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Contract Research Report

HYOSUNG Japan Inc.

Evaluation of SARS-CoV-2 inactivation effect

of an antiviral Mask washed 20 times

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Please find below our report on the topic declared in the contracted research project.

Notes

1. Purpose of the test: To clarify whether the antiviral effect of the antiviral Mask washed 20 times can inactivate SARS-CoV-2.

2. Test product: antiviral Mask (after 20 washes)

3. Investigated virus: Novel coronavirus (SARS-CoV-2)

VeroE6/TMPRSS2 cells were infected with the novel coronavirus, and those in which cytopathic effects was confirmed were recovered and cryopreserved in a freezer at -80°C. The cells subjected to two repeated freezing and thawing sessions were centrifuged, and the supernatant was concentrated and purified using an ultrafiltration membrane. This was used as the investigated virus and cryopreserved in a freezer at -80°C until the test. SARS-CoV-2 was obtained from the National Institute of Infectious Diseases, and VeroE6/TMPRSS2 cells were obtained from the JCRB Cell Bank of the National Institutes of Biomedical Innovation, Health and Nutrition.

4. Outline of test methods: The test methodology was according to ISO18184 (JIS L1922).

① The investigated product was inoculated with the novel coronavirus and kept for 5 min, 20 min or 120 min.

② After each reaction time, the virus was extracted from the investigated product with a recovery solution (DMEM liquid medium containing EDTA).

③ The recovered solution was diluted ten times with PBS, and the viral infection titer was measured. The measurements were carried out using the plaque method, and the viral infection titer (PFU/mL) was calculated from the number of plaques.

④ The procedure was performed three times each according to the standard protocol.

The inactivity effect was calculated as shown below.

Inactivity effect (Mv) = log ($Ct/C_{0)}$- log ($Nt/N_{0)}$

= logCt/Nt

Ct: Infection titer of the control after t h

C_0: Infection titer of the control at 0 h

Nt: Infection titer of the test product after t h

N_0: Infection titer of the test product at 0 h

The decrease rate was calculated as follows from the logarithmic decrement value:

Decrease rate = $(1\text{-}1/10^{\text{logarithmic decrement value}}) \times 100\%$

All tests were conducted at a biosafety level 3 (BSL3) experimental facility on campus under appropriate pathogen containment measures.

5. Results

The test results are shown in Tables 1 and 2 and Figure 1.

With the unprocessed mask, the virus decreased from 6.5×10^6 PFU/mL to 4.8×10^5 PFU/mL after 120 min, and this was considered to be spontaneous attenuation.

On the other hand, in the antiviral nonwoven mask washed 20 times, inactivation of the virus was confirmed by a slight reduction after 5 min, reduction to 4.8×10^4 PFU/mL after 20 min, and further reduction to the detection limit value of 2.5×10^2 PFU/mL or less after 120 min. The rate of decrease was also high at 99.948% at 120 min. Furthermore, the inactivation effect Mv value at 120 min reached 3 or more; therefore, the investigated product was considered to be sufficiently effective.

Table 1. Changes in viral infection titer during the tests

	0 min	5 min	20 min	120 min
Unprocessed mask	6.5E+06	-	-	4.8E+05
Mask washed 20 times (average)	6.5E+06	4.5E+06	4.8E+04	<2.5E+02
Standard deviation	2.8E+06	5.0E+05	6.7E+04	0.0E+00

Detection limit: 2.5E+02

Table 2. Inactivation effect of the investigated product

	0 min	5 min	20 min	120 min
Inactivation effect (Mv)	-	0.07	2.06	3.29
Rate of decrease (%)	-	15.625%	99.136%	99.948%

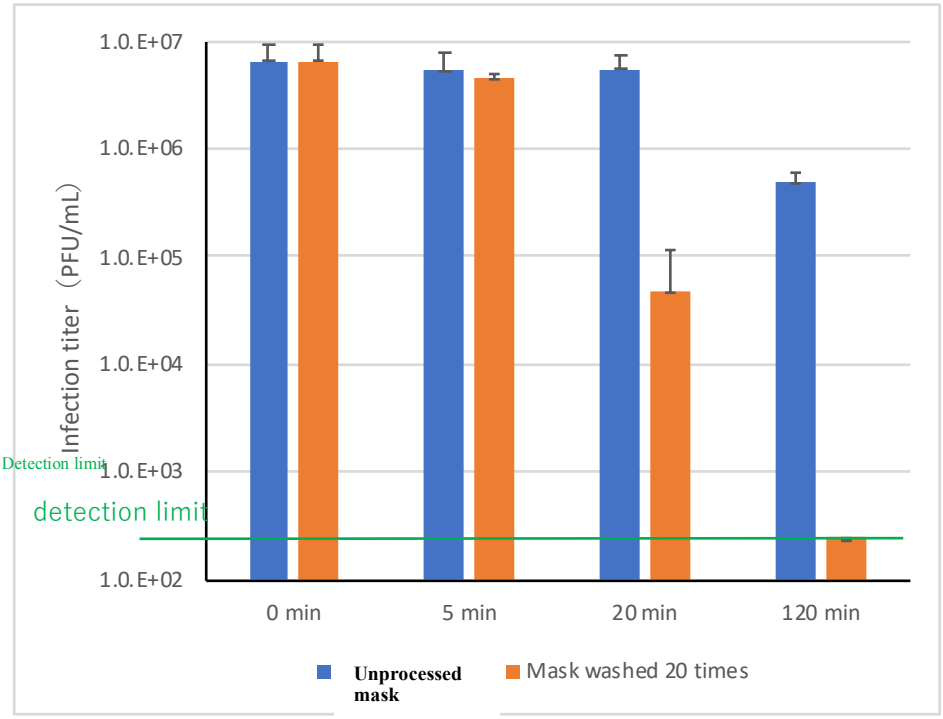

Figure 1. Changes in viral infection titer during testing

Summary

The antiviral mask examined in this study has been validated to be effective against SARS-CoV-2. This mask can reduce contact infection through hands when the mask is removed by hand.

The inactivation up to below the detection limit was achieved after 120 min. This mask is considered to be one that may reduce contact infection even when repeatedly used following washing.

We verify that the results of this test are as documented in this report.

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