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Impact of the Contamination Method on the Disinfection of N95 Respirators: Drops versus Aerosols

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ABSTRACT

The recent surge in the use of filtering facepiece respirators (FFRs) during the SARS-CoV-2 pandemic triggered economic and environmental concerns with regards to their safe reuse and/or disposal. Their decontamination through ultraviolet (UV) irradiation has proven efficient in bench tests. Nevertheless, no study has yet investigated to what extent the decontamination method's performance was impacted by the contamination method. In this study, *Bacillus subtilis* spores were inoculated in three suspensions used to contaminate coupons of FFRs via aerosols nebulisation or 2 μL drops deposition. The contaminated coupons were then exposed to UV irradiation in a monochromatic UVC lamp collimated beam reactor. The results revealed that contamination and decontamination were more efficient for drops (maximum 0.72 log losses and 3 log inactivation at 150 mJ cm^{-2}) than for aerosols (maximum 2.47 log losses and 1.75 log inactivation at 150 mJ cm^{-2}). Inactivation was greater in coupons contaminated using artificial saliva, followed by phosphate buffer solution, and finally artificial saliva with mucin which also presented the highest fraction of resistant spores, based on kinetic modeling. Disinfection was determined sensitive to the method of contamination ($p < 0.001$). However, the composition of the contaminating suspension was the most important performance predictor for decontamination by UV irradiation ($p = 9.2 \times 10^{-10}$).

Keywords: Filtering facepiece respirators, UV irradiation, Artificial saliva, Kinetic modeling

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1 INTRODUCTION

Almost three years after the emergence of SARS-CoV-2, the safe reuse or disposal of soiled personal protective equipment persists as an issue that burdens health institutions and jeopardizes public health and the environment. There is therefore an interest to identify and validate appropriate and effective disinfection methods. Several studies have presented and compared disinfection methods for respirators such as ultraviolet (UV) irradiation (Lindsley *et al.*, 2015; Mills *et al.*, 2018; Zhao *et al.*, 2020; Barancheshme *et al.*, 2021; Huber *et al.*, 2021; Kayani *et al.*, 2021; Nicolau *et al.*, 2021; Su *et al.*, 2021), hydrogen peroxide (Smith *et al.*, 2021), UV and ozone (Hasani *et al.*, 2021), UV and hydrogen peroxide (Hasani *et al.*, 2021), and ethanol (Smith *et al.*, 2021). In particular, UV irradiation is a promising disinfection technique as it was proven fast and effective for the reuse of respirators both in laboratory (Zhao *et al.*, 2020) and clinical studies (Baluja *et al.*, 2020). This eventually led to the design and validation of several irradiation devices (Baluja *et al.*, 2020; Neelakandan *et al.*, 2020; Bentancor *et al.*, 2021).

UV irradiation is generally preferred because it does not require the use, purchase, or storage of chemicals. It is also relatively easily applied and can be designed to disinfect multiple filtering

facepiece respirators (FFRs) simultaneously (Baluja *et al.*, 2020; Zhao *et al.*, 2020) without evidence of compromising the respirator's fit and material quality (Zhao *et al.*, 2020). Several studies have explored the application of different UV doses, pathogens or surrogates (influenza virus H1N1 (Zhao *et al.*, 2020), *Bacillus subtilis* spores (Barancheshme *et al.*, 2021), MS2 coliphage (Fisher and Shaffer, 2010)), suspension media (artificial saliva (Barancheshme *et al.*, 2021), saline solution (Hasani *et al.*, 2021), sterile water (Hasani *et al.*, 2021), sebum (Mills *et al.*, 2018), yeast extract and glucose broth (Vo *et al.*, 2009)), contamination methods (droplets (Barancheshme *et al.*, 2021) and aerosols (Zhao *et al.*, 2020)), and respirator material assessment (Zhao *et al.*, 2020) to prove the advantage of decontamination with UV. Among these different testing criteria, the media and contamination methods are key factors to make sure that the test conditions are representative of an actual contamination and may eventually dictate the suitability and success of the decontamination method.

With respect to SARS-CoV-2, its transmission occurs through the inhalation or deposition on exposed mucous membrane of particles generated when an infected individual talks, breathes, sneezes or coughs, or by immediate contact with infected secretions (Jayaweera *et al.*, 2020). Aerosols produced by anthropogenic respiratory activities fall within a wide particle size range; however, pathogens are carried in smaller particles that are typically less than 5 μm (Fennelly, 2020). The SARS-CoV-2 virus, in particular, was identified in aerosols between 10 nm and 1 μm as well as in particles greater than 2.5 μm (Huber *et al.*, 2021). It is important to note that the emitted liquid particles can convert to smaller ones via evaporation (Jayaweera *et al.*, 2020). As a result, the virus is suspended with (Fennelly, 2020) or shielded by (Barancheshme *et al.*, 2021) other components of the suspension used for contamination such as salts or proteins, which can hinder the efficiency of the decontamination by UV irradiation. This is because during UV irradiation, nucleic acids absorb the germicidal UV light (254 nm wavelength), which damages the pathogen's genetic material and prevents it from multiplying. The same light can also be absorbed by other organics in the suspension, which eventually compromises the efficiency of the process (Anderson, 2020).

