# MicronView BAMS (BioAerosol Monitoring System)

## Validation Report Summary


**Guidance Documents:**

- **USP <1223>** : Validation of Alternative Microbiological Methods
- **EP 5.1.6** : Alternative Methods for Control of Microbiological Quality
- **PDA TR 33 (2013)** : Evaluation, Validation, and Implementation of Alternative and Rapid Microbiological Methods

MICRONVIEW

# TABLE OF CONTENTS

MICRONVIEW

# MICRONVIEW BAMS VALIDATION REPORT SUMMARY

## 1. INTRODUCTION

This report presents the abridged validation results of the MicronView BAMS (BioAerosol Monitoring System), a device for real-time environmental microbial monitoring. The validation process is critical to demonstrate the reliability of BAMS as an alternative microbiological method, ensuring it meets rigorous industry standards for accuracy and performance.

The BAMS uses advanced laser-induced fluorescence technology to detect viable airborne particles by exciting metabolic compounds, such as NADH and riboflavin, with a 405 nm laser. This produces fluorescence directly related to the number of biological particles in the air, enabling real-time monitoring and trend analysis. In parallel, BAMS employs Mie scattering to measure particle size distribution and total airborne particles, providing comprehensive viable and non-viable environmental data.

To validate the BAMS as a rapid microbial detection method (RMM), the study followed the guidelines of USP <1223> and EP 5.1.6, using the Andersen six-stage sampler (referred to throughout this report as Andersen) as the reference instrument. Key validation parameters assessed include:

- **Accuracy -**
- **Precision -**
- **Linearity -**
- **Specificity -**
- **Limit of Detection -**
- **Limit of Quantification -**
- **Range -**
- **Ruggedness -**
- **Robustness -**
- **Equivalency -**

This report highlights the results and significance of these tests, demonstrating BAMS's capabilities as a reliable and efficient tool for microbiological quality control.

## 2. RESULTS SUMMARY

The BAMS validation results are comprehensively summarized in Table 2-1, which provides an overview of how the BAMS met each of the specified validation criteria. The summary table illustrates that BAMS successfully fulfilled all requirements outlined in USP <1223> and EP 5.1.6 for the validation of alternative microbiological methods, demonstrating its compliance with industry standards and its reliability as a rapid microbial detection method.

Table 2-1: BAMS Validation Results Summary

| Test | Organisms/Materials Tested | Acceptance Criteria | Test Result |
|---|---|---|---|
| Uniformity Test | N/A | The difference between sampling points must be ≤15% | Pass<br>Both low and high concentrations meet the acceptance criteria |
| Accuracy | S. aureus | The recovery of viable particles using the BAMS must be ≥ 70% of the recovery using the Andersen sampler. | Pass |
| | E. coli | | All five organisms meet the acceptance criteria |
| | M. luteus | | |
| | B. subtilis | | |
| | C. albicans | | |
| Precision | S. aureus | The relative standard deviation (RSD) of the BAMS must be less than or equal to the Andersen sampler, with both ≤ 35%. | Pass |
| | E. coli | | All five organisms meet the acceptance criteria |
| | M. luteus | | |
| | B. subtilis | | |
| | C. albicans | | |
| Linearity | S. aureus | The coefficient of determination ($R^2$) must be greater than or equal to 0.65, indicating an acceptable linear relationship. | Pass |
| | E. coli | | All five organisms meet the acceptance criteria |
| | M. luteus | | |
| | B. subtilis | | |
| | C. albicans | | |
| Specificity | S. aureus | The BAMS must successfully detect a broad range of organisms, including Gram-positive and Gram-negative bacteria, bacterial spores, and fungi (yeasts and molds). | Pass |
| | E. coli | | All tested microbes can be detected. |
| | M. luteus | | |
| | B. subtilis | | |
| | C. albicans | | |
| | P. chrysogenum | | |
| Interferent Testing | SiO2-0.56μm | / | The false viable count percentage of all tested interferents does not exceed 10%. |
| | SiO2-1.56μm | | |
| | SiO2-3.15μm | | |
| | Disposable rubber surgical gloves | | |
| | Disposable medical masks | | |
| | Dust-free cloth | | |
| | Dust-free paper | | |
| | Disposable non-woven clean clothes | | |
| | 70% IPA | | |
| | 7.5% H2O2 | | |
| | 75% Ethanol | | |

