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论文题目： 通过假病毒界定新型冠状病毒抗原检测试剂盒最低检出限的研究

通过假病毒界定新型冠状病毒抗原检测试剂盒最低检出限的研究

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Abstract

Coronavirus disease (COVID-19) refers to a disease caused by the novel coronavirus SARS-CoV-2. Since the first discovery in 2019, the disease has spread rapidly into a pandemic. To control the pandemic, the detection of the SARS-CoV-2 virus to determine infection is essential. Currently, testing for SARS-CoV-2 infection mainly relies on qPCR-based nucleic acid testing. However, nucleic acid testing takes hours to complete, and has high requirements for instrumentation and operation, thus takes great cost to complete. As antigen-testing has low requirements for instrumentation and operation and can provide results within 15 minutes, it is commonly used for initial testing. However, sensitivity of current antigen-testing kits is about 70%, which cannot be used as a solid basis for diagnosis. To make antigen-testing results more reliable, it is important to explore factors that influence the sensitivity of antigen-testing kits.

Currently, studies have shown viral load affects sensitivity of antigen-testing, but the minimum detection limit of antigen-testing kits in China has not been reported. Besides, human tissues and various medicines can possibly affect sensitivity of antigen detection. Therefore, this study will conduct antigen-testing with pseudoviruses, explore the effect of medicines and human tissue on antigen-testing, and test the minimum detection limit of antigen detection kits.

In this study, psuedoviruses featuring SARS-CoV-2 N protein are used to prepare antigen-testing samples. Large-scale concentration screening is conducted with antigen-testing kits. Based on the critical point of detection in the previous screening, a small-scale concentration gradient is established. Further antigen-testing is carried out to determine the minimum detection limit of antigen-testing kits. In the small-scale screening, different medicines

are added to the sample to simulate real-life sampling, and to explore the effects of medicines on antigen-testing. The samples used for the test were tested by qPCR to confirm the accuracy of the antigen-testing.

The innovation of this study is the use of psudeoviruses to prepare standard samples, thus determining the minimum detection limit of antigen-testing kits and the effects of medicines on antigen-testing. The results of this study will reveal the minimum detection limit of antigen-testing kits, facilitating interpretation of antigen-testing results.

Keywords: SARS-CoV-2, antigen-testing, minimum detection limit, pseudoviruses

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1. Introduction

Coronavirus pneumonia (COVID-19) refers to a disease caused by the novel coronavirus SARS-CoV-2. Since the first identification in 2019, the disease spread rapidly, evolving into a global pandemic[1]. According to the World Health Organization, most patients are able to self-heal after infection, yet elder people and people with underlying medical conditions are more likely to develop severe symptoms. Moreover, patients of any age and any physical condition can potentially have severe or life-threatening conditions[2]. Because of the scale of its impact, COVID-19 is currently an area of concern for scientists around the world.

As the pandemic develops and SARS-CoV-2 mutates, asymptomatic infections emerged. Therefore, in the prevention and control of the epidemic, the detection of the SARS-CoV-2 virus to determine infection became critical. Currently, testing for SARS-CoV-2 infection relies heavily on RT-PCR-based nucleic acid testing[3]. This assay typically targets SARS-CoV-2 envelope proteins, RNA-dependent RNA polymerase, nucleocapsid protein gene sequences, or viral genome open reading frame ORF1a and ORF1b[4]. However, nucleic acid testing generally takes hours to complete, and the testing process has high requirements for instrumentation and operation, thus is slow in process and expensive in implementation.

Antigen-testing is widely used for initial screening of SARS-CoV-2 infection due to its low requirements for instrumentation and operations and the fact that results can be detected quickly within 15 minutes. Currently, antigen detection kits approved by National Medical Products Administration of China mainly use colloidal gold method[5]. By this method, viral antigens first combine with colloidal gold, and then combine with antigens embedded in T-line to display color. Usually, antigen-testing kits target SARS-CoV-2 N proteins[6]. However, the sensitivity of current antigen-testing kits is around 70%, thus cannot be used as a solid basis for

diagnosis[7]. In order to improve reliability of antigen-testing result, it is critical to explore the factors that influence the sensitivity of antigen-testing kits.

Minimum detection limit of antigen-testing kits refers to the lowest copies of viruses that can be detected by antigen-testing kits with an accuracy between 90% to 95%. Minimum detection limit of antigen-testing kits is crucial to the interpretation of antigen-testing results, indicating the range of viral load the tests are applicable to[8]. To provide a solid basis for the interpretation of antigen-testing results, it is crucial to investigate the minimum detection limit.

The use of various medicines can also potentially affect sensitivity of antigen-testing kits, thus altering the minimum detection limit. Recently, interferon-alpha2b, a substance produced by cells to resolve infections and commonly used to treat virus infections, is reported to be useful in treatment of COVID-19, where duration of detectable virus in the upper respiratory tract can be significantly reduced[9]. Levofloxacin, a commonly used medicine to treat infections, also evolved to be used in clinical treatment of pneumonia symptoms of COVID-19[10]. In addition, the use of budesonide nasal spray in treatment of COVID-19 patients with chronic respiratory diseases is also proposed[11]. Therefore, to simulate real-life situations with medicines affecting minimum detection limit of antigen-testing kits, it is important to consider the effect of medicines on sensitivity of antigen-testing kits.

Currently, studies have shown that a lower viral load decreases sensitivity of antigen detection[12], but the minimum detection limit of antigen-testing kits in China has not been reported. Meanwhile, the effects of medicines on antigen-testing kits sensitivity have not been investigated. Therefore, this study will conduct antigen-testing with pseudoviruses to investigate the minimum detection limit of antigen-testing kits and to explore the detection effect of antigen-testing kits under the influence of different medicines.

2. Methodology

2.1 Materials and Equipment

Material or Equipment	Manufacturer	Item Number
2019-nCoV Antigen-testing Kit (Colloidal Gold Method)	Shanghai Outdo Biotech Company	20220406
2019-nCoV Antigen-testing Kit (Colloidal Gold Method)	EasyDiagnosis Biology Company	C22030401
2019-nCoV Antigen-testing Kit (Colloidal Gold Method)	Bioscience Biotech Company	20220301
Psuedoviruses Featuring SARS-CoV-2 N Protein	Future therapeutics Biomedical Technology Company	FNRV2565
Human Interferon-alpha2b	Shanghai Jingke Chemistry Company	CYT-520
Levofloxacin	Daiichi Sankyo Pharmaceutical (Beijing) Company	BA01061
Budesonide Nasal Spray	Shanghai Johnson & Johnson Pharmaceuticals Company	VBBX2203014

2.2 Methods

2.2.1 Investigation of Minimum Detection Limit of Antigen-Testing Kits

2.2.1.1 Large Scale Screening

To detect minimum detection limit of SARS-CoV-2 antigen-testing kits of three brands, Outdo, EasyDiagnosis and Bioscience, a large-scale screening is carried out with large concentration gradients, exploring the general range of concentration where T-lines of antigen-testing kits diminish. Psuedoviruses with initial concentration 3.37×10^9 copies/ml is diluted

with SARS-CoV-2 virus lysate into samples with concentration 3.37×10^8 (diluted by 10 times), 3.37×10^6 copies/ml (diluted by 1000 times), 3.37×10^5 copies/ml (diluted by 1×10^4 times), 1.135×10^5 copies/ml (diluted by 2×10^4 times), 5.675×10^4 copies/ml (diluted by 4×10^4 times). For each sample, 70 μl is used for a single antigen-test, with the process repeated for all three brands. After antigen-testing kits are left under room temperature to react for 15 minutes, photos are taken, and T-line deepness of antigen-testing kits are recorded. Based on the result, further dilution is carried out and the same process is repeated until further diluted samples do not display T-line in antigen-testing.

2.2.1.2 Small-Scale Screening

To investigate the precise minimum detection limit of antigen-testing kits, a small scale screening needs to be carried out within closer concentration gradients, thus figuring out the marginal viral concentration at which the antigen-testing kit is able to display positive result, known as its minimum detection limit. Based on results in 2.2.1.1, the lowest detectable concentration and the highest undetectable concentration are selected. Based on the T-line deepness displayed, desired concentrations between the two selected concentrations are planned to be tested for antigen-testing kits produced by different manufacturers, as shown in *Table 2*.

