KR Biotech Co., Ltd.
Institute of Infectious Disease Control

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TEST REPORT

KR-2009-042-MTN01-C

Virucidal Activity Test

by LED Irradiation

KR BIOTECH Co., Ltd

Institute of Infectious Disease Control

Summary of the Experiment

○ **Test:** Virucidal Activity Test

○ **Test No:** KR-2009-042-MTN01-C

○ **Product Name** CLEAN SERIES MODULE

○ **Client**

Affiliation : MALTANI Corp.

Address : Malitani B/D, Yeoksam-Ro, Kangnam-Gu, Seoul 06196, Korea

○ **Institute**

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Sign **date** Sept. 17, 2020

KR BIOTECH Co., Ltd

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September 17, 2020

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1. Summary

This test was to measure the efficacy of virus killing of the CLEAN SERIES MODULE presented by MALTANI Corp. The SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus) virus was used as a test virus. After irradiating the COVID-19 virus with LED for a certain period of time, the test was conducted in such a manner as to check the activity of the virus. The virucidal activity was evaluated by infecting the host cell with the virus and then measuring by a 50% tissue culture infectious dose assay ($TCID_{50}$). As a result of confirming the virus reduction rate by the CLEAN SERIES MODULE of MALTANI Corp. under this test condition, it was confirmed that SARS-CoV-2 virus showed more than 99.99% killing effect as a result of treatment for 60 minutes at a distance 20 cm.

2. Outline of the test

2.1 Test schedule

Test start date: September 04, 2020

Test end date: September 11, 2020

2.2 Scope of test

This test method is to evaluate the efficacy of killing the COVID-19 virus by irradiating the LED (wavelength 405nm) provided by MALTANI Corp. at a distance 20 cm. The test method was set based on previously published papers (Refs. 11, 12, 13) because the guideline for testing virus killing by LED irradiation was not released.

3. Materials and Equipment

3.1 Test materials

The sample was provided by the client MALTANI Corp.

Fig 1. CLEAN SERIES MODULE of MALTANI Corp.

3.2 Culture media and reagents

(1) Dulbecco's Modified Eagle Medium (DMEM), Hyclone, US

(2) Dulbecco's Phosphate buffered saline (PBS), Invitrogen, US

(3) Fetal bovine serum (FBS), Gibco, US

(4) Trypsin-EDTA (0.25% Trypsin), Gibco, US

(5) Penicillin-Streptomycin, Gibco, US

(6) Ethyl Alcohol (EtOH), Duksan Pharmaceutical, South Korea

(7) Hydrochloric Acid (HCl), Daejung, South Korea

(8) Formaldehyde (HCHO), Duksan Pharmaceutical, South Korea

(9) Crystal Violet, JUNSEI, Japan

3.3 Equipment and facility

(1) Biological safety cabinet (sterile worktable), Thermo scientific, US

(2) Optical microscope, OPTINITY, China

(3) Centrifuge (LABOGENE1248), Zyrozen, South Korea

(4) Refrigerator (4℃), Samsung Electronics, South Korea

(5) Freezer (-20℃), Samsung Electronics, South Korea

(6) Cryogenic freezer (-80℃), Thermo scientific, US

(7) Constant temperature carbon dioxide gas incubator (37℃) BB15, Thermo scientific, US

(8) Vortex mixer KMC-1300V, Vision Science, South Korea

(9) Dry oven HM-28, Hanil Science, South Korea

(10) LN2 Tank (Locator JR Plus), Thermo scientific, US

(11) Water bath, Korea Science, South Korea

(12) Multi well plate reader, Epoch, US

(13) PE6000, Mettler Instrument, US

(14) BSL-3 (No. KCDC-09-3-01)

4. Methods

4.1 Host cell line and culture

The cell line Vero-E6 is isolated from renal epithelial cells extracted from African green monkeys. Since SARS-CoV-2 can be cultured and causes virus-infected cell lesion (Cytopathic effect), Vero-E6 is used as a host cell in this test for measuring the viral titer.

4.2 Virus

COVID-19 (SARS-CoV-2)

- The Corona Virus COVID-19 (SARS-CoV-2) was first emerged in Wuhan, China in December 2019, and currently in May 21, 2020, there are over 4.8 million people infected worldwide. In addition, over 310,000 people died from COVID-19, and it is still spreading seriously in the US and in South America, etc.

- COVID-19 is included in the beta-corona classification to have positive single-strand RNA as the genome, and it is a spherical form of virus with envelope.

