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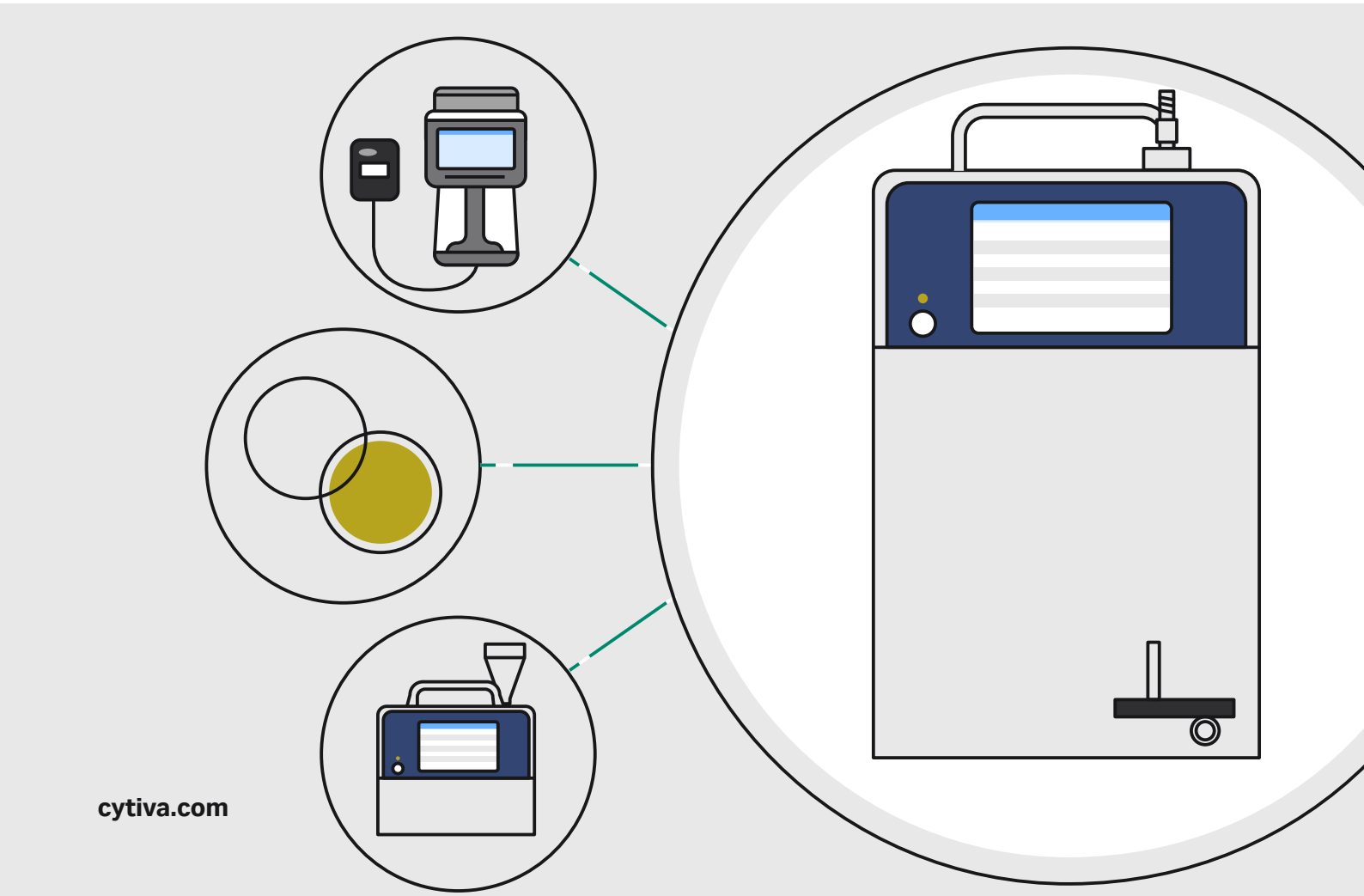
The evolution of environmental monitoring in pharmaceutical manufacturing: A shift towards bio-fluorescent particle counters



