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Article

Continuous airborne particle count in the pharmaceutical industrial environment: a fundamentals theoretical review

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In a pharmaceutical industrial environment, air quality control aims to find contaminations in the environment, which are used as an indicator of possible contamination of the product. Variables can be common quantities such as pressure differential, temperature, and humidity; or more complex quantities such as the number of particles suspended in the air and microbiological contamination. This set of controls in classified areas is called Environmental Monitoring. Monitoring the concentration of suspended particles in the air is detailed in the ISO 14644-2:2015 standard. This standard makes it mandatory for companies that have clean areas to control the number of particles suspended in the air as part of their environmental monitoring program and serves as a guide regarding the amount of concentration accepted for each type of clean area, as well as the classification of the clean room itself (ISO 14644-1). However, many features are not detailed in the ISO standard. The objective of this article was to review the main fundamentals related to particle counting in a pharmaceutical environment and to be used as a start point for professionals and academics starting studies in this area.

Keywords: Particle count. Definition of sampling points. Quality control. Risk assessment. environmental monitoring.

INTRODUCTION

The main objective of environmental monitoring is to minimize the risk of contamination of the product produced in a clean area. This goes beyond the environmental monitoring program itself but involves the entire design of the room and factory, as well as airflow type definitions, filters, and pressure cascades (Gorsky, 2019).

According to article 20 of IN 35 (ANVISA, 2019a), all sampling points of particles suspended in the air for environmental monitoring must be defined based on a formal risk assessment. Carrying out a formal risk assessment was already considered a good practice until then, but from the effective date of Normative Instruction 35 it became mandatory.

Still in IN 35 (ANVISA, 2019a), article 173 mentions the requirement for particle monitoring during the entire duration of critical Grade A processes (Grade A areas are typically used for the most critical aseptic processes). Sampling gained even more importance in the scope of quality control of environmental monitoring.

While ISO 14644-2:2015 establishes a strong foundation for particle counting in controlled settings, it overlooks various crucial aspects that are vital for efficient environmental monitoring in pharmaceutical production. Existing methods frequently fall short of providing a uniform strategy to identify key contamination sources, potentially resulting in inadequate control measures (Bogdanova, Chernykh, Bukovskaya, 2024).

ISO 14644-2:2015 also lacks thorough validation procedures for Heating, Ventilating and Air Conditioning (HVAC) systems, which are essential for ensuring air quality and managing contamination. The validation process should incorporate continuous monitoring

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and risk evaluations to confirm that HVAC systems function efficiently, a necessity that the standard does not adequately address (Aakash *et al*., 2024).

The standard does not provide direction on the trending and analysis of environmental monitoring data, which is essential for recognizing long-term contamination patterns and ensuring adherence to good manufacturing practices. Adequate data management strategies are vital for accurately interpreting monitoring outcomes and swiftly executing corrective measures (Sekuloska, Todorovski, Petreska, 2022).

The purpose of this article is to explore the required fundamentals regarding particle counting as continuous monitoring in a pharmaceutical environment in order to better understand the related principles and provide a smooth starter point in a continuous particle monitoring implementation.

METHODOLOGY

This theoretical review was conducted to explore the fundamental principles of continuous particle monitoring in pharmaceutical environments. To achieve this, a systematic approach was employed to identify, select, and analyse relevant scientific literature.

A thorough investigation was carried out utilizing esteemed scientific databases, such as PubMed, Google Scholar, and Scopus. Relevant keywords were pinpointed, including "continuous particle monitoring," "pharmaceutical manufacturing," "environmental monitoring," "risk assessment," and "quality control." Articles specifically addressing continuous particle monitoring within pharmaceutical contexts were selected. Preference was given to peer-reviewed articles published in reputable journals on the last 5 years. Books, conference proceedings, industry reports, and older publications were omitted unless they contained crucial information not found in peer-reviewed sources. Studies that concentrated on different forms of environmental monitoring or unrelated pharmaceutical processes were disregarded. The chosen articles underwent a critical evaluation to determine their methodological integrity, data quality, and conclusions. Essential information, encompassing research objectives, methodologies, results, and conclusions, was extracted from each article. The gathered data was thematically analysed to uncover common themes and patterns associated with continuous particle monitoring principles, implementation strategies, and regulatory requirements. The recognized themes were synthesized to create a comprehensive understanding of the topic, emphasizing key insights and knowledge gaps.

By adhering to this meticulous methodology, we aimed to deliver a reliable and informative review of continuous particle monitoring in pharmaceutical settings.

ENVIRONMENTAL MONITORING

According to the Brazilian Pharmacopoeia (ANVISA, 2019b), a sterile environment is an environment where there is no presence of viable microorganisms. Due to the difficulty in ensuring and guaranteeing the air quality of an area, good manufacturing practices and validation of the area through statistical methods are applied in order to demonstrate that the clean area has the necessary characteristics. However, as the state of the clean area is not a definitive condition, it is necessary that the environment is constantly monitored to link the cleanliness condition with the moment in which the drug batches were produced. This monitoring is called environmental monitoring (PDA, 2014).

Technical report number 13 of the PDA (2014) divides environmental monitoring into 8 major areas:

(1) Cleaning and Sanitizing or Disinfection.

(2) Sampling Location Selection.

(3) Sampling Frequency.

(4) Alert and Action Levels.

(5) Data Management.

(6) Characterization and Identification of Microorganisms.

(7) Investigations and Corrective Actions.

(8) Documentation.

The monitoring of suspended particles in the air interacts with all these areas. Regarding cleaning and sanitization, the use of the correct sanitizers can reduce the number of microorganisms and consequently reduce particle counts, but also prevent any excess sanitizer or disinfectant from being counted as potential contamination particles. It is important to highlight that the concept of a clean area (purpose of sanitizers and disinfectants) is achieved after qualifying the area in terms of the count of particles suspended in the air (PDA, 2014).