| | | | |
|---|---|---|---|
| LOD | S. aureus | The BAMS calculated LOD must be statistically equivalent to or better than the Andersen sampler under test conditions. | Pass |
| | E. coli | | LOD for BAMS is statistically equivalent to the Andersen LOD, with a limit of 4 CFU/$m^3$. |
| | M. luteus | | |
| | B. subtilis | | |
| | C. albicans | | |
| | P. chrysogenum | | |
| LOQ | S. aureus | The BAMS calculated LOQ must be statistically equivalent to or better than the Andersen sampler under test conditions. | Pass |
| | E. coli | | LOQ for BAMS is 24-26 CFU/$m^3$ under test conditions. |
| | M. luteus | | |
| | B. subtilis | | |
| | C. albicans | | |
| | P. chrysogenum | | |
| Range | S. aureus | / | The minimum tested concentration is 4 CFU/$m^3$, and the maximum is 24,382 Bio-particles/$m^3$. |
| | E. coli | | |
| | M. luteus | | |
| | B. subtilis | | |
| | C. albicans | | |
| Ruggedness | M. luteus | The BAMS must meet accuracy and precision requirements under varying conditions. | Pass |
| | | | The results meet the accuracy and precision requirements. |
| Robustness | Temperature Challenge | The BAMS must demonstrate stable performance with:<br>- Flow rate deviation within ± 3%.<br>- Scattering counting efficiency meeting ISO 21501-4 standards.<br>- Fluorescence counting efficiency of 45% ± 10% for 0.5µm particles. | Pass |
| | Humidity Challenge | | |
| Equivalency | All test organisms and materials | The BAMS must meet all validation test requirements and align with quantitative method equivalency requirements specified in USP <1223>. | Pass |
| | | | The system meets all equivalency requirements stated in USP <1223>. |

## 3. TEST SYSTEM

The BAMS validation test setup is illustrated in Figures 3-1A and 3-1B, providing a comprehensive overview of the experimental configuration. Prior to commencing the validation testing, an aerosol uniformity assessment was conducted to verify the consistency of bioaerosol concentrations across the two sampling ports. This step is critical to ensure the reliability and comparability of the data collected during the validation process. The results of the uniformity test, depicted in Figure 3-2, demonstrate that the variation in aerosol concentration between the two sampling ports is within acceptable limits, with a difference of less than 15%. This consistency validates the suitability of the test setup for the comparison of the BAMS with the Andersen sampler.


Figure 3-1A: Test System Layout

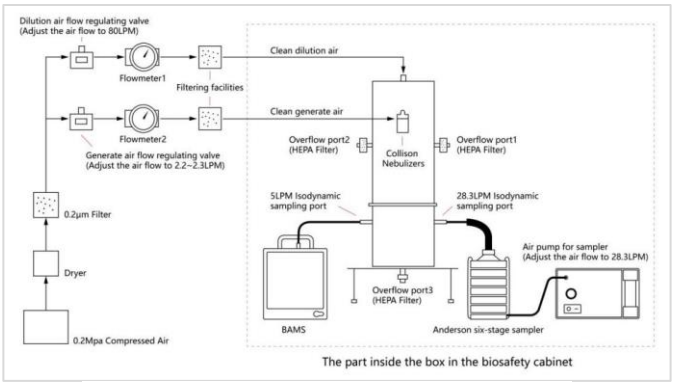


Figure 3-1B: Test System Flow Diagram

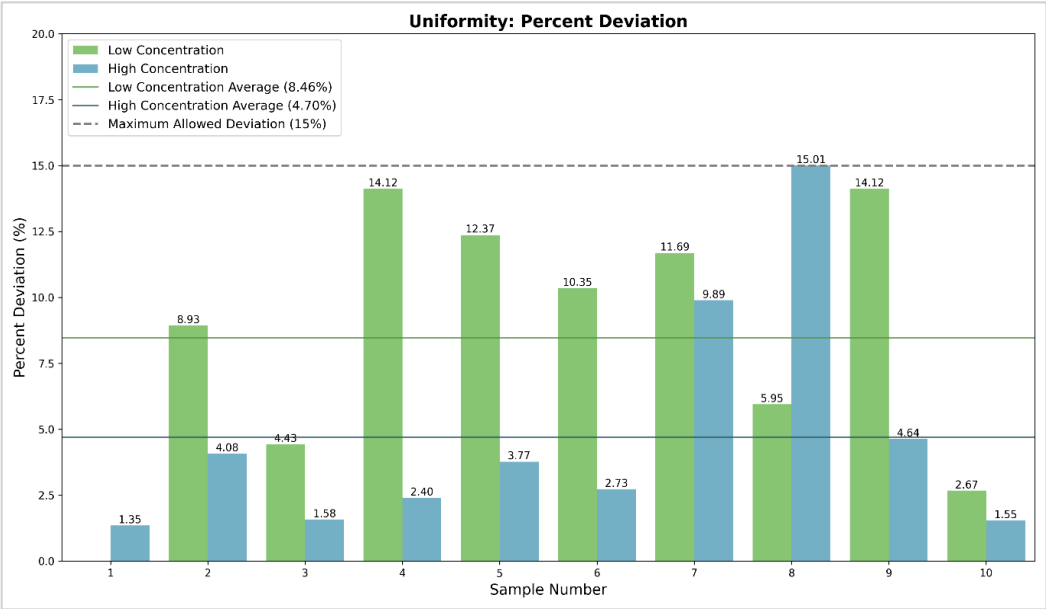


Figure 3-2: Uniformity Results

# 4. RESULTS DISCUSSION

## 4.1 ACCURACY

**Description:**
The accuracy test was conducted to evaluate the agreement between results obtained using the BAMS and those obtained using the Andersen sampler. This evaluation is crucial for ensuring that the BAMS provides results that are sufficiently close to those of the established reference method, confirming its suitability for use in airborne microbial sampling.

