Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Fast inactivation of coronavirus in filtering-facepiece respirators in a reflective cylindrical UV-C chamber

Marilia Wellichan Mancini a,*, Luciana Almeida-Lopes a, Paulo Sérgio Bossini a, Gislaine Santos Jacintho b, Junko Tsukamoto b, Clarice Weis Arns b

a Institute of Research and Education in the Health Area (NUPEN), São Carlos, SP, Brazil
b Animal Virology Laboratory, Department of Genetics, Evolution, Microbiology and Immunology, Institute of Biology, University of Campinas, Campinas, SP, Brazil

ARTICLE INFO

Keywords: COVID-19 UV-C light FFR N95 PFF2 Reuse Coronavirus MHV-3 Photobiology Photomedicine UV-C, Microbiology Light/Light source

ABSTRACT

Objective: We report on the development and characterization of a UV-C (λ = 200 – 280 nm, λ peak = 254 nm) chamber designed for the rapid disinfection of N95 class filtering-facepiece respirators contaminated with SARS-CoV-2 coronaviruses. The device was evaluated against Betacoronavirus strain MHV-3 and its virucidal capacity was evaluated as a function of different applied UV-C doses (UV-C exposure times of 60 s, 120 s, 180 s, and 240 s) using two types of respirators geometry (shell and two-panel shapes, 3M 8801 H and 9920 H, respectively), at eight points of the respirators.

Background: Most chemical disinfection methods are not recommended for N95 masks. UV-C light provided by UVGI lamps (254 nm) is an effective physical agent against viruses and bacteria due to direct photochemical harming effect on DNA/RNA, and can provide rapid disinfection for personal protective equipment such as N95/PFF2 masks.

Results: The device reached a mean elimination rate of 99.9999% of MHV-3 inoculated into all the assessed different points on the tested PFF2 respirator models in a UV-C cycle of just 60 s. Statistical analysis performed through Pearson chi-square test showed no correlation between the viral infectivity reduction and the viral inoculation point (p = 0.512) and the tested respirator models (p = 0.556). However, a correlation was found between the exposure time and the viral infectivity reduction (p = 0.000*), between UV-C and no UV-C exposure. All the tested UV-C exposure times (60 s, 120 s, 180 s, and 240 s) provided the same reduction in infection rates. Therefore, 60 s was confirmed as the minimum exposure time to achieve a 99.9999% or 6 Log reduction in MHV-3 coronavirus infection rates in the PFF2 samples tested in the device.

Conclusions: We conclude that the assessed UV-C chamber for the inactivation of MHV-3 coronavirus in N95/PFF2 standard masks can be a promising tool for effective and rapid disinfection of coronaviruses, including SARS-CoV-2 virus.

1. Introduction

The Covid-19 disease caused by the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a pandemic by the World Health Organization (WHO) on 11 March 2020. Due to the severe worldwide shortage of personal protective equipment (PPE) resulting from the widespread outbreak of the disease, protective filtering-facepiece respirators (FFR), i.e., disposable single use N95 masks and equivalent standards, had to be reutilized, increasing the risk of contamination of healthcare workers with SARS-CoV-2 virus. In response to this critical situation, healthcare institutions resorted to using UV-C to decontaminate N95 respirators for reuse.

Within the entire UV range (200 – 400 nm) of the optical electromagnetic spectrum, UV-C (200 – 280 nm) is the most effective against pathogenic, bacteria, viruses and fungi species. The peak wavelength of light radiation emitted by low-pressure Hg lamps (254 nm) lies in the UV-C region close to the maximum of the absorption spectrum of nucleic acids (265 nm), which explains its high inactivation effectiveness [1-3]. UV-C-induced inactivation takes place from the absorption by RNA/DNA and direct production of crosslinks between adjacent nucleic acids, damaging DNA and hindering its replication by the photochemical fusion of adjacent pyrimidines into covalently linked dimers, which
then become non-pairing bases [4,5].

UV-C is reportedly an effective and feasible technology for the inactivation of SARS-CoV-2 [6–10]. UV-C light of wavelength 254 nm is demonstrably effective against influenza virus deposited in the form of aerosol or droplets on N95 respirators, but it has shown to be dependent on the N95 model [11–14].

UV-C decontamination methods are wavelength and dose-dependent, and also hinge upon the type of exposed material and morphology. Moreover, environmental conditions such as humidity and temperature, as well as dirt and stains also alters the effectiveness of those methods. Another factor influencing achievable virus inactivation rates of the entire respirator is shadows arising from non-flat surfaces.

In this work, we developed and investigated a 254 nm UV-C chamber designed for the disinfection of N95 masks for the rapid SARS-CoV-2 inactivation, and validated it using the Betacoronavirus MHV-3 as a SARS-CoV-2 surrogate [15–17]. MHV-3 shares the genus Betacoronavirus and has a positive-sense single-stranded RNA. MHV-3 and SARS-CoV-2 have a similar genome size, since the MHV-3 is approximately 25 – 31 kb while that of SARS-CoV-2 is 29.8 – 29.9 kb causing them to present similar UV-C light susceptibility [17–19].

