PARTICLE MEASURING SYSTEMS®

a spectris company

Environmental Monitoring Handbook for Pharmaceutical Manufacturers

Without measurement there is no control

Environmental Monitoring Handbook for Pharmaceutical Manufacturers

HEADQUARTERS

5475 Airport Blvd
Boulder, Colorado 80301 USA
T: +1 303 443 7100

Instrument Service & Support
T: +1 800 557 6363

Customer Response Center
T: +1 877 475 3317
E: info@pmeasuring.com

www.pmeasuring.com
info@pmeasuring.com

PARTICLE MEASURING SYSTEMS®
a spectris company

GLOBAL OFFICES

AUSTRIA
T: +43 512 390 500
E: pmsaustria@pmeasuring.com

BENELUX
T: +32 10 23 71 56
E: pmsbelgium@pmeasuring.com

BRAZIL
T: +55 11 5188 8227
E: pmsbrazil@pmeasuring.com

CHINA
T: +86 21 6113 3600
E: pmschina@pmeasuring.com

FRANCE
T: 33(0)1 60 10 32 96
E: pmsfrance@pmeasuring.com

GERMANY
T: +49 6151 6671 632
E: pmsgermany@pmeasuring.com

ITALY
T: +39 06 9053 0130
E: pmssrl@pmeasuring.com

JAPAN
T: +81 44 589 3498
E: pmsjapan@pmeasuring.com

KOREA
T: +82 31 286 5790
E: pmskorea@pmeasuring.com

MEXICO
T: +52 55 2271 5106
E: pmsmexico@pmeasuring.com

NORDIC
T: +45 707 028 55
E: pmsnordic@pmeasuring.com

PUERTO RICO
T: +1 787 718 9096
E: pmspuertorico@pmeasuring.com

SINGAPORE
T: +65 6496 0330
E: pmssingapore@pmeasuring.com

SWITZERLAND
T: +41 71 987 01 01
E: pmsswitzerland@pmeasuring.com

TAIWAN
T: 886-3-5525300 Ext: 301
E: pmstaiwan@pmeasuring.com

Table of Contents

List of Tables and Figures

Introduction

Welcome to this first of our E-Books on life science applications. This first book will focus on the overall topic of environmental monitoring; it presents new information and also pulls from our extensive library of applications notes. As we head into a new era of requirements with the release of the much anticipated EU GMP Annex 1 2022 along with the adoption of a CCS, this book is a timely addition to any library.

We will cover the fundamentals of particle counting and cleanroom designs, and this will help establish the baseline of the technology being used to manufacture pharmaceutical and life science products in controlled areas. It will also explain how to demonstrate that control.

The second section will look at the standards applicable to production areas and the expectation of regulatory bodies governing release to market of aseptic drugs, advanced therapies, medical devices, and non-sterile products.

And finally, once the fundamental principles point the way, the standards and requirements define what is necessary to fulfill quality attributes; we will look at the instrumentation and techniques required to satisfy those needs.

If you have any questions regarding the content or have a desire to learn more about these topics, please contact your local Particle Measuring Systems' office, or us directly at **info@pmeasuring.com**.

Frank Panofen
Director & General Manager Life Sciences Division

Fundamentals

Fundamentals: Cleanrooms

Pharmaceutical Cleanroom Design Basics

To satisfy the assurances required by regulatory agencies, pharmaceutical products are manufactured in a controlled environment. Cleanrooms are an example of a controlled environment and are employed to reduce the contamination risk and variability of potential production environment. As controlled environments, cleanrooms can be regulated to meet specific standards. GMP regulations require that these environments are rigorously monitored to ensure that there is full and constant awareness of current environmental conditions for both viable and nonviable contamination.

A cleanroom is the fundamental starting point for contamination control. 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.

There are essentially two basic types of cleanrooms:

- **Turbulent dilution of contamination**
  Turbulent airflow cleanrooms utilize the exchange rate of filtered air to dilute any contamination down to an acceptable threshold. HEPA filtered air is delivered via a central system through diffusion panels in the ceiling at a rate of between 10 to 40+ room volumes per hour (Air Exchange Rate).This value is a function of the operations and number of personnel within each area. Return air is recirculated and uses a percentage of fresh air to maintain comfort.

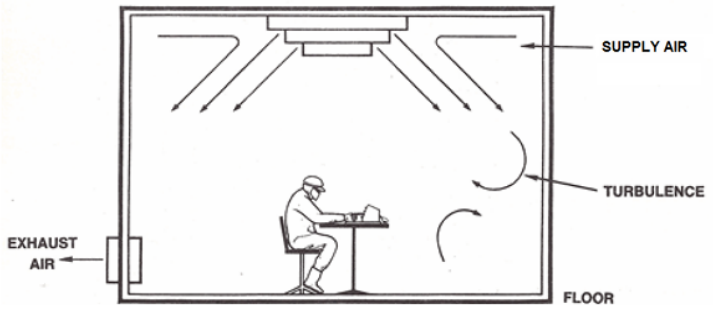


FIGURE A-1 TURBULENT DILUTION OF CONTAMINATION

- **Unidirectional Air wash**
  Unidirectional air cleanroom utilizes the velocity of air to act as shroud to prevent extraneous particles from a potential contaminating source to impact adjacent areas.

Where there are particles generated by the process or operators, the airflow also is designed to sweep particles away from the critical zone; this can be affirmed by the smoke airflow visualization test. Flowrates are optimally 0.36 – 0.54 m/s, however other airflow velocities can be used.

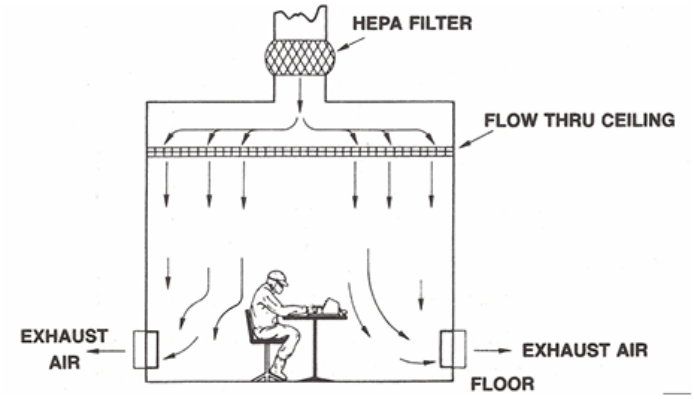


FIGURE A-2 UNIDIRECTIONAL AIR WASH

Within the cleanroom, operations will typically take place within HEPA filtered benches, allowing for the manual manipulation of product, containers, and processing equipment. Flow direction within these benches is either horizontal or vertical. Horizontal flow goes directly toward the operator, sweeping any operator-borne contaminants out of the critical zone. If the flow is vertical, the air flows down over the process ensuring a shroud is maintained of the critical areas.

The vertical flow also allows for fill and finish equipment to be automated and enclosed with a designed space.

The filling machine can be open to access from above and utilize the unidirectional airflow within the room to ensure isolation of the process to the outside activities within the general room environment.

Restriction of access to the critical processing zone can be limited using fixed or flexible curtains; this offers a certain degree of isolation of the critical area verses the general room environment where operators are able to intervene with the process should it be required.

Alternatively, a dedicated filter can be employed to deliver filtered air only to the processing equipment. This allows for a lower grade of air for the background areas and a higher degree of separation between the critical and the background areas.

This design can be expanded and scaled up to create

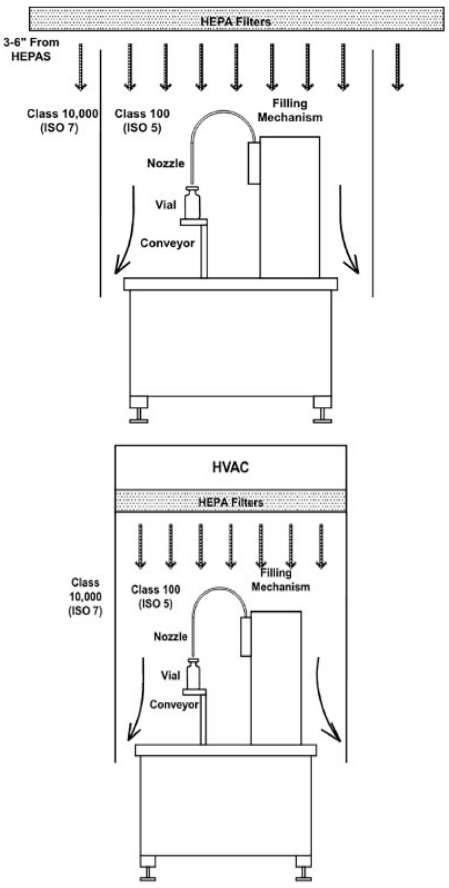


FIGURE A-4 FILLING MECHANISM DIAGRAMS

restricted access barrier protection (RABS) where access to the critical zone can only be performed using gloved aces ports. Full isolation can be achieved using fully closed isolator systems. These also allow for enclosed sanitation and sterilization processing.

The nature of activities and choice of cleanroom used will also affect the type of environmental monitoring required. The higher the access of operators to a process, the greater the risk of contamination. This is because personnel are the single largest contributor of airborne contaminants within a cleanroom.

Author

Mark Hallworth
Life Sciences Senior GMP Scientist
Life Science Division
Particle Measuring Systems

Mark Hallworth is the Life Sciences Regional Manager for Particle Measuring Systems. He has lectured for pharmaceutical societies throughout Europe, Asia, and the US on nonviable particle and facility monitoring and the implications of validating those systems. He can be reached at **mhallworth@pmeasuring.com**.

Editor: Noelle Boyton

Fundamentals: Particle Counters

A Simple Guide to How Aerosol Particle Counters Work

Overview

Aerosol particle counters are built to rapidly count and size contaminant particles in cleanroom air and other controlled environments. Users tend to see this as a device where a button is pressed and absolute results tumble out. However, when measuring anything small, it is important to be aware of the technology within the instrument to understand the relevance of the generated data, to put the operation of particle counters in context, and to be aware of the benefits and limitations of the technology. **No measurement is absolute—**all measurements are relative to the measuring technique employed. For example, if particles are measured with a scanning electron microscope, it would not be a surprise to get slightly different results than from a different technology. The instruments/technologies might even produce vastly different results between these two at certain sizes because each particular technology is producing a unique response.

Particle Types

Particles exist in a tremendous range of sizes, shapes, and compositions; for example, inside a cleanroom we could measure flakes of skin, small pieces of silicon or metal, or fungal spores. The sources can be very broad.

FIGURE B-1 LASAIR® PRO AEROSOL PARTICLE COUNTER

Particle Size

The size of particles is measured in micrometers (i.e., microns, µm), which is 10^{-6} meters (or a millionth of a meter and a thousandth of a millimeter). State of the art semi-conductor facilities measure in nanometers (nm), which is 10^{-9} meters (or a millionth of a millimeter); 1 micrometer is 1000 nm. However, many readers may be more interested in the 0.5 and 5 micron particles which are relevant to the pharmaceutical, health care, and medical device industries. To put size into perspective, visible particles are around 50 microns (e.g., a human hair would be 50 to 150 microns), non-visible particles such as bacteria are somewhere between sub 1 and 15 microns, and plant spores and pollens fall between the visible and non-visible at around 10 to 100 microns.

Most particles are non-uniform in structure, and this poses a question: How is the size qualified? If you ask those working in industry, some would say it is based on the longest length, others the

volume, and some an equivalent hole size through which a particle could pass. There would be many different answers, and it is probably fair to say that none of them would be wrong, provided that they are qualified.

Particle counters determine the size of particles by matching a signal response generated by the contaminant particle to an equivalent size of latex sphere. Users often look at the size and number distribution reported by an instrument and treat this data as absolute without recognizing that there are a number of operating variables, i.e., physical properties, refractive index, orientation, etc. All these factors play a part in the size indicated by the counter, and therefore, they play a role in the size channel in which the particle is counted.

Calibration Reference Standard using Latex Spheres

Aerosol particle counters are calibrated for size by sampling mono-dispersed (i.e., single size) polystyrene latex spheres (PSLs) nebulized into the flow of HEPA/ULPA grade filtered air. The instrument is adjusted for each test particle size used, and a calibration curve is generated within the instrument.

The sizing response from environmental particles is referenced by the instrument as an equivalent to a perfectly spherical latex sphere and counted in one particular size range or channel. As a result, any false sizing could affect not only the stated size but also the size channel into which the particle is allotted, affecting the number distribution.

How Particle Counters Work

All of the commonly used cleanroom airborne particle counters, regardless of their manufacturer, are based on the light scattering principle. Essentially, this means that they use a very bright light source to illuminate the particles. Nowadays, this source is a laser diode, where previously gas lasers and 'white light' halogen bulbs were used.

This very bright light source shines through an optical block. Within the optical block are mirrors and at least one photodetector. Sampled air is drawn through the laser beam by a small vacuum pump. As the particles in the air pass through the laser beam, the laser light interacts with particles and is scattered. The term 'scattering' means that the light undergoes a directional change. This change occurs in all directions: Forwards, backwards and sideways. Below is a schematic of how a sampled particle is read.

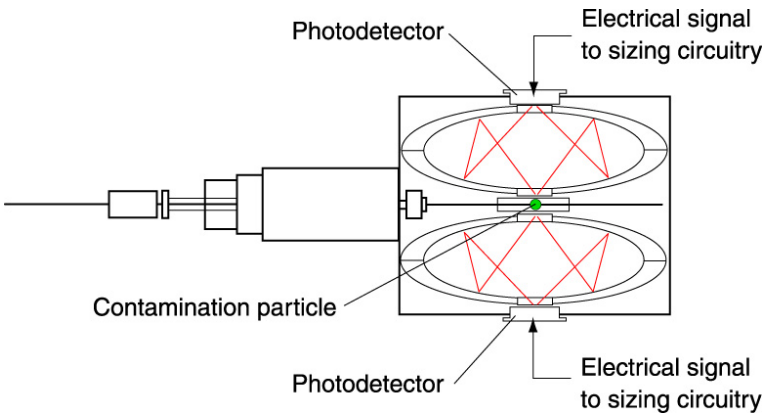


FIGURE B-2 PARTICLE COUNTER SCHEMATIC

The elliptical clam shapes in the diagram are mirrors. In a particle counter, they are silvered so that the reflecting surface is inside. As the light scatters, it is picked up by these mirrors, which focus the scattered light onto one or more photodetectors.

The photodetector converts the burst of light energy from each particle into a pulse of electrical energy. By measuring the height of the signal and referencing it to the calibration curve, we can determine the size of the particle, and by counting the number of pulses, we can determine quantity. It is relatively straightforward from that point to allocate particle numbers into size channels.

Light scattering is a general term and is composed of various different physical phenomena. Scattering is made up of:

1. **REFLECTED LIGHT** – when light hits a particle and is angularly deflected.
2. **REFRACTED LIGHT** – when light goes through the particle and its direction of travel is changed.
3. **DIFFRACTED LIGHT** – where the light comes close to the particle and is bent around it.

There may also be a degree of absorption (when a percentage of the light energy is retained by the particle), and in some instances, effects such as phosphorescence may occur from some particle types. Therefore, scattering is a combination of many physical properties relating to light, and the interaction of light and particles. The interaction of light and particles depends on the particle composition, its refractive index, and the difference between that particle and the background medium, (i.e., air).

In operation, the instrument compares the response it is getting from the particle signal to the calibration curve generated with latex spheres. What the instrument is actually doing is comparing the response from the interaction of that particle and the laser light and then relating it to the ideal latex sphere in a background of air. Users should be aware that particles with different scattering responses will size either smaller or larger relative to the latex standard.

For example, a silicon particle has a high reflectivity relative to the latex standard and is going to scatter a great deal of light. A particle of this material will size large compared to the standard. A particle that absorbs light or doesn't scatter very much light, such as a particle generated from a heat source, is going to size small relative to the latex standard. Therefore we are not looking at absolutes here. These sizing differences from the latex standard may assign the particles into larger or smaller size channels.

The orientation that the particle takes when passing through the laser beam will also have an effect on how it is sized. In an extreme example, if we sampled a rod shaped particle that exposes its full length to the laser beam, the light would strike the largest surface area and scatter a relatively large amount of light. If it only exposed one circular end, its relatively smaller cross sectional area would cause it to size as a small particle.

Conclusion

What particle counters do very well is allow users to take instantaneous samples and get a very good real time indication of the load of particles in a room or around a critical process. Alternative methods, such as using a filter, pump, or microscope, are time consuming, subjective, and labor-intensive.

Aerosol particle counters have been, and continue to be, crucial and beneficial in the development, operation, and advancement of cleanroom production environments. They are fast, well defined, and non-subjective, and modern instruments are now extremely stable, robust, and simple to use. By using the scientific principles of light scattering and comparison to a calibrated reference, these devices ensure repeatable, reliable data.

Author

Allen Humphries

Editor: Noelle Boyton

Introducing the new

PRO SERIES

Helping you meet all contamination control regulations including:

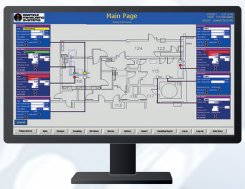
• EU Annex 1 • 21 CFR Part 11 • ISO 14644-1/2

PARTICLE COUNTERS

Portable and Remote

MICROBIAL MONITORS

Portable and Remote



DATA MANAGEMENT

Processors and Software

ADVISORY SERVICES

PARTICLE MEASURING SYSTEMS®
a spectris company

For more information contact
www.pmeasuring.com
info@pmeasuring.com

Applications

Applications: Cleanroom Standards

ISO 14644 Revisions Summary

Abstract

The recent revision of ISO 14644-1 and -2 has introduced several changes for cleanroom classification and monitoring guidelines. This paper will highlight the major changes in the new ISO 14644-1 compared to the previous version as well as the possible impact on the Pharmaceutical EU GMP Annex 1 and FDA Aseptic Processing Guideline.

Introduction

Over the last five years, the ISO Technical Committee 209 has been working on the revision of the basic airborne cleanliness classification, 14644-1 and -2.

The ISO community voted in favor of the revision to update and improve the standard specifically to:

- Simplify the classification process and, if possible, remove the need to evaluate the 95% upper confidence limit (UCL) for low sample location numbers (currently required for 2/9 of cleanroom locations)
- Review the classification procedure and make it more applicable to cleanroom operation. In this situation, the contamination is not expected to be evenly distributed: an assumption the current statistical approach makes
- Generally, update the standard as required to current thinking and industry requirements
- Avoid any radical change to the principles of the current ISO cleanliness classes 1-9

The same technical committee has also been working on the revision of the ISO 14644-2:2000 in conjunction with the revision of ISO 14644-1. The ISO community voted in favor of the revision to improve the ISO 14644-2:2000 standard to:

- Simplify and clarify requirement and guidance tables that specify frequency of testing and monitoring of cleanrooms used to demonstrate continued compliance with the cleanliness classification
- Refine how these intervals may be extended, provided that automated monitoring systems show the cleanroom is under control
- Provide new guidance on aspects that should be considered when configuring a monitoring system for a cleanroom

On October 29th 2015, during the last voting session, the revised 14644-1 and -2 Standards were approved by a significant majority of the member nations participating in the ISO/TC 209 committee.

Definitions

To simplify ISO 14644-1:2015, summaries are provided below of sections relevant to the revisions [1].

ISO 14644-1:2015
Cleanrooms and Associated Environments
Part 1. Classification of air cleanliness by particle concentration

This section specifies classes of air cleanliness for the world's cleanrooms and controlled environments in terms of the number of particles expressed as a concentration in air volume. To determine the class, a specified testing method is required, which includes selection of sampling locations.

ISO 14644-1 Introduction

ISO 14644-1 represents the first chapter of a series of documents, which describe the method, procedures, and limits to be applied in the cleanroom design, operation and controls. This standard is used in different industries including Microelectronics, Pharmaceuticals, Aerospace, Medical Devices, Healthcare, and Food Production.

It specifies classes of air cleanliness for the world's cleanrooms and controlled environments in terms of the number of particles expressed as a concentration in air volume. To determine the class, a specified testing method is required, which includes a strategic selection of sampling locations.

ISO 14644-1 Scope

The scope of this International Standard is to provide guidelines, specifications, and rules to be used for cleanroom certification in terms of airborne particle concentration. ISO 14644-1 addresses all consideration for classification purposes that have cumulative distributions based on threshold (lower limit) sizes ranging from 0.1 µm to 5 µm. Lower particle size concentration limits (nanoparticles), are addressed in ISO 14644-12.

