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Test Requested	To assess the impact of the air purifier on Influenza A (H1N1) virus in a decay test
Sample Description	Roger Little
Number of Samples	1
Date of Receipt	17 September 2020
ASC Code	ASC004019
Report Number	ASCR092436
Report Date	07 December 2020

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1. Purpose

This report outlines the results following the assessment of the Stadler Form Roger Little air purifier in removing airborne Influenza A (H1N1) from a 28.5 m³ environmental test chamber.

2. Test Item Description

The Roger Little air purifier was sent by Stadler Form to **airmid healthgroup** and was received on 17th September 2020 (Figure 2.1).



Figure 2.1. Stadler Form Roger Little air purifier tested at **airmid healthgroup**

3. Materials and Methods

3.1. Materials

- Influenza A (H1N1; A/PR/8/34)
- Influenza A Virus Capture ELISA
- Influenza A Virus Transport Medium

3.2. Influenza A

Influenza virus infection is one of the most common, highly contagious infectious diseases and it can occur in people of any age. Influenza A viruses are transmitted through direct contact, indirect contact, large respiratory droplets and aerosols (droplets nuclei).

Influenza viruses belong to the Orthomyxoviridae family and are divided into types A, B and C. Influenza types A and B are responsible for epidemics of respiratory illness that are often associated with increased rates of hospitalization and death. During the 20th century, the only influenza A subtypes that circulated extensively in humans were (H1N1) Spanish Flu; (H1N2); (H2N2) Asian Flu; and (H3N2) Hong Kong Flu. A new strain of influenza A, H1N1 emerged in 2009 called 'Swine Flu' as it originated in swine and spread to humans. More

recently in 2013, a new strain of Avian Influenza A, H7N9 has infected people in China and is believed to be from exposure to infected poultry.

All known subtypes of influenza type A viruses have been isolated from birds and can affect a range of mammalian species. As with humans, the number of influenza A subtypes that have been isolated from other mammalian species is limited. Influenza type B viruses almost exclusively infect humans.

In this case, the influenza type A virus has been used for the testing.

4. Protocol

4.1. Test Conditions

Testing of the Stadler Form Roger air purifier was conducted in a 28.5 m³ environmental test chamber. The chamber was preconditioned to 20 °C (± 3 °C) and 55 % (± 5 %) relative humidity before the commencement of the tests. After each run, the chamber was sterilised by operating a UV germicidal lamp, installed in the ceiling of the chamber, for at least 60 minutes. The air was extracted from the test chamber through HEPA filters, and fresh HEPA filtered air was resupplied. The chamber was then cleaned by washing with 5% Virkon multi-purpose disinfectant solution.

4.2. Air Purifier Control and Test Runs

Six decay tests were performed in the environmental chamber consisting of:

- Three inactive control runs without the air purifier
- Three active test runs with the Roger Little air purifier operating at the max airflow

For the active test runs the air purifier was placed on the floor in the centre of the chamber.

For the inactive control runs, the same procedure was performed except in the absence of the air purifier. Three replicates per sample timepoint were collected during each run.

In both the active and inactive runs, viable Influenza A virus was aerosolised into the chamber for up to 20 minutes. The amount of Influenza A aerosolised was dependent on the virus stock used, however, 100 - 200 µg of virus antigen was introduced into the test chamber for each run. The viral aerosol was mixed in the chamber by a ceiling fan, which was operating at low speed for the duration of the test.

