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各 位

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关于杀毒除菌装置「ReSPR」使铝表面 新型冠状病毒(SARS-CoV-2)失去活性的确认

关于Recomm Co.Ltd(总部:东京都涉谷区,代表取缔役社长 伊藤 秀博)销售的杀毒除菌装置「ReSPR」,美国Wisconsin大学兽医学部的Osorio研究所实验结果表明其可以使新冠病毒失去活性,特此公告。

正文

1.《关于杀毒除菌装置「ReSPR」使铝表面新型冠状病毒(SARS-CoV-2)失去活性的确认》的报告

「ReSPR」的制造商ReSPR TECHNOLOGIES INC.(总部:巴拿马共和国, Christophe Suchy、以下简称「ReSPR公司」)为了确认杀毒除菌装置「ReSPR」对新冠病毒的灭活性,于2020年6月中旬向美国Wisconsin大学兽医学部的Osorio研究所委托实验验证。

实验在9月中旬确认可以使新冠病毒失去活性之后,又进行了多种不同暴露时长的实验验证,实验从开始一共历时约5个月。美国Wisconsin大学兽医学部的Osorio研究所于2020年12月8日发布了本公告所附的实验报告,同日ReSPR公司接收了这份报告,我司本月11日接收到此报告书。我司应ReSPR公司要求,在该公司公布实验结果后披露信息,今日特此公告。此外, Wisconsin大学也参与了东京大学、国立国际医疗研究中心、国立传染病研究所的关于猫之间呼吸器的新型冠状病毒的共同研究。

2.关于对业绩的影响

关于2021年9月期相关业绩的影响,正在进一步确认中,如有需要更新的信息,我司会立即公示。

报 告

RESPR HVAC 设备对铝表面 SARS-CoV-2 的灭活

Cristhian Salas - Jorge Osorio

2020 年 12 月 8 日

摘要

设计可用于人类日常活动的 SARS-CoV-2 灭活有效方法，有助于减少 COVID-19 等传染病的转移和传播。RESPR 技术已证明能够有效地减少空气和表面的病原体和过敏原。它被用于释放氧化颗粒的设备，以净化人们吸入的空气。我们测试了不同暴露时间下 RESPR HVAC 设备在铝表面上的 SARS-CoV-2 灭活效果。采用噬菌斑测定法，在有该设备的情况下，经过 8 个不同暴露时间点(从 10 分钟到 2880 分钟)后，测量 SARS-CoV-2 滴度。RESPR HVAC 设备显示 1440 分钟后铝表面 SARS-CoV-2 传染性颗粒减少了 99.991%。

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材料和方法

材料感染和标本采集

将 RESPR HVAC 设备置于生物安全柜(BSC)内并开启。将事先用 70%乙醇消毒并在紫外线下照射 25 分钟的 24mm×24mm 的无菌铝箔片分别置于 BSC 内的培养皿中，并在室温下保存。使用微量移液管将 200 μ l 的 1×10^5 PFU 的 SARS-CoV-2 的接种物放置在每个铝片上并延伸。每个处理重复 3 次，并准备足够的样品来评估 8 个暴露时间（15、30、60、120、360、720、1440 和 2880 分钟）（表 1）。

在每个暴露时间之后，将 2ml 收集培养基（含 2%FBS 的 DMEM）添加到每个有盖培养皿中，初始稀释度为 1:11，并使用微量移液器将其重悬 4~5 次，从而洗净铝材料；收集病毒悬浮液，混合均匀，并等分至 1ml 离心管中。将收集到的每个样品立即贴上标签，在 -80°C 下保存，以备滴定检测。