The designed chamber for rapid disinfection of FFR (N95/PFF2 masks) to coronavirus for the simultaneous treatment of eight piece per UV-C cycle aims for a virus inactivation rate of at least 99.99% (4 Log reduction). The device was optically characterized with regard to irradiance (power density of fluence rate, mW/μm² and total energy density or fluence, J/cm²), as well as humidity percentual and temperature, with regard to the proposed UV-C experimental exposure times.

We tested different exposure times (UV-C doses) of 60 s, 120 s, 180 s, and 240 s to determine the dose dependence of the virus inactivation rate and the minimum time required for MHV-3 virus eradication on PFF2 (S) masks (reduction ≥ 99.99% or 4 Log) achieved by the chamber device. Eight inoculation position sites (a, b, c, d, e, f, g, and h) per FFR were tested for two N95/PFF2 masks models PFF2 (S) models 8810 H – 3M and 9920 H - 3M, shell-shaped and foldable, two-panel shaped masks. The Person’s Chi-squared test was applied to verify the relations between the respirator models (8801H e 9920H), exposure time (0 s, 60 s, 120 s, 180 s, and 240 s) and inoculation site (a, b, c, d, e, f, g, and h) in the viral infectivity reduction rate. A viralic rate up to 99.99999% (6 Log) was accomplished in just 60 s, regardless of the virus inoculation point and of tested FFR PFF2 models, as shown by the statistical analysis.

2. Materials and methods

2.1. UV-C chamber device for N95/PFF2 disinfection

A cylindrical chamber composed of a reflective aluminum alloy cavity containing an arrangement of five low-pressure Hg lamps was designed for fast inactivation of coronavirus SARS-CoV-2 on N95 masks. The cylindrical shape assembly was used due to symmetry concerns, for homogeneous irradiation of masks in different sites within the cavity. The device was developed and characterized by DMC Importação e Exportação de Equipamentos LTDA, São Carlos – SP, Brazil (Project Finep N° 03.20.0018.00).

The device is equipped with five lamps with wavelength 254 nm, 95 W power (27 W output UV-C 200-280 nm radiometric power), 533.0 mm x 40.0 mm L x d. The external cylindrical chamber has an outer diameter of 500 mm and a total height of 800 mm. The control (turn on/off) of the lamps, as weas II monitoring measurements inside the chamber (temperature, humidity, and light field irradiance and fluence) are performed by an embedded software. Dosimetric parameters within the chamber are measured and shown in real-time by the software during the irradiation process, and also temperature and humidity. Within the chamber a built-in metallic holder has the capacity for the simultaneous disinfection of eight masks per cycle. Data are transmitted via radio frequency (RF) using a ZigBee protocol for remote communication.

Fig. 1 shows the UV-C chamber device. Temperature and humidity are measured inside the cavity by a sensor placed at the inner cylindrical reflective wall. Irradiance and fluence were measured using both a UV photodiode and a calibrated UV-C meter sensor model Radiometer 7.1 for UV-C (GUVC10GS7.1-LA9) – GenuV, situated inside the closed chamber.

2.2. UV-C dosimetric parameters

The applied UV-C doses investigated here were based on literature pointing out for the need for at least 1000 mJ/cm² at 254 nm to be applied to each point of a respirator in order to achieve a ≥ 99.99% disinfection rate of N95 masks against SARS-CoV-2 [12,14,20]. This work aimed to obtain a minimum coronavirus inactivation rate of ≥ 99.99% (virucidal effect) of each of the investigated respirator models and points infected with the MHV-3 coronavirus, which was used as a SARS-CoV-2 surrogate.

Fig. 2 presents the UV-C irradiance (mW/cm²) and fluence (mJ/cm²) as a function of exposure time inside the chamber, measured at the site regarding the minimum point of concavity of the mask. Tested interaction times between the UV-C light and the virus on the mask’s surfaces were 60, 120, 180 and 240 s. Before starting a disinfection cycle, the lamps in the chamber were turned on to heat it to a temperature of 55° C. Irradiance is time-dependent because UVGI Hg lamps have time-dependent emission of UV-C output power. Fig. 2 shows time-dependent irradiance and fluence (dose) for a total exposure time of 240 s. Measured irradiance at 60 s is approximately 7.88 mW/cm², which corresponds to a applied dose of 472.8 mJ/cm². For a total exposure time of 240 s, the applied dose at the aforementioned point is approximately 1560 mJ/cm².

2.3. UV-C safety aspects

Safety requirements for UV-C establish the maximum irradiance and dose for daily exposure, defined by the threshold limit value (TLV) by the ISO 15858:2016 standard for UV-C Devices – Safety information – Permissible human exposure [21]. The TLV is a function of the UV-C wavelength between 180 nm and 400 nm incoherent optical radiation [22]. Personal protective equipment is mandatory for the protection of both skin and eyes against accidental exposure to UV-C and against the hazard risk of DNA impairment resulting from long-term exposure to UV-C [23].

