Environmental Monitoring

Particle Counts Are Easy

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Manufacturing pharmaceutical and biotechnology products requires that the appropriate level of quality be designed and constructed into the facility — and systems — that support the production process. As a result of recent 483 observations and warning letters, the FDA's current compliance focus is on inadequate facility design and environmental and personnel monitoring. One can only conclude that the skill level, training, and attitude of the personnel involved in the cases cited were inadequate with respect to the obvious requirement to minimize particulate, microbial, and pyrogen contamination.

A sample of FDA observations on environmental monitoring violations culled from warning letters available to the public on the FDA website includes the following:

PRODUCT: ALL REGULATED PRODUCTS

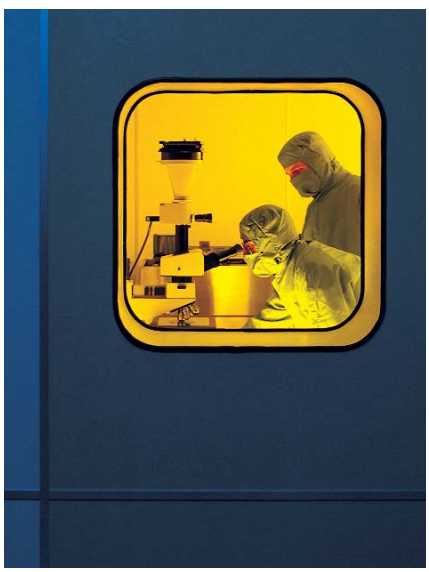
PROCESS FOCUS: MANUFACTURING

WHO SHOULD READ: PROJECT MANAGERS, MANUFACTURING, QA/QC, REGULATORY AFFAIRS

KEYWORDS: ENVIRONMENTAL MONITORING, CLEAN ROOM, CONTAMINATION CONTROL, VIABLE, NONVIABLE, PARTICULATE, MICROBIOLOGICAL

LEVEL: INTERMEDIATE

- "Monitoring is not conducted routinely nor concurrently with manufacturing . . . sampling should be done daily during both shifts, both inside and outside of the LAF (laminar air flow) areas, and sample times should be varied to cover all parts of the production period."
- "Sampling frequencies and locations must be defined."
- "Microbial air samples under laminar flow modules are collected only under static conditions."
- "Less than 10% of the microbial air samples were collected after noon, although production routinely continues until 3:00 PM"
- "Room air microbial samples are collected with the RCS (rotary centrifugal sampler) on a tripod at a height of five feet, which does not represent working level in these rooms. There is no trending of data."
- "There has been no daily monitoring of aseptic areas and LAF modules for nonviable particulates on a day of production basis."
- "HEPA filters have been certified with DOP (di-octyl phthalate) and a particle counter and not with a photometer."
- "Air velocities have been reported as average and do not show the individual readings."
- "HEPA filters need to be DOP tested at least twice a year, not once a year as is currently being done."
- "Some of the validations of air quality in rooms were done only under static conditions without personnel. Also no smoke pattern studies on the LAF have been performed to show effects of curtain movements on laminar air flows."
- "The firm has not set alert and action limits for most environmental samples . . . firm needs to identify all organisms isolated from aseptic area until a database is established for normal flora found in the production environment (with frequency distribution) for use in evaluating sterility test results."
- "Fail to eliminate objectionable organisms from interior surfaces of transfer carts in which sterilized unsealed containers of drug product are exposed."
- "No validation studies have been conducted to assure the microbial settle plates are capable of supporting microbial growth after



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the stated three-hour exposure time in Class 100 rooms."

- "Class 10,000 areas have a viable limit of 1.4 CFU (colony forming units)."
- "The quality control unit did not assure that adequate systems and controls were in place to monitor the functioning of, and to detect malfunctions of, the air handling systems used to control and assure aseptic conditions in aseptic manufacturing areas."

How do those types of situations develop? Whereas from time to time the European Union may provide some specific recommendations for meeting cGMPs, the FDA normally does not dictate how a specific outcome is to be achieved.

