Study Summary Article

Efficacy of Novaerus NV200 Room Air Purifier against Aerosolized MS2 bacteriophage

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Article Info

Testing Lab:

Aerosol Research and Engineering Laboratories, Inc.
Project #: 10867.80
Client: Novaerus

Keywords:

· *NV200*
· *MS2 bacteriophage*
· *Bioaerosol Efficacy*

Compliance:

This study was conducted in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Conflict of Interest:

Aerosol Research and Engineering Laboratories, Inc. have no affiliations with, or involvement in any capacity, with Novaerus's financial interests such as; membership, employment, stock ownership, or other equity interest.

Background: This in-vitro study characterized the decontamination efficacy of the Novaerus NV200 device against aerosolized *MS2* bacteriophage. MS2 has historically been used as a surrogate for influenza and, more recently, as a surrogate for SARS-CoV-2. The Novaerus NV200 is a device based on patented NanoStrike technology designed to reduce airborne pathogens. The effectiveness of the system was assessed in a 1m^3 bioaerosol chamber for a single (1) RNA based virus, MS2, which was tested in triplicate.

Methods: MS2 bacteriophage was aerosolized into a sealed 1m^3 environmental chamber containing the Novaerus NV200 system. Midget impingers and an Aerodynamic Particle Sizer (APS) were used to determine chamber bioaerosol concentrations at pre-determined sampling times. All impinger samples were serially diluted, plated, and enumerated in triplicate to yield viable bioaerosol concentration at each sampling point and time. Chamber control trial data was subtracted from Novaerus NV200 trial data to yield net LOG reduction in the chamber for viable bioaerosol and concentrations.

Results: Three trials were conducted to evaluate the Novaerus NV200 system's efficacy at removing viable MS2 bacteriophage from the air in the test chamber. The Novaerus NV200 device achieved a 4.07 +/- 0.13 net LOG reduction in MS2 bioaerosol in a 180-minute time period.

Conclusion: The Novaerus NV200 system performed well with a 99.99% net reduction in viable bioaerosol concentration within a 180 minute period. Testing was conducted using aerosolized MS2 bacteriophage. This testing confirms that, in theory, the Novaerus NV200 system should show efficacy at reducing the risk of viral respiratory infection.

Overview

This study was conducted to evaluate the efficacy of the Novaerus NV200, which is based on Novaerus's NanoStrike technology, at removing viable bioaerosols from the air. This device is also known commercially as the NV330, Protect 200, and the WellAir Nano. A picture of the device can be found in **Figure 1**.

Testing was conducted in a 1m^3 custom bioaerosol exposure chamber. The Novaerus NV200 device's effectiveness was tested against the MS2 bacteriophage in order to evaluate the system's net LOG reduction of viable bioaerosol within the chamber. Testing was conducted in triplicate trials plus a control trial to demonstrate the capability of reducing viable bioaerosol concentrations. There were a total of four (4) independent trials in this study.

During the control trial, the NV200 system remained inside the test chamber but was not switched on. During test trials, the system was switched on after initial chamber concentration sampling and remained operating until the completion of the trial. MS2 bacteriophage was aerosolized into the test chamber and impinger samples were collected at set time points throughout each trial.

Figure 1: Novaerus NV200 Device.

Test Location and Conditions

Testing was conducted at Aerosol Research and Engineering labs located at 15320 S. Cornice Street in Olathe, Kansas 66062. Laboratory conditions were approximately76°F (24°C) with 41% relative humidity.

Testing Chamber

The primary aerosol exposure chamber, containing the Novaerus NV200 device, is a sealed $1m^3$ environmental chamber constructed of 3/8" Lexan and outfitted with all necessary pass-through and sub-system sampling ports. The chamber is equipped with HEPA filtered house air in order to maintain a clean background environment prior to all testing. HEPA filtered house air also allows for rapid air flushing of the chamber after completion of each exposure to reduce aerosol concentrations in the chamber before conducting subsequent trials. A diagram of the chamber is shown if Figure 2.

During the aerosolization of the microorganism, the chamber was operated in a pressure balanced push/pull between the aerosol inlet and vacuum to eliminate over or under pressure in the chamber. The chamber was operated at a slightly negative pressure, -0.3 inH_2O, for technician safety. Once aerosolization of the challenge organism at the beginning of each trial was complete, the inlet and vacuum balance were cut off and the chamber sat idly until air sample collections.

