Assessment of the Air Sniper Inflow unit in removing airborne Escherichia virus MS2 in a multi-chamber set-up of two 28.5 m^3 environmental test chambers linked via ducting

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Customer Address	3855 64 Ave SE, Calgary, AB T2C 2V5, Canada
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Sample Description	Air Sniper Inflow Air Purifier
Number of Samples	1
Date of Receipt	05 October 2020
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1. Purpose

To test the Air Sniper Inflow unit against aerosolised *Escherichia* virus MS2 in a multi-chamber setup consisting of two 28.5m^3 environmental test chambers linked via ducting to allow for air to recirculate between the chambers.

2. Test Item Description

The Air Sniper Inflow unit received by airmid healthgroup on 5th October 2020 was installed in an Environmental Test Chamber (Figure 2.1).

Figure 2.1. Air Sniper Inflow unit installed inside the chamber at airmid healthgroup

3. Materials and Methods

3.1. Bacteriophage MS2 (MS2)

Bacteriophage MS2 (MS2) is a non-enveloped virus that infects *Escherichia coli* and some other closely related bacteria but has not been shown to infect eukaryotes. Like SARS-CoV2, MS2 is a single-stranded RNA virus. However, at approximately 27 nm in diameter, MS2 is much smaller than the 120 nm diameter SARS-CoV-2 virus. Because MS2 has similar aerosol characteristics to human viruses, it is often used in air purifiers and air filtration tests as a surrogate for viruses of similar or larger dimensions [1]. For example, MS2 has been used as a surrogate for Norovirus, including studies where MS2 has been aerosolized [2] and where viral inactivation by ultraviolet light has been assessed [3, 4]. MS2 is one of the bioaerosols recommended for air filtration tests by the EPA [5]. A study of the effect of UV exposure on virus aerosols found that MS2 was more resistant than the murine hepatitis virus (MHV) coronavirus to UV air disinfection [6]. Aerosols of the MHV coronavirus were found to be 7 – 10 times more susceptible than MS2 [6]. Therefore, MS2 is a conservative surrogate for coronaviruses in this type of testing. However, as stated by the FDA: "*…currently there is limited published data about the wavelength, dose, and duration of UVC radiation required to inactivate the SARS-CoV-2 virus*" [7].

Based on the requirements for aerosolisation, the use of ultraviolet light as the antiviral technology and its suitability as a surrogate for some human viral pathogens, MS2 was used as the challenge microorganism in this study.

3.2. 28.5 m^3 Environmental Test Chambers

Testing was conducted using two 28.5 m3 test chambers purpose-built to comply with the American Society for Testing and Materials (ASTM) standard. The test chambers are connected via modular ducting (supply and return) and allow for recirculation of air between the two chambers. Both chambers have HEPA filtered supply air and can maintain selected temperature and humidity levels at a wide range of air change rates. The air change rate in the chambers can be controlled within a range of 0.5 to 20 air changes per hour. The chambers are constructed using powder-coated stainless steel with all materials complying with low volatile organic compound (VOC) emission requirements. Both chambers comply with cleanroom standards, are sealable from the exterior environment and are accessed via an anteroom with interlocking doors.

Chamber A – upstream, contained the Air Sniper inline unit and supply fan.
Chamber B – downstream, contained a post-exposure duct section (Figure 3.1).

