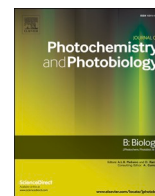




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Assessment of saliva interference with UV-based disinfection technologies

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ABSTRACT

Worldwide shortages of personal protective equipment during COVID-19 pandemic has forced the implementation of methods for decontaminating face piece respirators such as N95 respirators. The use of UV irradiation to reduce bioburden of used respirators attracts attention, making proper testing protocols of utmost importance. Currently artificial saliva is used but its comparison to human saliva from the UV disinfection perspective is lacking. Here we characterize UV spectra of human and artificial saliva, both fresh and after settling, to test for possible interference for UV-based disinfection. ASTM 2720 artificial saliva recipe (with either porcine or bovine mucin) showed many discrepancies from average ($N = 18$) human saliva, with different mucins demonstrating very different UV absorbance spectra, resulting in very different UV transmittance at different wavelength. Reducing porcine mucin concentration from 3 to 1.7 g/L brought UVA_{254} in the artificial saliva to that of average human saliva (although not for other wavelengths), allowing 254 nm disinfection experiments. Phosphate saline and modified artificial saliva were spiked with 8.6 log CFU/ml *B. subtilis* spores (ATCC 6633) and irradiated at dose of up to 100 mJ/cm², resulting in 5.9 log inactivation for a saline suspension, and 2.8 and 1.1 log inactivation for ASTM-no mucin and ASTM-1.7 g/L porcine mucin 2 μL dried droplets, respectively. UVC irradiation of spores dried in human saliva resulted in 2.3 and 1.5 log inactivation, depending on the size of the droplets (2 vs 10 μL, respectively) dried on a glass surface. Our results suggest that in the presence of the current standard dried artificial saliva it is unlikely that UVC can achieve 6 log inactivation of *B. subtilis* spores using a realistic UV dose (e.g. less than 2 J/cm²) and the ATSM saliva recipe should be revised for UV decontamination studies.

1. Introduction

The rapid spread of coronavirus disease 2019 (COVID-19) caused the shortage of filtering facepiece respirators (FFR). The latter is an important personal protective equipment (PPE) to reduce the transmission of respiratory pathogens amongst healthcare workers. N95 respirators are one of the most common FFR that are approved by the National Institute for Occupational Safety and Health (NIOSH) and designed to capture more than 95% of airborne particles larger than 0.3 μm [1]. These respirators can effectively remove infectious microorganisms from the air [2].

Although many PPE respirators are meant to be disposed after single-use, the shortage of respirators forced many healthcare workers and front-liners to reuse them. The Center for Disease Control and Prevention (CDC) published strategies to optimize the supply of N95 respirators

and suggested the limited reuse of N95 FFR after decontamination [3]. Filtration performance, fit test, damage, and contamination from soiling are factors that limit the number of time a FFR can be reused [3]. Based on the CDC's guidance, vapor hydrogen peroxide (VHP), ultraviolet germicidal irradiation (UVGI), and moist heat are the most promising FFR decontamination methods.

An effective decontamination method should meet the following criteria: remove target virus (SARS-CoV-2) effectively with minimum damage to the respirators filtration and fit. Also, the method should be safe, i.e. with no adverse effect on human health [3]. Ideally, the method should also be simple to put in place. Previous studies on the use of UV as an FFRs decontamination method showed promising results. Decontamination of six N95 FFR models using UVC required a UV_{254nm} dose of 100 mJ/cm² at the internal filtering medium to achieve 3 log reduction of MS2 virus in the absence of soiling agent [4]. The possibility to

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achieve a significant reduction of influenza virus viability (more than 3 log) has also been demonstrated [5]. Even though influenza is more sensitive to UVC than MS2, a higher UV dose (1.1 J/cm²) was needed to achieve 3 log inactivation in the presence of soiling agents such as artificial skin oil or saliva. The presence of soiling agents was shown to reduce the germicidal capability of UV by 1–2 log [6].

Respiratory pathogens are usually discharge in a mixture of saliva and sputum droplets, and indeed saliva was found to contain SARS-CoV-2 viruses [7]. Natural saliva is a complex mixture of proteins and salts, which are expected to interfere with UV light propagation and therefore negatively impacts the effectiveness of UVGI technologies. Saliva composition includes various electrolytes such as sodium, potassium, calcium, magnesium, bicarbonate, and phosphate as well as immunoglobulins, proteins, enzymes, mucin, and nitrogenous compounds, such as urea and ammonia [8]. The ATSM 2720E standard for artificial saliva recommends a recipe which uses a series of minerals as well as 3 g/L of mucin as the main organic constituent. Mucins are a family of high molecular weight proteins produced by epithelial tissues of most animals.