The deposition of 1–10 μL drops on the surface of respirators has commonly been used to contaminate FFRs in past researches given the ease of this method compared to aerosols deposition (Heimbuch *et al.*, 2011; Barancheshme *et al.*, 2021; Rezaei and Netz, 2021). One study reported on the use of a high UV dose of 18 kJ m^{-2} to inactivate H1N1 viruses suspended in artificial saliva with 3 g L^{-1} mucin and deposited on FFRs via 15 μm droplets (by a droplet loader) or 0.8 μm aerosols (by a laboratory scale aerosol tunnel) (Heimbuch *et al.*, 2011). Under these conditions, close inactivation levels were obtained for droplets (4.9 log inactivation) and aerosols (4.7 log inactivation). However, the size difference between aerosols and droplets is much smaller than the difference between aerosols and larger liquid drops (1–10 μL) commonly used to contaminate FFR. Thus, multiple contamination methods are used to simulate real life means of FFR contamination without being necessarily accurate and representative. They are likely to end up under- or over-estimating the efficiency of the tested decontamination technique. To the best of our knowledge, no study has yet investigated if drops are more or less difficult to decontaminate than aerosols.

The main objective of this study was to investigate the effect of the contamination method (deposited drops versus aerosolized particles) on the level of decontamination of N95 FFRs by exposure to UV irradiation. Several suspension media utilized to contaminate FFRs by drops or aerosols were compared, and the most statistically significant factors for FFRs decontamination were subsequently identified.

2 MATERIALS AND METHODS

2.1 Suspension Media Preparation

In this study, spores of *Bacillus subtilis* (0.8–0.12 μm) were selected to contaminate N95 respirators (Particulate Healthcare Respirator, 1860, 3M, Saint Paul, MN, USA). *B. subtilis* spores are known to moderately resist decontamination (Setlow, 2006). Actually, bacterial spores require a significantly higher UV dose (12.6 mJ cm^{-2}) than SARS-CoV-2 (1.27 mJ cm^{-2}) for 90% inactivation (Anderson, 2020; Ma *et al.*, 2021). A stock culture of *B. subtilis* spores (ATCC 6633, prepared as

per the method detailed elsewhere (Barbeau *et al.*, 2004)) was thoroughly mixed for 24 hours with a magnetic stirrer, at room temperature, then filtered through a 10-µm membrane to remove aggregates and titrated to determine its concentration as colony forming units per milliliter (CFU mL^{-1}). Spores from this culture (7.96 log CFU mL^{-1}) were then spiked to attain a theoretical spore concentration of 6.0 log CFU mL^{-1} into three different media: artificial saliva, artificial saliva containing mucin, and a phosphate buffer saline solution (PBS). These suspension media were chosen instead of natural human saliva which has a very complex composition that varies between individuals and can differ for the same individual depending on the time of the day, rendering this saliva impossible to replicate (Gal *et al.*, 2001). In order to maintain consistent experimental conditions, the same experimental protocol for the preparation of artificial saliva was used in this study (adapted from ASTM 2720E). A small modification to the protocol was made with the concentration of mucin, a large organic molecule produced by epithelial cells and a major mucus component (McGuckin *et al.*, 2015). A concentration of 1.7 g L^{-1} of mucin (rather than the 3.0 g L^{-1} suggested in the original recipe) was added to the artificial saliva to ensure that its UV absorbance at 254 nm is as close as possible to that of natural saliva, as recommended in Barancheshme *et al.* (2021). Artificial saliva without mucin was also prepared. Finally, phosphate-buffered saline (PBS) was used as a reference medium since it does not contain interfering organic compounds, but a high concentration of salts. The chemical compositions of all three suspensions are detailed in Table S1. The spiked solutions were stored at 4°C until utilised. Prior to any FFRs contamination experiment, the spore-containing medium was stirred for 1 hour or stirred overnight at room temperature to ensure its homogeneity. Preliminary tests were also performed to evaluate the impact of the agitation time on the initial concentration of spores in the suspension.