2015 Revision

ISO 14644-1:2015 – New maximum concentration limits

TABLE C-1 Selected airborne particulate cleanliness classes

ISO 14644-1:2015 Classification Number (N)	MAXIMUM CONCENTRATION LIMITS (PARTICLES/m³)					
	0.1 µm	**0.2** µm	**0.3** µm	**0.5** µm	**1.0** µm	**5.0** µm
ISO CLASS 1	10					
ISO CLASS 2	100	24	10			
ISO CLASS 3	1 000	237	102	35		
ISO CLASS 4	10 000	2 370	1 020	352	83	
ISO CLASS 5	100 000	23 700	10 200	3 520	832	
ISO CLASS 6	1 000 000	237 000	102 000	35 200	8 320	298
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

The ISO classification is based on a new table (Table C-1), which uses the current and well-known formula for the intermediate decimal classes:

$$C_n = 10^N \times \left(\frac{K}{D}\right)^{2.08}$$

where

C_n is the maximum permitted concentration (particles per cubic meter) of airborne particles that are equal to and greater than the considered particle size.

N is the ISO classification number, which shall not exceed a value of 9 or be less than 1

D is the considered particle size, in micrometers, that is not listed in Table C-1

K is a constant, 0.1, expressed in micrometers

The foremost concern in the Life Science industry is the removal of the ≥ 5 µm particle concentration in ISO Class 5 clean areas (for classification purposes) when compared to the ISO 14644-1:1999 version.

In the 1999 version, the limit is 29 particles per cubic meter as reported on the table below (Table C-2) [2]. This change to the ISO/DIS 14644 standard is a major concern for a number of reviewers.

TABLE C-2 Selected airborne particulate cleanliness classes

ISO 14644-1:1999 Classification Number (N)	Maximum concentration limits (particles/m3)					
	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1.0 µm	5.0 µm
ISO CLASS 1	10					
ISO CLASS 2	100	24	10			
ISO CLASS 3	1 000	237	102	35		
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	298
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

The reasons for the de-emphasis on the ≥ 5 µm ISO Class 5 limit include:

- Sampling and statistical limitations for particles in low concentrations make this classification inappropriate.
- Sample collection limitations for both particles in low concentrations and sizes greater than 1 µm make classification at this particle size inappropriate, due to potential particle losses in the sampling system.

ISO 14644-1:2015 – Sample locations

In order to achieve the goals of the ISO community, the significant changes with ISO 14644-1 are related to the revision of the classification method, summarized as follows.

Number of sample locations

- A new table has been developed for the determination of the number of sample locations, replacing:

$$\text{Location number} = \sqrt{m^2 \times \text{room area}}$$

from the ISO 14644-1:1999 version of the standard.

- For all room sizes above 6 m^2, the new table (Table C-3 below) results in an increase of required sample locations.
- The ISO 14644-1:1999 standard required the UCL 95% (Upper Confidential Limit) calculation for sample locations between 2 and 9.
- The new table has been pre-calculated to eliminate the need for this calculation. The new method, when successfully applied, assures that at least 90% of the room is compliant at a 95% confidence limit.

TABLE C-3 Number of sample locations required

AREA OF ZONE (m^2)	ISO 14644-1:1999	ISO 14644-1:2015
2	2	1
4	2	2
6	3	3
8	3	4
10	4	5
24	5	6
28	6	7
32	6	8
36	6	9
52	8	10
56	8	11
64	8	12
68	9	13
72	9	14
76	9	15
104	11	16
108	11	17
116	11	18
148	13	19
156	13	20
192	14	21
232	16	22
276	17	23
352	19	24
436	21	25
636	24	26
1000	32	27
>1000	N/A	SEE FORMULA A.1

$$N_L = 27 \times \left(\frac{A \times m^2}{1000} \right)$$

Formula A.1: Formula used to determine the number of sampling locations

The determination of each sampling location will be based on a semi-random sampling technique, based on a "hypergeometric" distribution, which is the statistical model for sampling without replacement.

This is a significant change from current practice, meaning that each time a zone is classified, the sample locations may be different. If a company has determined through a risk assessment that certain locations need to be examined specifically, then these should be applied in addition to the randomly selected locations.

Recognizing that the ≥ 5.0 micron class limit for ISO 5 has been removed in the revised standard, parties wishing to use the standard for classifying the environments EU GMP Grade A and B "at rest" will have to use the macro-particle limit table retained in the standard.

Instrument Calibration

Another important change in the new revised Standard is represented by the need to use ISO 21501-4 compliant particle counters.

The paragraph B.2.2 of the (previous) ISO 14644-1:1999 required using "calibrated" particle counters, not mentioning any specific calibration technique.

> **B.2.2 Instrument calibration**
> The instrument shall have a valid calibration certificate; the frequency and method of calibration should be based on current accepted practice.

The newly released version of ISO 14644-1:2015 specifically requires the use of ISO 21501-4 compliant instruments, as stated in paragraph A.2.2.

> **A.2.2 Instrument calibration**
> The particle counter shall have a valid calibration certificate: the frequency and method of calibration should be based upon current accepted practice as specified in ISO 21501-4.
>
> *Note: Some particle counters cannot be calibrated to all of the required tests in ISO 21501-4. If this is the case, record the decision to use the counter in the test report.*

Not all instruments will be able to meet the requirements described in A.2.2, and the use of non-compliant instruments will require an additional explanation for authorities.

More information about the ISO 21501-4 requirements can be found on a separate application note, available on the Particle Measuring Systems website, Knowledge Page: Understanding ISO 21501-4.

Conclusion

The new changes described here will impact cleanroom classifications, and any company that needs to comply with this standard will be required to update their internal SOP in order to meet the new ISO 14644 requirements.

ISO 14644-1:2015 was published on Nov. 1st, 2015 and will be effective starting from Jan. 1, 2016. All users who want to be compliant with this standard will be required to take any necessary action before the end of 2016.

All Particle Measuring Systems instruments will have updates applied in order to fully meet these new ISO requirements.

For more information about the new ISO 14644-1:2015, instrument firmware upgrades, or expert consultancies, contact your Particle Measuring Systems local representative or use our website to Contact Us.

References

1. International Standards Organization. C*leanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration*, ISO Standard No. 14644-1:2015 (2015).
2. International Standards Organization. *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness*, ISO Standard No. 14644-1:1999 (1999).

Author

Daniele Pandolfi

Global Product Line Manager for Data Management/Aerosol
Life Science Division
Particle Measuring Systems

Daniele Pandolfi has had experience in particle counter instrumentation and cleanroom contamination control for over ten years. While building strong customer relationships, he has helped many people solve their cGMP issues. Outside of work, he is a semi-professional photographer, an enthusiast of electronics, a lover of new technology, and a keen traveler. He can be reached at **dpandolfi@pmeasuring.com**

Editor: Noelle Boyton

Applications: EU GMP Annex 1

The New EU GMP Annex 1 Revision: Impact on Environmental Monitoring Programs

Fundamentals

Applications

Cleanroom Standards ISO14644-1 ISO14644-2

EU GMP Annex 1

Cleanroom Classification

Cleanroom Monitoring

Risk Assessment

Solutions

Introduction

In August 2022, a new revision of the EU GMP Annex 1 regulatory standard of the EU Guideline for Good Manufacturing Practice for sterile drug products and drug substances was released by the European Commission, replacing the most recent draft from 2020 and the existing revision from 2008 [1,2]. The deadline for operational use of the new standards is August 25, 2023: a year after the release. These requirements regulate the manufacturing of sterile drugs made in and imported to the EU. Pharmaceutical manufacturing is performed in controlled environments to reduce contamination, and changes recently announced by Annex 1 focus more on strategic control than on measurement of quality. This new revision better aligns the manufacturing principles contained in the Annex 1 to those presented by the World Health Organization (WHO), Pharmaceutical Inspection Cooperation Scheme (PIC/S), and US Food and Drug Administration (FDA).

The new revision is a complete rewrite of the existing Annex 1 from 2008 [2] and almost quadruples the length. It divides the document into 10 newly defined sections. One major sectioning change is the separation and differentiation of Certification (Section 4) and Monitoring (Section 9), which allows for expanded guidance and distinction between premise design/ qualification and ongoing routine monitoring. There is a new section that discusses the concept of contamination control strategy (CCS). This section shifts to a new paradigm of incorporating CCS as a central holistic approach to how each aspect of contamination interacts with the facility as a whole. There is also a new section that discusses and identifies Quality Risk Management (QRM) as a central principle to defining processes, operations, and limits, and it ties to CCS to balance process against risk. Additionally, as laid out in the new revision, regulations for Environmental Monitoring is essentially the same with a few enhanced descriptions to better align with QRM.

FIGURE D-1 EUROPEAN COMMISSION LOGO

Annex 1 Structure

The new structure provides a comprehensive understanding of where to find relevant content. While the former 2008 version was not well organized and became a patchwork of changes over time, the new authors compiled the content in a logical, easy-to-follow way.

Quality and Manufacturing Officers interested in environmental monitoring will readily find relevant content primarily in Chapter 5 “Premises” and Chapter 9 “Viable and nonviable environmental and process monitoring”.

Cleanroom Classification and Qualification

In the Annex 1 revision, most of the relevant principles of the ISO 14644-1:2015 standard are included (Section 5.26) [3]. The initial number of sampling sites, their even distribution and related sampling volume in critical zones (Grade A and B) rely on this standard.

However, it is clearly stated that these are only the minimum requirements sufficient to classify a cleanroom. All further decisions must be based on process knowledge and risk assessment. Consequently, it will become very difficult in the future to defend why lesser parameters for the qualification were chosen, especially for inspection of manufacturing environment sampling. This ties deeply with the statement in Section 5.28 of the ISO 14644-1:2015 standards, where "Clean room qualification (including classification) should be clearly differentiated from operational process environmental monitoring". A clear differentiation needs to be made between each phase of a clean environment's lifetime.

Let's bring this concept into a simple equation:

INITIAL CLASSIFICATION ≠ RE-QUALIFICATION ≠ PROCESS MONITORING

The classification of a cleanroom, covered in the ISO 14644-1:2015 standard, is based on particle load. There are no microbial limits given for this part of the process, but there has been a major change towards the 2008 version of the guideline:

In chapter 5.25, particles of the size equal to or bigger than 5 µm have been removed from the classification and qualification limit table for Grade A, but kept in the recommended limits for monitoring of the process environment.

The reasons for the de-emphasis on the 5 µm limit in Grade A class includes:

- Harmonization of the European Requirements with the recent release of ISO 14644-1, where the 5 µm limit in ISO Class 5 has already been removed.
- Sampling and statistical limitations for particles in low concentrations make this classification inappropriate.
- Sample collection limitations for both particles in low concentrations and sizes greater than 1 µm make classification at this particle size inappropriate, due to potential particle losses in the sampling system.

De-emphasis of the 5 µm limit only refers to the cleanroom classification process. The 5 µm particles still represent an important indicator of possible contamination during the manufacturing process and, therefore, must be kept under control continuously during filling and manufacturing.

Discrepancy in the treatment of 5 µm particles between classification and monitoring are of foremost concern and may cause discussion on the possible risk of not considering certain particle sizes during initial qualification. These sizes will need to be within certain limits during monitoring.

The language pertaining to the responsibility of defining alert and action levels and limits has been made stronger and clearly refers to the cleanroom user, who must define the appropriate

values based on a formal risk assessment and data trending analysis. This change emphasizes the expectation of regulators that manufacturers set their action and alert limits based on historical data, process knowledge and a risk-based approach. In addition, it is important not only to define particle limits, but also an appropriate alarm strategy which encourages the evaluation of ISO 14644-2 and its recommended practices (paragraph B.3.4) [4].

The following strategies consider the importance of evaluating an alert or alarm situation using a series of events rather than a single spot value.

Strategy 1

Establish a trigger threshold value based on a series of consecutivey higher readings.

For example: 3 consecutive 1-minute reading all above a specified level.

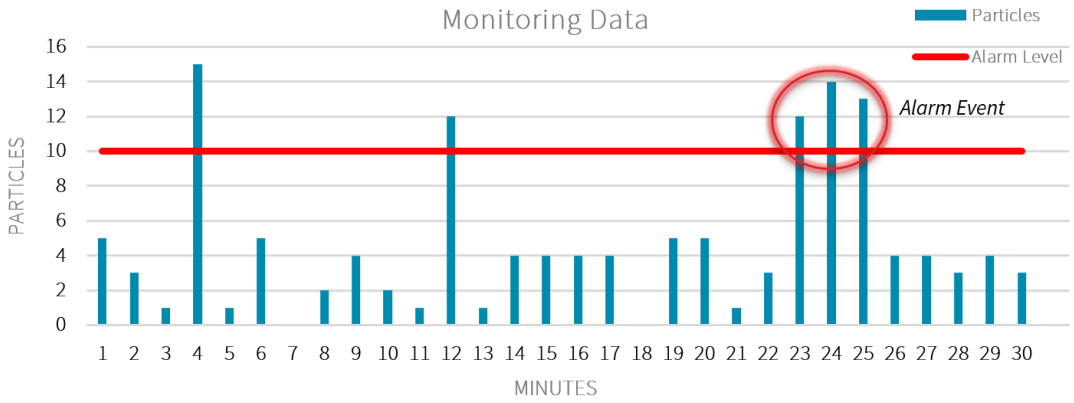


FIGURE D-2 GRAPHICAL REPRESENTATION OF STRATEGY 1

Strategy 2

Establish a trigger threshold value based on a high frequency of elevated readings. This method is commonly referred to as "x out of y, " where "x" is the number of events and "y" is the number of minutes.

For example: 3 out of the last 10 readings/minutes are above the specified alarm threshold.

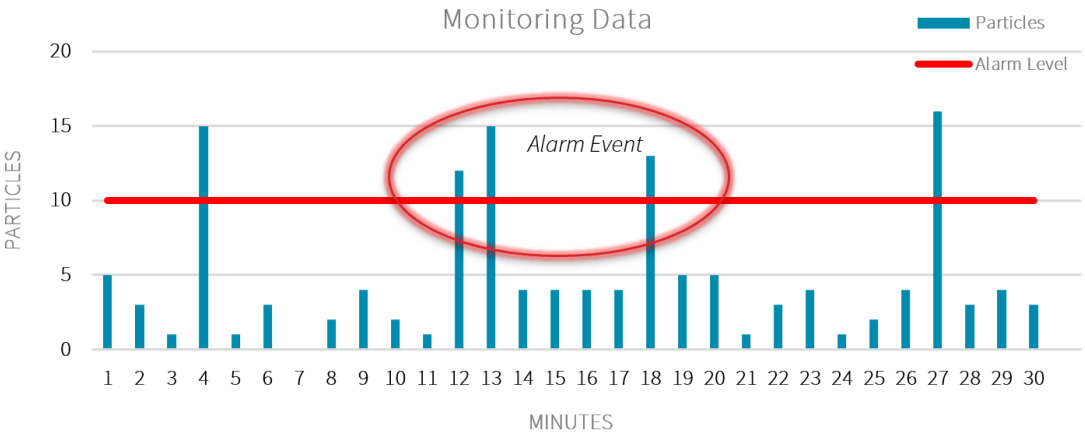


FIGURE D-3 GRAPHICAL REPRESENTATION OF STRATEGY 2

More information about monitoring guidelines dictated by ISO 14644-2:2015 can be found in the Cleanroom Monitoring Chapter of this book.

Re-qualification Frequency

Section 5.29 gives manufacturers a challenge: Bi-annual re-qualification of critical zones (Grade A and B) are becoming a standard of the industry. It is already a widespread practice, but many pharmaceutical companies have differing strategies that will need to be thoroughly explained in upcoming inspections. Modern technologies, including real-time methods for viable counts, that minimize downtimes caused by the re-qualification process and increase productivity will become more crucial to the success of pharmaceutical companies.

NOTE ***Throughout the new revision, Grade A and B environments are considered almost equal in the way they are treated for cleanliness.***

Annex 1 and Microbial Impurities

Microbial impurities can be divided into “viable” and “nonviable” particles. “Nonviable” impurities are inert and will not contain any microorganisms, and readers of the new regulation should be educated on all technical terms used to prevent misunderstandings of the document.

Laser-based particle counting, typically used for determining “nonviable” levels in a critical pharmaceutical environment, displays both “nonviable” and "viable" particles, which is comprised of "viable and culturable" and “viable but not culturable” (VBNC) impurities. The following equation represents what is seen by laser-based particle counting:

NONVIABLE = INERT + VIABLE AND CULTURABLE + VIABLE BUT NOT CULTURABLE

This equation is nonsense but its components have widespread usage, and therefore difficult to change. At a minimum, people using this terminology should be aware of the potential drawbacks from the habitual use of this language.

Cleanrooms for pharmaceutical use are not classified by microbiological parameters, but rather on the nonviable/inert aspect. Therefore, microbiological considerations start when the rooms are qualified for intended use. As in the 2008 version, this is called the “in operation” stage and proposed action limits can be found in Table 2 of the document. Although the values in the table look familiar, some major changes have been made:

- The values for Grade A zones are now set to "no growth" and not <1.
- No averaging of results is allowed.

Consequently, manufacturers are no longer allowed to “average out” non-welcome results by looking at a scale of multiple measurements.

Each single result should be considered and cause a deviation resulting in a full investigation. However, this is only true for the qualification of the “in operation” stage and does not apply for the monitoring of the process. True routine “in operation” monitoring limits can only be based on historical data and locations, frequency, volume and duration of monitoring on a risk-based approach and data generated during the qualification, as stated in Section 9.5. This may create some confusion between the qualification stage's “in operation” and the routine monitoring program's “in operation”.

Sections 9.7 and 9.27 follow previously established standards in terms of viable sampling which can be found in other regulatory documents.

> 9.7 The monitoring of grade A should demonstrate the maintenance of aseptic processing conditions during critical operations. Monitoring should be performed at locations posing the highest risk of contamination to the sterile equipment surfaces, containers, closures and product. The selection of monitoring locations and the orientation and positioning of sampling devices should be justified and appropriate to obtain reliable data from the critical zones.

FIGURE D-4 EU GMP ANNEX 1 2022, SECTION 9.7

In essence, sampling should be done as close as possible to the critical area in Grade A environments, but without posing any risk to the process and sampling itself. To do both has been a long-lasting dilemma, and often requires a specialized approach using technologies such as single use.

The frequency of viable sampling has received an almost revolutionary renewal in the revision to Annex 1, and it has become integrated with increasing control over the process by scientifically sound rationale. One significant change is that viable sampling should be performed continuously during routine process monitoring, as stated in Section 9.24. It will no longer be acceptable to have only small, snapshot sampling that does not characterize the entire manufacturing process. This concept was applied in the 2008 version for “nonviable” counts and has now been expanded into “viable” counts, creating some short-term challenges for manufacturers. Continuous data generation can only be achieved by either real-time methods or long-term, traditional viable sampling that is quasi-continuous. The right combination of methods will become critical in the decision-making process, and it will be an area of interest to see how inspectors push these requirements into the field and how manufacturers will respond. As always, the reasoning for all decisions must be documented and based on risk assessment and historical/scientific data.

> 9.24 Continuous viable air monitoring in grade A (e.g. air sampling or settle plates) should be undertaken for the full duration of critical processing, including equipment (aseptic set-up) assembly and critical processing. A similar approach should be considered for grade B cleanrooms based on the risk of impact on the aseptic processing. The monitoring should be performed in such a way that all interventions, transient events and any system deterioration would be captured and any risk caused by interventions of the monitoring operations is avoided.

FIGURE D-5 EU GMP ANNEX 1 2002, SECTION 9.24

Interestingly these strategies also apply to personnel monitoring (Section 9.25). At the moment, manufacturers tend to avoid multiple samplings of operators in order to prevent contamination build-up and the subsequent risk to the process and products. A possible solution could be the implementation of more sampling techniques that are not susceptible for residuals, such as the use of swabs instead of contact plates.

A greater emphasis on the qualification and monitoring of personnel is seen in Chapter 7. The stricter requirements may result in operators taking longer to contribute to the productivity of the company (until they are fully qualified). Good personnel will become an even more precious resource in the manufacturing of sterile drugs.

Grade A and B zones are now considered almost equivalent in how they are treated from a monitoring perspective, and Sections 9.31 and 9.43 impose on manufacturers the need to identify all microorganisms found in these environments down to the species level. This new requirement emphasizes:

1. The importance of Grade B in final product quality.
2. The need for investigations in both cleanrooms.
3. The need for understanding the instruments used in these zones and their capability to contribute to contamination.

Trending of environmental data, which has already been implemented on a worldwide scale, has finally found its representation in Section 10.10.

> 10.10 Environmental monitoring data and trend data generated for classified areas should be reviewed as part of product batch certification/release. A written procedure should be available that describes the actions to be taken when data from environmental monitoring are found out of trend or exceeding the established limits. For products with short shelf life, the environmental data for the time of manufacture may not be available; in these cases, the compliance should include a review of the most recent available data. Manufacturers of these products should consider the use of rapid/alternative methods.

