# Detection Report of Inactivated Novel Coronavirus on Antiviral Medical Mask

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# Effect Detection of Inactivated Novel Coronavirus (SARS-CoV-2) on Antiviral Medical Mask

## 1 Experiment Purpose

To detect whether the novel coronavirus (SARS-CoV-2) on the submitted materials can be inactivated , and calculate the efficiency of inactivating the virus.

## 2 Samples for Detection

The submitted samples were the antiviral medical masks treated with Goldshield® antimicrobial provided by Guilin HBM, and the control samples were untreated ordinary medical masks.

## 3 Cells and Viruses

Vero E6 cells: African green monkey kidney (Vero) cells, with culture conditions for Vero E6 cells: cultured in 5% $CO_2$ humidified incubator at 37 ℃. As the culture medium, DMEM medium containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin (Beyotime #C0222) was used. The cells were cultured for 2-3 days and passaged when they were overgrown.

Virus: novel coronavirus, SARS-CoV-2.

## 4 Experiment Principle

SARS-CoV-2 virus can be rapidly replicated in Vero E6 cells infected by it and released into the cell culture supernatant. After 3 days of culture, obvious cytopathic effect (CPE) can be observed. Thus it can be judged whether there is replication of SARS-CoV-2 in the cell culture wells, and then the $TCID_{50}$ value of the virus in the samples can be calculated.

# 5 Experiment Process

## 5.1 Preparation of test samples

5.1.1 The outermost layer of material of a medical mask was cut with the size of about 4cm×4cm, which was then cut into small pieces of 0.5cm×0.5cm. Then the small piece of material was placed into a 2ml Eppendorf tube, and 0.1mL virus stock solution was added. Three samples of antiviral masks and three samples of ordinary masks were taken as parallel replicates, totally 6 samples.

5.1.2 0.1mL virus stock solution was simultaneously taken as virus control sample.

5.1.3 The Eppendorf tube was placed for incubation at 37°C for 20 minutes, and taken out every 4 minutes to shake thoroughly for 1 minute.

5.1.4 After sampling, all samples were immediately diluted at a ratio of 1:10 with infected medium (DMEM medium containing 2% fetal bovine serum) for virus titer determination.

## 5.2 Determination of virus titer

In titer determination, the most commonly used 50% tissue culture infective dose method was adopted to detect the $TCID_{50}$ of the virus.

5.2.1 The supernatant of Vero E6 cells inoculated into the cell culture plate was removed and added with 100 μL DMEM medium containing 2% fetal bovine serum.

5.2.2 The samples collected in 5.1.4 were diluted with the infected medium by 10 folds with a total of 6 concentration gradients.

5.2.3 The diluted virus samples were added into the cell wells of the 96-well plate, 100 μL per well, 4 replicate wells per gradient. For sample dilution and arrangement, see Plate 1 and Plate 2. The cells were placed in the incubator for further culturing.

Plate 1:

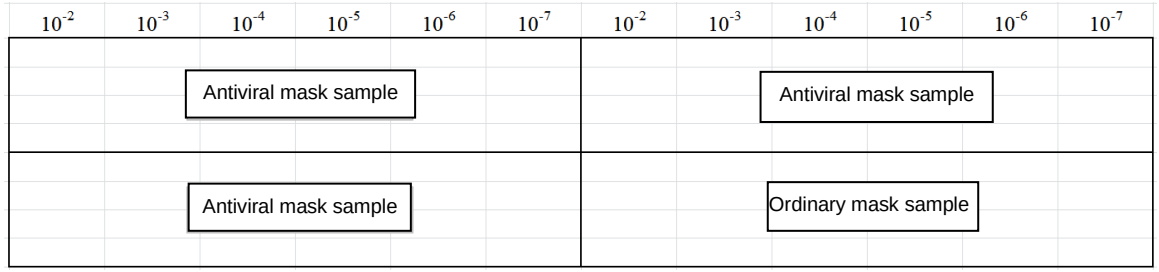
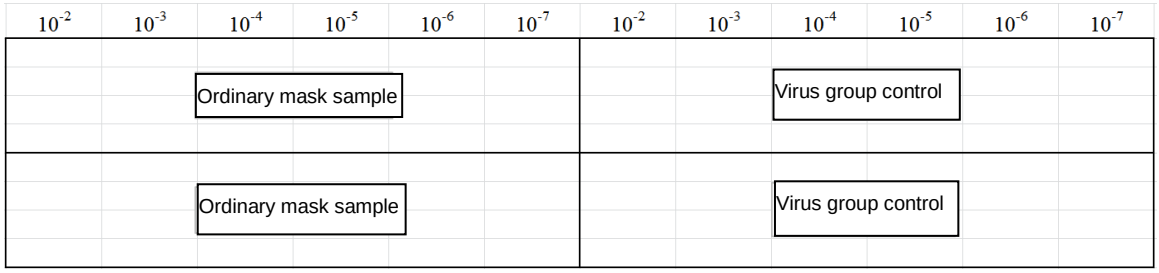



Plate 2:




5.2.4 After the cells were cultured for 3 days, the cytopathic effect (CPE) was observed under the microscope. The positive cell wells were marked as "+", otherwise they were marked as "-". The Karber method was used to calculate the virus $TCID_{50}$ value in the samples.

