



SEOUL  
NATIONAL  
UNIVERSITY

BIOLOGICAL SCIENCES  
Laboratory of Virology

## TEST REPORT

### TEST TITLE

Test report for “Inhibit effectiveness on BCoV of R&F  
Chemical antimicrobial film”

### TEST METHOD

Virus Suppression Test for the Surface of Synthetic Resin and Non-Porous Surface

### TEST FACILITY

Department of Virology, Veterinary College,  
Seoul National University

### REPORT ISSUED DATE

May 28, 2020

Industrial-Academic Cooperation  
Group of Seoul National University  
(Official Seal)





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I. Test requester

D. I. Park (Peter), CEO of R&F Chemical Co., Ltd

II. Test director

Park yong-ho, Professor at College of veterinary medicine at Seoul National University

Signature 

III. Testing purpose

To check the inhibitory efficiency against the coronavirus, we want to measure the inhibitory efficiency of the antibacterial plastic work-piece (film) developed and tested by R&F Chemical Co., Ltd

IV. Official Goods

Negative Sample : Non-treated Polybag

Positive Sample : R&F Anti-microbial Film

V. Testing method

1) Test method for inhibitory efficiency against bovine corona virus

► Use of anti-virus liquor with a titer of  $6.0 \log_{10} \text{TCID}_{50}/\text{ml}$  or more that is cultured stepwise in the MDCK cell separated from the oral biotoxin.

► Dilution of the virus was adequately followed by the instruction and purpose.

2) Viral content calculation

Expose a virus pre-calculated by titration concentration to an antibacterial film.

- Exposure time: 0.5hr, 1hr, 4h, 24hr

- Number of control and experimental samples: Control 1ea, Experimental Group 1ea



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### **3) Quantitative analysis of Virus**

- Collect all virus inhibitory candidate materials and inoculate them with DMEM with 5 wells of undiluted solution,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ , respectively, on 96well plate.
- Observe MDCK cell for 5 days after inoculation of 60-70% 96 well plate
- Observing the unique denatured effects of BCoV over time
- Examined virus concentration reduction effect compared to virus suppression candidate material and control group

### **4) Determination of virus proliferation**

- Repeated the experiment three times to calculate the virus titer using the Kaerber method.
- Effective concentration of dilute drainage identified as inactivation of virus inhibition candidate material.
- In this case, the effective deactivation capacity of the public announcement virus is the average value of the results of the three-repeated test.



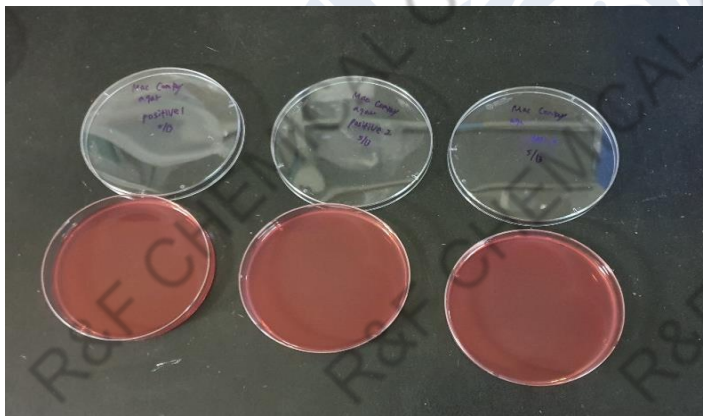
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## VI. Pre-pollution measurement

- Antimicrobial film (plastic) and one of negative control(Non-treated);
- Results of bacterial testing of antimicrobial films and one control film used in the experiment

