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Short Report

Monitoring SARS-CoV-2 decontamination by dry heat and ultraviolet treatment with a swine coronavirus as a surrogate

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SUMMARY

The critical need for reliable methods to validate decontamination protocols for personal protective equipment (PPE) for re-use during the SARS-CoV-2 pandemic is limited by the need for specialized containment facilities to handle the virus. Hence, we have herein validated the use of a swine coronavirus as a surrogate, and tested the effectiveness of dry heat and ultraviolet (UV) rays for PPE decontamination. Exposure of experimentally contaminated N95 masks and hospital gowns to 60°C for 20 min, and UVC at 1800 mJ/cm^2 resulted in a 4-log reduction and inactivation of the surrogate virus. This study provides a novel alternative to validate PPE reprocessing methods.

Introduction

The extraordinary magnitude of the SARS-CoV-2 pandemic and its associated hospitalizations have resulted in severe shortages of personal protective equipment (PPE), and increased risk to front-line workers. The acute demand for PPE has necessitated re-use of PPE, especially of N95 respirators, triggering an increased interest in reliable protocols for reprocessing PPE for the medical community [1]. The current Centers for Disease Control (CDC) and US Food and Drug Administration agency (FDA) guidelines recommend a 3-log reduction in coronaviral loads, and that the processes used should not compromise the integrity of the product [2,3].

Several approaches such as autoclaving, microwaving, exposure to ultraviolet germicidal irradiation (UVGI), dry or moist heat, bleach, ethanol and hydrogen peroxide are currently under exploration for reprocessing PPE [1]. However, due to the difficulties associated with culturing SARS-CoV-2, and the need for biosafety level 3 (BSL3) containment to

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handle the virus, a majority of approaches described in published literature are validated with surrogate agents such as bacteriophage MS2, influenza A viruses or even bacteria [1–4]. Although enveloped RNA viruses are relatively more amenable to disinfection when compared with non-enveloped viruses such as MS2 or spore-forming bacteria, the use of a coronavirus as a surrogate will provide greater reliability in translating experimental protocols to field settings.

Hence, the primary objective of this study was to use porcine epidemic diarrhoea virus (PEDV), a swine coronavirus, as a surrogate to assess the effectiveness of dry heat and UVGI for reprocessing N95 masks and disposable hospital gowns. Unlike SARS-CoV-2, PEDV is an enteric swine coronavirus. However, PEDV can be purchased from the National Veterinary Services Laboratory (Ames, IA, USA) can be easily cultured and handled in BSL2 facilities. It is also structurally similar to SARS-CoV-2 [5,6]. In this study, we demonstrate for the first time that experimental contamination of N95 masks and hospital gowns with PEDV, followed by dry heat or UVGI treatment resulted in a 4-log reduction in viral loads and complete inactivation of PEDV. The protocols described advance the reliable validation of PPE reprocessing methods, and are in compliance with current federal guidelines.

Methods

This study was conducted in compliance with the policies and procedures as approved by the N. Dakota State University's institutional biosafety committee under protocol B19017.

Experimental contamination of PPE

The PEDV strain CO2013 (National Veterinary Services Laboratory (NVSL), Ames, IA, USA) was cultured and quantified as previously described [6]. Virus cultures were stored at -80°C until use. Hospital gowns (Fluid Resistant Isolation Gown, Tronex® Inc., USA) and N95 masks (Health Care Particulate Respirator, N951860, 3M Inc., USA) were cut into 1-inch squares (coupons) and placed in sterile glass beakers or plastic Petri plates. The fluid-resistant gowns were composed of 100% pure polypropylene spunbond fabric. The N95 masks were composed of four layers of material. The inner and outer surfaces were composed of spunbound polypropylene, the second layer of cellulose/polyester and the third layer of melt-blown polypropylene. The PEDV virus culture was resuspended to 1×10^5 $TCID_{50}$/mL, and 100 μL was layered over the coupons in duplicate with a pipettor. The coupons were incubated at 4°C for 1 h before treatment. Other experimental controls included untreated virus as a baseline control, virus extracted from untreated experimentally contaminated coupons to determine whether the PPE material had an inhibitory effect on the virus, and treated virus culture which was not layered on the coupons to determine the effect of the treatment alone on the virus.

Dry heat treatment

The coupons, which were experimentally contaminated with PEDV as described above, were placed in sterile glass beakers and exposed to 60°C for 20 min in a forced air laboratory oven (VWR® Forced Air Ovens, VWR, USA). Following the heat treatment, the coupons were moved into tubes containing 1 mL of PEDV growth media (Dulbecco's modified Eagles' media (DMEM), containing 7% tryptose phosphate broth and 0.1% trypsin).