A classic example comes to mind when considering the number of unidirectional flow aseptic fill cleanrooms that, once built, have proven unable to meet validation requirements. In most of those situations, the aseptic fill application was treated as if it were simply a Class 100 particle count requirement without regard for the critically important airflow patterns required to ensure that exposed products and components are protected from contamination. Aseptic processing operations must be performed within separate, defined areas to prevent microbial and/or cross contamination.

Monitoring and Surveillance

Nonviable particulate and viable microbiological surveillance are used to evaluate the design and control of a cGMP-manufacturing environment. The nonviable particulate monitoring program is used to verify the maintenance of air classifications called for in a facility design. Particulate monitoring should be performed routinely using statistical sampling procedures that are appropriate for each individual room, piece of equipment, and process.

In general, a comprehensive environmental monitoring program should include scheduled monitoring of airborne viable and nonviable particulates; pressure differentials; direction of air flow; temperature and humidity; and surface microbial contaminants on personnel and equipment, work tables, floors, and walls. Some companies are beginning to monitor chemical contamination (airborne molecular contamination involving, for example, sulfuric oxides, nitrous oxides, ozone, and volatile organic compounds) and site-specific contaminants (such as chlorine, organophosphates, and ammonia) when and where such concerns exist.

Properly designed, controlled, and maintained HVAC systems, along with appropriate facility monitoring systems, are crucial for demonstrating and maintaining control. Facility monitors must rapidly detect and record any change that might lead to a compromised environment, and the monitoring system must alert personnel of such changes immediately.

Airborne nonviables should be monitored and controlled in all critical and controlled environments. Viables monitoring should be frequent, and in aseptic areas the personnel should be routinely monitored as well. Typical microbial flora should be identified and the resulting records input into a historical database for trend analysis. Alert and action levels are developed based on the resulting trends and product protection requirements. Definitive procedures for investigating contamination events must be developed.

Allowable airborne viable counts vary with air classification and among the different regulatory agencies. An environmental monitoring program should include routine testing of critical process support services including clean, dry compressed air; gases; and process water (RO/DI, USP water, and WFI).

Air and surfaces in critical areas should be monitored for particulate quality daily during all production shifts because shedding by personnel is typically the primary source of contamination. Table 1 lists airborne viable limits compared with various air (nonviable) classification levels. It is not meant to imply any relationship between these two metrics, however,

Table 1: Comparing airborne viable limits among various nonviable air classification levels

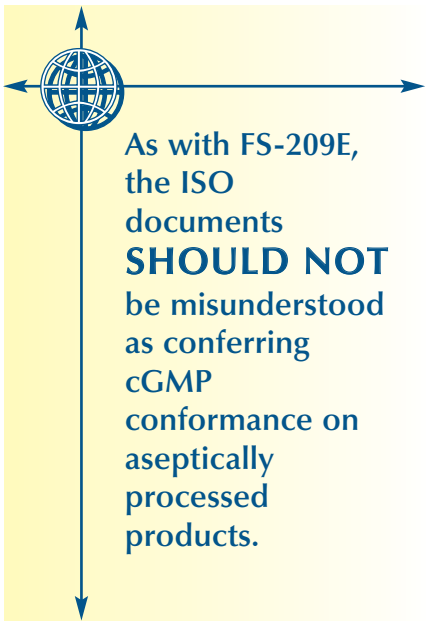
Authority	ISO 8 (Class 100,000)	ISO 7 (Class 10,000)	ISO 5 (Class 100)
FDA CDER June 1987 aseptic processing guidelines cfu/10 ft^3	25		<1
Draft USP <1116>, February 1997 "Microbiology" cfu/ft^3 (cfu/m^3)	<2.5 (<100)	<0.5 (<20)	<0.1 (<3)
EC Annex 1, 1997 cfu/m^3 (cfu/10 ft^3)	100 (30)	10 (3)	<1 (0.3)

Table 2: Comparing ISO 14644 with FS-209E: equivalence is almost exact at 0.5 µm but differs somewhat at other particle sizes.

