

# Determination of the Novaerus NV900 System's Efficacy against Various Bioaerosols

## Abstract

*This in vitro study characterized Novaerus NV900 decontamination efficacy against various aerosolized biologicals. Novaerus NV900 is designed to neutralize airborne bacteria, viruses, and fungal spores in order to sanitize enclosed rooms and associated equipment. This study evaluated the efficacy against multiple species of aerosolized biologicals in an environmental chamber.*

*The efficacy of the system was assessed for four (4) aerosolized biologicals: Staphylococcus epidermidis, MS2 bacteriophage, Aspergillus Niger fungus, and Bacillus subtilis endospores. The study consisted of a total of Twelve (12) separate trials; one control run plus challenge trials for each of the four (4) aerosolized biologicals.*

*NV900 System's efficacy of reduction of S. epidermidis viability, after correcting for control run losses, were 2.92 +/- 0.2 logs (average +/- standard deviation) in 6 hours. The reduction for viral bioaerosol concentrations within the chamber were 4.44 +/- 0.06 logs (Avg +/- STdev) in 5 hours for bacteriophage MS2. The A. niger fungal spores resulted in viable bioaerosol concentration reduction within the chamber of 1.94 +/- 0.15 logs (Avg +/- STdev) in 4 hours. The NV900 performance against aerosolized B. subtilis endospores was less than other aerosolized microorganisms yet the NV900 still showed viable bioaerosol concentration reduction within the chamber of 0.87 logs (Avg +/- STdev) in 6 hours.*

*This study was conducted in compliance with FDA Good Laboratory Practices (GLP) as defined in 40 CFR, Part 160.*

## Overview

This study was conducted to evaluate the ability of the Novaerus NV900 produced by Novaerus Inc. (Raleigh, NC), to neutralize airborne bioaerosols. Testing was conducted in a controlled stainless steel aerosol chamber. The Novaerus NV900 effectiveness against four separate Bio-safety level 1 (BSL1) organisms was compared to control runs in order to evaluate the system's effective LOG reduction of viable bioaerosols when compared to the control runs. The test plan incorporated challenging the NV900 device in a closed environmental chamber to determine the destruction rate of the NV900 against various airborne microorganisms. The preliminary effectiveness of the NV900 was

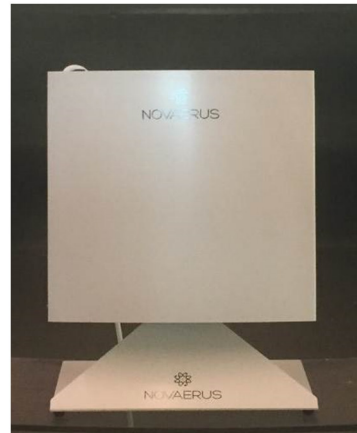
evaluated against a single vegetative bacterium, one RNA virus, a fungal spore and a bacterial endospore as simulants for a broader range of pathogenic organisms.

Testing was conducted to characterize a single NV900 unit against the four separate and distinct organisms in independently repeated tests to demonstrate the capability of the NV900 to reduce viable bioaerosol concentrations. The testing for the NV900's effectiveness was conducted in triplicate for the vegetative bacteria and duplicate trials for the virus and mold spore and a single trial for bacillus endospores.



NV900 Unit

Picture:



Device Features

Manufacturer: Novaerus Ltd.  
 Model: NV900

Notes: Plasma Air Disinfection

*Figure 1: Novaerus Mobile Disinfection Unit (NV900).*

**Bioaerosol Testing Chamber**

A large sealed aerosol test chamber was used to replicate a potentially contaminated room environment and to contain any potential release of aerosols into the surrounding environment.

The aerosol test chamber is constructed of 304 stainless steel and is equipped with three viewing windows and an air-tight lockable chamber door for system setup and general ingress and egress. The test chamber internal dimensions are 9.1ft x 9.1ft x 6.8ft, with a displacement volume of 563 cubic feet, or 15,933 liters.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitor, external humidifiers (for humidity control), lighting system, multiple sampling ports, aerosol mixing fans, and an HEPA filtered exhaust system that are operated with wireless remote control. For testing, the chamber was equipped with four 3/8 inch diameter stainless steel probes for aerosol sampling, a 1 inch diameter port for bio-aerosol dissemination into the chamber using a Collison 24-jet nebulizer for the bacteriophages and vegetative cells, or a dry powder eductor for the fungal and bacterial spores.

A ¼ inch diameter probe was used for continuous aerosol particle size monitoring via a TSI Aerodynamic Particle Sizer (APS) model 3321. All sample and dissemination ports were inserted approximately 18 inches from the interior walls of the chamber to avoid wall effects and at a height of approximately 40 inches from the floor.

The aerosol sampling and aerosol dissemination probes are stainless steel and bulk headed through the chamber walls to provide external remote access to the aerosol generator and samplers during testing.

The test chamber is equipped with two high-flow HEPA filters for the introduction of filtered purified air into the test chamber during aerosol evacuation/purging of the system between test trials and a HEPA filtered exhaust blower with a 500 ft<sup>3</sup>/min rated flow capability for rapid evacuation of remaining bioaerosols.

A magnehelic gauge with a range of 0.0 +/- 0.5 inch H<sub>2</sub>O (Dwyer instruments, Michigan City IN) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles.

