Efficacy of Novaerus NV 1050 System against A. Niger Endospores

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Background: This in vitro study characterized the decontamination efficacy of the Novaerus NV1050 system against aerosolized *Aspergillus Niger* spores. Novaerus's NV1050 system is designed to neutralize airborne bacteria, viruses, and fungal spores in order to sanitize rooms and associated equipment. This study evaluated the efficacy against aerosolized *A. Niger* spores. The study included two (2) trials against A. Niger spores as well as a control run.

Methods: A. Niger spores were aerosolized into the sealed bioaerosol chamber using a dry powder disseminator. AGI impingers were used to capture chamber bioaerosol concentrations. All impinger samples were serially diluted, plated and enumerated in triplicate to yield viable bioaerosol concentration at each sampling point and time. Chamber control trial data was subtracted from Novaerus trial data to yield net log reduction in the chamber for each bioaerosol challenge.

Results: The NV 1050 system was shown to be relatively high, at 30 minutes the NV 1050 system showed a net LOG reduction of 3.85 LOG for Trial 1 and 4.35 LOG for Trial 2. The average 30 minute net LOG reduction was 4.10 LOG. The net LOG reduction at 60 minutes showed a 4.28 LOG reduction for both trials due to reaching detection limit, actual LOG reduction is theoretically much higher at 60 minutes in a small room environment.

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

Overview

This study was conducted to evaluate the ability of the Novaerus NV1050 device by Novaerus Inc. to neutralize airborne bioaerosols. Testing was conducted in a controlled stainless steel aerosol chamber. The NV1050 device effectiveness was tested against one Bio-safety level 1 (BSL1) organism and was compared to a control run in order to evaluate the system's effective net LOG reduction of viable bioaerosols when compared to the control runs.

The test plan incorporated challenging the NV1050 device in a closed environmental chamber to determine the destruction rate of A. Niger due to the NV1050 system.

Testing was conducted to characterize the single NV1050 unit against *Aspergillus Niger* to demonstrate the capability of the NV1050 device to reduce viable bioaerosol concentrations. **Table 1** shows a complete testing matrix for all testing conducted.

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 562 cubic feet, or 15,914 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 a HEPA filtered exhaust system that are operated with wireless remote control.

