DEPARTMENT OF VETERANS AFFAIRS
Veterans Health Administration
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UNDER SECRETARY FOR HEALTH'S INFORMATION LETTER

MICROBIOLOGICAL ASSESSMENT OF PHARMACEUTICAL CLEANROOMS

1. This VHA Information Letter provides recommendations for microbiological assessment of pharmacy cleanrooms and the testing of biocabinet or isolator interiors.

a. United States Pharmacopeia (USP), Chapter 797, "Pharmaceutical Compounding, Sterile Preparations," specifies the monitoring of microbiological concentrations on surfaces and air for sterile pharmaceutical compounding.

b. USP, Chapter 1116, "Microbiological Evaluation of Cleanrooms and Other Controlled Environments," provides additional guidance.

c. The Food and Drug Administration (FDA) provides regulatory requirements and recommendations for the drug manufacturing industry.

d. The American Conference of Governmental Industrial Hygienists (ACGIH) specifies practices for accurate monitoring of indoor airborne microbial concentrations.

e. The International Organization for Standardization (ISO) 14644 requires acceptable airborne particulate concentrations and assessment for pharmaceutical cleanrooms.

2. USP 797 specifies a written plan and schedule for microbiological monitoring procedures in pharmaceutical cleanrooms. Monitoring locations are selected to test the areas most prone to contamination during compounding. Typically, this would include area sampling in the proximity of Class II biocabinets or barrier isolators (ISO Class 5 containment). The plan needs to specify sampling for the controlled environments of the pharmacy, including the interior of biocabinets and barrier isolators, compounding rooms (ISO Class 7 buffer areas), and anterooms (ISO Class 8 support areas) located outside the buffer area. Each sampling site (air, swab, or contact) needs to have predetermined alert and action levels as part of the written plan. Any sample result exceeding the action level requires immediate corrective action which may include full-scale cleaning, ventilation, or High Efficiency Particulate Air (HEPA) filter assessment and suspension of drug compounding until retesting verifies compliance.

3. GUIDANCE

a. **Collection of Microbiological Samples in Cleanrooms.** Microbiological sampling should be incorporated into the cleanroom commissioning process to verify engineering controls and provide baseline data prior to compounding activities. Air sampling should be conducted monthly for low-medium risk compounding and weekly for high-risk compounding. Cleanroom microflora will originate from staff, supplies and product contamination, air backwash through entrances and room filter leakage. Air samples should be collected following any required cleanroom air purge time that has been established by the pharmacy unit. The air purge time ensures the necessary number of air changes have been completed to reduce airborne contaminants prior to starting compounding activities. Electric pump air sampling (active air sampling) may be conducted using slit, sieve or centrifugal impact samplers. Samples are collected on nutrient media and incubated to promote microbial growth. The plate colonies are counted and reported as the number of colony forming units per cubic meter of air (cfu/M^3). The use of settling plates (open plates exposed for >1 hour) is suggested by USP 797. Settling plates have a higher error rate due to the uncontrolled sampling method and susceptibility to indoor air currents. USP recommends that settling plates only be used during sampling with electric pump samplers to allow for the comparison of data. The ACGIH documents high variability in airborne microbiological assessment data. Data variability is affected by media selection and storage, plate handling (temperature extremes during shipping to culture labs), incubation temperature and species viability. Species competition on the plate also affects total plate count.

b. **Culture Plate Media and Incubation.** USP recommends plate media and incubations times for general culture. Sampling should be conducted using non-selective malt extract agar or trypticase soy agar (TSA). Malt extract can be used for fungi culture with incubation at 20-25°C for up to 7 days. USP 797 recommends TSA media incubated at 30-35 °C for 48 hours (bacteria, yeast and mold). USP 1116 recommends incubation temperatures of 22.5°C (± 2.5°C) or 32.5°C (±2.5 °C) for 48-72 hours. More importantly, the plate media, incubation temperature and sample time should be consistent to ensure appropriate comparison of data. The decision to change culture media should be phased-in gradually and include side-by-side plate sampling for data comparison. Media may also be selected to assess specific microbiological species (i.e., gram-negative or positive bacteria).

c. **Airborne Microbiological Concentrations.** Current USP 797 does not recommend specific airborne microbiological concentration limits for cleanrooms.

