Test Report

Inactivation Effects of the Polypropylene (PP) Spun Bond Nonwoven Fabrics Containing Copper Oxide (Materials for Peijiu Face Masks) against SARS-CoV-2

Tested Samples:

- PP Spun Bond Nonwoven Fabric Containing Copper Oxide (Salmon Pink, Cat. No. SbPpPhAb, L001, MD25), 25 gsm
- PP Spun Bond Nonwoven Fabric Containing Copper Oxide (Orange, Cat. No. SbPpPhAb, L003, ML25), 25 gsm
- PP Spun Bond Nonwoven Fabric (Blue Control, without Copper Oxide), 25 gsm
- PP Spun Bond Nonwoven Fabric (White Control, without Copper Oxide), 25 gsm

Manufacturers & Sponsors:

- Shanghai Peijiu Medical Technology Limited Company (Peijiu)
- Dongguan Lingjin Precision Manufacturing Co. LTD (Lingjin)

Date Samples Received: May 11th, 2020

Pathogen Tested:

- SARS-CoV-2, clinical isolate

Testing Laboratory:

- State Key Laboratory of Emerging Infectious Diseases (The University of Hong Kong)

Lab Director & Principal Investigator:

- Prof. Yi GUAN

State Key Laboratory of Emerging Infectious Diseases (The University of Hong Kong) 新發傳染性疾病國家重點實驗室(香港大學)

Date Tested: May 15th - June 28th, 2020

Report Completion Date: July 20th, 2020 (Chinese version)
August 9th, 2020 (English version)

SKLEID 新發傳染性疾病國家重點實驗室(香港大學) STATE KEY LABORATORY OF EMERGING INFECTIOUS DISEASES

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1. Aims of study

The following tests were carried out on the materials for the face masks jointly developed by Peijiu and Lingjin:

1) To test whether the PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu mask materials) can inactivate SARS-CoV-2;
2) To evaluate the inactivation effects of PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu mask materials) on SARS-CoV-2 with different contact time;
3) To compare the efficacy of the PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu mask materials) and the traditional medical face mask materials (PP Spun Bond Nonwoven Fabrics without Copper Oxide) in inactivating SARS-CoV-2.

2. Materials for the tests

1) Cells

Vero cells (ATCC) were preserved in our laboratory and passaged once every 2-3 days. The original medium was discarded, and 2~3 ml of 0.25% trypsin-EDTA solution was added to disassociate the adherent cells for 30 sec - 1 min, as observed under the microscope. Then 4~5 ml of fresh medium was used to resuspend the cells, followed by centrifuging and removal of the supernatant. After resuspension with appropriate volume of medium, the cells were transferred into a new culture flask and maintained in a 5% CO_2 incubator at 37°C for 2-3 days.

2) Virus strain

The SARS-CoV-2 clinical isolate AP8/2020 was amplified in Vero cells and stored at -80°C. The titer of the virus stock was 3.58×10^6 $TCID_{50}$ / ml or 3.617×10^6 PFU / ml.

3) PP Spun Bond Nonwoven Fabrics

- PP Spun Bond Nonwoven Fabric Containing Copper Oxide (Salmon Pink, Cat. No. SbPpPhAb, L001, MD25), 25 gsm
- PP Spun Bond Nonwoven Fabric Containing Copper Oxide (Orange, Cat. No. SbPpPhAb, L003, ML25), 25 gsm
- PP Spun Bond Nonwoven Fabric (Blue Control, without Copper Oxide), 25 gsm
- PP Spun Bond Nonwoven Fabric (White Control, without Copper Oxide), 25 gsm

3. Methods

1) Determination of the virus titer (plaque assay)

Vero cells were seeded on a 6-well plate with 80% confluence (about 10^6 cells/ well), culturing for 24h, and then washed twice with PBS. The virus stock was serially diluted to 10^{-1}, 10^{-2} .....and 10^{-8} dilutions, and 400 µL of each virus suspension was inoculated into the cells. For each dilution, there were replicate tests with two blank controls. Cell plates inoculated with the virus were incubated in a 5% CO_2 incubator at 37°C for 1 h, with gentle shaking every 15 min.

