KR Biotech Co., Ltd.
Institute of Infectious Disease Control

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TEST REPORT

KR-2106-122-SPC01

Virucidal Activity test

KR BIOTECH CO., Ltd.

Institute of Infectious Disease Control

Summary of the Experiment

○ **Test:** Virucidal Activity Test

○ **Test No:** KR-2106-122-SPC01

○ **Test Material:** Inst2 Mask

○ **Client**

Affiliation : SPOCOM CO., LTD

Address : 101-3 Donghwagongdan-ro, Munmak-eup, Wonju-si, Gangwon-do, Republic of Korea

○ **Institute**

Affiliation : KR BIOTECH Co., Ltd. (ISO13485:2016)

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Written : Hansam Cho / Ph.D. **Sign**

Approved : Young Bong Kim / Ph.D. Director

Sign **date** July 13, 2021

KR BIOTECH Co., Ltd

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July 13, 2021

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1. Summary

This test was conducted to measure the efficacy of the virus-killing of the 'Inst2 Mask' textile presented by SPOCOM CO., LTD. The SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus) virus was used as a test virus, and the textile was treated with the virus culture solution and contacted for a period of time. Then the test was conducted by confirming the activity of the virus. The virucidal activity of the virus was confirmed by infecting the host cell with the virus and then measuring by a 50% tissue culture infectious dose assay ($TCID_{50}$). As a result of treating the 'Inst2 Mask' textile of SPOCOM CO., LTD for 1, 3, 10, 13 minutes, it was confirmed that 55.574%, 61.573%, 81.273 %, 98.684 % of COVID-19 was killed, respectively.

Test sample		The common logarithm average of infectivity titer value		The antiviral activity value log(Va)-log(Vc)	The antiviral activity (%)
Standard textile	Immediately after inoculation	log(Va)	5.833	—	—
	after 1 minute	log(Vb)	5.778	—	—
Inst2 Mask		log(Vc)	5.555	0.278	55.574

	Vertification of this test (1minute)		Test sample	Test result	Vertification
	Test item	Criterion value			
a	**The virus infective titer of inoculated concentration for the test ($LogTCID_{50}$/ml)**	—	—	6.651	—
b	**Vertification of cytotoxicity effect**	No damage	Inst2 Mask	No damage	Pass
			control textile	No damage	
	Vertification of cell sensiticity of virus and inactivation of antiviral activity	≤0.5	Inst2 Mask	0.167	Pass
c	**Logarithm reduction value of infective titer of control specimen**	≤1.0	—	0.056	Pass

Test sample		The common logarithm average of infectivity titer value		The antiviral activity value log(Va)-log(Vc)	The antiviral activity (%)
Standard textile	Immediately after inoculation	log(Va)	5.833	—	—
	after 3 minutes	log(Vb)	5.722	—	—
Inst2 Mask		log(Vc)	5.500	0.333	61.573

Vertification of this test (3 minutes)			Test sample	Test result	Vertification
Test item		Criterion value			
a	**The virus infective titer of inoculated concentration for the test ($LogTCID_{50}$/ml)**	—	—	6.651	—
b	**Vertification of cytotoxicity effect**	No damage	Inst2 Mask	No damage	Pass
			control textile	No damage	
	Vertification of cell sensiticity of virus and inactivation of antiviral activity	≤0.5	Inst2 Mask	0.167	Pass
c	**Logarithm reduction value of infective titer of control specimen**	≤1.0	—	0.111	Pass

Test sample		The common logarithm average of infectivity titer value		The antiviral activity value log(Va)-log(Vc)	The antiviral activity (%)
Standard textile	Immediately after inoculation	log(Va)	5.833	—	—
	after 10 minutes	log(Vb)	5.444	—	—
Inst2 Mask		log(Vc)	5.167	0.667	81.273

Vertification of this test (10 minutes)			Test sample	Test result	Vertification
Test item		Criterion value			
a	**The virus infective titer of inoculated concentration for the test ($LogTCID_{50}$/ml)**	—	—	6.651	—
b	**Vertification of cytotoxicity effect**	No damage	Inst2 Mask	No damage	Pass
			control textile	No damage	
	Vertification of cell sensiticity of virus and inactivation of antiviral activity	≤0.5	Inst2 Mask	0.167	Pass
c	**Logarithm reduction value of infective titer of control specimen**	≤1.0	—	0.389	Pass

2. Outline of the test

2.1 Test schedule

Test start date: June 25, 2021

Test end date: July 02, 2021

2.2 Scope of test

This test method was performed to verify the anti-viral efficacy of the textile by verifying the activity of the virus after processing the SARS-CoV-2 culture solution on the requested textile for a certain period of time. An antiviral test was conducted for the COVID-19 virus by establishing a test method referring to ISO18184:2019 criteria.