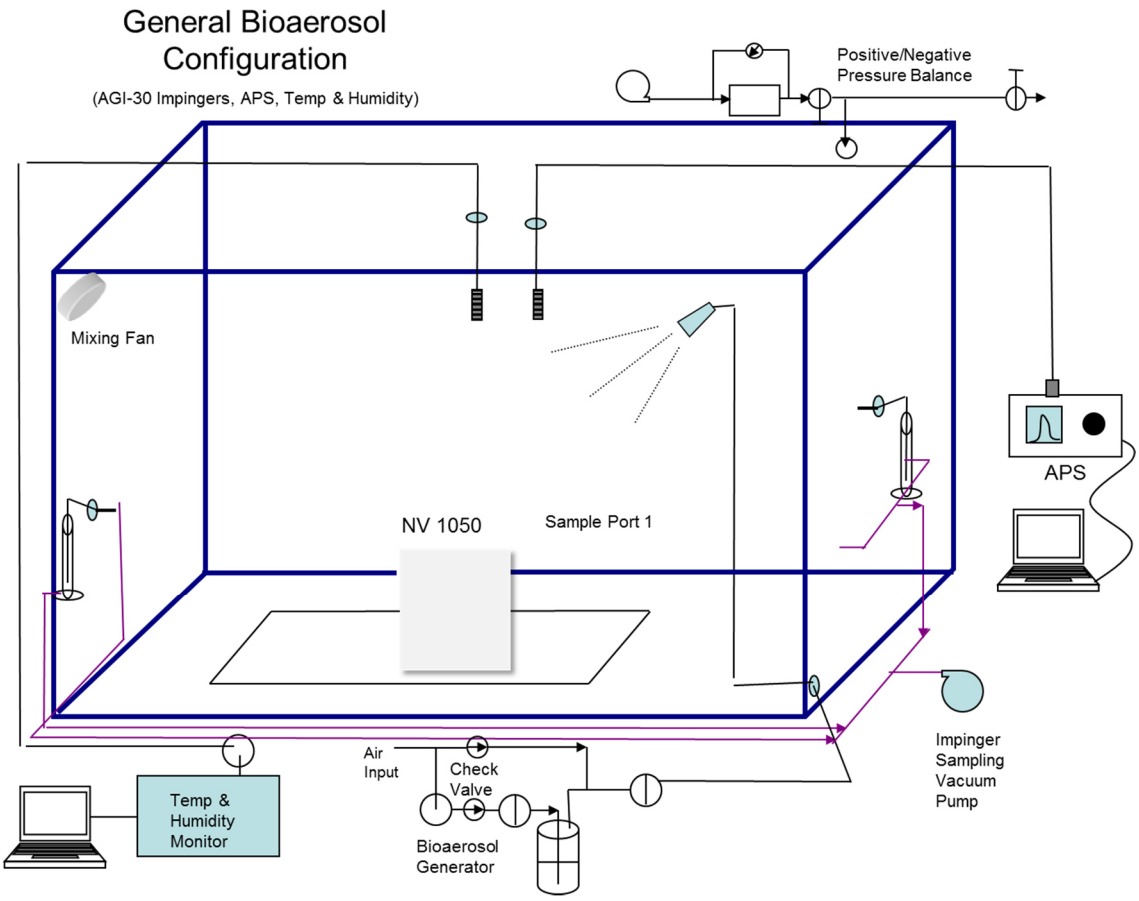

Figure 1: *Bio-Aerosol Test Chamber Flow Diagram.*

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 dry powder inductor for the Aspergillus Niger spores.

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

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

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

A magnehelic gauge with a range of 0.0 +/- 0.5 inch H_2O (Dwyer instruments, Michigan City IN) was used to monitor and balance the system pressure during aerosol generation, aerosol purge and testing cycles. **Figure 1** shows a Flow Diagram of the bioaerosol chamber.

Bioaerosol Sampling and Monitoring System

Two AGI impingers (Ace Glass Inc. Vineland NJ) were used for bio-aerosol collection of all biological aerosols to determine chamber concentration. The AGI-30 impinger vacuum source was maintained at a negative pressure of 18 inches of Hg

Novaerus NV 1050 Test Matrix

Trial	Run	Species (gram, description)	ATCC Ref	Target Monodispersed Particle Size	Challenge Conc. (#/L)	Total Trial Time (min)	Impinger Sample Time (min)	Sampling	Plating and Enumeration
1 2 3	Control Challenge Challenge	*Aspergillus niger* *(mold, spore forming)*	13835	<5.0um	2.5×10^5	60	0,30,60	APS, Impingers	all samples in triplicate

***Table 1**: NV 1050 Test Matrix*

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.

Aerosol particle size distributions and count concentrations were measured in real-time through the duration of all control and NV1050 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 (240 minutes) with 1 minute sampling intervals. A general flow diagram of the aerosol test system is shown in **Figure 1.**

Species Selection

Species selection is based on Biological Safety Level 1 (BSL1) surrogates for a wide range of BSL3 pathogenic organisms. It is routine in the bioaerosol field to use surrogate species to test performance against BSL3 organism decontamination. *A. Niger* endospores are used as a surrogate for several toxic black mold species

such as *Stachybotrys chartarumand*.

Endospore 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 approximately 1×10^9 cfu/ml. The spore suspension was serial diluted, plated on TSA plates and incubated at 30°C for 48 hours.

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 potato dextrose agar plates. The plated cultures were incubated for 24-48 hours and enumerated and recorded.

Control Testing

To accurately assess the NV1050 unit, test chamber a pilot control trial was performed with the *Aspergillus Niger* over a 120 minute period without the NV1050 in operation to characterize the 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 NV1050 challenge testing and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire pilot control test period.

