Evaluation of the Performance of the Illumisoft Sanilume Air Device

Report prepared by

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Introduction

The overall objective of the experiments was to determine the performance of the Illumisoft Sanilume, SL-36KT-75, 120V Option Air device using a steady state experimental protocol in an aerobiology test chamber. The device is an upper room UVC air sanitiser which combines UVC with a high-capacity fan.

Experimental Methodology

Although the testing is being carried out at the request of Illumisoft Lighting Canada, there was a requirement that to satisfy the requirements of Health Canada the testing needed to be conducted using specific bacteria and viruses.

Test Bacteria #1

Health Canada requirement for claims regarding air testing: *Staphylococcus aureus* (ATCC 6538)
Bacteria used in testing: *Staphylococcus aureus* (ATCC 6538)

Test Bacteria #2

Health Canada requirement for claims regarding air testing: P*seudomonas aeruginosa* (ATCC 15442)
Bacteria used in testing: *Pseudomonas aeruginosa* (ATCC9027)

Due to the unavailability of *Pseudomonas aerugin*osa (ATCC 15442), the closest substitute that could be obtained was *Pseudomonas aeruginosa* (ATCC9027). However, published studies presented by Malayeri et al (2021) have established the UVC inactivation dosage of both strains of Pseudomonas aeruginosa is the same (Table 1).

Table 1. UV dose required for a given log reduction for various strains of Pseudomonas aeruginosa (Malayeri et al (2021)

		Fluence (UV dose) (mJ/cm^2) for a given log reduction without photoreactivation								
Bacterium	Lamp Type	1	2	3	4	5	6	Proto-col?	Notes	Reference
Pseudomonas aeruginosa										
ATCC 9027	LP	*3.8*	*6.5*	*10*	*17*			no		Abshire & Dunton 1981
ATCC 10145	LP	*4.6*						no		Abshire & Dunton 1981
ATCC 14207	LP	*3.7*						no		Abshire & Dunton 1981
ATCC 15442	LP	*3.8*						no		Abshire & Dunton 1981
ATCC 27853	LP	*4.9*						no		Abshire & Dunton 1981
ATCC 27853	LP	0.8	1.6	2.3	3.1			yes		Clauß 2006

Test Viruses

The test facility and associated microbiology laboratories in the School of Civil engineering at the University of Leeds can be used aerosolise up to Category 2 microorganisms but cannot be used to aerosolise live viruses. As a surrogate for live virus the testing was carried out using Phi-6 Bacteriophage (DSM 21518, host bacterium *pseudomonas syringae).*

Health Canada provided an extremely limited list of acceptable viruses including poliovirus type 1, CHAT strain (ATCC VR-1562), human adenovirus type 5 (ATCC VR-5), bovine parvovirus (ATCC VR-767) and canine parvovirus (ATCC VR-2017). However, a number of published studies have reported the UVC inactivation dosage of Phi 6 as well as human adenovirus type 5 (ATCC VR-5). Guo et al (2010) (Figure 1) reported that for Human Adenovirus Type 5 (ATCC VR-5) the UVC inactivation dose was at 254 nm was 152 and 19 MJ/cm^2 for a 4 and 3 log reduction respectively.

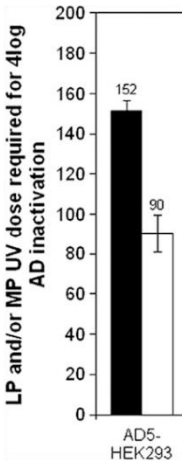

Figure 1 UVC inactivation dose required to achieve a 4 log reduction of Human Adenovirus Type 5 (ATCC VR-5) (Guo et al, 2010)

There have been many studies recently quantifying the 254 nm UVC inactivation dose for aerosolised SARS-CoV2 including the data reported by Marita et al (2021) shown in Figure 2. Their data showed that a 3-log reduction could be achieved with a dose of 4.3 MJ/cm^2.

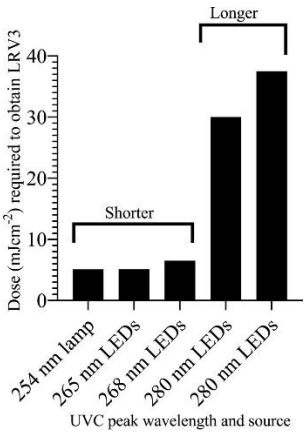

Figure 2 UV dose required to achieve a 3-log reduction in SARS-CoV2 (Marita et al, 2021)

Ma et al (2021) reported inactivation data for Bacteriophage Phi-6 (Figure 3) and demonstrated a log 3 dosage of approximately 100 MJ/cm^2 when using UVC at 254nm

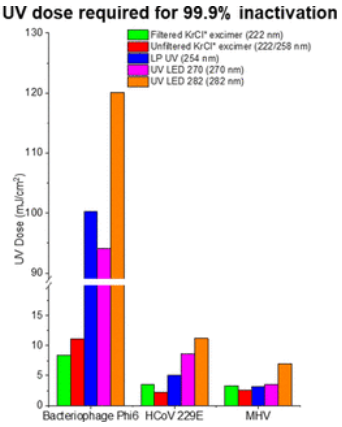

Figure 3 Inactivation of Phi-6 Bacteriophage at different UVC wavelengths (Ma et al, 2021)

In summary, the data taken from previously published data shows the following inactivation dosages. This data shows that Phi-6 Bacteriophage is more resistant to UVC than either SARS-CoV2 or Human Adenovirus Type 5 and strongly supports the use of the bacteriophage as a test virus surrogate.

