TECHNICAL PAPER

EU GMP Annex 1 Update 2008 Airborne Particle Counting

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Airborne Particle Counting for Pharmaceutical Facilities: Update 2008, EU GMP Annex 1

Lighthouse Worldwide Solutions

On February 14th, 2008, The European Commission updated Volume 4 EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use, Annex 1: Manufacture of Sterile Medicinal Products.

This update comes into operation on March 1st, 2009.
(With the provisions on capping of freeze – dried vials implemented by March 1st, 2010)

Cleanroom and Clean Air Device Classification:
The formal cleanroom testing for classification should be done per the EN ISO 14644-1 standard.

Another key point stated in this update is that classification should be clearly differentiated from the operational process of environmental monitoring.

The maximum permitted airborne particle concentration is given in table 1.

Table 1: Updated limits in particle counts per Grade.

	Maximum permitted number of particles per m^3 equal to or greater than the tabulated size			
	At rest		**In operation**	
Grade	**0.5µm**	**5.0µm**	**0.5µm**	**5.0µm**
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

Comparison Annex 1, to ISO 14644-1
To note the Particle Classifications are NOT Exactly per ISO 14644-1, but the amount of difference is listed in Table 2 for Grades C and D (note areas in BOLD)

Table 2

	At Rest				Operational			
	0.5		5.0		0.5		5.0	
Grade	Annex 1	ISO 14644-1 (Class) Count	Annex 1	ISO 14644-1 (Class) Count	Annex 1	ISO 14644-1 (Class) Count	Annex 1	ISO 14644-1 (Class) Count
A	3,520	(5) 3,520	20	(4.8) 20	3,520	(5) 3,520	20	(4.8) 20
B	3,520	(5) 3,520	29	(5) 29	352,000	(7) 352,000	2,900	(7) **2,930**
C	352,000	(7) 352,000	2,900	(7) **2,930**	3,520,000	(8) 3,520,000	29,000	(8) **29,300**
D	3,520,000	(8) 3,520,000	29,000	(8) **29,300**	Not Defined		Not Defined	

For cleanroom certification testing of Grade A zones, a minimum sample volume of $1m^3$ should be taken per sample location.

It should be noted that this follows the sampling guidelines of EN ISO 14644-1. For Grade A, the airborne particle classification is *ISO 4.8 and is dictated by the limit for particles ≥5.0µm, which is 20 particles per cubic meter.

*(EN ISO 14644-1 allows intermediate classifications with the minimum increment of 0.1)

For Grade B (at rest) the airborne particle classification is ISO 5 for both particle sizes and in operation, the airborne particle classification is ISO 7 for both particle sizes.

For Grade C at rest, the airborne particle classification is ISO 7 and in operation the airborne particle classification is ISO 8.

For Grade D (at rest) the airborne particle classification is ISO 8 and it is not defined for in operation.

For the classification of a cleanroom or clean air device, EN/ISO 14644-1 defines:

- The minimum number of sample locations
- The sample size (volume) based on the class limit of the largest particle size under consideration
- The means of evaluating the data

Minimum Number of Sample Locations
The minimum number of sample locations for cleanroom certification testing per EN ISO 14644-1 is a simple calculation

NL=√A

Where NL is the minimum number of sample locations (rounded up to the nearest whole number), and A is the area of the cleanroom or clean zone in square meters.

Minimum Sample Size (Volume)
The minimum sample volume for cleanroom certification testing per EN ISO 14644-1 is determined by largest considered particle size for that particular environment. The Grade A environment at 5.0µm is classified as an ISO 4.8 cleanroom or clean air device. This is where the 1 cubic meter of air / location comes from.