To date, no studies have evaluated the optical spectral characteristics of human or artificial saliva recipes, as given in ASTM 2720E. The goal of this study was to compare the characteristics of artificial saliva and human saliva with respect to their UV absorbance spectrum and interference to UVGI in order to confirm if the recipe proposed in ASTM 2720E was adequate to serve as soiling agent during the validation of a UVGI technology.

2. Material and Methods

2.1. Human Saliva Sampling

A total number of 18 volunteers including 8 females and 10 males took part in the study. Eight samples were collected in the Israel and ten samples were collected in Canada to measure the effect of regional variability. For all samples of saliva, the UV absorption spectrum and protein composition were determined. Duplicates were used for all analysis. The volunteers had diverse ages and ethnicity and saliva was collected at random time of the day during normal work hours.

2.2. Artificial Saliva Preparation

The artificial saliva was prepared based on the ATSM 2720E standard recipe (Table S2, supplementary materials). Saliva with 1.7 or 3.0 g/L of porcine mucin were tested. As discussed later, the lower mucin concentration was selected to match the average UVA₂₅₄ of human saliva. The spectral characteristics of two types of mucin (bovine and porcine, respectively M3895 and M2378 from Sigma-Aldrich, USA) were also compared during this project.

2.3. Saliva Spectral Analysis

A Nanodrop 2000 (Thermo Scientific) was used to measure saliva UV absorbance spectra from 200 to 700 nm. Samples volumes were 0.5, 1.0 or 2.0 μL. UV spectrum of eight saliva samples including three females and five males were determined in duplicate before and after 20 min of settling prior to the analysis. UVA at 222, 254, and 280 nm were extracted from the spectra for data presentation.

2.4. Impact of Evaporation and Settling on UV Spectral Analysis

The impact of settling was assessed by measuring saliva samples before and after 60 min settling at room temperature. The goal was to validate if the samples needed to be mixed before analysis. The influence of evaporation was evaluated at room temperature by placing two 50 μL (collected from a 54 yr-old male) each in a 4-cm polystyrene Petri dish equipped with a loose cover (to allow evaporation while preventing

airborne contamination). At time 0, 20 min, 40 min, and 60 min each drop was mixed with a micropipette tip and a 2 μL subsample was analyzed using the Nanodrop.

2.5. UV Transmittance of Dried Saliva

UV transmittance (UVT₂₅₄) measurement of dried saliva was done using a radiometer (IL400A, International Light Inc.) by evaluating UV light attenuation. Saliva droplets with increasing volumes (2, 10 and 50 μL) and variable characteristics (human and artificial saliva with/without porcine mucin) were dried on a UV-transparent microscope coverslip (75875–478, VWR, USA). The human saliva was provided by a female donor. The coverslips were then placed over the radiometer reading area and installed under a low-pressure collimated beam apparatus. The irradiance (mW.cm⁻²) was measured with (*I*) or without (*I*₀) the presence of the dried saliva residue on the coverslip. The irradiated area had a diameter of 7 mm. UVT transmittance was calculated as:

$$UVT (\%) = \frac{I}{I_0} \times 100 \quad (1)$$

In order to increase the precision of the UVT calculations, the irradiances were calculated by plotting the cumulative UV doses (as mJ.cm⁻²) against time (s) in such a way that:

$$I = \frac{Dose}{t} \quad (2)$$

Eq. 1 was modified to account for the proportion of the irradiated area which was covered by the dried residue (Eq. 3):

$$UVT (\%) = \frac{I_0 - \left[(I_0 - I) \times \frac{A_{total}}{A_{residue}} \right]}{I_0} \quad (3)$$

This modification renders the UVT calculation independent of the area covered by the dried residue (*A*_{residue} in mm²) compared to the total irradiated area (*A*_{total} = 38.49 mm²). For smaller droplets of 2 μL, the precision of the irradiance measurement was improved by placing three droplets in the irradiated area while for larger droplets, only one droplet was present.

The UV absorbance of the dried residues from artificial saliva were approximated using the Beer-Lambert law.

$$UVA_{254} (cm^{-1}) = \frac{-\text{Log} [UVT]}{depth} \quad (4)$$

The depth of dried residue was estimating by calculating the volume of dried residue (known precisely for artificial saliva) which we divided by its area (measured experimentally) (Eq. 5). As the residue is expected to be partially composed of voids, an internal porosity of 50% was arbitrarily assumed in all calculations.

$$Depth (\mu m) = \frac{1}{A \times porosity} \times \frac{\sum_{i=1}^n m_i / \rho_i}{10^6} \quad (5)$$

Where *m*_{*i*} (g) and *ρ*_{*i*} (g/m³) are the mass and density of compound *i*, *n* is the total number of ingredients in the saliva recipe (*n* = 11), *A* is the area of the dried residue (m²), the porosity (50%) and 10⁶ is the conversion from m to μm.