**Acceptance Criteria:**
The performance of the BAMS was assessed against the following criteria:

1. The average recovery rate of the BAMS must not be less than 70% of the recovery rate achieved by the Andersen sampler. In other words, the ratio of the BAMS bio-particle concentration to the Andersen colony-forming unit (CFU) concentration (both normalized to concentration/cubic meter) should be no less than 0.70.

These criteria align with the requirements of USP <1223>, which emphasizes that the accuracy of an alternative microbiological method must be demonstrated by showing sufficient agreement to a compendial reference method.

**Results Discussion:**
A comprehensive accuracy study was performed using five types of microorganisms:

- **Staphylococcus aureus** (Gram-positive bacterium)
- **Escherichia coli** (Gram-negative bacterium)
- **Micrococcus luteus** (Gram-positive bacterium)
- **Bacillus subtilis** (Gram-positive, spore-forming bacterium)
- **Candida albicans** (fungus/yeast)

For each microorganism, five different concentration levels were tested, with ten replicates performed at each concentration level to ensure statistical robustness. The experimental design, including the tested microorganisms, concentrations, sampling times, and replicates, is outlined in Table 4-1 (this table is applicable to accuracy, precision, linearity, and specificity of microorganisms tests).

Table 4-1 Accuracy Test Parameters

| Microorganism | Target Concentration (Bio-particles/m$^3$) | Sampling Time (s) | Replicates |
|---|---|---|---|
| Staphylococcus aureus （G+） | 6,000 | 30 | 10 |
| Escherichia coli （G-） | 9,000 | 30 | 10 |
| Micrococcus luteus （G+） | 12,000 | 30 | 10 |
| Bacillus subtilis （G+, spores） | 15,000 | 20 | 10 |
| Candida albicans (Fungi-Yeast) | 20,000 | 20 | 10 |
| Penicillium chrysogenum (Fungi-Mold) | 6,000 | 30 | 10 |
| | 20,000 | 20 | 10 |

The results showed that, for all five microorganisms tested, the ratio of BAMS bio-particle concentration to Andersen CFU concentration exceeded 0.70 across all replicates and concentrations. The overall average concentration ratios for the five microorganisms ranged from 0.98 to 2.23, as presented in Figure 4-1-1. The BAMS consistently achieved recovery rates that meet or exceed the acceptance criteria.

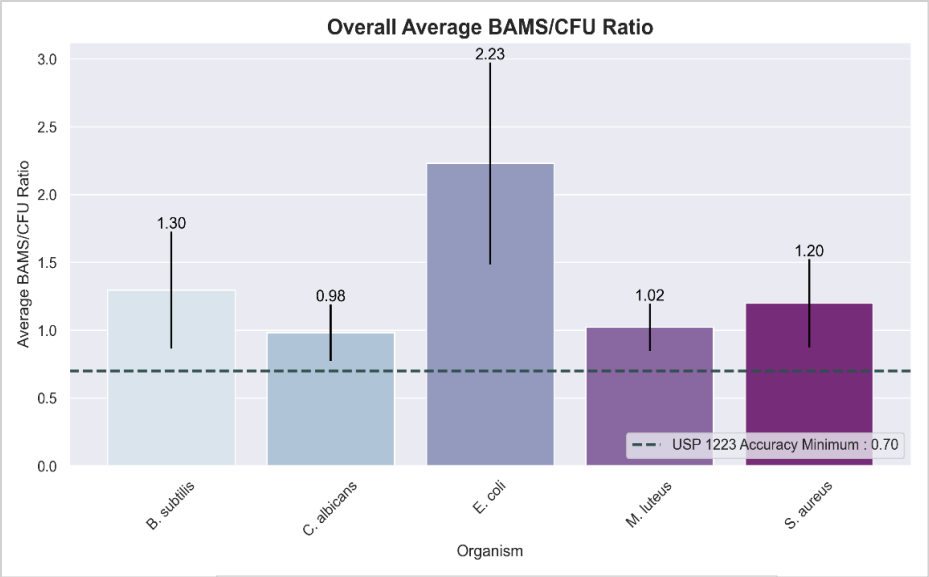


Fig 4-1-1 Overall Average Accuracy Ratio Results

**Conclusion:**
The BAMS accuracy test results confirm that the instrument meets the acceptance criteria specified in USP <1223> for all test microbial species. With concentration ratios consistently above the required threshold and overall averages well within the acceptable range, the BAMS has demonstrated its capability to provide accurate and reliable microbial recovery results comparable to or better than those of the Andersen sampler.

## 4.2 PRECISION

**Description:**
The precision test was performed to evaluate the degree of agreement among individual test results when the procedure was repeatedly applied to multiple samples of laboratory-prepared microorganism suspensions across the test range. Precision measures the consistency and repeatability of results and is typically expressed as the relative standard deviation (RSD).

According to USP <1223>, precision testing requires the analysis of at least five suspensions, with each suspension tested in at least ten replicates to calculate the RSD. This ensures statistical reliability in determining the repeatability of the method.