Sampling site selection seeks a representative placement with respect to particle counts. The aim is for particle count values to exceed the predetermined threshold in situations of potential contamination, not during routine process operations. At this stage, possible improvements in the process are often identified, in cases where common production operations generate particles that indicate potential contamination (PDA, 2014).

Sampling frequency will vary according to the criticality of the process or product, and the classification of the area. Alert and action levels are provided by ANVISA as shown in Table I, present in IN 35, but they must be reviewed periodically according to the characteristics of the area being monitored. The data generated by monitoring airborne particles, in addition to being collected, needs to be analyzed and interpreted. The correct interpretation of these data will be essential in decision-making on the quality of the batch produced

TABLE I - Sampling limits for qualification of clean areas (Adapted from ANVISA, 2019b)

Grade	**Maximum number of particles/m³ equal to or greater than tabulated size**			
	At Rest		In Operation	
	0,5µm	5,0µm	0,5µm	5,0µm
A	3.520	20	3.520	20
B	3.520	29	352.000	2.900
C	352.000	2.900	3.520.000	29.000
D	3.520.000	29.000	Not Defined	Not Defined

The extent and expectations of characterization and identification of microorganisms will be defined based on the classification of the area with respect to concentrations of suspended particles in the air. Particle excursions above the previously established action level must be investigated, and the necessary corrective measures are taken. And last but not least, all events that occur during particle count monitoring must be properly documented so that they can be tracked and audited. In summary, the count of particles suspended in the air is a fundamental part of environmental monitoring (PDA, 2014).

Statistical techniques are crucial for interpreting environmental monitoring data, facilitating thorough analysis and informed decision-making. For example, Trend Analysis entails reviewing environmental monitoring data over time to uncover patterns or fluctuations in the levels of viable and non-viable particles. By scrutinizing these trends, manufacturers can assess whether their facility is effectively maintaining microbial control and adhering to regulatory requirements. In terms of Setting Action and Alert Limits, statistical approaches assist in defining thresholds for permissible levels of microorganisms.

Action limits (ANVISA, 2019a) and alert limits (ANVISA, 2019a) are monitoring tools that help in corrective measures, whereas alert limits act as notifications that conditions may be nearing unacceptable levels. This forward-thinking strategy is vital for sustaining control within the manufacturing environment. Visual representations of environmental monitoring data can help in swiftly detecting trends and anomalies. Methods such as control charts can be utilized to illustrate the stability of the environment and the efficiency of cleaning and sanitization efforts. In conclusion, grasping the concepts of viable and non-viable particles, along with employing statistical techniques for data interpretation, is critical for ensuring the cleanliness and safety of pharmaceutical manufacturing settings (Sekuloska, Todorovski, Petreska, 2022).

In terms of international regulations, the 2021 PIC/S GMP guide follows the same direction as IN 35, indicating the same values for aerosol particle counts and correlating the quality of the area with other environmental characteristics such as temperature and relative humidity from the air (PIC/S, 2021).

Pressure cascades (Figure 1), for example, are prepared in the design stage of clean rooms. However, a poor-quality pressure cascade can make environmental monitoring very difficult. The purpose of the pressure cascade is to segregate areas with different classifications through pressure and ensure unidirectional airflow in the most critical classified areas. In addition to a known good practice, the use of pressure cascades is a WHO recommendation. Briefly, the pressure cascade is a pressure control system in adjacent areas, to ensure that the pressure in the most critical areas is always higher than the pressure in the less critical areas. This ensures that the air direction is always maintained from the most critical area to the least critical area, thus preventing particles with contaminants from being moved from the less critical areas (where the clean area criteria are more lenient) to the more critical areas (Britto, 2012)

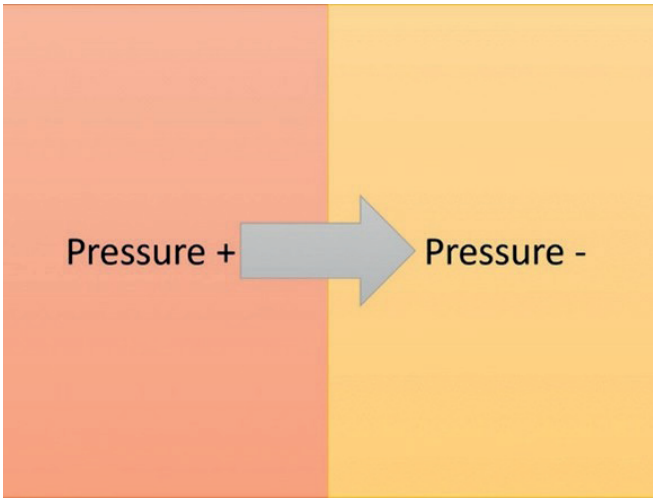

FIGURE 1 - Example of pressure cascade air movement.

AIRBORNE PARTICLE COUNT

The amount of airborne particles is a criterion detailed in ISO 14644 as an indicator of contamination in a cleanroom. The standard details which particle sizes and concentrations must be controlled, as the classification of clean areas concerning particle counts (ISO, 2015). Environments that have a low concentration of particles according to the limits and trends indicated in the ISO 14644 standard present a lower risk of contamination of the medicine produced in the area in question. Even if the measurement of airborne particles is considered a measurement of non-viable particles (or total, depending on the author), this variable is a strong indicator of microbiological contamination (Sutton, 2010).

In the realm of microbiological monitoring, especially within pharmaceutical manufacturing, it is vital to grasp the difference between viable and non-viable particles to guarantee product safety and quality. Viable particles are defined as microorganisms that are alive and have the ability to reproduce. This category encompasses bacteria, fungi, and viruses that could potentially lead to contamination and spoilage of products. The surveillance of viable particles is critical since they can result in product contamination, compromising

the safety and effectiveness of pharmaceutical items. The existence of viable microorganisms may signal a breakdown in the sterility and quality control processes of the production environment. Common techniques for identifying viable particles include culture-based methods, where samples are incubated to promote growth, and molecular techniques such as PCR that detect living organisms through their genetic material. Non-viable particles are characterized as those that are deceased or unable to reproduce. These can consist of remnants from microorganisms, including cell walls or fragments, alongside inert particles such as dust or other contaminants. Although non-viable particles may not directly threaten product safety, their presence can indicate inadequate cleaning practices or insufficient environmental control. They can also obstruct the detection of viable particles, resulting in deceptive outcomes in microbiological monitoring. Non-viable particles are frequently identified using methodologies like microscopy or filtration techniques, which aid in evaluating the cleanliness of the production environment (Bogdanova, Chernykh, Bukovskaya, 2024).

