

## Particle Monitoring Requirements in Pharmaceutical Cleanrooms

All drugs must be manufactured in accordance with the current Good Manufacturing Practice (cGMP) regulations. Pharmaceutical manufacturers must demonstrate compliance with the regulations at every stage before a drug can be released to market. This paper explains the various microcontamination requirements of pharmaceutical manufacturing, defines a clean manufacturing environment, and shows how to prove contamination control over an environment.

### Why do I need a Particle Counter?

All drugs must be manufactured in accordance with the current Good Manufacturing Practice (cGMP) regulations which state that cleanroom validation must be performed and impose limitations for production environments. In the United States these regulations are governed by the Food and Drug Administration (FDA) as the 21st Code of the Federal Regulations (CFR). In Europe, European Commission (EC) guidelines must be met. The pharmaceutical company manufacturing the product must, therefore, prove that they have been in compliance with the regulations at every stage before a drug can be released to market and ultimately the end user.

The US cGMP regulations govern various drugs manufacture activities including

- Organization and Personnel [21 CFR 211 Subpart B]
- Buildings and Facilities [21 CFR 211 Subpart C]
- Production and Process Controls [21 CFR 211 Subpart F]

A pharmaceutical company must have a quality control department that has the responsibility for drug approval independent of the production department. This department is responsible for the routine quality assurances that:

*Establishes documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.*  
FDA definition in 'General Principles of Validation, May 1987.

To satisfy the requirements, the products are manufactured in a controlled environment. Cleanrooms are employed to reduce the variability of potential production environments and as controlled environments can be regulated to meet specific standards. GMP regulations require that these environments be rigorously monitored to ensure that there is full and constant awareness of current environmental conditions, both for viable and non-viable contamination.

### Cleanrooms

A cleanroom is the fundamental starting point for contamination control. In the old Federal Standard 209E, a cleanroom is defined as "a room in which air filtration, air distribution, utilities, materials of construction, and equipment are maintained in a controlled manner." Operational procedures are defined and regulated for airborne particle concentrations to meet appropriate particulate cleanliness classifications. ISO 14644-1 is the international standard of defining cleanroom contamination levels.

Pharmaceutical cleanrooms are classified according to the particle concentration of the air that is required to meet the cleanliness criteria for the manufacturing process being performed. Using the ISO standards, the higher the classification number, the lower the particle concentration. Originally cleanrooms were classified according the number of particles per cubic foot at 0.5 microns ( $\mu\text{m}$ ). The determination of the cleanroom class is a process based on actual statistically valid measurements, as described in the following section.



**Particle Count Room Classification**

There are three measurement phases involving particle counting in cleanrooms:

- As-Built: a completed room with all services connected and functional, but without production equipment or personnel within the facility.
- At Rest: all the services are connected, all the equipment is installed and operating to an agreed manner, but no personnel are present.
- Operational: all equipment is installed and is functioning to an agreed format, and a specified number of personnel are present, working to an agreed procedure.

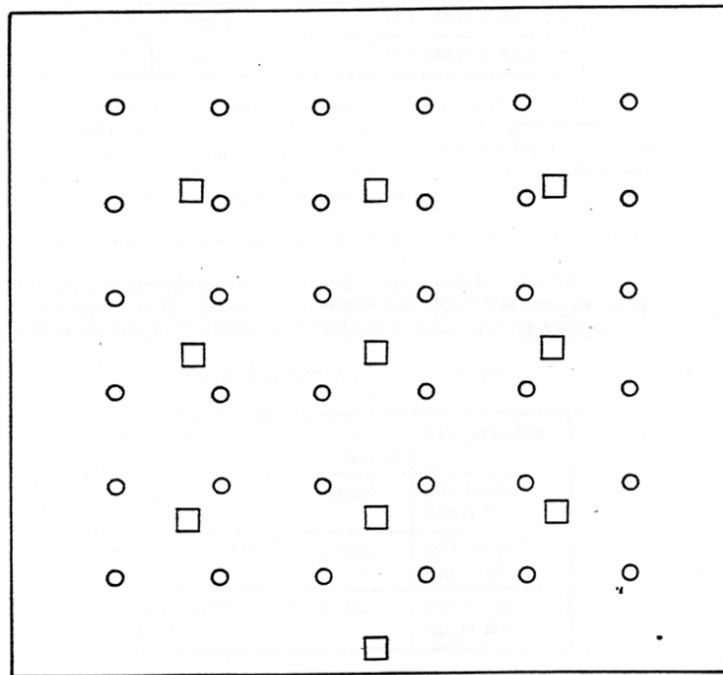
The airborne particle count test is performed by counting particles at defined grid locations within the as-built cleanroom. The test points should be equally spaced throughout the room and at work height to demonstrate the quality of the air cleanliness as it approaches the work area. Equipment location may result in modifications to the standard grid pattern.

