GenBody Inc.
(주)젠바디

Technical File

Performance Evaluation

Product: **GenBody COVID-19 Ag**

Date: **2020-12-01**

Prepared by / date	Reviewed by / date	Approved by / date
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2020.12.01	2020.12.01	2020.12.01

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0. Revision history

Revision History		
Rev. O	2020. 06. 02	Release of the performance evaluation result for the GenBody COVID-19 Ag
Rev. 1	2020. 06. 15	Updated information
Rev. 1.1	2020. 09. 30	Updated information
Rev. 1.2	2020. 11. 12	Updated information
Rev. 2	2020. 12. 01	Updated information

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1 Performance Evaluation Plan

1.1 Purpose

To confirm the performance and effectiveness of GenBody COVID-19 Ag through the performance evaluation test and clinical trial designed with reference to the CLSI guideline

1.2 Responsibility

- Test specialist name: Seo seul ki at the GenBody Biotech Institute
- Team leader/first reviewer: Jedae Moon at the GenBody Biotech Institute.

1.3 Test guidance / regulation documents

- GenBody Inc.'s performance evaluation test guide document for diagnostic kit
- European harmonised standard EN13612:2002 and EN23640:2015,
- NCCLS (EP17-A2, EP06-A, EP07-A2, MM17-A, EP05-A3, EP12-A2, EP10-A3, EP09-A2)

1.4 Information of the test diagnostic kit

- Kit name: GenBody COVID-19 Ag
- Catalog No.: COVAG025
- Batch No: 3 Lots (FMFOS25201, FMFOS25202, FMFOS25203)

1.5 Intended use

GenBody COVID-19 Ag kit is an immunochromatographic assay for the qualitative detection of SARS-CoV-2 antigen in nasopharyngeal and oropharyngeal swab from human.

1.6 Information of instruments

Not applicable

1.7 Information of specimen

Human nasopharyngeal swab and oropharyngeal swab

1.8 Test Design

Test Item		Reference for Test Method
Analytical Sensitivity	Limit of Detection	EP17-A2
Analytical Specificity	Cross Reactivity	EP07-A2
	Substance	MM17-A
Interfering substance		EP07-A2
Whole system failure rate		EP05-A3
Precision assay		EP05-A3
Reproducibility assay	Inter-Operator	EP05-A3
	Intra-Instrument	EP05-A3
	Inter-batch	EP05-A3
Clinical evaluation	Diagnostic sensitivity	EP12-A2
	Diagnostic specificity	EP12-A2

2 Analytical performance evaluation

2.1 Analytical Sensitivity / LoD

The Limit of Detection (LoD) of the GenBody Ag kit was determined using serial dilutions of the heat-inactivated SARS-CoV-2 (USA-WA1/2020). The limiting dilutions were prepared in viral transport media as shown in the table below according to the following procedure.

Details about Cut-off values and LoD setting in this study

GenBody COVID-19 Ag is a qulitative analysis reagent, and since it was performed by visual reading during the LoD test, the **LoD point and the cutoff value are set identically.**

Detail explanation)

To find LoD, The first step is find the numerical value of LoB. Since our kit is interpret with naked eyes, there is no ways to calculate a numerical value for the LoB and this leads to can not calculate the LoD and LoQ .

LoD was set as the minimum point at which a positive results was observed with the naked eye (i.e. cut-off value) by serial dilution of positive standard material.

2.1.1 Protocols

- Material: SARS-CoV-2 Iisolate: USA-WA1/2020) Culture Fluid (Heat inactivated) (Zeptomatrix corp.). Lot No. 324163.

No	Serial dilution	Titer
P0 (stock)	1/1x	3.55.E+05
P1	1/300x	1.18.E+03
P2	1/400x	8.88.E+02
P3	1/500x	7.10.E+02
P4	1/600x	5.92.E+02
P5	1/700x	5.07.E+02
P6	1/800x	4.44.E+02

- Method: serial dilution of material spiked in matrix

- No. of tests: 20 times per sample - Test Kit: GenBody COVID-19 Ag - Protocol: According to the manual of GenBody COVID-19 Ag, as blow;
  Dilutions of the heat-inactivated virus were prepared in VTM in triplicate, and 50 μL of each dilution was added to a swab provided in the GenBody Ag kit. The swabs were then inserted into appropriate collection tubes containing 400 μL of GenBody extraction solution. In the next step, 100 μL of the diluent was added to the device and results were recorded after 15-20 minutes.

