Bio-Fluorescent Particle Counter (BFPC) Continuous Environmental Viable Particle Monitoring Strategy for Aseptic Filling

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1 Introduction

Aseptic filling has undergone tremendous development in the past decades, from manned cleanrooms to modern isolator systems, which provide a significant improvement in containment and minimizing contamination risks. Isolator systems allow for a validated aseptic environment where operators are completely separated via gloves, and material transfers in and out are performed through validated technologies. As recommended in the EU GMP Annex 1 [1], isolators provide a safe environment against potential sources of endotoxin/pyrogen, particulate, and microbial contamination for aseptic filling. This is also reflected in the current trend of the pharmaceutical industries toward gloveless and robotic isolator technology.

Nevertheless, traditional monitoring approaches, originally developed for manned clean rooms, are still applied. Monitoring with traditional methods have limitations regarding the sensitivity of the methods and pose a risk to the drug product by interventions and handling steps connected to sampling, sample transport to the laboratory, and evaluation. Modern technologies, for example robotic isolators, and the advancement of pharmaceutical products, such as personalized medicines in cell and gene therapies, drive the need for fast and reliable viable particle monitoring.

Reduction of conventional monitoring can be achieved by the implementation of new methods, such as using Bio-Fluorescent Particle Counters (BFPCs), which provide many advantages in the pharmaceutical industry. BFPCs detect and count the total biological particles in real time, significantly reducing contamination risks by eliminating the human interventions required for the manual sampling steps connected with traditional methods. Furthermore, BFPCs can detect adverse trends as they occur, with the possibility to react immediately.

The adoption of Environmental Monitoring (EM) through modern technologies, such as BFPCs, has proven to be a long process and requires a solid data set and validation strategy to be accepted by the authorities. This paper provides key points to consider during the implementation of BFPCs as a routine EM tool in modern isolators.

2 Continuous Real-Time Monitoring Using a Quality Risk Management Approach as Part of the Contamination Control Strategy

A holistic approach for process analysis and understanding is needed when developing a proper Contamination Control Strategy (CCS). EM constitutes an integral part of each CCS. To date, the information from traditional viable monitoring is generally provided at the end of the control chain, due to the time involved in evaluating the data. Real-time systems, conversely, allow for receipt of fast feedback on the process control, making them perfectly suited to the purpose of the CCS, while also reducing the risks involved in performing the monitoring itself.

From the perspective of EM, moving toward a holistic approach means correlating the data obtained from non-viable particle monitoring with the data obtained from viable monitoring. This correlation becomes a key investigative step in case of out-of-limit values. Real-time systems not only allow for this correlation, but the data produced can be used to implement timely mitigations at the same time as the information is communicated by the system. This aspect supports a parametric release of the batch and/or could even save a batch from being rejected.

The CCS is intended to assess the effectiveness of all the controls (design, procedural, technical, and organizational) and monitoring measures employed to manage risks associated with contamination. Implementation of BFPCs meet the expectation as written in the EU GMP Annex 1 [1]:

> *"The use of appropriate technologies (e.g. Restricted Access Barrier Systems (RABS), isolators, robotic systems, rapid/alternative methods and continuous monitoring systems) should be considered to increase the protection of the product from potential extraneous sources of endotoxin/pyrogen, particulate and microbial contamination…"*

This will lead to a deeper understanding of processes and allow for implementation of the necessary mitigations and technologies to develop the best possible strategy for maintaining environmental control and product safety.

3 Qualification and Validation Requirements

BFPCs provide fundamentally different signals from viable airborne particles compared to traditional, growth-based methodologies. It is noted that the reported BFPC signal output, Auto Fluorescence Units (AFUs), are not comparable to the traditional Colony Forming Units (CFUs). Therefore, a more innovative approach in terms of qualification of such devices is required, which needs to be accompanied by a switch in the mindset of classical equipment qualification structures. Reliance on the supplier to provide data or to work with the end user is needed to meet the deliverables of the qualification activity, as outlined in Table 3.1.

