Diagnostic Performance Evaluation Report of Test Kit against SARS-CoV-2 Variants

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Version: 1.0

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1. Abstract

Product name: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based) (hereinafter described as "Antigen Detection Test")

Manufacturer: Nanjing Vazyme Medical Technology Co., Ltd. (hereinafter described as "Vazyme")

2. Study Purpose

To validate and evaluate the efficacy and performance for the above-mentioned product on frequent mutations of the SARS-CoV-2 virus (listed in Table 1 as follow), as manufacturer Vazyme keeps continuous attention on Covid-19 and makes necessary verification to evaluate the Antigen Detection Test can specifically interact with the recombinant nucleocapsid protein (N protein), RBD domain (RBD) and spike mutant protein (S protein) from the SARS-CoV-2 variant receptor.

Table 1 SARS-CoV-2 variant type [1]

No.	SARS-CoV-2 variant type		Date of designation
1	Alpha	B.1.1.7	18-Dec-2020
2	Beta	B.1.351	18-Dec-2020
3	Gamma	P.1	11-Jan-2021
4	Delta	B.1.617.2	VOI:4-Apr-2021 VOC:11-May-2021
5	Epsilon	B.1.427/B.1.429	5-Mar-2021
6	Eta	B.1.525	17-Mar-2021
7	Kappa	B.1.617.1	4-Apr-2021
8	Omicron	B.1.1.529	VUM: 24-Nov-2021 VOC: 26-Nov-2021

9	Lambda	C.37	14-Jun-2021

3. Study Materials

The materials used in the experiment include the antigen detection kit and the other materials shown in Tables 2 to 4.

Table 2. Materials used for recombination research of wild-type and mutant proteins of N protein

SARS-CoV-2 variant	Target viral	Sample name	Mutation site [2]	Concentration tested
Wild-type	N protein	WN	–	0.1 ng/mL,1 ng/mL, 10 ng/mL and 100 ng/mL
B.1.1.7		MN1	D3L, R203K, G204R, S235F	
B.1.351		MN2	P13S, T205I	
P.1		MN3	P80R, R203K, G204R, I292T	
B.1.525		MN4	A12G, T205I	
B.1.429+B.1.427		MN5	T205I, T391I	
B.1.617 [1]		MN6	R203M, D377Y	
B.1.617.2 [3]		MN7	D63G, R203M, D377Y	
B.1.1.529		MN8	P13L, Δ31-33, RG203KR	

Table 3. Materials used for recombination research of wild-type and mutant proteins of S protein

SARS-CoV-2 variant	Target viral protein	Sample name	Mutation site	Concentration tested
Wild-type	S protein	WS	–	0.1 ng/mL, 1 ng/mL, 10 ng/mL and 100 ng/mL
B.1.1.7		MS1	H69del, V70del, Y144del, N501Y, A570D, P681H, T716I, S982A, D1118H	

B.1.351	MS2	D614G, D80A, D215G, E484K, N501Y, A701V, L18F, R246I, K417N, Δ242-244	
B.1.526	MS3	L5F, T95I, D253G, E484K, S477N, D614G, A701V	
B.1.525	MS4	A67V, D614G, E484K, F888L, Δ 69, Q52R, Q677H, Δ V70, Δ Y144	
B.1.429+B.1.427	MS5	D614G, L452R, S13I, W152C, W258L	
Fin-796H	MS6	T95I, E484K, D614G, P681H, ΔY144	
B.1.1.529 [4]	MS7	A67V, Δ69-70, T95I, G142D, Δ143-145, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K,	

Table 4. Materials used for recombination research of wild-type and mutant proteins of RBD

SARS-CoV-2 variant	Target viral	Sample name	Mutation site	Concentration tested
Wild-type	RBD	WRBD	–	0.1 ng/mL,

B.1.1.7 B.1.351 P.1		MRBD1	N501Y
B.1.351		MRBD2	K417N
B.1.351 P.1		MRBD3	E484K
B.1.429+B.1.427		MRBD4	L452R
B.1.617		MRBD5	L452R, E484Q
B.1.617.2		MRBD6	L452R, T487K
C.37		MRBD7	L452Q, F490S
B.1.1.529		MRBD8	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H

4. Study Design and Evaluation Criteria

4.1 Study Design

Both sensitivity and repeatability studies are carried out to evaluate if Antigen Detection Kit (Colloidal Gold-Based) can detect both recombinant N protein, RBD and S protein wild-type and mutant proteins equally well consistently at low (0.1 ng/mL or 1 ng/mL), intermediate (10 ng/mL), and high (100 ng/mL) concentrations. For sensitivity study, 0.1 ng/mL, 1 ng/mL, 10 ng/mL and 100 ng/mL of wild-type and mutant proteins are tested one time respectively. For repeatability study, 1 ng/mL and 100 ng/mL of wild-type and mutant proteins are tested ten times respectively.