2.2 Contamination Procedures

2.2.1 Contamination by aerosols

Aerosols were generated using the nebulization system in Fig. 1. The system was originally designed and built at Institut de Recherche Robert-Sauvé en Santé et Sécurité au Travail (IRSST), Montreal, Canada (Marchand *et al.*, 2008; Marchand, 2010). It was mounted, in the fume hood in the CREDEAU Laboratory, at Polytechnique Montréal and operated according to the conditions

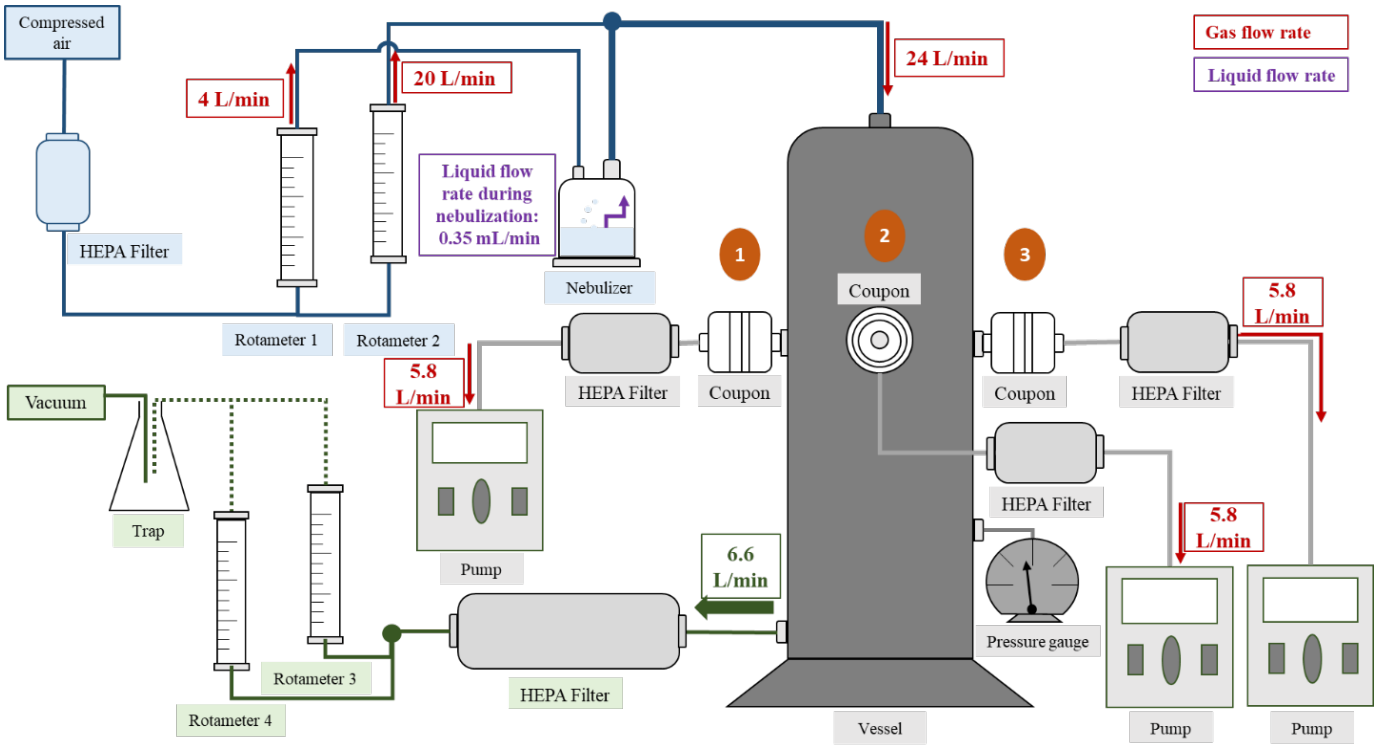


Fig. 1. Detailed schematic of the process of respirator contamination with aerosols.

detailed in Table S2. The N95 respirators were pre-cut into 37 mm coupons with a punch cutter. Each coupon was placed along with an absorbing pad in a 37 mm clear press-fit cassette (SKC Ltd., Dorset, UK) with the blue side facing up. The cassette was firmly closed and wrapped with parafilm to ensure good sealing. High efficiency particulate air (HEPA) filters (Cytiva, Marlborough, MA, USA) were fitted in the system in order to remove any unwanted residues at the air intake and control lab contamination. Three sampling pumps (Leland Legacy, SKC Ltd., Dorset, UK) were connected to the cassettes and were operated at 5.8 L min^{-1}. This value is equivalent to 9 cm s^{-1} (considering the 37 mm coupon). It is comparable to the recommended rate for testing surgical masks (9.8 cm s^{-1} according to the European standard for medical face masks EN 14683) and is within the filter penetration limit (95 L min^{-1} according to the European Standard for respiratory protective devices EN 149). Prior to every contamination assay, the system was run using sterile ultrapure water for 45 minutes. The air flow rate was adjusted using rotameters at the inlet and outlet.

During operation, the nebulizer (Model 9302, TSI Inc., Shoreview, MN, USA) was filled with 75 mL of the contaminated suspension or sterile ultrapure water and agitated on a magnetic stirrer to maintain homogeneity. Compressed air was introduced to the nebulizer to generate aerosols that traveled to the coupons through the main vessel measuring 4 L, where the pressure was maintained slightly negative. Each round of nebulization lasted for 13 minutes after which the pumps were tuned off; all valves were closed; and the cassettes were carefully opened in a sterile environment to retrieve the contaminated coupons. With every spore-containing suspension, the three recuperated coupons were cut in halves: one half coupon was used as a control to determine the initial spore concentration, while the other half was decontaminated with UV. Controls were immediately transferred to 50 mL sterile tubes containing the extraction solution (details in Section 2.4).

