Study Report for

One Step Test for SARS-CoV-2 Antigen (Colloidal Gold)

on SARS-CoV-2 Variants

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Content

1 Purpose

The purpose of this report was to determine the impact of different SARS-CoV-2 variants on the One Step Test for SARS-CoV-2 Antigen (Colloidal Gold).

One Step Test for SARS-CoV-2 Antigen (Colloidal Gold) was developed by Getein Biotech, Inc. This test is intended for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in human nasal swab samples from patients who are suspected of COVID-19 within the first 7 days of symptom onset, or for screening of individuals without symptoms.

2 Experimental materials

2.1 Trial reagent

Product name: One Step Test for SARS-CoV-2 Antigen (Colloidal Gold)

Lot no.: 6SC22001W (Manufacturing date: February 9th, 2022)

Manufacturer: Getein Biotech, Inc.

2.2 Reagent for negative sample

Product name: Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2

Specifications: 50 reactions per kit

Manufacturer: BGI Genomics Co. Ltd.

PCR System: ABI 7500 Fast Real-Time PCR System with software v2.0.6

Viral RNA extraction kit: QIAamp Viral RNA Mini Kit (cat. #52904)

3 Experimental materials

The different SARS-CoV-2 variant full-length nucleocapsid proteins (NPs) were recombinant expressed, purified, and measured molecular weights through mass spectrum, so the amino acid mutations were verified. The different recombinant SARS-CoV-2 variant samples were established by spiking different recombination NPs (list in table below) into the pooled negative sample matrix obtained from multiple healthy volunteers eluted in virus transport media (VTM) and confirmed as SARS-CoV-2 negative by RT-

PCR kit (Name: Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2, Manufacturer: BGI Genomics Co. Ltd.).

Table 1 NPs information

No.	Virus (Lineage)	Cat No.	Manufacturer
1	SARS-CoV-2	HP811-7K	Nanjing Getein Bio-Medical Co., Ltd
2	B.1.1.7	HP811-8K	
3	B.1.351 / B.1.429 / B.1.427	HP811-9K	
4	P.1	HP811-10K	
5	B.1.617/ XD	HP811-11K	
6	B.1.617.1	HP811-12K	
7	B.1.617.2	HP811-13K	
8	B.1.617.3	HP811-14K	
9	P.2	HP811-15K	
10	A.23.1 with E484K-1	HP811-16K	
11	A.23.1 with E484K-2	HP811-17K	
12	B.1.1.7 with E484K	HP811-18K	
13	B.1.525 (previously designated UK1188)	HP811-19K	
14	B.1.1.318	HP811-20K	
15	P.3	HP811-21K	
16	AV.1	HP811-22K	
17	B.1.1.529/ BA.1/ BA.1.1/ XF	HP811-23K	
18	C.37	HP811-24K	
19	B.1.621	HP811-25K	
20	B.1.526	HP811-26K	
21	BA.2/ BA.3/ BA.5/ XE/BA.2.11/BA.2.12.1/BA.2.13	HP811-28K	
22	BA.4	HP811-29K	
23	B.2.9.1	HP811-30K	
24	C.63.3	HP811-31K	
25	AY.42	HP811-32K	
26	BA.2.75/ BA.2.10	HP811-33K	
27	BE.3	HP811-34K	
28	BE.1	HP811-35K	
29	BA.4.6	HP811-36K	
30	BA.5.2.1	HP811-37K	

Wet-testing were conducted for all variants of concern, no matter how many the mismatch percentage is.

4 Experimental process

4.1 In silico analysis

Search the sequences of SARS-CoV-2 variants from GISAID database, find one sequence for each variant lineage and conduct in silico analysis. Calculate the mismatch percentage of each variant according to the following formula:

Mismatch percentage = (number of mutation sites/419) *100%

Mismatch percentage of the epitope sequence = (number of mutation sites of the epitope sequence /137) *100%

4.2 Wet testing

Determine the minimum detected concentration of recombinant SARS-CoV-2 NP. An initial minimum detected concentration was performed using 5-fold serial dilutions (four dilutions in total) of the recombinant SARS-CoV-2 NP starting at a test concentration of 300 pg/mL. These dilutions were tested in triplicate. The lowest concentration at which all (3 out of 3 replicates) were positive was chosen for the next study. The minimum detected concentration was further refined using a 2-fold dilution series (four dilutions in total). These dilutions were also tested in triplicate. The lowest concentration (3 out of 3 replicates) was treated as the tentative minimum detected concentration. The final minimum detected concentration was determined to be the lowest concentration resulting in positive detection of nineteen (19) out of twenty (20) replicates.

