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 an air purifier on Human parainfluenza virus Type 3
Sample Description	Novaerus air cleaner device (NV1050) & replacement filters (3 x HEPA, 3 x Carbon and 3x pre filters)
Number of Samples	1 NV1050 and 3 sets of replacement filters
Date of Receipt	06 April 2018 (air purifier) 01 May 2019 (filters)
ASC Code	ASC003569 and ASC003752
Report Number	ASCR092355v2
Report Date	30 October 2019

Contents

1. Purpose

This report evaluates the ability of the NV1050 air purifier, manufactured by Novaerus, to remove Human parainfluenza virus type 3 (HPIV3) (renamed human respirovirus 3), a surrogate for Measles virus, when aerosolised into a 28.5 m^3 environmental test chamber.

2. Background

Human parainfluenza virus 3 (human respirovirus 3, HPIV3) was used as a surrogate for Measles morbillivirus (MeV). MeV causes measles disease in humans. Measles is one of the most contagious diseases known. The basic reproduction number, which indicates the number of secondary cases that can be generated from an index case in a susceptible population, is estimated to be more than 15 and could be much higher. MeV infections are characterized by a maculopapular rash, dry cough, coryza, fever, conjunctivitis, and photophobia. The virus is transmitted via aerosol droplets. For safety reasons, this virus cannot be aerosolised and therefore the use of a surrogate is suggested.

HPIV3 belongs to the same family and subfamily as MeV, i.e. *Paramyxoviridae* and *Orthoparamyxovirinae* (Figure 2.1). All paramyxoviruses are enveloped particles 150 to 300 nm in diameter. The tube-like, helically symmetrical nucleocapsid contains a monopartite, single-stranded, negative-sense RNA genome and an RNA-directed RNA polymerase. The nucleocapsid associates with the matrix protein at the base of a double-layered lipid envelope. The spikes on the envelope contain two glycoproteins, a viral attachment protein, and a fusion protein.

The paramyxoviruses can be distinguished by the gene order for the viral proteins and by the biochemical properties for their viral attachment proteins. In parainfluenza viruses, the viral protein spikes have hemagglutinating and neuraminidase activities. MeV lacks neuraminidase but has hemagglutinating activity. Paramyxoviruses tend to be labile and can be rapidly inactivated, e.g. by heat, organic solvents, detergents, ultraviolet, or visible light, and low pH value.

On the basis of the above description and the nature of testing, it is believed that HPIV3 represents a valid surrogate candidate for MeV.

