ARE Labs 16m^3 Chamber Homogeneity and Validation Report

Applicant:

Internal Validation Report

Testing Lab:

ARE Labs Inc.
12880 Metcalf Avenue
Overland Park, Kansas 66213

Objective

The primary objective of this validation is to formally verify and document that aerosolized microorganisms are distributed homogeneously throughout the ARE Labs 16 m³ bioaerosol test chamber. This study aims to demonstrate, with a high degree of confidence, that samples collected from any of the four designated sampling ports are statistically equivalent and representative of the entire chamber environment. Successful validation is critical to ensuring the consistency, accuracy, and reliability of all client and internal bioaerosol testing projects.

Scope

This validation report applies specifically to the 16 m³ bioaerosol test chamber located at ARE Labs' Overland Park facility. The procedures, data, and conclusions herein certify the chamber's performance with respect to the spatial uniformity of airborne viral particles under standard operating conditions, including active internal air mixing.

Background

In bioaerosol efficacy testing, the fundamental assumption is that the challenge aerosol is uniformly mixed within the test environment. Inhomogeneous concentrations, or "hot spots," can lead to significant errors, where the measured efficacy of a device or process is skewed by sampling from an area of artificially high or low concentration. This can invalidate test results and lead to incorrect conclusions. Therefore, as a matter of good laboratory practice, it is essential that such test environments be properly characterized. This validation serves as the formal internal qualification of the 16 m³ chamber, providing objective evidence that it functions as a well-mixed system suitable for testing.

Acceptance Criteria

To successfully pass this validation, the average aerosol concentration measured at each of the four sampling ports must be within ±20% of the overall mean concentration calculated from all four ports across the full set of experimental runs.

This criterion is based on established principles in aerosol science and ARE Labs' internal quality standards. The ±20% range is considered stringent enough to ensure no significant spatial concentration gradients exist. It is also practical, as it accounts for the inherent variability of the complete measurement process, which includes both aerosol sampling and the subsequent microbiological analysis. For context, the United States Pharmacopeia (USP) General Chapter <1223> acknowledges that conventional plate counts can exhibit a Relative Standard Deviation (%RSD) of 15% to 35%. Therefore, a homogeneity tolerance of ±20% is a robust and appropriate standard, confirming that the chamber itself contributes minimally to overall experimental variance. Meeting this criterion provides high confidence that the chamber is a truly homogeneous system.

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, a digital internal temperature and humidity monitor, external humidifiers (for humidity control), a lighting system, multiple sampling ports, a central 26-inch ceiling fan for aerosol mixing, and a 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, and a ¼ inch diameter probe for continuous aerosol particle size monitoring. 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 four 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.

