

Study Summary Article

Efficacy of the M40 Air Purifier against a Broad Range of Respirable Microorganisms: High Speed Broad Range Efficacy and Low Speed Select Species Efficacy

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

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Conflict of Interest:

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

ABSTRACT

Background: Due to the high rate of infectious disease transmission through aerosol exposure to pathogenic microorganisms, systems designed to reduce the levels of airborne pathogens, and other bio particulates in room air, have been attracting significant attention. This in-vitro study characterized the efficacy of the M40 air purification device to reduce respirable bioaerosol levels for six broad-ranged species of microorganisms from room air. The species selected are recognized surrogates for more dangerous pathogenic organisms. In this study, the species tested were: MS2, a non-enveloped ssRNA virus that is a common surrogate for influenza viruses and is a tentative surrogate for SARS-CoV-2; Phi X 174, a non-enveloped DNA virus that is a surrogate for herpes and smallpox viruses; Methicillin Resistant Staphylococcus epidermidis (MRSE), a gram-positive bacterium that serves as a surrogate for many pathogenic gram-positive bacteria, including Methicillin Resistant Staphylococcus aureus (MRSA); Escherichia coli, a gram-negative bacterium that is a well-known pathogen itself; Bacillus subtilis endospores, which serve as a model for the many bacterial species that produce highly resistant endospores; and the spores from Aspergillus brasiliensis, one of the most common sources of toxic black mold.

This is a two part study designed to demonstrate the efficacy of the M40 and M50 air purifiers. In this part, the M40 was tested in its intended final commercial form. The results of that study are presented in this report. The M40 study was further divided into two phases;

Phase I: The highest level fan speed (Speed 3), which is anticipated to be the speed most used in routine use, was used for this phase of the testing. Additionally, a full contingent of six microorganisms were used to test the system. It consisted of a total of twenty-four (24) live bioaerosol trials; six species with three test trials and one control trial each. In addition to the microorganism tests an inert particles trial and control was performed to determine the reduction of PSL's (polystyrene latex microspheres) by the device in a sealed chamber.

Phase II: The lowest fan speed available on the unit (Speed 1), which is the setting with the lowest room turnover rate, was used for this phase of testing to assess two species, the MS2 bacteriophage (virus) and the endospores from *Bacillus subtilis.* These represented the most difficult to remove microbes. All bioaerosol trials were run in triplicate. In addition to the microorganism test at the lowest fan speed an inert particles trial was also performed to determine the reduction of PSL's (polystyrene latex microspheres) by the device in a sealed chamber.

Methods: Each microorganism was aerosolized into a sealed environmental bioaerosol chamber, containing the M40 air purifier, using a Collison 24-Jet Nebulizer or dry powder feeder. All the bioaerosols had a mass median aerodynamic diameter (MMAD) ranging from 0.7-4.0 μ m (species dependent). Bioaerosol samples were taken at multiple time points throughout each trial, in order to quantify the reduction rate capability of the air purification device. Impinger samples were serially diluted, plated, incubated, and enumerated in triplicate to yield viable bioaerosol concentrations for each sampling point. Chamber control trial data, or natural decay, was subtracted from the device trial data to yield the net LOG reduction for each of the bioaerosol challenges.

Results: Phase I – The M40 unit, set to 'Speed 3', was effective at reducing all six organisms by a net log of 4.09 or greater (equivalent to 99.99% or greater) within 30 minutes.

Results: Phase II – The M40 unit, set to 'Speed 1', was effective at reducing both organisms by a net log of 4.0 or greater (equivalent to 99.99% or greater) within 90 minutes.

Conclusions: Based on the results of Phase I and Phase II testing, all fan speeds are effective in reducing airborne microorganisms within a short period of time. However, the higher fan speed achieved a >4.0 net log reduction in 30 minutes vs the lower fan speed which took 90 minutes.



Introduction

This study was conducted to evaluate the efficacy of the M40 room air purifier, manufactured by Medify, at reducing aerosolized bio organisms. The M40 device is an air filtration device, equipped with ozone free ionizer and a true HEPA filter. It is designed to reduce a broad range of gram-positive and gram-negative bacteria, RNA and DNA viruses including SARS-CoV-2, bacterial and mold fungal spores, and airborne particles in room air. The M40 device is designed for commercial and residential applications. The test plan incorporated challenging the M40 device, in a closed environmental chamber, to determine the reduction rate and extent of two separate aerosolized viruses, two separate aerosolized bacteria, and two types of spores. A picture of the M40 device is shown in Figure 1.



Figure 1: M40 Air System Device: Portable HEPA filtration with ozone free ionizer. Integrated pre-filter and carbon filter for further polishing of treated air. Multi-speed capable.

Study Overview

The effectiveness of the M40 device was evaluated against an RNA virus, a DNA virus, a gram negative bacteria, a gram positive bacteria, a spore forming bacteria, and a mold spore.

Testing was conducted to characterize a single M40 unit against six organism types to demonstrate the capability of the M40 device, when operating at its highest fan speed, to reduce viable bioaerosol concentrations, therefore theoretically reducing the chances of airborne infection. Two of these organisms, MS2 and *Bacillus subtilis* endospores, were tested

at one additional fan speed, Speed 1, to demonstrate efficacy at multiple fan speeds.

Phase I: Speed 3, Broad Range Efficacy Testing:

The Phase I component consisted of testing all six species in the Speed 3 of the M40 device which is intended to be the most commonly used setting. A single trial and control were also performed to characterize the reduction of PSL's in a sealed room. This demonstrated the broad efficacy of the device.

Phase II: Speed 1 Efficacy Testing

Phase II of the testing consisted of running two selected species, MS2 and *B. subtilis* endospores, run with the device at Speed 1. These two organisms were chosen because of their hardiness and particle size. In addition, a single inert particle reduction trial was performed to characterize the device efficacy at removing non-living aerosolized particulates. Testing the efficacy of the device at different air flow rates demonstrated the device's overall capability.

Test Device Description

The M40 device is equipped with multi-step filtration including a pre-filter, carbon filter, HEPA filter, and an ionizer that increases kill efficiency. The pre-filter is used to captures large dust particles and other debris followed by an activated carbon filter intended to remove volatile organic compounds (VOC's). An integrated High Efficiency Particulate Air (HEPA) filter removes respirable particles (>0.1 μ m). The ionizer kills any microorganisms the get by the filter system. The device is equipped with four blower speeds: Speed 1, 2, and 3.



Figure 2: Stainless Steel Bioaerosol Test Chamber used for all M40 Testing. Chamber is equipped with HEPA in/out, multiple bioaerosol sampling ports, decontamination and pressure balance. Exterior picture.



General Large Chamber Bioaerosol Configuration

(AGI-30 Impingers, APS, Temp & Humidity)

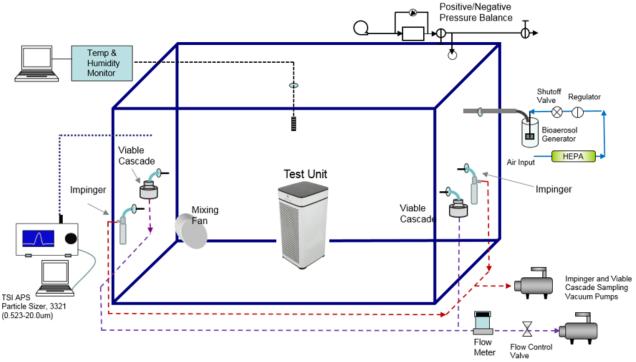


Figure 3: Bio-Aerosol Test Chamber Flow Diagram. Chamber includes bioaerosol induction, multiple bioaerosol sampling ports, Particle size monitoring, internal mixing fans, temperature and humidity controls. Main system HEPA Evacuation System not pictured.

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 9.1 ft x 9.1 ft x 7 ft, with a displacement volume of 579 cubic feet, or 16,000 liters. Figure 2 shows the bioaerosol chamber used for all testing in this study.

The chamber is equipped with filtered HEPA inlets, digital internal temperature and humidity monitors, heaters and humidifiers, lighting system, multiple sampling ports, aerosol mixing fans, and a HEPA filtered exhaust system that are operated with wireless remote control. For testing, the chamber is equipped with four 3/8-inch diameter stainless steel probes for aerosol sampling and a 1-inch diameter port for bio-aerosol dissemination into the chamber using a Collison 24-jet nebulizer or dry powder eductor for the aerosolization of the microorganisms and spores, respectively.

In order to avoid wall effects, all sample and dissemination ports were inserted approximately 18 inches in from the interior walls of the chamber and at a height of approximately 40 inches from the floor to avoid wall effects. 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 evacuation of remaining bioaerosols. A Magnehelic gauge with a range of -0.5 to 0.5 inches of H₂O (Dwyer instruments, Michigan City IN) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles.

Environmental Controls

For increased stability of bioaerosols, relative humidity inside the chamber is kept at 65% +/- 5% using a PID humidity controller in combination with an ultra-sonic humidifier to nebulize filtered DI water. Temperature controls maintain chamber trial conditions at typical ambient conditions of $74^{\circ}F$ +/- $2^{\circ}F$.



Bioaerosol Generation System

All test bioaerosols were disseminated using a Collison 24-jet nebulizer (BGI Inc. Waltham MA), similar to the one shown in **Figure 4**, with the exception of the *A. brasiliensis* spores which were aerosolized using a dry powder eductor. The aerosolization of bioaerosols were driven by purified, filtered house air supply. A pressure regulator allowed for control of disseminated particle size, use rate and sheer force generated within the Collison nebulizer. Prior to testing, the Collison nebulizer flow rate and use rate were characterized using an air supply pressure of approximately 40-60 psi, which produced an output volumetric flow rate of 50-80 L/min with a fluid dissemination rate of approximately 1.25 mL/min. The flow of the Collison nebulizer was flow characterized by using a calibrated TSI model 4040 mass flow meter (TSI Inc., St Paul MN).



Figure 4. 6-Jet Collison nebulizer. Glass and 304 stainless steel construction, BGI Industries.

Bioaerosol Sampling and Monitoring System

Two AGI impingers (Ace Glass Inc. Vineland NJ) were used for bioaerosol collection of all biological aerosols to determine chamber concentrations. The two AGI Impingers were placed at opposite corners of the chamber in order to represent an entire room sample. The mixing fans inside the chamber worked to ensure a homogenous air mixture inside the chamber.



Figure 5: SKC Single Stage BioStage Viable Cascade Impactor used for bacterial and spore sampling for select time points during bioaerosol trials. LOD is >0.01 cfu/L.

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 flows were characterized using a calibrated TSI model 4040 mass flow meter. A general flow diagram of the aerosol test system is shown above in Figure 3.

During testing with less resilient organisms or those which fall out of the air more easily, sample collections were also obtained using a pair of viable cascade impactors. A viable cascade impactor (SKC Inc., Valley View, PA) is comprised of an inlet cone, a precision-drilled 400-hole impactor stage, and a base that holds a standard-size agar plate (Figure 5). A high flow pump pulls microorganisms in the air through the holes (jets) at 30 liters per minute, where they are collected directly onto the agar surface. This method is the most sensitive for the detection of organisms at low concentrations.

TSI AERODYNAMIC PARTICLE SIZER

A TSI Aerodynamic Particle Sizer (APS) model 3321 (TSI Inc., Shoreview, MN) was used to measure aerosol concentrations and particle size during trials. The APS provided real-time aerodynamic particle characterization with a size range from 0.54-20.0 µm with 52 size bins of resolution. Sampling is continuous with a data export interval of 1 second. The APS has a continuous flow rate of 5 liters per minute (LPM). A picture of the APS is shown in Figure 6.



Figure 6. TSI Aerodynamic Particle Sizer (APS) model 3321 used to measure total particle concentration and particle size distribution of the challenge bioaerosol. Range 0.54-20.0 µm aerodynamic diameter, with 1 particle/L detection limits.

Species Selection

Due to safety concerns for bioaerosol testing, organism selection was based on Biological Safety Level 1 (BSL1) species which served as surrogates for more dangerous pathogenic (BSL2 & BSL3) organisms.

Viral Challenges:

Virus MS2 is a viral single-stranded, non-enveloped RNA bacteriophage that has been used historically as a surrogate for influenza viruses. MS2 has also recently been used as a tentative surrogate for SARS-CoV-2 in numerous published bioaerosol studies. Phi-X174 (Φ-X174) is a viral, single-stranded, non-enveloped, DNA bacteriophage traditionally used as a surrogate for viral species such as herpes simplex and smallpox.



The US FDA guidance document, Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency, states that lipid enveloped viruses, such as coronaviruses, are the least resistant microorganisms to disinfectants. It is presumed that this susceptibility is similar for other chemical, physical and catalytic methods of destruction.

MS2 and Phi X 174 are non-enveloped viruses, which makes them more resistant to disinfection than lipid viruses, and therefore, should represent a "worst case scenario" when compared to actual lipid-enveloped RNA viruses like SARS-CoV-2. Figure 7 is a graphic from the FDA document, COVID Sterilizers, Disinfectant Devices, and Air Purifiers Guidance, demonstrating resistance to disinfection.

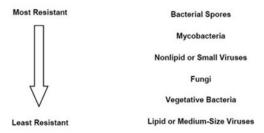


Figure 7: FDA graphic demonstrating general resistance to disinfection for various microorganisms. FDA, Guidance Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers during the Coronavirus Disease 2019 (COVID-19). Pg. 7. March 2009. SARS-CoV-2 (lipid or medium-Sized Virus), MS2 (non-lipid small virus).

Vegetative Bacteria Challenges:

The vegetative bacteria organisms used for this study included Methicillin Resistant *Staphylococcus epidermidis* (MRSE) (ATCC 12228). *Staphylococcus epidermidis* is a grampositive bacterium and BSL1 simulant for a wider range of medically significant pathogens including Methicillin Resistant *Staphylococcus aureus* (MRSA).

Escherichia coli was selected as the gram-negative vegetative bacterium for this study. (ATCC 15597). E. coli is a bacterium commonly used in various forms of testing as it is a common pathogen found in a multiplicity of places, and it can survive on many surfaces, and it may cause serious illness (potentially lethal) itself.