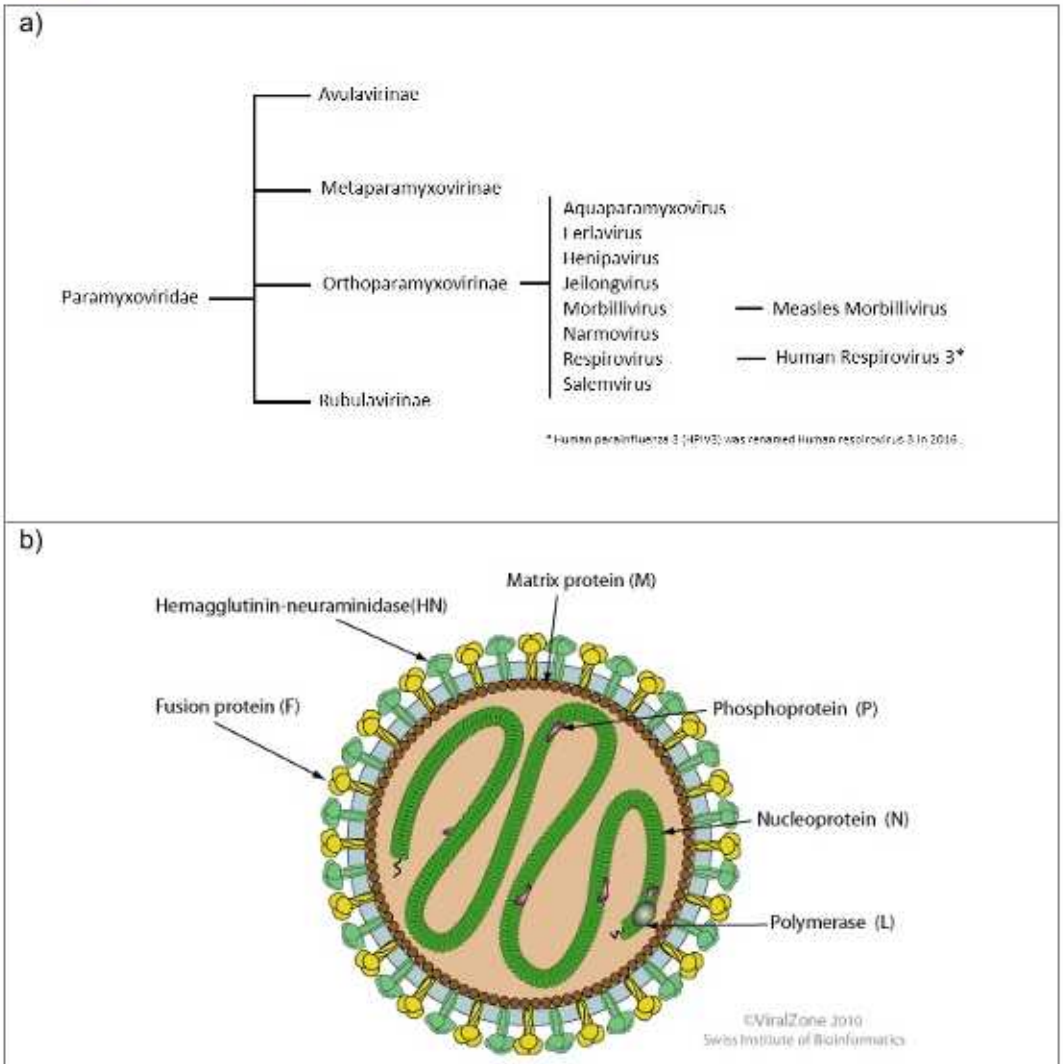


Figure 2.1 a) Classification of representative members of the paramyxovirus family. b) *Paramyxoviridae* structure scheme.

3. Test Item Description

The Novaerus (NV1050) air purifier was received by **airmid healthgroup** on 06 April 2018 (Figure 3.1). Replacement filters (3 x HEPA, 3 x Carbon and 3 x pre filters) were received by **airmid healthgroup** on 01 May 2019.

Figure 3.1. Novaerus (NV1050) air purifier tested at **airmid healthgroup**

4. Protocol

4.1. Test conditions:

4.1.1. The impact of the Novaerus (NV1050) air purifier on aerosolised HPIV3 (strain MK-3) was assessed in the 28.5 m^3 environmental test chamber.

4.1.2. The test chamber was pre-conditioned to 20 ± 3 °C and 55 ± 5% relative humidity. During testing the chamber air handling unit was shut down, which reduces the number of air changes to as close to zero as possible.

4.2. Test Procedure & Analysis:

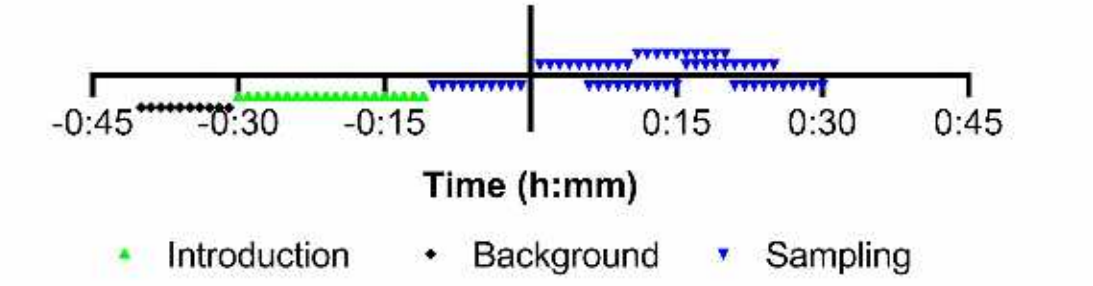
4.2.1. A total of 6 runs were performed to test the impact of operating the Novaerus (NV1050) air purifier on aerosolised HPIV3 (3 Test and 3 Control runs).

4.2.2. During the test runs the air purifier was placed in the centre of the test chamber and operated at full speed mode. During the control runs the air purifier was switched off.

