BIOCREDIT COVID-19 Ag Test

: Analytical Sensitivity Report using Inactivated SARS-CoV-2 mutation

Authorization	Dept. & Name	Signature	Date
Prepared by	R & D / S. C. Kwon		2021.04.19
Reviewer	QMR / J. Han		2021.04.19
Approved by	R & D / K. M. Min		21.04.19

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1. Purpose

To assess the analytical limit of detection (LoD) of BIOCREDIT COVID-19 Ag for inactivated SARS-CoV-2 mutations.

2. Tested by/Verified by

Tested by Se-Chang Kwon (RapiGEN, Inc.)

Verified by In-Ho Park (Yonsei University College of Medicine)

3. Test Date

Apr. 15, 2021 ~ Apr. 16, 2021

4. Materials and Method

4.1. Materials

4.1.1. Test product

Two independent lot products were used in this study (Table 1).

Table 1. Test kit

Product	Lot No.	Mfg.date	Cat No
BIOCREDIT COVID-19 Ag	H073014EV	2021.03.09	G61RHA20C
	H073016EV	2021.03.11	

4.1.2. Test materials

The mutated SARS-CoV-2 viruses were provided by Korean Centers for Disease Control and Prevention (KCDC).

Table 2. Test specimen

Variants	Country identified	Clade	Conc. (PFU/ml)	Source
B.1.1.7	United Kingdom (UK)	GR	1.0×10^6	NCCP 43381 SARS-CoV-2
B.1.351	South Africa	GH	7.0×10^6	NCCP 43382 SARS-CoV-2

4.1.3. Test equipment

Table 3. Test equipment

Type	Equipment No.	Calibration date	Calibration valid date
2~20 ㎕	R-11-0030	2020.10.26	2021.10.25
10~100 ㎕	R-11-0034	2020.10.26	2021.10.25
20~200 ㎕	R-11-0033	2020.10.26	2021.10.25
200~1000 ㎕	R-11-0031	2020.10.26	2021.10.25

4.2. Method

4.2.1. Test condition

This test was conducted at Yonsei University College of Medicine, and the temperature and humidity conditions were verified using a thermo-hydrometer.

Table 4. Test condition

Test site	Temperature and humidity
Yonsei University College of Medicine	Minimum 18℃, 28% RH Maximum 20℃, 25% RH

4.2.2. Preparation of cell culture-derived virus

Virus: Two SARS-CoV-2 variants (B.1.1.7 and B.1.351) were provided from KCDC pathogen bank.

Put the cryopreserved base virus in the water bath at a temperature of 37 °C and keep it for rapid defrosting. Drain the growth medium from the 75-cm^2 flask with the cultured Vero cells (3 x 10^6 cells) in the monolayer. Add 5 ml of DPBS the flask. Wash the surface of the cultured cells and drain the DPBS. Repeat the washing procedure 2 times. Put the defrosted base SARS-CoV-2 virus in the new test tube, dilute with the serum-free DMEM medium and adjust the concentration of virus to 10^3 PFU to 10^4 PFU per ml. Inoculate 2 ml of the adjusted base SARS-CoV-2 virus on the surface of cell in the flask and spread to the whole surface. Put the flask in the CO_2 incubator at a temperature of 37°C and keep it for 1 h to adsorb the virus to the cells. Put 12 ml of 2% FBS-DMEM with 1x penicillin/streptomycin in the flask. Put the flask in the CO_2 incubator at a temperature of 37°C for 2 to 3 days to multiply the SARS-CoV-2 virus. Observe the cytopathic effect by a microscope and judge the multiplication of virus. If the multiplication of virus is confirmed, put the

multiplied virus suspension in the centrifugal tube. Centrifuge the multiplied virus suspension by using a centrifuge at a temperature of 4°C and 1 000×g for 15 min. Take the supernatant suspension from the centrifugal tube after the centrifugation. This is to be the SARS-CoV-2 virus suspension. Divide the suspension into test tubes appropriately and cryopreserve at −80°C in the freezer. Check the concentration of the virus if it is more than 10^7 PFU/ml by plaque titer assay. If the concentration is less than 1 x 10^7 PFU/ml, prepare it from beginning.

4.2.3. Inactivation of cell culture-derived virus

Inactivated viruses were prepared by incubation for 1 hour in a heat block at 65°C. Plaque Assay was conducted to confirm heat inactivation.

4.2.4. Test method

4.2.3.1. Determination of LoD

① Nasopharyngeal smear, which was confirmed as negative, was provided by Lee biosolutions.

② The variants of SARS-CoV-2 was spiked into the solution of ① to a concentration of 1×10^6 PFU/ml, and then diluted it to a concentration of 2.44×10^2 PFU/ml by a 12 steps dilution method as shown in Table 5.

③ The 120 ㎕ of sample prepared in ② was added to the device sample well (S).

④ Read results in 15 minutes.

⑤ The tests were performed with 2 Lots.