Executive summary

Assuring the sterility of advanced sterile injectable drug products is critical to addressing drug manufacturing efficiency, global drug shortages, and the need for increasingly effective medicines. As therapeutic modalities grow in complexity and variety, continuous environmental monitoring methods are gaining traction. Among these, biofluorescent particle counters (BFPCs) are an appropriate and powerful technology that enhances performance compared with traditional environmental monitoring methods and provides operational and cost benefits to drug manufacturers of all types. BFPCs also enable compliance and readiness under evolving regulatory regimes, including EU Annex 1 guidelines.

Introduction

From December 2023 through November 2024, 26% of sterile injectable drug recalls were due to microbial contamination (1). While the total value of these recalls wasn't disclosed, it's likely in the tens of millions of US dollars. Premarket manufacturing batch losses aren't accounted for in this tally but are likely to represent substantial lost value. Loss of products due to contamination also contributes to drug shortages, which have been a continuing challenge for healthcare over the last several decades (2).

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Aseptic filling is a critical step in maintaining sterility and protecting the value of drug products. Developing and maintaining a comprehensive contamination and control strategy (CCS) for preventing and detecting contamination during aseptic filling is one of the most challenging aspects of the biomanufacturing process. As newer, more complex, and less stable therapeutic modalities like mRNA-based drugs, cell and gene therapies, and others enter the market, the obstacles to maintaining a sufficient CCS will increase. The regulatory landscape is adapting to help manufacturers anticipate and keep pace with an evolving pharmaceutical landscape. One example is the recent revisions to the European Union Good Manufacturing Practice Annex 1 Guidelines for the manufacture of sterile medicinal products (Annex 1), which took effect in August 2023 (3).

Annex 1 provides specific guidance for sterility monitoring that can be difficult to achieve without modern technologies and approaches, including continuous active environmental monitoring that can detect and measure both viable and nonviable particulate contamination (3; §9). Annex 1 also recommends that "adoption of suitable rapid or automated monitoring systems should be considered by manufacturers to expedite the detection of microbiological contamination issues and to reduce the risk to product (3; §9.28).

Biofluorescent particle counters (BFPCs) are a rapid microbiological method that can help manufacturers meet the increasing regulatory demands for continuous environmental monitoring (EM) during aseptic filling. BFPCs offer many advantages compared with traditional environmental monitoring where microbial growth must be detected visually. These advantages include shorter time to detection, real-time continuous monitoring, increased sensitivity, a lower false negative rate, and simultaneous detection of viable and nonviable particles, among others (4). Because BFPCs evaluate aseptic environments in real time, they can help reduce the lag time between the finish and release of a manufacturing batch.

In this white paper we will provide an overview of the current EM landscape for sterility assurance for injectable drug manufacturing, a description of BFPCs and their value for EM, how BFPCs are designed to meet regulatory guidelines such as EU Annex 1, and an overview of the BFPC validation process.

Current technology landscape for environmental monitoring

The evolution of technologies to support CCS is a chain of fit-for-purpose solutions leading to the continuous EM strategies of today. The first laminar flow cleanroom was invented in 1962 to eliminate fine particulate matter during the manufacture of micro-scale nuclear weapons components (5). Cleanrooms eventually evolved into various other forms as a response to scientific or other industry needs. Laminar flow hoods can protect research samples from operator contamination, while biosafety cabinets protect users from contamination coming from microbiological samples. Restricted access barriers systems (RABS) are the next step in the technology chain, protecting food and drug products from operator contamination. Eventually, isolators were designed to reduce operator contact with products, for example inside an aseptic filling chamber. The pinnacle of today's technology is the robotic gloveless isolator, which handles the aseptic filling process through automation and robotics to dramatically reduce contact between operator and drug product.