Post operation, the system was disinfected by nebulizing sterile ultrapure water containing 10% bleach for 30 minutes followed by sterile ultrapure water for 15 minutes. It is worth noting that every contamination assay by aerosolized particles deposition was followed by another contamination assay by drops deposition (described in the next section) using the same spore-containing solution. A portable laser aerosol spectrometer (11-R Mini-LAS, Grimm GmbH, Ainring, Germany; instrument detection range: 0.25–32 μm) was used to measure the size of the dried aerosols generated by the nebulization of artificial saliva, artificial saliva with mucin, and PBS. The particle sizer was connected to the main vessel through a desiccation column at the same outlet that holds the cassette and thus provided dry particle sizes.

2.2.2 Contamination by drops

A volume of 10 μL of the contaminated medium was transferred with a micropipette to the coupon using five times a 2 μL volume deposited randomly on the coupon. The contaminated coupon was left to dry inside the biosafety cabinet for 1 hour. For every spore-containing suspension, four coupons were contaminated with one control coupon directly transferred to a 50 mL sterile tube containing the extraction solution, while the other three coupons were used for UV decontamination experiments.

2.3 Decontamination by UV Irradiation

A monochromatic UVC lamp collimated beam reactor was used to decontaminate coupons of FFRs. The irradiance (typically 0.085–0.095 mW cm^{-2}) was measured with a spectroradiometer (IL1400A, International Lights Inc., Peabody, MA, USA), and the irradiation time was adjusted according to the desired UV dose as per Eq. (1). Multiple contamination assays were performed, and three UV doses were tested (50, 100, and 150 mJ cm^{-2}). Three contaminated half coupons (for aerosolized particles l) or full coupons (for deposited drops) were independently placed in three different sterile glass petri dishes at 68 cm distance from the UV lamp, and were irradiated with the same UV dose. After irradiation, the coupons were transferred to 50 mL sterile tubes containing the extraction solution for further processing.

$$\text{UV dose (J cm}^{-2}\text{)} = \text{Irradiance (W cm}^{-2}\text{)} \times \text{time (s)} \quad (1)$$

2.4 Extraction Method and Microbiological Analysis

In order to extract the deposited spores, all contaminated coupons were immersed in 10 mL of a sodium bicarbonate solution (1.5 M $NaHCO_3$, filtered over a sterile 0.22 μm membrane), and the tubes were then vortexed for 5 minutes after which all the liquid was collected and transferred to 15 mL sterile tubes (Barancheshme *et al.*, 2021). The extraction solutions were used to prepare serial dilutions in buffered water, which were utilised for spore enumeration according to the method adapted from Barbeau *et al.* (2004). The results were reported as colony forming units per milliliter CFU mL^{-1}. As such, 1 mL of each solution was filtered through sterile 0.45 μm filter paper that was then deposited in a petri dish containing a petri pad (Fisher Scientific, Pittsburgh, PA, USA) soaked in Trypticase Soy Broth (TSB). The petri dishes were placed in two airtight bags and pasteurised at 75°C for 15 minutes, after which they were incubated at 35°C for 24 hours. Red colonies were enumerated, and only the dilutions resulting in 30 to 300 colonies per petri dish were accounted for. The CFU loading per coupon was calculated as per Eq. (2) for coupons contaminated by aerosol particles and Eq. (3) for those contaminated by drops deposition.

$$\text{CFU coupon}^{-1} = \left(\text{Liquid flow rate}\left(\text{mL}_\text{l}\ \text{min}^{-1}\right) \times \text{Time of Nebulization}\ (\text{min}) \times \text{Conc. of suspension nebulized}\left(\text{CFU mL}_\text{l}^{-1}\right) \times \text{Gasflow rate by coupon}\left(\text{L}_\text{g}\ \text{min}^{-1}\right)\right) \Big/ \text{Total gas flow rate}\left(\text{L}_\text{g}\ \text{min}^{-1}\right) \quad (2)$$

$$\text{CFU coupon}^{-1} = \text{Conc. of suspension}\left(\text{CFU mL}^{-1}\right) \times \text{Volume transferred from the suspension}\ (\text{mL}) \quad (3)$$

2.5 Data Analysis

Data acquired from the particle sizer were mined and analyzed using Microsoft Excel®. Statistical analysis was performed using Microsoft Excel® or R Statistical Software (v3.6.0; R Core Team (2019)). Three-factor ANOVA tests (Analysis of Variance) were performed to determine whether the type of contaminated suspension, the means of contamination, and/or the UV dose had an impact on the level of spore inactivation (expressed in log). The interactions between these factors and the extent of inactivation were assessed according to the corresponding *p*-values: only the interactions with p-values less than or equal to 0.05 were considered statistically significant.

