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Effective ultraviolet C light disinfection of respirators demonstrated in challenges with *Geobacillus stearothermophilus* spores and SARS-CoV-2 virus

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SUMMARY

Background: The global COVID-19 pandemic, accompanied by spikes in the number of patients in hospitals, required substantial amounts of respiratory protective devices (respirators), thereby causing shortages. Disinfection of used respirators by applying ultraviolet C (UVC) light may enable safe reuse, reducing shortages.

Aim: To determine whether UVC disinfection is applicable to enable repeated safe reuse of respirators.

Methods: The UVC chamber, equipped with low-pressure mercury discharge lamps emitting at 254 nm, was used to determine the sporicidal and virucidal effects. Respirators challenged with spores and viruses were exposed to various UVC energy levels. Deactivation of the biological agents was studied as well as UVC effects on particle filtration properties and respirator fit.

Findings: A 5 \log_{10} reduction of *G. thermophilus* spore viability by a UVC dose of 1.1 J/cm² was observed. By simulating spores present in the middle of the respirators, a 5 \log_{10} reduction was achieved at a UVC dose of 10 J/cm². SARS-CoV-2 viruses were inactivated by 4 \log_{10} upon exposure to 19.5 mJ/cm² UVC. In case UVC must be transmitted through all layers of the respirators to reach the spores and virus, a reduction of >5 \log_{10} was achieved using a UVC dose of 10 J/cm². Exposure to a six-times higher UVC dose did not significantly affect the integrity of the fit nor aerosol filtering capacity of the respirator. **Conclusion:** UVC was shown to be a mild and effective way of respirator disinfection allowing for reuse of the UVC-treated respirators.

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Introduction

The current COVID-19 pandemic crisis caused a large demand for personal protective equipment, especially respiratory protective devices (respirators) [1]. Apart from shortages of respirators, considerable amounts of energy and resources are needed for production and disposable use, resulting in massive quantities of plastic waste [2,3]. In view of the shortages, some countries released guidelines for using respirators by healthcare workers and public [4,5]. To mitigate shortage, decontamination procedures were explored to allow for safe reuse [6-9]. A recent study demonstrated that steam sterilization at 121 °C was not a generic option for respirator disinfection and appeared detrimental to many respirator brands [8]. In-house testing was therefore advised to verify respirator guality, for which simple tests have been established [8,10]. Disinfection by UVC irradiation might be less harmful to respirator integrity.

UVC is effective in inactivating micro-organisms, microbial spores, and viruses including SARS-CoV-2 [9,11–15]. Typically, UVC light is generated by low-pressure (LP) mercury discharge lamps, that emit a 254 nm peak wavelength, close to the optimal UVGI wavelength of 260–265 nm. Wavelengths in the UVC range of 200–280 nm are suitable to deactivate micro-organisms [16]. The mechanism of UVC deactivation of viruses and bacteria is primarily a photoreaction, which causes cross-linking of nucleic acids, impeding transcription and replication of DNA [17–19]. UVC light also induces photochemical reactions in proteins, resulting in loss of host cell recognition of viruses and damage to membranes and envelopes [16].

In this study, the UVC light has been applied on test samples using a closed cabinet, BioShift[™] UVC disinfection chamber (Signify, Eindhoven, the Netherlands). This is a system intended for object disinfection. The experimental work on this UVC disinfection chamber is aiming to provide an insight into the efficacy of UVC for respirator disinfection. In addition, it should provide results on the effect of UVC on the filtering capabilities of respirators to assure safe reuse of respirators for healthcare workers.

For this study, bacterial spores of *Geobacillus stearothermophilus* were used as a target because they represent a more robust biological agent compared to vegetative growing micro-organisms and viruses [20,21]. In addition, the virucidal effects of UVC against SARS-CoV-2 were explored in this study because of being the main reason to disinfect respirators for reuse in the current pandemic.

