

Determination of the ODOROX[®] MDU/Rx[™] System's Efficacy against Various Bioaerosols

Abstract

This in vitro study will characterize the ODOROX[®] MDU/Rx[™] System decontamination efficacy against various aerosolized biologicals. The ODOROX[®] MDU/Rx[™] System is designed to neutralize surface and 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 bacteria, virus, and spores in a large environmental chamber.

The efficacy of the system was assessed for each of the five (5) following aerosolized biologicals: Staphylococcus epidermidis, Erwinia herbicola, MS2 bacteriophage, Phi-X174 bacteriophage, and Aspergillus Niger fungus spores. The study consisted of a total of twenty (20) separate trials; one control run plus triplicate challenge trials for each of the five (5) aerosolized biologicals.

MDU/Rx[™] System's efficacy of reduction of S. epidermidis viability, after correcting for control run losses, were 5.0 +/- 0.2 logs (average +/- standard deviation) in 1 hour. The system's efficacy against E. herbicola bioaerosol, after correcting for control trial viability losses, were 5.0 +/- 0.5 log (Avg +/- STdev) in 1.5-2.0 hours trial time. The reduction for viral bioaerosol concentrations within the chamber were 4.9 +/- 0.3 logs and 4.0 +/- 0.1 logs (Avg +/- STdev) in 2 hours or less for bacteriophage MS2 and PhiX174 respectively. The A. niger fungal spores resulted in viable bioaerosol concentration reduction within the chamber of 4.7 +/- 0.3 logs (Avg +/- STdev) in 1 hour.

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 ODOROX[®] Mobile Disinfection Unit (MDU/Rx[™]) Hydroxyl Air Processor, produced by HGI Industries Inc. (Boynton Beach, FL), to neutralize airborne bioaerosols. Testing was conducted in a controlled stainless steel environmental chamber. The ODOROX[®] MDU/Rx[™] effectiveness against five separate Biosafety 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 test device in a closed environmental chamber to

determine the destruction rate of the MDU/Rx[™] against airborne microorganisms. The system's effectiveness was evaluated against two vegetative bacteria, two viruses, and a fungal spore used as simulants for a broader range of pathogenic organisms.

Testing was conducted to characterize a single MDU/Rx[™] unit against the five separate and distinct organisms in independently repeated tests to demonstrate the capability of the MDU/Rx[™] to reduce viable bioaerosol concentrations by four logs (99.99% deactivation) compared to control runs. The testing for the MDU/Rx[™]'s effectiveness was conducted in triplicate and compared to a single control run.

ODOROX® MDU/Rx™ System

Picture:



Device Features

Manufacturer: HGI Industried Inc.
 Model: Mobile Disinfection Unit (MDU)

Notes: Hydroxyl Air Processor

Figure 1: ODOROX® Mobile Disinfection Unit (MDU/Rx™).

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 Fox dry powder eductor for the fungal 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 ft³/min rated flow capability for rapid evacuations of remaining bioaerosols.

A magnehelic gauge with a range of 0.0 +/- 0.5 inch H₂O (Dwyer instruments, Michigan City IN) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles.

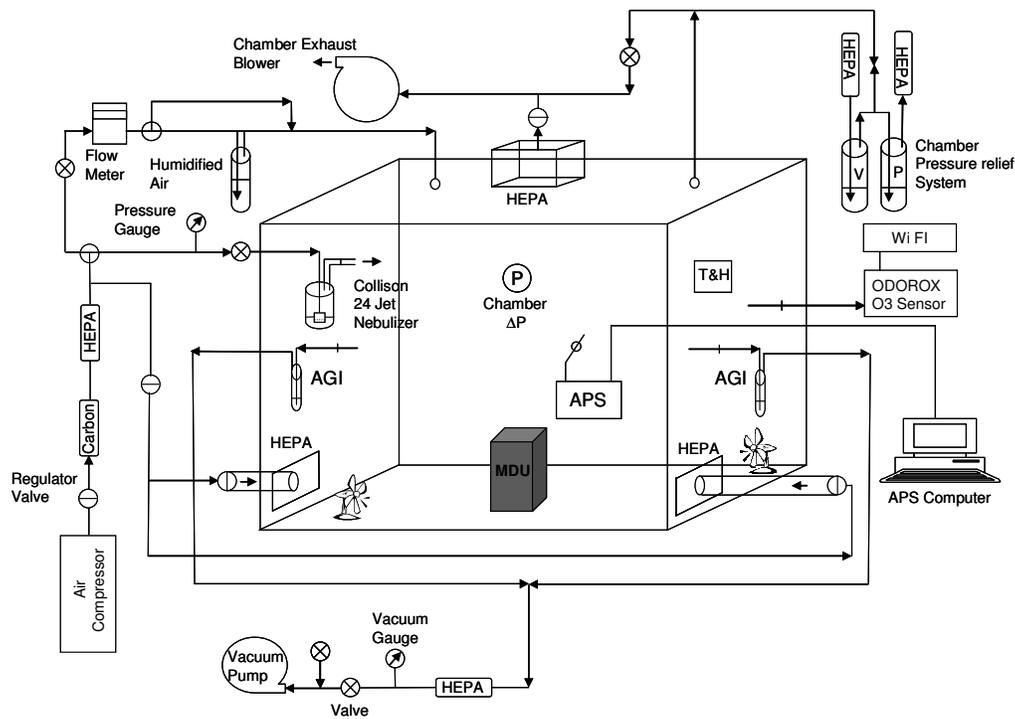


Figure 2: Bio-Aerosol Test Chamber Flow Diagram.

Bioaerosol Generation System

Test bacteriophage and vegetative bioaerosols were disseminated using a Collison 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 Collison nebulizer.

Prior to testing, the Collison 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 Collison nebulizer was flow characterized using a calibrated TSI model 4040 mass flow meter (TSI Inc, St Paul MN).

A Fox dry powder eductor was used for the dissemination of dry *A. niger* spores 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 fungal spores 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 MDU/Rx™ 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

Two vegetative bacteria were chosen for the study as simulants for a broad range of pathogenic bacteria. The first vegetative organism used for this study was *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*.

Erwinia herbicola (ATCC 39049), renamed *Pantoea agglomerans*, is a Gram-negative bacterium which is commonly used as a simulant for *Francisella tularensis* and *Yersinia pestis* (bubonic plague).

Two representative BSL1 viruses were chosen to evaluate the MDU/Rx™'s performance against both RNA and DNA 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 simulant for pathogenic RNA viruses.

Phi-X174 (ATCC 13706-B1) *bacteriophage* is a circular single stranded DNA based virus that infects the bacterium *Escherichia coli*. Phi-X174 was selected as a simulant for DNA based pathogenic 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 1×10^9 cfu/gram.

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 1×10^9 cfu/ml for both *Staphylococcus epidermidis* and *Erwinia herbicola* 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 were 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 was discharged by centrifugation. MS2 stock yields were greater than 1×10^{11} plaque forming units per milliliter (pfu/ml) with a single amplification procedure. Phi-X174, due to its much lower burst size, required multiple amplification steps to produce satisfactory viral yields. After amplification the cells were lysed and the cellular debris was separated from the liquid media and discarded. Phi-X174 viral yields were plated and enumerated and yielded viable concentrations greater than 1×10^8 pfu /ml in the stock used for aerosolization.

Fungal Spore Culture & Preparation

A. niger fungal spores were obtained in purified bulk powder form at a concentration of 1×10^9 cfu/g. To verify the bulk powder spore concentration, an aliquot of weighed dry powder was prepared in suspension in PBS + 0.005% Tween 80 at a mass: volume ratio to obtain a concentration of 1×10^9 cfu/ml. The spore suspension was serially diluted, plated on TSA plates and incubated at 30°C for 48 hours.

Plates were enumerated and bulk powder spore concentration was verified to be in the range of 1×10^9 cfu/g. Calculations were performed to obtain mass use needed to generate aerosol test challenge chamber concentrations in the range of 1×10^6 cfu/L for testing the ODOROX® MDU/Rx™ System.