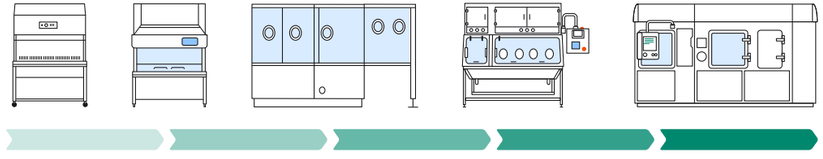


Fig 1. The evolution of technologies to support CCS

When any of these technologies are used for aseptic filling, the major concerns for manufacturers are contamination control and EM of the filling environment to assure regulatory bodies that products have been manufactured aseptically. The traditional method for EM has evolved over the years but generally depends on a compendial approach to intermittent sampling. Typically, viable particulate sampling is achieved through a combination of active air sampling instruments, passive air sampling with settle plates to collect contaminant fallout, and contact sampling with swabs and agar plates that are pressed directly onto work surfaces. These techniques involve sample culturing to visually detect viable colony forming units (CFUs), which can take anywhere from 2–14 days depending on the microorganism. Nonviable particulates (e.g., dust, skin flakes, microplastics) are generally monitored using portable machines for airborne sampling, often based on light-scattering detection technology.

As biopharmaceutical manufacturing technologies have matured, both the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) have shifted their EM emphasis toward continuous improvement, stronger data requirements, and the adoption of modern technologies to meet more stringent standards for aseptic manufacturing and filling. Also, FDA guidance for process analytical technology frameworks for quality assurance in pharmaceutical manufacturing emphasizes technologies that are in-line with manufacturing and focused on gains in efficiency and consistency (6).

This regulatory momentum toward more rapid, stringent, continuous, and consistent contamination detection methods has led to the development of several modern approaches for EM. Methods being developed or used include the detection of microbial autofluorescence (molecular detection), respiration (CO_2 detection), genetic material (PCR testing), and enzymes (H_2O_2 detection) to name a few (4). The use of autofluorescence detection in BFPCs enables an EM process that is continuous, real-time, and rapid, designed to meet the highest criteria in both EMA and FDA guidance.

General Technology	Mode of Action (3)	CCS Element* Applicable To
Intrinsic fluorescence and Mie scatter	Measurement of total and biologic particles in air or water through detection of intrinsic fluorescence	Personnel and training, facility, process, investigations
Fluorescence (e.g., Viability staining	Measurement of total particulate and viable cells in air or water through detection of extrinsic fluorescence	Personnel and training, facility, process, raw materials, investigations
Bioluminescence	Measurement of viable organisms in sterile and non-sterile samples	Process, raw materials, investigations
Enzyme Indicators	Measurement of bio-decontamination process using gaseous hydrogen peroxide	Process, investigations
Respiration Methods	Measurement of sample changes resulting from microbial respiration (e.g., CO2-related changes in color/fluorescence, pressure changes)	Process
Raman	Spectral signature of each particle is obtained for the identification and enumeration of organisms through comparison to a library of known microorganism signatures	Facility, process, investigations
Flow Cytometry	Measurement of intrinsic or extrinsic fluorescence to enumerate viable counts	Personnel and training, facility, process, raw materials, investigations
Solid Phase Cytometry	Viability or species-specific stains are used with resulting fluorescence detection to enumerate bioburden	Process, raw materials, investigations
Polymerase Chain Reaction	Detection of specific species for testing water, wastewater, in-process samples and raw materials	Process, raw materials
Automated Colony Detection	Colony- forming unit enumeration through detection of auto-fluorescence and growth using optics/camera	Personnel and training, facility, process, raw materials

Table 1: General MMM Technologies and Their Applicability to Elements of a CCS
* This table is a compilation of information taken from technologies listed in the Microbiology Consultants RMM Product Matrix (3). Some methods fall under or use multiple general technologies (e.g., use of fluorescence in flow cytometry).

The pharmaceutical industry has long relied on traditional methods to monitor product sterility, safety, and quality. Most injectable drug manufacturers still use traditional methods for sterility assurance in manufacturing. But reliance on these methods has drawbacks during the aseptic filling step.