- Acceptance criteria:
  The lowest concentration at which 19 out of 20 replicates (i.e., more that 95% agreements) revealed a positive band in the T line was selected for LoD.

- Result analysis

Sample		P1	P2	P3	P4	P5	P6
Number of repeats	1	Pos	Pos	Pos	Pos	Pos	Pos
	2	Pos	Pos	Pos	Pos	Pos	Pos
	3	Pos	Pos	Pos	Pos	Pos	Pos
	4	Pos	Pos	Pos	Pos	Pos	Pos
	5	Pos	Pos	Pos	Pos	Pos	Pos
	6	Pos	Pos	Pos	Pos	Pos	Pos
	7	Pos	Pos	Pos	Pos	Pos	Pos
	8	Pos	Pos	Pos	Pos	Pos	Neg
	9	Pos	Pos	Pos	Pos	Pos	Pos
	10	Pos	Pos	Pos	Pos	Pos	Pos
	11	Pos	Pos	Pos	Pos	Pos	Pos
	12	Pos	Pos	Pos	Pos	Pos	Pos
	13	Pos	Pos	Pos	Pos	Pos	Neg
	14	Pos	Pos	Pos	Pos	Pos	Pos
	15	Pos	Pos	Pos	Pos	Pos	Pos
	16	Pos	Pos	Pos	Pos	Pos	Pos
	17	Pos	Pos	Pos	Pos	Pos	Pos
	18	Pos	Pos	Pos	Pos	Pos	Pos

	19	Pos	Pos	Pos	Pos	Pos	Pos
	20	Pos	Pos	Pos	Pos	Pos	Pos

Pos: positivie result, Neg: negative result

Sample	Serial dliution	Agreement to expected result	Titer
P1	1/300x	20/20 100%	1.18.E+03
P2	1/400x	20/20 100%	8.88.E+02
P3	1/500x	20/20 100%	7.10.E+02
P4	1/600x	20/20 100%	5.92.E+02
P5	1/700x	**20/20 100%**	5.07.E+02
P6	1/800x	18/20 90%	4.44.E+02

2.1.2 Conclusion

- The GenBody Ag kit Limit of Detection was confirmed by testing the selected dilution (P5, 5.07x 10^2 $TCID_{50}$/ml) in 20 replicates

2.2 Additional Analytical Sensitivity / Hook effect (prozone effect)

High dose Hook Effect or Prozone Effect on GenBody COVID-19 Ag kit was examined using increasing levels of inactivated SARS-CoV-2 (ZeptoMetrix). 2-fold dilutions of the heat inactivated virus were prepared in triplicate. No evidence of high hook effect was observed up to 1.15 x 10^7 $TCID_{50}$/ml of inactivated SARS-CoV-2.

2.2.1 Protocols

- Material:

Sort	Product name	Concentration	Abbreviation

Positive material	SARS-CoV-2 isolate: USA-WA1/2020) Culture Fluid (Heat inactivated), #0810587CFHI	Low (2.5x LoD)	P6

- Method: serial dilution of material spiked in matrix

No	Serial dilution	Titer ($TCID_{50}$/ml)
P1-H (stock)	1/1x	1.15×10^7
P2-H	1/2x	5.75×10^6
P3-H	1/4x	2.86×10^6
P4-H	1/8x	1.43×10^6
P5-H	1/16x	7.15×10^5
P6-H	1/32x	3.59×10^5
P7-H	1/64x	1.80×10^5

- No. of tests: 2 repeats per sample
- Test Kit: GenBody COVID-19 Ag
- Protocol: followed by the manual of GenBody COVID-19 Ag
- Criteria of Hook effect: If intensity of the test was not further increased (= reaching to plateau point), specify the interval as the hook effect point.
- Result

Sample		P1-H	P2-H	P3-H	P4-H	P5-H	P6-H	P7-H
Number of repeats	1	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	2	Pos	Pos	Pos	Pos	Pos	Pos	Pos
Plateau effect		Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed

Pos: positive result, Neg: negative result

2.3 Analytical Specificity /Analytical Specificity(Interfering substances testing)

The cross reactivity of GenBody COVID-19 Ag kit with a list of potential interfering substances was evaluated. Each substance was tested in triplicate in the absence and presence of inactivated SARS-CoV-2 virus at the LoD level (2.5x LoD, $7.18*10^3$ $TCID_{50}$/ml). The GenBody COVID-19 Ag kit protocol was precisely followed to test each substance. Each agent was added to a swab provided by the kit and spiked into 400 µL of the GenBody extraction solution. Then 4 drops (~100 µL) of the prep was introduced to the Antigen device. To test

the interfering substance cross reactivity in the presence of SARS-CoV-2, inactivated virus at the 2.5x LoD concentration was added to the swab at the same time as each substance.

2.3.1 Protocols - Material:

Sort	Product name	Concentration	Titer
Positive material	SARS-CoV-2 isolate: USA-WA1/2020) Culture Fluid (Heat inactivated), #0810587CFHI	Low (2.5x LoD)	$7.18*10^3$ $TCID_{50}$/ml
Negative material	Extraction solution	N/A	N/A

- Method: Material spiked in matrix
- No. of tests: Triplecate per sample
- Test Kit: GenBody COVID-19 Ag (Lot No.: FMFOS25201)
- Protocol: followed by the manual of GenBody COVID-19 Ag
- Test guidance: Interference Testing in Clinical Chemistry ; Approved Guideline-Second Edition, EP07-A2, NCCLS

2.3.2 Results

Interfering Substance	Concentration (mg/dL)	+SARSCoV-2			-SARS-CoV-2		
Viral Transport Medium (VTM)	50%	+	+	+	-	-	-
Whole blood	5%	+	+	+	-	-	-
NasoGEL (NeilMed)	5% v/v	+	+	+	-	-	-
Phenylephrine (Nasal Drop)	10% v/v	+	+	+	-	-	-
Acetylsalicylic acid	20 mg/ml	+	+	+	-	-	-
Beclomethasone	0.5 mg/ml	+	+	+	-	-	-
Benzocaine (Vicks)	5%	+	+	+	-	-	-
Flunisolide	3 mg/ml	+	+	+	-	-	-
Guaiacol glyceryl ether	20 mg/ml	+	+	+	-	-	-
Menthol	10 mg/ ml	+	+	+	-	-	-

Oxymetazoline (Afrin)	15% v/v	+	+	+	-	-	-
Tobramycin	40 mg/ml	+	+	+	-	-	-
Zanamivir	3.3 mg/ml	+	+	+	-	-	-
Oseltamivir phosphate (Tamiflu)	12 mg/mL	+	+	+	-	-	-
Cromolyn (Nasal Spray)	40 mg/ ml	+	+	+	-	-	-
Homeopathic (Alkalol)	5% v/v	+	+	+	-	-	-
Zicam Cold Remedy	5% v/v	+	+	+	-	-	-
mucous	35%	+	+	+	-	-	-

+: Positive signal -: Negative signal

2.3.3 Conclusion

No endogenous interference or cross reactivity with the GenBody COVID-19 Ag test

device was observed among the substances used for this study.

2.4 Cross-reactivity

A cross reactivity evaluation between GenBody COVID-19 Ag kit and a broad range of high prevalence respiratory pathogens and normal flora agents that might coexist with SARSCoV-2 virus in a pooled nasopharyngeal (NP) swab was performed. The final concentration of each organism is documented in the table below. Furthermore, the Human Nasal Matrix (HNM) was tested as a negative matrix. Each microorganism and HNM was tested in triplicate in the absence and presence of inactivated SARS-CoV-2 virus at the LoD level (2.5x LoD, $7.18*10^3$ $TCID_{50}$/ml). The GenBody COVID-19 Ag kit protocol was precisely followed to test each microorganism. Each microbial agent was added to a swab provided by the kit and spiked into 400 µL of the extraction solution. Then 4 drops (~100 µL) of the prep was introduced to the Antigen device, per the IFU instructions. To test the microbial cross reactivity in the presence of SARS-CoV-2, inactivated virus at the LoD concentration was added to the swab at the same time as each microbial agent (or negative matrix).