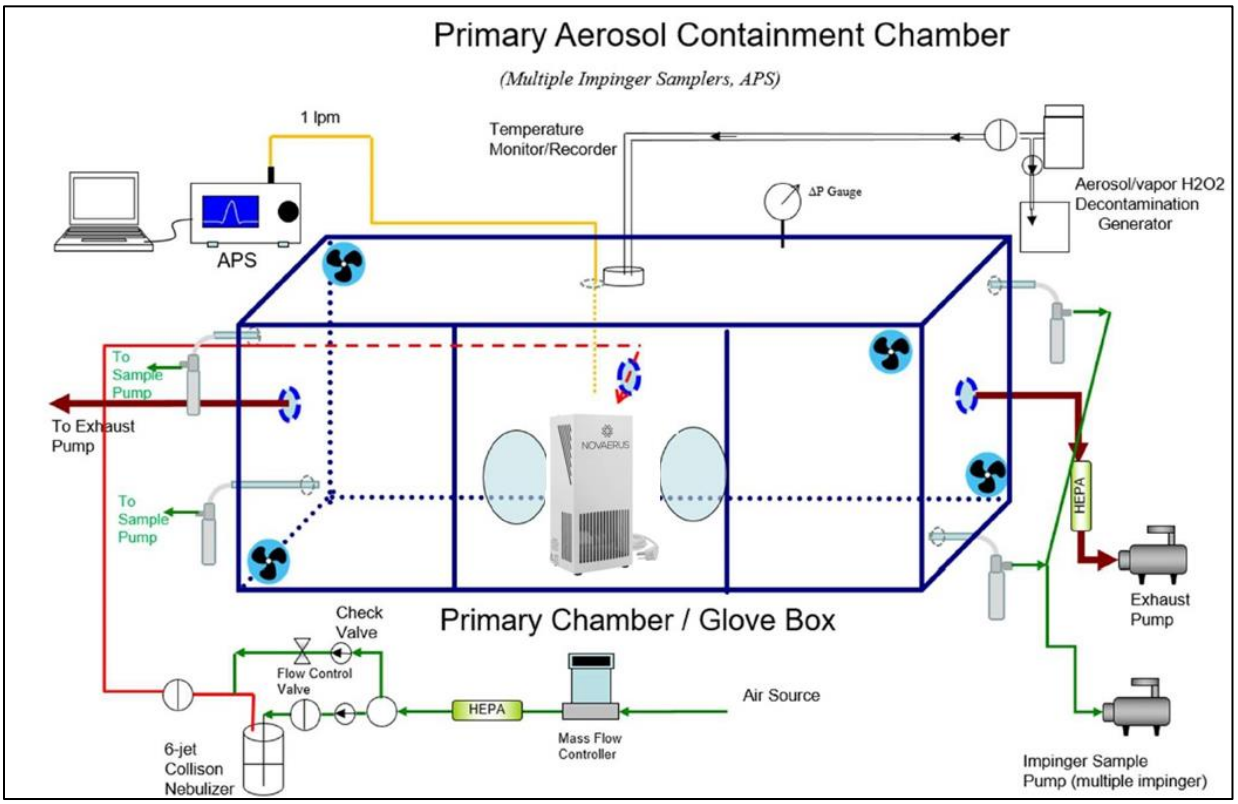

Figure 2: Test Chamber Flow diagram for testing.

The chamber is equipped with four (4) mixing fans to ensure spatial homogeneity of bioaerosols during their aerosolization and sampling. These fans were switched on during the aerosolization of the bioaerosol into the chamber and remained on for the duration of the trials to ensure homogeneity.

Bioaerosol Generation System

Test bioaerosols were disseminated using a Collison 6-jet nebulizer (BGI Inc., Waltham MA) driven by purified filtered house air supply. A pressure regulator allowed for control of disseminated particle size, use rate, and sheer force generated within the Collison nebulizer.

Figure 3: BGI Collison Stainless Nebulizer. (6-Jet version pictured).

Prior to testing, the Collison nebulizer flow rate and use rate were characterized using an air supply pressure of approximately 35 psi, which obtained an output volumetric flow rate of approximately 50 lpm with a fluid dissemination rate of approximately 1 ml/min. The Collison nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul MN). A picture of the nebulizer type used in the trials is pictured in **Figure 3**.

Bioaerosol Sampling and Monitoring System

A midget impinger (Chem Glass Inc., Vineland NJ) was used for bioaerosol collection of biological aerosols to determine the chamber concentration. This impinger was connected to the bioaerosol chamber via a sample port located near the center of the exposure box.

The impinger vacuum source was maintained at a negative pressure of 18 inches of Hg during all characterization and test sampling to assure critical flow conditions. The sample impingers were flow characterized using a calibrated TSI model 4040 mass flow meter.

The impingers were filled with 5 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 was shown to increase the impinger collection efficiency and de-agglomeration of all microorganisms for proper plate counts. Impingers were taken in duplicate and pooled for an overall average of chamber concentration.

TSI Aerodynamic Particle Sizer

A TSI Aerodynamic Particle Sizer (APS) model 3321 (TSI Inc., Shoreview, MN) was used to measure aerosol concentrations and particle size during trials. The APS provided real-time aerodynamic particle characterization with a size range from 0.54-20.0 µm with 52 size bins of resolution. Sampling is continuous with a data export interval of 1 second. The APS has a continuous flow rate of 5 liters per minute (LPM). A picture of the APS is shown in **Figure 4**.

Figure 4. TSI Aerodynamic Particle Sizer (APS) model 3321 used to measure total particle concentration and particle size distribution of the challenge bioaerosol. Range 0.54-20.0 µm aerodynamic diameter, with 1 particle/L detection limits.

Species Selection

Species selection was based on Biological Safety Level 1 (BSL1) surrogates for BSL2 and BSL3 pathogenic organisms. MS2 is a viral RNA bacteriophage that is commonly used as a surrogate for the influenza virus, and more recently, for SARS-CoV-2. The CDC estimates that the influenza virus is responsible for 140,000 to 810,000 hospitalizations and 12,000 to 61,000 deaths annually.

Test Matrix

To accurately test the Novaerus NV200 device, triplicate challenge trials were performed in the test chamber. In order to characterize the device's performance while taking into account the natural losses of the bioaerosol in the chamber, a control trial was run. A testing matrix for the device can be found in **Figure 4.**

Test Matrix

Trial	Run	Pathogenic Organism	Surrogate Species (aerosol description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1 2 3 4	Control Challenge Challenge Challenge	*Influenza* , (tentative surrogate for *Sars-cov2*)	*MS2 bacteriophage (E. coli phage)*	15597-B1	<1.0µm	10^4-10^6	150	0, 30, 60, 90, 120, 150	Impingers	all samples in triplicate
5 6	Control Challenge	*NA*	Polystyrene Latex Microspheres	*NA*	0.5 to 4.0µm	10^4-10^6	120	Continuous	APS	*NA*

Figure 5: Test Matrix for Aerosol Trials.