After 1 h of absorption, virus suspension was removed from the cell plates and discarded. Low melting point agarose prepared in 2 ml of DMEM was then added to the cells, and set at 4°C for 15 min before the plates were placed back into 37 °C and 5% CO_2 incubator for 72 h. After the incubation, cell plates were fixed with 4% formaldehyde for 2 h, followed by removal of the gel and staining with crystal violet for 20 min. The plates were then air-dried and the plaque forming unit (PFU) was calculated using the following formula: PFU / ml = number of plaques counted/ (dilution factors × inoculation volume).

2) Inactivation effects of the dry nonwoven fabric materials on SARS-CoV-2 (Test 1)

The four tested materials were cut into 2 cm × 2 cm size and UV-sterilized for 2 hours. The fabric pieces were then placed into the culture dishes, and 0.2 ml virus stock was dropped onto each piece of the materials. With a cover, the dishes were kept at room temperature for 1 hour. Then 20 ml SCDLP medium was added to resuspend the virus with repeated vortex. The supernatant was used for determination of the PFU titers. A blank control group without the use of any fabric material was included in this test. Viral titers were measured after treatment with the fabrics and the inhibition rate was calculated using the following formula: Inhibition

rate (%) = (virus titer of the control group - virus titer of the test group) / virus titer of the control group × 100.

3) Inactivation effect of different nonwoven fabrics on SARS-CoV-2 with different titers and contact times (Test 2)

The four fabric materials were cut into 2 cm × 2 cm pieces respectively. After 2 hours of UV treatment, each fabric piece was further cut into 0.5 cm × 0.5 cm size within the biosafety cabinet using sterile techniques and put into a 2 ml sample tube with tweezers, and then the ultraviolet disinfection was conducted for another 30 min.

The original virus stock was 10× serially diluted with EBSS medium and titers of 10^5, 10^4, 10^3, 10^2, 10^1 PFU / ml were obtained. Screw-cap microcentrifuge tubes were then allocated into five groups, with Groups 1~4 each containing one fabric material, whereas Group 5 serving as a blank control (without fabrics). For each of these five groups, three further subgroups were assigned, which represented the different contact time (i.e. 10 min, 30 min and 60 min respectively) between the virus and the fabric samples.

As such, 1.5 ml of the serially diluted virus were added into each tube, vortexed and co-incubated with fabrics at 37°C for 10, 30 or 60 min as designated. Vortex was conducted every 15 min to mix the samples well. Supernatant was then collected at the set time point for PFU determination.

At each time point, the viral titer was recorded after co-incubation with each of the designated materials, and the inhibition rate was calculated as follows: Inhibition rate (%) = (virus titer of the control group - virus titer of the test group) / virus titer of the control group × 100.

4. Results

1) Test 1: Inactivation effects of the dry fabric materials on SARS-CoV-2

As shown in Figure 1, the inactivation effects of the two PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu Orange and Salmon Pink mask materials) against high titers of SARS-CoV-2 were over 50% higher than those of the ordinary PP Spun Bond Nonwoven Fabrics (traditional blue and white medical mask materials).

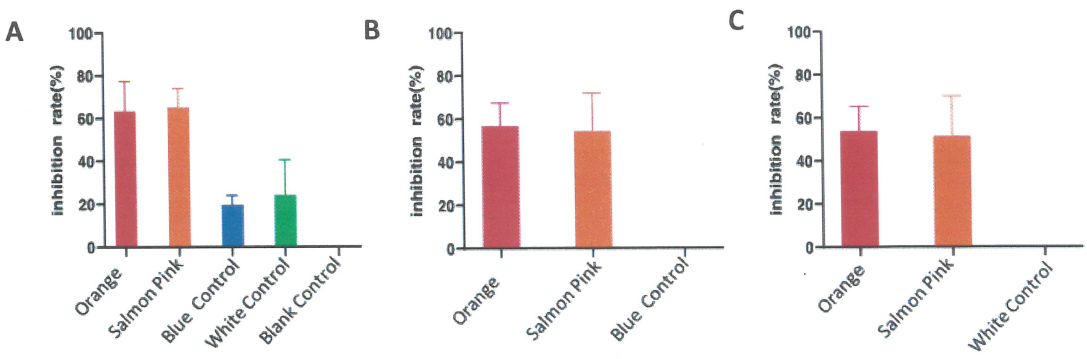

Figure 1. Inactivation effects of the dry polypropylene spunbonded nonwoven fabric materials on SARS-CoV-2