- In March 11, 2020, WHO declared pandemic on this virus, and there is no medicine or vaccine in the present. The resistance to the disinfectant is in mid-grade, but the spreading power is very high to have serious impact globally.

Severe acute respiratory syndrome-related coronavirus (SARS-CoV-2)

- Classification: Coronaviridae family, Betacoronavirus
- Virus genome: ss-RNA
- envelope: Yes
- Resistance: middle
- Titer: 6.81 x 10^6 $TCID_{50}$/mL

4.3 Virucidal test

This test is to evaluate the efficacy of killing the COVID-19 virus by irradiating the LED (wavelength 405nm) provided by MALTANI Corp. Virus killing test by LED irradiation was established based on previously published papers (Refs. 11, 12, 13).

① One day before the test, prepare Vero-E6 cells in a 96 well plate.

② Add 100μl of SARS-CoV-2 virus (6.81 x 10^6 $TCID_{50}$/ml) to the petri dish, and cover with quartz cover and then irradiate LED for 30 minutes, 60 minutes, and 90 minutes at a distance of 20 cm.

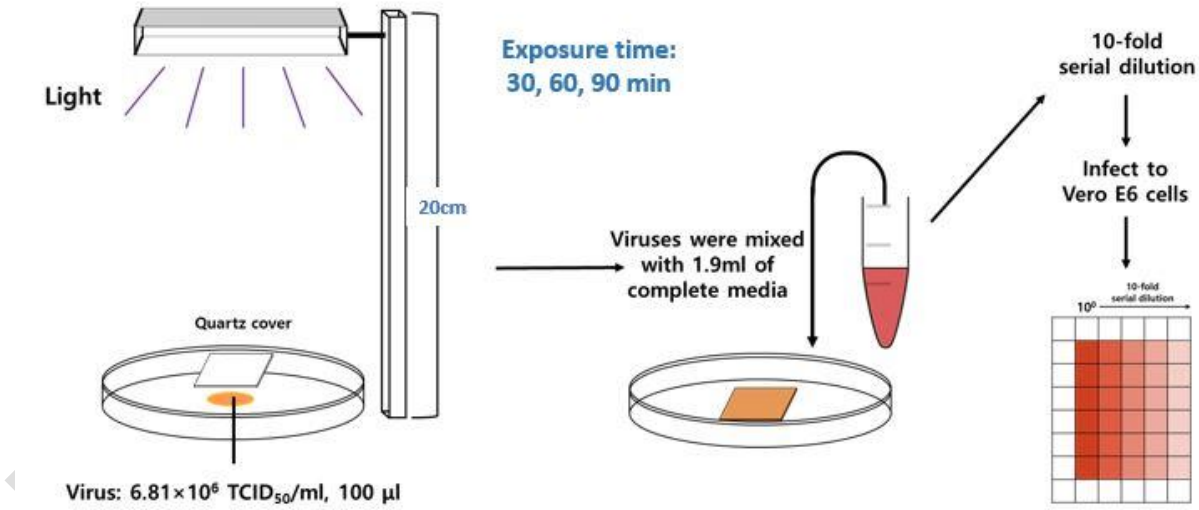
③ Each virus irradiated by time mix with 1.9ml of culture medium, and prepare a 10^n step dilution solution.

④ Each diluent was infected with Vero-E6 cells, and cultured at 5% CO_2 at 37°C. As a control, a virus not irradiated with LED was serially diluted in the same manner.

⑤ After 3 days of culture, cytopathic effect (CPE) was observed under a microscope.

⑥ Crystal violet staining reagent was treated with cells and stained at room temperature for 30 minutes.

⑦ The titer of the virus was calculated by counting the number of stained wells.



4.4 Data reading and calculation

4.4.1 Virucidal Test

To evaluate the virus killing efficacy, each diluent was inoculated into a host cell, and virus titers of the control group and the test group were measured after 3 days.

The number of wells stained with Crystal violet dyeing reagent was counted to calculate the titer by Sperman-Karber method. Virus titers were calculated according to 4.4.2 and reduction rates were determined according to 4.4.3.

4.4.2 Calculate viral titer

The virus titers can be confirmed by observing the morphological changes (CPE) of cultured cells caused by virus growth for a period of time. The virus infectious value is obtained by inoculating, cultivating, and observing the cultured cells seeded in a plurality of incubators by preparing a 10^n dilution series of the virus solution. After the CPE observation for a certain period of time (four days post infection), the virus infection value ($TCID_{50}$) is calculated according to ICH Q5A (R1), which is indicated by taking the commercial log value.