The number of measurements taken at each test point depends on the cleanroom class and the statistical requirements specified in the standards. The standards also state that the data should permit defining the classification level with 95% confidence level. It is recommended that a particle counter capable of 0.5 µm sensitivity be used for the definition of classes ≥ ISO Class 5.

To calculate the minimum number of samples required:  $\text{Area (m}^2\text{)}^{0.5}$

Minimum sample volume is determined by  $\frac{20 \times 1000}{\text{Class limit}}$

Total required sample time (minutes):  $\frac{\text{Minimum volume} \times \text{minimum number samples}}{28.3}$



○ Nonunidirectional Airflow, FS209E Class 4.5

□ Nonunidirectional Airflow, 14644-1, Class ISO 6

The following table shows the ISO14644-1 cleanroom classifications.

Table 1 Selected airborne particulate cleanliness classes for cleanrooms and clean zones						
ISO classification number (N)	Maximum concentration limits (particles/m <sup>3</sup> of air) for particles equal to and larger than the considered sizes shown below (concentration limits are calculated in accordance with 3.2)					
	0,1 µm	0,2 µm	0,3 µm	0,5 µm	1 µm	5 µm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1 000	237	102	35	8	
ISO Class 4	10 000	2 370	1 020	352	83	
ISO Class 5	100 000	23 700	10 200	3 520	832	29
ISO Class 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO Class 7				352 000	83 200	2 930
ISO Class 8				3 520 000	832 000	29 300
ISO Class 9				35 200 000	8 320 000	293 000

**NOTE:** Uncertainties related to the measurement process require that concentration data with no more than three significant figures be used in determining the classification level.

Pharmaceutical cleanrooms typically operate at Class 5 (most aseptic areas), Class 7 (surrounding areas), or Class 8 (support areas).

### Pharmaceutical Cleanroom Utilization

We can prove that a cleanroom meets a standard using a particle counter. The room classification achieved also dictates which production activities can be performed in the cleanroom. A document produced by the FDA and published in 2004 defines two areas. A “critical” area is where the sterilized drug product, containers, and closures are exposed to environmental conditions that must be designed to maintain product sterility, and a “supporting” clean area is where nonsterile components, formulated products, in-process materials, equipment, and container/closures are prepared. The environmental requirements for these two areas are given in the FDA Guide as follows:

**Critical area:** This area is defined as critical because it contains sterilized products that, if exposed, are vulnerable to contamination. To maintain product sterility, it is essential that the environment in which aseptic operations are conducted be controlled and maintained at an appropriate quality. One aspect of environmental quality is the particle content in the air. Particles are significant because they can enter a product as an extraneous contaminant and can also contaminate it biologically by acting as a vehicle for microorganisms.