## 6 Detection Results

0.1mL SARS-CoV-2 virus solution was incubated with antiviral medical mask or ordinary medical mask for 20 minutes to obtain the treated virus mixture, and the virus group control was set. After the virus mixture was diluted with infected medium, it was added into Vero E6 for culturing. The results observed under the microscope after 72 hours are shown in Plate 1 and Plate 2. The titer of the virus mixture was calculated by the Karber method, and the titer of the virus group control samples was $2.60\times10^{7}TCID_{50}$/mL. The titer after the virus was incubated with antiviral medical mask was declined below the detection line, that is, the virus titer in the treated mixture was less than $3.16\times10^{4}TCID_{50}$/mL, and the titer after the virus was incubated with ordinary medical mask was $9.26\times10^{6}$ $TCID_{50}$/mL (Table 1 and Figure 1).

Plate 1:

| $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CT | CT | - | - | - | - | CT | CT | - | - | - | - |
| CT | CT | - | - | - | - | CT | CT | - | - | - | - |
| CT | CT | - | - | - | - | CT | CT | - | - | - | - |
| CT | CT | - | - | - | - | CT | CT | - | - | - | - |
| CT | CT | - | - | - | - | + | + | + | + | - | - |
| CT | CT | - | - | - | - | + | + | + | + | - | - |
| CT | CT | - | - | - | - | + | + | + | + | + | - |
| CT | CT | - | - | - | - | + | + | + | + | - | - |

Plate 2:

| $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| + | + | + | + | - | - | + | + | + | + | + | - |
| + | + | + | + | - | - | + | + | + | + | + | - |
| + | + | + | + | + | - | + | + | + | + | + | - |
| + | + | + | + | + | + | + | + | + | + | + | - |
| + | + | + | + | + | - | + | + | + | + | + | - |
| + | + | + | + | - | - | + | + | + | + | + | - |
| + | + | + | + | - | - | + | + | + | + | - | - |
| + | + | + | + | + | - | + | + | + | + | + | - |

Note: “+” indicates that cytopathic effect was observed, which is SARS-CoV-2 virus positive well; “-” indicates virus negative well. CT (cell toxicity) indicates that the sample is toxic to cells, and the cell survival rate is below 10%.

Table 1 Titer of virus samples after treatment

| | Antiviral medical mask | Ordinary medical mask | Virus control |
|---|---|---|---|
| **Log ($TCID_{50}$/ml)** | ⩽4.5 | 6.97±0.23 | 7.42±0.09 |
| **$TCID_{50}$/ml** | $\leqslant 3.16\times10^{4}$ | $9.26\times10^{6}$ | $2.60\times10^{7}$ |

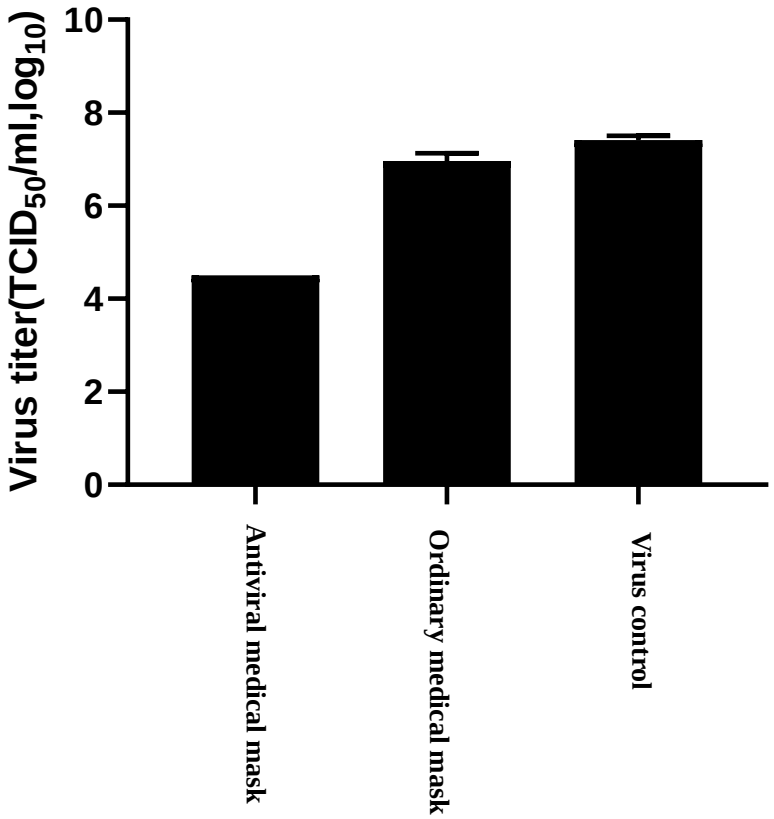



Figure 1 Titer of virus samples after treatment

## 6.3 Conclusion and analysis

According to the above results, the titer of the virus group control was $2.60\times10^{7}TCID_{50}$/mL, while the titer of the virus solution after incubation with the antiviral medical mask fell below the detection line, that is, $3.16\times10^{4}$ $TCID_{50}$/mL, down by 2.92 log value compared with the control group, indicating a virus inactivation rate of the antiviral medical masks was 99.88%.