Customer Name	Colorobbia Consulting S.r.l
Customer Address	via Pietramarina, 53, Sovigliana-Vinci (FI), 50053, Italia
Contact	Elia Rinaldi
Test Requested	Removal of MS2 Virus in a single pass assessment

		date received	*airmid code*
Sample Description	Photocatalytic polycarbonate filter	5 September 2022	*ASC004328*

Report Number	ASCR092602 V1.1
Report Date	4 October 2022

Contents

1. Purpose

To assess the ability of the Colorobbia Consulting patented photocatalytic polycarbonate filter in removing MS2 virus in a single pass test.

2. Test Item Description

The photocatalytic polycarbonate filter (Figure 2.1) was received by airmid healthgroup on 5 September 2022 (internal airmid code ASC004328).

Figure 2.1. Colorobbia Consulting photocatalytic polycarbonate filter at airmid healthgroup

3. Materials

3.1. Bacteriophage MS2 (MS2)

MS2 is a well-studied surrogate virus that infects *Escherichia coli* and some other closely related bacteria but has not been shown to infect eukaryotes (e.g., animals and humans).

Like SARS-CoV-2 and Influenza A H1N1, MS2 is a single-stranded RNA virus (SARS-CoV-2 and MS2 are positive-sense RNA, and H1N1 is negative-sense RNA). However, at approximately 25 nm in diameter, MS2 is much smaller than the 120 nm diameter SARS-CoV-2 virus and the 80 – 120 nm diameter H1N1 virus.

All viruses consist of genetic material within a protein capsid that acts as a shell to protect the DNA or RNA genome. Influenza viruses and Coronaviruses have an additional protective layer made up of a lipid membrane known as an envelope. By contrast, MS2 is nonenveloped. Studies have found that in wastewater, enveloped viruses are inactivated faster than most nonenveloped viruses [1]. Nonenveloped viruses such as MS2 also remain infectious on surfaces for longer periods than enveloped viruses like influenza [2].

Due to its viability and resistance (relative to enveloped viruses) to disinfection, the nonenveloped MS2 is frequently used as a surrogate for enveloped viruses in such infection control studies, where it is considered to represent a “worst case scenario” [3].

Because MS2 has similar aerosol characteristics to human viruses, it is often used in air purifier and air filtration tests as a surrogate for viruses of similar or larger dimensions [4]. For example, MS2 has been used as a surrogate for Norovirus, including in studies where MS2 has been aerosolised [5] MS2 is one of the bioaerosols recommended for air filtration tests by the EPA [6].

3.2. 28.5 m^3 Environmental Test Chambers

Testing was conducted using two 28.5 m^3 test chambers (Figure 3.1) purpose-built to comply with the American Society for Testing and Materials (ASTM) standard. The test chambers are connected via modular ducting. 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 emission requirements. Both chambers comply with cleanroom standards, are sealable from the exterior environment and are accessed via an anteroom with interlocking doors.

- *Chamber B* – upstream, contained the polycarbonate filter and blower fan.
- *Chamber A* – downstream, contained a post-exposure duct section.

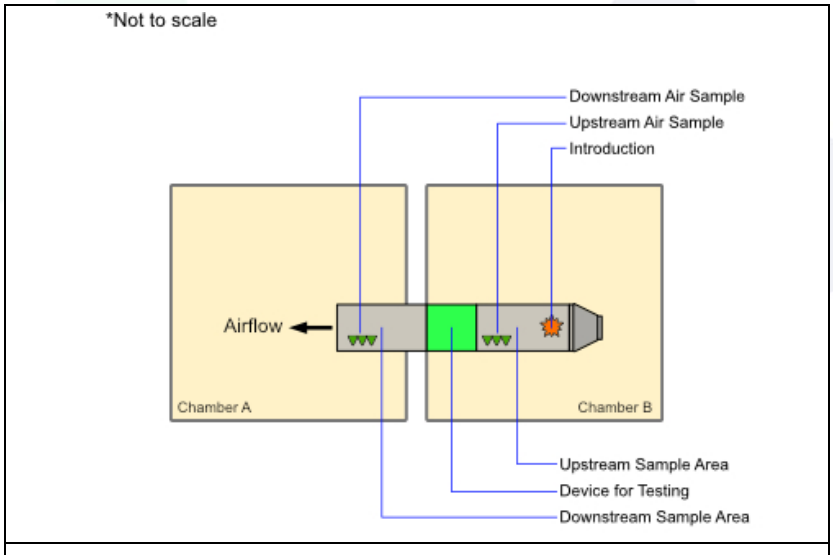

Figure. 3.1. Chamber configuration for the single-pass assessment

3.3. Test Conditions

All testing was conducted in environmental test chambers preconditioned to 20 ± 3°C and 50 ± 5% relative humidity before each test.