Methods

UVC chamber and UVC irradiance

The BioShift UVC disinfection chamber generates UVC by Philips low-pressure (LP) mercury discharge lamps, emitting a narrow spectral line at 254 nm. The cabinet is closed by doors with locks, that will prevent opening during disinfection cycles, to guarantee safe use. For measuring the UVC light irradiance, a ILT2400-UVGI-NB sensor, calibrated for narrow band sources such as LP mercury lamps, was used. The irradiance was measured for the various positions at which spore or virusloaded stainless steel discs or respirators were placed. These irradiance levels are used for dose calculations. The dose (in mJ/cm²) was calculated from the irradiance level (in mW/ cm²) \times time (in seconds).

UV transmission of respiratory masks

To eradicate absorbed biological agents in and on the respirator fabric and on the non-exposed side of the respirator by UVC, one must rely on the penetration of UVC radiation into the respirator matrix. For the selected respirators, the respirator material is a stack of multiple layers. The effectiveness of disinfection has been studied by using G. stearothermophilus spores and SARS-CoV-2 viruses in the UVC chamber. Full stacks and parts of stacks of the respirator material have been analysed with UVC light spectroscopy using a Varian Cary 5 UV-vis-NIR spectrometer which measures transmission and reflection and calculates absorption at 254 nm. This provides information on the optical properties and predicts the effectiveness of UVC disinfection of other respirator types not included in the study. These measurements yield information on the irradiance levels (mW/cm²) to which the bacterial spores and viruses are exposed on the various locations in and on the respirators.

Two different FFP2 respirator brands (respirator 1 and respirator 2) were analysed. Biological agents as spores and viruses might theoretically migrate into the respirator material because of fluid capillary diffusion. The presence of biological agents in between the two double layers of the respirators represents a worst-case location in view of being inactivated by the transmitted UVC. Therefore, it was decided to decompose the respirators into individual layers and in dual-layer stacks (face-facing stack and environmental-facing stack) to represent the suggested hypothesis of spore or virus infiltration into the mask layers and accumulation at interfaces.

Bacterial strain and culturing

For spore preparation, *G. stearothermophilus* DSM 1550 was confluently cultured at 55 °C on Nutrient Agar (Thermo Fisher, Loughborough, UK) supplemented with 53 mg/L CaCl₂·2H₂O and 55 mg/L MgSO₄·H₂O for five days. Spores present on the surface were resuspended in 5 mL peptone physiological salt solution supplemented with 0.1% Tween 80. This suspension was centrifuged at 4000 g for 10 min, the pellet was washed with demineralized water and stored in demineralized water at -20 °C. The vegetative cells were deactivated by heating for 10 min at 80 °C. Spore counts were determined by plating serial dilutions on Tryptone Soy Agar medium (Thermo Fisher) after incubation for 48 h at 55 °C.

Exploring sporicidal effects of UVC on G. stearothermophilus spores

The effects of UVC on the *G. stearothermophilus* spores were explored by applying 50 μ L spore suspensions of about 4.5×10^6 cfu/mL on stainless steel discs with 20 mm diameter and drying the suspension on the surface at 45 °C for 15 min. The spore-loaded discs were exposed to UVC irradiation of the two upper UVC tubes in the BioShift chamber for 8, 60, and 180 min under the following conditions in triplicate: (i) as such, not covered with respirator material; (ii) covered by all four layers of respirators 1 and 2; (iii) covered by the two outer layers of the two individual respirators; (iv) covered by the two inner layers of the two individual respirators.

UVC disinfection applied on respirator material simulating post-breathing use

To simulate post-breathing spore contamination, a 50 μ L spore suspension of 4.5 \times 10⁷ cfu/mL was applied as a spot on the inside or outside of the two types of respirator, dried during 15 min at 45 °C and subsequently sucked into the respirator layers with an airspeed of 28 L/min, simulating inhaling or exhaling conditions. The spore-loaded respirator materials were exposed during 1 h to a dose of UVC irradiation in the BioShift of 10 J/cm². Negative controls that were not exposed to UVC were included. Each condition was tested in triplicate.