The purpose of particle counting within a clean area is to ensure that the area is within a standard established as minimally acceptable from a contamination point of view. Viable particles indicate microbiological contamination, and non-viable particles indicate potential microbiological contamination. As particle counters count any type of particle in suspension, whether viable or non-viable (hence the term total particles), there is no way to know exactly the amount of viable particles within each measurement. For this purpose, there are microbiological analysis processes, which normally take days or weeks, and deliver a more specific result from the point of view of contamination. This process, although more assertive, does not allow for real-time control. The need for real-time control is the main driver of total particle control (Dalmaso, 2019).

The choice for particle sizes of 0.5μm and 5μm is related to an understanding that particles that exceed these sizes are more often considered carriers of viable particles that generate microbiological contamination. Limits are different for "At Rest" and "In Operation" rooms. The term "At Rest" indicates the state of the clean area without human presence, while the term "In Operation" indicates the state of the clean area with operators (Ludwig, Silva, 2019).

Pharmaceutical standards have a level of acceptance regarding particulate matter suspended in the air, but this is not the only industry with this type of requirement. In some electronics industries, similar controls for airborne particulate matter are applied, but much more stringent. These industries are looking for even smaller particles (0.3μm or 0.1μm), and have standards with even stricter tolerances regarding quantity, with classification even for areas free of contamination, where the particle count must always be zero (Rahaman *et al*., 2008).

The amounts of particles suspended in the air defined in the sampling table refer to a concentration within $1m^3$. As the equipment used for environmental monitoring commonly uses a flow rate of 28.3 liters/minute (1 foot/minute), it is necessary that the particle values are accumulated or normalized so that they present a value consistent with the measurement units established in the standard. These calculations (whether summation or normalization) make manual control of particles complex. For this reason, the most recent standards call for environmental monitoring systems that can provide this data without the chance of human error. This is another reason why the limits established in the standard should be considered only as a guide, since the way of calculating the particle count will change the considered volume, and the considered volume needs to be normalized to meet the value equivalent to the defined value in norm (Hartigan, 2016).

The sampling limits defined in the standards are commonly called action limits. These limits, when reached, require immediate intervention in the process. This intervention can be a justification detailing the reason for the particle excursion, corrective action in the area, or even the partial or total disposal of the batch in production. In addition to action thresholds, there are alert thresholds that are also used. The purpose of

alert thresholds is to inform the user that the particle concentration in the area is high before the particle count excursion above the action threshold occurs. This approach makes the process safer, as it allows preventive rather than corrective intervention, protecting the integrity of the product as the process can be interrupted before the action limit is reached. Both limits must be reviewed periodically, always seeking the use of new limits that are more adherent to the process and that make the process safer and more efficient (Campanella, 2017).

To define new limits, statistical methods are used, such as histograms, evaluation of normal or non-normal distribution, and control charts. The objective of these methods is always to identify trends in the collected values, so that "noise values" can be discarded, and to identify which values best represent the behavior of the area. It is a good practice recognized and demanded by many regulatory agencies that the behavior of clean areas is constantly evaluated within the scope of particle counting, not only to define new limits but also to identify areas for improvement. The improvement in these values may indicate that there was an improvement in the quality of the area, either by choosing points with more assertive representation or by identifying new processes that generate less particulate matter (Campanella, 2017).

Regarding the collection of particles, some points are important to guarantee the representativeness of the sampled values. The first point is the type of geometric structure used to capture the particles. The movement of particles into the tube occurs through the combination of 4 physical forces: gravitational force, electrostatic forces, diffusion forces, and thermophoretic forces. The result of these forces applied to the particles distorts the result sent into the tube if a common cylindrical tip is used. To correct this distortion, an isokinetic tip is required which ensures that the particles move into the tube without distortion (PMS, 2018).

It is very common that, due to physical limitations, particle counters need to be placed in locations far from the probe used to perform the collection. When this occurs, the particles transported by the probe need to reach the particle counter, and in some cases, the loss related to this transport cannot be ignored. In Figure 2, it is indicated that the distance is as small as possible and that the bends, when necessary, maintain the largest possible radius to minimize the impact with the tube walls. It is also indicated that the material used in these tubes is an appropriate material, with the lowest possible internal friction to minimize the deposition of particles on the tube walls (PMS, 2018).

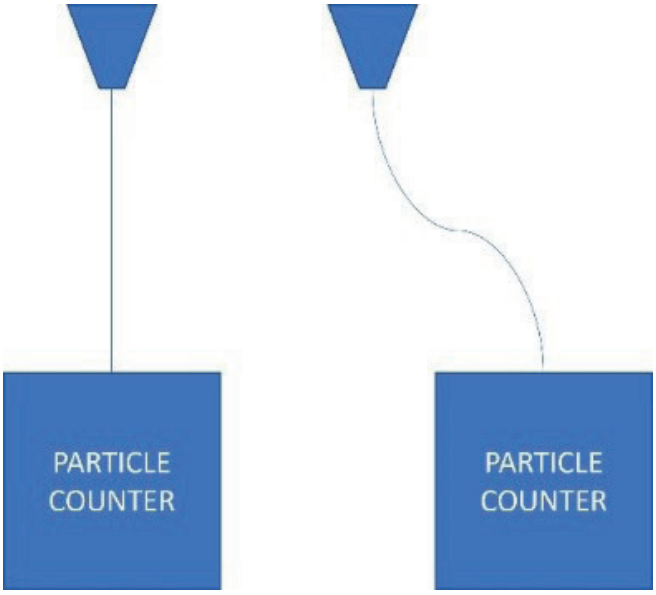

FIGURE 2 - Examples of placement of particle counters and probes.