Viral Culture & Preparation

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown overnight in Tryptic Soy Broth. The liquid cell suspension was infected during the logarithmic growth cycle with the MS2 bacteriophage. After an appropriate incubation time (approximately 24 hours), the cells were lysed and the cellular debris separated by centrifugation.

MS2 stock yields were greater than 1 x 10^{11} plaque forming units per milliliter (pfu/mL) with a single amplification procedure. This stock MS2 viral solution was then diluted with PBS to approximately 1 x 10^{10} plaque forming units per milliliter (pfu/mL) for use in the Collison nebulizer.

Challenge Bioaerosol Aerodynamic Diameter

Bioaerosol particle size distributions were measured with a TSI Aerodynamic Particle Sizer model 3321 (APS) for all challenge species. The particle size distribution was taken shortly after aerosolization for each species via sampling through a sample probe into the test chamber. The APS has a dynamic measurement range of 0.54 to 20.0 µm and was programmed to take consecutive real-time one-minute aerosol samples. Data were logged in real-time to an Acer laptop computer, regressed, and plotted.

The aerodynamic particle size distribution for all challenge bioaerosols are shown to be within the respirable range for regional alveolar tract deposition and show a low geometric standard deviation (GSD), indicating that a monodispersed aerosol was generated in the chamber for each of the challenge species. The aerodynamic particle size distributions for MS2 can be found in **Figure 6,** shown above.

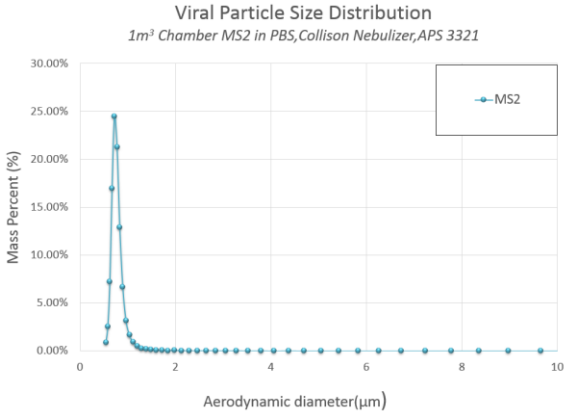

Figure 6: Aerodynamic Particle Size Distribution of RNA virus MS2 in the test chamber. MMAD for MS2 species averaged approximately 0.7 µm.

Bioaerosol Plating and Enumeration

Impinger and stock biological cultures were serially diluted and plated in triplicate (multiple serial dilutions) using a standard spread plate assay technique onto tryptic soy agar plates in a class 2 biosafety cabinet. The plated cultures were incubated for 24 hours, enumerated and recorded for data analysis.

Control Testing Method

To accurately assess the Novaerus NV200 unit, test chamber pilot control trials were performed with MS2 bacteriophage for 180 minute periods without the system in operation to characterize the biological challenge aerosol for aerosol delivery/collection efficiency, decay rate and viable concentration over time.

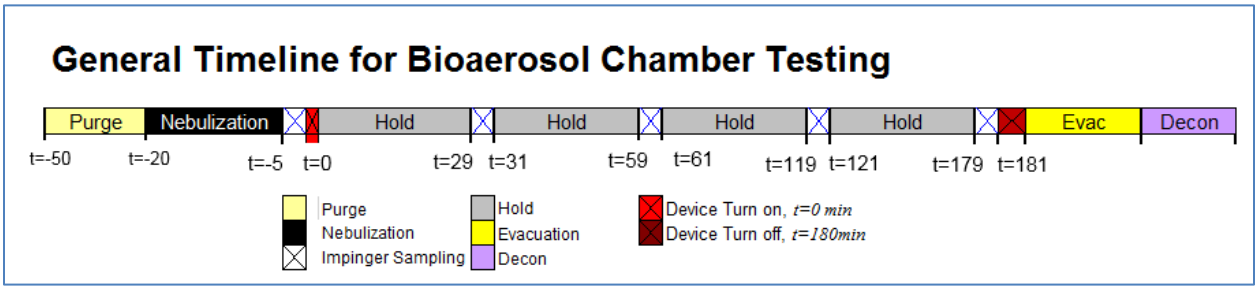

Figure 7: General trial timeline for bioaerosol testing.

Control testing was performed to provide baseline comparative data in order to assess the actual viable bioaerosol reduction from the NV200 challenge testing and verify that chamber concentrations persisted above the required concentrations over the entire pilot control test period.

Novaerus NV200 Testing Method

For each control and challenge test, the Collison nebulizer was filled with approximately 50 mL of biological or particulate stock and operated at 35 psi for a period of 5 minutes. For control and system trials, the impinger was filled with 5 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 has been shown to increase the impinger collection efficiency and de-agglomeration of micro-organisms.

The chamber mixing fans were turned on during bioaerosol generation to assure a homogeneous bioaerosol concentration in the test chamber prior to the first impinger sample. For the remainder of both control and test trials, mixing fans remained on to ensure bioaerosol homogeneity.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and challenge test by sampling with a midget impinger located near the center of the chamber. Impinger samples were collected for 2 or 5 minutes depending on which time point the sample was taken. Longer samples were taken towards the end of each test in order to collect enough viable bioaerosol for plating and enumeration.

Aliquots of impinger samples were collected and then used for plating. Impingers were rinsed 6x with sterile filtered water between each sampling interval, and re-filled with sterile PBS using sterile graduated pipettes for sample collection.

For device testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the trial length of 180 minutes. Subsequent impinger samples were taken at intervals of 30 and 60 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the system over time.

