Chapter 7. Performance Evaluation

GenBody Inc.
(주)젠바디

Technical File

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. This device is intended to be used on patient within 7 days of symptom onset. This device is intended to be used by a healthcare professional.

1.6 Information of instruments

Confiscope G20

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
	Inter-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 cut-off 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.)

No	Serial dilution	Titer
P0 (stock)	1/1x	$3.55 * 10^5$
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	Neg
	2	Pos	Pos	Pos	Pos	Pos	Neg
	3	Pos	Pos	Pos	Pos	Pos	Neg
	4	Pos	Pos	Pos	Pos	Pos	Neg
	5	Pos	Pos	Pos	Pos	Pos	Neg
	6	Pos	Pos	Pos	Pos	Pos	Neg
	7	Pos	Pos	Pos	Pos	Pos	Neg
	8	Pos	Pos	Pos	Pos	Pos	Neg
	9	Pos	Pos	Pos	Pos	Pos	Neg
	10	Pos	Pos	Pos	Pos	Pos	Neg
	11	Pos	Pos	Pos	Pos	Pos	Neg
	12	Pos	Pos	Pos	Pos	Pos	Neg
	13	Pos	Pos	Pos	Pos	Pos	Neg
	14	Pos	Pos	Pos	Pos	Pos	Neg
	15	Pos	Pos	Pos	Pos	Pos	Neg
	16	Pos	Pos	Pos	Pos	Pos	Neg
	17	Pos	Pos	Pos	Pos	Pos	Neg
	18	Pos	Pos	Pos	Pos	Pos	Neg
	19	Pos	Pos	Pos	Pos	Pos	Neg
	20	Pos	Pos	Pos	Pos	Pos	Neg

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	0/20 0%	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	1.15 x 10^7 $TCID_{50}$/ml	P1-H (Stock)

- Method: serial dilution of material spiked in matrix

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

of repeats	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: positivie result, Neg: negative result

- Conclusion

High dose Hook Effect or Prozone Effect on GenBody COVID-19 Ag kit was examined using increasing levels of inactivated SARS-CoV-2 up to 1.15 x 10^7 $TCID_{50}$/ml. 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.

2.3 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)	+SARS-CoV-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 SARS-CoV-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	+SARS-CoV-2			-SARS-CoV-2		
Adenovirus (e.g. C1 Ad. 71) - Type 7A	1.41×10^5 $TCID_{50}$/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 belew 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: singal 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 nagative (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, FMFOS25202, FMFOS25203

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	P2`	$2.87 * 10^4$

		(10x LoD)		
		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 Matrix equivalency for collection media

2.10.1 Description

Equivalence between transport media (cf., collection media) was evaluated using cultured virus (USA-WA1 /2020) spiked into nasopharyngeal swabs to prepare contrived low positive (2.5x LoD) to high positive (40x LoD) sample for each collection media. Each transport media was compared with GenBody extraction solution, which is considered to be as a positve control according to previous results.

2.10.2 Protocols

- Material:
  The substances listed below were tested using the GenBody COVID-19 Ag Kit (Lot No.: 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`	$1.15* 10^5$
		Low (2.5x LoD)	P3`	$7.18 * 10^3$
Negative material	Extraction solution	N/A	N	N/A

Sort	Product name	Manufacturer	Cat. No.	Abbreviation
Reference (Positive control)	Extraction solution	GenBody Inc.	N/A	T1
UTM	BD universal viral transport (UVT)	BD Diagnostics, *USA*	220220	T2
UTM	REST™ CTM (Clinical Virus Transport Medium)	NobleBio, *Korea*	UTM-001B	T3
UTM	Asan Transport Medium (II)	Asan Pharm, *Korea*	AM608-09	T4
UTM	UTM-RT	Copan, *Italy*	330C.DHI	T5
UTM	Universal Transport media	HAN CHANG MEDIC, *Korea*	HC-UTM	T6
NAP	eNAT medium	Copan, *Italy*	608CS01M	T7
NAP	NAPT midium	NobleBio, *Korea*	NATC-NFS1-N1P	T8