3 RESULTS AND DISCUSSION

3.1 Aerosol Size Distribution

Data pertaining to the dry particle size distribution of aerosols generated from the nebulization of the three different suspensions are illustrated in Fig. 2. The average particle count was computed for each particle size during the nebulization period. Aerosols obtained from the nebulization of artificial saliva with or without mucin were very similar in particle size distribution. Aerosols obtained from PBS exhibited a wider particle size distribution with more submicron particles (local extremum at 0.65 μm). It is hard to comment on the potential particle size differences below 0.3 μm and that is due to limitations associated with the instrument's capacity to accurately and distinctively detect particles close in size to its minimum detection limit. As a reference, The NIOSH (National Institute for Occupational Safety and Health) testing system uses 0.30 μm as the aerodynamic diameter for challenge aerosols following the most penetrating particles size prediction (Eninger *et al.*, 2008). For comparison, the size of each 2 μL drop was measured in previous work on a glass surface and estimated as 1500 μm, assuming a sphere (Barancheshme *et al.*, 2021).

3.2 Assessment of the Contamination Procedures

Preliminary tests were conducted to evaluate the reproducibility of the coupons contamination methods using drops deposition or aerosols nebulization. Spore concentrations were measured

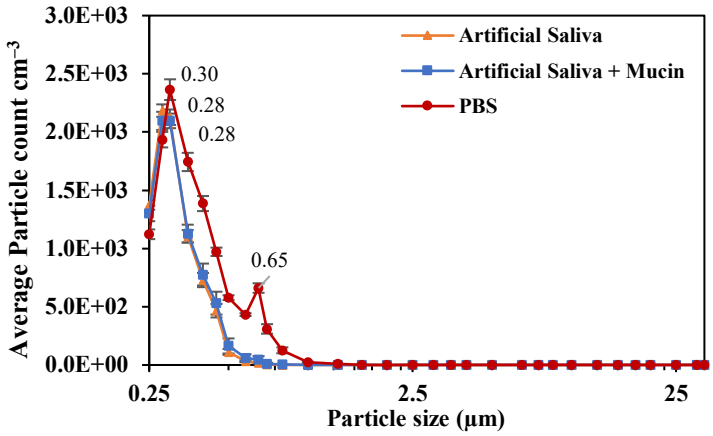


Fig. 2. Dry particle size distribution for aerosols generated from the nebulization of the three different media at 4 L min^{-1} gas flow rate and 0.35 mL min^{-1} liquid flow rate.

on contaminated coupons prior to their decontamination and compared to the theoretical concentrations. Greater losses and higher variability were observed for coupons contaminated by aerosols from suspensions mixed for 1 hour, while smaller spore losses (0.08–0.72 log) were obtained on coupons contaminated by drops (Fig. 3). Also, mixing the spore suspension overnight reduced losses and variability. The three-factor ANOVA analysis confirmed these observations by revealing a significant association between losses and methods of contamination (p = 6.97 × 10^{-7}) and between losses and mixing time (p = 0.007) (Table S3).

Nebulization is a more complex means of contamination than the simple direct drop deposition. It entails the conversion of the liquid suspension into mist. The compressed air entering the nebulizer expands through an orifice and creates a high-velocity jet. By venturi effect the liquid is sucked up and atomized. The small liquid particles carried by compressed air form an aerosol that transports spores to the coupon, while the fluid in particles evaporates in few milliseconds (Morawska, 2006). Accordingly, there are two possibilities to explain the observed losses during

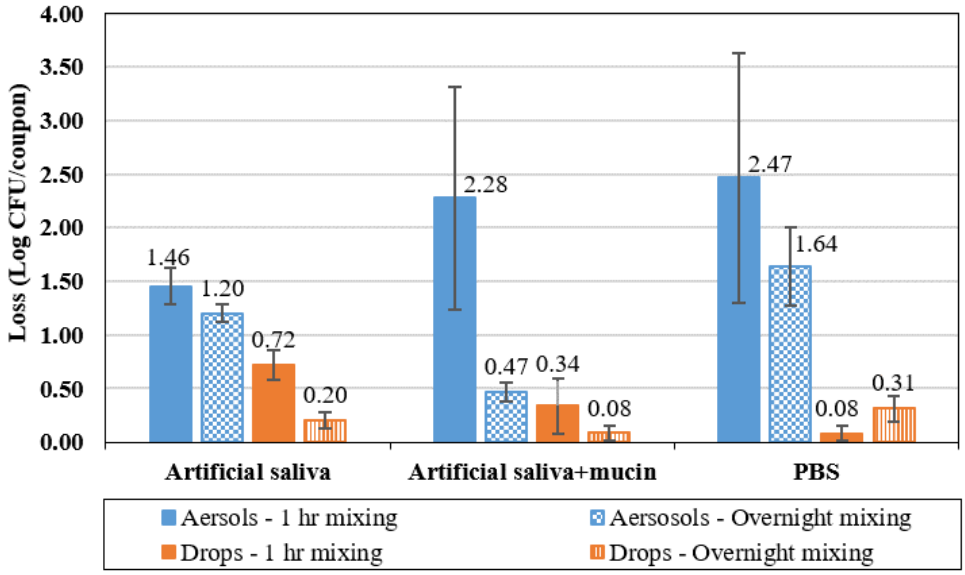


Fig. 3. Losses of spores (log CFU coupon^{-1}) incurred during the contamination by aerosols and by drops deposition using the three different suspensions (Artificial saliva, Artificial saliva + Mucin, and PBS) prepared by mixing for 1 hour or overnight. The theoretical spore concentration was 4.93 log CFU coupon^{-1}.