3. Materials and Equipment

3.1 Test materials

The sample was provided by the client SPOCOM CO., LTD.

3.2 Culture media and reagents

(1) Dulbecco's Modified Eagle Medium (DMEM), Hyclone, US

(2) Dulbecco's Phosphate buffered saline (PBS), Invitrogen, US

(3) Fetal bovine serum (FBS), Gibco, US

(4) Trypsin-EDTA (0.25% Trypsin), Gibco, US

(5) Penicillin-Streptomycin, Gibco, US

(6) Ethyl Alcohol (EtOH), Duksan Pharmaceutical, South Korea

(7) Hydrochloric Acid (HCl), Daejung, South Korea

(8) Formaldehyde (HCHO), Duksan Pharmaceutical, South Korea

(9) Crystal Violet, JUNSEI, Japan

3.3 Equipment and facility

(1) Biological safety cabinet (sterile worktable), Thermo scientific, US

(2) Optical microscope, OPTINITY, China

(3) Centrifuge (LABOGENE1248), Zyrozen, South Korea

(4) Refrigerator (4℃), Samsung Electronics, South Korea

(5) Freezer (-20℃), Samsung Electronics, South Korea

(6) Cryogenic freezer (-80℃), Thermo scientific, US

(7) Constant temperature carbon dioxide gas incubator (37℃) BB15,
Thermo scientific, US

(8) Vortex mixer KMC-1300V, Vision Science, South Korea

(9) Dry oven HM-28, Hanil Science, South Korea

(10) LN2 Tank (Locator JR Plus), Thermo scientific, US

(11) Water bath, Korea Science, South Korea

(12) Multi well plate reader, Epoch, US

(13) PE6000, Mettler Instrument, US

(14) BSL-3 (No. KCDC-09-3-01)

4. Methods

4.1 Host cell line and culture

The cell line Vero-E6 is isolated from renal epithelial cells extracted from African green monkeys. Since SARS-CoV-2 can be cultured causes virus-infected cell lesion (Cytopathic effect), Vero-E6 is used as a host cell in this test for measuring the viral titer.

4.2 Virus

COVID-19 (SARS-CoV-2)

- The Corona Virus COVID-19 (SARS-CoV-2) was first emerged in Wuhan, China in December 2019, and currently, in May 21, 2020, there are over 4.8 million people infected worldwide. In addition, over 310,000 people died from COVID-19, and it is still spreading seriously in the US and in South America, etc.

- COVID-19 is included in the beta-corona classification to have positive single-strand RNA as the genome, and it is a spherical form of the virus with envelope.

- On March 11, 2020, the WHO declared pandemic on this virus, and there is no medicine or vaccine in the present. The resistance to the disinfectant is in mid-grade, but the spreading power is very high to have a serious impact globally.

