

SIDSCO Biomedical Co., Ltd

Final Report of Contract

「 Evaluation of Test Samples against anti-SARS-CoV-2 pseudovirus infectivity *in vivo* 」

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Test Facility: SIDSCO Biomedical Co., Ltd

Test location : 4F., No. 63, Luke 2nd Rd., Luzhu Dist.,
Kaohsiung City 821

Sponsor: Light-Salt Testing Co., Ltd

Sponsor location : 3F., No. 63, Luke 2nd Rd., Luzhu Dist.,
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Study director:

2. Sponsor Information

2.1. Sponsor : Light-Salt Testing Co., Ltd

Sponsor location : 3F., No. 63, Luke 2nd Rd., Luzhu Dist., Kaohsiung City 821

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3. Abstract

This project aimed to evaluate the samples (CV19-XH) from Light-Salt Testing Co., Ltd. against SARS-CoV-2 pseudovirus infectivity *in vivo*. CV19-XH were intratracheally injected into Balb/c mice before SARS-CoV-2 pseudovirus infection. After 10 hours of drug administration, mice were intratracheally injected with SARS-CoV-2 pseudovirus. The luminescence intensity of SARS-CoV-2 pseudovirus was analyzed by IVIS spectrum *in vivo* imaging system. The results showed that group of healthy control exhibited no luminescence signal. The SARS-CoV-2 pseudovirus-infected mice exhibited significant luminescence signal. Compared to SARS-CoV-2 pseudovirus group, CV19-XH-treated mice exhibited lower level of luminescence signals. The result of statistical analysis indicated that the luminescence levels between the sample-treated groups and SARS-CoV-2 pseudovirus-infected group exhibited significant difference.

4. Information of test and reference item

4.1 Test item: CV19-XH

4.2 Reference item: Saline

5. Materials and methods

5.1. Animal, chemicals, dose, group

5.1.1 Animal: 6-week-old female Balb/c mice

5.1.2 Virus: SARS-CoV-2 pseudovirus

5.1.3 Dose: 1 % CV19-XH

5.1.4 Groups:

- | | | |
|----|---|--------|
| a. | Health control (HC) | 4 mice |
| b. | SARS-CoV-2 pseudovirus (virus) | 4 mice |
| c. | CV19-XH (1%) 10 hr + SARS-CoV-2 pseudovirus (10 hr) | 4 mice |

5.2. Methods

5.2.1 Twelve of 6-week-old female BALB/c mice were allowed to adapt to the laboratory housing for 1 to 2 weeks. After adaption, 4 mice were receiving CV19-XH (50 μ l) by intratracheally injection at 10 hours before SARS-CoV-2 pseudovirus (10^{10} PFU/mouse) using intra-tracheal injection. Mice then received 100 μ l of luciferin using intraperitoneal injection at 24 hours post infection, and the luminescence signal at lung was detected and analyzed by IVIS[®] Spectrum In VIVO Imaging System (PerkinElmer Inc.) (4 mice per photo). The exposure time was 1 min.

5.2.2 Analyzing items:

- a) luminescence intensity of mice.

6. Results

The results showed that the luminescence signals were obviously observed in lung of the pseudovirus (virus) group; no luminescence signal was observed in health control (HC) group; the luminescence signals were observed in part of mice in the pseudovirus + CV19-XH groups (Figure 1). The raw data of luminescence signals which was acquired from the quantification software of IVIS spectrum system were listed in table 1. Raw data from the IVIS Spectrum was used for calculation of statistically significant using Prism (GraphPad). The Scatter Plot was used to show the signal intensities of each mouse (Figure 2).

To graphically depicting the dispersion of data of each group, the boxplot was used to show the quartiles and median, and the outliers were plotted as individual points (Figure 3). There is no outlier shown in the figure. The descriptive analysis of each group signal in definite signal showed following results: HC group: median = 0 (first quartile = 0, third quartile = 0); virus group: median = 256610 (first quartile = 244413, third quartile = 368030); 10 hr group: median = 0 (first quartile = 0, third quartile = 7113).