Table 3.1: Recommended Flow and Responsibilities for a BFPC Qualification

Qualification Activity	Responsibility	
	User	Supplier
Risk Assessment	X	
User Requirement Specification (URS)	X	
Design Qualification (DQ)	X	X
Functional Design Specification (FDS)	X	X
Installation Qualification (IQ)		X
Operational Qualification (OQ)		X
Performance Qualification (PQ) / (Method Validation)	Refer to Section 3.2.1 Primary Validation and Section 3.2.2 PQ	
Maintenance	X	X

3.1 Qualification

Within the scope of initial qualification are the activities performed by the supplier with respect to the User Requirements Specification (URS) provided by the user. The URS is a key document that details the requirements to meet the needs of the intended users, specific to the business process and operations. This is an effort that must include input from relevant stakeholders such as Quality, Regulatory, Engineering, and IT. The details within this document inform about the activities to perform for validation and implementation.

The aspects related to Design Qualification (DQ) and Functional Design Specification (FDS) are a collaboration between user and supplier. Ideally, the inclusion of a BFPC instrument is incorporated at the beginning of a new filling line project, rather than retrofitting into an existing filling line.

Retrofitting into an existing line is difficult, as BFPCs need space in the sub-construction of an isolator and specific requirements, for example, tubing length and the impact of bends, have to be considered. If retrofitting must be considered, extended evaluation is required regarding the requirements of the EU GMP Annex 1 [1].

When planning the installation of a BFPC instrument, suitable sample tubing (taking into consideration of particle sedimentation), the sample tube length, tube section, number of bends and bend radius, instrument flowrate, tube materials, and surface finish, should be reviewed as a function of sampling design. The sample probe should also consider isokinetic and isoaxial sampling as variations of configuration.

Tubing length should be minimized to reduce the loss of sedimented particles, with large particles being of significant importance due to the relationship of viability and size. Tubing length should ideally be less than 1 m. Where tubing bends are required, the bend radius needs to be maximized relative to the tubing diameter (long radius bends). It is recommended that the bend radius be optimized at a minimum of 140 mm since this reduces impaction and attrition of particles on bends. The location of the BFPC should ideally be below the sample probe inlet as this improves the transit of large particles. Where these parameters are exceeded, or where other restrictions to flow are encountered, appropriate evaluation of particle loss is needed.

Installation Qualification/Operational Qualification (IQ/OQ) activities are often performed by the supplier. These activities must be completed prior to Performance Qualification (PQ). IQ/OQ protocols should follow the standard requirements per *ISPE GAMP® 5: A Risk-Based Approach to Compliant GxP Computerized Systems (Second Edition)* [2] to ensure correct installation of the equipment and confirm that system functionalities perform according to predetermined limits per operational procedures.

As BFPCs have a higher sensitivity through detection of biologic fluorescence that is independent of growth, supporting studies are necessary to evaluate potential interference factors and materials. The supplier should provide data on common potential interferences that would trigger a false positive result, such as 70% Isopropyl Alcohol (IPA). Additionally, the user should supplement this data at the site of intended use and expose the instrument to particles normally present in the respective environment (e.g., product, disinfectants, other materials, or nearby equipment). The combination of supplier and user generated data provide an understanding of the system and background influences. This is the basis for the development of an implementation and alarm handling concept.

3.2 Validation

In this section, validation refers to the primary validation performed by the supplier and the PQ executed by the user that incorporates the implementation concept.

3.2.1 Primary Validation by the Supplier

It is broadly recognized in the industry that there is no standardized, safe, and precise way in a pharmaceutical Quality Control (QC) laboratory or manufacturing environment to create a homogeneously distributed aerosol with viable organisms. These types of trials require special chambers or settings and a high level of expertise, which is often only found at the instrument vendor's site. Therefore, the main validation elements should rely on the data and documentation provided by the vendor of such devices and described in the company's equipment validation guidance.

Validation should be performed in accordance with USP <1223> [3] and Ph. Eur. 5.1.6 [4] and meet the expectations of regulatory authorities. However, both regulatory authorities and users need to recognize that BFPCs operate by a different mode of detection that is expressed by AFUs instead of CFUs, which are not equivalent. This constitutes a paradigm change. There are still some limitations and hurdles to be solved as there is still less guidance for these types of new technologies. Therefore, several industry working groups, together with suppliers and regulators, are working to tackle these hurdles and limitations and to raise acceptance; see "Challenges Encountered in the Implementation of Bio-Fluorescent Particle Counting Systems as a Routine Microbial Monitoring Tool" [5].

3.2.2 Performance Qualification by the User

Part of the system PQ is an alarm handling concept, which describes the measures taken in case of the occurrence of viable (AFU) signals and is an integral part of the implementation strategy.