First, sample culturing is retrospective, requiring the hold of manufacturing batches until acceptable incubation periods have elapsed, up to a few weeks. This additional time isn't ideal for newer, less stable therapeutic modalities, especially those requiring cryogenic storage.

Second, intermittent sampling is an all-or-nothing approach. Positive microbial growth that indicates potential contamination can't be pinpointed to a specific time during the aseptic filling process. A positive result also requires an extensive investigation to characterize it and may lead to disposal of the entire manufacturing batch. Also, sample culturing methods provide only a snapshot of the filling environment, because they sample either a very narrow timepoint or a very small area of the environment, as with swabs and contact plates.

Sample culturing also runs the risk of false negatives, because not all viable microbes are culturable on traditional growth media. False negatives carry the potential for future drug

recalls when contamination is discovered in the clinical setting – or even worse, harm or death if not discovered before administering the therapeutic.

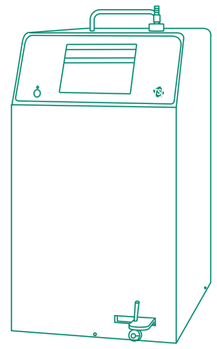
Lastly, traditional sampling methods require separate systems to sample viable and nonviable particulate contamination. This increases the potential variables in a CCS, as well as the resources and costs. In fact, traditional methods tend to be extremely costly for manufacturers who don't have onsite laboratories to incubate and analyze cultured samples.

In contrast to traditional EM methods, BFPCs are automated, continuous, and operate in real time to detect both viable and nonviable particulates. Currently, the adoption of BFPCs is more prevalent among larger pharmaceutical companies and biotech companies, but progress in adoption has been somewhat slow. Among 11 member companies in the BioPhorum biopharmaceutical community who are actively integrating BFPCs into their EM strategies, 82% are still in the validation stage, and only one company has achieved full implementation (7). However, the design and configuration of BFPCs makes them well-adapted for the future direction of EM for aseptic filling.

For example, recommendations from the Commercial Lines of the Future workstream of the Fill Finish Phorum, a pharmaceutical industry consortium facilitated by BioPhorum include (8):

1. "The development of closed assets where the risk of contamination is removed by using automation to both complete the filling operation (including supporting process steps such as line setup, format parts change and settle plate handling) and manage other activities in the cleanroom, with further reduction in costs associated with environmental monitoring (EM)/particulate monitoring (PM) activities. (§2.0)"
2. "The implementation of continuous processing and right-time release through application of process analytical technology (PAT), in-line testing and in real time; adopting rapid microbiological testing methods (§2.0)."

Closed filling technologies, such as robotic gloveless isolators, are designed to reduce the operator interventions inherent in traditional options such as RABS. Robotic gloveless isolators are well-suited to supporting goal #1 above; using them in conjunction with the automated functionality of BFPCs offers further support. The real-time continuous monitoring afforded by BFPCs aligns with goal #2 and allows "right-time release" of products.



Advantages of BFPCs for EM during aseptic filling

Unlike traditional particle counters and active air samplers that classify particles solely based on size, BFPCs use laser-diffraction technology—more specifically, laser interferometry—to provide a more nuanced analysis of particulate contamination. Laser interferometry allows BFPCs to differentiate between viable microbial contaminants—such as bacteria and fungi—and nonviable particles like dust, skin flakes, and microplastics.

BFPCs operate on the principles of light scattering and autofluorescence. When a particle passes into the sampling channel and through a laser beam (for biopharmaceutical testing, this is typically a 405-nm wavelength blue laser), it scatters light, which allows the instrument to determine its presence and size. This provides a total particulate count including viable and nonviable particles.

Viable, biological particles contain specific biomolecules—tryptophan, reduced nicotinamide-adenine dinucleotides (NADH), and riboflavin—which auto fluoresce when exposed to the detection laser. BFPCs are equipped with fluorescence detectors, enabling them to detect viable particles, or autofluorescence units (AFUs), separate from non-AFUs, which are counted as nonviable particles.