A sensor inside the chamber monitors the irradiance during each irradiation cycle to ensure the proper dose is supplied to inactivate the coronavirus. Moreover, personnel are not exposed to UV-C light, as the system is a sealed closed cavity and the disinfection process stops immediately when the door is opened. There are no glass windows or any other UV-C transmissive materials, thus reducing the possibility of operational risks posed by UV-C methods.

2.4. Virus and cells

Murine coronavirus strain MHV-3, belonging to the genus Betacoronavirus, was used as a surrogate for SARS-CoV-2, were maintained in Dulbecco’s Modified Eagle Medium (DMEM, Vitrocell, Brazil) supplemented with 2% fetal bovine serum (FBS) and cultured at 37° C with 5% CO₂. The L929 cell lineage NCTC clone 929 [L-929, derivative of strain 1] (ATCC® CCL-1™) was cultured in DMEM supplemented with 10 % FBS at 37° C, 5% CO₂. Coronavirus titration was performed using the 50% Tissue Culture Infectious Dose (TCID₅₀) method.

2.5. Maximum non-toxic concentration (MNTC)

The maximum non-toxic concentration (MNTC) in the cells was determined in the experiment to ensure that the UV-C radiation was harmless to cells and to ascertain the inhibition of the virus. For
preparation of the series concentrations/dilutions to be used in the virucidal assays, it was also determined the MNTC. To check feasible morphological variation of cells by UV-C radiation, one part of hard water and one part of test interference (3 g/L albumin and fetal bovine serum) are mixed with suspension virus tested by UV-C. Serial dilutions (10^3 – 10^9) were prepared in the cells were cultured in microplates with 96 wells (monolayes, 90% confluence) and incubated for 48 h at 37 °C, 5% CO₂. The results were obtained using an inverted optical microscope and proceed by quantal tests (endpoint titration).

2.6. FFR samples

The utilized FFR samples to assess the virucidal performance of the chamber were PFF2 (S) models 8810 H – 3M and 9920 H - 3M, in compliance with Brazilian standard NBR ABNT 13698:2011 - Respiratory protective devices - Filtering half mask to protect against particles. Morphologically, the 8801H model is shell-shaped, whereas 9920H is a folding two panel mask (Fig. 3).

2.7. Antiviral assays for FFR coronavirus decontamination

All the virus assays were performed in a Biosafety Level 2 laboratory at the Laboratory of Animal Virology – University of Campinas – UNICAMP, following the methodology described in the ISO 18184/2019-06-25: “Textiles – Determination of antiviral activity of textile products” with pertinent adaptations. This study compared two different FFR conformations in order to take into account the different respirator geometries, and several virus inoculation sites were examined to investigate the virus disinfection rate as a function of their position in the masks. Droplets were deposited at eight points per mask. Eight points were dropled on each mask, for the two models, five points on the outer surface, one central point on the inner surface, and one point on each of the two elastic bands.

A 15 μL (100 TCID₃₀) MHV-3 droplet was applied to each point on each mask, on the external surface in the upper (a) and lower (c) hemispheres, a central spot (b) (maximum concavity site), on the right (d) and left (e) side s; and on the right (g) and left (f) elastic bands. A droplet was applied at the internal surface, the droplet was applied in the central spot of the inner surface (h), regarding the minimum concavity of the respirator (Fig. 3).

The droplets were immediately covered and the virus spread over the specific area. The respirators were immediately positioned inside the chamber and submitted to the UV-C disinfection process. Tested irradiation times (UV-C cycles) were 60 s, 120 s, 180 s, and 240 s. After the UV-C treatment, the respirators were stored at -80 °C for the subsequent antiviral assays.

A total of 20 respirators were assessed, with 16 respirators containing the virus droplets (8 on each model) and submitted to UV-C and 4 masks (2 of each model) with the virus droplets and not exposed to UV-C, which served as a control.

Each respirator was cut into circles regarding the spots containing the virus droplets. Each circular piece was placed in a Falcon tube containing DMEM 20 mL and agitated in a vortex equipment. Next, the treated virus solutions were serially diluted, poured into confluent L929 cells and incubated for 48 h at 37 °C in a humidified incubator with 5% CO₂. The cytopathic effect (CPE) was scored by using an inverted optical microscope. The coronavirus titer was calculated based on the method of Reed and Muench (1938) [24]. All the virus titer levels were determined in four repetitions. Table 1 describes citotoxicity, interference, neutralization and a 0.7% formaldehyde virucidal controls.

2.8. Statistical analysis

Statistically significant differences regarding the inactivation rates were investigated as a function of the virus application points, UV-C exposure times (in seconds) and PFF2 respirator models. The Person’s Chi-squared test was applied to verify the possible relations between the respirator models (8801H and 9920H), exposure time (0 s, 60 s, 120 s, 180 s, and 240 s) and inoculation point (a, b, c, d, e, f, g, and h) and their effects on the viral infectivity reduction rate. To this end, the data were categorized into virucidal (viral inactivation rate ≥ 99.99%) and non-virucidal (viral inactivation rate < 99.99%). The significance level was considered as 5% (p ≤ 0.05). The data were analyzed by using the SPSS software version 17.0.

