



Address: 5252 Hollister St Suite 520 Houston TX 77040 USA
Tel: 281-513-7010

**The study of the Novel Coronavirus (2019-nCoV / SARS-CoV-2) N protein detection kit
performance Validation**

Product name: New Coronavirus (2019-nCoV / SARS-CoV-2) N Protein Detection Kit
(Fluorescence Immunochromatography)

Manufacturer: Beijing Savant Biotechnology Co., Ltd.

Address: 14#, 4F, No. 2 Kechuang East 5th Street, Tongzhou District, Beijing, China

CLIA Laboratory: Genview Diagnosis Inc.

Address: 5252 Hollister Street Suite 520 Houston TX 77040 USA

Operator: Dr. Jacky Deng

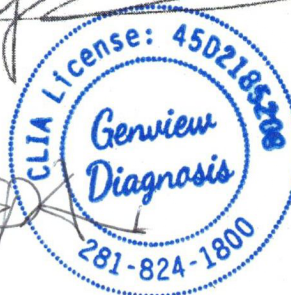
Signature:

CLIA Laboratory Director: Dr. Bosong Dai

Signature:

Data: 01/08/2021

CONFIDENTIAL



1. Purpose

For performance evaluation, verify the cross-reactivity with the reagent in study.

2. Material

2.1. All viruses are order from ZeptoMetrix Corporation (see attachment for detailed information)

Table 1 Detail of pathogens or organisms

No.	Pathogens or microorganisms	Type	Source	N>=5	Titer U/ml or TCID ₅₀ /ml
1	Human coronavirus 229E	Strain 229E	zepto metrix	1	5.62×10^5 TCID ₅₀ /ml
2	Human coronavirus OC43	Strain OC43	zepto metrix	1	4.17×10^5 TCID ₅₀ /ml
3	Human coronavirus NL63	Strain NL63	zepto metrix	1	1.41×10^5 TCID ₅₀ /ml
4	Adenovirus (e.g. C1 Ad. 71)	Type:1(Species C)	zepto metrix	6	4.17×10^5 TCID ₅₀ /ml
5		Type:4(Species E)	zepto metrix		4.17×10^5 TCID ₅₀ /ml
6		Type:7A(Species B)	zepto metrix		4.17×10^5 TCID ₅₀ /ml
7		Type:8(Species D)	zepto metrix		$1 \times 10^{5.15}$ TCID ₅₀ /ml
8		Type:31(Species A)	zepto metrix		1.41×10^5 TCID ₅₀ /ml
9		Type:41(Species F) Strain:Tak	zepto metrix		4.57×10^6 TCID ₅₀ /ml

10	Human Metapneumovirus (hMPV)	hMPV-3 Type: B1 Strain:Peru2-2002	zepto metrix	4	3.89×10^5 TCID ₅₀ /ml
11		hMPV-4 Type: B2 Strain:Peru1-2002	zepto metrix		1.05×10^6 TCID ₅₀ /ml
12		hMPV-9 Type: A1 Strain:IA3-2002	zepto metrix		1.17×10^5 TCID ₅₀ /ml
13		hMPV-27 Type: A2 Strain:IA27-2004	zepto metrix		3.80×10^6 TCID ₅₀ /ml
14	Parainfluenza virus 1-4	Type 1	zepto metrix	5	5.01×10^5 TCID ₅₀ /ml
15		Type 2	zepto metrix		1.51×10^6 TCID ₅₀ /ml
16		Type 3	zepto metrix		1.70×10^5 TCID ₅₀ /ml
17		Type 4A	zepto metrix		4.17×10^5 TCID ₅₀ /ml
18		Type 4B	zepto metrix		1.70×10^5 TCID ₅₀ /ml
19	Influenza A	H1N1 Strain:New Caledonia/20/99	zepto metrix	3	1.41×10^5 TCID ₅₀ /ml
20		H3N2 StrainLWisconsin/67/05	zepto metrix		1.41×10^5 TCID ₅₀ /ml
21	Influenza B	Strain:Florida/02/06	zepto metrix		1.26×10^6 TCID ₅₀ /ml
22	Enterovirus	Type 71 Isolate:2003 Isolate	zepto metrix	2	4.57×10^6 TCID ₅₀ /ml
23		Type:68 Isolate:2007 Isolate	zepto metrix		1.51×10^6 TCID ₅₀ /ml
24	Respiratory syncytial virus	Type:A(RSV-A) Isolate:2006 Isolate	zepto metrix	2	5.01×10^5 TCID ₅₀ /ml
25		Type:B(RSV-B) Strain:CH93(18)-18	zepto metrix		1.70×10^5 TCID ₅₀ /ml