*Air in the immediate proximity of exposed sterilized containers/closures and filling/closing operations would be of appropriate particle quality when it has a per-cubic-meter particle count of no more than 3,520 in a size range of 0.5 µm and larger when counted at representative locations normally not more than 1 foot away from the work site, within the airflow, and during filling/closing operations. This level of air cleanliness is also known as Class 100 (ISO Class 5).*

(Guidelines on Sterile Drug Products Produced by Aseptic Processing, CDER, FDA 2004)

Particle Measuring Systems recommends that measurements to confirm air cleanliness in critical areas be taken at sites where there is the most potential risk to the exposed sterilized product, containers, and closures. The particle counting probe should be placed in an orientation demonstrated to obtain a meaningful sample. Regular monitoring should be

performed during each production shift. We recommend conducting nonviable particle monitoring with a remote counting system. These systems are capable of collecting more comprehensive data and are generally less invasive than portable particle counters.

HEPA-filtered air should be supplied in critical areas at the point-of-use. The air flow should be at a velocity sufficient to sweep particles away from the filling/closing area and maintain unidirectional airflow during operations.

**Supporting Clean areas:** Classification of a supporting clean area is explained by the FDA as follows:

*The nature of the activities conducted in a supporting clean area determines its classification. It is recommended that the area immediately adjacent to the aseptic processing line meet, at a minimum, Class ISO 7 standards under dynamic (operational) conditions. Manufacturers can also classify this area as Class ISO 6 or maintain the entire aseptic filling room at Class ISO 5. An area classified at a Class ISO 8 air cleanliness level is appropriate for less critical activities (e.g., equipment cleaning).*

(Guidelines on Sterile Drug Products Produced by Aseptic Processing, CDER, FDA 2004)

### Environmental Monitoring

To meet the required cGMP compliance, an area has to demonstrate that it meets the specifications required for drug manufacturing. A clean manufacturing environment needs to be rigorously monitored to ensure that there is full and constant awareness of current conditions, including the detection of periodic events which could be catastrophic if gone unnoticed. Constant monitoring creates a continuous flow of information, resulting in a large quantity of data which can be used to watch for trends.

The manufacturing facility should therefore have a comprehensive environmental monitoring program, which includes monitoring for non-viable and viable airborne particulates, surface viable contamination and, in the aseptic areas, personnel [21 CFR 211.42]. These procedures should address frequencies and locations for the monitoring sample points, warning and alarm limits for each area, and corrective actions which need to be undertaken if any of the areas show a deviation from expected results. Actions taken when limits are exceeded should include investigation into the source of the problem, the potential impact on the product, and any measures required to prevent a recurrence.

In general, less frequent monitoring is required in areas of a lower classification (ISO Class 8 or unclassified rooms). This reduced frequency monitoring performed in "controlled" environments (ones with some level of particulate controls) should be of the same integrity as that sampled in the highest classification.

During a quality or regulatory audit the specifications for viable and non-viable particulates will be reviewed. Focus is placed on the viable monitoring as this will potentially have the greatest impact on the final product. Rooms are, however, classified for both, with the levels of viable particulates being a function of the room classification, determined by non-viable monitoring.

### EC Based Cleanroom Classes

Room classification	Maximum concentration limits (particles/m <sup>3</sup> of air) for particles / Airborne Viabiles (cfu/m <sup>3</sup> )		
	0.5 µm	5.0 µm	Viable cfu/m <sup>3</sup> And / 90 mm settle plate
A – Operational	3,500	1	<1 <1 / 4 hrs
B – Operational	350,000	2000	<10 <5 / 4 hrs
C – Operational	3,500,000	20,000	<100 <50 / 4 hrs
D – Operational	Not defined	Not Defined	<200 <100 / 4 hrs

Manufacturers must determine that ISO Class 5 conditions have been validated and are maintained in areas where sterile product and components, including container/closure systems, are exposed; ensure that if limits are exceeded, an investigation is conducted and appropriate action is taken; and perform microbial identification, especially in aseptic areas, and watch for trends.

When a product is to be exported to Europe, it must comply with the EC guidance on room classification. The EC requires that a room be classified in accordance with the ISO14644-1 test methodology but apply the limits as set out in Annex 1. To achieve this standard, a minimum of 1m<sup>3</sup> of sample must be taken per room; this is to satisfy the statistical confidence that the target of <1 at 5.0 µm has been achieved.

