Standards for classification of cleanrooms (February 2005)

Cleanrooms are classified by the cleanliness of their air. This is done according to ISO 14644-1. This is an international standard, having been adopted by the European Union in 1999, and the USA in 2001. However, the most easily understood classification of cleanrooms is the obsolete Federal Standard 209 of the USA; it is still widely used.

1 Federal Standard 209

The first Federal Standard 209 was published in 1963 in the USA, and titled "Cleanroom and Work Station Requirements, Controlled Environments". It was revised in 1966 (209A), 1973 (B), 1987 (C), 1988 (D) and 1992 (E), and withdrawn in 2001. The cleanroom class limits, given in the earlier 209 A to D versions, are shown in table 1. The class of a cleanroom is found by measuring the number of particles ≥ 0.5 μm in one cubic foot of room air, and determining which class limit is not exceeded; this is the cleanroom classification.

Table 1 Federal Standard 209 class limits

Class	**Particles / ft^3**				
	≥ 0.1 μm	≥ 0.2 μm	≥ 0.3 μm	**≥ 0.5 μm**	≥ 5.0 μm
1	35	7.5	3	**1**	NA
10	350	75	30	**10**	NA
100	NA	750	300	**100**	NA
1,000	NA	NA	NA	**1,000**	7
10,000	NA	NA	NA	**10,000**	70
100,000	NA	NA	NA	**100,000**	700

the nomenclature shown in table 1 is likely to be used for many years.

2 ISO Standards

A range of cleanroom standards is being produced by the International Organization for Standardization (ISO). Various committees of experts, nominated by countries throughout the world, are writing these standards.

The standards that have been published, or are being written at the time of publication of this information are as given below. Information about the current status of these standards is given at www.s2c2.co.uk/docs/StandardsUpdate-B2004.html

2.1 ISO 14644

This consists of the following parts, under the general title ‘Cleanrooms and Associated Controlled Environments’:

Part 1: Classification of air cleanliness

This gives the airborne particle limits for different classifications of cleanrooms. This standard also gives the methods that should be used to measure the airborne particles when testing a cleanroom to determine its class.

Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1

This gives information, including time intervals, for testing a cleanroom to demonstrate continual compliance with the ISO 14644-1 standard.

Part 3: Test methods

This gives a description of the test methods that should be used to test the cleanroom to show that it is working correctly.

Part 4: Design, construction, and startup

This gives general guidance as to how a cleanroom should be designed, constructed and made ready for handing over to the user.

Part 5: Operation

This gives general advice on how to run a cleanroom.

Part 6: Terms and definitions

This is a collection of all the definitions of terms used in the ISO cleanroom standards.

Part 7: Separative enclosures (clean air hoods, gloveboxes, isolator, mini environments)

This gives information on clean air devices.

Part 8: Molecular contamination

This gives information on gaseous contamination in cleanrooms.

2.2 ISO 14698

This consists of the following parts under the general title 'Cleanrooms and Associated Controlled Environments–Biocontamination Control':

Part 1: General principles and methods

This gives information on how to establish a risk system and methods for measuring microorganisms in the cleanroom.

Part 2: Evaluation and interpretation of biocontamination data

This gives information on how to deal with the results obtained from measuring micro-organisms in a cleanroom.

Both of these standards are necessary for those involved with microorganisms in cleanrooms.

2.3 ISO 14644-1

ISO 14644-1 gives a method to classify cleanrooms by the concentration of airborne particles, the classification being based on the following equation:

$$Cn = 10^{N} \times \left[\frac{0.1}{D}\right]^{2.08}$$

where:

Cn is the maximum permitted concentration (in particles/m^3 of air) of airborne particles that are equal to, or larger, than the considered particle size. Cn is rounded to the nearest whole number, using no more than three significant figures.

N is the ISO classification number, which shall not exceed the value of 9. Intermediate ISO classification numbers may be specified, with 0.1 the smallest permitted increment of N.

D is the considered particle size in μm.

0.1 is a constant with a dimension of μm.

From equation 1, the maximum permitted airborne concentration of particles, i.e. the class limit can be calculated for any given particle size. Shown in Table 2 are the classes selected by ISO 14644-1 to illustrate class limits.

Table 2 Selected ISO 14644-1 airborne particulate cleanliness classes for cleanrooms and clean zones

ISO Classification number	**Maximum concentration limits (particles/m^3 of air) for particles equal to and larger than the considered sizes shown below**					
	≥0.1μm	≥0.2μm	≥0.3μm	≥0.5μm	≥1μm	≥5.0μm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1 000	237	102	35	8	
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	293
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

It should be noted that there is a crossover to the Federal Standard 209 classes. If the particle concentration/m^3 in the ISO standard is divided by 35.2 the count is converted to counts/ft^3. The equivalent Federal Standard 209 classification is then found at the 0.5 μm size, e.g. an ISO Class 5 is equivalent to Federal Standard 209 Class 100. A comparison in given in table 3.