4.2 Evaluation Criteria [5]

According to the Test Procedure described in the product manual, read the results after 10 minutes. The result is invalid after 15 minutes.

Negative result: Only one red quality control line (C-line) is visible (Please see the Figure 1)

Positive result: two clear red lines are visible; one is quality control line (C-line) and the other is the T test line. (Please see the Figure 1)

Invalid result: there is no red line or there is only T test line, but no quality control line (C-line), suggesting that the item has a test error or the test result is invalid, and the item should be retested. (Please see the Figure 1)

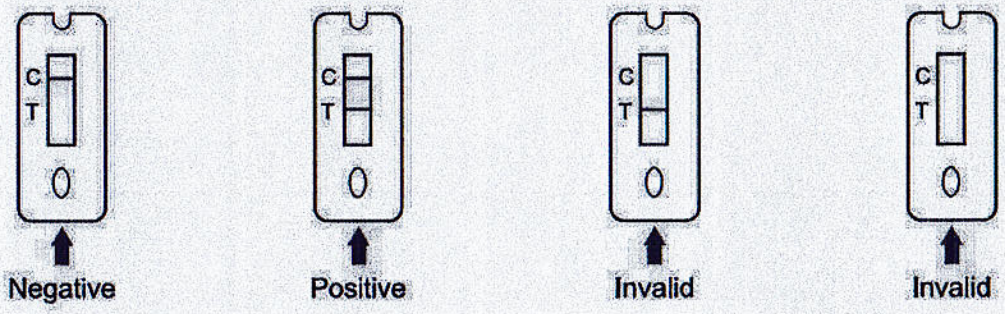

Figure 1 Reference Diagram of Test Result

Interpretation criteria for sensitivity and repeatability, the result summarized through the line color of the cassette is defined as follow:

- Recombinant N protein wild-type protein at 0.1 ng/mL: +
- Recombinant N protein wild-type protein at 1 ng/mL: ++
- Recombinant N protein wild-type protein at 10 ng/mL: +++
- Recombinant N protein wild-type protein at 100 ng/mL: ++++
- Recombinant RBD wild-type protein at 0.1 ng/mL: -
- Recombinant RBD wild-type protein at 1 ng/mL: +
- Recombinant RBD wild-type protein at 10 ng/mL: ++
- Recombinant RBD wild-type protein at 100 ng/mL: +++
- Recombinant spike wild-type protein at 0.1 ng/mL: -
- Recombinant spike wild-type protein at 1 ng/mL: +
- Recombinant spike wild-type protein at 10 ng/mL: ++
- Recombinant spike wild-type protein at 100 ng/mL: +++

5. Study Result

5.1 Test result of N protein (referred to Table 5)

The test results show that under the testing of the recombinant wild-type and mutant proteins of N protein, the test concentration of the kit can reach 0.1ng/mL. The cassette line color rendering intensity is consistent, which means the result meets the evaluation criteria. Furthermore, this means the Antigen Detection Kit can detect the wild-type N protein of the new crown and the N

protein of different mutants without any difference.

Table 5. Sensitivity test results based on 0.1ng/mL, 1ng/mL, 10ng/mL and 100ng/mL wild-type and mutant N protein

Tested protein		Concentration			
		100 ng/mL	10 ng/mL	1 ng/mL	0.1 ng/mL
N protein	WN	++++	+++	++	+
	MN1	++++	+++	++	+
	MN2	++++	+++	++	+
	MN3	++++	+++	++	+
	MN4	++++	+++	++	+
	MN5	++++	+++	++	+
	MN6	++++	+++	++	+
	MN7	++++	+++	++	+
	MN8	++++	+++	++	+

5.2 Test result of S protein (referred to Table 6)

The test results show that under the testing of the recombinant wild-type and mutant proteins of S protein, the test concentration of the kit can reach 1ng/mL. The cassette line color rendering intensity is consistent, which means the result meets the evaluation criteria. Furthermore, this means the Antigen Detection Kit can detect the wild-type S protein of the new crown and the S protein of different mutants without any difference.

Table 6. Sensitivity test results based on 0.1ng/mL, 1ng/mL, 10ng/mL and 100ng/mL wild-type and mutant S protein

Tested protein		Concentration			
		100 ng/mL	10 ng/mL	1 ng/mL	0.1 ng/mL
S protein	WS	+++	++	+	-
	MS1	+++	++	+	-
	MS2	+++	++	+	-
	MS3	+++	++	+	-
	MS4	+++	++	+	-
	MS5	+++	++	+	-
	MS6	+++	++	+	-
	MS7	+++	++	+	-

5.3 Test result of RBD (referred to Table 7)

The test results show that under the testing of the recombinant wild-type and mutant proteins of RBD, the test concentration of the kit can reach 1ng/mL. The cassette line color rendering intensity is consistent, which means the result meets the evaluation criteria. Furthermore, this

means the Antigen Detection Kit can detect the wild-type RBD of the new crown and the RBD of different mutants without any difference.