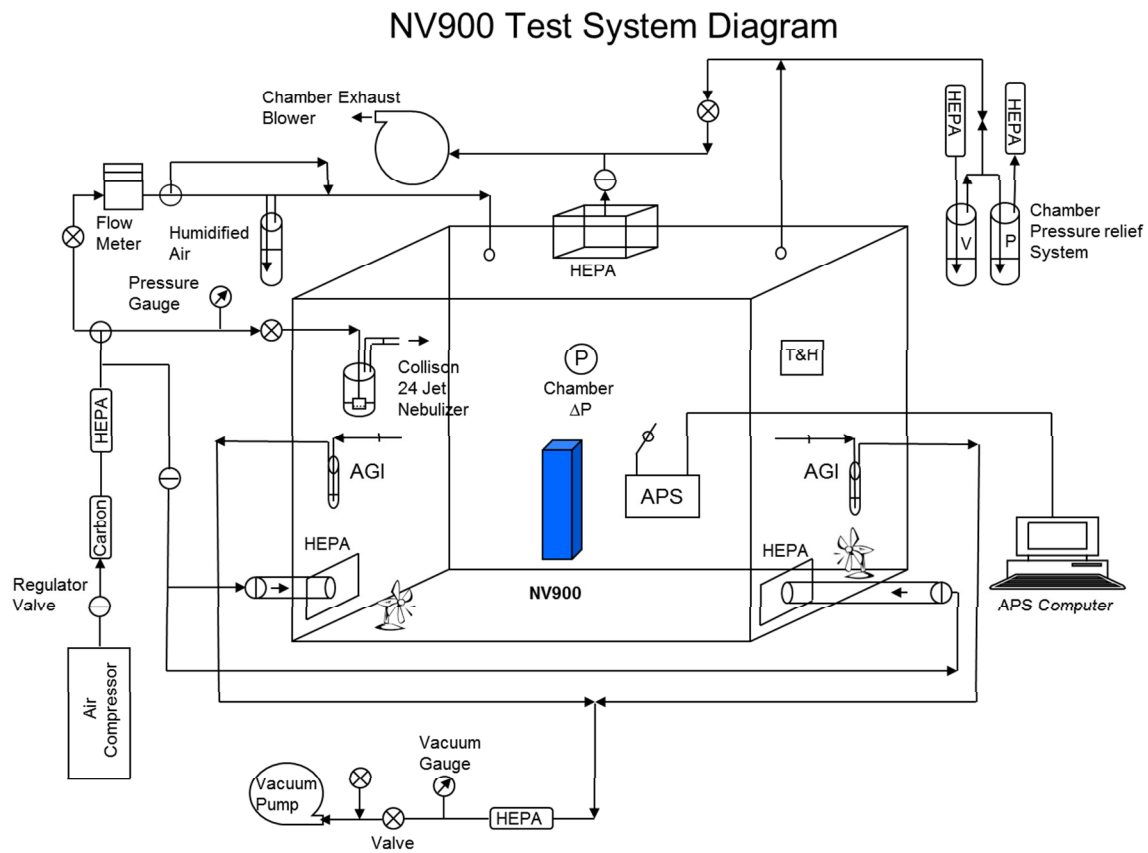


Figure 2: Bio-Aerosol Test Chamber Flow Diagram.

## Bioaerosol Generation System

Test bacteriophage and vegetative bioaerosols were disseminated using a Collision 24-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 shear force generated within the Collision nebulizer.

Prior to testing, the Collision nebulizer flow rate and use rate were characterized using an air supply pressure of approximately 28-50 psi, which obtained an output volumetric flow rate of 50-80 lpm with a fluid dissemination rate of approximately 1-2 ml/min. The Collision nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc, St Paul MN).

A dry powder eductor was used for the dissemination of dry *A. niger* spores and Bacillus

endospores using purified filtered house air. Eductor air supply pressure was regulated at 50 psi with a volumetric flow rate of 30 lpm.

## Bioaerosol Sampling and Monitoring System

A pair of AGI impingers (Ace Glass Inc. Vineland NJ) was used for bio-aerosol collection of viral and vegetative aerosols. The spores trial samples were collected with a 47mm 0.22um Tisch Scientific MCE in line filter with sample flow rates controlled and monitored using a valved Emerson 1/3 hp rotary vane vacuum pump (Emerson Electric, St. Louis, MO) equipped with a 0-30 inHg vacuum gauge (WIKA Instruments, Lawrenceville, GA).

The AGI-30 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 AGI-30 sample

impingers were flow characterized using a calibrated TSI model 4040 mass flow meter. Filter sample flow rates were maintained and monitored at 12.5 lpm using an in line calibrated TSI model 4040 mass flow meter.

Aerosol particle size distributions and count concentrations were measured in real-time through the duration of all control and NV900 trial runs using a model 3321 Aerodynamic Particle Sizer (APS) (TSI Inc, St Paul, MN). The APS sampled for the entire duration of all trials (2-6 hours) with 1 minute sampling intervals. A general flow diagram of the aerosol test system is shown above in Figure 2.

### Species Selection

A single vegetative bacteria as simulant for a common hospital born pathogenic bacteria. *Staphylococcus epidermidis* (ATCC 12228). *Staphylococcus epidermidis* is a Gram-positive bacterium and simulant for a wider range of medically significant pathogens such as *Staphylococcus aureus*.

A single virus were chosen to evaluate the NV900's performance against RNA based viruses. *MS2 bacteriophage* (ATCC 15597-B1) is positive-sense, single-stranded RNA virus that infects the bacterium *Escherichia coli* and other members of the Enterobacteriaceae family. MS2 is routinely used as a surrogate for pathogenic RNA viruses.

*Aspergillus niger* (ATCC 16404) or *A. niger* is one of the most common species of the genus *Aspergillus*. *A. niger* is routinely defined as a troublesome black mold and has been attributed to many respiratory problems for infants, elderly and immune compromised individuals. Purified *A. niger* spores were obtained in bulk dry powder with an approximate concentration of  $1 \times 10^9$  cfu/gram.