The average data of total FLUX [p/s] of virus group and 10 hr sample group were 355833 and 7113, respectively. There was significant difference between these two groups. ($p < 0.05$)

7. Figures

Figure 1. Luminescence images of each group without quantification.

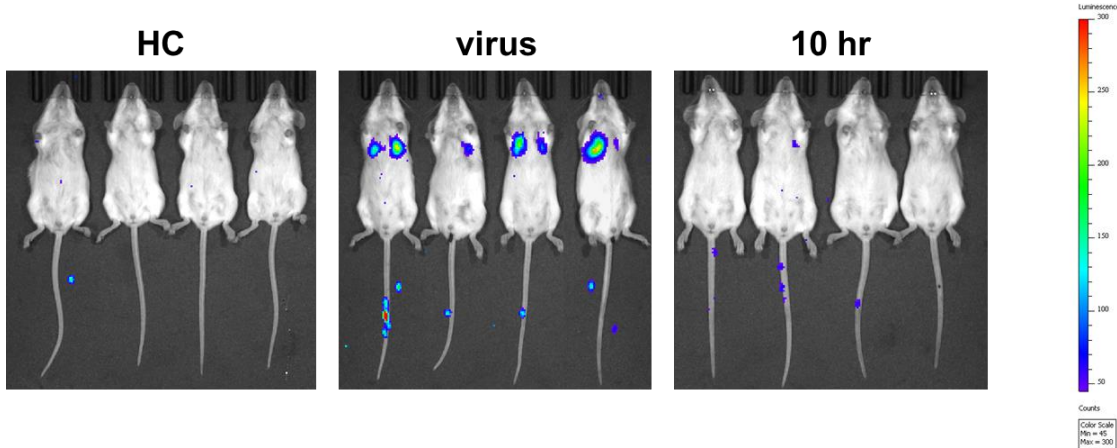


Figure 2. Luminescence intensities of each group (exhibited by scatter plot).

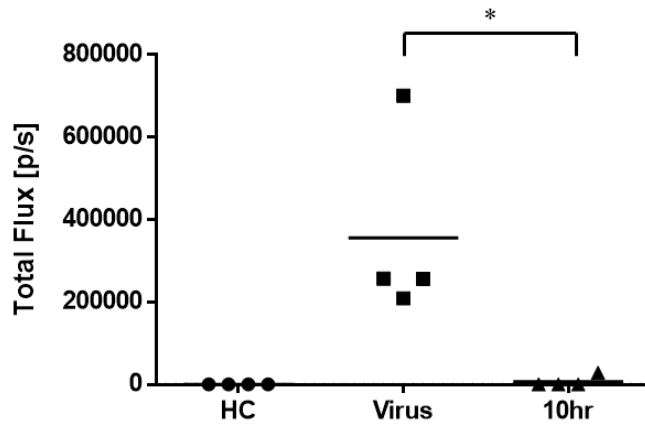


Figure 3. Luminescence quartile of each group (exhibited by box plot).

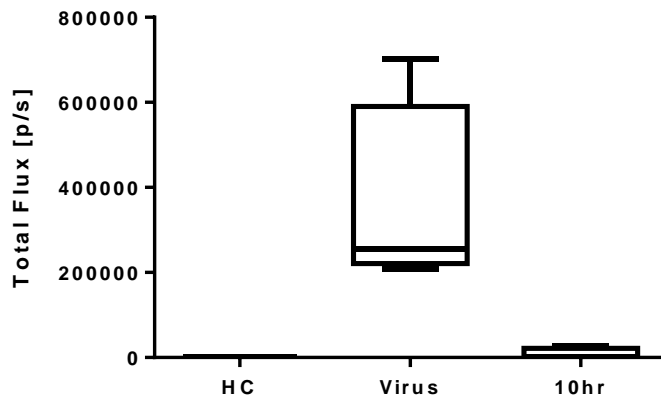


Table 1. Raw data of luminescence of each mice.

	HC	Virus	10 hr
Mouse#1	0	256940	0
Mouse#2	0	208810	28450
Mosue#3	0	256280	0
Mouse#4	0	701300	0



Laboratory supervisor :

Date: 2021/06/11