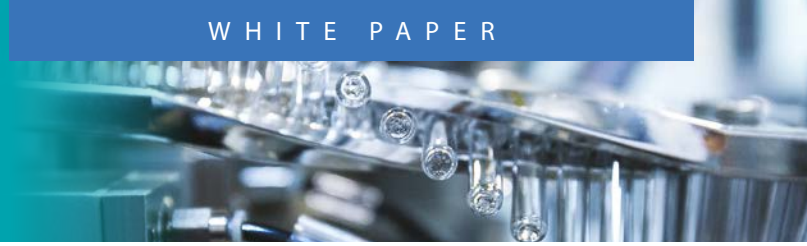
Selecting The Locations Of Particle Monitoring For The Fill-Finish Process



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Abstract

Aseptic manufacturing is required for many parenteral drugs especially biological products, but this process has the potential to introduce contaminants at several points in the manufacturing process. There are strict regulations and guidelines provided by the US Food and Drug Administration (FDA) and European regulatory bodies to manage aseptic cleanrooms, such as advising and mandating Good Manufacturing Practices (GMP) to reduce the risk of contaminants entering the drug processing environment.

One of the processes of drug production that has a high risk of contamination is during the final stages of the fill-finish manufacturing process whereby vials are being filled with drug product, which are sometimes lyophilized.

Understanding the high-risk critical areas during the fill-finish process and, identifying and monitoring particle exposure appropriately is crucial to avoid introducing any contaminants into a drug product that could pose life-threatening health risks to a patient.

This white paper focuses on the monitoring of particles (viable and non-viable) during the fill-finish process of drug manufacturing. It describes the current regulatory guidelines and considerations for the location of airborne particle monitors in the equipment design of the processing area.

Introduction

Recent years have seen a growing focus on new biologic therapies and vaccine production, most of which are administered parenterally. A recent 2018 market report stated that '50% of the drugs in the clinical pipeline are comprised of biologics'[1]. The presence of foreign particulate contamination in parenteral drugs poses a risk to patients and continues to be a leading cause of drug recalls.

Terminal sterilization, where only the final product is subjected to sterilization, is the preferred decontaminating method for injectable products. However, not all products, particularly biological formulations, can withstand the high temperatures necessary to achieve full sterilization.

In contrast, aseptic processing requires sterilization of each component as part of the manufacturing process and not the final product. This is a more challenging set-up with a risk of contamination at several steps in the aseptic filling process but enables products sensitive to terminal sterilization, to be aseptically manufactured.

Process of Fill-Finish

Fill-finish is the final operation in the manufacture of aseptic products. At this late stage of production, the biopharmaceutical product is extremely valuable, therefore product loss or contamination would be costly.

The vial journey in the fill-finish process depicted in Figure 1 below starts by being washed, sterilized and depyrogenated before being filled with a drug product. From here, it will have a stopper added and, in some cases, the product will be lyophilized. Lastly, the product filled vial will be capped, externally washed, and loaded onto a tray for inspection, labeling and packaging.