High titers of virus suspension (> 10^6 PFU / ml) were dropped onto the polypropylene spunbonded nonwoven fabrics. One hour later, the virus was resuspended with culture medium and titrations were determined. **(A)** Inhibition of the virus upon treatment with different fabrics, when compared with the blank control group. **(B)** Virus inhibition by the two PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu face mask materials), when compared with the ordinary blue PP Spun Bond Nonwoven Fabric (traditional medical mask material). **(C)** Virus inhibition by the two PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu face mask materials), when compared with the white PP Spun Bond Nonwoven Fabric (traditional medical mask material).

2) Test 2: Inactivation effects of different nonwoven fabrics on different titers of SARS-CoV-2 with different contact times

As shown in Figures 2, 3, 4 and 5, (1) the inactivation effects of both PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu mask materials, both Orange and Salmon Pink) on SARS-CoV-2 were significantly higher than that of the ordinary PP Spun Bond Nonwoven Fabric (traditional blue or white medical mask materials). (2) At the same viral titer, the longer the contact time lasted, the better the effects of virus inactivation could be observed. (3) PP Spun Bond Nonwoven Fabrics Containing Copper Oxide shown remarkable inhibition effects on SARS-CoV-2 at the titers no higher than 10^4 PFU/ml, where the virus inactivation efficiency can reach around 100% even though the contact time only lasted for 10 minutes. When the viral titer reached 10^5 PFU/ml, the inactivated efficiency can still approach 100% within 60 minutes. (4) Although the ordinary blue and white PP Spun Bond Nonwoven Fabrics also shown some inhibitory effects on low titers of SARS-CoV-2, little inactivation could be seen

with higher titers. (5) At a viral titer no higher than 10^4 PFU/ml, the inactivation effects of PP Spun Bond Nonwoven Fabrics Containing Copper Oxide were more than 80% better than that of the traditional blue or white PP Spun Bond Nonwoven Fabric without copper. When the virus titer reached 10^5 PFU/ml, the inactivation effects of PP Spun Bond Nonwoven Fabrics Containing Copper Oxide were also 50-90% better than that of the blue or white ones.