During control runs, a single low velocity fan located in the corner of the bioaerosol test chamber was turned on for the duration of trial to ensure a homogenous aerosol concentration within the aerosol chamber. The mixing fan was used for all control runs and was turned off during NV 1050 decontamination trials. The fungal endospore test samples were pooled and mixed prior to plating and enumeration

General Timeline for Bioaerosol Chamber Testing

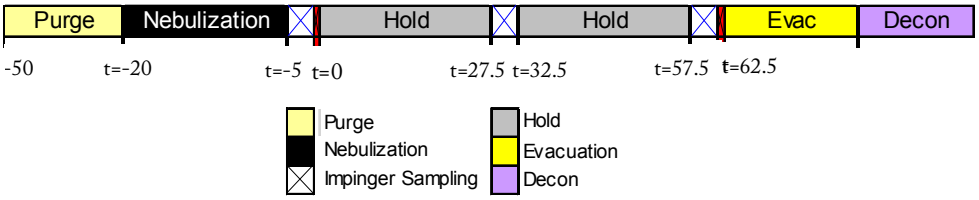

Table 1: General *Trial Timeline for NV1050 Decontamination Trials.*

NV 1050 Testing

For control and NV1050 trials, the impingers were filled with 20 mL of sterilized PBS (addition of 0.005% v/v Tween 80) for bioaerosol collection. The addition of Tween 80 was shown to increase the impinger collection efficiency and de-agglomeration of all microorganisms.

The chamber mixing fan was turned on during bioaerosol dissemination to assure a homogeneous bioaerosol concentration in the test chamber prior to the first impinger sample.

Following bioaerosol generation, baseline bioaerosol concentrations were established for each pilot control and NV1050 test by sampling simultaneously with two AGI-30 impingers located at opposite corners of the chamber. AGI samples were collected for 5 minutes at intervals of 30 & 60 minutes throughout the entire period. **Table 2** above shows the general timeline for each NV1050 live bioaerosol challenge trial.

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 NV1050 biological testing, the unit was turned on immediately following a time 0 baseline sample and operated for the entirety of the test (up to 4 hours). Subsequent impinger samples were taken at intervals of 5 to 10 minutes and samples enumerated for viable concentration to measure the effective viable bioaerosol reduction during operation of the NV1050 system over time.

Test chamber temperature and humidity were recorded at the initiation and completion of each test. All samples were plated in triplicate on potato dextrose agar media over a minimum of a 3 log dilution range.

Plates were incubated for viable colony forming units (cfu) for the fungal spores. Plates were incubated and enumerated for viable counts to calculate aerosol challenge concentrations in the chamber and reduction of viable microorganisms.

Post-Testing Decontamination and Prep

Following each test, the chamber was air flow evacuated/purged for a minimum of twenty minutes between tests and analyzed with the APS for particle concentration decrease to baseline levels between each test. The impingers were cleaned at the conclusion of each day of testing by soaking in a 5% bleach bath for 20 minutes. The dry powder disseminator and impingers were then submerged in a DI water bath, removed, and spray rinsed 6x with filtered DI water until use.

Bioaerosol Particle Size Data

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

Data was logged in real time to an Acer laptop computer, regressed, averaged and plotted. The aerosol particle size distribution for A. Niger is shown **Figure 2.**

The particle size distribution for Aspergillus Niger is 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.