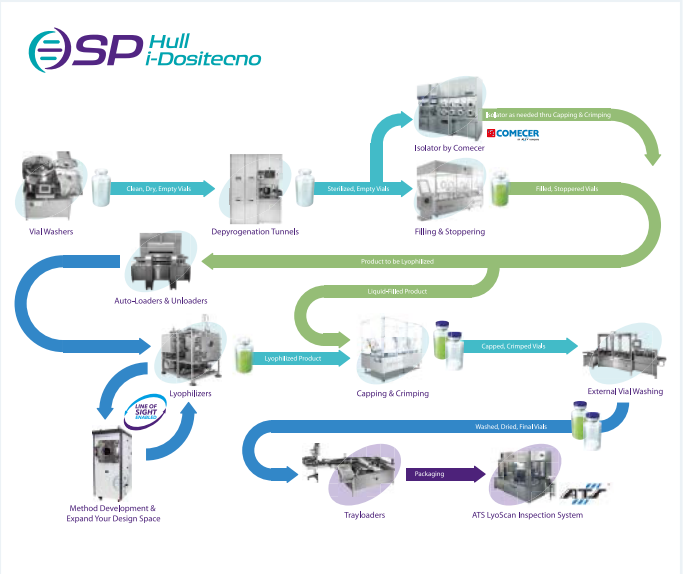


Figure 1: *Your Complete Vial Journey – From Bulk API to Production*

Accuracy, control, and monitoring must all be addressed in the fill-finish process.

Environmental Monitoring

Environmental monitoring should identify potential routes of contamination promptly so that actions can be taken before extensive product contamination occurs. Monitoring of the fill-finish process requires the monitoring of all interactive elements of the operation, such as between personnel, sterile filtered product, the fill-finish equipment system, cleanroom and support facilities, and sterilized filling components.

The integrity of these elements is undermined by airborne or surface particles in the environment, predominantly from equipment generated particles and human contact, both of which are the focus of particle monitoring. An average human sheds ten million particles a day and transports particles on their clothes and shoes every time they move around the cleanroom especially areas of skin exposures because of poor aseptic gowning techniques. Other parameters, such as temperature, humidity, air velocity and direction, and pressure differential can also influence the movement of particles in the cleanroom setting. For example, the pressure cascade enables air to flow from the cleanest area, usually the filling area, to the least clean area so any contaminants will flow away from the filling process.

Contamination during the filling, stoppering and capping processes can be reduced by a simple physical barrier between the filling operation and operators - such as an isolator or restricted access barrier system (RABS) which reduces human contact with the sterile products - or more sophisticated automated or semi-automated equipment.

Isolator systems which utilize glove box technology to minimize operator contact with the drug product are typically biodecontaminated by vaporized hydrogen peroxide. The equipment within a typical isolator is divided into an upper ISO 5 compliant isolator area and a lower technical area separated by a barrier plate. They are a popular alternative to the classic barrier equipment installation but can be complex to install, validate and operate, and require significant capital investment. The RABS is a barrier method using glove box technology in a conventional aseptic core cleanroom. The advantage of the RABS is lower investment cost and speed of validation. However, RABS can be more costly to operate and impose a higher aseptic risk than isolator systems.

Even if the system is a fully automated enclosed system, there are times when unscheduled interventions may be required and the classified area is breached for human intervention, so regular monitoring for potential contamination is crucial to provide confidence in the sterility of the final product.

Regulations

The vital nature of aseptic manufacturing for parental drugs necessitates global regulatory requirements. Each country of the world has its own regulatory guidance which is not yet harmonized but they all recognize the vulnerable stages of production and measure similar parameters irrespective of manufacturing scale. The FDA states the importance of maintaining an environment of extremely high-quality during the fill-finish stages of production where containers are at a vulnerable state of exposure to environmental particles.

Good Manufacturing Practice (GMP) guidelines in both the USA (21 CFR parts 201, 211 and 600-680)[2] and Europe (EudraLex GMP volume 4)[3] require that there is extensive monitoring and continuous data collection at every stage of the fill-finish process. Monitoring needs to be performed initially during facility performance qualification (PQ) to classify the working area, periodically for requalification, and routinely during operation.

The GMP guidelines also state that the whole process of fill-finish needs to take place in a 'clean' environment in accordance with ISO standards (ISO 14644[4], 21501[5] and 14698[6] (EN17141[7])). These standards classify clean air by the content of particles in the air and recommend action levels of microbiological quality. ISO 14644 and 21501 standards describe how to evaluate contamination in a cleanroom in terms of airborne particles. These guidelines clarify the classification of these particles and testing methods used to monitor them and provide guidelines on the design of the cleanroom which include the location, performance, and calibration of particle counters. Assessment and control of biocontamination is also covered by ISO 14698 (in the EU, this has been superseded by EN17141).