### **Monitoring Particulate Levels in Cleanrooms**

After a room has been verified as meeting ISO 14644-1 for room particle levels and has established cleanliness suitable for product manufacture, it must be proven that the cleanroom can be maintained at that level. The cGMP guides from both the EU and the FDA define what levels of particulates are allowed for sterile manufacturing and how and when monitoring should take place.

#### ***Portable Particle Counter***

A compact approach to maintaining cleanliness is to build data storage into a portable particle counter. The data can then be exported to a computer for statistical manipulation. However, there is an increasing need to monitor more locations, more regularly than can be easily achieved using a portable counter. This need is being driven by the desire to reduce operational costs, increase confidence in good manufacturing practices, and fulfill regulatory requirements.

#### ***Facility Monitoring System***

One way of achieving the monitoring levels required is to install a Facility Monitoring System (FMS) which includes particle sensors. An FMS is either a single continuous particle counter installed into a critical location, or an arrangement of instruments linked to a central monitoring computer suitable for making the measurements required. The computer controls the intake of data from the particle counters, logs and displays the information, and reports to the operator any changes in conditions or trends.

Inputs to the FMS may be from facility sources other than particle counters. This leads to a full, independent environmental monitoring system which can accept data from viable monitoring, differential pressure sensors, air velocity, and temperature/relative humidity sensors.

An automated FMS will provide increased vigilance while decreasing the labor requirements to make measurements, manually transfer data to interpretive applications, and produce reports to support product release. ISO 14644-2 says that a Class 5 cleanroom with an installed continuous particle monitoring system can be revalidated every 24 months. However, if a continuous system is not in place, revalidation must occur every 6 months.

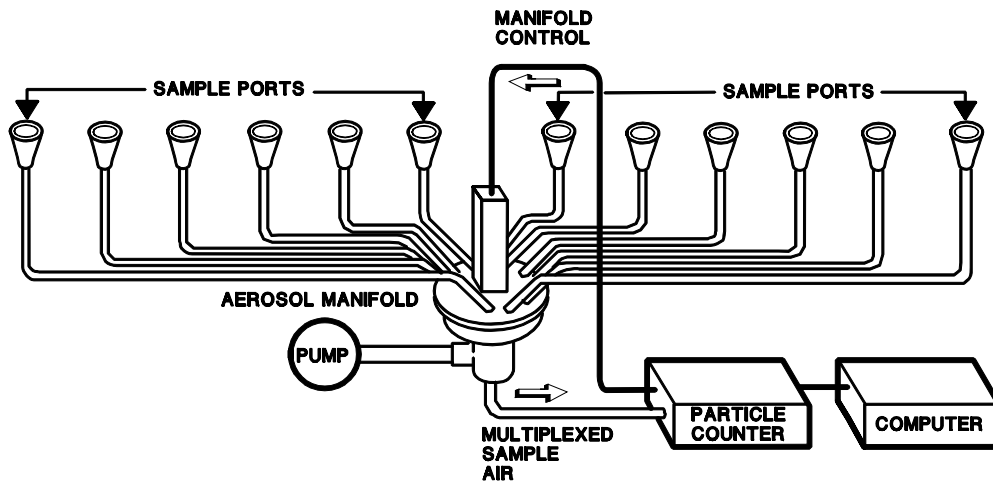
If the system is well planned, fast detection of potential problems in operating conditions will occur enabling counter measures to be taken rapidly. Any significant trends in operating conditions over a long-term, can be monitored. Statistical analysis of data should allow for closer control and identification of normal and abnormal conditions.