Trial	Run	Species (gram, description)	ATCC Ref	Target Mondsperized Particle Size	Challenge Conc. (#/ft ³)	Total Trial Time (min)	Impinger Sample Time (min)	Sampling
1 2 3 4	Control Challenge Challenge Challenge	<i>E. coli</i> (+, vegetative)	11229	2.5 um	10 ⁴ -10 ⁶	120	0,30,60,90,120	APS, Ozone, Impinger Train
5 6 7 8	Control Challenge Challenge Challenge	<i>Staphylococcus epidermidis</i> (+, vegetative)	12228	1.5-2.0 um	10 ⁴ -10 ⁶	120	0,30,60,90,120	APS, Ozone, Impinger Train
9 10 11 12	Control Challenge Challenge Challenge	<i>Phi-X174 phage</i> (<i>E. coli</i> phage)	13706	<1.0um	10 ⁴ -10 ⁶	120	0,30,60,90,120	APS, Ozone, Impinger Train
13 14 15 16	Control Challenge Challenge Challenge	<i>MS2 bacteriophage</i> (<i>E. coli</i> phage)	15597-B1	<1.0um	10 ⁴ -10 ⁶	120	0,30,60,90,120	APS, Ozone, Impinger Train
17 18 19 20	Control Challenge Challenge Challenge	<i>Aspergillus niger</i> (mold, spore forming)	16404	3.5-4.0 um	>10 ⁶	120	0,30,60,90,120	APS, Ozone, Filter Samples

Table 1: Test Matrices for all trials.

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 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 TSA agar plates. The plates were incubated at 30°C for 24-48 hours and enumerated.

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.

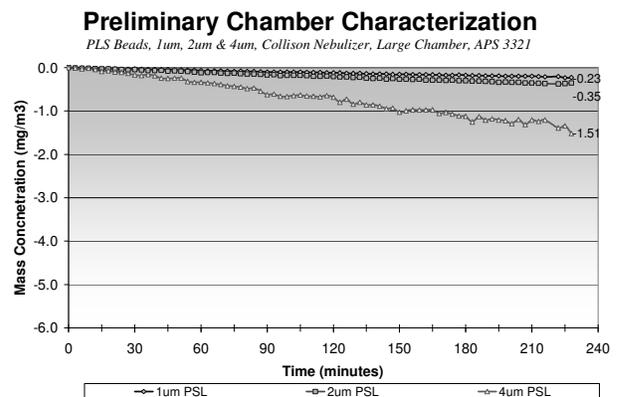


Figure 3: Chamber Characterization using various sizes of PSL beads.

Control Testing

To accurately assess the ODOROX[®] MDU/Rx[™] unit, a test chamber pilot control test was performed with each biological over 4 to 6 hour periods without the ODOROX[®] MDU/Rx[™] 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 MDU/Rx[™] challenge testing and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire pilot control test period.

During control runs, two low velocity fans located in the corners of the bioaerosol test chamber were turned on for the duration of impinger sampling and filter sampling to ensure that a homogenous aerosol sample was collected. The two impingers used for bacteriophage and vegetative 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 for 5 minutes prior to plating.

ODOROX[®] MDU/Rx[™] Testing

Four challenge biological organisms: *Staphylococcus epidermidis* (ATCC 12228), *Erwinia herbicola* (ATCC 39049), MS2 bacteriophage (ATCC 15597-B1), *Phi-X174* bacteriophage (ATCC 13706-B1), and *Aspergillus niger* (ATCC 16404) were used for testing the viable reduction capacity of the ODOROX[®] MDU/Rx[™] unit against the broad spectrum bioaerosols. Aerosol decontamination testing was performed in triplicate for each biological with the addition of a pilot control test for each organism (20 total tests). 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

approximately 40 mL of biological stock and operated at 28-50 psi (organism dependent for a period of 20 minutes. For control and MDU/Rx[™] trials, the impingers were filled with 20 mL of sterilized PBS (addition of 0.01% v/v Tween 80 for Phi-X174) for bioaerosol collection. The addition of Tween 80 was shown to increase the impinger collection efficiency of Phi-X174. For *A. niger* control and MDU/Rx[™] trials, the Fox eductor was filled with approximately 2 grams of gravimetrically weighed purified dry spores and operated at 50psi for 5 minutes.

Chamber mixing fans were turned on during bioaerosol dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to the first impinger or filter sample. Mixing fans were not used for subsequent MDU/Rx[™] AGI or filter sampling because the MDU/Rx[™] system has its own internal mixing fan.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and ODOROX[®] MDU/Rx[™] test by sampling simultaneously with two AGI-30 impingers or filters located at opposite sides of the chamber. AGI samples were collected for 5 minutes and filter samples were collected for 10 minutes with subsequent 5 or 10 minute samples taken at intervals of 30 minutes throughout the entire period. Table 2 below shows the general timeline for each MDU/Rx[™] 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 3x 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 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.

General Timeline for Bioaerosol Chamber Testing

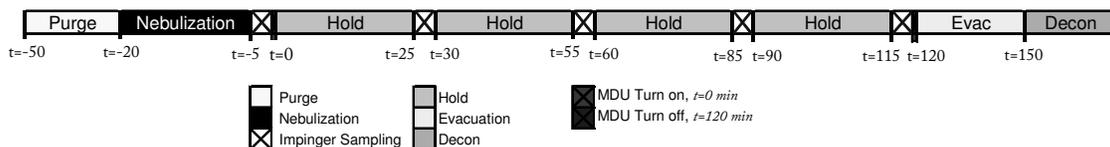


Table 2: General Trial Timeline for MDU/Rx[™] Decontamination Trials.

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

For ODOROX® biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test (120 minutes). 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 ODOROX® system over time.

Test chamber temperature and humidity were recorded at the initiation and completion of each test.

The Collison 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 bacterial and fungal spore 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.

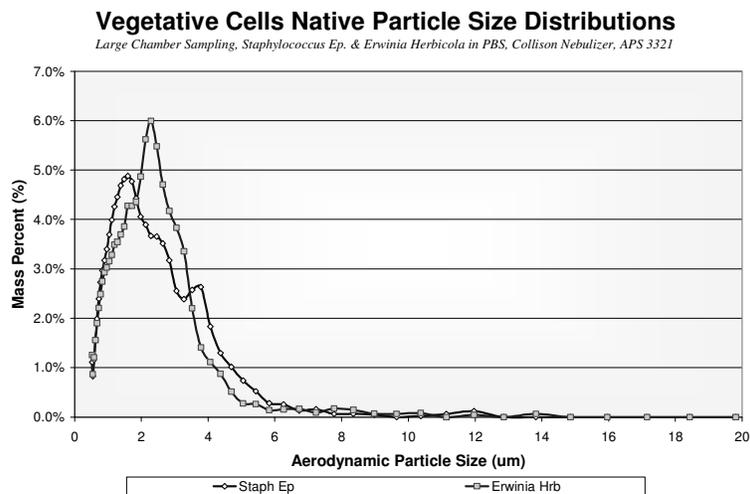


Figure 3: Vegetative Cells Particle Sized Distribution in Test Chamber.

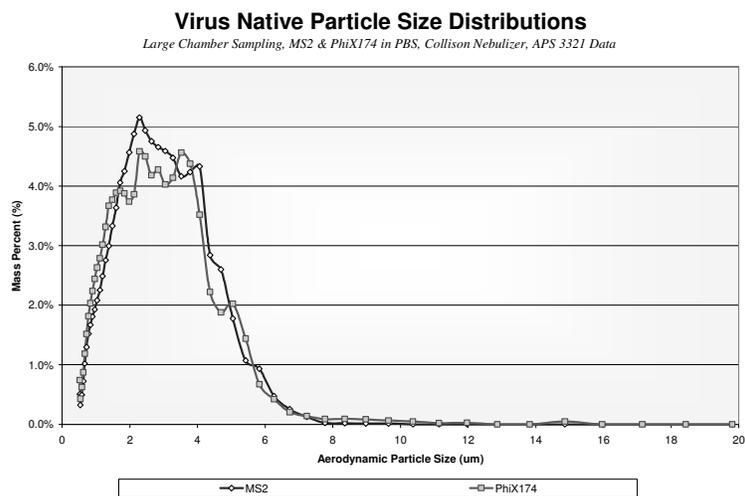


Figure 4: Viral Particle Size Distribution in Test Chamber.

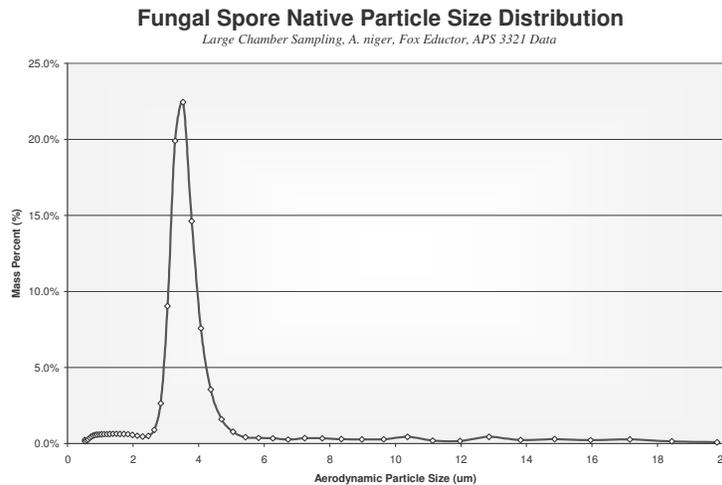


Figure 5: Fungal Spores Particle Size Distribution in Test Chamber.

