

Technical Report No. 13 (Revised)

Fundamentals of an Environmental Monitoring Program

2014



PDA Fundamentals of an Environmental Monitoring Program Technical Report Team

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Disclaimer: The task force for this report consisted of members representing global companies to ensure that the methods, terminology, and practices reflect international and not just U.S., procedures. Technical peer reviews were completed by prominent environmental monitoring scientists.

The content and views expressed in this technical report are the result of a consensus achieved by the authoring task force and are not necessarily views of the organizations they represent.

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ISBN: 978-0-939459-67-4

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1.0 Introduction

Environmental monitoring is adjunct to a sterility assurance program and is used to evaluate the effectiveness of microbial controls used in the manufacture of sterile pharmaceutical products.

PDA first published guidance on environmental monitoring in the form of *Technical Report No. 13* in 1990, and revised the guidance in 2001. This is the second revision of that guidance.

The task force chose to reference the ISO cleanroom classifications as benchmark recommendations throughout the Technical Report. However, cleanroom classifications expectations are different per region. Regulatory and compendial classifications have been identified in **Tables 3.0-1** and **3.0-2** for the United States of America, the European Union, and Japan.

1.1 Purpose

This document was created to aid in the establishment of an environmental control and monitoring program that is meaningful, manageable, and defensible. This revision updates microbiological and particulate control concepts and principles as they relate to facilities involved in the manufacture of sterile pharmaceutical products and other designated controlled environments. It expands on PDA's 2001 revision of *Technical Report No. 13* to reflect substantial changes to regulatory guidelines, international standards, and scientific advances in environmental monitoring procedures and equipment.

This document should be viewed as technical guidance; it is not intended to establish any voluntary or mandatory standards.

1.2 Scope

This document serves as a resource on controlled environmental test methods, and although some nonviable particulate information is included, the report's primary focus is microbiological control for sterile product manufacturing.

This document addresses international standards and regulatory guidances, elements of an environmental monitoring program, and environmental monitoring by application. Current guidelines for typical environmental monitoring frequencies and levels for pharmaceutical water are covered in the appendix.

1.2.1 Exclusions

1.2.1.1 Bioburden Monitoring

Product or component bioburden monitoring is not considered part of all environmental monitoring programs and is therefore outside of the scope of this technical report. Incubation media, times, and conditions are also not addressed in this document, as individual monitoring circumstances and requirements will vary and most regulatory expectations are that the sampling conditions should be justified and validated.

1.2.1.2 Other Environmental Control Support Activities

In order to ensure a consistently acceptable controlled environment, a comprehensive environmental control program should be supported by:

- Sound facility design and maintenance
- Established documentation systems
- Validated/qualified sanitization/disinfection procedures
- Reliable process controls

- Good housekeeping practices
- Effective area access controls
- Consistent sample collection and analysis
- Effective training, certification/qualification, and evaluation programs
- Quality assurance of materials, facilities, and equipment

These support elements are not covered in this technical report.

2.0 Glossary of Terms

Action Level

An established microbial or airborne particle level that, when exceeded, indicates a process is outside of its normal operating range. A response to such an excursion should involve a documented investigation and corrective actions based on the results of that investigation.

Alert Level

An established microbial or nonviable particle level giving early warning of potential drift from normal operating conditions; not necessarily grounds for definitive corrective action but typically requires follow-up investigation (1).

Airborne Particulate Count (Total Particulate Count)

The total number of particles of a specific size per unit volume of air.

Airborne Viable Particulate Count (Total Airborne Aerobic Microbial Count)

The recovered number of colony-forming units per unit volume of air.

Aseptic Filling

Part of aseptic processing in which a presterilized bulk product is filled and/or packaged into sterile containers and closed in a cleanroom.

Aseptic Processing

Handling of sterile product, containers, and/or devices in a controlled environment in which the air supply, materials, equipment, and personnel are regulated to maintain (product) sterility.

Bioburden

The total number of microorganisms per unit of material prior to sterilization.

Campaign

A series of consecutive production batches manufactured without intervening cleaning and sterilization.

Colony-Forming Unit (CFU)

A single macroscopic colony formed after the successful growth of one or more microorganisms to a solid microbiological growth medium.

Cleaning

Chemical or physical means used to remove soil and/or microorganisms from surfaces.

Cleanroom

A room designed, maintained, and controlled to prevent particle and microbiological contamination of a drug product or medical device. A cleanroom is assigned and reproducibly meets an appropriate air cleanliness classification.

Continuous Monitoring

A process of data collection in which conditions are monitored continuously throughout the operation. In most U.S. applications, this definition implies “during production.”

Controlled Area

An area constructed and operated in such a manner that some attempt is made to control the introduction of potential contamination (an air supply approximating to Grade D may be appropriate), and the consequences of accidental release of living organisms. The level of control exercised should reflect the nature of the organism employed in the process. At a minimum, the area should be maintained at a pressure positive to the immediate external environment and allow for the efficient removal of small quantities of airborne contaminants.

