Study Report of GenBody COVID-19 Ag with SARS-CoV-2 Variant (B.1.1.529)

Jan 5th, 2022

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I. Protocol Summary

Objective
To investigate the analytical sensitivity of GenBody COVID-19 Ag with SARS-CoV-2 variants (Omicron).

Applicant
Company Name: GenBody Inc.
Address: 3-18, Eopseong 2-gil, Seobuk-gu, Cheonan-si, Chungcheongnamnam-do, Republic of Korea.

Information of study site
1) Institution: Kunkuk University (Seoul, Korea)
2) Investigator: Hee-Jung Lee, Ph.D, Professor
3) Contact: ziniga@konkuk.ac.kr

Investigational device
GenBody COVID-19 Ag (Lot. No.: FVFPS26211)

Test period
Jan 4th, 2022 ~ Jan 5th, 2022

Evaluation method
Followed by the manual of GenBody COVID-19 Ag

II. Introduction

º Investigational device: GenBody COVID-19 Ag kit
º Test period: Jan 4th, 2022. ~ Jan 5th, 2022.
º Objective: To investigate the analytical sensitivity of GenBody COVID-19 Ag with SARS-CoV-2 variants (Omicron, B.1.1.529).

III. Materials and Methods

º SARS-CoV-2 virus

1) Virus: SARS-CoV-2 virus were provided by KDCA (Korean Disease Control and Prevention Agency). All the handlings and experiments with these viruses were performed in BSL3 laboratory in Konkuk University.
   *Note: Please refer Appendix 1. for more detailed information of the virus resourcing.
2) Diluent: Nasopharyngeal Clinical Matrix provided by GenBody.
   Lot Number: FMVA0321
3) Information of the SARS-CoV-2 virus.

Substance	SARS-CoV-2 (NCCP 43408) / Omicron variant / B.1.1.529 lineage
Characteristic	1) Media: DMEM + 1% penicillin-streptomycin + 2% FBS 2) Host cell: Vero E6 3) Culture conditions: 37℃, 5% CO_2 4) Virus titration: by cytopathic effects (CPE), PFU (Plaque forming unit)
Depositor and comments	1) Storage: cryotube, frozen virus with culture media (-70℃) 2) Classification: Betacoronavirus (SARS-CoV-2) 3) Isolated strain: hCoV-19/Korea/KDCA447321/2021 4) Isolation pathway: The variant virus was cell cultured and isolated from SARS-CoV-2 patient. 5) Clades: GH 6) Isolation year: 2021 7) Viral titer: 2.1 x 10^5 PFU/mL

Substance	SARS-CoV-2 (NCCP 43326), wild type, first isolated in Korea
Characteristic	1) Media: DMEM + 1% penicillin-streptomycin + 2% FBS

	2) Host cell: Vero E6 3) Culture conditions: 37℃, 5% CO_2 4) Virus titration: by cytopathic effects (CPE), PFU (Plaque forming unit)
Depositor and comments	1) Storage: cryotube, frozen virus with culture media (-70℃) 2) Classification: Betacoronavirus (SARS-CoV-2) 3) Isolated strain: 4) SARS CoV-2/human/KOR/KCDC03-NCCP43326/2020 5) Isolation pathway: The variant virus was cell cultured and isolated from SARS-CoV-2 patient who entered to Korea from China. 6) Clades: S 7) Isolation year: 2020 8) Viral titer: 1.5 x 10^6 PFU/mL

º Test Procedure

1. Preparation for the test

1) Preparation of the GenBody COVID-19 Ag kit (Lot number: FVFPS26211)

① Refer to the user manual for preparation of GenBody COVID-19 Ag kits.

② Check the components of the kit (Test Device, Extraction Solution, Extraction Tubes, Dropper Tips, Nasopharyngeal swab, Positive external control swab, Negative external control swab, Package Insert).

2) Preparation of the specimens

Frozen sample: Defrost on ice or at low temperature in advance and place the thawed sample at room temperature prior to testing (~30 minutes).

2. Description of test steps

① Dilute the virus with clinical matrix.

② Add the Extraction Solution up to the Fill line indicated on the Extraction Tube.

③ Add 50 μL of the positive sample to a swab.

④ Insert the swab samples into the Extraction Solution.

⑤ Mix by squeezing the tube and simultaneously rotating the swab 8 – 10 times.

⑥ Place the disposable dropper cap on the Extraction Tube.

⑦ Remove the foil-pouch and place Test Device on a level surface.