Mold Spores and Bacterial Endospore Challenges:

Aspergillus brasiliensis (ATCC 16404), formerly known as A. niger, is one of the most common species of the genus Aspergillus. A. brasiliensis is routinely defined as a surrogate for various toxic black mold species such as Stachybotrys chartarum. Many respiratory problems found in infants, the elderly and immunocompromised individuals are attributed to mold. Purified A. brasiliensis spores were used in bulk, dry

powder form with an approximate concentration of 1 x 10^9 cfu/gram.

Bacillus subtilis (ATCC 49760), endospores were used as a surrogate for Bacillus anthracis (Anthrax), a biological agent used for bioterrorism/biowarfare research. It also serves as a surrogate for other pathogenic endospore forming species such as Clostridioides difficile, a common and difficult to eliminate hospital pathogen. Bacillus subtilis, a sub-species of Bacillus atrophaeus, is a gram-positive bacterium found in soil and in the gastrointestinal tract of ruminants and humans. B. subtilis is rod-shaped, and forms a tough, highly resistant endospore, which allows it to tolerate extreme environmental conditions.

Challenge Bioaerosol Aerodynamic Diameter

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

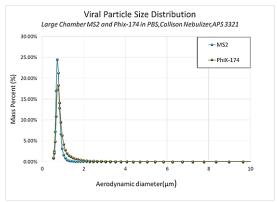


Figure 8: Aerodynamic Particle Size Distribution of RNA virus MS2 and DNA virus PhiX-174 in the test chamber. MMAD for both viral species averaged approximately 0.7 µm.

The aerodynamic particle size distribution for all challenge bioaerosols are shown to be within the respirable range for regional alveolar tract deposition and show a low geometric standard deviation (GSD), indicating that a monodispersed aerosol was generated in the chamber for each of the challenge species. The aerodynamic particle size distributions for MS2 and Phi X174 can be found in Figure 8, shown above.

The bioaerosol particle size distributions for *S.epidermidis* and *E.coli* are shown in **Figure 9**. The particle size distribution for *A.brasiliensis* and *B.subtilis* are found in **Figure 10**.

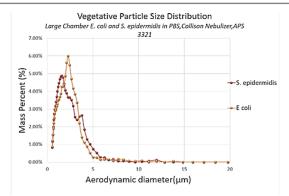


Figure 9: Aerodynamic Particle Size Distribution of *S. epidermidis* and *E. coli* in the test chamber. MMAD for each species was approximately 2.4-2.6 µm.

The particle size distribution of the spore species are noticeably larger than that of the vegetative bacteria. This makes these species easier to filter out of the air, however they are much more resilient when it comes to physical destruction.

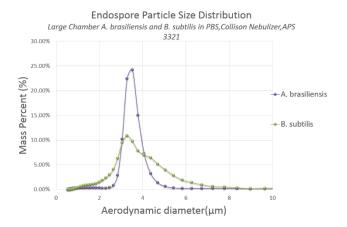


Figure 10: Aerodynamic Particle Size Distribution of *A. brasiliensis* and *B. subtilis* in the test chamber. MMAD for *B. subtilis* is approximately 2.4 μm with *A. brasiliensis* having a MMAD of approximately 4.0 μm.

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 separated by centrifugation. MS2 stock yields were greater than 1×10^{11} plaque forming units per milliliter (pfu/mL) with a single amplification procedure. This stock MS2 viral solution was then diluted with PBS to approximately 1×10^{10} plaque forming units per milliliter (pfu/mL) for use in the Collision nebulizer. The Phi-X174 stock was prepared in the same manner however, in order to achieve a high enough

concentration the Phi-X174 underwent a double amplification procedure.

Vegetative Cells Culture & Preparation

Pure strain seed stocks were purchased from ATCC (American Type Culture Collection, Manassas VA). For ATCC reference numbers see **Table 1** below on page 8. Working stock cultures were prepared using aseptic 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 around 1 x 10¹⁰ cfu/ml.

Stock cultures were centrifuged for 10 minutes at 3000rpm in an LD-3 centrifuge 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.

Fungal Spore Culture & Preparation

A. brasiliensis 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. This aliquoted spore suspension was plated prior to testing to verify concentration.

Bacillus subtilis freeze-dried spores were purchased from ATCC with a stock concentration of 1 x 10^{11} cfu/gram. One gram of dry spores was suspended in a 250mL solution of 50/50 91% Isopropyl alcohol and PBS + 5% Tween to assist in deagglomeration. This suspension was sonicated for 40 minutes in order to bring all powder into solution. This aliquoted spore suspension was plated prior to testing to verify concentration.

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 24-48 hours depending on the species and enumerated and recorded.



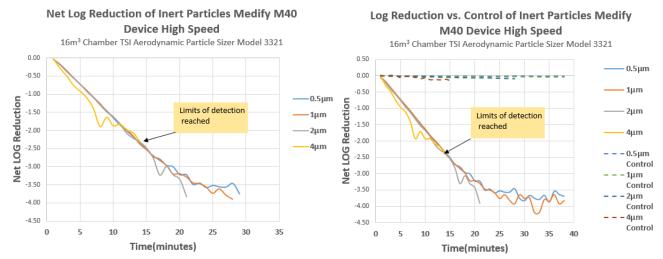


Figure 11: PSL Microspheres Chamber Trials for the Control and M40 Device at High Speed. First figure (Left) shows the net log reduction of four different inert particles normalized to 100% for T=0 sample for both control and M40 trials. Second figure (Right) show the log reduction of inert particles by the M40 unit.

When working with microorganisms at extremely low concentrations the viable cascade sampling was used. This method samples the chamber by pulling 30 liters per minute through the cascade device directly onto an agar plate. Enumeration of colonies grown depends on the concentration of the sample. Colony counts totaling up to 400 can then be adjusted using the positive conversion table. This table is based on the principle that, as the number of viable particles being impinged on a given plate increases, the probability of the next particle going into an "empty hole" decreases. This can be corrected statistically using the conversion formula of Feller, W (1950).

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 at the conclusion of the trials with aerosol/vaporous hydrogen peroxide (35%). The Collison nebulizer and impingers were cleaned at the conclusion of each day of testing by soaking in a 5% bleach bath for 20 minutes. The nebulizer and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

Data Analysis

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 the M40 device to achieve at least a 4 LOG

(99.99%) 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 are normalized with time zero enumerated concentrations. Subsequent samples are normalized and plotted to show the loss of viability over time.

Result: Inert Particle Characterization

PSL microsphere trial data were used to estimate nebulization efficiencies, particle stability, determine sample collection times, and aerosol persistence prior to bioaerosol testing. In order to estimate total bioaerosol trial times, sampling frequency and sample duration for the bioaerosol challenges testing with the M40 device was conducted using PSL microspheres. The removal efficacy of polystyrene latex microspheres (PSL microspheres) were used to characterize simple particle capture efficiency.

Polydispersed PSL microspheres with aerodynamic diameters of 0.5 - 4.0 μm were nebulized in PBS and chamber concentrations were recorded using the APS over time. The APS recorded individual particle count from 0.54 to 20.0 μm in size with 52 separate size bins of resolution. Two pre-trials were conducted: a negative control with the test unit "off" and a single positive control with the test unit turned "on" after aerosolization. All trials were performed with chamber mixing fans "on" during the entirety of the trial. Results show a sharp drop in the particle number concentration with the M40 in operation (note the log scale of the y-axis). Figure 11 (left) shows the net log reduction for the unit and control trial, while Figure 11 (right) shows the LOG reduction for 0.5, 1.0, 2.0 and 4.0 μm PSL microspheres.

all samples in

triplicate



D:-1--:-1 T--4 M-4-:-

Biologic	cal Test Mat	rix								
Trial	Run	Pathogenic Organism	Surrogate Species (gram, description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1	Control									
2	Challenge	Pathogenic E.	Eschericia Coli	15597	2.5-3.0um	10 ⁴ -10 ⁶	30	0, 10, 20, 30	APS, Impingers,	all samples in
3	Challenge	coli sp.	(-, vegetative)	13397	2.3-3.0uiii	10 -10	30	0, 10, 20, 30	Viable Cascade	triplicate
4	Challenge									
5	Control	Methicillin								
6	Challenge	resistant	Staphylococcus Epidermidis	12228	2.5-3.0um	10 ⁴ -10 ⁶	30	0, 10, 20, 30	APS, Impingers,	all samples in
7	Challenge	staphylococcus	(+, vegetative)	12226	2.5 5.0diii	10 -10	30	0, 10, 20, 30	Viable Cascade	triplicate
8	Challenge	aureus								
9	Control	Influenza ,								
10	Challenge	(tentative	MS2 bacteriophage	15597-B1	<1.0um	10 ⁴ -10 ⁶	30	0, 10, 20, 30	APS, Impingers	all samples in
11	Challenge	surrogate for	(E. coli phage)	13397 B1	<1.0uiii	10 -10	30	0, 10, 20, 30	Ai 5, implingers	triplicate
12	Challenge	Sars-cov2)								
13	Control									
14	Challenge	Herpes simplex	Phi X 174	13706-B1	<1.0um	10 ⁴ -10 ⁶	30	0, 10, 20, 30	APS, Impingers	all samples in
15	Challenge	and Smallpox	(E. coli phage)	13700 B1	<1.ouiii	10 -10	30	0, 10, 20, 30	711 b, impingers	triplicate
16	Challenge									
17	Control									
18	Challenge	Toxic Black	Aspergillus brasiliensis	16404	<5.0um	10 ⁴ -10 ⁶	30	0, 10, 20, 30	APS, Impingers,	all samples in
19	Challenge	Molds (spore)	(mold, spore forming)	10404	S.Juili	10 -10	30	0, 10, 20, 30	Viable Cascade	triplicate
20	Challenge									

Table 1: Phase I Test Matrix for the M40 air purification system.

<3.5 um

 $10^4 - 10^6$

30

49760

Phase I Methods: Bioaerosol Efficacy Testing

C. difficile &

Bacillus

anthracis

(spore)

Bacillus subtilis endospore

(Bacillus Spores)

Method Controls:

Control Challenge

Challenge

Challenge

23

To accurately assess the M40 unit, test chamber pilot control trials were performed with all organisms over a 60minute time period to characterize the biological challenge aerosol delivery/collection efficiency, and concentration over time. Control testing was performed to provide baseline comparative data in order to assess the actual reduction from the M40 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 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 runs and was turned off during M40 decontamination trials. The two impingers used for bioaerosol collection were pooled and mixed prior to plating and enumeration. A complete test matrix for Phase I bioaerosol trials can be found in Table I above.

Methods: M40 Testing

For each control and challenge test, the Collison nebulizer was filled with approximately 40 mL of biological stock and operated at 40 psi for a period of 20 minutes. Then, the impingers were filled with 20 mL of sterilized PBS with an addition of 0.005% v/v Tween 80 for bioaerosol collection. The addition of Tween 80 was used in order to increase the impinger collection efficiency and de-agglomeration of all microorganisms. The chamber mixing fan was turned on during bioaerosol dissemination to assure a homogeneous

bioaerosol concentration in the test chamber prior to taking the first impinger sample (T=0).

0, 10, 20, 30

APS, Impingers

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and M40 test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. AGI samples were collected for 2 to 10 minutes at intervals of 10 or 30 minutes throughout the entire test period.

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

For M40 biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test. Subsequent impinger samples were taken at various time points throughout the trial. These samples were enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the M40 device over time.

All samples were plated in triplicate on tryptic soy agar media over a minimum 3 log dilution range. Plates were incubated for 24-48 hours and enumerated for viable plaque forming units (pfu) or colony forming units (cfu) to calculate aerosol challenge concentrations in the chamber and reduction of viable microorganisms.



Triplicate Average Net Log Reduction Broad Range Bioaerosol Challenge

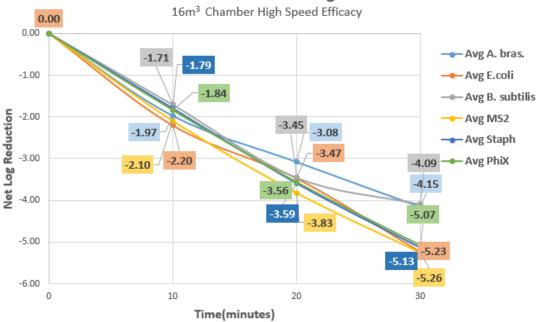


Figure 12: Phase I Net LOG Reduction for the M40.

Phase I Results - High Speed

Phase I of this study was performed to evaluate the M40 device efficacy at reduction of bioaerosols in a controlled room. Reduction of viable bioaerosols by a net 4 logs or 99.99% is the minimum requirement for FDA approved use. The species of organisms used were chosen specifically for their ability at gauging device efficacy against the most common encountered organisms.

When tested against the MS2 bacteriophage, the device showed a net log reduction of 5.26 +/- 0.22 in 30 minutes. When tested against Phi X 174 the device achieved a net log reduction of 5.07 +/- 0.27 in 30 minutes. When tested against

Staphylococcus epidermidis the device reached a net log reduction of 5.13 +/- 0.11 in 30 minutes. The other bacterial species tested *Escherichia coli* reached 5.23 +/- 0.16 in 30 minutes. The *Aspergillus brasiliensis* spores showed an average net reduction of 4.15 +/- 0.04 net logs in 30 minutes. The bacterial endospore from *Bacillus subtilis* reached a net log reduction of 4.15 +/- 0.13 net log in 30 minutes. Net log reduction data can be found above in Figure 12 and Table 2.

Phase I Conclusion

Overall, the Speed 3 yielded consistent reduction throughout, reaching a net 4 log reduction for all species. The duration of time needed to reach a net 4 log reduction was 30 minutes for all species.

