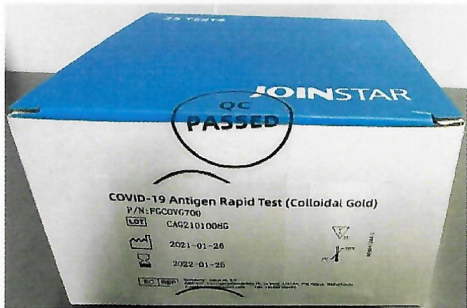
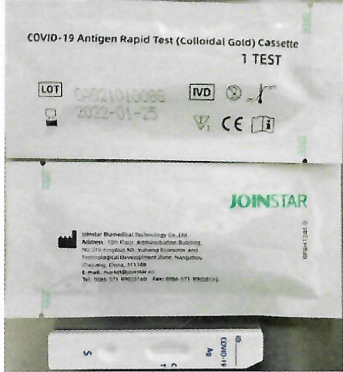


Standard Operating Procedure



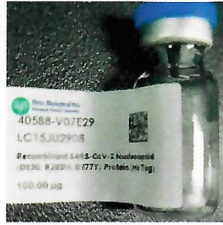
Operation Name	Sensitivity test of Joinstar COVID-19 Antigen Rapid Test (Colloidal Gold) to SARS-CoV-2 Variants	Document number	hCoV-(R20210704)		
Drafter	张咪	Proofreader	吴王荣	Reviewer	范春雷 Prof. Chunlei Fan
Number of pages	9	Implementation department	CELL BIOLOGY	Personnel allocation	2

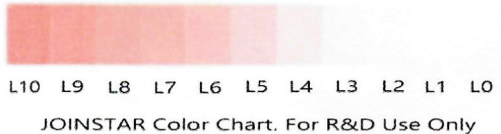
Experiment A	Sample source and grouping design	Experimenter	张咪	Experimental time	2021.07.04
Procedures	Standards		Detection reagent		
Samples	1. SARS-CoV-2 N-Nucleocapsid Recombinant Protein (Hangzhou bioeast biotech. Co., Ltd. , Cat: SC2N301, Lot#20200430), [Wild Type]		COVID-19 Antigen Rapid Test (Colloidal Gold): Joinstar Biomedical Technology Co.,Ltd; Lot#: CAG2101008G  		
	2. SARS-CoV-2 (2019-nCoV) Nucleocapsid(D3L, R203K, G204R, S235F)-His Recombinant Protein (Sino Biological , Cat: 40588-V07E7, Lot#ME15MY0502), [Alpha B.1.1.7 (U.K.)]				
	3. SARS-CoV-2 (2019-nCoV) Nucleocapsid (T205I) Protein (His Tag)(Sino Biological, Cat: 440588-V07E9, Lot#ME15MA0312), [Beta B.1.351 (South Africa)] [Epsilon B.1.427/B.1.429 (U.S.A)]				
	4. SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein (His Tag)(Sino Biological, Cat: 40588-V07E29, Lot#LC15JU2908), [Delta B.1.617.2(India)]				
	5. SARS-CoV-2 (2019-nCoV) Nucleocapsid (P80R) Protein (His Tag)(Sino Biological, Cat: 40588-V07E11, Lot#ME15MA0313), [Gamma P.1 (Brazil)]				



Grouping design:

Group		Results																	
Negative control	Standard concentration (ng/ml)	0																	
	Test strip number	01			02			03			04			05			06		
	Chromatic value																		
1. SARS-CoV-2 N-Nucleocapsid Protein [Wild Type]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01		
	Test strip number	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	Chromatic value																		
2. 2019-nCoV Nucleocapsid (D3L, R203K, G204R, S235F) Protein [Alpha B.1.1.7 (U.K.)]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01		
	Test strip number	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
	Chromatic value																		
3. 2019-nCoV Nucleocapsid (T2051) Protein [Beta B.1.351 (South Africa) Epsilon B.1.427/B.1.429 (U.S.A.)]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01		
	Test strip number	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
	Chromatic value																		
4. SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein [Delta B.1.617.2 (India)]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01		
	Test strip number	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78
	Chromatic value																		
5. 2019-nCoV Nucleocapsid (P80R) Protein [Gamma P.1 (Brazil)]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01		
	Test strip number	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
	Chromatic value																		

