

# Approach of using a household device in decontaminating respirators with ultraviolet C during the scarcity in the COVID-19 pandemic

**Darunee Chotiprasitsakul**

Mahidol University Faculty of Medicine Ramathibodi Hospital

**Taya Kitiyakara**

Mahidol University Faculty of Medicine Ramathibodi Hospital

**Anan Jongkaewwattana**

Biotec: National Center for Genetic Engineering and Biotechnology

**Pitak Santanirand**

Mahidol University Faculty of Medicine Ramathibodi Hospital

**Atisak Jiaranaikulwanich**

Mahidol University Faculty of Medicine Ramathibodi Hospital

**Chureetat Prahsam**

National Metal and Materials Technology Center

**Naruporn Monmaturapoj**

Assistive Technology and Medical Devices Research Center

**Phattira Wadwongsri**

Bangkok RIA Laboratory

**Nunnarin Jeendum**

Bangkok RIA Laboratory

**Siriorn P. Watcharananan** (✉ [orndocor@yahoo.com](mailto:orndocor@yahoo.com))

Mahidol University Faculty of Medicine Ramathibodi Hospital <https://orcid.org/0000-0002-8464-9709>

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## Research

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# Abstract

## Background

Reusing N95 respirators with a practical and easy decontamination protocol might be a solution for the shortage of N95 respirators. We aimed to study the reliability and safety of household UV-C devices for the decontamination of N95 respirators.

## Methods

We investigated a baby bottle ultraviolet C (UV-C) sterilizer box for N95 decontamination. Swine coronavirus (PEDV) was sprayed on the N95 and the virus was cultivated after UV-C sterilization. Bacterial culture from used N95 respirator was performed before and after UV-C exposure. Scanning electron Microscopy (SEM) was used to observe the structural change after UV-C exposure. The radiation was measured by radiometer. The fit test was performed on 3 participants before use, and after reuse 4 and 6 times.

## Results

The PEDV sprayed on the N95 respirator was inactivated by the UV-C level of 0.930–0.932 mW/cm<sup>2</sup>. Nearly all bacterial colonies had disappeared after the 10 min of the UV-C exposure. No significant change to the structure of the N95 polymer fibers after 240 min of UV-C exposure was observed by SEM. Three and two participants passed the fit test after the fourth and sixth time of reuse, respectively.

## Conclusions

During the COVID-19 pandemic crisis, UV-C products may be practical and safe options for decontaminating N95 respirators, if the energy of UV-C is in the range known to deactivate the virus.

## Introduction

The COVID-19 pandemic has led to an unexpectedly expanded number of infected cases as well as a shortage of standard protective equipment for health care workers. The strain on resources in the healthcare setting is an extremely challenging problem. Amid the COVID 19 crisis and the shortage of protection, reusing the masks, particularly N95 respirators, a core component of the personal protective equipment among the healthcare workers, seems to be the reasonable practice. The ideal reuse strategy must disinfect pathogenic viruses and bacteria, while conserving the effectiveness of N95 respirators. Another challenge is that the method should be feasible, quick, easy, and practical for all hospitals.

A prior study had shown that SARS-CoV was mostly inactivated by dry heat of 65°C for 20 min or greater, and inactivated by ultraviolet C (UV-C) by 4016  $\mu\text{W}/\text{cm}^2$  at a distance of 3 cm for 15 min [1]. Another study also showed an effectiveness of viral inactivation by heat using temperature at 56°C and 60°C [2].

We investigated the possibility of reusing N95 respirators after decontamination by the UV-C sterilizer using household devices. A baby bottle UV-C sterilizer box was tested to ensure the safety for implementation in the hospitals (Fig. 1).

## Methods

### Ultraviolet C (UV-C) sterilization

The UV-C sterilization was done for N95 respirator (3M models 9010). A baby bottle UV-C sterilizer box (UV CARE<sup>â</sup>, Utronix company, Bangkok, Thailand) with the interior dimensions of 10-in (W)  $\times$  10-in (L)  $\times$  9.8-in (H), and 1 unit of 4-watt lamp of UV-C (254-nm wavelength) was used for the study. The function modes included UV sterilization (10/15/20 mins sterilization, 1 min air exchange), UV sterilization plus heat “auto mode” (30/40/50 mins drying, 10 mins sterilization, 1 min air exchange), and heat (30/40/50 mins heat, 1 min air exchange). The temperature during the 50 mins of heat mode (auto mode) was measured every 5 mins for the entire experiment.