The use of long tubes or tubes with more pronounced curvatures can mean a significant increase in the loss of particles that reach the particle counter in relation to the particles that were collected in the probe. Larger particles (and considered more critical) are the most affected. In the comparative example of pipes with 2m, 3m, and 4.5m at a linear flow of 28.3 liters/minute, it is possible to identify the loss of particles larger than 5.0µm from ~17% to ~58%. In cases where the use of long tubes is the only option for sampling, this loss needs to be considered in the value used as an action limit (PMS, 2018).

CLASSIFICATION AND SUBDIVISION OF AREAS

The Brazilian Pharmacopoeia (ANVISA, 2019b) details that the clean areas used for the production of medicines are classified according to the concentration of suspended particles in the air present in the same. The standard that regulates this classification is ISO 14644-1. The standard presents a table that lists the classification of the room (ISO Class 1, 2, 3 (...)) as the highest according to the amount of particles/m³ in the room, with the ISO Class 1 classification being considered the cleanest room, that is, with a lower concentration of particles/m³. Other nomenclatures for the classifications are applied (Class A, B, C, (...) and ISO 5, 6, 7 (...)), but the logic remains the same: indicate through a class the degree of cleanliness of the area. Area classifications are performed considering some criteria such as critical points, people flow, material flow, and operation areas.

It is important to highlight that classification and monitoring are completely different things. Sampling points defined for area classification are not the same as monitoring points. In the case of classification, the objective is to evaluate the characteristics of the area seeking a quality of particle count sufficient to reach a specific classification in a determined moment, while the monitoring seeks to evaluate the capacity of the area to maintain the characteristics during the process. The external factors that affect classification and monitoring are also different since during classification the characteristics of the "In Operation" state use simulated operations or media fills, not actual production (PIC/S, 2021).

For classification, the cleanroom is first divided by segregation (Figure 3), using a machine enclosure, curtains, or other material that serves as an air separator (Eaton, 2019).

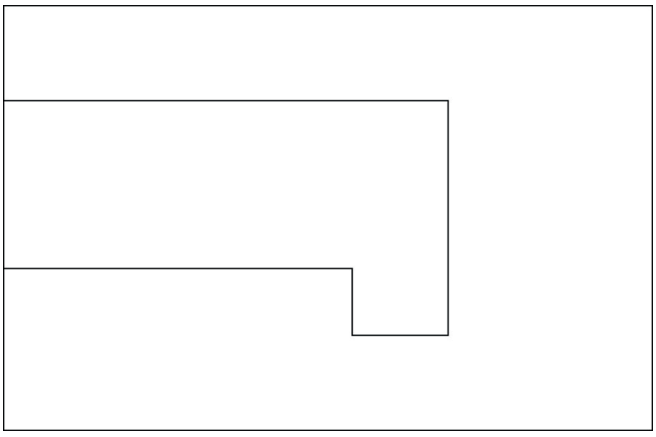

FIGURE 3 - Example of a clean room with segregation between machine and room.

After this segregation, both divisions are treated separately. In each of the segregations, the areas are divided into quadrants (Figures 4 and 5), so that each quadrant can be evaluated individually (Eaton, 2019).

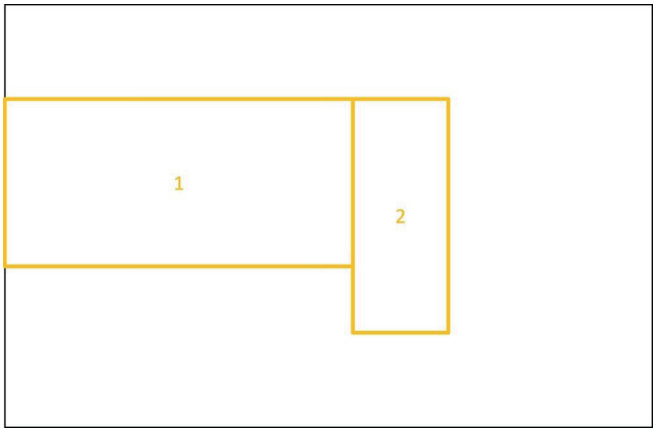

FIGURE 4 - Example of machine quadrant separation.

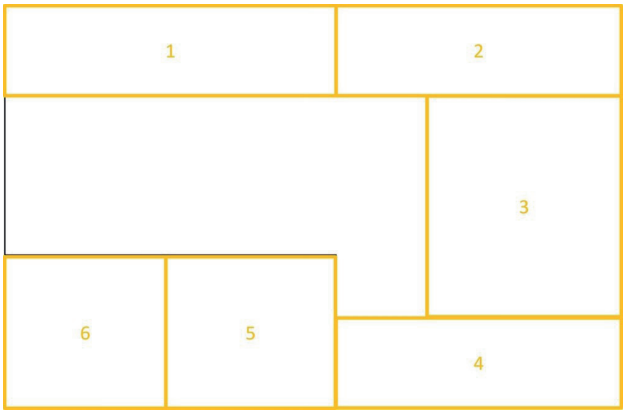

FIGURE 5 - Example of separating quadrants in a room.

Each part of the segregated area is evaluated according to its area in square meters (m²). The area in m² is compared with a table in the ISO 14644 standard to define how many minimum sampling points need to be used in its classification (Table II). The position of the sampling points within the quadrant is defined according to the distance from the critical point(s) of the quadrant. The resulting value for the number of sampling points in each sub-area is obtained through a ratio between the sub-area area and the minimum number of samplings present in the table (Eaton, 2019).