**Acceptance Criteria:**
The precision of the BAMS was assessed using the following criteria:

1. The RSD for the BAMS system must not exceed the RSD of the traditional culture-based reference method (Andersen sampler).
2. The RSD for both the BAMS and Andersen methods must not exceed 35% at any concentration level tested.

**Results Discussion:**

For each microorganism, five different concentration levels were tested, with ten replicates performed for each concentration level to ensure comprehensive data collection.

The precision results revealed that the RSD values for both the BAMS (reported as Bio-particles/$m^3$) and the Andersen sampler (reported as CFU/$m^3$) were below the acceptance threshold of 35% for all test conditions. Furthermore, the RSD values for the BAMS were consistently lower than those of the Andersen sampler at all tested concentrations, demonstrating the superior precision of the BAMS device.

Figure 4-2-1 provides a summary of the precision results, highlighting the comparative performance of the BAMS and Andersen systems across the tested range. These results highlight the reliability of the BAMS for delivering consistent and reproducible microbial recovery data under varied conditions.

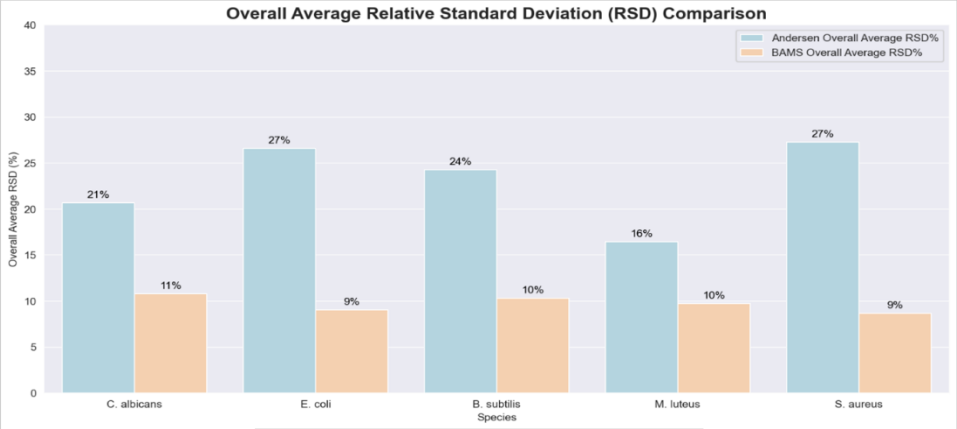


Fig 4-2-1: Overall Average RSD Results

**Conclusion:**

The precision test results confirm that the BAMS meets the acceptance criteria specified in USP <1223>. The RSD values for all tested concentrations of all five microorganisms were within the allowable range, and the BAMS demonstrated greater precision than the Andersen sampler. This establishes the BAMS as a highly consistent and reliable method for quantifying airborne microorganisms in controlled environments.

## 4.3 LINEARITY

**Description:**

The linearity test evaluates BAMS's ability to accurately measure and correlate microorganism concentrations across a range of levels. A correlation analysis was performed between the average values of CFU/$m^3$ obtained from Andersen six-stage samplers and the average values of BAMS (Bio-particles/$m^3$) at five concentration levels.

**Acceptance Criteria:**

The coefficient of determination ($R^2$) must be greater than or equal to 0.65 to demonstrate an acceptable linear relationship between the BAMS results and the reference Andersen method.

**Results Discussion and Conclusion:**

As summarized in Table 4-3-1, the coefficient of determination ($R^2$) for all tested microorganisms exceeded the threshold of 0.65, indicating a correlation between the BAMS and Andersen results. These findings confirm that BAMS meets the linearity acceptance criteria and demonstrates reliable performance across the tested concentration range.

Table 4-3-1: Linearity Results Summary

| Linearity Results Summary | | |
|---|---|---|
| **Organism** | **Minimum Linearity Requirement** | **$R^2$ Result** |
| C. albicans | $R^2 \geq 0.65$ | 0.9993 |
| E. coli | | 0.8714 |
| B. subtilis | | 0.7953 |
| M. luteus | | 0.9924 |
| S. aureus | | 0.9465 |

## 4.4 SPECIFICITY

### 4.4.1 SPECIFICITY OF MICROORGANISMS

**Description:**
The specificity of microorganisms test assessed BAMS's ability to detect a variety of microorganisms commonly found in airborne environments. The tested microorganisms included:

- **Staphylococcus aureus** (Gram-positive bacterium)
- **Escherichia coli** (Gram-negative bacterium)
- **Micrococcus luteus** (Gram-positive bacterium)
- **Bacillus subtilis** (Gram-positive, spore-forming bacterium)
- **Candida albicans** (fungus/yeast)
- **Penicillium chrysogenum** (mold, spore-forming fungus)

**Acceptance Criteria:**
BAMS must demonstrate the ability to detect a broad spectrum of microorganisms relevant to the environments in which it is deployed, including bacteria, spores, and fungi.

**Results Discussion and Conclusion**:

The results, summarized in Table 4-4-1, confirm that BAMS successfully detects all tested microorganism types, including Gram-positive and Gram-negative bacteria, bacterial spores, yeast, and mold spores. These findings validate that BAMS meets the specificity requirements, ensuring its effectiveness in diverse environmental conditions.