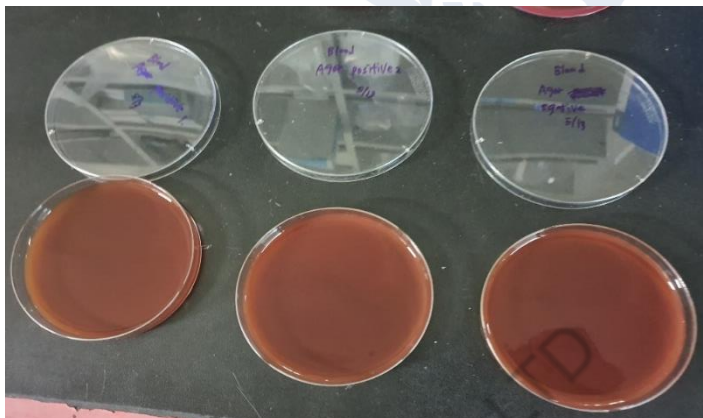


Mix film with DMEM diluent and incubate in Blood agar and MacConkey agar



(1) Check the colony of MacConkey agar after cultivation in incubator for 48hrs

“No-Colony” : Not contaminated



(2) Check the colony of blood agar after cultivation in incubator for 48hrs

“No-Colony” : Not contaminated

### ➤ Pre-pollution Measurement Results

Both Positive and Negative Control antimicrobial films are free from bacterial contamination



## VII. Measurement of virus titration

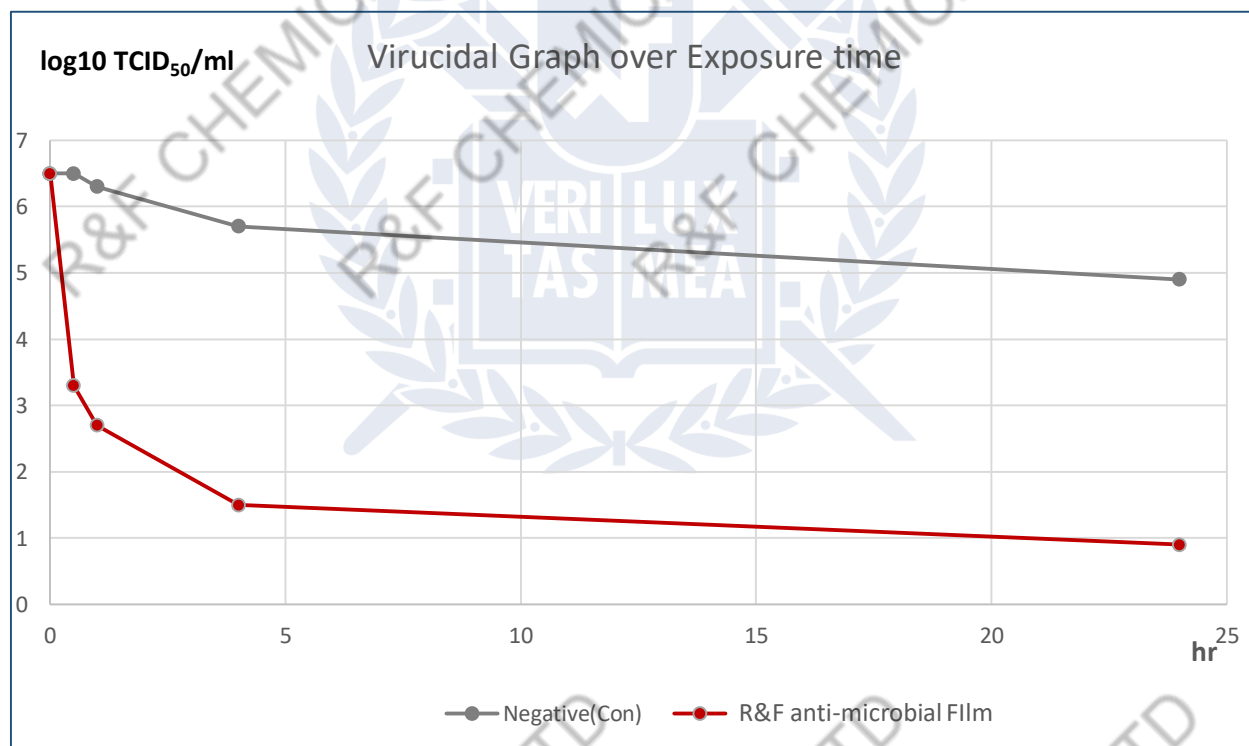
1) Type of sample : Positive(Anti-microbial film) and Negative Control Film

2) Exposure time of film: 0.5hr, 1hr, 4hr, 24hr

3) Titer: Converted to  $\log_{10}$  TCID<sub>50</sub>/ml and calculated

\* TCID<sub>50</sub> : concentration concept by dilution of viruses that infect 50% of inoculated cells