Bioaerosol Type	Species (gram, description)	Number of Trials	Total Trial Time(minutes)	Data Type	Trial 1	Trial 2	Trial 3	Average
	MS2 bacteriophage			Net Log Reduction	-5.30	-5.46	-5.02	-5.26+/-0.22
Virus	(RNA E. coli phage Sars-CoV2 surrogate)	3	30	Net % Reduction	99.9995%	99.9997%	99.9991%	99.9994% +/- 0.0003%
Virus	Phi X 174 bacteriophage	2	30	Net Log Reduction	-5.19	-5.26	-4.77	-5.07+/-0.27
Virus	(DNA E. coli phage)	3		Net % Reduction	99.9994%	99.9994%	99.9983%	99.999% +/- 0.0006%
Bacterial	Staphylococcus Epidermidis	3	30	Net Log Reduction	-5.23	-5.14	-5.02	-5.13+/-0.11
Dacterial	(+, vegetative)			Net % Reduction	99.9994%	99.9993%	99.9990%	99.9992% +/- 0.0002%
Bacterial	Escherichia coli	3	30	Net Log Reduction	-5.41	-5.18	-5.09	-5.23+/-0.16
Bacteriai	(-, vegetative)			Net % Reduction	99.9996%	99.9993%	99.9992%	99.9994% +/- 0.0002%
Bacterial	Bacillus subtilis	2	20	Net Log Reduction	-4.08	-4.05	-4.14	-4.09+/-0.04
Bacteriai	(vegetative, spore forming)	3	30	Net % Reduction	99.9917%	99.9912%	99.9928%	99.9919% +/- 0.0008%
Mold	Aspergillus brasiliensis	2	30	Net Log Reduction	-4.29	-4.13	-4.04	-4.15+/-0.127
Mold	(mold, spore forming)	3	30	Net % Reduction	99.9949%	99.9926%	99.9908%	99.9928% +/- 0.002%

Table 2: Phase I Executive Summary



Trial	Run	Fan Setting	Pathogenic Organism	Surrogate Species (gram, description)	ATCC Ref	Target Monodisperse d Particle Size	Challenge Conc. (#/L)	Trial Time (min)	Sample Time (min)	Sampling	Plating and Enumeration
1 2 3	Challenge Challenge Challenge	Speed 1	Influenza, (tentative surrogate for Sars-cov2)	MS2 bacteriophage (E. coli phage)	15597-B1	<1.0um	10 ⁴ -10 ⁶	90	0, 30, 60, 90	APS, Impingers	all samples in triplicate
4 5 6	Challenge Challenge Challenge	Speed 1	C. difficile & Bacillus anthracis (spore)	Bacillus subtilis endospore	49760	<3.5 um	10 ⁴ -10 ⁶	90	0, 30, 60, 90	APS, Impingers	all samples in triplicate

Table 3: Phase II Test Matrix for Multi-speed bioaerosol testing.

Phase II Methods: Bioaerosol Efficacy Testing

The second part of this study used the same methods as the first with the exception of the device fan speed setting being different. For the 'Speed 1' testing, a net 4 log reduction was reached in a longer amount of time probably due to the decreased rate of filtration by the device. The more air being pulled through the device the faster the air in the chamber is being cleared of bioaerosol and particulates. The test matrix for Phase II is picture above in Table 3.

Phase II Results-Speed 1

Phase II of this study was designed to test the efficacy of the device at different fan speeds. The device had a total of two speeds tested with two of the test organisms. MS2 and *Bacillus subtilis* endospores were chosen for the multi speed tests for their resilience to natural decay. Testing with hardier organisms was crucial in determining efficacy.

The MS2 bacteriophage tested on Speed 1 was observed to have a net reduction of 5.29 +/- 0.13 logs in 90 minutes.

Bacillus subtilis spores tested on Speed 1 were observed to have a net reduction of 4.37 +/- 0.11 logs after 90 minutes. When the device was tested with inert particles ranging from 0.5 to 4.0 μ m on Speed 1 there was a reduction of over 4 net log in 45 minutes. The results for inert particle reduction testing can be found in Figure 13 below.

Similar reductions across all fan speeds demonstrated a robust performance of the M40 device. Net log and net percent reductions are shown in **Table 4** and **Figure 14** on the following page.

Phase II Conclusion:

Phase II demonstrated consistent results achieving a net 4 log reduction of the two selected species of microorganisms. Speed 1 yielded an average reduction of net 4 log reduction in only 90 minutes. The effectiveness of the M40 device at two of the operating speeds was key to demonstrating the device's efficacy.

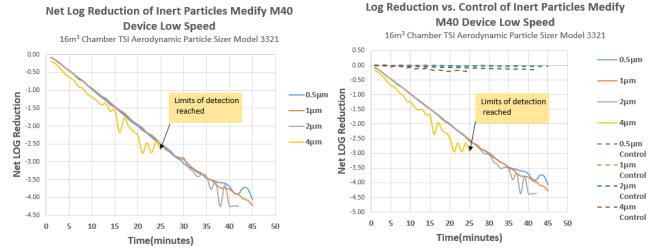


Figure 13: PSL Microspheres Chamber Trials for the Control and M40 Device at High Speed. First figure (Left) shows the net log reduction of four different inert particles normalized to 100% for T=0 sample for both control and M40 trials. Second figure (Right) show the log reduction of inert particles by the M40 unit.



Average % NET Reduction and NET LOG Reduction of Viable BioAerosols

Device Setting	Species (gram, description)	Number of Trials	Total Trial Time(minutes)	Data Type	Trial 1	Trial 2	Trial 3	Average
Speed 1	MS2 bacteriophage	2	90	Net Log Reduction	-5.41	-5.28	-5.16	-5.29+/-0.13
Speed 1	(DNA E. coli phage)	3		Net % Reduction	99.9996%	99.9995%	99.999%	99.9995% +/- 0.0002%
	Bacillus subtilis	2	00	Net Log Reduction	-4.28	-4.35	-4.49	-4.37+/-0.11
Speed 1	(vegetative, spore forming)	3	90	Net % Reduction	99.995%	99.996%	99.997%	99.9957% +/- 0.001%

Table 4: Phase II Executive Summary Triplicate net reduction data from each trial performed

Deviations and Data Analysis:

No deviations from the protocol were noted throughout the trials.

Because of the nature of the data and the normal variation, there was no need to perform statistical analyses. All results were ≤0.30 standard deviations from the mean.

In accordance with ARE Labs standard practice and in compliance with GLPs, all data were verified for accuracy.

Overall Study Summary:

In conclusion, the M40 device achieved a net 4 log reduction of all bioaerosols within a relatively short period of time. The device proved to be highly effective in reducing the aerosol bioburden of a broad range of microbial species. It is anticipated that such a reduction should reduce the likelihood of individuals contracting airborne infectious diseases in any enclosed environment, medical or otherwise.

M40 Low Speed vs. High Speed Trials: Net LOG Reduction Trial Averages

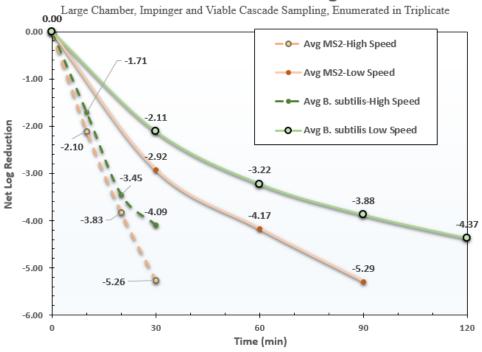


Figure 14: M40 device net log reduction of average triplicate at 'Speed 1' and 'Speed 3' of MS2 and B. subtilis



References

Feller, W. (1950). An introduction to probability theory and its applications. Wiley.

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.

U.S. Department of Health and Human Services Food and Drug Administration. *Enforcement Policy for Sterilizers,*Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency Guidance for Industry and Food and Drug Administration Staff. March 2009



Analytical Testing Facility

Aerosol Research and Engineering Labs, Inc. 15320 S. Cornice Street Olathe, KS 66062

Project

10940.10

Study Director

Jamie Balarashti Aerosol Research and Engineering Laboratories

GLP Statement

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

Conflict of Interest Statement

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

Study Director:	
Jani/fr	8/9/2021 Date
Jamie Balarashti	
Study Director	
ARE Labs Inc.	
Principal Investigator:	
5/12	
Sean McLeod	8/9/2021
Principal Investigator	Date
ARE Labs, Inc.	



