DEPARTMENT OF VETERANS AFFAIRS
Veterans Health Administration
Washington DC 20420

IL 10-2006-009
In Reply Refer To: 10NB

July 10, 2006

UNDER SECRETARY FOR HEALTH'S INFORMATION LETTER

AIRBORNE PARTICLE ASSESSMENT OF PHARMACEUTICAL CLEANROOMS

1. The United States Pharmacopeia, Chapter 797 (USP 797), "Pharmaceutical Compounding, Sterile Preparations," specifies International Organization for Standardization (ISO) 14644 as the standard for acceptable cleanroom airborne particulate concentrations and assessment procedures. Specifically, USP 797 requires sterile compounding in a ISO Class 7 room environment. The particulate count method and sampling procedures are provided in ISO 14644-1. ISO 14644-1 Annex B and C are the minimum standards necessary to meet the ISO designation and USP 797. This information letter reviews the ISO requirements to meet the Class designation standard.

2. The following table provides the ISO maximum allowable concentration limits for particles larger than (>) 0.5 microns. USP 797 requires ISO Class 5 cleanliness for compounding cabinets and isolators, ISO Class 7 for compounding rooms (buffer areas), and ISO Class 8 for anterooms (support areas) located outside the buffer area.

Table 1 : ISO 14644 - 1 Airborne Particulate Cleanliness Classes
Concentration Limits for Particles > 0.5 microns

ISO Class	Particles per cubic meter (M^3) of air	Particles per cubic foot (ft^3) of air
5	3,520	100
6	35,200	1,000
7	352,000	10,000
8	3,520,000	100,000

3. GUIDANCE

a. **General ISO Testing.** Particulate counts need to be incorporated into the cleanroom commissioning process. The initial particulate data closely reflects the quality of air supplied. USP 797 requires ISO particulate sampling every six months and when renovations occur. During sampling, cleanroom activities need to be designated as "operational" or "at-rest" (ISO 14644-1, 3.1). Operational testing verifies the particulate count during normal work procedures. No cleanroom activities should occur during "at-rest" testing. Specific activities in the unit need to be recorded during sampling. The operation of the room controls need to be documented

during the sampling period (airflow volumes or velocity tests, air pressure differentials, containment or filter leakage).

b. **Test Report and Equipment.** The ISO 14644 standard specifies the test report needs to include the name of testing organization; ISO reference; test and sample locations; identification of the test equipment and calibration certificate; any use of an equivalent test method (deviation from the ISO test method); test results; data analysis; and particle concentration data (ISO 14644-1, 4.4). Test reports need to state assessment compliance with ISO (Annex B and C) or the equivalent methodology. The ISO standard specifies the use of a light scattering particle counter capable of displaying or recording particle count and size (ISO 14644-1, B.2). On-site calibration is performed per manufacturer specifications. Sample probe inlet shall be positioned into the direction of room air flow or face upward if air flow direction is not controlled or predictable (ISO 14644-1, B.4).

c. **Sample Plan.** The ISO 14644-2 standard specifies a written plan designating the sample locations; minimum air volume and sample time; number of samples per location; time interval between each sample; particle size(s) and limits; and procedures to address high particle counts if the room exceeds the ISO particle limits. The standard also provides additional guidance for permanent installation of continuous particle detectors. Sample locations should be evenly distributed throughout the room and positioned at the height of the work activity. The owner or unit chief may specify additional sample locations (ISO 14644-1, B.4). Specific guidance on sampling locations is also provided in Chapter 18, ASHP, Compounding Sterile Preparations (see subpar. 4d.).

d. **Sample Number.** The ISO standard uses the size of the cleanroom to determine the sufficient number of sample locations (ISO 14644-1, B.4).