Figure 7 outlines the general timeline for the testing procedure with the Novaerus NV200 system. All samples were plated in triplicate on tryptic soy agar media over a minimum of a 3 log dilution range.

Plates were incubated and enumerated for viable plaque forming unit (pfu) counts to calculate bioaerosol challenge concentrations in the chamber and reduction of viable microorganisms. This testing method was designed to assess the viable bioaerosol reduction in the test chamber, it did not directly assess the inactivation of the microorganism.

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of thirty minutes and analyzed with a TSI Aerodynamic Particle Sizer (APS) for particle concentration decrease to baseline levels. At the conclusion of testing, the chamber was decontaminated using 35% vaporous, food grade hydrogen peroxide.

The Collison nebulizer and impingers were cleaned at the conclusion of each day of testing by soaking in a 5% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

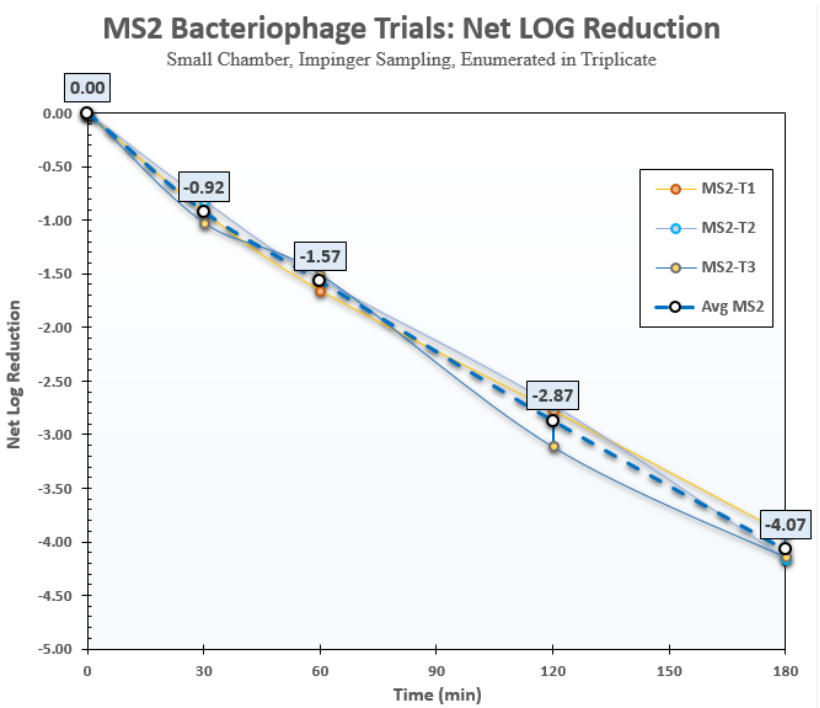

Figure 8: Net LOG reduction of MS2 in challenge trials.

Data Analysis

The data analysis shows the results of the triplicate trials conducted for this study, as well as an average at each time point for the group. All trials show individual and group average +/- standard deviations for Net LOG reduction on a per trial basis. The values depicted on each graph represents the group average at that time point.

Novaerus NV200 Results

MS2 bacteriophage cultures were initiated the day prior to testing and grew to a concentration greater than $1e^{10}$ cfu/ml. The control trial experienced a 0.81 LOG reduction of MS2 after 180 minutes of sample collections using the midget impingers. At the 180 minute time point, the device showed an average 4.07 net LOG reduction of viable MS2 bacteriophage. A graph of the reduction capabilities of the device, as well as an average for all the trials, can be found in **Figure 8** above.

Summary

The Novaerus NV200 device performed well, in the $1m^3$ test enclosure, achieving a bioaerosol reduction of 4.07 +/- 0.13 net LOG of viable airborne MS2 concentration within a 180-minute period. A comparative graph showing the chamber concentration over time for the MS2 trial and the control averages can be found in **Figure 10** on the following page. The results for the trials including group averages and standard deviation can be found in a summary table in **Figure 9**.

Novaerus NV200 MS2 Trial Summary Data

Bioaerosol Type	Species (description)	Trial Name	Reduction Type	Trial Time (minutes)			
				30	60	120	180
Virus	*MS2 Bacteriophage* (RNA E. coli phage)	*MS2-T1*	*Net Log Reduction*	-0.92	-1.65	-2.77	-3.92
			Net % Reduction	88.06%	97.79%	99.83%	99.9881%
Virus	*MS2 Bacteriophage* (RNA E. coli phage)	*MS2-T2*	*Net Log Reduction*	-0.82	-1.55	-2.73	-4.16
			Net % Reduction	84.8169%	97.1785%	99.8118%	99.9931%
Virus	*MS2 Bacteriophage* (RNA E. coli phage)	*MS2-T3*	*Net Log Reduction*	-1.02	-1.51	-3.11	-4.14
			Net % Reduction	90.52%	96.89%	99.92%	99.9927%
All Trial Averages			***Net Log Reduction***	**-0.92 +/- 0.1**	**-1.57 +/- 0.08**	**-2.87 +/- 0.21**	**-4.07 +/- 0.13**
			Net % Reduction	***87.8% +/- 2.86%***	***97.28% +/- 0.46%***	***99.86% +/- 0.06%***	***99.991% +/- 0.003%***

Figure 9: Summary Data Table for Net LOG Reduction of the Novaerus NV200.