Table 4-4-1: Specificity Results Summary

| Specificity Results Summary | | | |
|---|---|---|---|
| **Organism** | **Organism Characteristics** | **BAMS** | **Andersen** |
| C. albicans | Fungi - Yeast | √ | √ |
| E. coli | G- Bacteria | √ | √ |
| B. subtilis | G+ Bacterial (Spores) | √ | √ |
| M. luteus | G+ Bacteria | √ | √ |
| P. chrysogenum | Fungi - Mold (Spores) | √ | √ |
| S. aureus | G+ Bacteria | √ | √ |

## 4.4.2 POTENTIAL FALSE POSITIVE INTERFERENCE TESTING

**Description:**
Interferent testing was conducted to ensure BAMS can accurately differentiate between biological particles and non-biological interferents. The goal was to confirm that potential interferents, such as dust or other particles, are correctly identified and categorized without being misinterpreted as viable particles to an unacceptable degree. The test included common cleanroom materials and substances such as:

- Disposable medical masks
- Disposable non-woven clean clothes
- Disposable rubber surgical gloves
- Dust-free cloth and paper
- $SiO_2$ particles (0.56µm, 1.56µm, 3.15µm)
- 7.5% hydrogen peroxide ($H_2O_2$)
- 70% isopropyl alcohol (IPA)
- 75% ethanol

**Results Discussion**:

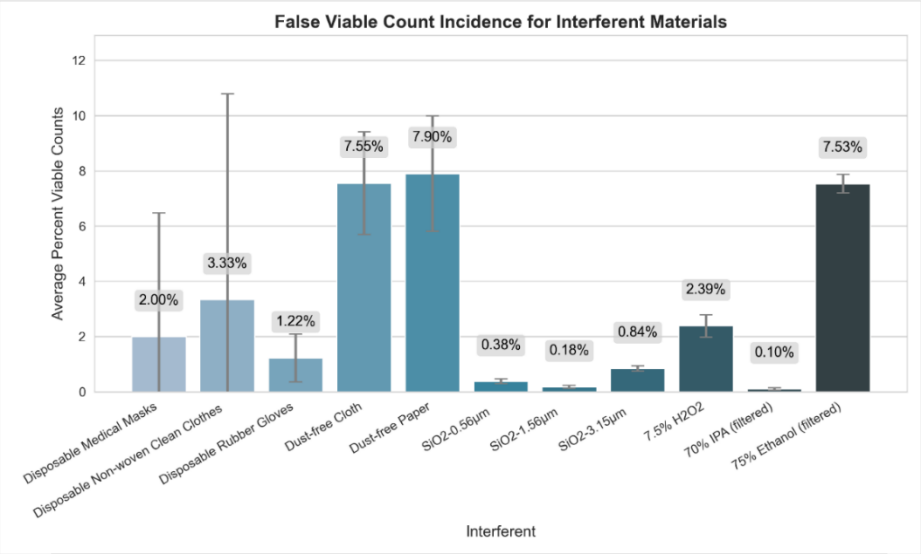


Fig 4-4-1: False Viable Count Incidence for Potential Interferent Materials

The test results, shown in Figure 4-4-1, indicate that BAMS demonstrates a very low rate of false-positive interference, with viable count rates ranging from 0.10% to 7.90% for the tested materials. However, specific findings highlighted some materials with higher viable count rates:

- **Dust-free cloth and paper**: These materials are not sterile and may contain trace microbial contamination. Such contamination could explain their higher viable count rates, emphasizing the importance of avoiding non-sterile materials during sampling in critical environments. These materials are typically not found in sterile environments.
- **75% Ethanol**: This volatile compound may interact with the laser-induced fluorescence system in BAMS, leading to higher false-positive readings. To minimize interference, an alternative disinfection agent, such as IPA or $H_2O_2$, is recommended during active sampling.

The **B/P ratio** (viable particle to total particle ratio) was evaluated as part of this analysis. A B/P ratio exceeding 10% indicates significant interference, making the material unsuitable for use during sampling. Materials with a B/P ratio below 10% exhibit low false-positive interference but should still be handled with caution, particularly in areas with strict viable particle count requirements (e.g., Grade A cleanrooms).

**Conclusion:**

Interferent testing confirms that BAMS provides reliable differentiation between biological particles and non-biological interferents. However, to ensure optimal performance, materials causing higher interference or those that are non-sterile should be avoided during active sampling, especially in critical environments. The recommendations provided support effective and accurate monitoring with BAMS.

## 4.5 LIMIT OF DETECTION (LOD)

**Description:**
This test served to confirm that BAMS can detect the minimum concentration of microorganisms under the specified test conditions. The LOD value for BAMS was determined based on the established LOD value of the Andersen sampler. Several key microorganisms that are commonly found in airborne environments were selected for testing, including:

- **Staphylococcus aureus** (Gram-positive bacterium)
- **Escherichia coli** (Gram-negative bacterium)
- **Micrococcus luteus** (Gram-positive bacterium)
- **Bacillus subtilis** (Gram-positive, spore-forming bacterium)
- **Candida albicans** (fungus/yeast)
- **Penicillium chrysogenum** (mold, spore-forming fungus)

The process and methodology for this testing are outlined in Figure 4-5-1.

