BIOCREDIT COVID-19 Ag Test

: Analytical Sensitivity Report using mutated SARS-CoV-2 recombinant protein

Authorization	Dept. & Name	Signature	Date
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1. Purpose

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

2. Tested by/Verified by

Tested by Se-Chang Kwon

Verified by Seo-woo Lee

3. Test Date

May 25, 2021 ~ May 26, 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 product

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 recombinant protein were provided by Fapon (China).

Table 2. Test specimen

Variants	Country identified	Conc. (mg/ml)	Source
B.1.618	India	0.58	FPZ0688
B.1.617.1	India	1.04	FPZ0689
B.1.429	California	1.92	FPZ0691

4.1.3. Test equipment

Table 3. Test equipment

	Type	Equipment No.	Calibration date	Calibration valid date
Micro pipette	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
Thermo-hydrometer	N/A	P-11-0058	2020. 07.13	2021.07.12

4.2. Method

4.2.1. Test condition

This test was conducted at RapiGEN Biotechnology Research Institute, and the temperature and humidity conditions were verified using a thermo-hydrometer

Table 4. Test condition

Test site	Temperature and humidity
Rapigen Biotechnology Research Institute	Minimum 18℃, 21% RH
	Maximum 20℃, 25% RH

4.2.2. Test method

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

② The variants of SARS-CoV-2 recombinant protein was spiked into the solution of ① to a concentration of 50 ng/ml, and then diluted it to a concentration of 0.01 ng/ml 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

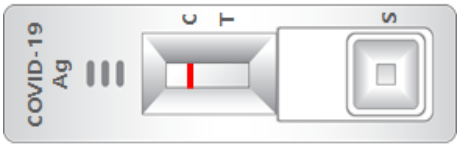
Sample No.	Conc. (ng/ml)	Sample No.	Conc. (ng/ml)
1	50	8	0.39
2	25	9	0.19

3	12.5	10	0.09
4	6.25	11	0.04
5	3.12	12	0.02
6	1.56	13	0.01
7	0.78	14	0

5. Results

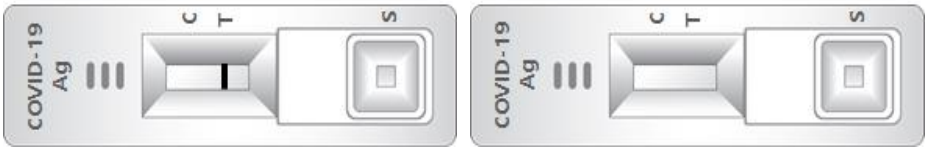
5.1. Interpretation of results

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).

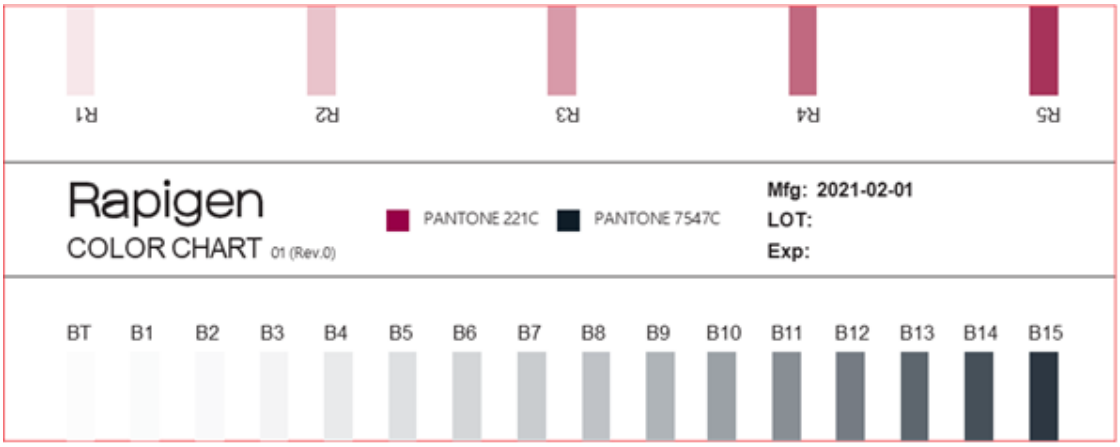


Figure 1. RapiGEN Color Chart. Strong positive (B11~B15); Medium positive (B6~B10); Weak positive (B1~B5); Negative (Black trace (BT) or Not detected (ND)).

5.3. Test results

5.3.1. B.1.618 (India)

Results showed that the established tentative LoD was confirmed as 0.04 ng/ml (Table 6). In addition, it was confirmed that the variant recombinant protein showed strong positive (B11~B15) at concentration of 12.5 to 50 ng/ml, medium positive (B6~B10) at concentration of 1.56 to 6.25 ng/ml, weak positive (B1~B5) at concentration of 0.04 to 0.78 ng/ml, and negative (BT, ND) to under concentration.