BFPC sampling instruments are placed in critical areas of the filling apparatus—for example next to the filling pedestal in a robotic gloveless isolator or near a stoppering chamber—and oriented in the direction of the airflow to maximize sampled air volume. This captures EM data at the point where contaminants have the greatest chance to impact the sterility of a drug product.

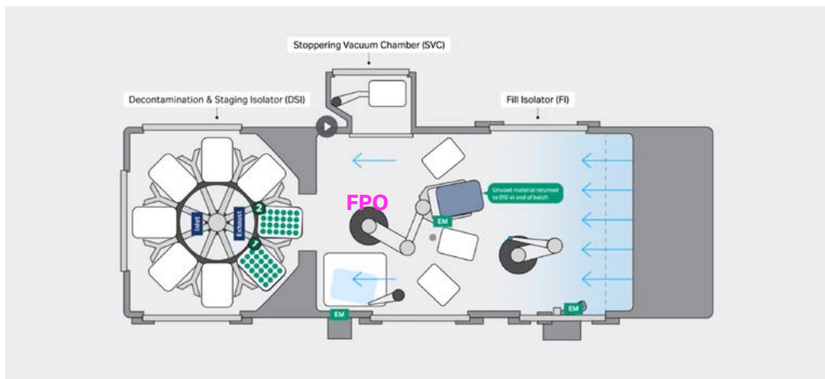


Fig 2. Position of BFPCs in filling sequence

For pharmaceutical manufacturers, the use of BFPCs for EM offers several advantages over growth-based sampling.

Improved sensitivity for viable particle sampling:
BFPCs detect and quantitate biological particles with higher sensitivity than traditional methods. This offers a substantial advantage, especially in environments where product quality is paramount (e.g., when manufacturing biologically vulnerable modalities such as allogeneic cell therapies.) BFPCs have demonstrated both lower false positive and false negative rates versus traditional EM methods (9). Using BFPCs dramatically reduces false negatives resulting from viable contaminants that can't be cultured in traditional growth media (10).

Real-time monitoring:
BFPCs provide continuous real-time EM data, allowing for immediate feedback on potential viable contaminants. In contrast, traditional EM methods involve time-consuming incubation periods and may result in long delays before manufacturing batch release. Real-time monitoring enables early warning and timely intervention, as well as quicker batch release. With BFPCs, pharmaceutical companies can make timelier decisions and take quicker corrective actions to better align product quality and compliance with regulatory standards.

Dual-mode sampling:
BFPCs avoid the need for separate systems to detect viable and nonviable particulate contamination. Using one system can reduce the associated costs of a second instrument to improve cost efficiency.

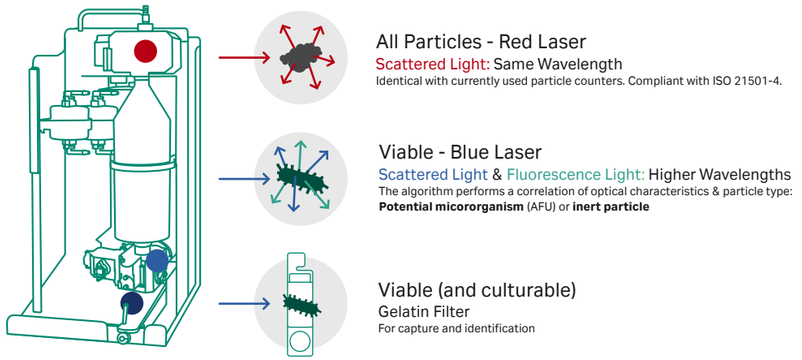


Fig 3. Positioning of the red/blue lasers and gelatin filter in the BFPC

Operational cost savings:
Real-time monitoring with BFPCs can lead to significant savings, potentially preventing batch losses of 85%–90% compared to traditional EM methods, according to a 2024 study commissioned by Cytiva. For a pharmaceutical batch value around $3 million, these savings can be substantial. Because BFPCs monitor in real time, only the nest of containers in the filling chamber during a positive count runs the risk of being rejected, instead of losing the entire manufacturing batch.