2.6. UVC Inactivation of *B. subtilis* Spores Dried in Various Saliva

B. subtilis spores (ATCC 6633) were produced according to the method described by Barbeau et al. in 2005 [9]. Purified spores were filtered through a 10 μm filter to remove large aggregates that might be present in the stock suspension. Spores were spiked at a final concentration of 8.6 log CFU/mL in the following background liquid suspensions: (i) phosphate buffer saline (PBS), artificial saliva ATSM 2720 (ii)

with porcine mucin adjusted at 1.7 g/L, (iii) without porcine mucin, and (iv) in human saliva (female donor). The disinfection test in PBS was done in liquid phase to quantify the spores intrinsic resistance. In contrast, disinfection assays conducted in artificial and human saliva were realized after drying of 10 μL ($n = 1$) or 2 μL ($n = 5$) droplets for 1 h on microscopic quartz coverslips. Testing the area of the droplets aimed to assess the interference of a deep dried saliva residue on UVC performance. UV disinfection assays were conducted in a monochromatic UVC lamp collimated beam reactor. Cumulative UV doses (0–250 mJ/cm^2) were measured for each individual coverslip being irradiated using a radiometer (IL400A, International Light inc.). Irradiation times (0–70 min) varied depending on the target UVC dose and saliva composition. Liquid phase UV dosages (i.e. tests in PBS) were adjusted according to the standard methodology described in Bolton and Linden (2003). No correction factors were applied to dried saliva UV dosages. Spores found in dried saliva were extracted from the quartz surface by immersing the coverslip in 10 mL of 1.5 mM NaHCO_3 buffer and vortexing for 5 min. The extraction technique of spores dried on quartz glass proved to be efficient and repeatable as the average initial spore concentration measured on the positive controls was $10^{6.19 \pm 0.02}$ CFU/coverslip while the expected spore concentration was $10^{6.60 \pm 0.05}$ (i.e. 39% recovery), suggesting no important loss and good extraction. Spores in the extraction buffer were analyzed using the modified procedure proposed by Barbeau et al. in 2007 [10]. Briefly, samples are filtered on a 0.45 μm membrane which is then deposited in a petri dish equipped with a pad soaked with 1.4 mL of TSB. Petri dishes are inserted in two Ziploc bags before pasteurizing them for 15 min in a water bath set at 75 $^\circ\text{C}$. Colonies are counted after 24 h of incubation at 35 $^\circ\text{C}$. Results are reported as CFU/mL of extraction buffer. Log removals were calculated using duplicate results of untreated vs treated samples.

An overview of all experimental tests completed can be found in Table S1.

3. Results

3.1. Precision of UVA Measurements

UV absorbance spectra were measured in duplicate for three settled and unsettled human saliva using either a volume of 0.5, 1.0 or 2.0 μL on the Nanodrop spectrophotometric pedestal. The means, standard deviations and coefficient of variations were calculated. Table S3 presents the average coefficient of variance for 0.5, 1.0 or 2.0 μL of sample at different wavelengths. Based on the average coefficient of variations, the precision of the UVA_{254} measurement was highest using a test volume of 2 μL . Consequently, all results presented in this study were based on a test volume of 2 μL . Detailed UV spectrum (from 210 to 410 nm) of the 18 human saliva samples are presented in Supplementary Material

(Fig. S1).

3.2. Impact of Sample Handling on UV Spectra Measurements

The first step of this study was dedicated to understanding how sample collection and preservation could impact UV spectra measurements. Amongst potential sources of analytical errors, we considered that measurements might be impacted by settling during sample storage prior to its analysis. Secondly, evaporation of human saliva could potentially increase UVA_{254} . The impact of settling was assessed in triplicate on one human saliva by measuring UVA spectra before and after 60 min of settling (Fig. 1A). A volume of about 2 mL was left in a 15 mL closed tube (to minimize evaporation). UVA largely declined in the supernatant after 1 h of settling. For example, UVA_{254} was reduced from 9.4 to 5.9 cm^{-1} , an observation coherent with the fact that precipitates were visually observed at the bottom of the vial. The impact of saliva evaporation was tested by leaving 50 μL of saliva in a covered petri dish for up to one hour (Fig. 1B). For this test, increasing UVA spectra was noted for evaporation time above 20 min. After this period, the UVA was seen to increase significantly. For example, the average UVA_{254} increased from 9.9 to 11.8 cm^{-1} after 60 min of evaporation. For the rest of the project, we measured UVA spectra on fresh human saliva only a few minutes after collection to eliminate biases related to sample handling. We recommend that saliva be analyzed as fresh as possible, without sedimentation and sampled in a small, closed container to minimize air gap leading to evaporation.