⑧ Add 4 drops of the solution to the center of the sample well of the Test Device.

⑨ Interpret the test results after 15 – 20 minutes.

3. Test procedure for Limit of Detection (LoD)

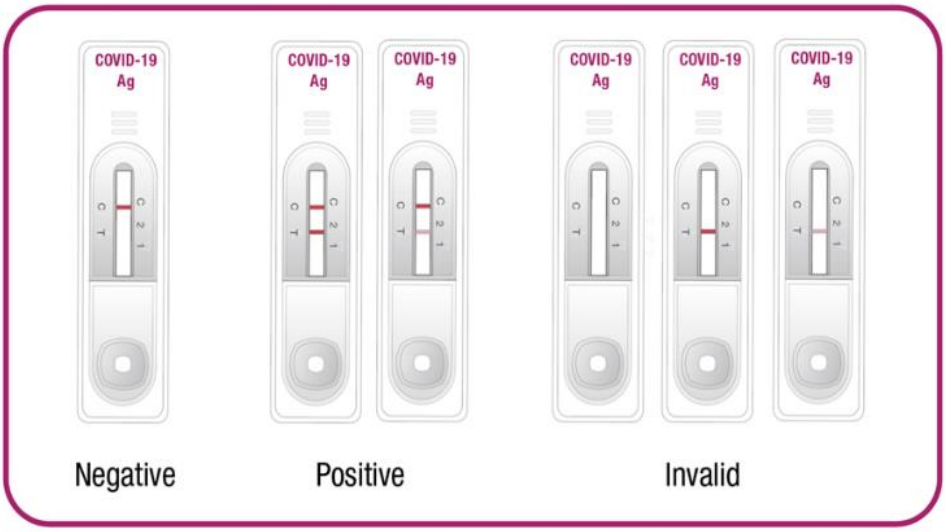
① To confirm the test kit performance (quality control), test with the positive and negative external control swabs according to the test procedure of GenBody COVID-19 Ag.

② LoD range finding: Test with 10-fold serial diluted virus, according to the 'Assay procedure' described in Package Insert. Test should be performed with at least 3 replicates. Select the above 1 point from the lowest concentration at which all 3 replicates produced positive results as 'LoD determination' step.

③ LoD Determination: Test with the virus which were 2-fold serial diluted from the selected concentration, according to the 'Assay procedure' described in Package Insert. Test should be performed with 20 replicates. Determine the lowest detectable concentration at which 19 of 20 replicates are positive.

4. Interpretation of Test Results

Followed by the manual of GenBody COVID-19 Ag



- Positive result:
  Two reddish purple lines appear in the test window, one on the test line position (T) and the other on the control line position (C).
  Note: The Test line (reddish purple line) may vary in shade and intensity (light or dark, weak or strong) depending on the concentration of antigen detected. The intensity of the Control line should not be compared to that of the Test line for the interpretation of the test result. Even a light or faint Test line should be interpreted as a positive result.

- Negative result:
  Only one reddish purple line on the control line (C) position appears with no line on the test line position (T).

- Invalid result:
  If a line does not appear on the control line position (C) in 15 minutes, the test result is invalid. Re-test with a new GenBody COVID-19 Ag Test Device.

IV. Results

1. Analytical sensitivity (LoD, detection limit) of the kit with Omicron variant (B.1.1.529 lineage).

In the previous LoD range finding step (10-fold dilution study), 2.1 x 10^3 PFU/mL was selected for further 2-fold dilution study. The 2.1 x 10^3 PFU/mL of SARS-CoV-2 (NCCP 43408, Omicron variant, B.1.1.529 lineage) was 2-fold serially diluted with the clinical matrix. These diluted solutions were tested 20 times of each, and then interpreted the results to find out the lowest level of the concentration at which 19 of 20 replicates are positive. This study showed the LoD of the kit to be 1.31 x 10^2 PFU/mL.

No.	Concentration (PFU/mL)	Replicates	Number of positive results	Positive ratio (%)
1	2.1 x 10^5	3	3	100
2	2.1 x 10^4	3	3	100
3	2.1 x 10^3	3	3	100
4	2.1 x 10^2	3	3	100
5	2.1 x 10^1	3	0	0
6	2.1 x 10^0	3	0	0

Table 1. Result of LoD range finding for Omicron variant.

No.	Concentration (PFU/mL)	Replicates	Number of positive results	Positive ratio (%)
1	2.10 x 10^3	20	20	100
2	1.05 x 10^3	20	20	100
3	5.25 x 10^2	20	20	100
4	2.63 x 10^2	20	20	100
5	1.31 x 10^2	20	20	100
6	6.56 x 10^1	20	0	0

Table 2. Result of LoD determination for Omicron variant.