nebulization including losses in tubes and the difficulty to recover spores embedded within the respirators material. Some aerosolized spore-containing particles may interact with tubes connecting the nebulizer to the main vessel, even before reaching the coupon in the cassette. Moreover, depending on the filter material and particle size, respirators retain particles via different mechanisms: diffusion, interception, impaction, electrostatic interaction (Zhao *et al.*, 2020). The nebulization method used in this study led to the deposition of aerosolized particles and their filtration through the N95 coupon in the cassette, unlike the drops deposition method that occurred on the coupon's surface and did not rely on the airflow through the N95 fabric. In the former case, which actually better represents the real life application of FFRs, the pressure difference favors the penetration of aerosolized particles inside the filter's layers. These may not have been effectively recovered by the extraction method and hence culminated in more losses. On the other hand, contamination by drops deposition is a simpler process that infers a direct transfer of a small volume from the suspension to the coupon's surface and therefore, it was less prone to losses. It is important to note that optimization of the nebulization method was out of the scope of this study.

High losses were noticeable after the nebulization of spore-containing PBS probably due to the high salinity in this suspension (0.137 M NaCl, the equivalent of nine times that of NaCl in artificial saliva (0.015 M)) (Table S1). When aerosols deposit on the coupon's surface, the salts concentration increases due to the liquid evaporation. This results in a toxic environment for most microorganisms, as observed with the influenza virus (Yang *et al.*, 2012). For comparison, high losses of 1.2 log (for MS2 coliphages in PBS) and 2.5 log (for *E. coli* in PBS) have been reported in a study applying nebulization to contaminate respirators (particle size: 5 μm) (Bernardy *et al.*, 2022). These results are coherent with the expected relative resistance of spores, viruses and bacteria to environmental stressors.

The least losses were observed with both aerosol (0.47 log) and drop deposition (0.08 log) assays using spore-containing artificial saliva with mucin after overnight mixing. It is actually believed that artificial saliva with mucin is the closest to real human saliva and is thus the most realistic option for testing UV decontamination (Barancheshme *et al.*, 2021).

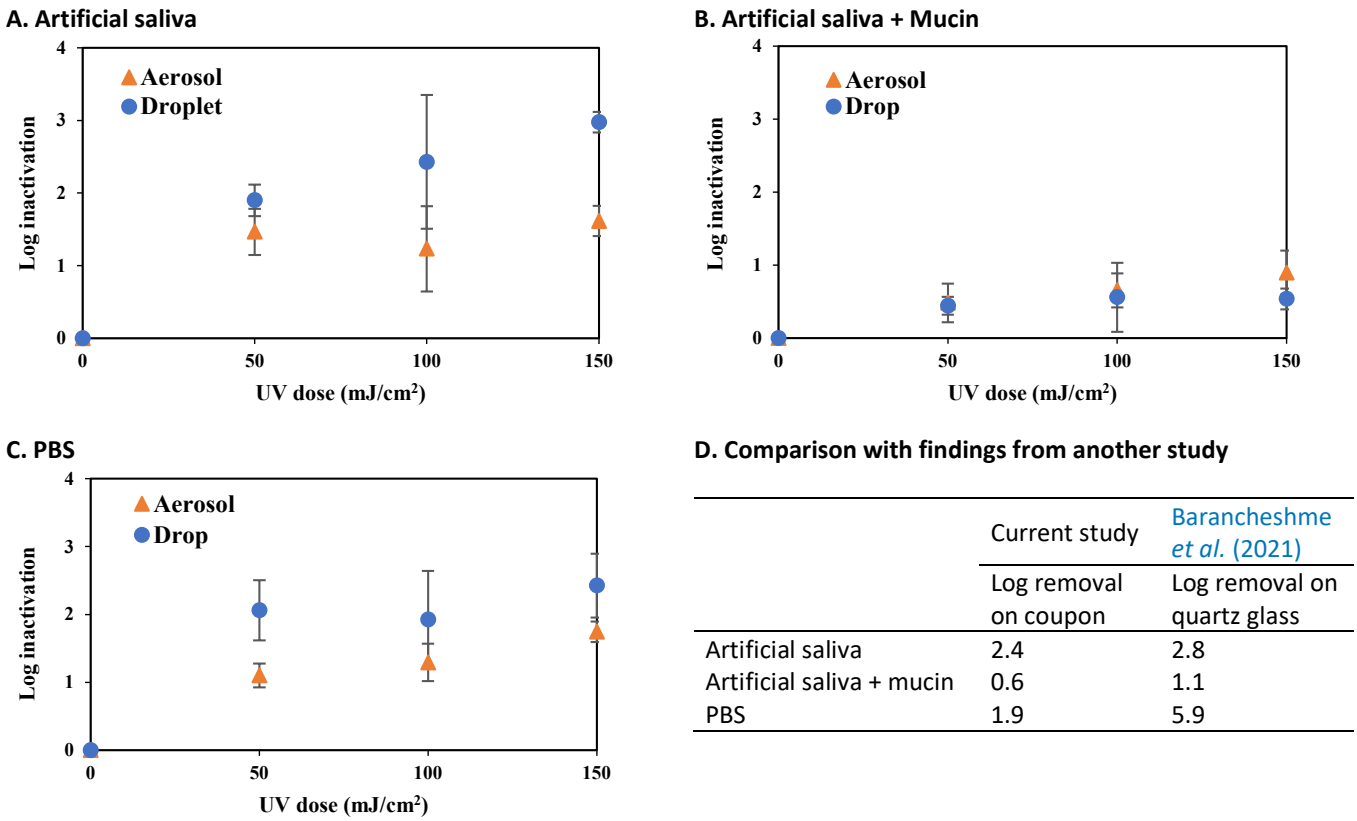
3.3 Evaluation of the Efficacy of UV Irradiation for the Decontamination of Respirators Contaminated by Aerosols Versus Drops