3.3. Comparison of UV Spectra of Artificial and Human Saliva

The average UV spectra of human saliva ($N = 18$) is compared in Fig. 2 with ASTM 2720E artificial saliva recipe prepared with either 3 g/L of bovine mucin or porcine mucin. Human saliva is composed of 3 distinct peaks located at 220, 230 and 280 nm. The average UVA_{254} of human saliva was calculated as $6.74 \pm 3.25 \text{ cm}^{-1}$. The ASTM artificial saliva prepared with 3 g/L of bovine mucin did not provide a sufficiently strong UV absorbance ($< 0.1 \text{ cm}^{-1}$ throughout the measured range) to correctly mimic human saliva. In comparison, ASTM with 3 g/L porcine mucin had a high UVA_{254} (11.8 cm^{-1}) but lacked the distinct peak observed at 280 nm in human saliva. These preliminary observations suggest that UV interference following soiling with ASTM2720E recipe will result in a different protective behavior as compared to human saliva. One would also need to consider the emission UV spectrum being tested (e.g. UV_{254} vs UV_{280}) before selecting an adequate artificial saliva recipe. For $\text{UV}_{254\text{nm}}$ disinfection, a reduced concentration of 1.7 g/L porcine mucin provided an equivalent $\text{UVA}_{254\text{nm}}$ than the average human saliva (i.e. 6.8 cm^{-1}) (see arrow on Fig. 2).

The differences in the absorbance spectra of the different mucins and

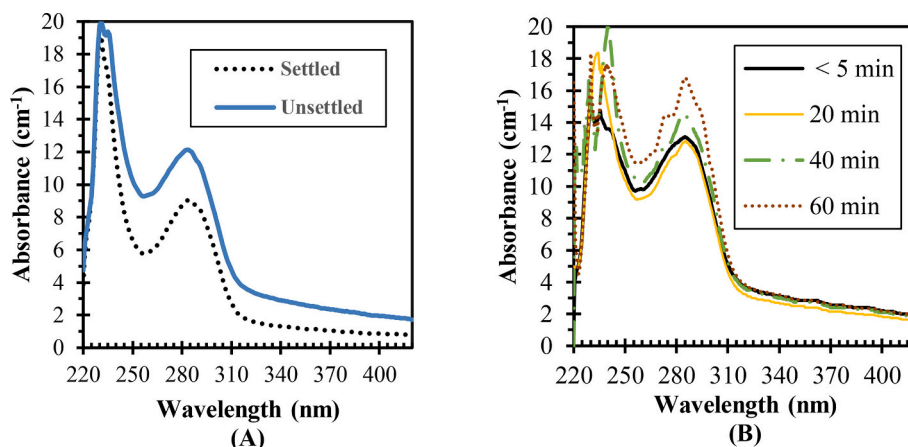


Fig. 1. Impact of (A) 60-min of settling and (B) 5 to 60 min evaporation on UV spectra measurements of human saliva ($N = 1$). $T = \text{room temperature} = 21\text{--}23 \text{ }^\circ\text{C}$.

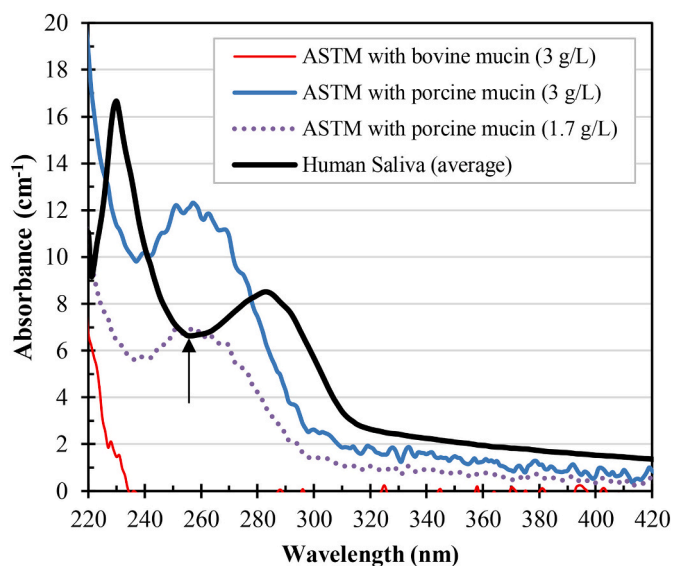


Fig. 2. Comparison of ASTM2720 artificial saliva (prepared with either 1.7, 3.0 g/L of porcine mucin or 3.0 g/L of bovine mucin) with the average human saliva UV spectra ($N = 18$). The arrow points to $\lambda = 254$ nm.

of the different saliva preparations can be explained by variations in proteins composition. In proteins only three amino acids are responsible for the bulk of UVA_{254} , namely Tryptophan, Tyrosine, and Phenylalanine, having absorbance coefficients of 2670, 321 and $184 \text{ M}^{-1} \cdot \text{cm}^{-1}$ respectively at this wavelength [17], thus difference in the content of these amino acids could result in large difference in the UV absorbance. Given that bovine mucin did not show any UV absorbance at 254 nm, it was no longer used in the rest of our experiments.