UV germicidal irradiation

The coupons, which were experimentally contaminated with PEDV as described above, were placed in sterile Petri plates at a height of 8.5 cm below a UVC lamp (CTUV-25, 25 W, 110 V, Coospider Inc., USA). A fluence dose of 6 mJ/s was delivered at this position, as measured by a UVC light meter (UVC-254SD, Lutron Inc., USA). Samples were exposed for 1 min (360 mJ/cm^2), 3 min (1080 mJ/cm^2), 5 min (1800 mJ/cm^2) or 7 mins (2520 mJ/cm^2) each, on either side of the coupon, and processed as described as below.

Viability of the treated virus

To extract residual virus, the tubes containing the coupons were vortexed for 1 min, incubated at 4°C for 15 min, and again pulse vortexed for 5 min and the media collected. Six-well tissue culture plates and eight-well chamber slides containing Vero cell monolayers were infected with virus extracted from the treated coupons and the control samples. After 48 h of incubation in a CO_2 incubator at 37°C, viral inactivation was assessed by the absence of viral replication using a PEDV-specific immunofluorescence assay (IFA), as previously described [5,7]. In treatments where virus-specific florescence was detected, the log reduction in viral titres due to the treatment was quantified by the $TCID_{50}$ method. To determine whether the inactivation was complete, samples were subjected to a viral amplification test by passaging three times in Vero cells, followed by visualization with the IFA.

Statistical analysis

The mean, standard deviation and one-way analysis of variance (ANOVA) were calculated. Pair-wise comparisons were carried out using a Student's *t*-test. All analysis was carried out using Excel 2016 (Microsoft Inc., USA).

Results

To ensure that contact with the PPE by itself did not significantly affect viral titres, PEDV was extracted from the contaminated but untreated coupons by vertexing and incubating at 4°C for 15 min. The eluted PEDV produced a clear fluorescent signal when tested by IFA (data not shown). Titres of the extracted samples ($10^{4.79 \pm 0.06}$ and $10^{5.08 \pm 0.35}$ $TCID_{50}$/mL for the N95 and gown samples, respectively) were not significantly different from the untreated control, indicating that any significant effects noted were due to the treatments and not the experimental process used. As expected, back titration of the untreated virus control showed that it did not differ significantly from the original stock culture. The viability and titers of the untreated virus control were maintained over the passages tested (Table I). The $TCID_{50}$ method used for the quantification of PEDV had a lower detection limit of 1×10^2 $TCID_{50}$/mL [8].

Under the experimental conditions used, exposure to dry heat at 60°C for 20 min inactivated PEDV, both on the

Table I
Virus amplification test for inactivation

Untreated control	P-0* Log_{10} $TCID_{50}$ /mL	P-1* $Log_{10}TCID_{50}$ /mL	P-2* $Log_{10}TCID_{50}$ /mL	P-3* $Log_{10}TCID_{50}$ /mL
	+	+	+	+
	5.16 ± 0.23	5.05 ± 0.45	5.32 ± 0.16	5.54 ± 0.16
PEDV contaminated N-95 mask				
Heat (60°C, 20 min)	-#	-#	-#	-#
UV – 7min	-#	-#	-#	-#
UV – 5 min	-#	-#	-#	-#
UV – 3 min	-#	-#	-#	-#
UV – 1 min	-#	-#	-#	-#
PEDV-contaminated gown				
Heat (60°C, 20 min)	-#	-#	-#	-#
UV – 7 min	-#	-#	-#	-#
UV – 5 min	-#	-#	-#	-#
UV – 3 min	-#	+# 3.33 ± 0.08	Discontinued	Discontinued
UV – 1 min	-#	+# 3.68 ± 0.12	Discontinued	Discontinued

+, Positive viral detection by an immunofluorescence assay; -, negative for viral detection by an immunofluorescent assay; PEDV, porcine epidemic diarrhoea virus.

* P-passage number in cell culture following heat or ultraviolet C treatment.

Significantly different from the untreated control at the same passage $P \leq 0.05$, Student's *t*-test.

experimentally contaminated PPE coupons as well as in the control samples, which were not layered on coupons but exposed to heat. Viral replication was not detected in the dry-heat-treated samples even after amplification by three serial passages in cell culture (Table I).