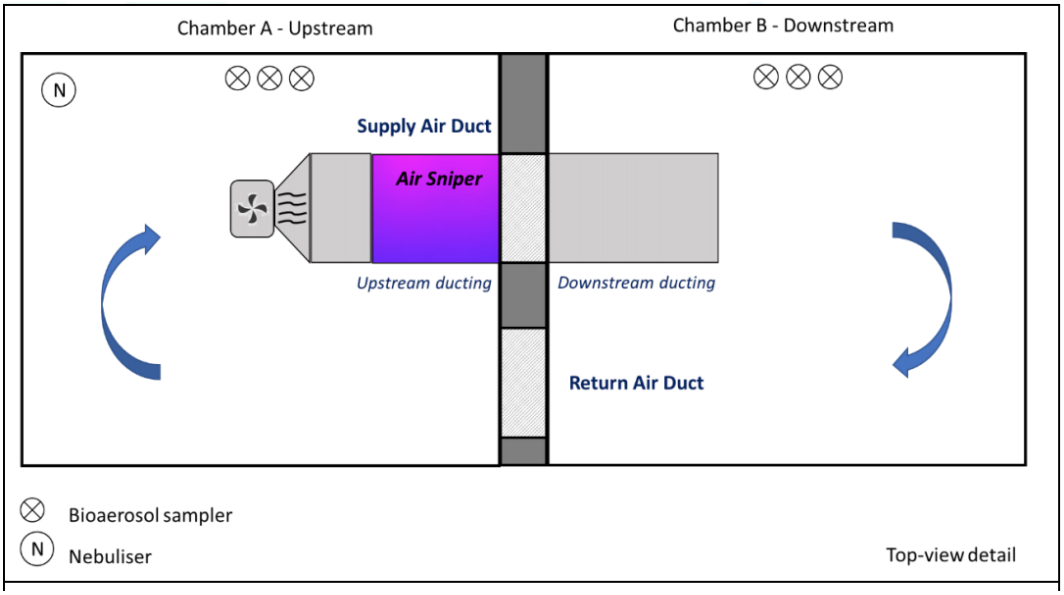

Figure 3.1. Multi-chamber Test Setup for MS2 Testing

4. Procedure

Six decay tests were performed as outlined below using a bespoke duct supplied by Air Sniper:
Active Test: conducted in triplicate *with* the Air Sniper Inflow unit operating
Inactive Control: conducted in triplicate *without* the Air Sniper Inflow unit operating

- The chambers were preconditioned before testing to 20°C (±3°C) and 50% RH (±5%). A UV-C light in the ceiling of the test chambers sterilised the surfaces for 1 h before testing.
- The Air Sniper Inflow unit with a bespoke duct and blower fan was installed in Chamber A and a duct was installed in Chamber B downstream (Figure 3.1). A fan, calibrated to 1200 CFM using a hotwire thermal anemometer (testo 405i, Smart Probe), was used to blow air through the ducting in both test and control runs.
- Background air samples were taken using SKC BioSamplers 1.0 m from the floor at 11 l/m.
- MS2 was aerosolised upstream (into Chamber A) for up to 20 minutes. Mixing fans were operated to promote the homogenous distribution of the aerosol throughout the test chambers.
- To estimate the concentration of the virus after aerosolisation, triplicate air samples (T_0, -5 to 0 minutes) were collected in both test chambers.
- In active test runs, at time zero (i.e., immediately after the T_0 sample was taken) the Air Sniper Inflow unit was operated. The blower fan was turned on 1 minute after the Air Sniper unit began operating. Inactive control runs were conducted identically to the active test but without Air Sniper Inflow unit operating.
- Triplicate air samples were collected from each chamber at the following timepoints:
    - 2.5 to 7.5 minutes ($T_{7.5}$)
    - 10.0 to 15.0 minutes (T_{15})
    - 55.0 to 60.0 minutes (T_{60})
- After each run, all air samples were transferred to the laboratory for analysis. The chambers were cleaned, sterilised using UV-C lights and preconditioned for the next run.

4.1. Sample Analysis

Samples collected from the test chambers were analysed by plaque assay, which assesses the infectivity of the sampled virus. By applying samples to a Petri plate pre-prepared with a lawn of *E. coli*, the concentration of viable virus in that sample can be determined by quantifying the number of plaques formed after incubation. The concentration of infective MS2 virus was denoted as the number of plaque-forming units per cubic meter of air (PFU/m^3). These values were reported logarithmically (Log_{10}). By comparing the concentration of virus in the samples collected in the test runs to the control runs, the efficacy of the device to remove airborne virus from the environmental test chambers over time can be determined.