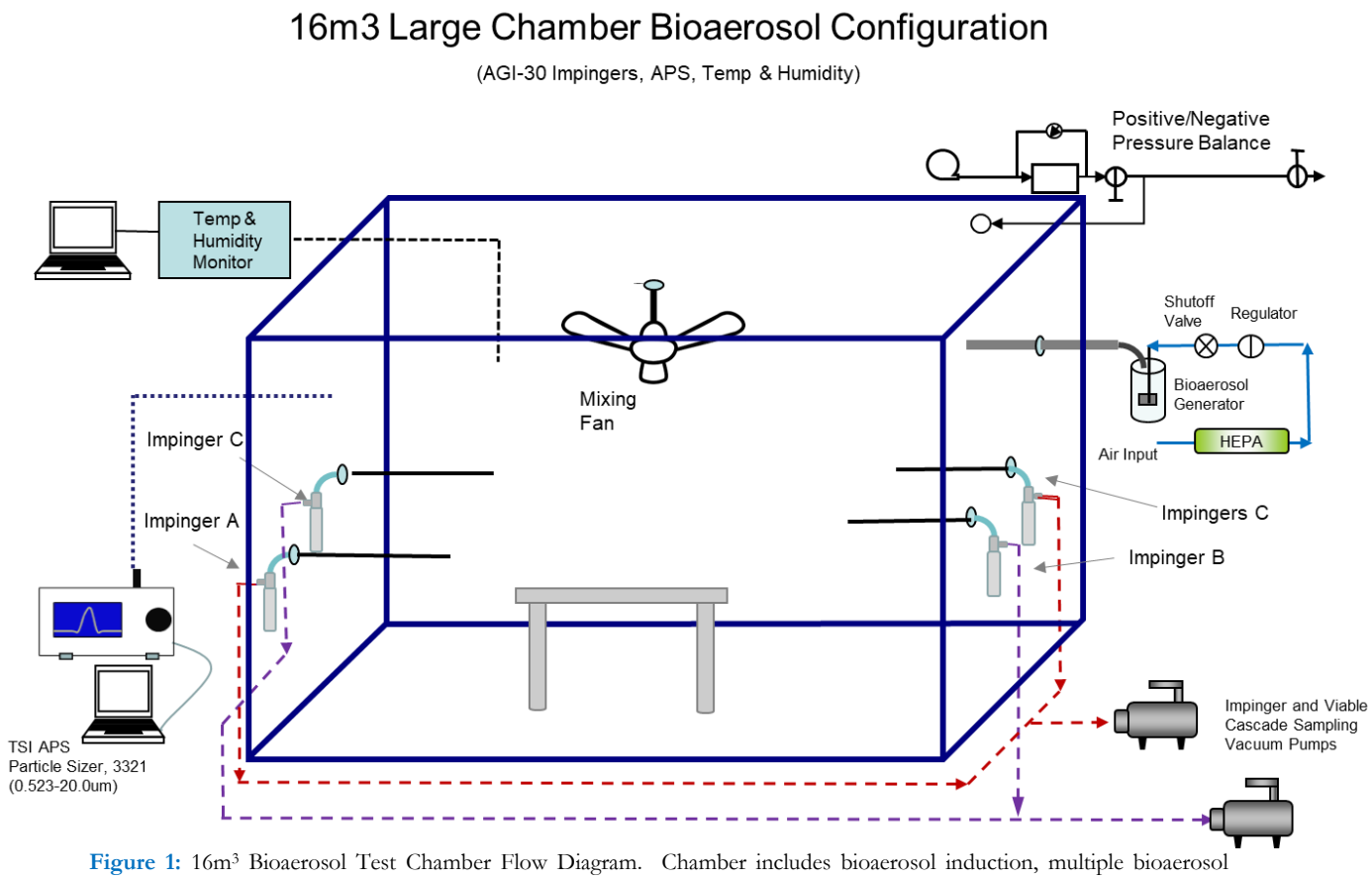

Figure 1: 16m^3 Bioaerosol Test Chamber Flow Diagram. Chamber includes bioaerosol induction, multiple bioaerosol sampling ports, particle size monitoring, internal central mixing fan, and temperature and humidity controls. Main system HEPA evacuation system not pictured.

Figure 2: The 30 m^3 bioaerosol testing chamber at ARE Labs adheres to AHAM AC-5 standards and ASHRAE 241 criteria. The chamber is equipped with HEPA filtered air in/out, multiple bio aerosol sampling ports, decontamination, and pressure balance.

A general flow diagram of the aerosol test system is shown in **Figure 1** above. A Magnehelic gauge (Dwyer instruments, Michigan City IN), with a range of -0.5 to 0.5 inches of H_2O, is used to monitor and balance the system pressure during aerosol generation, aerosol purge, and testing cycles. **Figure 2** shows the 16m^3 bioaerosol chamber used for all testing in this study.

Test Equipment

The test equipment included a 24-jet Collison nebulizer (BGI Inc.) connected to the 1-inch dissemination port to generate the challenge aerosol. Bioaerosol samples were collected using six calibrated All-Glass Impingers, model AGI-30 (Ace Glass Inc.), connected to the 3/8-inch sampling probes. Each impinger was operated at a critical flow of 12.5 LPM, maintained by a vacuum source set to -18 inches of Hg. To confirm the particle size distribution prior to the validation runs, a TSI Model 3321 Aerodynamic Particle Sizer (APS) was connected to the 1/4-inch monitoring port.