3.37×10^9 copies/ml pseudoviruses is diluted with virus lysate into samples with planned concentrations. For each sample, 70 μl is used for a single antigen test, with 5 repeats. After antigen-testing kits are left under room temperature to react for 15 minutes, photos are taken, and T-line deepness of antigen-testing kits are recorded. Based on the results, further repeats are conducted until a sample with detection rate 95% is discovered.

Antigen-Testing Kit Brand	Selected Concentration Gradient
Outdo	1.872×10^5 copies/ml (Diuted by 1.8×10^4 times)
	1.685×10^5 copies/ml (Diluted by 2×10^4 times)
	1.605×10^5 copies/ml (Diluted by 2.1×10^4 times)
EasyDiagnosis	8.024×10^4 copies/ml (Diluted by 4.2×10^4 times)
	7.489×10^4 copies/ml (Diluted by 4.5×10^4 times)
	7.326×10^4 copies/ml (Diluted by 4.6×10^4 times)
Bioscience	1.465×10^5 copies/ml (Diluted by 2.3×10^4 times)
	1.348×10^5 copies/ml (Diluted by 2.5×10^4 times)
	1.204×10^5 copies/ml (Diluted by 2.8×10^4 times)

Table 2. Concentration Gradients Selected for Small-Scale Screening in Different Antigen-Testing Kits

2.2.2 Investigation of Effects of Medicines on Sensitivity of Antigen-Testing Kits

2.2.2.1 Levofloxacin

Samples of the following virus concentrations and Levofloxacin concentrations are prepared as shown in *Table 3*. Each sample is diluted by 2 times with virus lysate, and then 70 µl is used for a single antigen test, with 3 repeats and tested with all three types of antigen-testing kits. After antigen-testing kits are left under room temperature to react for 15 minutes, photos are taken, and T-line deepness of antigen-testing kits are recorded.

	Levofloxacin 0.25 mg/ml		**Levofloxacin 0.50 mg/ml**		**Levofloxacin 0.75 mg/ml**	
Viral Load 500x	Solution	Volume	Solution	Volume	Solution	Volume
	10x Virus	80 µl	10x Virus	80 µl	10x Virus	80 µl
	Levofloxacin	20 µl	Levofloxacin	40 µl	Levofloxacin	60 µl
	ddH_2O	1.9 ml	ddH_2O	1.88 ml	ddH_2O	1.86 ml

Viral Load 2000x	Solution / Volume 10x Virus 20 µl Levofloxacin 20 µl ddH_2O 1.96 ml	Solution / Volume 10x Virus 20 µl Levofloxacin 40 µl ddH_2O 1.94 ml	Solution / Volume 10x Virus 20 µl Levofloxacin 60 µl ddH_2O 1.92 ml
Viral Load 11000x	Solution / Volume 10x Virus 4 µl Levofloxacin 22 µl ddH_2O 1.974 ml	Solution / Volume 10x Virus 4 µl Levofloxacin 44 µl ddH_2O 1.952 ml	Solution / Volume 10x Virus 4 µl Levofloxacin 66 µl ddH_2O 1.93 ml

Table 3. Components of Samples with Designated Levofloxacin and Virus Concentrations

2.2.2.2 Budesonide Nasal Spray (BNS)

Samples of the following virus concentrations and Budesonide Nasal Spray concentrations are prepared as shown in *Table 4*. Each sample is diluted by 2 times with virus lysate, and then 70 µl is used for a single antigen test, with 3 repeats and tested with all three types of antigen-testing kits. After antigen-testing kits are left under room temperature to react for 15 minutes, photos are taken, and T-line deepness of antigen-testing kits are recorded.

	Budesonide Nasal Spray 5% (v/v)	**Budesonide Nasal Spray 10% (v/v)**	**Budesonide Nasal Spray 15% (v/v)**
Viral Load 500x	Solution / Volume 10x Virus 80 µl BNS 200 µl ddH_2O 1.72 ml	Solution / Volume 10x Virus 80 µl BNS 400 µl ddH_2O 1.52 ml	Solution / Volume 10x Virus 80 µl BNS 600 µl ddH_2O 1.32 ml

Viral Load 2000x	Solution / Volume 10x Virus / 20 µl BNS / 200 µl ddH_2O / 1.78 ml	Solution / Volume 10x Virus / 20 µl BNS / 400 µl ddH_2O / 1.58 ml	Solution / Volume 10x Virus / 20 µl BNS / 600 µl ddH_2O / 1.38 ml
Viral Load 11000x	Solution / Volume 10x Virus / 4 µl BNS / 220 µl ddH_2O / 1.776 ml	Solution / Volume 10x Virus / 4 µl BNS / 440 µl ddH_2O / 1.556 ml	Solution / Volume 10x Virus / 4 µl BNS / 660 µl ddH_2O / 1.336 ml

Table 4. Components of Samples with Designated Budesonide Nasal Spray and Virus Concentrations

2.2.2.3 Interferon-alpha2b (IFN)

Samples of the following virus concentrations and interferon-alpha2b concentrations are prepared as shown in *Table 5*. Each sample is diluted by 2 times with virus lysate, and then 70 µl is used for a single antigen test, with 3 repeats and tested with all three types of antigen-testing kits. After antigen-testing kits are left under room temperature to react for 15 minutes, photos are taken, and T-line deepness of antigen-testing kits are recorded.

	Interferon-alpha2b 20 pm/ml	**Interferon-alpha2b 50 pm/ml**	**Interferon-alpha2b 100 pm/ml**
Viral Load 500x	Solution / Volume 10x Virus / 80 µl IFN / 80 µl ddH_2O / 1.84 ml	Solution / Volume 10x Virus / 80 µl IFN / 200 µl ddH_2O / 1.72 ml	Solution / Volume 10x Virus / 80 µl IFN / 400 µl ddH_2O / 1.52 ml

Viral Load 2000x	Solution / Volume 10x Virus / 20 µl IFN / 80 µl ddH_2O / 1.9 ml	Solution / Volume 10x Virus / 20 µl IFN / 200 µl ddH_2O / 1.78 ml	Solution / Volume 10x Virus / 20 µl IFN / 400 µl ddH_2O / 1.58 ml
Viral Load 11000x	Solution / Volume 10x Virus / 4 µl IFN / 88 µl ddH_2O / 2.072 ml	Solution / Volume 10x Virus / 4 µl IFN / 220 µl ddH_2O / 1.976 ml	Solution / Volume 10x Virus / 4 µl IFN / 440 µl ddH_2O / 1.756 ml

Table 5. Components of Samples with Designated Inteferon-alpha2b and Virus Concentrations

2.2.3 qPCR Testing to Confirm Sample Viral Load

2.2.3.1 Nucleic Acid Extraction

2.2.3.1.1 Lysis and Binding

Transfer 200 µl sample to a new 1.5 ml centrifuge tube, add 400 µl protease K, 400 µl lysate, 200 µl isopropanol, and 20 µl magnetic bead suspension, vortex to mix, and heat at 55°C for 15 minutes (inverted every 5 minutes). The centrifuge tube is then placed on a magnetic separator for 1 min and the supernatant is aspirated with pipette.

2.2.3.1.2 Wash

Add 600 µl of wash solution I, vortex for 10 seconds to resuspend the beads sufficiently, and place the centrifuge tube on magnetic separator until the solution is clear, then remove the supernatant with a pipette and remove the centrifuge tube. Add 600 µl of Washing Buffer II (check whether absolute ethanol has been added), vortex for 10 seconds so that the magnetic beads are fully resuspended and place the centrifuge tube on the magnetic separator until the

solution is clear, remove the supernatant with the pipette and remove the centrifuge tube, repeating the step once.

2.2.3.1.3 Drying

Keep the centrifuge tube on a magnetic separator and air dry it in an ultra-clean table until there is no obvious gloss on the surface of the beads.

2.2.3.1.4 Elution

Add 60 μl of Nuclease-free Water, vortex for 1 minute so that the magnetic beads are fully resuspended and heat the solution at 55 °C for 5 min. Place the centrifuge tube on a magnetic separator until the solution is clear, then transfer the supernatant to a new centrifuge tube, which is the purified pathogenic genome.