表 1. 评估处理

病毒剂量	评估时间（分钟）	处理
1×10^5 PFU/200 μ l	15、30、60、120、360、 720、1440 和 2880	RESPR HVAC

病毒灭活定量

将回收的病毒悬浮液在混合板中一式两份稀释（10 倍，3 种稀释度：1 / 10、1 / 100、1 / 1000），然后添加到 96 孔 Vero E6 接种板中。将板在 37°C 下培养 1 小时。丢弃接种物，加入 2% 羧甲基纤维素覆盖层，在 37°C 下培养 24 小时。然后丢弃覆盖层，对板进行清洗并在 -20°C 下固定 10 分钟（使用丙酮-甲醇溶液）。固定后，将板用 PBS-T 洗涤 2 次，并加入一抗（人抗冠状病毒 IgG，1: 2000）并在 37°C 下培养一夜。然后丢弃一抗，并且将板用 PBS-T 洗涤 2 次。加入二抗（羊 IgG 抗人 HRP 标记，1:2000），在 37°C 下培养 2 小时。去除二抗后，将板用 PBS-T 洗涤 2 次，并用色原底物显影噬菌斑。使用 Immunospot Image 分析仪和开源软件 Viridot 对噬菌斑进行计数，以确定病毒滴度。使用以下公式计算滴度降低百分比：

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

式中: Percent reduction 为降低百分比；A 为未经处理(对照)的病毒滴度或初始滴度； B 为处理后的病毒滴度。

结果

如预期,病毒滴度随时间下降,其平均值见表 1 和图 1;感染 24 小时后(1440 分钟)滴度降低至 45FFU/mL。表 1。将 24mm×24mm 铝箔片暴露于 RESPR HVAC 设备后,在感染后不同时间点(0 ~ 2880 分钟)收集 SARS-CoV-2 接种物的平均滴度和标准偏差。

时间 (分钟)	平均 (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

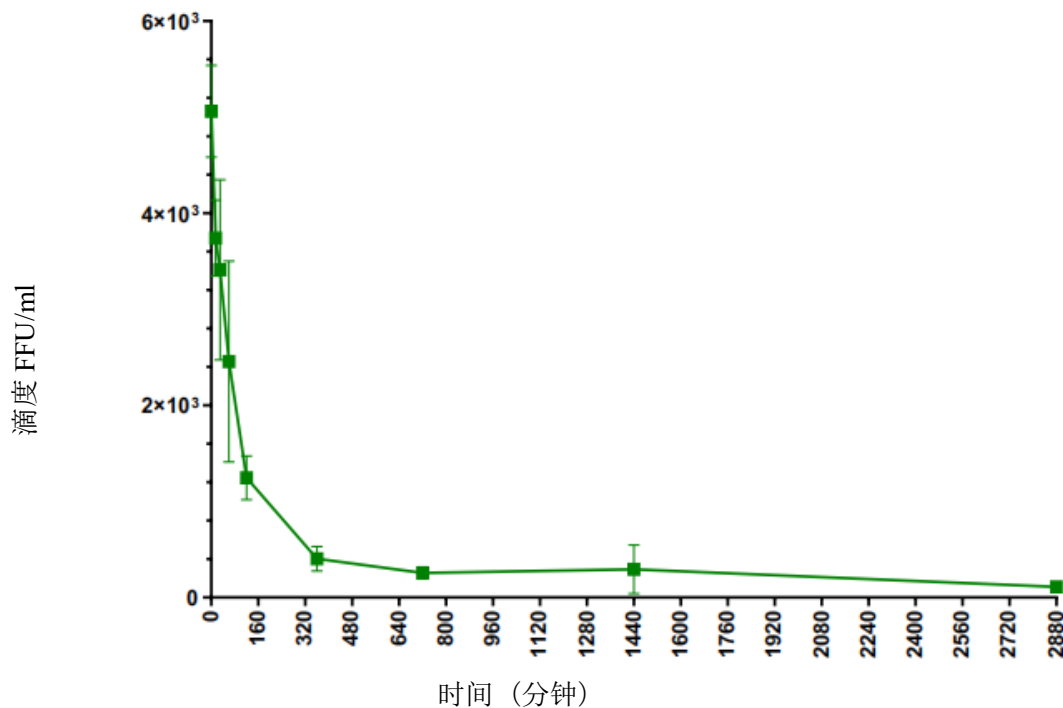


图 1. 将 24mm×24mm 铝箔片暴露于 RESPR HVAC 设备后,在感染后不同时间点(0 ~ 2880 分钟)收集 SARS-CoV-2 接种物的平均滴度和标准偏差。