26	Rhinovirus	Type: 1A	zepto metrix	1	1.41×10^5 TCID ₅₀ /ml
27	<i>Haemophilus influenzae</i>	Type: B Eagan	zepto metrix	1	5.43×10^8 CFU/ml
28	<i>Streptococcus pneumoniae</i>	Z022 19F	zepto metrix	1	4.16×10^8 CFU/ml
29	<i>Streptococcus pyogenes</i>	Z018	zepto metrix	1	4.07×10^9 CFU/ml
30	<i>Candida albicans</i>	Z006	zepto metrix	1	4.50×10^8 CFU/ml
31	<i>Bordetella pertussis</i>	A639	zepto metrix	1	6.43×10^9 CFU/ml
32	<i>Mycoplasma pneumoniae</i>	M129	zepto metrix	1	2.70×10^8 CFU/ml
33	<i>Chlamydia pneumoniae</i>	Z022, 19F	zepto metrix	1	4.16×10^8 CFU/ml
34	<i>Legionella pneumophila</i>	Philadelphia	zepto metrix	1	1.42×10^{10} CFU/ml
35	<i>Mycobacterium tuberculosis</i>	H37Ra-1	zepto metrix	1	6.32×10^7 CFU/ml
36	SARS-CoV-2 N Protein, nucleocapsid protein		RayBiotech	1	1mg/ml

2.2. Reagent

Table 2

	Reagent
Manufacturer	Beijing Savant Biotechnology Co., Ltd.
Product	New Coronavirus (2019-nCoV / SARS-CoV-2) N Protein Detection Kit (Fluorescence Immunochromatography)

2.3. Positive reference

Recombinant SARS-CoV-2 Nucleocapsid Protein purchased from RayBiotech (CODE: 230-01104).

3. Method

3.1 Pre-experiment: The recombinant N protein was diluted to 100ng/ml with the sample preservation solution provide with kit, the blank (sample preservation solution) and 100ng/ml samples were tested at the same time. If the blank sample was negative and the 100ng/ml sample was positive, the operation was continued 3.2 – 3.4. If not, find out cause.

3.2 The concentration of the sample to be tested (table 1) should be no less than 1×10^5 TCID₅₀/mL or 1×10^5 pfu/ml. If dilution is needed, the sample preservation solution in the kit shall be used;

3.3 The test was performed in three times. Please refer to the instructions for use of the kit.

3.4 If the test result is positive, there is a cross reaction between the reagent and the sample; If the test result is negative, there is no cross-reaction between the reagent and the sample.

4. Results and analysis

4.1. Results

Table 3 Results of the pre-experiment

NO.	Substance	Result		
		1#	2#	3#
1	Sample preservation solutin	Negative	Negative	Negative
2	100ng/ml SARS-CoV-2 N Protein	Positive	Positive	Positive
3	50ng/ml SARS-CoV-2 N Protein	Positive	Positive	Positive

Table 4 Results of cross-reactivity

NO.	Substance	Type	Tested Concentration/ Titer	Result		
				1#	2#	3#
1	Human coronavirus 229E	Strain 229E	5.62×10^5 TCID₅₀/ml	Negative	Negative	Negative
2	Human coronavirus OC43	Strain OC43	4.17×10^5 TCID₅₀/ml	Negative	Negative	Negative
3	Human coronavirus NL63	Strain NL63	1.41×10^5 TCID₅₀/ml	Negative	Negative	Negative
4	Adenovirus	Type:1(Specie	4.17×10^5 TCID₅₀/ml	Negative	Negative	Negative

