Microbial Detection Efficiency of Biofluorescent Particle Counter (BFPC) Technology versus Conventional Culture-Based Microbial Air Recovery Methods for Environmental Monitoring

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BACKGROUND

Current Environmental Monitoring (EM) programs rely on conventional culture-based methods to assess the state of microbial control within classified spaces. Methods include passive viable air sampling (settle plates) and active viable air sampling by air impaction devices. These require 5-6 days of sample incubation and enumeration by an analyst before results are available. BFPC technology enables continuous monitoring and real-time detection of airborne microbial contamination. BFPC technology has the potential to replace conventional viable sampling methodologies as well as non-viable particulate monitoring.

The goal of this Proof-of-Concept study was to determine viability for deployment across Lilly manufacturing sites.

Benefits:

- Continuous, real-time results
- Reduction of human interventions
- Potential real-time discard strategies within Grade A environments

SCIENTIFIC PRINCIPLE

Air drawn through inlet at controlled flow rate

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Particles subjected to laser excitation

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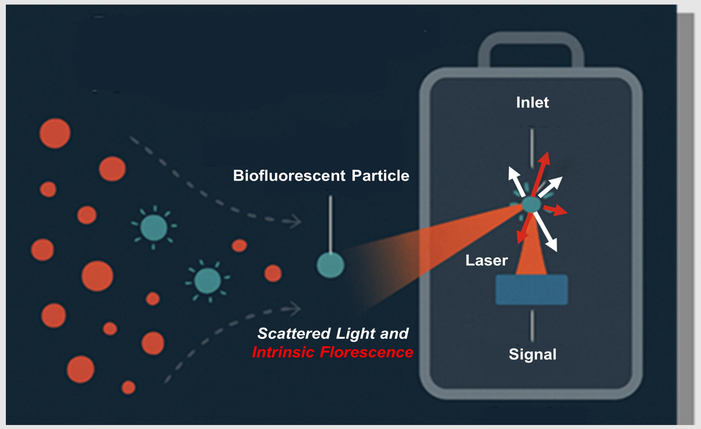
Non-biological particles scatter light; biological particles generate intrinsic fluorescence from metabolic biomolecules (NADH, riboflavin, etc.)



PROOF-OF-CONCEPT

BIOAEROSOL EXPERIMENTAL DESIGN

In partnership with Microchem Laboratory…

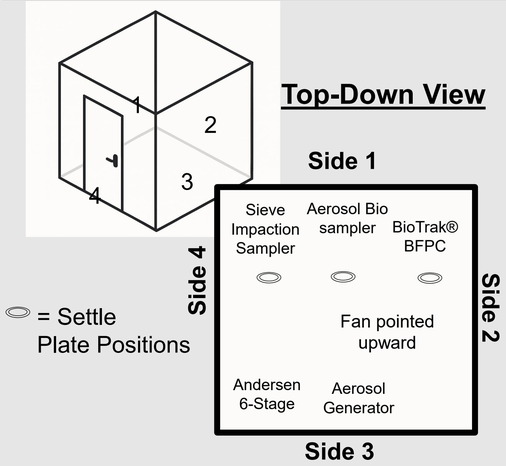
TSI's BioTrak® BFPC was chosen as the test device. To evaluate the relative detection efficiency of TSI's BioTrak® BFPC compared to conventional methods, testing was performed in a bioaerosol chamber engineered to maintain consistent airborne microbial concentrations within a homogeneous environment. Instead of introducing the inoculum directly through the device inlet, the bioaerosol was generated in situ within a room-sized chamber to enable quantitative rather than comparative results. Five representative microorganisms were tested individually over 15-minute periods; the duration was selected due to the inherent challenges and short viability of airborne microbes. All results were normalized to one cubic meter of air based on the homogeneous aerosol.

POC STRATEGY

The POC strategy was designed to generate quantitative data associated with the recovery efficiency of conventional culture-based sampling methods, and to determine if the BioTrak® BFPC performance is equivalent or superior to conventional methods.

Per European Commission. (2022). *EudraLex: The rules governing medicinal products in the European Union, Volume 4: EU Guidelines for Good Manufacturing Practice for Medicinal Products and Veterinary Ise: Annex 1 – Manufacture of Sterile Medicinal Products* "Sampling methods and equipment used should be fully understood and procedures should be in place for the correct operation and interpretation of results obtained. Supporting data for the recovery efficiency of the sampling methods chosen should be available."

