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TEST REPORT

KR-2102-094-SPE01

Virucidal Activity Test

KR BIOTECH CO., Ltd.

Institute of Infectious Disease Control

Summary of the Experiment

○ **Test:** Virucidal Activity Test

○ **Test No:** KR-2102-094-SPE01

○ **Product Name** H-Ioncluster module (AIOII)

○ **Client**

Affiliation : A subsidiary of Sudo Premium Engineering Co., Ltd.

Address : 13, Banpo 4-gil, Seocho-gu, Seoul, Republic of Korea

○ **Institute**

Affiliation : KR BIOTECH Co., Ltd. (ISO13485:2016)

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Sign **date** March.09, 2021

KR BIOTECH Co., Ltd

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March 09, 2021

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1. Summary

This test was conducted to measure the efficacy of the virus-killing of the H-Ioncluster module (AIOII) presented by A subsidiary of Sudo Premium Engineering Co., Ltd. The SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus) virus was used as a test virus, and the test sample was contacted with the virus culture solution for a period of time. Then the test was conducted by confirming the activity of the virus. The virucidal activity was confirmed by infecting the host cell with the virus and then measuring by a 50 % tissue culture infectious dose assay ($TCID_{50}$). Under this test condition, H-Ioncluster module (AIOII) showed 93.176%, 98.530% killing effect on the 60, 120 minutes condition, respectively against SARS-CoV-2

2. Outline of the test

2.1 Test schedule

Test start date: Febuarary 18, 2021

Test end date: Febuarary 26, 2021

2.2 Scope of test

This test method was performed to verify the anti-viral efficacy of the H-Ioncluster module (AIOII) by verifying the activity of the virus after processing the SARS-CoV-2 culture solution on the requested sample for a certain period of time.

3. Materials and Equipment

3.1 Test materials

The sample was provided by the client A subsidiary of Sudo Premium Engineering Co., Ltd.

Fig 1. The H-Ioncluster module (AIOII)

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

SARS-CoV-2

- The 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.

- The SARS-CoV-2 is included in the beta-corona classification to have positive single-strand RNA as the genome, and it is a spherical form of the virus with envelope.

- On March 11, 2020, the 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 a serious impact globally.

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

- Classification: Coronaviridae family, Betacoronavirus
- Virus genome: (+)ssRNA
- Envelope: Yes
- Resistance: middle
- Titer: 3.16 x 10^6 $TCID_{50}$/mL

4.3 Virucidal Test

This test was conducted for the virus-killing test by H-Ioncluster module (AIOII).

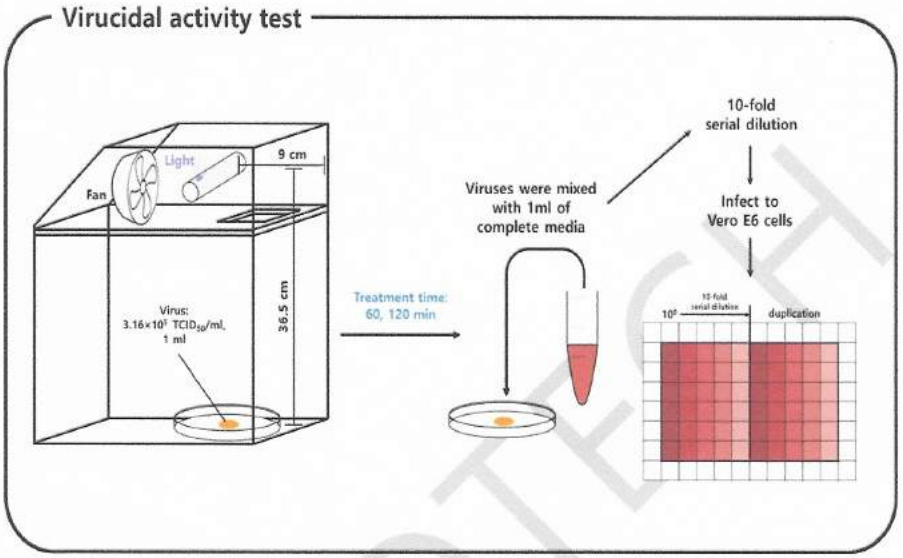

Fig 2. Outline diagram of virucidal activity test

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

② Put 1ml of diluted SARS-CoV-2 virus (3.16 x 10^5 $TCID_{50}$/ml) in 4 petri dishes. And place 2 petri dishes in the chamber, other 2 petri dishes place to the outside of the chamber. Use the petri dish outside the chamber as a control group.

③ After 1 and 2 hours of operation, take out one petri dish, respectively. In order to make 10^n diluent, add 1ml of culture medium to the petri dish, mix it well, and obtain the virus.

④ Each diluent was infected with Vero-E6 cells, and cultured at 5% CO_2 at 37°C.

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 titer 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 after infection), the virus titer ($TCID_{50}$) 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 the 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)

- 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

Table 1. Virus titer calculation

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

Virus	Titer(initial)	Treatment	Control	Test
SARS-CoV-2	6.500	60 min	5.134	3.968
		120 min	4.717	2.884

Table 2. Virus reduction rate

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

Virus	Treatment	Log reduction (LR)
SARS-CoV-2	60 min	1.166
	120 min	1.833

$LR = L_U - L_T$

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 (%)
H-Ioncluster module (AIOII)	SARS-CoV-2	60 min	1.166	93.176%
		120 min	1.833	98.530%

* Interpretation of results

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

The initial virus titer of SARS-CoV-2 is 6.500 log_{10} $TCID_{50}$/ml and the titers of the control group are confirmed 5.134 , 4.717 log_{10} $TCID_{50}$/ml at 60, and 120 minutes operation, respectively.

The virus titers after operation of H-Ioncluster module (AIOII) are confirmed 3.968., 2.884 $TCID_{50}$/ml on 60, 120 minutes, respectively. According to this result, the H-Ioncluster module (AIOII) showed the virus reduction rate 1.166, 1.833 at 60, 120 minutes operation, respectively. As a result, it has been confirmed that the H-Ioncluster module (AIOII) has 93.176%, 98.530% virus-killing efficacy at 60, 120 minutes operation condition, respectively.

6. Conclusion

The H-Ioncluster module (AIOII) of A subsidiary of Sudo Premium Engineering Co., Ltd had 93.176%, 98.530% virus killing effect for 60, 120 minutes operating condition, respectively against SARS-CoV-2.

7. References

(1) ASTM E1052-11, Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension

(2) Schmidt, N. J. et. Al., Diagnostic Procedures for Viral, Rickettsial and Chlamydial infection, 7th edition, Am. Pub. Hlth. Assoc., Washington, DC, 1995.

(3) BS EN 14476:2013 A1:2015, Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area

(4) Test method for the evaluation of virucidal efficacy of three common liquid surface disinfectants on a simulation environmental surface. Appl Microbiol, 28(1974), pp.748-752

(5) In vitro evaluation of antiviral and virucidal activity of a high molecular weight hyaluronic acid. Virology Journal 8, Article number:141(2011)

(6) Virucidal and Neutralizing Activity Tests for Antiviral Substances and Antibodies 10.21769/BioProtoc.2855 Vol 8, Iss 10, May 20, 2018

(7) Guidelines for disinfectants for external use (non-pharmaceutical products) Effectiveness Evaluation Act 2014.8. Food and Drug Safety Evaluation Institute

(8) Sterilization. Disinfectant Efficacy Test Method Data Collection 2018. 12. National Institute of Environmental Science

(9) ISO21702:2019, Measurement of antiviral activity on plastics and other non-porous surfaces

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