- Human Adenovirus Type 5 – 19 mJ/cm^2
- SARS-CoV2 – 4.3 mJ/cm^2
- Phi-6 Bacteriophage – 100 mJ/cm^2

Culture Preparation

S. aureus and *P. aeruginosa* were prepared by inoculating 100ml of sterile Tryptone Soya Broth (Oxoid, UK). The broth was then incubated at 37°C for 24 hours and shaken at 100rpm. After 24 hours incubation the culture is assumed to be at the boundary between the exponential and stationary growth phases. After incubation the culture was centrifuged and re-suspended in sterile Ringers solution and the concentration of bacteria in the culture was determined.

P. syringae was cultured overnight at 25°C on tryptone soya agar before being transferred to 100ml of sterile tryptone Soya broth and incubated overnight at 25°C at 150rpm. Bacteriophage Phi-6 was cultured in tryptone soya broth with P. syringae in the exponential growth phase and incubated at 25°C overnight with shaking at 150rpm. The culture was then centrifuged at 3220 rpm for 10 min at 4°C and the supernatant filtered using a polyether sulphate membrane with a 0.22 µm pore size, to remove intact bacteria or bacterial debris. The resulting filtrate containing the bacteriophage Φ6 was stored at 4°C. The concentration of Phi-6 in the stock culture was determined using the double agar method. The stock culture was serially diluted in phage buffer. 200 µL of *P. syringae* and 500 µL of Phi-6 were added to molten top agar (30 g L-1 Tryptone Soya Broth, 6 g L-1 agar) mixed thoroughly and poured onto sterile tryptone Soya Agar plates. Once set, the plates were incubated overnight at 25°C and the number of plaques counted

Aerobiology Chamber Set Up

The experiments described in this report were carried out in the aerobiological test chamber at the University of Leeds, which consists of a 35.6 m^3 hermetically sealed negatively pressurised chamber (with room dimensions: 4.25m long, 3.35m wide and 2.5m high) in which the air flow rate, temperature and relative humidity can be constantly controlled and monitored. The experiments were carried out with the ventilation system set at 6 AC/hr at ambient temperature (approx. 20°C) and relative humidity (approx. 50%). Figure 4 shows the setup of the chamber and the location of the aerosolization and sampling points and the test device

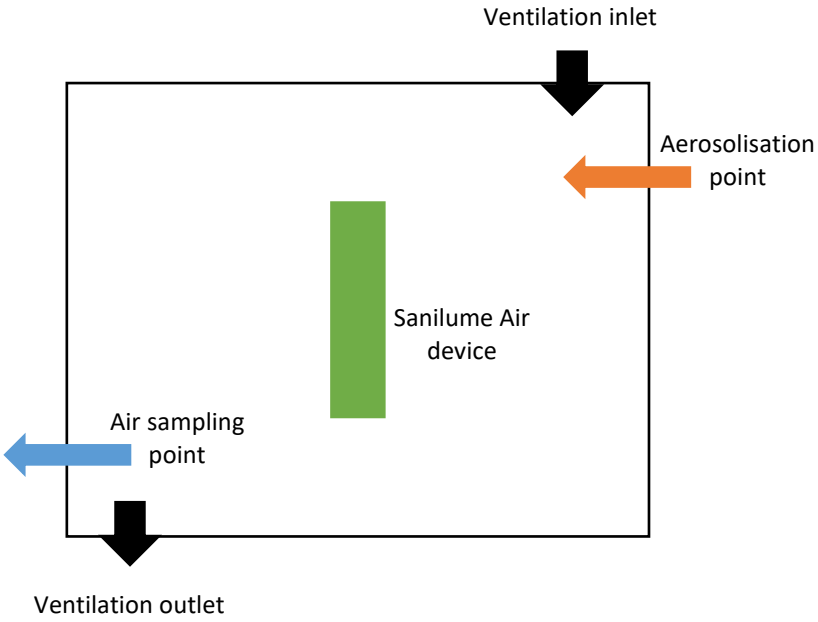

Figure 4 Experimental set up of the aerobiology chamber

During the experiments the phage aerosols were generated using a 6-jet Collison nebuliser operating at a flow rate of 12 l/min and at a pressure of 20 psi (Plate 1A). The microorganisms was suspended in 100ml of sterile distilled water or phage buffer at a concentration of approximately 10^7 pfu or cfu/ml. This was connected to the chamber via a 25 mm diameter pipe which allowed the microbial aerosol to be delivered into the chamber. Air samples were collected through a plastic pipe located immediately in front of the extract grille. This pipe was connected to a six stage Andersen sampler (Plate 1B) loaded with Tryptone Soya Agar plates (inoculated with 300µl of the host bacteria for Phi-6 sampling). During the sampling process air passed through the sampler and the microbial aerosols were deposited onto the agar plates. The sampling time was set at 1 minute at a flow rate of 28 l/min.