(1) The minimum number of sample locations is determined by: $N = \sqrt{A}$

N = Number of air sample locations
A = Floor area (square meters (M^2))

(2) Using the ISO method, the number of sample locations and samples are:

Table 2 – Number of Air Samples and Locations

ISO Floor space M^2 (ft^2)	Number of sample locations	Number of air samples per location
< 4 (43)	**1**	**3*** ***NOTE:*** *A single sample location incorporates three air samples to demonstrate ISO compliance (ISO 14644-1, B.4.3.4).*
>4-9 (>43-97)	**2**	**1**
>9-16 (>97-172)	**3**	**1**
>16-25 (>172-269)	**4**	**1**
>25-36 (>269-387)	**5**	**1**

e. **Sample Volume.** The ISO standard specifies a single sample volume for each sample location. Samples need to have sufficient air volume for a minimum detection of 20 particles. The sample volume should also be a minimum of 2 liters with a minimum 1 minute sample time (ISO 14644-1, B.4.2). ISO 14644 specifies sample volume as: $V = \frac{20}{C} \times 1000$

V = minimum sample volume (liters)
C = Class Limit (number of particles per M^3 from Table 1)

(2) Using the ISO method, the air sample volumes for Class 7 and 8 particle sizes are:

Table 3 - Air Sample Volumes

Class (particle size)	Air Volume *NOTE: In practice, particle detectors normally have a high air flow rate and will quickly exceed the minimum air volume requirements. Therefore, samplers are usually operated for 1 minute at the sample location to meet the ISO 1 minute requirement.*
7 (0.5µm)	**0.06 liters**
7 (1.0µm)	**0.24 liters**
7 (5.0µm)	**6.8 liters**
8 (0.5µm)	**0.006 liters**
8 (1.0µm)	**0.024 liters**
8 (5.0µm)	**0.68 liters**

f. **Air Sample Data.** ISO sample results are recorded for the particle size and sample location, and are expressed as particles per cubic meter of air (p/M^3). When two or more samples are collected at the same sample location, the sample results need to be averaged to represent the location. The cleanroom meets the ISO classification if the averages of the sample location concentrations (p/M^3) do not exceed the allowable ISO limits of Table 1 (ISO 14644-1, B.5).

g. **Data Analysis.** Under the ISO standard, cleanroom compliance assessments using a single sample location or ten or more sample locations require only the computation of the average particle concentration and the standard deviation. For two to nine sample locations, the average particle concentration, standard deviation, and 95 percent Upper Confidence Limit (95 percent UCL) need to be calculated. The average particle concentration and the 95 percent UCL should not exceed the allowable ISO particle limit for each particle size (ISO 14644-1, B.5.2.2).

h. **Non-compliance.** Should the cleanroom assessment fail the ISO classification, additional sample locations may be added and the cleanroom retested. All sample locations need to be evenly distributed within the cleanroom floor space (ISO 14644-1, B.6.1).

(1) The result of the 95 percent UCL calculation may cause non-compliance. The ISO standard allows a single, non-random outlier sample to be excluded and the 95 percent UCL is recalculated. A minimum of three values need to remain in the 95 percent UCL calculation.

(2) Exclusion of the outlier sample is acceptable for procedural error, equipment malfunction, erroneous measurement, or unusually low particle count. Under the ISO standard, the subsequent recalculation is considered the final analysis. (ISO 14644-1, B.6.2).

(3) The failure to meet the ISO classification should trigger the review of operations, ventilation system performance and suspension of drug compounding until retesting verifies compliance.

4. REFERENCES

a. International Organization for Standardization, ISO14644-1 (Annex A-F), Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness, 1999.

b. International Organization for Standardization, ISO 14644-2, Cleanrooms and associated controlled environments – Part 2: Specifications for testing and monitoring to prove compliance with ISO 14644-1.

c. United States Pharmacopeia, USP29-NF24, Chapter 797, Pharmaceutical Compounding-Sterile Preparations. http://www.usp797.org/index.html

d. American Society of Health-System Pharmacists, Compounding Sterile Preparations, Chapter 18: Environmental Monitoring, 2005.

5. INQUIRIES. Questions regarding this information letter may be addressed to Safety and Technical Services (10NB) at (202) 273-5870.

Jonathan B. Perlin, MD, PhD, MSHA, FACP
Under Secretary for Health

DISTRIBUTION: CO: E-mailed 7/10/06
FLD: VISN, MA, DO, OC, OCRO, and 200 – E-mailed 7/10/06