*Bacillus subtilis* is a Gram positive bacterium found in soil and the gastrointestinal tract of ruminants and humans. *Bacillus subtilis* is a member of the genus *Bacillus* and is a commonly used in research as a surrogate for *B. anthracis*. *B. subtilis* is rod-shaped, and can form a tough, protective endospore, which allows it to tolerate extreme environmental conditions.

### Vegetative Cells Culture & Preparation

Pure strain seed stocks were purchased from ATCC (American Type Culture Collection, Manassas VA). Working stock cultures were prepared using sterile techniques in a class 2 biological safety cabinet and followed standard preparation methodologies. Approximately 250mL of each biological stock was prepared in tryptic soy liquid broth media, and incubated for 24 – 48 hours with oxygen infusion (1cc/min) at 37°C. Biological stock concentrations were greater than  $1 \times 10^9$  cfu/ml for *Staphylococcus epidermidis* using this method.

Stock cultures were centrifuged for 20 minutes at 5000 rpm in sterile 15mL conical tubes, growth media was removed, and the cells re-suspended in sterile PBS buffer for aerosolization. Aliquots of these suspensions were enumerated on tryptic soy agar plates (Hardy Diagnostics, Cincinnati OH) for viable counts and stock concentration calculation. For each organism, test working stocks were grown in sufficient volume to satisfy use quantities for all tests conducted using the same culture stock material.

### Viral Culture & Preparation

Pure strain viral seed stock and host bacterium were obtained from ATCC. Host bacterium was grown in a similar fashion to the vegetative cells in an appropriate liquid media. The liquid media was infected during the logarithmic growth cycle with the specific bacteriophage. After an appropriate incubation time the cells were lysed and the cellular debris discharged by centrifugation. MS2 stock yields were greater than  $1 \times 10^{11}$  plaque forming units per milliliter (pfu/ml) with a single amplification procedure.

### Fungal Spore Culture & Preparation

*A. niger* fungal spores in purified bulk powder form at a concentration of  $1 \times 10^9$  cfu/g. To verify the bulk powder spore concentration, an aliquot of weighed dry powder was prepared in suspension in PBS + 0.05% Tween 80 at a mass: volume ratio to obtain a concentration of  $1 \times 10^9$  cfu/ml. The spore suspension was serially diluted, plated on potato-dextrose plates and incubated at 35°C for 48 hours.

Plates were enumerated and bulk powder spore concentration was verified to be in the range of  $1 \times 10^9$  cfu/g. Calculations were performed to obtain

mass use needed to generate aerosol test challenge chamber concentrations in the range of  $1 \times 10^6$  cfu/L for testing.

### **Bacillus Subtilis Spore Culture & Preparation**

*B. Subtilis* spores in purified bulk powder form at a concentration of  $1 \times 10^{11}$  cfu/g were used for all trials.

Calculations were performed to obtain mass use needed to generate aerosol test challenge chamber concentrations in the range of  $1 \times 10^6$  cfu/L for testing. Bulk powder spore concentration, an aliquot of weighed dry powder was prepared in suspension in ethanol + PBS + 0.005% Tween 80 at a mass: volume ratio to obtain sufficient chamber concentrations.

### **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. The plated cultures were incubated for 24 hours and enumerated and recorded.

Bacteriophage samples and stock were plated using the small drop plaque assay techniques outlined by A. Mazzocco, T. Waddell, E Lingohr and R. Johnson. The plates were then incubated 8-12 hours and enumerated. All colonies and plaques counts were manually enumerated and recorded.

Bulk powder working stock *Aspergillus* spores were concentration verified prior to testing using the small drop technique. Test spore sample filters were placed in 50ml conical tubes and spores were extracted in 20 ml of sterile PBS buffer + 0.005% Tween 80. Samples were plated using the small drop technique on potato-dextrose agar plates. The plates were incubated at 35°C for 24-48 hours and enumerated.

Trial	Run	Species (gram, description)	ATCC Ref	Target Mondsperised Particle Size	Challenge Conc. (#/ft <sup>3</sup> )	Total Trial Time (min)	Impinger Sample Time (min)	Sampling	Plating and Enumeration
1	Control	<i>Staphylococcus epidermidis</i> (+, vegetative)	11229	2.5 um	$10^4$ - $10^5$	360	0, 60, 120, 180, 240, 300, 360	APS, Impingers	all samples in triplicate
2	Challenge								
3	Challenge								
4	Challenge								
5	Control	<i>MS2 bacteriophage</i>	15597-B1	1.0-1.5um	$10^4$ - $10^6$	240	0, 60, 120, 180, 240, 300, 360	APS, Impingers	all samples in triplicate
6	Challenge								
7	Challenge								
8	Control	<i>Aspergillus niger</i> (mold, spore forming)	13835	<5.0um	$10^4$ - $10^6$	240	0, 60, 120, 180, 240, 300, 360	APS, Impingers	all samples in triplicate
9	Challenge								
10	Challenge								
11	Control	<i>Bacillus subtilis endospore</i> ( <i>Bacillus Spores</i> )	16404	<3.5 um	$10^4$ - $10^6$	240	0, 60, 120, 180, 240, 300, 360	APS, Impingers	all samples in triplicate
12	Challenge								

**Table 1: Bioaerosol Test Matrices for all trials.**

### Novaerus - Polystyrene Latex Beads - Large Chamber

LOG Scale, Normalized Airborne Concentration, Poly Styrene Latex Beads (PSL), TSI APS 3321

