1 Physical Inspection

3.1.1 Appearance

Table 1-1 Kit appearance test results

LOT	Appearance
20200513	Cassette should be flat and free of burrs on the edge. Appearance of Cassette should be neat, uniform in color and no stains. Appearance is complete in an airtight Foil bag. Markings on the package are correct. Appearance of packaging should be tidy. Symbols, wordage and logo should be clear. Complete contents and its packaging should not be damaged.
20200519	Cassette should be flat and free of burrs on the edge. Appearance of Cassette should be neat, uniform in color and no stains. Appearance is complete in an airtight Foil bag. Markings on the package are correct. Appearance of packaging should be tidy. Symbols, wordage and logo should be clear.
20200526	Cassette should be flat and free of burrs on the edge. Appearance of Cassette should be neat, uniform in color and no stains. Appearance is complete in an airtight Foil bag. Markings on the package are correct. Appearance of packaging should be tidy. Symbols, wordage and logo should be clear.

3.1.2 Strip width

Use vernier calipers to detect Test strip, test results as table 1-2:

Table 1-2 Test strip width test results

LOT	Width（mm）			
	1	2	3	Average
20200513	3.00	3.00	3.01	3.00
20200519	3.01	3.01	3.00	3.01
20200526	3.02	3.01	3.00	3.01

3.1.3 Liquid Migration Speed

Use a stopwatch to measure the liquid transit time, the time required for the liquid to drip into the sample hole and start counting until the liquid completely passes the observation window (t), the distance from the center of the sample hole to the end edge of the observation window (L), and calculate L / t is the moving speed. Repeat 3 test cards and take the average value.

Table 1-3 Liquid Migration Speed Test Results

LOT	t（s）				v（mm/min）
	1	2	3	Average	
20200513	110.9	111.5	106.8	109.73	16.40
20200519	105.7	108.9	110.5	108.37	16.61
20200526	108.6	106.8	105.6	107.00	16.82

3.1.4 Conclusion

The physical properties of the three batches of kits meet the expected requirements. The test strip width is 3.00mm, 3.01mm, 3.01mm, respectively, all larger than 2.5mm; Liquid migration speed is 16.40mm/min, 16.61mm/min, 16.82mm/min, all larger than 10mm/min.

3.2 Positive Coincidence Rate

3.2.1 Requirements

The test operator should be familiar with the detection method and operational instrument. The kits used for the test are three batch numbers and are within the validity period.

3.2.2 Method

There are 8 positive reference samples (P1 ~ P8) was diluted for 1:10 by sample extraction separately. Three batches of kits tested the diluted samples (P1 ~ P8) according to the instructions and recorded the test results.

3.2.3 Results

The test results are shown in Table 1-4. The results show that the positive coincidence rate is 100%.

Table 1-4 Positive coincidence rate results

Lot No. / Samples	Dilution Ratio	20200513	20200519	20200526
P1	1:10	1+	1+	1+
P2	1:10	2+	2+	2+
P3	1:10	2+	2+	2+
P4	1:10	2+	2+	2+
P5	1:10	3+	3+	3+
P6	1:10	2+	2+	2+
P7	1:10	1+	1+	1+
P8	1:10	3+	3+	3+
Coincidence Rate	/	8/8	8/8	8/8

3.3 Negative Coincidence Rate

3.3.1 Requirements

The test operator should be familiar with the detection method and operational instrument. The kits used for the test are three batch numbers and are within the validity period.

3.3.2 Method

Three batches of kits tested the original concentration of 20 negative reference samples (N1 ~ N20) according to the instructions and recorded the test results.

3.3.3 Results

The test results are shown in Table 1-5. The results show that the negative coincidence rate is 100%.

Table 1-5 Negative coincidence rate test results

LOT / Reference	Dilution Ratio	20200513	20200519	20200526
N1	Original	-	-	-
N2	Original	-	-	-
N3	Original	-	-	-
N4	Original	-	-	-
N5	Original	-	-	-
N6	Original	-	-	-
N7	Original	-	-	-
N8	Original	-	-	-
N9	Original	-	-	-
N10	Original	-	-	-
N11	Original	-	-	-
N12	Original	-	-	-
N13	Original	-	-	-
N14	Original	-	-	-
N15	Original	-	-	-
N16	Original	-	-	-
N17	Original	-	-	-
N18	Original	-	-	-
N19	Original	-	-	-
N20	Original	-	-	-
Negative Coincidence	/	20/20	20/20	20/20

3.4 Repeatability

3.4.1 Requirements

The test operator should be familiar with the detection method and operational instrument. The reference samples used for the test are three batch numbers and are within the validity period.