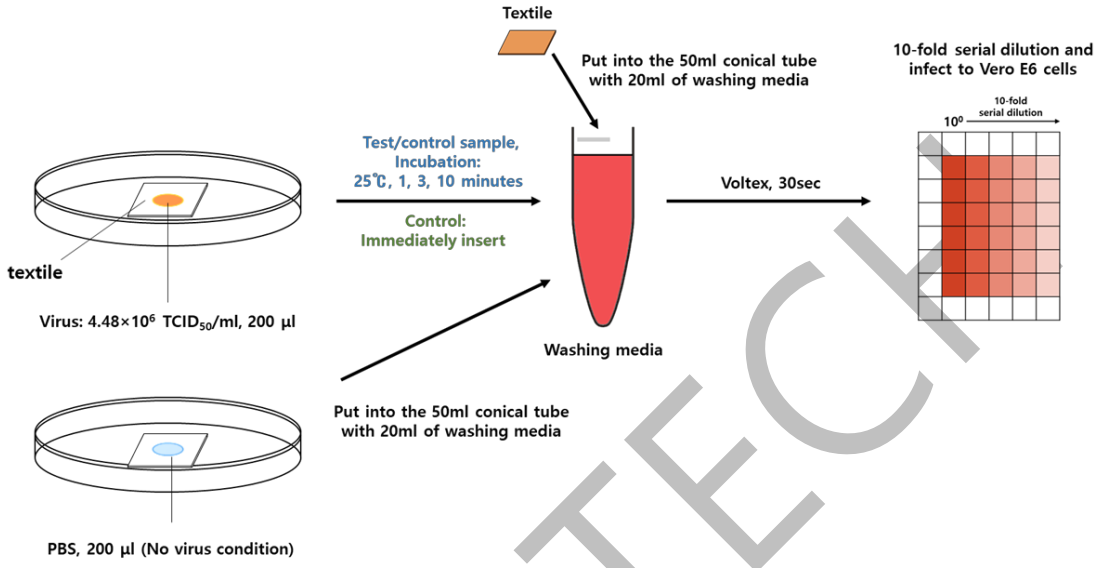
Severe acute respiratory syndrome-related coronavirus (SARS-CoV-2)

- Classification: Coronaviridae family, Betacoronavirus
- Virus genome: (+)ss-RNA
- Envelope: Yes
- Resistance: middle
- Titer: 4.48 x 10^6 $TCID_{50}$/mL

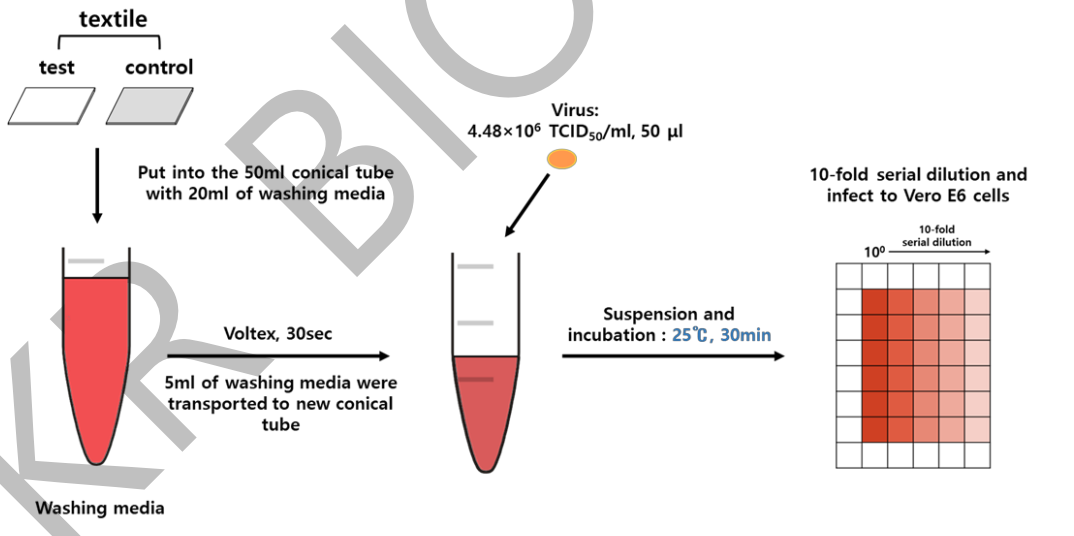
4.3 Virucidal Test

This test was conducted on the basis of ISO18184, the virus killing test by textile.

Schematic diagram of antiviral activity test



Verification of cytotoxicity by cell sensitivity to virus and the inactivation of antiviral activity



① Before the antiviral test, textile specimens were prepared as 20 mm × 20 mm size.

② One day before the test, prepare Vero-E6 cells in a 96 well plate.

③ Put the test or control textile specimens into petri dish. And 200 µl of SARS-CoV-2 viruses (4.48 x 10^6 $TCID_{50}$/mL) were treated to the textile and incubated

for 1, 3, and 10, 13 minutes at 25°C, respectively.