3. Results

Cytotoxicity test results relating to the maximum non-toxic concentration (MNTC) in masks without virus and submitted to a serial dilution in DMEM tested cells indicated that the highest titer was non-toxic to L-929 cells. Table 1 describes the citotoxicity, interference, neutralization and a 0.7% formaldehyde virucidal controls.

Table 2 summarizes the viral titers (TCID₃₀/ml) and reduction of infectivity after virucidal solution control (formaldehyde at 0.7%) and
the viral infectivity result for formaldehyde 0.7% virucidal control (TCID50/ml) in four repetitions.

Table 3 shows the viral infectivity reduction (Log10) and percent activity (%) (TCID50/mL) in PFF2 masks inoculated with coronavirus MHV-3 for different exposure times to UV-C, as a function of the inoculation point (a, b, c, d, e, f, g and h), and UV-C exposure time (t = 60, 120, 180, and 240 s), in comparison with virus and no UV-C irradiation (t = 0 s) for FFR models 8801H (Table 3a) and 9920H (Table 3b).

The results are expressed in terms of the percental viral inactivation in comparison to the untreated viral control (virus titer), as mean ± standard deviation. Logarithm infectivity reduction was determined through the percentual inactivation rate, by the expression log10(A/B) = log10(A) − log10(B), where A = 100 and B = x, the percentual of viable viral particles before and after the UV-C intervention, respectively, log10(100/(100 − x)).

Table 4 presents the results of the statistical analysis for the

| Negative Cellular control (2 × 10^5 cells/mL) in DMEM medium, without virus, and no test sample. |
| Virus control Virus titer (10^10 to 10^15) and cells culture in DMEM medium. |
| Control with virus, no UV-C exposure Inoculated masks with virus submitted to no UV-C exposure. |
| Positive test Virus presence, each tested sample and cellular lineage in DMEM medium. |
| Virucidal control 0.7% formaldehyde and cellular lineage in DMEM medium. |
| Cytotoxicity Control Cellular control (2 × 10^5 cells/mL) in DMEM medium, without virus and with test samples. |
inactivation rate as function of the virus inoculation point (a, b, c, d, e, f, g and h) on the mask surfaces, UV-C radiation exposure time (in seconds) and tested FFR PFF2 models (3M 8801H and 3M 9920H). In special, virus inoculation points distribution and different (shell-shaped and foldable, two panel models) FFR models were included to take into account geometry spatial issues regarding UV-C exposition. Exposure time to UV-C (dose dependence) was assessed in order to determine the minimum time for virucidal effect with a 6 Log infectivity reduction.

Masks submitted to a total of 10 UV-C irradiation cycles of 60 s each (total irradiation time of 10 min) were tested according to the specifications of Brazilian technical standards ABNT NBR 15052:2004 and 14873:2002 for bacteriological filtering efficiency (BFE) against to 50 TCID50/mL MHV-3 (Log6.00) (mean ± S.D.) and exposition times 10, 30, and 60 min, regarding doses of 0.33, 0.99, and 1.98 J/cm². They found a 3 Log viral infectivity reduction Log2.5 - Log0.33

4. Discussion

N95 FFRs are usually single-use personal protective equipment, but their high demand under an infectious disease outbreak may lead to their reuse. The COVID-19 pandemic surge gave rise to a critical shortage of PPE, particularly N95 FFRs.

With regard to the whole UV (100–400 nm) range of the optical electromagnetic spectrum, the UV-C part is the most effective against pathogenic viral, bacterial, and fungal species. The UV spectrum components or wavelength ranges, UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (100–280 nm), have been studied and applied to disinfection purposes for different pathogenic strains for many decades, as one of the most efficient physical methods [25].

UV-C light is a high photon energy part of the optical spectrum which can be easily provided by artificial sources as low pressure mercury lamps (wavelength mission within 200–280 nm, peak wavelength λp = 254 nm). Its viral inactivation action is based on the DNA/RNA direct damage by means of photochemical interaction.

Fischer et al. applied an UV-C light source for SARS-CoV-2 inactivation on N95 masks. The emission wavelength range was 260–285 nm, irradiance 0.55 mW/cm² and exposition time 10, 30, and 60 min, regarding doses of 0.33, 0.99, and 1.98 J/cm². They found a 3 Log viral titer reduction for 1.98 J/cm² (60 min exposure time) [20].

Ozog et al. investigated the performance of an UV-C device (254 nm) to N95 respirators inoculated with the SARS-CoV-2 in four different locations of five N95 investigated models. They applied a dose of 1.5 J/cm² to both sides of the respirators (irradiance 16.5 mW/cm²) at the apex position (90.9 s) and observed the virus inactivation to be dependent on the respirator model and virus inoculation site [26].