Plate 1. Six jet Collison nebuliser (A) and Six stage Andersen Sampler (B)

The Sanilume Air device was suspended from the ceiling in the centre of the chamber at a location mid-way between the floor and the ceiling as shown in plates 2, 3 and 4.

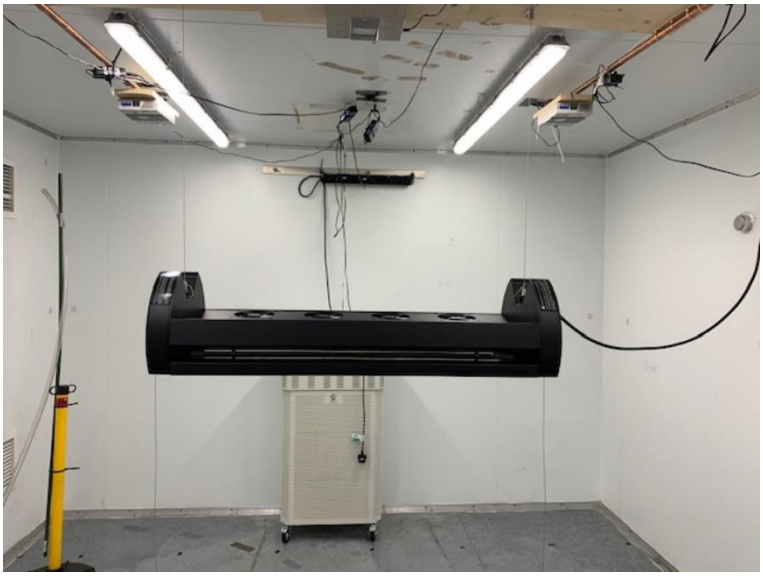

Plate 2 Sanilume Air device suspended from the ceiling in the chamber at mid-height

Plate 3 Close up of the Sanilume Air device set on aperture 1 (Suitable for rooms with 8' 6" ceiling heights)

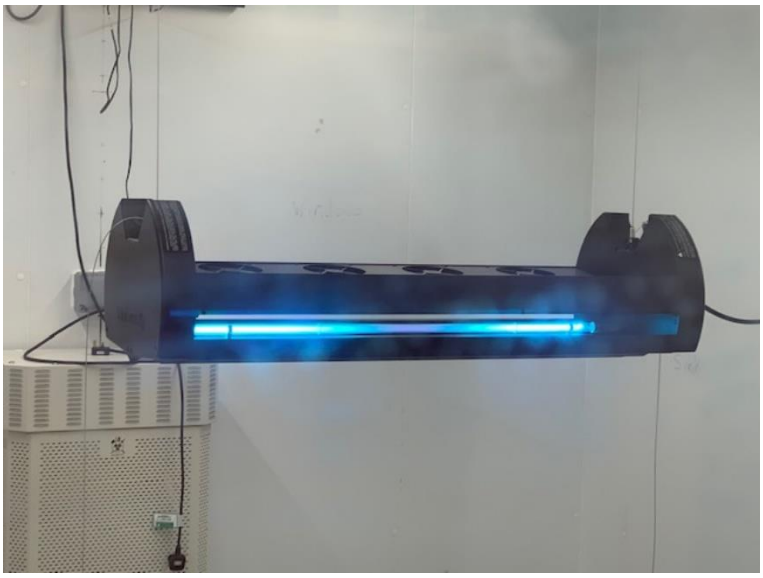

Plate 4 Sanilume Air device operating in the aerobiology chamber

Steady State Test Protocol

The test chamber was set up as shown in Plate 2 prior to the start of each experimental run and the chamber door closed and locked and both the sampling port and the nebuliser port sealed. The air fans were then switched on and operated at maximum speed (approx. 15AC/hr) for up to 30 minutes in order to ensure the chamber was sterile. During this purging period the nebuliser remained switched off. During the initial purging period the pre-sterilised nebuliser was prepared and filled with 100ml of microbial suspension at a concentration of approximately 10^7 pfu or cfu/ml. The nebuliser was then connected to the inlet tube ready for the start of the experiment.

After the initial purging period the ventilation rate was reduced to 6 AC/hr and nebulisation of the microbial culture then began and the concentration in the test chamber was allowed to stabilize for

30 minutes. A total of ten replicate samples were then taken at approximately 2-minute intervals during which time the test device remained switched off and these are the control samples. Once all ten samples had been taken the test device was then switched on and left for 30 minutes for the concentration of microorganisms in the air inside the chamber to reach steady state once again. A total of ten replicate samples were then taken at approximately 2-minute intervals during which time the test device was switched on and these are the test samples.

