Customer Name	Novaerus (Ireland) Ltd.
Customer Address	DCU Alpha, Old Finglas Road, Glasnevin, Dublin 11
Contact	Felipe Soberon
Test Requested	To assess the impact of the Air Cleaner on Influenza A virus in a decay test
Sample Description	Novaerus air cleaner device (NV1050) & x3 replacement filters (Ozone filter, Kompaktfilter & Megalam panel filter)
Number of Samples	1
Date of Receipt	06/04/2018
ASC Code	ASC003569
Report Number	ASCR092274
Report Date	25/04/2018

Contents

1. Purpose

This report assesses the performance of the Novaerus (NV1050) air purifier in removing Influenza A from a sealed test chamber.

2. Test Item Description

The Novaerus (NV1050) air purifier was received in airmid healthgroup on 06/04/2018 (Figure 2.1).

Figure 2.1. Novaerus (NV1050) 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 and highly contagious infectious diseases and 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 influenza type A virus has been used for the testing.

4. Protocol

4.1. Test Conditions

Testing of the Novaerus (NV1050) air purifier, called A/C in this report, was conducted in a 28.5 m^3 environmental test chamber. The chamber was preconditioned to 20±3ºC and 50±10% relative humidity prior to commencement of the tests. After each run the chamber was sterilized by operating a UV germicidal lamp, which is fitted in the ceiling of the chamber, for at least 60 min followed by a full dump of the air from the chamber. The chamber was then decontaminated by washing with 5% Virkon multi-purpose disinfectant solution. Circulation of HEPA-filtered conditioned air in the chamber was allowed to proceed until the next experimental run.

4.2. Air Cleaner Control and Test Runs

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

- Three control runs with the A/C off.
- Three test runs with the A/C operating at max speed.

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

For the control runs, the procedure was performed in the absence of the air cleaner. Three or two replicates per sample time point were collected during each run.

In each control or test run, Influenza A viable virus was aerosolized into the chamber for 30 minutes. About 100 µg of virus were introduced for each run in the chamber. The aerosol was mixed in the airstream of the ceiling fan during the nebulization.

4.3. Sampling Time Points

Three SKC BioSamplers collected air samples at 1 m height for 10 minutes at a rate of 12.5 l/min at the following time points:

The first sampling point was taken during the last 10 minutes of the 30 minutes of virus aerosolisation (-10 – 0 min).

i. -10 – 0 min
ii. 10 – 20 min
iii. 20 – 30 min
iv. 30 – 40 min
v. 40 – 50 min

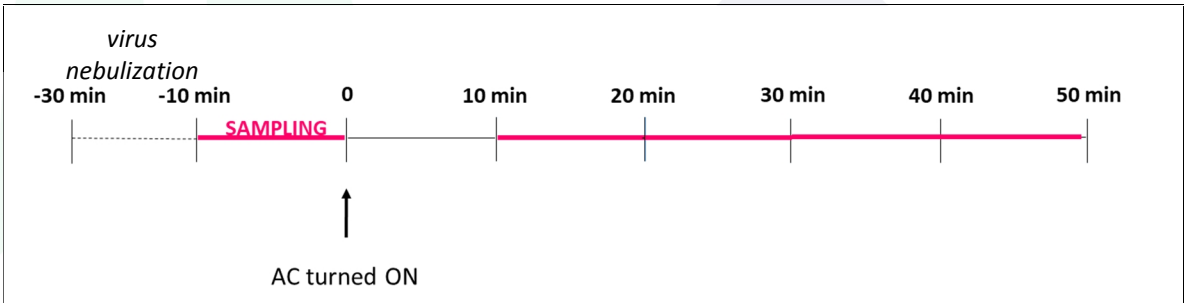

Figure 4.1. Sampling scheme. Sampling time points are indicated by pink lines.

For the test runs, the A/C was switched on at t = 0 minutes and was operated for the duration of the run post-virus introduction (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 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:

$$\textbf{\% Virus Reduction} = 100 - \frac{\text{InfA ng/m}^3 \text{ with A/C operating (t = t}x\text{)}}{\text{InfA ng/m}^3 \text{ without A/C operating (t = 0 min)}} \times 100$$

tx = 0, 20, 30, 40 or 50 min, depending on the time point

The sampling pump activity was checked with a rotometer before and after each run to ensure the sampling air volume was constant.

5. Results and Discussion

The recovery concentrations of Inf A in the three control runs (without the A/C operating) and in the three test runs (with the A/C operating) are reported in Tables 5.1 and 5.2. Each result is the average of three replicates sampled at the indicated time, unless specified otherwise. The Inf A concentration was determined by ELISA in units of ng/ml and then converted into ng/m^3, i.e. nanograms of Inf A per cubic metre of air sampled by the SKC BioSamplers.

Table 5.1. Average Inf A concentration detected in the control runs (SD: standard deviation).

	Inf A ng/m^3		
	Control 1	**Control 2**	**Control 3**
-10 – 0 min	2388.00[(2)] SD=725.21	1948.27 SD=335.33	1928.00 SD=426.70
10 – 20 min	1650.40[(2)] SD=69.01	1502.40[(2)] SD=24.89	2113.60[(2)] SD=260.22
20 – 30 min	1404.80[(2)] SD=88.25	1131.20[(2)] SD=554.37	1408.80[(2)] SD=69.01
30 – 40 min	1154.67 SD=94.04	1505.07 SD=21.13	1200.00 SD=90.67
40 – 50 min	898.40 SD=342.80	1289.60 SD=632.95	813.33 SD=213.48

[(2)] This result is based on the average of two replicates

Table 5.2. Average Inf A concentration detected in the test runs (SD: standard deviation).