④ After incubation, put the textile specimens into the 50ml conical tube filled with 20ml of washing media (containing 0.7% tween 20), and vortexing for 30 sec. And then, 10-fold serial dilute with the washing media. As other control, virus treat to control textile and wash immediately and 10-fold serial dilute with the washing media as same condition.

⑤ For the cytotoxicity test of textile, 200 µl of PBS were treated to the test or control textile specimens, and 10-fold serial dilute with the washing media as same condition.

⑥ For washing test of antiviral material in the textile, put the textile specimen into the 50ml conical tube filled with 20ml of washing media, and vortexing for 30 sec.

⑦ 50 µl of SARS-CoV-2 viruses (4.48 x 10^6 $TCID_{50}$/mL) were treated to the 5ml of textile washing media and mixed. Next, incubate 30min at 25°C.

⑧ 10-fold serial dilute with the washing media as same condition.

⑨ Each diluent was treated to Vero-E6 cells and cultured at 5 % CO_2 at 37 °C.

⑩ After 3 days of culture, the cytopathic effect (CPE) was observed under a microscope.

⑪ Crystal violet staining reagent was treated with cells and stained at room temperature for 30 minutes.

⑫ The titer of the virus was calculated by counting the number of stained wells.

4.4 Data reading and calculation

4.4.1 Virucidal Test

To evaluate the virus killing efficacy, each diluent was inoculated into a host cell, and virus titers of the control group and the test group were measured after 3 days.

The number of wells stained with Crystal violet dyeing reagent was counted to

calculate the titer by Sperman-Karber method. Virus titers were calculated according to 4.4.2 and reduction rates were determined according to 4.4.3.

4.4.2 Calculate viral titer

The virus titers can be confirmed by observing the morphological changes (CPE) of cultured cells caused by virus growth for a period of time. The virus titer is obtained by inoculating, cultivating, and observing the cultured cells seeded in a plurality of incubators by preparing a 10^n dilution series of the virus solution. After the CPE observation for a certain period of time (three days after infection), the virus titer ($TCID_{50}$) is calculated according to ICH Q5A (R1), which is indicated by taking the commercial log value.

The number of wells determined to be positive is cumulatively calculated from the high diluent side to obtain the cumulative positive rate (%) of each diluent.

$$TCID_{50}: N=10^{[(A-50)/(A-B)]-(a)}$$

How to calculate viral titer

1) Calculate the cumulative for the number of well, which had decided to be positive from high diluted solution and obtain the cumulated positivity rate (%) of each diluted solution.
2) Obtain 50% of cumulative positivity rate, and cumulative positivity rate of high diluted solution is called as A; cumulative positivity rate of low diluted solution is called as B; and the natural logarithm value of diluted solution with A obtained is called as a.
3) Obtain the viral titer according to the following formula.

However, if overall well became negative even for the diluted solution having the lowest magnification, assume that overall well become positive in the diluted solution that is one step lower than that diluted solution and then calculate; add a sign of inequality to obtained

value and then write down. And make the valid number to have 2 digits by rounding the 3rd number of calculated value for valid digit number of viral titer.

4.4.3 How to calculate the viral reduction factor (Ri)

- Viral titer appeared in the experimental group before the combustion: 10^A
  Total amount of test solution before the combustion: V^A
  - ➔ Viral titer of test solution before the combustion $V^A \times 10^A = N_A$
- Viral titer appeared in the experimental group after the combustion: 10^B
  Total amount of test solution after the combustion: V^B
  - ➔ Viral titer of test solution after the combustion $V^B \times 10^B = N_B$

Viral titer (Ri) of test solution is

$$10^{Ri} = V^A \times 10^A / V^B \times 10^B = N_A / B_A$$

$$Ri = \log_{10}(N_A / B_A) = \log_{10} N_A - \log_{10} N_B$$

5. Results

Table 1. Cytotoxicity test of textile

(unit: $log_{10}CC_{50}$/ml)

Specimen	Occasion	Titer	Average
Inst2 Mask	1	No damage	—
	2		
	3		
NTC textile	1	No damage	—
	2		
	3		

Table 2. Washing test of antiviral material in the textile

(unit: $log_{10}TCID_{50}$/ml)

Specimen	Occasion	Titer	NTC-Test
Inst2 Mask	1	5.833	0.334
	2	5.500	0.167
	3	5.500	0.000
NTC textile	1	6.167	**Average**
	2	5.667	**0.167**
	3	5.500	