3.4. Natural Variability of Human UV Absorbance Spectra

The 222 nm wavelength has been proposed to effectively inactivate viruses while mitigating adverse human health effects [11]. The 254 nm is the common wavelength produced by low pressure Hg lamps while high UV absorbance is expected at 280 nm due to light absorption by proteins. UV LED systems are also commercialized at this wavelength [12]. Defining one reference saliva as a soiling agent is complicated by the fact that saliva characteristics are known to vary significantly, even amongst one individual. To better illustrate human saliva variability, Fig. 3 presents the UVA statistical distribution of the 18 human samples investigated at three wavelengths, demonstrating ~ 6 -fold variability at

each wavelength (the detailed statistical parameters are provided in supplementary materials Table S4). The datasets were shown to be log-normally distributed. Median UVA were not statistically different amongst men and women ($p > 0.05$) or between the Israeli and Canadian participants ($p > 0.05$, data not shown). Ca/P and Na/K ratios are salivary markers which differ between men and women [13]. However, variability in UVA spectra are expected to originate from the protein content rather than dissolved minerals which bear low UVA. Finally, the available number of participants was not large enough to test for differences in the composition of saliva as a function of age and ethnicity.

3.5. UV Transmittance of Dried Saliva

Measuring the UVA of liquid saliva is an important information required in preparation of a UV disinfection test using saliva as soiling agent. However, in the context of N95 respirator decontamination, one can assume that saliva will most commonly be found as a dried residue following its evaporation on the surface of the respirator. To the best of our knowledge, UV transmittance and absorbance of dried saliva have never been reported before. UVT_{254} across a dried residue is expected to depend on the salt as well as the organic content (mucin in the case of artificial saliva). Although most dissolved salts exhibit low UVA_{254} absorbance (e.g. a 1% NaCl solution has a UVA_{254} of only 0.002 cm^{-1}) [14], once crystallized or precipitated by evaporation, they are expected to shield organisms due to light scattering, making it important to assess not only the UVA_{254} of liquid saliva but also consider the UVT_{254}/UVA_{254} of dried residues. Apart from saliva composition, we hypothesized that larger liquid droplets would yield a thicker dried residue which would be more detrimental to UV light penetration. Table 1 presents the UVT_{254} and UVA_{254} measured for droplets of 2, 10 and 25 μL saliva of varying composition. Fig. S2 presents an example of irradiance measurements (I and I_0) performed for one 10 μL dried droplet of human saliva. Please note that several past studies have used droplets of 1–2 μL to contaminate N95 respirators prior to their treatment with UVC, heat or H_2O_2 [5,6,15].

As expected, larger droplets produced a wider and thicker dried residue which is estimated to be in the order of several micrometers deep. The UVT_{254} of a single droplet of ASTM2720 artificial saliva declined from 80.8%, 63.4% and 48.5% when droplets initial volumes increased from, 2 to 10 and 25 μL . In comparison, a 10 μL droplet of dried human saliva or artificial saliva without mucin provided UVT of 84.4% and 100%, respectively. Such result indicates that even though the revised artificial saliva recipe had a lower mucin concentration (1.7 vs 3 g/L), it was still a conservative soiling agent compared to the average human saliva. Finally, UVA of dried artificial saliva was estimated using Beer-Lambert law which accounts for the depth of the

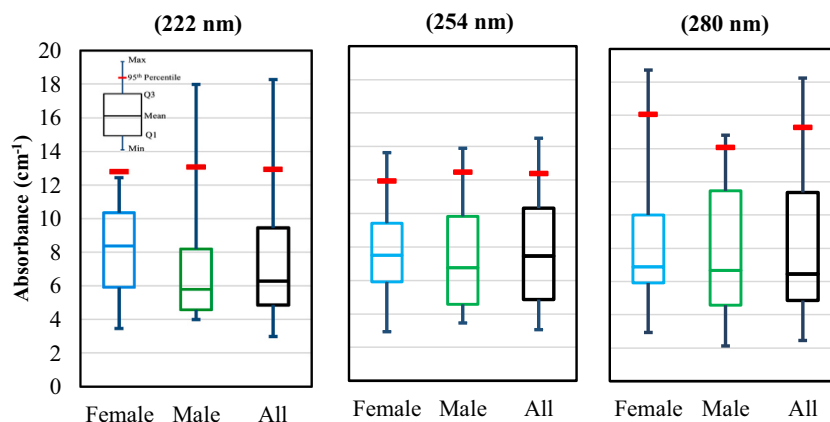


Fig. 3. Variability of human saliva UVA measured at 3 characteristic wavelengths (222, 254 and 280 nm). See inset for the description of the whisker-box information.