2.4.1 Protocols

- Material: described in above.
- No. of tests: Triplecate per sample
- Test Kit: GenBody COVID-19 Ag
- Protocol: followed by the manual of GenBody COVID-19 Ag
- Test result: No cross reactivity was observed among all tested microorganisms with the mouse Anti-SARS-CoV-2 NP monoclonal antibody present in the test device.

2.4.2 Result

Microorganism	Concentration	+SARSCoV-2			-SARSCoV-2		
Adenovirus (e.g. C1 Ad. 71) - Type 7A	1.41 x 10^5 $TCID_{50}$/mL	+	+	+	-	-	-

Enterovirus (e.g. EV68)	5.01×10^5 $TCID_{50}$/mL	+	+	+	-	-	-
Human Metapneumovirus (hMPV)	3.80×10^6 $TCID_{50}$/mL	+	+	+	-	-	-

Influenza A H1N1 (New Cal/20/99)	1.15×10^7 $TCID_{50}$/mL	+	+	+	-	-	-
Influenza B (Florida/02/06)	1.41×10^5 $TCID_{50}$/mL	+	+	+	-	-	-
Parainfluenza virus 1	9.12×10^8 $TCID_{50}$/mL	+	+	+	-	-	-
Parainfluenza virus 2	4.17×10^5 $TCID_{50}$/mL	+	+	+	-	-	-
Parainfluenza virus 3	6.61×10^6 $TCID_{50}$/mL	+	+	+	-	-	-
Parainfluenza virus 4A	$1 \times 10^{6.58}$ $TCID_{50}$/mL	+	+	+	-	-	-
Respiratory syncytial virus -Type A	3.80×10^6 $TCID_{50}$/mL	+	+	+	-	-	-
Rhinovirus (Type 1A)	$1 \times 10^{6.58}$ $TCID_{50}$/mL	+	+	+	-	-	-
Bordetella pertussis	1.13×10^{10} CFU/mL	+	+	+	-	-	-
Candida albicans	6.27×10^8 CFU/mL	+	+	+	-	-	-
Chlamydia pneumoniae	2.12×10^8 IFU/mL	+	+	+	-	-	-
Haemophilus influenzae	5.43×10^8 CFU/mL	+	+	+	-	-	-
Legionella pneumophila	1.63×10^{10} CFU/mL	+	+	+	-	-	-
Mycobacterium tuberculosis	6.86×10^7 CFU/mL	+	+	+	-	-	-
Mycoplasma pneumoniae	3.16×10^8 CCU/mL	+	+	+	-	-	-
Pneumocystis jirovecii (PJP) -S. cerevisiae Recombinant	3.45×10^8 CFU/mL	+	+	+	-	-	-
Pseudomonas aeruginosa	3.44×10^9 CFU/mL	+	+	+	-	-	-
Staphylococcus epidermis	9.27×10^9 CFU/mL	+	+	+	-	-	-

Streptococcus pneumoniae	4.16×10^8 CFU/mL	+	+	+	-	-	-
Streptococcus pyogenes	1.64×10^9 CFU/mL	+	+	+	-	-	-
Streptococcus salivarius	8.17×10^8 CFU/mL	+	+	+	-	-	-
MERS-coronavirus	3.55×10^5 $TCID_{50}$/mL	+	+	+	-	-	-
Human coronavirus 229E	4.17×10^5 $TCID_{50}$/mL	+	+	+	-	-	-
Human coronavirus OC43	1.26×10^6 $TCID_{50}$/mL	+	+	+	-	-	-
Human coronavirus NL63	1.41×10^5 $TCID_{50}$/mL	+	+	+	-	-	-
SARS-coronavirus (in PBS)	1×10^8 PFU/mL	+	+	+	-	-	-
SARS-coronavirus (Vero E6 Cell DMEM)	1×10^8 PFU/mL	+	+	+	-	-	-
Pooled human nasal wash	100%	+	+	+	-	-	-
Human coronavirus HKU1	N/A	Not Tested			Not Tested		

Due to the lack of availability to Human coronavirus HKU1 in Korea, *In silico* analysis was performed via the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool BLAST to investigate the potential sequence homology between SARS-CoV-2 and HKU1 nucleocapsid phosphoproteins. As shown below, the comparison analysis revealed a 36% homology across 82% of the sequences tested, thus the cross reactivity cannot be ruled out.