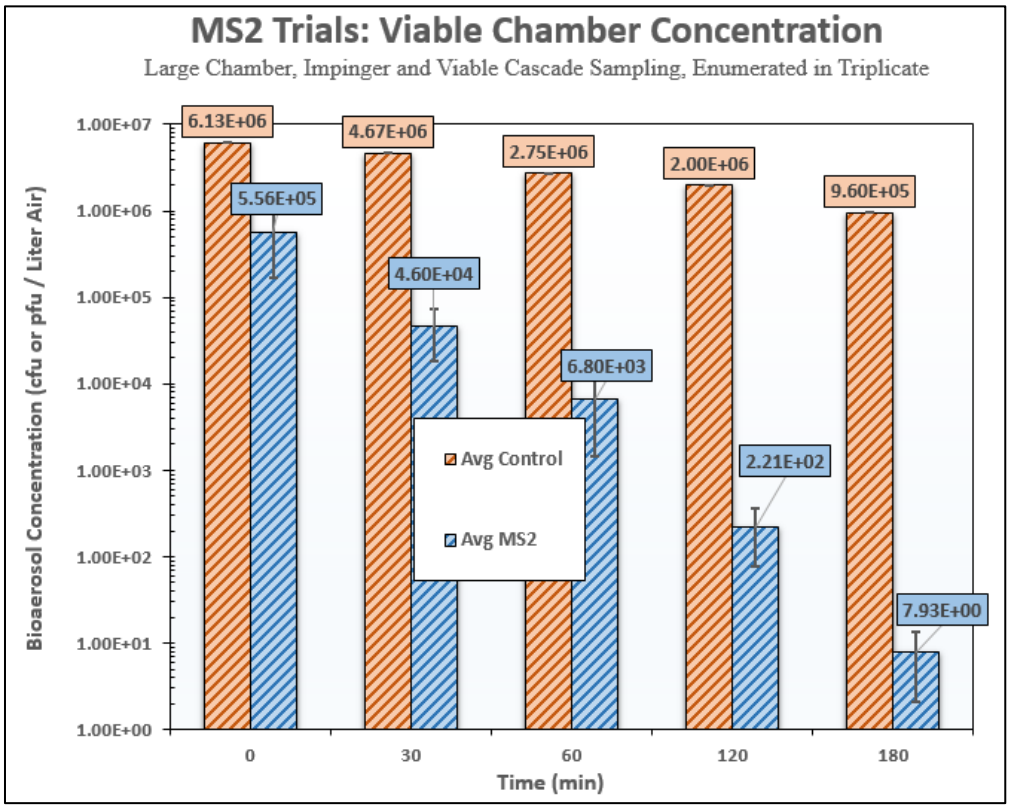

Figure 10: Viable Chamber Concentrations Averages for the Novaerus NV200 trials.

References

T. Reponen, K. Willeke, V. Ulevicius et al. *Techniques of Dispersion of Microorganisms in Air.* Aerosol Science and Technology. 27: 1997. pp. 405-421.

Ding and Wing. *Effects of Sampling Time on the Total Recovery rate of AGI-30 Impingers for E. coli*. Aerosol and Air Quality Research, Vol. 1, No. 1, 2001, pp. 31-36.

Analytical GLP Certificate

Aerosol Research and Engineering Labs, Inc.
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Project

10824.17

Study Director

Jamie Balarashti
Aerosol Research and Engineering Laboratories

GLP Statement

We, the undersigned, herby certify that the work described herein was conducted by Aerosol Research and Engineering Laboratories in compliance with FDA Good Laboratory Practices (GLP) as defined in 21 CFR, Part 58.

Study Director:

Jamie D. Balarashti
Study Director
ARE Labs, Inc.

8/20/2021
Date

Principal Investigator:

Sean McLeod
Principal Investigator
ARE Labs, Inc.

8/20/2021
Date

Appendix A: Raw Data

Trial Information

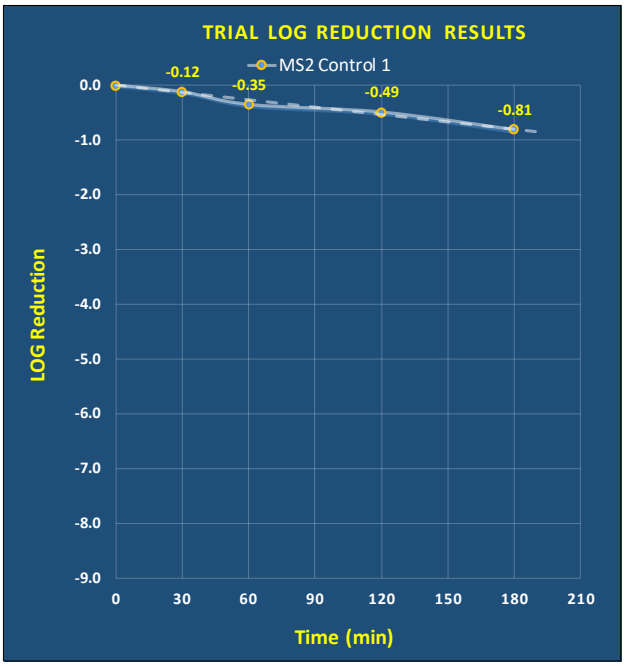
TEST DATE:	Monday, August 16, 2021
TRIAL PERFORMED BY:	JCT
TRIAL NUMBER:	Control 1
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 Control 1

Device Information

MANUFACTURER:	NA
UNIT MODEL:	NA
FAN SPEED (CFM):	NA
UNIT SERIAL #:	na
FITER ID #:	na
FILTER LOT #:	na

General Testing Conditions

TEST CHAMBER VOLUME (m^3):	1
NEBULIZER CONDITIONS:	Collison 6-Jet; approx. 10 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	na
TRIAL COMMENTS/NOTES	na