FIGURE D-6 EU GMP ANNEX 1 2022, SECTION 10.10

Conclusion

The new Annex 1 revision provides insight into upcoming regulatory trends. In terms of environmental monitoring content, there is significant emphasis placed on manufacturers basing their decisions on scientifically sound and historical data while applying a risk-based approach.

From a microbiology and particle contamination perspective, this document may push modern, relevant and scientifically sound monitoring methods into the pharmaceutical world, in addition to a reasonable trending approach to data analysis.

The overall quality of products is sure to increase with the released revision, and a stronger and deeper understanding of cleanroom performance inside each single company should be fostered by allowing continuing discussion and evaluation of the collected sampling data, and development of user set alert and action levels. Monitoring plans should be proactively revised using growing knowledge of the process and risk assessment tools. Rather than considering each single monitoring session as isolated from the whole program, consider it part of a bigger picture.

For a more detailed section by section explanation of the new Annex 1 revision check out this paper!

Review of Annex 1 2022

References

1. European Commission. *The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products*, Annex 1 (2022).
2. European Commission, EudraLex. T*he Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products (corrected version),* Annex 1 (2008).
3. International Standards Organization. *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration,* ISO Standard No. 14644-1:2015 (2015).
4. International Standards Organization. *Cleanrooms and associated controlled environments — Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration*, ISO Standard No. 14644-2:2015 (2015).

Authors

Daniele Pandolfi

Global Product Line Manager for Data Management/Aerosol
Life Science Division
Particle Measuring Systems

Daniele Pandolfi has had experience in particle counter instrumentation and cleanroom contamination control for over ten years. While building strong customer relationships, he has helped many people solve their cGMP issues. Outside of work, he is a semi-professional photographer, an enthusiast of electronics, a lover of new technology, and a keen traveler. He can be reached at **dpandolfi@pmeasuring.com**

Frank Panofen, PhD

Director and General Manager Life Sciences
Life Science Division
Particle Measuring Systems

Dr. Panofen has a Diploma in Chemistry from the University of Bielefeld and a PhD in molecular and cell biology from the University of Osnabrück. He has expansive experience in the field of applied pharmaceutical microbiology and serves as the Sterility Assurance/ Microbiology Product Line Manager at Particle Measuring Systems. Frank has been an invited speaker at international conferences including ECA and PDA, with a strong regulatory background in pharmaceuticals. He is a certified Microbiological Laboratory Manager from ECA. He can be reached at **fpanofen@pmeasuring.com.**

Editor: Noelle Boyton

Who defines your Contamination Control Strategy?

The experts you need to support you at every step of your process including risk analysis and EM data interpretation.

Advisory Services

Helping you to achieve the highest level of contamination control and sterility assurance

Training

Preparing your employees
ensure success at every step

Performance Optimization

Designing quality into your operations using gold standard PMS tools & processes

- Environmental MonitoringRisk/ Gap Aalysis
- Cleaning and Disinfection
- Aseptic Process Simulation
- Sterility Assurance Audits
- Environmental Monitoring Trend Analysis
- Gowning
- New Filling Line QbD
- Crisis Management
- Utilities
- Process Risk Assessment

PARTICLE MEASURING SYSTEMS®
a spectris company

For more information contact

www.pmeasuring.com
info@pmeasuring.com

Applications: Cleanroom Classification

Cleanroom Classification for Pharmaceutical Applications

Pharmaceutical products are manufactured to meet exacting standards of both efficacy and quality. All aspects of quality are reviewed considering the risks associated with the delivery method (injected, ingested etc.) and the manner in which they were produced (aseptic, terminally sterilized, or under lesser controlled conditions). This chapter looks at two parts of that process: the quality of the environment in which the product is manufactured and the standards that surround the particle concentration limits that determine what a controlled environment consists of.

This chapter examines the standards for physical testing (EN ISO 14644-1:2015 [1]) and those standards which apply in regulatory guidance (EU GMP Annex 1 [2]).

ISO 14644-1

In 1999, the new ISO 14644 room classification suite of standards became active, the first of which was ISO 14644-1, which determined the method by which a room should be classified and the maximum allowable particles within a fixed volume of air. The reader should note that although ISO 14644 has been adopted globally for cleanroom classification, there are differences for routine monitoring, particularly between ISO 14644 and EU and WHO GMP.

The certification state of the cleanroom must be defined in advance of testing; three states exist within the context of ISO 14644-1:

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 limits for the cleanroom concentration of particles greater than a prescribed size are defined and presented in Table E-1 (below).

TABLE E-1 Airborne particulate cleanliness classes for cleanroom and clean zones (as indicated in ISO 14644)

ISO 14644-1:2015 Classification Number (N)	MAXIMUM CONCENTRATION LIMITS (PARTICLES/m³)					
	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1.0 µm	5.0 µm
ISO CLASS 1	10					
ISO CLASS 2	100	24	10			
ISO CLASS 3	1 000	237	102	35		
ISO CLASS 4	10 000	2 370	1 020	352	83	
ISO CLASS 5	100 000	23 700	10 200	3 520	832	
ISO CLASS 6	1 000 000	237 000	102 000	35 200	8 320	298
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

These limits have been defined in accordance with the calculation from the standard using the following formula for the intermediate decimal classes:

$$C_n = 10^N \times \left(\frac{K}{D}\right)^{2.08}$$

where

C_n is the maximum permitted concentration (particles per cubic meter) of airborne particles that are equal to and greater than the considered particle size.

N is the ISO classification number, which shall not exceed a value of 9 or be less than 1

D is the considered particle size, in micrometers, that is not listed in Table E-1

K is a constant, 0.1, expressed in micrometers

The relationship of particle size to its abundance within a population is therefore a function of $\left(\frac{1}{D}\right)^{2.08}$, and if the particle size is plotted against its concentration on a log/log scale, the slope of the curve for each class is 2.08; this relationship is shown in the table above.

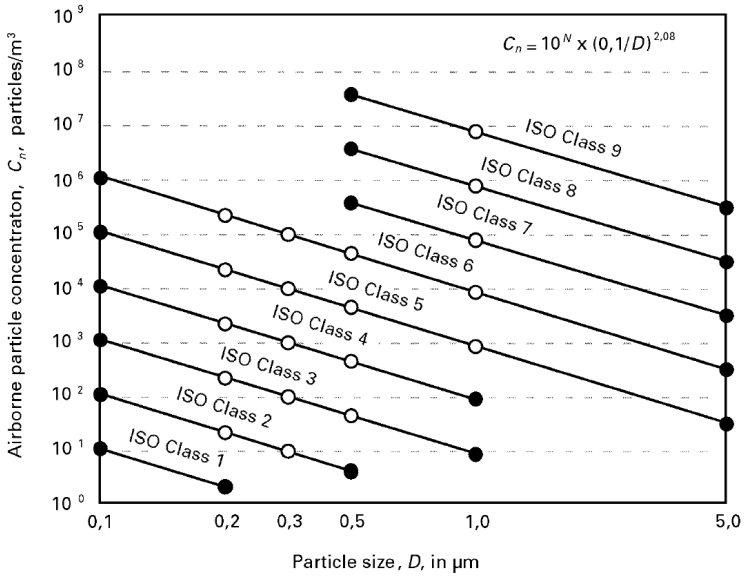


FIGURE E-1 GRAPHICAL REPRESENTATION OF ISO CLASS CONCENTRATIONS LIMITS FOR SELECTED CLASSES.

The designation for cleanroom or clean zone certification should also include the following elements:

- The room classification number expressed as “ISO Class N”.
- The occupancy state.
- The considered particle size. It is also possible to certify a cleanroom at multiple sizes; if this is the case then the sample volume requirement for the largest particle size is used.

Example:

An example would be: unidirectional airflow device x is an ISO Class 5 clean zone at 0.5 µm (3520 n/m^3), operational state.

The clean zone now needs to be tested to prove the statement; the ISO 14644-1 standard identifies each of the component steps required to prove compliance.

Assume we have a clean air device that we want to use for aseptic preparation area. This area needs to meet ISO Class 5 at 0.5 µm (≡ 10,000 /ft^3) in the operational state, how do we go about the process of determining the classification of this are?

The room is 12 m by 5 m (60 m^2) and has a worktable in the center of the room.

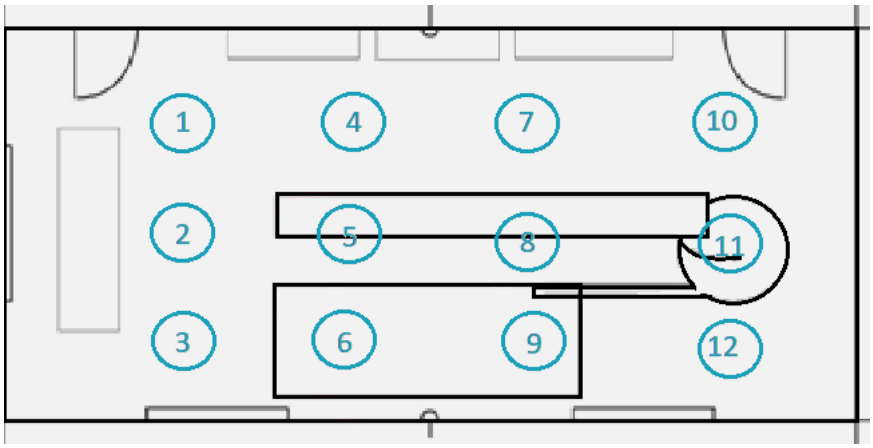


FIGURE E-2 DIAGRAM SHOWING THE LOCATIONS FOR CLASSIFICATION WITHIN THE CLEAN ZONE

Step 1. Calculate the maximum permitted particle concentration

$$C_n = 10^5 \times \left(\frac{0.1}{0.5}\right)^{2.08} = 3517 \quad \text{rounded to } 3520 \text{ n}/m^3$$

FIGURE E-3 MAXIMUM PERMITTED PARTICLE CONCENTRATION CALCULATION

C_n	3520 n/m³	is the maximum permitted concentration
N	5	is the ISO classification number
D	0.5 µm	is the considered particle size, in micrometers
K	0.1 µm	is a constant, 0.1, expressed in micrometers

Step 2. Determine the number of sample locations from the Table below

TABLE E-2 Number of sample locations required

Area of Zone (m²)	ISO 14644-1:1999	ISO 14644-1:2015
2	2	1
4	2	2
6	3	3
8	3	4
10	4	5
24	5	6
28	6	7
32	6	8
36	6	9

Area of Zone (m²)	ISO 14644-1:1999	ISO 14644-1:2015
52	8	10
56	8	11
64	8	12
68	9	13
72	9	14
76	9	15
104	11	16
108	11	17

Step 3. Calculate the sample volume

$$V_s = \left(\frac{20}{C_{n,m}}\right) \times 1000$$

$$V_s = \left(\frac{20}{3520}\right) \times 1000 = 5.68 \text{ liters}$$

From ISO 14644-1:2015, this formula finds a sample valume of 5.68 L, and for a standard particle counter with a flowrate of 28.3 L/min, a 1-min sample would surpass the criteria of minimum sample volume.

So, to meet specification we shall take a 1 minute sample at each of the 12 locations.

Step 4. Take measurements at each location (1 minute per sample) and record results

TABLE E-3 Illustration of locations within the example clean zone and measurement results

LOCATION	NUMBER /m³
1	708
2	885
3	1522
4	2336
5	3363
6	2584
7	2301
8	2089
9	1344
10	2013
11	1756
12	1897

Step 5. Define report

$$\text{Max from all locations} = 3,363 \text{ n}/m^3 < 3,520 \text{ n}/m^3 \text{class limit} = \text{PASS}$$

This area meets the specification for an ISO Class 5 clean zone at 0.5 µm and can now be used for the purpose that it was designed for. Room classification will need to be repeated on a frequency defined by ISO 14644-2 [3].

EU GMP Annex 1

The European Union GMP guidance for sterile manufacture was revised in 2003 to accommodate the changes from various cleanroom standards to a single unified cleanroom standard [4], ISO4644-1. The front page makes note of this:

> *"Annex 1 of the EC Guide to Good Manufacturing Practice (GMP) provides supplementary guidance on the application of the principles and guidelines of GMP to sterile medicinal products. The guidance includes recommendations on standards of environmental cleanliness for clean rooms. The guidance has been reviewed in the light of the international standard EN/ISO 14644-1 and amended in the interests of harmonisation but taking into account specific concerns unique to the production of sterile medicinal products."*

Specifically, the means by which a cleanroom was certified needed to comply with the rules and format of the ISO14644-1 guidance, but, the ISO standard was modified with respect to sterile medicinal products. To that end a table of cleanroom certification values that roughly translated to ISO 14644-1 was defined.

For clarity a series of notes appended the table, unfortunately the first of which, Note 'a', caused certain confusion. This confusion was remedied in the 2008 release of the EU GMP Annex 1 which clearly outlines three phases that need to be performed [5]. Each cleanroom and clean air device should first be classified; it should then be monitored to verify that conditions are being maintained relative to product quality and that the data accrued from said monitoring be reviewed in the light of risk to finished product quality.

TABLE E-4 EU GMP Annex 1:2008 room classification table

EU GMP Grade	At Rest		In Operation	
	Maximum number of particles permitted/m³ equal to or greater than the tabulated size			
	0.5 µm	5.0 µm	0.5 µm	5.0 µm
A	3520	20	3520	20
B	3520	29	352,000	2900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	NOT DEFINED	NOT DEFINED

The reader will note, as indicated above, that the limits in the EU GMP table differ slightly to those in the ISO 14644 standard. To perform the required certification, it is important to know the workings of ISO 14644-1, how to certify a cleanroom in accordance with that standard, rules on number of sample points, rules on sample point location, rules on volume of sample to be taken at each location, and the rules on statistical analysis of cleanroom data that need to be followed. However, rather than use the table for classification limits prescribed in ISO 14644-1, one should be using the table shown above, as printed in the revised guidance document.

Other expectations are also defined by the GMP. These can be expectations such as having a sample volume for Grade A of 1m³ per sample location or using a minimum sample tubing length due to the high precipitation of 5.0 µm particles in transport tubing (ideally no sample tubing should be used). Also, recertification of the cleanroom should follow the guidance given in ISO 14644-2: once per year for ISO Grade 6 and greater and once per six months for ISO Grade 5. Concessions are made for extending the ISO Grade 5 areas if a continuous monitoring system has been implemented. Suitable times to perform certification are media fills or simulated filling runs.

References

1. International Standards Organization. *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration,* ISO Standard No. 14644-1:2015 (2015).
2. European Commission. *The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products*, Annex 1 (2022).
3. International Standards Organization. *Cleanrooms and associated controlled environments — Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration*, ISO Standard No. 14644-2:2015 (2015).
4. European Commission. *EC Guide to Good Manufacturing Practice Revision to Annex 1: Manufacture of Sterile Medicinal Products*, Annex 1 (2003).
5. European Commission, EudraLex. T*he Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products (corrected version),* Annex 1 (2008).

Author

Mark Hallworth
Life Sciences Senior GMP Scientist
Life Science Division
Particle Measuring Systems

Mark Hallworth is the Life Sciences Regional Manager for Particle Measuring Systems. He has lectured for pharmaceutical societies throughout Europe, Asia, and the US on nonviable particle and facility monitoring and the implications of validating those systems. He can be reached at **mhallworth@pmeasuring.com**.

Editor: Noelle Boyton

Applications: Cleanroom Monitoring

Understanding ISO Standards: ISO 14644-2:2015 Cleanroom Monitoring

Fundamentals

Applications

Cleanroom Standards ISO14644-1 ISO14644-2

EU GMP Annex 1

Cleanroom Classification

Cleanroom Monitoring

Risk Assessment

Solutions

Abstract

This chapter underlines the major changes between the previous version of ISO 14644-2 and the latest, second edition, dated December 12, 2015. This chapter discusses a specific approach to the best practices to be adopted for an efficient and compliant cleanroom monitoring process.

Introduction

In combination with the revision to *Part 1: Classification of air cleanliness by particle concentration* of ISO 14644-1:2015 [1], the ISO Technical Committee TC 209 has been working on the update to the basic airborne cleanliness monitoring guidelines contained in ISO 14644-2 [2].

The TC 209 community voted in favor of the revision to update and improve the standard specifically to:

- Simplify and clarify requirements and guidance tables that specify frequency of testing and monitoring of cleanrooms used to demonstrate continued compliance with the cleanliness classification
- Refine how these intervals may be extended, provided that automated monitoring systems show the cleanroom is under control
- Provide new guidance on aspects that should be considered when configuring a monitoring system for a cleanroom

The new standard's introduction also lists the goals of the ISO 14644-2, when successfully applied:

- Emphasize the needs and advantages of a planned cleanroom contamination monitoring
- Provides the method for a correct particles contamination alarm and warning limits setting, based on a careful evaluation of data trends
- Define the differences between a simple periodic cleanroom control and a more intensive/complex monitoring strategy
- Enhance the installation and process knowledge, as to improve the risk assessment evaluation and a faster reaction to any unexpected cleanroom performance
- Concretely reduce the operation cost by preventing production loss

Terminology

Before we get into the specific requirements, the understanding of ISO Standard terminology is essential. Below is a list of the most important terms and their definitions:

Monitoring

Monitoring is an observation of the process made in accordance with a specific method, able to provide clear evidence of cleanroom performance.

Monitoring can be *continuous*, *sequential*, or *periodic*.

Action Level

User set level that, when exceeded, will require immediate intervention, root cause investigation, and corrective actions.

Alert Level

User set level that is defined to provide early warning of a drift from normal conditions. This level should be used to prevent action level conditions.

Risk Assessment

The appropriate creation of risk assessment documentation is a basic requirement for implementing a monitoring plan.

The new ISO 14644-2:2015 requires a well-developed risk assessment document as a tool to correctly understand the process, the critical areas/locations, possible sources of contamination, and any element/event that may compromise or negatively impact the cleanroom performance, product quality, or operation cost.

ICH Q9 – QUALITY RISK ASSESSMENT

The 2005 *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use* is one of the best guidelines available for proper risk assessment development, review, and application. It is well integrated with good manufacturing practices required by the Pharmaceutical Industry Standards.

ICH
harmonisation for better health

PDA - PARENTAL DRUG ASSOCIATION

Fundamentals of an Environmental Monitoring Program, Technical Report No.13 is also a useful document when approaching monitoring plan development, and it addresses the necessity of a meaningful, manageable, and defensible monitoring program.

PDA®
Parenteral Drug Association

Other useful tools to be considered for reliable risk assessment development are HACCP, FMEA/ FMECA, PHA, FTA, and/or HAZOP.

A responsible understanding of the production process and installation performance aids in the prevention of unexpected out of specification conditions and also assists in achieving energy saving targets.

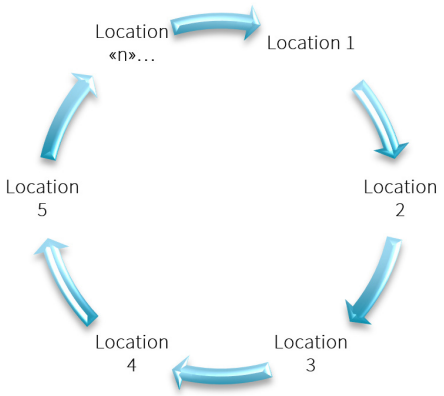
Monitoring Plan

Particle concentration control is mandatory for cleanroom classification (ISO 14644-1:2015) and monitoring (ISO 14644-2:2015). It provides clear evidence of cleanliness level and the capability of cleanrooms or clean zones to accomplish required production performance.

CLEANROOM MONITORING CAN BE:

SEQUENTIAL

Sequential monitoring is normally performed by using multiplexing manifold systems. This practice is well accepted in semiconductor industries where small particle size (no greater than 1 micron) are monitored.



The sequential monitoring method typically utilizes long transport tubes, which is unacceptable in Pharmaceutical Manufacturing cleanrooms where larger particles are considered and the risk of particle loss in tubing isn't negligible.

Read more about Particle Loss in Tubing.

CONTINUOUS

The continuous method uses multiple particle counters, one per individual location.

This monitoring approach provides a continuous flow of data over time and is normally active during the entire production phase.

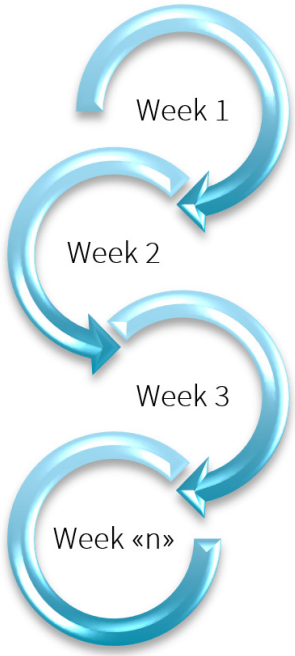
The correct use of this control method allows for the immediate evaluation of unexpected contamination events, thereby allowing for swift performance of corrective actions.