ISO Classification Number	Maximum concentration limits (particles/m^3 air) for particles equal to and larger than the considered sizes shown below						FS-209E
	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1.0 µm	5.0 µm	
Class 1	10	2					
Class 2	100	24	10	4			
Class 3	1000	237	102	35	8		1
Class 4	10,000	2370	1020	352	83		10
Class 5	100,000	23,700	10,200	3520	832	29	100
Class 6	1,000,000	237,000	102,000	35,200	8320	293	1000
Class 7				352,000	83,200	2930	10,000
Class 8				3,520,000	832,000	29,300	1,000,000
Class 9				35,200,000	8,320,000	293,000	

Table 3: European Union airborne particulate classifications

Room Grade	AT REST Maximum permitted particles per m³ (≥) 0.5 µm	AT REST 5 µm	IN OPERATION 0.5 µm	IN OPERATION 5 µm
A	3500	1	3500	1
B	3500	1	350,000	2000
C	350,000	2000	3,500,000	20,000
D	3,500,000	20,000	not defined	not defined



because generally no good correlation can be made between airborne particulates and microorganisms.

International Standards Organization Rules

On 29 November 2001, the US General Services Administration (GSA) officially announced that Federal Standard 209E (FS-209E), "Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones," had been canceled and is now superseded by the ISO standards for cleanrooms and associated controlled environments: ISO14644-1, "Classification of Air Cleanliness," and ISO 14644-2, "Specifications for Testing and Monitoring to Prove Continued Compliance with ISO 14644-1."

The GSA action resulted from a recommendation made by IEST Working Group CC-100 to "sunset" Federal Standard 209E in favor of the ISO documents. FS-209E was a very useful standard that defined the minimum acceptable criteria for US government contracts across virtually all industries. It had become widely accepted for use in private contracting as well and served as the de facto key reference to consult when quantifying the particulate cleanliness of a clean space. The ISO documents are equally useful and should serve to promote global industrial harmonization for cleanroom cleanliness classification.

Table 2 compares the standards. The class designations have changed from FS-209E to ISO 14644-1. Along with an obvious change to the metric measures for air volume, ISO 14644-1 adds three additional classes: two cleaner than Class 1 and one dirtier than Class 100,000.

ISO also forces the contractual partners to specify the particle size of interest and the state of cleanroom occupancy for certification: "as-built" (a completed room with all services connected and functional, but without production equipment or personnel), "at-rest" (a condition in which all services are connected, all equipment is installed and operating in an agreed manner, but no personnel are present), or "operational" (all equipment is installed and functioning to an agreed level, and a specified number of personnel are present working to a certain procedure).

Particle count tests for classifications less than or equal to ISO 5 will be required every six months. For classifications greater than ISO 5, testing will be required every 12 months. Air pressure differences as well as airflow recertification for all classes will be required every 12 months. And further, installed filter leakage for all classes is recommended every 24 months, as is containment leakage testing, recovery testing, and airflow visualization. Intervals between retests can be extended provided that frequent, periodic monitoring of the working environment is performed and demonstrates conclusively that effective controls exist.

As with FS-209E, the ISO documents should not be misunderstood as conferring cGMP conformance on aseptically processed products. There is no change in the FDA regulations and guidance. Inherent to the ISO documents is the recognition that minimum requirements do not constitute a universally applicable "one size fits all" solution. Neither FS-209E nor ISO 14644 provides all the information needed for developing a protocol to qualify an HVAC system.

For aseptic processing, clean area classification should not be based only on the nominal test grid locations prescribed by 14644-2 (which typically calls for fewer initial testing points than did FS-209E). Consider as well various locations carefully chosen for the risk each poses to the operation and the product and also microbiological monitoring data obtained from those and other appropriate locations in each critical area.

Table 4: Recommended microbial monitoring limits in operation (European Union)

Room Grade	Air cfu/m³	90-mm settle plate cfu/4 hours	55-mm contact plate cfu/plate	Glove Print cfu/glove
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	
D	200	100	50	

An environmental monitoring program that emphasizes a risk-based approach and provides the impetus for review of trends will contribute significantly to demonstrating facility control. Rather than using solely a grid-like approach to identifying sample locations throughout a processing facility, effective environmental monitoring programs will identify sample locations based on careful risk analysis. The choice of sampling locations and associated frequency of sampling should reflect an understanding of the locations' relationship to the overall operations being performed in the facility. Manufacturers that pay proper attention to surveying locations of highest potential hazard to the product can best detect potentially significant contamination vectors. Otherwise, an emerging or existing contamination route could be overlooked, delaying detection of problems.

