



December 22, 2020

Dear Sirs,

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Name of company: **Recomm Co., Ltd.**
Representative name:
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(No. of Code : 3323 TSE JASDAQ Standard)
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By virus removal / sterilization device "ReSPR"
confirmed SARS-CoV-2 inactivation on aluminum surfaces

Regarding the virus removal and sterilization device "ReSPR" that RECOMM Co., Ltd. (Headquarters: Shibuya-ku, Tokyo, President & CEO Hidehiro Ito, hereinafter referred to as "we") is selling, we inform you that the SARS-CoV-2 inactivation was confirmed as the result of the demonstration experiment at the Osorio Laboratory, School of Veterinary Medicine, Wisconsin University,

Notice:

1. About "SARS-CoV-2 inactivation on aluminum surfaces by the virus removal and sterilization device 'ReSPR' " report
ReSPR TECHNOLOGIES INC. (Headquarters: Republic of Panama, President Christophe Suchy, hereinafter referred to as "ReSPR INC.") commissioned the Osorio Laboratory, School of Veterinary Medicine, Wisconsin University, to conduct the demonstration experiment in order to confirm the SARS-CoV-2 inactivation by the virus removal and sterilization device "ReSPR".
After confirming the inactivation of the SARS-CoV-2 in mid-September, the demonstration experiment was carried out over about 5 months from the start of the experiment, such as evaluating the exposure time in several patterns. The laboratory published the attached demonstration experiment report on December 8, 2020, and ReSPR INC. received it on the same day. We received it from ReSPR INC. on the 11th. We informed you today because ReSPR INC. requested us to disclose the verification results after posting them on its websites.
The Wisconsin University also participated in the joint research on the 'Transmission of SARS-CoV-2 in Domestic Cats' of the University of Tokyo, the National Center for Global Health and Medicine, and the National Institute of Infectious Diseases, which was announced in June 2020.
2. Impact on business performance
The impact on the consolidated financial results for the fiscal year ending September 2021 is currently under scrutiny and will be disclosed promptly if becoming necessary to disclose.

REPORT:

SARS-CoV-2 inactivation on aluminum surfaces by RESPR HVAC device

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12/08/2020

ABSTRACT

Designing effective methods of SARS-CoV-2 inactivation that can be applied in daily human activities can help diminish the transfer and spread of infectious diseases such as COVID-19. RESPR technology has shown to be effective in reducing pathogens and allergens from the air and from surfaces. It is used in devices that release oxidizing particles to purify the air that people inhale. We tested the SARS-CoV-2 inactivation efficacy of a RESPR HVAC device at different exposure times on aluminum surfaces. A plaque assay was used to measure SARS-CoV-2 titers after 8 different exposure time points (from 10 minutes to 2880 minutes) with the presence of the device. The RESPR HVAC device showed a reduction of 99.991% of the SARS-CoV-2 infectious particles on the aluminum surface after 1440 minutes.

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MATERIALS AND METHODS

Materials infection and sample collection

RESPR HVAC device was placed inside a Biosafety cabinet (BSC) and turned on. Sterile aluminum foil pieces of 24mm x 24mm previously disinfected with 70% ethanol and exposed to UV light for 25 minutes, were individually placed in a petri dish inside the BSC and were kept at room temperature. A 200 μ l inoculum of 1×10^5 PFU of SARS-CoV-2 was placed and extended on each aluminum piece using a micropipette tip. Three replicates were prepared per treatment and enough samples were prepared to evaluate 8 exposure times (15, 30, 60, 120, 360, 720, 1440 and 2880 minutes) (Table 1).

Following each exposure time, 2ml of collection media (DMEM with 2%FBS) was added to each petri dish, making an initial dilution of 1:11, and the aluminum material was washed out by resuspending four to five times using a micropipette; the viral suspension was collected, mixed for homogeneity and aliquoted into 1ml centrifuge tubes. Each collected sample was immediately labeled and stored at -80°C for titration assays.