4.3. Sampling Time Points

Three SKC BioSamplers collected air samples at 1 m height for 10 minutes at a rate of 11.8 l/min at the following time points:

- -10 to 0 min (AS-1)
- 05 to 15 min (AS-2)
- 20 to 30 min (AS-3)
- 50 to 60 min (AS-4)

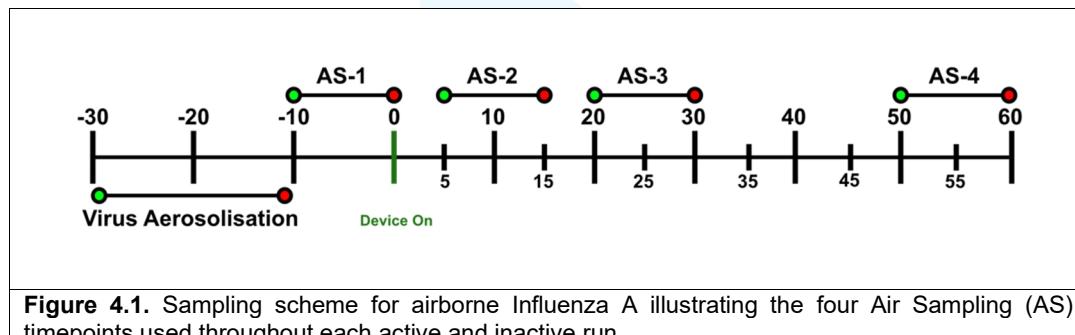


Figure 4.1. Sampling scheme for airborne Influenza A illustrating the four Air Sampling (AS) timepoints used throughout each active and inactive run.

For the active test runs, the air purifier was operated remotely at $t = 0$ minutes and remained operating for the duration of the test (Figure 4.1). At the end of the test, the samples were removed from the BioSamplers and transferred to sterile 40 ml tubes that were immediately placed on ice and then stored in the laboratory at -20°C until analysis.

4.4. Sample Analysis

Influenza A quantification was performed by ELISA. The ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique that uses antibodies with high specificity to detect and quantify substances, such as peptides and proteins, called antigens. The NCP-ELISA validated at **airmid healthgroup** detects and quantifies Influenza A nucleoprotein (NPA). In this report, the abbreviation "**Inf A**" is used to refer to the virus quantified by the ELISA detection of the **NPA antigen**. The concentration of Inf A in each sample is reported in this report as ng per m^3 of sampled air.

Virus reduction percentage was calculated according to the formula below:

$$\% \text{ Virus Reduction} = 100 - \frac{\text{Inf A ng/m}^3 \text{ with device operating } (t = tx \text{ min})}{\text{Inf A ng/m}^3 \text{ without device operating } (t = 0 \text{ min})} \times 100$$

** tx = sample timepoint*

5. Results and Discussion

The recovery concentrations of Inf A in the three inactive control runs and the three active test runs are reported in Tables 5.1 and 5.2. Each result is the average of three replicates sampled at the indicated time. The Inf A concentration was determined by ELISA in units of ng/ml and then converted into ng/m³, i.e. nanograms of Influenza A per cubic metre of air sampled by the SKC BioSamplers.

Table 5.1. Average Influenza A concentration measured in the inactive control runs (ng/m³)