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 Collison nebulizer, impingers, and filter holders were cleaned at the conclusion of each day of testing by soaking in a 10% bleach bath for 20 minutes. The nebulizer, impingers and filter holders were then submerged in a DI water bath, removed, and spray rinsed 10x 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. Data was logged in real time to an Acer laptop computer, regressed, and plotted. Representative aerosol particle size distributions showing the mass median aerodynamic diameter (MMAD) of each bioaerosol are shown in Figures 3 (page 7) and figure 4 and 5 (page 8).

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 6, shows a summary of the MMAD and GSD for each challenge organism.

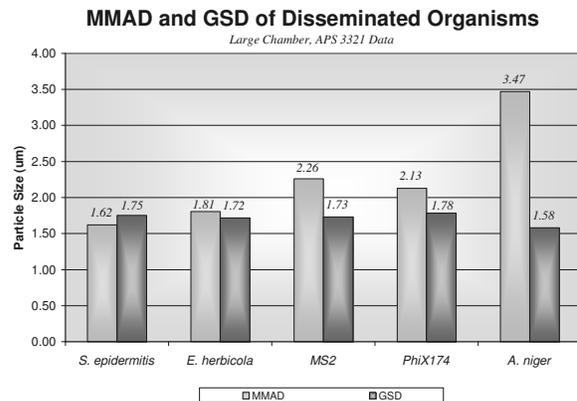


Figure 6: MMAD and GSD of Bioaerosols

MDU/Rx™ 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 MDU/Rx™ 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, and 11).

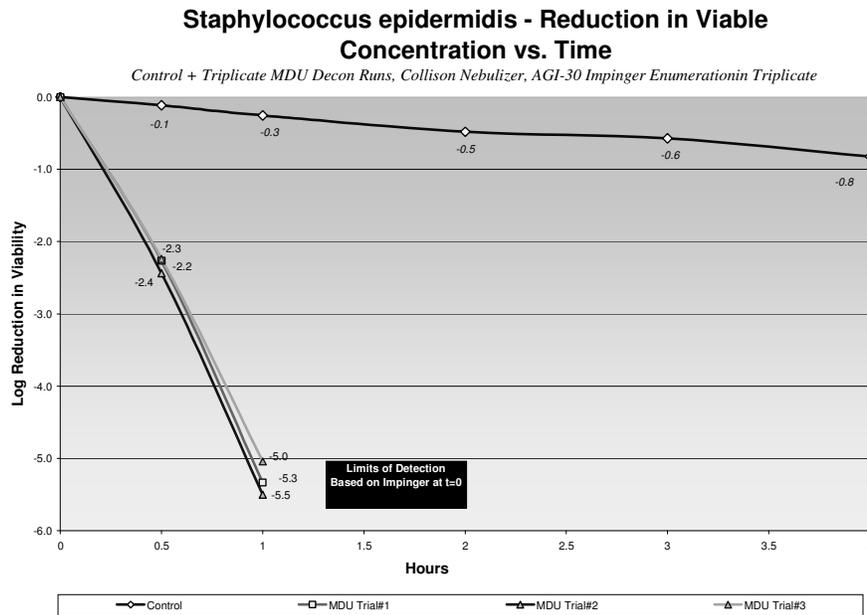


Figure 7: *S. epidermidis* Control and MDU/Rx™ trial Log Reduction in Viable Concentration.

It was demonstrated that for *S. epidermidis* stability of the organism during the control runs was excellent even at extremely high concentrations. Chamber viable aerosol concentrations were greater than 1×10^5 cfu/liter or 2.8×10^6 cfu/ft³ for all trials.

The viable concentration within the aerosol chamber decreased over a period of 4 hours and showed a loss in viable aerosol of approximately 0.8 logs for the control run. In contrast, the MDU/Rx™ trials showed a viable bioaerosol reduction of 5.0, 5.3 and 5.3 logs for each trial in 1 hour. After 1 hour all subsequent impinger samples (t=90 and 120 minutes)

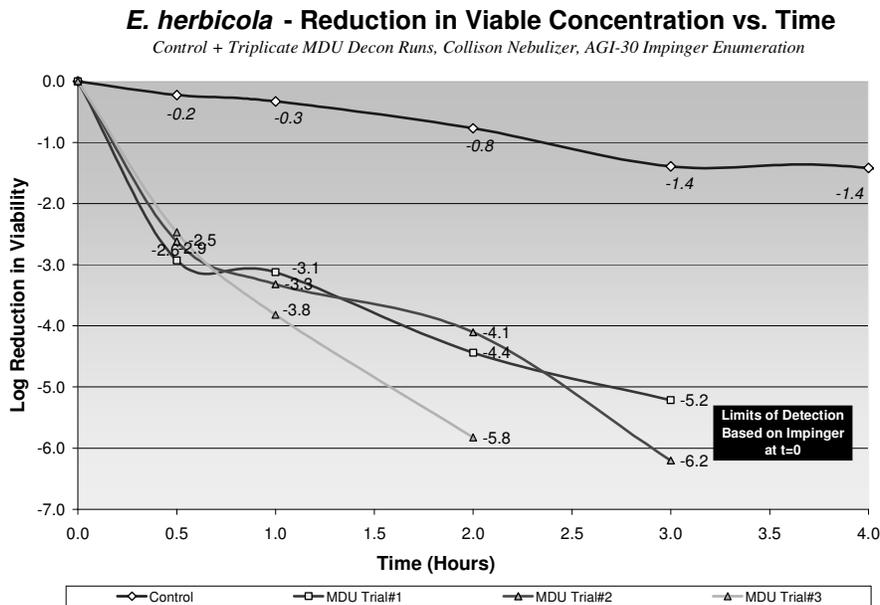


Figure 8: *E. herbicola* Control and MDU/Rx™ trial Log Reduction in Viable Concentration.

plated neat showed no colony growth. Total viable reduction of airborne *S. epidermidis* was 5.0 +/- 0.2 logs (Avg. +/- STdev) above the control run at 1 hour. Limits of detection were not able to resolve the continued reduction at the 90 and 120 minute mark. Figure 7, shows the results of the control and triplicate Staphylococcus MDU/Rx™ trial runs.

E. herbicola stability was similar to that of the *S. epidermidis*. The control run showed that over a 4 hour period, approximately 1.4 log reduction in viable aerosol was observed. Chamber initial aerosol concentrations were high for all MDU/Rx™ trials and averaged 3.62×10^5 cfu/l or 1.02×10^7 cfu/ft³ for the t=0 impinger sample.

The triplicate MDU/Rx™ trials showed a viable *E. herbicola* reduction of 6.2, 5.2 and 5.8 logs within 90-120 minutes. Figure 8, shows the results of the control and triplicate *Erwinia herbicola* MDU/Rx™ trial runs. The MDU/Rx™ unit was able to reduce the viable bioaerosol concentrations 5.0 +/- 0.5 logs (Avg +/- STdev) over the control runs in approximately 180 minutes.

MDU/Rx™ 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 MDU/Rx™ live challenge triplicate runs.

Testing results with MS2 bacteriophage (figure 9) showed that the MDU/Rx™ showed viable reductions of 6.2, 5.9 and 6.6 log for the triplicate trials. This was in contrast to the control run which showed a 0.9 log reduction after 3 hours. The adjusted viable reduction after subtracting the control run reduction showed that the MDU/Rx™ reduced the viable MS2 aerosol by 4.9 +/- 0.3 logs (Avg. +/- STdev) in the 90-180 minutes timeframe.

Similar results were observed for the DNA phage Phi-X174. The MDU/Rx™ trials (figure 10) showed a 5.0, 5.1 and 5.1 log reduction in 2 hours compared to the control which had a 1.1 log reduction in the same timeframe. MDU/Rx™ showed a net of 4.0 +/- 0.1 log (Avg. +/- STdev) reduction above the baseline control trial.