Table 3. Virus titration immediately after virus treatment to the control textile

(unit: $log_{10}TCID_{50}$/ml)

Specimen	Occasion	Titer	Average
NTC textile	1	5.833	**5.833**
	2	6.167	
	3	5.500	

Table 4-1. Virus killing test result (1 minute incubation condition)

(unit: $log_{10}TCID_{50}$/ml)

Specimen	Occasion	Titer	Average
Inst2 Mask	1	5.667	**5.555**
	2	5.500	
	3	5.500	
NTC textile	1	5.667	**5.778**
	2	5.833	
	3	5.833	

Table 4-2. Virus killing test result (3 minutes incubation condition)

(unit: $log_{10}TCID_{50}$/ml)

Specimen	Occasion	Titer	Average
Inst2 Mask	1	5.500	**5.500**
	2	5.500	
	3	5.500	
NTC textile	1	5.500	**5.722**
	2	5.833	
	3	5.833	

Table 4-3. Virus killing test result (10 minutes incubation condition)

(unit: $log_{10}TCID_{50}/ml$)

Specimen	Occasion	Titer	Average
Inst2 mask	1	5.167	**5.167**
	2	5.000	
	3	5.332	
NTC textile	1	5.500	**5.444**
	2	5.332	
	3	5.500	

Table 5. Virus reduction rate

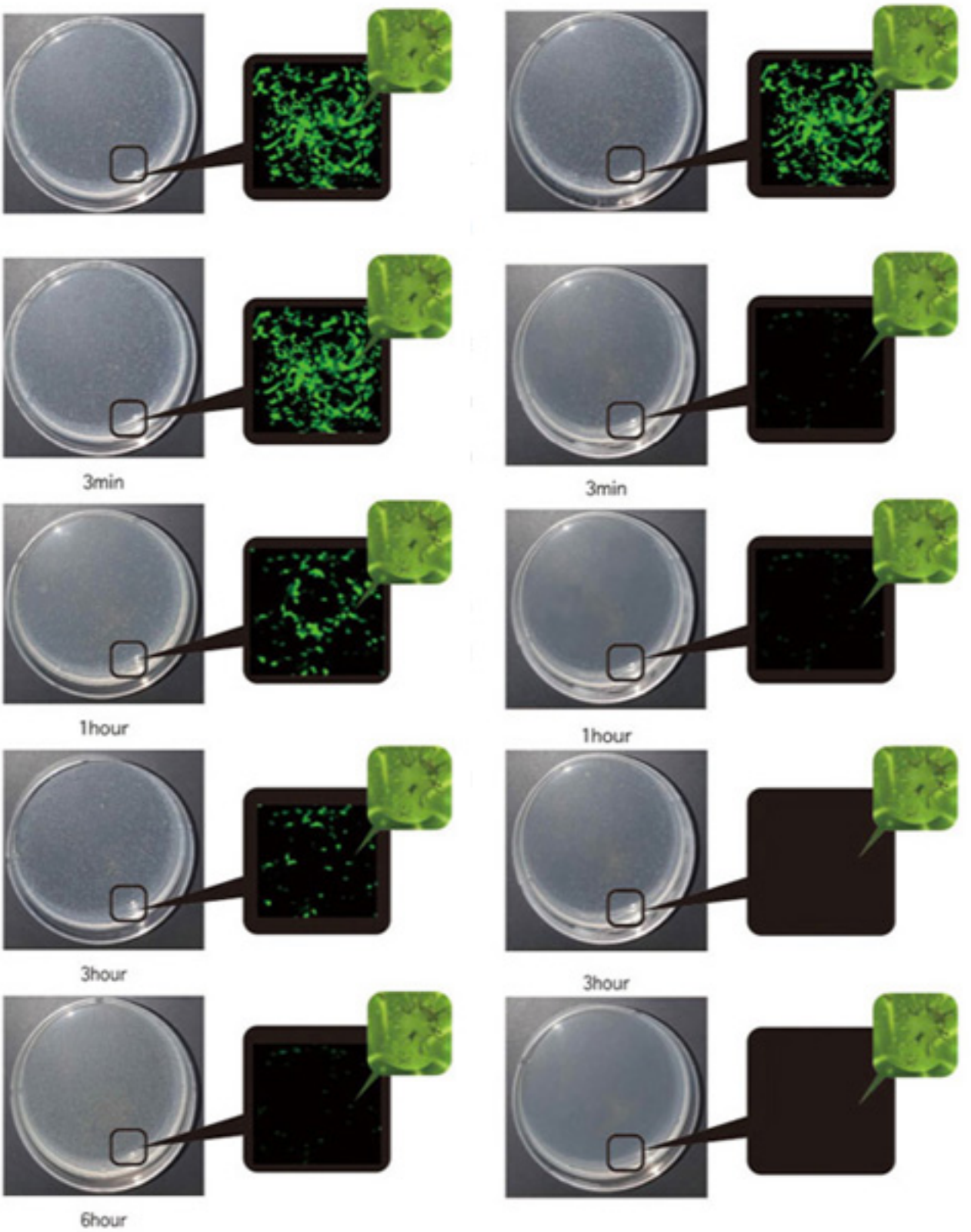
(unit: $log_{10}TCID_{50}/ml$)