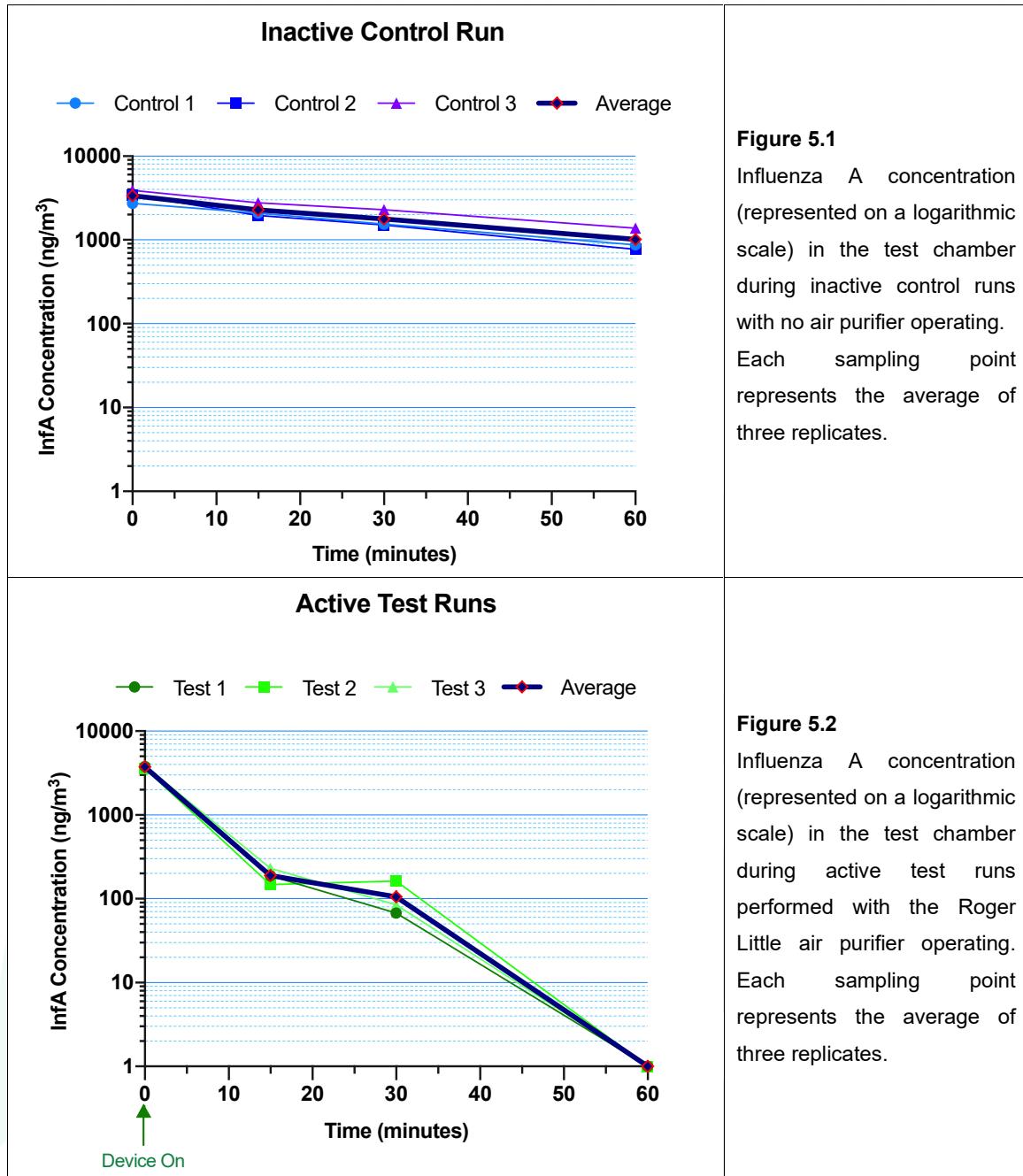
Timepoint	Control 1	Control 2	Control 3	Average (n=3)
-10 – 0	2729.1	3447.4	3900.1	3358.9
5 – 15	2099.3	1973.9	2770.5	2281.2
20 – 30	1546.7	1511.5	2294.5	1784.2
50 – 60	874.9	773.3	1386.7	1011.6

Table 5.2. Average Influenza A concentration measured in the active test runs (ng/m³)

Timepoint	Test 1	Test 2	Test 3	Average (n=3)
-10 – 0	3800.9	3594.9	3898.4	3764.7
5 – 15	188.4	147.5	231.5	189.1
20 – 30	67.5	164.0	85.1	105.5
50 – 60	<LOD	<LOD	<LOD	<LOD