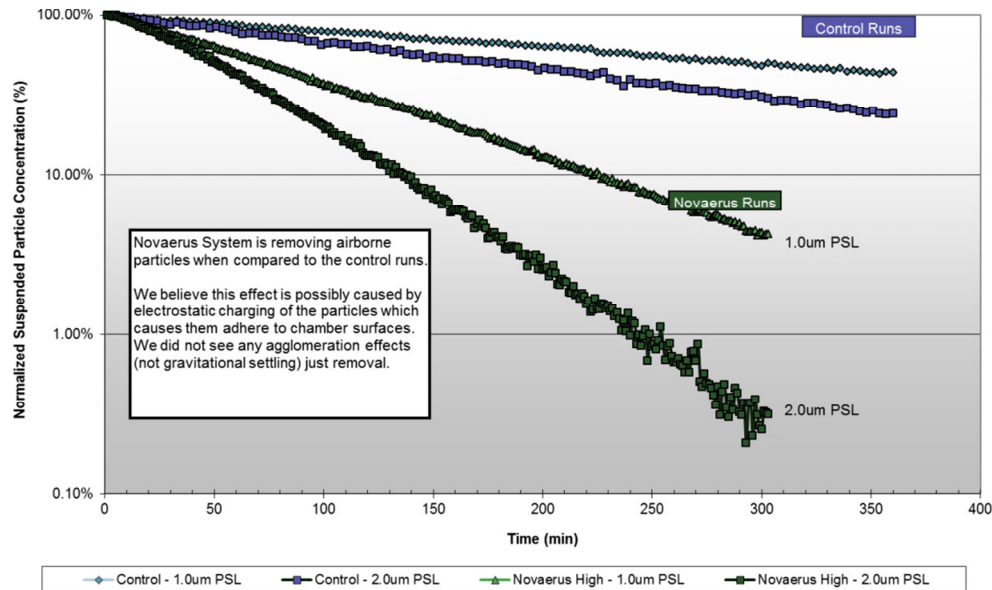


Figure 3: Preliminary PSL Trials Chamber and NV900 Characterization.

### Chamber Characterization

In order to calculate the dissemination efficiency and stability of the bioaerosol, polystyrene latex beads (PSL beads) were used to characterize the various aspects of the chamber system. PSL beads with aerodynamic diameters of 1.0µm, 2.0µm and 4.0µm were nebulized and chamber concentrations were recorded using the APS. Nebulization efficiencies, particle stability and AGI-30 collection efficiencies were used to estimate generation efficiencies, dissemination times, sample times and aerosol persistence prior to bioaerosol testing.

### Control Testing

To accurately assess the NV900 unit, a test chamber pilot control trials were performed with each biological over 4 to 6 hour periods without the NV900 in operation to characterize each biological challenge aerosol for particle size distribution, aerosol delivery/collection efficiency, and viable concentration over time. Control testing was performed to provide baseline comparative data in order to assess the actual reduction from NV900 challenge testing and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire pilot control test period.

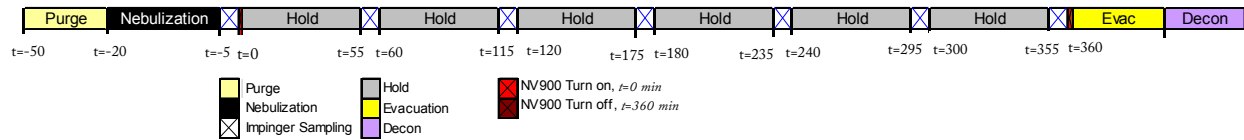
During control runs, a single low velocity fan located in the corner of the bioaerosol test chamber was turned on for the duration of trial to ensure a homogenous aerosol concentration within the aerosol chamber. The mixing fan was used for all control and NV900 decontamination trials. The two impingers used for bacteriophage, vegetative, and bacterial endospore test sampling were pooled and mixed prior to plating and enumeration. Filter samples used for fungal spore test sampling were extracted in 20ml of PBS buffer + 0.005% Tween 80 and vortexed prior to plating.

### Novaerus NV900 Testing

Four challenge biological organisms: *Staphylococcus epidermidis* (ATCC 12228), MS2 bacteriophage (ATCC 15597-B1), *Aspergillus niger* (ATCC 16404), and *Bacillus Subtilis* were used for testing the viable reduction capacity of the Novaerus NV900 unit against the broad spectrum bioaerosols. Aerosol decontamination testing was performed for each biological with the addition of a pilot control test for each organism. The complete test matrix for the study is shown in Table 1 (page 5).

For each control and challenge test, excluding *A. niger*, the Collison nebulizer was filled with

## General Timeline for Bioaerosol Chamber Testing



**Table 2:** General Trial Timeline for NV900 Decontamination Trials.

approximately 40 mL of biological stock and operated at 28-50 psi for a period of 15 or 25 minutes (organism dependent). For control and NV900 trials, the impingers were filled with 20 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 *A. niger* control and NV900 trials, the eductor was filled with approximately 2 grams of gravimetrically weighed purified dry spores and operated at 50psi for 5 minutes.

Chamber mixing fan were turned on during bioaerosol dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to the first impinger or filter sample.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and Novaerus NV900 test by sampling simultaneously with two AGI-30 impingers or filters located at opposite sides of the chamber. AGI samples were collected for 5 or 10 minutes (organism dependent) and filter samples were collected for 10 minutes with subsequent 5 or 10 minute samples taken at intervals of 30-60 minutes throughout the entire period. Table 2 below shows the general timeline for each NV900 live bioaerosol challenge trial.

Collected impinger samples were pooled and mixed at each sample interval for each test, and an aliquot pulled for plating and enumeration of viable concentration. 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. Filter samples used for *Aspergillus* spore only aerosol collection were placed in sterile 50 ml conical tubes, extracted in 20ml of PBS + 0.005% Tween 80 and an aliquot pulled for plating and enumeration of viable concentration.

The filter holders were rinsed with isopropyl alcohol, dried with filtered compressed air, and reloaded with a sterile filter between each sample point.