Using diluent to dilute repeatable reference product of SARS-CoV-2 Antigen Saliva Rapid Test Kit (R) for 1:10, 1:100, then marked R1 and R2 as the low positive and moderately positive samples to be tested. One negative reference sample N1 was tested as negative specimen.

3.4.2 Method

Using sample extraction buffer to dilute repeatable reference samples (R) for 1:10, 1:100, then marked R1 and R2.

Table 1-6 Sample (R1&R2) preparation

	R (ul)	sample extraction buffer (ul)
R1	100	900
R2	10	990

Three batches of kits tested repetitive reference (R1/R2/N1). Repeat the test 10 times for each batch and perform the test according to the instructions. The quantity of + are shown the degree of color to record the test results.

3.4.3 Test Results

The test results are shown in Table 1-7. All the tests show consistent result, and there is no obvious difference in color.

Table 1-7 Repeatability Test Results

LOT / Times	20200513			20200519			20200526		
	R1	R2	N1	R1	R2	N1	R1	R2	N1
1	2+	1+	-	2+	1+	-	2+	1+	-

2	2+	1+	-	2+	1+	-	2+	1+	-
3	2+	1+	-	2+	1+	-	2+	1+	-
4	2+	1+	-	2+	1+	-	2+	1+	-
5	2+	1+	-	2+	1+	-	2+	1+	-
6	2+	1+	-	2+	1+	-	2+	1+	-
7	2+	1+	-	2+	1+	-	2+	1+	-
8	2+	1+	-	2+	1+	-	2+	1+	-
9	2+	1+	-	2+	1+	-	2+	1+	-
10	2+	1+	-	2+	1+	-	2+	1+	-
Conclusion	The test results are consistent, and there is no obvious difference in color.								

3.5 Limit of Detection

3.5.1 Establishment of the Limit of Detection

Detection limit reference material L: SARS-CoV-2 inactivated viral sample (BetaCoV/Wuhan/IPBCAMS-WH-01/2019) was purchased from Biocome Co, Ltd.

Material and method:

Based on the Technical Key Points for Coronavirus (COVID-19) Antigen-antibody Detection Reagent Registration Review (Trial), the limit of detection (LOD) for the SARS-CoV-2 Antigen Rapid Test Kit was established using limiting dilutions of the viral sample inactivated by gamma irradiation. The viral sample is supplied at a concentration of 3.2×10^5/mL TCID50 as the detection limit reference material L. In this study, designed to estimate the LOD of the assay when using saliva sample, The starting material is spiked into a volume of pooled human saliva obtained from healthy donors and confirmed negative for SARS-CoV-2. Using sample extraction buffer to dilute reference materials L for 1:10, 1:100, 1:1000 and 1:0000, then marked as L1~L4. Test with three bathes of kits and each dilution was tested in three replicates. Record the results. The positive result was represented by '+', and the negative result was represented by '-'. The test results are shown in the table below.

Table 1-8 LOD samples preparation

Samples	Dilution Ratio	Virus Concentration (TCID 50 /mL)
L	Original	3.2×10^5
L1	1:10	3.2×10^4
L2	1:100	3.2×10^3
L3	1:1000	3.2×10^2
L4	1:0000	3.2×10^1

Table 1-9 LOD test results for SARS-CoV-2 Antigen Saliva Test Strip

Lot / Sample	Dilution Ratio	Virus Concentration (TCID 50 /mL)	20200513			20200519			20200526		
			1	2	3	1	2	3	1	2	3
L	Original	3.2×10^5	5+	5+	5+	5+	5+	5+	5+	5+	5+
L1	1:10	3.2×10^4	4+	4+	4+	4+	4+	4+	4+	4+	4+
L2	1:100	3.2×10^3	3+	3+	3+	3+	3+	3+	3+	3+	3+
L3	1:1000	3.2×10^2	2+	2+	2+	2+	2+	2+	2+	2+	2+
L4	1:10000	3.2×10^1	-	-	-	-	-	-	-	-	-

Results analysis: the sample L1, L2, L3 all showed positive results and sample L4 gave 3 negative results. As a result, the concentration of L3 was chosen for further dilution. Using sample extraction buffer to dilute L3 for 1:2, 1:4, 1:8, then marked as L5~L7.Test with three bathes of kits and each dilution was tested in three replicates. Record the results. The positive result was represented by '+', and the negative result was represented by '-'. The test results are shown in the table below.