Microorganism	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Human coronavirus HKU1	197	197	82%	9e-63	36.74%	Query_ 54967

2.5 Whole System Failure

2.5.1 Protocols

- Test purpose: Conducting a Reliability Demonstration Test

- Material: each sample spiked in matrix. Samples concentration below under table.

Sort	Product name	Concentration	Titer
Positive material	SARS-CoV-2 isolate: USA-WA1/2020) Culture Fluid (Heat inactivated), #0810587CFHI	Low (2.5x LoD)	$7.18*10^3$ $TCID_{50}$/ml
Negative material	Extraction solution	N/A	N/A

- No. of Tests: signal per run, 100 tests of each
- Test Kit: GenBody COVID-19 Ag (Lot No.:FMOS25201)
- Protocol: Followed by GenBody COVID-19 Ag manual

2.5.2 Results

- Whole System Failure: Results was determined within intensity
- Whole system Faliure rate= 0% (False negative (or positive) detection number 0/100 tests)

Sample name	Tests(n)	False positive (n)	False nagative (n)
Nagative material	100	0	
Positive material	100		0

2.5.3 Conclusion

No abnormality was confirmed within test.

2.6 Precision assay

2.6.1 Protocols

- Material:

Product name	GenBody COVID-19 Ag
Manufacturer	GenBody Inc.
Cat. No/ Lot. No	COVAG025/ FMFOS25201

Sort	Product name	Concentration	Abbreviation	Titer ($TCID_{50}$/ml)
Positive material	SARS-CoV-2 isolate: USA-WA1/2020) Culture Fluid (Heat inactivated), #0810587CFHI	High (40x LoD)	P1`	$1.15* 10^5$
		Moderate (10x LoD)	P2`	$2.87 * 10^4$
		Low (2.5x LoD)	P3`	$7.18 * 10^3$
Negative material	Extraction solution	N/A	N	N/A

- No. of Tests: Triplicates per run, 2 run a day; 5 days
- Protocol: followed by the manual of GenBody COVID-19 Ag

2.6.2 Results

- Precision : Results was determined within intensity

Lot No. FMFOS25201										
Sample name	Day1		Day2		Day3		Day4		Day5	
Repeat	1	2	1	2	1	2	1	2	1	2
NC	-	-	-	-	-	-	-	-	-	-
P1`	+	+	+	+	+	+	+	+	+	+
P2`	+	+	+	+	+	+	+	+	+	+
P3`	+	+	+	+	+	+	+	+	+	+

Lot No. FMFOS25202										
Sample name	Day1		Day2		Day3		Day4		Day5	
Repeat	1	2	1	2	1	2	1	2	1	2
NC	-	-	-	-	-	-	-	-	-	-
P1`	+	+	+	+	+	+	+	+	+	+
P2`	+	+	+	+	+	+	+	+	+	+

P3`	+	+	+	+	+	+	+	+	+	+

Lot No. FMFOS25203										
Sample name	Day1		Day2		Day3		Day4		Day5	
Repeat	1	2	1	2	1	2	1	2	1	2
NC	-	-	-	-	-	-	-	-	-	-
P1`	+	+	+	+	+	+	+	+	+	+
P2`	+	+	+	+	+	+	+	+	+	+
P3`	+	+	+	+	+	+	+	+	+	+

Positive signal: + Negative signal: -

2.7 Reproducibility / Inter-Operator

- Material

Product name	GenBody COVID-19 Ag
Manufacturer	GenBody Inc.
Cat. No/ Lot. No	COVAG025/ FMFOS25201

Sort	Product name	Concentration	Abbreviation	Titer ($TCID_{50}$/ml)
Positive material	SARS-CoV-2 isolate: USA-WA1/2020) Culture Fluid (Heat inactivated), #0810587CFHI	High (40x LoD)	P1`	$1.15* 10^5$
		Moderate (10x LoD)	P2`	$2.87 * 10^4$
		Low (2.5x LoD)	P3`	$7.18 * 10^3$
Negative material	Extraction solution	N/A	N	N/A

- No. of Tests: Duplicates per run, 2 run a day; 5 days
- Protocol: followed by the manual of GenBody COVID-19 Ag

2.7.1 Test Result

- Results was determined within intensity

Day 1				
Sample name	Operator 1		Operator 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Day 2				
Sample name	Operator 1		Operator 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Day 3				
Sample name	Operator 1		Operator 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Day 4		
Sample name	Operator 1	Operator 2

Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Day 5				
Sample name	Operator 1		Operator 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Positive signal: + Negative signal: -

2.8 Reproducibility / Inter-site

2.8.1 Protocols

- Material:

Product name	GenBody COVID-19 Ag
Manufacturer	GenBody Inc.
Cat. No/ Lot. No	COVAG025/ FMFOS20201

Sort	Product name	Concentration	Abbreviation	Titer ($TCID_{50}$/ml)
Positive material	SARS-CoV-2 isolate: USA-WA1/2020) Culture Fluid (Heat inactivated), #0810587CFHI	High (40x LoD)	P1`	$1.15* 10^5$
		Moderate (10x LoD)	P2`	$2.87 * 10^4$

		Low (2.5x LoD)	P3`	$7.18 * 10^3$
Negative material	Extraction solution	N/A	N	N/A

- No. of Tests: Duplicates per run, 2 run a day; 5 days
- Protocol: followed by the manual of GenBody COVID-19 Ag

2.8.2 Test Result

- Results was determined within intensity

Day 1				
Sample name	Site 1		Site 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Day 2				
Sample name	Site 1		Site 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Day 3				
Sample name	Site 1		Site 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Day 4				
Sample name	Site 1		Site 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Day 5				
Sample name	Site 1		Site 2	
Repeat	1	2	1	2
NC	-	-	-	-
P1`	+	+	+	+
P2`	+	+	+	+
P3`	+	+	+	+

Positive Signal: + Negative signal: -

2.9 Reproducibility / Total analysis

Item	Results
With-in run	Confirmed
With-in day	Confirmed
Between run	Confirmed
Inter-Operator	Confirmed
Inter-batch	Confirmed
Inter-site	Confirmed

2.10 Studies to support Point of Care claim

In the effort to begin demonstration of test efficacy in a non-professional setting and with an eye towards eventual PoC claim, a variety of studies were performed as described below.

(1) Materials

The substances listed below were tested using the GenBody COVID-19 Ag Kit (lot No.: FMFOS25201).

Sort	Product name	Concentration	Abbreviation
Positive material	SARS-CoV-2 isolate: USA-WA1/2020) Culture Fluid (Heat inactivated), #0810587CFHI	Low (2.5x LoD)	P6
Negative material	Extraction solution	N/A	N

(2) Objects and procedures:

For each step outlined in the Instructions for Use (IFU), we have determined potential deviations that may arise in a non-professional setting as below. Test performance was assessed after these deviations were introduced.

IFU Step	Description	Potential User Deviation with Study Objective to Support Point of Care Claim
Non-IFU Step	In addition to deviations from the individual instructions, it was also hypothesized that drop volume could differ significantly from user to user. A study was performed to assess the variability of drop volume with results as below.	
1	Place the device on a flat surface	A non-flat surface may be employed. -Testing of this deviation is described in the 'inverted stability' section above
2	Add the Extraction Solution to the Fill line indicated on the Extraction Tube (~400µL).	A volume significantly disparate from 400 µL may be utilized. - Testing was performed with multiple alternative volumes as described below.
3	Insert the nasopharyngeal swab samples into the extraction solution, mix by squeezing the tube and simultaneously rotating the swab 8 - 10 times.	The extraction solution may not be mixed as described per the IFU. - Testing was performed to determine device performance with various degrees of mixing as described below.
4	Add 4 drops (~100 µL) of the solution to the center of the sample well of the test device	A volume significantly disparate from 100 µL may be introduced into the sample well. -Testing was performed with multiple samples well volumes and described below
5	Read the test between 15 - 20 minutes after the addition of the sample.	Test interpretation may be performed at a time other than the recommended 15-20 minutes -Testing was performed at a variety of time elapsed points as described below

- Designation of the results: +/-; +: positive, -: negative
- Acceptance criteria: When the control line appears, the positive standard should be positive and the negative standard should be negative in all test results.

(3) Results

A. Non-IFU Step

For each drop number, the precise volume was calculated and averaged over 5 repetitions by 5 individuals. The average volume per drop of Extraction Solution is reported.