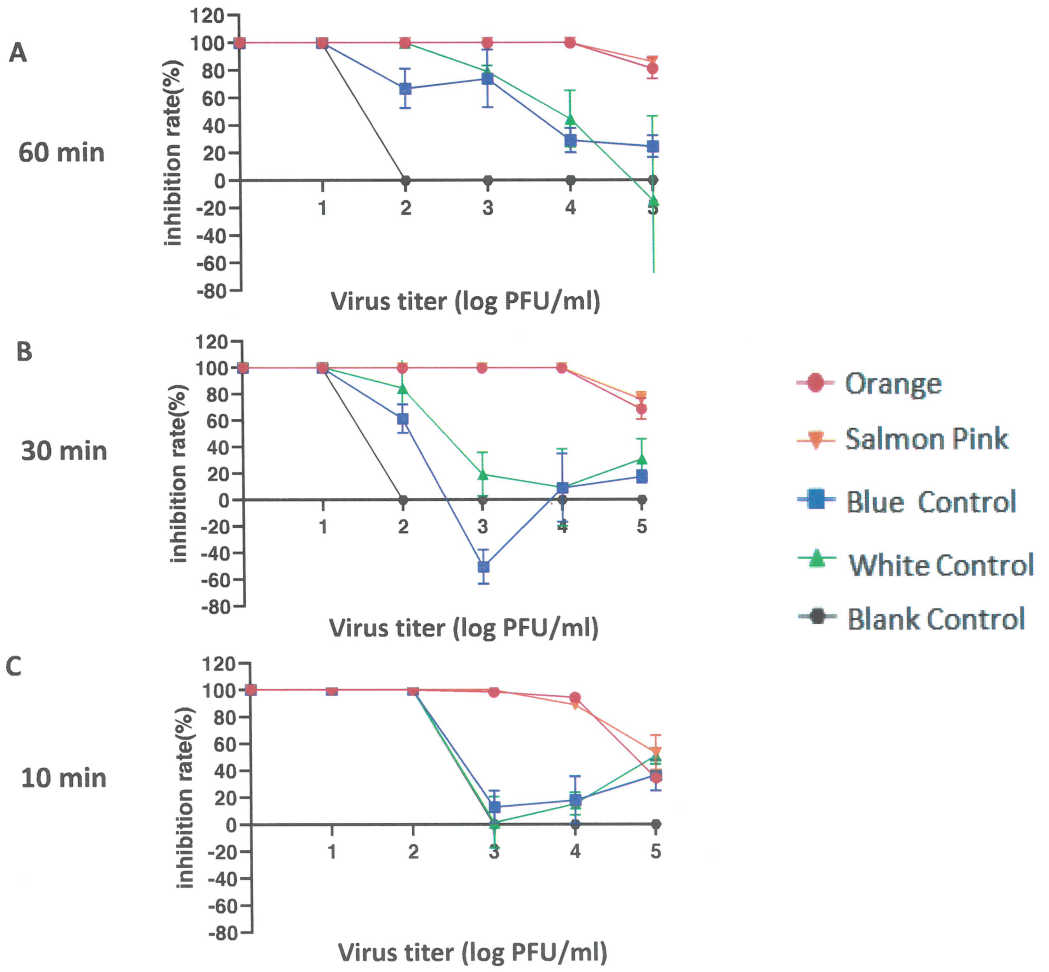

Figure 2. Inactivation effects of the polypropylene spunbonded nonwoven fabrics on SARS-CoV-2 solution

The tested materials were cut into pieces and co-incubated with different titers of diluted virus (10^1-10^5 PFU / ml) for a certain period of time. The remaining virus titers were determined respectively. The reduction and inhibition of the virus titers were calculated and compared with the blank control group with no fabrics. Results were shown after the tested materials were incubated with the virus for **(A)** 60 minutes; **(B)** 30 minutes; or **(C)** 10 minutes.