The agar plates were incubated at 37°C for 24 hours (25°C for 48 hours for the Phi-6) after which the number of colonies/plaques on each plate were counted. All the counts were then subjected to positive hole correction to account for multiple impaction (Macher 1989). The corrected counts for each sample were multiplied to give a count per m^3 of test chamber air. Each set of samples represents ten replicates taken during steady state, the first ten being the concentration without the test device operating and the second ten with the test device operating. The mean was taken of the ten replicate samples to give a mean concentration with and without the test device operating. This allowed the mean reduction in concentration to be calculated and used to give an indication as to the efficacy of the test device against the three different microorganisms. For each microorganism, two tests were carried out at two different apertures.

Results

Table 2 provides a summary of the results from the six steady state tests carried out in the aerobiology chamber using the Illumisoft Sanilume Air device with three different microorganisms and at two different apertures. The lowest aperture setting is suitable for rooms with ceiling heights greater than 8’ 6” from an occupant safety perspective, and the second aperture setting is suitable for rooms with ceiling heights greater than 22’ from an occupant safety perspective. There is a linear relationship between recommended aperture setting for a given ceiling height (between the first and second aperture settings) and the recommended ceiling heights (between 8’6” and 22’). Individual experimental results are presented in graphs in the appendix.

The performance of the device was determined from the difference between the initial steady state concentration of airborne microorganisms when the device was not operating and the second steady state concentration and is expressed as percent reduction and log reduction. This data has then been used to calculate the equivalent air change rate.

The data in Table 2 shows that the device is capable of achieving significant reductions in airborne concentrations for all three microorganisms and at both aperture settings. When used to inactivate *Staphylococcus aureus* the device achieved a reduction of 97.3% at aperture one and this reduction increased to 99.2% when the maximum aperture was used. This is equivalent to log reductions and equivalent air changes of 1.57, 216 and 2.07, 744 respectively.

For *Pseudomonas aeruginosa* the performance of the device overall was higher than with *Staphylococcus aureus* at both aperture setting. With aperture 1 the inactivation was 98.3% which is equivalent to a 1.77 log reduction and 347 equivalent air changes. When the aperture was increased to its maximum the inactivation increased to 99.7% equivalent to a log reduction of 2.5 and 1994 equivalent air changes. The superior performance against *Pseudomonas aeruginosa* compared to *Staphylococcus aureus* would be expected given that it has been demonstrated that gram negative bacteria are more susceptible to UVC at 254nm compared to gram positive bacteria.

When the device was tested against Phi-6 bacteriophage the overall performance at both apertures was still significant but was very slightly lower that that seen for the bacteria and in particular *Pseudomonas aeruginosa*. At aperture 1 the device achieves an inactivation of 97.1% which increased

to 99.1% when the aperture was increased. These results are equivalent to log reductions of 1.54 and 2.03 and equivalent air changes of 201 and 661 respectively.

Based on the UV inactivation data presented earlier for Human Adenovirus Type (ATCC VR-5) (Guo et al, 2010) and SARS-CoV2 (Marita et al, 2021) it is clear that the dose required to achieve a 3-log reduction in bacteriophage Phi-6 is approximately 5 and 23 times higher respectively. Therefore, based on the inactivation results achieved for Phi-6 in these experiments, it can be assumed that the Illumisoft Sanilume Air upper room UV device, if tested under the same conditions would achieve a significantly higher inactivation rate for these two viruses.

Table 2. Performance of the Illumisoft Sanilume Air upper room UV device in 35.6 m^3 aerobiology test chamber.

Test organism	Aperture	Mean Concentration (cfu/m^3)		Percent reduction	Log Reduction	eACH
		Before	After			
Staphylococcus aureus	1	17548	477	97.3	1.57	216
	2	19568	165	99.2	2.07	744
Pseudomonas aeruginosa	1	21907	368	98.3	1.77	347
	2	23569	75	99.7	2.50	1994
Phi-6 bacteriophage	1	11717	339	97.1	1.54	201
	2	11791	111	99.1	2.03	661

To put the performance of the Illumisoft Sanilume Air upper room UV device into context Table 3 shows the inactivation rates of other UV devices tested under the same conditions in the same aerobiology chamber. The tests shown in Table 3 were performed using *Serratia marscecens* as the test microorganism which has a UV susceptibility constant (or Z value) of 0.4449 m2/J compared to 0.5721 and 0.3476 m2/J for *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively and is therefore comparable. The UV susceptibility constant is usually calculated experimentally and is a function of the linear relationship between the UV dose applied and the natural logarithm of the survival fraction and is used to describe the behaviour of microorganisms when exposed to UVC irradiation (Beggs et al, 2020). Knowing the UV susceptibility of a microorganism allows reasonably accurate prediction of what will happen if the microorganism is exposed to UVC irradiation.