Corrective Action

A response taken to remediate the effect of an excursion or product failure.

Critical Area/Critical Zone

An area designed to maintain sterility of materials where sterilized product, containers, closures, and equipment may be exposed to the environment.

Critical Surface

A surface within a critical area that may come in direct contact with sterilized products, containers, or closures.

Disinfection

The chemical or physical inactivation of a bioburden on inanimate surfaces. Typically this requires a minimum three-log (3-log) reduction of vegetative microorganisms and two-log (2-log) reduction for bacterial spore be achieved in validation (2).

D-value

The time in minutes at a specific temperature required to reduce the population of a specific microorganism by 90% [or one (1) log] in defined conditions [e.g., method of sterilization (dry heat versus steam), solute, or carrier].

Dynamic Monitoring

Monitoring of an environment during normal operations, that is, when the usual equipment is operating and personnel are present, and the process or simulated process is ongoing. Per the EU and ISO documents this is synonymous with operational condition (including the equipment operating and personnel present).

Environmental Control Parameters

Conditions and corresponding measurements as associated with facilities and equipment used in the control of a manufacturing area that may impact the identity, strength, quality, or purity of a product. Among such parameters are airflow rates and patterns, pressure differentials, materials and personnel flow, temperature and relative humidity, as well as nonviable and viable particulates.

Frequent Monitoring

A process of collecting data in which conditions are monitored at a defined frequency not exceeding sixty minutes during operation. In most U.S. applications, this means “during production.”

Grid Profiling

A process of dividing areas of equivalent classifications into grids for the purpose of uniformly assessing contamination characteristics in that area. This process is usually confined to the validation of new facilities and not routine monitoring.

Isolator

Isolator, Closed

A decontaminated unit meeting ISO 5 conditions that provides uncompromised, continuous, isolation of its interior from the surrounding environment. Any air exchange with the surrounding environment takes place only through microbially retentive filters.

Isolator, Open

A decontaminated unit meeting ISO 5 conditions that provides uncompromised, continu-

ous isolation of its interior from the surrounding environment. It may transfer air directly to the surrounding environment through openings (e.g., “mouseholes”) that preclude the ingress of microbial contamination.

Microbial Characterization

The description of microorganisms based on their cellular morphology, Gram reaction, and key diagnostic tests (e.g., Gram-positive coagulase-negative cocci).

Microbial Classification

The arrangement of microorganisms into taxonomic groups based on their similarities and relationships.

Microbial Identification

The determination of the genus, and species when possible, to which a laboratory or manufacturing isolate belongs.

Nonviable

A term used in reference to particulates that are not capable of living, growing, or developing and functioning successfully (“unable to divide” or “not capable of reproducing”).

Parametric Release

A sterility release program based on effective control, monitoring, and documentation of a validated sterile-product manufacturing process where sterility release is based on demonstrated achievement of critical operational parameters in lieu of end-product sterility testing (3).

Process Control Parameters

Conditions and corresponding measurements associated with the manufacturing process that may affect the identity, strength, quality, potency, and purity of a product. Examples of parameters of concern include bioburden, process rate, weight, volume, temperature, and pressure.

Restricted Access Barrier System (RABS)

Aseptic processing systems (ISO 5) intended to substantially reduce human-borne contamination within the aseptic environment where sterile product, containers, closures, and equipment are exposed by the use of separative devices and defined mechanical features and operating procedures (4).

Risk Analysis

The estimation of the risk associated with the identified hazards (5,6).

Sanitization

Reduction of microbial contaminants to safe levels as judged by public health requirements for the specific country.

Spore

A bacterial dormant form that is highly resistant to adverse conditions. Fungal spores are not highly resistant; their susceptibilities are closer to vegetative microorganisms.

Static Monitoring

Monitoring of the environment in the absence of normal operations. This includes having the equipment installed and operational when no personnel are present. Per the EU and ISO standards, this is synonymous with “at rest.”

Sterilization

Validated process used to render product free from viable microorganisms (7).

Strain

A specific isolate of a species that is maintained in pure culture and is serotypically, genotypically, or chemotaxonomically characterized to differentiate it from other strains of the same species. The strain is representative of the species and provides a reference for the species based on its historic isolation, characterization, and deposition in recognized culture collections.

2.1 Acronyms

API — Active pharmaceutical ingredient

HEPA Filter — High-efficiency particulate air filter

Terminal Sterilization

The application of a lethal agent to sealed, finished drug products for the purpose of achieving a predetermined sterility assurance level (SAL) of usually less than 10^{-6} (i.e., a probability of a nonsterile unit of less than one in a million). A process where the material is sterilized in its final packaged configuration.

