

Customer Name	Novaerus (Ireland) Ltd.
Customer Address	DCU Alpha, Old Finglas Road, Glasnevin, Dublin 11
Contact	Felipe Soberon
Customer PO number	
Test Requested	To assess the impact of Air cleaner on <i>Mycobacterium smegmatis</i>
Sample Description	Novaerus air cleaner device (NV1050), 3 replacement filters (Ozone filter, Kompaktfilter & Megalam panel filter)
Number of Samples	1
Date of Receipt	06/04/2018
ASC Code	ASC003569
Report Number	ASCR092288
Report Date	06.07.2018

Contents

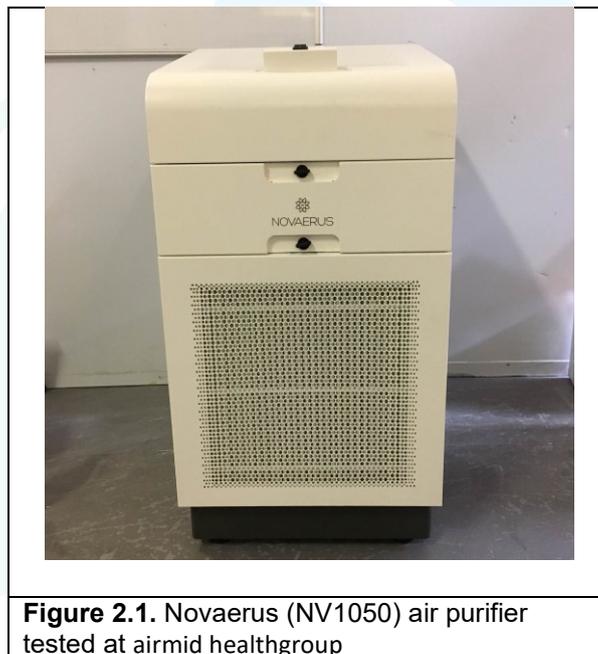
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1. Purpose

The purpose of the testing is to assess the performance of the Novaerus (NV1050) air purifier in removing aerosolised *Mycobacterium smegmatis*, a surrogate for *Mycobacterium tuberculosis*.

2. Test Item Description

The Novaerus (NV1050) air purifier was received by **airmid healthgroup** on 06/04/2018 (Figure2.1).



3. Background

Novaerus (NV1050) air purifier, called A/C in this report, was submitted to **airmid healthgroup** for testing against *Mycobacterium smegmatis*, a surrogate for *Mycobacterium tuberculosis*.

Mycobacterium genus contains several types of pathogenic species. Most common infectious diseases caused by this genus are Tuberculosis (TB) & Buruli ulcer. Most of the pathogenic species and sub-species in this genus share >90% of their DNA. *M.*

tuberculosis species causes TB in both humans and animals. Tuberculosis can be transferred by direct or indirect contact, aerosols and droplets. *M. tuberculosis* is resilient and can adapt and survive during harsh conditions, including hypoxic and nutrient-deprived conditions. People living or working in buildings with inadequate ventilation are more prone to the disease.

M. smegmatis was proposed as a surrogate for *M. tuberculosis* because the latter is a hazardous group 3 contaminant, which requires a biosafety level 3 work setting. Compared to *M. tuberculosis*, *M. smegmatis* is a fast-growing organism and belongs to hazard group 2. Apart from its ability to grow faster, *M. smegmatis* has a similar cell wall structure, almost similar cell size and shares 2000 similar proteins with *M. tuberculosis* [1] [2].

4. Protocol

4.1. Test conditions:

- 4.1.1 The impact of Novaerus (NV1050) air purifier on aerosolised *M. smegmatis* was conducted in a 30 m³ environmental testing chamber.
- 4.1.2 Test chamber was preconditioned to 20 ± 3 °C and 55 ± 5% relative humidity. These conditions were maintained throughout the test and control runs.
- 4.1.3 Prior to each run, the test chamber was decontaminated by scrubbing the walls and surfaces using 5 % virkon and then rinsing with water to remove excess virkon.
- 4.1.4 Apart from using virkon, the chamber was sterilised using UV lamp, for at least 120 mins with full air dump.
- 4.1.5 Bio-stage impactors, used for the air sampling, were autoclaved after each run.

4.2 Test Procedure & Analysis:

- 4.2.1 A total of 6 runs were performed to test the impact of A/C on aerosolised *M. smegmatis*. (3 Test and 3 Control runs)
- 4.2.2 During the test runs A/C was placed in the centre of the test chamber and operated at full speed mode. During the control runs the A/C was switched off.

- 4.2.3 The culture of the test organism was prepared at optimal conditions and diluted to a pre-determined concentration of 10^5 colony forming units (CFU).
- 4.2.4 The *M. smegmatis* was nebulised into the chamber and mixed with a ceiling fan.
- 4.2.5 Biostage impactors were used to sample the air @ 25 litres per minute (lpm). The sampling points for both test and control runs were as follows:

Air sampling time-points		
Test Stage	Time-point (t)	Sample Duration (min)
I	-2 - 0	2
II	11 -15	4
III	25 - 30	5

- 4.2.6 At each sampling point, triplicate air samples were collected.
- 4.2.7 For the test runs, A/C was turned ON at t=0 min and operated throughout the test period.
- 4.2.8 At the end of the test, the test chamber was set to full air dump. The agar plates were removed from the biostage impactors and incubated at 37 °C for 24 hrs.
- 4.2.9 After 24 hrs the number CFU's on the plates were counted, corrected for positive hole correlation and converted to CFU per cubic meter of air sampled (CFU/m³).
- 4.2.10 CFU/m³ results were then converted to Log₁₀ values for the graph.