	R&F Anti-microbial Film				Negative control			
	Titer ( $\log_{10}$ TCID <sub>50</sub> /ml)				Titer ( $\log_{10}$ TCID <sub>50</sub> /ml)			
	1st	2nd	3rd	Median	1st	2nd	3rd	Median
0.5h	3.3	3.5	3.1	3.3	6.5	6.5	6.5	6.5
1h	2.9	2.5	2.7	2.7	6.5	6.3	6.1	6.3
4h	1.5	1.7	1.3	1.5	5.7	5.9	5.5	5.7
24h	0.9	0.9	0.9	0.9	4.9	4.9	4.9	4.9

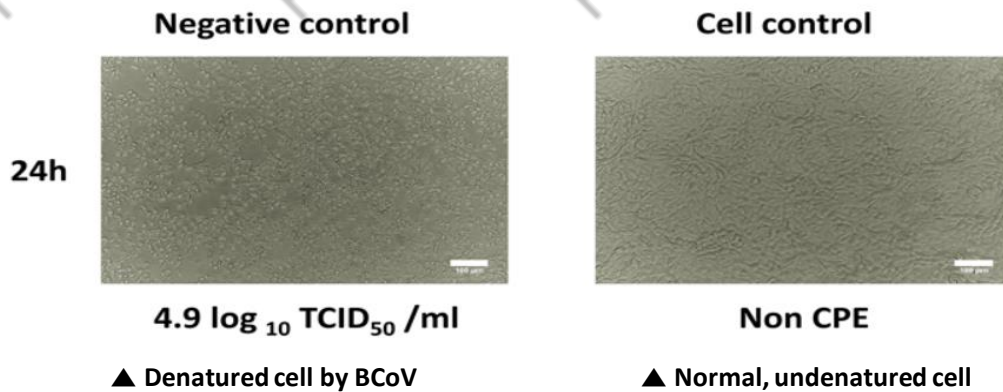




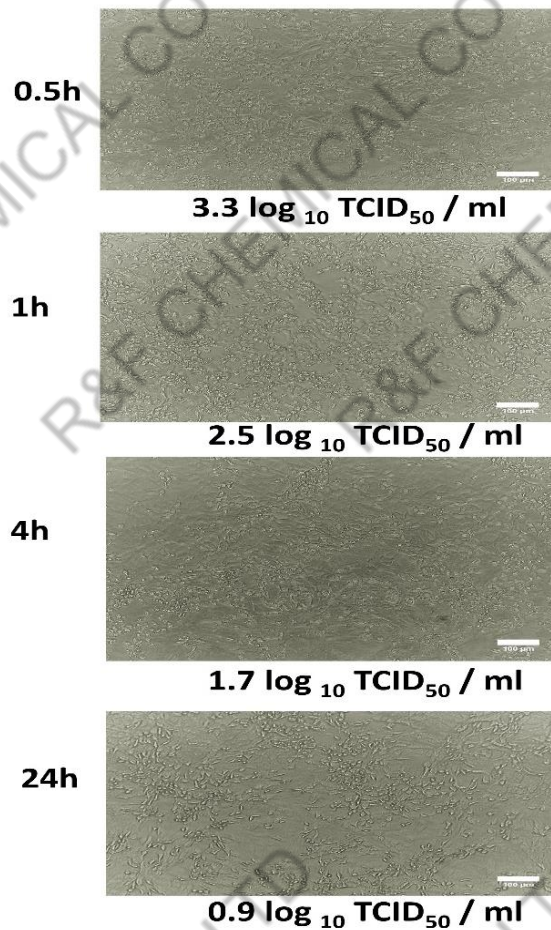


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### VIII. Result of MDCK Cytopathetic effect (CPE)