### Quantitation of viral titers for the UV-C disinfection method

A Porcine Epidemic Diarrhea virus (PEDV), a swine coronavirus, harmless to humans and, previously shown to possibly transmit via an aerosol route [3] was used for evaluating the inactivation of virus on the N95 respirators. This virus has been successfully used to investigate several aspects of coronavirus replication [4]. For the assessment of viral infection, recombinant PEDV bearing a fluorescent mCherry gene in its genome (PEDVmCherry) was used [5].

To investigate the effect of UV-C radiation on the infectivity of PEDV bearing the mCherry gene (PEDVmCherry), droplets of Opti-MEM containing PEDVmCherry on the N95 respirator were allowed to be exposed to UV-C for 20 mins before being adsorbed onto Vero E6 cells. The 200  $\mu\text{L}$  ( $10^4$  tissue culture infectious dose [ $\text{TCID}_{50}$ ] per mL) of the recombinant PEDVmCherry was sprayed on the outer layer of N95 respirators and placed at a distance of 5 cm from the UV-C bulb. The N95 respirators were divided into 4 groups; 1. no UV-C exposure, undisturbed at room temperature for 1 hour, 2. only heat mode of the sterilizer box for 50 mins, 3. only UV-C mode of the sterilizer box for 20 mins, 4. combination mode of heat 50 mins and UV-C 10 mins.

Samples of N95 respirator were incubated in 2 mL of Opti-MEM to release the virus at room temperature for 1 hour, then overnight at 4°C. The 500  $\mu\text{L}$  of Opti-MEM containing released virus was adsorbed onto Vero E6 cells for 2 hours, washed with phosphate buffer saline, maintained in Opti-MEM<sup>â</sup> (Gibco<sup>â</sup>, Thermo Fischer Scientific Inc., Waltham, MA, USA) with trypsin (2  $\mu\text{g}/\text{mL}$ ) and incubated for 48 hours at

37°C. Hoechst dye was used to stain the nuclei of the infected cells. A fluorescence microscope was used to observe the cytopathic effect and visualize the mCherry expression in infected cells.

### **Bacterial culture for the UV-C disinfection method**

A used N95 respirator was swabbed before and after decontamination with UV-C. The respirator was hung in the sterilizing box at around 5-8.5 cm directly below the UV-C bulb. The colonies were cultured on chocolate, blood and MacConkey agars. Colonies of bacteria were counted and identified by MALDI-TOF mass spectrometry (MALDI-Biotyper<sup>®</sup>, Bruker Cooperation, Germany) after 24-48-hour incubation.

### **Scanning Electron Microscope (SEM) before/after heat and UV-C exposure**

The samples of N95 respirator were cut and placed on SEM sample holder before sputter coated with thin gold layer. Their structures as well as the contained fibers were examined using scanning electron microscope with a 1000-time magnification (SEM, Hitachi S-3400N).

### **Measurement of radiation in the UV-C sterilizer box**

The emitted UV-C radiation at the middle and corner of the box, at distances 5 cm to 15 cm below the bulb was measured by a UV radiometer (LT Lutron YK-37UVSD SD Card Data logger UV light meter). The radiated power was calculated using Stefan–Boltzmann law.

### **Qualitative fit testing for N95 respirators**

The fit test procedure was done on N95 respirators (3M models 1870) as previously described [6]. Three participants who had no prior experience with respirator-fit testing underwent the initial fit test. The masks were reused after 10 mins decontamination with UV-C each time. The follow-up fit test was assessed after decontaminating and reusing the N95 respirators after four and six times.

## **Results**

### **Effect of UV-C radiation on the infectivity of a pig coronavirus**

While unexposed PEDV mCherry gave rise to robust mCherry expression, undetectable level of mCherry were observed in the UV-treated group. When the sample was incubated in the heat mode alone, viral infectivity was hardly affected (Fig. 2). The temperature measured during that time reached above 56°C only 9 mins (6-15 mins after the heat mode started) while it was 54-55°C for the rest of the time.

### **Effect of UV-C radiation on bacterial growth**

All the bacteria that grew were grown from swabs taken from the facial side of the respirator. They were normal skin and oral flora. All but one were destroyed by 10 mins of UV-C exposure in the sterilizer box.