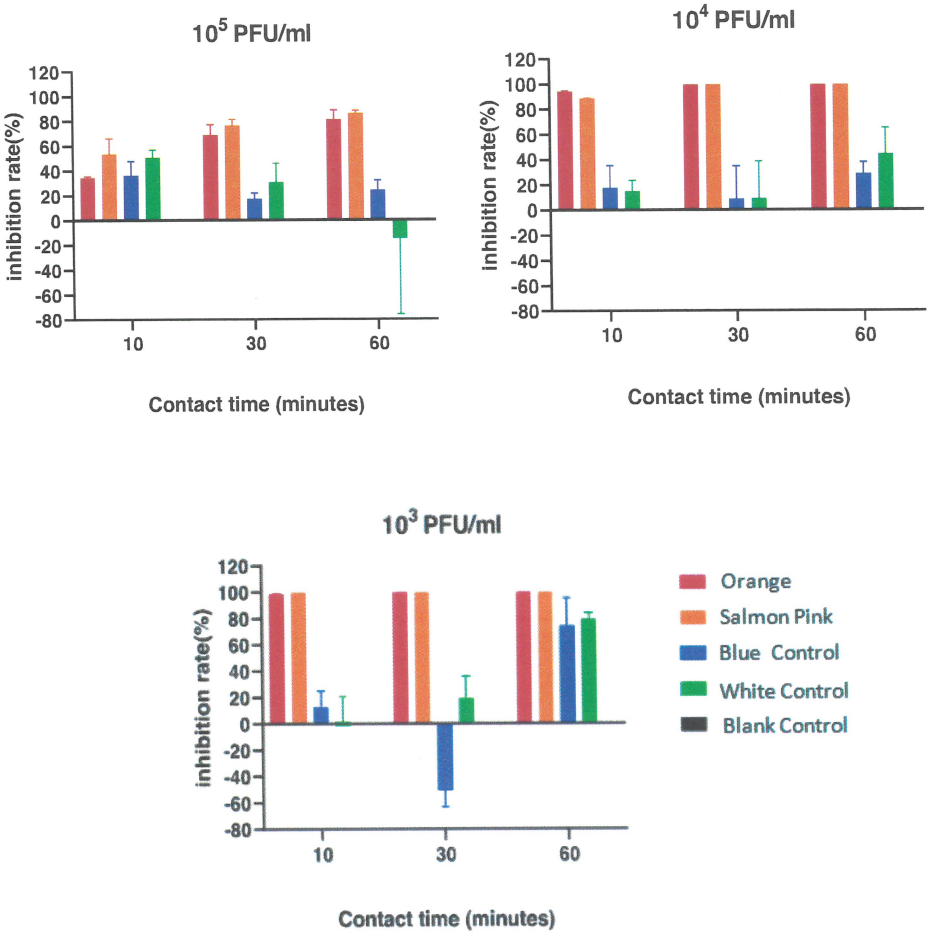

Figure 3. Inactivation effects of the different polypropylene spunbonded nonwoven fabrics against different titers (10^3 ~ 10^5 PFU/ml) of SARS-CoV-2 solution

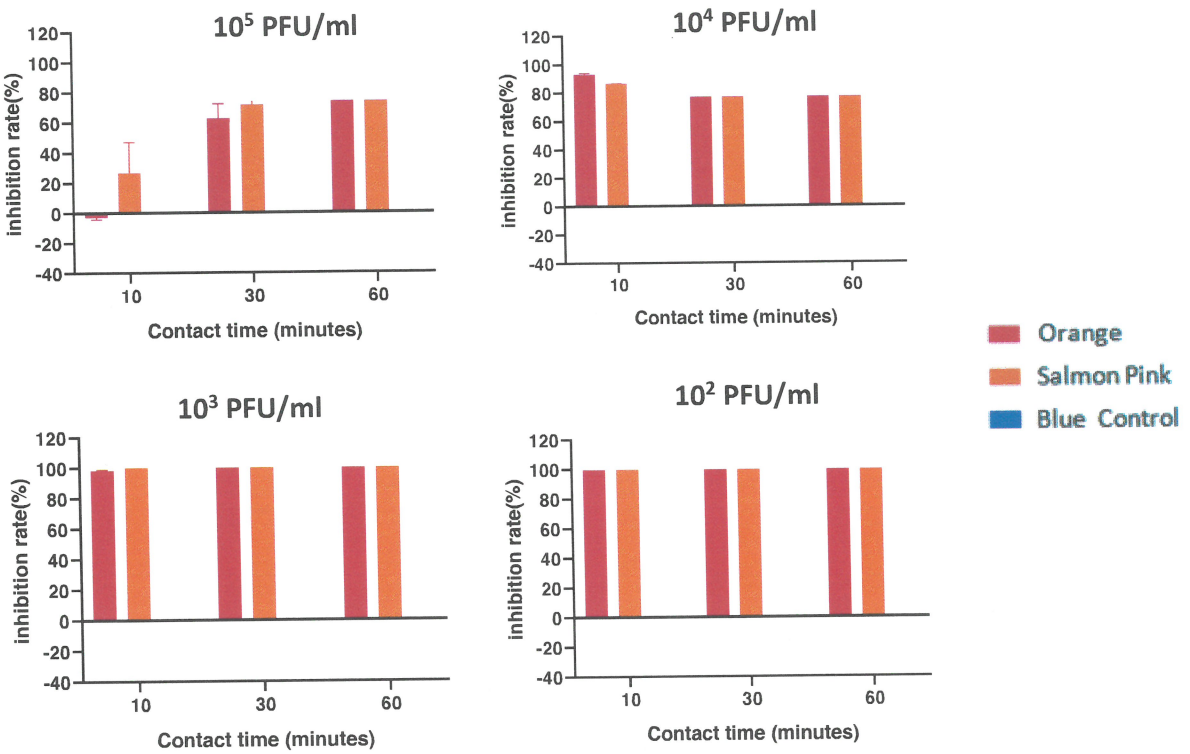

Figure 4. Inactivation effects of the PP Spun Bond Nonwoven Fabrics Containing Copper Oxide on different titers of SARS-CoV-2 (10^2~10^5 PFU/ml), compared with the ordinary blue PP Spun Bond Nonwoven Fabric control