5. Results

Tables 5.1 & 5.2 summarise the concentration of MS2 measured in the *Upstream* and *Downstream* test chambers in control runs, while Tables 5.3 & 5.4 summarise the test run results. Figure 5.1 presents the average of the triplicate test and control runs in each chamber.

Table 5.1. MS2 in the ***Upstream*** chamber during ***control*** runs (without Air Sniper unit operating)

	PFU/m³					Log₁₀ of Average PFU/m³
	Run 1	Run 2	Run 3	Average	Standard deviation	
T_0	1.50E+08	2.48E+08	5.00E+08	**2.99E+08**	*1.81E+08*	**8.48**
$T_{7.5}$	3.13E+07	2.28E+07	9.39E+07	**4.94E+07**	*3.88E+07*	**7.69**
T_{15}	1.35E+07	2.23E+07	5.55E+07	**3.04E+07**	*2.21E+07*	**7.48**
T_{60}	1.84E+06	5.27E+06	1.79E+07	**8.33E+06**	*8.45E+06*	**6.92**

Table 5.2. MS2 in the ***Downstream*** chamber during ***control*** runs (without Air Sniper unit operating)

	PFU/m³					Log₁₀ of Average PFU/m³
	Run 1	Run 2	Run 3	Average	Standard deviation	
T_0	3.97E+06	2.99E+06	1.06E+07	**5.86E+06**	*4.14E+06*	**6.77**
$T_{7.5}$	6.61E+07	6.48E+07	1.65E+08	**9.85E+07**	*5.72E+07*	**7.99**
T_{15}	8.15E+06	2.87E+07	7.45E+07	**3.71E+07**	*3.40E+07*	**7.57**
T_{60}	4.52E+06	1.02E+07	2.15E+07	**1.21E+07**	*8.63E+06*	**7.08**

Table 5.3. MS2 in the ***Upstream*** chamber during ***test*** runs (with Air Sniper unit operating)

	PFU/m³					Log₁₀ of Average PFU/m³
	Run 1	Run 2	Run 3	Average	Standard deviation	
T_0	2.20E+08	2.27E+08	5.30E+08	**3.26E+08**	1.77E+08	**8.51**
$T_{7.5}$	2.06E+07	3.85E+06	4.23E+07	**2.23E+07**	1.93E+07	**7.35**
T_{15}	5.21E+05	9.36E+05	1.04E+06	**8.33E+05**	2.75E+05	**5.92**
T_{60}	≤LOD	≤LOD	≤LOD	**≤LOD**	N/a	**≤LOD**

Table 5.4. MS2 in the ***Downstream*** chamber during ***test*** runs (with Air Sniper unit operating)

	PFU/m³					Log₁₀ of Average PFU/m³
	Run 1	Run 2	Run 3	Average	Standard deviation	
T_0	9.45E+06	4.76E+07	3.67E+05	**1.91E+07**	2.50E+07	**7.28**
$T_{7.5}$	4.73E+05	2.73E+06	1.24E+05	**1.11E+06**	1.41E+06	**6.04**
T_{15}	≤LOD	1.21E+04	≤LOD	**1.01E+04**	1.75E+03	**4.00**
T_{60}	≤LOD	≤LOD	≤LOD	**≤LOD**	N/a	**≤LOD**

≤LOD: less than the limit of detection (9.09E+03 PFU/m³ or 3.96 Log_{10} PFU/m³) Averages were calculated using the 9.09E+03 PFU/m³ limit of detection
N/a: not applicable