Table 1-10 LOD Samples Preparation

Samples	Dilution Ratio	Virus Concentration (TCID 50 /mL)
L3	1:1000	3.2×10^2
L5	1:2000	1.6×10^2
L6	1:4000	8.0×10^1
L7	1:8000	4.0×10^1

Table 1-11 LOD test results for SARS-CoV-2 Antigen Saliva Test Strip

Lot / Sample	Dilution Ratio	Virus Concentration (TCID 50 /mL)	20200513			20200519			20200526		
			1	2	3	1	2	3	1	2	3
L3	1:1000	3.2×10^2	2+	2+	2+	2+	2+	2+	2+	2+	2+
L5	1:2000	1.6×10^2	1+	1+	1+	1+	1+	1+	1+	1+	1+
L6	1:4000	8.0×10^1	-	-	-	-	-	-	-	-	-
L7	1:8000	4.0×10^1	-	-	-	-	-	-	-	-	-

Results analysis: The sample L3 and L5 showed positive results. Sample L6 and L7 gave all negative results.

Therefore, sample L5 (diluting the detection limit reference sample L by 2000 times) was determined as the minimum detection limit concentration level.

3.5.2 Verification of the Limit of Detection

Material and method:

Using sample extraction buffer to dilute the detection limit reference material L (3.2×10^5/mL $TCID_{50}$) for 1:2000 and marked as L5 for the verification of the limit of detection Sample L5 was tested with three bathes of kits and repeat the test 20 times for each batch. Test and record the result. The positive detection rate should be above 90%. The positive result was represented by '+', and the negative result was represented by '-'. The test results are shown in the table below.

Table 1-12 LOD verification results for SARS-CoV-2Antigen Saliva Test Strip

Lot / Sample	20200513 No. Positive/Total			20200519 No. Positive/Total			20200526 No. Positive/Total			The Total Positive Rate%
	1	2	3	1	2	3	1	2	3	
L5	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	1+ 20/20	100%

Results analysis: The results showed that the positive detection rate of sample L5 in three batches was 100%, which meets the requirements.

Based on the evaluation of experiments, sample L5 which is diluted by the detection limit reference sample L (3.2 x 10^5/mL $TCID_{50}$) by 2000 times was determined as the minimum detection limit concentration level.

Table 1-13 LOD result for SARS-CoV-2 Antigen Saliva Test Strip

Reference sample **L**	LOD concentration	No. Positive/Total	The total positive rate%
3.2 ×10^5 $TCID_{50}$/mL	1.6×10^2 $TCID_{50}$/mL	180/180	100%

3.6 Analytical Specificity

All potential Cross-Reacting Organisms were purchased from Genetimes Technology, Inc.

3.6.1 Requirements

The test operator should be familiar with the detection method and operational instrument. The kits used for the test are three batch numbers and are within the validity period.

3.6.2 Method

The SARS-CoV-2 antigen saliva test kits were used to detect High prevalence of respiratory pathogens, the cross-reactivity validation between antigen (bacteria, viruses, yeast) and SARS-CoV-2 was performed in each type. Each of the samples were tested in duplicate in the absence and presence of heat inactivated SARS-CoV-2 virus (3.2×10^2 $TCID_{50}$/mL) and the test results were recorded.

3.6.3 Results

Table 1-14 Cross-reactivity

Potential Cross-Reacting Organism	Concentration Tested	Results			
		With inactive SARS-CoV-2 virus		Without inactive SARS-CoV-2 virus	
Human coronavirus 229E (Heat-Inactivated)	1.0 x 10^5 U/mL	2+	2+	-	-
Human coronavirus OC43	1.0 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Human coronavirus NL63	1.0 x 10^5 $TCID_{50}$/mL	2+	2+	-	-

Adenovirus type 3	1.0 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Human Metapneumovirus	1.0 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Parainfluenza virus 1	1.0 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Parainfluenza virus 2	1.0 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Parainfluenza virus 3	5.2 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Parainfluenza virus 4	1.6 x 10^4 $TCID_{50}$/mL	2+	2+	-	-
Influenza A	2.5 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Influenza B	2.9 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Human enterovirus 71	4.0 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Respiratory syncytial virus	4.0 x 10^5 $TCID_{50}$/mL	2+	2+	-	-
Rhinovirus	1.1 x 10^5 PFU/mL	2+	2+	-	-
Haemophilus influenza	1.4 x 10^6 CFU/mL	2+	2+	-	-
Streptococcus pneumoniae	1.0 x 10^6 CFU/mL	2+	2+	-	-
Streptococcus pyogenes	1.6 x 10^6 CFU/mL	2+	2+	-	-
Candida albicans	1.8 x 10^6 CFU/mL	2+	2+	-	-
Pooled human saliva	100%	2+	2+	-	-
Bordetella pertussis	1.4 x 10^6 CFU/mL	2+	2+	-	-
Mycoplasma pneumoniae	1.0 x 10^6 CFU/mL	2+	2+	-	-
Chlamydia pneumoniae	1.0 x 10^6 IFU/mL	2+	2+	-	-
Legionella pneumophila	1.0 x 10^6 CFU/mL	2+	2+	-	-