TABLE II - Table of number of samples per area (Adapted from ISO, 2015)

Area of cleanroom (m²) less than or equal to	Minimum number of sampling locations to be tested (N_L)
2	1
4	2
6	3
8	4
10	5
24	6
28	7
32	8
36	9
52	10
56	11
64	12
68	13
72	14
76	15
104	16

108	17
116	18
148	19
156	20
192	21
232	22
276	23
352	24
436	25
636	26
1000	27
> 1000	See Note 3

Note 1 If the value of the area considered is intermediate between two values in the table, the largest value in the table must be used.

Note 2 For cases of unidirectional air flow, the area should be considered as the cross-section of air moving perpendicular to the direction of air flow. For all other cases, the area shall be considered as the horizontal plane area of the clean area or clean zone.

Note 3 When the area of the cleanroom or clean zone is greater than 1000m², apply the following formula to determine the minimum number of locations required:

$$NL=27 \times [A/1000]$$

Where NL is the minimum number of sampling locations to be rounded up to the next whole number, and A is the cleanroom area in m².

RISK ASSESSMENT

For area classification or for defining the environmental monitoring program, a risk assessment is necessary to detail which are the points where the product is exposed to greater risk and to outline a strategy that will mitigate this risk. In a risk assessment, several aspects are considered, such as conditions of the room at rest or in operation, the flow of materials, the flow of people, and points of exposure of the product to the environment (Eaton, 2019).

IN 35 (ANVISA, 2019a) brings several different approaches to risk. In more than a dozen articles, the normative instruction draws attention to some type of risk. What is common in almost all articles is that the risk that is sought to be mitigated is the risk of microbiological contamination. The greater the exposure of the product to the environment, the greater the risk of the product being contaminated. The areas considered to be at greater risk are normally the areas where the product is exposed (after filling and before sealing, for example) or where any component that will have direct contact with the product is exposed (reservoir lids, for example). The risk is only considered smaller after the

complete sealing of the product.

Within the context of a clean area, various types of risk analysis can be applied. Risk assessment of a machine or system with respect to non-compliance with a user specification, risk assessment to determine optimal cleaning and decontamination procedures, risk assessment of raw material disposal, and risk assessment of cross-contamination, among others. others. The concept is the same: identifying risks, analyzing risks according to a specific methodology, and defining which risks are subject to mitigation and which require further action (PIC/S, 2021).

In the case of risk analysis for defining sampling points, the objective is directly linked with potential microbiological contamination. The objective is to mitigate the risk of microbiological contamination occurring inside the clean area without it being detected. This is an important point worth mentioning: the purpose of particle counting is not to prevent contamination but to ensure that, in the event of potential contamination, it is detected. In this way, there is a guarantee that the contaminated product can be identified and will not be delivered to the final consumer. To identify possible microbiological contamination, sampling points for airborne particles need to be positioned in such a way as to be representative. The risk analysis for defining sampling points seeks to evaluate each candidate point for sampling point in relation to its representativeness. A representative point is a point where there is no particulate excursion during routine operations, but which excursions whenever some non-standard event occurs in the clean area (Eissa, 2016).

One of the most used tools for risk assessment is the FMEA. This tool evaluates the risk through 3 factors: Detectability, occurrence, and severity. Detectability evaluates the probability of risk detection, the greater the chance of the risk not being detected, the higher the index. Occurrence assesses the likelihood that the risk will occur. The greater the chance of the risk occurring, the higher the index. Severity evaluates the impact of the occurrence of the risk if it happens. The more severe the impact, the higher the index. The 3 factors are multiplied together, generating a single RPI index. This index is classified according to the value as low, medium, or high. Usually, risks classified as medium or high require a more robust mitigation action. The ideal risk analysis does not have any risk classified as medium or high, as shown in Table III (PMS, 2017).

TABLE III - Default FMEA example

Risk	Discoverability	Occurrence	Severity	RPI	Classification
Operator bumping into probe	1	1	1	1	Low
Operator Adjust Feeder	1	1	3	3	Medium
Door opening during process	1	3	3	9	High

The FMEA for defining sampling points evaluates each candidate using a specific approach based on contamination risk (Table IV). Detectability (D) evaluates the probability of detecting potential contamination at that point through particle counting. The Occurrence is divided into two probabilities, the probability of contamination of the environment (P1) and the probability of contamination of the product (P2). Severity (G) assesses the severity of contamination based on the consequences of contamination reaching the final product. The RPN is the result of multiplying all these indices (P1 x P2 x G x D), with the risk classification presented in Table V (PMS, 2017).

TABLE IV - Example of a 4-factor risk matrix (Adapted from PMS, 2017)

Risk Matrix							
G: Severity		P1: Probability of environmental contamination				D: Detection	
		Low	Medium	High	Very High		
		1	2	3	4		
		P2: Probability of product contamination					
		1	2	3	4		
Very High	4	16	64	144	256	4	Low
High	3	9	36	81	144	3	Medium
Medium	2	4	16	36	64	2	High
Low	1	1	4	9	16	1	Very High

TABLE V - Risk rating (PMS, 2017)

1 – 8	Low
9 – 36	Medium
37 - 256	High

After classifying all points according to the FMEA, a threshold (X) is defined. If the RPN is greater than X, the point must be sampled. If the value is less than X, there is no need for sampling (Table VI). Another way to assess the need for sampling is through risk classification, where the risk is related to the criticality of the point for defining the need for sampling or not (PMS, 2017). The areas considered most critical hat e

TABLE VI - Risk categories (PMS, 2017)

Risk Category	Description
Unacceptable XX - YY	High risks that are above the acceptable threshold need to be reduced through risk control measures. A sampling point is required.
Acceptable / Low according to practical rationale XX - YY	Medium risk is acceptable when justified. A sampling point is required according to the criticality assessment of the area and process.
Acceptable XX - YY	Low risk is acceptable. No sampling point is required.

The areas considered most critical are the areas where sterile products are handled. In these areas, any activities involving the manipulation of products, connections, or other sterile items may allow particles to come into direct contact with the product. As the particles can act as a transport vehicle for microorganisms, this exposure can lead to contamination of the product. In addition to risk identification, it is important to ensure that all stakeholders are aware of all risks. This usually occurs through proper documentation of the risk analysis so that it can be consulted by all interested parties. After documenting the risk analysis, it is good practice to establish risk analysis review routines. The state of the area, flows and risks are very dynamic and a small change can generate a big impact (Lawson, 2018).