Dilute the above NPs into the same concentration (as the final minimum detected concentration) with the pooled negative sample matrix. Test diluted SARS-CoV-2 variant protein samples using trial reagent and record the test results. Compare the test results of SARS-CoV-2 variant NPs with SARS-CoV-2 NP to study the impact of variants on performance of trial reagent.

5 Test result

5.1 In silico analysis results

Table 2 In silico analysis results

No.	Virus (Lineage)	Date of in silico analysis	Mismatch percentage	Mismatch percentage of the epitope sequence
1	SARS-CoV-2	/	/	/
2	B.1.1.7	2021.11.25	0.24%	0.00%
3	B.1.351 / B.1.429 / B.1.427	2021.11.25	0.72%	0.00%
4	P.1	2021.11.25	0.48%	0.73%
5	B.1.617**/ XD**	2021.11.25 **/ 2022.03.31**	0.48%	0.00%
6	B.1.617.1	2021.11.25	0.72%	0.00%
7	B.1.617.2	2021.11.25	0.72%	0.00%
8	B.1.617.3	2021.11.25	0.72%	0.73%
9	P.2	2021.11.25	0.24%	0.73%
10	A.23.1 with E484K-1	2021.11.25	0.95%	0.00%
11	A.23.1 with E484K-2	2021.11.25	0.95%	0.00%
12	B.1.1.7 with E484K	2021.11.25	0.95%	0.00%
13	B.1.525 (previously designated UK1188)	2021.11.25	0.95%	0.00%
14	B.1.1.318	2021.11.25	0.72%	0.00%
15	P.3	2021.11.25	0.72%	0.73%
16	AV.1	2021.11.25	0.95%	0.73%
17	B.1.1.529/ BA.1/ BA.1.1**/ XF**	2022.03.31 **/ 2022.05.17**	0.95%	2.19%
18	C.37	2021.11.25	0.24%	0.00%
19	B.1.621	2021.11.25	0.95%	0.00%
20	B.1.526	2021.11.25	1.67%	0.00%
21	BA.2/ BA.3/ BA.5/ XE**/BA.2.11/BA.2.12.1/BA.2.13**	2022.03.31 **/ 2022.06.03**	1.91%	2.19%
22	BA.4	2022.03.31	0.48%	2.92%
23	B.2.9.1	2022.06.03	2.15%	2.92%
24	C.36.3	2022.07.07	0.72%	0.00%
25	AY.42	2022.07.07	0.95%	0.73%
26	BA.2.75/ BA.2.10	2022.07.22	0.95%	2.19%
27	BE.3	2022.07.29	2.15%	3.65%
28	BE.1	2022.09.14	1.67%	2.92%
29	BA.4.6	2022.10.14	1.91%	2.92%
30	BA.5.2.1	2022.10.14	0.95%	0.00%

Please refer to in silico analysis report for more detailed information.

5.2 Wet testing results

5.2.1 Initial minimum detected concentration of SARS-CoV-2 NP

Table 3 The test results of initial minimum detected concentration

SARS-CoV-2 NP Concentration (pg/mL)	Dilutions	Test results			Statistical result
		Rep1	Rep2	Rep3	
300	/	+	+	+	3/3 positive
60	5	+	+	+	3/3 positive
12	25	+	+	+	3/3 positive
2.4	125	-	-	-	0/3 positive

Note: "-" means negative; "+" means positive.

According to the test results, 12 pg/mL was the lowest concentration at which all (3 out of 3 replicates) were positive and chosen as the initial minimum detected concentration.

5.2.2 Tentative minimum detected concentration of SARS-CoV-2 NP

Table 4 The test results of tentative minimum detected concentration

SARS-CoV-2 NP Concentration (pg/mL)	Dilutions	Test results			Statistical result
		Rep1	Rep2	Rep3	
12	/	+	+	+	3/3 positive
6	2	+	+	+	3/3 positive
3	4	+	-	-	1/3 positive
1.5	8	-	-	-	0/3 positive

Note: "-" means negative; "+" means positive.

According to the test results, 6 pg/mL was chosen as the tentative minimum detected concentration.

5.2.3 Final minimum detected concentration of SARS-CoV-2 NP

Select 6 pg/mL as the tentative concentration and test 20 replicates.

Table 5 The test results of final minimum detected concentration

Rep	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10
Result	+	+	+	+	+	+	+	+	+	+
Rep	Rep11	Rep12	Rep13	Rep14	Rep15	Rep16	Rep17	Rep18	Rep19	Rep20
Result	+	+	+	+	-	+	+	+	+	+
Statistical result	19/20 positive									

Note: "-" means negative; "+" means positive.

Based on the above results, the final minimum detected concentration of SARS-CoV-2 NP was confirmed as 6 pg/mL.