BIOAEROSOL Sample ID and Summary Data	S1	S3	S5	S6	S7
SAMPLING TIME (min)	0	30	60	120	180
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	6.133E+06	4.667E+06	2.747E+06	2.000E+06	9.600E+05
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)					
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**6.133E+06**	**4.667E+06**	**2.747E+06**	**2.000E+06**	**9.600E+05**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**76.0870%**	**44.7826%**	**32.6087%**	**15.6522%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**23.9130%**	**55.2174%**	**67.3913%**	**84.3478%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.12**	**-0.35**	**-0.49**	**-0.81**

Impinger Sampling Conditions

SAMPLING TIME (min)	0	30	60	120	180
IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5	12.5

Dilution Range #1						
	DILUTION RATIO (10^x)	**-5**	**-4**	**-4**	**-4**	**-4**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	9 8 6	62 58 55	42 33 28	28 27 20	14 12 10
	PLATE AVERAGE COUNT (# / drop)	7.67	58.33	34.33	25.00	12.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	7,666,667	5,833,333	3,433,333	2,500,000	1,200,000
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	6.13E+06	4.67E+06	2.75E+06	2.00E+06	9.60E+05

Figure 1a: *MS2 Control*

Trial Information

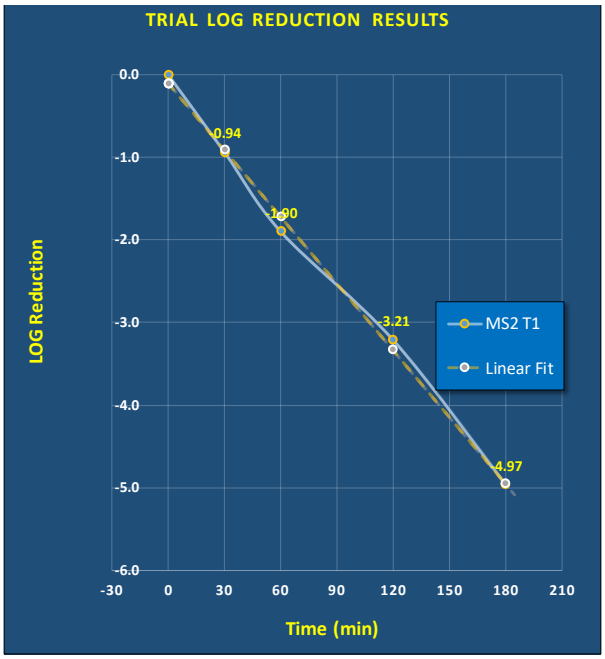
TEST DATE:	Thursday August 5th 2021
TRIAL PERFORMED BY:	ZTC
TRIAL NUMBER:	T1
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T1

Device Information

MANUFACTURER:	Novaerus
UNIT MODEL:	NV200
FAN SPEED (CFM):	N/A
UNIT SERIAL #:	na
FITER ID #:	na
FILTER LOT #:	na

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	1
NEBULIZER CONDITIONS:	Collison 6-Jet; approx. 10 min neb
SAMPLING METHOD:	Impinger
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	70
OTHER INSTRUMENTS:	na
TRIAL COMMENTS/NOTES	na



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	**0**	**30**	**60**	**120**	**180**
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.231E+05	1.422E+04	1.556E+03	7.556E+01	1.333E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	**54.21%**				
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**1.231E+05**	**1.422E+04**	**1.556E+03**	**7.556E+01**	**1.333E+00**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**11.5523%**	**1.2635%**	**0.0614%**	**0.0011%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**88.4477%**	**98.7365%**	**99.9386%**	**99.9989%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-0.94**	**-1.90**	**-3.21**	**-4.97**

Impinger Sampling Conditions

	SAMPLE TIME (min)	**0**	**30**	**60**	**120**	**180**
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0
	IMPINGER FLOW RATE (lpm)	7.5	7.5	7.5	7.5	7.5
Dilution Range #1	**DILUTION RATION (10^x)**	**-3**	**-2**	**-1**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	12 12 14	14 10 8	13 10 12	6 5 6	1 0
	PLATE AVERAGE COUNT (# / drop)	12.67	10.67	11.67	5.67	0.50
	IMPINGER CONCENTRATION (cfu or pfu/ml)	126,667	10,667	1,167	57	1
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**1.69E+05**	**1.42E+04**	**1.56E+03**	**7.56E+01**	**1.33E+00**
Dilution Range #1	**DILUTION RATION (10^x)**	**-2**	**-1**	**-2**	**-2**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	55 58 61				
	PLATE AVERAGE COUNT (# / drop)	58.00				
	IMPINGER CONCENTRATION (cfu or pfu/ml)	58,000				
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	**7.73E+04**				

Figure 2a: *MS2 T1*

Trial Information

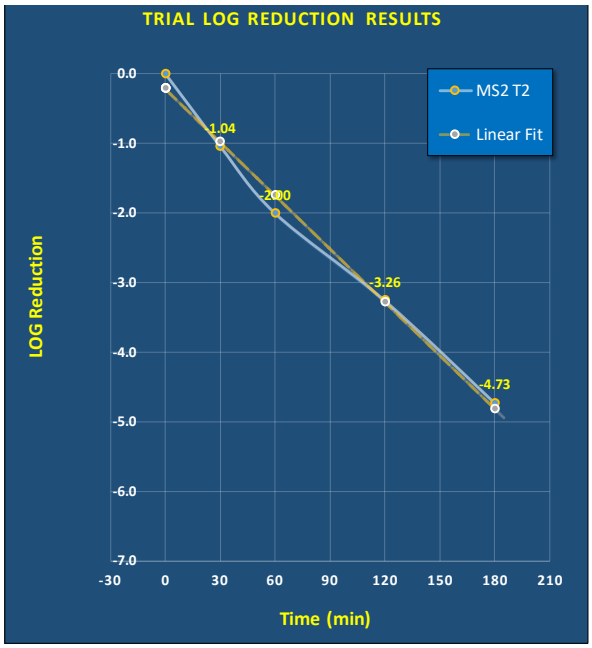
TEST DATE:	Tuesday, August 17, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T2
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T2