### R&F anti-microbial Film



- Confirmed that cellular degeneration is inhibited in anti-microbial film



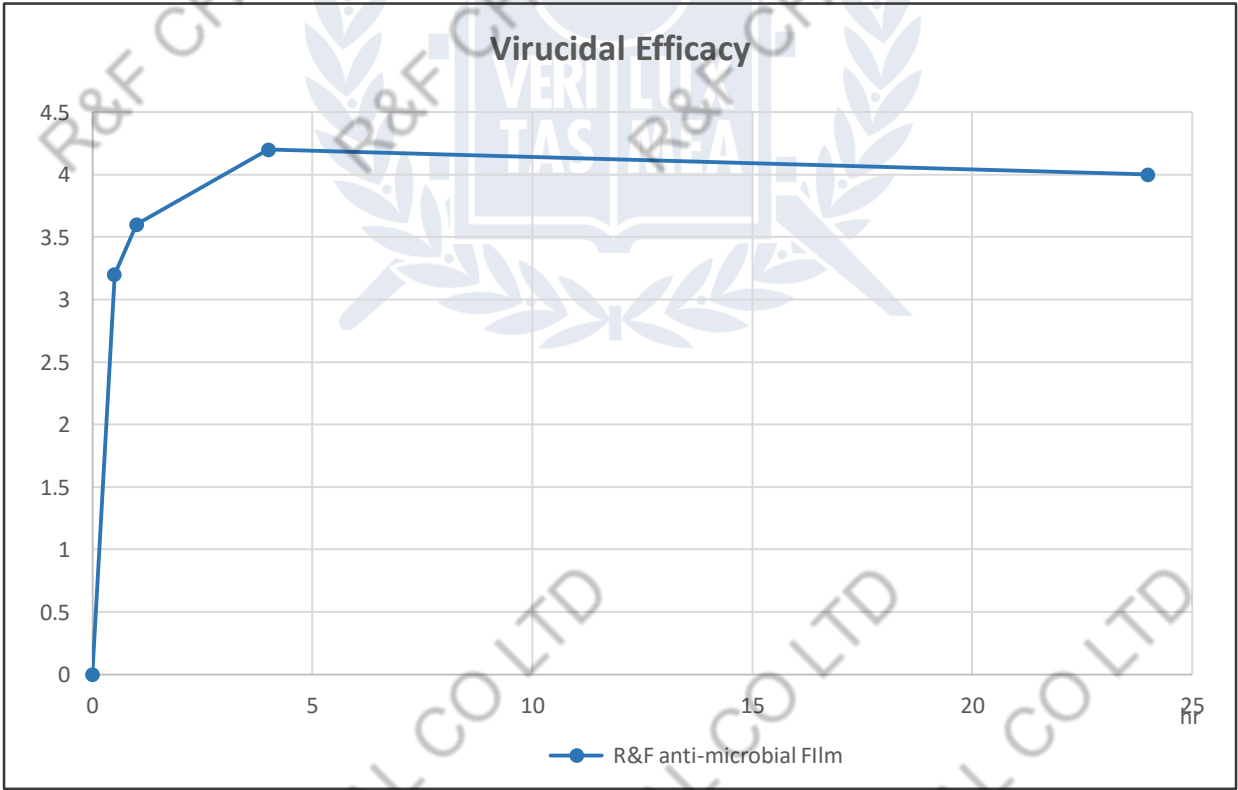
IX. Virus Inhibition Ability Judgment Criteria

Equation for Virus inhibitory efficiency= Negative (log<sub>10</sub>TCID<sub>50</sub>/ml) – Positive (log<sub>10</sub>TCID<sub>50</sub>/ml)

► At 4.0 or higher, virus self-disinfectivity is recognized.

(The standard for virus suppression varies, between 3.0 and 4.0 levels, depending on the regulatory authority.)

Final inhibitory efficiency of two species of antimicrobial film against BCoV	
Inhibitory efficiency criteria (log10 TCID <sub>50</sub> /ml); Inhibitory ability %	
Exposure time	R&F Anti-microbial Film
0.5h	3.2; 99.937%
1h	3.6; 99.975%
4h	4.2; 99.994%
24h	4.0; 99.990%





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**IX. Final conclusions on the requested sample (antibacterial film)**

- 1. Anti-microbial film samples exhibit inhibitory effect against bovine coronavirus.**
- 2. For the anti-microbial film, 4.0 (99.99%) of self-disinfection was observed when the exposure time is more than 4 hr.**

**\* Self-disinfectant means that the substance itself has the ability to suppress pathogenic bacteria without the need for disinfectants or disinfection.**

**\*\*4.0 means that it has 10,000 times the sterilization power of the coronavirus.  
(Compared to non-antibacterial treated materials)**

**Approximately 99.94% of  
inhibitory efficiency against virus  
was detected at 30 minutes.**

**99.99% of inhibitory  
efficiency against virus was  
detected at 4hr**