In addition to particle counting points, risk analysis is also responsible for identifying which points should be monitored through air impaction and surface monitoring. Air impaction uses active air sampling to impact ambient air into a plate that will assess microbiological contamination through plate incubation. Swabs and contact pads are used for surface monitoring. Like microbiological impaction plates, swabs or contact plates are also incubated after sampling and therefore are not considered real-time monitoring (PMS, 2017).

Also within the context of microbiological contamination control, in some cases, it is necessary to use alternative methods, especially when there is no possibility of certifying the production batch before the

product is administered due to the short shelf life of the medicine or due to other similar reasons. In these cases, rapid microbiological methods are used to obtain data (PIC\S, 2021).

CRITICAL POINT

The Brazilian Pharmacopoeia (ANVISA, 2019b) highlights the importance of monitoring close to the product being produced, as well as monitoring the air and surfaces that come into direct contact with the product. A critical point, critical surface, or critical location is the point on the production line where the product is exposed. When monitoring takes place close to a critical point, it is concluded that the state of microbiological contamination of the surface and air that had contact with the product is the same as that of the product itself. In this way, it is possible to evaluate the microbiological contamination through the critical point, without the need for direct evaluation of the product.

Serra *et al*. (1999) brings the concept of Critical Controls Points (CCP) within the context of the food production environment. Even though this article was published more than 20 years ago, at a time when contamination still did not have as many controls as it does today, the use of a control point to indirectly identify contamination is clear. This strategy guarantees a 100% non-invasive inspection, which guarantees that the samples will not be destroyed and that there will be no contamination caused by the monitoring itself (Serra *et al*., 1999).

Critical processes from the point of view of microbiological contamination are processes in which, due to the need of the production process, there is a need to expose the product to the environment. To minimize the risk of contamination, IN 35 (ANVISA, 2019a) in articles 20 and 21 requires that these processes be monitored with a frequency directly proportional to the criticality of the area or process. This monitoring is done through particle counting or air sampling and surface monitoring.

The critical point extends for the distance where it remains representative, i.e., in addition to the critical point itself, the surroundings of the point where there is an equivalent representation of the particles are also considered part of the critical point from the point of view of monitoring particle counts. In this respect, it is important to consider the direction of the air, since any point between the origin of the air and the critical point will not be representative, as it represents the air before being in contact with the critical point. Therefore, the representation of the critical point does not occur in a uniform radius around the critical point, but rather follows the direction of the air after the contact of the air with the critical point (Eaton, 2019).

The relationship between the critical point and the surroundings of the point changes when talking about the production of radiopharmaceuticals. In this case, there is special care with all the air that passes through the critical point, as it may contain radioactive isotopes and cause cross-contamination if it is sucked in by equipment that is taking the air to an external environment. In this type of production line, the areas also have inverted pressure, to ensure that no radioactive particles from inside the room are taken to the external environment and cause radioactive contamination (Gouveia *et al*., 2015).

One of the objectives of a risk analysis is to identify the critical points. In addition to the points where there is direct contact with the product, a critical point can also be any location or point that for some reason offers a high risk of contamination concerning the end user of the medicine. The use of the FMEA method to obtain the RPN value, especially in the severity item, seeks to assess the criticality of the point, that is, how severe a microbiological contamination that occurs at that point can be if the contamination is not properly detected (Eissa, 2016).

LAMINAR FLOW AND FIRST AIR

Inside a cleanroom, one of the most important points to consider is the air. Even if it is not visible to

human eyes, the air is essential inside the cleanroom to avoid contamination. In this regard, the number and placement of air inlets and outlets can make all the difference in the search for a cleaner room. The objective is that the air enters the filtered environment, passes through the critical point to "wash" the product, and is directed directly to the air outlet (Whyte *et al*., 2018).

As illustrated in Figure 6, if the critical point is not positioned between the air inlet and outlet, the air will leave the room without having passed through the critical point. Without the critical point receiving a constant flow of air, it is exposed to all contaminations that may be in the environment (Whyte *et al*., 2018).

FIGURE 6 - Air flow that does not pass the critical point.

Air laminarity ensures that air is directed toward the critical surface without it coming into contact with any other surface, object, or person. If the air outlets are correctly positioned, but the flow is not laminar, the air will pass through other areas before reaching the critical point. In this way, the air that will reach the critical point with the objective of "washing" and guaranteeing the protection of the product will already be potentially contaminated, as it had contact with other areas before having contact with the critical point or not even touching the critical point, as can be seen in Figure 7 (Whyte *et al*., 2018).

FIGURE 7 - Non-laminar air flow.

As with any environment, it should be assumed that the particle concentration in the cleanroom is not uniform. In this way, collecting particles at different locations within the cleanroom may indicate different results. By ensuring that you first have contact with the critical point, you ensure that the critical point is exposed to the best quality air within the room (Hartvig, Jensen, Hansen, 2011).

As a concept of laminarity for clean areas, air from a homogeneous and unidirectional source with a velocity between 0.36 and 0.54 m/s is considered (PIC/S, 2021). The ideal laminar flow placement is directly above the critical point, with sufficient coverage to ensure air laminarity throughout the entire length of the critical area. In this way, the air that will come into contact with the critical point will be completely filtered and clean air will come directly from the air inlet into the room, avoiding contact of the critical point with microorganisms, as shown in Figure 8. The fact that air

is filtered at constant speed being blown at the critical point also prevents air from other sources with lower velocities from coming into contact with the critical point (Whyte *et al*., 2018).