<LOD: Less than the limit of detection

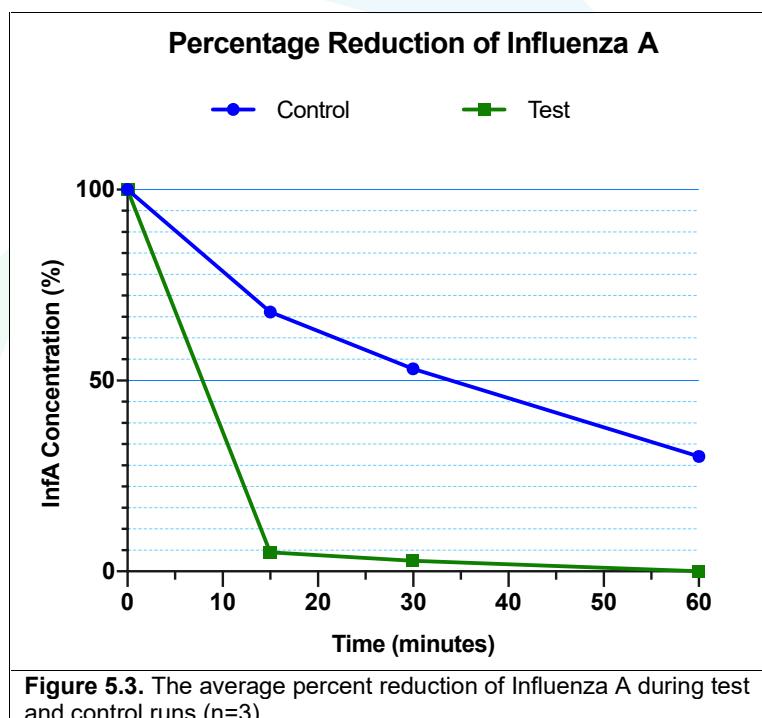
Figures 5.1 and 5.2 show the trend of Inf A levels over time in the three control and test runs, respectively. The rapid reduction in Inf A concentration observed in the test runs (Figure 5.2) could not be attributed to natural decay due to forces exerted on the virus particles, i.e. inertia, diffusion. In the three test runs, between 50 and 60 minutes of the air purifier operating, the Influenza A concentration had dropped below the detection limit of the assay used to quantify the virus. The differences among the same sampling time points in the runs can be ascribed to the virus stock used to perform the runs and the sampling process itself. As reported by Fabian et al. in 2009, for laboratory studies SKC BioSamplers represent the most efficient airborne virus particle sampling tool in terms of virus infectivity preservation and collection efficiency. Despite this, the BioSampler recovery efficiency is about 79% for particles sized > 0.3 µm, which may lead to variation in the collected concentrations of Inf A particles sized ~ 0.1 µm.



The data presented here show that within the first 60 minutes of the Stadler Form Roger Little air purifier operating at the highest fan speed, the Influenza A concentration in the test chamber was reduced to less than 0.156 ng/ml (the detection limit of the assay performed to quantify the collected airborne virus).

Figure 5.3 shows the percentage reduction in Inf A levels (calculated per the formula cited above in Section 4.4) during the control and test runs. Fluctuations in virus concentration were observed

during control runs. Statistical fluctuations are unavoidable, especially for a test like the one described in this report. Several factors affect the outcome of the result. The sampling process and the assay bring variability, and one must not forget that the virus, adapted to the ideal 'survival' environment of the human body, is aerosolized into an indoor space with certain physical characteristics, where physical forces such as inertia and diffusion are applied on the viral particles throughout the test duration (Hind 1999, U.S. EPA 2010, Lee et al. 2011). The aerosolised virus may also adhere to the chamber surfaces after a certain period or move to areas of the chamber with a lower or null concentration of virus, with a consequent variation in the number of particles collected by the SKC BioSamplers over an extended period. In contrast, a 99.9% decrease in Inf A levels is observed in the test runs 60 minutes after the air purifier is turned on.



6. Conclusion

The Stadler Form Roger Little air purifier was demonstrated to be effective in reducing airborne Influenza A aerosols in the test chamber, achieving 99.9 % airborne virus reduction after 60 minutes of operation at the highest air flow rate. These results indicate that in the presence of an operational unit the Influenza A concentration in the test chamber was reduced to levels below 0.156 ng/ml, the detection limit of the assay performed to quantify the collected airborne virus.

7. References

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End of Report