Among the coupons exposed to UVC, viral replication was not detected in passage 0 for either the hospital gown or N95 mask coupons, indicating that a UVGI dose of 360mJ/cm^2 was sufficient to substantially reduce coronaviral titres. Virus extracted from the UVC-treated N95 mask coupons remained non-viable even after amplification by three serial passages in Vero cells. However, a minimum UVGI dose of 1800 mJ/cm^2 was required for the complete inactivation of PEDV on hospital gown coupons, under the conditions used in this study, as coupons exposed for 1 min (360 mJ/cm^2) and 3 mins (1080 mJ/cm^2) contained viable virus which was detected in the first serial passage in cell culture (Table I). Viral viability did not differ significantly between the UVC-treated control samples which were not layered on the PPE coupons and the samples from the experimentally contaminated PPE coupons, as the incompletely inactivated PEDV was also detected in the first serial passage of the control samples.

Discussion

Although hospital gowns are currently not in short supply in the US, they were selected to represent impervious material contaminated with infective coronavirus, and as not much is known regarding their durability when exposed to heat, or the survival of SARS-CoV-2 on hospital gowns. A wide range of time and temperature combinations ranging from 56°C to 92°C from 5 min to 60 min are reported to be effective for the inactivation of enveloped viruses in liquid media [3]. Current FDA and CDC guidelines [3] recommend 70°C for 30 min for reprocessing N95 masks, as the presence of biological fluids such as blood, mucous or sputum on PPE surfaces can significantly influence the effectiveness of heat-treatment processes [9]. However, other published studies on SARS-CoV-1 [10] indicate that lower temperatures and times could be sufficiently effective. While the need for an overabundance of caution cannot be underestimated, in this study, we elected to use 60°C for 20 min based on available data regarding inactivation of PEDV [5], and taking into consideration the practicality of achieving and maintaining high temperatures for the large-scale decontamination of PPE. The 4-log reduction achieved in this study using PEDV as a surrogate meets the current guidelines recommending a 3-log reduction in viral loads after treatment, and supports the use of PEDV as a suitable surrogate for heat-treatment methods. Data from other published studies show that exposure of N95 masks to a temperature of 60°C for 30 min, which is a longer duration of exposure than the 20 min used in this study, did not damage the integrity of the masks for three decontamination cycles [11,12]. While integrity testing was not carried out after the heat treatment in this study, based on published data, it is unlikely that the conditions used will compromise the integrity of the N95 masks.

The use of UVGI is an established method for the inactivation of viruses, and is scalable for field use. However, the low penetration capability, requirement for operator protection against UV rays, the needs to carefully calibrate doses delivered due to the variability in UV sources, and operating conditions such as distance from the source are disadvantages of using UVGI for decontamination. Therefore, validation of treatment conditions with a reliable surrogate virus is especially critical for UVGI. The dose of UVC required for inactivation in this study is consistent with the currently recommended dose of $\geq$1 J/cm^2 required to achieve a 3-log reduction in viral load for N95 masks [2,13]. However, a higher dose was required to decontaminate the hospital gown coupons, likely due to poor penetration of coloured solutions by UVC as the PEDV culture was resuspended in DMEM containing phenol red, and remained largely unabsorbed on the surface of the material.

Data from several other published studies demonstrates that exposure of N95 masks to UVGI doses greater than $\geq$1 J/cm^2 does not affect the integrity or performance of N95 masks for more than two decontamination cycles [9,11]. While the lack of integrity testing is a limitation of this study, based on the above-cited studies, the doses of UVGI used in this study are unlikely to affect the integrity and performance of N95 masks.

Several other agents including *Staphylococcus aureus*, bacteriophages MS2, Phi6 and PhiX174, a murine coronavirus (murine hepatitis virus) and a human coronavirus (Hu-CoV229) have been used as surrogates for SARS-CoV2 in published studies on decontamination [4,14—17]. Similar to this study, in one study where the porcine respiratory coronavirus (PRCV) was used as a surrogate, a UVGI dose of 1.3 J/cm^2 was sufficient to reduce viral loads on N95 masks by four-fold. A dry heat temperature of 102°C for 60 min was used for decontamination of PRCV in this study. While these dry heat conditions were more stringent than the parameters used for PEDV in this study, the data suggests that both PRCV and PEDV can serve as good surrogates for SARS-COV-2 [18].

In conclusion, the described study provides the first proof-of-concept data for the use of PEDV as a reliable surrogate for SARS-CoV-2 for the validation of reprocessing protocols for PPE. Our findings also have important implications to meet the increasing demand for reliable decontamination of environments such as hospital premises, classrooms, electronic equipment, aircraft, and meat production facilities during the ongoing pandemic.

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Author contributions

G.S.: acquisition of data, analysis and interpretation; J.J.: conception, critical review and revision of the article; T.N.: conception, critical review and revision of article; T.P.: acquisition of data, critical review and revision of the article; S.R.: conception, design, analysis and interpretation of the data, drafting the manuscript.

Conflict of interest statement

None declared.

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