Regulatory compliance:
Annex 1 guidelines emphasize viable and nonviable EM and continuous data-driven monitoring. This can be especially important for advanced therapeutic medicinal products like drugs that incorporate viral or virus-like delivery vehicles, bispecific antibody products, and cell therapies of any kind. Annex 1 requires manufacturers to justify their CCS based on the specific requirements of the drug they're manufacturing. For more complex, less stable drug modalities and formulations, it can be difficult to justify traditional EM methods. BFPCs greatly reduce the burden of qualification of aseptic strategy under Annex 1.

Qualifying and validating BFPCs

Manufacturers often point to the complexity of qualifying non-traditional EM methods as a major hurdle to their adoption. Most require external support, which complicates and lengthens the adoption process. The change management required for manufacturers to adopt rapid microbiological methods for EM, such as BFPCs, requires an updated approach to improve and enable the qualification and validation process for new BFPC users.

There are five key steps in this process:

- **BFPC vendor primary validation:** The vendor-provided package should demonstrate the ability of the BFPC to detect viable particles. It should also show that the BFPC is comparable or noninferior to the traditional methods of monitoring viable particles using a standalone instrument.
- **Baseline studies:** This establishes the expected level of viable counts when using a BFPC within an aseptic filling process.
- **Interference study:** This process demonstrates that materials used in the aseptic filling of an injectable drug—such as a nest, tub, container, adhesive—have a low probability of causing false positives during BFPC-mediated EM.
- **Manufacturer baseline and interference study:** In this step, the earlier interference and baseline studies are repeated during operational or performance qualification. The objective is to verify that the BFPC behaves as expected consistently during the actual manufacturing process.
- **Demonstrating comparability and changing CFU acceptance levels to AFU acceptance levels:** While Annex 1 "encourages adoption of rapid microbial methods (§9.28)," the guidance continues to include defined limits of detection based on CFUs (3). As such, AFU-CFU comparability must be established for regulatory bodies to be able to evaluate EM data from batches aseptically filled under BFPC-mediated EM strategies. This can be difficult, because AFUs are more sensitive to viable particles than CFUs. Comparability studies establish to regulatory bodies that the sensitivity of AFU data generated by BFPCs meets the CFU-based limits found in guidance documents, such as Annex 1. This step also establishes the baseline AFU levels for evaluating future aseptic filling processes.

  Comparability can be demonstrated using baseline and interference studies conducted and provided by the BFPC vendor. Or it can be demonstrated by biomanufacturers performing their own parallel testing to determine the distribution or range of CFUs based on the AFU results. The most conservative approach for changing acceptance levels is to continue to use the existing CFU levels and extend this to AFU data from the BFPC.

The qualification and validation approach outlined here reduces end-user testing, addresses concerns about false positives, and helps manufacturers build comfort in the use of BFPCs, by correlating results with traditional EM methods. It also helps accelerate the path towards production following good manufacturing practices (GMP) and the collective end goal as an industry–delivering therapies more quickly to people who need them.

Fig 4. Rapid qualification and validation process for BFPCs in Cytiva robotic, gloveless isolators. TSI is the current BFPC vendor.

Conclusion

The journey towards adopting rapid microbiological methods in the pharmaceutical industry has been gradual, with hurdles still in place. However, there's a growing recognition of, and regulatory inertia toward, the need for real-time monitoring solutions. BFPCs are an available and powerful solution to enable real-time EM for sterility assurance in pharmaceutical manufacturing. With continued collaboration between technology providers and pharmaceutical manufacturers, the industry is likely to see a shift towards more efficient and effective environmental monitoring practices, with BFPCs as a core technology. Companies that embrace these advances stand to enhance product quality, improve compliance, and achieve substantial operational savings.

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