5. Results and Discussion:

The number of colony forming units recovered from both the test and control runs were corrected to the positive hole correlation and converted to the number CFU's per metre cube of air sampled. The values are reported in Tables 5.1 and 5.2. It is noted that there was some variation in the initial number of *M. smegmatis* CFU's obtained for each run. This is difficult to control as a fresh stock of an overnight culture of the organism had to be used for each run. The average particle size of the aerosolized *M. smegmatis* is approximately 3 µm, but larger and smaller particles are also present in the aerosol.

Table 5.1: Average number of CFU/m³ recovered from the 3 Control runs

Time-point	Control 1	Control 2	Control 3	Average
-2 - 0 min	12465.89	17060.00	16308.73	15278.21
11 - 15 min	7601.00	9194.96	9604.45	8800.14
25 - 30 min	5413.45	6564.85	5626.47	5868.26

Table 5.2: Average number of CFU/m³ recovered from the 3 Test runs

Time-point	Test 1	Test 2	Test 3	Average
-2 - 0 min	9806.00	20846.00	15907.21	15519.74
11 - 15 min	365.00	932.00	853.75	716.92
25 - 30 min	280.00	627.00	497.46	468.15

A marked reduction in the recovery of *M. smegmatis* in the test runs compared to the control runs can be observed after 15 min of A/C operation. However, there is some natural decay in the control runs, which is to be expected for an aerosol of this size.

The % Reduction of *M. smegmatis* in the test and control runs, at the 15 and 30 minute time-points, was calculated as a percentage of the initial concentration obtained for each run. In the test runs an average reduction of 95% was achieved after 15 min of A/C operation. This increased to 97% after 30 min of A/C operation.

Figure 5.1 is the logarithmic representation of the average number of CFU/m³ recovered at each time-point for each of the control and test runs.

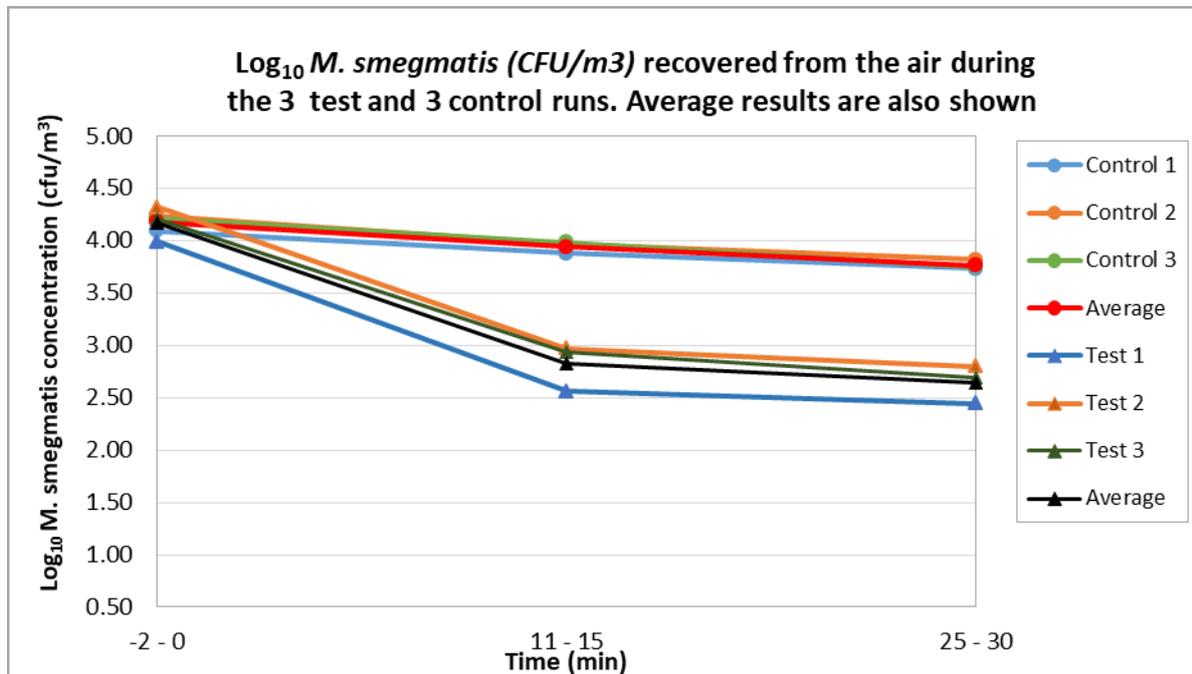


Figure 5.1: Log₁₀ concentration of *M. smegmatis* recovered in the test chamber in the 3 control runs with the A/C off and in the 3 test runs with A/C on. The average for the test and control runs is also shown.

As can be seen from Figure 5.1, there was a marked reduction in the concentration of *M. smegmatis* sampled from the air in the 3 test runs compared to the 3 control runs.

5 Conclusion

The results achieved during the testing show that the Novaerus (NV1050) was able to reduce the concentration of *M. smegmatis* artificially aerosolised by 95% within the first 15 minutes and this rose to 97% after 30 minutes of A/C operation. Some unofficial results suggest to **airmid** that the levels of *M. smegmatis* remaining in the air after 1 hour of A/C operation could be even further reduced. Novaerus may want to consider extending the testing period to 1 hour.

6 References

1. **Tshilombo KV, Warren R, Sampson S.** Survival and Re-aerosolization in Dust of Mycobacterium smegmatis -a Surrogate for Mycobacterium tuberculosis Declaration of Originality.
2. **Lauten EH, Pulliam BL, DeRousse J, Bhatta D, Edwards DA.** Gene Expression, Bacteria Viability and Survivability Following Spray Drying of Mycobacterium smegmatis. *Materials (Basel)* 2010;3:2684–2724.

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