For NV900 biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test (up to 6 hours). Subsequent impinger samples or filter samples were taken at intervals of 30 to 60 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the NV900 system over time.

Test chamber temperature and humidity were recorded at the initiation and completion of each test. The Collision nebulizer stock volume and use rate were also measured gravimetrically. Impingers were tared on a microbalance, and reweighed after each sample period for net collection media mass and accurate calculation of collected concentration. All samples were plated in triplicate on tryptic soy agar media over a minimum of a 3 LOG dilution range.

Plates were incubated for viable plaque forming units (pfu) formation for the viral phase of the study, and colony forming units (cfu) for fungal spore, and bacterial endospore phases of the study. Plates were incubated and enumerated for viable counts to calculate aerosol challenge concentrations in the chamber and reduction of viable microorganisms.

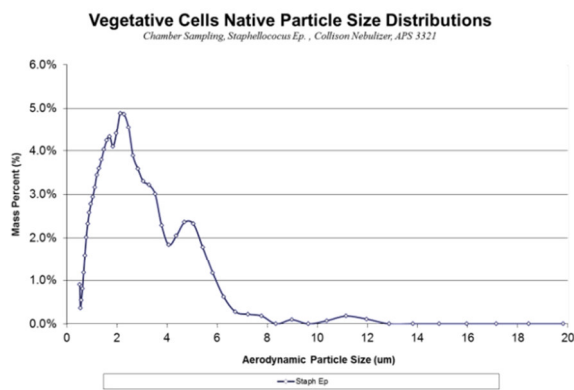
### Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of twenty minutes between tests and analyzed with the APS for particle concentration decrease to baseline levels between each test. The chamber was decontaminated between live microorganism trials with vaporous hydrogen peroxide. The Collision nebulizer, impingers, and filter holders were cleaned at the conclusion of each day of testing by soaking in a 5% bleach bath for 20 minutes. The nebulizer, impingers and filter holders

were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

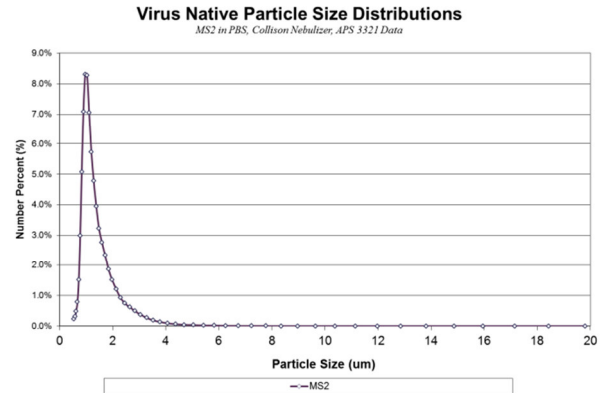
### Bioaerosol Particle Size Data

Aerosol particle size distributions were measured with the APS. The APS has a dynamic measurement range of 0.5 to 20µm and was programmed to take consecutive real time one minute aerosol samples throughout the duration of each aerosol trial.



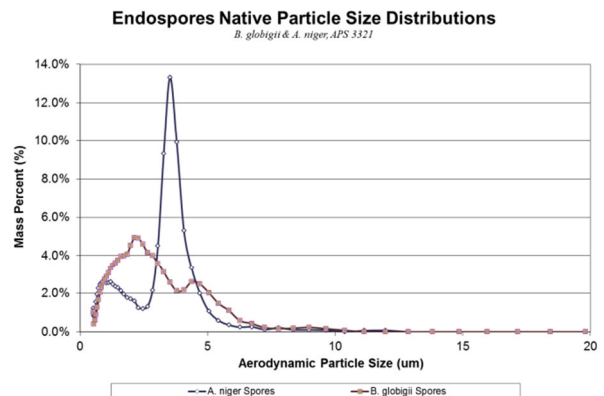
**Figure 3:** Vegetative Cells Particle Sized Distribution in Test Chamber.

Data was logged in real time to an Acer laptop computer, regressed, and plotted. Aerosol particle size distributions showing each bioaerosol are shown in Figures 3 and figure 4 and 5.



**Figure 4:** Viral Particle Size Distribution in Test Chamber.

The particle size distributions for each bioaerosol are shown to be within the respirable range for alveolar region tract lung deposition and show a low geometric standard deviation (GSD) indicating a monodispersed aerosol was generated into the test chamber.



**Figure 5:** Fungal Spores and Endospore Particle Size Distribution in Test Chamber.

### NV900 Vegetative Bioaerosol Results

Results from the control trials were graphed and plotted to show natural viability loss over time in the chamber. These control runs served as the basis to determine the time required for NV900 to achieve a 4 LOG reduction in viable bioaerosol above the natural losses from the control runs. The control and trial runs are plotted showing LOG reduction in viable bioaerosol for each organism. All data is normalized with time zero ( $t=0$  minutes) enumerated concentrations. Subsequent samples are normalized and plotted to show the loss of viability over time (Figures 7, 8, 9, 10, 11 and 12).