Antiviral activity UVC on SARS-CoV-2

SARS-CoV-2 virus was propagated on VERO E6 cell line as described by Heilingloh *et al.* with minor modifications [14]. Dulbecco's modified Eagle's medium (Gibco # 41966-029) was supplemented with 5% v/v fetal calf serum and contained 100 IU/mL penicillium and 100 μ g/mL streptomycin. The load of viral infectious particles was determined by 50% endpoint titre in serial dilution assay in combination with 50% tissue culture infective dose (TCID₅₀) calculation by the Spearman–Karber method [22]. For testing, 50 μ L virus suspensions were loaded on the 20 mm stainless steel discs and dried on the surface for 1 h at room temperature prior to UVC exposure. The virus-loaded discs were placed in a transport box with a UVC transparent quartz screen for biologically safe handling. UVC irradiance levels in the transport box were ~1.3 mW/cm² when placed in the UVC cabinet.

The SARS-CoV-2-loaded discs were exposed to 1.3 mW/cm² UVC in duplicate to 0, 15, and 60 s in the transport box placed in the BioShift chamber. In addition, the virus-loaded discs covered individually with material of respirator 1 and respirator 2 were exposed under identical conditions in the transport box for 125 min, thereby being exposed to 10 J/cm² UVC. To verify the limit of UVC energy needed to realize almost complete eradication of the virus on the discs covered by respirator material 1, UVC exposure was done for 5.5 min in the transport box in the BioShift chamber. The difference between the recovered infectious counts (TCID₅₀) of non-exposed virus and UVC-exposed viruses was used to calculate the reduction in infectivity of the virus.

Comparative testing of filter efficiency and fit of respirator material pre and post UVC treatment

The quality of the respirators 1 and 2 were evaluated pre and post UVC treatment for face-fitting and total inward leakage of particles by the particle penetration test using the method described by van Wezel *et al.* [10].

Results

UVC measurements: calibration/UVC transmission through respirators

Table A.1 shows details of UVC measurements including the comparison for the two dual-layer stacks for the two respirator

brands, indicating that the 254 nm optical properties of the masks are different. The sum of percentage transmission, reflection, and absorption is 100%. Respirator 1 shows transmission of 35.9% and reflection of 23% for the face-facing stack, and 12.1% transmission and 16.9% reflection for the environment-facing stack. Much lower transmission of UVC was measured for respirator 2, being 4.8% and 4.6% for the face-facing and environment-facing material stacks, respectively. The corresponding reflection was 14.5% and 30.4%, respectively.

UVC effect on G. thermophilus spore viability

The UVC power released by the BioShift to the dried suspension of *Geobacillus* spores on stainless steel discs was homogeneously distributed and ~2.2 mW/cm² (±0.1). The spores present on the discs were rapidly inactivated by a 5 log₁₀ reduction within 8 min. The decimal reduction in spore viability (D-value) was 1.6 min. In terms of UVC energy effects, a 5 log₁₀ reduction is realized by UVC irradiance of ~1056 mJ/cm².

In case the disc with dry spores was covered by all layers of respirator 1, a D-value of 5 min was observed instead of 1.6 min. This increase in D-value is caused by the reduction of energy of UVC due to absorption and reflection by the respirator material. Only 4.3% of the UVC energy was shown to be transmitted through the respirator material (Table A.1).

To simulate the worst-case condition in which the spores are most far away from UVC irradiance when respirators are illuminated with UVC from both sides, spores should be placed in the middle of the respirator. This was simulated by separating the inner two from the outer two layers of the respirator and using those partial respirators for covering the spores on the discs. Given these conditions, intermediate D-values were observed of 2.4 and 1.9 min, respectively. Taking therefore 12.1% and 35.9% transmission through the inner and outer two layers into consideration, a decimal reduction of viable spores reaching the stainless steel surface of the discs covered with the inner and outer two layers of respirator 1 was achieved by about 38 and 90 mJ/cm² UVC.