	Inf A ng/m^3		
	Test 1	**Test 2**	**Test 3**
-10 – 0 min	2456.00 SD=359.94	3028.27 SD=314.94	2823.20[(2)] SD=48.65
10 – 20 min	<LOD[(2)]	<LOD[(2)]	<LOD[(2)]
20 – 30 min	<LOD[(2)]	<LOD[(2)]	<LOD[(2)]
30 – 40 min	<LOD	<LOD	<LOD
40 – 50 min	<LOD	<LOD	<LOD[(2)]

[(2)] This result is based on the average of two replicates
<LOD=below the limit of detection (1.28 ng/m^3)

Figures 5.1 and 5.2 show the trend of Inf A levels over time in the three control and test runs, respectively. During the control runs (Figure 5.1) a slight decay in Inf A concentration was observed. In contrast 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, after 10 to 20 minutes of A/C operation, the Inf A concentration had dropped below the detection limit of the assay used to quantify the virus. The slight differences in Inf A concentration among the same sampling time points in the runs can be ascribed to 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.

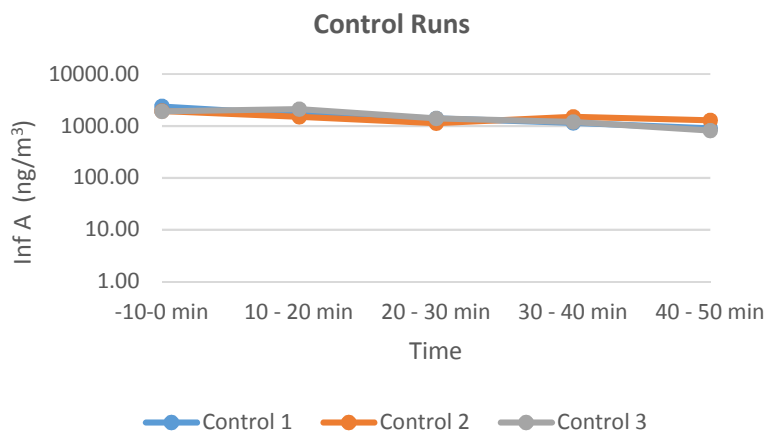

Figure 5.1
Influenza A concentration (represented on a logarithmic scale) in the test chamber during control runs performed without the A/C operating. Each sampling point represents the average of three replicates (unless specified otherwise, see Table 5.1).

Figure 5.2
Influenza A concentration (represented on a logarithmic scale) in the test chamber during test runs performed with the A/C operating. Each sampling point represents the average of three replicates (unless specified otherwise, see Table 5.2).

The data presented here show that within the first 20 minutes of Novaerus (NV1050) operating at max speed, the Influenza A concentration in the test chamber was reduced to less than the limit of detection of the assay (<0.156 ng/ml).

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 from 30 to 50% were observed during control runs. Statistical fluctuations are unavoidable, especially for a test like the one described in this report and the 'linearity' of the control runs can hardly be controlled. Several factors affect the outcome of the final result. The sampling process and the assay bring their own variability, and one must not forget that the virus, adapted to the ideal 'survival' environment of the human body, is nebulised 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 nebulised virus may also adhere to the chamber surfaces after a certain period of time or move to areas of the chamber with lower or null concentration of virus, with a consequent variation in the number of particles collected by the SKC BioSamplers over an extended period of time. In contrast a 99.9% decrease in Inf A levels is observed in the test runs at the first sampling time after the Air Cleaner is turned on.

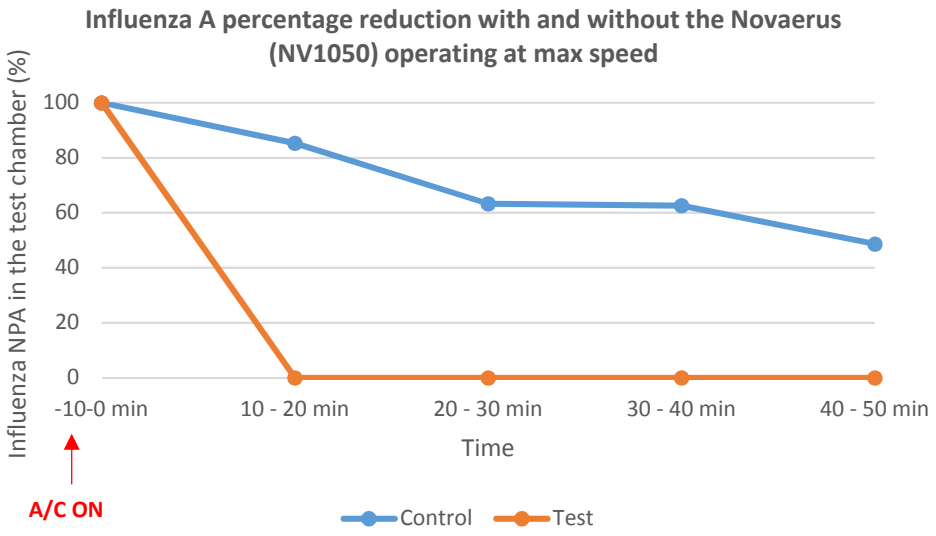

Figure 5.3 Average Influenza A percentage reduction during test and control runs (n=3).

6. Conclusions

The Novaerus (NV1050) was demonstrated to be effective in reducing airborne Influenza A aerosols in the test chamber, reaching 99.9% airborne virus reduction within the first 10 - 20 minutes of operation at max speed. Influenza A was not detectable by ELISA in the samples collected after 20 minutes of Novaerus (NV1050) operation. These results indicate that in the presence of an operational unit the Influenza A concentration in the test chamber was reduced to levels below the detection limit of the assay performed to quantify the collected airborne virus.

7. References

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End of Report