3.3.1 Impact of UV dose on inactivation

The results of decontamination tests are summarized in Fig. 4. UV irradiation was obviously more efficient in coupons contaminated by drops than in coupons contaminated by aerosols. One explanation could be the ease of accessibility to the contaminated particles: drops deposited on the FFR's surface are more accessible to UV irradiation than aerosolized particles penetrating the FFR's layers where they are substantially protected from the UV light.

The lowest inactivation (0.5–0.9 log at 150 mJ cm^{-2}) was observed for coupons contaminated with spores in artificial saliva and mucin (Fig. 4(B)) where the difference between drops and aerosols could not be discerned and was not statistically significant (p = 0.30). The lower inactivation in artificial saliva with mucin is attributed to the shielding effect that mucin has on spores, whereby spores become enmeshed by this protein and guarded from irradiation. Moreover, the addition of mucin incurs an increase in the UV absorbance decreasing the overall treatment efficiency. A suspension with higher concentration of mucin (3 g L^{-1}) was previously used to contaminate respirators with MS2 and as a result, a remarkably higher UV dose of 1800 mJ cm^{-2} was necessary to attain an average inactivation of 4.8 log on aerosol-contaminated respirators (Heimbuch *et al.*, 2011).

The inactivation results in PBS (1.7–2.4 log at 150 mJ cm^{-2}, Fig. 4(C)) were close to those in artificial saliva without mucin in coupons contaminated with aerosolized particles; and in both cases, an increase in the UV dose did not entail a linear improvement in the log inactivation. Yet, the inactivation level in artificial saliva was slightly higher than that in PBS when the contamination was performed by drops deposition (2.97 log and 2.42 log inactivation respectively at 150 mJ cm^{-2}). For comparison, one study applied UV doses up to 1500 mJ cm^{-2} to attain up to 2.75 log and 3 log inactivation of MS2 phages and *E. coli* in PBS, respectively (Bernardy *et al.*, 2022). The lower efficiency



D. Comparison with findings from another study

	Current study	Barancheshme *et al.* (2021)
	Log removal on coupon	Log removal on quartz glass
Artificial saliva	2.4	2.8
Artificial saliva + mucin	0.6	1.1
PBS	1.9	5.9

Fig. 4. Correlation between the log inactivation of spores and the UV dose applied on coupons contaminated with aerosols or droplets in (A) Artificial saliva, (B) Artificial saliva + Mucin, (C) PBS. (D) Comparison with findings from another study using the same means of contamination: drops deposition, and a UV dose of 100 mJ cm^{-2}.

of inactivation in PBS can be associated with the negative impact of high salinity. Higher salinity results in higher ionic strength, which decreases the forces of repulsion between microorganisms and favors the formation of clusters. When evaporation happens in the case of contamination by drops deposition, dried residues form at the surface of the FFR where they coat microorganisms (Heimbuch *et al.*, 2011) and impede UV radiation from reaching all targets.

Increasing the UV dose provided modest improvement in the performance, as tailing kinetics were observed for 5 out of 6 tested conditions. The only noticeable improvement was in the case of drops of artificial saliva where inactivation increased from 1.9 log to almost 3.0 log with the increase in UV dose from 50 to 150 mJ cm^{-2}. As expected, decontaminating FFR coupons was more difficult than inactivating spores dried on quartz glass (Barancheshme *et al.*, 2021) (Fig. 4(D)). Glass is an inert material, and the spores that were deposited as droplets on its surface remained on the surface and were more easily targeted by UV light during decontamination, especially in the absence of a shielding agent like the mucin protein. As such, our results were in line with the reported impact of clumps or clusters formation (the case of PBS) as well as adsorption onto or enmeshment within particles (the case of artificial saliva with mucin) on decreasing the rate of inactivation by UV irradiation (Hill *et al.*, 2021).