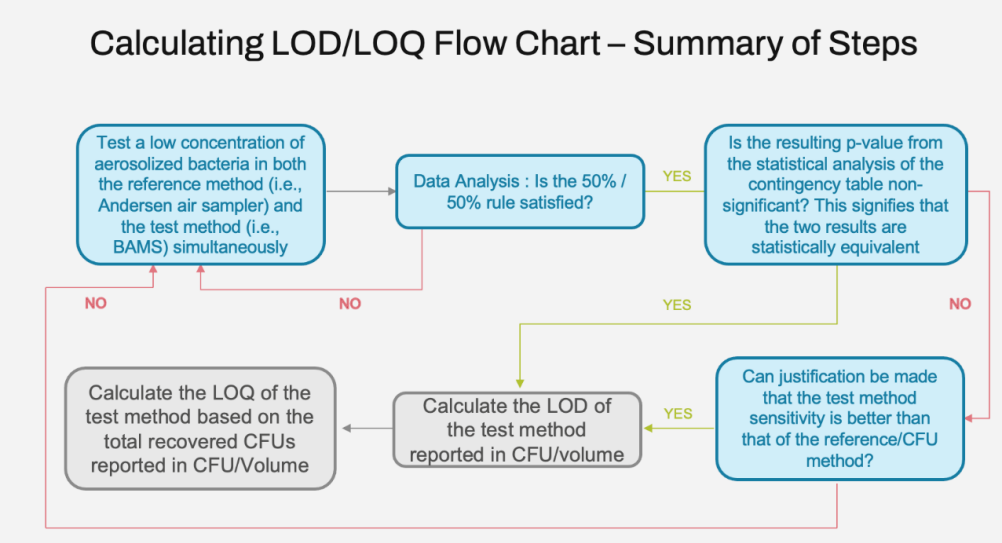


Fig 4-5-1: LOD/LOQ Flow Chart

**Acceptance Criteria:**
The calculated LOD for BAMS must be statistically equivalent to or better than the LOD for the Andersen sampler.

**Results Discussion and Conclusion:**

The LOD test results, shown in Table 4-6-1, indicate that the LOD range for the six tested microorganisms was 4-5 CFU/$m^3$. Statistical analysis using Fisher's Exact Test demonstrated that the LOD results for BAMS are statistically equivalent to those of the Andersen sampler. These findings confirm that BAMS meets the acceptance criteria and can reliably detect microorganisms at low concentrations, aligning with the performance of the reference method.

## 4.6 LIMIT OF QUANTIFICATION (LOQ)

**Description:**
The purpose of this test was to demonstrate BAMS's ability to accurately quantify the lowest number of microorganisms under specified experimental conditions. The LOQ is defined as the minimum concentration of microorganisms that can be enumerated with acceptable accuracy and precision. The LOQ for BAMS was determined based on the results of the LOD test.

**Acceptance Criteria:**
The calculated LOQ for BAMS must be equivalent to or better than the LOQ of the Andersen sampler.

**Results Discussion and Conclusion**:

The LOQ test results, presented in Table 4-6-1, show that the LOQ for the six tested microorganisms ranged from 24-26 CFU/$m^3$. These findings confirm that BAMS meets the acceptance criteria for LOQ, demonstrating its capability to quantify microorganisms at low concentrations with accuracy and precision comparable to or better than the reference method.

Table 4-6-1: LOD/LOQ Results Summary

| Limit of Detection and Quantification Results Summary | | | | | |
|---|---|---|---|---|---|
| **Species** | **Statistical Test Used** | **Resulting P-Value** | **Significance Level P=0.05** | **LOD (CFU/$m^3$)** | **LOQ (CFU/$m^3$)** |
| E. coli | Fisher's Exact | 0.24 | Statistically Equivalent | 5 | 26 |
| M. luteus | Fisher's Exact | 0.34 | Statistically Equivalent | 5 | 24 |
| B. subtilis | Fisher's Exact | 0.14 | Statistically Equivalent | 5 | 25 |
| C. albicans | Fisher's Exact | 0.14 | Statistically Equivalent | 4 | 24 |
| S. aureus | Fisher's Exact | 0.24 | Statistically Equivalent | 4 | 24 |
| P. chrysogenum | Fisher's Exact | 0.06 | Statistically Equivalent | 5 | 25 |

## 4.7 RANGE

**Description:**

The purpose of this test was to verify the sampling range of airborne microorganisms detectable by BAMS. The range was determined as the interval between the lowest and highest concentrations of microorganisms that met the criteria for accuracy, precision, linearity, and LOD in previous tests.

**Results Discussion:**

The test results, summarized in Table 4-7-1, indicate that the highest concentration meeting the requirements for accuracy, precision, and linearity was 24,382 Bio-particles/$m^3$, while the lowest value determined through LOD testing was 4 CFU/$m^3$.