Figure 2: *Complete Fill Finish Line in An Isolator*

Particles monitored in the fill-finish process are categorized by size. In the FDA guidelines, anything above 0.5 microns (µm) at either rest or in operation is recorded. This would reveal bacteria (0.5 – 15 µm) or fungal spore (> 0.8 µm) contamination. The European guidelines further divide this category into particle sizes of 0.5 – 5 µm and ≥ 5 µm and distinguish between rest (stopped for at least 15 minutes) and in operation.

Other factors that will influence the quality of contamination detection are related to the sampling protocols. Determining the number of samples, the frequencies of the samples, and the location within the workspace is another critical decision to be rationalized.

Once the vulnerable areas of potential contamination during the fill-finish process have been determined, methods to identify and monitor airborne particles are established.

Monitoring of Airborne Particles

The regulations for aseptic manufacturing classify airborne particles as extraneous contaminants (non-viable, NVP) or viable biological microorganisms. Viable microorganisms are those that can cause illness to a patient, but NVP can also be harmful when injected into a patient.

Monitoring Methods

There are several different methods to direct, capture and measure these airborne particles. Most of these work by actively monitoring the airflow, or passively via the incubation of agar plates, or a combination of both. The chosen method needs to demonstrate high-efficiency by the good physical capture of particles, and, in the case of viable particles, the agar plates must retain their biological ability to grow. Regulations also specify how the monitoring system should be qualified and calibrated, and how to maintain sterility of the equipment e.g., using sterilizable stainless steel or single-use material.

1. Viable Particle Monitoring

Viable particles can be actively monitored via an air sampler which captures particles from one cubic meter of air passed onto media (water or gel) for subsequent incubation over 48 hours. Each batch is then quarantined until the media has been inspected and analyzed for growth of colony-forming units (CFUs). In the event of positive bacterial growth, a formal investigation is required.

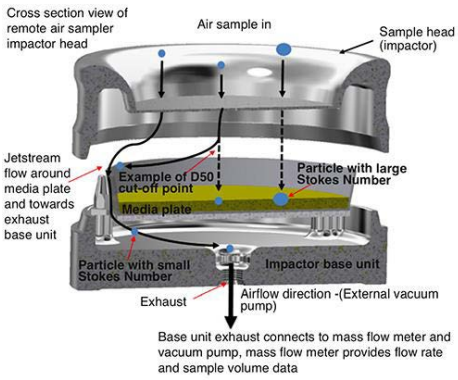
In contrast, passive monitoring utilizes settling plates of agar that are exposed in the environment and capture particles over a 4-hour period, after which they are incubated and analyzed.

Air samplers vary according to how the particles are monitored and by the rate of sampling, precision, and recovery. All have advantages and disadvantages in a laboratory and production setting and no one model overcomes all the limitations.

Results from active instruments are considered quantitative, while those from passive settling plates are qualitative or at best partly quantitative.

- An impaction air sampler draws air in through small holes in the sampler and deposits microorganisms onto an agar plate. Air is drawn in by a vacuum pump and in the case of the *slit-to-agar air sampler* (STA), this is through a standardized slit, whereas in a *sieve impactor* it is through a perforated cover with predetermined sized holes (Figure 3). These are the two most popular air samplers as they are small and relatively easy to place in any area. Sterility can be maintained in the entire unit by steam sterilization if it is made from stainless steel or using single-use components. In a closed system, such as in an isolator or RABS, sieve impactors are the most beneficial as they are available as self-contained, portable units and systems for full integration with the aseptic line.