4. Procedure

4.1. Test set up

- For the **active test** runs only, the polycarbonate filter was installed and sealed into the test duct (Figure 3.1). The LED lights proximal to the polycarbonate filter were operated.
- A blower fan was operated at a speed of 2.6 m/s.
- MS2 virus was aerosolised into the test duct upstream of the polycarbonate filter.
- Air was sampled from the upstream and downstream sections of the duct simultaneously.
- At the end of each run, all samples were transferred to the laboratory for analysis.
- The environmental test chambers and test ducting duct were decontaminated with UV lights before starting the next run. The air within the test chamber was ventilated using HEPA-filtered air and the chamber was preconditioned.
- Inactive control runs were conducted identically, except the Colorobbia polycarbonate filter was not installed into the test duct.
- Both the active test runs, and inactive control runs were performed in triplicate
- The single-pass efficiency of the filter to remove airborne virus was determined by comparing the concentration of the virus in the upstream and downstream sections of the ducting in both the active test runs and the inactive control runs.

4.2. Sample Analysis

Samples were analysed by plaque assay, which assesses virus infectivity. By applying samples to a pre-prepared lawn of *E. coli,* the concentration of infectious virus in that sample can be determined by quantifying the number of plaques formed after incubation. The concentration of infective MS2 virus is denoted as the number of plaque-forming units per cubic meter of air (PFU/m^3). These values are reported logarithmically (Log_{10}).

5. Results

The concentrations of MS2 virus recovered in inactive control and active test runs in both upstream and downstream ducts are presented in Table 5.1 below. The table also includes the percentage reduction in the concentration of virus recovered downstream relative to that recovered upstream.

Table 5.1. MS2 Virus in inactive control runs and active test runs

		MS2 Bacteriophage (PFU/m^3)				*Standard Deviation*	**Log_{10} of Average**	**% Reduction** *(downstream vs upstream)*
		Run 1	Run 2	Run 3	**Average**			
Control *(No Filter)*	*Upstream*	1.98E+08	3.69E+08	8.89E+07	**2.19E+08**	±1.41E+08	**8.34**	*N/a*
	Downstream	1.31E+08	2.18E+08	7.51E+07	**1.41E+08**	±7.20E+07	**8.15**	**35.3%**
Test *(Filter installed, Light On)*	*Upstream*	5.31E+08	3.19E+08	4.06E+08	**4.19E+08**	±1.07E+08	**8.62**	*N/a*
	Downstream	1.97E+08	1.56E+08	1.51E+08	**1.68E+08**	±2.51E+07	**8.23**	**59.9%**

6. Conclusion

The single-pass efficiency testing of the Colorobbia polycarbonate filter demonstrated the following:

- In the inactive control runs (no polycarbonate filter present) the concentration of MS2 virus was 8.34 Log_{10} upstream and 8.15 Log_{10} downstream, a reduction of **35.3%.**
- In the active test runs (polycarbonate filter present with LED operating) the concentration of MS2 virus was 8.62 Log_{10} upstream of the filter and 8.23 log_{10} downstream, a reduction of **59.9%.**
- When considering the decay observed in the control runs, the overall reduction due to the Colorobbia polycarbonate filter is **24.6%.**

7. References

[1]. Yinyin Ye, Robert M. Ellenberg, Katherine E. Graham, and Krista R. Wigginton. Survivability, Partitioning, and Recovery of Enveloped Viruses in Untreated Municipal Wastewater. Environmental Science & Technology 2016 50 (10), 5077-5085.

[2]. Boone, S. A., & Gerba, C. P. (2005). The occurrence of influenza A virus on household and day care center fomites. Journal of Infection, 51, 103-109.

[3]. Fisher E, Shaffer R. Survival of Bacteriophage MS2 on Filtering Facepiece Respirator Coupons. Applied Biosafety. 2010;15(2):71-76

[4]. 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

[5]. 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

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

Report written by:

Dana Ziuzina, PhD

Senior Laboratory Scientist

Report reviewed by:

Vivienne Mahon

Digitally signed by Vivienne Mahon
Reason: I have reviewed this document
Location: airmid healthgroup
Date: 2022.10.12 16:49:14 +01'00'

Vivienne Mahon, PhD.

Chief Scientist/Quality Director

End of Report