EN ISO 14644-1 states that, for each sample location, sample a sufficient volume of air that a minimum of 20 particles would be detected if the particle concentration for the largest considered particle size were at the class limit for the designated ISO Class. The Sample volume or Vs per location is determined by the equation below:

$$V_s = \frac{20}{C_{n,m}} \times 1\,000$$

Where :

- Vs is the minimum single sampling volume per location, expressed in liters
- Cn,m is the class limit (number of particles per cubic meter) for the largest considered particle size, specified for the relevant class.
- 20 is the defined number of particles that could be counted if the particle concentration were at the class limit.

(See EN ISO 14644-1 Section B.4.2 for details)

Per ISO 14644-1 the minimum sample volumes for each of the Grade areas in liters is listed in Table 3, based upon the largest considered particle size.

Table 3

	At Rest	In Operation
Grade	**Minimum Sample Volume**	**Minimum Sample Volume**
A	1000	1000
B	690	*7
C	*7	*2
D	*2	Not defined

*the volume sampled at each location shall be at **least 2 liters, with a minimum sample time of 1 minute.**

The minimum sample volume presents an unusual situation where the minimum number of sample locations is 1. Per EN ISO 14644-1 where only one sampling location is required, take a minimum of three single sample volumes at that location. For a small Grade A environment where only one sample location is needed, a minimum of 3 cubic meters of air must be tested (see EN ISO 14644-1 Annex B 3.4.3 for details).

For the classification testing, portable particle counters with a short length of sample tubing should be used. This requirement exists because of the loss of large sized particles (≥5.0µm) in the sample tubing. Though not clearly stated in Annex 1, Lighthouse Worldwide Solutions recommends keeping the length of this tube (when possible) to less than 3 meters.

Isokinetic sample probes must be used in unidirectional airflow systems. Lighthouse Worldwide Solutions recommends using an isokinetic probe in non-unidirectional airflow systems as well.

The in-operation classification may not always be possible during normal operations. This classification may be demonstrated in simulated operations, during media fills or normal operations.

Demonstrating Continued Compliance
In order to demonstrate continued compliance, EN ISO 14644-2 provides information on this testing. The frequency of such testing is in the Table 4.

Table 4

Classification	Maximum Time Interval	Test Method
≤ISO Class 5	6 Months	Annex B in ISO 14644-1: 1999
>ISO Class 5	12 Months	Annex B in ISO 14644-1: 1999
NOTE: Particle count tests will normally be performed in the operational state, but may also be performed in the at-rest state in accordance with the designated ISO classification		

Grade A and B cleanrooms or clean air devices must be re-tested every 6 months. Grade C and D must be recertified at a minimum of every year.

Cleanroom and Clean Air Device Monitoring:
Particle monitoring during the operational process is different from the particle monitoring for classification of the environment.

All Grade environments should be routinely monitored while they are in operation. The locations selected for this particle monitoring should be chosen based upon a formal risk analysis and the results taken during the cleanroom classification testing of the cleanroom or clean air device.

Grade A Particle Monitoring

In Grade A zones, particle monitoring should be done during the entire operation of a critical process, including equipment assembly aand setup stages. Justifiable exceptions are where the contaminates in the process would damage the particle counter or present a hazard. Examples of such a hazard are when working with live organisms or radiological hazards.

Grade A zone should be monitored continuously in such a manner that any interventions in the process, transient events, or air system deterioration is detected, recorded and, if necessary, appropriate alarms or warnings are triggered.

Though not specifically stated in Annex 1, dedicated fixed point remote particle counters with 1 minute sample times have been demonstrated to adequately monitor Grade A Environments. These devices provide adequate sampling frequency to detect all occurrences.

Some operations may generate particles or droplets from the product, thus making the demonstration of low levels of particles $\geq 5.0\mu m$ not possible. Annex 1 states this is acceptable, especially at the point of fill, when filling is in process. It is still necessary to monitor these locations, both at rest and during setup.

Grade B Particle Monitoring
Particle monitoring of the Grade B zone should be similar to Grade A; however, the sample frequency may be decreased. The importance of the Grade B monitoring is determined by the degree of segregation between the Grade A and B zones.