However, a few colonies of *Bacillus beringensis* continued to be cultured from swabs taken from the folds of the respirator after 30 mins of UV-C exposure.

### **Effect of UV-C on SEM characterization**

The contour of N95 respirator fiber, structure and spaces between the fibers remained unaffected after a cumulative of 240 mins UV-C exposure (Fig. 3).

### **Radiometric analysis**

A significant variation in the UV-C level at different points in the box was detected, depending on the closeness and angle from the bulb. At the position of testing the recombinant PEDVmCherry coronavirus, 5 cm below the bulb, the level was 0.930-0.932 mW/cm<sup>2</sup>. The lowest level was found at the top corner of the box at 0.055 mW/cm<sup>2</sup>. The minimum and maximum level measured at the floor of the box, measured at the corner and the centre respectively, were 0.26 mW/cm<sup>2</sup> and 0.30 mW/cm<sup>2</sup>.

### **Qualitative fit testing for N95 respirators after reuse**

All 3 subjects passed the fit test at the first time, and the fourth time after reuse. Two subjects passed and one subject failed the fit test after the sixth time of reuse.

## **Discussion**

The strategy of reusing respirators might be a very reasonable practice to conserve available supplies for further use during this unprecedented threat of COVID-19. Nevertheless, ineffective sterilization of respirators can result in contamination, transmission and self-inoculation of mucous membranes resulting in COVID-19 infection among the reusers. To our knowledge, there has only been limited data that describe experimental models of coronavirus inactivation on the N95 respirator. SARS-CoV-2 is primarily transmitted through respiratory droplets and contact routes [7–9] but airborne transmission is possible even in the absence of aerosols-generating procedures [10]. Therefore, the safety of healthcare workers caring for the COVID-19 cases during the current SARS-CoV-2 pandemic has been a major concern. Previous studies have shown that heat effectively inactivated SARS-CoV and MERS-CoV at the lowest temperature range 56–65 °C, after an average time of 30 min [2, 11, 12].

Prior to the present study, published literature showed that viruses could be inactivated by UV-C but the reported effective dose of UV-C varied between studies. The UV-C exposure dose required for 90% inactivation (D90) for coronaviruses including SARS-CoV varied between studies from 3-3046 J/m<sup>2</sup> in air or liquid media [1, 13–17]. However, the relatively high UV-C dose of 1,000–18,000 J/m<sup>2</sup> was used for inactivating the majority of influenza viruses and coliphage on contaminated N95 facepieces [18–20].

Our study found that the emitted radiation in different parts of the UV-C sterilizer box ranged between 0.05–0.93 mW/cm<sup>2</sup>. The effective time of viral inactivation by UV-C in our study was observed at 20 min.

The calculation of UV dose in the sterilizer box, excluding the corners of the box, for 20 min and 10 min were 3,600 – 11,160 J/m<sup>2</sup>, and 1,800-5,580 J/m<sup>2</sup>, respectively.

Although heat alone had no effect on virus, the combination of heat 54–55°C and UV-C 10 min showed no detectable viruses. The fluctuation in temperature may have been the reason for the poor viral inactivation in the heat mode. Alternatively, this could be interpreted as the effectiveness of 10-mins UV-C exposure or the synergistic effect of heat combined with UV-C. However, the MERS-CoV was still detectable after heat at 56°C after 30 min in a previous study [11]. Therefore, it is likely that 10-min UV-C is sufficient for the inactivation of the virus.

UV radiation degrades polymers. This may result in the loss of protective function of N95 respirators. However, a study using relatively high UV irradiation at 120 J/cm<sup>2</sup> (1,200,000 J/m<sup>2</sup>) found minimal increase in particle penetration (1.25%) and had little effect on the flow resistance. This high UV dose decreased the strength of the layers of respirator material by 5–42% depending on the models and layer of respirators, but it had very small effect on the strength of the straps [21]. Although we did not test particle penetration or flow resistance in our study, we used the physical appearance of the fibers, as determined by SEM, and fit testing as secondary measures to imply any changes in respirator function that might occur after UV-C decontamination. The SEM showed no change in the fiber arrangement and spaces between fibers after 240 min of UV-C exposure, compared to the structure of unexposed UV-C samples.