It is clear from the data in Table 3 that the Illumisoft Sanilume Air upper room UV device is the best performing UV device that has been tested in the aerobiology chamber at the University of Leeds to date. Regardless of the test microorganism or the aperture used, the eACH achieved by the device are significantly higher than those achieved by the other upper room UVC devices tested previously.

Table 3. Comparable performance of upper room UVC devices testing in the aerobiology chamber at 6 AC/hr using Serratia marcescens

Device	Percent reduction	eACH
Passive short wall mounted upper room device (1 x 36W lamp)	5%	0.003
Passive long wall mounted upper room device (2 x 36W lamps)	13%	0.98
Passive ceiling mounted upper room device (4 x 18W lamps)	12%	0.82
Long and short wall device in combination	74%	17.1
Enclosed UV device with fan - wall mounted	10%	0.33
Enclosed UV device with fan – ceiling mounted	13%	0.98
Enclosed UV device with fan – mounted close to aerosol source	63%	9.7

Given what we know about the UV susceptibility constant described previously, it is possible to use the values for *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* to estimate what the performance of the devices presented in Table 3 would be by calculating the ratio between the UV susceptibility constants for *Serratia marcescens* and the two test microorganisms used in these experiments.

- UV susceptibility constant ratio between Pseudomonas aeruginosa and Serratia marcescens = 0.5721 / 0.4449 = 1.285
- UV susceptibility constant ratio between *Staphylococcus aureus* and Serratia marcescens = 0.3476 / 0.4449 = 0.781

Table 4 shows the comparable performance of the upper room UVC devices estimated for Pseudomonas aeruginosa and Staphylococcus aureus using the UV susceptibility constant ratios of 1.285 and 0.781 respectively. **The calculated data clearly shows that the Illumisoft Sanilume Air upper room UV device achieved a significantly higher inactivation of airborne *Pseudomonas aeruginosa* and *Staphylococcus aureus* compared to what would be achieved by the other upper room UVC devices that have previously been tested in the aerobiology chamber**.

Table 4. Comparable performance of upper room UVC devices testing in the aerobiology chamber at 6 AC/hr estimated for *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA)

Device	eACH (PA)	eACH (SA)
Passive short wall mounted upper room device (1 x 36W lamp)	0.004	0.002
Passive long wall mounted upper room device (2 x 36W lamps)	1.26	0.77
Passive ceiling mounted upper room device (4 x 18W lamps)	1.05	0.64
Long and short wall device in combination	21.97	13.36
Enclosed UV device with fan - wall mounted	0.42	0.26
Enclosed UV device with fan – ceiling mounted	1.26	0.77
Enclosed UV device with fan – mounted close to aerosol source	12.46	7.58
Sanilume Device Aperture Setting #1	347	216
Sanilume Device Aperture Setting #2	1994	744

Key Observations

Based on the results presented above there are a number of key observations regarding the performance of the Illumisoft Sanilume Air device:

- **The device is capable of achieving an extraordinary reduction in the concentration of airborne microorganisms when tested under steady state conditions in the 32m^3 aerobiology test chamber**
- The inactivation achieved by the device is slightly higher for the gram-negative *Pseudomonas aeruginosa* compared to gram positive *Staphylococcus aureus* and Phi-6 bacteriophage
- The inactivation achieved is significantly higher for all the test microorganisms at the maximum aperture compared to aperture 1
- Given the significantly higher dose required to achieve a 3-log inactivation for bacteriophage Phi-6 compared to both Human Adenovirus Type (ATCC VR-5) and SARS-CoV2 it can safely be assumed that the Illumisoft Sanilume Air device would be capable of achieving significantly higher rates for these two viruses when testing under the same conditions.

- **Based on calculated data, the Illumisoft Sanilume Air device was capable of achieving significantly higher inactivation of airborne Pseudomonas aeruginosa and Staphylococcus aureus compared to what would be achieved by the other upper room UVC devices that have previously been tested in the aerobiology chamber at the University of Leeds to date**
- **The Illumisoft Sanilume Air device is the best performing UV device that has been tested in the aerobiology chamber at the University of Leeds to date**

