New coronavirus (SARS-CoV-2)

Inactivation (removal) effect confirmation test report (1)

Test condition

Test sample	**Hypochlorite water preparation** Content (Chlorous acid $HClO_2$ = 68.46) as 0.8% (at the time of manufacture) Free chlorine concentration (as Cl = 35.45) 200 mg / L or more.
Virus strain	SARS-CoV-2 (2019-nCoV / Japan / AI / I-004 / 2020) strain (Distributed by the National Institute of Infectious Diseases)
Host Cell	VeroE6 / TMPRSS2 cells (JCRB1819)
FBS concentration in virus solution	0%
Medium for virus culture	Dulbecco's Modified Eagle Medium (DMEM) (FUJIFILM Wako Pure Chemical Industries)
Virus titer detection method	$TCID_{50}$ method
Virus solution: sample solution ratio	1:9
Initial virus concentration	approximately $2x10^7$ $TCID_{50}$ / mL

Sample Preparation

Test sample: The chlorinated water preparation was diluted with distilled water using a polystyrene.
The reaction with the virus was also carried out in a polyvinyl styrene tube. In addition, this virus solution and the diluted solution of the test sample: chlorite water preparation is mixed at a ratio of (1: 9).

Preparation of virus solution

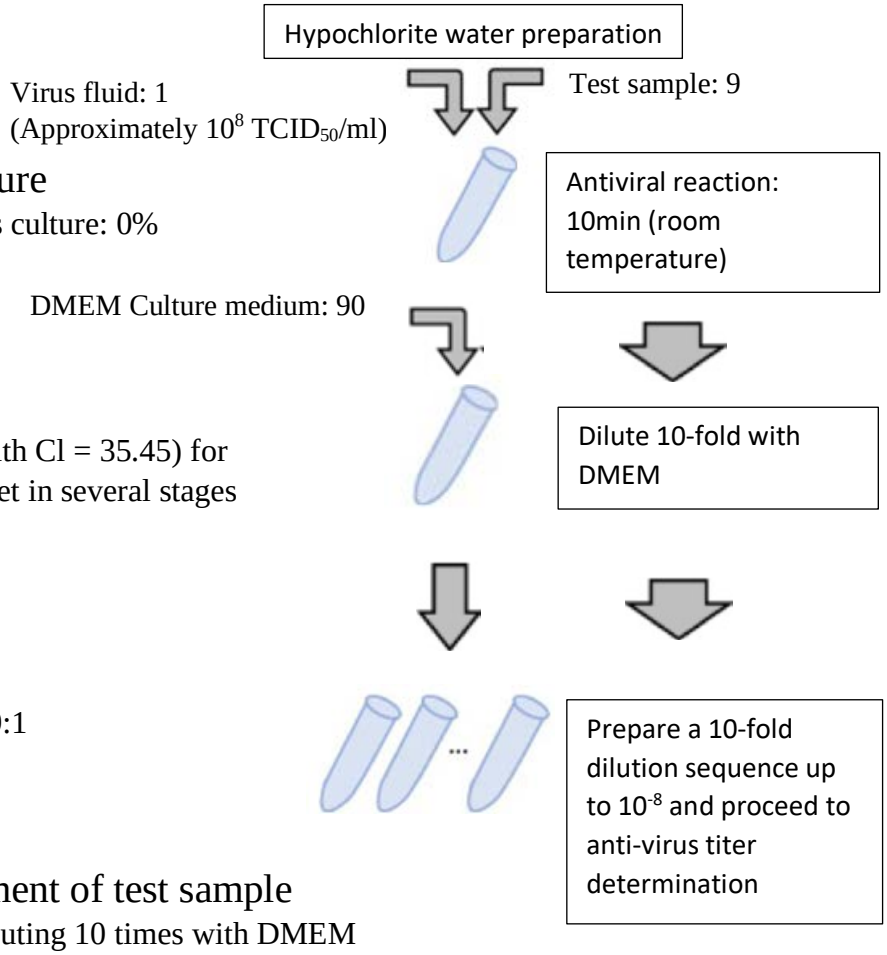
VeroE6 / IMPRSS2 cells (10-cm dish) were inoculated with the virus solution so that the moi was about 0.01, and after 1 hour, the inoculum was aspirated and removed, and serum-free DMEM (5 ml) was added and cultured. When the cytopathic effect spread throughout the cells and the cells began to peel off, the culture supernatant was collected, and the cells were completely removed by low-speed centrifugation and a 5-um filter to prepare a virus solution.

Virus inactivation (removal) effect confirmation test method

The virus solution and the reagent were mixed at a ratio of (1: 9), reacted at room temperature for a predetermined time, and then diluted 10-fold with serum-free DMEM to stop the reaction. Further 10-fold serial dilution was performed from 10 to 8. Next, Vero E6 / TMPRSS2 cells (96 well plate), four wells were inoculated with each diluted virus solution at 50µl / well, and after 1 hour, the cells were aspirated and removed, replaced with 100µl / well serum-free DMEM, and cultured. After 3 days, the presence or absence of infection in each well was determined, and the 50% infection dilution was calculated by the Behrens Karber method to determine the viral load [50% Tissue culture infectious dose ($TCID_{50}$)/ml].