### Bioaerosol Decontamination Characterization

*Staphylococcus epidermidis*, Control + Novaerus Decon Run, AGI-30 Impinger Enumeration in Triplicate

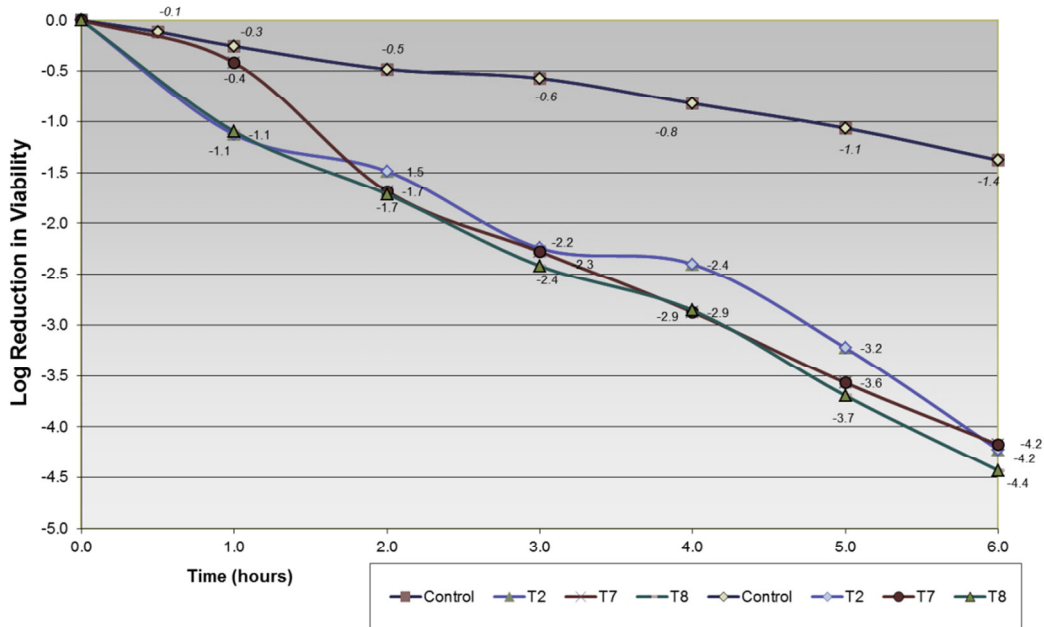


Figure 7: *S. Epidermidis* Control and NV900 trial LOG Reduction in Viable Concentration.

It was demonstrated that stability for *S. epidermidis* during the control runs was excellent even at extremely high concentrations. Chamber viable aerosol concentrations were greater than  $1 \times 10^4$  cfu/liter or  $2.8 \times 10^5$  cfu/ft<sup>3</sup> for all trials.

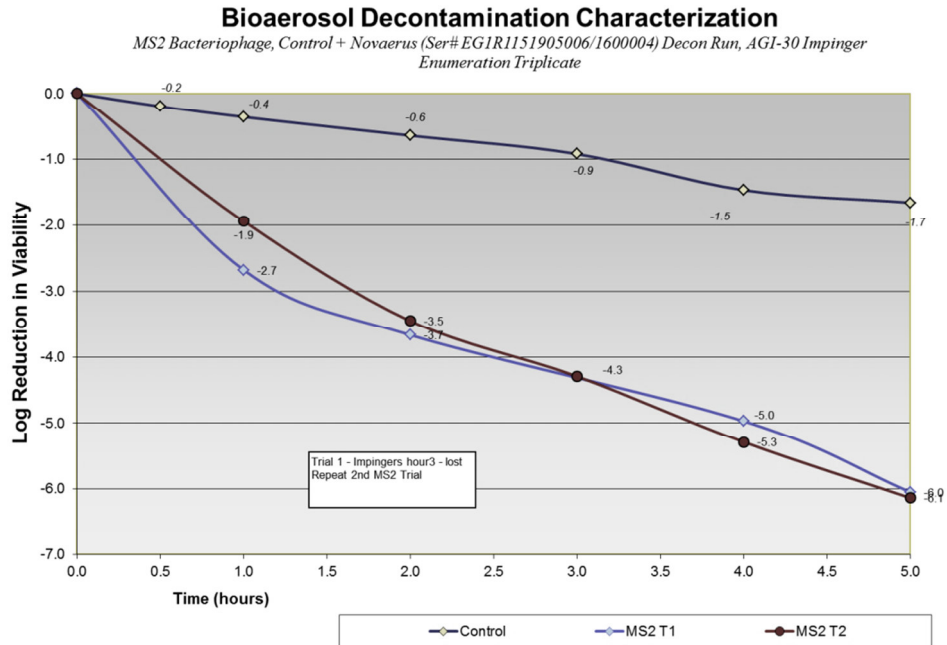
The viable concentration within the aerosol chamber decreased over a period of 6 hours and showed a loss in viable aerosol of approximately 1.4 logs for the control run. In contrast, the NV900 trials showed a viable bioaerosol reduction of 4.2, 4.2 and 4.4 logs for each trial in 6 hour.

Total viable reduction of airborne *S. epidermidis* was  $2.90 \pm 0.14$  logs (Avg.  $\pm$  STdev) above the control run at 6 hour. Figure 7, shows the results of the control and triplicate *Staphylococcus* NV900 trial runs.