Results analysis: With human coronavirus 229E, human coronavirus OC43, human coronavirus NL63, adenovirus, human metapneumovirus, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, influenza A, type B Influenza, enterovirus, respiratory syncytial virus, rhinovirus, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, Candida albicans, Bordetella pertussis, Mycoplasma pneumonia, pneumonia Chlamydia, Legionella pneumophila, etc. have no cross reaction.

3.7 Interfering Substances

The following interfering substances were introduced into the pooled human saliva obtained from healthy donors and confirmed negative for SARS-CoV-2. Each of the interfering substances listed were tested in duplicate in the absence and presence of heat inactivated SARS-CoV-2 virus (3.2×10^2 $TCID_{50}$/mL). The SARS-CoV-2 Antigen Saliva Rapid Test Kit at the concentrations listed below had no effect to the performance of the test kit.

Table 1-15 Interfering Substances Test Results

Substance	Active Ingredient	Concentration	Results			
			With inactive SARS-CoV-2virus		Without inactive SARS-CoV-2 virus	
Endogenous	Whole Blood	1.2 % v/v	2+	2+	-	-
	Mucin	2.0 % w/v	2+	2+	-	-
Nasal Drops	Sodium Chloride	5% v/v	2+	2+	-	-
Nasal Spray	Fluticasone Propionate	0.3 ng/mL	2+	2+	-	-
	Gluconic Acid Zinc	5 % w/v	2+	2+	-	-
	Fluconazole	5 % w/v	2+	2+	-	-
	Oxymetazoline	12 % v/v	2+	2+	-	-
	Cromolyn	15 % v/v	2+	2+	-	-
Sore Throat Phenol Spray	Phenol	15 % v/v	2+	2+	-	-
Throat Lozenge	Benzocaine, Menthol	0.15% w/v	2+	2+	-	-
Anti-viral Drug	Tamiflu (Oseltamivir Phosphate)	1.3 mg/mL	2+	2+	-	-
	Ribavirin	12.9 mg/mL	2+	2+	-	-
Antibacterial, Systemic	Tobramycin 4.0	4.0 ug/mL	2+	2+	-	-
Human Saliva	Toothpaste	5% v/v	2+	2+	-	-
	Mouthwash	5% v/v	2+	2+	-	-

3.8 Hook Effect

Base on the data from LoD analysis study, no high dose hook effect was observed when tested with up to a concentration of 3.2x 10^5 TCID50/mL of inactivated SARS-CoV-2 virus with the test kit.

Table 1-16 Hook Effect Results

Lot / Sample	Dilution Ratio	Virus Concentration (TCID 50 /mL)	20200513			20200519			20200526		
			1	2	3	1	2	3	1	2	3
L	Original	3.2×10^5	5+	5+	5+	5+	5+	5+	5+	5+	5+
L1	1:10	3.2×10^4	4+	4+	4+	4+	4+	4+	4+	4+	4+
L2	1:100	3.2×10^3	3+	3+	3+	3+	3+	3+	3+	3+	3+
L3	1:1000	3.2×10^2	2+	2+	2+	2+	2+	2+	2+	2+	2+
L5	1:2000	1.6×10^2	1+	1+	1+	1+	1+	1+	1+	1+	1+

The verification result of the highest concentration value without hook effect is as follows:

Table 1-17 The Highest Concentration Value Without Hook Effect

SARS-CoV-2	The Highest Concentration Value Without Hook Effect
	3.2×10^5 $TCID_{50}$/mL

3.9 Validation of Test Reading Time

The diluted samples (P1 ~ P8) and original concentration of 20 negative reference samples (N1~N20) were tested for selecting and validating of a test reading time. Monitoring and observing the reaction process at 5min, 10min, 15min and 20min. the phenomena and results were recorded in below table.