Table 6. Detection limit test result for B.1.618

Lot No.	Sample No.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
H073014EV	B14	B13	B12	B10	B8	B7	B5	B3	B3	B2	B1	BT	ND	ND
H073016EV	B14	B13	B12	B10	B7	B7	B5	B4	B3	B2	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%

5.3.2. B.1.617.1 (India)

Results showed that the established tentative LoD was confirmed as 0.04 ng/ml (Table 7). In addition, it

was confirmed that the variant recombinant protein showed strong positive (B11~B15) at concentration of 6.25 to 50 ng/ml, medium positive (B6~B10) at concentration of 1.56 to 3.12 ng/ml, weak positive (B1~B5) at concentration of 0.04 to 0.78 ng/ml, and negative (BT, ND) to under concentration.

Table 7. Detection limit test result for B.1.617.1

Lot No.	Sample No.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
H073014EV	B15	B15	B13	B11	B9	B6	B5	B4	B3	B2	B1	BT	ND	ND
H073016EV	B15	B14	B12	B12	B8	B6	B5	B4	B3	B2	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%

5.3.3. B.1.429 (California)

Results showed that the established tentative LoD was confirmed as 0.09 ng/ml (Table 8). In addition, it was confirmed that the variant recombinant protein showed strong positive (B11~B15) at concentration of 12.5 to 50 ng/ml, medium positive (B6~B10) at concentration of 3.12 to 6.25 ng/ml, weak positive (B1~B5) at concentration of 0.09 to 1.56 ng/ml, and negative (BT, ND) to under concentration.

Table 8. Detection limit test result for B.1.429

Lot No.	Sample No.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
H073014EV	B15	B13	B12	B9	B8	B4	B3	B3	B2	B1	BT	ND	ND	ND
H073016EV	B15	B14	B12	B8	B8	B5	B4	B3	B2	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%

6. Conclusions

BIOCREDIT COVID-19 Ag can detect mutant recombinant protein of India (B.1.618, B.1.617.1) and California (B.1.429) variant, and LoD of each mutant virus is 0.04 and 0.09 ng/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

FAPON

Certificate of Analysis

Name:	COVID-19 Nucleocapsid protein recombinant antigen(B.1.618)
Catalog No.:	FPZ0688
Lot No.:	20210510
Manufacturing Date:	20210510
Expiry Date:	20240509
Concentration:	0.58mg/ml
Source:	E.coli
Format:	Clear and transparent liquid
Amino acid sequence:	1-419aa
Specific fragment:	Nucleocapsid protein
Molecular weight:	45KD
Buffer:	10mMPB+150mMNaCl，pH7.4
Preservative:	/
Purification method:	Column chromatography
Purity:	≥90%
Application:	Lateral flow/ELISA/CMIA
Storage:	-20±5℃

Remark:

1.The raw material can be stored at 2~8℃ for 15 days during transportation;

2.Avoid repeating freeze-thaw cycles.

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Fapon Biotech Inc.
No.5 Huallan Road, Taiwan High-tech industrial park, Songshan Lake, Dongguan, Guangdong, China 523808
0769-22998886

FAPON

Certificate of Analysis

Name:	COVID-19 Nucleocapsid protein recombinant antigen(B.1.617.1)
Catalog No.:	FPZ0689
Lot No.:	20210509
Manufacturing Date:	20210509
Expiry Date:	20240508
Concentration:	1.04mg/ml
Source:	E.coli
Format:	Clear and transparent liquid
Amino acid sequence:	1-419aa
Specific fragment:	Nucleocapsid protein
Molecular weight:	45KD
Buffer:	10mMPB+150mMNaCl，pH7.4
Preservative:	/
Purification method:	Column chromatography
Purity:	≥90%
Application:	Lateral flow/ELISA/CMIA
Storage:	-20±5℃

Remark:

1.The raw material can be stored at 2~8℃ for 15 days during transportation;

2.Avoid repeating freeze-thaw cycles.

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Fapon Biotech Inc.

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Certificate of Analysis

Name:	COVID-19 Nucleocapsid protein recombinant antigen(B. 1. 429)
Catalog No.:	FPZ0691
Lot No.:	20210113-1
Manufacturing Date:	20210113
Expiry Date:	20240112
Concentration:	1.92mg/ml
Source:	E.coli
Format:	Clear and transparent liquid
Amino acid sequence:	1-419aa
Specific fragment:	Nucleocapsid protein
Molecular weight:	45KD
Buffer:	10mMPB+150mMNaCl，pH7.4
Preservative:	/
Purification method:	Column chromatography
Purity:	≥90%
Application:	Lateral flow/ELISA/CMIA
Storage:	-20±5℃

Remark:

1.The raw material can be stored at 2~8℃ for 15 days during transportation;

2.Avoid repeating freeze-thaw cycles.

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