Pharmaceutical aseptic production is required to perform continuous particle monitoring during the entire production time. This methodology provides trend information that is useful for alarm and action level evaluation.

PERIODIC

The periodic method consists of performing particle monitoring at a scheduled frequency (i.e., once per week) to demonstrate continuous compliance of the cleanroom from one classification test to the next.

ISO 14644-2:2015 allows periodic monitoring to be performed, provided the user clearly specifies the test frequency.



Considerations

The ISO 14644-2:2015 standard lists the main items to be taken into account when developing an airborne particle monitoring system:

- Understand the contamination sources and their impact on the activity in the cleanroom
  - Locate particle counter probes as close as possible to critical zones
- Potential adverse impact of the sampling system on the process or the process environment (e.g. possible effects of the rate of the extraction of the sample volume in small volume environments)
- Airborne particle collection efficiency, suitability to monitor the selected particle size(s), and accessibility for maintenance, calibration, and repair
- Air sample flow rate and volume
- Frequency and duration of the collection of each air sample (determined by the sampling rate)
- Sample probe configuration and orientation with respect to airflow (e.g. isokinetic or anisokinetic)

HOW LONG CAN MY TUBING BE?

- The use of long sample transport tubes must be avoided when intending to evaluate particle concentrations at sizes greater than 1 µm. ISO requires the adherence to maximum tubing lengths as specified by the particle counter manufacturer, which is typically between 1.5 and 2 meters.
- In case longer sample tubing is needed, the particle loss rate should be evaluated by measuring the number of particles that remain trapped in the transportation tubes.
  - Some method examples can be found in *How to Conduct Particle Transport Tests*.

WHAT'S THE RIGHT ISOKINETIC PROBE POSITION?

- The sampling probe (i.e. the Isokinetic Probe or ISP) provides the advantage of harmonizing the sampling flow speed with the typical laminar flow patch speed (90 ft/min or 0.45 m/s) for the purpose of not creating any potential adverse impact of the sampling system on the process with respect to the ISO requirements.

- The position of these probes must be evaluated in the risk assessment and should not be located right under the filtration system (unless specifically required).
- The position of the sampling probe must be representative of the cleanroom performance and should be placed as close as possible to where the production occurs and where risk to the product is highest. Also, it must be clearly determined and included in monitoring SOPs to allow for reproducible sampling operations.

WHICH MONITORING METHOD SHOULD I USE?

ISO 14644-2:2015 does not specifically provide a link between the ISO class of the cleanroom and the recommended monitoring method. That means that it is up to the user to choose the most appropriate method based on their specific manufacturing requirements and risk assessment.

Life science industries may also consider other standards to correctly and reasonably set up their monitoring method and frequency. For example, the World Health Organization(WHO) issued a document titled *Environmental Monitoring of Clean Rooms in Vaccine Manufacturing Facilities* in November 2012 which includes instruction on determining the best monitoring frequency approach based on cleanroom cleanliness grade class (see Table F-1 below).

TABLE F-1 Monitoring Frequencies for In Operation Routine Particulate Sampling

Classification	In Operation (Dynamic) Routine Particulate Sampling
Grade A (filling operation)	For the full duration of operation
Grade B	Daily (working days)
Grade C	Weekly
Grade D	Not required
UDAF work stations in B	Daily (working days)
UDAF work stations in C	Weekly
UDAF work stations in D	Monthly
UDAF in UNC areas	Runtime re-qualification of UDAF is sufficient.

Table taken from *Environmental Monitoring of Cleanrooms in Vaccine Manufacturing Facilities*. WHO, 2012.

How to Set Action and Alert Limits

The determination of action and alert limits is extremely important and must be supported by the risk assessment as well as a consistent quantity of historical sampling data. In addition, they must be supported by a consistent amount of data collected during previous monitoring controls.

ISO 14644-2:2015 states the importance of a long term evaluation as well as a yearly assessment of limits, methods, and frequency. While not necessarily requiring a change, the assessment is an important exercise in the critical evaluation of a monitoring plan. For old plans especially, it should be questioned whether they are still applicable and consistent with the actual performance, activities, and needs of the cleanroom.

The standard provides some important recommendations, as well as applicable strategy to keep in mind when setting alert and action limits. One with high significance is provided in the paragraph B.3.1.3, quoted below:

> ***B.3.1.3*** *When setting alert and action levels, it is important to be sensitive to the high variability of airborne particle concentrations with time and at different locations. In particular, special care shall be taken when considering alert and action levels for cleanliness classes ISO Class 5 and cleaner with low concentrations of particles. In these circumstances, the occurrence of "nuisance alarms" due to false counts and/or natural variability of particle concentration is more likely and should be avoided by careful selection of alert and action levels. Frequent "nuisance" alarms should be avoided as they can lead to alarms being ignored by users.*

This concept is frequently ignored when setting alert or action alarms for large particle sizes in areas of high cleanliness, and this neglect may result in extensive and useless investigation to determine the reason of alarms.

Good methods to deal with large particle sizes (i.e. 5 µm) in ISO 5 cleanrooms are described in paragraph B.3.4 of the ISO 14644-2:2015 standards.

The following strategies consider the importance of evaluating an alert or alarm situation by taking into consideration a series of events rather than a single spot value.

Strategy 1:

Establish a trigger threshold value based on a series of consecutively high readings. For example: 3 consecutive, 1 minute readings all above a specified level.

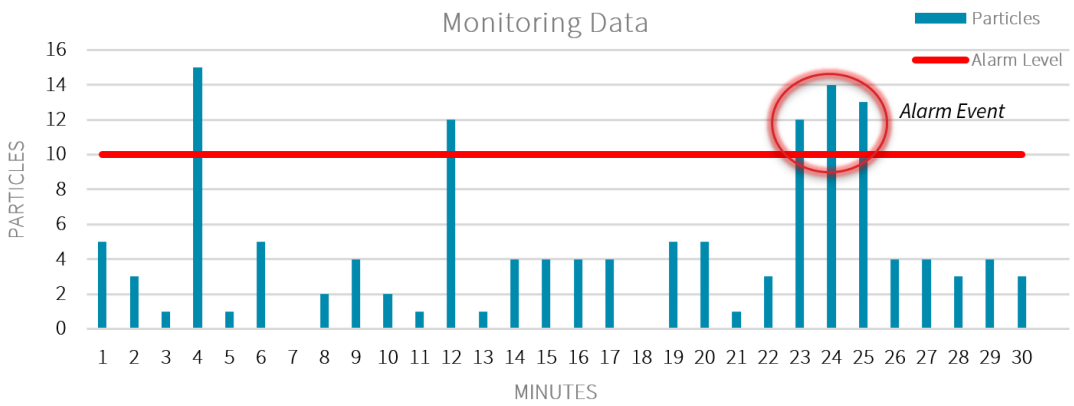


FIGURE F-1 GRAPHICAL REPRESENTATION OF STRATEGY 1

Strategy 2:

Establish a trigger threshold value based on a high frequency of elevated readings. This method is commonly referred as “x out of y”, where “x” is the number of events and “y” is the number of minutes.

For example, 3 out of the last 10 readings/minutes are above the specified alarm threshold.

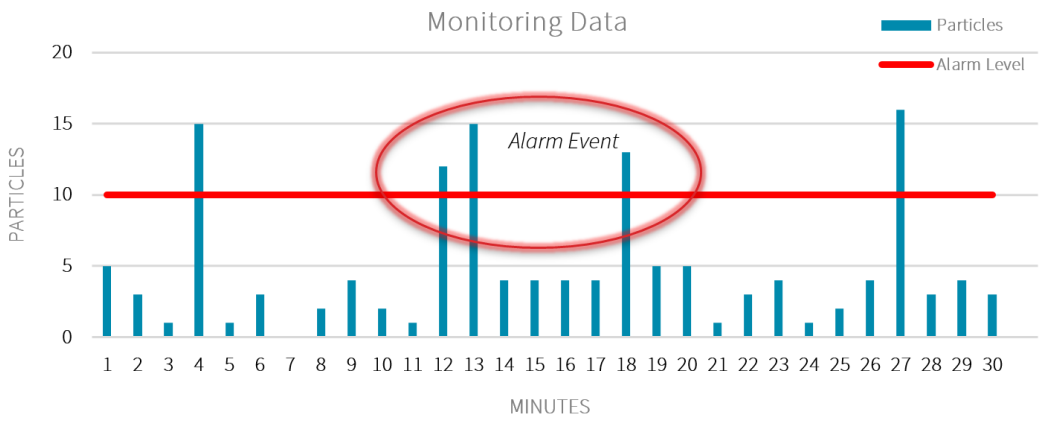


FIGURE F-2 GRAPHICAL REPRESENTATION OF STRATEGY 2

ISO 14644-2:2015 COMPLIANT – LEARN FROM FDA 483 AND TECHNICAL REPORTS

Being compliant with any standard requires experience, knowledge, and a critical approach, enabling the harmonization of regulation requirements with specific production environments. Jumping on this task can be done well in advance by studying the observations of FDA inspectors made to users in similar situations.

Several 483 warning letters have been redacted over the last years, and many of them are strictly linked with monitoring plans' lack of compliance. Some of them are summarized in Scott Sutton's *The Environmental Monitoring Program in a GMP Environment*.

Some issued letters may refer to basic concepts when read by cleanroom experts, which remain a useful component when preparing a demonstration of an efficient, compliant, and defensible monitoring program.

The following warning letter states the need of having a monitoring plan in place, a description of the location to be tested, and a specific sampling method. Monitoring results are considered insufficient if they don't support and link to a clear and approved plan.

> *…Regarding the increased non-routine surveillance monitoring performed to further evaluate the Building 37 Flu manufacturing facility, there was no plan in place specifying the locations to be tested, method of sampling, and actions to be taken when microbial contamination was noted…*

The FDA emphasizes the need to develop a monitoring plan based on risk analysis. Compliance with the reference standards before implementation must be verified. The following is an excerpt of the warning letter:

> *…The [redacted] method used for increased surveillance monitoring of the environment has not been qualified…*

One warning letter, dated 2001, requires the cleanroom user to proactively and critically review the sampling historical data, as it must be referenced to correctly set up the appropriate alert and action levels. This requirement was likely difficult to accomplish in 2001, but is now easily achievable using the appropriate software platform (CFR 21 Part 11 compliant), capable with a particle counter's data storage. Here is an excerpt of the letter:

> *…the alert and action limits established for the manufacturing areas are not based on historical data taken from the EM Program…*

FIGURE F-3 LASAIR® PRO AEROSOL PARTICLE COUNTER: CFR 21 PART 11

Conclusion

ISO 14644-2:2015 is not only a new standard to be compliant with but is also a beneficial tool to use in achieving mature cleanroom environmental control.

The standard's main goal is to cultivate and promote a strong knowledge of cleanroom performance inside every company. This goal is accomplished by the enforcement of continuing discussion and evaluation of collected sampling data, the development of user-set alert and action levels, and the proactive revision of monitoring plans and risk assessments based on the ongoing developments of monitoring activities rather than considering each single monitoring session as isolated from the entire program.

ISO 14644-2:2015 was published on December 15, 2015, and all users who want to be compliant with this standard are required to take necessary action immediately. For more information about ISO 14644-2:2015 or other specific questions concerning cleanroom contamination control, contact your Particle Measuring Systems local representative or navigate to our Contacts web page.

References

1. International Standards Organization. *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration,* ISO Standard No. 14644-1:2015 (2015).

2. International Standards Organization. *Cleanrooms and associated controlled environments — Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration,* ISO Standard No. 14644-2:2015 (2015).

Author

Daniele Pandolfi

Daniele Pandolfi is the Global Product Line Manager, Aerosol in Particle Measuring Systems' Life Science Division. He has over ten years' experience in particle counter instrumentation and cleanroom contamination control, built building close customer relationships. He helps customers to solve their cGMP issues. He can be reached at **dpandolfi@pmeasuring.com.**

Applications: Risk Assessment

Risk Assessment as a Process Quality Assurance Tool

Abstract

Environmental contamination control is a critical component of sterile pharmaceutical manufacturing, and Risk Management is necessary to ensure that the correct control practices are in place. The Risk Management process is a series of steps, including risk assessment, that allows for a deeper understanding of the manufacturing environment. Removing, reducing, or monitoring activities associated with a product or process to mitigate risk may be the result of such an assessment. This qualitative risk assessment can be transformed into a quantitative evaluation using modern risk analysis tools and procedures; these tools deliver the fully documented rationale behind the path chosen.

The Concept of Risk in Pharmaceutical Production

In the released revision of EU GMP Annex 1, the importance of risk management (Risk Management) is highlighted as an appropriate tool for ensuring the quality of a process. The revision is explicit about the necessity of risk management for sterile drug manufacturers, and it also widely recommends risk management for other product types, especially where control of microbial, particle, and pyrogen contamination is required (e.g., certain liquids, creams, ointments, and low bacterial intermediates) [1].

The production and use of a drug (medicine) and its components necessarily involves a certain degree of risk. It is important to understand that product quality must be maintained throughout the product life cycle. This ensures that the attributes important to the quality of the drug (Critical Quality Attributes) remain consistent throughout the development and production phases of the drug. According to ICH (*International Conference on Harmonisation)* Q6A, *drug quality* refers to the suitability of a drug substance or drug product for its intended use [2].

In general, a risk management procedure focuses on analyzing each process in a product life cycle with the intent to perform an assessment, mitigation, and review of the associated risks over time. As defined in ICH Q9, *risk assessment* is a systematic process of organizing information to support risk decisions that are made as part of a risk management process [3].

When talking about pharmaceutical quality, the term “process” can take on different meanings. It can refer to any of the stages of development, production, testing, inspection, distribution, up to and including drug delivery.

ICH harmonisation for better health

FIGURE G-1 ICH (INTERNATIONAL CONFERENCE ON HARMONISATION) LOGO

Additionally, it can include the design, qualification, and validation of equipment, instruments, and facilities. Thus, a process is any activity that may directly or indirectly affect the quality of the final product. It is immediately apparent that the scope of pharmaceutical quality risk management is very broad.

Before delving into risk management procedures, it is good to reflect on some key distinctions, starting with the difference between *hazards* and *problems*. Problems are related to the perception or implementation of a process [4], whereas a hazard is understood as an intrinsic property or quality that has the potential to cause damage to the process and, thus, to the end customer.

Therefore, risk is a probabilistic concept; it is the combination of the probability of a certain event occurring and the ability of this event to cause damage. This is further complicated by the inability to detect this risk at the time of occurrence. From a technical point of view, risk is the product of probability and severity [3], where detectability - if introduced - should consider the risk that the detection system will fail. The notion of risk thus implies the existence of a source of danger and the possibility of it turning into harm [5].

It is from this moment in the analysis of Risk Management that the risk is effectively actualized and translated into something scientific and documented. From this point forward, the tests and evaluations of the risk assessment must be transformed into something visual and quantitative so that both the capacity to manage the risk and the source of the risk can be understood externally.

The final risk assessment tool is the identification of hazards and the scientific analysis and evaluation of the risks associated with exposure to them. This includes severity of damage or harm to health, including logistical damage that may result from loss of product quality or availability.

It is essential to have both a robust quality management system and good manufacturing practices in place to reduce the risks to product quality, patient safety, and company reputation to an acceptable level.

There are two primary principles of quality risk management:

- Risk assessments should be based on scientific knowledge and driven by patient protection.
- The level of commitment, formality, and documentation of the quality risk management process should be commensurate with the level of risk.

Risk Assessment

The risk assessment process consists of different steps ranging from the identification of hazards to the analysis and evaluation of the risks associated with exposure to these hazards. Figure G-2 shows the steps developed during the analysis.

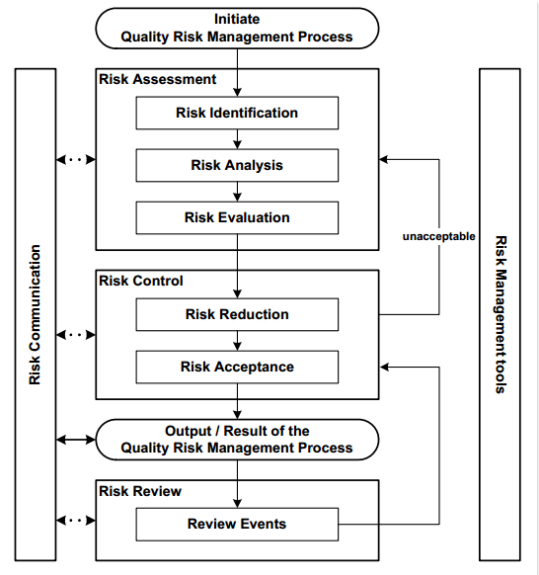


FIGURE G-2 STEPS FOR RISK IDENTIFICATION AND ANALYSIS

The risk identification step is composed of the systematic use of information to identify potential sources of harm (hazards) and possible consequences (impact/effect). The identification of these harms and consequences is based on historical data, theoretical analysis, informed opinions, stakeholder concerns, brainstorming sessions, etc. This information is necessary to deepen the knowledge of processes.

These three basic questions are often useful as an aid to clearly defining risk:

1. What could go differently than expected?
2. What is the probability (likelihood) of it going differently from the expectation?
3. What are the consequences (severity)?

Risk analysis is the estimation of the risk associated with identified hazards. It is a qualitative or quantitative process of linking the probability and severity of harm (severity being a measure of the possible consequences of a hazard) by evaluating the design/measures that have control over their occurrence and detection [3]. Analyzing the degree of risk leads to defining the appropriate tools or actions for its management over time. With some risk management tools, the ability to detect damage (detectability) may also be considered as a factor influencing the overall risk estimate.

Risk assessment then leads to the comparison of the estimated risk with certain risk criteria. This is done through a quantitative or qualitative scale that determines its significance and, subsequently, defines a threshold of acceptability. When risk is expressed quantitatively, a numerical probability is used. Alternatively, risk can be expressed using qualitative descriptors, such as "high," "medium," or "low," which should be defined in as much detail as possible. The purpose of risk control is to reduce the risk to an acceptable, defined level.

Therefore, the assessment should lead to either an acceptance of the risk itself (risk control), if the level is acceptable, or to a reduction of the risk, if it is not an acceptable level.

The risk control process might include actions to:

- decrease the probability of occurrence of the identified hazards and risks
- decrease the severity of the identified hazards and risks
- increase the detectability of the identified hazards and risks

It must always be kept in mind that the implementation of risk reduction measures may introduce new risks into the system (induced risk) or increase the significance of other already existing risks (correlated risk). Therefore, it may be appropriate to review the assessment after the implementation of a risk reduction process to identify and evaluate any possible changes. The frequency of any review should be based on the level of risk. The risk review may include reconsideration of risk acceptance decisions [3, 4].

Acceptance is only possible when it is scientifically proven that the final quality of the process is not critically impacted by the identified or residual risk. For some types of damage, even the best quality risk management practices may not eliminate all of the risk. In these circumstances, the application of an appropriate risk management strategy reduces the risk to quality to a specified (acceptable) level. This acceptable level will depend on many parameters and should be decided on a case-by-case basis. Various processes, including a cost-benefit analysis, can be used to understand the optimal level of risk control, while always in compliance with regulatory and normative requirements [3].

Risk Management and Possible Approaches

Several scientific methods are currently available for use in risk analysis. These can be used independently or in conjunction with each other to achieve the most constructive outcomes. For example, using a combination of the HACCP method with FMEA could lead to the production of more comprehensive documents than either would on its own. We cover three of these below.

HACCP

The HACCP approach is a systematic, proactive, and preventive tool for ensuring product quality, reliability, and safety [7]. HACCP stands for Hazard, Analysis, Critical, Control, Points, and it is based on the development of a "Decision Tree." Through this "decision Tree", the HACCP approach facilitates the identification of critical and non-critical areas of the process under analysis.

FMEA

The FMEA approach is a systematic analysis of potential failure modes aimed at preventing failures. It is a process of preventive action implemented before introducing new products, modifications, or processes. FMEA stands for Failure, Modes, Effect, Analysis, and ideally, FMEA analyses are conducted in the process/product design or development phases, however, it can also be very useful when applied to existing products and processes. This

approach is applicable in a variety of areas including the pharmaceutical manufacturing and assembly. It consists of several steps that include reviewing the process, identifying potential error modes, listing the potential effects of each error mode, assigning severity/occurrence and detection to each effect, and calculating Risk Priority Number (RPN) to prioritize mitigating actions that eliminate or reduce the risk.