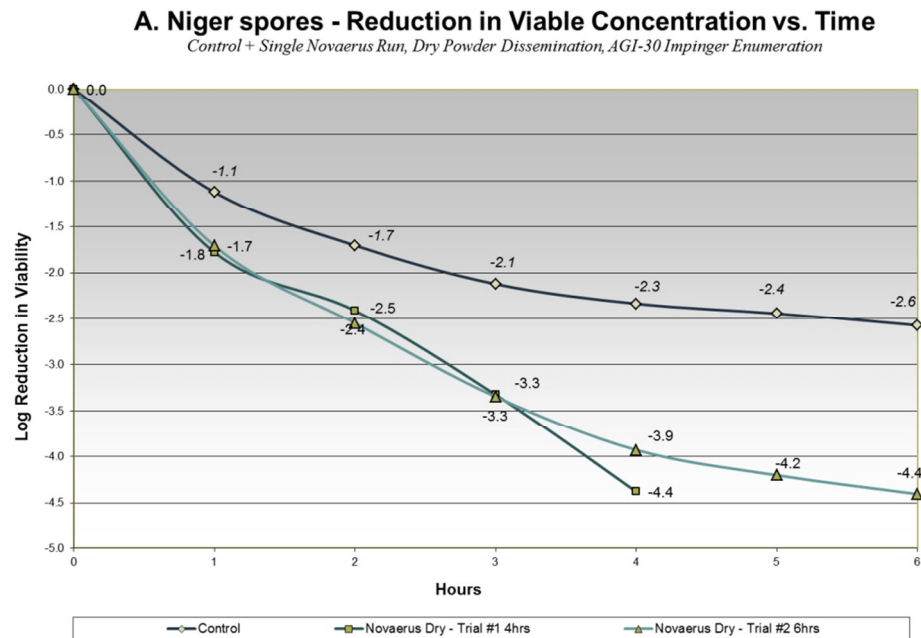
### NV900 Viral Bioaerosol Results

Results from the control trials were graphed and plotted in a similar fashion to vegetative cell bioaerosol testing with the control runs plotted alongside the NV900 live challenge runs.

Testing results with MS2 bacteriophage (figure 8) showed that the NV900 showed viable reductions of 6.0 and 6.1 LOG for the duplicate trials. This was in contrast to the control run which showed a 1.7 LOG reduction after 6 hours. The adjusted viable reduction after subtracting the control run reduction showed that the NV900 reduced the viable MS2 aerosol by  $4.44 \pm 0.06$  logs (Avg.  $\pm$  STdev) in the 360 minutes timeframe.



**Figure 8:** Bacteriophage MS2 Control and NV900 trial LOG Reduction in Viable Concentration.



**Figure 9:** *Aspergillus niger* spores Control and NV900 trial LOG Reduction in Viable Concentration.

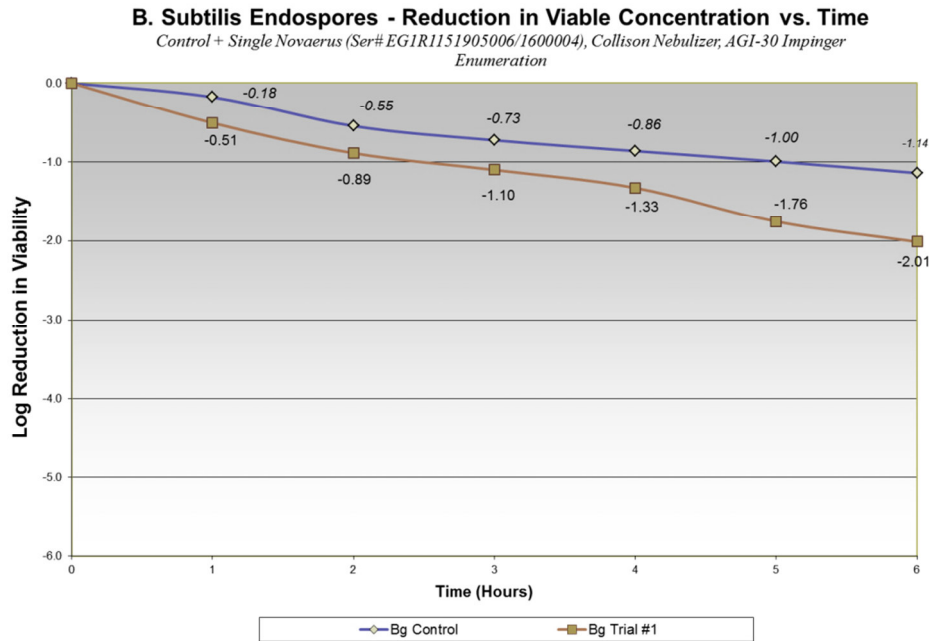


Figure 10: B. Subtilis Control and NV900 Trial LOG Reduction in Viable Concentration.

### NV900 Aspergillus Spore Bioaerosol Results

*A. niger* stability was poor over the 6 hour control trial with a measured loss of a loss 2.6 LOG after 6 hours. This could possibly be due to a net surface charge on the bioaerosol due to the dry powder dissemination technique. However, stability was adequate to show 2.05 LOG net reduction during the 4 hour trial NV900 trial.

NV900 testing showed a net of 1.94 +/- 0.15 LOG (Avg. +/- STdev) reduction above the baseline control trial.

### NV900 Endospore Bioaerosol Results

*B. subtilis endospore* stability was excellent over the 6 hour control run period. The control run showed that over a 6 hour period, approximately 1.14 LOG reduction in viable aerosol was observed. Chamber initial aerosol concentrations were high for all NV900 trials an averaged  $1.27 \times 10^6$  cfu/l for the  $t=0$  impinger samples.

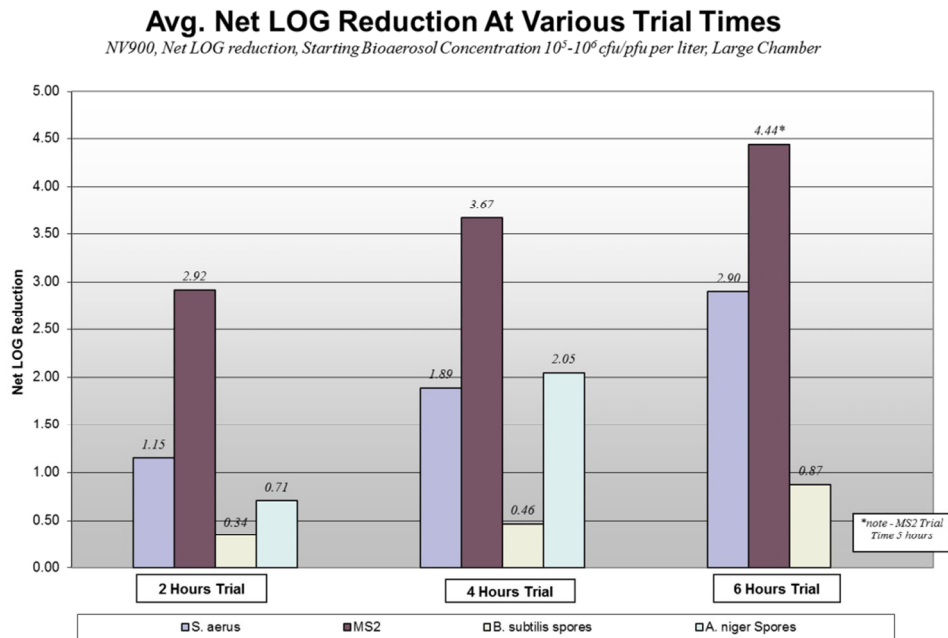
Test results shown in figure 10 for *B. Subtilis* reflect the NV900 trials showed only a 2.01 LOG reduction in 6 hours, compared to the control which had a 1.14 LOG reduction in the same timeframe. NV900 testing showed a net of 0.87 LOG reduction above the baseline control trial for the single bioaerosol challenge.