References

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Appendix

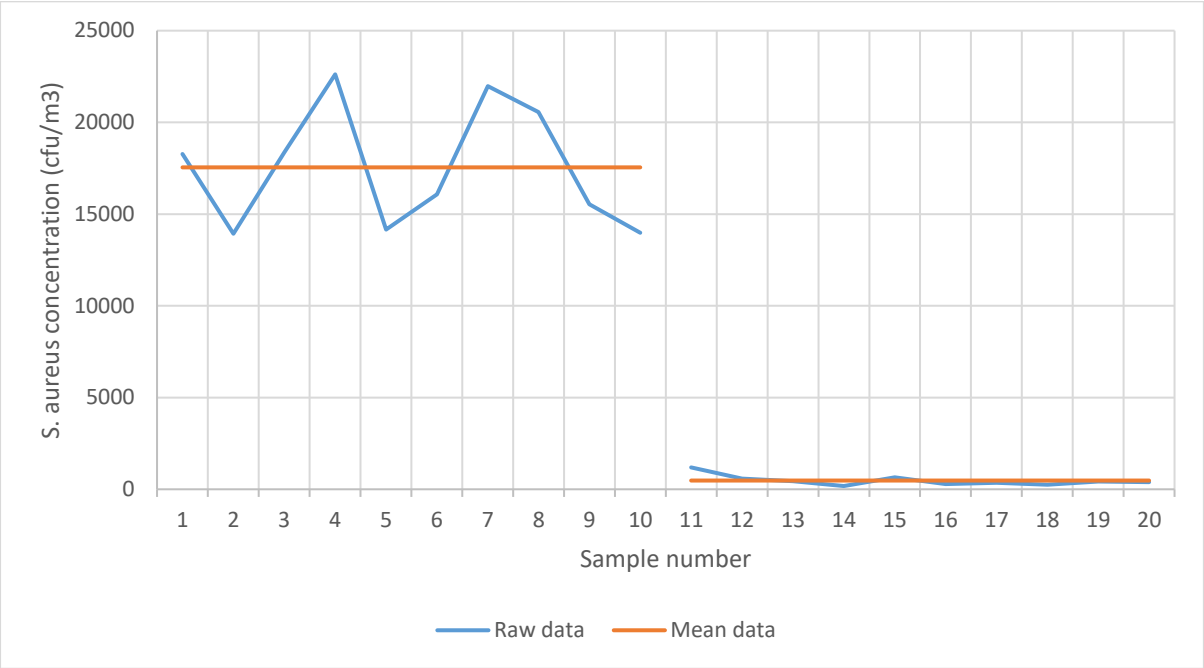

Figure 3 Staphylococcus aureus inactivation using aperture 1

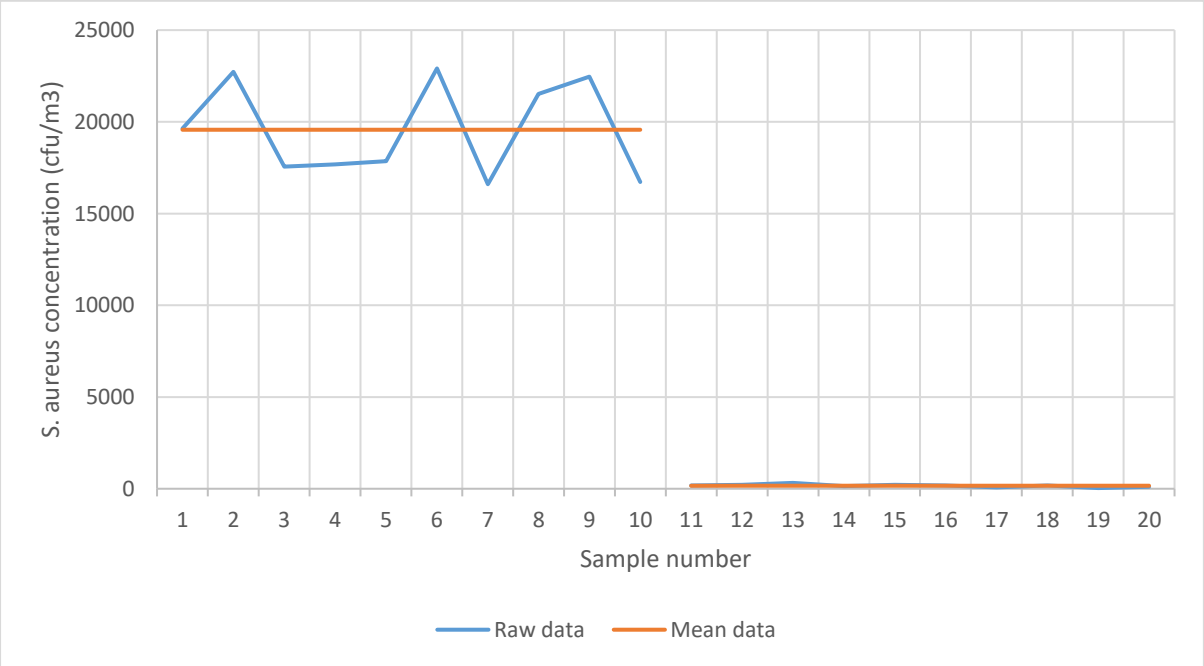

