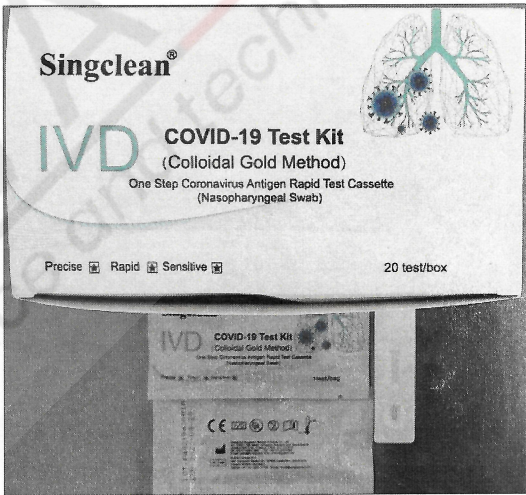
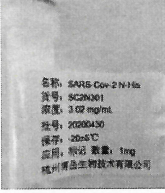


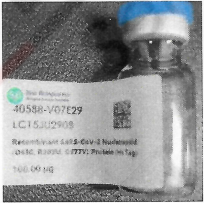
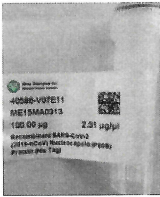


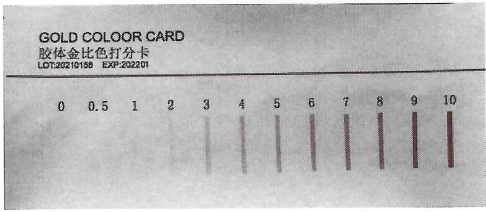
| Standard Operating Procedure | | | | | |
|-------------------------------------|--|----------------------------------|------------------------|-----------------------------|--------------------------|
| Operation Name | Sensitivity test of Singclean® COVID-19 Test Kit(Colloidal Gold Method) to SARS-CoV-2 Variants | | Document number | nCoV-(R20210729) | |
| Drafter | 张咪 | Proofreader | 吴圣东 | Reviewer | 范磊磊 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 | 2021.07.28 |
|-------------------|---|--------------|---|-------------------|------------|
| Procedures | Standards | | Detection reagent | | |
| Samples | 1. SARS-CoV-2 N-Nucleocapsid Recombinant Protein (Hangzhou bioeast biotech. Co., Ltd. , Cat: SC2N301, Lot#20200430), [Wild Type] | | Singclean® COVID-19 Test Kit(Colloidal Gold Method), One Step Coronavirus Antigen Rapid Test Cassette (Nasopharyngeal Swab): Hangzhou Singclean Medical Products Co.,Ltd; Lot#: P49210430A1A  | | |
| | 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 (T2051) 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 | | | 05 | | | 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 | | | 05 | | | 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 (T205I) Protein [Beta B.1.351 (South Africa) Epsilon B.1.427/B.1.429 (U.S.A.)] | Standard concentration (ng/ml) | 5 | | | 1 | | | 05 | | | 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 | | | 05 | | | 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 | | | 05 | | | 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 | 2021.07.20 |
|-------------------------------|---|---|---|-------------------|------------|
| 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] |  <p>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.</p> | | | |
| | 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.)] |  <p>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.</p> | | | |
| | 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)] |  <p>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.</p> | | | |
| | 5. SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein (His Tag)(Sino Biological, Cat: 40588-V07E29, Lot#LC15JU2908), [Delta B.1.617.2(India)] |  <p>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.</p> | | | |
| | 6. SARS-CoV-2 Nucleocapsid (P80R) Protein (His Tag)(Sino Biological, Cat: 40588-V07E11, Lot#ME15MA0313), [Gamma P.1 (Brazil)] |  <p>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.</p> | | | |

| | | |
|--|---|--|
| | 7. Chroma determination color card |  |
| | 8. Before the experiment, check the pipetting gun (tip) and other consumables are sufficient and sterilized | |

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|---------------------|-------------|--------------------------|------------|--------------------|--------------------------|
| Experiment C | Test report | Experimental time | 2021.07.28 | Reviewer | 范春雷 Prof. Chunlei Fan |
| Experimenter | 张咪 | Delivery time | 2021.07.28 | Review time | 2021.07.29 |