Challenge Aerosol Preparation

The challenge aerosol was prepared using MS2 bacteriophage, a non-enveloped ssRNA virus selected for its environmental resilience and common use as a surrogate in viral studies. A high-titer stock of MS2 (>1x10^9 PFU/mL) was suspended in a solution of phosphate-buffered saline (PBS) and tryptic soy broth (TSB) with 100 uL of antifoam A concentrate to ensure stable aerosolization. The pressure of the Collison nebulizer was adjusted to generate a target Mass Median Aerodynamic Diameter (MMAD) of approximately 0.7 um, a size that falls within the most penetrating particle size range for filters**.**

Bioaerosol Sampling System

Six total AGI-30 impingers (Ace Glass Inc. Vineland NJ) were used for bioaerosol collection to determine chamber concentrations and compare port uniformity. Four impingers were sampled simultaneously for each trial. The mixing fan inside the chamber was left on low speed for the entirety of the testing period to ensure a homogenous air mixture inside the chamber. A picture of the AGI-30 is shown in **Figure 3** below.

Figure 3: AGI-30 Impinger, Ace Glass Inc. Vineland NJ.

Experimental Design & Sampling Procedure

A rotational matrix was designed to eliminate potential bias from any individual impinger or port location. The procedure began with chamber preparation, where the chamber was purged with HEPA-filtered air until the particle count returned to baseline (<1 particle/L). The internal mixing fan was activated and run continuously throughout the experiment. Following preparation, the MS2 suspension was nebulized into the chamber until a stable target concentration was achieved. For sample collection, a rotational sampling scheme was employed, and for each run, all four active impingers were operated simultaneously for a 2-minute sampling duration.

The six impingers were rotated through the four ports over a series of test runs to ensure that each impinger collected samples from each port. For example:

- **Run 1:** Impingers 1, 2, 3, 4 were placed in Ports A, B, C, D.
- **Run 2:** Impingers 5, 6, 1, 2 were placed in Ports A, B, C, D.
- **Run 3:** Impingers 3, 4, 5, 6 were placed in Ports A, B, C, D.
- This scheme continued until the full matrix was complete.

Microbiological Analysis and Data Calculation

A redundant analysis protocol was implemented where the collection fluid (PBS) from each impinger was divided and provided to two staff scientists who performed analyses independently. Each scientist performed standard 10-fold serial dilutions and subsequent plaque assays in triplicate using E. coli as the host bacterium, incubating plates until plaques were quantifiable. The plaque counts from the triplicate plates were averaged for each scientist and used to calculate the airborne concentration in plaque-forming units per cubic meter (PFU/m3) for each port, accounting for sample volume and air flow rate. The results from both scientists were then averaged for a final port concentration. For the final homogeneity analysis, a mean concentration was calculated from the four active ports in each test run. The performance of each port was then expressed as a percentage of this run average. Finally, all percentage values for each port across all runs were averaged to yield the final results presented in this report.

Chamber Validation Results

The validation study successfully demonstrated a high degree of aerosol homogeneity across all four sampling ports. The final averaged results, compiling all runs and analyses, show that the concentration at each port was well within the established acceptance criteria. The individual port averages were 109.6% (Port A), 90.1% (Port B), 104.2% (Port C), and 96.2% (Port D) relative to the overall mean concentration. This narrow distribution confirms that the chamber's internal mixing fan effectively eliminates spatial concentration gradients. The final averaged results for each port are summarized graphically in **Figure 4** with **Figure 5** showing each trials variation from the average.