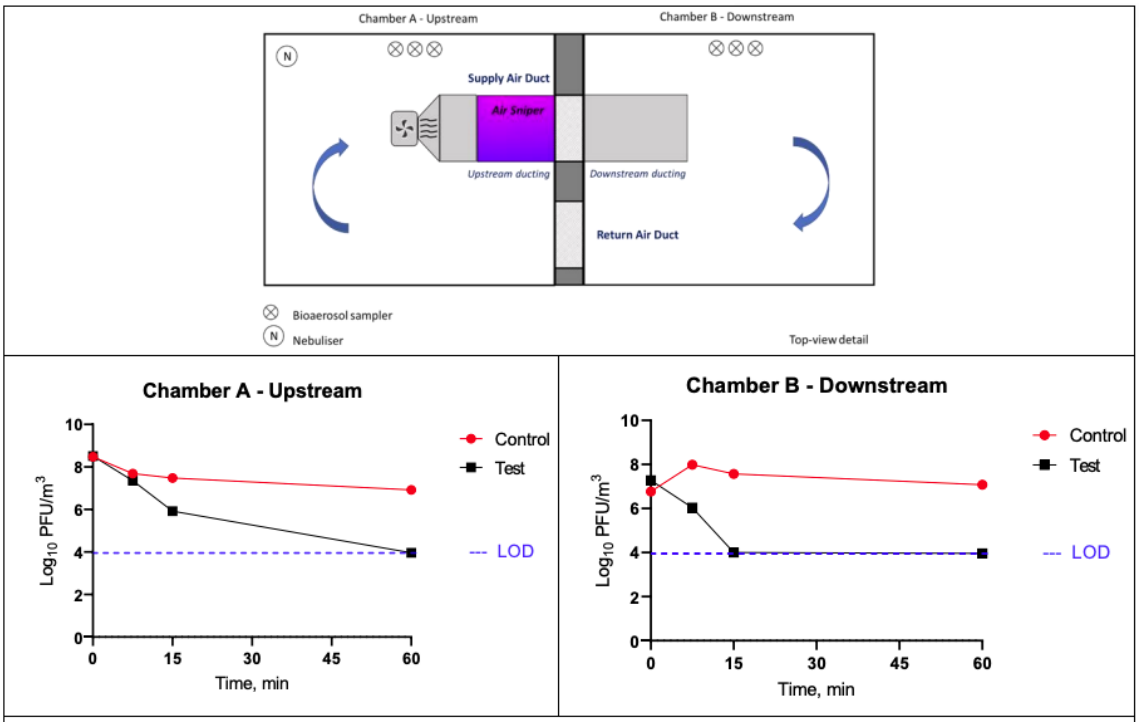

Figure 5.1. Airborne MS2 measured in test chambers A and B.

In chamber **A**, over 60 min in the control runs the concentration of MS2 reduced from an average of 8.48 to 6.92 Log_{10} PFU/m^3, whereas in the test runs MS2 was not detected (limit of detection 3.96 Log_{10} PFU/m^3) after 60 minutes.

In chamber **B**, in the control runs the concentration of MS2 varied between 6.77 Log_{10} PFU/m^3 at T_0 and 7.08 Log_{10} PFU/m^3 recorded for T_{60}. In the test runs, the concentration of MS2 reduced from 7.28 Log_{10} PFU/m^3 recorded at T_0 to 4.00 Log_{10} PFU/m^3 after 15 min and was below the detection limit at T_{60}.

Figure 5.2 presents an average concentration of MS2 (Log_{10} PFU/m^3) in the upstream and downstream chambers for control (Tables 5.1 & 5.2) and test (Tables 5.3 & 5.4) runs.