Experimental procedure

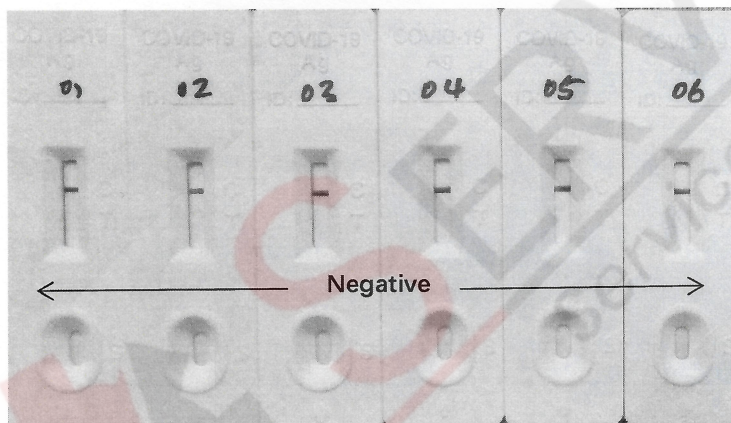
In this experiment, the sensitivity of Singleclean® COVID-19 Test Kit(Colloidal Gold Method), One Step Coronavirus Antigen Rapid Test Cassette (Nasopharyngeal Swab)(Lot#: P49210430A1A) produced by Hangzhou Singleclean Medical Products Co.,Ltd was determined by SARS-CoV-2 Nucleocapsid Recombinant Protein (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:

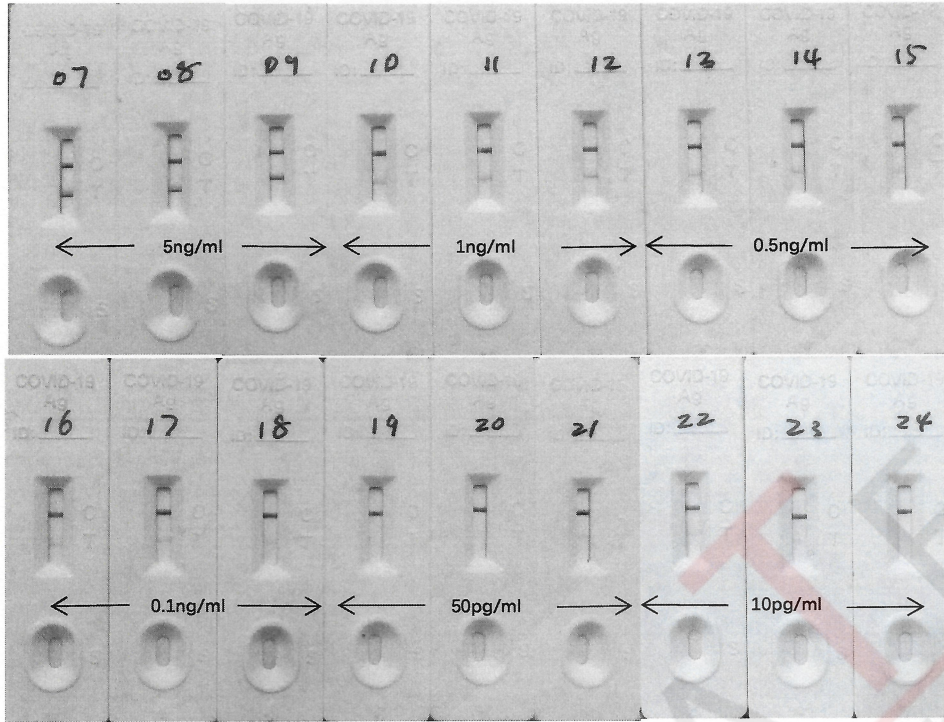
1. Take 96 test strips of Lot#P49210430A1A , grouped and numbered according to the grouping table, and placed flat on the desktop;
2. Add sample according to the contents of the grouping table, 100µl/well;
3. The results were observed and photographed after standing at room temperature for 15-20 minutes.