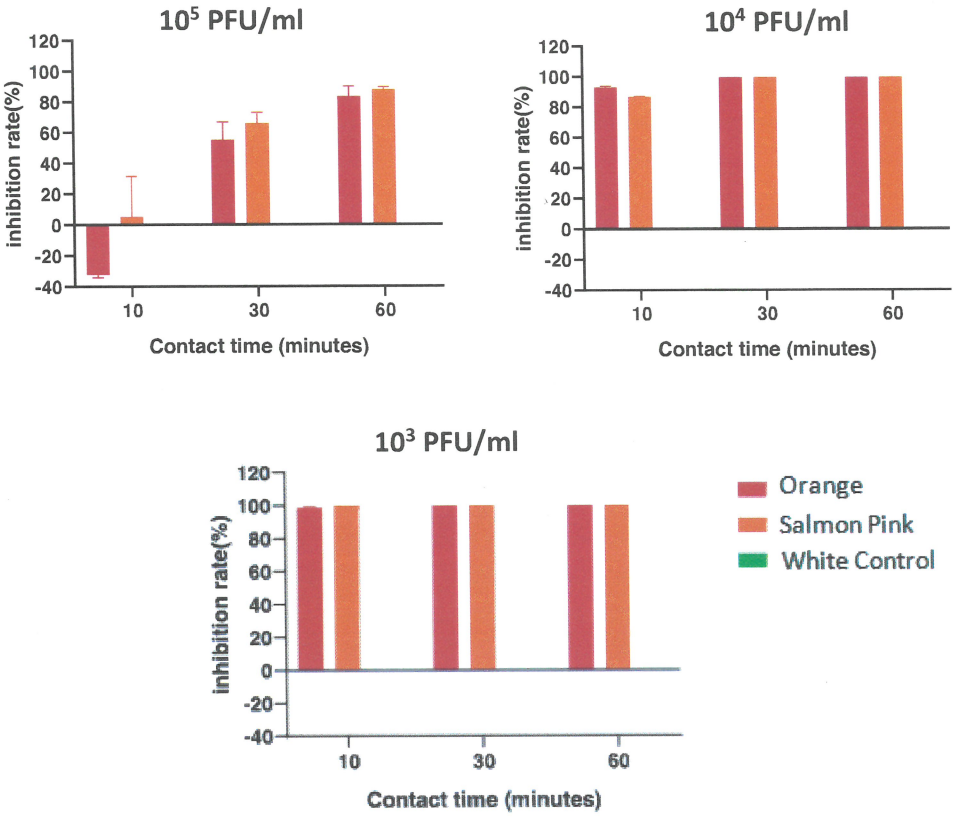

Figure 5. Inactivation effects of the PP Spun Bond Nonwoven Fabrics Containing Copper Oxide on different titers of SARS-CoV-2 (10^3~10^5 PFU/ml), compared with the ordinary white PP Spun Bond Nonwoven Fabric control

5. Conclusion

1) Both PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu Orange and Salmon Pink mask materials) shown remarkable inactivation effects on SARS-CoV-2 at all titers, which were also significantly stronger than that of the ordinary PP Spun Bond Nonwoven Fabric (traditional blue or white medical mask materials). For SARS-CoV-2 at a titer ranging from 10^2 to 10^4 PFU/ml, the inactivation efficiency of Peijiu materials could reach 100%, which was 80% higher than those of the ordinary PP Spun Bond Nonwoven Fabrics; and for SARS-CoV-2 at a higher titer (10^5 PFU/ml), the inactivation efficacy was 50-90% higher than those of the ordinary PP Spun Bond Nonwoven Fabrics.

2) At the same titer, the longer the contact time between the PP Spun Bond Nonwoven Fabrics Containing Copper Oxide (Peijiu mask materials, both Orange and Salmon Pink) and the virus lasted, the more significant inactivation effects were shown, which were also significantly higher than that of the ordinary PP Spun Bond Nonwoven Fabric (traditional blue or white medical mask materials). For SARS-CoV-2 with a titer no higher than 10^4 PFU/ml, the inactivation efficiency could reach approximately 100% within 10 minutes. For virus with 10^5 PFU/ml titer, the efficiency was also close to 100% when treated for 60 minutes.