2.2.3.2 qPCR Testing

To quantify viral loads of diluted samples, qPCR is conducted to detect LUC2 gene included in SARS-CoV-2 pseudoviruses.

2.2.3.2.1 Preparation of DNA Template

Prepare two 1.5 ml centrifuge tube, add 26 μl supernatant collected in 2.2.3.1.4 to each tube. Add 1 μl DNase I (RNase-free) and 3μl DNase I Reaction Buffer to one of the tubes; add 4μl DNase I Reaction Buffer to the other tube. Incubate both tubes under 37℃ for 30 minutes. Add 0.3μl EDTA to the first tube and heat under 75℃ for 10 minutes, store under -10℃ for future usage.

2.2.3.2.2 Preparation of Testing Samples

For each sample, add solutions shown in *Table 6* to a well of a 96-well double-sided centrifugal plate, with three repeats for each sample.

Solution	Volume
2x AceQ Universal SYBR qPCR Master Mix	10 µl
Forward Primer (10 µM)	0.4 µl
Reverse Primer (10 µM)	0.4 µl
DNA Template	2 µl
ddH_2O	7.2 µl

Table 6. Reaction System for qPCR-Testing

2.2.3.2.3 qPCR Running

The 96-well double-sided centrifugal plate is centrifuged for 1 minute and samples are collected to PCR tubes. PCR tubes are placed in PCR instrument with the program shown in *Table 7*.

Temperature	Time	
50 °C	5 min	
95 °C	3 min	
95 °C	10 sec	40 Cycles
60 °C	30 sec	
95 °C	15 sec	
60 °C	1 min	
95 °C	15 sec	

Table 7. Reaction Program for qPCR-Testing

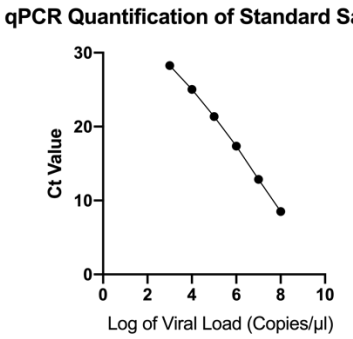
3. Results

3.1 Minimum Detection Limit of Antigen-testing Kits

3.1.1 qPCR Confirm Concentration of Tested Samples

qPCR-testing for antigen-testing samples and standard samples is conducted to detect viral loads in the tested samples. Test results are compared to standard samples test results. Due to technological limitations, only standard samples with viral load between 2.15×10^5 and 2.15×10^{10} copies/μl are available. Therefore, tested samples with viral load beyond this range cannot be compared to the standard samples to confirm if diluted viral load matches actual viral load. However, the Ct values of all tested samples are still applicable for future calculations. The qPCR results are shown in *Figure 1* and will be used for later calculations.

A



Viral Load (Copies/μl)	Mean Ct Value
2.15 x 10^5	28.27
2.15 x 10^6	25.00
2.15 x 10^7	21.35
2.15 x 10^8	17.37
2.15 x 10^9	12.87
2.15 x 10^{10}	8.52

B

Viral Load (Copies/μl)	Mean Ct Value
3.37 x 10^5 (Dilution 10X)	15.93
3.37 x 10^3 (Dilution 1000X)	26.28
3.37 x 10^2 (Dilution 10000X)	31.03
1.872 x 10^2 (Dilution 18000X)	31.61
1.685 x 10^2 (Dilution 20000X)	31.47
1.605 x 10^2 (Dilution 21000X)	32.71
1.465 x 10^2 (Dilution 23000X)	32.62
1.348 x 10^2 (Dilution 25000X)	32.88
1.204 x 10^2 (Dilution 28000X)	29.91
8.425 x 10 (Dilution 40000X)	32.43
8.024 x 10 (Dilution 42000X)	29.36
7.489 x 10(Dilution 45000X)	32.10
7.326 x 10 (Dilution 46000X)	32.70
6.74 x 10 (Dilution 50000X)	32.83
5.617 x 10 (Dilution 60000X)	35.41

Figure 1. qPCR Quantification of Tested Samples

Figure 1 is obtained by qPCR-testing of antigen-testing samples and purchased standard samples. The Ct value refers to the cycle threshold, or the number of amplification cycles for the sample to undergo from which the fluorescence signal crosses the threshold line. Viral load refers to the copies of viruses included in the sample.

3.1.2 Antigen-Testing Results for Investigation of Minimum Detection Limit

3.1.2.1 Minimum Detection Limit of Outdo Antigen-Testing Kits

To investigate the minimum detection limit of Outdo antigen-testing kits, large-scale screening with a large range of viral concentration gradients is conducted, as shown in *Figure 2A*. In the large-scale screening, it is discovered that Outdo antigen-testing kits still display a visible T-line of deepness 0.8 with viral load 1.1794×10^4 copies (diluted by 20000 times), yet display invisible T-line of deepness 0 with viral load 5.8795×10^3 copies (diluted by 40000 times). This reveals that the minimum detection limit of Outdo antigen-testing kits should lie between the two, leading to a small-scale screening.

In the small-scale screening, samples with viral loads between 5.8795×10^3 copies and 1.1794×10^4 copies are tested, as shown in *Figure 2B*, *Figure 2C*, and *Figure 2D*. Among the three selected concentration gradients, samples with viral load 1.1795×10^4 copies display one negative result out of a total of 20 tests, with the other 19 results displaying visible T-line deepness. This means an accuracy of 95%, making it a valid minimum detection limit. Moreover, as seen in *Figure 2C*, tests of samples with viral load 1.31056×10^4 copies provide 5 positive results out of all 5 tests, with 4 of the samples displaying a relatively strong positivity of T-line deepness 1, making it likely to be higher than the minimum detection limit. Meanwhile, as seen in *Figure 2D*, tests of samples with viral load 1.23333×10^4 copies provide 5 positive results with 2 displaying T-line deepness lower than 0.5, showing weak sensitivity to viruses, making it likely to be lower than the minimum detection limit.

In conclusion, the minimum detection limit of Outdo antigen-testing kits should be higher than 1.23333×10^4 copies (Ct = 31.61) and lower than 1.31056×10^4 copies (Ct = 32.71), estimated to be 1.1795×10^4 copies (Ct = 31.47).