Table 5. Concentration of SARS-CoV-2 variants

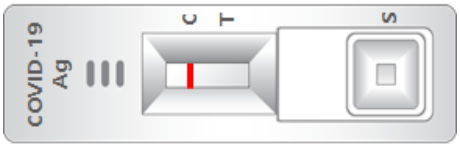
Sample No.	Conc. (PFU/ml)	Sample No.	Conc. (PFU/ml)
1	1×10^6	8	7.81×10^3
2	5×10^5	9	3.90×10^3
3	2.5×10^5	10	1.95×10^3
4	1.25×10^5	11	9.76×10^2
5	6.25×10^4	12	4.88×10^2

6	3.12×10^{4}	13	2.44×10^{2}
7	1.56×10^{4}	14	0

5. Results

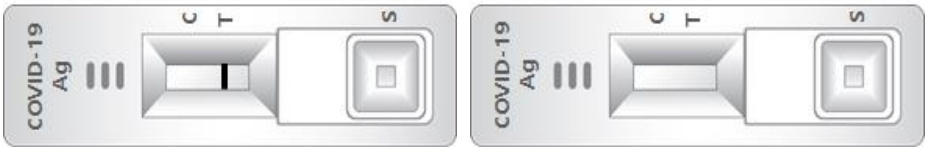
5.1. Interpretation of results

1) Negative: The presence of only one red band at the control line (C) within the result window indicates a negative result.



2) Positive: Two bands appear, one red control line (C) and one black test line (T) within the result window indicates a positive result.

3) Invalid: If the control line fails to appear within the result window or presence of only one black band at the Test line (T), the result is considered invalid.



5.2. Sensitivity criteria

The test line intensity was evaluated by referring to the RapiGEN color chart (Figure 1).

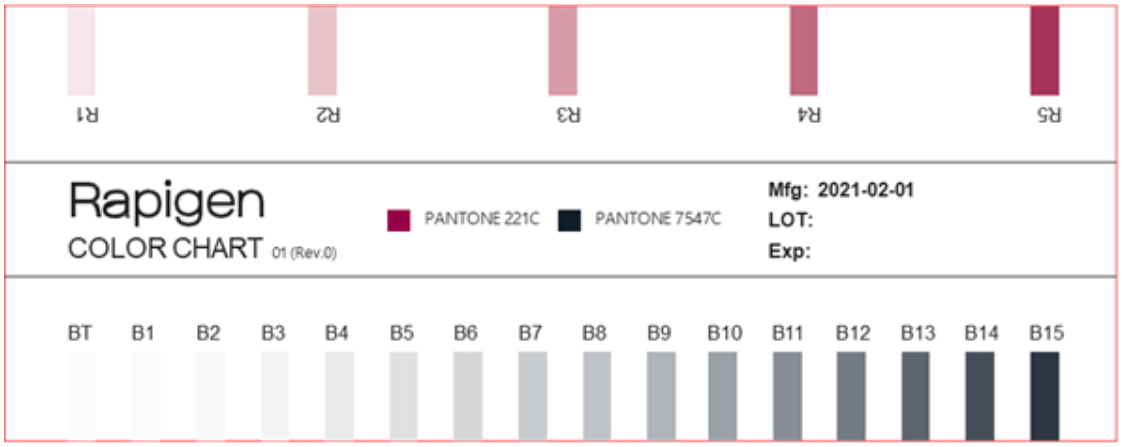


Figure 1. RapiGEN Color Chart. Strong positive (B11~B15); Medium positive (B6~B10); Weak positive (B1~B5); Negative (Black trace (BT) or Not detected (ND)).

5.3. Test results

5.3.1. B.1.1.7

Results showed that the established tentative LoD was confirmed as 1.95 x 10^3 PFU/ml (Table 6). In addition, it was confirmed that the cell culture-derived virus showed strong positive (B11~B15) at concentration of 1×10^6 to 5×10^5 PFU/ml, medium positive (B6~B10) at concentration of 2.5×10^5 to 1.25×10^5 PFU/ml, weak positive (B1~B5) at concentration of 6.25×10^4 to 1.95×10^3 PFU/ml, and negative (BT, ND) to under concentration.

Table 6. Detection limit test result for B.1.1.7

Lot No.	Sample No.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
H073014EV	B14	B11	B10	B8	B4	B3	B2	B1	B1	B1	BT	ND	ND	ND
H073016EV	B14	B12	B10	B7	B5	B3	B2	B1	B1	B1	BT	ND	ND	ND
Number of Positive	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	0/2	0/2	0/2
Positivity	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	0%	0%	0%	0%

5.3.2. B.1.351

Results showed that the established tentative LoD was confirmed as 9.76 x 10^2 PFU/ml

(Table 7). In addition, it was confirmed that the cell culture-derived variant virus showed strong positive (B11~B15) at concentration of 1×10^6 to 2.5×10^5 PFU/ml, medium positive (B6~B10) at concentration of 1.25×10^5 to 6.25×10^4 PFU/ml, weak positive (B1~B5) at concentration of 3.12×10^4 to 9.76×10^2 to PFU/ml, and negative (BT, ND) to under concentration.

Table 7. Detection limit test result for B.1.351.