Drop Count	Average volume per drop in 5 replicates/individual (μL)					Average Volume Per Individual (μL)	Coefficient of Variation (%)	Average Volume Per Drop
	5 Individuals							
	1	2	3	4	5			
1 drop	23.80	23.60	24.00	24.00	22.60	23.60	5.62	23.60
2 drops	49.00	47.00	49.20	48.60	50.40	48.84	4.32	24.42
3 drops	75.40	68.20	74.60	76.40	76.80	74.28	4.87	24.76
4 drops	97.40	94.20	99.40	100.40	102.20	98.72	4.94	24.68
5 drops	124.80	122.60	124.80	124.40	127.00	124.72	2.36	24.94
6 drops	156.40	145.00	151.40	150.60	153.20	151.32	3.17	25.22
Total Mean Value							2.28	24.60

Conclusion

The average volume of extraction solution per drop was 24.60 μL, and the coefficient of variation of each drop was 2.28% from 5 individuals.

B. IFU Step #1. Place the device on a flat surface

Please see Section 2.11 above for 'Inverted Stability' study, which concludes this section of the study.

C. IFU Step #2. Add the Extraction Solution to the Fill line indicated on the Extraction Tube (~400 μL).

It was hypothesized that different users may add variable volumes of Buffer to the extraction tube. Thus, device performance was assessed using different buffer volumes with results as below.

Buffer volume (µL)	Substance to be tested: P6 (Titer: 2.5x LoD)					Agreement to Expected Results
	Replicates					
	1	2	3	4	5	
300	Positive	Positive	Positive	Positive	Positive	100%
350	Positive	Positive	Positive	Positive	Positive	100%
400	Positive	Positive	Positive	Positive	Positive	100%
450	Positive	Positive	Positive	Positive	Positive	100%
500	Positive	Positive	Positive	Positive	Positive	100%

Conclusion

No change in performance efficacy was observed despite departure of buffer volume from the recommended 400 µL within a range of 300 to 500 µL.

D. Insert the nasopharyngeal swab samples into the extraction solution, mix by squeezing the tube and simultaneously rotating the swab 8 - 10 times.

It was hypothesized that different users may not adequately mix the solution as directed. Thus, device performance was assessed with varying numbers of rotations.

Number of swab rotations	Substance to be tested: P6 (Titer: 2.5x LoD)					Agreement to expected Results
	Replicates					
	1	2	3	4	5	
2	Positive	Positive	Negative	Negative	Negative	40%
4	Positive	Positive	Positive	Positive	Positive	100%
6	Positive	Positive	Positive	Positive	Positive	100%
8	Positive	Positive	Positive	Positive	Positive	100%
10	Positive	Positive	Positive	Positive	Positive	100%

Conclusion

Test performance was maintained with varying degrees of swab rotations unless the swab was rotated fewer than 4 times.

E. Add 4 drops (~100 µL) of the solution to the center of the sample well of the test device

It was hypothesized that users may add a variable number of drops of the solution mixture to the test well. Thus, device performance was assessed after varying numbers of drops were added to the test well.

Drops Count	Substance to be tested: P6 (Titer: 2.5x LoD) Replicates					Agreement to expected Results
	1	2	3	4	5	
1 drop	Invalid	Invalid	Invalid	Invalid	Invalid	0%
2 drops	Positive	Positive	Positive	Positive	Positive	100%
3 drops	Positive	Positive	Positive	Positive	Positive	100%
4 drops	Positive	Positive	Positive	Positive	Positive	100%
5 drops	Positive	Positive	Positive	Positive	Positive	100%
6 drops	Positive	Positive	Positive	Positive	Positive	100%

Conclusion

When 2-6 drops of extraction mixture were added to the test well, no change in performance efficacy was observed. However, when 1 drop of the extraction mixture was added to the test well, the volume was insufficient to migrate to the test line, and the test was ineffective.