Trend Analysis

A review performed in response to an alert or action condition. This review provides an analysis of specific environmental monitoring data to identify adverse trends.

Vegetative Cell

Cells in an actively growing state. Some microorganisms can only be vegetative, while others are sporeformers and can be in a vegetative or spore (dormant) state.

VBNC — Viable but not culturable

3.0 Environmental Classifications: Regulatory Expectations

An environmental monitoring program should be designed and implemented in conformance with the requirements of the government agencies regulating (mandatory requirements) the manufacturing site and international standard-setting organizations (e.g., some mandatory or legally enforceable and some voluntary).

If the intent is to serve the international markets, the most stringent requirements should be evaluated as the basis of an environmental monitoring program.

This section compares regulations instituted for environmental monitoring by these authorities and standards setting organizations:

- ISO (voluntary)
- U.S. FDA
- U.S. Pharmacopoeia (mandatory)
- European Pharmacopoeia (mandatory)
- European Commission
- MHLW (Japan)
- Japan Pharmacopoeia (mandatory)
- World Health Organization

Although the regulations and guidelines are similar to each other in many respects, there are important differences among them in terms of the information each provides, particularly with respect to cleanroom classifications.

The most commonly accepted international cleanroom standard is ISO 14644-1, Cleanrooms and Associated Controlled Environments—Part 1: Classification of Air Cleanliness, 1999 (8). ISO class designations are based on the number of particles greater than a specified size (0.1–5 µm) per cubic meter of air sampled. ISO 14644-1 defines classes from 1 to 9, with ISO 1 being the cleanest. ISO classes 5 through 8 are used in the pharmaceutical industry for sterile-product manufacture and other areas where airborne particulate control is required.

Some international and national bodies have based their cleanroom requirements for sterile manufacturing on the ISO standard, thus its use here as the benchmark for other regulations and standards. The USP has adopted the ISO cleanliness classes in USP <1116> Microbial Control and Monitoring of Aseptic Processing Environments (9).

No international regulation or consensus standard except for the People's Republic of China requires ISO-class cleanrooms for the manufacture of all types of nonsterile products. China requires ISO 8 tested at rest for areas where nonsterile product is exposed during manufacture. However, the EU GMP regulations require ISO 8 classification for the manufacture only for inhalants. In other jurisdictions, firms are free to apply the ISO classes to nonsterile manufacturing or to specify equivalent or modified conditions based on their product requirements.

It is important to note that ISO does not specify the operating state of the area being classified (i.e., as built, at rest, or in operation), nor does it specify the particle size thresholds to be employed. In addition, the ISO standard deals only with total particulate count and does not discern between viable and nonviable particles. These decisions are left to the regulating or advisory body to specify. Therefore, each regulatory or standard-setting body has comparable but somewhat different applications of the ISO standard. In general, however, the international pharmaceutical community has provided guidance according to the following scheme (in operation):

- ISO 5: Aseptic processing zone; sterile product and/or packaging component is exposed. Unidirectional airflow required
- ISO 7: Area immediately surrounding the aseptic processing zone (ISO 6 may be employed but is neither required nor recommended)
- ISO 8: Nonsterile formulation, materials, and component preparation; filling area for terminally sterilized product

The European Union (as specified in EU GMPs Annex 1) and the World Health Organization (as specified in WHO/TRS 957 Annex 4) both use an alphabetic classification of Grades A to D (10,11). For each grade, a $\geq 0.5 \mu\text{m}$ and $\geq 5 \mu\text{m}$ particle count limit is specified (see Table 3.0-1 for details). In addition, these limits are set for both “at rest” and “in operation” states. Thus, each grade has up to two corresponding ISO classes, as follows:

- Grade A: ISO 4.8 at rest and in operation
- Grade B: ISO 5 at rest, ISO 7 in operation
- Grade C: ISO 7 at rest, ISO 8 in operation
- Grade D: ISO 8 at rest, undefined in operation

Note that the Grade A ISO equivalent is Class 4.8 based on the reduced maximum count of particles $\geq 5.0 \mu\text{m}$ per cubic meter from 29 (ISO 5) to 20 (ISO 4.8).

Japan has adopted similar classifications in its *Guidance on the Manufacture of Sterile Pharmaceutical Products by Aseptic Processing*. One minor difference is that the Japan guidance refers to the Grade A classification as ISO 5 rather than ISO 4.8, although the limit for particles $\geq 5 \mu\text{m}$ technically corresponds to ISO 4.8. The Japan Pharmacopeia also uses the Grade A–D classification but does not specify counts $\geq 5.0 \mu\text{m}$ and refers to the U.S. FDA guidance for equivalency in operation (12).