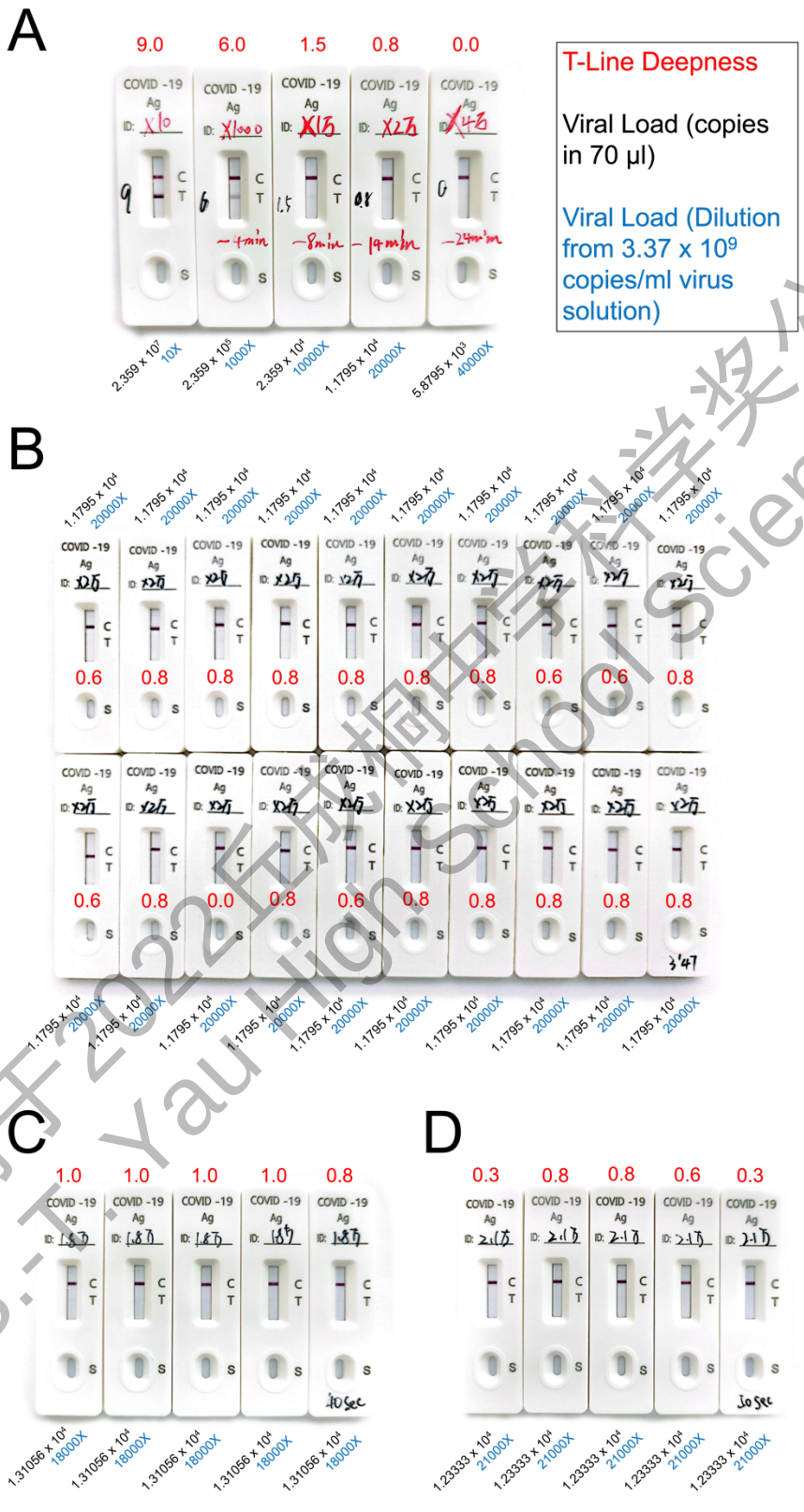


Figure 2. Antigen-Testing Results for Outdo Antigen-Testing Kits Minimum Detection Limit

Figure 2 displays antigen-testing results in investigation of minimum detection limit of antigen-testing kits produced by Outdo. The red labels reveal the T-line deepness read based on the color card scale of 2019-nCoV antigen testing kits, the larger the value, the deeper the T-line, and the more sensitive the antigen-testing kit is to the sample. The black labels indicate the viral load of samples in terms of the copies of viruses added to the antigen testing kit. The blue labels indicate the viral load of samples in terms of dilution rate from the original solution with virus concentration 3.37×10^9 copies/ml. Figure 2A is the antigen-testing results of large-scale screening, where a large range of viral concentration gradients are tested. Figure 2B, C and D are the antigen-testing results of small-scale screening for samples with viral loads 1.1795×10^4 copies, 1.31056×10^4 copies and 1.23333×10^4 copies respectively.

3.1.2.2 Minimum Detection Limit of EasyDiagnosis Antigen-Testing Kits

To investigate the minimum detection limit of EasyDiagnosis antigen-testing kits, large-scale screening with a large range of viral concentration gradients is conducted, as shown in *Figure 3A*. In the large-scale screening, it is discovered that EasyDiagnosis antigen-testing kits still display a visible T-line of deepness 0.8 with viral load 5.8756×10^3 copies (diluted by 40000 times), yet display a hardly visible T-line of deepness 0.1 with viral load 4.712×10^3 copies (diluted by 50000 times) and display invisible T-line of deepness 0 with viral load 3.9257×10^3 (diluted by 60000 times). This reveals that the minimum detection limit of EasyDiagnosis antigen-testing kits should lie between the two, leading to a small-scale screening.

In the small-scale screening, samples with viral loads between 4.712×10^3copies and 5.8756×10^3 copies are tested, as shown in *Figure 3B*, *Figure 3C*, and *Figure 3D*. Among the three selected concentration gradients, samples with viral load 5.2422×10^3 copies display one negative result out of a total of 20 tests, with the other 19 results displaying visible T-line deepness. This means an accuracy of 95%, making it a valid minimum detection limit. Moreover, as seen in *Figure 3C*, tests of samples with viral load 5.6167×10^3 copies provide 5 positive

results out of all 5 tests, making it likely to be higher than the minimum detection limit. Meanwhile, as seen in *Figure 3D*, tests of samples with viral load 5.1283 x 10^3 copies provide 2 negative results out of the total of 5 tests, making it likely to be lower than the minimum detection limit.

In conclusion, the minimum detection limit of EasyDiagnosis antigen-testing kits should be higher than 5.1283 x 10^3 copies (Ct = 29.36) and lower than 5.6167 x 10^3 copies (Ct = 32.71), estimated to be 5.2422 x 10^3 copies (Ct = 32.10).

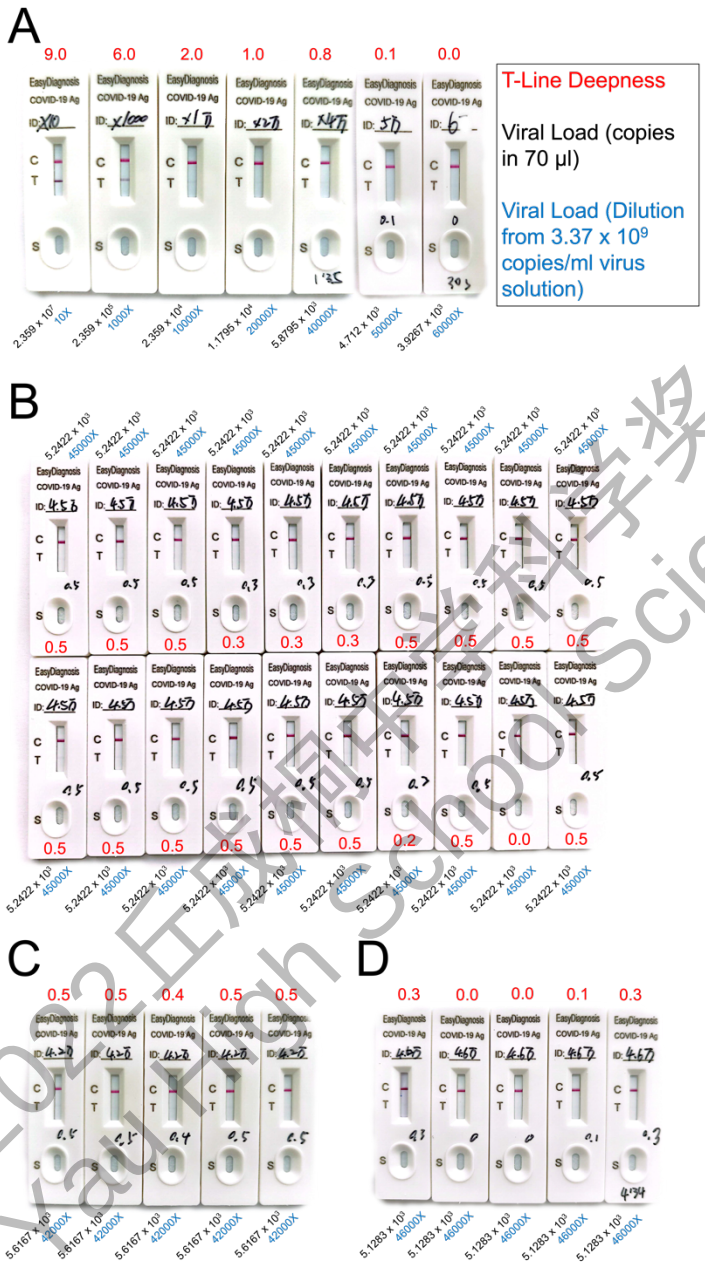


Figure 3. Antigen-Testing Results for EasyDiagnosis Antigen-Testing Kits Minimum Detection Limit

Figure 3 displays antigen-testing results in investigation of minimum detection limit of antigen-testing kits produced by EasyDiagnosis. The red labels reveal the T-line deepness read based on the color card scale of 2019-nCoV antigen testing kits, the larger the value, the deeper the T-line, and the more sensitive the antigen-testing kit is to the sample. The black labels indicate the viral load of samples in terms of the copies of viruses added to the antigen testing kit. The blue labels indicate the viral load of samples in terms of dilution rate from the original solution with

virus concentration 3.37 x 10^9 copies/ml. Figure 3A is the antigen-testing results of large-scale screening, where a large range of viral concentration gradients are tested. Figure 3B, C and D are the antigen-testing results of small-scale screening for samples with viral loads 5.2422 x 10^3 copies, 5.1283 x 10^3 copies and 5.6167 x 10^3 copies respectively.