Risk Priority Number (RPN)

The calculation of the Risk Priority Number represents the result obtained from the evaluation of three parameters: "Severity" (the severity of the risk), "Detection" (the ability to be able to detect the risk), and "Occurrence" (the probability that risk may occur and recur within the process under analysis). For each of these parameters, variables are evaluated and then multiplied to obtain the final evaluation. (RPN = Severity x Detection x Occurrence) [6]. Once the variables for each individual parameter have been established, the maximum and minimum scores can be calculated to define the ranges. A risk value of "low" would be assigned if the score value falls within the lowest range, "medium" if the score value falls within the intermediate range, and "high" if the RPN value falls within the highest range. The division into different risk ranges is necessary for the completion of the next risk control step. Assigning a risk value to one range rather than another contributes to the decision-making process of risk reduction or acceptance. If the total risk identified is medium or high, then corrective or preventive risk control actions must be applied to the process to lower its value (i.e., risk of impact on quality). Additionally, where possible, the risk value should be reduced to an acceptable threshold value. Being in the "low" range determines the acceptance of the risk as being below an established acceptable threshold and not requiring corrective or preventive action because it is already under control.

If we take the fill and finishing of a product as an example, a good strategy for preventing environmental contamination of the sterile production process becomes essential in ensuring the quality of the finished product. The risk assessment is therefore a necessary and fundamental tool for pharmaceutical companies to utilize to strengthen the quality assurance process, combined with the instrumentation used to practically perform what is theoretically assessed. Good data historicization and adequate data representation can be parameters to be considered to facilitate the risk control and review process.

The appropriate use of quality risk management can facilitate (but does not obviate) the industry's obligation to comply with regulatory requirements [3].

Conclusions

An effective approach to risk management can further ensure delivery of a high quality drug or medicine to the patient by providing a proactive means to identify and control potential quality issues during drug development and manufacturing. The main goal of any risk management should be the protection of the end-user of the product, and product quality is the definitive measure of the success of a quality risk strategy that identifies and maintains the safety of the

end customer. A fully documented Risk Assessment enables pharmaceutical companies to obtain high-quality products and comply with regulatory guidelines and requirements.

Ongoing product quality management is ensured through microbiological and particle control of air and surfaces according to a defined monitoring plan. It is also important to keep in mind the interdependence of the risk management and risk assessment approaches.

It can be useful to utilize external organizations for guidance. These experts can help the company define and manage their risks by sharing information gathered in clear and systematic ways. The communication can take place at any point in the process and the information shared could concern the existence, nature, form, likelihood, severity, acceptability, control, treatment, detectability, or other aspects of the quality risks as required.

References

1. European Commission. *The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products*, Annex 1 (2022).
2. EMEA: European Medicines Agency, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. *Q6A Specifications: Test Procedures And Acceptance Criteria For New Drug Substances And New Drug Products: Chemical Substances*, ICH Q6A (1999).
3. EMEA: European Medicines Agency, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. *Quality Risk Management*, ICH Q9 (2005).
4. International Standards Organization, International Electrotechnical Commission. *Safety aspects — Guidelines for their inclusion in standards*, ISO/IEC Guide No. 51:1999 (1999).
5. Italy Government Agencies. Article 2, Legislative Decree No. 81 (2008)
6. Robin McDermott, Raymond J. Mikulak, Michael R. Beauregard. *The Basics of FMEA*, ISBN 0527763209 (1996).
7. World Health Organization. *WHO Application of Hazard Analysis and Critical Control Point (HACCP) Methodology in Pharmaceuticals,* WHO Technical Report Series No 908, Annex 7 (2003).

Author

Marco Castaldo

Head of Advisory Team
Particle Measuring Systems

Marco has a diverse background in the pharmaceutical field, including expertise in the areas of Sterility Assurance, Quality Assurance and Compliance. He gained extensive experience in large, multinational companies before becoming a Consultant and a Project Manager. Marco helps pharmaceutical companies with projects including: validation of new technologies, reduction of microbiological contamination, and internal audits for aseptic behaviors. As Head of the Advisory Team for Particle Measuring System, he is focused on supporting pharmaceutical companies worldwide to improve their sterility assurance approach and strategy. He can be reached at **mcastaldo@pmeasuring.com.**

Editor: Noelle Boyton

Complete Cleanroom Contamination Monitoring

DATA MANAGEMENT

Contamination Monitors

Remote and Mobile

- *Particle Counters*
- *Microbial Samplers*

Services

- Advisory
- GMP
- Calibration and Maintenance
- Validation and Installation
- Project Management

STERILITY ASSURANCE

Environmental Monitoring Systems

- *Control System*
- *Data Collection Software*
- *Sampling Automation*
- *Data Management*

Training and Education

- *Particle College*
- *Onsite Customized*
- *Webinars*
- *Knowledge Center and Library*
- *Industry Education*

DATA INTEGRITY

PARTICLE MEASURING SYSTEMS®
a spectris company

For more information contact
www.pmeasuring.com
info@pmeasuring.com

Solutions

Solutions: Total Particle Portable Monitoring Best Practices

Pharmaceutical Requirements for Portable Monitoring in Cleanrooms

All pharmaceutical and biotech products must be manufactured in accordance with the current Good Manufacturing Practice (cGMP) regulations. Environmental monitoring of these pharmaceutical manufacturing areas meets the requirement for contamination control of an environment, which is essential in defining clean manufacturing and demonstrating the necessary controls are working.

A pharmaceutical company must have a quality department that 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. [1]*

To satisfy these requirements, the products are manufactured in controlled environments/ cleanrooms. Cleanrooms are employed to reduce the variability of production environments, and as controlled environments they can be tested and proven to meet specific standards. GMP require that these environments be rigorously tested and monitored to ensure that there is full and constant awareness of current environmental conditions for both viable and nonviable contamination.

A cleanroom is the fundamental starting point for contamination control; 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. [2]*

Operational procedures are defined and regulated for airborne particle concentrations to meet appropriate particulate cleanliness classifications. The International Standard, ISO 14644-1 [3] is the current international standard of defining cleanroom contamination levels.

Pharmaceutical cleanrooms are classified according to required particle concentrations of the air that meet the cleanliness criteria for the manufacturing process being performed. The determination of the cleanroom class is a process based on statistically valid measurements, and its a function of the filtration and operations status of the room; it is, in essence, a calibration of the room to ensure it meets its intended classification. It is not, primarily, a function of risk of application.

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 cleanroom. The test points should be equally spaced throughout the room and at work height to demonstrate the quality of the air cleanliness at the work area.

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

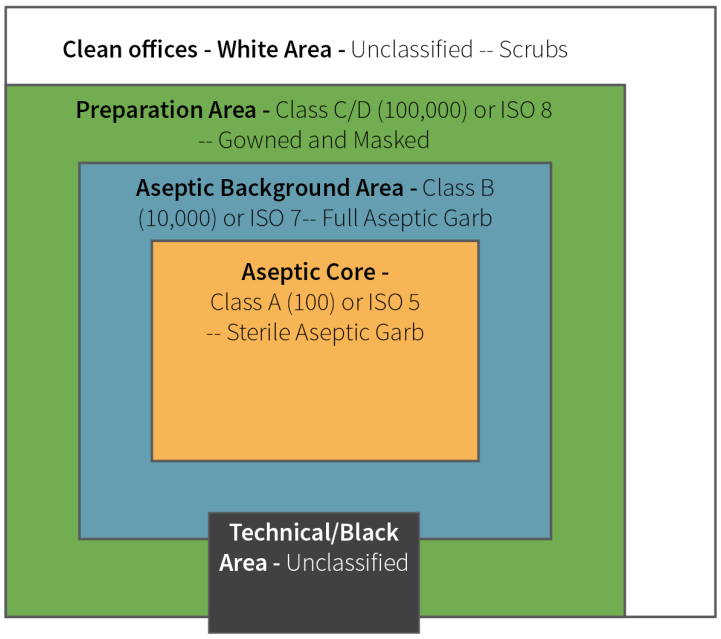


FIGURE H-1 DIAGRAM OF TYPICAL ISO RATING IN AREAS OF CLEANROOM

Pharmaceutical Cleanroom Utilization

Once a cleanroom has been tested to compliance to cleanroom classification (typically using a Light Scattering Aerosol Particle Counter (LSAPC)), the classification achieved dictates which production activities can be performed in that cleanroom or clean air device. The FDA defines two areas.

1. Critical Areas
2. Supporting Clean Areas

Critical area: This area is defined as critical because it contains products that are vulnerable to contamination if exposed. To maintain product assurance, 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).*
>
> *We recommend 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. [4]*

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). [4]*

Environmental Monitoring

Once a cleanroom or clean air device has been proven to meet the requirements for cleanliness from a certification perspective, it must also demonstrate that this control can be maintained throughout production periods. The 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 nonviable and viable airborne particulates, surface viable contamination, and, in the aseptic areas, personnel. 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 7, ISO 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.

For both critical areas and supporting clean areas, a portable particle counter can be used. Portable particle counters are chosen based on several influencing factors for ergonomics and suitability for the areas to be tested.

- **Size channels** required. There is a traditional requirement for two primary channels for monitoring, 0.5 µm and 5.0 µm. However these sizes can be complimented with additional sizes both smaller and larger than the given range. The expansion of sizes allows for further investigation for out of tolerance trends, as events may generate particles within a specific band indicative of a failure mode.

- **Sample flow rate** of 1 CFM (1 cubic-foot/minute = 28.3 liters/min) is the most suitable for the majority of cleanroom activities, including certification. This flowrate has suitable particle transport qualities that allow for up to 2 m of tubing to be used without significant losses of the larger sized particle counts. Higher flowrates are available (up to 100 LPM); these very high flowrates allow for rapid qualification testing of clean environments where low populations of the 0.5 µm particles exist. The higher flowrate gives a statistically significant sample volume in a shorter period.

- **Mains or battery-operated** functionality allow for ease of portability and ergonomics of the instrument chosen. Batteries should last for the duration of required testing or be available to be live exchanged without compromising data quality.

- **Mobility**, either by hand or cart: the technician may require several instruments in order to perform environmental monitoring. If small spaces are required to be tested, then the instrument can be placed in the clean air device, or tubing can be used to transport the sample from within an environment to the particle counter.

- **Accessories** such as a local display for controls and alarming and a built-in printer for sample point generated reports may also be required. Electronic transfer of data to a central software or LIMS may be required as thermal paper has limitations for durability and transposition requirements for reporting.

A portable instrument is typically used throughout the facility by moving it from location to location after each measurement. The data record for each sample point should consist of the following information:

Primary Data

- Sample Date and time
- Sample location
- Channel data and results and units
- Instrument status (hardware alarms)

Additional information to support the primary data includes.

- Operator taking sample
- Instrument identification (serial number)
- Alarm threshold excursions
- Sampling parameters
- Occupancy state

This data is then either printed locally or exported to a software management application for further review.

Reviews of data should be performed in three phases. Short term, medium term, and long term trending.

- **Short term trends** look at sample to sample relationship and stability of a process; it also allows for 'n' out of 'm' nuisance controls where samples might reflect the process and not first air.
- **Medium term trends** allow for a day-to-day or batch-to-batch review. Trend data reviews at this time scale show if a particular process or occupancy influences particle burden during production
- **Long term trends** are every 3, 6, or 12 months and may identify a gradual decline in the effectiveness of installed controls.

The reporting and trending requirements feed into the Contamination Control Strategy (CCS) of a site and should be performed and documented routinely.

Introduction

As environmental system designers, we are often asked where to place sample points for particle monitoring whether it be performed in a pharmaceutical cleanroom or clean device (RABS, isolator, etc.).

The answer is not always straightforward. There are several guidance documents that offer advice on what processes need to be monitored and also advice on suitable distances from the process being monitored. The goal of this chapter is to identify the considerations, establish the most suitable location for monitoring a process, and build a scientific rationale for that decision.

Particle counting in pharmaceutical applications can be clearly segregated into one of three categories: certification, qualification, and monitoring. Each category requires a different approach.

Certification: Measuring a cleanroom to a standard. The only standard recognized worldwide is ISO 14644-1, which defines how a cleanroom performs and its ability to show uniformity across the entire space. This is done irrespective of the activities performed in it.

Qualification: The process of analyzing risk assessment for the activities in the room. Qualification follows grid methodology testing methods. Particle counts are measured in both operational and at-rest states; however, the operational data is the most valid.

Monitoring: The ongoing sampling of the cleanroom on a frequency relative to the degree of control required to prove management over risk to the finished product. The number of sample points and their locations are determined by risk assessment and the qualification and certification processes.

Certification

As mentioned above, cleanroom certification is based on ISO 14644-1 standards. The specifics of the assessment may vary slightly between FDA and EU GMP regulations, but the underlying methodology is standard.

Certification demonstrates that the entire area meets a specific ISO classification by particle concentration. That is, irrespective of the final use of the room, only the design and implementation of the filtration system are considered. The international standard means that a cleanroom tested to meet compliance for ISO 5 standards will meet that standard independent of geography and regulatory aspects (i.e., FDA or EU GMP). This provides a universal standard to show that a cleanroom level has been established. Particle Measuring Systems' products, including the Airnet® II and IsoAir® Pro-E Aerosol Particle Sensors, comply with new ISO standards set in 2015. The interactive software of the Lasair® Pro Aerosol Particle Counter can even step the user through the certification process.

There are many different resources to prove ISO compliance and this paper will not cover these in depth. However, using the example of a classic filling machine (Grade A/ISO 5) within a Grade B (ISO 7) background area, the basic rules of testing can be demonstrated.

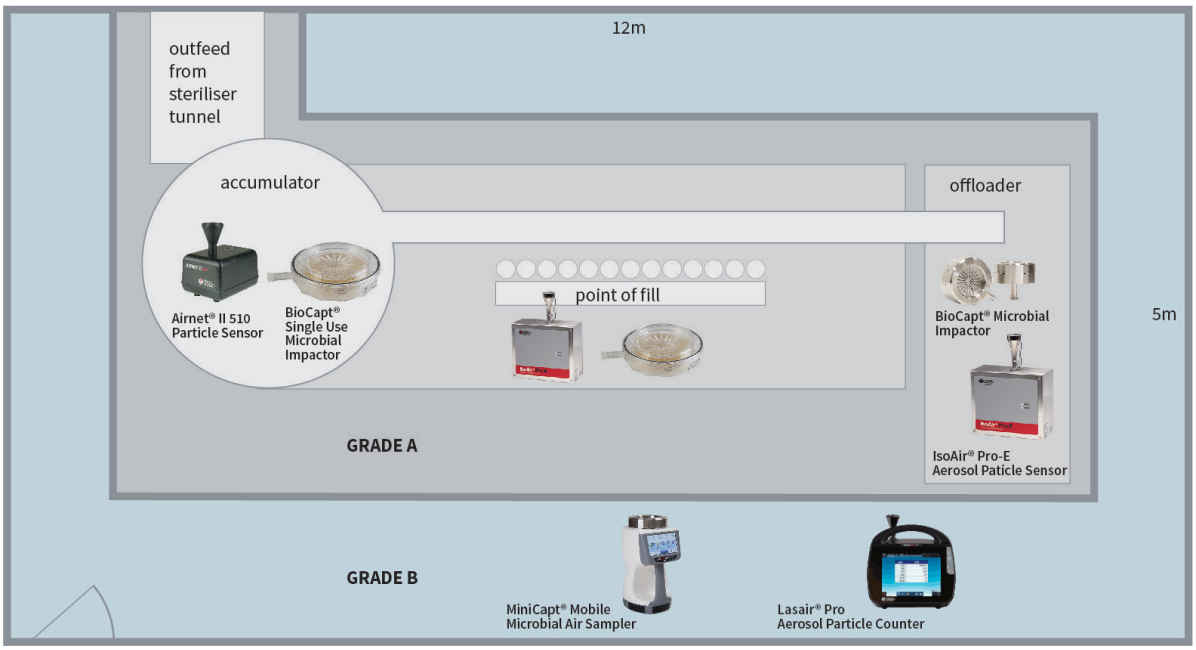
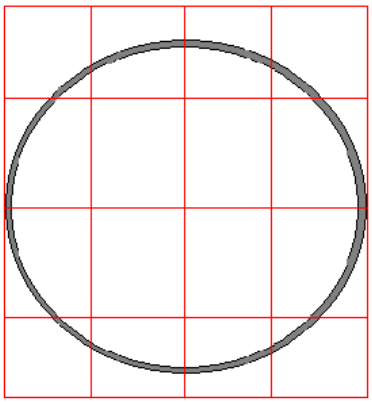


FIGURE H-2 BIOCAPT® SINGLE-USE AND STAINLESS STEEL MICROBIAL IMPACTORS, AND THE MINICAPT® MOBILE MICROBIAL AIR SAMPLER COMPLY WITH ISO 14698-1, CERTIFICATION STANDARDS SET FOR BIO-CONTAMINATION CONTROL

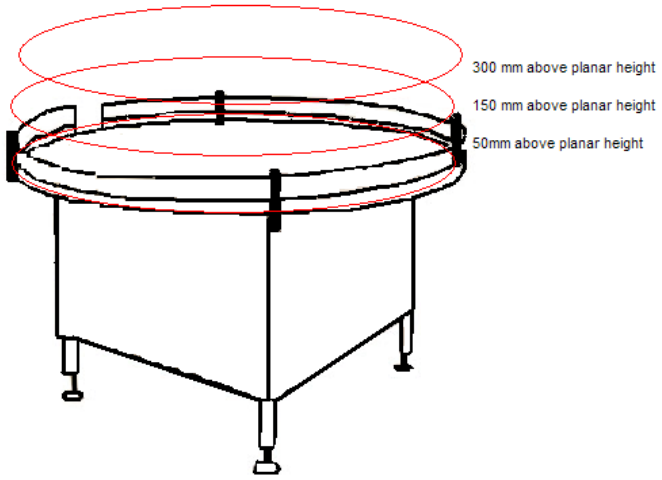
1. The number of sample points is based on a statistical function of the area. Calculate the area of Grade A/ISO5. Find the number of required sample locations in the table.
   - Calculate the area of Grade A/ISO5. Find number of required sample locations in the table.
   - Calculate the area of Grade B/ISO7. Find number of required sample locations in the table.
2. Sample point placement for the Grade A (ISO5) area:
   - The sample points must all be equidistant and at work height, irrespective of the activity at the location of their placement.
   - Samples are taken in a grid pattern at the identified locations. Derive the minimum number of sampling locations, NL, from ISO 14644-1 Table A.1. This table provides the number of sampling locations related to the area of each cleanroom or clean zone to be classified and provides at least 95% confidence that at least 90% of the cleanroom or clean zone area does not exceed the class limits.
   - PASS/FAIL criteria are calculated for ISO and EU GMP Annex 1. It is recommended to have both sets of data, as the FDA requires ISO14644-1 and the EU requires Annex 1 data points (although the EU data would suffice for the FDA).
3. Sample point placement for the Grade B (ISO7) area:
   - Repeat the steps used for the Grade A (ISO5) area.
   - It may be more difficult to determine the locations of the sample points due to the unusual shape of a room. Derive the minimum number of sampling locations, NL, from ISO 14644-1 Table A.1. This table provides the number of sampling locations related to the area of each cleanroom or clean zone to be classified and provides at least 95% confidence that at least 90% of the cleanroom or clean zone area does not exceed the class limits.
4. A final report is created and marks the end of the certification phase.

Qualification

The qualification phase considers the risks to the quality of the finished product. Each activity must be considered and measured. Continuing with the example of the filling line, let us consider the accumulator table at the exit of the sterilizer tunnel. The risk is that glassware (vials/bottles) are exposed to the open environment and operator. Therefore, contamination can fall into clean vials/bottles prior to filling. Operator intervention and the shifting of glassware causes turbulent air movement on the table, impacting contamination risk to the exposed vials/bottles. Therefore, it is an area of contamination risk and the following actions should be taken:



4 x 4 grid pattern over area



1. Divide the area of risk into a 3 x 3 or a 4 x 4 grid. If the activity can occur at several levels, then each level (working height, +150 mm from work height, and +300 mm from work height) must be considered.
2. Take a particle sample at the center of each of the grid squares at each level.
   - Samples are taken during 'At Rest' and 'Operational' states. It may be required to work around an activity or operator to gain suitable data.
   - Slight movement of sample points within the grid square is acceptable. A location is invalid if found to impede normal activities.
3. When all samples are taken this will provide a particle map of the pharmaceutical activity.

Each of the key functions within the cleanroom (filling point, stoppering, general background activities, etc.) should be analyzed accordingly.