*UTM: Universal transport media,
NAP: Nucleic Acid Preservation Media (Transport media for Nucleic Acid Amplification test, also know as

NAAT media)

- Method: Material spiked in matrix (extraction solution and each collection media)
- No. of tests: (5 repititions per samples) per matrix
- Test Kit: GenBody COVID-19 Ag (Lot No.: FMFOS25201)
  Protocol: Dilutions of the heat-inactivated virus were prepared in various media described in below table. 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.
- Test guidance: Interference Testing in Clinical Chemistry ; Approved Guideline-Second Edition, EP07-A2, NCCLS.
- Acceptance criteria: Select virus collection media that correlated the positive control (i.e., Diluted in GenBody extraction solution) in 5 repititions.

2.10.3 Results

Equivalency		Standard materials			Agreements to expected results
		P1	P3	N	
Matrix	T1	Positive (5/5)	Positive (5/5)	Negative (0/5)	References
	T2	Positive (5/5)	Positive (5/5)	Negative (0/5)	Positive: (10/10), 100% Negative: (0/5), 100%
	T3	Positive (5/5)	Positive (5/5)	Negative (0/5)	Positive: (10/10), 100% Negative: (0/5), 100%
	T4	Positive (5/5)	Positive (5/5)	Negative (0/5)	Positive: (10/10), 100% Negative: (0/5), 100%
	T5	Positive (5/5)	Positive (5/5)	Negative (0/5)	Positive: (10/10), 100% Negative: (0/5), 100%
	T6	Positive (5/5)	Positive (5/5)	Negative (0/5)	Positive: (10/10), 100% Negative: (0/5), 100%
	T7	Positive (4/5)	Positive (1/5)	Negative (0/5)	Positive: (5/10), 50% Negative: (0/5), 100%
	T8	Positive (3/5)	Positive (0/5)	Negative (0/5)	Positive: (3/10), 30% Negative: (0/5), 100%

*x/y; x: number of positive results, y: number of tests.

2.10.4 Conclusion

The performance of various virus collecting media was evaluated using the inactivated SARS-CoV-2 at 2.5x and 40x LoD concentration. It was shown that there is no difference from positive control (Diluted in GenBody extraction solution) with the other virus collecting media, except NAP Transport media. It was demonstrated that NAP transport media had occured false-negative results

in several tests, so it will recommended ‘do not use the NAP transport media‘ in IFU.
This test was performed with the exact calaulated concentration for produce the diluents, but in practical use, the dilution ratio may vary depending on the VTM brands. In this regard, it will be stated in IFU that ‘Using samples from VTM, it may cause inaccurate results due to decreasing the sensitivity of the test‘.

3 Clinical Evaluation/Diagnostic sensitivity & specificity

3.1 Test Protocol

-Study method

Tests were performed according to instruction for use of 'GenBody COVID-19 Ag' test with residual nasopharyngeal swabs in VTM (Viral Transport Medium) from 75 positive and 276 negative patients confirmed by real-time PCR method

- Material : 351 clinical samples (75 positive specimens, 276 negative specimens/ see under each result)
- 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 Evalution Qualitative Test Performance ; Approved Guideline, EP12-A2, NCCLS
- Result analysis : Each target in positive samples were tested Real-Time PCR kit
- * User Protocol for Evalution Qualitative Test Performance ; Approved Guideline, EP12-A2, NCCLS

3.2 Test Result

		Reference (Real-Time PCR)		Total
		Positive	Negative	
GenBody COVID-19 Ag	Positive	72	2	74
	Negative	3	274	277
Total		75	276	351

- Sensitivity = 96.00% (95% CI = 88.75% to 99.17%)
- Specificity = 99.28% (95% CI = 97.41% to 99.91%)

4 Performance claim in IFU

Sample type: *Nasopharyngeal swab and oropharyngeal swab from human*