Experiment B	Experiment preparation	Experimenter	张咪	Experimental time	2021.07.04
Procedures	Reagents and Instruments		Preparation and matters needing attention		
Experiment preparation	1. Sample diluent			Sample diluent provided by Joinstar	
	2. SARS-CoV-2 N-Nucleocapsid Recombinant Protein (Hangzhou bioeast biotech. Co., Ltd. , Cat: SC2N301, Lot#20200430), [Wild Type]			The concentration of the original solution was 3.02 mg/mL and diluted with sample diluent to 5ng/ml, 1ng/ml, 0.5ng/ml, 0.1ng/ml, 50pg/ml, and 10pg/ml respectively.	
	3. SARS-CoV-2 Nucleocapsid (D3L, R203K, G204R, S235F)-His Recombinant Protein (Sino Biological , Cat: 40588-V07E7, Lot#ME15MY0502), [Alpha B.1.1.7 (U.K.)]			The concentration of the original solution was 1.33mg/mL and diluted with sample diluent to 5ng/ml, 1ng/ml, 0.5ng/ml, 0.1ng/ml, 50pg/ml, and 10pg/ml respectively.	
	4. SARS-CoV-2 Nucleocapsid (T205I) Protein (His Tag)(Sino Biological, Cat: 440588-V07E9, Lot#ME15MA0312), [Beta B.1.351 (South Africa)] [Epsilon B.1.427/B.1.429 (U.S.A)]			The concentration of the original solution was 1.48 mg/mL and diluted with sample diluent to 5ng/ml, 1ng/ml, 0.5ng/ml, 0.1ng/ml, 50pg/ml, and 10pg/ml respectively.	
	5. SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein (His Tag)(Sino Biological, Cat: 40588-V07E29, Lot#LC15JU2908), [Delta B.1.617.2(India)]			500 µL of sterile water was added to the vial to prepare a stock solution of 0.2 mg/ml, and then diluted with sample diluent to 5ng/ml, 1ng/ml, 0.5ng/ml, 0.1ng/ml, 50pg/ml, and 10pg/ml respectively.	
	6. SARS-CoV-2 Nucleocapsid (P80R) Protein (His Tag)(Sino Biological, Cat: 40588-V07E11, Lot#ME15MA0313), [Gamma P.1 (Brazil)]			The concentration of the original solution was 2.31 mg/mL and diluted with sample diluent to 5ng/ml, 1ng/ml, 0.5ng/ml, 0.1ng/ml, 50pg/ml, and 10pg/ml respectively.	

	7. Chroma determination color card	 <p>L10 L9 L8 L7 L6 L5 L4 L3 L2 L1 L0 JOINSTAR Color Chart. For R&D Use Only</p>
	8. Before the experiment, check the pipetting gun (tip) and other consumables are sufficient and sterilized	

Experiment C	Test report	Experimental time	2021.07.04	Reviewer	范春雷 Prof. Chunlei Fan
Experimenter	张咪	Delivery time	2021.07.05	Review time	2021.07.06

Experimental procedure

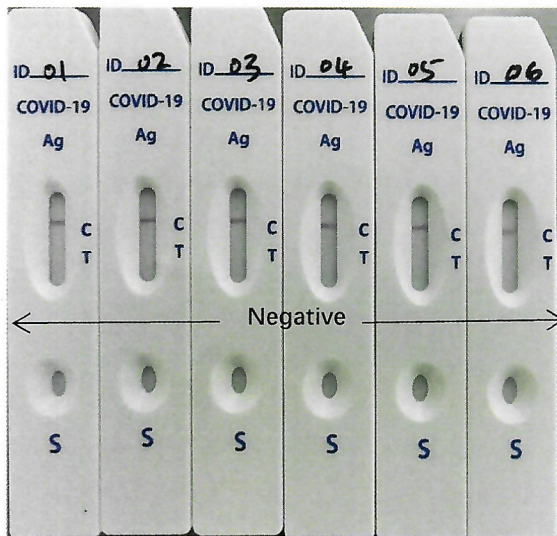
In this experiment, the sensitivity of the COVID-19 Antigen Rapid Test (Colloidal Gold) (Lot#: CAG2101008G) produced by Joinstar Biomedical Technology Co.,Ltd was determined by SARS-CoV-2 Nucleocapsid Recombinant Protein (Hangzhou bioeast biotech. Co., Ltd., Cat#: SC2N301, Lot#20200430),[Wild Type]; SARS-CoV-2 Nucleocapsid (D3L, R203K, G204R, S235F)-His Recombinant Protein (Sino Biological , Cat#: 40588-V07E7, Lot#ME15MY0502),[Alpha | B.1.1.7 (U.K.)]; SARS-CoV-2 Nucleocapsid (T205I) Protein (His Tag)(Sino Biological, Cat: 440588-V07E9, Lot#ME15MA0312), [Beta | B.1.351 (South Africa)][Epsilon | B.1.427/B.1.429 (U.S.A)]; SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein (His Tag)(Sino Biological, Cat: 40588-V07E29, Lot#LC15JU2908), [Delta | B.1.617.2(India)]; SARS-CoV-2 Nucleocapsid (P80R) Protein (His Tag)(Sino Biological, Cat: 40588-V07E11, Lot#ME15MA0313), [Gamma | P.1 (Brazil)].