Sieve Impactors - Principle Credit: Lighthouse Worldwide Solutions



- Steam Sterilizable (Stainless Steel)
- Require Aseptic Assembly

Single Use Sieve Impactor Credit: Particle Measurement Systems

- Single Use
- No Aseptic Assembly

Figure 3: *Type of Sieve Impactor for Viable Monitoring*

- An alternative to this type of air sampler is a *centrifugal-based monitor* that draws air into the sampler head through a rotating vane mechanism (centrifugal propeller sampler). The microorganisms are thrown out of the air and onto strips of agar through a centrifugal force. Although it is convenient and flexible, there are limitations and doubt as to whether it can collect all viable particle sizes and therefore reducing efficiency.
- Another mechanism to collect airborne viable particles uses a *filter* to capture any microorganisms from the air which is transferred to a culture medium and incubated. If the filter is made from gelatin, it avoids desiccation and damage to the microorganisms that can occur with other forms of membranes, although gelatin membranes are relatively fragile to handle.
- It is also possible to capture particles from the air that impact into a liquid medium. Known as *impingers*, these air samplers consist of a specially designed tube, made from glass or perfluoroalkoxy (PFA). Air flows through the tube and the particles impact a specified liquid. Aliquots of the liquid are then plated onto agar to determine the microbial content. Colonies develop on the medium where the organism impinges.

Most of these methods rely on the culture of microorganisms, but these approaches have limitations, the most significant of which is the time required to culture the microorganisms. The data is therefore always retrospective and doesn't allow for immediate corrective action. Another hazard of cultured methods is the desiccation or dehydration of the media which will kill the microorganisms before they have had the chance to grow.

Over the past few years, some new technologies have been developed for sampling airborne particles that can determine the numbers in real-time and can also detect microorganisms that cannot be recovered using conventional microbiological agar plate methods. *Laser-induced fluorescence (LIF)* is a real-time airborne microbial detection technique based on the scattering of light. It also has the benefit of being able to detect and distinguish between viable and non-viable particles since microorganisms contain relatively high concentrations of fluorescent molecules and therefore will scatter light at different wavelengths. There is, however, a risk of false positives, since not all particles that fluoresce are colony-forming.

2. Non-Viable Particle Monitoring

Although detecting viable microorganisms is of obvious significant concern to the manufacturing of drug products and patient safety, non-biological foreign matter e.g., dust and particulates can also be harmful to a patient. It has been reported that the presence of non-viable particles can also reduce product yield when a cleanroom has been breached after rejection, so it is vital to monitor these airborne contaminants in addition to viable particles.

A light scattering device can be used to identify the size and number of total viable and non-viable particles and provide immediate results in real-time. A beam is shot from a laser diode and as it encounters the contaminating particle, it scatters the laser. This scattering is presented on a photodetector which sends an electronic signal in relation to the size and number of the particles (Figure 4). Whereas the viable particle monitors measure actual microorganisms, non-viable particle monitors measure particles calibrated against latex spheres and therefore actual particle sizes may differ by +/- 20%.

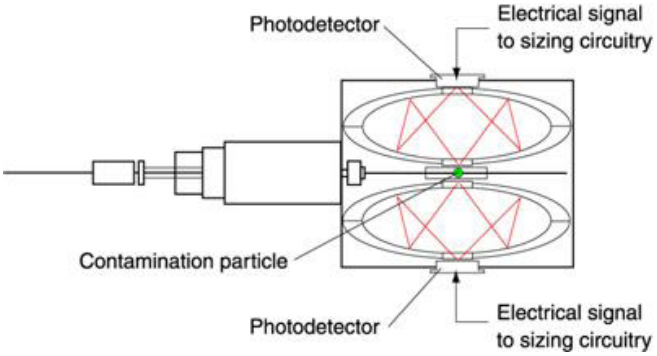


Figure 4: *Light Scattering Particle Counter - Principle* Credit: PMT (GB)

When installing a particle monitor, the speed of the airflow and length of tubing need to be considered according to the guidelines for GMP. The ISO 14644-2[4] standard states the tubing length should be less than 1 meter with minimal bends to minimize the loss of the larger ≥ 5.0 µm particles. Particle loss in tubing can also be caused by the lack of turbulent airflow identified by a dimensionless Reynolds number (Re) which is expressed in terms of tube dimension, speed of airflow and fluid properties. The Re number can predict the flow pattern of a fluid. It is suggested for particle monitoring in a fill-finish process, a Re of between 3,000 and 5,500 would provide appropriate turbulent flow.