Dunn et al. [27] characterized a UV-C (λp = 254 nm) chamber for SARS-CoV-2 inactivation in FFRs by measuring irradiance (mW/cm²) values considering the FFR geometry inside the assessed device, as well as they estimate the irradiation time for a dose if at least 1.0 J/cm² dose obtaintion, considered as the minimum dose for SARS-CoV-2 inactivation. Nevertheless, no data provided on the virus inactivation in FFRs by this chamber.

Golowkine et al. [28] evaluated the efficacy of UV-C inactivation of SARS-CoV-2 on N95 coupons by using a UV-C light emitting diodes (LEDs) based device, with emission peak wavelength λp = 272 nm and full width at half maximum (FWHM) of 9.5 nm, calibrated to deliver an irradiance of 1.0 mW/cm² across all sites of the mask. Five points on the external surface of the masks were inoculated with a 50 ml (8 × 10⁷ TCID50/ml) of virus stock. It was tested the decontamination level on the two 3M 1860 and 3M 8210 shell shaped models. The tested UV-C exposition times were 300 and 600 s (5 and 10 min, respectively).
Table 3b
Viral infectivity reduction (Log_{10}) and percentual activity (%) (TCID_{50}/mL) to coronavirus MHV-3 inoculated PFF2 (model 3M - 9920H) to different UV-C exposure times, as function of the virus inoculation site (a – h), in comparison with virus and no UV-C irradiation.

| Virus inoculation site | infectivity reduction TCID_{50}/mL MHV-3 (Log_{10}) | Inactivation rate TCID_{50}/mL MHV-3 (%) (Mean ± S.D.) |
|------------------------|-----------------------------------------------|--------------------------------------------------|
| on 9920H model         |                                               |                                                  |
| a - outer surface      | 5.9375 ± 0.1083                                | 99.9966 ± 0.0004                                 |
| b - outer surface      | 5.9375 ± 0.1083                                | 99.9988 ± 0.0000                                 |
| c - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| d - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| e - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| f - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| g - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| h - inner surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| UV-C exposure time: 60 s |                                               |                                                  |
| a - outer surface      | 5.9375 ± 0.1083                                | 99.9988 ± 0.0000                                 |
| b - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| c - outer surface      | 5.9375 ± 0.1083                                | 99.9988 ± 0.0000                                 |
| d - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| e - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| f - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| g - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| h - inner surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| UV-C exposure time: 120 s |                                               |                                                  |
| a - outer surface      | 5.9375 ± 0.1083                                | 99.9988 ± 0.0000                                 |
| b - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| c - outer surface      | 5.9375 ± 0.1083                                | 99.9988 ± 0.0000                                 |
| d - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| e - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| f - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| g - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| h - inner surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| UV-C exposure time: 180 s |                                               |                                                  |
| a - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| b - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| c - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| d - outer surface      | 5.9375 ± 0.1083                                | 99.9988 ± 0.0000                                 |
| e - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| f - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| g - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| h - inner surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| UV-C exposure time: 240 s |                                               |                                                  |
| a - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| b - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| c - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| d - outer surface      | 5.9375 ± 0.1083                                | 99.9988 ± 0.0000                                 |
| e - outer surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| f - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| g - elastic band       | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| h - inner surface      | 6 ± 0.0000                                     | 99.9999 ± 0.0000                                 |
| UV-C exposure time: none (control with virus without UV-C, 240 s) | | |
| a - outer surface      | 3 ± 0.0000                                     | 99.875 ± 0.0433                                  |
| b - outer surface      | 3 ± 0.0000                                     | 99.9 ± 0.0000                                    |
| c - outer surface      | 2.9 ± 0.1083                                    | 99.875 ± 0.0433                                  |
| d - outer surface      | 3 ± 0.0000                                     | 99.9 ± 0.0000                                    |
| e - outer surface      | 2.9 ± 0.1083                                    | 99.875 ± 0.0433                                  |
| f - elastic band       | 3 ± 0.0000                                     | 99.9 ± 0.0000                                    |
| g - elastic band       | 3 ± 0.0000                                     | 99.9 ± 0.0000                                    |
| h - inner surface      | 3 ± 0.0000                                     | 99.875 ± 0.0433                                  |

These authors observed the decontamination efficacy provided by the tested device to be dependent on the assessed mask model, material and location of the contamination placed on the mask surface. For the 3M 1860 model they found a maximum average log reduction of 4.95 and for the two exposed exposure times (300 and 600 s) and minimum mean reduction of 1.10 and 1.63 (300 and 600 s), corresponding to the aluminum foil at the center of the mask and strap, respectively. For the model 3M 8210 a maximum Log reduction of 4.92 and 4.86, respectively, to 300 and 600 s exposure times to the aluminum foil central location and 3.24 and 1.10 reduction referring to strap location for 300 s and 600 s exposure times.