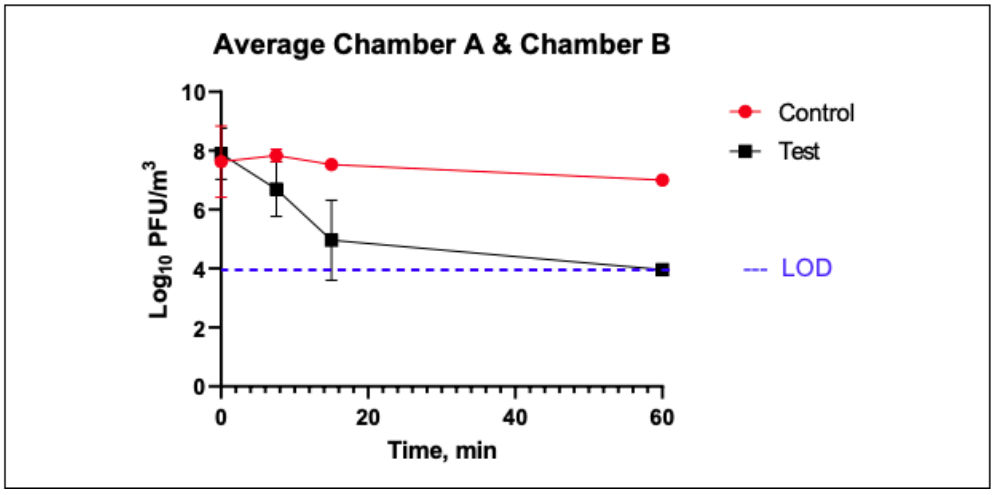

Figure 5.2. The average concentration of airborne MS2 determined in test chambers A and B.
LOD: limit of detection 3.96 Log_{10} PFU/m^3

In the test runs the average concentration of MS2 in chamber A + chamber B was 7.90 ±0.87 at time 0, reduced to 6.70 ±0.92 after 7.5 minutes of Air Sniper Inflow unit operation and 4.96 ±1.36 after 15 min of unit operation. MS2 levels were below the detection limit (LOD ≤3.96 Log_{10} PFU /m^3) after 60 min of Air Sniper Inflow unit operation.
In the control runs the concentration of MS2 reduced by 0.84 Log_{10} units over the 60 minutes.

Table 5.5 presents a sum of the MS2 concentrations in the upstream (Table 5.1) and downstream (Table 5.2) chambers for each control run. Table 5.6 presents a sum of the MS2 concentrations in the upstream (Table 5.3) and downstream (Table 5.4) chambers for each test run. Both tables also include the resulting average/standard deviation and corresponding Log_{10} values. The summed data are also represented in Figure 5.3.

Table 5.5. Sum of the concentrations of MS2 (Chamber A + Chamber B) in ***control*** runs

	PFU/m³					***Log₁₀ of Average PFU/m³***
	Run 1	**Run 2**	**Run 3**	**Average**	*Standard deviation*	
T_0	1.54E+08	2.51E+08	5.11E+08	**3.05E+08**	*1.84E+08*	**8.48**
$T_{7.5}$	9.74E+07	8.77E+07	2.58E+08	**1.48E+08**	*9.59E+07*	**8.17**
T_{15}	2.17E+07	5.10E+07	1.30E+08	**6.76E+07**	*5.60E+07*	**7.83**
T_{60}	6.35E+06	1.55E+07	3.94E+07	**2.04E+07**	*1.70E+07*	**7.31**

Table 5.6. Sum of the concentrations of MS2 (Chamber A + Chamber B) in ***test*** runs

	PFU/m³					***Log₁₀ of Average PFU/m³***
	Run 1	**Run 2**	**Run 3**	**Average**	*Standard deviation*	
T_0	2.29E+08	2.75E+08	5.31E+08	**3.45E+08**	*1.63E+08*	**8.54**
$T_{7.5}$	2.11E+07	6.58E+06	4.25E+07	**2.34E+07**	*1.81E+07*	**7.37**
T_{15}	5.30E+05	9.48E+05	1.05E+06	**8.43E+05**	*2.76E+05*	**5.93**
T_{60}	≤LOD	≤LOD	≤LOD	**≤LOD**	N/a	**≤LOD**

Resulting limit of detection (1.82E+04 PFU/m^3 or 4.26 Log_{10} PFU/m^3).
Averages were calculated using the 1.82E+04 PFU/m^3 limit of detection
N/a: not applicable