Virus inactivation (removal) effect confirmation test outline

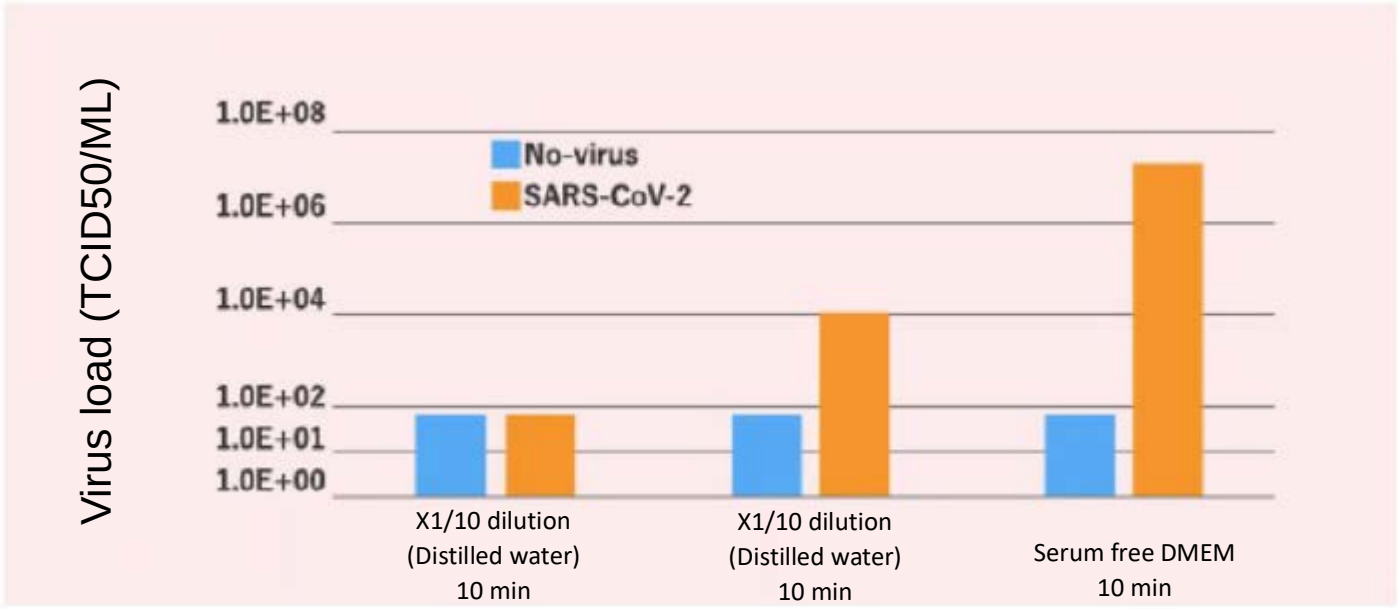
- Host cell culture and virus culture
  - FBS concentration during virus culture: 0%
- Preparation of test sample
  - Free chlorine concentration (with Cl = 35.45) for chlorinated water preparation set in several stages with distilled water
- Antiviral reaction
  - Test sample: Virus solution = 9:1
  - Room temperature 10 minutes
- Removal / neutralization treatment of test sample
  - Immediately up to 10^{-8} after diluting 10 times with DMEM create a 10-step dilution sequence



Results

[Virus inactivation (removal) effect confirmation test]

Test reagents and reaction conditions		(Configuration) Free chlorine concentration Cl = 35.45	No-virus (Blank)	SARS-CoV-2	Log	Δ Log	Rate of inactivation (%)
Test	x1/10 dilution (Distilled water), 10 min	20 mg/L	6.3.E+01	6.3.E+01	1.8	-5.50	>99.999%
Test	x1/20 dilution (Distilled water), 10 min	10 mg/L	6.3.E+01	1.1.E+04	4.1	-3.25	99.945%
Cont.	Serum free DMEM, 10 min	0 mg/L	6.3.E+01	2.0.E+07	7.3	Standard	-



Test sample: Hypochlorite aqueous preparation 1/10 diluted solution (free chlorine concentration (Cl = 35.45) 20 mg / L) reduced viral infectivity to below the detection limit (6.3 × 10) [5.5 Log or higher (5.5 Log or higher) 99.999%) was removed]. This infectious titer is the detection limit, and no virus-infected cells have been observed. Therefore, the actual infectious titer is lower than this, and it can be considered that this virus has been completely eliminated (inactivated). In addition, the virus infectivity titer was 1/1000 or less compared to the untreated (serum-free DMEM) even with the test sample: chlorinated water preparation 1/20 diluted (free chlorine concentration (Cl = 35.45) 10 mg / L). It has been reduced [3.25Log (99.945%) has been removed], and it can be said that there is a removal (inactivation) effect * on SARS-CoV-2.

* The evaluation of the effect is based on the evaluation standard of the 2015 Norovirus Inactivation Condition Survey Report (National Institute of Health Sciences).

The results of this test are a report of the test results conducted by Professor Takemasa Sakaguchi, Department of Virology, Graduate School of Medical Sciences, Hiroshima University.
It was created by the Sankei Group based on the book (unpublished).