Table 4-7-1: Range Results Summary

| | Range Results Summary | |
|---|---|---|
| | **Detected Value** | **Test Source** |
| Minimum | **4** (CFU/$m^3$) | Limit of Detection |
| Maximum | **24382** (Bioparticles/$m^3$) | Accuracy/Precision/Linearity |

**Conclusion:**

This test establishes the validated counting range for BAMS as **4 CFU/$m^3$ to 24,382 Bio-particles/$m^3$**. Notably, in practical applications, BAMS has the capability to detect single bio-particles, extending its usability beyond the validated range for enhanced sensitivity in specific environments.

## 4.8 RUGGEDNESS

**Description:**

The ruggedness test aimed to evaluate the consistency and reliability of BAMS under varying

experimental conditions, including different testing times, analysts, and sampling equipment. The test used *Micrococcus luteus* at a concentration of approximately 15 Bio-particles/L, with each test repeated ten times.

**Acceptance Criteria:**

1. The accuracy and precision results must meet the requirements outlined in Section 4.1 (accuracy) and Section 4.2 (precision).
2. The ratio of the results between the two tests must not be less than 0.70.

**Results Discussion**:

The results, summarized in Table 4-8-1, indicate that both tests met the accuracy and precision requirements. The ratio of BAMS counts for the same aerosol concentration between the two tests was close to 1, with an average ratio of 1.06 for Test 1/Test 2 and 0.96 for Test 2/Test 1.

Statistical analysis further validated the consistency of the results:

- A paired sample t-test comparing the 10 BAMS results (Bio-particles/$m^3$) from the two tests showed no significant difference (**p=0.38**).
- Similarly, a paired sample t-test for the 10 Andersen results (CFU/$m^3$) from the two tests also showed no significant difference (**p=0.87**).

Table 4-8-1: Ruggedness Results Summary

| **Ruggedness : Comparison Ratio** | | |
|---|---|---|
| Test | Requirement | Ratio Result |
| **Cross-test Ratio** (BAMS Test 1 / BAMS Test 2) | ≥0.70 | 1.06 |
| **Cross-test Ratio** (BAMS Test 2 / BAMS Test 1) | | 0.96 |
| **Accuracy Test 1** (BAMS Test 1 / Andersen Test 1) | | 1.06 |
| **Accuracy Test 2** (BAMS Test 2 / Andersen Test 2) | | 1.03 |
| **Ruggedness : Precision** | | |
| Test | Precision: RSD% Requirement | Precision RSD % Result |
| BAMS Test 1 | ≤ 35% | 8.0% |
| Andersen Test 1 | | 13.0% |
| BAMS Test 2 | | 17.1% |
| Andersen Test 2 | | 28.4% |
| **Ruggedness : Statistical Analysis** | | |
| Instrument | Statistical Significance Requirement | p-value Result |
| BAMS Test 1 vs. Test 2 | p-value non-significant (> 0.05) as determined by paired t-test | 0.38 |
| Andersen Test 1 vs. Test 2 | | 0.87 |

**Conclusion:**

The ruggedness test demonstrated that BAMS provides consistent and reliable results across varying experimental conditions, meeting all specified criteria.

## 4.9 ROBUSTNESS

**Description:**

The robustness test was conducted to evaluate BAMS's ability to maintain stable performance despite small variations in method parameters. This assessment ensures the reliability of BAMS during use in real-world conditions. Key parameters tested included flow rate, scattering counting efficiency, and fluorescence counting efficiency under challenging environmental conditions:

- **High and low temperatures**: 35℃ and 5℃
- **High relative humidity**: 90% RH

**Acceptance Criteria:**

1. **Flow rate**: Deviation must not exceed ±3% after exposure to temperature and humidity variations.
2. **Scattering counting efficiency**: Must comply with ISO 21501-4 standards:
    - 50% ± 20% for the minimum detectable particle size of 0.5μm
    - 100% ± 10% for particle sizes 1.0μm (1.5 to 2 times larger than the minimum detectable size).
3. **Fluorescence counting efficiency**: Must meet internal standards of 45% ± 10% for the minimum detectable particle size of 0.5μm.

**Results Discussion:**

The results, presented in Table 4-9-1, confirm that BAMS successfully passed all critical performance tests for flow rate, scattering counting efficiency, and fluorescence counting efficiency under the tested conditions of 5℃, 35℃, and 90% relative humidity.

