Japan Textile Products Quality and Technology Center

TEST REPORT

18th March 2021

APPLICATION

Test applicant : Mitsubishi Electric Corporation
Test sample : Air Purifying Filter with NanoEL AB
Test item : Antiviral Activity Test for Textile Product
Date of application : 10th February 2021

TEST METHOD

Antiviral activity of the test sample is tested mainly based on JIS L 1922 「Textiles -- Determination of antiviral activity of textile products」

○The Summary of Antiviral Activity Test for Textile Products

- Virus strain : Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); JPN/TY/WK-521 (Distributed from National Institute of Infectious Diseases, Japan)
- Host cell : VeroE6/TMPRSS2 JCRB1819
- Growth medium : Dulbecco's modified Eagle's medium (low-glucose) ; DMEM (SIGMA, Cat#D6046) Minimum Essential Medium Eagle ; EMEM (SIGMA, Cat#M4655)
- Fetal Bovine Serum (FBS) (NICHIREI, Cat#174012)

- Control specimen : 0.4 g of Cotton 100% woven fabric without fluorescent brighteners or other finish sourced from JTETC
- Antiviral test specimen : 0.4 g of Air Purifying Filter with NanoEL AB
- Wash-out solution : 1/10 SCDLP diluted with 2% FBS-containing DMEM
- Contacting time : 2 h, 8 h at the temperature of 25 ℃
- Measurement of viral infectivity titer : Plaque assay

○Antiviral activity test

1. Preparation of test virus suspension
1-1. Drain a growth medium from a flask with cultured VeroE6/TMPRSS2 in the monolayer.
1-2. Wash the surface of the cultured cells with EMEM and drain the medium.
1-3. Inoculate SARS-CoV-2 suspension on the surface of cell in the flask and spread to the whole surface.
1-4. Put the flask in the CO_2 incubator at 37 ℃ and keep it for 1 h to adsorb the virus to the cells.
1-5. Add the appropriate amount of EMEM to the flask.
1-6. Put the flask in the CO_2 incubator at the temperature of 37 ℃ for 1 to 3 days to multiply SARS-CoV-2.
1-7. Observe the cytopathic effect under an inverted microscope and judge the multiplication of the virus. If the multiplication of the virus is confirmed, then, Centrifuge the multiplied virus suspension by using the centrifuge at 4 ℃ and 1,000 ×g for 15 min.

1-8. Take the supernatant suspension from the centrifugal tube after the centrifugation.
1-9. The virus suspension was proceeded with 10-fold dilution using distilled water as diluent.
1-10. The concentration of the virus suspension for the test after 10-fold dilution should be adjusted to a titer of 1×10^7 PFU/ml to 5×10^7 PFU/ml. This is to be the test virus suspension.

2. Inoculation of virus to the samples
Inoculate exactly 0,2 ml of the test virus inoculum to the several points of 0.4 g of specimen in the vial containers by pipette for all. Then put the caps on all vial containers and close them.

3. Contact
Put the vials in the incubator and keep for the chosen contact time at the temperature of 25 ℃.

4. Wash-out of virus after contacting
After contacting for each chosen contact time, add 20 ml of wash-out solution in the vial containers, then put the caps on the containers, close them and agitate them by Vortex mixer for 5 s and 5 times to wash out the virus from the specimens.

5. Virus infective titer measurement
Determine the virus infectivity titer by plaque assay.

○ Control test
1. Verification of cytotoxic effect
1-1. Put control specimens and antiviral test specimens in the vial containers.
1-2. Add 20 ml of wash-out solution in all containers. Then, put the caps on the containers and agitate them by Vortex mixer for 5 s and 5 times.
1-3. Observe if cells damage or not, by plaque assay.

2. Verification of cell sensitivity to virus and the inactivation of antiviral activity
2-1. Put control specimens and antiviral test specimens in the vial containers.
2.2. Add 20 ml of wash-out solution in all containers. Then, put the caps on the containers and agitate them by Vortex mixer for 5 s and 5 times.
2-3. Take 5 ml of washing out solution to new tubes.
2-4. Add 50 μl of virus suspension prepared to be a concentration of 5.0×10^4 PFU/ml into the tubes.
2-5. Keep them at 25 °C for 30 min.
2-6. Determine virus infective titer by plaque assay.