For new filling lines, an EM concept is established that includes the definition of sampling points based on a risk assessment as part of the overall CCS. When intending to implement new technologies like BFPCs, sampling positions for both measurement systems (traditional and BFPC) must be evaluated and defined. The number of sampling positions should fulfill the expectations of regulatory authorities, although there are no specific requirements with respect to the number of sampling positions for viable particle monitoring in the current guidelines or standards. For customized lines, it is advisable to implement a traditional method for viable particle monitoring that can easily be dismantled after final implementation of the new system. For non-viable particle counting, the BFPC system can be used from the beginning as a regular particle counter.

There is a parallel phase to be performed during the implementation phase of the new technology (BFPC/traditional air sampling method) under ISO 5/Grade A. This should be discussed and agreed upon with the respective authorities in advance. Thereby an alarm handling concept, the time period, and/or the number of batches for the parallel studies should be presented. The data collected in parallel with the traditional method serves to gain experience with the new technology directly at the intended location for use and should show that the BFPC method is equivalent or better compared to the traditional one.

For the alarm handling concept, procedures should be established in the event a viable signal is detected (AFU alarm). Information from data collected during the qualification phase and interference/background measurement studies in the respective environment should be taken into account. A procedure describing how to react to individual AFU signals should be in place, such as stopping the filling process, removing open primary containers, starting an investigation, and/or starting the decontamination cycle of the isolator where possible.

EM results from the entire filling process should be taken into consideration during the investigation (from passive air sampling, surface sampling, and glove sampling, if applicable). When applicable, secondary collection devices, such as used to perform traditional monitoring, may be used to assist in analysis to support further investigations. The validity of the data collected from these secondary systems should be considered and should not comprise a function in the diagnosis of AFU data.

The alarm handling concept also depends on the type of environment (classical cleanroom, RABS, isolator) and line (standard line or robotic line without human interventions). Final approval for the implementation of the new BFPC method and the removal of the old (traditional) monitoring systems should be discussed with the authorities based on the assessed data collected during the parallel study.

4 Conclusion

With the advances in isolator technology, the impact of personnel on the critical ISO 5/Grade A manufacturing environment is greatly reduced. One of the remaining risks is the introduction of agar plates and handling of the monitoring equipment via gloves in modern isolators. Advanced EM methods include technologies such as bio-fluorescent particle counting systems, which do not require interventions, thereby do not compromise the aseptic environment itself.

Within isolator environments, the statistical assurance of 0 CFU takes on elevated importance. The use of traditional techniques to demonstrate this assurance can only be expanded to a limit. For an elevated demonstration of statistical control, the use of real time, quantitative indicators such as AFU detection gives a better confidence of control. This can be supplemented with traditional data where applicable, such as for investigations when significant events compromising environmental control are detected.

BFPC systems provide continuous, real-time monitoring and feedback as soon as a potential microorganism is collected from the critical ISO 5/Grade A environment. However, pharmaceutical companies must still ensure that these systems are properly qualified and that particle limits are appropriately established. BFPCs will gain more and more importance with the industry moving forward to real-time release and the need for finished products to be administered in a timely manner, while still stable.

5 Acronyms and Abbreviations

AFU	Auto Fluorescence Unit
BFPC	Bio-Fluorescent Particle Counting
CCS	Contamination Control Strategy
CFU	Colony Forming Unit
DQ	Design Qualification
EM	Environmental Monitoring
FDS	Functional Design Specification
IPA	Isopropyl Alcohol
IQ	Installation Qualification
ISO	International Organization for Standardization
IT	Information Technology
OQ	Operational Qualification
PQ	Performance Qualification
QC	Quality Control
URS	User Requirements Specification

6 References

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2. *ISPE GAMP® 5: A Risk-Based Approach to Compliant GxP Computerized Systems,* International Society for Pharmaceutical Engineering (ISPE), Second Edition, July 2022, www.ispe.org.

3. USP <1223> Validation of Alternative Microbiological Methods, United States Pharmacopeial Convention, www.usp.org.

4. Ph. Eur. 5.1.6 Alternative Methods for Control of Microbiological Quality, European Pharmacopoeia, EDQM Council of Europe, www.edqm.eu/en/news/european-pharmacopoeia.

5. Scott, A., et al., “Challenges Encountered in the Implementation of Bio-Fluorescent Particle Counting Systems as a Routine Microbial Monitoring Tool,” *PDA Journal of Pharmaceutical Science and Technology,* January 2022, doi: 10.5731/pdajpst.2021.012726, https://journal.pda.org/content/early/2022/07/15/pdajpst.2021.012726.