Virus titer[1)] (Immediately)	Incubation time	Virus titer[2)] (specimen treated)	Virus reduction(LR)	Percentage(%)
5.917	1 minute	5.555	**0.278**	**55.574**
	3 minutes	5.500	**0.333**	**61.573**
	10 minutes	5.167	**0.667**	**81.273**
	13 minutes	5.167	**0.837**	**98.684**

$LR = L_U - L_T$

1) : L_U , Virus titer immediately after virus treatment to the control textile

2) : L_T , Virus titer after incubation with the specimen textile



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* Interpretation of results

Log reduction	Percent (%) reduction
≥1	≥90 %
≥2	≥99 %
≥3	≥99.9 %
≥4	≥99.99 %
≥5	≥99.999 %

The initial virus titer of SARS-CoV-2 for the virus killing test is 6.651 $log_{10}TCID_{50}$/ml. Textile washing solution with virus, textile washing solution without virus contact, and solution washed immediately after virus contact with textile were used as a control for this virucidal test. First, 'Inst2 Mask' textile and NTC textile washing solutions without virus contact showed no cytotoxicity. The virus reduction rate was evaluated based on titer of solution washed immediately after virus contact with NTC textile (5.833 $log_{10}TCID_{50}$/ml). After 1, 3, 10, 13 minutes contact, the virus titer of the 'Inst2 Mask' textile

was 5.555, 5.500, 5.167 $log_{10}TCID_{50}$/ml, and the resulting virus reduction in the 'Inst2 Mask' textile was 0.278, 0.333, 0.667 $log_{10}TCID_{50}$/ml, respectively. Reduction values of NTC textile at 1, 3, 10 minutes contact condition were 0.056, 0.111, 0.389 $log_{10}TCID_{50}$/ml respectively, and these were passed the verification standard. Washing test of antiviral material in the textile, the difference between the NTC textile and 'Inst2 Mask' textile was 0.167 $log_{10}TCID_{50}$/ml, therefore, the 'Inst2 Mask' textile washed solution in each conditions were found to have no antiviral material. These results indicate that the antiviral material in the textile was not cleaned by the washing media, and that the washing solution in the specimen material itself did not affect the test.

As a result, it was found that 'Inst2 Mask' textile has 55.574, 61.573, 81.273% of viral reduction effect in the 1, 3, 10, 13 minutes contact condition to SARS-CoV-2.

This test evaluated the antiviral efficacy on average of the results of three repetitions.

6. Conclusion

The averages of SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus) virus reduction rate (virucidal rate) for 'Inst2 Mask' textile of SPOCOM CO., LTD under the test guideline were 0.278, 0.333, 0.667 at 1, 3, 10, 13 minutes contact condition . As a result,

'Inst2 Mask' showed 55.574, 61.573, 81.273% virus killing efficacy at 1, 3, 10, 13 minutes contact condition, respectively.

7. References

(1) ASTM E1052-11, Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension

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(8) Sterilization. Disinfectant Efficacy Test Method Data Collection 2018. 12. National Institute of Environmental Science

(9) ISO18184:2019, Determination of antiviral activity of textile products

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