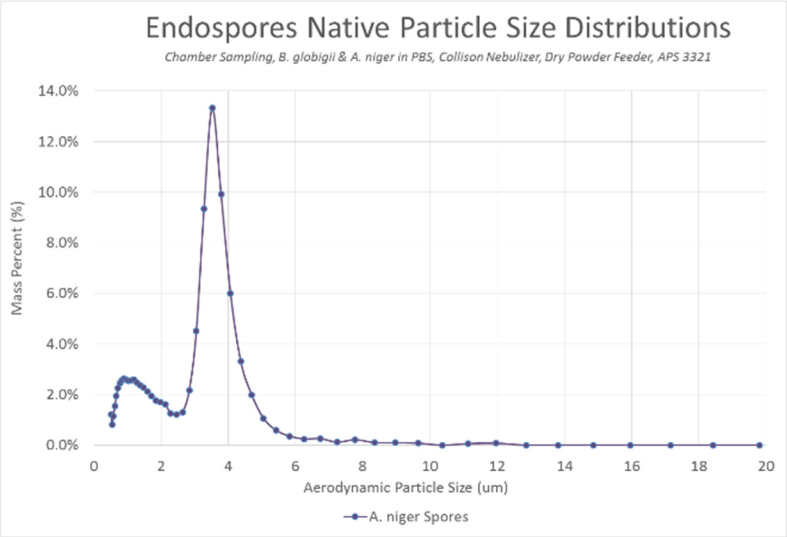

Figure 2: *Endospores (A. niger) Particle Size Distribution in Test Chamber.*

Data Analysis

Results from the control trial were graphed and plotted to show natural viability loss over time in the chamber. These control run served as the basis to determine the time required for the NV1050 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.