3.1.2.3 Minimum Detection Limit of Bioscience Antigen Testing Kits

To investigate the minimum detection limit of Bioscience antigen-testing kits, large-scale screening with a large range of viral concentration gradients is conducted, as shown in *Figure 4A*. In the large-scale screening, it is discovered that Bioscience antigen-testing kits still display a visible T-line of deepness 0.8 with viral load 1.1794 x 10^4 copies (diluted by 20000 times), yet display invisible T-line of deepness 0 with viral load 5.8795 x 10^3 copies (diluted by 40000 times). This reveals that the minimum detection limit of Bioscience antigen-testing kits should lie between the two, leading to a small-scale screening.

In the small-scale screening, samples with viral loads between 5.8795 x 10^3 copies and 1.1794 x 10^4 copies are tested, as shown in *Figure 4B*, *Figure 4C*, and *Figure 4D*. Among the three selected concentration gradients, samples with viral load 9.436 x 10^3 copies display one negative result out of a total of 20 tests, with the other 19 results displaying visible T-line deepness. This means an accuracy of 95%, making it a valid minimum detection limit. Moreover, as seen in *Figure 4C*, tests of samples with viral load 1.0256 x 10^4 copies provide 5 positive results out of all 5 tests, with 4 of the samples displaying a relatively strong positivity of T-line deepness 1, making it likely to be higher than the minimum detection limit. Meanwhile, as seen in *Figure 4D*, tests of samples with viral load 8.425 x 10^3 copies provide 4 results out of all 5 tests, with one displaying T-line deepness 0.1, showing weak sensitivity to viruses, making it likely to be lower than the minimum detection limit.

In conclusion, the minimum detection limit of Bioscience antigen-testing kits should be higher than 8.425 x 10^3 copies (Ct = 29.91) and lower than 1.0256 x 10^4 copies (Ct = 32.62), estimated to be 9.436 x 10^3 copies (Ct = 32.88).

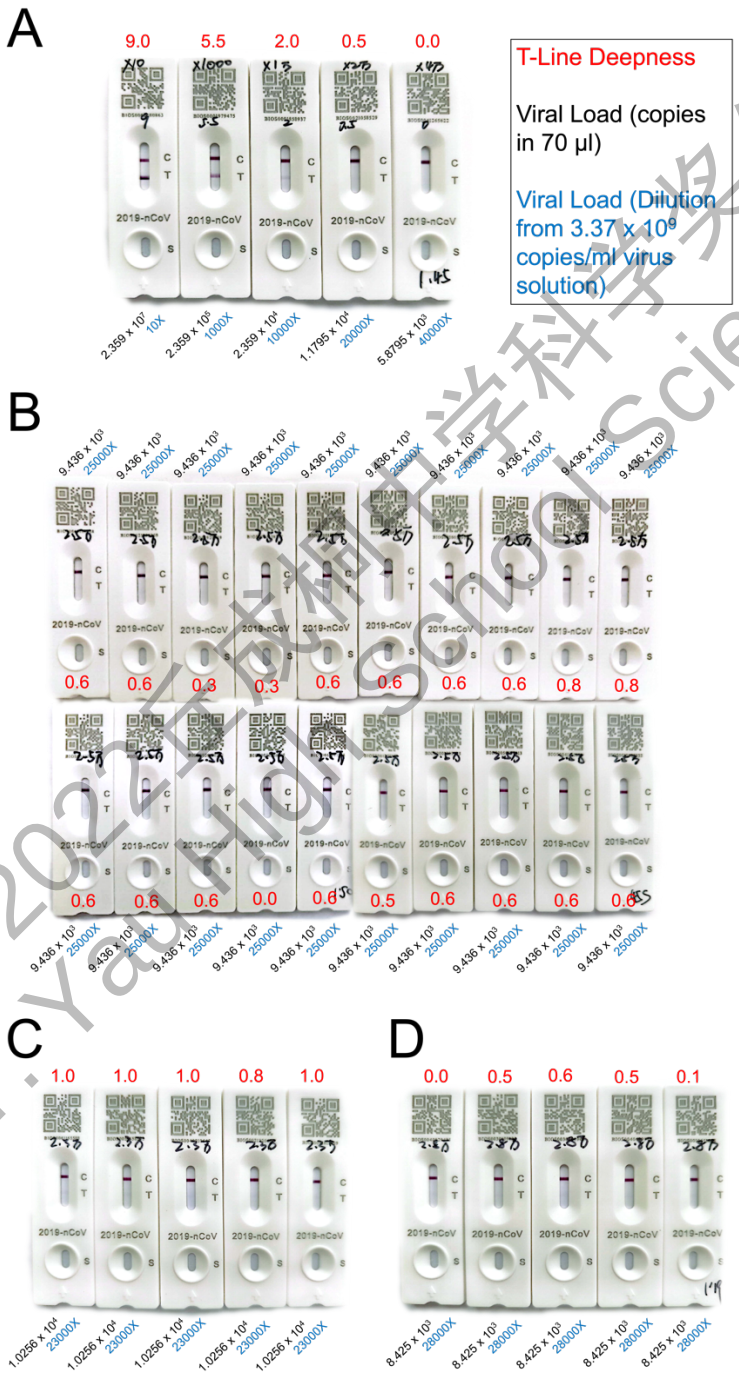


Figure 4. Antigen-Testing Results for Bioscience Antigen-Testing Kits Minimum Detection Limit

Figure 4 displays antigen-testing results in investigation of minimum detection limit of antigen-testing kits produced by Bioscience. The red labels reveal the T-line deepness read based on the color card scale of 2019-nCoV antigen testing kits, the larger the value, the deeper the T-line, and the more sensitive the antigen-testing kit is to the sample. The black labels indicate the viral load of samples in terms of the copies of viruses added to the antigen testing kit. The blue labels indicate the viral load of samples in terms of dilution rate from the original solution with virus concentration 3.37×10^9 copies/ml. Figure 4A is the antigen-testing results of large-scale screening, where a large range of viral concentration gradients are tested. Figure 4B, C and D are the antigen-testing results of small-scale screening for samples with viral loads 9.436×10^3 copies, 1.0256×10^4 copies and 8.425×10^3 copies respectively.

3.2 Effect of Levofloxacin on Antigen-Testing Kits Sensitivity

To investigate the effect of Levofloxacin on antigen-testing kits sensitivity, Levofloxacin of different concentrations and viruses of different concentrations are mixed together, and antigen tests are collected, with T-line deepness recorded, as shown in *Figure 5*.

In general, the addition of Levofloxacin decreases sensitivity of antigen-testing kits to viruses, with a few discrepancies showing a slight increase: Outdo antigen-testing kits viral load 1.1795×10^5 copies Levofloxacin concentration 50 mg/ml, Outdo antigen-testing kits viral load 2.1445×10^4 copies Levofloxacin concentration 25 mg/ml, EasyDiagnosis antigen-testing kits viral load 1.1795×10^5 copies Levofloxacin concentration 25 mg/ml, EasyDiagnosis antigen-testing kits viral load 1.1795×10^5 copies Levofloxacin concentration 50 mg/ml, EasyDiagnosis antigen-testing kits viral load 2.1445×10^4 copies Levofloxacin concentration 25 mg/ml, Bioscience antigen-testing kits viral load 1.1795×10^5 copies Levofloxacin concentration 50 mg/ml.

As displayed in *Figure 5A*, *Figure 5B* and *Figure 5C*, the effect of Levofloxacin varies by concentration, and shares a common trend among the three brands. With viral load 4.718×10^5 copies (diluted by 500 times) and viral load 1.1795×10^5 copies (diluted by 2000 times),

Levofloxacin significantly decreases T-line deepness at concentration 0.25 mg/ml, indicating a reduction in antigen-testing kit sensitivity. In addition, there is a significant increase in T-line deepness from Levofloxacin concentration 0.25 mg/ml to 0.50 mg/ml. However, with viral load 4.718×10^5 copies, Levofloxacin concentration 0.75 mg/ml samples display T-line deepness almost equivalent to that 0.50 mg/ml, whereas viral load 1.1795×10^5 copies, a significant decrease is observed. With viral load 2.1445×10^4 copies, samples of different Levofloxacin concentrations display similar T-line deepness, showing a general decreasing trend with the increase of Levofloxacin concentration, with samples with Levofloxacin concentration 0.50 mg/ml usually displaying the lowest T-line deepness.