5.2.4 Test results of SARS-CoV-2 variant NPs

Table 6 The test results of SARS-CoV-2 Variant NPs

SARS-CoV-2 Variant NPs	Date of wet analysis	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8	Rep9	Rep10	Statistical result
		Rep11	Rep12	Rep13	Rep14	Rep15	Rep16	Rep17	Rep18	Rep19	Rep20	
HP811-8K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	-	+	+	+	+	
HP811-9K	21.12.09	+	+	+	+	-	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	
HP811-10K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	-	+	+	+	+	+	
HP811-11K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	-	+	+	+	+	
HP811-12K	21.12.09	+	+	+	-	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	
HP811-13K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	-	+	+	+	+	

HP811-14K	21.12.09	+	+	-	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	
HP811-15K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	-	+	+	+	+	+	+	
HP811-16K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	-	+	+	+	+	+	
HP811-17K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	-	+	+	+	+	
HP811-18K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	-	+	
HP811-19K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	-	+	+	
HP811-20K	21.12.09	+	+	+	+	+	-	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	
HP811-21K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	-	+	+	+	+	
HP811-22K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	-	+	+	+	+	+	+	
HP811-23K	22.04.14	+	+	-	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	
HP811-24K	21.12.09	+	+	+	+	+	+	-	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	
HP811-25K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	-	+	+	+	+	+	
HP811-26K	21.12.09	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	-	+	+	+	+	+	+	+	+	
HP811-28K	22.04.14	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	-	+	
HP811-29K	22.04.14	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	-	+	+	+	+	+	+	+	
HP811-30K	22.06.17	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	-	+	+	
HP811-31K	22.07.21	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	-	+	+	+	+	+	+	+	+	
HP811-32K	22.07.21	+	+	+	+	-	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	
HP811-33K	22.08.05	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	-	+	+	
HP811-34K	22.08.12	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	-	+	+	+	+	+	+	

HP811-35K	22.09.21	+	+	+	+	+	+	+	-	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	
HP811-36K	22.10.14	+	+	+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	-	+	
HP811-37K	22.10.14	+-		+	+	+	+	+	+	+	+	19/20 positive
		+	+	+	+	+	+	+	+	+	+	

Note: "-" means negative; "+" means positive.

According to the above results, the LoDs of all SARS-CoV-2 variant NPs were showed in below table.

Table 7 The test results of SARS-CoV-2 Variant NPs

Virus (lineage)	LoDs	Virus (lineage)	LoDs	Virus (lineage)	LoDs
SARS-CoV-2	6 pg/mL	A.23.1 with E484K-1	6 pg/mL	B.1.621	6 pg/mL
B.1.1.7	6 pg/mL	A.23.1 with E484K-2	6 pg/mL	B.1.526	6 pg/mL
B.1.351 / B.1.429 / B.1.427	6 pg/mL	B.1.1.7 with E484K	6 pg/mL	BA.2 / BA.3 / BA.5 / XE / BA.2.11 / BA.2.12.1 / BA.2.13	6 pg/mL
P.1	6 pg/mL	B.1.525 (previously designated UK1188)	6 pg/mL	BA.4	6 pg/mL
B.1.617/ XD	6 pg/mL	B.1.1.318	6 pg/mL	B.2.9.1	6 pg/mL
B.1.617.1	6 pg/mL	P.3	6 pg/mL	C.63.3	6 pg/mL
B.1.617.2	6 pg/mL	AV.1	6 pg/mL	AY.42	6 pg/mL
B.1.617.3	6 pg/mL	B.1.1.529 / BA.1/ BA.1.1/ XF	6 pg/mL	BA.2.75	6 pg/mL
P.2	6 pg/mL	C.37	6 pg/mL	BE.3	6 pg/mL
BE.1	6 pg/mL	/	/	/	/

6 Conclusion

From the above results, trial reagent can detect all recombinant SARS-CoV-2 variant NPs at minimum detected concentration (6 pg/mL) and show no drop off in sensitivity when compared with respect to the following variants – B.1.1.7, B.1.351, P.1, B.1.429, B.1.427, B.1.617, B.1.617.1, B.1.617.2 , B.1.617.3, P.2, A.23.1 with E484K-1, A.23.1 with E484K-2, B.1.1.7 with E484K, B.1.525 (previously designated UK1188), B.1.1.318, P.3, AV.1, B.1.1.529 (Omicron), C.37, B.1.621, B.1.526, BA.1, BA.1.1, BA.2, XF, XD, XE, BA.3, BA.4, BA.5, BA.2.11, BA.2.12.1, BA.2.13, B.2.9.1, C.63.3, AY.42, BA.2.75, BE.3 BE.1, BA.4.6 and BA.5.2.1.