Device Information

MANUFACTURER:	Novaerus
UNIT MODEL:	NV200
FAN SPEED (CFM):	N/A
UNIT SERIAL #:	na
FITER ID #:	na
FILTER LOT #:	na

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	1
NEBULIZER CONDITIONS:	Collison 6-Jet; approx. 5 min neb
SAMPLING METHOD:	Impingers
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	40
OTHER INSTRUMENTS:	na
TRIAL COMMENTS/NOTES	na



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	30	60	120	180
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	6.667E+05	6.056E+04	6.611E+03	3.667E+02	1.244E+01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	0.00%	44.29%	30.00%		40.00%
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**6.667E+05**	**6.056E+04**	**6.611E+03**	**3.667E+02**	**1.244E+01**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**9.0833%**	**0.9917%**	**0.0550%**	**0.0019%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**90.9167%**	**99.0083%**	**99.9450%**	**99.9981%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.04**	**-2.00**	**-3.26**	**-4.73**

Impinger Sampling Conditions

SAMPLE TIME (min)	0	30	60	120	180
IMPINGER FILL VOL (ml)	5.0	5.0	5.0	5.0	5.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	7.5	7.5	7.5	7.5	7.5

Dilution Range #1						
	DILUTION RATIO (10^x)	**-5**	**-4**	**-3**	**-1**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	4 1 1	4 1 2	2 2 3	10 11 12	1 8 5
	PLATE AVERAGE COUNT (# / drop)	2.00	2.33	2.33	11.00	4.67
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,000,000	233,333	23,333	1,100	47
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	6.67E+05	7.78E+04	7.78E+03	3.67E+02	1.56E+01

Dilution Range #1						
	DILUTION RATIO (10^x)	**-4**	**-3**	**-2**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	28 20 12	14 13 12	14 18 17		14
	PLATE AVERAGE COUNT (# / drop)	20.00	13.00	16.33		14.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,000,000	130,000	16,333		28
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	6.67E+05	4.33E+04	5.44E+03		9.33E+00

Figure 3a: *MS2 T2*

Trial Information

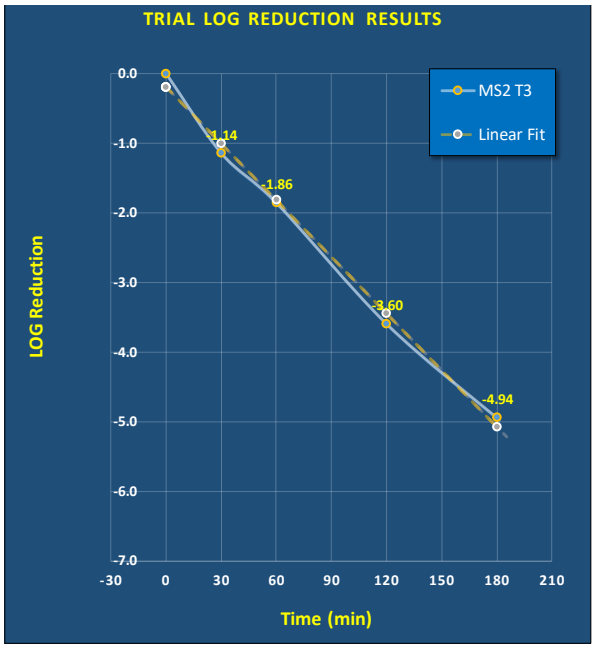
TEST DATE:	Tuesday, August 17, 2021
TRIAL PERFORMED BY:	SMM
TRIAL NUMBER:	T3
TEST ORGANSIM:	MS2
TRIAL NAME ID (GRAPHS/TABLES):	MS2 T3

Device Information

MANUFACTURER:	Novaerus
UNIT MODEL:	NV200
FAN SPEED (CFM):	N/A
UNIT SERIAL #:	na
FITER ID #:	na
FILTER LOT #:	na

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m^3):	1
NEBULIZER CONDITIONS:	Collison 6-Jet; approx. 5 min neb
SAMPLING METHOD:	Impingers
CHAMBER MIXING FAN:	yes
TEMP (F):	74
RH (%):	40
OTHER INSTRUMENTS:	na
TRIAL COMMENTS/NOTES	na



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	30	60	120	180
IMPINGER USED (y / n)	y	y	y	y	y
VIABLE CASCADE USED (y / n)	n	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	8.778E+05	6.333E+04	1.222E+04	2.222E+02	1.000E+01
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)					
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	24.44%	37.14%			50.00%
VIABLE CONSISTENCY CHECKS (% agreement)					
IMP & VIABLE CROSS CHECK (% agreement)					
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	**8.778E+05**	**6.333E+04**	**1.222E+04**	**2.222E+02**	**1.000E+01**
RELATIVE PERCENT REMAINING FROM T=0 (%)	**100.0000%**	**7.2152%**	**1.3924%**	**0.0253%**	**0.0011%**
RELATIVE PERCENT REMOVAL FROM T=0 (%)	**0.0000%**	**92.7848%**	**98.6076%**	**99.9747%**	**99.9989%**
LOG REDUCTION FROM T=0 (log_{10})	**0.00**	**-1.14**	**-1.86**	**-3.60**	**-4.94**