In this work, we investigated the UV-C (λ = 254 nm) method applied to SARS-CoV-2 variants and strains for the disinfection of FFR for safe reuse. To this end, we used the coronavirus strain MHV-3 on PFF2 masks, using an UV-C chamber designed for the simultaneous disinfection of eight FFR per each disinfection cycle. Various doses were tested aiming to achieve an inactivation rate of at least ≥ 99.99% (virucidal dose).

Table 3 presents the reduction in infectivity of MHV-3 inoculated on the tested PFF2 masks (models 8801H and 9920H, 3M, Brazil), submitted to irradiation by the UV-C chamber Steritray – DMC, for the different exposure times (60, 120, 180 and 240 s) to UV-C light interaction. The corresponding dose dependence of the UV-C exposure times is shown in the Fig. 2.

The total fluence in a given FFR sample point is lamp power, temperature and exposition time dependent. We have measured the total irradiance and fluence to the point of minimum concavity from inner respirator surface of the assessed PFF2 masks exposed to treatment against coronavirus by the investigated UV-C chamber (Fig. 2), starting the UV-C operation with standardized chamber temperature at 55 °C.

Thus, the validated UV-C method described herein appears to be a promising and convenient method for decontamination of N95, PFF2 and other filtering respirator masks belonging to the class N95. This inexpensive method is effective and fast and can be employed for both and large scale decontaminations.

The disinfection of materials and inanimate surfaces using UV-C is largely three-fold dependent on the light wavelength and dose, and the exposed material type. Hence all the points of a FFR must receive the minimum dose (mJ/cm^2) for effective disinfection. Other important factors are humidity, depth and geometry of the object to be UV-C disinfected. Geometry may provide a gradient of received doses and occasionally shadows. Hence, the implementation of any UV-C decontamination method for N95 masks requires careful consideration of the face mask model, including the type of material, design, and the implementation of filtration tests at the maximum permissible irradiation cycles (without UV-C photo damage).

In this work we assessed the coronavirus inactivation rate as a function of irradiation time (dose-dependence), position of the virus inoculated on the respirators, and filtration performance of the tested PFF2 models (8801H and 9920H, 3M, shell-shaped and two panel-shaped, respectively), after a total of 10 UV-C cycles of 60 s per cycle (10 min).

Increasing the total UV-C dose may improve decontamination, but UV radiation can degrade polymers in a dose-dependent manner.
Moreover, the regular handling of the respirator itself on a very regular basis may degrade the characteristics of the filters, hindering the filtration performance and imposing safety concerns. The exposure of humans and animals to UV-C is unsafe because it damages DNA. Therefore, it is required the use of PPE for eyes and skin protection. In the case of tested UV-C chamber, the UV-C exposure damages DNA. Therefore, it is required the use of PPE for eyes and skin protection. Germicidal ultraviolet irradiation, the process of adding dirt agents on coronavirus. Considered a safe option for the N95/PFF2 masks disinfection against to SARS-CoV-2 virus.

4.1. Study limitations

The virucidal activity was not tested with masks containing substances that simulate body and respiratory fluids such as mucin (synthetic saliva) and NaCl (synthetic sweat) [29]. According to the AST E3135-18 standard for analysis of the antimicrobial activity of the gemicidal ultraviolet irradiation, the process of adding dirt agents on the microorganisms can reduce the effectiveness of the UV antimicrobial activity. There are reports in the literature of other forms of virus deposition on the tissue surface, such as aerosol. It is known that the respiratory symptoms of the virus, such as sneezing and coughing, result in the generation of virus-containing particles, in a continuous size from 1 to 500 μm [30], these particles disperse occupying a large area of the tissue, not focusing on certain specific points under the conditions tested in the present experiment.

With regard to the utilized methodology, this study assessed only two FFR models (8801H and 9920H, 3M). In order to be applied to a specific FFR, the validation method must be performed in order to assure the minimum disinfection rate for virucidal effect, as further required morphology and filtration maintenance tests must be applied as considering the intended maximum number of UV-C cycles.

Different models of FFRs use distinct conformations regarding the morphology of the facepiece, as well as present different layers and material components, introducing the necessity of validation regarding UV-C disinfection to coronavirus. Another important issue is checking the disinfection rate for the elastic bands in addition to the concave and convex faces of the respirator to ensure proper disinfection for reuse, to take into account concavity aspects and shadows to UV-C irradiation and hence expected disinfection action, with a given minimum inactivation rate.

5. Conclusions

In conclusion, we developed and assessed an UV-C chamber based on UVGI lamps (λ = 200 – 280 nm, λpeak = 254 nm) designed for the inactivation of coronavirus, including SARS-CoV-2 on N95 and PFF2 masks.

The device designed for rapid coronavirus disinfection and was assessed for its virucidal capacity as a function of applied UV-C dose (exposure times) for two PFF2 masks models (8801H and 9920H - 3 M, Brazil), at eight points distributed on each piece model.