Table 1-18 Test Reading Time Results

Sample	Dilution Ratio	5min		10min		15 min		20min	
		Reaction membrane	Result	Reaction membrane	Result	Reaction Membrane	Result	Reaction Membrane	Result
P1	1:10	In reaction	N/A	In reaction	N/A	clean	1+	clean	1+
P2	1:10	In reaction	N/A	In reaction	N/A	clean	2+	clean	2+
P3	1:10	In reaction	N/A	In reaction	N/A	clean	2+	clean	2+
P4	1:10	In reaction	N/A	In reaction	N/A	clean	2+	clean	2+
P5	1:10	In reaction	N/A	In reaction	N/A	clean	3+	clean	3+
P6	1:10	In reaction	N/A	In reaction	N/A	clean	2+	clean	2+
P7	1:10	In reaction	N/A	In reaction	N/A	clean	1+	clean	1+
P8	1:10	In reaction	N/A	In reaction	N/A	clean	3+	clean	3+
N1	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N2	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N3	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N4	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N5	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N6	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N7	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N8	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-

N9	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N10	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N11	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N12	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N13	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N14	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N15	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N16	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N17	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N18	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N19	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-
N20	Original	In reaction	N/A	In reaction	N/A	clean	-	clean	-

Results analysis: The results showed that the 15-20 min was the most suitable test reading time.

3.10 Operating Temperature and Humidity

Requirement: Testing conditions recommended in the IFU (Humidity≤60%, Temp: 20℃-30℃ Please use immediately when the humidity＞60%) are validated.All negative specimens should be non-reactive and all positive specimens should be reactive with same and uniform coloration.

Study design: The sealed test kits and reagents were placed in a specific environment condition for 30mins. The diluted positive samples (P1 ~ P8) and original concentration of 10 negative reference samples (N1 ~ N10) were tested for validating of temperature and humidity list on the table. One batch of kits (Lot 20200513) was used to test the samples according to the instructions under specific environment condition. Monitoring and observing the reaction process. The phenomena and results were recorded.

Table 1-19 Operating condition of temperature and humidity

Conditions	Temperature	Humidity
C1	15℃	40%
C2	25℃	40%
C3	35℃	40%
C4	25℃	70%

Table 1-20 Test Operating Temperature and Humidity

Sample	Dilution Ratio	C1	C2	C3	C4
P1	1:10	1+	1+	1+	1+
P2	1:10	2+	2+	2+	2+
P3	1:10	2+	2+	2+	2+
P4	1:10	2+	2+	2+	2+
P5	1:10	3+	3+	3+	3+
P6	1:10	2+	2+	2+	2+
P7	1:10	1+	1+	1+	1+
P8	1:10	3+	3+	3+	3+
N1	Original	-	-	-	-
N2	Original	-	-	-	-
N3	Original	-	-	-	-
N4	Original	-	-	-	-
N5	Original	-	-	-	-
N6	Original	-	-	-	-
N7	Original	-	-	-	-
N8	Original	-	-	-	-
N9	Original	-	-	-	-
N10	Original	-	-	-	-

In conclusion, Testing condition recommended in the IFU (Humidity≤60%, Temp: 20℃-30℃Please use immediately when the humidity＞60%) is validated. The kit performance is insensitive to environment condition.

3.11 Specimen volume

Requirement: Testing specimen volume recommended in the IFU (6 drops) are validated. All negative specimens should be non-reactive and all positive specimens should be reactive with same and uniform coloration.

Study design: The diluted samples (P1 ~ P8) and original concentration of 10 negative reference samples (N1 ~ N10) were tested for validating of specimen volume. Monitoring and observing the reaction process at different specimen volume 5 drops, 6 drops, 7 drops and 8 drops. One batch of kits (Lot 20200513) was used to test the samples according to the instructions. The phenomena and results were recorded.

Table 1-21 Test Validation of Specimen Volume

Sample	Dilution Ratio	5 drops	6 drops	7 drops	8 drops
P1	1:10	N/A	1+	1+	N/A
P2	1:10	N/A	2+	2+	N/A
P3	1:10	N/A	2+	2+	N/A
P4	1:10	N/A	2+	2+	N/A
P5	1:10	N/A	3+	3+	N/A
P6	1:10	N/A	2+	2+	N/A
P7	1:10	N/A	1+	1+	N/A
P8	1:10	N/A	3+	3+	N/A
N1	Original	N/A	-	-	N/A
N2	Original	N/A	-	-	N/A
N3	Original	N/A	-	-	N/A
N4	Original	N/A	-	-	N/A
N5	Original	N/A	-	-	N/A
N6	Original	N/A	-	-	N/A
N7	Original	N/A	-	-	N/A
N8	Original	N/A	-	-	N/A
N9	Original	N/A	-	-	N/A
N10	Original	N/A	-	-	N/A

In conclusion, testing specimen volume recommended in the IFU (6 drops) is validated.

3.12 Stability of Specimen

3.12.1 Specimen type

SARS-CoV-2 Antigen Saliva Rapid Test Kit has been evaluated with saliva specimens. The storage condition of specimen was evaluated.