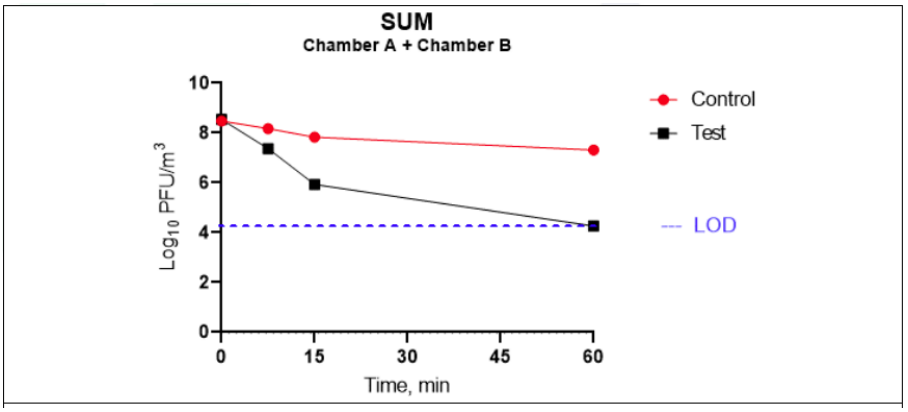

Figure 5.3. Sum of the concentrations of airborne MS2 determined in test chambers A and B.
LOD: limit of detection 4.26 Log_{10} PFU/m^3

When considering the summed concentration of MS2 in both test chambers, over 60 min in the control runs the concentration reduced from an average of 8.48 Log_{10} PFU/m^3 to 7.31 Log_{10} PFU/m^3, while in the test runs it reduced from an average of 8.54 Log_{10} PFU/m^3 to below the limit of detection (4.26 Log_{10} PFU /m^3) (Table 5.3, Figure 5.3).

6. Conclusion

The data in this report demonstrated that the sum concentration of MS2 in both chamber A and B reduced from 8.48 Log10 PFU/m^3 to 7.31 Log_{10} PFU/m^3 after 60 minutes in the inactive control runs, a reduction of 93.3%.

In contrast, in the active test runs with the Air Sniper unit operating the concentration of airborne MS2 reduced from 8.54 Log_{10} PFU/m^3 to less than the limit of detection (4.26 Log_{10} PFU/m^3) after 60 minutes, which corresponds to a reduction of >99.9%.

7. References

[1] John Zhang, Ph.D.; Doug Huntley; Andy Fox; Bryan Gerhardt; Al Vatine; John Cherne. Study of Viral Filtration Performance of Residential HVAC Filters. ASHRAE Journal, Vol. 62, no. 8, August 2020

[2] Tung-Thompson G, Libera DA, Koch KL, de los Reyes FL III, Jaykus L-A (2015) Aerosolization of a Human Norovirus Surrogate, Bacteriophage MS2, during Simulated Vomiting. PLoS ONE 10(8): e0134277. https://doi.org/10.1371/journal.pone.0134277

[3] G.W. Park, K.G. Linden, M.D. Sobsey (2010) Inactivation of murine norovirus, feline calicivirus and echovirus 12 as surrogates for human norovirus (NoV) and coliphage (F+) MS2 by ultraviolet light (254 nm) and the effect of cell association on UV inactivation. Letters in Applied Microbiology (Volume 52, Issue 2, Pages 162-167). https://doi.org/10.1111/j.1472-765X.2010.02982.x

[4] Jung Eun Lee, GwangPo Ko (2013) Norovirus and MS2 inactivation kinetics of UV-A and UVB with and without TiO2. Water Research (Volume 47, Issue 15, Pages 5607-5613). https://doi.org/10.1016/j.watres.2013.06.035

[5] EPA. 2006. “Generic Verification Protocol for Biological and Aerosol Testing of General Ventilation Air Cleaners.” Cooperative Agreement R-83191101. U.S. Environmental Protection Agency

[6] Christopher M. Walker and GwangPyo Ko. Effect of ultraviolet germicidal irradiation on viral aerosols. Environ. Sci. Technol. 2007, 41, 5460–5465 https://doi.org/10.1021/es070056u

[7] UV Lights and Lamps: Ultraviolet-C Radiation, Disinfection, and Coronavirus https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/uv-lights-and-lamps-ultraviolet-c-radiation-disinfection-and-coronavirus

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