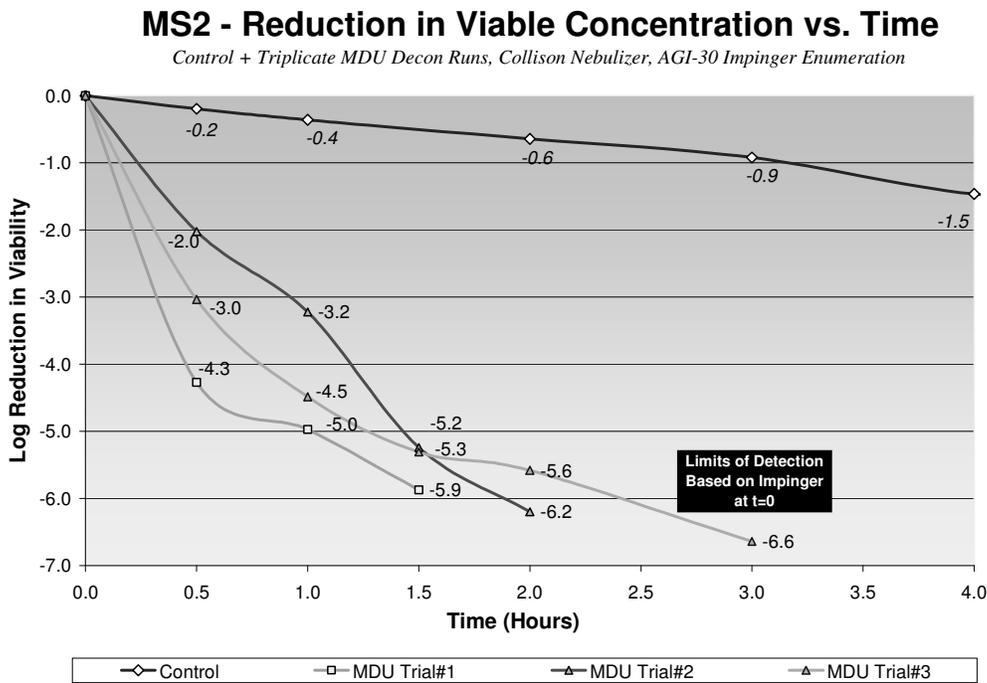


Figure 9: Bacteriophage MS2 Control and MDU/Rx™ trial Log Reduction in Viable Concentration.

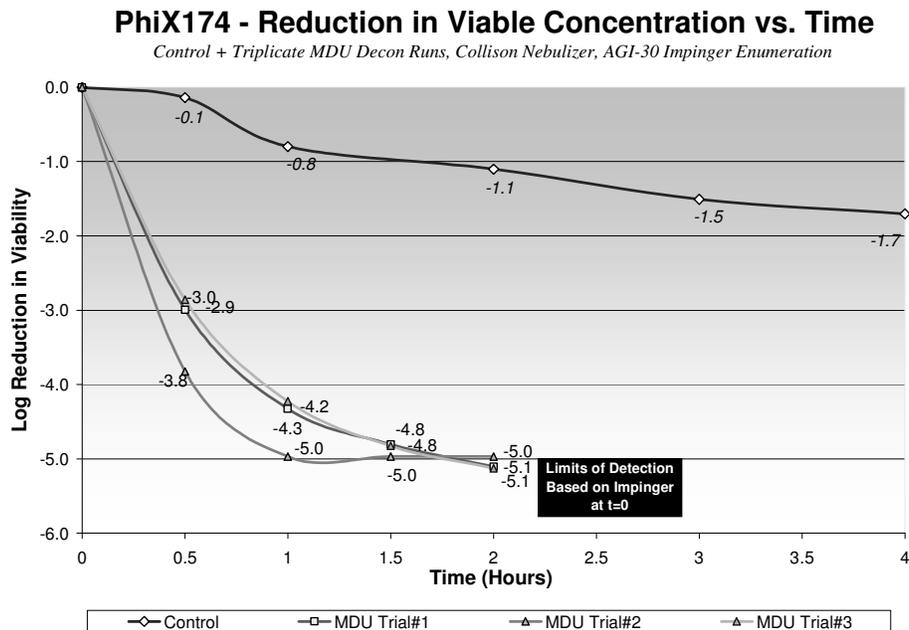


Figure 10: Bacteriophage MS2 Control and MDU/Rx™ trial Log Reduction in Viable Concentration.

MDU/Rx™ Spore Bioaerosol Results

Test results shown in figure 11 for *A. niger* reflect the MDU/Rx™ trials showed a 5.8, 5.3 and 5.7 log reduction in 1.5 hours or less compared to the

control which had a 1.1 log reduction in the same timeframe. MDU/Rx™ testing showed a net of 4.7 +/- 0.3 log (Avg. +/- STdev) reduction above the baseline control trial.

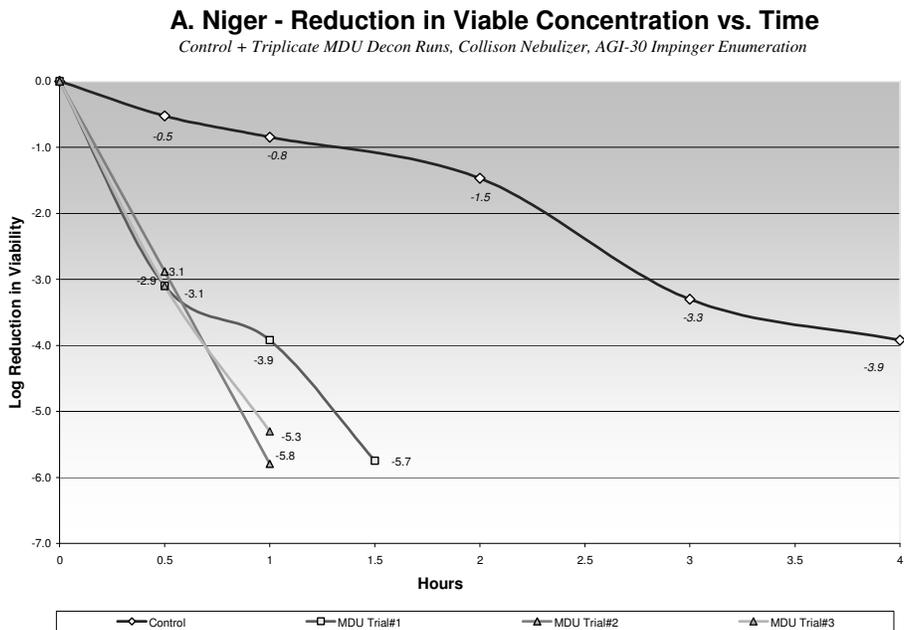


Figure 11: Aspergillus niger Control and MDU/Rx™ trial Log Reduction in Viable Concentration.

Summary of Findings

Test results show that the ODOROX[®] MDU/Rx[™] system 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.

MDU/Rx[™] System’s efficacy of reduction of *S. epidermidis* viability, after correcting for control run losses were 5.0 +/- 0.2 logs (average +/- standard deviation) in 2 hours or less.

MDU/Rx[™] System’s efficacy against *E. herbicola* bioaerosol, after correcting for control run viability losses, were 5.0 +/- 0.5 log (Avg +/- STdev) in 2 hours or less.

MS2’s reduction in viable bioaerosol concentrations within the chamber, after correcting for control run viability losses, were 4.9 +/- 0.3 logs (Avg +/- STdev) in 2 hours or less. Although, a single MDU/Rx[™] trial was also ran for 3 hours and this showed a viability loss of 5.7 logs after correcting for control run losses.

PhiX174’s reduction in viable aerosol, once again correcting for control run losses, yielded 4.0 +/- 0.1 logs (Avg +/- STdev) in 2 hours.

A. niger reduction in viable aerosol, once again correcting for control run losses, yielded 4.7 +/- 0.3 logs (Avg +/- STdev) in 1.5 hours.

In summary the ODOROX[®] MDU/Rx[™] showed 4 logs or greater reduction in viable bioaerosols for all biological challenges in 3 hours or less for all tested organisms.

Figure 12 shows the log reduction in all bioaerosols after correction for control run viability losses.