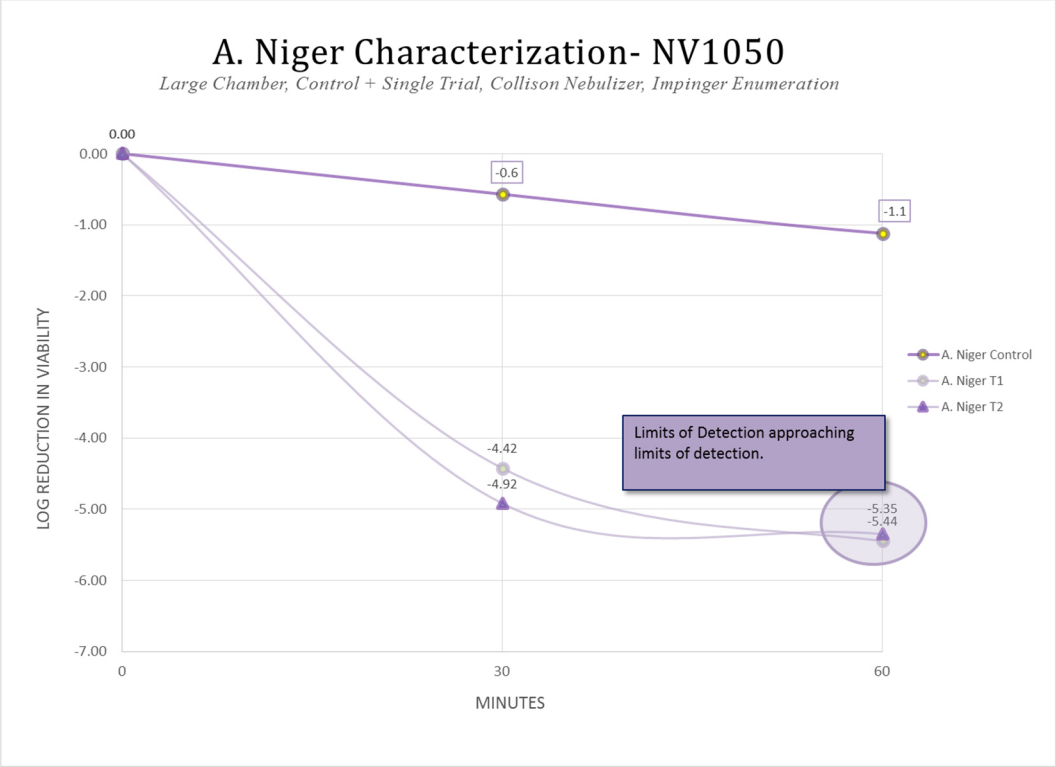

Figure 3: *A. Niger Endospore NV 1050 Efficacy*

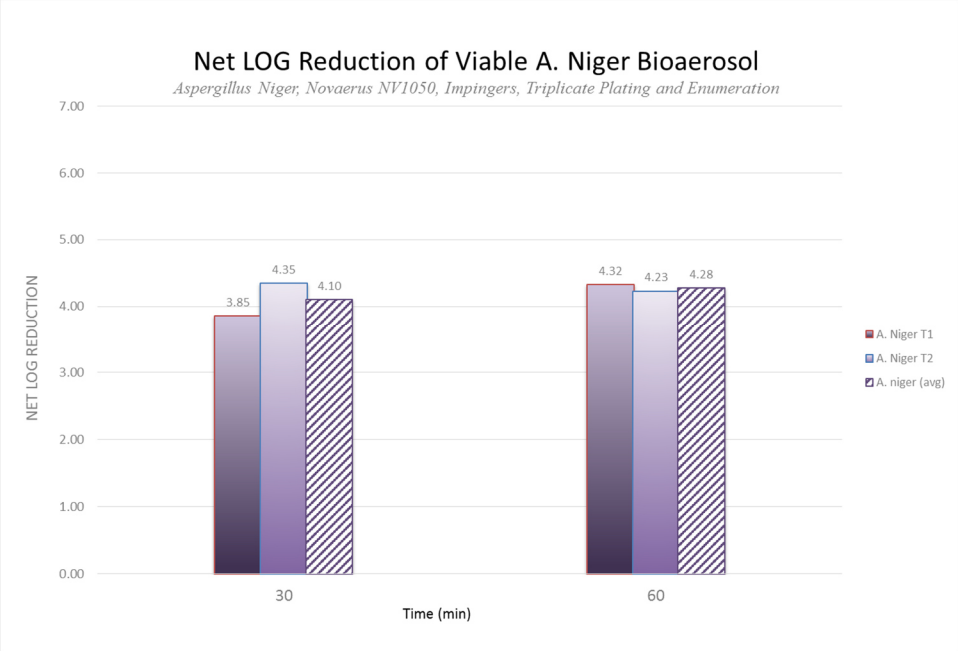

***Figure 4**: NV 1050 net LOG Reduction for Trials 1 and 2 with averages.*

Summary of Results

When tested against A. Niger endospores the NV 1050 system was shown to be extremely effective at removing viable mold spores from the air. At 30 minutes the NV 1050 system showed a net LOG reduction of 3.85 LOG for Trial 1 and 4.35 LOG for Trial 2. The average 30 minute net LOG reduction was 4.10 LOG. It should be noted that trials were conducted for a total of 240 minutes however at 60 minutes limits of detection were reached. Plate counts were approximately 1-2 cfu/ml in the impinger samples. This corresponds to approximately 0.15-0.3 cfu/liter of air in the chamber. Due to low plate counts actual LOG reduction is theoretically much higher at 60 minutes. Future trials could be performed using viable cascade impactors for the sampling method to increase limits of detection by an additional 1.5-2.0 LOGs.

Overall the NV 1050 system is extremely effective at reduction of viable airborne mold spores from room environments. The results show 99.9921% in 30 minutes.

These results are represented in **Table 2** below and in **Figures 3 and 4.**

Average NET LOG and Percent Reduction of Bioaerosols

Bioaerosol Type	Species (gram, description)	Surrogate	Number of Trials	Net Reduction	
				30 Minutes	60 Minutes
Spores	*Aspergillus niger*	*Black Mold*	*2*	4.10	4.28
	(mold, spore forming)			99.9921%	99.9947%

Table 2: Average Net LOG and Percentage Reduction of Bioaerosols.

References

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

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

Analytical Testing Facility

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Project

10824.10

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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.

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Appendix A: Calculations

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

- Plating and enumeration of the biological to derive the concentration of the stock suspension (C_s) in pfu/mL or cfu/mL, or cfu/g for dry powder.
- Collison 24 jet nebulizer use rate (R_{neb}) (volume of liquid generated by the nebulizer/time) at 28 psi air supply pressure = 1.0 ml/min.
- Collison 24 jet Generation time (t) = 20 or 30 minutes, test dependent.
- Chamber volume (V_c) = 15,993 Liters

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

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

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_p) is calculated as:

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

AGI – 30 impinger or 47mm filter collection calculation:

- Viable aerosol concentration collection (C_a) = cfu or pfu/L of chamber air.
- Viable Impinger concentration collection (C_{Imp}) = cfu or pfu/mL from enumeration of impinger sample or filter sample.
- Impinger sample collection volume (I_{vol}) = 20 mL collection fluid/impinger, or extraction fluid for filter.
- AGI–30 impinger or filter sample flow rate (Q_{imp}) = 12.5 L/min.
- AGI–30 impinger or filter sample time (t) = 5 or 10 minutes, test dependent.

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

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

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

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