3.12.2 Objective

To determine requirements for specimen stability for two different storage temperature ranges, 2~8℃ and -20℃, transport condition and in-use condition. This study investigated the stability of saliva samples.

3.12.3 Method

3.12.3.1 Specimen Material

Saliva specimens were obtained by spiking negative pooled human saliva matrix obtained from healthy donors with appropriate amount of viral sample inactivated by gamma irradiation. The negative specimen marked as S0 was obtained by the pooled negative saliva matrix. 10 positive saliva specimens containing inactivated SARS-CoV-2 virus was marked as S1~S10. All the 11 specimens were tested for each storage condition by SARS-CoV-2 Antigen Saliva Rapid Test Kit (Lot 20200513).

3.12.3.2 Saliva specimen storage at 2～8℃

After the first test, keep the saliva specimens stored at 2-8°C. Use SARS-CoV-2 Antigen Saliva Rapid Test Kit for testing at 24, 48, and 72 hours respectively. Repeat the test 3 times for each sample. Record the test results, use the first test result as the benchmark, and the coloration was uniform.

3.12.3.3 Saliva specimen storage at -20℃

After the first test, keep the saliva specimens stored at -20°C. Use SARS-CoV-2 Antigen Saliva Rapid Test Kit for testing at 15, 30, and 40 days respectively. Repeat the test 3 times for each sample, record the test results. Use the first test result as the benchmark, and the color results should be consistent.

The test results of saliva specimens after repeated freezing and thawing 1, 2, 3 and 4 times were compared with the results before freezing and thawing.

3.12.3.4 Saliva specimens cold-chain transportation

After the first test, packing the saliva specimens for cold-chain transportation (2～8℃). Use SARS-CoV-2 Antigen Saliva Rapid Test Kit for testing at 4, 8, 16 and 24 hours respectively. Repeat the test 3 times for each sample. Record the test results, use the first test result as the benchmark, and the coloration was uniform.

3.12.3.5 Saliva Specimens In-Use Stability

After the saliva specimens were collected, Use SARS-CoV-2 Antigen Saliva Rapid Test Kit for testing at 0, 1, 2 and 3 hours respectively. Repeat the test 3 times for each sample. Record the test results, use the first test result as the benchmark, and the coloration was uniform.

3.12.4 Results

Table 1-22 Test Results Of Saliva Specimens Storage At 2～8℃

Time / Samples	0h	24h	48h	72h
S0	-	-	-	-
S1	**2+**	**2+**	**2+**	**1+**
S2	**2+**	**2+**	**2+**	**2+**
S3	**1+**	**1+**	**1+**	**1+**
S4	**3+**	**3+**	**3+**	**2+**
S5	**2+**	**2+**	**2+**	**2+**
S6	**3+**	**3+**	**3+**	**3+**
S7	**2+**	**2+**	**2+**	**2+**
S8	**1+**	**1+**	**1+**	**1+**
S9	**1+**	**1+**	**1+**	**1+**
S10	**2+**	**2+**	**2+**	**2+**
Conclusion	Saliva specimens storage at 2～8℃ for up to 48hours prior to test had no significant influence on measuring SARS-CoV-2 antigen.			

Table 1-23 Test Results Of Saliva Specimens Storage At -20℃

Time / Samples	0h	15days	30days	40days
S0	-	-	-	-
S1	**2+**	**2+**	**2+**	**1+**
S2	**2+**	**2+**	**2+**	**2+**
S3	**1+**	**1+**	**1+**	**1+**
S4	**3+**	**3+**	**3+**	**3+**
S5	**2+**	**2+**	**2+**	**2+**
S6	**3+**	**3+**	**3+**	**2+**
S7	**2+**	**2+**	**2+**	**1+**
S8	**1+**	**1+**	**1+**	**1+**
S9	**1+**	**1+**	**1+**	**1+**
S10	**2+**	**2+**	**2+**	**2+**
Conclusion	Saliva specimens storage at -20℃ for up to 30days prior to test had no significant influence on measuring SARS-CoV-2 antigen.			

Table 1-24 Test results of saliva specimens free/thaw cycles at -20℃

Cycles / Samples	0	1 times	2 times	3 times	4 times
S0	-	-	-	-	-
S1	**2+**	**2+**	**2+**	**2+**	**1+**
S2	**2+**	**2+**	**2+**	**2+**	**1+**
S3	**1+**	**1+**	**1+**	**1+**	**1+**
S4	**3+**	**3+**	**3+**	**3+**	**2+**
S5	**2+**	**2+**	**2+**	**2+**	**2+**
S6	**3+**	**3+**	**3+**	**3+**	**2+**
S7	**2+**	**2+**	**2+**	**2+**	**1+**
S8	**1+**	**1+**	**1+**	**1+**	**1+**
S9	**1+**	**1+**	**1+**	**1+**	-
S10	**2+**	**2+**	**2+**	**2+**	**2+**
Conclusion	Saliva specimens after repeated freezing and thawing for 2 times had no significant influence on measuring SARS-CoV-2 antigen.				