Impinger Sampling Conditions

	S1	S2	S3	S4	S5
SAMPLE TIME (min)	0	30	60	120	180
IMPINGER FILL VOL (ml)	5.0	5.0	5.0	5.0	5.0
IMPINGER SAMPLING TIME (min)	2.0	2.0	2.0	2.0	2.0
IMPINGER FLOW RATE (lpm)	7.5	7.5	7.5	7.5	7.5

Dilution Range #1						
	DILUTION RATIO (10^x)	**-5**	**-4**	**-3**	**-1**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**100**
	ENUMERATED PLATE COUNTS (# / drop)	2 3 4	3 2 2	2 4 5	7 8 5	3 2 1
	PLATE AVERAGE COUNT (# / drop)	3.00	2.33	3.67	6.67	2.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	3,000,000	233,333	36,667	667	20
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.00E+06	7.78E+04	1.22E+04	2.22E+02	6.67E+00

Dilution Range #1						
	DILUTION RATIO (10^x)	**-4**	**-3**	**-2**	**0**	**0**
	DROPLET SIZE (µl)	**100**	**100**	**100**	**100**	**500**
	ENUMERATED PLATE COUNTS (# / drop)	18 28 22	17 11 16			20
	PLATE AVERAGE COUNT (# / drop)	22.67	14.67			20.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,266,667	146,667			40
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	7.56E+05	4.89E+04			1.33E+01

Figure 4a: *MS2 T3*

Appendix B: Calculations

To evaluate the viable aerosol delivery efficiency and define operation parameters of the system, calculations based on (theoretical) 100% efficacy of aerosol dissemination were derived using the following steps:

- Plating and enumeration of the biological to derive the concentration of the stock suspension (C_s) in pfu/mL or cfu/mL, or cfu/g for dry powder.
- Collison 24 jet nebulizer liquid use rate (R_{neb}) (volume of liquid generated by the nebulizer/time) at 30 psi air supply pressure = 1.0 ml/min.
- Collison 24 jet Generation time (t) = 20 or 30 minutes, test dependent.
- Chamber volume (V_c) = 15,993 Liters
- Nebulizer Generation efficiency (ε) (usually around 10%)

Assuming 100% efficiency, the quantity of aerosolized viable particles (V_P) per liter of air in the chamber for a given nebulizer stock concentration (C_s) is calculated as:

$$\text{Nebulizer: } V_P = \frac{C_s \cdot R_{neb}}{V_c} t \cdot \varepsilon$$

Midget impinger or 47mm filter collection calculation:

- Viable aerosol concentration collection (C_a) = cfu or pfu/L of chamber air.
- Viable Impinger concentration collection (C_{Imp}) = cfu or pfu/mL from enumeration of impinger sample or filter sample.
- Impinger sample collection volume (I_{vol}) = 20 mL collection fluid/impinger, or extraction fluid for filter.
- Midget impinger or filter sample flow rate (Q_{imp}) = 7.5 L/min.
- Midget impinger or filter sample time (t) = 5 or 10 minutes, test dependent.

For viable impinger or filter aerosol concentration collection (C_a) = cfu or pfu/L of chamber air:

$$C_a = \frac{\mathrm{C_{Imp}} \cdot \mathrm{I_{vol}}}{\mathrm{Q_{imp}}} \mathrm{t}$$

Appendix C: PSL Test Results

PSL testing yielded a slight reduction when compared to the control losses. Reduction of the 2.0 and 4.0µm particles was observed to be 0.45 logs, whereas there was little to no reduction of 1.0 µm particles and 0.23 logs reduction of 0.5 µm particles. Results are represented graphically in **Figure 1C** above.

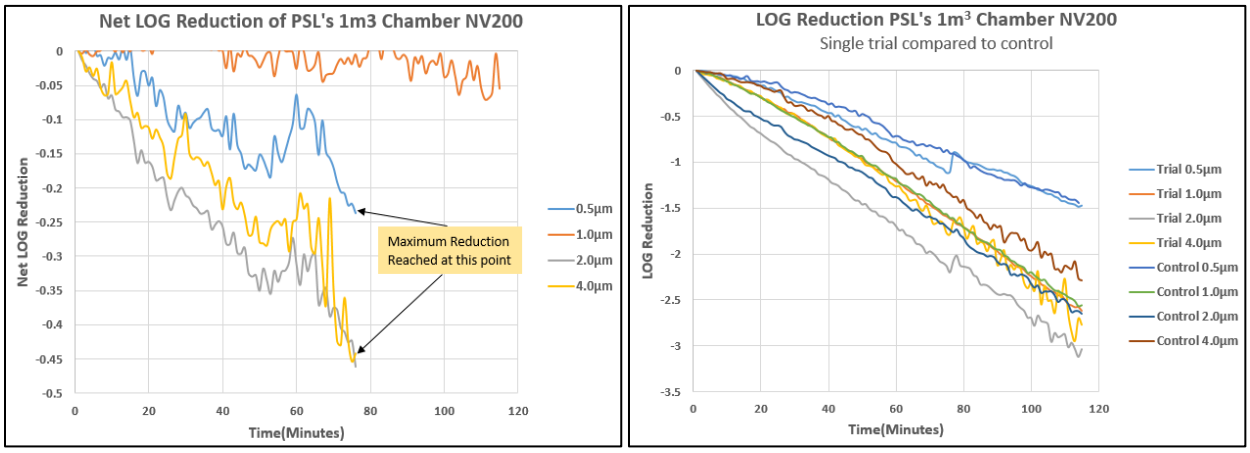


Figure 1C: Net LOG reduction and LOG reduction of particulates by NV200 device in 1m³ chamber.