4.2.3. HPIV3 stock was nebulised into the chamber.

4.2.4. Duplicate air samples were collected at each timepoint. The sampling points for both test and control runs were as follows:

Air Sampling Details	Timepoint (min)	Duration (min)
Background	Prior to nebulisation	10
Nebulise	-30 to -10	20
Sample	-10 to 0	10
Air Purifier Switched-On (Test Runs)		
Sample	0 to 10	10
Sample	5 to 15	10
Sample	10 to 20	10
Sample	15 to 25	10
Sample	20 to 30	10



4.2.5. For the test runs, the air purifier was turned on remotely at t=0 min and operated throughout the test period.

4.2.6. After each run, the test chamber was decontaminated by exposing the walls and floor to 5% Virkon and rinsing with water. The chamber surfaces were then exposed to UV lamps for at least 120 mins with full air dump.

4.2.7. Viral RNA was extracted from samples using the QiaAMP Viral RNA mini kit (Qiagen). The RNA was quantified using the GeneSig HPIV3 Standard kit and Precision Plus 2x RT-qPCR Master Mix. qPCR was performed using the LightCycler Nano (Roche).

4.2.8. Analysis of qPCR data was performed using the LightCycler Nano software, which utilized a standard curve to quantify HPIV3 RNA copy number per µl of sample. The mean was taken of the sample replicates. From this, the total HPIV3 RNA copy number in each sample was obtained, taking into account the volume of original sample and the volumes used in the RNA extraction process. The mean total RNA copy number/m^3 of air was then calculated. Values entered as "<LOD" in Tables 5.1 and 5.2 below were treated as "0" for the graph and for statistical analyses.

4.2.9. The percentage reduction in total RNA copy number/m^3 in the test runs was calculated from the mean data obtained at each time-point (tx) and expressed as a % of the mean data obtained at $t0$ using the following formula:

$$\%\ Reduction = \left(\frac{(RNA\ copy\ no./m^3 t0 - RNA\ copy\ no./m^3 tx)}{RNA\ copy\ no./m^3\ t0}\right) \times 100$$

5. Results and Discussion:

The performance of the Novaerus (NV1050) air purifier at removing aerosolised HPIV3 from the test chamber was assessed using qPCR and the average of the total HPIV3 RNA copy number/m^3 of air was calculated. The results for each control run, without the air purifier, and for each test run, with the air purifier operating, are presented in Tables 5.1 & 5.2, respectively. The mean results from the three runs were obtained and log transformed as shown in the last column of each table.

Table 5.1. Total HPIV3 copy number/m^3 recovered from the three control runs

TIME	CONTROL 1	CONTROL 2	CONTROL 3	MEAN	Log_{10}
-10 - 0 MIN	2.20E+08	2.02E+08	1.37E+08	1.86E+08	8.3
0 - 10 MIN	7.77E+07	1.01E+08	3.78E+08	1.86E+08	8.3
5 - 15 MIN	5.36E+07	7.11E+07	3.26E+08	1.50E+08	8.2
10 - 20 MIN	7.53E+07	9.02E+07	3.49E+08	1.72E+08	8.2
15 - 25 MIN	2.49E+08	1.24E+08	1.95E+08	1.89E+08	8.3
20 - 30 MIN	7.59E+07	1.19E+08	4.92E+08	2.29E+08	8.4

Table 5.2. Total HPIV3 copy number/m^3 recovered from the three test runs

TIME	TEST 1	TEST 2	TEST 3	MEAN	Log_{10}
-10 - 0 MIN	1.51E+08	2.96E+08	3.30E+08	2.59E+08	8.4
0 - 10 MIN	2.38E+06	3.19E+08	9.03E+07	1.37E+08	8.1
5 - 15 MIN	2.87E+06	2.69E+07	<LOD	9.94E+06	7.0
10 - 20 MIN	3.32E+06	<LOD	<LOD	1.11E+06	6.0
15 - 25 MIN	2.15E+05	6.49E+06	<LOD	2.24E+06	6.3
20 - 30 MIN	1.05E+06	<LOD	<LOD	3.48E+05	5.5

<LOD = less than limit of detection

The Log_{10} of HPIV3 RNA copy number/m^3 was plotted as shown in Figure 5.1 to compare the effect of operating the NV1050 air purifier over time versus the control (natural decay).