Table 1-25 Test Results of Saliva Specimens Cold-Chain Transportation

Time / Samples	0h	4h	8h	16h	24h
S0	-	-	-	-	-
S1	**2+**	**2+**	**2+**	**2+**	**2+**
S2	**2+**	**2+**	**2+**	**2+**	**2+**
S3	**1+**	**1+**	**1+**	**1+**	**1+**
S4	**3+**	**3+**	**3+**	**3+**	**3+**
S5	**2+**	**2+**	**2+**	**2+**	**2+**
S6	**3+**	**3+**	**3+**	**3+**	**3+**
S7	**2+**	**2+**	**2+**	**2+**	**2+**
S8	**1+**	**1+**	**1+**	**1+**	**1+**
S9	**1+**	**1+**	**1+**	**1+**	**1+**
S10	**2+**	**2+**	**2+**	**2+**	**2+**
Conclusion	Saliva specimens cold-chain transportation for up to 24hours prior to test had no significant influence on measuring SARS-CoV-2 antigen .				

Table 1-26 Test Results of Saliva Specimens In-Use Stability

Time / Samples	0h	1h	2h	3h
S0	-	-	-	-
S1	**2+**	**2+**	**2+**	**2+**
S2	**2+**	**2+**	**2+**	**2+**
S3	**1+**	**1+**	**1+**	**1+**
S4	**3+**	**3+**	**3+**	**3+**
S5	**2+**	**2+**	**2+**	**2+**
S6	**3+**	**3+**	**3+**	**3+**
S7	**2+**	**2+**	**2+**	**2+**
S8	**1+**	**1+**	**1+**	**1+**
S9	**1+**	**1+**	**1+**	**1+**
S10	**2+**	**2+**	**2+**	**2+**
Conclusion	After the saliva specimens were collected for up to 3 hours prior to test had no significant influence on measuring SARS-CoV-2 antigen.			

In conclusion, specimen stability studies showed that sample of human saliva should be processed as soon as possible after sample collection. If the test cannot be

performed immediately, the sample should be stored in a sealed state, stored at 2~8℃ for 48 hours, and stored below -20℃ for 1 month. Repeated freezing and thawing for more than two times are not recommended. The transportation and shipping time more than 24 hours is not recommended. After samples collected, recommend the test in 2 hours.

3.13 Comparative Test Report

3.13.1 Method

In this trial, 505 clinical samples of saliva and nasopharyngeal swabs were selected. There were 105 positive samples and 400 negative samples.

The SARS-CoV-2 Antigen Saliva Rapid Test Kit and the PCR test were detected simultaneously, and the clinical sensitivity, clinical specificity, and total coincidence rate were calculated. An CE marked real-time polymerase chain reaction (PCR) system from Sansure Biotech for the detection of SARS-CoV-2 was used as the comparator method for this study.

3.13.2 Statistics Result

(1) Statistics of test reagent results and PCR results on nasopharyngeal swab samples

SARS-CoV-2 Antigen Saliva Rapid Test	PCR Results		Total
	Positive	Negative	
Positive	99	4	103
Negative	6	396	402
Total	105	400	505

Analysis of compliance rate

Clinical sensitivity: 94.29%（95%CI: 87.98%-97.87%）
Clinical specificity: 99.00%（95%CI: 97.46%-99.73%）
Total coincidence rate: 98.02%（95%CI: 96.39%-99.05%）

3.13.3 Statistics of test reagent results based on Ct counts

The performance of SARS-CoV-2 Antigen Saliva Rapid Test Kit with positive results stratified by the PCR method Ct counts were assessed to more understand the correlation of assay performance to the PCR Ct value, estimating the viral amount present in the clinical sample. As showed in the following table, the positive agreement of the SARS-CoV-2 Antigen Saliva Rapid Test Kit is higher with samples of a Ct count ≤30.

Table 1-27 Performance against the PCR Method – by Ct Counts

		PCR Method	
		Positive （Ct≤30）	Positive （Ct>30）
SARS-CoV-2 Antigen Saliva Test Strip	Positive	77	22
	Negative	2	4
	Total	79	26
	Positive Coincidence Rate	97.47% （91.15%-99.69%）	84.62% （65.13%-95.64%）

3.13.4 Statistics of test reagent results based on system onset

In this research, there are 105 positive specimens confirmed by the PCR method, 46 specimens with an onset period of 0-3 days, 59 specimens with an onset period of 4-7 days.