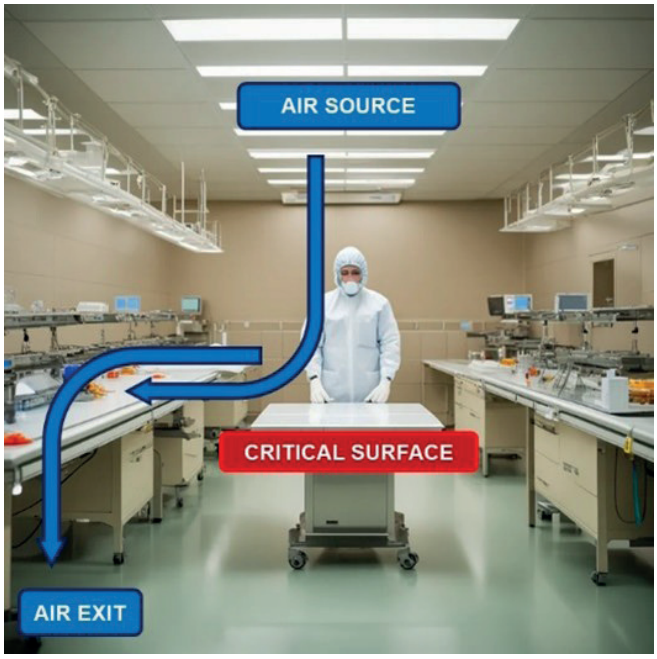

FIGURE 8 - Laminar air flow.

All clean areas need to have control over heating, ventilation, and air conditioning. Systems that perform this control are commonly referred to as HVAC systems. HVAC systems are responsible, among other things, for ensuring that ventilation occurs correctly within a clean area. Depending on the classification of the room, the ventilation requirements change, but ISO-14644 requires the use of unidirectional laminar flow for Grade A areas. Unidirectional laminar flow ensures that the first air the filter blows is directed directly to the critical point (Chen *et al*., 2019).

TURBULENCE

All air entering a Grade A cleanroom comes from a laminar air source. When this air encounters an obstacle that causes it to lose its laminarity, there is the concept of turbulence. Turbulence implies non-laminar air or air recirculation through the same point. The original air is blown through the filter, which means it is contamination-free air. When this air passes through an obstacle that causes it to lose its laminarity, the air is carrying the particles present in the obstacle. In this way, any turbulence generated before the air reaches the critical point consequently leads to potential contamination of the critical point (Rouaud, Havet, 2002).

According to Whyte *et al*. (2018), when a high particle count is identified, this count is actually in the sampled air, and not necessarily in the product that is at the critical point. Considering the path that the air takes and correlating it with time, it is possible to know where this contamination will be in N units of time. For example, considering an air speed of 1m/s, in a room with a distance of 3m between the air outlet and the critical point, the filtered air will take 3s to reach the critical point, as shown in Figure 9.

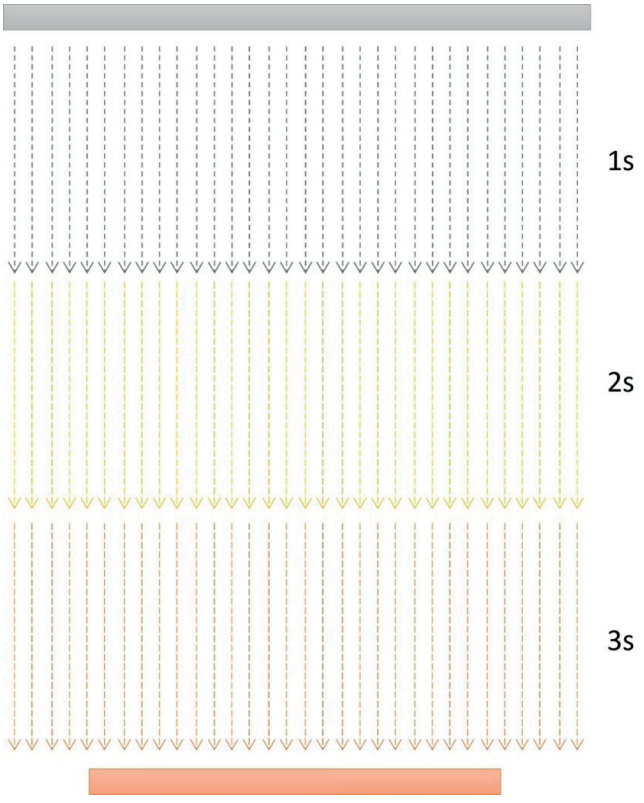

FIGURE 9 - Example of air movement in laminar flow.

RECOVERY TIME

The time required for the contamination generated at the critical point to be removed from the room through the air outlet is called the recovery time. As the suspension of particles in the air is normally not an isolated event, and as recirculation is frequent within the room due to the constant change in the positioning of operators and equipment, it is common for the recovery time in a clean room to take a few minutes (White, 2018).

Some strategies used to improve air laminarity and minimize turbulence are related to the use of enclosures with curtains. In this case, the air intake uses the upper part, just above the critical point, and the air maintains laminarity, aided by the curtain. The curtain does not extend to the floor, leaving enough space for the air that passed the critical point can exit the enclosed area. In this way, recirculation is avoided, and turbulence avoided (Shao *et al*., 2022).

In addition to air velocity, another important variable is the number of air changes that occur in a given period. The amount of air changes is related to the recovery time of the room, and it is the time required for contamination to leave the environment. The amount of air changes in a period is inversely proportional to the concentration of contamination in the environment. Therefore, the more air exchanges in a given period occur in the area, the less time is required for the concentration of contamination in the area to reach zero (Whyte *et al*., 2017).

SMOKE TEST

One of the elements used in the composition of the risk analysis is the smoke test. The smoke test consists of placing visible smoke in the cleaned area and observing the airflow, to understand the possible paths that possibly contaminated air can take and identify the air flow movement patterns within the room. With this information, it is possible to predict which locations are most likely to have contact with a contaminant particle suspended in the air and monitor this critical location. Through this monitoring, we seek to avoid exposing sterile products in areas with a higher risk of contamination (PDA, 2015).