Phase I Additional Figures: Speed 3 LOG and Net LOG Reduction Graphs



M40 E. coli High Speed Trials: LOG Reduction

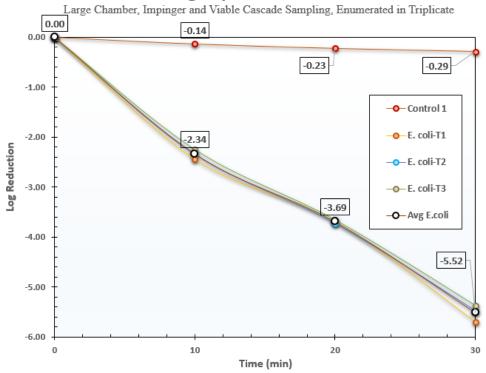


Figure 1A: E.coli M40 LOG Reduction

M40 *E. coli* High Speed Trials: Net LOG Reduction

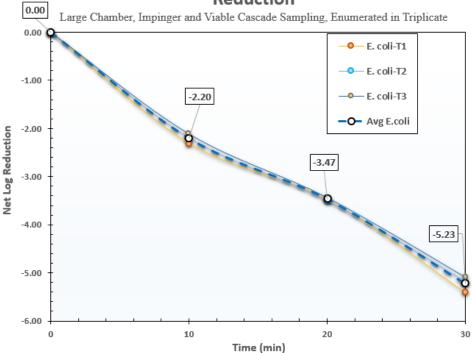


Figure 2A: E.coli M40 Net LOG Reduction



M40 A. brasiliensis High Speed Trials: LOG Reduction

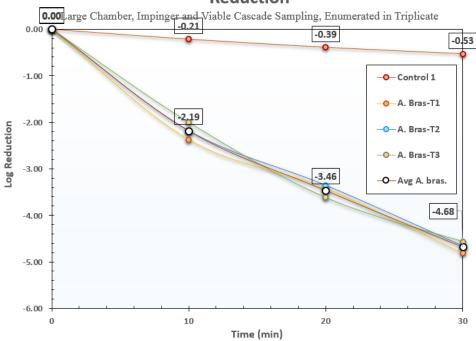


Figure 3A: A.brasiliensis M40 LOG Reduction

M40 A. brasiliensis High Speed Trials: Net LOG Reduction

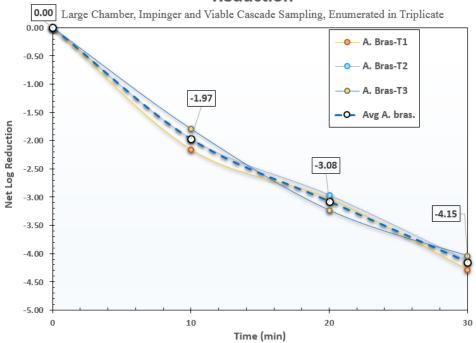


Figure 4A: A.brasiliensis M40 Net LOG Reduction



M40 *B. subtilis* High Speed Trials: LOG Reduction

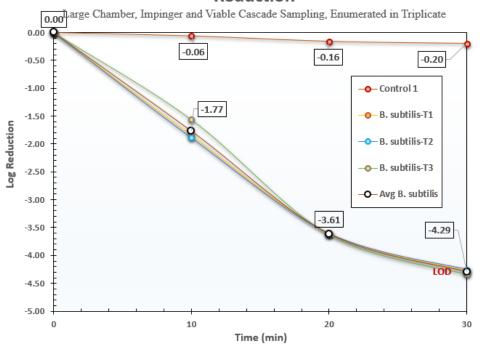


Figure 5A: B. subtilis M40 LOG Reduction

M40 *B. subtilis* High Speed Trials: Net LOG Reduction

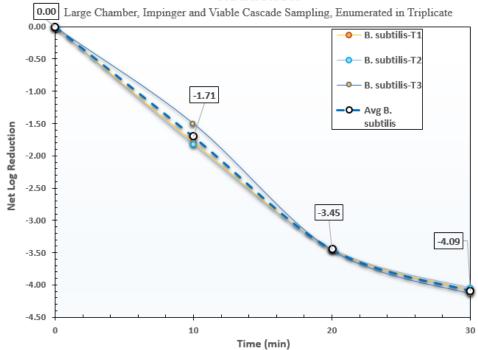


Figure 6A: B. subtilis M40 Net LOG Reduction



MS2 M40 High Speed Trials: LOG Reduction

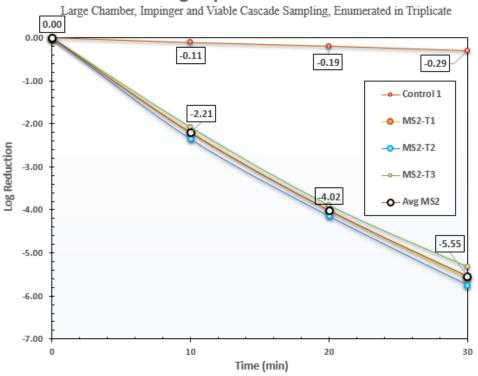


Figure 7A: MS2 LOG Reduction for the M40

MS2 M40 High Speed Trials: Net LOG Reduction

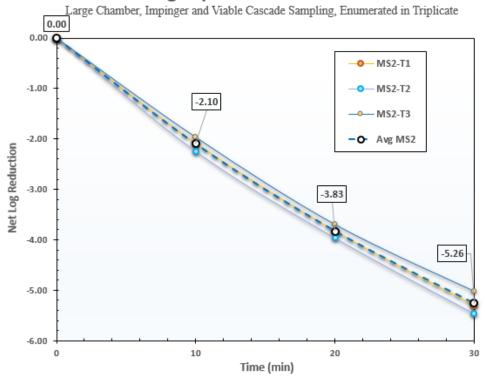


Figure 8A: MS2 Net LOG Reduction for the M40



M40 PhiX 174 High Speed Trials: LOG Reduction

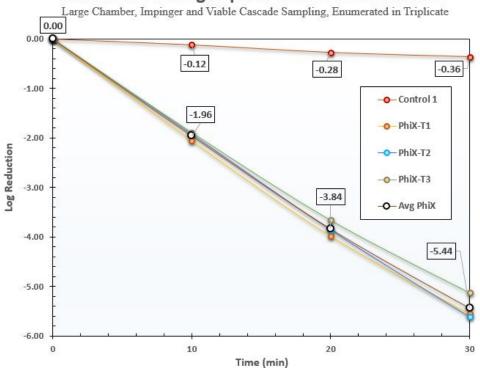


Figure 9A: Phi X 174 M40 LOG Reduction

M40 PhiX 174 High Speed Trials: Net LOG Reduction

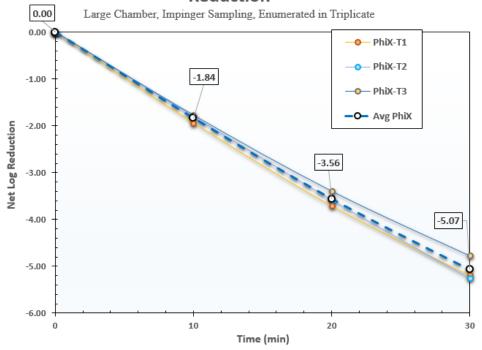


Figure 10A: Phi X 174 M40 net LOG Reduction



M40 High Speed *Staph. epi.* Trials: LOG Reduction

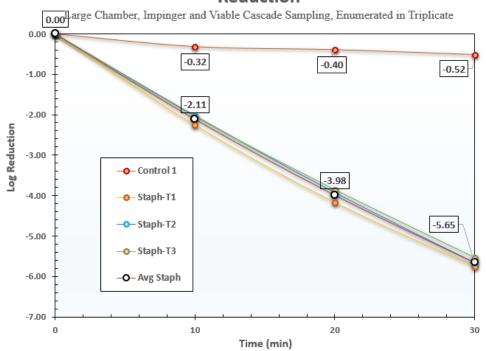


Figure 11A: Staph M40 LOG Reduction

M40 High Speed *Staph. epi.* Trials: Net LOG Reduction

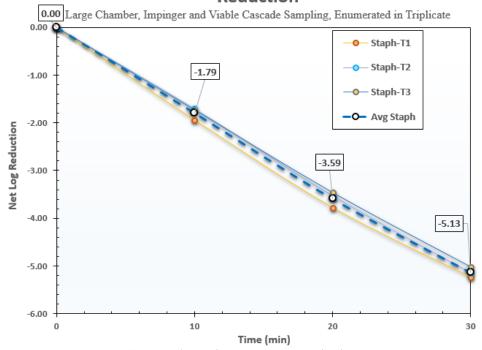


Figure 12A: Staph M40 net LOG Reduction



Phase II Results: Speed 1 Reduction Graphs by Organism



MS2 M40 Low Speed Trials: LOG Reduction

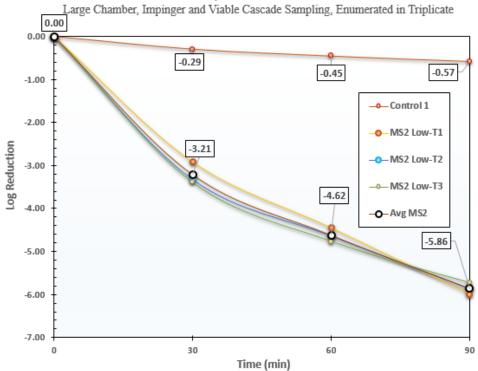


Figure 1B: M40 MS2 LOG Reduction Speed 1

MS2 M40 Low Speed Trials: Net LOG Reduction

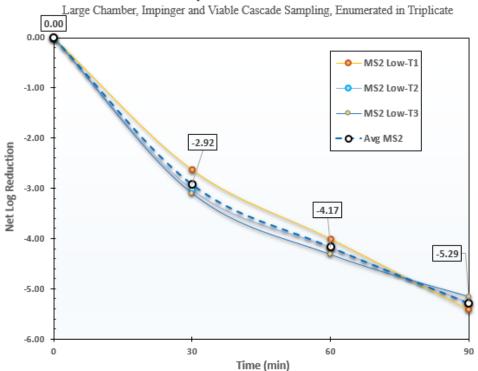


Figure 2B: M40 MS2 net LOG Reduction MS2 Speed 1



M40 B. subtilis Low Speed Trials: LOG Reduction

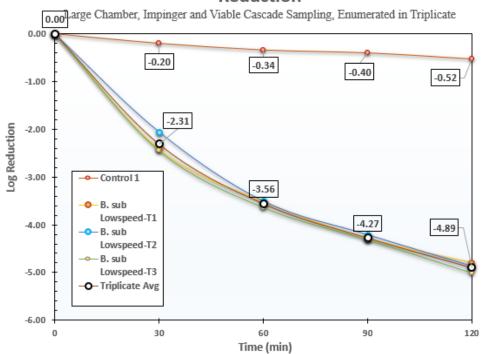


Figure 3B: M40 LOG Reduction Bacillus subtilis Speed 1

M40 B. subtilis Low Speed Trials: Net LOG Reduction

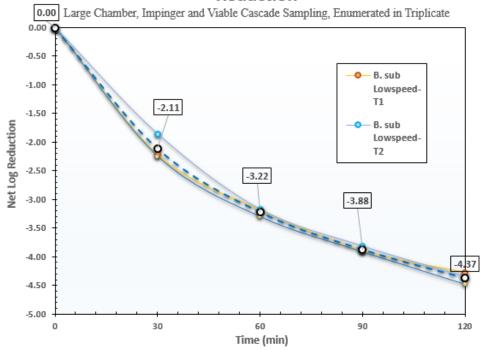


Figure 4B: M40 net LOG Reduction Bacillus subtilis Speed 1



Phase I Raw Data



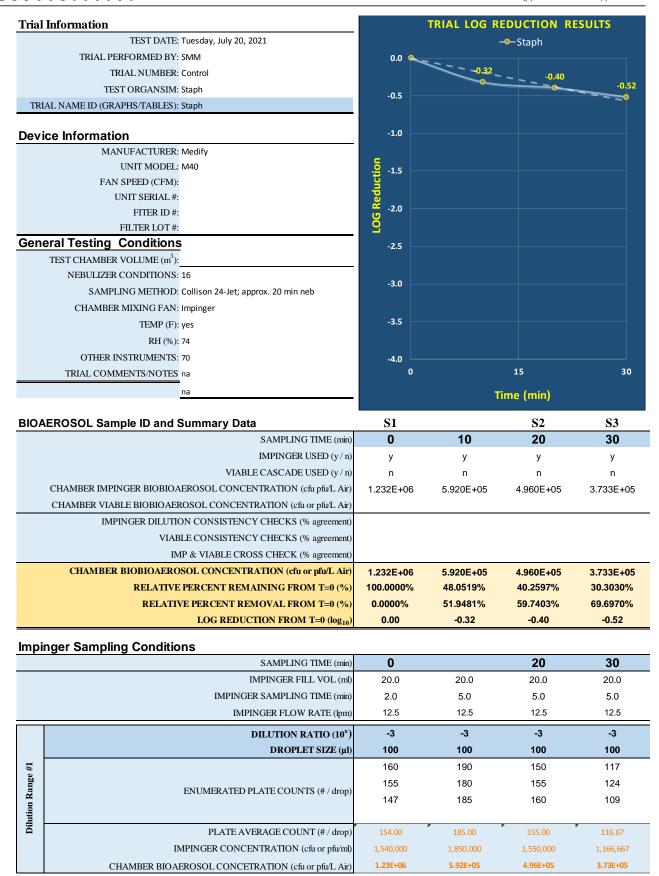
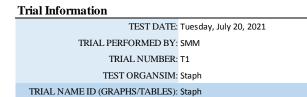


Figure 1B: S. epidermidis Control





Device Information

MANUFACTURER: Medify
UNIT MODEL: M40
FAN SPEED (CFM):
UNIT SERIAL #:
FITER ID #:
FILTER LOT #:

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m³): 16

NEBULIZER CONDITIONS: Collison 24-Jet; approx. 20 min neb

SAMPLING METHOD: Impinger

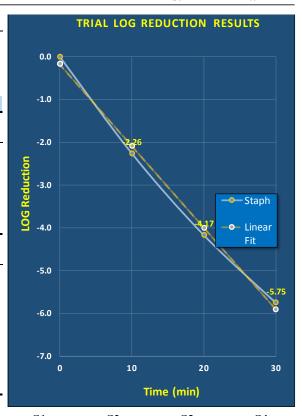
CHAMBER MIXING FAN: yes

TEMP (F): 74

RH (%): 70

OTHER INSTRUMENTS: na

TRIAL COMMENTS/NOTES na



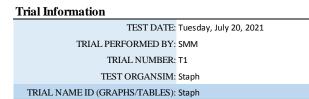
BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4
SAMPLE TIME (min)	0	10	20	30
IMPINGER USED (y / n)	У	у	у	у
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.280E+06	7.040E+03	8.640E+01	2.276E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)				
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.280E+06	7.040E+03	8.640E+01	2.276E+00
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	0.5500%	0.0068%	0.0002%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	99.4500%	99.9933%	99.9998%
LOG REDUCTION FROM T=0 (log ₁₀)	0.00	-2.26	-4.17	-5.75

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	10	20	30
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5
	DILUTION RATIO (10 ^x)	-4	-2	0	0
	DROPLET SIZE (μl)	100	100	100	750
#1		18	24	29	3
Range	ENUMERATED PLATE COUNTS (# / drop)	24	24	23	7
	ENUMERATED PLATE COUNTS (# / dtop)	30	18	29	6
Dilution					
Dil	PLATE AVERAGE COUNT (# / drop)	24.00	22.00	27.00	5.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,400,000	22,000	270	7
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.28E+06	7.04E+03	8.64E+01	2.28E+00

Figure 2B: S. epidermidis Trial 1





Device Information

MANUFACTURER: Medify
UNIT MODEL: M40
FAN SPEED (CFM):
UNIT SERIAL #:
FITER ID #:
FILTER LOT #:

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m³): 16

NEBULIZER CONDITIONS: Collison 24-Jet; approx. 20 min neb

SAMPLING METHOD: Impinger

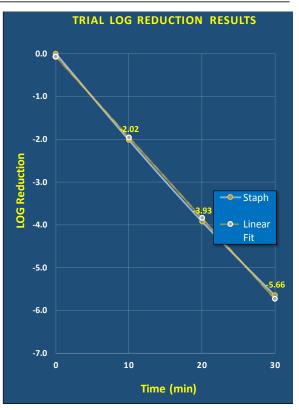
CHAMBER MIXING FAN: yes

TEMP (F): 74

RH (%): 70

OTHER INSTRUMENTS: na

TRIAL COMMENTS/NOTES
na



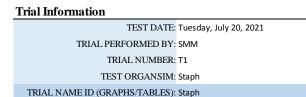
BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4
SAMPLE TIME (min)	0	10	20	30
$IMPINGER\ USED\ (y \ / \ n)$	у	У	У	у
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.689E+06	1.600E+04	2.005E+02	3.698E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)				
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.689E+06	1.600E+04	2.005E+02	3.698E+00
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	0.9474%	0.0119%	0.0002%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	99.0526%	99.9881%	99.9998%
LOG REDUCTION FROM $T=0$ (log ₁₀)	0.00	-2.02	-3.93	-5.66

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	10	20	30
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5
	DILUTION RATIO (10 ^x)	-4	-2	0	0
	DROPLET SIZE (μl)	100	100	100	750
#1		36	52	56	11
ange	ENUMERATED PLATE COUNTS (# / drop)	25	43	64	10
n R	ENOWIERATED FLATE COUNTS (# / diop)	34	55	68	5
Dilution Range #1					
Di	PLATE AVERAGE COUNT (# / drop)	31.67	50.00	62.67	8.67
	IMPINGER CONCENTRATION (cfu or pfu/ml)	3,166,667	50,000	627	12
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.69E+06	1.60E+04	2.01E+02	3.70E+00

Figure 3B: S. epidermidis Trial 2





Device Information

MANUFACTURER: Medify
UNIT MODEL: M40
FAN SPEED (CFM):
UNIT SERIAL #:
FITER ID #:
FILTER LOT #:

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m³): 16

NEBULIZER CONDITIONS: Collison 24-Jet; approx. 20 min neb

SAMPLING METHOD: Impinger

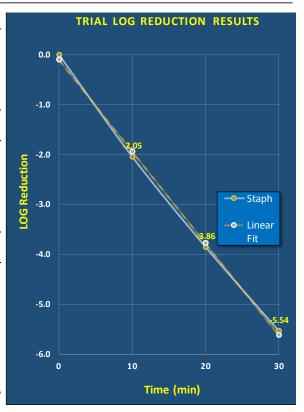
CHAMBER MIXING FAN: yes

TEMP (F): 74

RH (%): 70

OTHER INSTRUMENTS: na

TRIAL COMMENTS/NOTES na



BIOAEROSOL Sample ID and Summary Data	S1	S2	S3	S4
SAMPLE TIME (min)	0	10	20	30
$IMPINGER\ USED\ (y\ /\ n)$	У	У	У	У
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.476E+06	1.323E+04	2.059E+02	4.267E+00
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfw/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)				
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.476E+06	1.323E+04	2.059E+02	4.267E+00
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	0.8964%	0.0140%	0.0003%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	99.1036%	99.9860%	99.9997%
LOG REDUCTION FROM T=0 (\log_{10})	0.00	-2.05	-3.86	-5.54

Impinger Sampling Conditions

	SAMPLE TIME (min)	0	10	20	30
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	3.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5
Dilution Range #1	DILUTION RATIO (10 ^x)	-4	-2	0	0
	DROPLET SIZE (μl)	100	100	100	750
	ENUMERATED PLATE COUNTS (# / drop)	28	48	76	11
		25	42	60	11
		30	34	57	8
Di	PLATE AVERAGE COUNT (# / drop)	27.67	41.33	64.33	10.00
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,766,667	41,333	643	13
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.48E+06	1.32E+04	2.06E+02	4.27E+00