The minimal required UVC energy to eradicate all spores when covered by the respirator materials has been determined. In this setting, worst case conditions for UVC to reach the spores present in the middle of the respirators are mimicked. The viability of spores covered by the different layers of the respirators was determined and plotted as a function of the energy of the UVC dose (Figure A.1). A gradual reduction of spore viability was observed with increasing UVC dose. At energy levels >10 J/cm² viable spores were not detected, indicating full eradication of the original 5.6 log₁₀ viable spores. The minimum amount of energy of 10 J/cm² to eradicate all spores required an exposure time of about 1.3 h in the BioShift Chamber.

UVC effect on G. thermophilus spore viability when sucked into the respirator material

Spore inactivation by UVC in a more practical setting was studied in which spores were captured in the respirator via inhaling and exhaling simulation. In these cases, $10 \text{ J/cm}^2 \text{ UVC}$ in the BioShift chamber resulted in $\geq 5.5 \log_{10}$ reduction for respirator 1 and $\geq 4.6 \log_{10}$ reduction for respirator 2 (Table A.2).

The effect of UVC on SARS-CoV-2

The effect of UVC on SARS-CoV-2 viruses applied on the surface of stainless steel discs was also explored in the BioShift. In 15 s, a $4 \log_{10}$ reduction of infectious SARS-CoV-2 viruses was achieved, implying a decimal reduction in 3.8 s, which equals a decimal reduction by a UVC dose of 4.9 mJ/cm².

The virus-loaded discs covered individually with all layers of respirator 1 and respirator 2 were exposed to 125 min UVC in the transport box in the BioShift chamber. This time setting did yield a UVC dose in the transport box of 10 J/cm² (Table A.3). A 5.7 log₁₀ reduction of SARS-CoV-2 was obtained below respirator 1. In this case a transmission of 419 mJ/cm² UVC was realized. For respirator 2, 10 J/cm² resulted in a 5.1 log₁₀ reduction of SARS-CoV-2, given a transmission of a 21.5 mJ/cm² dose UVC through the respirator material. A 4.75 log₁₀ TCID₅₀ reduction of SARS-CoV-2 covered with the full stack of respirator 1 material was achieved in 5.5 min, resulting in a transmission-based calculated dose level of 18 mJ/cm².

Effect of UVC on quality of respirators

A UVC dose of 60 J/cm^2 , equivalent to six times the UVC dose to deactivate bacterial spores in the respirators, did not damage the respirators to the extent that particle penetration was impaired (Figure A.2). Moreover, a negative effect on the fit was not detected.

Discussion

Ultraviolet light released by the BioShift chamber has to penetrate the respirator material to deactivate all biological agents. Information on transmission and reflection of UVC allows for calculating the absorbed UVC fraction in the material stacks. Calculation of UVC light reductions at the material interfaces and bulk of the materials dictates deactivation effects on bacterial spores and viruses that reside at the various locations in/on the respirators. This is well reflected by the outcome of bacterial spore and virus inactivation experiments in which these biological agents are covered or trapped into the respirator materials. Since the biological agents included in the disinfection experiments contain cell debris from lysed and killed cells (RNA, DNA, protein, cell wall material and membrane lipids), these conditions also reflect to a large extent the presence of respiratory secretions.

