date of issue: September 8, 2020

Test report

Verification results of inactivating activity of ALTANT against the COVID-19(SARS-CoV-2)

Experiment period: July 1, 2020-September 8, 2020

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[E-Tech Co., Ltd. translated this report from "Japanese version" to "English version"]

[Material]

・Virus solution

The JPN / TY / WK-521 strain provided by the National Institute of Infectious Diseases was used. For the experiment,

a viral growth medium (VGMCoV-2 ; see below for composition, but not amphotericin B) containing SARS- CoV-2 was used as the virus solution (virus titer: approx. 6.75 $\log_{10}$ $TCID_{50}$ / ml).

・Cells used, medium

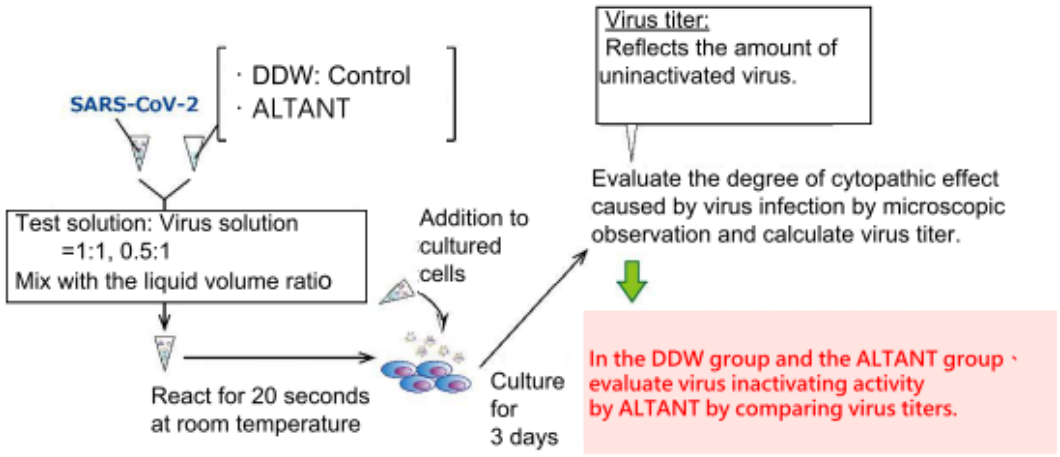
VeroE6 / TEMPRSS2 cells donated by the National Institute of Infectious Diseases were used. As the cell growth medium, modified Dalveco supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 μ g / ml canamycin, 2 μ g / ml amhotericin B, and 500 μ g / ml G418. Eagle's medium (DMEM) was used. As VGM, DMEM supplemented with 1% fetal bovine serum, 2 mM L-glutamine, 100 μ g / ml kanamycin, and 2 μ g / ml amphotericin B was used. On the day of the test, VereE6 / TEMPRSS2 cells were inoculated into a 96-well plate in advance so that the cells became 90-100% confluent, washed once with serum-free MEM immediately before the experiment, and then 180 μ l / well was added to VGM.

[Test solution]

・ALTANT

[Evaluation method]

ALTANT and virus solution (SARS-CoV-2 / VGM) were mixed in a screw cap tube so that the liquid volume ratio was 1: 1 or 0.5: 1, and pipetting was performed 10 times or more. At that time, a group in which distilled water (DDW) and virus solution were mixed was also placed as a reference group. The test was conducted with 3 tubes in 1 group (n=3). After reacting the mixture at room temperature for 20 seconds, 20 μ l of the mixture was added to a 96 well plate pre-inoculated with VeroE6 / TMPRSS2 cells (from each tube to 2 wells). After that, 10-fold step dilution was performed on a 96-well plate. After culturing in a CO2 incubator at 37 ° C for 3 days, the cytopathic effect caused by virus infection / proliferation was observed under a microscope, and based on this, the virus titer ($TCID_{50}$ / ml) was observed using the Behrenskelber method. Was calculated. The virus titers were compared between the DDW group and the ALTANT group, and the virus inactivating activity of ALTANT was evaluated.



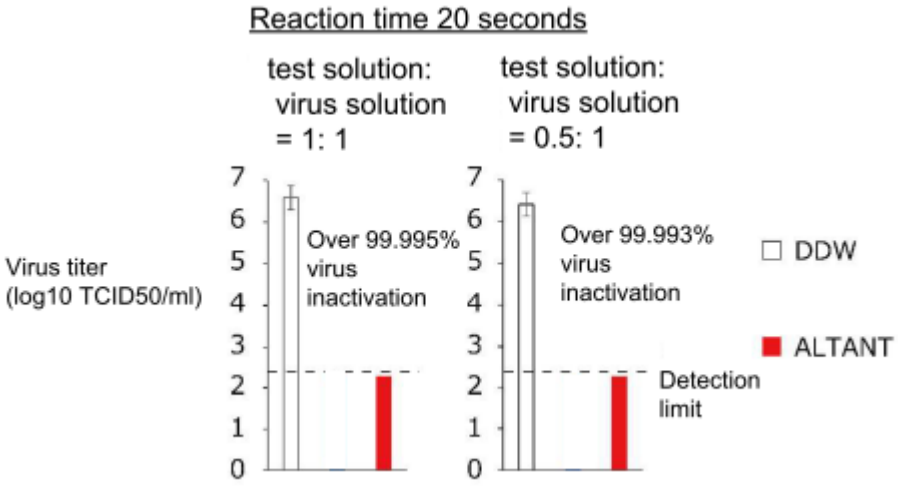
[Result]

Reaction time 20 seconds, test solution: virus solution = 1: 1, 0.5: 1

(≤2.25: below the detection limit)

		Mixing ratio 1: 1		Mixing ratio 0.5: 1	
		DDW	ALTANT	DDW	ALTANT
Virus titer ($log_{10}TCID_{50}$/ml)	Tube 1	6.75	≦2.25	6.25	≦2.25
	Tube 2	6.25	≦2.25	6.75	≦2.25
	Tube 3	6.75	≦2.25	6.25	≦2.25
	Mean ±standard deviation	6.58 ±0.29	≦2.25 ±0.00	6.42 ±0.29	≦2.25 ±0.00
	Difference between the mean values of the DDW group and the ALTANT group	-	≧4.33	-	≧4.17
Virus inactivation rate in the ALTANT group compared to the DDW group (%)		-	≧99.995	-	≧99.993

Average value graph



When the mixture ratio of the test solution and the virus solution was 1: 1 or 0.5: 1, the virus titer in the ALTANT group was below the detection limit in the reaction time of 20 seconds. Over 99.99% of viruses were inactivated compared to the DDW group.