与初始接种量(5.06×10^3 PFU/ml)相比, SARS-CoV-2 滴度的总降低量在 1440 分钟暴露后达到 99.991%(图 3)。

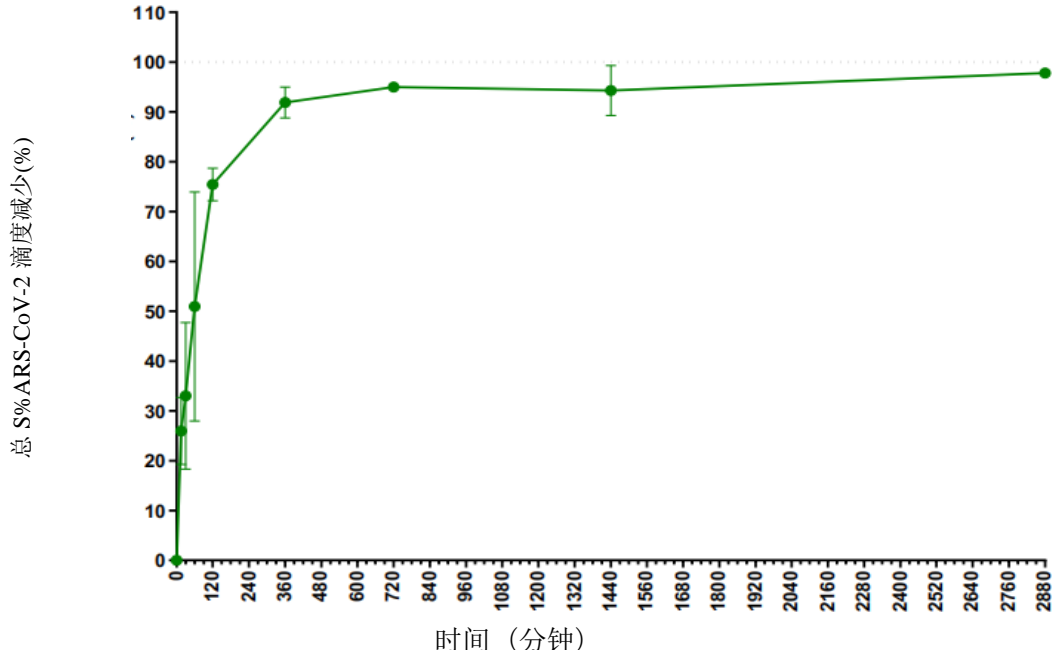


图 3.从暴露于 RESPR HVAC 设备的 24mm×24mm 铝箔片感染后, 在不同时间点 (0~ 2880 分钟) 收集的 SARS-CoV-2 接种物的总减少量 (%)。

结论

在使用 RESPR HVAC 设备时, 铝表面的 SARS-CoV-2 感染颗粒在暴露 1440 分钟后最大减少了 99.991%。超过 97.8%的这种减少是在首次暴露后 360 分钟检测到的。

(原文)

REPORT:

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

Cristhian Salas - Jorge Osorio

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 200µl 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