Graph data summary

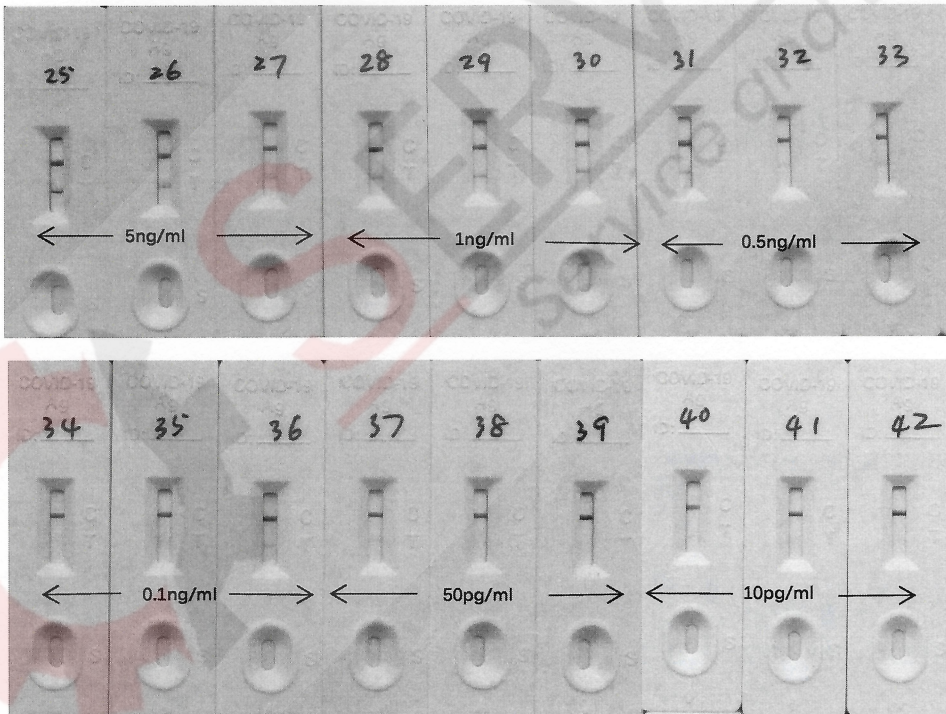
1. Negative control



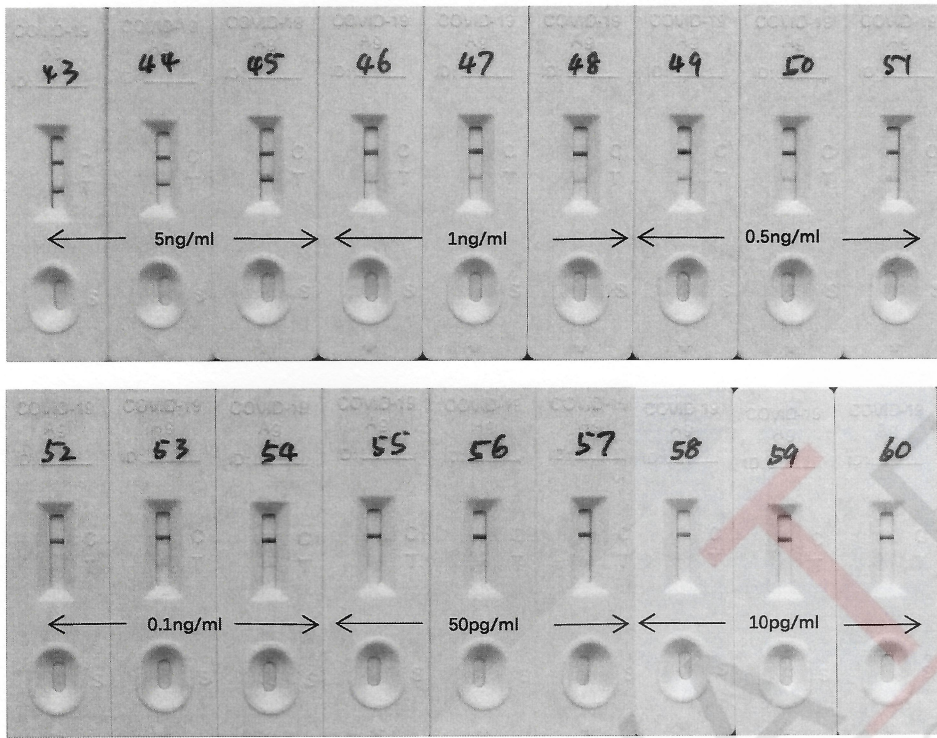
2. SARS-CoV-2 Nucleocapsid Recombinant Protein (Hangzhou bioeast biotech. Co., Ltd., Cat#: SC2N301, Lot#20200430), [Wild Type]



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)]