Particulate and microbiological methods used to support an environmental monitoring program are expected to be reliable and to include provisions for significant sample sizes that provide an accurate representation of airborne particulates and microorganisms. Environmental monitoring SOPs should identify each sampling location and list sampling frequency, timing (e.g., during operation or at end of process), and technique; equipment tested; action and alert levels; and corrective plans for when those action and alert levels are exceeded. Review and trend results periodically. Periodic reassessment of your sampling plans is a good idea, too.

The FDA's aseptic processing guideline discusses locations for monitoring nonviable particulates and states that samples should be taken "not more than one foot away from the worksite and upstream of the airflow during filling/closing operations." Measurement of airflow velocity is a requirement of both US and EU authorities. Both describe the proper sweeping action of the air — away from the critical point of fill — but leave sample locations for airflow velocity to each individual company to implement based on the operations being performed within a critical area. The control of airflow velocity demonstrated by a suitable and appropriate unidirectional flow of air that "washes" surfaces within each critical zone is essential for proper performance and more important than any superimposed velocity value.

Table 5: USP guidelines for surface microbial cleanliness (cfu/contact plate)

Classification	Personnel Cleanliness Gloves	Clothing	Surface Cleanliness
ISO 5	3	5	3 (including floors)
ISO 7	10	20	5 10 (floors)

Table 6: Air classifications from PQRI based on data measured in the vicinity of exposed articles during periods of activity. Alternative biological standards may be established where justified by the nature of the operation. Use of settling plates is optional. Samples from class 100 environments should normally yield no biological contaminants.

Clean Area Classification	ISO Designation	>0.5-µm Particles/m^3	Microbiological Action Levels cfu/m^3	Microbiological Settling Plates Action Levels (90-mm, cfu/4 hrs.)
100	5	3520	1	1
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

In the European Union

European inspectors want to see a program of monitoring in which ventilation systems are running and other equipment is present "in an operational condition." EU authorities also require monitoring at both the 0.5-µm and 5.0-µm threshold levels. The apparent rationale for monitoring at the 5.0-µm level is based on a premise that microbiological contamination in classified environments exists in conjunction with larger particles.

Table 3 provides the EU airborne particulate classifications. Just to make life interesting, no direct correlation can be made between these EU particulate classifications and the ISO standard — although the numbers are very close except in the case of the 5.0-µm particles, for which the European Union is clearly more stringent (1 particle per cubic meter compared with 29/m^3 for ISO Grade 5).

Definitions and Acronyms

483: FDA form 483, which is used to report inspectional observations suggesting noncompliance with regulatory guidelines

cGMP: current good manufacturing practice

HVAC: heating, ventilation, and air conditioning

ISO: International Standards Organization

RO/DI: reverse-osmosis, deionized water

USP: United States Pharmacopeial Convention, Inc.

WFI: water for injection

The EC guide states the following: "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." The guide also makes the following statement with respect to the limit of one particle equal to or larger than 5 µm in size per cubic meter of air: "These areas are expected to be completely free from particles of size greater than or equal to 5 microns.

Table 7: Typical minimum recommendations for location and frequency of viables and nonviables monitoring

Air Cleanliness Classification	NONVIABLES MONITORING Type of Operation	In Operation	VIABLES MONITORING Settle Plates	Air Sampler
ISO 5 (Grade A)	Critical aseptic filling and dispensing areas	Not less than once per shift	At least four hours per shift	Once each shift
ISO 5 (Grade B)	Areas immediately adjacent to Grade A areas (aseptic) corridors and rooms, areas where sterile components and products are stored, gown room exit to aseptic area, etc.	Not less than once each shift for areas adjacent to Grade A; not less than once daily for all other Grade B areas	Once each shift	Once each shift for areas adjacent to Grade A (optional), Once daily otherwise
ISO 7 (Grade C)	Nonaseptic filling of products for terminal sterilization, areas for preparation of equipment, components, and solutions for sterilization	Routine sampling, once weekly, minimally with recertification	Once weekly	Once weekly
ISO 8 (Grade D)	Areas for washing and handling of components and equipment gown room entrance area	Routine sampling, typically monthly, minimally with recertification	Monthly	Monthly

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 cleanroom qualification, it should be shown that the area can be maintained within the defined limits."