The inclusion of an isokinetic port on a scattering device ensures larger macroparticles (> 5.0 µm) are captured and not prevented from being pulled into the inlet. The port also ensures particles travel at the same speed as the aesthetic environment (0.45 m/sec or 90 ft/min) which is an ISO 5 requirement.

Location of Particle Monitoring Systems

Understanding the high-risk areas of particle exposure and monitoring these appropriately can be a difficult but important decision as it directly impacts product sterility and patient safety. Inadequate risk assessments can lead to poor monitoring results with badly positioned monitors misrepresenting contamination status. As the users of the aseptic line must justify the location of the particle monitors and rationalize their decision to the regulatory agencies, it is prudent to consider the space required for these monitors early in the project design.

In aseptic manufacturing of drug products, the critical areas with the greatest risk of contamination are within the fill-finish process, but it is also important to consider the dynamics of the cleanroom (overall room size, air handling systems, and equipment location) as possible sources of contamination as well.

Within the cleanroom, an open barrier door system provides the greatest risk of contamination and not considered best practice, but if an open barrier or RABS system is unavoidable, additional risk-based qualification control measures are necessary. As isolators remain closed throughout the fill-finish process, they provide the lowest contamination risk.

Within the isolator/RABS system, zones can be allocated to the filling lines so that risk assessment can be targeted, and any compromised area can be isolated and corrected without disruption of other areas. The critical zones with the greatest contamination risk are defined as the areas where vials are exposed to the environment, such as exiting from the depyrogenation tunnel, during filling, stoppering, loading into the lyophilizer (if required), and capping. When considering the location of the particle monitors during this process, the ISO 14644 standard (updated in 2015[4]) provides a reference table that specifies the number of locations required based on room size and is calculated using a statistical approach that considers a heterogeneous distribution of particles at each location (hypergeometric model of particle distribution). Within the critical zones, the monitors are placed in areas selected by risk.

For the monitoring of viable particles, monitoring needs to be performed within 12 in (30 cm) of the point of filling whereas, for the monitoring of non-viable particles, at least one monitor needs to be placed within each zone based on risk assessments. If using settle plates, these are typically placed adjacent to the viable/ non-viable devices.

Figure 6 presents an example of particle monitoring in an aseptic fill-finish process. Enclosures are separated with mouse holes that have been introduced to separate the risk zones. An isolator is used

