Report No. : 20KB-060860-2

Japan Textile Products Quality and Technology Center

TEST REPORT

4th September 2020

APPLICATION

Test applicant : [redacted]

Test sample : Anti-Bacteria Film
(①Transparent : untreated test material、②Transparent : treated test material)

Test item : Antiviral Activity Test for non porous surfaces

Date of application : 8th June 2020

TEST METHOD

Antiviral activity of each sample is tested mainly based on ISO21702
「Measurement of antiviral activity on plastics and other non-porous surfaces」

○ The Summary of Antiviral Activity Test for non porous surfaces

- Virus strain : *Influenza A virus* (H1N1):
  A/PR/8/34;TC adapted ATCC VR-1469
- Host cell : MDCK cell ATCC CCL-34
- Contacting time : 24 h at the temperature of 25 °C
- Wash-out solution : SCDLP medium
- Measurement of viral infectivity titer : Plaque assay

○ Antiviral activity test

1.Preparation of test virus inoculum

1-1. Drain the growth medium from the flask with the cultured MDCK cells in the monolayer.

1-2. Wash the surface of the cultured cells with EMEM and drain the medium. Repeat the washing procedure 2 times.

1-3. Inoculate the influenza virus suspension prepared to be a concentration of 10^3 to 10^4 PFU/ml 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 the temperature of 34 °C and keep it for 1 h to adsorb the virus to the cells.

1-5. Put EMEM containing 1.5 ppm Trypsin derived from beef pancreas in the flask.

1-6. Put the flask in the CO_2 incubator at the temperature of 34 °C for 1 to 3 days to multiply the influenza virus.

1-7. Observe the cytopathic effect under an inverted microscope and judge the multiplication of influenza virus. If the multiplication of influenza virus is confirmed, then, centrifuge the multiplied virus suspension by using the centrifuge at the temperature of 4 °C 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. This is to be the test influenza virus inoculum.

2. Preparation of test specimens

2-1. Prepare flat 50 mm × 50 mm specimens of the treated test material and the untreated test material and put specimens in the sterile Petri dish. Put the treated side of the product on top when placed in the sterile Petri dish.

2-2. The surface of the test material was wiped with gauze immersed in ethanol at a purity greater than 99% and drid.

2-3. After sterilization, close with the lid of the Petri dish.

3. Inoculation of virus to the samples

3.1. Inoculate 0,40 ml of the test virus inoculum onto the test surface.

3.2. Cover the test inoculum with a piece of PE film that measures 40 mm × 40 mm and gently press down on the film so that the test inoculum spreads to the edges. Make sure that the test inoculum does not leak beyond the edges of the film. After the specimen be inoculated and the cover film applied, close with the lid of the Petri dish.

4. Contact

Keep each of the Petri dish with the inoculated test specimens at 25 °C and a relative humidity of not less than 90 % for 24 h.

5. Wash-out of virus after contacting

After contacting for 24 h, add 10 ml of wash-out solution in the Petri dish, then wash the surface of specimens with pipetting the wash-out solution to recover the virus from the specimens.

6. Virus infective titer measurement

Determine the virus infectivity titer by plaque assay.

○ Control test

1. Verification of cytotoxic effect

1-1. Add 10 ml of either the wash-out solution to each Petri dish with the test specimens.

1-2. Wash the specimens with pipetting the wash-out solution.

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. Add 10 ml of either the wash-out solution to each Petri dish with the test specimens.

2.2. Wash the specimens with pipetting the wash-out solution.

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 4.0×10^{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

Test Virus : ***Influenza A virus*** **(H1N1)**
A/PR/8/34;TC adapted ATCC VR-1469

Test virus suspension : 1.5×10^7 PFU/ml

Test Sample		Common logarithm average of Infectivity titer(PFU/cm^2) (Note 2)	**Antiviral activity value [*R*]** (Note 3)
①Anti-Bacteria Film (Transparent : untreated test material) (Note 1)	Immediately after inoculation [U_0]	5.48	—
	After contacting for 24h [U_t]	4.44	
②Anti-Bacteria Film (Transparent : treated test material)	After contacting for 24h [A_t]	< 0.80	≧ 3.6

(Note 1) Anti-Bacteria Film (Transparent : untreated test material) is used for "untreated test specimen".
(Note 2) PFU : plaque forming units (Note 3) Antiviral activity value $R = U_t - A_t$

○Result of control test

Test Virus : ***Influenza A virus*** **(H1N1)**
A/PR/8/34;TC adapted ATCC VR-1469

Test virus suspension : 4.2×10^4 PFU/ml

Test Sample	Cytotoxic effect	Cell sensitivity to virus: Common logarithm average of Infectivity titer (PFU/mL) (Note 2)		Judgement of control test
①Anti-Bacteria Film (Transparent : untreated test material)	negative	[S_u]	2.54	satisfied
②Anti-Bacteria Film (Transparent : treated test material)	negative	[S_t]	2.59	satisfied
SCDLP medium (Note 4)	negative	[S_n]	2.65	

(Note 4) SCDLP medium is used for "negative control".

【Conditions for control test】
Cytotoxic effect : negative
Cell sensitivity to virus : $|S_n - S_u| \leq 0.5$
$|S_n - S_t| \leq 0.5$

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