Table 4-9-1: Robustness Results Summary

| Robustness : Low Temperature (5˚C) | | |
|---|---|---|
| **Test** | **Requirement** | **Result** |
| Flow Rate Deviation Percent | ≤ 3% | 0.47% |
| 0.5μm Counting Efficiency Percent | 50% +/- 20% | 47.47% |
| 1.0μm Counting Efficiency Percent | 100% +/- 10% | 98.23% |
| Fluorescent Counting Efficiency Percent | 45% +/- 10% | 43.30% |
| **Robustness : High Temperature (35˚C)** | | |
| **Test** | **Requirement** | **Result** |
| Flow Rate Deviation Percent | ≤ 3% | 1.06% |
| 0.5μm Counting Efficiency Percent | 50% +/- 20% | 37.22% |
| 1.0μm Counting Efficiency Percent | 100% +/- 10% | 93.37% |
| Fluorescent Counting Efficiency Percent | 45% +/- 10% | 43.06% |
| **Robustness : High Humidity (90% RH)** | | |
| **Test** | **Requirement** | **Result** |
| Flow Rate Deviation Percent | ≤ 3% | 0.47% |
| 0.5μm Counting Efficiency Percent | 50% +/- 20% | 44.25% |
| 1.0μm Counting Efficiency Percent | 100% +/- 10% | 102.58% |
| Fluorescent Counting Efficiency Percent | 45% +/- 10% | 51.06% |

**Conclusion:**

These findings demonstrate that BAMS performs reliably in environmental conditions that are significantly more extreme than those typically found in cleanrooms (18-26℃, 45-60% RH). Therefore, BAMS's robustness ensures consistent and trustworthy performance in routine operational settings.

## 4.10 EQUIVALENCY

**Description**:

Equivalency between two analytical procedures is established when their results are sufficiently similar for the intended purpose. Demonstrating equivalency involves meeting pre-specified criteria to validate the similarity between the alternative and compendial methods. Four approaches are available to establish equivalency for alternative analytical methods:

1. **Acceptable Procedures:** Meeting minimum performance or acceptance criteria without requiring direct equivalence to the compendial method.
2. **Performance Equivalence:** Demonstrating equivalent or superior results compared to the compendial method based on validation criteria such as accuracy, precision, specificity, LOD, LOQ, robustness, and ruggedness. This can include using calibration curves to confirm correlation within the product specification range.
3. **Results Equivalence:** Showing that the alternative and compendial methods yield equivalent numerical results.
4. **Decision Equivalence:** Ensuring that pass/fail outcomes are consistent between the alternative and compendial methods.

For quantitative microbiological procedures, traditional equivalency may not always be demonstrable due to differences in units or numerical results (e.g., CFU vs. bio-particles/$m^3$). Therefore, two criteria are emphasized:

- **Precision:** The alternative method must exhibit at least acceptable repeatability.
- **Correlation:** Results from the alternative procedure must correlate highly with those from the compendial method, ensuring quantitative acceptance criteria in CFU can be calibrated to the alternative method's units.

**Results Discussion:**

BAMS satisfies the precision and correlation requirements, as well as additional performance equivalency criteria:

- **Precision:** As detailed in Section 4.2, BAMS exhibited lower RSD values in bio-particles/$m^3$ compared to the Andersen method (CFU/$m^3$), indicating greater consistency in results.
- **Correlation:** Section 4.3 confirms that bio-particles/$m^3$ results obtained by BAMS are highly correlated with CFU/$m^3$ results from the Andersen method, enabling calibration between the two measurement units.
- **Accuracy:** Section 4.1 demonstrates that BAMS is equivalent or superior in microbial counting compared to the compendial method.
- **Specificity:** Section 4.4 highlights BAMS's ability to detect a wide range of microorganisms with very low false-positive interference rates for cleanroom materials.

- **Limit of Detection (LOD) and Limit of Quantification (LOQ):** Sections 4.5 and 4.6 confirm that BAMS's LOD and LOQ are statistically equivalent to those of the Andersen sampler.
- **Range:** As described in Section 4.7, BAMS has a significantly higher upper detection range compared to the compendial method.
- **Ruggedness:** Section 4.8 shows BAMS's repeatability under varied conditions is equivalent to the compendial method.
- **Robustness:** Section 4.9 highlights BAMS's superior performance under extreme environmental conditions (e.g., 5°C, 35°C, 90% RH).

**Conclusion:**

BAMS is equivalent to or exceeds the capabilities of the traditional plate-counting method (Andersen six-stage sampler) in terms of performance, precision, and correlation. Its ability to provide highly consistent and reliable results, coupled with robustness under varied environmental conditions, makes it a superior alternative for quantitative microbiological analysis.

## 5 VALIDATION SUMMARY

The validation of the MicronView BAMS confirms its reliability and effectiveness as an alternative method for airborne microbial monitoring. Through rigorous testing, the system met or exceeded all acceptance criteria for accuracy, precision, linearity, specificity, LOD, LOQ, range, ruggedness, and robustness, as outlined in USP <1223>.

BAMS showed strong alignment with the traditional Andersen method, delivering consistent, precise results with high correlation. Its ability to perform reliably under a variety of environmental conditions highlights its robustness and suitability for cleanroom monitoring. The BAMS is also able to minimize the effect of false positive interferent materials on data through its advanced algorithm, as shown in the specificity results.

With laser-induced fluorescence technology, BAMS provides fast and accurate detection of airborne microorganisms, offering a valuable tool for maintaining strict microbial quality standards in regulated industries. Additionally, its ability to continuously capture real-time data makes it an excellent tool for trend analysis, allowing users to monitor patterns and detect potential issues proactively.

This validation demonstrates that BAMS is well-suited for enhancing efficiency and compliance in critical environments, providing reliable real-time data for microbial control.