Table 1

UV transmittance of droplet of variable initial volume and characteristics.

Saliva type	Droplet characteristics		Irradiated area		Dried residue Characteristics**				
	Volume (μL)	UVA_{254} (cm^{-1})	Residue (mm^2)	Total (mm^2)	Depth ^{&} (cm)	I_0 (mW/cm^2)	I (mW/cm^2)	UVT (in %)	$\text{UVA}^{\&\&}$ (cm^{-1})
Human	10	6.7	21.81	38.49	NA	0.79	0.72	84.4%	NA
ASTM2720E without porcine mucin	10	0.03	16.32	38.49	3.6	0.59	0.63	>100%	NA
ASTM2720E with 1.7 g/L of porcine mucin	2	6.4	16.20*	38.49	2.2	0.85	0.78	80.8%	421
	10		18.55	38.49	3.2	0.68	0.56	63.4%	628
	25		30.97	38.49	4.8	0.68	0.40	48.5%	655

&: Calculated assuming 50% porosity.

&&: Calculated using Beer-Lambert law: $\text{UVA}_{254} = -\text{Log}(\text{UVT}_{254})/\text{depth}$.

NA: Not available.

* : Cumulative area of 3 droplets of 2 μL ,

** : I = Irradiance with saliva, I_0 = irradiance without saliva.

residue. The values were in the range of 421–655 cm^{-1} , 666–102 102 times higher than the UVA_{254} of the liquid. At such high level of UVA, it is unlikely that the Beer-Lambert law still holds true due to variations in the refractive index of the residue.

3.6. Impact of Saliva Type on the Inactivation of *B. subtilis* Spores with UVC

B. subtilis spores suspended in PBS buffer were inactivated by UVC as a baseline. Up to 6 log inactivation was confirmed with a UVC dose of 100 mJ/cm^2 . The intrinsic resistance of the spores tested in this study was 36 mJ/cm^2 for 2 log inactivation, within the expectation for this organism (USEPA, 2006). The kinetic exhibited a small shoulder followed by a log-linear inactivation representative of a Chick-Watson kinetic. Tailing in PBS was observed only above 5.5 log inactivation, a result which suggests a very low level of spores clumping in the stock suspension (which had been filtered through a 10 μm membrane).

Using a UV_{254} dose of 100 mJ/cm^2 , inactivation of spores dried in artificial saliva were 2.8 log (no mucin) and 1.1 log (1.7 g/L of mucin). Using dried human saliva, inactivation reached 2.3 or 1.5 log depending on the size of the droplets dried on the glass surface (2 vs 10 μL), in good agreement with the hypothesis that a larger droplet leads to more interference. Artificial saliva droplets of 2 μL prepared without mucin addition were the easiest dried residue to decontaminate but still presented an important tailing at approximately 4.0–4.5 log inactivation. This confirms the significant protection offered by the 2.53 g/L of dried mineral salts present in the mucin-less ASTM2720 recipe, even though our measurement of UVT (cf Table 1) did not suggest a significant interference. The female saliva used for the inactivation assay had a UVA_{254} of 6.7 cm^{-1} , which was coincidentally identical to the average UVA_{254} measured during our survey. Spores inactivation in this specific dried human saliva (2 μL droplets) initially behaved similar to artificial saliva without mucin but produced an earlier tailing phenomenon at 2.5–3.0 log, most likely reflecting the higher level of clumping achieved in the human saliva. The slowest kinetic and tailing were observed when 2 μL droplets of dried ASTM artificial saliva with 1.7 g/L of mucin were used as soiling agent. The result was coherent with the fact that this suspension exhibited the highest UVA_{254} (10 cm^{-1}) which includes a contribution of about 3.3 cm^{-1} from the spores themselves. Finally, the impact of droplet size was assessed by inactivating spores dried in human saliva droplets of 2 or 10 μL . The larger droplets were more difficult to inactivate than the smaller ones as tailing inactivation at 250 mJ/cm^2 was reduced from 3.0 log (2 μL) to 2.0 log (10 μL) (Fig. 4).