Inactivation of organisms by UV-C depends on the type of organisms, energy of the light source, distance between light source and object surface, and the time of exposure. All these factors must be integrated in order to determine the optimal disinfection strategy. Our approach using a UV-C sterilizer box, with a 4 watt bulb, at the distance of 5-8.5 cm, for at least 10 min should be enough to inactivate the coronaviruses, according to our test result, and previous studies.

There were several limitations in our study. We did not perform the viral and SEM test on the strap of N95 respirator. Viral inactivation requires direct UV-C exposure of the contaminated surface. It is important to ensure that all contaminated surfaces are exposed to the UV-C radiation. Previous study suggested that UV-C had minimal effect on the respirator straps. A UV-C dose of 590 J/cm<sup>2</sup> (5,900,000 J/m<sup>2</sup>) decreased the strap strength by 10–21% [21]. The filtration efficiency after reuse was not conducted in our study. However, no structural integrity change was observed using SEM in this study, and the fit testing has been shown to correlate well with the particle-size-selective protection factors [22]. Following the recommended guidelines, a seal check must always be performed prior to each reuse.

Our disinfection strategy of using a home device has several advantages. UV-C sterilization devices are already available in the markets. The process takes only 10–20 min. Users take responsibility for the disinfection process and keep their own N95 respirators. The disinfection process is easy, as no new invention or additional workers are required for the process.

We have implemented N95 respirator extended use and decontamination program using UV-C sterilizer boxes at our institution since March 19, 2020. The consumption rate of N95 respirators reduced by a factor of four within two weeks. We have distributed these devices to many hospitals in Thailand, especially rural hospitals, through a public donation network. The ministry of public health has adopted the idea of UV-C decontamination in the national strategy.

## **Conclusions**

This study provides the evidence that household utilities such as an UV-C sterilizer box could be repurposed for N95 respirator decontamination, granting the safe reuse of the respirators for the healthcare workers during the pandemic of COVID-19.

## **Declarations**

## **Availability of data and materials**

The data set supporting the conclusions in this article is available from the corresponding author on reasonable request.

### **Ethics approval**

Institutional review board approval was not required as this research did not involve human or animal subjects. This study was conducted to facilitate the mitigation of respirators shortages during the COVID-19 crisis in Thailand.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

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## **Authors' contributions**

DC, TK and SW designed the study and drafted the manuscript. AnJ, PS, AtJ, CP, NM, PW and NJ conducted the experiments and collected the data. All authors have read approved the final manuscript.

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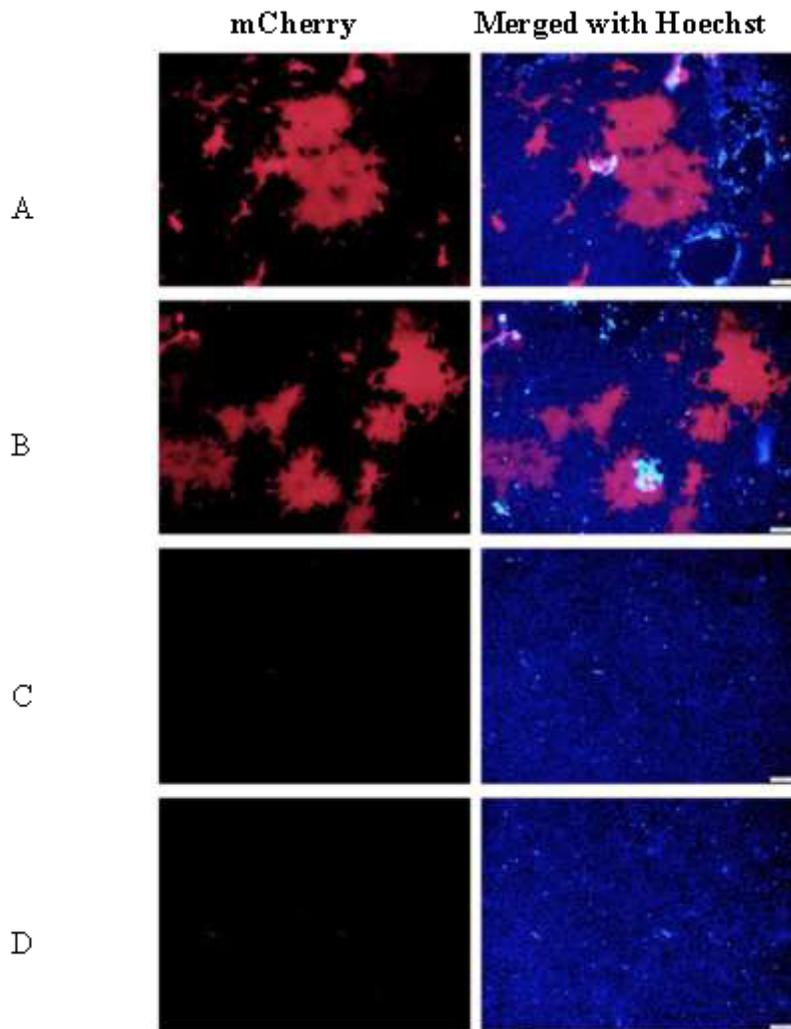
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## Figures