Lot No.	Sample No.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
H073014EV	B15	B14	B11	B9	B8	B5	B4	B3	B2	B1	B1	BT	ND	ND
H073016EV	B14	B14	B12	B10	B8	B5	B4	B3	B2	B1	B1	BT	ND	ND
Number of Positive	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	0/2	0/2
Positivity	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	0%	0%	0%

6. Conclusions

BIOCREDIT COVID-19 Ag can detect mutant viruses of UK and South African variant, and LoD of each mutant virus is 1.95×10^3 and 9.76×10^2 PFU/ml, respectively.

7. References

[1] Corona 19 In Vitro Diagnosis Medical Device License and Review Guidelines, KFDA, 2020

[2] Instructions and requirements for Emergency Use Listing (EUL) submission: In vitro diagnostics detecting SARS-CoV-2 nucleic acid and rapid diagnostics tests detecting SARS-CoV-2 antigens

[3] CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

8. Attachments

8.1. Certificate of SARS-CoV-2 variant viruses

질병관리청 국립보건연구원

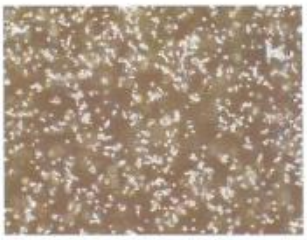
Information Sheet

NCCP

SARS-CoV-2 (NCCP43381)

◈ **배양 조건**

배지 : DMEM + 1% penicillin-streptomycin + 2% FBS
감수성세포 : Vero E6
배양 조건 : 37℃, 5% CO_2
배양기간 : 3일
세포반응 : 세포병변효과(CPE); 세포형태가 둥글어지며 사멸



◈ **자원세부정보**

- 보존형태 : 동결된 바이러스 배양액이 포함된 튜브(-70℃), 500㎕
- 자원분류 : (Beta Coronavirus)속 (SARS-COV-2)종
- 분리주 : hCoV-19/Korea/KDCA51463/2021
- 분리경로 : 영국에서 국내 입국한 코로나바이러스감염증-19 확진자의 인후도말 검체에서 세포배양을 통해 바이러스 분리
- Clades : GR (영국변이주)
- 분리년도 : 2021
- 역 가 : 1.0 x 10^6 PFU/ml

◈ **생물안전등급(Biosafety Level) : 3**

근거 : 유전자재조합실험지침 제6조 물리적밀폐(보건복지부고시 제2020-140호)

◈ **NCCP 자원 품질보증**

- 분양자원에 대한 이의신청은 인수 후 30일 이내에 정보시트에 제시된 정보에 따라 저장 또는 배양된 경우에만 해당됨
- 기타 다른 배지에 배양된 자원의 경우 자원에 대해 책임지지 않음

(28160)충북 청주시 흥덕구 오송읍 오송생명2로 200 국립중앙인체자원은행 3층 병원체자원관리과
Tel. 043-719-6670 Fax. 043-719-6680 E-mail. nccpbank@korea.kr

질병관리청 국립보건연구원

Information Sheet

NCCP

동결자원 재생법 및 유의사항

◆ 동결자원 재생법

- 동결된 바이러스가 들어있는 튜브를 아이스박스를 이용하여 옮김
- 튜브를 37℃ water bath를 이용하여 해동
- 튜브의 내용물이 완전히 녹음과 동시에 cryovial을 아이스박스로 옮김
- 준비된 monolayer 감수성 세포에 바이러스 배양액 적정량을 접종하여 바이러스를 배양

◆ 주의사항

국가병원체자원은행에서 제공하는 병원체자원은 분양신청서에 기재한 연구목적 및 시설에서만 사용하여야 하며, 자세한 내용은 분양신청서 참조

◆ 유의사항

- 모든 실험은 Biosafety level 3 실험실 내 생물안전작업대(BSC)에서 실험복과 장갑을 착용한 후 실시해야 함
- 재생이 안되거나 오염된 자원의 경우 인수 후 30일 이내에 국가병원체자원은행으로 연락바람

(28160)충북 청주시 흥덕구 오송읍 오송생명2로 200 국립중앙인체자원은행 3층 병원체자원관리과

Tel. 043-719-6670 Fax. 043-719-6680 E-mail. nccpbank@korea.kr

질병관리청 국립보건연구원

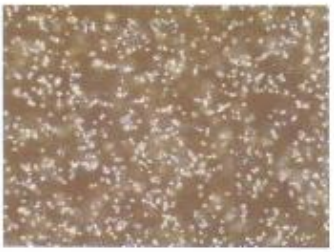
Information Sheet

NCCP

SARS-CoV-2 (NCCP43382)

◈ 배양 조건

배지 : DMEM + 1% penicillin-streptomycin + 2% FBS
감수성세포 : Vero E6
배양 조건 : 37℃, 5% CO_2
배양기간 : 3일
세포반응 : 세포병변효과(CPE); 세포형태가 둥글어지며 사멸



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- Clades : GH (남아공변이주)
- 분리년도 : 2021
- 역 가 : 7.0 x 10^6 PFU/ml

◈ 생물안전등급(Biosafety Level) : 3

근거 : 유전자재조합실험지침 제6조 물리적밀폐(보건복지부고시 제2020-140호)

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