**Japan Textile Products Quality and Technology Center**

# TEST REPORT

**16th September 2022**

## APPLICATION

Test applicant : New Island Printing Group Co., Ltd.
Test sample : Paper sheet
Test item : Antiviral Activity Test for non porous surfaces
Date of application : 16th June 2022

## TEST METHOD

ISO21702「Measurement of antiviral activity on plastics and other non-porous surfaces」

○ The Summary of Antiviral Activity Test for non porous surfaces

- Virus strain : Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Variant (Omicron) ; hCoV-19/Japan/TY38-873/2021
  * 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)
- Type of cover film : Polyethylene film
- Untreated test material : ①Paper sheet (OST-MV) (Untreated sample)
- Treated test material : ②Paper sheet (OST-MV122) (Treated sample)
- Method of cleaning the surface of test sample : UV irradiation on both sides for 30 minutes
- Wash-out solution : 1/10 SCDLP medium diluted with 2% FBS-containing DMEM
- Contacting time : 24 hours at the temperature of 25 ℃
- Shape of test piece : 50 mm (length) × 50 mm (width)
- Shape of cover film : 40 mm (length) × 40 mm (width)
- Volume of test virus suspension : 0.4 mL
- Measurement of viral infectivity titer : Plaque assay

○ Antiviral activity test

1.Preparation of test virus inoculum

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 $1\times10^7$ PFU/mL to $5\times10^7$ PFU/mL. This is to be the test virus suspension.

2. Preparation of test specimens
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.

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 to $6.0 \times 10^4$ 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 Variant (Omicron) ; hCoV-19/Japan/TY38-873/2021

Test virus suspension : $2.1 \times 10^7$ PFU/mL

| Test Sample | | Common logarithm value of Infectivity titer ($PFU/cm^2$) (Note 2) | | | Antiviral activity [$R$] (Note 3) |
|---|---|---|---|---|---|
| | | | Common logarithm | Common logarithm average | |
| ①Paper sheet (OST-MV) (Untreated sample) (Note 1) | Immediately after inoculation [$U_0$] | n1 | 5.50 | 5.51 | |
| | | n2 | 5.52 | | |
| | | n3 | 5.51 | | |
| | After contacting for 24h [$U_t$] | n1 | 3.48 | 3.49 | |
| | | n2 | 3.50 | | |
| | | n3 | 3.49 | | |
| ②Paper sheet (OST-MV122) (Treated sample) | After contacting for 24h [$A_t$] | n1 | < 0.80 | 0.80 | 2.7 |
| | | n2 | 0.80 | | |
| | | n3 | < 0.80 | | |

(Note 1) ①Paper sheet (OST-MV) (Untreated sample) is used for "untreated test specimen".

(Note 2) PFU : plaque forming units

(Note 3) Antiviral activity $R = U_t - A_t$

○ Result of control test

Virus strain : SARS-CoV-2 Variant (Omicron) ; hCoV-19/Japan/ TY38-873/2021

Test virus suspension : $4.5 \times 10^4$ PFU/mL

| Test Sample | Cytotoxic effect | Cell sensitivity to virus | | Judgement of control test |
|---|---|---|---|---|
| | | Common logarithm average of Infectivity titer (PFU/mL) (Note 2) | | |
| ①Paper sheet (OST-MV) (Untreated sample) (Note 1) | negative | [$S_u$] | 2.62 | satisfied |
| ②Paper sheet (OST-MV122) (Treated sample) | negative | [$S_t$] | 2.65 | satisfied |
| Negative control (Note 4) | negative | [$S_n$] | 2.65 | |

(Note 4) 1/10 SCDLP medium diluted with 2% FBS-containing DMEM 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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