We concluded that the UV-C device evaluated here exerts a rapid virucidal effect against the SARS-CoV-2 coronavirus with a rate inactivation rate of 99.99999% for the tested materials. No statistically significant difference was observed for the MHV-3 inactivation rate provided by the UV-C chamber as a function of the virus inoculation points (distributed on the outer and inner surfaces and on the elastic bands), and of tested PFF2 conformation (shell and two panel). On the other hand, a statistically significant difference was detected in the exposure time dependence. It was observed relation between the exposure time and the viral infectivity reduction (p = 0.000*). The correlation occurs between UV-C exposure and non-exposure, as the viral infectivity reductions are the same for all experimental times (60 s, 120 s, 180 s, and 240 s). Given that no difference was found as a function of the exposure time, 60 s was confirmed as the minimum irradiation time for a 6 Log reduction in MHV-3 coronavirus infectivity.

Finally, UV-C treated PFF2 respirators by the device were also assessed as a function of their morphology and filtration maintenance capacity performance after ten consecutive 60 s cycles and their characteristics were preserved (total exposure time of 10 min).

Both PFF2 models used in the virucidal assays was tested according to standards referring to PFF2 masks for filtering bacteriological efficiency against Staphylococcus aureus and Staphylococcus Epidermidis, and testing regarding visual inspection, breath resistance, penetration through the filter (NaCl), flammability and have shown conformity.

We conclude that the UV-C chamber assessed for inactivation of coronaviruses in N95/PFF2 standard masks can be a feasible and usable tool for the effective and rapid disinfection of coronaviruses including SARS-CoV-2 virus.

Contributors

M.W. Mancini: Study design, formal analysis, discussion, funding acquisition.
M.W. Mancini and L. Almeida-Lopes wrote the manuscript.

P.S. Bossini: formal analysis, discussion.

J. Tsukamoto and G. S. Jacinto conducted the viruses experiments and cell culture assays.

C. W. Arns: Supervision to the microbiology tests and discussion of the results.

All the authors have read and agreed to the final version of the manuscript for publication.

Declaration of Competing Interest

M.W. Mancini works in R&D at the company DMC Importação e Exportação de Equipamentos Ltda. in São Carlos, SP, Brazil. DMC developed and supplied the STERITRAY prototype characterized optically and microbiologically in this research work. This research was supported by the Brazilian Research and Projects Financing Agency – FINEP in a special program against Covid-19. STERITRAY is registered at ANVISA – Brazilian regulatory agency under N° 80030819017. The other authors declare no conflicts of interest.

Acknowledgments

We thank the Brazilian Agency FINEP - Financiadora de Estudos e Projetos for its financial support (Project No 03.20.0018.00) and for company DMC for developing and manufacturing the UV-C chamber device. We also thank G. Gagliardi and M. Prado for technical help. JT, APM and GSJ were supported by CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico and CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. CWA is a CNPq research fellow.

References

[1] M. Heilingh, K. Hönès, P. Vatter, C. Linigerfelder, Ultraviolet irradiation doses for coronavirus inactivation - review and analysis of coronavirus photoinactivation studies, GMS Hyg. Infekt. Control. 15 (2020) 01-08.

[2] N.G. Reed, The history of ultraviolet germicidal irradiation for air disinfection, Public Health Rep. 125 (1) (2010) 15-27.

[3] W. Kowalski, Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface Disinfection, Springer-Verlag Berlin Heidelberg, 2009.

[4] M.E. Darnell, K. Subbarao, S.M. Feinstone, D.R. Taylor, Inactivation of the coronavirus that causes severe acute respiratory syndrome, SARS-CoV-2, J. Virol. Methods 121 (1) (2004) 85-91.

[5] D. Perdiz, P. Grot, M. Mezzina, O. Nikaido, E. Moustacchi, E. Sage, Distribution and repair of bipyrimeridine photoproducts in solar UV-irradiated mammalian cells. Possible role of deoxypurines in solar mutagenesis, J. Biol. Chem. 275 (35) (2000) 26752-26762.

[6] A. Gidari, S. Sabbatini, S. Bastianelli, S. Pierucci, C. Busti, D. Bartolini, A. Gidari, S. Sabbatini, S. Bastianelli, S. Pierucci, C. Busti, D. Bartolini, A.

[7] A. Hamzavi, Skin and eye protection against ultraviolet C from ultraviolet germicidal irradiation devices during the COVID-19 pandemic, Int. J. Dermatol. 60 (4) (2021) 391-393.

[8] L.J. Reed, H. Muench, A simple method of estimating fifty per cent endpoints, Am. J. Hyg. 22 (4) (1938) 453-497.

[9] M.W. Mancini, L. Almeida-Lopes, G.S. Jacinto, J. Tsukamoto, C.W. Arns, Prompt inactivation of coronavirus using a 280 nm light-emitting diode cluster device, Photobiodmol. Photomed. Laser Surg. 40 (4) (2022) 273–279.