For the United States, the FDA’s 2004 *Guidance for Industry Sterile Drug Products Produced by Aseptic Processing: Current Good Manufacturing Practice* and USP General Information Chapter <1116> Microbiological Control and Monitoring of Aseptic Processing Environments both discuss the application and environmental requirements for cleanrooms used for aseptic processing (9,13). These include ISO Classes 5–8 and their corresponding Federal Standard 209E classes (e.g., ISO 5/Class 100). Limits are set only for particles $\geq 0.5 \mu\text{m}$. Both are silent on the state of the area to be tested but clearly imply areas to be “in operation.”

NOTE: Both the FDA guidance and USP mention ISO Class 6 as being applicable to the area immediately surrounding the critical aseptic processing zone (ISO 5). However, both stop short of recommending this application. Federal Standard 209E has been retired; however, the FDA guidance still uses the class references defined therein.

ISO 14644-1 not only defines the airborne particulate levels for the various ISO classes but also specifies the sample plan for classifying an area. The International Organization for Standardization published a revision to ISO 14644-1 in 2010 in the form of a Draft International Standard. This version is not finalized and has yet to be formally recognized by the international pharmaceutical regulatory community. However, it contains two significant changes that are likely to remain in the final version that environmental monitoring experts should be aware of. First, the sampling plan for area classification has been changed to provide a higher statistical assurance of room performance. Second, the particulate limit at $\geq 5.0 \mu\text{m}$ for ISO Class 5 has been deleted due to the difficulty in measuring such low counts accurately. Firms are advised to monitor the development of the revised ISO Standard 14644-1 and also to stay abreast of changing regulations as a result of these revisions.

Common factors among the various guidelines and regulations described earlier include the requirement that the most critical ISO 5 zone, where aseptic conditions must be maintained, requires unidirectional airflow. ISO 14644-1 describes unidirectional flow as 0.45 meters per second (90 ft/min) plus or minus 20% measured 150 to 300 mm from the supply filter face. (Note that EU Annex 1 recommends this measurement be taken as close to the work surface as practical) (10).

In addition, all of the authorities recommend an air pressurization scheme to ensure airflow from the cleaner zone to the less clean. The broadly acceptable guidance value is a differential pressure (ΔP) of 10–15 Pa (0.04–0.06 inches H_2O) between zones of differing class. Several of the guidance documents describe the use of air locks to maintain this differential while doors are in use. Where an ISO 5 unidirectional zone is placed within an ISO 6 or ISO 7 background, this pressure differential is not required (10).

Table 3.0-1 summarizes the area classifications for sterile manufacturing specified by various regulatory and standard-setting bodies. **Table 3.0-2** summarizes monitoring requirements for the various environments according to the relevant authorities. Note that the summary of requirements is condensed and is limited to routine monitoring. It excludes requirements for area qualification or requalification. More complete guidance may be found in the references cited.

Table 3.0-1 Cleanroom Standards—Airborne Particulate Limits (particles/m³)

Particle Size	ISO 14644	U.S. FDA (Aseptic Processing Guidance)	USP <1116>	EU Annex 1 and WHO Annex 4	Japan (Aseptic Processing Guidance)	JP XVI
	ISO 5	Class 100 ^{1,2}	ISO 5/Class 100	Grade A Grade B (at rest)	Grade A Grade B (at rest)	Grade A Grade B (at rest)
≥0.5 μm	3,520	3,520 ³	3,520	3,500	3,520	3,520
≥5 μm	29	Not specified	Not specified	20 ⁴	20	Not specified
	ISO 6	Class 1000	ISO 6/Class 1000	NA	NA	NA
≥0.5 μm	35,200	35,200	35,200	NA	NA	NA
≥5 μm	290	Not specified	Not specified	NA	NA	NA
	ISO 7	Class 10,000	ISO 7/Class 10,000	Grade B (in operation) Grade C (at rest)	Grade B (in operation) Grade C (at rest)	Grade B (in operation) Grade C (at rest)
≥0.5 μm	352,000	352,000	352,000	350,000	352,000	352,000
≥5 μm	2,900	Not specified	Not specified	2,900	2,900	Not specified
	ISO 8	Class 100,000	ISO 8/Class 100,000	Grade C (in operation) Grade D (at rest) ⁵	Grade C (in operation) Grade D (at rest)	Grade C (in operation) Grade D (at rest)
≥0.5 μm	3,520,000	3,520,000	3,520,000	3,500,000	3,520,000	3,520,000
≥5 μm	29,000	Not specified	Not specified	29,000	29,000	Not specified

1. Class 100 and Grade A are defined as requiring unidirectional airflow by all applicable guidelines.
2. Obsolete U.S. Federal Standard 209E classification added for continuity.
3. Class titles for U.S. FDA and USP indicate equivalent particle counts per cubic foot.
4. ISO 4.8 based on reduced limit for particles ≥5 μm.
5. Grade D operational particulate counts depend on the operation and are not defined by any guideline.