The number of wells determined to be positive is cumulatively calculated from the high diluent side to obtain the cumulative positive rate (%) of each diluent.

$$TCID_{50}: N=10^{[(A-50)/(A-B)]-(a)}$$

How to calculate viral titer

1) Calculate the cumulative for number of well which had decided to be positive from high diluted solution and obtain the cumulated positivity rate (%) of each diluted solution.
2) Obtain 50% of cumulative positivity rate and cumulative positivity rate of high diluted solution is called as A; cumulative positivity rate of low diluted solution is called as B; and the natural logarithm value of diluted solution with A obtained is called as a.
3) Obtain the viral titer according to the following formula.

However, if overall well became negative even for the diluted solution having the lowest magnification, assume that overall well become positive in the diluted solution that is one

step lower than that diluted solution and then calculate; add a sign of inequality to obtained value and then write down. And make the valid number to have 2 digits by rounding the 3rd number of calculated value for valid digit number of viral titer.

4.4.3 How to calculate the viral reduction factor (Ri)

Regarding the combustion process, the viral reduction factor (Ri) can be calculated with natural logarithm for ratio of viral titer in the test solution, whether the sample underwent combustion process or not for test solution. However, in case of reduction of viral titer in the test solution is less than 10^1 ($\log_{10}$= natural logarithm value 1), it is not determined as the reduction of viral titer and not used for calculation of viral clearance factor.

How to calculate the viral reduction factor (Ri)

- Viral titer appeared in the experimental group before the combustion: 10^A
  Total amount of test solution before the combustion: V^A
  - ➔ Viral titer of test solution before the combustion $V^A \times 10^A = N_A$
- Viral titer appeared in the experimental group after the combustion: 10^B
  Total amount of test solution after the combustion: V^B
  - ➔ Viral titer of test solution after the combustion $V^B \times 10^B = N_B$

Viral titer (Ri) of test solution is

$$10^{Ri} = V^A \times 10^A / V^B \times 10^B = N_A / B_A$$

$$Ri = \log_{10}(N_A / B_A) = \log_{10} N_A - \log_{10} N_B$$

5. Results

5.1 Virucidal test

The initial virus titer of SARS-CoV-2 for the test is 6.83 log_{10} $TCID_{50}$/ml.

The titer of the control group was 6.22 $log_{10}TCID_{50}$/ml after 30 minutes and 60 minutes, 5.80 $log_{10}TCID_{50}$/ml after 90minutes as a result of calculating the titer through cell infection using a sample that was not irradiated with a LED as a control. After irradiating the LED of the requested CLEAN SERIES MODULE to the virus solution for 30 minutes, 60 minutes, and 90 minutes the titers were calculated through cell infection, and the titers of the test groups were 3.80, 1.80 and 1.80 log_{10} $TCID_{50}$/ml, respectively. Therefore, the reduction rate of SARS-CoV-2 by LED irradiation was confirmed to be 4.42 after 60 minutes, and the virus killing efficacy was 99.99% or more.

Table 1. Virus titer calculation

(unit: $log_{10}TCID_{50}$/ml)

Virus	Treatment	Virus titer	Control (PBS)	Test
SARS-CoV-2	30 min	6.83	6.22	3.80
	60 min	6.83	6.22	≤1.80
	90 min	6.83	5.80	≤1.80

Table 2. Virus reduction rate

Virus	Treatment	Log reduction (LR)
SARS-CoV-2	30 min	2.42
	60 min	≥4.42
	90 min	≥4.00

$LR = L_U - L_T$

L_U : Virus titer of the control (untreated)

L_T : Virus titer of the test (treated)

Table 3. Virucidal test results

Product	Virus	Treatment	Virus reduction (log)	Virus reduction (%)
CLEAN SERIES MODULE	SARS-CoV-2	30 min	2.42	99.62%
		60 min	≥4.42	≥99.99%
		90 min	≥4.00	≥99.99%

* Interpretation of results

Log reduction	Percent (%) reduction
≥1	≥90 %
≥2	≥99 %
≥3	≥99.9 %
≥4	≥99.99 %
≥5	≥99.999 %

6. Conclusion

The SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus) virus reduction rate (virucidal rate) by CLEAN SERIES MODULE of MALTANI Corp. under guideline test conditions was 4.42 after 60 minutes treatment at a distance of 20 cm, confirming the virus killing efficacy of 99.99% or more.

7. References

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