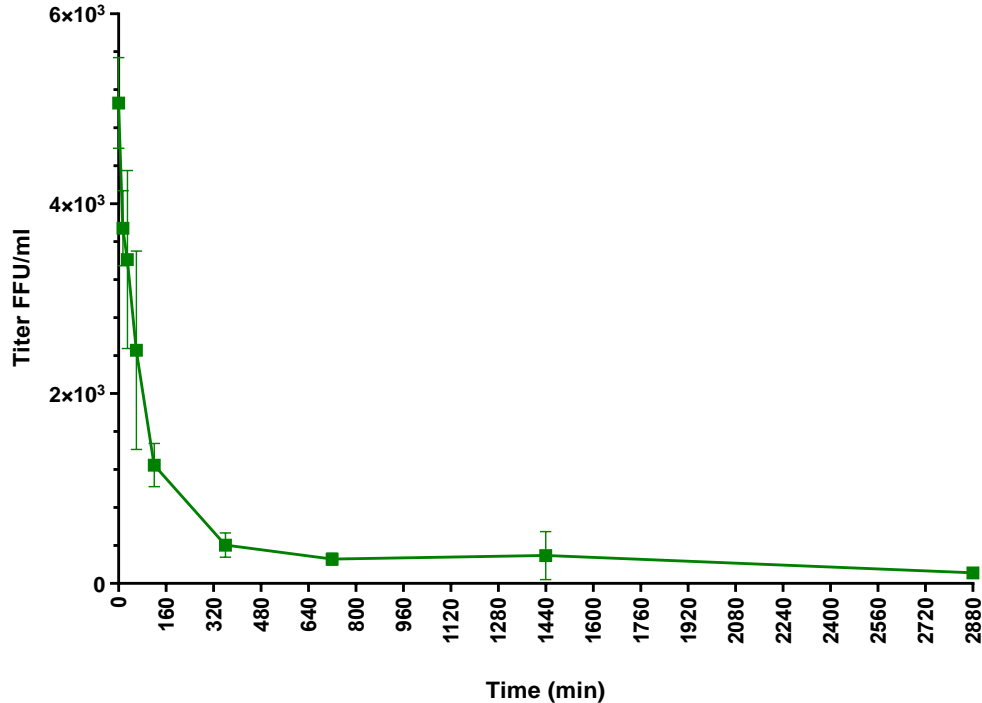


Figure 1. Mean titers and standard deviation of SARS-CoV-2 inoculum collected at different time points (from 0 to 2880 minutes) after infection from 24mm x 24mm aluminum foil pieces exposed to a RESPR HVAC device.

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).

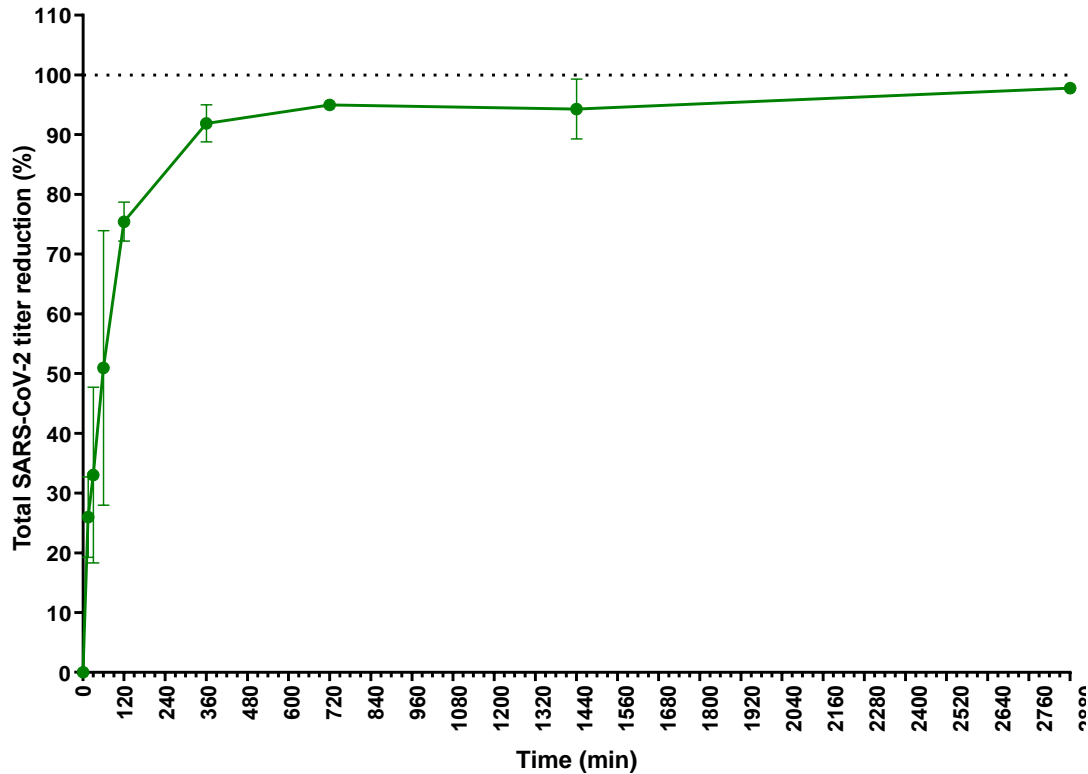


Figure 3. Total reduction (%) of SARS-CoV-2 inoculum collected at different time points (from 0 to 2880 minutes) after infection from 24mm x 24mm aluminum foil pieces exposed to a RESPR HVAC device.

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.