		s C)				
5	Adenovirus	Type:4(Species E)	4.17×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
6	Adenovirus	Type:7A(Species B)	4.17×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
7	Adenovirus	Type:8(Species D)	$1 \times 10^{5.15}$ TCID ₅₀ /ml	Negative	Negative	Negative
8	Adenovirus	Type:31(Species A)	1.41×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
9	Adenovirus	Type:41(Species F) Strain:Tak	4.57×10^6 TCID ₅₀ /ml	Negative	Negative	Negative
10	Human Metapneumovirus 3 (hMPV)	hMPV-3 Type: B1 Strain:Peru2-2002	3.89×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
11	Human Metapneumovirus 4 (hMPV)	hMPV-4 Type:B2 Strain:Peru1-2002	1.05×10^6 TCID ₅₀ /ml	Negative	Negative	Negative
12	Human Metapneumovirus 9 (hMPV)	hMPV-9 Type:A1 Strain:IA3-2002	1.17×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
13	Human Metapneumovirus 27 (hMPV)	hMPV-27 Type:A2 Strain:IA27-2004	3.80×10^6 TCID ₅₀ /ml	Negative	Negative	Negative
14	Parainfluenza virus 1	Type 1	5.01×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
15	Parainfluenza virus 2	Type 2	1.51×10^6 TCID ₅₀ /ml	Negative	Negative	Negative
16	Parainfluenza virus 3	Type 3	1.70×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
17	Parainfluenza virus 4	Type 4A	1.04×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
18	Parainfluenza virus 4B	Type 4B	1.00×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
19	Influenza A	H1N1 Strain:New Caledonia/20/99	1.00×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
20	Influenza A	H3N2 StrainLWisconsin/67/05	1.41×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
21	Influenza B	Strain:Florida/02/06	1.26×10^5 TCID ₅₀ /ml	Negative	Negative	Negative
22	Enterovirus	Type 71 Isolate:2003 Isolate	4.57×10^6 TCID ₅₀ /ml	Negative	Negative	Negative

23	Enterovirus	Type:68 Isolate:2007 Isolate	1.51×10⁵ TCID₅₀/ml	Negative	Negative	Negative
24	Respiratory syncytial virus	Type:A(RSV-A) Isolate:2006 Isolate	5.01×10⁵ TCID₅₀/ml	Negative	Negative	Negative
25	Respiratory syncytial virus	Type:B(RSV-B) Strain:CH93(18)-18	1.70×10⁵ TCID₅₀/ml	Negative	Negative	Negative
26	Rhinovirus	Type: 1A	1.00×10⁵ TCID₅₀/ml	Negative	Negative	Negative
27	<i>Haemophilus influenzae</i>	<i>Type:B Eagan</i>	5.43×10⁸ CFU/ml	Negative	Negative	Negative
28	<i>Streptococcus pneumoniae</i>	<i>Z022 19F</i>	4.16×10⁸ CFU/ml	Negative	Negative	Negative
29	<i>Streptococcus pyogenes</i>	<i>Z018</i>	4.07×10⁹ CFU/ml	Negative	Negative	Negative
30	<i>Candida albicans</i>	<i>Z006</i>	4.50×10⁸ CFU/ml	Negative	Negative	Negative
31	<i>Bordetella pertussis</i>	<i>A639</i>	6.43×10⁸ CFU/ml	Negative	Negative	Negative
32	<i>Mycoplasma pneumoniae</i>	<i>M129</i>	2.70×10⁷ CFU/ml	Negative	Negative	Negative
33	<i>Chlamydia pneumoniae</i>	<i>Z022,19F</i>	4.16×10⁸ CFU/ml	Negative	Negative	Negative
34	<i>Legionella pneumophila</i>	<i>Philadelphia</i>	1.42×10⁹ CFU/ml	Negative	Negative	Negative
35	<i>Mycobacterium tuberculosis</i>	<i>H37Ra-1</i>	6.32×10⁶ CFU/ml	Negative	Negative	Negative

4.2. Analysis

It is verified that the test results of the pathogen/microorganism samples in Table 1 are all negative, indicating that there is no cross-reaction between the reagent and sample.

5. Conclusion

The results of cross-reactivity between the reagents and the pathogen/microorganism in Table 1 were all negative, showed that the specificity of the reagents was good and there was no cross reaction.

Appendix