Table 3 Comparison between selected equivalent classes of FS 209 and ISO 14644-1

ISO 14644-1 Classes	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8
FS 209 Classes	Class 1	Class 10	Class 100	Class 1000	Class 10 000	Class 6 100 000

It should be appreciated that the airborne particle concentration of a given cleanroom is dependent on the particle generating activities going on in the room. When a cleanroom is first built and the room is empty, a very low particle concentration can be achieved, this closely reflecting the quality of air supplied. If the room has production equipment in it that is operating, there should be a greater particle concentration, but the greatest concentration occurs when the room is in full production. The classification of the room may therefore be carried out one, or more, of these different occupancy states. The occupancy states defined in ISO 14644-1 are as follows:

As built: the condition where the installation is complete with all services connected and functioning but with no production equipment, materials or personnel present.

At-rest: the condition where the installation is complete with equipment installed and operating in a manner agreed upon by the customer and supplier, but with no personnel present.
Operational: The condition where the installation is functioning in the specified manner, with the specified number of personnel present and working in the manner agreed upon.

The ISO 14644-1 standard gives a method by which the standard of a cleanroom may be ascertained by measuring airborne particles. The method for determining the number of sampling locations, the sample volumes, and the acceptance criteria, is given in the standard.

3 Pharmaceutical Cleanroom Classification

Cleanrooms used for pharmaceutical manufacturing have their own standards. The two most widely used are those published by the European Union and the USA.

3.1 European Union Guide to Good Manufacturing Practice

The most recent pharmaceutical standard used in Europe came into operation on January 1997. It is called 'The rules governing medicinal products in the European Union. Volume 4. Good manufacturing practices - Medicinal products for human and veterinary use'. It is available for free down load at: http://pharmacos.eudra.org/F2/eudralex/vol-4/home.htm

It is often called the European Union Guide to Good Manufacturing Products (EU GGMP), and is called that in these notes. The most relevant part of the EU GGMP is Appendix 1, which was revised in 2003. Shown in Table 4 is the airborne classification given in Appendix 1 of the EU GGMP, as revised in 2003.

Table 4 Airborne classification in the EU GGMP

	Maximum permitted number of particles/m^3 equal to or above (a)			
Grade	**at rest (b)**		**in operation (b)**	
	0.5 µm (d)	5 µm	0.5 µm (d)	5 µm
A	3 500	1(e)	3 500	1(e)
B(c)	3 500	1(e)	350 000	2 000
C(c)	350 000	2 000	3 500 000	20 000
D(c)	3 500 000	20 000	not defined (f)	not defined (f)

Notes:
(a) Particle measurement (are) 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.

Author's note: 1m^3 is the total sample volume in the area sampled.

(b) The particulate conditions given in the table for the "at rest" state should be achieved after a short "clean up" period of 15-20 minutes (guidance value) in an unmanned state after completion of operations. The particulate conditions for grade A "in operation" given in the table should be maintained in the zone immediately surrounding the product whenever the product or open container is exposed to the environment. It is accepted that it may not always be possible to demonstrate conformity with particulate standards at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself.

(c) In order to reach the B, C and D air grades, the number of air changes should be related to the size of the room and the equipment and personnel present in the room. The air system should be provided with appropriate terminal filters such as HEPA for grades A, B and C.

(d) The guidance given for the maximum permitted number of particles in the "at rest" and "in operation" conditions correspond approximately to the cleanliness classes in the EN/ISO 14644-1 at a particle size of 0.5 μm.

(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.

(f) The requirements and limits will depend on the nature of the operations carried out.

The EU GGMP contains the following suggestions for the manufacture of sterile medicinal products:

Terminally sterilised products

'Preparation of components and most products should be done in at least a grade D environment in order to give low risk of microbial and particulate contamination, suitable for filtration and sterilisation. Where the product is at a high or unusual risk of microbial contamination, (for example, because the product actively supports microbial growth or must be held for a long period before sterilisation or is necessarily processed not mainly in closed vessels), then preparation should be carried out in a grade C environment.

Filling of products for terminal sterilisation should be carried out in at least a grade C environment.

Where the product is at unusual risk of contamination from the environment, for example because the filling operation is slow or the containers are wide-necked or are necessarily exposed for more than a few seconds before sealing, the filling should be done in a grade A zone with at least a grade C background. Preparation and filling of ointments, creams, suspensions and emulsions should generally be carried out in a grade C environment before terminal sterilisation.'

Aseptic preparation

'Components after washing should be handled in at least a grade D environment. Handling of sterile starting materials and components, unless subjected to sterilisation or filtration through a micro-organism-retaining filter later in the process, should be done in a grade A environment with grade B background.