Figure 4 Staphylococcus aureus inactivation using aperture 2

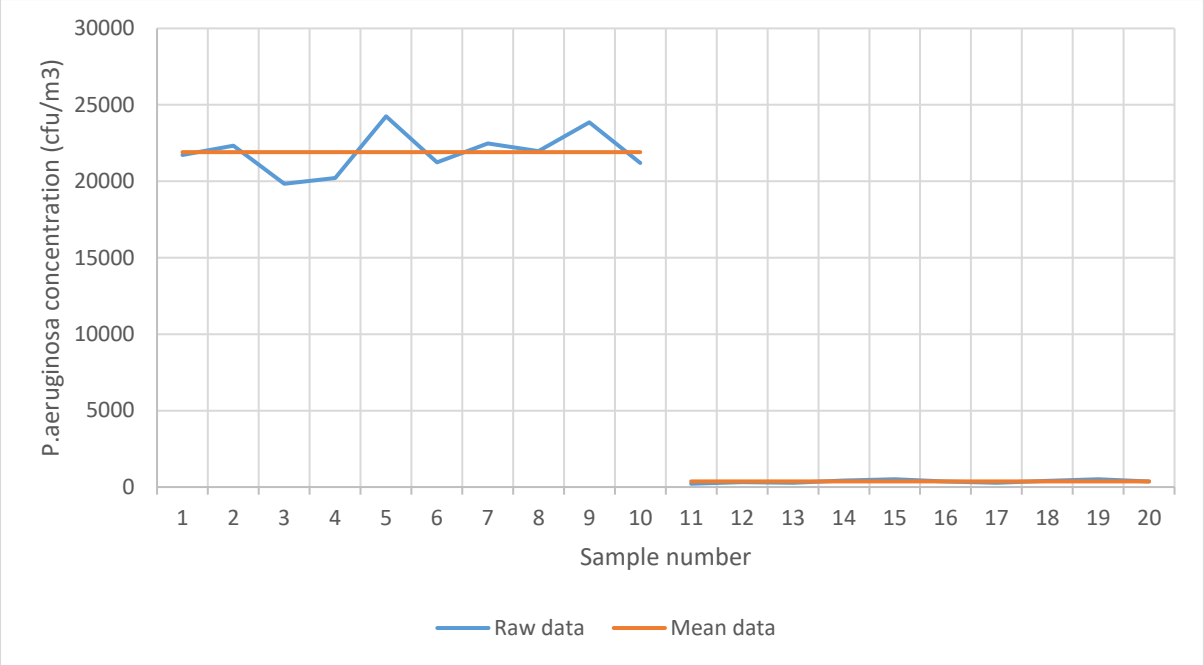

Figure 3 Pseudomonas aeruginosa inactivation using aperture 1

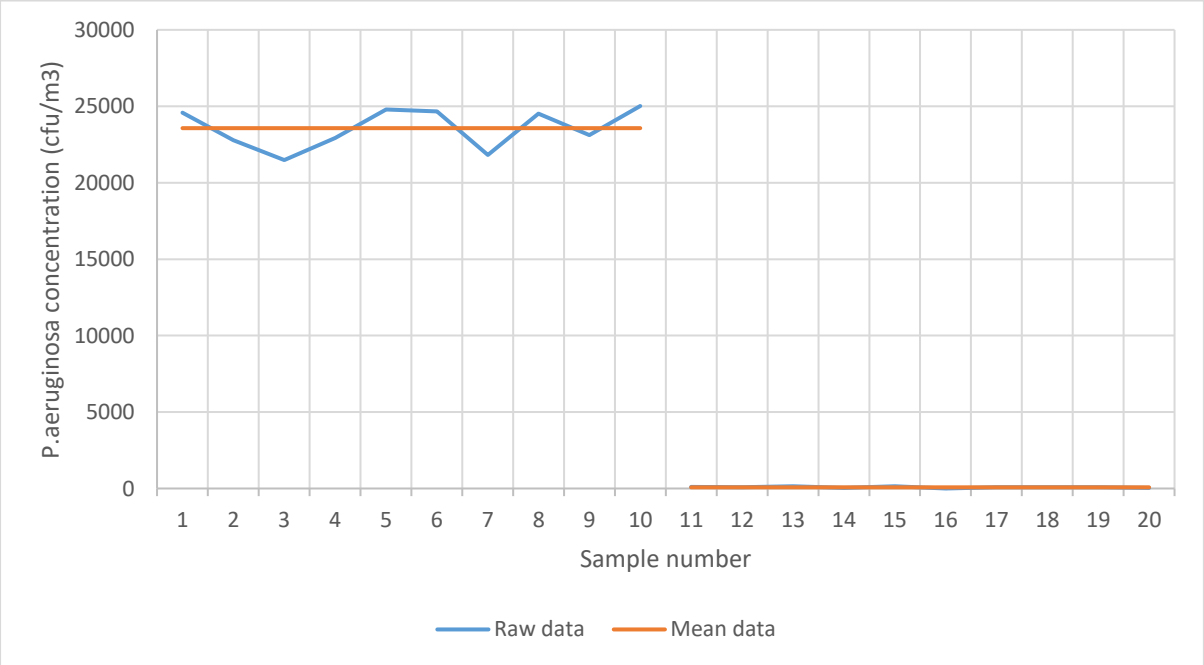