Figure 4B: S. epidermidis Trial 3



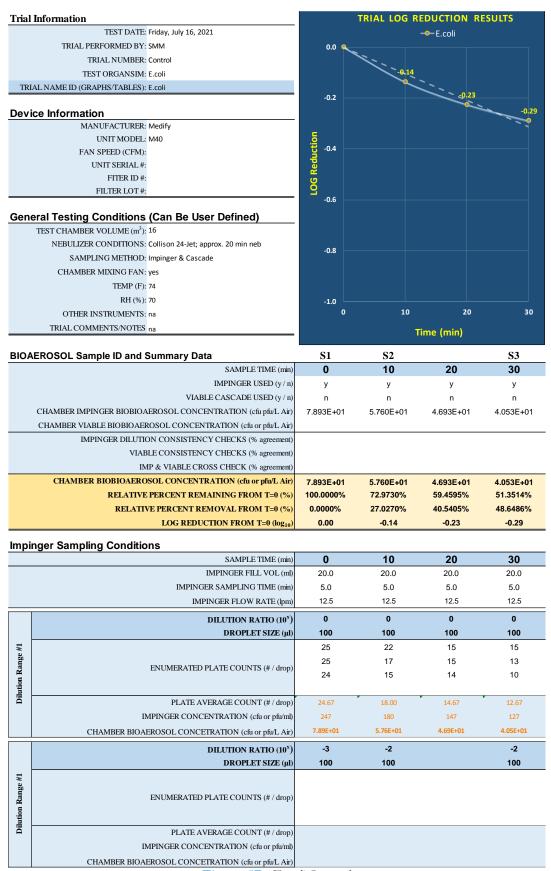


Figure 5B: E. coli Control



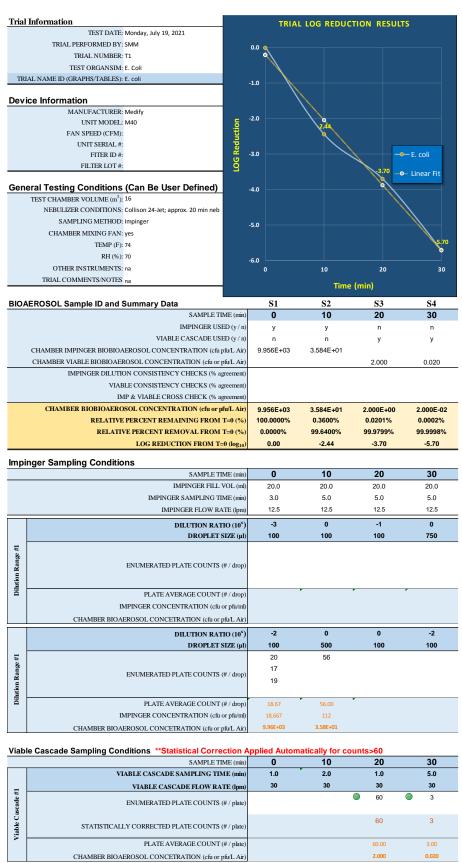


Figure 6B: E. coli Trial 1



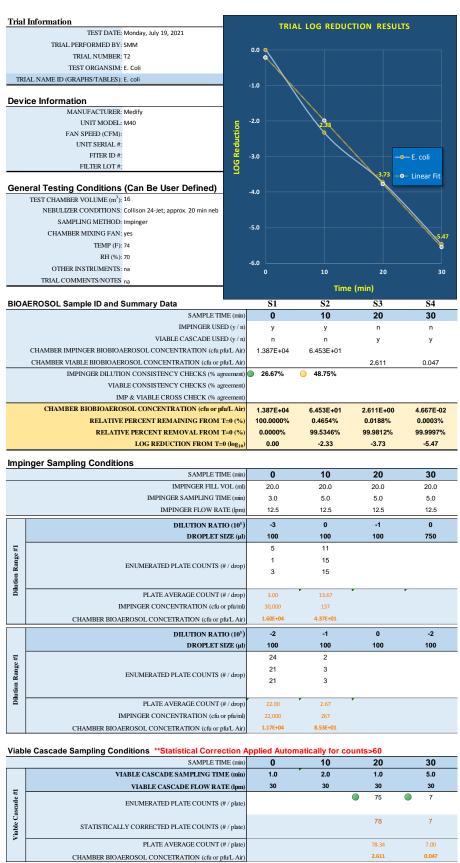


Figure 7B: E. coli Trial 2



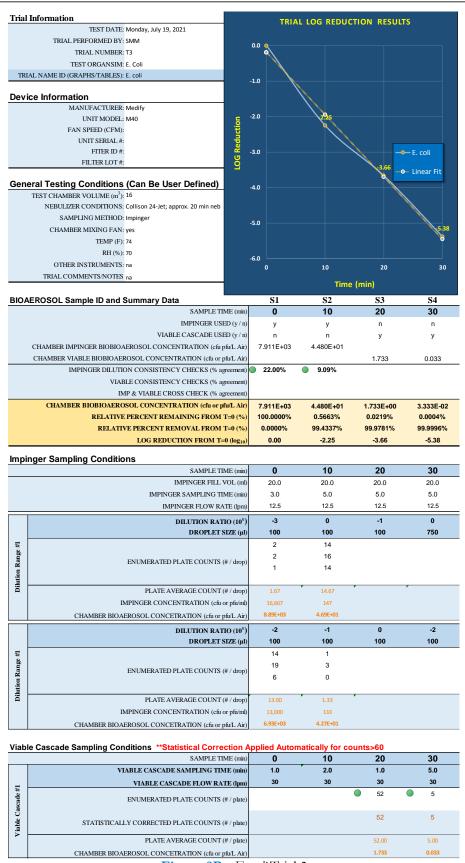


Figure 8B: E. coli Trial 3



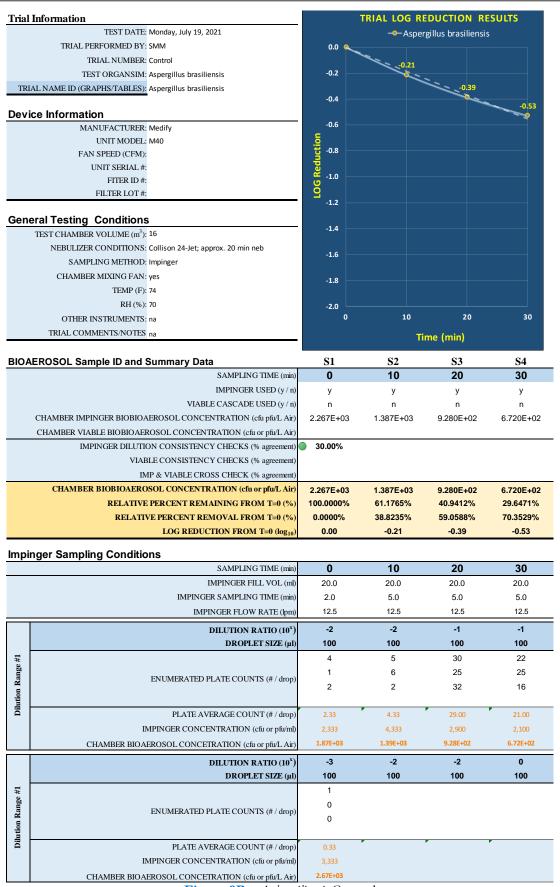


Figure 9B: A. brasiliensis Control



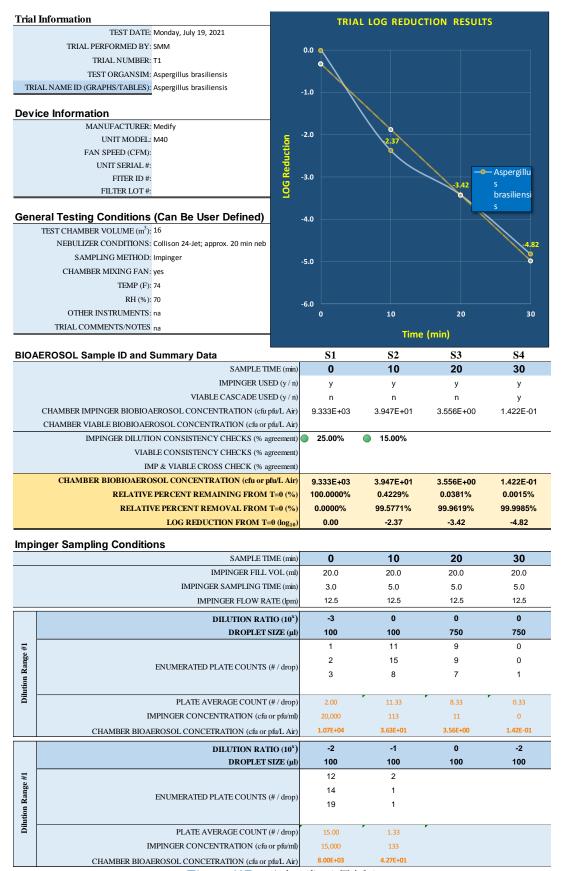


Figure 10B: A. brasiliensis Trial 1



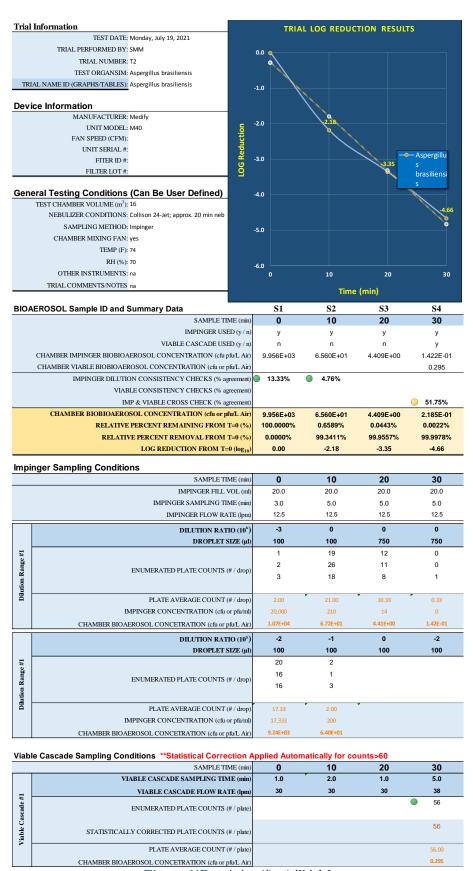


Figure 11B: A. brasiliensis Trial 2



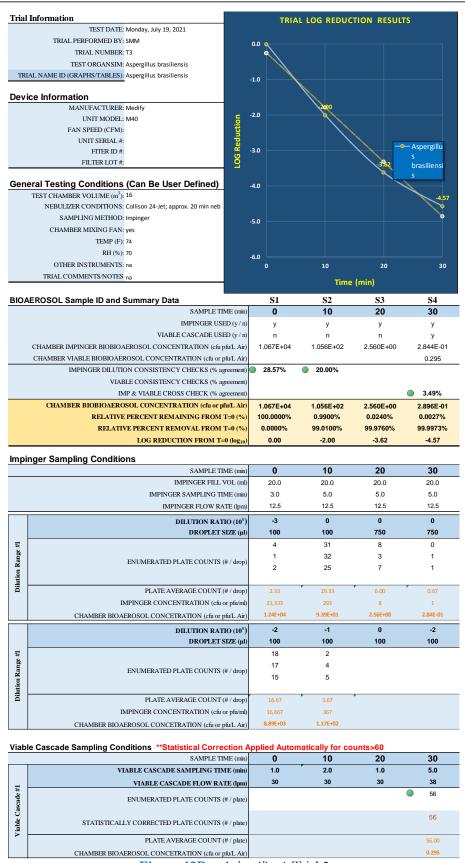


Figure 12B: A. brasiliensis Trial 3





TEST DATE: Friday, July 30, 2021

TRIAL PERFORMED BY: ZC

TRIAL NUMBER: T1

TEST ORGANSIM: B. subtilis

TRIAL NAME ID (GRAPHS/TABLES): B. subtilis

Device Information

MANUFACTURER: Medify

UNIT MODEL: M40

FAN SPEED (CFM):

UNIT SERIAL #:

FITER ID #:

FILTER LOT #:

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m³): 16

NEBULIZER CONDITIONS: Collison 24-Jet; approx. 20 min neb

SAMPLING METHOD: Impinger

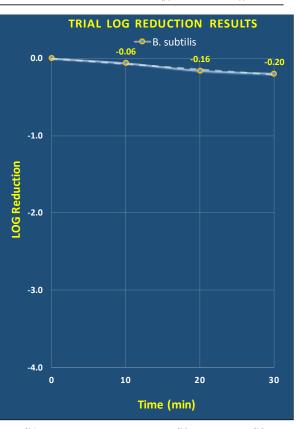
CHAMBER MIXING FAN: yes

TEMP (F): 74

RH (%): 70

OTHER INSTRUMENTS: na

TRIAL COMMENTS/NOTES na



BIOAEROSOL Sample ID and Summary Data	S1		S2	S3
SAMPLE TIME (min)	0	10	20	30
$IMPINGER\ USED\ (y\ /\ n)$	у	у	У	у
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	7.253E+05	6.293E+05	5.013E+05	4.587E+05
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)				
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	7.253E+05	6.293E+05	5.013E+05	4.587E+05
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	86.7647%	69.1176%	63.2353%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	13.2353%	30.8824%	36.7647%
LOG REDUCTION FROM T=0 (log ₁₀)	0.00	-0.06	-0.16	-0.20

lmpi	nger Sampling Conditions				
	SAMPLE TIME (min)	0		20	30
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	5.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5
	DILUTION RATIO (10 ^x)	-4	-4	-4	-4
	DROPLET SIZE (μl)	100	100	100	100
#1	ENUMERATED PLATE COUNTS (# / drop)	20	18	18	18
Range		23	21	14	16
n Re		25	20	15	9
Dilution					
Di	PLATE AVERAGE COUNT (# / drop)	22.67	19.67	15.67	14.33
	IMPINGER CONCENTRATION (cfu or pfu/ml)	2,266,667	1,966,667	1,566,667	1,433,333
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	7.25E+05	6.29E+05	5.01E+05	4.59E+05