Preparation of solutions which are to be sterile filtered during the process should be done in a grade C environment; if not filtered, the preparation of materials and products should be done in a grade A environment with a grade B background.

Handling and filling of aseptically prepared products should be done in a grade A environment with a grade B background.

Prior to the completion of stoppering, transfer of partially closed containers, as used in freeze drying should be done either in a grade A environment with grade B background or in sealed transfer trays in a grade B environment.

Preparation and filling of sterile ointments, creams, suspensions and emulsions should be done in a grade A environment, with a grade B background, when the product is exposed and is not subsequently filtered.'

The air classification required for a cleanroom that contains an isolator depends on the design of the isolator, and its application. The room that houses the isolator should be controlled, and for aseptic processing be at least grade D.

Blow/fill/seal equipment used for aseptic production, which is fitted with an effective grade A air shower, may be installed in at least a grade C environment, provided that grade A/B clothing is used. The environment should comply with the viable and non-viable limits 'at rest', and the viable limit only when in 'operation'. Blow/fill/seal equipment used for the production of products for terminal sterilisation should be installed in at least a grade D environment.

Microbiological sampling is required to demonstrate the microbiological cleanliness of the cleanroom during operation and the recommended limits are given in table 4.7.

Table 5 Recommended limits for microbial contamination (a)

Grade	**Air Sample** cfu/m^3	**Settle Plates** (diam. 90 mm), cfu/4 hours (b)	**Contact Plates** (diam. 55 mm), cfu/plate	**Glove Print** 5 fingers cfu/glove
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Notes
(a) These are average values.
(b) Individual settle plates may be exposed for less than 4 hours.

3.2 Guidance for Industry - Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (2004)

This document is produced by the Food and Drug Administration (FDA) in the USA and was published in 2004. It is available for download at:
http://www.fda.gov/cder/guidance/5882fnl.pdf

Many pharmaceutical companies export their products to the USA. To do so they are inspected and approved by the FDA. They must comply with the FDA Guidance.

The FDA Guidance has a similar table to the EU GGMP for the required airborne particle and microbial conditions for aseptic processing. However, the FDA place emphasis on ensuring that the processing areas conform to the classification in the operational conditions. These conditions are given in the following table:

Table 6 – Air classification [a]

Clean Area Classification (0.5 μm particles/ft3	ISO Designation[b]	≥ 0.5μm particles/m^3	Microbiological Active Air Action Levels [c] (cfu/m3)	Microbiological Setting Plates Action Levels [c,d] (diam. 90mm; cfu/4hours)
100	5	3,520	1[e]	1[e]
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

a- All classification based on data measured in the vicinity of exposed materials/articles during periods of activity

b- ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO particle concentration is equal to Class 100 and approximately equals EU Grade A

c- Values represent recommended levels of environmental quality. You may find it appropriate to establish alternate microbiological action levels due to the nature of the operation or methods of analysis

d- The additional use of settling plates is optional

e- Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.

The FDA Guidance document identifies two clean areas of particular importance: the 'critical area' and the 'supporting clean areas' associated with it.
A 'critical area' is described in the FDA document as:

> '*one in which the sterilized dosage form, containers, and closures are exposed to environmental conditions that must be designed to maintain product sterility. Activities conducted in such areas include manipulations (e.g. aseptic connections, sterile ingredients additions) of these sterile materials prior to and during filling and closing operations'*.

In a critical area, air is measured at *'representative locations not more than 1 foot away from the work site, within the airflow'*, and should have a count of particles ≥ 0.5 μm of no more than 3520/ m^3 during filling/closing operations.

The 'supporting clean areas' are described in the Guidance as follows:

> *'Many support areas function as zones in which nonsterile components, formulated products, in-process materials, equipment, and container/closures are prepared, held, or transferred.'*

The FDA recommends that the area immediately adjacent to the aseptic processing line meet, at a minimum, Class 10,000 (ISO 7) standards during production. They also say that 'manufacturers can also classify this area as Class 1,000 (ISO 6) or maintain the entire aseptic filling room at Class 100 (ISO 5). An area classified at a Class 100,000 (ISO 8) air cleanliness level is appropriate for less critical activities (e.g. equipment cleaning)'.

In Appendix 1 of the guidance there is information about isolators. The recommended clean air requirements are that the interior of the isolator should meet Class 100 (ISO 5) standards and the environment round the isolator should be based on the design of the interfaces e.g. transfer ports. They point out that a Class 100,000 (ISO 8) is commonly used. Similarly in Appendix 2 information is given about blow-fill technology. The FDA consider that the environment round the BFS machinery should meet Class 100,000 (ISO 8) or better. The air in the critical area i.e. where sterile products or materials are exposed (parison formation, container moulding or filling steps should achieve Class 100 (ISO 5) microbiological standards, during operations, but a well designed system should also normally achieve Class 100 (ISO 5) airborne particle levels.