[10] R.J. Fischer, D.H. Morris, N. van Doremalen, S. Sarchette, M.J. Matson, T. Bushmaker, C.K. Yinda, S.N. Seifert, A. Gamble, B.N. Williamson, S.D. Judson, E. de Wit, J.O. Lloyd-Smith, V.J. Munster, Effectiveness of N95 respirator decontamination and reuse against SARS-CoV-2 Virus, Emerg. Infect. Dis. 26 (9) (2020) 2253-2265.

[11] ISO 15858: 2016 UV-C devices - safety information - permissible human exposure.

[12] The International Commission on Non-Ionizing Radiation Protection – ICNIRP. Guidelines on limits of exposure to ultraviolet radiation of wavelengths between 180 nm and 400 nm (incandescent optical radiation), Health Phys. 87 (2) (2004) 171–186.

[13] A.B. Lyons, S. Narla, A.E. Torres, A. Parks-Miller, I. Kohli, D.M. Ozog, H.W. Lim, I. Am. Hamzavi, Skin and eye protection against ultraviolet C from ultraviolet germicidal irradiation devices during the COVID-19 pandemic, Int. J. Dermatol. 60 (4) (2021) 391-393.

[14] L.J. Reed, H. Muench, A simple method of estimating fifty per cent endpoints, Am. J. Epidemiol. 27 (3) (1938) 493-497.

[15] M.W. Mancini, L. Almeida-Lopes, G.S. Jacinto, J. Tsukamoto, C.W. Arns, Prompt inactivation of coronavirus using a 280 nm light-emitting diode cluster device, Photobiodmol. Photomed. Laser Surg. 40 (4) (2022) 273–279.

[16] D.M. Ozog, J.Z. Sexton, S. Narla, C.T. Ng, M. Marzuki, P.S. Jeslyn Wong, B. Tan, A. Lee, C.

[17] C.G. Wang, Z. Li, S. Liu, C.T. Ng, M. Marzuki, P.S. Jeslyn Wong, B. Tan, A. Lee, C.

[18] E. Haramoto, P. Gyawali, A. Korajkic, B.R. McMinn, J.F. Mueller, S.L. Simpson, W.

[19] S. Narla, A.E. Torres, S. Douthwaite, S.D. Goldenberg, D.J. Weber, C.

[20] D.M. Ozog, J.Z. Sexton, S. Narla, C.T. Ng, M. Marzuki, P.S. Jeslyn Wong, B. Tan, A. Lee, C.

[21] L.J. Reed, H. Muench, A simple method of estimating fifty per cent endpoints, Am. J. Epidemiol. 27 (3) (1938) 493-497.

[22] R.J. Fischer, D.H. Morris, N. van Doremalen, S. Sarchette, M.J. Matson, T. Bushmaker, C.K. Yinda, S.N. Seifert, A. Gamble, B.N. Williamson, S.D. Judson, E. de Wit, J.O. Lloyd-Smith, V.J. Munster, Effectiveness of N95 respirator decontamination and reuse against SARS-CoV-2 Virus, Emerg. Infect. Dis. 26 (9) (2020) 2253-2265.

[23] ISO 15858: 2016 UV-C devices - safety information - permissible human exposure.

[24] The International Commission on Non-Ionizing Radiation Protection – ICNIRP. Guidelines on limits of exposure to ultraviolet radiation of wavelengths between 180 nm and 400 nm (incandescent optical radiation), Health Phys. 87 (2) (2004) 171–186.

[25] A.B. Lyons, S. Narla, A.E. Torres, A. Parks-Miller, I. Kohli, D.M. Ozog, H.W. Lim, I. Am. Hamzavi, Skin and eye protection against ultraviolet C from ultraviolet germicidal irradiation devices during the COVID-19 pandemic, Int. J. Dermatol. 60 (4) (2021) 391-393.

[26] L.J. Reed, H. Muench, A simple method of estimating fifty per cent endpoints, Am. J. Epidemiol. 27 (3) (1938) 493-497.

[27] M.W. Mancini, L. Almeida-Lopes, G.S. Jacinto, J. Tsukamoto, C.W. Arns, Prompt inactivation of coronavirus using a 280 nm light-emitting diode cluster device, Photobiodmol. Photomed. Laser Surg. 40 (4) (2022) 273–279.

[28] D.M. Ozog, J.Z. Sexton, S. Narla, C.T. Ng, M. Marzuki, P.S. Jeslyn Wong, B. Tan, A. Lee, C.

[29] C.G. Wang, Z. Li, S. Liu, C.T. Ng, M. Marzuki, P.S. Jeslyn Wong, B. Tan, A. Lee, C.

[30] E. Haramoto, P. Gyawali, A. Korajkic, B.R. McMinn, J.F. Mueller, S.L. Simpson, W.

[31] S. Narla, A.E. Torres, S. Douthwaite, S.D. Goldenberg, D.J. Weber, C.

[32] D.M. Ozog, J.Z. Sexton, S. Narla, C.T. Ng, M. Marzuki, P.S. Jeslyn Wong, B. Tan, A. Lee, C.