Table 1. Evaluated treatments

Virus dose	Exposure time (min)	Treatment
1x10 ⁵ PFU/200µl	15, 30, 60, 120, 360, 720, 1440 and 2880	RESPR HVAC

Viral-inactivation quantification

The recovered virus suspension was diluted (10-fold, 3 dilutions: 1/10, 1/100, 1/1000) in a mixing plate in duplicate and added to 96 well Vero E6 seeded plates. Plates were incubated for 1 hour at 37°C. Inoculum was discarded and a 2% carboxymethylcellulose overlay was added and incubated for 24 hours at 37°C. Next, the overlay was discarded, plates washed and fixed for 10 minutes at -20°C (using acetone-Methanol solution). Following fixation, plates were washed two times with PBS-T and a primary antibody (IgG Human anti-Coronavirus, 1:2000) was added and incubated overnight at 37°C. The primary antibody was then discarded, and plates were washed twice with PBS-T. A secondary antibody (Goat IgG Anti-Human HRP conjugated, 1:2000) was added and left to incubate for 2 hours at 37°C. After removing the secondary antibody, plates were washed twice with PBS-T and plaques were developed with a Chromogen substrate. Plaques were counted using Immunospot Image analyzer and open-source software Viridot to determine the viral titer. The titer reduction percentage was calculated using the following formula:

$$\text{Percent reduction} = \frac{(A - B) \times 100}{A}$$

Where: A is the virus titer with no treatment (Control) or the initial titer; and B is the viral titer after treatment.

RESULTS

Viral titers decreased with time as expected, mean values are reported in table 1 and Figure 1; 24 hours after infection (1440 minutes) the titer was reduced up to 45 FFU/mL.

Table 1. Mean titers and standard deviation of SARS-CoV-2 inoculum collected at different time points (from 0 to 2880 minutes) after infection from 24mm x 24mm aluminum foil pieces exposed to a RESPR HVAC device.

Time (m)	Mean (FFU/mL)	DS
0	5060	479.4789
15	3740	396.6106
30	3410	939.8404
60	2456.67	1045.482
120	1246.67	228.9833
360	110.333	127.0171
720	57.67	63.50853
1440	45.333	0
2880	10.67	0

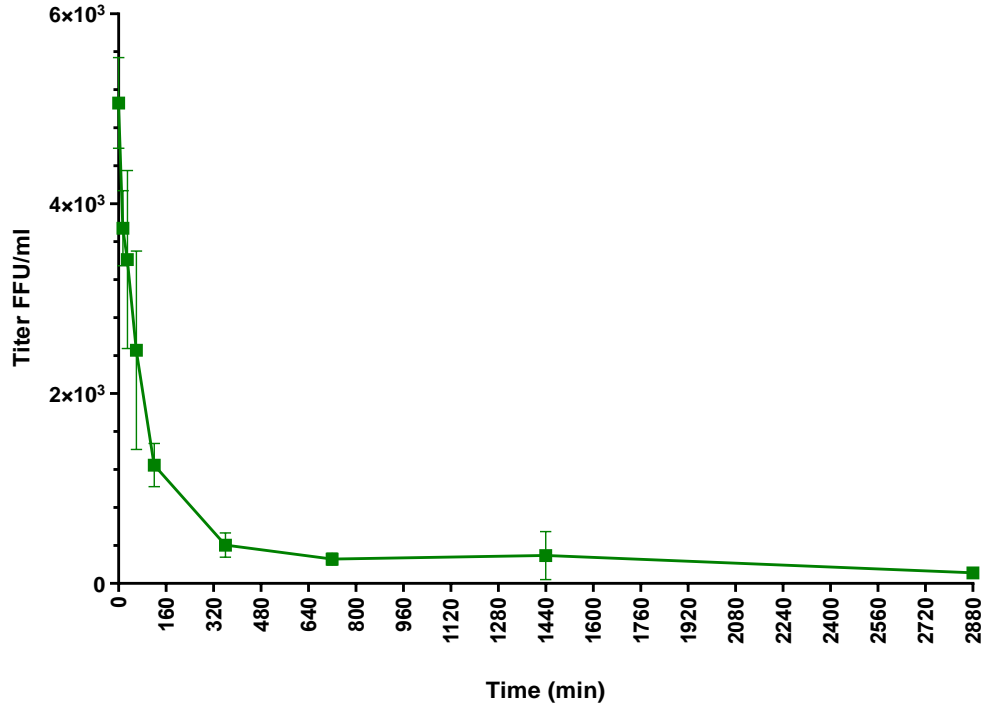


Figure 1. Mean titers and standard deviation of SARS-CoV-2 inoculum collected at different time points (from 0 to 2880 minutes) after infection from 24mm x 24mm aluminum foil pieces exposed to a RESPR HVAC device.