As first-order kinetic fits were considered not suitable due to the important tailing present on the dose-response curves, Eq. (4) was used to describe the two stages of inactivation: (i) the first stage corresponds to the rapid inactivation of susceptible spores, that can also be referred to as single organisms (with inactivation rate constant k_1, and fraction of susceptible spores $1 - f_1$); and (ii) the second stage corresponds to the slower inactivation of resistant spores, (with inactivation rate constant k_2, and fraction of shielded spores f_1). The latter type of spores were not readily accessible for irradiation being shielded by proteins, salts or in aggregates. We have tested this model recently to describe UV inactivation of spores contaminating N95 respirators (Gibson *et al.*, 2021). Evidently, this model is a simplification of the actual inactivation condition where a

continuous distribution of inactivation rate constant is expected rather than a simple binomial distribution. However, a more complex modeling approach was limited by the low number of data available to describe each inactivation curve (N = 4).

$$\text{Log inactivation} = (f_1 \times k_2 \times \text{Dose}) + ([1 - f_1] \times k_1 \times \text{Dose}) \quad (4)$$

The fraction of susceptible and resistant spores and k_1 *and* k_2 were calculated by fitting Eq. (4) to experimental data using nonlinear regression (Table 1). Results demonstrated that the inactivation kinetic of the resistant fraction of spores in drops was 2.0 to 8.7 times lower than the susceptible fraction (Table 1). The artificial saliva with mucin suspension generated the highest fraction of resistant spores in both drops and aerosols (0.92 and 0.88, respectively), while those fractions in artificial saliva without mucin and PBS suspensions were almost similar (0.62–0.72). Remarkably, the experimentally determined k_1/k_2 ratio for UV disinfection of spore-containing drops of artificial saliva with mucin (8.7) was close to the ratio obtained for 10 µL spore-containing drops from human saliva (8.3) used in a Monte Carlo simulation in our former study (Gibson *et al.*, 2021).

3.3.2 Statistical analysis: factors affecting FFRs decontamination by UV

Results from a three-way ANOVA analysis confirmed that the type of suspension medium, the method of contamination (aerosol versus drops), and the UV dose all significantly impacted the spore inactivation (Table S4). A strong interaction effect between the type of suspension and the method of contamination (p = 0.003) was also observed mainly for contaminated drops from artificial saliva without mucin and PBS. However, in the presence of mucin, the method of contamination (drops vs aerosols) became a secondary performance predictive factor probably due to the very low level of performance achieved during decontamination (< 1 log).

4 CONCLUSION

This study explored two methods of contamination of FFRs using *Bacillus subtilis* spores in three different suspension media along with the subsequent decontamination via UV irradiation. Results demonstrated that contaminating coupons of FFRs through drops deposition was more efficient than through the deposition of aerosolized particles and that the former also led to better levels of decontamination. The disinfection of FFRs was thus sensitive to the contamination method. Also, the level of spores' inactivation was greater in coupons contaminated with artificial

Table 1. Inactivation constants for susceptible and resistant *B. subtilis* spores in drops and aerosols of artificial saliva, artificial saliva with mucin and PBS.

Suspensions		Units	Drops	Aerosols
Artificial saliva	f_1	–	0.62	0.71
	k_1	$cm^2\ mJ^{-1}$	0.0326	0.0234
	k_2	$cm^2\ mJ^{-1}$	0.0160	0.0081
	k_1/k_2	–	2.0	2.9
Artificial saliva with Mucin	f_1	–	0.92	0.88
	k_1	$cm^2\ mJ^{-1}$	0.0245	0.0333
	k_2	$cm^2\ mJ^{-1}$	0.0028	0.0027
	k_1/k_2	–	8.7	12
PBS	f_1	–	0.66	0.72
	k_1	$cm^2\ mJ^{-1}$	0.0288	0.0343
	k_2	$cm^2\ mJ^{-1}$	0.0137	0.0043
	k_1/k_2	–	2.1	7.9

f_1 = Fraction of resistant spores.
k_1 = inactivation constant for susceptible spores (first stage).
k_2 = inactivation constant for resistant spores (second stage).

saliva without mucin, followed by PBS, and finally artificial saliva with mucin. The latter also presented the highest fraction of resistant spores in drops and in aerosols. In order to simulate real life contamination by applying contaminated aerosols, method optimization is required to maximize the concentration of the targeted microorganisms and their distribution within the layers of the respirator. More contamination and decontamination studies using other microorganisms are also recommended.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version at https://doi.org/10.4209/aaqr.230018

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