Figure 3 Pseudomonas aeruginosa inactivation using aperture 2

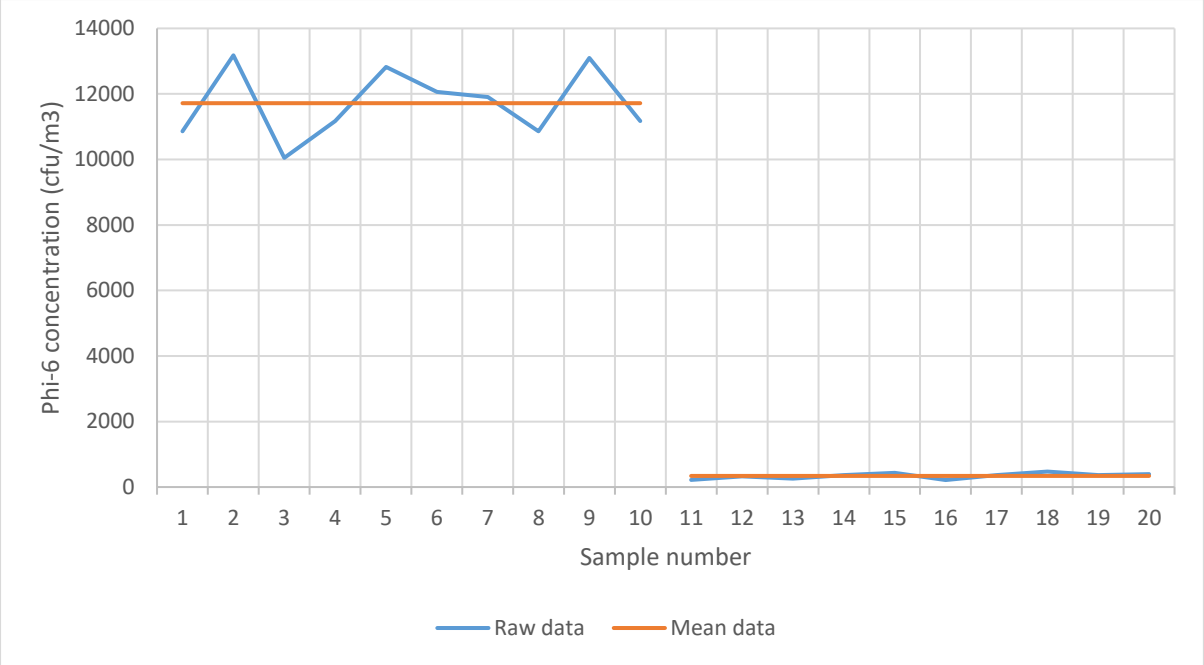

Figure 3 Phi-6 inactivation using aperture 1

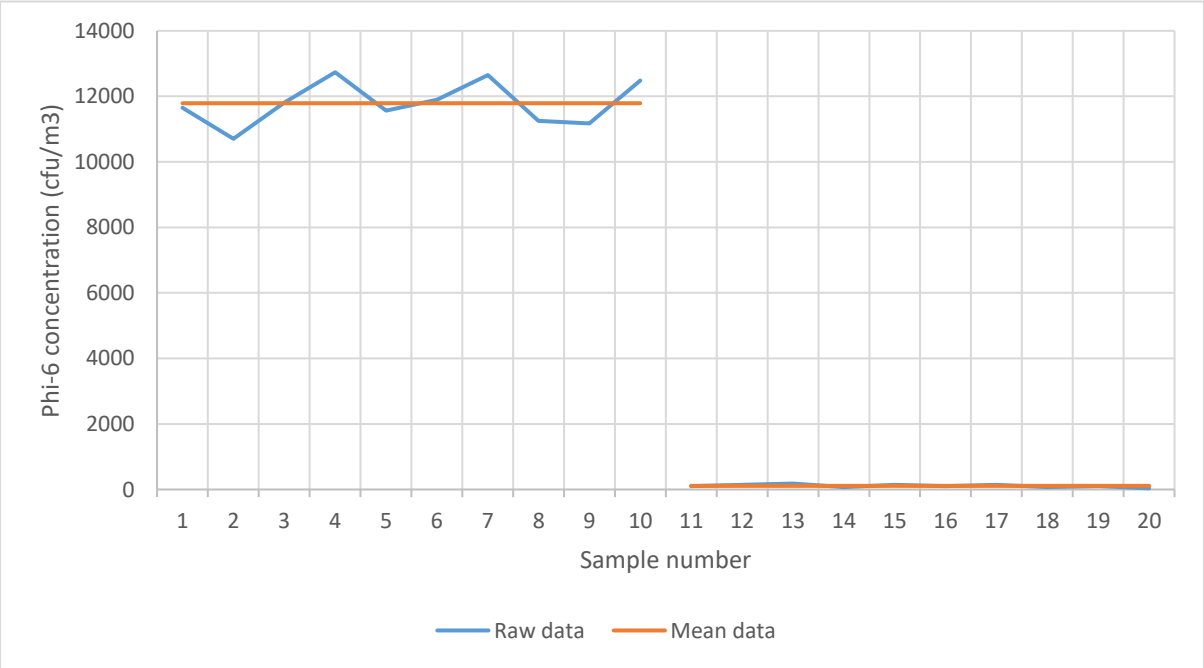

Figure 3 Phi-6 inactivation using aperture 2