The role of soiling agent is of paramount importance on the inactivation kinetic given the significant tailing observed in the dose-response curves. As the saliva evaporates, microorganisms are expected to clump with each other and/or salts crystals and proteins. Fig. 5 presents pictures of various saliva droplets before and after desiccation on a ruler to estimate the area covered the dried residue. The 10 and 2 μL droplets

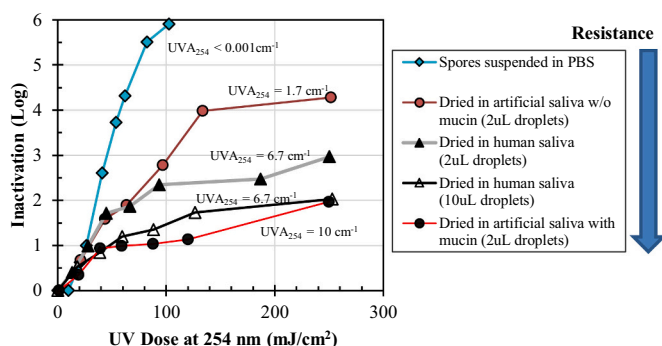


Fig. 4. Inactivation of *B. subtilis* spores by UV_{254} suspended in PBS buffer or dried on quartz glass in human or ASTM270E artificial saliva with 1.7 g/L of mucin. The initial volume of droplets is given in μL . The UVA_{254} of the liquid suspension prior to drying is given on the graph. The average precision on log inactivation presented is ± 0.04 log. The precision of spore analysis was 0.03 log/mL.

had diameters of roughly 4 and 2 mm before drying (Fig. 5A and B). Human and artificial dried saliva (Fig. 5C, D) exhibited a very different morphology. The human saliva was clearly more translucent with very small crystals in it while the artificial saliva produced a whitish and opaque residue with a larger spatial heterogeneity.

4. Discussion

Soiling agents are commonly used during disinfection studies to mimic realistic conditions expected to prevail during decontamination. Covering organisms with artificial skin oil after they have been dried on a N95 respirator has been used by Mills et al. in 2018 [5]. However, the repeatability of this procedure for UVGI testing is questionable giving the difficulty in covering the dried organisms with a uniform depth of soiling agent. Artificial saliva is in our opinion a more robust approach to define soiling as the organisms can be spiked directly in the saliva prior to its direct deposition on a surface within droplets or through aerosols.

During this study, we tested the direct droplet deposition technique. Given the fact that the droplet size was seen to impact UVC inactivation of spores, it would be of interest for future studies to compare the impact of the droplet deposition to aerosol techniques on UV disinfection. Aerosols are expected to penetrate deeper within the layers of a N95 respirator than a large droplet of 2 μL (diameter: ~ 1500 μm). On the other hand, the mass of dried residue left after desiccation of a 2 μm droplet will be more important than what can be found in 1–25 μm aerosols. Heimbuch et al. in 2011 [2] did perform a comparison of two contamination techniques which yielded droplets of 15 μm vs aerosols of

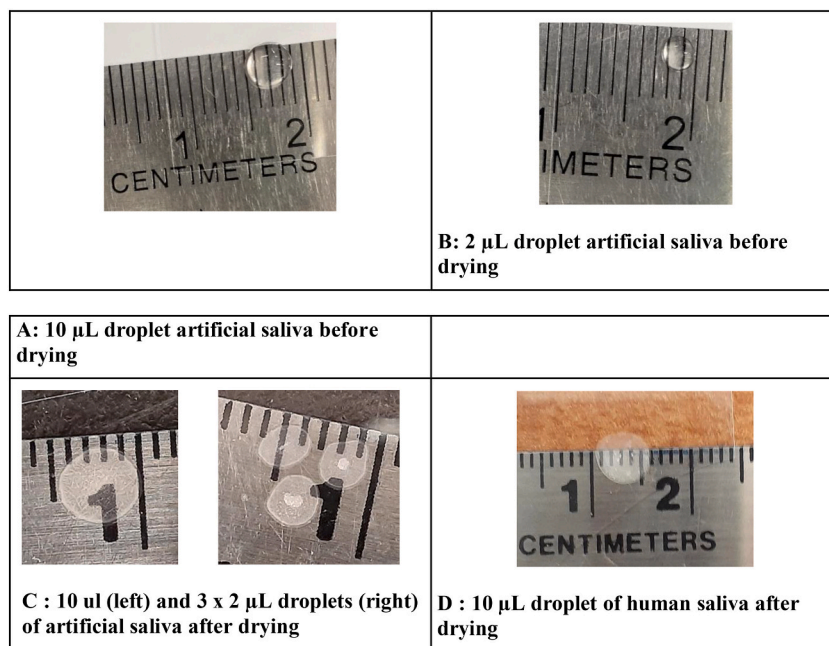


Fig. 5. Pictures of saliva droplets. Artificial saliva: ASTM with 1.7 g/L of porcine mucin. Human saliva ($UVA_{254} = 6.7 \text{ cm}^{-1}$).