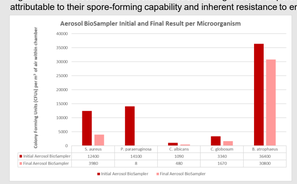
Per U.S. Food and Drug Administration. (2004). *Guidance for Industry: Sterile drug products produced by aseptic processing – Current good manufacturing practice* "Other suitable microbiological test methods (e.g., rapid test methods) can be considered for environmental monitoring, in-process control testing, and finished product release testing after it is demonstrated that the methods are equivalent or better than traditional methods (e.g., USP)."

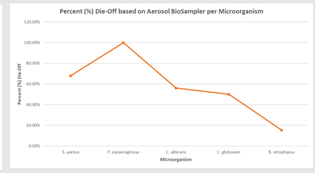


Equipment	Samples	Purpose
Aerosol BioSampler	Three (3) replicates for Concentration initial and Concentration final	Positive control for bioaerosol generation
Representative Sieve Air Impaction Sampler	Three (3) replicates performed in series	Determination of recovery efficiency
Andersen 6-Stage Sampler	One replicate, 6 stages	Positive Control for bioaerosol, sizing and total viable count
BioTrak® BFPC	15-minute sample	Test device; determination of detection efficiency
N/A	Settle Plates near each device	Determination of recovery efficiency

RESULTS & CONCLUSIONS

Figures 1 and 2: *P. aeruginosa* exhibited the greatest reduction in viability post-aerosolization, consistent with the known susceptibility of Gram-negative organisms to desiccation and mechanical stress during airborne dispersion. Conversely, *B. atrophaeus* demonstrated the lowest die-off percentage, likely attributable to their spore-forming capability and inherent resistance to environmental stressors.





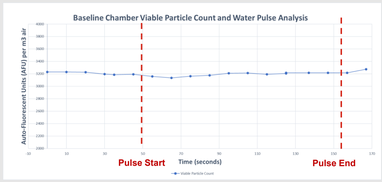


Figure 2: Sterile distilled water, used as the bioaerosol carrier, was assessed for potential interference with BioTrak® BFPC detection. Baseline chamber microbial levels were recorded prior to aerosolization and monitored post-introduction of the carrier. No deviation from baseline was observed, confirming absence of interference.

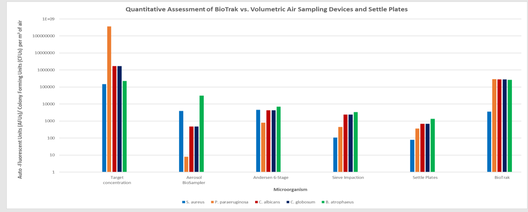
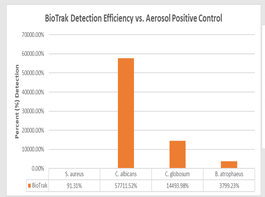


Figure 3: Settling plate data collected from the Aerosol BioSampler position was normalized to a 4-hour sampling period and volumetric sampling device data was normalized to one cubic meter. Devices were evaluated in parallel to enable quantitative comparison. The target concentration was the theoretical concentration, while the aerosol Biosampler and Andersen 6-Stage Biosampler functioned as a positive control to validate chamber aerosol levels. BioTrak® BFPC counts for all organisms fell between the target and control values and were generally higher than the culture-based methods. This may be attributed to BioTrak BFPC's ability to detect both viable and non-viable cells (as demonstrated in a separate study utilizing UV-C inactivation).



Figures 4, 4.1 & 5, 5.1: BioTrak® BFPC showed the highest percent detection, likely due to its sensitivity and ability to detect both viable and non-viable cells. The sieve impaction device and settle plates demonstrated lower overall recovery, with improved detection associated with larger sized microorganisms. Sieve impaction devices allow smaller particles to follow the airstream and may bypass the impaction, and smaller organisms are less likely to impact on settle plates since these organisms are more likely to stay suspended in the air. There was high variability associated with detection and recovery of *P. paraeruginosa* due to aerosol survivability of the organism.

Relative Average Detection & Recovery Efficiencies

Comparator	BioTrak® BFPC	Sieve Impaction Sampler	Settling Plate
Aerosol Positive Control	3614.63%	43.58%	10.91%

Results were compared to the positive controls to calculate relative detection and recovery efficiencies in this study.

Overall Conclusions:

- BFPC demonstrated significantly higher recover rates compared to conventional methods
- BFPC can detect dead cells, which conventional methods are unable to identify
- In real-world Grade A environments, the concentration of dead cells is expected to be low
- BFPC is best suited for use in Grade A environments