Experimental procedure:

1. Take 97 test strips of Lot#CAG2101008G , grouped and numbered according to the grouping table, and placed flat on the desktop;
2. Add sample according to the contents of the grouping table, 50µl/well;
3. The results were observed and photographed after standing at room temperature for 15-20 minutes.

Graph data summary

1. Negative control



2. SARS-CoV-2 Nucleocapsid Recombinant Protein (Hangzhou bioeast biotech. Co., Ltd., Cat#: SC2N301, Lot#20200430), [Wild Type]



3. SARS-CoV-2 (2019-nCoV) Nucleocapsid(D3L, R203K, G204R, S235F)-His Recombinant Protein (Sino Biological, Cat: 40588-V07E7, Lot#ME15MY0502), [Alpha | B.1.1.7 (U.K.)]

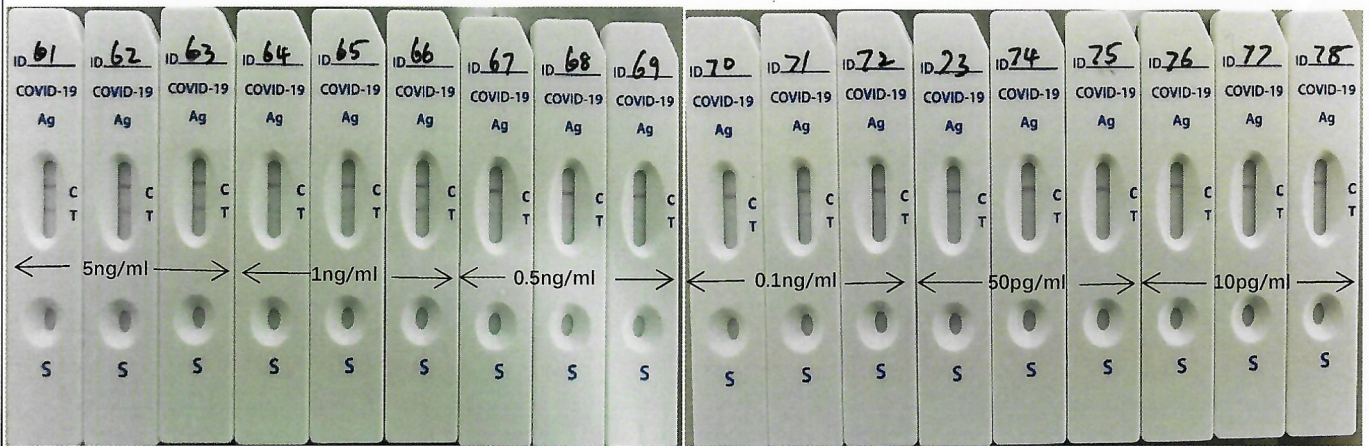


4. SARS-CoV-2 (2019-nCoV) Nucleocapsid (T205I) Protein (His Tag)(Sino Biological, Cat: 440588-V07E9, Lot#ME15MA0312), [Beta | B.1.351 (South Africa)] [Epsilon | B.1.427/B.1.429 (U.S.A)]