Figure 13B: B. subtilis Control



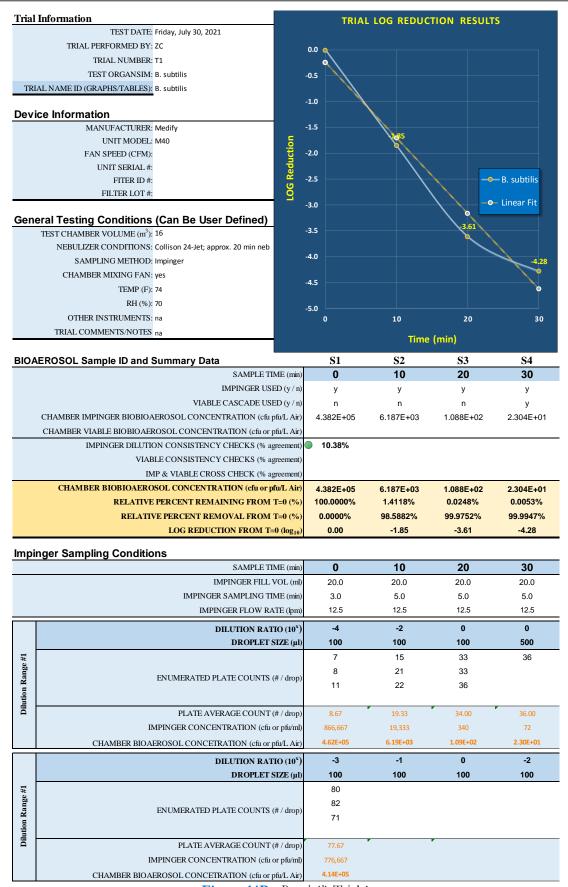


Figure 14B: B. subtilis Trial 1



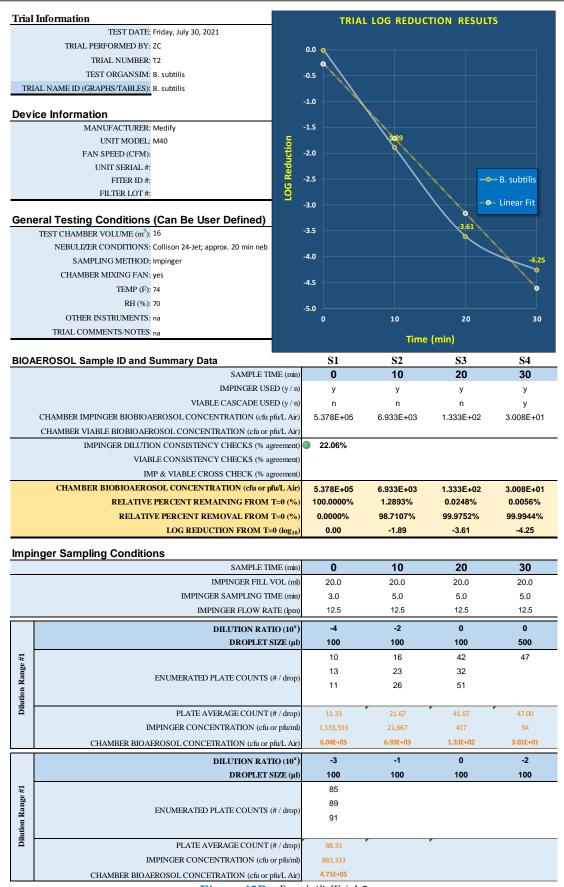


Figure 15B: B. subtilis Trial 2



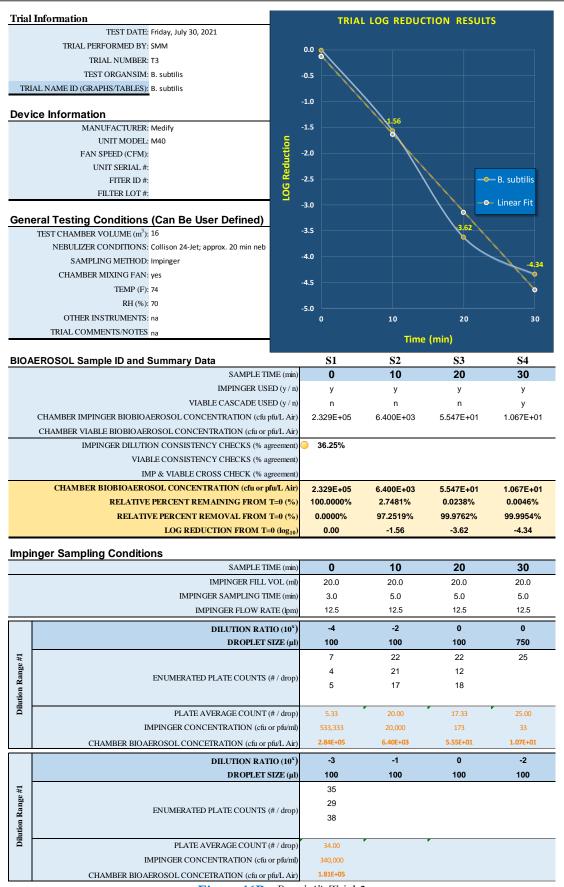


Figure 16B: B. subtilis Trial 3



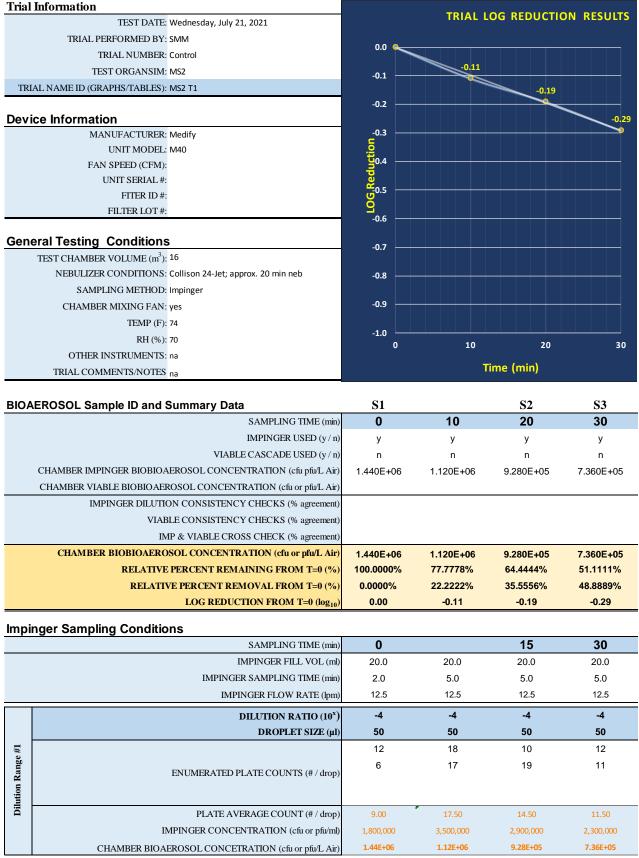


Figure 17B: MS2 Control



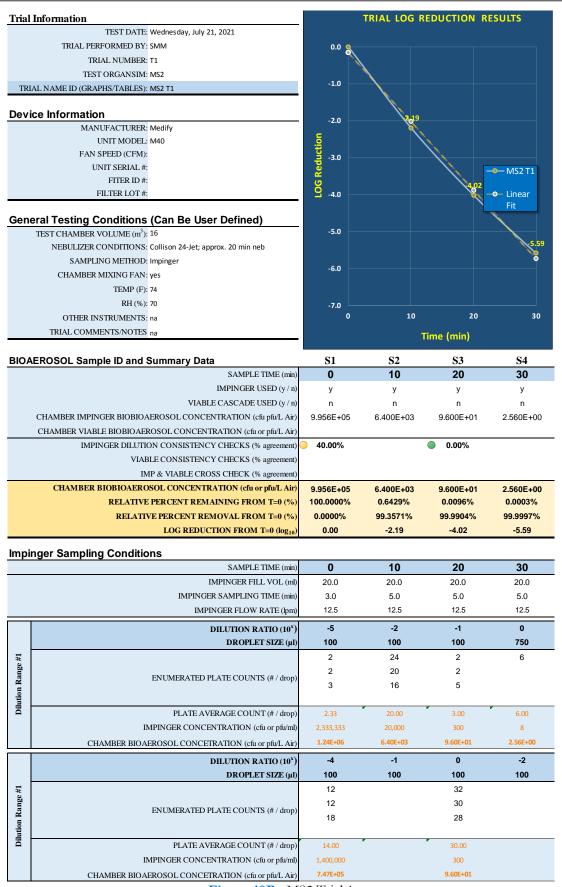


Figure 18B: MS2 Trial 1



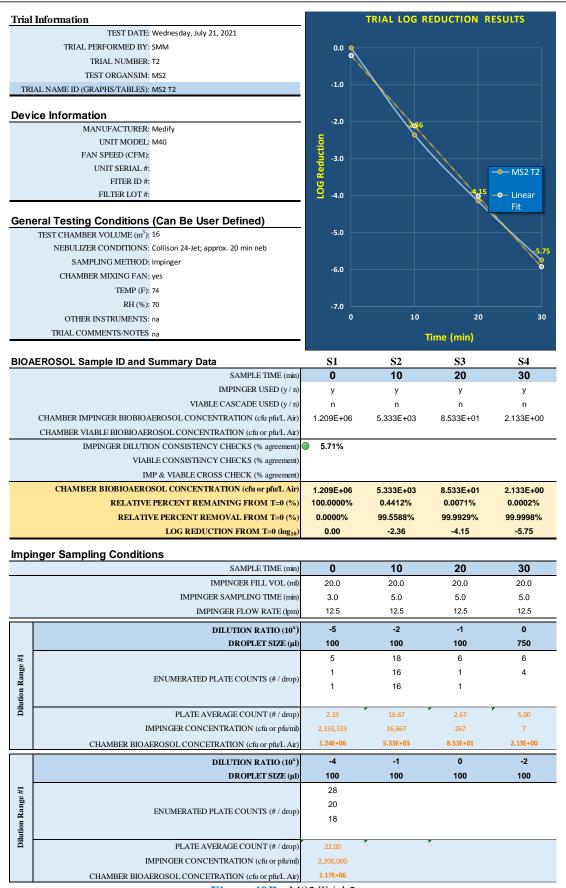


Figure 19B: MS2 Trial 2



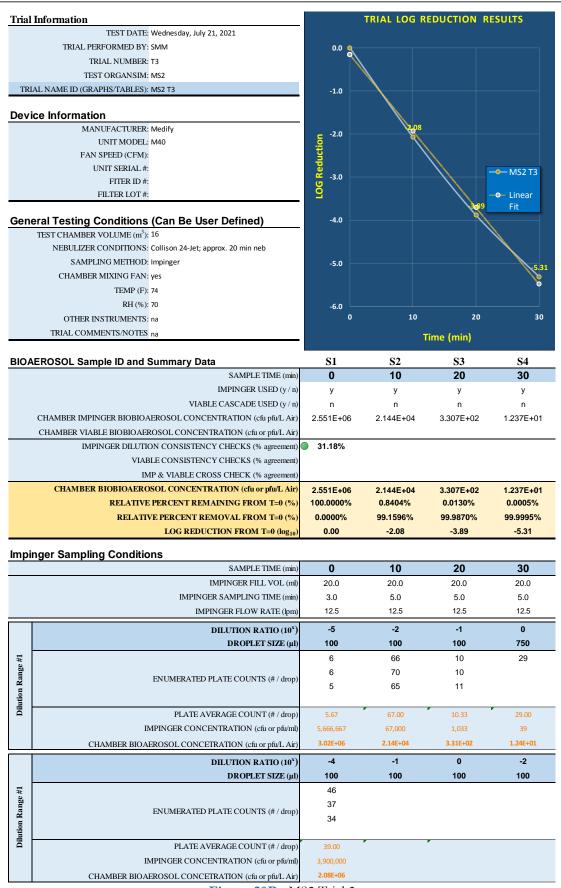
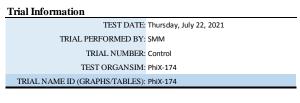


Figure 20B: MS2 Trial 3





Device Information

MANUFACTURER: Medify
UNIT MODEL: M40
FAN SPEED (CFM):
UNIT SERIAL #:
FITER ID #:
FILTER LOT #:

General Testing Conditions (Can Be User Defined)

TEST CHAMBER VOLUME (m³): 16

NEBULIZER CONDITIONS: Collison 24-Jet; approx. 20 min neb

SAMPLING METHOD: Impinger

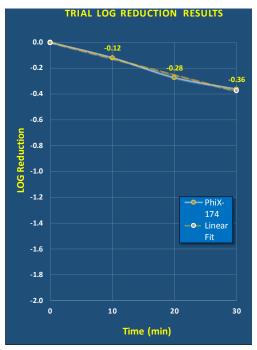
CHAMBER MIXING FAN: yes

TEMP (F): 74

RH (%): 70

OTHER INSTRUMENTS: na

TRIAL COMMENTS/NOTES na



BIOAEROSOL Sample ID and Summary Data	S1			S2
SAMPLE TIME (min)	0	10	20	30
IMPINGER USED (y / n)	У	у	у	у
VIABLE CASCADE USED (y / n)	n	n	n	n
CHAMBER IMPINGER BIOBIOAEROSOL CONCENTRATION (cfu pfu/L Air)	1.307E+05	9.920E+04	6.933E+04	5.653E+04
CHAMBER VIABLE BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)				
IMPINGER DILUTION CONSISTENCY CHECKS (% agreement)	4.00%			
VIABLE CONSISTENCY CHECKS (% agreement)				
IMP & VIABLE CROSS CHECK (% agreement)				
CHAMBER BIOBIOAEROSOL CONCENTRATION (cfu or pfu/L Air)	1.307E+05	9.920E+04	6.933E+04	5.653E+04
RELATIVE PERCENT REMAINING FROM T=0 (%)	100.0000%	75.9184%	53.0612%	43.2653%
RELATIVE PERCENT REMOVAL FROM T=0 (%)	0.0000%	24.0816%	46.9388%	56.7347%
LOG REDUCTION FROM T=0 (log ₁₀)	0.00	-0.12	-0.28	-0.36