F. Read the test between 15 - 20 minutes after the addition of the sample.

It was hypothesized that test interpretation may be performed at a time other than the recommended 15-20 minutes. Thus, test interpretation was performed at a variety of time elapsed points as described below

Elapsed Time (minutes)	Substance to be tested: P6 (Titer: 2.5x LoD) and N (extraction solution)		
	Results window situation	Agreement to expected results in 5 replicates	
		P3	N
0	Unreadable due to uncleared background	Not applicable	
5	Uncleared Background is remained but readable	Positive (5/5) 100%	Negative (5/5) 100%

10	Some of the background is not clean. Easier to read compared to 5 minutes.	Positive (5/5) 100%	Negative (5/5) 100%
15	Accurate reading is possible without any interference	Positive (5/5) 100%	Negative (5/5) 100%
20		Positive (5/5) 100%	Negative (5/5) 100%
25	No problem in reading the results, but drying of membrane starts.	Positive (5/5) 100%	Negative (5/5) 100%
30		Positive (5/5) 100%	Negative (5/5) 100%
60		Positive (5/5) 100%	Negative (5/5) 100%
120	Background occurs again due to backflow. Reading the result itself is possible.	Positive (5/5) 100%	Negative (5/5) 100%

Conclusion

Between 5-15 minutes, a test line appeared as expected, though the background was noted to appear hazy. It was felt that this could possibly raise the risk of inaccurate interpretation. The likelihood of an accurate reading is felt to be maximized between 15-20 minutes. Beyond 20 minutes, the membrane continues to dry. Once again, a visible test line remained apparent, but the likelihood of an inaccurate reading is thought to be more likely beyond 25 minutes.

3 Clinical Evaluation/Diagnostic sensitivity & specificity

3.1 Test Protocol

-Study methood

Tests were performed according to instruction for use of 'GenBody COVID-19 Ag Test' with residual nasopharyngeal swab (NPS)s in VTM (Viral Transport Medium) from 3 different sites (2 in Republic of Korea and 1 in United States). Total amount of tested specimen were 141 positive patients confirmed by real-time PCR method and 365 negative patients confirmed by real-time PCR.

(1) Model name/manufacturer for VTM: ESwapTM 482C (COAPN Diagnotic Inc) T-SWAB TRANSPORTTM CTM (Noble Bio)

(2) Real-time PCR:

a) Conducted in Korea: Allplex 2019-nCoV assay (Seegene Inc, EUA approved) b) Conducted in U.S.:

1) Thermo Fisher Scientific TaqPath COVID-19 Combo Kit
2) CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

(3) Storage temperature for the specimens in VTM: stored at -70℃.

- Material: 506 clinical samples (141 positive specimens, 365 negative specimens/ see under each result). 495 samples were confirmed to be ≤ 30 Ct with Real-Time PCR, and 11 samples were over 30 Ct.
- No. of Tests: Single test per sample
- Test Kit: GenBody COVID-19 Ag
- Protocol: Follow GenBody COVID-19 Ag manual
- Test guidance: User Protocol for Evaluation Qualitative Test Performance ; Approved Guideline, EP12-A2, NCCLS
- Result analysis: Each target in positive samples were tested Real-Time PCR kit
- * User Protocol for Evaluation Qualitative Test Performance; Approved Guideline, EP12-A2, NCCLS

3.2 Results

1) Primary end point

CT ≤ 30		Real-time RTPCR		Total
		Positive	Negative	
GenBody COVID-19 Ag Test	Positive	122	3	125
	Negative	4	366	370
Total		126	369	495

-Positive Percent Agreement:

1) CT ≤ 30: 96.83% (95% CI: 92.07% to 99.13%)

2) CT > 30: Less than 50%

-Negative Percent Agreement: 99.18% (95% CI: 97.25% to 99.70%)

-Positive Predictive Value: 96.83% (95% CI: 92.00% to 98.78)

-Negative Predictive Value: 98.92% (95% CI: 97.21% to 99.59%)

2) Secondary end point

Days Post symptom onset	Asymptomatic	Day 1~6	Day 7
Positive	5	48	24
Negative	1	4	3
total	6	52	27

-Positive Percent Agreement

1) Asymptomatic: 83.3%

2) After 1~6 days post symptom onset: 92.31%

3) After 7 days post symptom onset: 88.89%

The clinical evaluation for the GenBody COVID-19 Ag Test for rapid detection of SARS-CoV-2 antigen was conducted at the Medical Center in South Korea or CMB labs in United States. Total 506 residual and selected specimens in VTM from persons, who were hospitalized or visited, were tested with comparing to commercialized molecular assays.

The GenBody COVID-19 Ag Test showed 96.83% of sensitivity and 99.18% of specificity.

4 Performance claim in IFU

Sample type: *Nasopharyngeal swab from human*