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Additional Investigations of UV-C Irradiation Schemes for Viral Decontamination of FFP2 Masks

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The reprocessing of personal protective equipment that is only intended for single use has been brought into focus by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, especially regarding respiratory masks.[1–4]

In a recent study by Vaupel et al.,[1] a reprocessing concept for filtering face piece 2 (FFP2) masks was developed, investigating different ultraviolet-C (UV-C) irradiation schemes and UV-C-doses. The study successfully proved the effectiveness of the developed method for bacterial decontamination, but viral decontamination was not investigated. Therefore, additional investigations for the effectiveness for viral decontamination of FFP2 masks are needed.

Methods

This study was performed in 2 parts. For the first part, 40 masks were used: 20 "Bluebec BB 203" (most common model in Vaupel et al.[1]) and 20 "3M Aura 9322+" (most common model in Döhla et al.[2]). These models were selected to be able to compare the results of this study with these 2 studies mentioned without having to consider possible differences between mask models. Ten masks of each model served as control group, while 10 masks of each model were decontaminated following the recommended scheme of 30 seconds inside irradiation and 30 seconds outside irradiation ("30/30").[1]

Further decontamination protocols were tested using 20 "Bluebec BB 203" masks only: 10 masks were reprocessed applying a 45 seconds inside irradiation, 45 seconds outside irradiation scheme ("45/45"). 10 masks were reprocessed using 60 seconds inside irradiation, followed by 60 seconds outside irradiation ("60/60"). The restriction to Bluebec was made because these masks were the only masks in use at the time of the study and were therefore available for research purposes.

Virological Contamination

Madin-Darby Canine Kidney (MDCK) epithelial cells (ATCC p69) were used to grow Influenza H1N1 (ATCC p3) as surrogate for enveloped respiratory viruses. The tissue culture infectious dose 50 ($TCID_{50}$) was calculated as 500/ml. The virological contamination was carried out according to Döhla et al.[2]

Irradiation

The irradiation of the masks was carried out as described by Vaupel et al.[1] In this study, the used irradiation times translate to 7.125 $\frac{J}{25\,cm^2}$ per side for 30/30 seconds of irradiation, to 10.687 $\frac{J}{25\,cm^2}$ per side for 45/45 seconds of irradiation and to 14.250 $\frac{J}{25\,cm^2}$ per side for 60/60 seconds of irradiation.

Examination via Cell culture

Cytopathic effect (CPE) observed in MDCK epithelial cells (ATCC p69) was used to confirm the presence of infectious virus. Details are provided by Döhla et al.[2] Deviating from this study, the cell cultures were investigated microscopically (CPE) and by influenza-specific immunofluorescence testing (IFT) after 24 h and 21 days instead of 36 h and 3 weeks, as in Döhla et al.[2] A negative IFT result after 21 days served as confirmation of a successful decontamination.

Results

In the first part of this study, it was shown that the radiation dose after 30/30 seconds was not sufficient to achieve complete decontamination for both mask models examined; on the contrary,

7 of 10 "Bluebec BB203" and 6 of 10 "3M Aura 9322+" were still contaminated. In the second part of the study, "Bluebec BB203" were irradiated with higher doses; complete decontamination of the masks was observed both after 45/45 seconds and after 60/60 seconds (Table 1).

Discussion

The results of this study indicate that 45/45 seconds of both side irradiation is an effective and efficient reprocessing concept for the viral decontamination of FFP2 masks. An irradiation time of 30/30 seconds did not lead to a sufficient decontamination for both mask models. As this result does not seem to be model dependent, only "Bluebec BB 203" were used for the following irradiations with 45/45 seconds and 60/60 seconds. Irradiation for 45/45 seconds or more was sufficient to successfully decontaminate each sample.

In comparison to other studies on the UV-C doses required for viral decontamination of respiratory masks, the doses indicated in the present study are higher.[5–7] While the method developed in the present study requires 45 seconds of irradiation per side (translating into 427.5 $\frac{mJ}{cm^2}$ per side), a similar study by Fisher and Shaffer[6] found a dose of 100 $\frac{mJ}{cm^2}$ to be sufficient for successful disinfection of MS2 bacteriophage in FFP2 masks. One reason for these differences may be the more realistic use of whole masks for irradiation in the present study instead of the circular excisions used by Fisher and Shaffer.[6] As discussed in Vaupel et al.,[1] the positioning of the masks and possible shaded zones could increase the UV-C dose required for decontamination.[8] The decontamination method developed in this study is intended for the efficient and rapid, high throughput reprocessing of whole masks in real life settings and not intended for the investigation of the lowest possible irradiation doses. Therefore, higher doses are plausible and necessary to ensure the effectiveness of the presented method.

Another method-specific reason for the comparatively high UV-C dose reported in the present study could be that the dried virus suspension with cell culture medium on the masks may have a shielding effect that leads to a higher required UV-C dosage.[9,10] This was already shown by Ratnesar-Shumate et al.,[9] who also demonstrated that the required UV-C dose for viral inactivation is significantly lower with simulated saliva than with culture medium. In a real-life setting, where the presented method is used to decontaminate worn FFP2-masks, that may contain saliva but no cell culture medium, the presented method would be even more effective.

In conclusion, the 30/30 seconds irradiation concept presented by Vaupel et al.[1] is not applicable for the decontamination of viable viruses. However, 45/45 seconds of irradiation proved to be effective to inactivate viable enveloped respiratory viruses like Influenza H1N1 on FFP2 masks. The combination of both studies indicates that a both side irradiation of 45 seconds per side (corresponding to a total of 21.38 $\frac{J}{25\,cm^2}$) is an efficient method to inactivate both bacteria and viruses on both sides of a mask. Further investigations, especially investigations on the same mask model (Bluebec BB 203)

Table 1. Mask models, irradiation schemes, PCR, and IFT results after irradiation

					Positive IFT (24 h)		Positive IFT (21 days)	
						third		third
						upper		upper
						middle		middle
Mask model	Irradiation scheme	Dose per side $\frac{J}{25\,cm^2}$	*n*	Positive PCR (24 h)	total	lower	total	lower
Bluebec BB 203	Control	0	10	10	10	10	10	10
						10		10
						10		10
	30/30	7.125	10	10	4	1	7	5
						2		5
						3		1
	45/45	10.687	10	10	0	0	0	0
						0		0
						0		0
	60/60	14.250	10	10	0	0	0	0
						0		0
						0		0
3M Aura 9322+	Control	0	10	10	10	10	10	10
						10		10
						10		10
	30/30	7.125	10	10	2	0	6	2
						1		2
						1		2

PCR, polymerase chain reaction; IFT, immunofluorescence testing.

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with parallel contamination of bacteria and viruses with larger sample sizes, are expedient to detect possible environmental influences, errors in handling, and other factors influencing the irradiation result.

Author contribution. F.V. developed and firstly described the irradiation method, irradiated the masks, and wrote the first draft of the manuscript. K.K. prepared the cell cultures, performed the virological tests, and revised the manuscript. N.T.M. planned and supervised the investigations and revised the manuscript. P.L.S. provided resources (irradiation chamber, staff, laboratory time slots) and supervised the virological tests. R.W. created the database, assisted in cell culture and irradiation, and supervised the documentation of the examinations. M.D. planned and supervised the investigations, analysed the data, and revised the manuscript.

Competing interest. All authors state that they have no competing interests regarding this study.

Ethical approval. No personal data were collected, processed, or saved for this study. No experiments were conducted on or with humans or animals. The irradiated masks are legally considered waste, which means that there are no longer any property rights. Therefore, IRB approval was not necessary from the author's point of view.

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