To contrast between different brands, EasyDiagnosis antigen-testing kits display T-line deepness at Levoloxacin concentration 0.25 mg/ml at viral load 1.1795×10^5 copies equivalent to that of 0 Levofloxacin concentration, which is different from the sharp decrease displayed by the other two brands. In addition, EasyDiagnosis antigen-testing kits generally show less changes in T-line deepness compared to the other two brands.

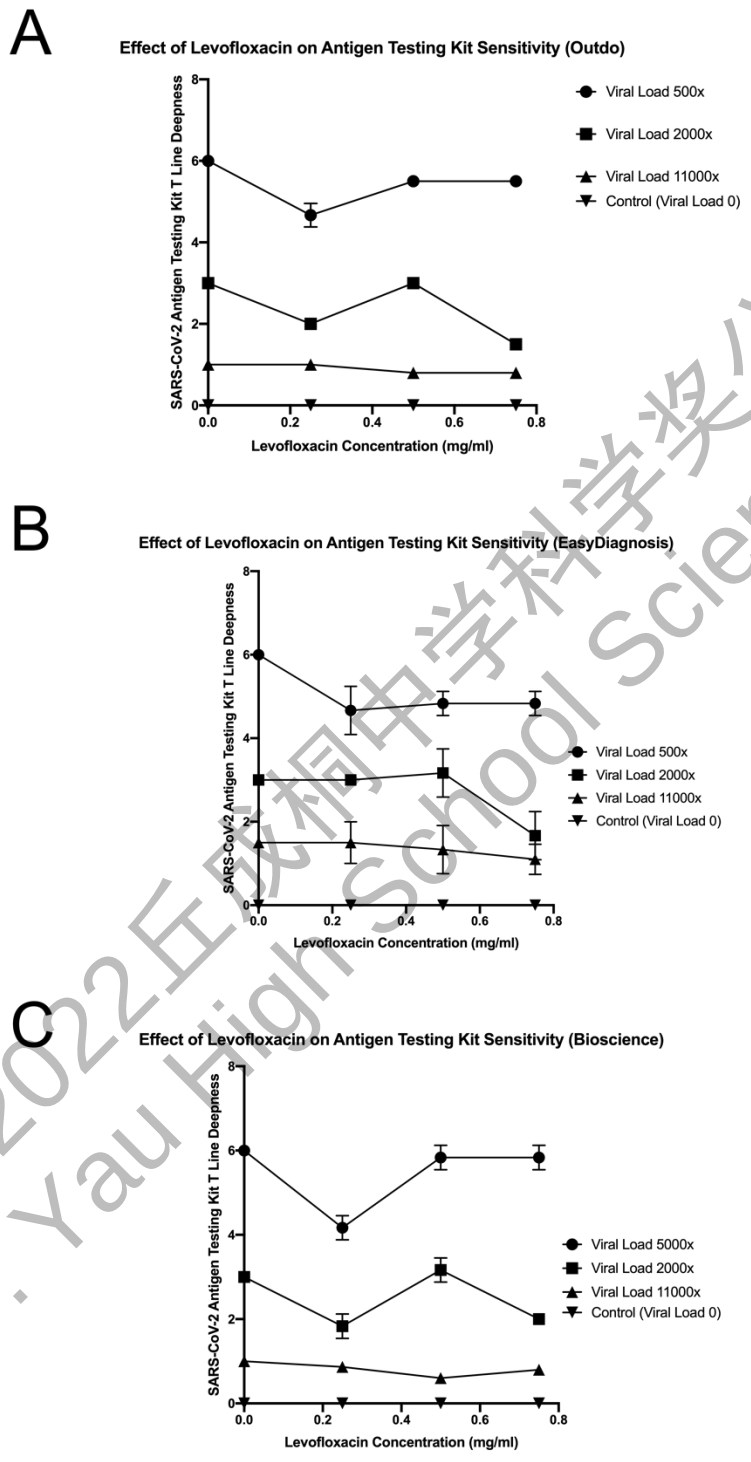


Figure 5. Effect of Levofloxacin on Antigen-Testing Kit Sensitivity

Figure 5 displays the T-line deepness recorded in antigen-testing with samples of different concentrations of Levofloxacin and viruses. The x-axis records the Levofloxacin concentrations, and the y-axis records the recorded

T-line deepness. The higher the T-line deepness value, the deeper the T-line is, the more sensitive the antigen-testing kit is to the sample. The lines in the graph with round points refer to recorded results of samples with viral load 4.718×10^5 copies (diluted by 500 times); the lines with square points refer to recorded results of samples with viral load 1.1795×10^5 copies (diluted by 2000 times); the lines with triangle points refer to recorded results of samples with viral load 2.1445×10^4 copies (diluted by 11000 times); the lines with flipped triangle points refer to recorded results of samples with viral load 0 (control group).

3.3 Effect of Budesonide Nasal Spray on Antigen-Testing Kits Sensitivity

To investigate the effect of Budesonide nasal spray on antigen-testing kits sensitivity, Budesonide nasal spray of different concentrations and viruses of different concentrations are mixed together, and antigen tests are collected, with T-line deepness recorded, as shown in *Figure 6*.

In general, the addition of Budesonide nasal spray decreases sensitivity of antigen-testing kits to viruses and shows a general decrease in T-line deepness as the concentration of Budesonide nasal spray increases. However, there is a slight increase in T-line deepness from Budesonide nasal spray concentration 10% to 15% when viral load is 2.1445×10^4 copies. In addition, the difference in T-line deepness decreases with the decrease in viral load. The general trends are all shared among all three brands.

To contrast between different brands, EasyDiagnosis antigen-testing kits display the sharpest decrease in T-line deepness with the increase in Budesonide nasal spray concentration, whereas Outdo antigen-testing kits display the flattest.