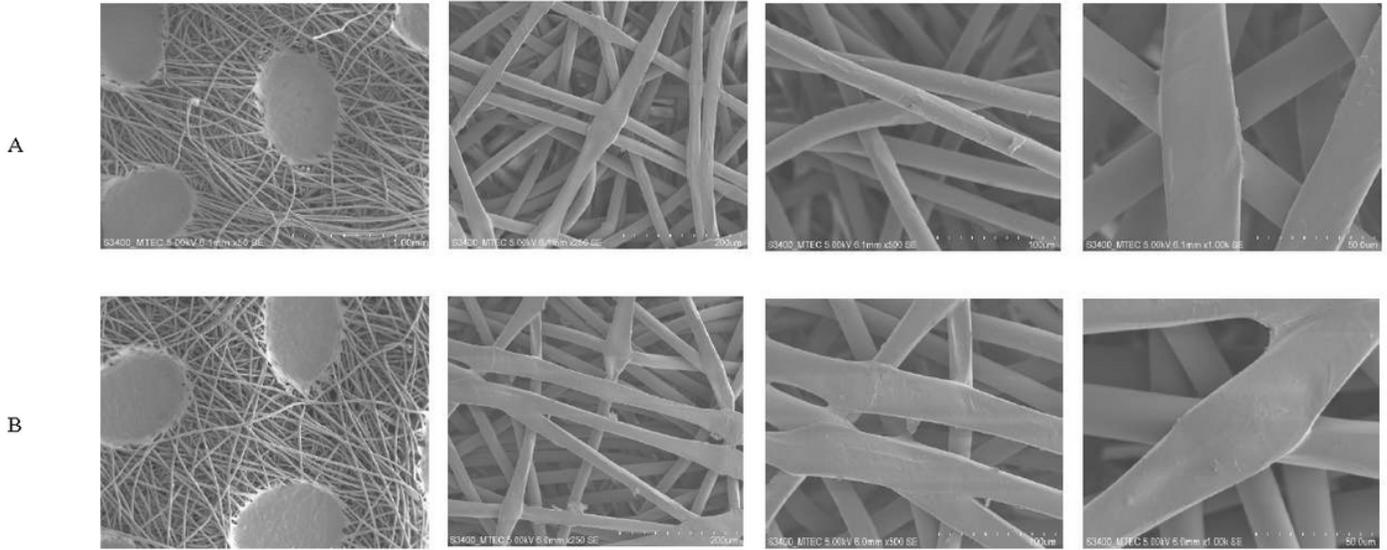
**Figure 1**

Baby bottle UV-C sterilizer box for decontamination of N95 respirators.



**Figure 2**

The N95 respirator was sprayed with the recombinant porcine epidemic diarrhea virus bearing the mCherry gene. The mCherry expression and cytopathic change stained by Hoechst dye was visible as red color in A) no UV-C exposure and B) 50 mins of heat in the UV-C sterilizer box. No mCherry expression and cytopathic change was detected in C) 20 mins of UV-C exposure and D) 50 mins of heat combined with 10 mins of UV-C in the UV-C sterilizer box.



**Figure 3**

Scanning Electron Microscope showing the structure of N95 respirator fiber after UV-C exposure (from left to right, x50, x250, x500, x1000 magnification power). A) Before UV-C exposure B) After 240 mins of UV-C exposure; no change in the structural findings.