There are three basic approaches to obtaining automated particle counts:

- Manifold system (using multiple tubes via multi-port scanning) linked to a particle counter
- Individual particle sensors
- Combination of manifolds and particle sensors

#### **Manifold System**

This solution is very common and consists of a central manifold with up to 32 sample tubes radiating from a central manifold location. Each tube is capable of drawing a sample from up to 38 m from the manifold into a single particle counter. The data from each of the monitored sample points is reported back to a central monitoring software package. The software is a validated package, which reports the data to the users in multiple formats. These formats include real-time current values, spreadsheet viewing of historical data and live time plots. To report problems to field operators a system of local alarm devices, paging, and email alerting of out of condition warnings and alarms is employed.



Advantages of a manifold system:

- Low cost per sample point. This system requires a particle counter, aerosol manifold, and a length of flexible tubing for each position monitored.
- Low maintenance and calibration costs; only a single particle counter per manifold to calibrate and service.

Disadvantages of a manifold system:

- Only samples one location at a time, and transient events may be missed. To offset this, sample sequencing may be biased to monitor the most critical locations more often.
- Loss of particles of 5  $\mu\text{m}$  and greater in the tubes may occur due to sedimentation and impaction. However, a properly designed system that maintains turbulent flow in the tubes to eliminate unnecessary valves and minimizes sharp bends.

#### Individual Particle Sensors

Increasingly, to ensure that continuous monitoring is preserved, dedicated, locally-mounted particle sensors are being used. A sensor consists of a small enclosure, housing an optical system, a light source (laser diode), and signal generation electronics. The sensors often require an external vacuum source and signal communication cable to transmit data to the central monitoring computer.

Advantages of individual sensors:

- Automated, so lower personnel costs
- Continuous monitoring and reporting of data, therefore detecting short-lived particle bursts
- Simple, low-cost installation
- Ease of relocation to alternative positions; easy to rotate out for servicing
- Highest level of confidence

Disadvantages of individual sensors:

- More instruments
- Higher initial equipment cost
- Higher maintenance cost

### Combination System

An alternative to the above choices (manifold or individual sensors) is a combination which uses the advantages of both systems. In this solution, the majority of sampling is monitored using the manifold system, while specific critical locations are continuously sampled by individual sensors.

### **Special Considerations**

There are various applications that require monitoring as part of process control but have special considerations; two different applications are identified below:

#### ***Sterilizing Tunnels***

These are typically classified as the first part of the aseptic environment, and therefore ISO Class 5. As a classified environment, it must be regularly monitored.

One of the monitoring functions of a particle system is to gauge the presence of viable particles. In this case, the environment is sterile due to high temperatures; therefore the first potential for viable organisms to exist is within the cooling zone where temperatures drop to a point which may support viable organisms. If sampling of the hot zone must be performed, then the sample must be cooled to a sufficient temperature acceptable by the particle counter. This is typically less than 35°C. The only method of cooling the sample down is to use a cooling probe.

#### ***Lyophilization***

The process under which products are manufactured using lyophilization requires a standard aseptic manufacturing filling operation, sterilization of the vials, classic filling in either open or isolator fill lines and subsequent semi-stoppering of the final product. The environment where the freeze dryer is loaded needs to be a sterile area due to the non-closure of the final stopper prior to freeze drying; additional controls to prevent contamination should be in place. The stopper is placed on top of the vial and is ultimately seated in the Lyophilizer. As a result, the contents of the vial are subject to contamination until they are actually sealed.

The required monitoring for critical areas should be continuous and within the zone immediately surrounding the product whenever the product or open container is exposed to the environment. The monitoring locations should therefore be as close as practically possible to the exposed product or semi-stoppered vials. Where a significant distance exists between the end of the filling line and semi-stoppering and the loading door of the lyophilizer, the product should be maintained within an ISO Class 5 environment and monitored at intervals throughout this distance.

### **Summary:**

Particle monitoring is required to meet cGMP requirements. Cleanrooms must be validated to demonstrate a cleanroom classification and monitored to prove that product is not contaminated. The level of monitoring varies with the classification of the cleanroom dependant on the use of the area. Monitoring particulate levels in cleanrooms can be approached using several different methods; the best method depends on the function and classification of the cleanroom.

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