Impinger Sampling Conditions

	SAMPLE TIME (min)	0		20	30
	IMPINGER FILL VOL (ml)	20.0	20.0	20.0	20.0
	IMPINGER SAMPLING TIME (min)	2.0	5.0	5.0	5.0
	IMPINGER FLOW RATE (lpm)	12.5	12.5	12.5	12.5
	DILUTION RATIO (10 ^x)	-4	-3	-3	-3
	DROPLET SIZE (μl)	100	100	100	100
#	ENUMERATED PLATE COUNTS (# / drop)	3	30	20	15
ange		1	30	21	20
n R		1	33	24	18
Dilution Range #1					
Ö	PLATE AVERAGE COUNT (# / drop)	1.67	31.00	21.67	17.67
	IMPINGER CONCENTRATION (cfu or pfu/ml)	166,667	310,000	216,667	176,667
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.33E+05	9.92E+04	6.93E+04	5.65E+04
	DILUTION RATIO (10 ^x)	-3			-2
	DROPLET SIZE (μl)	100			100
#		16			
ınge	THURST ATTER BY ATTE COLLEGE (II / I	14			
n Ra	ENUMERATED PLATE COUNTS (# / drop)	18			
Dilution Range #1					
Ω	PLATE AVERAGE COUNT (# / drop)	16.00			•
	IMPINGER CONCENTRATION (cfu or pfu/ml)	160,000			
	CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)	1.28E+05			