0.5 μm (median diameters). No significant difference in the ability of UVC to inactivate MS2 coliphages deposited on N95 respirators was observed. However, the difference in these two techniques was less important than we would expect between aerosols and a large droplet of 1500 μm diameter. Future studies should assess if the large droplet deposition leads to an overly conservative decontamination condition compared to aerosols. This question is of interest given that the direct deposition of a 2 μL droplet would be much easier to implement as a standard practice than the use of an aerosol-generating apparatus. It is worth noting that ASTM2720E standard describes such equipment in its appendix. In comparison, ASTM3135 standard (Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil) recommends using 1 μL droplet deposition on test carriers and the use of artificial skin oil and artificial saliva as soiling agents (tested separately).

Defining a standard artificial saliva as a representative soil for UVGI testing poses some challenges. Human saliva has a complex composition that varies from one person to another [16] and also during the day for one given individual [13]. For example, the dynamics of mineral composition of saliva in 20 men and 20 women of the same age group have been studied during 24 h. The maximum mineral content occurred between 7 and 8 a.m. and 18–19 p.m. while the saliva pH changes were matched with dynamics of the Ca/P ratio with a maximum ratio at 9:00 am and 15–18 p.m. Also, the results showed that mineral composition of saliva significantly depends on the gender [13]. The saliva mineral content of men and women were significantly different in pH ($p < 0.001$), concentrations of inorganic phosphorus ($p < 0.001$), and Ca/P ratio ($p = 0.011$) [13]. In a case study investigating the characteristics of 90 unstimulated saliva [16], age was the most important factor impacting saliva composition (flow rate, protein concentration, amylolytic and lipolytic activity) were quantified. Ethnicity was also shown to impact protein concentration while gender did not. In the study presented here, UVA measured at characteristic wavelengths followed a log-normal distribution. Nevertheless, UVA_{254} could not fully predict the interference of saliva given that crystalized salts were also shown to interfere with UVC inactivation.

The current ASTM2720E recipe, even after reducing mucin concentration from 3 to 1.7 g/L, provided conservative decontamination conditions compared to a representative human saliva. This result could be

expected given that the recipe was never developed to serve as a soiling agent for UVC decontamination per se. This opens the question of what could be an adequate artificial saliva recipe? All dried residues were shown to generate dose-response curves with important tailing. When targeting high log inactivation, the tailing effect will become the controlling factor of the overall UV performance. Therefore, we propose that the tailing behavior (i.e. the plateau observed at high UV doses) of the targeted organisms should match what is achieved in human saliva. This decision is made more complex by the fact that the high concentration of organisms needed to demonstrate 6 log inactivation are likely to act as an interference to UV light penetration by themselves, especially after drying. Therefore, we anticipate that tests using a lower initial concentration of organisms on the N95 respirator could lead to an apparent improved performance of UVGI. This phenomenon could be considered by standardizing the UVA of the artificial saliva after its spiking with the target organisms. Further studies are ongoing in group to assess the impact of saliva components on UVC interference, including the interference of organisms.

5. Conclusions

The aim of this study was to compare the characteristics of artificial saliva and human saliva with respect to their UV absorption spectrum in order to confirm if the recipe proposed in ASTM 2720 was adequate to serve as soiling agent during the validation of a UVGI technology. Our evaluation demonstrated that:

- Significant discrepancies exist between the ASTM 2720E artificial saliva recipe and the human saliva measured in this study ($N = 18$).
- Reducing porcine mucin from 3 to 1.7 g/L allowed to yield an equivalent UVA_{254} in the artificial saliva and human saliva but did not correct for other wavelength.
- Apart from mucin, crystalized salts, and the spiked microorganisms at 8.6 log/mL also created interference to UV inactivation.
- For a UV dose of 100 mJ/cm^2 , UV inactivation was maximal (5.9 log) in PBS buffer. Inactivation of spores suspended in dried artificial saliva were 2.8 log (no mucin) and 1.1 log (1.7 g/L of mucin). Using dried human saliva, inactivation reached 2.3 or 1.5 log depending on

the size of the droplets dried on the glass surface (2 vs 10 μL). Using smaller droplets is recommended.

- The interference of dried saliva on spores UVC inactivation was coherent with the measurements of the UV transmittance of the dried residue.
- In the presence of a conservative dried artificial saliva, it is unlikely that UVC can achieve 6 log inactivation of *B. subtilis* spores using a realistic UV dose (e.g. less than 2 J/cm^2).

Future research should compare dose-response curves for surfaces contaminated by aerosols deposition rather than direct droplet deposition. It would also be desirable to revisit the ASTM saliva recipe in order to have a better match of its absorbance profile over the entire UV spectrum.

Authors contribution

F. Baranchesme and B. Barbeau co-wrote the first draft which was revised by the entire group of authors. J. Philibert, Y. Gercham and N. Noam-Amar performed the laboratory experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jphotobiol.2021.112168>.

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