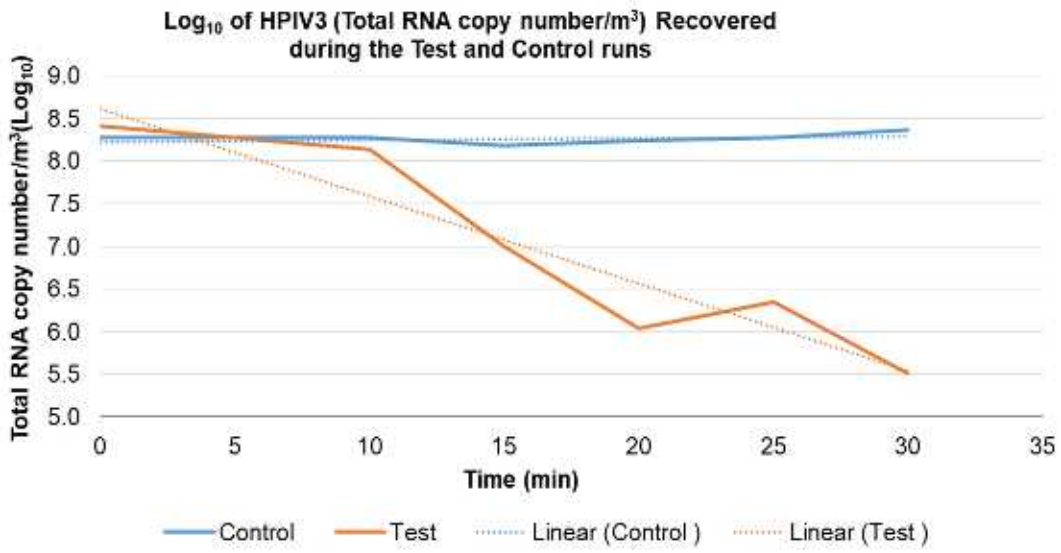


Figure 5.1. The effect of operating the Novaerus air purifier on aerosolised HPIV3. Lines represent the mean values obtained at each time point for control (blue) and test (orange) runs.

The total RNA copy number/m^3 was used to calculate the percentage reduction in RNA copy number using the formula in 4.2.9 as a result of the Novaerus (NV1050) air purifier operating.

Table 5.3. Percent reduction in total HPIV3 RNA copy number/m^3 air as a result of the Novaerus (NV1050) air purifier operating over 30 minutes

TIME (MIN)	% REDUCTION
-10 - 0	**n/a**
0 - 10	**47.01**
5 - 15	**96.16**
10 - 20	**99.57**
15 - 25	**99.14**
20 - 30	**99.87**

6. Conclusion

In assessing the performance of the Novaerus NV1050 air purifier in removing airborne HPIV3 within a 28.5 m^3 environmental test chamber, we found that the air purifier was able to significantly decrease the amount of aerosolized HPIV3 over time.

When the unit was switched off in the control runs, the levels of airborne HPIV3 remained high with very little natural decay over time. In the test runs, with the Novaerus NV1050 air purifier operating, the unit was able to reduce the aerosolized HPIV3 by 47% within the first 10 minutes and achieved over 99% reduction of airborne virus within 30 minutes as detected by qPCR.

7. References

1) Henrickson. Parainfluenza viruses Clin Micro Rev, 2003, 16: 242–264
2) Rima and Duprex. Morbilliviruses and human disease J Pathol 2006; 208: 199–214
3) Enders G. Paramyxoviruses. In: Baron S, editor. Medical Microbiology. 4th edition. 1996. Chapter 59
4) Rima, Bertus & Collins, Peter & Easton, Andrew & Fouchier, Ron & Kurath, Gael & Lamb, Bob & Maisner, Andrea & Rota, Paul & Wang, Linfa & Kuhn, Jens. (2016). Implementation of taxon-wide non-Latinized binomial species names in the family Paramyxoviridae.

Report written by:

Tristan Russell, PhD.

Senior Scientific Officer

Report reviewed by:

Natasha Gordon

Digitally signed by Natasha Gordon
DN: cn=Natasha Gordon, o=airmid healthgroup limited, ou=Laboratory, e=ngordon@airmidhealthgroup.com
Reason: I am approving this document
Location:
Date: 2016-10-30 18:02

Natasha Gordon, PhD.

Senior Scientific Officer

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