Monitoring

The location of the monitoring points must be based upon a formal risk assessment using tools such as but not limited to Failure Mode and Effects Analysis (FMEA) or Failure Mode, Effects, & Criticality Analysis (FMECA), with data from the certification and qualification testing. Other factors, such as equipment interference, mounting points, operator impedance, and operator intervention, contribute to selecting the final location for the sample probe. In the current regulatory environment, a risk assessment is absolutely required. Without a risk assessment, poor

or incorrect sampling methodology can lead to data unreliably associated to the process. It could also lead to potential impact to finished product quality. Without the option of correlating events, the lack of connection between location and sample frequency can lead to long investigations for out of tolerance events.

There are several steps to defining a risk-based environmental monitoring plan:

1. **Process understanding:** You must study personnel and material flows within the assessed area in addition to the production operations. This will give an understanding as to how the system is used and what evidence there is to support its state of control, such as:

   - Current monitoring practices
   - Historical data
   - Smoke studies

   This Gemba walk of the process and rooms is necessary to define the scope of monitoring required and to aid in applying a process that fits with an organization's internal practices. Figure H-3 is an example.

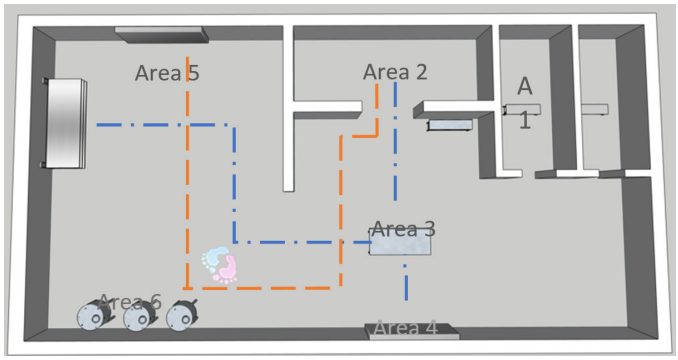


FIGURE H-3 EXAMPLE GEMBA WALK PATHS

2. **Definition of critical areas:** Identification using Hazard Analysis Critical Control Point (HACCP) helps which critical areas require environmental monitoring, and identifies areas which meet the needs of a critical sample location.

3. **Evaluation of sampling methods:** You need to make a determination between traditional methods such as volumetric air samplers, newer technologies such as Rapid Microbiological Methods, or manual collection techniques such as swabbing and contact plates. Also, determine if the chosen method needs to be portable, continuous, remote, etc.

4. **Definition of potential sample locations:** Determine a single sample location within each critical area, following this criteria (as shown in the adjacent figure):

   - Check the available space around the critical area.
   - Measure the size of probes and plate holders.
   - Determine the accessibility to the location for operator maintenance.
   - Assess the interaction between the process operation with personnel and material flows.
   - Calculate the probability of potential contamination events.

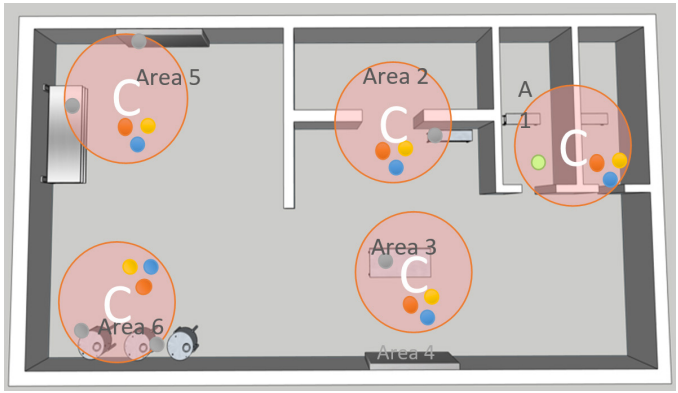


FIGURE H-4 EXAMPLE SAMPLE LOCATIONS

5. **Definition of critical control points (CCP):** Each individually considered location is evaluated according to the FEMA method to rank and identify critical sample locations.
6. **Define sampling parameters:** The sample frequency is found based on the criticality of operations along with any additional criteria, such as incubation parameters and mitigating measures that might be put into place prior to establishing a monitoring plan.

Sampling practicalities include elements such as:

- The isokinetic sample probe should face into the air stream.
- The minimum length of tubing should be used.
  - Although different manufacturers claim specific lengths of tubing can be used with their particle counter, this is typically a function of vacuum pump dynamics and not of particle transportation. Particles that are 0.5 µm move freely in long lengths of tubing. However, 5.0 µm particles do not have this same mobility. As 5.0 µm particles are a greater concern, the tubing should be maintained at its shortest recommended lengths (For more information on tubing, read, "Particle Loss in Transport Tubing" by Particle Measuring Systems).
  - Particle Measuring Systems quotes maximum tubing lengths based upon the same conditions of airflow and has a recommended maximum length of 3 m. However, for pharmaceutical particle systems we advise a reduced recommended length of 2 m to ensure transportation of the larger particles.

From the FDA's Aseptic Processing cGMP Guideline:

> *"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 3520 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 5)."*

The frequency of sampling should reflect the risks and follow from the FDA guidelines on sterile manufacturing and the EU GMP Annex 1. Particle monitoring should be automated and maintained in a continuous state when glassware and products are exposed.

References

1. Federal Drug Administration. *General Principles of Validation,* (1987).
2. Institute of Environmental Sciences. *Federal Standard Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones*, Federal Standard 209E (1999).
3. International Standards Organization. *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration,* ISO Standard No. 14644-1:2015 (2015).
4. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER,) Center for Biologics Evaluation and Research (CBER), Office of Regulatory Affairs (ORA). *Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice,* (2004).

Author

Mark Hallworth
Life Sciences Senior GMP Scientist
Life Science Division
Particle Measuring Systems

Mark Hallworth is the Life Sciences Regional Manager for Particle Measuring Systems. He has lectured for pharmaceutical societies throughout Europe, Asia, and the US on nonviable particle and facility monitoring and the implications of validating those systems. He can be reached at **mhallworth@pmeasuring.com**.

Editor: Noelle Boyton

Solutions: Microbiological Portable Monitoring Best Practices

Comparing Microbiological Air Monitoring Techniques for Critical Environments

Abstract

This paper discusses the use of active air and passive air settle plate monitoring for routine and continuous pharmaceutical manufacturing. Scientific literature supporting settle plates and active air sampler monitoring effectiveness is evaluated to support the transition from passive to active air monitoring.

Introduction

Contamination is monitored continuously with the latest in cleanroom technologies. However, traditional growth-based solutions, limited to 4-hour sampling periods, are still the most common method used for microbial monitoring. To provide evidence that a continuous sampling of cleanroom air is performed, pharmaceutical manufacturers widely use settle plates even if scientific and regulatory experts agree that they are a non-quantitative and non-validatable method. Compare this to active microbial air samplers, validated to run for a prolonged period in continuous mode to sample one cubic meter of air.

FIGURE I-1 EXAMPLE OF ACTIVE MICROBIAL AIR DEVICE: MINICAPT MOBILE® MICROBIAL AIR SAMPLER

Method Efficiency

ISO 14698:2003's Annex B describes a technique for determining collection efficiency of microbial air samplers, broken into two separate types [1]:

- **Physical efficiency** is the ability of the sample to collect various sizes of particles.
- **Biological efficiency** is the efficiency of the sample in collecting microbe-carrying particles.

Physical efficiency is the same for inanimate particles, particles carrying a microorganism, or particles that are microorganisms. Biological efficiency is expected to be lower than physical efficiency because it depends on the survival of the collected microorganisms and the growth medium. Annex B is mainly concerned with physical efficiency.

In a 2005 publication, a highly accredited author, Dr. William Whyte, concluded settle plates were a "fundamental method of measuring the number of microbe-carrying particles that will deposit onto a given area in a given time. There is therefore no need to determine its collection efficiency" [2]. In 2016, the European Journal of Parenteral & Pharmaceutical Science in partnership with Whyte and T. Eaton reassessed and suggested improvements to EU cGMP's Annex 1, specifically for how airborne concentration and settle plate counts of MCPs contribute to the grade of a pharmaceutical cleanroom [3]. Using more accurate deposition velocities, the EU cGMP maximum concentrations can be revised to provide more accurate settle plate counts.

ISO 14698:2003's Annex A specifies the selection of the microbial air sampling device to be dependent on the purpose of sampling. In addition, the device should have an impact velocity (speed of the air hitting the culture medium) that is a compromise between:

1. A high enough velocity to allow the entrapment of viable particles down to approximately 1 µm, and
2. A low enough velocity to ensure viability of particles by avoiding mechanical damage or the break-up of clumps of bacteria or micromycetes.

The ISO standard's recommendation has generally been a sampler at or near 50 percent physical recovery at 1 µm (a D50 of 1 µm). From a microbiological stance, 1 µm is the size of most common species of individual bacteria. Fungal particles are usually 2 to 5 µm, and *Bacillus anthracis* spores have a size range from 0.65 to 2.0 µm.

The Importance of Design

If a stream of gas undergoes a sharp change in direction, the particles it transports will tend to continue in their original direction. Particles having different dimensions and densities will follow different trajectories and may be collected separately. When a jet of air is accelerated through a nozzle, the particles it transports are carried at the same speed as the fluid and follow its flow line. If the fluid flow lines rapidly change direction at the nozzle output, the particle trajectories will appreciably depart from the airflow lines, depending on the inertia associated with the particles. In other words, the particles will tend to run in a straight line, and if they find a surface in their path, they can adhere to it.

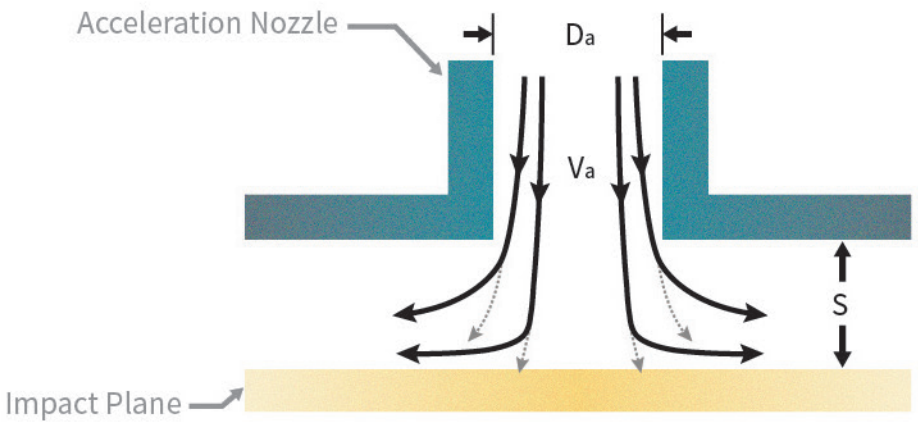


FIGURE I-2 AIRFLOW MODEL OF AN ACTIVE AIR SAMPLER

Active air impactors are designed to sample particles in the air or other gas through a collision with a solid surface. The impactor's geometry is optimized to allow laminar flow into the nozzle (e.g., Re < 2300), with a velocity as high as possible and a D50 as low as possible.

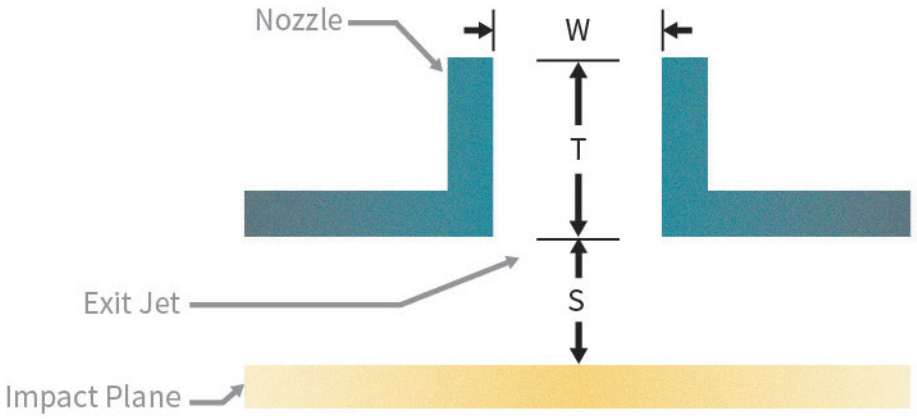


FIGURE I-3 ACTIVE AIR SAMPLER GEOMETRY

Comparing Monitoring Methods

Due to modern cleanroom clothing features, the microbial-carrying particles detected in aseptic areas with operators are mainly in the size range of 0.5 µm to 5 µm. For these reasons, continuous microbial active air sampling in Grade A is recommended, replacing the combination of settle plates and single or intermittently used active air sampling activities. The following table summarizes the differences between techniques.

TABLE I-1 Technique Comparison

COMPONENT	SETTLE PLATES	CONTINUOUS MICROBIAL ACTIVE AIR SAMPLING
Continuous monitoring	BOTH CAN BE USED TO MONITOR ALL PHASES OF PRODUCTION.	
Measuring the concentration of microorganisms in the air	MEASURES THE NUMBER OF MICROORGANISMS SETTLING FROM THE AIR ONTO A KNOWN SURFACE AREA WITHIN A KNOWN TIME IN A TURBULENT ENVIRONMENT.	MEASURES THE TOTAL NUMBER OF MICROORGANISMS IN A QUANTIFIABLE VOLUME OF AIR.
Quantitative method	NOT A QUANTITATIVE METHOD. RESULTS ARE OFTEN THE NUMBER OF CFUS PER SETTLE PLATE, WITH THE SIZE OF THE PLATE AND THE TIME EXPOSED OFTEN NOT REPORTED.	A QUANTITATIVE METHOD. THE RESULTS CAN BE ANALYZED IN TERMS OF TIME AND AIR QUALITY.
Detect low concentrations of microorganisms	DOES NOT DETECT LOW CONCENTRATIONS OF MICROORGANISMS AND PROVIDES VERY LOW SENSITIVITY IN GRADE A DUE TO HIGH AIRFLOW.	DETECTS LOW CONCENTRATIONS OF MICROORGANISMS.
Position in the filling machine	BOTH CAN BE PLACED CLOSER THAN TRADITIONAL VOLUMETRIC SAMPLERS TO CRITICAL AREAS WHERE THE PRODUCT IS EXPOSED TO AIR. BOTH CAN BE PROVIDED IN STERILE FORM AND ARE SMALLER THAN TRADITIONAL VOLUMETRIC SAMPLERS.	
Comparison of microbiological and particulate data	THE CORRELATION OF DATA IS NOT DEFINABLE DUE TO THE TWO METHODS' DIFFERENCES.	CORRELATION OF DATA IS POSSIBLE BECAUSE AIRFLOW SPEED OF THE TWO SYSTEMS IS SIMILAR.
Validation	NOT VALIDATED.	VALIDATED ACCORDING TO ISO 14698-1.

Table 1 highlights that active air sampling is an improved approach compared to both settle plates and traditional volumetric air samplers in Grade A continuous monitoring applications.

Monitoring in Different Cleanroom Areas

When patient safety is key, the qualification of pharmaceutical cleanrooms is a necessary step. Particularly, microbiological qualification verifies the cleanliness level of rooms where medicines are manufactured. Following qualification, companies must design a monitoring plan that demonstrates air quality is in accordance with specifications established during qualification. Through monitoring, microbiological contamination can be controlled and minimized.

Grade A (ISO 5) areas include the product and materials in contact with the product, including the surrounding environment (i.e., air). For this reason, they are considered extremely critical and subject to continuous monitoring with high air frequencies during all production phases. Grade B (ISO 7) areas are used to protect Grade A areas and include the presence of operators in a variable number depending on the production process. Here, the purpose of microbiological monitoring is to verify the level of microbiological contamination is within specifications. The microbial trend of these areas must always be constant or slightly decreasing.

Settle plates are not recommended in Grade A areas because they do not detect low concentrations of microorganisms and offer low sensitivity with their high airflow rate. Settle plates are only acceptable in Grade B, C, and D areas where less turbulent air movement allows for microbe-carrying particles to be deposited at a higher rate.

Continuous microbiological monitoring of Grade A air is already required by cGMP and implemented for total particle monitoring. It provides key information on the amount and size of total particles present in the air at a given sampling point. It is therefore extremely important to have a strategy for both particle and biological monitoring that utilize validated methodologies quantifying both the microorganisms and particles present in a sampling area. Doing so will aid in determining potential correlation between events and provide the data necessary for root cause investigations.

Conclusion

The settle plate method is a non-validated, non-quantifiable method that does not take into account a microorganism's recovery rate. This method should not be used in Grade A (ISO 5) where high air speeds are common and air changes are frequent. These conditions make it difficult or impossible for microorganisms and particles containing microorganisms to settle. Settle plates have more viability in static environments.

Continuous microbiological monitoring of air in critical areas should be performed with validated methodologies with a recovery rate as high as possible. Regulations will keep pushing standards higher, and this strategy promotes better process knowledge while substantially increasing sterility assurance of the released product.

References

1. International Standards Organization. *Cleanrooms and associated controlled environments — Biocontamination control — Part 1: General principles and methods,* ISO Standard No. 14698:2003 (2003).

2. Whyte, W. (2005) 'Collection efficiency of microbial methods used to monitor cleanrooms'. European Journal of Parenteral and Pharmaceutical Sciences, 10 (2). pp. 3-7. ISSN 0964-4679

3. W Whyte (School of Engineering, University of Glasgow, Glasgow G12 8QQ) and T Eaton (AstraZeneca, Macclesfield, Cheshire, SK10 2NA) 'Deposition velocities of airborne microbe-carrying particles' - European Journal of Parenteral & Pharmaceutical Sciences 2016; 21(2): 45-49

Authors

Gilberto Dalmaso, PhD.

Anna Campanella, PhD.

Paola Lazzeri

Editor: Noelle Boyton

Solutions: FMS

Designing an Environmental Monitoring Solution for GMP applications

Introduction

With the release of the new EU GMP Annex 1revision, a review of current practices is required to ensure that the installed monitoring system, chosen to meet the Annex 1 needs, complies with the requirements. This chapter will review the needs of Annex 1 with systems designs currently being installed [1].

Cleanroom Classification

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. The determination of the cleanroom class is a process based on actual statistically valid measurements, and a function of the filtration and operations status of the room, it is in essence a calibration of the room to ensure it meets its intended classification, it is not, primarily, a function of risk of application [2].

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 cleanroom. The test points should be equally spaced throughout the room and at work height to demonstrate the quality of the air cleanliness at the work area.

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

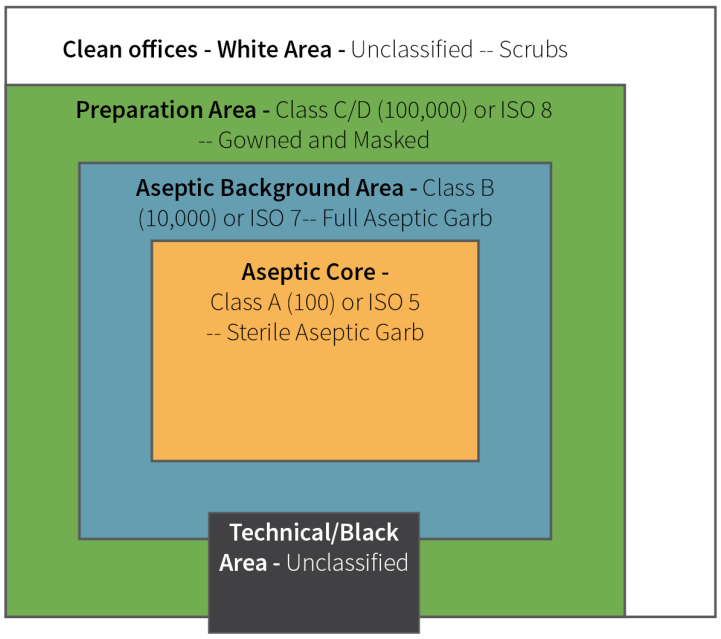


FIGURE J-1 DIAGRAM OF TYPICAL ISO RATING IN AREAS OF CLEANROOM

Pharmaceutical Cleanroom Utilization

Once a cleanroom has been tested to compliance to cleanroom classification typically using a Light Scattering Aerosol Particle Counter (LSAPC), the classification achieved dictates which production activities can be performed in that cleanroom or clean air device. The FDA defines two areas.

1. **Critical Areas** contains products that, if exposed, are vulnerable to contamination, these areas are designated Grade A (ISO5), within the Annex 1 document. To maintain product assurance, it is essential that the environment in which aseptic operations are conducted be controlled and maintained at an appropriate quality.
2. **Supporting Clean Areas** are used for all other activities outside the critical core, these are designated as grade B/C/D within the Annex 1 and are typically a lower risk to finished product contamination.

Once a cleanroom or clean air device has been proven to meet the requirements for cleanliness from a certification perspective, it must also demonstrate that this control can be maintained throughout production periods. The 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 nonviable and viable airborne particulates, surface viable contamination and, in the aseptic areas, and personnel. 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 an investigation into the source of the problem, the potential impact on the product, and any measures required to prevent a recurrence.