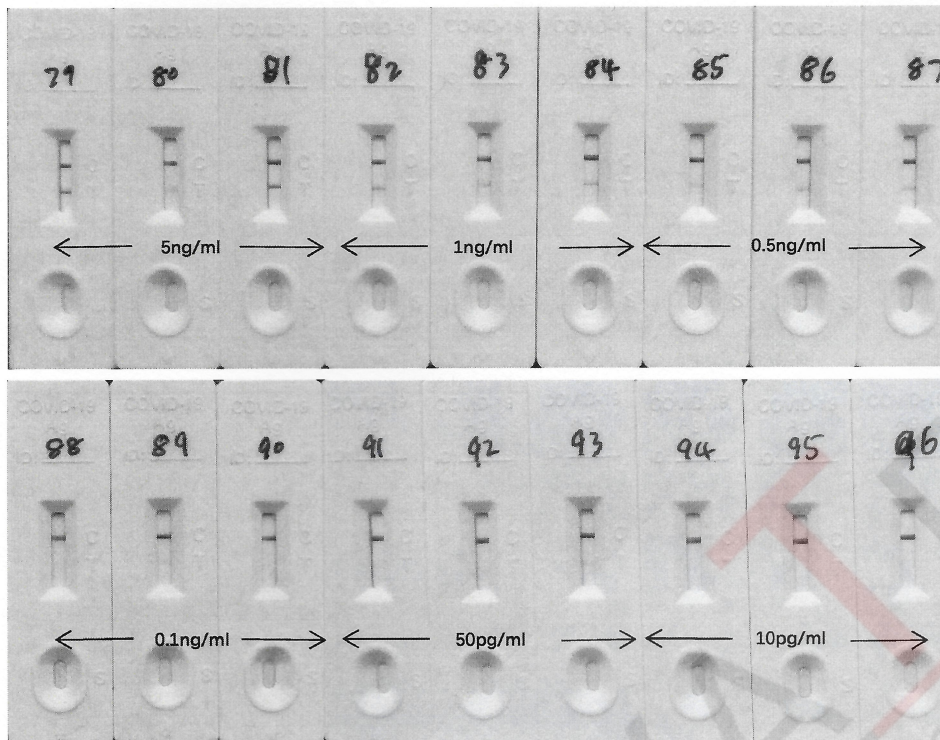


5. SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) Protein (His Tag)(Sino Biological, Cat: 40588-V07E29, Lot#LC15JU2908),
[Delta | B.1.617.2(India)]



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 | 10 | 10 | 10 | 7 | 7 | 7 | 6 | 6 | 6 | 5 | 5 | 5 | 2 | 2 | 4 | 4 | 3+ | 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 | 10 | 10 | 8 | 9 | 9 | 8 | 7 | 4 | 5 | 3 | 5 | 4 | 5 | 3+ | 4 | 2 | 3 | 3 |
| 7. 2019-nCoV) Nucleocapsid (T2051) | Standard concentration | 5 | | | 1 | | | 0.5 | | | 0.1 | | | 0.05 | | | 0.01 | | |

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|---|--------------------------------|----|----|----|----|----|----|----|----|----|-----|----|----|------|----|----|------|----|----|
| Protein | (ng/ml) | | | | | | | | | | | | | | | | | | |
| [Beta B.1.351 (South Africa)] | Test strip number | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 |
| [Epsilon B.1.427/B.1.429 (U.S.A)] | Chromatic value | 10 | 10 | 10 | 8 | 9 | 8 | 9 | 7 | 6 | 3 | 4 | 3+ | 6 | 3+ | 3 | 2 | 1 | 3 |
| 8. SARS-CoV-2 Nucleocapsid (D63G, R203M, D377Y) | Standard concentration (ng/ml) | 5 | | | 1 | | | 05 | | | 0.1 | | | 0.05 | | | 0.01 | | |
| Protein | Test strip number | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 |
| [Delta B.1.617.2(India)] | Chromatic value | 10 | 10 | 10 | 8 | 9 | 7 | 3 | 5 | 5 | 4 | 5 | 5 | 4 | 2 | 2 | 1 | 1 | 1 |
| 9. 2019-nCoV Nucleocapsid (P80R) | Standard concentration (ng/ml) | 5 | | | 1 | | | 05 | | | 0.1 | | | 0.05 | | | 0.01 | | |
| Protein | Test strip number | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 |
| [Gamma P.1 (Brazil)] | Chromatic value | 9 | 5 | 10 | 8 | 7 | 7 | 5 | 6 | 7 | 1 | 3 | 1 | 4 | 3 | 3 | 1 | 1 | 1 |

Conclusion:

The Singclean® COVID-19 Test Kit(Colloidal Gold Method), One Step Coronavirus Antigen Rapid Test Cassette (Nasopharyngeal Swab) (Lot#: P49210430A1A) produced by Hangzhou Singclean Medical Products 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.

Experiments prove that product of Singclean COVID-19 Test Kit (Colloidal Gold Method), One Step Coronavirus Antigen Rapid Test Cassette (Nasopharyngeal Swab) can detect the above-mentioned COVID-19 Variants.