Summary MDU/Rx Log Reduction Over Control

Large Chamber, Average +/- ST. Dev Log Reduction, Triplicate MDU/Rx Trials

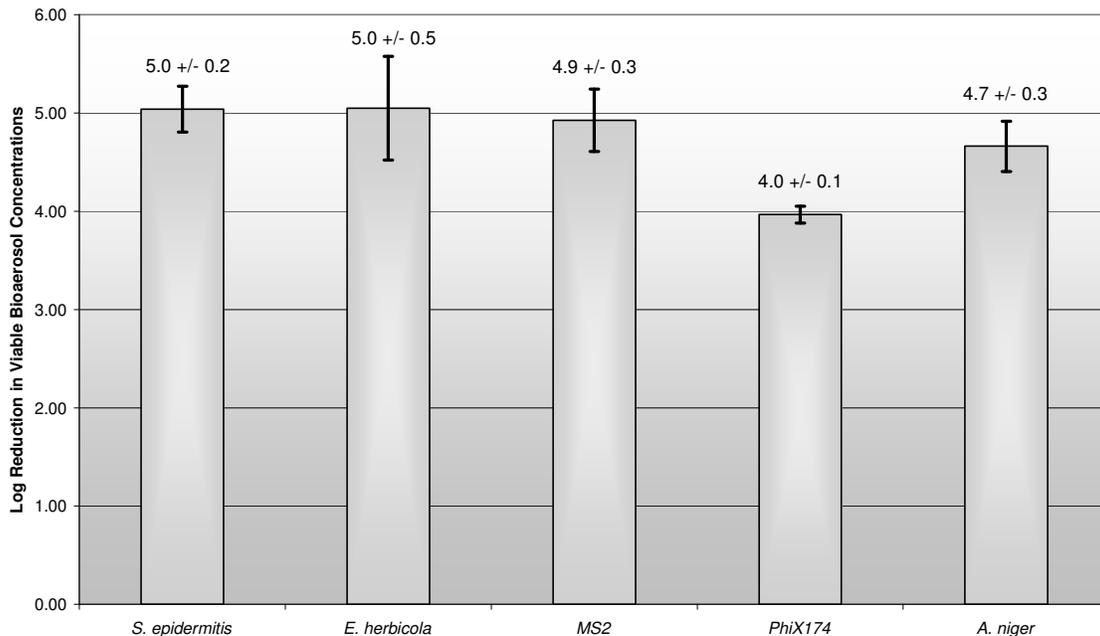


Figure 12: Summary of log reduction of Viable Bioaerosol concentration for MDU/Rx[™] over Control.

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.

Flint et al. *Principles of Virology*. Principles of Virology (ASM). Chapter 2 Virological Methods. Vol. 2. 2008.

J.F. Heidelberg et al. *Effects of Aerosolization on Culturability and Viability of Gram-Negative Bacteria*. Applied and Environmental Microbiology. Sept 1997, p 3585-3588.

A. Mazzocco et al. *Enumeration of Bacteriophages Using the Small Drop Plaque Assay System*. Bacteriophages: Methods and Protocols, Vol. 1: Isolation, Characterization and Interactions. vol. 501. 2009. pp. 81-95.

P Hyman et al. *Practical Methods for Determining Phage Growth Parameters*. Bacteriophages: Methods and Protocols, Vol. 1: Isolation, Characterization and Interactions. vol. 501. 2009. pp. 175-201.

A. Furiga, G. Pierre, et al. *Effects of Ionic Strength on Bacteriophage MS2 Behavior and Their Implications of the Assessment of Virus Retention*. University of Toulouse. 2007.

Analytical Testing Facility

Aerosol Research and Engineering Labs, Inc.
10870 Benson Drive Suite 2120
Overland Park, KS 66210

Project #

10805.1

Study Director

Jamie Balarashti
Aerosol Research and Engineering Laboratories

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.

Study Director:

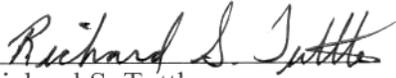


Jamie D. Balarashti
Study Director
ARE Labs, Inc.

11/14/2014

Date

Principal Investigator:



Richard S. Tuttle
Principal Investigator
ARE Labs, Inc.

11/14/2014

Date

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:

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

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

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

Appendix B

Plating and Enumeration Tables

S. Epidermidis Stock and Control Run Plating Results

Staph, Large Chamber Control, 10/14/2014 Data							Total Chamber	100% eff Chamber	Averaged Chamber	Average Chamber
Stock Enumeration Results							Volume Liters	Theoretical Concentration (t=0)	Measured Concentration (t=0)	Average Chamber Dissemination Efficiency (t=0)
Sample hour AGI	Plate dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/mL	Actual concentration cfu/mL			
		1	2	3						
Generation Stock	6x	TNTC	TNTC	TNTC		0	0.00E+00			
	7x	52	55	53	53	533	5.33E+09			
	8x	3	4	4	4	37	3.67E+08			
Average								2.85E+09		
							15936	3.90E+06	8.11E+04	2.08%

Impinger Enumeration Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3						
0	3x	331	353	296	327	3267	3.27E+05	6.53E+06	1.0E+05	
	4x	39	40	21	33	333	3.33E+05	6.67E+06	1.1E+05	
	5x	0	3	0	1	10	1.00E+05	2.00E+06	3.2E+04	
Average								5.07E+06	8.11E+04	100.0%

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3							
0.5	2x	TNTC	TNTC	TNTC	N/A	N?A	N/A	N/A	N/A	N/A	
	3x	206	148	168	174	1740	1.74E+05	3.48E+06	5.6E+04	68.7%	
	4x	19	28	18	22	217	2.17E+05	4.33E+06	6.9E+04	85.5%	
Average								3.91E+06	6.25E+04	77.1%	-0.11
1	2x	TNTC	TNTC	TNTC							
	3x	96	128	122	115	1153	1.15E+05	2.31E+06	3.7E+04	45.5%	
	4x	14	16	20	17	167	1.67E+05	3.33E+06	5.3E+04	65.8%	
Average								2.82E+06	4.51E+04	55.7%	-0.25
2	2x	TNTC	TNTC	TNTC							
	3x	87	99	86	91	907	9.07E+04	1.81E+06	2.9E+04	35.8%	
	4x	7	8	8	8	77	7.67E+04	1.53E+06	2.5E+04	30.3%	
Average								1.67E+06	2.68E+04	33.0%	-0.48
3	2x	TNTC	TNTC	TNTC							
	3x	62	74	42	59	593	5.93E+04	1.19E+06	1.9E+04	23.4%	
	4x	5	11	7	8	77	7.67E+04	1.53E+06	2.5E+04	30.3%	
Average								1.36E+06	2.18E+04	26.8%	-0.57
4	2x	TNTC	TNTC	TNTC							
	3x	33	46	accident	40	395	3.95E+04	7.90E+05	1.3E+04	15.6%	
	4x	2	4	5	4	37	3.67E+04	7.33E+05	1.2E+04	14.5%	
Average								7.62E+05	1.22E+04	15.0%	-0.82
5	2x	TNTC	TNTC	TNTC							
	3x	15	21	25	20	203	2.03E+04	4.07E+05	6.5E+03	8.0%	
	4x	2	3	2	2	23	2.33E+04	4.67E+05	7.5E+03	9.2%	
Average								4.37E+05	6.99E+03	8.6%	-1.06
6	2x	123	121	111	118	1183	1.18E+04	2.37E+05	3.8E+03	4.7%	
	3x	11	9	accident	10	100	1.00E+04	2.00E+05	3.2E+03	3.9%	
	4x	1	1	1	1	10	1.00E+04	2.00E+05	3.2E+03	3.9%	
Average								2.12E+05	3.40E+03	4.2%	-1.38

S. Epidermidis MDU/RX Trial #1 Plating Results

Staph Ep. - MDU Trial #1 - Impinger Enumeration Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3						
0	3x	296	282	355	311	3110	3.11E+05	6.22E+06	1.0E+05	
	4x	42	41	28	37	370	3.70E+05	7.40E+06	1.2E+05	
	5x	4	3	5	4	40	4.00E+05	8.00E+06	1.3E+05	
<i>Average</i>							7.21E+06	1.15E+05	100.0%	

0

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3							
0.5	1x	206	148	168	174	1740	1.74E+03	3.48E+04	5.6E+02	0.5%	
	2x	19	28	18	22	217	2.17E+03	4.33E+04	6.9E+02	0.6%	
<i>Average</i>							3.91E+04	6.25E+02	0.542%	-2.27	
1	Neat	1	0	1	1	7	6.67E+00	1.33E+02			
	1x	1	0	0	0	3	3.33E+00	6.67E+01	1.1E+00	0.0%	
	2x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
<i>Average</i>							6.67E+01	5.33E-01	0.0005%	-5.33	
2	Neat	0	0	0	0	0	0.00E+00				
	1x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
<i>Average</i>							0.00E+00	0.00E+00	0.0000%	N/A	

S. Epidermidis MDU/RX Trial #2 Plating Results

Staph Ep. - MDU Trial #2 - Impinger Enumeration Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3						
0	3x	316	343	352	337	3370	3.37E+05	6.74E+06	1.1E+05	
	4x	34	41	36	37	370	3.70E+05	7.40E+06	1.2E+05	
	5x									
							<i>Average</i>	7.07E+06	1.13E+05	100.0%

0

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3							
0.5	1x	146	138	127	137	1370	1.37E+03	2.74E+04	4.4E+02	0.4%	
	2x	11	14	11	12	120	1.20E+03	2.40E+04	3.8E+02	0.3%	
		2	1	0	1						
							<i>Average</i>	2.57E+04	4.11E+02	0.3635%	-2.44
1	Neat	0	0	1	0	3	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
							<i>Average</i>	2.22E+01	3.56E-01	0.0003%	-5.50
2	Neat	0	0	0	0	0	0.00E+00				
	1x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
							<i>Average</i>	0.00E+00	0.00E+00	0.0000%	#NUM!