The Grade B zone should be monitored so that changes in the particle levels or any system deterioration would be detected, and alarm and warnings triggered and recorded if limits are exceeded.

Particle Monitoring
For the particle monitoring system, fixed point remote particle counters, or sequential sampling systems (manifold), attached to a particle counter or a combination may be used.

Regardless of the system used, it must be appropriate for the particle size considered. When using any system, the loss of $\geq 5.0\mu m$ particles in the sample tubing is a concern. The length of the tubing used, the number of bends and the radius of these bends must be considered.

The system should take into account any risk from the materials used in the operation. An example of the risk is with live organisms or radioactive materials.

The sample size of sample volume for monitoring using an automated system does not necessarily the same sample volume used for the formal classification of the cleanroom or clean air device. Whereas the classification of a Grade A environment requires a

minimum sample (per location) of 1 cubic meter of air, for environmental monitoring a smaller sample volume may be used.

Particle Monitoring ≥ 5.0µm particles
Particles that are ≥ 5.0µm are important as they can provide early indication of failure in the environmental system. Occasional ≥ 5.0µm particle counts may be false counts because of electronic noise, stray light or the sudden release of particles from the sample tubing. But Annex 1, states that consecutive or regular counting of low levels of ≥ 5.0µm particles is an indication of a possible contamination event and should be investigated.

These counts might indicate a failure of the HVAC system, filling equipment failure or poor practices during routine operation or equipment set up.

Clean up Period
For all Grades, the clean-up time between the "operational state" and the "at-rest" state should be 15-20 minutes in an unmanned state after the completion of operations. This period is referred to as a guidance value.

Particle Monitoring of Grade C and D Areas.
These areas should be monitored for particles in operation and, for Grade C, also at rest. The frequency of such monitoring is determined by quality risk management. The alert/action limits are dependent on the types of operations carried out.

Examples of operations carried out for products are listed in Table 5.

Table 5

Grade	Examples of operations for Terminally Sterilized products.
A	Filling of products, when unusually at risk
B	Background of Grade A
C	Preparation of solutions, when unusually at risk. Filling of products
D	Preparation of solutions and components for subsequent filling
Grade	**Examples of operations for Aseptic Preparations.**
A	Aseptic preparation and filling.
B	Background of Grade A
C	Preparation of solutions to be filtered.
D	Handling of components after washing.

Isolators:
The background classification for the isolator environment depends on the isolator design and the type of application. The background should be a controlled environment. For an aseptic process it should at least be a Grade D.

The transfer of materials into and out of the unit is one of the greatest potential sources of contamination, as the area inside the isolator is the local zone for high risk manipulations.

Routine monitoring of isolators should be carried out and include frequent leak testing of the glove/sleeve system.

Finishing of Sterilized Products
Partially stoppered freeze drying vials should be maintained under Grade A conditions at all times until the stopper is fully inserted (this part of Annex 1 will be effective March 1, 2010).

Crimping of the cap should take place as soon as possible after the stopper has been inserted. As the crimping process can generate large numbers of non-viable particles, it should be done at a different station.

For aseptic processing, vial capping can be done as an aseptic process or as a clean process outside the aseptic area. When vials leave the aseptic area for capping in the clean area, they should be protected by Grade A conditions up to the point of leaving the Aseptic area, thereafter stoppered vials should be protected with a Grade A air supply until the cap has been crimped. Note that a Grade A air supply is differentiated from a Grade A environment.

About Lighthouse Worldwide Solutions
Lighthouse offers particle counting and contamination monitoring solutions for pharmaceutical facilities. These offerings range from a complete line of portable particle counting systems, remote particle counters, facility monitoring systems, active viable microbiological sampling systems, temperature sensors, relative humidity sensors, differential pressure sensors, air velocity sensors as well as software systems and validation and installation services for these systems.

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