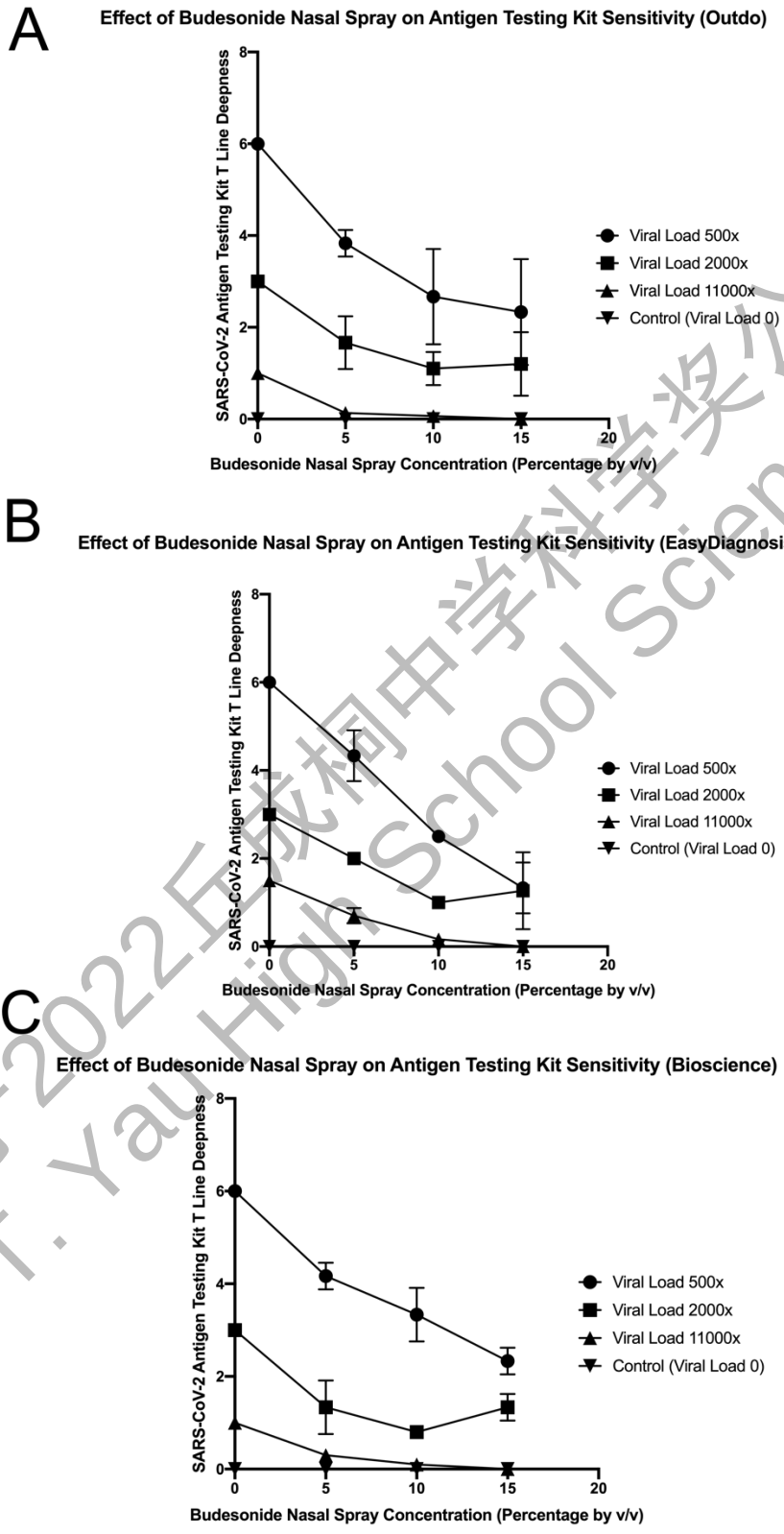


Figure 6. Effect of Budesonide Nasal Spray on Antigen-Testing Kit Sensitivity

Figure 6 displays the T-line deepness recorded in antigen-testing with samples of different concentrations of Budesonide nasal spray and viruses. The x-axis records the Budesonide nasal spray concentrations, and the y-axis records the recorded T-line deepness. The higher the T-line deepness value, the deeper the T-line is, the more sensitive the antigen-testing kit is to the sample. The lines in the graph with round points refer to recorded results of samples with viral load 4.718 x 10^5 copies (diluted by 500 times); the lines with square points refer to recorded results of samples with viral load 1.1795 x 10^5 copies (diluted by 2000 times); the lines with triangle points refer to recorded results of samples with viral load 2.1445 x 10^4 copies (diluted by 11000 times); the lines with flipped triangle points refer to recorded results of samples with viral load 0 (control group).

3.4 Effect of Interferon-alpha2b on Antigen-Testing Kits Sensitivity

To investigate the effect of interferon-alpha2b on antigen-testing kits sensitivity, interferon-alpha2b of different concentrations and viruses of different concentrations are mixed together, and antigen tests are collected, with T-line deepness recorded, as shown in *Figure 7*.

In general, the addition of interferon-alpha2b decreases sensitivity of antigen-testing kits to viruses, with a few discrepancies showing a slight increase: Outdo antigen-testing kits viral load 1.1795 x 10^5 copies interferon-alpha2b concentration 20 pg/ml, Outdo antigen-testing kits viral load 2.1445 x 10^4 copies interferon-alpha2b concentration 50 pg/ml, EasyDiagnosis antigen-testing kits viral load 4.718 x 10^5 copies interferon-alpha2b concentration 20 pg/ml, EasyDiagnosis antigen-testing kits viral load 4.718 x 10^5 copies interferon-alpha2b concentration 100 pg/ml, EasyDiagnosis antigen-testing kits viral load 1.1795 x 10^5 copies interferon-alpha2b concentration 20 pg/ml, EasyDiagnosis antigen-testing kits viral load 1.1795 x 10^5 copies interferon-alpha2b concentration 50 pg/ml, EasyDiagnosis antigen-testing kits viral load 1.1795 x 10^5 copies interferon-alpha2b concentration 100 pg/ml, Bioscience antigen-testing kits viral load 4.718 x 10^5 copies interferon-alpha2b concentration 20 pg/ml, 1.1795 x 10^5 copies interferon-alpha2b concentration 20 pg/ml.

As displayed in *Figure 7A*, *Figure 7B* and *Figure 7C*, the effect of interferon-alpha2b varies among different brands. In general, there is a decreasing trend in T-line deepness with the increase of interferon-alpha2b concentration when viral load is 1.1795×10^5 copies. The other trends remain specific to the brand.

To contrast between different brands, Outdo antigen-testing kits display a decreasing trend in T-line deepness with the increase of interferon-alpha2b concentration at viral load 4.718×10^5 copies. Bioscience antigen-testing kits display a decrease in T-line deepness from interferon-alpha2b concentration 50 pg/ml to 100 pg/ml, differing from the trend displayed by the other two brands. The trend of T-line deepness for interferon-alpha2b with viral load 2.1445×10^4 varies for different brands, showing a flat trend in Outdo antigen-testing kits, an increasing trend with the increase of interferon-alpha2b concentration in EasyDiagnosis antigen-testing kits, and a decreasing trend with the increase of interferon-alpha2b concentration in Bioscience antigen-testing kits.