S. Epidermidis MDU/RX Trial #3 Plating Results

Staph Ep. - MDU Trial #3 - Impinger Enumeration Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3					
0	3x	256	289	315	287	2.87E+05	5.73E+06	9.2E+04	
	4x	27	20	14	20	2.03E+05	4.07E+06	6.5E+04	
	5x								
					<i>Average</i>	4.90E+06	7.84E+04	100.0%	

0

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3						
0.5	1x	209	185	167	187.0	1.87E+03	3.74E+04	6.0E+02	0.8%	
	2x	16	14	11	13.7	1.37E+03	2.73E+04	4.4E+02	0.6%	
	3x	2	1	0	1.0	1.00E+03	2.00E+04	3.2E+02	0.4%	
					<i>Average</i>	2.82E+04	4.52E+02	0.5764%	-2.24	
1	Neat	1	0	1	0.7	6.67E+00	1.33E+02	2.1E+00	0.0%	
	1x	0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
					<i>Average</i>	4.44E+01	7.11E-01	0.0009%	-5.04	
2	Neat	0	0	0	0.0	0.00E+00				
	1x	0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
					<i>Average</i>	0.00E+00	0.00E+00	0.0000%	#NUM!	

E. herbicola Control Run Plating Results

Erwinia Large chamber control							Total Chamber	100% eff Chamber	Averaged Chamber	Chamber	
STOCK Enumeration							Volume Liters	Theoretical Concentration (t=0)	Measured Concentration (t=0)	Dissemination Efficiency (t=0)	
Sample hour AGI	Plate dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Actual concentration cfu/mL				
		1	2	3							
Generation Stock	6x	71	86	68	75	750	7.50E+08	15936	2.00E+06	2.23E+04	1.11%
	7x	13	11	14	13	127	1.27E+09				
	8x	2	1	2	2	17	1.67E+09				
Average											

Impinger Enumeration

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3							
0	3x	62	10	66	46	460	4.60E+04	9.20E+05	1.5E+04		
	4x	8	11	10	10	97	9.67E+04	1.93E+06	3.1E+04		
	5x	0	1	1	1	7	6.67E+04	1.33E+06	2.1E+04		
	Average								1.40E+06	2.23E+04	100.0%
0.5	2x	TNTC	TNTC	TNTC	N/A	N/A	N/A	N/A	N/A	N/A	
	3x	39	34	36	36	36333	3.63E+04	7.27E+05	1.2E+04	52.1%	
	4x	5	4	5	5	46667	4.67E+04	9.33E+05	1.5E+04	66.9%	
	Average								8.30E+05	1.33E+04	59.5%
1	2x	320	352	295	322	32233	3.22E+04	6.45E+05			
	3x	35	34	38	36	35667	3.57E+04	7.13E+05	1.1E+04	51.1%	
	4x	3	2	4	3	30000	3.00E+04	6.00E+05	9.6E+03	43.0%	
	Average								6.53E+05	1.05E+04	47.1%
2	2x	134	145	152	144	14367	1.44E+04	2.87E+05			
	3x	19	15	17	17	17000	1.70E+04	3.40E+05	5.4E+03	24.4%	
	4x	0	1	1	1	6667	6.67E+03	1.33E+05	2.1E+03	9.6%	
	Average								2.54E+05	3.79E+03	17.0%
3	2x	68	75	81	75	7467	7.47E+03	1.49E+05			
	3x	4	7	6	6	5667	5.67E+03	1.13E+05	1.8E+03	8.1%	
	4x	0	0	0	0		0.00E+00	0.00E+00	0.0E+00	0.0%	
	Average								8.76E+04	9.07E+02	4.1%
4	2x	35	25	44	35	3467	3.47E+03	6.93E+04			
	3x	3	1	accident	2	2000	2.00E+03	4.00E+04	6.4E+02	2.9%	
	4x	1	0	0	0	3333	3.33E+03	6.67E+04	1.1E+03	4.8%	
	Average								5.87E+04	8.53E+02	3.8%
5	2x										
	3x										
	4x										
6	1x	37	46	43	42	420	4.20E+02	8.40E+03	1.3E+02	0.6%	
	2x	5	4	5	5	467	4.67E+02	9.33E+03	1.5E+02	0.7%	
	3x						0.00E+00	0.00E+00	0.0E+00	0.0%	
	Average								5.91E+03	9.46E+01	0.4%

E. herbicola MDU/Rx Trial #1 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3						
0	3x	28	35	39	34	340	3.40E+05	6.80E+06	1.1E+05	
	4x	14	15	10	13	130	1.30E+06	2.60E+07	4.2E+05	
	5x					0				
						<i>Average</i>	1.64E+07	2.62E+05	100.0%	

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3							
0.5	Neat	153	194	184	177	1770	1.77E+03	3.54E+04	5.7E+02	0.2%	
	1x	16	16	19	17	170	1.70E+02	3.40E+03	5.4E+01	0.0%	
	2x	1	2	0	1	10					
						<i>Average</i>	1.94E+04	3.10E+02	0.118%	-2.93	
1	Neat	-	-	-	-	-	-	-	-	-	
	1x	8	11	10	10	97	9.67E+02	1.93E+04	3.1E+02	0.1%	
	2x	5	1	2	3	27	2.67E+02	5.33E+03	8.5E+01	0.0%	
						<i>Average</i>	1.23E+04	1.97E+02	0.075%	-3.12	
2	Neat	8	12	7	9	90	9.00E+01	1.80E+03	2.9E+01	0.0%	
	1x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
						<i>Average</i>	6.00E+02	9.60E+00	0.004%	-4.44	
3	Neat	0	0	3	1	10	1.00E+01	2.00E+02	3.2E+00	0.0%	
	1x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x										
						<i>Average</i>	1.00E+02	1.60E+00	0.001%	-5.21	

E. herbicola MDU/Rx Trial #2 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3						
0	3x	316	343	352	337	3370	3.37E+05	6.74E+06	1.1E+05	
	4x	34	41	36	37	370	3.70E+05	7.40E+06	1.2E+05	
	5x									
<i>Average</i>								7.07E+06	1.13E+05	100.0%

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3							
0.5	Neat	84	70	77	77	770	7.70E+02	1.54E+04	2.5E+02	0.2%	
	1x	11	8	9	9	933	9.33E+02	1.87E+04	3.0E+02	0.3%	
<i>Average</i>								1.70E+04	2.73E+02	0.241%	-2.62
1	Neat	11	11	10	11	107	1.07E+02	2.13E+03	3.4E+01	0.0%	
	1x	2	3	2	2	233	2.33E+02	4.67E+03	7.5E+01	0.1%	
	2x	0	0	0	0	0					
<i>Average</i>								3.40E+03	5.44E+01	0.048%	-3.32
2	Neat	8	11	6	8	83	8.33E+01	1.67E+03	2.7E+01	0.0%	
	1x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
<i>Average</i>								5.56E+02	8.89E+00	0.008%	-4.10
3	Neat	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	1x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x										
<i>Average</i>								0.00E+00	0.00E+00	0.000%	#NUM!

E. herbicola MDU/Rx Trial #3 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3					
0	3x	256	264	232	251	2.51E+06	5.01E+07	8.0E+05	
	4x	16	20	22	19	1.93E+06	3.87E+07	6.2E+05	
	5x	-	-	-	-	-	-	-	
					<i>Average</i>	4.44E+07	7.10E+05	100.0%	

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3						
0.5	Neat	TNTC	TNTC	TNTC	-	-	-	-	-	
	1x	41	39	44	41.3	4.13E+03	8.27E+04	1.3E+03	0.2%	
	2x	11	14	8	11.0	1.10E+04	2.20E+05	3.5E+03	0.5%	
					<i>Average</i>	1.51E+05	2.42E+03	0.341%	-2.47	
1	Neat	33	42	28	34.3	3.43E+02	6.87E+03	1.1E+02	0.0%	
	1x	3	6	1	3.3	3.33E+02	6.67E+03	1.1E+02	0.0%	
	2x	-	-	-	-	-	-	-	-	
					<i>Average</i>	6.77E+03	1.08E+02	0.015%	-3.82	
2	Neat	0	0	1	0.3	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0.0	-	-	-	-	
	2x	-	-	-	-	-	-	-	-	
					<i>Average</i>	6.67E+01	1.07E+00	0.0002%	-5.82	
3		0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
		0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
		0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
					<i>Average</i>	0.00E+00	0.00E+00	0.0000%	#NUM!	