The smoke test is a USP requirement and seeks to identify the behavior patterns of the air inside the clean room. It is carried out through smoke generators inserted just below the laminar flow, making it possible to visualize the path taken by the air generated in the laminar flow. As it is a regulatory requirement, smoke tests are usually documented on video, which facilitates later analysis, whether in audits or to implement improvements (Rhoads, Exner, Wagner, 2020).

The Brazilian Pharmacopoeia also mentions the smoke test:

"An example method for conducting particle challenge testing for the system is to increase the ambient particle concentration by using smoke around critical work areas and visualizing air movements. The presence of vortices and turbulent zones can be visualized and the airflow pattern can be finely tuned to eliminate or minimize unwanted effects. This assessment is done under simulated production conditions, but with equipment and personnel on-site" (ANVISA, 2019b, p. 705).

The use of smoke tests, in addition to being important to achieving the related standards, is essential to clarify the understanding of the behavior of the airflow in the clean area.

DATA INTERPRETATION

Environmental monitoring produces an abundance of data that, when analyzed properly, can generate significant insights into the cleanliness and regulation of a pharmaceutical manufacturing setting. Descriptive statistics, including mean, median, and standard deviation, provide a foundational comprehension of the data's distribution. By visualizing trends over time through trend analysis, potential contamination problems can be detected at an early stage. Control charts, as upper and lower control limits, assist in monitoring process stability and prompt investigations

when necessary (Sekuloska, Todorovski, Petreska, 2022).

Establishing appropriate action and alert limits, based on statistical analysis, guarantees prompt responses to deviations from acceptable microbial levels. Hypothesis testing offers a comprehensive framework for assessing the efficacy of interventions, such as new cleaning protocols or adjustments to equipment. Regression analysis also helps in revealing relationships between variables, leading to improved environmental control strategies (Sekuloska, Todorovski, Petreska, 2022).

Statistical techniques are essential for evaluating data gathered during air validation procedures. They assist in assessing the efficiency and effectiveness of air systems, ensuring adherence to regulatory standards. Statistical methods can be utilized in risk evaluation to measure uncertainties and potential failures within air systems. This aids in making educated decisions regarding system performance and necessary enhancements. Ongoing surveillance of air systems can gain from statistical process control methodologies. These methodologies utilize statistical techniques to oversee and regulate processes, ensuring that the air systems function within designated parameters (Aakash *et al*., 2024).

Employing these statistical methods is crucial for deriving meaningful insights from environmental monitoring data. By analyzing trends, establishing suitable limits, and applying various statistical techniques, pharmaceutical manufacturers can proficiently manage microbial risks, uphold a better manufacturing environment, and ultimately improve product quality (Sekuloska, Todorovski, Petreska, 2022).

SPECIAL CASES

Sterile Powder Filling

One of the most complex processes for carrying out monitoring is the filling of sterile powders. This is because the product is suspended in the air during the production process. Even though the product is sterile, the particle counter identifies the particles of the product and does not differentiate sterility. The result of this process is a false positive count, which can hide real contaminations (Hallworth, 2020).

For the filling of sterile powders, two different methodologies can be applied. The first is to look for a sampling location that is farthest from the product source, far enough away not to damage the particle counter, but close enough to remain representative. However, this approach is not always feasible (Sutton, 2010).

The other approach, supported by the EU and FDA, is sampling during assembly and after the dust is no longer suspended in the area. In this way, the state of cleanliness of the area is checked immediately before the start of the production process and immediately afterward. This strategy is used because if data are collected during production, the result will not be representative and the data will not be used for analysis (Hallworth, 2020).

Delimitation between different area classifications

Demarcations between areas of different classifications are essential to ensure airflow and protect the area with the most critical class from receiving air from the less critical area. Some types of delimitations allow the containment of air by pressure, others ensure air laminarity, protecting the most critical area from turbulence, and others are only responsible for directing the air in the desired direction (Britto, 2012).

The better the quality of the delimitation, that is, the greater the protection that the delimitation provides to guarantee that the air from the less critical area does not enter the more critical area, the lesser the need for monitoring the less critical area. Areas that have a more efficient separation between the different classes of room or machine tend to guarantee better quality air within the most critical area, as they limit the source of contamination to mainly the air source (which must have

a HEPA filter) and the products or materials entering the area. Both sources of contamination normally have independent quality and sterility controls, which almost completely mitigate the risk of contamination. This result can be proven through particle counting (ANVISA, 2019a).

Open construction machine

Open construction machines are the most common type of machines used in the pharmaceutical industry. Mainly due to the acquisition cost and ease of maintenance, the industry has great difficulty in replacing these machines with more technological equipment. While not state-of-the-art in terms of aseptic process technology, open construction machines can still be very efficient if correctly designed and coupled with the right production process.

Isolators and Restricted Access Barrier System (RABS)

Isolators and Restricted Access Barrier System (RABS) allow the product to be completely isolated from the outside environment. This occurs through sealed physical barriers (sealed doors) in some cases with gloves for handling the product inside the machine. RABS and insulators commonly have a unique laminar flow as their front is not exposed to ambient air. In this way, there is a guarantee that there will be no turbulence since the variables that generate external turbulence are removed. In some cases, the RABS or isolator is still pressurized using antechambers to ensure pressurization. In this way, the sense of air is also guaranteed (Peters, 2007).

The isolated technology present in this group of machines is considered ideal concerning protection against microbiological contamination. Even though this is an advanced technology, no machine construction is completely safe or contamination-proof. For this reason, RABS and insulators must still be monitored, through microbiological monitoring and particle counting. However, monitoring external contamination sources is not necessary, as there is no way for contamination to reach the inside of the machine without loss of integrity. Another item that must be additionally considered with this type of technology is the integrity of the items that separate the insulator from the rest of the room, such as gloves, doors, and sealing connections (PDA, 2014).

RABS and isolators have a more efficient barrier against microbiological contaminations when compared to open construction machines (PDA, 2015).

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CONFLICTS OF INTEREST

The authors confirm that there are no conflicts of interest.

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