Spores of *G. stearothermophilus* dried on a stainless steel surface showed a decimal reduction in viability upon UVC radiation for 1.6 min. However, when spores were covered by all layers of the respirator 1 material, the decimal reduction required 5 min of exposure to UVC. This showed that increased UVC energy levels are needed to yield the same \log_{10} reduction of bacterial viability when covered with respirator material, or longer exposure time. Transmission of UVC through layers dictates the efficiency of disinfection. Considering that spores end up in the middle of the respirators, the minimal level of UVC irradiance dose to be applied on the mask, whether respirator 1 or 2, should be $\geq 10 \text{ J/cm}^2$. Bacterial presence in the respirator material via simulated inhaling or exhaling required

 $\geq\!10$ J/cm^2 UVC dose by 59 min exposure to yield a $\geq\!4\log_{10}$ reduction of spores.

One can debate whether the choice of spores of *G. stearothermophilus* as the worst-case target can prove respirator safety upon UVC disinfection. Information about the sensitivity of spores in liquid suspension towards UVC radiation showed that *G. stearothermophilus* was not the most UVC-resistant species despite its extreme temperature resistance [12,21,23]. However, spores of the typical pathogenic bacterium *Bacillus cereus* appeared to be more sensitive to UVC [21]. Moreover, vegetative bacterial cells are even more UVC sensitive than bacterial spores [24,25]. This implies that spores of the *Geobacillus* strain are acceptable representatives as the worst-case target for respirator disinfection.

inclusion of Although the bacterial spores of G. stearothermophilus showed this species to be a suitable candidate as worst-case biological agent, SARS-CoV-2 was also included in this study. Virucidal activity of UVC radiation must also be proven for respirator disinfection for later safe use. Dried SARS-CoV-2 viruses on stainless steel discs showed a 4 log₁₀ reduction in TCID₅₀ upon 15 s of exposure to UCV, which represents about 19.5 mJ/cm² UVC. As seen for spore inactivation, there is an association between UVC transmission through the respirator material and the exposure time, thus the total level of UVC energy. Respirator 1 with 4.3% transmission and respirator 2 with only 0.2% UVC transmission yielded \geq 5 log₁₀ TCID₅₀ reduction of SARS-CoV-2 virus upon 419 and 21.5 mJ/cm², respectively.

Comparing the results of this study with recently published information on SARS-CoV-2 eradication by UVC showed that the 4 log_{10} reduction we observed upon exposure to 19.5 mJ/cm² is in a similar range to the previously seen 2.7 log₁₀ reduction by 3.4 mJ/ cm², the reduction of 3×10^6 dried SARS-CoV-2 units by 16 mJ/ cm^2 UVC, the 3 log₁₀ SARS-CoV-2 reduction by 3.7 mJ/cm², and the 5 \times 10⁶ SARS-CoV-2 reduction by 1048 mJ/cm² [13,14,26,27]. Considering the influenza virus H1N1, a UVC dose of 1 J/cm² was needed to inactivate a viral load of 10⁶ TCID₅₀. Taking this into consideration, it can be concluded that the UVC energy levels needed for a 5 log₁₀ sporicidal effect are sufficient to eradicate vegetative bacteria as well as viruses. Furthermore, the analysis of UVC transmission through the respirator materials can guide the UVC settings of the BioShift UVC chamber for sufficient inactivation of the biological load of the used respirators in a healthcare environment by healthcare workers.

Special attention should be paid to the construction of the respirators. A few respirators are on the market that have their strap passed through a folded part of the mask which forms a crevice that is probably less accessible for UVC. These types of respirator may not be a logical choice for UVC disinfection. Since UVC is also known to be destructive to organic chemical polymers, the effect of six times higher UVC energy (60 J/cm²) exposure of the respirators for disinfection was shown not to be destructive to an extent where particle penetration and fit of respirators were impaired to non-acceptable levels. UVC is therefore concluded to be a preferred alternative method for respirator disinfection as a means to prevent shortages in healthcare institutions, as it allows for safe respirator reuse from a microbiological and integrity perspective.

In conclusion, UVC was shown to be a mild though effective method for respirator disinfection, allowing for non-personal reuse of the UVC-treated respirators.

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Conflict of interest statement None declared.

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Appendix A. Supplementary data

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