MS2 Control Run Plating Results

MS2 Large chamber control						Total Chamber	100% eff Chamber	Averaged Chamber	Chamber	
Sample hour AGI	Plate dilution Factor	Plate counts 100ul			Average pfu/100ul	Actual concentration pfu/mL	Volume Liters	Theoretical Concentration (t=0)	Measured Concentration (t=0) pfu/L	Dissemination Efficiency (t=0)
		1	2	3						
Generation Stock	9x	14	15	18	16	15936	1.82E+08	7.95E+05	0.44%	
	10x	1	2	1	1					
	Average				1.33E+11					

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average pfu/100ul	Enumerated Concentration pfu/mL	Total pfu Collected (pfu) (per AGI)	Average Chamber Concentration (pfu/l)	Normalized Concentration (t=0, basis)
		1	2	3					
0	4x	27	22	30	26	2.63E+06	5.27E+07	8.4E+05	
	5x	4	1	2	2	2.33E+06	4.67E+07	7.5E+05	
	Average						4.97E+07	7.95E+05	100.0%

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul on Plate			Average Plate Count	Enumerated Concentration pfu/mL	Total pfu Collected (pfu) (per AGI)	Average Chamber Concentration (pfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3						
0.5	3x	TNTC	TNTC	TNTC	-	-	-	-	-	
	4x	19	23	23	22	2.17E+06	4.33E+07	6.9E+05	87.2%	
	5x	1	0	2	1	1.00E+06	2.00E+07	3.2E+05	40.3%	
Average						3.17E+07	5.07E+05	63.8%	-0.20	
1	3x	TNTC	TNTC	TNTC	-	-	-	-	-	
	4x	12	14	19	15	1.50E+06	3.00E+07	4.8E+05	60.4%	
	5x	1	1	0	1	6.67E+05	1.33E+07	2.1E+05	26.8%	
Average						2.17E+07	3.47E+05	43.6%	-0.36	
2	3x	TNTC	TNTC	TNTC	-	-	-	-	-	
	4x	5	4	8	6	5.67E+05	1.13E+07	1.8E+05	22.8%	
	5x	0	0	0	0	-	-	-	-	
Average						1.13E+07	1.81E+05	22.8%	-0.64	
3	3x	38	30	41	36	3.63E+05	7.27E+06	1.2E+05	14.6%	
	4x	3	2	2	2	2.33E+05	4.67E+06	7.5E+04	9.4%	
	5x	0	0	0	0	-	-	-	-	
Average						5.97E+06	9.55E+04	12.0%	-0.92	
4	2x	-	-	-	-	-	-	-	-	
	3x	11	14	6	10	1.03E+05	2.07E+06	3.3E+04	4.2%	
	4x	2	0	0	1	6.67E+04	1.33E+06	2.1E+04	2.7%	
Average						1.70E+06	2.72E+04	3.4%	-1.47	
5	2x	59	72	63	65	6.47E+04	1.29E+06	2.1E+04	2.6%	
	3x	7	7	6	7	6.67E+04	1.33E+06	2.1E+04	2.7%	
	4x	0	0	0	0	-	-	-	-	
Average						1.31E+06	2.10E+04	2.6%	-1.58	

MS2 MDU/Rx Trial #1 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3					
0	4x	10	11	14	12	1.17E+06	2.33E+07	3.7E+05	
	5x	1	2	1	1	1.33E+06	2.67E+07	4.3E+05	
	6x	-	-	-					
					<i>Average</i>	2.50E+07	4.00E+05	100.0%	

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3						
0.5	Neat	12	13	15	13	1.33E+02	2.67E+03	4.3E+01	0.0%	
	1x	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x									
					<i>Average</i>	1.33E+03	2.13E+01	0.005%	-4.27	
1	Neat	1	2	1	1	1.33E+01	2.67E+02	4.3E+00	0.0%	
	1x	0	0	0	0	-	-	-	-	
					<i>Average</i>	2.67E+02	4.27E+00	0.001%	-4.97	
1.5	Neat	0	1	0	0	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
					<i>Average</i>	3.33E+01	5.33E-01	0.0001%	-5.88	
2	Neat	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	1x	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
					<i>Average</i>	0.00E+00	0.00E+00	0.0000%	#NUM!	

MS2 MDU/Rx Trial #2 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3						
0	4x	TNTC	TNTC	TNTC	-	-	-	-	-	-
	5x	9	13	12	11	113	1.13E+07	2.27E+08	3.6E+06	-
	6x	2	1	2	2	17	1.67E+07	3.33E+08	5.3E+06	-
					<i>Average</i>		<i>2.80E+08</i>	<i>4.48E+06</i>	<i>100.0%</i>	

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Average cfu/1mL	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3							
0.5	3x	14	19	17	17	167	1.67E+05	3.33E+06	5.3E+04	1.2%	
	4x	1	1	1	1	100	1.00E+05	2.00E+06	3.2E+04	0.7%	
	5x	-	-	-	-	-	-	-	-	-	
					<i>Average</i>		<i>2.67E+06</i>	<i>4.27E+04</i>	<i>0.952%</i>	<i>-2.02</i>	
1	Neat	13	14	11	13	127	1.27E+02	2.53E+03	4.1E+01	0.0%	
	1x	1	2	2	2	167	1.67E+04	3.33E+05	5.3E+03	0.1%	
	2x	0	0	0	0	0	-	-	-	-	
					<i>Average</i>		<i>1.68E+05</i>	<i>2.69E+03</i>	<i>0.060%</i>	<i>-3.22</i>	
1.5	Neat	7	11	6	8	80	8.00E+01	1.60E+03	2.6E+01	0.0%	
	1x	0	0	0	0	0	-	-	-	-	
	2x	-	-	-	-	-	-	-	-	-	
					<i>Average</i>		<i>1.60E+03</i>	<i>2.56E+01</i>	<i>0.001%</i>	<i>-5.24</i>	
2	Neat	1	0	0	0	3	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	-	-	-	-	-	-	-	-	-	
					<i>Average</i>		<i>3.33E+01</i>	<i>5.33E-01</i>	<i>0.000%</i>	<i>-6.92</i>	

MS2 MDU/Rx Trial #3 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3					
0	4x	TNTC	TNTC	TNTC	-	-	-	-	
	5x	14	16	14	15	1.47E+07	2.93E+08	4.7E+06	
	6x	1	0	1	1	6.67E+06	1.33E+08		
					<i>Average</i>	2.13E+08	4.69E+06	100.0%	

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3						
0.5	1x	TNTC	TNTC	TNTC	-	-	-	-	-	
	2x	22	24	27	24.3	2.43E+04	4.87E+05	7.8E+03	0.2%	
	3x	1	4	3	2.7	2.67E+03	5.33E+04	8.5E+02	0.0%	
					<i>Average</i>	2.70E+05	4.32E+03	0.092%	-3.04	
1	Neat	47	37	34	39.3	3.93E+02	7.87E+03	1.3E+02	0.0%	
	1x	6	4	7	5.7	5.67E+02	1.13E+04	1.8E+02	0.0%	
	2x	-	-	-	-	-	-	-	-	
					<i>Average</i>	9.60E+03	1.54E+02	0.003%	-4.49	
1.5	Neat	3	4	6	4.3	4.33E+01	8.67E+02	1.4E+01	0.0%	
	1x	0	1	2	1.0	1.00E+02	2.00E+03	3.2E+01	0.0%	
	2x	-	-	-	-	-	-	-	-	
					<i>Average</i>	1.43E+03	2.29E+01	0.0005%	-5.31	
2	Neat	3	4	6	4.3	4.33E+01	8.67E+02	1.4E+01	0.0%	
	1x	0	1	0	0.3	3.33E+01	6.67E+02	1.1E+01	0.0%	
	2x	-	-	-	-	-	-	-	-	
					<i>Average</i>	7.67E+02	1.23E+01	0.0003%	-5.58	
3	Neat	0	0	1	0.3	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	-	-	-	-	-	-	-	-	
	2x	-	-	-	-	-	-	-	-	
					<i>Average</i>	6.67E+01	1.07E+00	0.0000%	-6.64	

Phi-X174 MDU/Rx Trial #1 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3					
0	2x	TNTC	TNTC	TNTC					
	3x	13	18	26	19	1.90E+05	3.80E+06	6.1E+04	
	4x	1	2	4	2	2.33E+05	4.67E+06	7.5E+04	
					<i>Average</i>	4.23E+06	6.77E+04	100.0%	