Table 1-28 Stratified Comparison Results of Samples at Different Stages Of Disease

Symptom Onset	Total Samples	SARS-Cov-2 Antigen Saliva Strip Detected Positive	Positive Coincidence Rate	95% Confidence Interval
0-3 days	46	44	95.65%	85.16% - 99.47%
4-7 days	59	55	93.22%	83.54% - 98.12%

3.13.5 Conclusion

SARS-CoV-2 Antigen Saliva Rapid Test Kit of Labnovation Technologies, Inc. was validated in RUMAH SAKIT UMUM DR.R.M. PATOMO BAGANSIAPIAPI. The

test process was carried out strictly according to the standard operating procedures. The clinical study demonstrates SARS-CoV-2 Antigen Saliva Rapid Test Kit has clinical sensitivity of 94.29%, clinical specificity of 99.00% and total consistent rate of 98.02%.

3.13.6 Quality Control

Good laboratory practice suggests the use of quality controls to ensure that test reagents are working and that the test is correctly performed. Labnovation SARS-CoV-2 Antigen Saliva Rapid Test Kit contain an internal control. For ensuring the authenticity and compliance, test process was controlled as follow:
A. The technician shall train the operator participating in test and conduct the unified judgment standard. The operator should be familiar with and understand the test method of this product, be familiar with product instruction of evaluation reagent, master the operation procedures and precautions in product instruction.
B. The distinct colored lines at the “Control” position is an internal procedural control. If the test flows and the reagents work, this line will always appear.
C. The clearing of background color from the result window is a negative background control. The background color in the window should be light pink to white within 15minutes. Background color should not hinder reading of the test.

3.14 Conclusion

The test results show that the positive coincidence rate for the three batches of SARS-CoV-2 Antigen Saliva Rapid Test Kit produced by our company is 100%, the negative coincidence rate is 100%. The repetitive test results (R1, R2) are all positive and the colors show no significant difference; The negative repetitive test results (N1) are all negative; the limit of detection reference is tested, and the results meet the requirements.
With human coronavirus 229E, human coronavirus OC43, human coronavirus NL63, adenovirus, human metapneumovirus, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, influenza A, type B Influenza, enterovirus, respiratory syncytial virus, rhinovirus, Haemophilus influenzae,

Streptococcus pneumoniae, Streptococcus pyogenes, Candida albicans, Bordetella pertussis, Mycoplasma pneumonia, pneumonia Chlamydia, Legionella pneumophila, etc. have no cross reaction.

Common interfering substances in the sample, such as blood, mucin, and pus, have no effect on the test results.

The limit of detection reference is tested and the LOD concentration is 1.6×102 TCID50/mL .

No hook effect was observed in the verification test at a concentration of $3.2x10^5$ TCID50/mL.

Specimen stability studies show that saliva specimen stored at 2~8℃for 48 hours, and stored below -20℃ for 1 month have no significant influence on measuring SARS-CoV-2 antigen. Repeated freezing and thawing for more than 2 times is not recommended.

Comparative analysis of coincidence rate of SARS-CoV-2 Antigen Saliva Rapid Test Kit and PCR Test in 505 samples shows that the SARS-CoV-2 Antigen Saliva Rapid Test Kit has clinical sensitivity of 94.29% , clinical specificity of 99.00% and total consistent rate of 98.02%, indicating that the kits have very high consistency with PCR Test.

Through the evaluation of various performance characteristics, the results show that the quality of the SARS-CoV-2 Antigen Saliva Rapid Test Kit (Immunochromatography) produced by our company is stable and meets the requirements of the products.

4. Conclusion

The experimental results of the performance evaluation of the reagent kit show that this product's precision (repeatability and reproducibility), positive coincidence rate, negative coincidence rate, limit of detection, hook effect, interference and cross reactivity (analysis specificity) and clinical evaluation all meet the design requirements, clinical needs and comply with WHO requirements.

Appendix

Main Raw Materials

	Control Line		Test Line	
	Capture antibody	Detection antibody	Capture antibody	Detection antibody
Name	Goat anti-mouse IgG	Mouse IgG	Anti-SARS-CoV-2 N Protein(4H2G1), mAb,Mouse	Anti-SARS-CoV-2 N protein(BN-M015), mAb,Mouse
Soruce	Goat	Mouse	Mouse	Mouse
Purification	ProteinA/G affinity column	ProteinA/G affinity column	Affinity purification chromation	Affinity purification chromation