Table 4 provides recommended EU microbial monitoring limits in operation. EU inspectors also place emphasis on the use of settle plates rather than the volumetric sampling favored by the US FDA. Such plates should be placed within critical zones and surrounding areas in addition to practicing volumetric air sampling when EU requirements are anticipated.

USP Guidance: The United States Pharmacopeial Convention, Inc. (USP) also provides some guideline levels for surface microbial cleanliness. Although its values do not constitute FDA GMP guidance, they are quite often taken under consideration in design of facility-specific monitoring programs. Table 5 provides more detail.

Some Helpful Suggestions

Can someone help us navigate this sea of environmental monitoring alphabet soup? Possibly. The Product Quality Research Institute's (PQRI's) Aseptic Processing Working Group has reviewed the FDA's preliminary concept paper entitled *Sterile Drug Products Produced by Aseptic Processing* (a long-awaited replacement for the 1987 *Guideline on Sterile Drug Products Produced by Aseptic Processing*). Its final report does address the inconsistency between ISO and the European Union as follows:

> Recommendation:
> • The document should be standardized to the ISO designations.
> • The air classification table should only use metric units for the microbial action levels.
> • Replace the term "limits" (refers to microbial counts) with "Action Levels."
> • Add Microbial Settling Plates to harmonize with EU Annex 1.

Table 6 summarizes the PQRI's recommended air classifications. The working group's rationale: Recommended modifications to the table harmonize it with international standards and reflect the most current published standards. Incorporation of the term "action levels" clearly conveys that the numbers provided are not product-related specifications but levels that when exceeded must be investigated. Note that ISO class 5 is approximately equal to EU Grade A.

In any event, both the European Union and the FDA require a sound sampling plan, and most companies today seem to feel that "more is better" when it comes to environmental monitoring. The design of the sampling plan must be covered in an approved SOP, and media controls must demonstrate sterility and growth control. Table 7 provides some typical minimum recommendations for the locations and frequency of monitoring for both nonviables and viables.

A risk-adjusted approach to the design of environmental monitoring will often lead to more frequent sampling than suggested by the minimum recommendations provided in Table 7. For example, many companies practice continuous particle monitoring in ISO 5 areas, and USP <1116> recommends particulate monitoring each shift in ISO 7 areas and twice-a-week sampling in ISO 8 areas. When observing an operation, the FDA assesses whether the facility/process design creates potential contamination routes:

- Does the design adequately incorporate appropriate separation and control measures for differing levels of air quality as required by each particular operation?
- Are material choices (composition of materials and surface quality) consistent with the need for cleaning, sanitization, and sterilization?
- Does the maintenance program appropriately address gradual breakdowns in facility infrastructure?

Additionally, some authorities may require monitoring of other parameters in conjunction with viables and nonviables. For example, the European Union requires continuous monitoring of pressure differentials under operational dynamic conditions in "Grade A" areas.

A final word on environmental monitoring as relates to batch release: The PDA in its Points to Consider for Aseptic Processing tells us,

> It is important to emphasize that environmental alert and action levels should not be considered as an extension of product specifications. Environmental levels are established for the purpose of detecting adverse changes or drifts in a validated (typically aseptic) processing environment. A 'cause and effect' relationship generally does not exist between environmental monitoring level excursions and product contamination. There is certainly an indirect or inferential relationship between microbiological environmental data and batch release, hence the data generated from microbiological environmental monitoring should clearly be reviewed as part of the batch release. The significance of action level excursions in environmental monitoring and its impact on batch release is based upon the outcome of a comprehensive investigation of all conditions that might impact the acceptability of the process and the batches produced by that process. The results of such an action level investigation may indeed lead to the rejection of a batch (e.g., problems at filling line plus action levels in multiple environmental monitoring programs) or may not lead to the rejection of a batch (e.g., isolated event, no definable cause, no action levels in multiple environmental monitoring programs, data acceptable before and after event).

For Further Reading

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