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3						
0.5	Neat	31	23	15	23	2.30E+02	4.60E+03	7.4E+01	0.1%	
	1x	1	2	3	2	2.00E+02	4.00E+03	6.4E+01	0.1%	
	2x									
					<i>Average</i>	4.30E+03	6.88E+01	0.102%	-2.99	
1	Neat	2	0	1	1	1.00E+01	2.00E+02	3.2E+00	0.0%	
	1x	0	0	0	0	-	-	-	-	
					<i>Average</i>	2.00E+02	3.20E+00	0.005%	-4.33	
1.5	Neat	1	0	1	1	6.67E+00	1.33E+02	2.1E+00	0.0%	
	1x	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
					<i>Average</i>	6.67E+01	1.07E+00	0.002%	-4.80	
2	Neat	0	0	1	0	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
					<i>Average</i>	3.33E+01	5.33E-01	0.001%	-5.10	

Phi-X174 MDU/Rx Trial #2 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3					
0	2x	TNTC	TNTC	TNTC	-	-	-	-	
	3x	14	11	18	14	1.43E+05	2.87E+06	4.6E+04	
	4x	2	1	2	2	1.67E+05	3.33E+06	5.3E+04	
					<i>Average</i>	3.10E+06	4.96E+04	100.0%	

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3						
0.5	Neat	2	1	1	1	1.33E+01	2.67E+02	4.3E+00	0.0%	
	1x	0	0	1	0	3.33E+01	6.67E+02	1.1E+01	0.0%	
	2x					-	-	-	-	
					<i>Average</i>	4.67E+02	7.47E+00	0.0151%	-3.82	
1	Neat	1	0	0	0	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x									
					<i>Average</i>	3.33E+01	5.33E-01	0.0011%	-4.97	
1.5	Neat	1	0	0	0	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x					-	-	-	-	
					<i>Average</i>	3.33E+01	5.33E-01	0.001%	-4.97	
2	Neat	0	0	1	0	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	2x	-	-	-	-	-	-	-	-	
					<i>Average</i>	3.33E+01	5.33E-01	0.001%	-4.97	

Phi-X174 MDU/Rx Trial #3 Plating Results

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)
		1	2	3					
0	2x	TNTC	TNTC	TNTC	-	-	-	-	
	3x	15	9	13	12	1.23E+05	2.47E+06	3.9E+04	
	4x	1	0	2	1	1.00E+05	2.00E+06	3.2E+04	
					<i>Average</i>	2.23E+06	3.57E+04	100.0%	

Sample Time (h)	Plate Dilution Factor	Plate counts 100ul			Average cfu/100ul	Enumerated Concentration cfu/mL	Total cfu Collected (cfu) (per AGI)	Average Chamber Concentration (cfu/l)	Normalized Concentration (t=0, basis)	Log Reduction (t=0, basis)
		1	2	3						
0.5	Neat	17	15	21	17.7	1.77E+02	3.53E+03	5.7E+01	0.2%	
	1x	2	1	1	1.3	1.33E+02	2.67E+03	4.3E+01	0.1%	
	2x	0	0	0	0.0	-	-	-	-	
					<i>Average</i>	3.10E+03	4.96E+01	0.139%	-2.86	
1	Neat	2	1	1	1.3	1.33E+01	2.67E+02	4.3E+00	0.0%	
	1x	0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	-	-	-	-	-	-	-	-	-	
					<i>Average</i>	1.33E+02	2.13E+00	0.006%	-4.22	
1.5	Neat	0	0	1	0.3	3.33E+00	6.67E+01	1.1E+00	0.0%	
	1x	0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	-	-	-	-	-	-	-	-	-	
					<i>Average</i>	3.33E+01	5.33E-01	0.0015%	-4.83	
2	Neat	0	0	0.5	0.2	1.67E+00	3.33E+01	5.3E-01	0.0%	
	1x	0	0	0	0.0	0.00E+00	0.00E+00	0.0E+00	0.0%	
	-	-	-	-	-	-	-	-	-	
					<i>Average</i>	1.67E+01	2.67E-01	0.0007%	-5.13	

A. niger Plating Results (TCID50 Technique)

A. niger Large Chamber MDU Testing															
47mm Filter Sample Plate Enumeration Results - Plated using TCID50 Small Drop Analysis															
	0	1x	2x	3x	4x	5x	cfu	cfu	20 ml Dilution	Sample Flow Rate	Sample Time	Sampling volume	Conc.	Conc.	Normalized Concentration
MDU Control	Neat	10x	100x	1000x	10000x	100000x	5ul	1000ul	Sample	L/min	Minutes	L	cfu/L	cfu/Fl3	(±0, basis)
0	NA	TNTC	TNTC	6 of 6	3 of 6	0 of 6	5.00E+04	1.00E+07	2.00E+08	12.5	10	125	1.60E+06	4.53E+07	100.00%
0.5	NA	TNTC	TNTC	6 of 7	1 of 7	1 of 7	1.50E+04	3.00E+06	6.00E+07	12.5	10	125	4.80E+05	1.36E+07	30.00%
1	NA	TNTC	7 of 7	5 of 7	2 of 7	0	7.10E+03	1.42E+06	2.84E+07	12.5	10	125	2.27E+05	6.43E+06	14.20%
2	NA	TNTC	7 of 7	2 of 7	0	0	1.70E+03	3.40E+05	6.80E+06	12.5	10	125	5.44E+04	1.54E+06	3.40%
3	NA	12 of 12	3 of 12	0	0	0	2.50E+01	5.00E+03	1.00E+05	12.5	10	125	8.00E+02	2.26E+04	0.05%
4	NA	6 of 6	1 of 5	0	0	0	6.00E+00	1.20E+03	2.40E+04	12.5	10	125	1.92E+02	5.43E+03	0.01%

MDU Trial #1															
	0	1x	2x	3x	4x	5x	cfu	cfu	20 ml Dilution	Sample Flow Rate	Sample Time	Sampling volume	Conc.	Conc.	Normalized Concentration
Test 1	Neat	10x	100x	1000x	10000x	100000x	5ul	1000ul	Sample	L/min	Minutes	L	cfu/L	cfu/Fl3	(±0, basis)
0	TNTC	12of 12	1.00E+05	2.00E+07	4.00E+08	12.5	10	125	3.20E+06	9.06E+07	100.0000%				
0.5	TNTC	10of10	2 of 10	2of10	1 of 10	NA	4.00E+01	8.00E+03	1.60E+05	12.5	10	125	1.28E+03	3.62E+04	0.0800%
1	6of10	2of10	0	0	0	0	6.00E+00	1.20E+03	2.40E+04	12.5	10	125	1.92E+02	5.43E+03	0.0120%
1.5	1of11	0	0	0	0	0	9.00E-02	1.80E+01	3.60E+02	12.5	10	125	2.88E+00	8.15E+01	0.0002%
2	0	0	0	0	0	0									0.0000%

MDU Trial #2															
	0	1x	2x	3x	4x	5x	cfu	cfu	20 ml Dilution	Sample Flow Rate	Sample Time	Sampling volume	Conc.	Conc.	Normalized Concentration
Test 2	Neat	10x	100x	1000x	10000x	100000x	5ul	1000ul	Sample	L/min	Minutes	L	cfu/L	cfu/Fl3	(±0, basis)
0	TNTC	TNTC	TNTC	12 of 12	6of12	3of12	5.00E+03	1.00E+06	2.00E+07	12.5	10	125	1.60E+05	4.53E+06	100.00%
0.5	12of12	8of12	0	0	0	0	6.60E+01	1.32E+04	2.64E+05	12.5	10	125	2.11E+03	5.98E+04	0.1320%
1	1of12	0	0	0	0	0	8.00E-02	1.60E+01	3.20E+02	12.5	10	125	2.56E+00	7.24E+01	0.0002%
1.5	0	0	0	0	0	0									0.0000%
2	0	0	0	0	0	0									0.0000%

MDU Trial #3															
	0	1x	2x	3x	4x	5x	cfu	cfu	20 ml Dilution	Sample Flow Rate	Sample Time	Sampling volume	Conc.	Conc.	Normalized Concentration
Test 3	Neat	10x	100x	1000x	10000x	100000x	5ul	1000ul	Sample	L/min	Minutes	L	cfu/L	cfu/Fl3	(±0, basis)
0	TNTC	TNTC	TNTC	10of10	7of10	2of10	7.00E+03	1.40E+06	2.80E+07	12.5	10	125	2.24E+05	6.34E+06	100.00%
0.5	TNTC	10of10	4of10	1of10	0	0	4.00E+01	8.00E+03	1.60E+05	12.5	10	125	1.28E+03	3.62E+04	0.0800%
1	4 of 12	0	0	0	0	0	2.50E-01	5.00E+01	1.00E+03	12.5	10	125	8.00E+00	2.26E+02	0.0005%
1.5	0	0	0	0	0	0									0.0000%
2	0	0	0	0	0	0									0.0000%