The total reduction of SARS-CoV-2 titer, calculated in relation to the initial inoculum ($\bar{X}=5.06 \times 10^3$ PFU/ml), reached 99.991% after 1440 minutes of exposure (Figure 3).

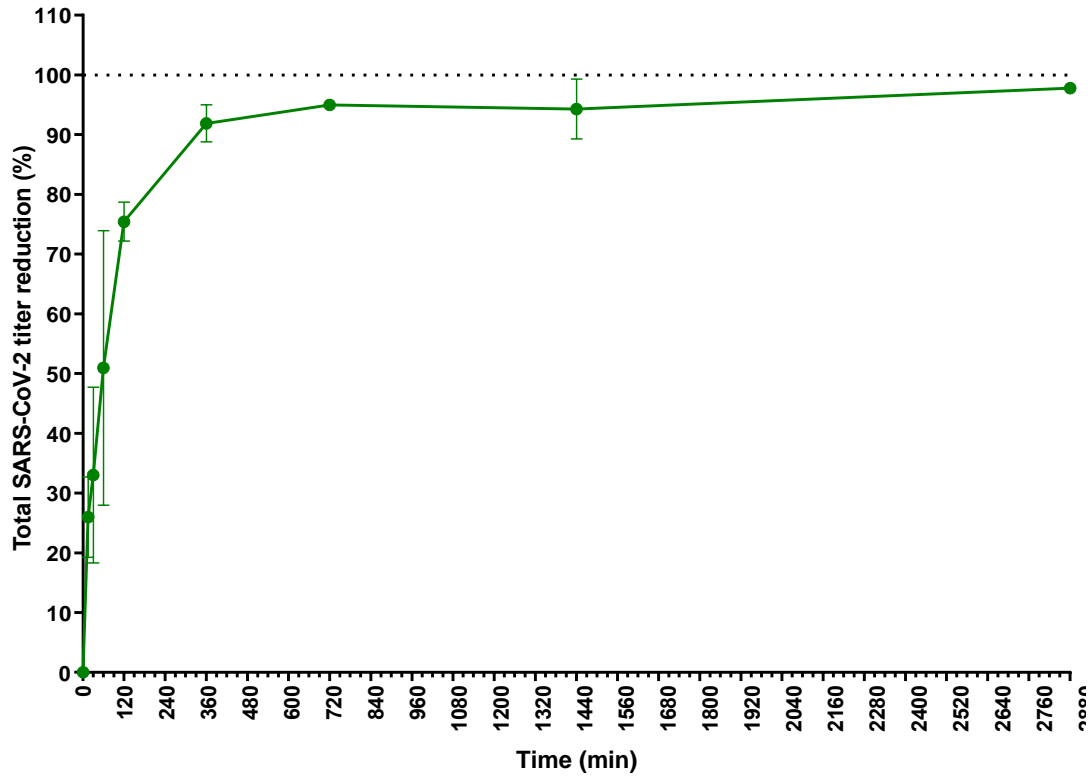


Figure 3. Total reduction (%) of SARS-CoV-2 inoculum collected at different time points (from 0 to 2880 minutes) after infection from 24mm x 24mm aluminum foil pieces exposed to a RESPR HVAC device.

CONCLUSIONS

While using the RESPR HVAC device, a maximum reduction of 99.991% of SARS-CoV-2 infectious particles on an aluminum surface was reached after 1440 minutes of exposure. More than 97.8% of this reduction was detected 360 minutes after the initial exposure.