Contamination Control Strategy

A Contamination Control Strategy (CCS) will include the environmental monitoring program and should be implemented across the facility. The CCS should define critical control points as part of a risk assessment and assess the effectiveness of the controls and monitoring measures used to manage risks associated with contamination. The CCS should be reviewed frequently, especially during the early phases of implementation, and it should be updated to drive continuous improvement of the monitoring and control methods, ultimately improving overall quality of process.

Elements that should be considered as part of a Contamination Control Strategy will include:

i. Design of the plant and processes.
ii. Premises and equipment.
iii. Personnel.
iv. Utilities.
v. Raw material controls.
vi. Product containers and closures.
vii. Vendor approval –key suppliers.
viii. Outsourced services, such as sterilization, ensure the process is operating correctly.
ix. Process risk assessment.
x. Process validation.
xi. Preventative maintenance.
xii. Cleaning and disinfection.
xiii. Monitoring systems - the introduction of scientifically sound, modern methods that optimize the detection of environmental contamination.
xiv. Prevention – trending, investigation, corrective, and preventive actions (CAPA).

The scope of a Facility Monitoring System should encompass those identified in the list above (i, ii, iii, ix, x, xii, xiii and xiv), many of the CCS considerations should be included in the Environment Monitoring (EM) program.

Environmental Monitoring Requirements

TABLE J-1 EU GMP Annex 1 room classification table (Annex 1 2022)

EU GMP Grade	At Rest		In Operation	
	Maximum number of particles permitted/m3 equal to or greater than the tabulated size			
	0.5 µm	5.0 µm	0.5 µm	5.0 µm
A	3520	NOT SPECIFIED[a]	3520	NOT SPECIFIED[a]
B	3520	NOT SPECIFIED[a]	352,000	2930
C	352,000	2,930	3,520,000	29,300
D	3,520,000	29,300	NOT PREDETERMINED[b]	NOT PREDETERMINED[b]

[a] Classification including 5µm particles may be considered where indicated by the CCS or historical trends.
[b] For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and routine data where applicable.

FIGURE J-2 FOOTNOTES TO ABOVE TABLE IN ANNEX 1 2022

Monitoring should be performed using suitable techniques that meet the needs of the Risk Assessment; for many of the monitoring requirements of lower classification areas, a portable instrument can be deployed and used. However, the grade A area should be continuously monitored (for particles ≥0.5 and ≥5 µm) with a suitable sample flow rate (at least 28.3 LPM / 1CFM) so that all interventions, transient events, and system deterioration is captured. The system should frequently correlate each individual sample result with alert levels and action limits at such a frequency that any potential excursion can be identified and responded to in a timely manner. Alarms should be triggered if alert levels are exceeded. Procedures should define the actions to be taken in response to alarms, including the consideration of additional microbial monitoring.

The requirement for continuous monitoring within the Grade A is satisfied by using point of use dedicated sensors; these are connected to a central monitoring software application that can send alarm outputs to operators within the cleanroom or messages to concern groups. These alert and alarm excursions are also permanently recorded in the audit trail of the system.

One aspect of the system that needs to be determined is the location of the sample point(s); these should be determined following a documented Environmental Monitoring Risk Assessment (EMRA) and include the following information:

- Sampling locations
- Frequency of monitoring
- Monitoring method used and
- Incubation conditions (e.g. time, temperature(s), aerobic and/or anaerobic conditions).

and be based on the following inputs from site:

- Detailed knowledge of; the process inputs and final product, the
- Facility, equipment,
- Specific processes,
- The operations involved,
- Historical monitoring data,
- Monitoring data obtained during qualification and
- Knowledge of typical microbial flora isolated from the environment.
- Air visualization studies should also be included

Suitable sample point locations are also impeded by:

- Physical installation of sample probe
- Physical installation of instrument
- Tubing length, bends and bends radii between the two

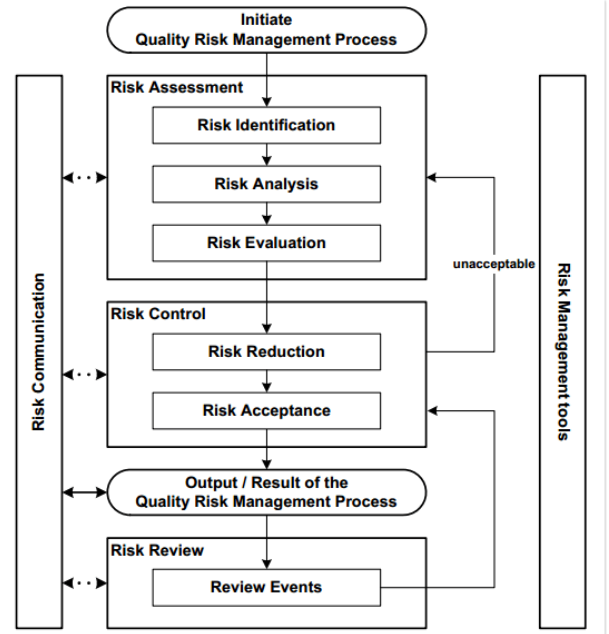


FIGURE J-3 STEPS FOR RISK IDENTIFICATION AND ANALYSIS

Typical Automated Continuous Monitoring System

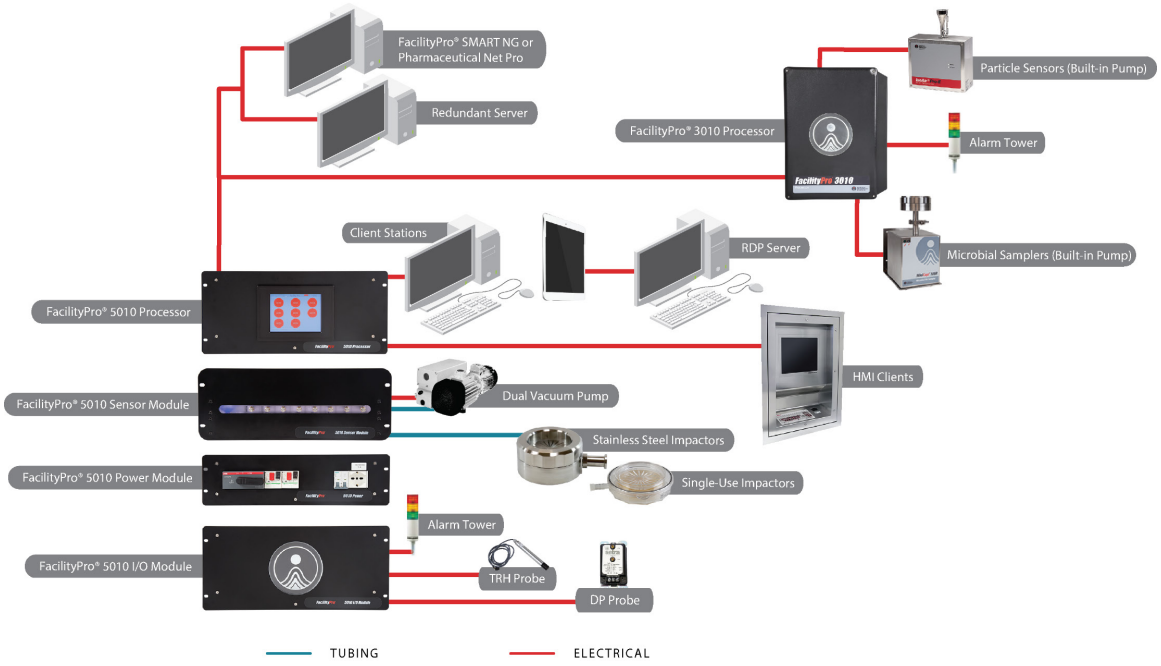


FIGURE J-4 EXAMPLE FACILITY MONITORING SYSTEM (FMS) SETUP

Instrumentation used in constructing an integrated solution will typically include:

Particle Counting – The need for continuous data requires a dedicated sensor at each location that samples continuously during the set-up and production phases of manufacturing. The sensor(s) send data back to a central processing component that is used to manage response processing, data buffering, and sensor controls. The sensor can have an internal pump or a remote vacuum source; both are controlled using the central interface within the software application.

Microbial Sampling – Where a risk has identified the need for total particle counting, there is an associated requirement for microbial sampling. The sample head only is placed within the environment, ensuring that any exhaust is managed by the system and not emitted locally within the critical space. Microbial samplers are fixed flowrate devices (typically 25 LPM), and this flow control is performed either locally (using a dedicated device) or centrally (using the same central vacuum source as the particle counter sensor sub-system). Start and stop controls are performed via the software interface.

Alarm Beacons – These additional devices allow for local annunciation (visible and audible) and can alert operators within the controlled space if a system is out of tolerance. Additional information can be achieved by situating a remote interface within the viewing space of the operators; these can also be interactive if they are within the clean core of the facility.

Central Software System - The system is designed with Industrial Automation architecture, which consists of a central processing system that collects data from field sensors and controls remote devices while communicating with a SCADA (Supervisory Control and Data Acquisition) software package. The following features should be available to interact effectively with the system and data reports.

Data and Status Information Displays - The main page is used for visualization of the facility layout with current data and status information for each sample point.

Data, status, and sampling information can also be viewed for each dedicated area on a single screen.

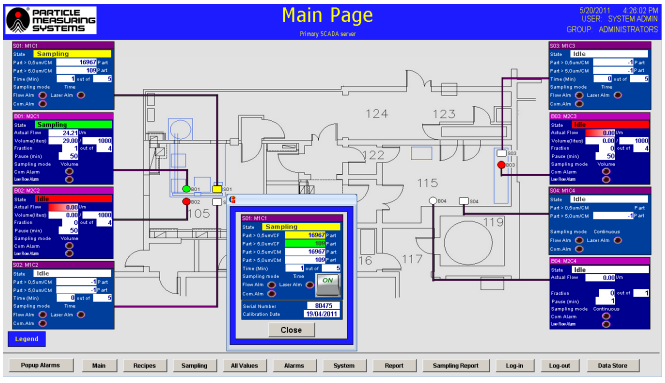


FIGURE J-5 MAIN PAGE OF FACILITY MONITORING SOFTWARE

User and Area segregation - According to CFR 21 Part 11 and Annex 11, single user access shall be controlled and managed to ensure each individual operated as described in the user Standard Operating Procedures and, according to the role, responsibility and training received.

The SCADA software should also ensure proper segregation of data whenever multiple departments are connected and controlled by the same supervising system.

Report Generator - The SCADA software requires a data report generator capable of providing human readable reports for all recorded data such as audit trails (events), data/statistical summaries, and trend charts. The system should be capable of retrieving data historically as defined in the site User Requirement Specification for the associated system. Using filters for data, time, location, and batch, data should be readily accessed and, where required, exported or printed to support the release of product.

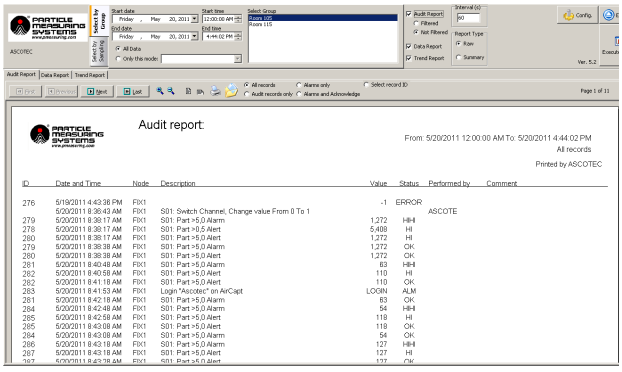


FIGURE J-6 REPORT GENERATOR

Alarms - The alarms display provides date, time, area, description, value, etc. for alarms and provides an alarm acknowledgment function. The alarms display also offers the capability to sort alarms by different criteria. Defining the alarm set point within the software is based upon the limits table in Annex 1 and based on historical data for each sample location.

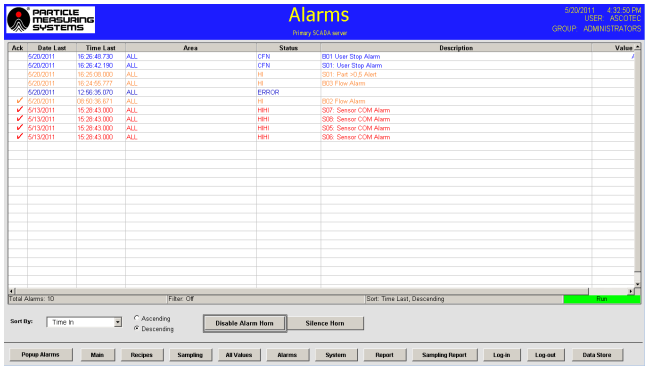


FIGURE J-7 ALARMS PAGE

Annex 1 (2022) also notes in Section 9, that

> *"The occasional indication of macro particle counts, especially ≥ 5 µm, within Grade A may be considered to be false counts due to electronic noise, stray light, coincidence loss etc. However, consecutive or regular counting of low levels may be indicative of a possible contamination event and should be investigated. Such events may indicate early failure of the room air supply filtration system, equipment failure, or may also be diagnostic of poor practices during machine set-up and routine operation"*

Therefore, when considering alarm rationale, it should also take into account the frequency of event and not singularly the magnitude; these factors should be considered within the CCS.

Additional information and alarming strategies can also be found in ISO 14644-2:2015, paragraph B.3.1; Environmental Monitoring Systems should allow for seamless and validated configuration of an "N of M" strategy to ensure sequences of out of specification events are promoted to alarms when the conditions are met.

As with all integrated systems, especially those using a central software package, the validation is a significant element on the timeline of any installed project. The review and circulation of documents can take several weeks where multiple departments are involved, and the start to finish time of a project should be discussed with the installation project team to ensure it meets the site requirements for shutdown accessibility.

Conclusion

Monitoring of pharmaceutical aseptic production environments is well established and the changes presented in the revision of Annex 1 (2022) do not change many aspects of the requirements for monitoring. The formal risk assessment and inclusion of data within a CCS create a more comprehensive addition to the continuous systems installed under past regulations. More emphasis is given to establishing the correct sample locations and techniques based on risk and reviewing data to support product release. This emphasis is however an enhancement to the documentation requirements more than the traditional expectations of a continuous system.

References

1. European Commission. *The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products,* Annex 1 (2022).

2. International Standards Organization. *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration,* ISO Standard No. 14644-1:2015 (2015).

Author

Mark Hallworth
Life Sciences Senior GMP Scientist
Life Science Division
Particle Measuring Systems

Mark Hallworth is the Life Sciences Regional Manager for Particle Measuring Systems. He has lectured for pharmaceutical societies throughout Europe, Asia, and the US on nonviable particle and facility monitoring and the implications of validating those systems. He can be reached at **mhallworth@pmeasuring.com**.

Editor: Noelle Boyton

Solutions: Alarm Rationale

Alarm Rationale for Continuous Particle Counting Systems

Introduction

Many of us have grown accustomed to performing routine environmental monitoring with a cart of equipment that takes samples at predetermined cleanroom locations. We collect these particle counts and microbial burdens with portable particle counters, dynamic air-samplers, and settle plates, and we take surface samples to adhere to gowning protocols. This process occurs regularly based on guidance from industry [1] with frequencies ranging from each shift up to monthly, depending on the associated risk to the final product.

FIGURE K-1 CRITICAL POINT IN CLEANROOM ENVIRONMENT

We approve conditions within controlled spaces such as cleanrooms by affirming the results taken from these 'spot reading' campaigns, where we aim for each individual sample to be:

- **As accurate and representative as possible**
- **As statistically significant as the conditions of the environment allow**
- **Appropriate so as to confirm controls are being maintained**

However, regulations require continuous particle monitoring in the most critical zones during production and prior to manufacturing, as this reflects a better demonstration of environmental control.

This shift to continuous monitoring can yield an ocean of data. Rather than using a portable instrument to, for example, collect three one-minute samples at each selected and defined location, data is now a continuous stream flowing from the sensor to a centralized monitoring system. This, in turn, continuously allows the state of control to be quantified and shows any shift between "normal" and "anomalous" conditions. Where previously we needed to prove control through discrete sample data, control is now the assumed state and out of tolerance (OOT) events become the discrete event requiring investigation.

This chapter is a description of current good manufacturing practices (cGMPs) and how they can be applied to a continuous monitoring system installed within a facility. This process should enable Quality and Production teams to establish new monitoring understandings and harmonization with standards. This review will look at the configuration of alert and action levels, potential reactions, and the reporting requirements of such systems.

Current Requirements in Industry

Forming a stance on what approach to take requires a look at the historical changes to GMP that define the current understanding and expectations of environmental monitoring (EM) in controlled areas.

> *"The purpose of the EM program is to document the state of control of the facility, not to determine the quality of the finished product."*
>
> SCOTT SUTTON, PH.D. - AUTHOR AND SPEAKER FOR THE PHARMACEUTICAL INDUSTRY

Cleanrooms are classified to a common standard. In 1999, the standard changed from the Federal Standard 209E:1992 (FS209E) [2] to a new metric standard, ISO 14644-1:1999 [3]. Specifically, the USA replaced FS209E in favor of the ISO 14644-1 on November 29, 2001. At this point, cleanrooms were now considered by their metric classification number (ISO 5, ISO 7, etc.) and not as the FS209E number (Class 100, Class 10000, etc.), and the monitoring requirements also became metrified. Data to meet the new standard must be normalized and displayed as counts per cubic meter (n/m^3), regardless of actual sample volume taken.

With the advent of a new ISO 14644 classification requirement, the EU GMP Annex 1 [4] was also updated. In 2003, a new version of the Annex 1 was released to reflect the ISO 14644 changes. The following statement/note was made on the front cover:

> **The guidance has been reviewed in the light of the international standard EN/ISO 14644-1 and amended in the interests of harmonization but taking into account specific concerns unique to the production of sterile medicinal products.**

A few points to note: Annex 1 has been amended to reflect the industrial certification standard ISO 14644-1, sterile medicinal products, and additional concerns that apply to more critical, sterile environments. A new table of required limits was also imposed:

TABLE K-1 Annex 1 (2003) particle cleanliness limits

EU GMP Grade	At Rest		In Operation	
	Maximum permitted number of particles permitted/m³ equal to or above			
	0.5 µm	5.0 µm	0.5 µm	5.0 µm
A	3500	1	3500	1
B	3500	1	350,000	2000
C	350,000	2,000	3,500,000	20,000
D	3,500,000	20,000	NOT DEFINED	NOT DEFINED

The limit for 5.0 µm, prior to the 2003 release, was zero due to the correlation between macro particles (> 5.0 µm) and the potential for microbial activity to sustain to colony forming units. Individual microbes either use moisture, dust, or biomass as an aid to offset desiccation. They can also slough off as larger components from operators. However, the original zero is technically impossible to achieve while following the ISO 14644-1 rules for sample volume, so the number needed to be the lowest possible integer to allow the formula to operate.

$$V_s = \left(\frac{20}{C_{n,m}} \right) \times 1000$$

FIGURE K-2 FORMULA IN ISO 14644-1:1999, SECTION B.4.2 ESTABLISHMENT OF SINGLE SAMPLE VOLUME PER LOCATION

This leads to a sample volume of 20 m³. Such a result is impractical for the classification of cleanrooms because significant time is required to fulfill the requirement. The Annex 1 clarified this in the footnotes listed below the table (see Figure K-4).

(a) Particle measurement based on the use of a discrete airborne particle counter to measure the concentration of particles at designated sizes equal to or greater than the threshold stated. A continuous measurement system should be used for monitoring the concentration of particles in the grade A zone, and is recommended for the surrounding grade B areas. For routine testing the total sample volume should not be less than 1 m^3 for grade A and B areas and preferably also in grade C areas.

(e) These areas are expected to be completely free from particles of size greater than or equal to 5 µm. As it is impossible to demonstrate the absence of particles with any statistical significance the limits are set to 1 particle / m^3. During the clean room qualification it should be shown that the areas can be maintained within the defined limits.

FIGURE K-3 EU GMP ANNEX 1 2003, TABLE 1, FOOTNOTES (A) AND (E)

Note (a) can be broken down into the following directives:

1. Use a particle counter.
2. Use a continuous measuring system for monitoring Grade A.
3. Take a 1 m³ sample for routine classification (testing) purposes.

In real terms, the zero had to be dropped to meet ISO 14644-1 statistical requirements, but there should be essentially no particles present greater than 5.0 µm. The notes were misinterpreted to read "use a particle counter and sample 1 m^3 for routine monitoring," ***which it does not***. This caused confusion and was clarified with a new classification table with the release of EU GMP Annex 1, coming into effect in 2009 [5]:

Clean room and clean air device classification

4. Clean rooms and clean air devices should be classified in accordance with EN ISO 14644-1. Classification should be clearly differentiated from operational process environmental monitoring. The maximum permitted airborne particle concentration for each grade is given in the following table.