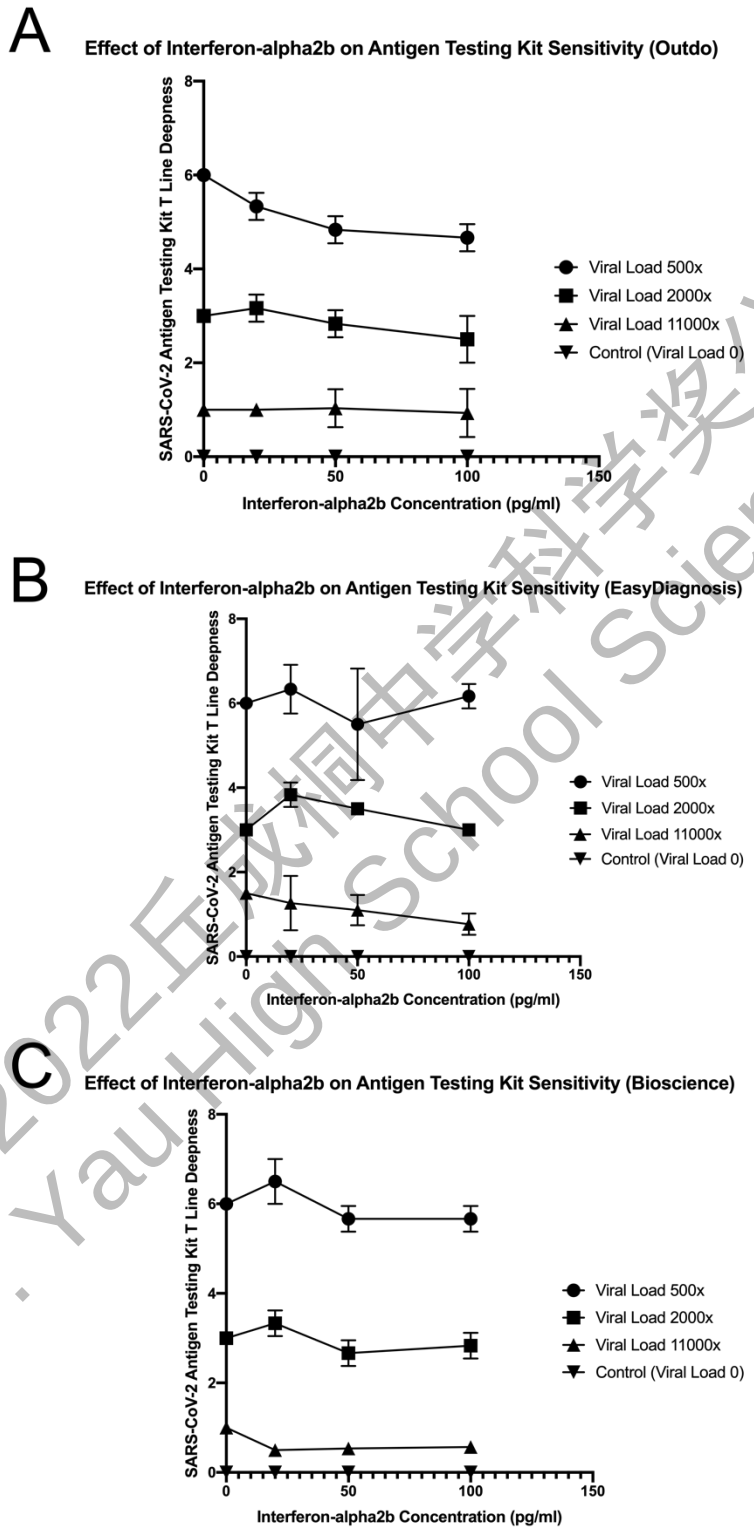


Figure 7. Effect of Interferon-alpha2b on Antigen-Testing Kit Sensitivity

Figure 7 displays the T-line deepness recorded in antigen-testing with samples of different concentrations of interferon-alpha2b and viruses. The x-axis records the interferon-alpha2b concentrations, and the y-axis records the recorded T-line deepness. The higher the T-line deepness value, the deeper the T-line is, the more sensitive the antigen-testing kit is to the sample. The lines in the graph with round points refer to recorded results of samples with viral load 4.718 x 10^5 copies (diluted by 500 times); the lines with square points refer to recorded results of samples with viral load 1.1795 x 10^5 copies (diluted by 2000 times); the lines with triangle points refer to recorded results of samples with viral load 2.1445 x 10^4 copies (diluted by 11000 times); the lines with flipped triangle points refer to recorded results of samples with viral load 0 (control group).

4. Discussion

4.1 Minimum Detection Limit of Antigen-Testing Kits

Through the use of psuedoviruses to conduct antigen-testing, the minimum detection limits of different antigen-testing kits are deduced.

The minimum detection limit of Outdo antigen-testing kits should be higher than 1.23333 x 10^4 copies (Ct = 31.61) and lower than 1.31056 x 10^4 copies (Ct = 32.71), estimated to be 1.1795 x 10^4 copies (Ct = 31.47). The minimum detection limit of EasyDiagnosis antigen-testing kits should be higher than 5.1283 x 10^3 copies (Ct = 29.36) and lower than 5.6167 x 10^3 copies (Ct = 32.71), estimated to be 5.2422 x 10^3 copies (Ct = 32.10). The minimum detection limit of Bioscience antigen-testing kits should be higher than 8.425 x 10^3 copies (Ct = 29.91) and lower than 1.0256 x 10^4 copies (Ct = 32.62), estimated to be 9.436 x 10^3 copies (Ct = 32.88).

The discovery of the minimum detection limit of antigen-testing kits can indicate the lowest viral load at which the antigen-testing kits provide valid results, providing basis in application of antigen-testing kits in clinical diagnosis.

4.2 Effect of Levofloxacin on Antigen-Testing Kits Sensitivity

The general decrease in T-line deepness with the addition of Levofloxacin indicates its effect in decreasing antigen-testing kits accuracy in testing, potentially due to its effect in decreasing antigen-testing kits sensitivity to viruses or its effect in altering viral structures. The discrepancies in the general decreasing trend might be due to the uncertainties in reading antigen-testing results and the binding of Levofloxacin molecules to the embedded antibodies on T-line.

The difference across antigen-testing kits might be due to the difference in antibodies embedded in T-line of antigen-testing kits, where some may be structurally more complement to Levofloxacin molecules, thus increasing the sensitivity. In addition, some antibodies may be easily altered by Levofloxacin molecules, thus decreasing the sensitivity.

The difference between T-line deepness caused by difference in Levofloxacin concentration may be due to the working concentration of Levofloxacin, where the most significant effect can be seen.

The variation of optimum Levofloxacin concentration based on different viral loads may be due to the reaction mechanisms between Levofloxacin and viruses.

In general, Levofloxacin leads to a decrease in antigen-testing kit sensitivity to viruses, with the most significant effect occur when the concentration is 75 mg/ml. This provides insights in real life, where patients taking Levofloxacin are less likely to display a positive antigen-testing result, especially when the viral load is close to the minimum detection limit of antigen-testing kits.

4.3 Effect of Budesonide Nasal Spray on Antigen-Testing Kits Sensitivity

The decreasing trend of T-line deepness corresponding to the increasing Budesonide nasal spray indicates that Budesonide nasal spray decreases antigen-testing kit accuracy, which may be due to its effect in affecting antigen-testing kits by affecting the embedded antibodies or altering viral structures.

As the viral load decreases, the difference in T-line deepness become less obvious, which is likely due to the limitations of reading antigen-testing kits T-line, where a lower viral load results in a shallower T-line, making differences hard to be observant.

In general, Budesonide nasal spray significantly reduces antigen-testing kits sensitivity, thus when applied to patients, will be less likely to display a positive test.

4.4 Effect of Interferon-alpha2b on Antigen-Testing Kits Sensitivity

The general decreasing trend of T-line deepness corresponding to the increasing interferon-alpha2b concentration reveals that interferon-alpha2b decreases antigen-testing kit accuracy, which is a result of either its influence on viral structure or influence functionality of antigen-testing kits. The exceptions showing slight increase may be due to the specific structure of antibodies embedded in T-line of antigen-testing kits, leading to possible bindings or structural interference.

In general, interferon-alpha2b holds a limited influence on antigen-testing kits sensitivity and may leave arbitrary effects on antigen-testing kits sensitivity.

4.5 Limitations of Study

This study holds several limitations. The reading of antigen-testing kits T-line deepness is dependent on perception of the observer, thus leading to discrepancies among different observers. In addition, pseudoviruses have different coating protein from SARS-CoV-2, thus the efficiency of virus lysate on the pseudoviruses may differ from that of SARS-CoV-2, affecting the minimum detection limit.

5. Conclusion and Prospect

5.1 Conclusion

In this study, the minimum detection limit of Outdo antigen-testing kits is estimated to be 1.1795×10^4 copies (Ct = 31.47); the minimum detection limit of EasyDiagnosis antigen-testing kits is estimated to be 5.2422×10^3 copies (Ct = 32.10); the minimum detection limit of Bioscience antigen-testing kits is estimated to be 9.436×10^3 copies (Ct = 32.88).

The addition of Levofloxacin may decrease antigen-testing kits sensitivity, especially at concentration of 25 mg/ml and 75 mg/ml. The addition of Budesonide nasal spray strongly decreases antigen-testing kits sensitivity, showing a decreasing trend in antigen-testing kits sensitivity with the increasing concentration of Budesonide nasal spray. Interferon-alpha2b holds a limited effect on antigen-testing kits, yet a high concentration may reduce the sensitivity of antigen-testing kits.

The innovation points of this study are:

1. The proposal of using pseudoviruses to prepare standard samples for antigen-testing, which provides an accurate basis for comparison.

2. The exploration of the specific effect of medicines on antigen-testing kits behavior, which is not explored yet.

3. The investigation of minimum detection limit of antigen-testing kits, which helps the interpretation of antigen-testing results.

5.2 Prospect

This study provides a valid estimation for antigen-testing kits minimum detection limit, specifically those produced by Outdo, EasyDiagnosis and Bioscience. To confirm the accuracy of the minimum detection limit of the antigen-testing kits, this study can be replicated with SARS-CoV-2 under appropriate operations. In the future, minimum detection limit of antigen-testing kits produced by other manufacturers can also be investigated through this method.

In this study, the general effect of Levofloxacin, Budesonide nasal spray, and interferon-alpha2b on antigen-testing kits sensitivity is investigated. Further experiments with different viral loads can be tested to determine the specific influence of the medicines on minimum detection limit of antigen-testing kits. In addition, a wider concentration gradient of medicines can be tested to further explore the trend in effect of medicines on antigen-testing kits sensitivity. Moreover, further examinations can be taken the explore the mechanism of the influence of the medicines on antigen-testing kits sensitivity. Additionally, other medicines and human tissues can be tested to determine the effect on sensitivity of antigen-testing kits.

This study lays a solid foundation for interpretation of minimum detection limit of antigen-testing kits and provides a primary exploration of effect of medicines on antigen-testing kits sensitivity. In the future, the study can be expanded to discuss the minimum detection limit

of other antigen-testing kits and the specific effects of medicines on antigen-testing kits, along with the underlying mechanisms.

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