### Summary of Findings

Test results show that Novaerus NV900 was extremely effective at reducing viability of bioaerosols in all conducted trials. Results from the control baseline viability tests show very stable viable aerosol persistence in the chamber with minimal losses in viability related to environmental conditions or chamber deposition.

NV900 System's efficacy of reduction of *S. epidermidis* viability, after correcting for control run losses, were 2.92 +/- 0.2 logs (average +/- standard deviation) in 6 hours. The reduction for viral bioaerosol concentrations within the chamber were 4.44 +/- 0.06 logs (Avg +/- STdev) in 5 hours for bacteriophage MS2. The *A. niger* fungal spores resulted in viable bioaerosol concentration reduction within the chamber of 2.05 +/- 0.15 logs (Avg +/- STdev) in 4 hours. The NV900 performance against aerosolized *B. subtilis* endospores was less than other aerosolized micro-organisms yet the NV900 still showed viable bioaerosol concentration reduction within the chamber of 0.87 logs (Avg +/- STdev) in 6 hours.

Figure 12 shows the average net LOG reduction in all bioaerosols trials after correction for control run viability losses. Table 3 shows the summary of results in tabulated form.



**Figure 12:** Summary of Net LOG reduction of Viable Bioaerosol concentration for NV900.

**Average NET LOG Reduction of BioAerosols by NV900**

Bioaerosol Type	Species (gram, description)	Surrogate	Number of Trials	Trial Time					
				1hr	2hr	3hr	4hr	5hr	6hr
Bacterial	<i>Staphylococcus epidermidis</i> (+, vegetative)	<i>Staphylococcus aerus</i>	3	0.63 +/- 0.4	1.15 +/- 0.12	1.74 +/- 0.09	1.89 +/- 0.27	2.43 +/- 0.24	2.9 +/- 0.14
Virus	<i>MS2 bacteriophage</i> (RNA E. coli phage)	<i>Influenza</i>	2	1.95 +/- 0.52	2.92 +/- 0.15	3.37	3.67 +/- 0.23	4.44 +/- 0.06	-
Spores	<i>Bacillus subtilis endospore</i> (Bacillus Spores)	<i>Anthrax</i>	1	0.33	0.34	0.37	0.46	0.76	0.87
Spores	<i>Aspergillus niger</i> (mold, spore forming)	<i>Black Mold</i>	2	0.66	0.71	1.20	1.94 +/- 0.15	-	-

**Average Percent Kill of BioAerosols by NV900**

Bioaerosol Type	Species (gram, description)	Surrogate	Number of Trials	Trial Time					
				1hr	2hr	3hr	4hr	5hr	6hr
Bacterial	<i>Staphylococcus epidermidis</i> (+, vegetative)	<i>Staphylococcus aerus</i>	3	67.82% +/- 31.7%	92.68% +/- 2.21%	98.16% +/- 0.37%	98.51% +/- 1%	99.59% +/- 0.25%	99.87% +/- 0.04%
Virus	<i>MS2 bacteriophage</i> (RNA E. coli phage)	<i>Influenza</i>	2	98.44% +/- 1.52%	99.88% +/- 0.04%	99.96%	99.98% +/- 0.01%	100% +/- 0%	-
Spores	<i>Bacillus subtilis endospore</i> (Bacillus Spores)	<i>Anthrax</i>	1	53.44%	54.42%	57.59%	65.59%	82.73%	86.63%
Spores	<i>Aspergillus niger</i> (mold, spore forming)	<i>Black Mold</i>	2	77.96%	80.32%	93.75%	99.10%	-	-

**Table 3:** Summary of Results.

## References

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**GLP Statement**

We, the undersigned, hereby 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 40 CFR, Part 160.


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## Appendix A: 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 use rate ( $R_{neb}$ ) (volume of liquid generated by the nebulizer/time) at 28 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

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:

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

Plating and enumeration of the biological to derive the concentration of the dry powder ( $C_p$ ) in cfu/g.

- Eductor use rate ( $M_p$ ) (Mass of powder generated by the eductor in grams)
- Chamber volume ( $V_c$ ) = 15,993 Liters

Assuming 100% efficiency, the quantity of aerosolized viable particles ( $V_p$ ) per liter of air in the chamber for a given dry powder stock concentration ( $C_p$ ) is calculated as:

$$\text{Eductor: } V_p = \frac{C_p \cdot M_p}{V_c}$$

AGI – 30 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.
- AGI-30 impinger or filter sample flow rate ( $Q_{imp}$ ) = 12.5 L/min.
- AGI-30 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:

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

The aerosol system viable delivery efficiency (expressed as %) is:

$$Efficiency = \frac{C_a}{V_p} \cdot 100$$