	Maximum permitted number of particles per m^3 equal to or greater than the tabulated size			
	At rest		**In operation**	
Grade	**0.5 µm**	**5.0µm**	**0.5 µm**	**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

5. For classification purposes in Grade A zones, a minimum sample volume of $1m^3$ should be taken per sample location. For Grade A the airborne particle classification is ISO 4.8 dictated by the limit for particles ≥5.0 µm. For Grade B (at rest) the airborne particle classification is ISO 5 for both considered particle sizes. . For Grade C (at rest & in operation) the airborne particle classification is ISO 7 and ISO 8 respectively. For Grade D (at rest) the airborne particle classification is ISO 8. For classification purposes EN/ISO 14644-1 methodology defines both the minimum number of sample locations and the sample size based on the class limit of the largest considered particle size and the method of evaluation of the data collected.

FIGURE K-4 EU GMP ANNEX 1 2008, #4 AND 5

This revision allowed for the formula result to meet the 1 m^3 sample for classification. It also clarified its application as ONLY for classification, resulting in the adoption of an ISO Class 4.8 for Grade A. Paragraph 4 states that classification should be clearly differentiated from operational process environmental monitoring.

So, what to do for the alert and action limits? There is guidance (hidden) in the 2008 revision. If we look at paragraph 20, it reflects current risk assessment practices that state process conditions should define the limits:

20. Appropriate alert and action limits should be set for the results of particulate and microbiological monitoring. If these limits are exceeded operating procedures should prescribe corrective action.

FIGURE K-5 EU GMP ANNEX 1 2008 TEXT, #20

Therefore, limits set by room classification are those "appropriate" to determining control over potential risk, based upon room dynamics such as number of personnel, product type, activity type, etc. These limits are frequency based due to small-number statistics, in line with USP <1> and USP <1116> where trend analysis is a better reflection of control than absolute numbers with questionable statistical significance. Paragraph 13 in the Annex 1 gives the following directive:

13. In Grade A and B zones, the monitoring of the ≥5.0 µm particle concentration count takes on a particular significance as it is an important diagnostic tool for early detection of failure. The occasional indication of ≥5.0 µm particle counts may be false counts due to electronic noise, stray light, coincidence, etc. However consecutive or regular counting of low levels is an indicator of a possible contamination event and should be investigated. Such events may indicate early failure of the HVAC system, filling equipment failure or may also be diagnostic of poor practices during machine set-up and routine operation.

FIGURE K-6 EU GMP ANNEX 1 2008 TEXT, #13

When reviewing an alert and action philosophy and its application to particle counting and environmental control, the RISK should define what constitutes "occasional" vs "consecutive or regular". In all cases, the limit is not prescribed as 1 count/m^3 or 20 counts/m^3 but as a "low level", which can only be defined by a specific site, room, or process operation.

2022 Revision to EU GMP Annex 1

A new revision of EU GMP Annex 1 was published in 2022 [6]. It echoes the revision to ISO 14644-1:2015 [3] where the 5.0 µm limit for ISO 5 classified spaces was dropped due to statistical significance of particles in the larger sizes.

Grade	Maximum limits for total particle ≥ 0.5 µm/m^3		Maximum limits for total particle ≥ 5 µm/m^3	
	at rest	in operation	at rest	in operation
A	3 520	3 520	Not specified [a]	Not specified [a]
B	3 520	352 000	Not specified [a]	2 930
C	352 000	3 520 000	2 930	29 300
D	3 520 000	Not predetermined [b]	29 300	Not predetermined [b]

FIGURE K-7 2022 ANNEX 1, TABLE 1

Classification is section 4 and monitoring for both total particle and microbial contaminants has been relocated to section 9, separating the approach required for each exercise.

Monitoring now requires a Contamination Control Strategy (CCS), which looks more holistically at the process of creating controlled spaces, HVAC, gowning, data review and monitoring then using required techniques. It states:

9.1 The site's environmental and process monitoring program forms part of the overall CCS and is used to monitor the controls designed to minimize the risk of microbial and particulate contamination. It should be noted that the reliability of each of the elements of the monitoring system (viable, non-viable and APS) when taken in isolation is limited and should not be considered individually to be an indicator of asepsis. When considered together, their reliability is dependent on the design, validation and operation of the system that they are monitoring.

FIGURE K-8 2022 ANNEX 1 TEXT, SECTION 9.1

Alert and action limit requirements are carried over from the 2008 version and clarified. They do not mention what number determines periodic events, nor do they define what number constitutes an actionable threshold. That is instead documented with the Risk Assessment and CCS.

9.9 Appropriate alert levels and action limits should be set for the results of viable and total particle monitoring. The maximum total particle action limits are described in Table 5 and the maximum viable particle action limits are described in Table 6. However, more stringent action limits may be applied based on data trending, the nature of the process or as determined within the CCS. Both viable and total particle alert levels should be established based on results of cleanroom qualification tests and periodically reviewed based on ongoing trend data.

9.10 Alert levels for grade A (total particle only) grade B, grade C and grade D should be set such that adverse trends (e.g. a numbers of events or individual events that indicate a deterioration of environmental control) are detected and addressed.

FIGURE K-9 2022 ANNEX 1 TEXT, SECTION 9.9-9.10

9.17 The grade A area should be monitored continuously (for particles ≥0.5 and ≥5 µm) and with a suitable sample flow rate (at least 28 litres ($1ft^3$) per minute) so that all interventions, transient events and any system deterioration is captured. The system should frequently correlate each individual sample result with alert levels and action limits at such a frequency that any potential excursion can be identified and responded to in a timely manner. Alarms should be triggered if alert levels are exceeded. Procedures should define the actions to be taken in response to alarms including the consideration of additional microbial monitoring.

FIGURE K-10 2022 ANNEX 1 TEXT, SECTION 9.17

Monitoring procedures should define the approach to trending. Trends can include, but are not limited to:

- Increasing numbers of action or alert level breaches
- Consecutive breaches of alert levels
- Frequent or regular isolated breaches of action limits that may have a common cause

Reporting

System reports should be considered as part of the CCS. As noted earlier, a room monitored using a portable device produces data that needs to reflect that control was maintained. As such, the data needs to be 'absolute' and shown in all reports. However, the transition to a continuous monitoring system now continuously demonstrates adherence to control parameters (defined by alert and action levels), and only instances where there is a loss of control require investigation. Overall trend reports and audit trails show when the system was out of control and any remedial actions taken as part of the SOP.

System reports should focus on:

1. The report boundary, what sensors are included, what time frame the report covers, and any additional batch information that uniquely identifies this period.
2. The audit trail, alert and action threshold excursions, remedial actions, user log in, start end time data, system changes, etc.
3. The trend report as a way to show data as an easy to read trend plot (graph) image, demonstrating no loss of data.
4. The location of the out of tolerance event that caused an action level to be exceeded. This period can be covered by an exception report to demonstrate that, prior to the event, all was running typically and that, post-event, normal conditions were restored.

Application of Strategy

Alert and action limits can be applied to reflect a trending condition referred to as an N:M, where the number of events (N) is within a population of events (M). For example, an N:M of 2:6 would refer to a rolling set of numbers where any 2 samples out of a total of any 6 samples would require an action, with the initial trigger setting the clock to review the next 6 samples for a second triggering event. If none are present, the counter is reset until the next activation event.

Using this application, the system alerts are converted to non-actionable events, where the trigger is only used for data review. To ensure the system does not trend adversely over longer periods, the non-actionable events allow for comparison to similar time periods (e.g., weeks, months, quarters) or to similar events (e.g., filling, batch, lot). This ensures the limits prescribed in the system are reliable triggers of adverse conditions, and the limits reflect a loss of control over the environment as it pertains to product quality.

The particle levels are set with each of the following:

1. **Alert** – If any value goes above the 'normal' operating condition values when normalized to cubic meters (x35), an alert event is recorded. If alert levels are exceeded, operating procedures should prescribe assessment and follow up, which includes consideration of an investigation and/or corrective actions to avoid any further deterioration of the environment. This is essentially a non-actionable event.
    - Data is reviewed periodically for adverse trending and can be correlated against the room production events to ensure that where potential contamination may occur; the future revisions can reflect the required changes.
2. **Alarm** – The frequency (N:M) is set such that a single location must be in ALERT for a determined frequency before triggering an ALARM event, beacon, siren, audit trail etc. When these events occur, operating procedures should prescribe a root cause investigation, an assessment of the potential impact to product, and requirements for corrective and preventive actions. This is an out of control condition.

Alert and Action Level Setting

It follows then that a determination of the level to be used to catalyze needs should be determined.

Guidance from the PDA Technical report 13 gives the following approaches:

- **Cutoff Value** – A 95% or 99% control limit based on data previously gathered from a single site or collection of similar sites.
- **Normal Distribution** – The mean and standard deviation of a set of data, where high counts exist. Where a predominance of zeros prevail, skewed data sets may occur and non-parametric rules apply.
- **Non-parametric Tolerance** – The statistical determination that a room meets compliance with at least 95% confidence.

A data review is required per sample location or group of sample locations which exhibit similar properties. The practice of normalization is more to put the data into an SI unit mentioned in

regulations than understanding process controls. For example, pressure can be used as either inches of water gauge (in H_2O), pascals (Pa), or millibar (mBar), but each has a different number value due to scaling. Data per minute gives a balance between measuring at a short enough interval for response rate, while providing enough sample volume (assuming a 28.3 LPM flowrate), and, therefore, associated potential counts and statistical confidence in the data.

We can also consider the extremes of each time period. Long sample periods (35.3 minutes) will give great statistical confidence in the data, but poor response to control conditions and very short sample periods (5 second intervals) will give great dynamic responses. Low confidence data is where the few counts yielded requires multiple records to gain statistical insights and leads back to low responses. As such, the raw data from the instrument can be used, given as counts per minute or counts per cubic foot. However, industry has found it more accommodating to reflect the standard in counts per cubic meter (n/m^3).

Regardless of units chosen to reflect the level at which an out of tolerance event occurs, the number of particles which trigger the event and the frequency with which an event is escalated to actionable needs to be determined.

References

1. Parenteral Drug Association. *Fundamentals of an Environmental Monitoring Program*, PDA Technical Report No. 13 (2016).
2. Institute of Environmental Sciences. *Federal Standard Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones,* Federal Standard 209E (1999).
3. International Standards Organization. *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration,* ISO Standard No. 14644-1:2015 (2015).
4. European Commission. *EC Guide to Good Manufacturing Practice Revision to Annex 1: Manufacture of Sterile Medicinal Products*, Annex 1 (2003).
5. European Commission, EudraLex. *The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products (corrected version),* Annex 1 (2008).
6. European Commission. T*he Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products,* Annex 1 (2022).

Author

Mark Hallworth
Life Sciences Senior GMP Scientist
Life Science Division
Particle Measuring Systems

Mark Hallworth is the Life Sciences Regional Manager for Particle Measuring Systems. He has lectured for pharmaceutical societies throughout Europe, Asia, and the US on nonviable particle and facility monitoring and the implications of validating those systems. He can be reached at **mhallworth@pmeasuring.com**.

Editor: Noelle Boyton

Solutions: Compressed Gas

Compressed Gas Monitoring of Microbes: The contamination Control Strategy in Pharmaceutical Manufacturing Environments

Abstract

Compressed gases, such as nitrogen, carbon dioxide, and oxygen, are used for a variety of applications in pharmaceutical manufacturing. These applications, such as aseptic packaging, purging, and filtration, are critical processes monitored for their efficacy. To avoid sampling compressed gases, it has been speculated by some manufacturers that the rapid decompression of a gas when exiting its container kills any microbial contamination. However, it has been shown by multiple studies that microbial survival is not impacted by the typical compression or decompression seen in pharmaceutical process gases.

Many GMP standards recommend sampling process gases for contamination before use in critical areas of manufacturing. This step, along with many others that make up a contamination control strategy, contributes to the purity and quality of the final product.

Introduction

The quality attributes of manufactured pharmaceutical products include the physical, chemical, and microbiological characteristics of the raw materials, excipients, active pharmaceutical ingredients (APIs), and final drug products. Here, absence of microbiological contamination is critical because it can dramatically impact a drug's safety. As a result, the cleanliness of compressed gases, which often come into contact with pharmaceutical products, is also critical.

For the variety of gases used in manufacturing, their compressed state refers to how they are contained. Compressed gases are typically sampled by taking a small amount from a gas line and drawing it into a smaller space (i.e., a sampler). The decrease in volume that the gas occupies increases its pressure. Gases are decompressed when exiting whatever is containing them for use in a manufacturing line. Process gases are therefore more likely to be decompressed before coming into contact with the product.

FIGURE L-1 CRITICAL POINT OF A FILLING LINE

Regulatory Requirements

The latest revision of *Annex 1, Manufacture of Sterile Medicinal Products*, introduced the concept of a "contamination control strategy" which limits particulate and microbial contamination and promotes process understanding [1]. A manufacturer's contamination control strategy must demonstrate:

- A thorough understanding of potential sources of contamination.
- Regular trend analysis is performed, ensuring appropriate critical quality attributes of high-risk utilities.
- Gases and other high-risk utilities that come in direct contact with the product or primary container are of appropriate chemical, particulate, and microbial quality.

Specifically, actions should be taken to ensure the sterility of process gases, including filtration through a sterilizing filter at the point where the gas is used in production and sterilization of any subsequent piping or tubing. Filtration should be part of batch standards with certification guaranteed before release. Integrity testing should be performed for both critical and non-critical gas filters. Care should be taken to avoid introducing moisture to filtration systems, as this promotes the growth of microbes. In lines 715 - 716, Annex 1 states:

> **"When gases are used in the process, microbial monitoring of the gas should be performed periodically at the point of use."**

In a similar vein, the FDA's *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing* makes specific mention of the need for purity in a compressed gas. Its microbiological and particle quality after filtration should also be equal to or better than where it's going [2]. ISO 8573 consists of nine separate parts pertaining to the quality of compressed air, with the first specifying the quality requirements and parts two through nine concerning the methods of testing for a range of contaminants. The test method for microbial monitoring of compressed gases is provided in ISO 8573-7.

All the data generated from testing should be recorded to show proof of conditions for any generated product. Auditors will want to see the end results of testing in addition to the systems in place to verify claims.

Microbial Survival

The microbial component and, more particularly, the sampling methodology of compressed gases has been the subject of extensive discussion. It was assumed that the sudden decompression of a compressed gas before sampling was considered to have a deleterious effect on microbial survival, thereby voiding sampling results. This claim has been proven false with extensive study.

In an FDA study from 2014, bacterial cultures in food products were found to require 2500 to 3000 bar of pressure to inactivate. Cells subjected to pressures less than 1000 bar had no significant loss in viability [3]. In another study, *Serratia* and *Carnobacterium* strains were found to survive

conditions similar to those found on Mars [4]. Lessened microbial growth and metabolism in vivo was only seen in pressures higher than 1000 bar. In fact, microbial cells have been found to survive volatile external pressures found in the harshest environments Earth has to offer, including those in deep-sea environments. In these extremes, microbes conserve their ability to sustain life and reproduce.

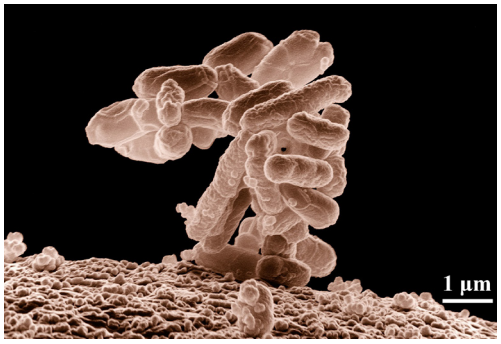


FIGURE L-2 *SERRATIA MARCESCENS*

In a study of decompression, *Escherichia coli* and *Corynebacterium xerosis* were shown to survive rapid decompressions from 300 to 0 bar [5]. In a typical compressed gas sampler, decompressions are on a much smaller scale (i.e., from around 10 bar to 1 at most). Only the viable counts of gram-negative, gas vacuolate bacteria types, such as *Marmoricola aquaticus*, *Prosthecomicrobium pneumaticum* and *Meniscus glaucopis* were shown to be affected by minimum decompression pressures from 25 to 50 bar [5].

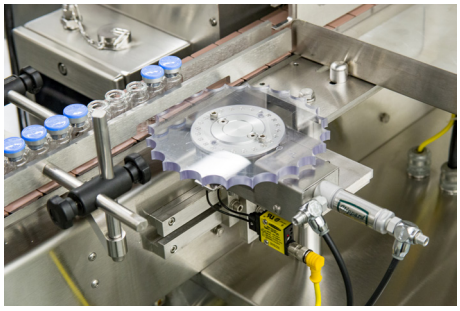


FIGURE L-3 TYPICAL DECOMPRESSION IN MOST PHARMA ENVIRONMENTS: 2.5 TO 1.1 BAR

Instrument Selection

The effects of compressed gas sampling on microbes are insignificant and should not affect instrument selection. Helpful parameters include ease of cleaning and disinfection, associated data management tools, and simplicity. Cost is also one to consider when the monitoring compressed gases is infrequent[6]. Different monitoring uses can occur at various frequencies:

- Classification frequency: Performed monthly or quarterly.
- Routine testing frequency: In Europe, twice a year. The US requires it to be once a year.

Investment into dedicated monitoring equipment can be hard to justify. Alternatives include hiring a service provider for scheduled sampling or using equipment that can be purchased with an accessory for compressed gas sampling (i.e., the MiniCapt® Mobile Microbial Air Sampler with compressed gas kit). Deciding on the most feasible solution will require a comparison of these solutions.

Conclusion

Regulations have maintained their stance on the importance of contamination control and continue to be a necessary tool to ensure products meet key standards for safety. GMPs are regularly updated and reflect the modernization and improvements made to manufacturing systems made in recent years. Knowing these guidelines and requirements will expand their reach; it is practical and responsible for companies to seek forward-thinking solutions to better their own processes. In the

case of microbial monitoring, taking steps to ensure sterility of equipment and process gases is a vital part of the contamination control strategy. The methods for monitoring contamination levels should not be determined with speculation and antiquated reasoning, but with constructive comparison and validated study.

Author

Frank Panofen, PhD

Director and General Manager Life Sciences
Life Science Division
Particle Measuring Systems

Dr. Panofen has a Diploma in Chemistry from the University of Bielefeld and a PhD in molecular and cell biology from the University of Osnabrück. He has expansive experience in the field of applied pharmaceutical microbiology and serves as the Sterility Assurance/ Microbiology Product Line Manager at Particle Measuring Systems. Frank has been an invited speaker at international conferences including ECA and PDA, with a strong regulatory background in pharmaceuticals. He is a certified Microbiological Laboratory Manager from ECA. He can be reached at **fpanofen@pmeasuring.com.**

Editors: Noelle Boyton and Briana Krueger

References

1. European Commission. *The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products,* Annex 1 (2022).
2. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER,) Center for Biologics Evaluation and Research (CBER), Office of Regulatory Affairs (ORA). *Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice*, (2004).
3. Federal Drug Administration. *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies - High Pressure Processing (HPP): A report of the Institute of Food Technologists for the Food and Drug Administration of the U.S. Department of Health and Human Services*, Journal of Food Science, Volume 65, Issue Supplement s8 (2000).
4. Nicholson, W. (2013). *Growth of Carnobacterium spp. from permafrost under low pressure, temperature, and anoxic atmosphere has implications for Earth microbes on Mars*. pnas 110(2):666- 671.
5. Hemmingsen, B. B. & Hemmingsen, E. A. (1980). *Rupture of the Cell Envelope by Induced Intracellular Gas Phase Expansion in Gas Vacuolate Bacteria*. Journal of Bacteriology. 143 no. 2:841-846.
6. Based on author's personal communications with customer

Contamination Control Advisory Solutions

Our Contamination Control Advisory Team can support you at every step, whether it be non-compliance issues, setting up a new process, GAP analysis, or training employees.

- Environmental MonitoringRisk/ Gap Aalysis
- Cleaning and Disinfection
- Aseptic Process Simulation
- Sterility Assurance Audits
- Environmental Monitoring Trend Analysis
- Gowning
- New Filling Line QbD
- Crisis Management
- Utilities
- Process Risk Assessment

Advisory Services

Helping you achieve the highest level of contamination control and sterility assurance.

Performance Optimization

Designing quality into your operations using gold standard PMS® tools and processes.

BioCapt® Single Use Microbial Impactor

Training

Preparing your employees to ensure success at every step.

Basic and Advanced

Coaching

PARTICLE MEASURING SYSTEMS®
a spectris company

For more information contact
www.pmeasuring.com
info@pmeasuring.com