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TEST RESULT

○Result of antiviral activity test

Virus strain : SARS-CoV-2; JPN/TY/WK-521
(Distributed from National Institute of Infectious Diseases, Japan)
Test virus suspension : 1.7×10^7 PFU/mL

Test Sample		Common logarithm value of Infectivity titer (PFU / vial) (Note 2)			Reduction value 【M】 (Note 4)	**Antiviral activity value (Mv)** (Note 3)
		Common logarithm		Common logarithm average		
Control specimen (Note 1)	Immediately after inoculation 【lg(V_a)】	n1	6.18	6.21		
		n2	6.24			
		n3	6.21			
	After contacting for 2h 【lg(V_b)】	n1	5.69	5.57	0.6	
		n2	5.69			
		n3	5.34			
	After contacting for 8h 【lg(V_b)】	n1	5.36	5.47	0.7	
		n2	5.48			
		n3	5.58			
Air Purifying Filter with NanoEL AB	After contacting for 2h 【lg(V_c)】	n1	2.30	2.63	—	3.6
		n2	2.90			
		n3	2.70			
	After contacting for 8h 【lg(V_c)】	n1	< 2.30	< 2.30	—	≧ 3.9
		n2	< 2.30			
		n3	< 2.30			

(Note 1) The cotton 100% woven fabric without fluorescent brighteners or other finish sourced from JTETC is used for "control specimen".

(Note 2) PFU : plaque forming units (Note 3) Antiviral activity value (Mv)= $\lg(V_a) - \lg(V_c)$

(Note 4) Reduction value (M) = $\lg(V_a) - \lg(V_b)$ (Judgement of test effectiveness: $M \leq 1.0$)

○Result of control test

Virus strain : SARS-CoV-2; JPN/TY/WK-521
(Distributed from National Institute of Infectious Diseases, Japan)
Test virus suspension : 5.8×10^4 PFU/mL

Test Sample	Cytotoxic effect	Cell sensitivity to virus	Judgement of control test
		Common logarithm average of Infectivity titer (PFU/mL) (Note 2)	
Control specimen (Note 1)	negative	2.76	
Air Purifying Filter with NanoEL AB	negative	2.75	satisfied

【Conditions for control test】

Cytotoxic effect : negative

Cell sensitivity to virus :

lg(Infectivity titer (PFU/mL) of control specimen) − lg(Infectivity titer (PFU/mL) of treated specimen) ≤0.5

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<Reference date>

○Real-time RT-PCR measurement of virus suspension used in this test

- Virus strain : SARS-CoV-2; JPN/TY/WK-521
  (Distributed from National Institute of Infectious Diseases, Japan)
- Virus suspension : $>10^8$ PFU/ml
- Real-time PCR device : Thermal Cycler Dice® Real Time System Ⅲ (TaKaRa)
- Detection Kit : SARS-CoV-2 Detection Kit －N1 set－ (Code NCV-301; Lot# 038200)
  (TOYOBO CO.,LTD. Biotech support Department)

○Result

As the results of real-time RT-PCR measurement, an amplification of viral RNA in virus suspension used in this test was confirmed (Fig.1).

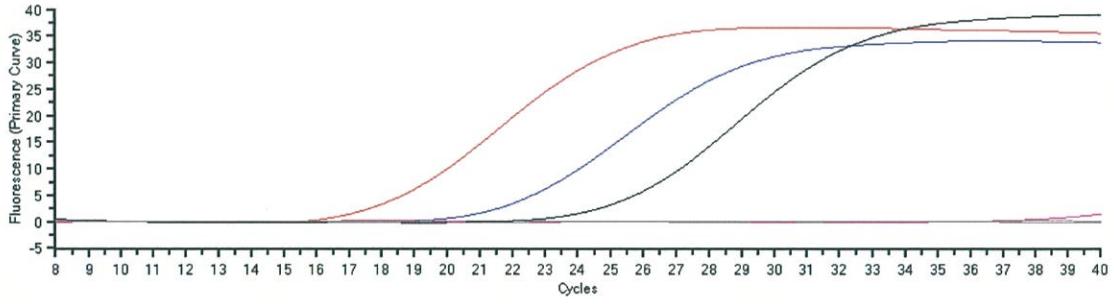

Fig.1. Real-time RT-PCR amplification plot

Red line shows the 10^{-2} dilution of virus suspension with PBS.
Blue line shows the 10^{-3} dilution of virus suspension with PBS.
Black line shows the 10^{-4} dilution of virus suspension with PBS.
Pink line shows the negative control; EMEM.

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