2. Analytical sensitivity (LoD, detection limit) of the kit with standard strain of SARS-CoV-2 (first isolated in Korea, Feb. 2020).

In the previous LoD range finding step (10-fold dilution study), 1.5 x 10^3 PFU/mL was selected for further 2-fold dilution study. The 1.5 x 10^3 PFU/mL of SARS-CoV-2 (NCCP 43326, first isolated, Feb. 2020) was 2-fold serially diluted with the clinical matrix. These diluted solutions were tested 20 times of each, and then interpreted the results to find out the lowest level of the concentration at which 19 of 20 replicates are positive. This study showed the LoD of the kit to be 1.88 x 10^2 PFU/mL.

No.	Concentration (PFU/mL)	Replicates	Number of positive results	Positive ratio (%)
1	1.5×10^6	3	3	100
2	1.5×10^5	3	3	100
3	1.5×10^4	3	3	100
4	1.5×10^3	3	3	100
5	1.5×10^2	3	3	100
6	1.5×10^1	3	0	0
7	1.5×10^0	3	0	0

Table 3. Result of LoD range finding for standard strain in Korea (Feb. 2020).

No.	Concentration (PFU/mL)	Replicates	Number of positive results	Positive ratio (%)
1	1.50×10^3	20	20	100
2	7.50×10^2	20	20	100
3	3.75×10^2	20	20	100
4	1.88×10^2	20	20	100
5	9.38×10^1	20	8	40
6	4.69×10^1	20	0	0

Table 4. Result of LoD determination for standard strain in Korea (Feb. 2020).

V. Conclusion

This study has been carried out to identify if GenBody COVID-19 Ag kit is capable of detecting SARS-CoV-2 variants (Omicron, B.1.1.529). We have performed its evaluation study in BSL3 laboratory at Konkuk University. We have obtained SARS-CoV-2 virus from Korean NIH under their strict regulations.

In this study, the detection limit of GenBody COVID-19 Ag was 1.31×10^2 PFU/mL for Omicron variant and 1.88×10^2 PFU/ml for standard strain in Korea (Feb. 2020).

[Appendix 1]

Background

1) Service program of Korean NCCP for SARS-CoV-2 variants

Korean NCCP (National Culture Collection for Pathogens) belonged to KDCA (Korean Disease Control and Prevention Agency) started to provide SARS-CoV-2 variants to researchers who are operating BioSafety Level 3 facility (http://nccp.cdc.go.kr/main.do).

2) Regulations for handling the variants

Konkuk University has a good BSL3 laboratory which facility can satisfy all the regulations (Appendix 2. Certificates of BSL3 facility in Konkuk University). According to the law of Article 16 of the Enforcement Decree of the Pathogen Resources Act 'Safety Management Standards for Pathogen Resources', all researchers who received the pathogenic viruses must comply with the Standards of Safety Management and Rules of Laboratory Biosafety.

[Appendix 2] Certificates of BSL3 facility in Konkuk University

■ 감염병의 예방 및 관리에 관한 법률 시행규칙 [별지 제15호서식] <신설 2018. 6. 12.>

허가번호(신고확인번호) 제KCDC-HP-09-3-01호

고위험병원체 취급시설 설치·운영

[ ] 허가서
[ ] 신고확인서
[ ] 허가사항 변경허가서
[√] 허가사항 변경신고확인서
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※ [ ]에는 해당되는 곳에 √표를 합니다.

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	시설종류 [√] 일반 [ ] 대형배양 [ ] 동물이용 [ ] 곤충이용		
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	안전관리등급 생물안전 3등급		
	취급 병원체의 명칭 Avian influenza virus:AIV, Middle east respiratory syndrome coronavirus:MERS-CoV		
	허가 조건 변경신고(대표자)(최초허가 2009. 2. 10.)		

「감염병의 예방 및 관리에 관한 법률」 제23조제2항·제3항·제4항, 같은 법 시행령 제19조의2제4항·제6항, 제19조의3제2항·제5항 및 제19조의4제2항, 같은 법 시행규칙 제20조의2제2항·제4항, 제20조의3제2항·제4항 및 제20조의4제2항에 따라 위와 같이 고위험병원체 취급시설의 설치·운영에 대한

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2020년 9월 24일

질병관리청장

210mm×297㎜(백상지 120g/㎡)