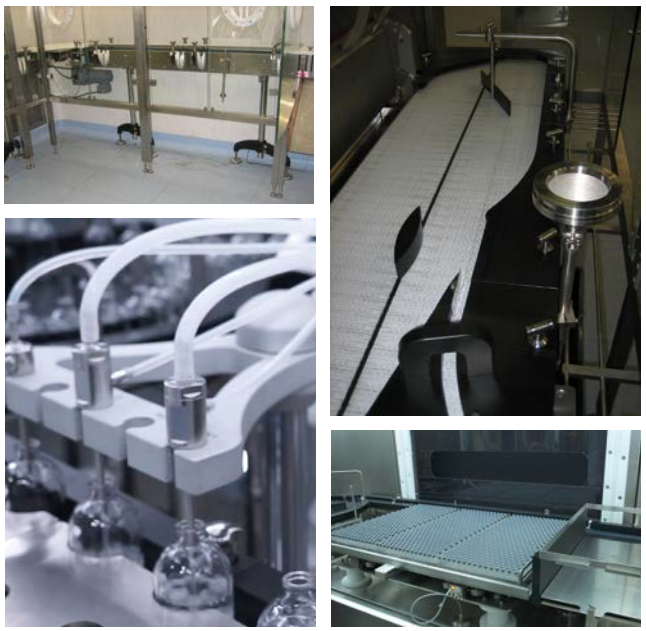


Figure 5: *Viable and non-viable particle monitors*

for vial filling and loading (as part of the lyophilization process) which are high-risk activities and incorporate viable, non-viable monitors and settle plates close to each critical point. A transfer conveyor transports the vials between these two activities and requires only a non-viable monitor as it is not considered a critical zone. A RABS is then used to transport the vials to the capping machine. Once the vials are capped, they exit the RABs and proceed to the external vial washer under clean room conditions.

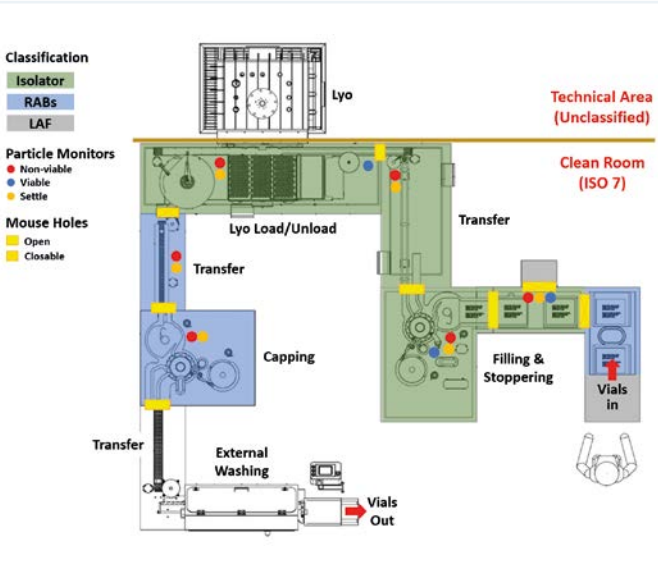


Figure 6: *Aseptic Fill-Finish Line Showing Zoning and Locations of Particle Monitors*

Since capping is not considered a critical zone, a viable sampling instrument is not required. Supports for settling plates are typically placed next to all non-viable monitors for use during validation and production activities, as defined by the risk assessment. Removing mouse holes between zones can reduce the quantity of non-viable monitors required but any intervention into the zone would then compromise a larger number of vials.

Although this example covers the main critical zones at risk of contamination during the fill-finish process, there are times when additional monitoring may be required. Extra monitoring could be advantageous at points of human entry when loading stoppers or for fault correction. Also, in areas that involve automated systems; moving robots could generate particles and create air flow disturbance increasing the risk of particles entering a vial. Such scenarios should be evaluated during the risk assessment and validation activities to determine whether particle monitoring is required.

Summary

When establishing a new fill-finish operation or upgrading a facility with new equipment, regulations for aseptic manufacturing can be difficult to navigate and it can be helpful to engage with suppliers of equipment early in the process to gain advice on the location of appropriate monitors for environmental contamination. Many suppliers have extensive experience in the designing of cleanrooms and locating possible high-risk contamination areas along with up-to-date knowledge of the appropriate regulations.

Prior formal risk assessment of transfer and deposition of particles in the specified environment can be important to base some of the initial location decisions on. There are mathematical models available that can calculate these values and are regularly used as part of a Quality-by-Design (QbD) approach to drug manufacturing.[9]

There are detailed regulations and guidelines from the US FDA, the EU and other national bodies on GMP that cover aseptic processing and provide information to help identify critical high-risk areas and describe methods used to protect the drug product from both viable and non-viable particle contamination. Understanding the characteristics and limitations of barrier systems and the various types of airborne particle monitors will help in making robust decisions for a fill-finish facility.

The world biopharmaceutical market is projected to grow ≥12%[8] over the next few years. Aseptic manufacturing and supporting technologies such as particle monitoring will be an essential element in providing safe and effective drugs for the future.

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