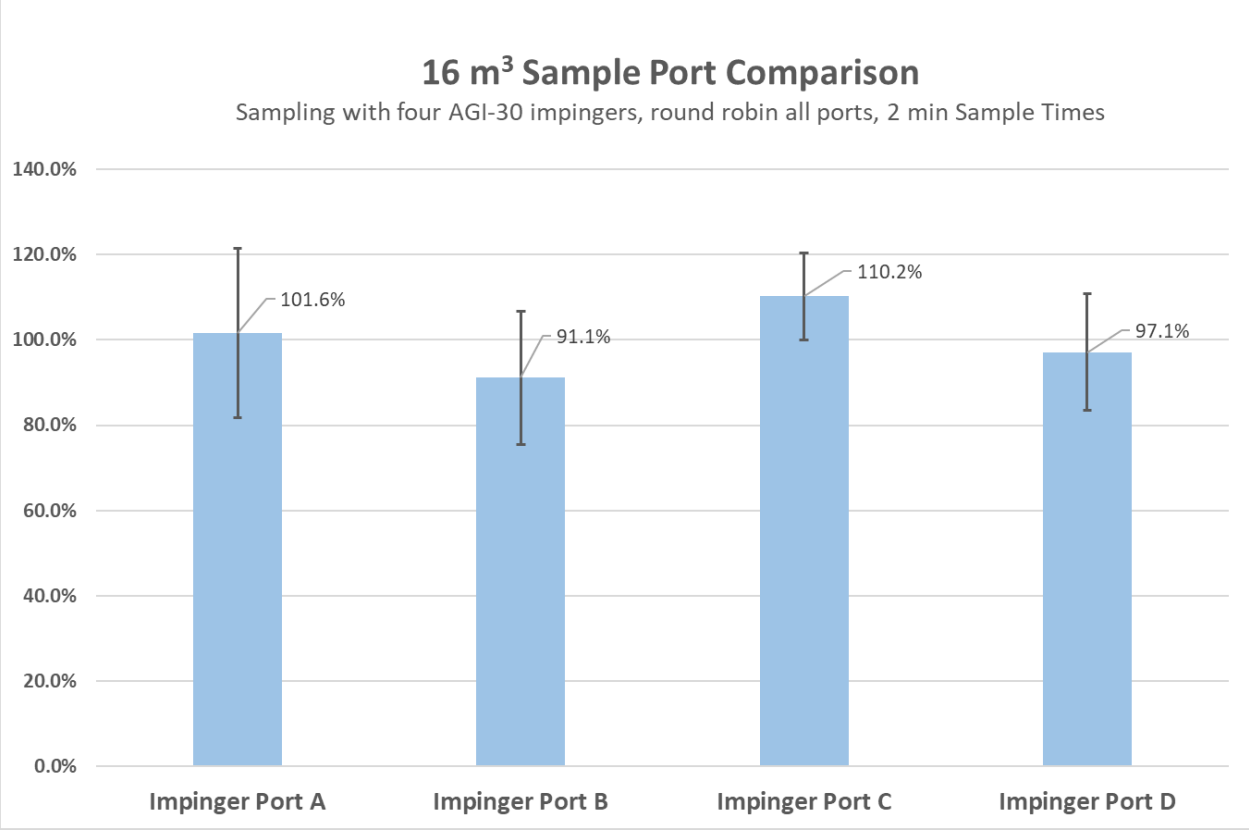

Figure 4. The graph displays the final averaged concentration for each port (A, B, C, and D) expressed as a percentage of the overall mean concentration from all tests. All ports are shown to be well within the +/-20% acceptance criteria..

Impinger Variation from Average

Sample	Impinger Port A	Impinger Port B	Impinger Port C	Impinger Port D
1	102.7%	97.8%	122.7%	76.8%
2	142.4%	71.2%	108.0%	78.3%
3	83.4%	94.7%	117.5%	104.4%
4	97.7%	90.4%	101.4%	110.5%
5	111.9%	68.3%	108.7%	111.1%
6	84.8%	84.2%	121.8%	109.3%
7	83.7%	114.3%	115.5%	86.5%
8	89.5%	110.9%	104.2%	95.4%
9	118.4%	88.1%	91.8%	101.7%
Average	**101.6%**	**91.1%**	**110.2%**	**97.1%**
St. Dev.	***19.9%***	***15.7%***	***10.2%***	***13.6%***

Figure 5. Individual Trial Data showing impinger variation from the average for each trial set..

Discussion

The results provide strong evidence that the 16 m³ chamber functions as a well-mixed environment. The largest positive deviation observed was +9.6% (Port A), and the largest negative deviation was -9.9% (Port B). Both of these extreme values, along with the more central results from Port C (+4.2%) and Port D (-3.8%), are comfortably inside the +/-15% acceptance window defined in the protocol.

The rigorous experimental design, featuring a systematic rotation of six impingers through four ports, effectively isolates the variable of interest—chamber location—from potential equipment bias. Furthermore, the use of two independent scientists for microbiological analysis adds a layer of redundancy that increases confidence in the final calculated concentrations. The data confirms that a sample taken from any single port can be considered representative of the entire chamber atmosphere, which is a prerequisite for conducting valid aerosol efficacy studies.

Conclusion

The ARE Labs 16 m³ bioaerosol test chamber PASSES the homogeneity validation. The chamber is formally qualified and certified for use in client and internal R&D studies that require a consistent, uniform, and representative aerosol challenge.