Table 7. Sensitivity test results based on 0.1ng/mL, 1ng/mL, 10ng/mL and 100ng/mL wild-type and mutant RBD

Tested protein		Concentration			
		100 ng/mL	10 ng/mL	1 ng/mL	0.1 ng/mL
RBD	WRBD	+++	++	+	-
	MRBD1	+++	++	+	-
	MRBD2	+++	++	+	-
	MRBD3	+++	++	+	-
	MRBD4	+++	++	+	-
	MRBD5	+++	++	+	-
	MRBD6	+++	++	+	-
	MRBD7	+++	++	+	-
	MRBD8	+++	++	+	-

In addition, the test is repeated for 10 times on wild-type and mutant proteins with concentrations of 1 ng/mL and 100 ng/mL, and the results meet the evaluation criteria. The specific data is shown in **Appendix A**.

6. Conclusion

In summary, for the SARS-CoV-2 variants listed above in "Clausula 2 Study Purpose", the antigen detection kit is able to detect SARS-CoV-2 variants.

At the equivalent concentrations, the color intensity of red T lines of mutant proteins is similar to those of wild-type protein. Acute Respiratory Syndrome Coronavirus 2(SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based) can be performed equally well, in a consistent manner against both recombinant N protein, RBD and S protein wild-type and mutant proteins that are tested in the studies. In addition, the analytical sensitivity of the Kit is 0.1 ng/mL of N protein, 1 ng/mL of RBD or S protein. And there's no invalid result.

7. Reference

1. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/
2. https://covdb.stanford.edu/
3. Jamie Lopez Bernal, Nick Andrews, Charlotte Gower.et.al. Effectiveness of COVID-19 vaccines against the B.1.617.2 variant. doi.org/10.1101/2021.05.22.2

4. Akatsuki Saito, Takashi Irie, Rigel Suzuki.et.al. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. Doi:10.1038/s41586-021-04266-9

5. Sandile Cele, Farina Karim, Gila Lustig.et.al. SARS-CoV-2 evolved during advanced HIV disease immunosuppression has Beta-like escape of vaccine and Delta infection elicited immunity. Doi: 10.1101/2021.09.14.21263564

6. Manual of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based)

Appendix A

Table 8: Result of repeatability study with 100 ng/mL of wild-type and mutant proteins for 10 times

Concentration / Tested protein		100 ng/mL									
		1	2	3	4	5	6	7	8	9	10
N protein	WN	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	MN1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	MN2	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	MN3	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	MN4	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	MN5	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	MN6	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	MN7	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	MN8	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Spike protein	WS	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MS1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MS2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MS3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MS4	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MS5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MS6	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MS7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
RBD of spike protein	WRBD	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MRBD1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MRBD2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MRBD3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MRBD4	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MRBD5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MRBD6	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MRBD7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	MRBD8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Table 9: Result of repeatability study with 1 ng/mL of wild-type and mutant proteins for 10 times

Concentratin / Tested protein		1 ng/mL									
		1	2	3	4	5	6	7	8	9	10
N protein	WN	++	++	++	++	++	++	++	++	++	++
	MN1	++	++	++	++	++	++	++	++	++	++
	MN2	++	++	++	++	++	++	++	++	++	++
	MN3	++	++	++	++	++	++	++	++	++	++
	MN4	++	++	++	++	++	++	++	++	++	++

	MN5	++	++	++	++	++	++	++	++	++	++
	MN6	++	++	++	++	++	++	++	++	++	++
	MN7	++	++	++	++	++	++	++	++	++	++
	MN8	++	++	++	++	++	++	++	++	++	++
Spike protein	WS	+	+	+	+	+	+	+	+	+	+
	MS1	+	+	+	+	+	+	+	+	+	+
	MS2	+	+	+	+	+	+	+	+	+	+
	MS3	+	+	+	+	+	+	+	+	+	+
	MS4	+	+	+	+	+	+	+	+	+	+
	MS5	+	+	+	+	+	+	+	+	+	+
	MS6	+	+	+	+	+	+	+	+	+	+
	MS7	+	+	+	+	+	+	+	+	+	+
RBD of spike protein	WRBD	+	+	+	+	+	+	+	+	+	+
	MRBD1	+	+	+	+	+	+	+	+	+	+
	MRBD2	+	+	+	+	+	+	+	+	+	+
	MRBD3	+	+	+	+	+	+	+	+	+	+
	MRBD4	+	+	+	+	+	+	+	+	+	+
	MRBD5	+	+	+	+	+	+	+	+	+	+
	MRBD6	+	+	+	+	+	+	+	+	+	+
	MRBD7	+	+	+	+	+	+	+	+	+	+
	MRBD8	+	+	+	+	+	+	+	+	+	+