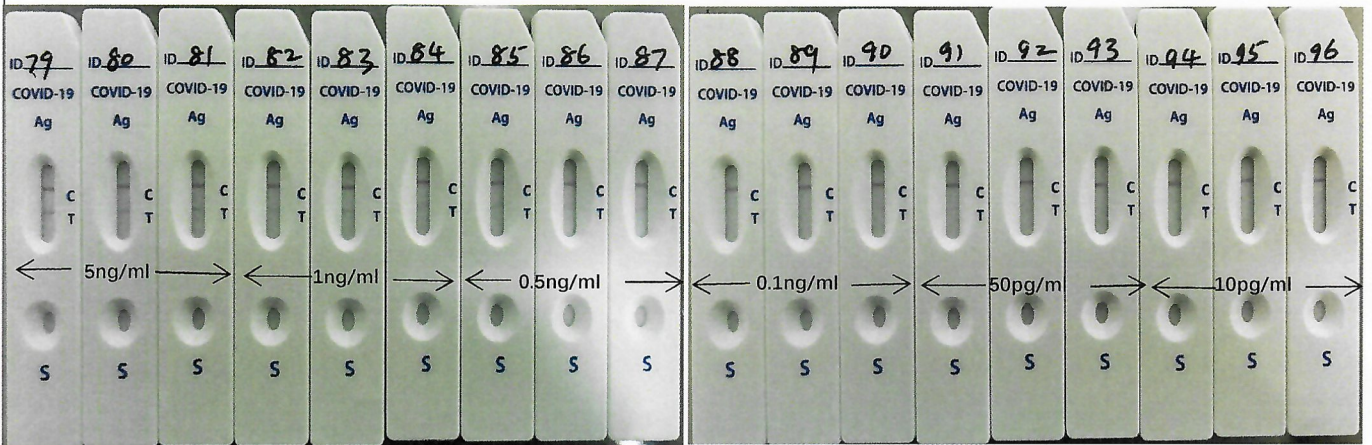
5. SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein (His Tag)(Sino Biological, Cat: 40588-V07E29, Lot#LC15JU2908),

[Delta | B.1.617.2(India)]



6. SARS-CoV-2 (2019-nCoV) Nucleocapsid (P80R) Protein (His Tag)(Sino Biological, Cat: 40588-V07E11, Lot#ME15MA0313),

[Gamma | P.1 (Brazil)]



Group		Results																							
Negative control	Standard concentration (ng/ml)	0																							
	Test strip number	01			02			03			04			05			06								
	Chromatic value	0			0			0			0			0			0								
1.SARS-CoV-2 N-Nucleocapsid Protein [Wild Type]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01								
	Test strip number	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24						
	Chromatic value	8	8+	8+	8	6	7	6	6	5	3	4	3	3	2	2	2	2	2						
6. 2019-nCoV Nucleocapsid(D3L,R203K, G204R,S235F) Protein [Alpha B.1.1.7 (U.K.)]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01								
	Test strip number	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42						
	Chromatic value	8+	8+	8+	7	7	7	5+	5+	5	4	4	4	2	3	3	4	2	3+						
7. 2019-nCoV Nucleocapsid (T205I) Protein [Beta B.1.351 (South Africa)] [Epsilon B.1.427/B.1.429 (U.S.A)]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01								
	Test strip number	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60						
	Chromatic value	8	9	9	8	7+	8	7+	7	7	5	6	6	5	6	5	2	2	2						
8. SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein [Delta B.1.617.2(India)]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01								
	Test strip number	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78						
	Chromatic value	8	8	9	7	7	8	5	5	6	5	6	6	5	3	5	2	2	2						
9. 2019-nCoV Nucleocapsid (P80R) Protein [Gamma P.1 (Brazil)]	Standard concentration (ng/ml)	5			1			0.5			0.1			0.05			0.01								
	Test strip number	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96						
	Chromatic value	8	8	8+	7	8	6	6	5	6	3	3+	4	4	3	4	2	2	2						

Conclusion:

The COVID-19 Antigen Rapid Test (Colloidal Gold) (Lot#: CAG2101008G) produced by Joinstar Biomedical Technology Co.,Ltd was sensitive to SARS-CoV-2 Nucleocapsid Recombinant Protein (Hangzhou bioeast biotech. Co., Ltd., Cat#: SC2N301, Lot#20200430),[Wild Type]; SARS-CoV-2 Nucleocapsid (D3L, R203K, G204R, S235F)-His Recombinant Protein (Sino Biological , Cat# : 40588-V07E7, Lot#ME15MY0502),[Alpha | B.1.1.7 (U.K.)]; SARS-CoV-2 Nucleocapsid (T205I) Protein (His Tag)(Sino Biological, Cat : 440588-V07E9, Lot#ME15MA0312), [Beta | B.1.351 (South Africa)][Epsilon | B.1.427/B.1.429 (U.S.A)];

SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein (His Tag)(Sino Biological, Cat: 40588-V07E29, Lot#LC15JU2908), **[Delta | B.1.617.2(India)]**; SARS-CoV-2 Nucleocapsid (P80R) Protein (His Tag)(Sino Biological, Cat: 40588-V07E11, Lot#ME15MA0313), **[Gamma | P.1 (Brazil)]**, with a sensitivity of 10pg/ml.