Figure 21B: Phi X Control



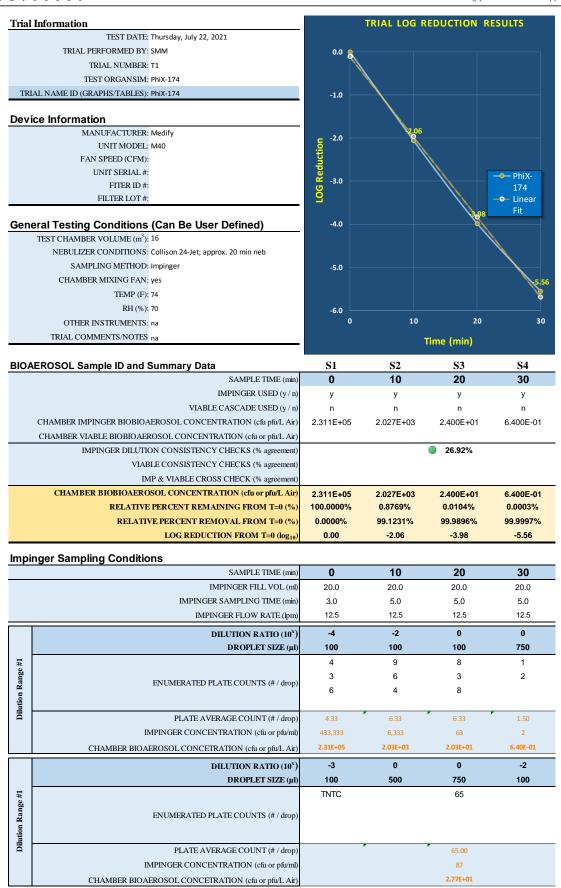


Figure 22B: Phi X Trial 1



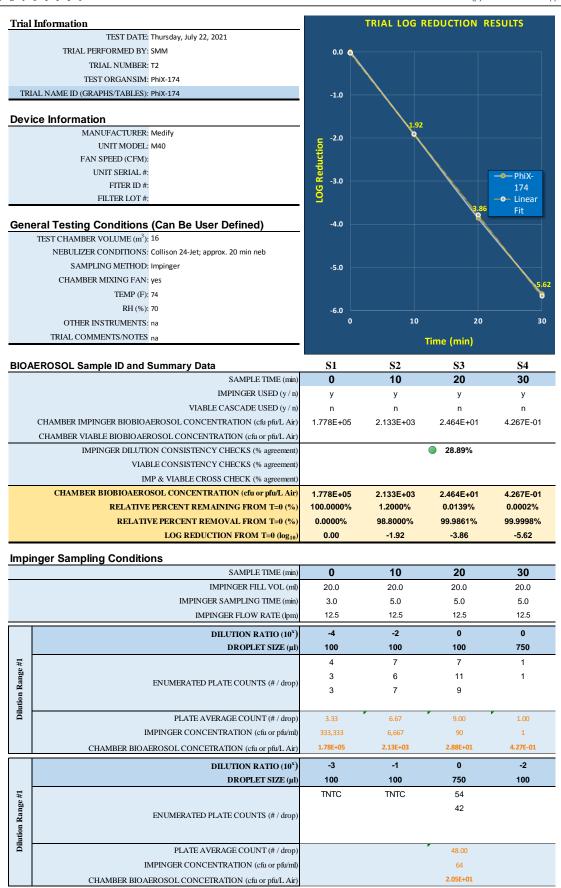


Figure 23B: Phi X Trial 2



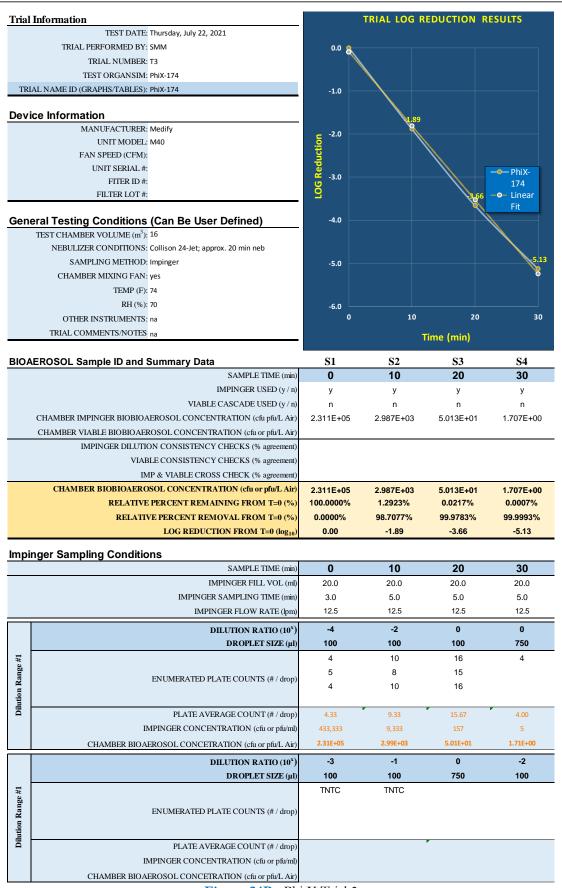


Figure 24B: Phi X Trial 3



Phase II Raw Data



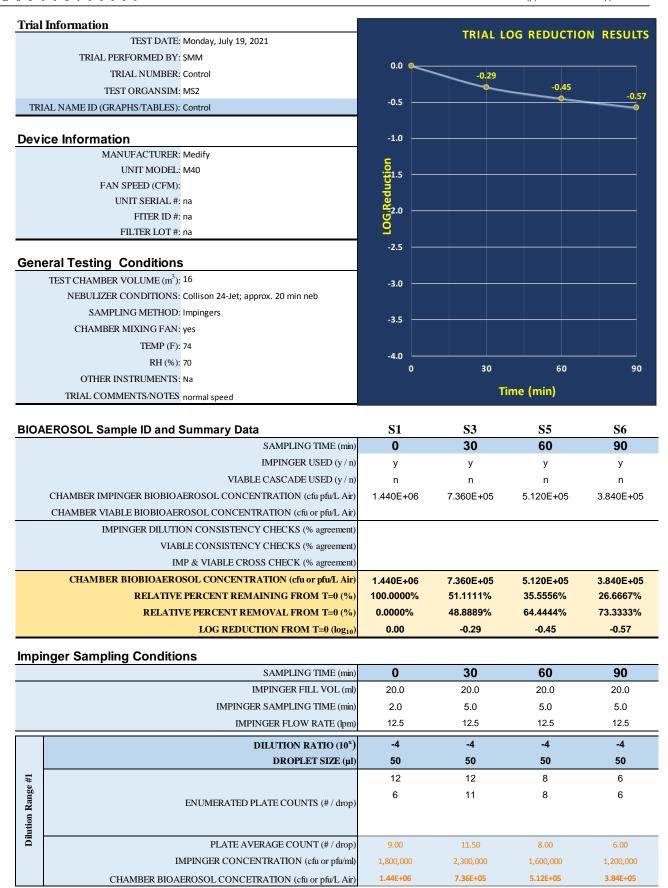


Figure 1C: MS2 Control



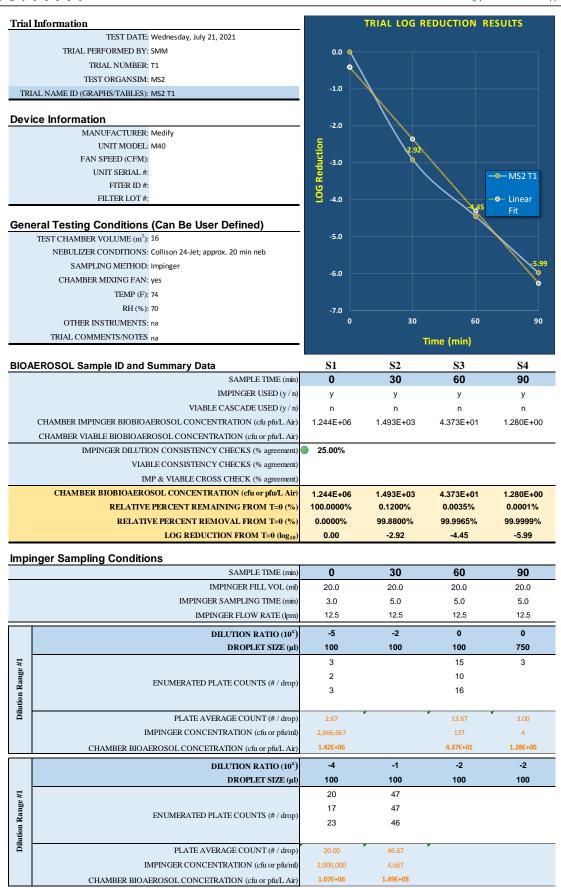


Figure 2C: MS2 Speed 1 Trial 1



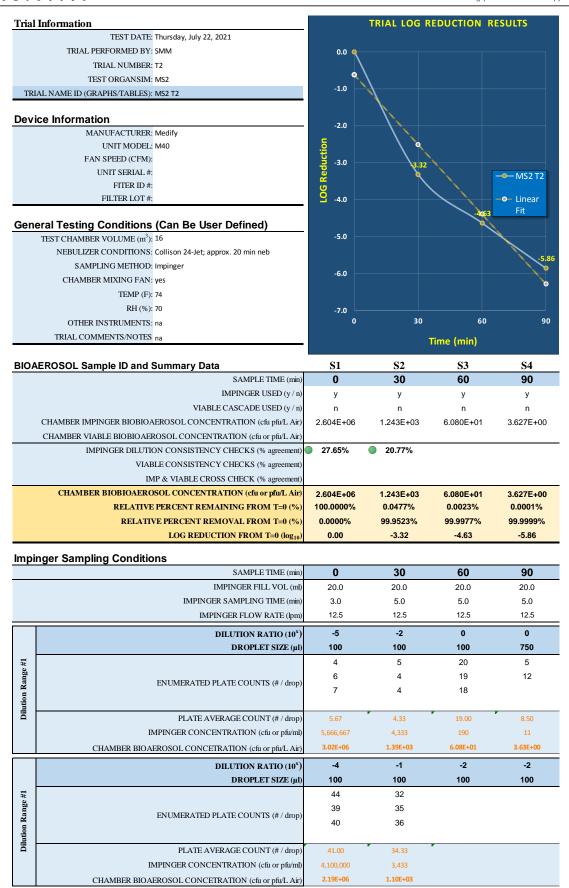


Figure 3C: MS2 Speed 1 Trial 2



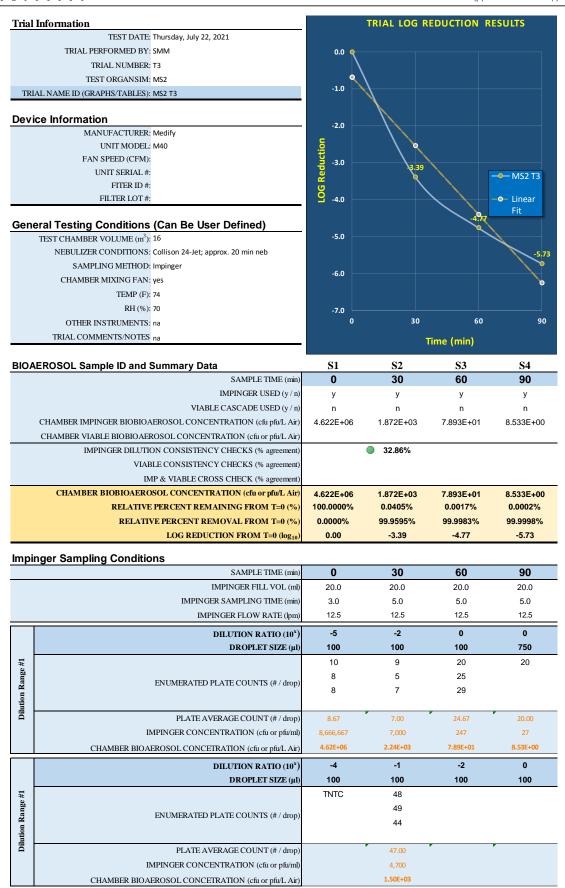


Figure 4C: MS2 Speed 1 Trial 3



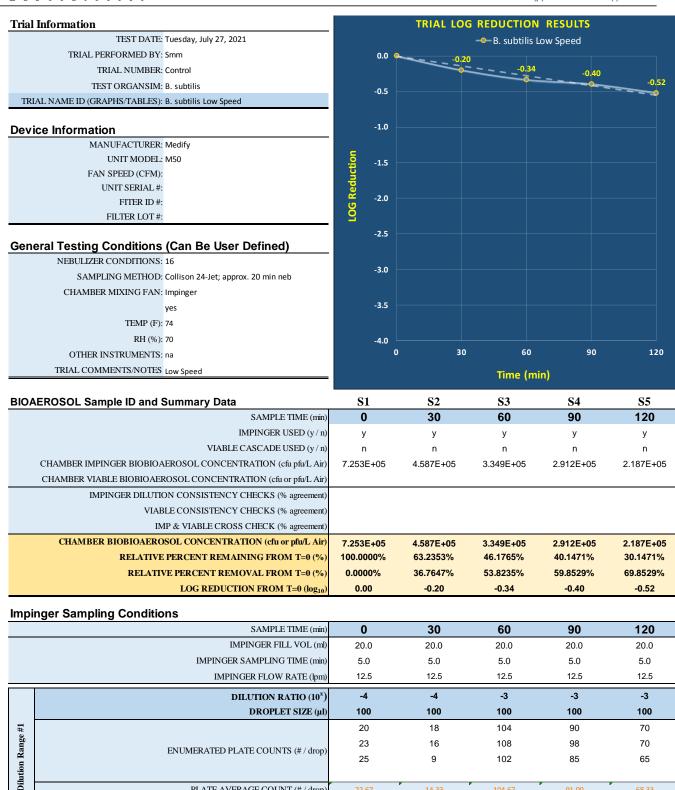


Figure 5C: B. subtilis Control

2.266.667

1,433,333

4.59E+05

104.67

1,046,667

3.35E+05

91.00

910,000

2.91E+05

PLATE AVERAGE COUNT (# / drop)

IMPINGER CONCENTRATION (cfu or pfu/ml)

CHAMBER BIOAEROSOL CONCETRATION (cfu or pfu/L Air)

68.33

683.333

2.19E+05



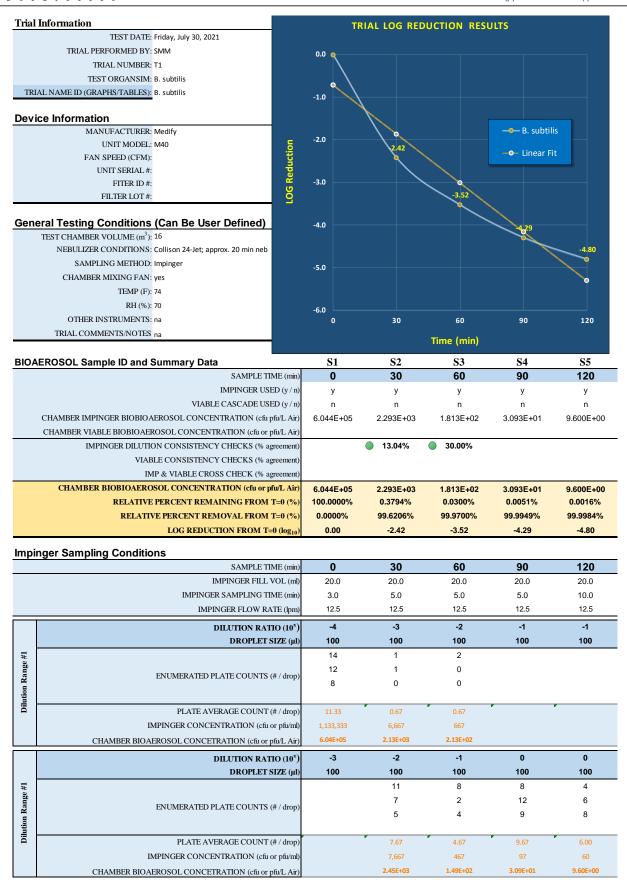


Figure 6C: B. subtilis Speed 1 Trial 1



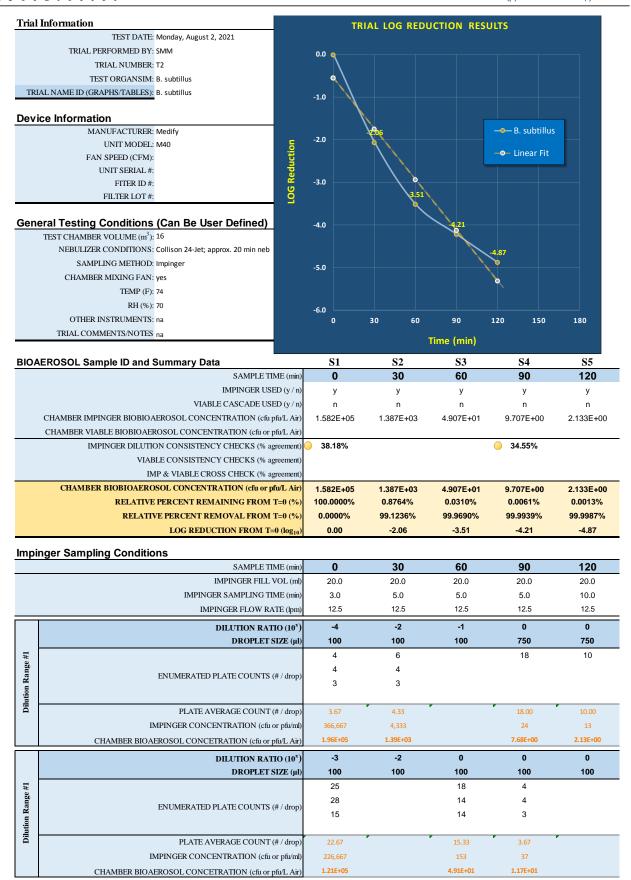


Figure 7C: B. subtilis Speed 1 Trial 2



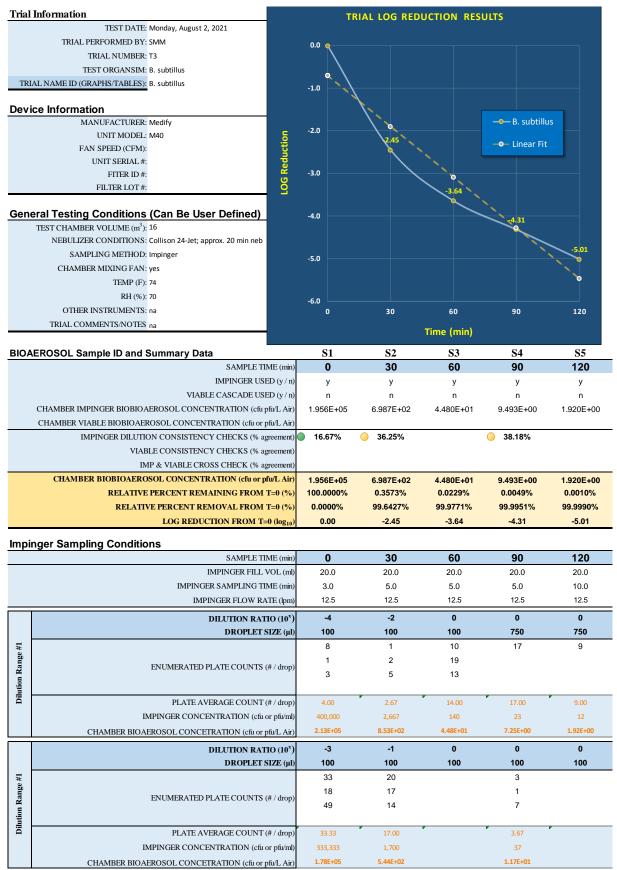


Figure 8C: B. subtilis Speed 1 Trial 3



Appendix D: 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:

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

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_D) is calculated as:

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 $(Q_{imp}) = 12.5 \text{ L/min}$.
- 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 =
$$\frac{C_a}{V_p} \cdot 100$$

The table below is based on the principle that, as the number of viable particles being impinged on a given plate increases, the probability of the next particle going into an "empty hole" decreases. This can be corrected statistically by using the conversion formula of Feller [4]:

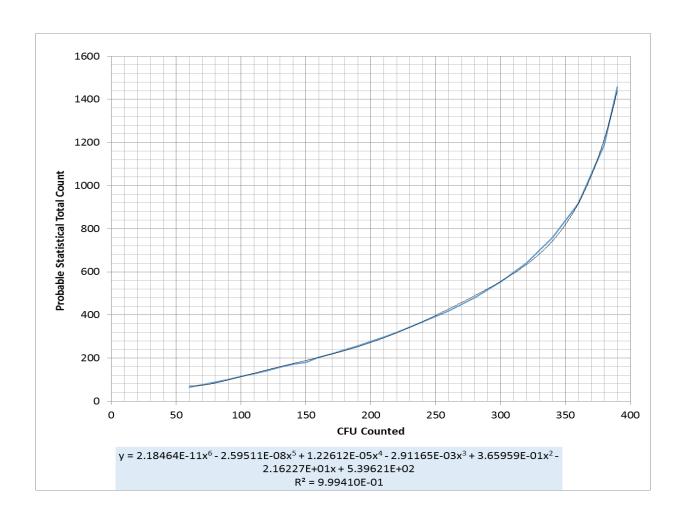
$$Pr = N [1/N + 1/N-1 + 1/N-2 + 1/N-r+1]$$

N is the number of holes (400) in the sampling head.

For easy use of this formula please refer to the table in chapter 17.2

For each colony count **r** a statistically corrected total count **Pr** can be easily seen in the table.







17.2 Positive hole conversion table for all MAS-100 air monitoring systems r = number of colony forming units counted on 100 mm petri dish Pr = probable statis

Pr = probable statistical total count

2 2 52 56 102 118 152 191 202 281 252 397 302 8 3 3 53 57 103 119 153 193 203 283 253 400 303 8 4 4 54 58 104 120 154 194 204 285 254 402 304 8 5 5 55 59 105 122 155 196 205 287 255 405 305 8 6 6 56 60 106 123 156 197 206 289 256 408 306 8 7 7 57 61 107 124 157 199 207 291 257 411 307 8	357 351 361 352 365 353 369 354 373 355 378 356 382 357 366 358 391 359	836 844 853 861 870 879
2 2 52 56 102 118 152 191 202 281 252 397 302 1 3 3 53 57 103 119 153 193 203 283 253 400 303 1 4 4 54 58 104 120 154 194 204 285 254 402 304 1 5 5 55 59 105 122 155 196 205 287 255 405 305 1 6 6 56 60 106 123 156 197 206 289 256 408 306 1 7 7 57 61 107 124 157 199 207 291 257 411 307 1	561 352 565 353 569 354 573 355 578 356 582 357 586 358 591 359	844 853 861 870 879
3 3 53 57 103 119 153 193 203 283 253 400 303 1 4 4 54 58 104 120 154 194 204 285 254 402 304 1 5 5 55 59 105 122 155 196 205 287 255 405 305 1 6 6 56 60 106 123 156 197 206 289 256 408 306 1 7 7 57 61 107 124 157 199 207 291 257 411 307 1	565 353 569 354 573 355 578 356 582 357 586 358 591 359	853 861 870 879
4 4 54 58 104 120 154 194 204 285 254 402 304 9 5 5 55 59 105 122 155 196 205 287 255 405 305 9 6 6 56 60 106 123 156 197 206 289 256 408 306 9 7 7 57 61 107 124 157 199 207 291 257 411 307 9	669 354 673 355 678 356 682 357 686 358 691 359	861 870 879
5 5 55 59 105 122 155 196 205 287 255 405 305 9 6 6 56 60 106 123 156 197 206 289 256 408 306 9 7 7 57 61 107 124 157 199 207 291 257 411 307 9	355 378 356 382 357 386 358 391 359	870 879
6 6 56 60 106 123 156 197 206 289 256 408 306 57 7 57 61 107 124 157 199 207 291 257 411 307	356 357 357 366 358 359 359	879
7 7 57 61 107 124 157 199 207 291 257 411 307	357 366 358 351 359	: 1
	358 359 359	
0 70 70 400 400 700 700 700 700 700	359	888
		897
		907
	360	917
	361	927
	362	937
	363	947
	364	958
	365	969
	366	981
	367	992
	368	1005
	369	1017
20 20 70 77 120 142 170 221 220 319 270 449 320 (370	1030
	371	1043
	372	1057
	373	1071
	374	1086
	375	1102
	376	1118
	377	1134
	378	1152
	379	1170
	380	1189
	7 01 381	1209
	706 382	1230
33 34 83 93 133 161 183 244 233 349 283 491 333 T	7 12 383	1252
	118 384	1276
35 37 85 95 135 164 185 248 235 353 285 497 335 1	24 385	1301
36 38 86 97 136 166 186 250 236 356 286 501 336 T	' 30 386	1327
37 39 87 98 137 167 187 252 237 358 287 504 337 T	387	1356
38 40 88 99 138 169 188 254 238 361 288 508 338 1	7 43 388	1387
39 41 89 101 139 171 189 255 239 363 289 511 339 7	7 49 389	1420
40 42 90 102 140 172 190 257 240 366 290 515 340	756 390	1456
41 43 91 103 141 174 191 259 241 368 291 519 341	7 63 391	1496
42 44 92 104 142 175 192 261 242 371 292 522 342	7 69 392	1541
43 45 93 106 143 177 193 263 243 373 293 526 343	76 393	1591
44 47 94 107 144 178 194 265 244 376 294 530 344 7	7 <mark>83</mark> 394	1648
	'91 395	1715
	7 <mark>98</mark> 396	1795
	397	1895
48 51 98 112 148 185 198 273 248 386 298 545 348 1	398	2028
49 52 99 114 149 186 199 275 249 389 299 549 349 4	399	2228
	328	