(1) The standard specifies the collection of microbial data and recommends corrective action when colony counts exceed the baseline by 50 percent (medium to high-risk compounding) and 100 percent (low-risk compounding).

(2) Alternatively, Hussong and Madsen provide an analysis of baseline deviation specific to cleanroom operations. The data trend is used to establish upper and lower confidence limits for cleanroom colony counts (see subpar. 4c). The FDA provides comparison between ISO class 7 and 8 environments and microbiological action levels using active air sampling and settling plates (see Table 1 and subpar. 4d).

(3) Kastango recommends less than (<) 2.5 colony forming units (cfu) per cubic foot (ft^3) airborne bioburden for cleanrooms and anterooms and <0.1 cfu/ft^3 for ISO Class 5 biocabinets and isolators (see subpar. 4f).

Table 1 – FDA Recommended Action Levels

ISO Class	Particles per cubic meter (M^3)(more than (>) 0.5 micron (µm))	Air Sampling Action Level: cfu/M^3 (cfu/ft^3)	Settling Plate Action Level: cfu at 4 hours
5	3,520	1 (0.028)	1
6	35,200	7 (0.2)	3
7	352,000	10 (0.28)	5
8	3,520,000	100 (2.83)	50

d. **Sampling Strategy for Cleanrooms.** Sampling sites need to be selected in high-traffic areas where the potential exists for product contamination and to verify levels at compounding work areas.

(1) Air samples need to be collected within 12 inches of all Class 5 containment work zones (12 inches outside the cabinet face), all cleanroom entrances, and the anteroom. ***NOTE:*** *Specific guidance on sampling locations is provided in Chapter 18, ASHP, Compounding Sterile Preparations (see subpar. 4f).* A minimum of one media plate or 20 percent of the total plates needs to be incubated as a control (non-exposed blank) for each assessment to check media contamination.

(2) Table 2 provides the recommended air sample volumes for estimated airborne microbiological concentrations. An air sample volume of 990 liters of air would meet the ACGIH recommended lower detection level(LDL=10 cfu/plate) and upper detection limits (UDL=1 cfu/ square centimeter (cm^2) of plate) for a ISO Class 7 room with 1-10 cfu/M^3.

Table 2 Air Sample Volumes to meet ACGIH Recommended Detection Limits
NOTE: *Based on 28 liters per minute air sampling rate; 78 cm^2 culture plate.*

Sample Time (minutes)	Sample Volume (liters)	LDL = 10 cfu/plate	UDL = 1 cfu/cm^2 of plate
15	420	15 cfu/M^3	130 cfu/M^3
20	560	8 cfu/M^3	65 cfu/M^3
25	700	4 cfu/M^3	33 cfu/M^3
30	840	2 cfu/M^3	17 cfu/M^3
35	990	1 cfu/M^3	9 cfu/M^3

40	1130	<1 cfu/M^3	5 cfu/M^3

e. Identification of Microbiological Species. Most laboratory services will provide Genus identification with total plate colony count. In certain applications it may be beneficial to determine whether the bacteria is gram positive or negative, or a full identification to species level of the organism may be required. Species identification may be appropriate during cleanroom commissioning for comparison to an action level event. Identification to species can assist in determining the source of contamination and corrective actions. Additionally, air sampling can be conducted in adjacent hallways or outdoor air to assess anterooms, or HEPA filter bypass.

f. **ISO Class 5 Microbiological Containment Testing.** The interior of ISO Class 5 (biocabinets and isolators) need to be monitored for microbiological air quality at a minimum of once a month, or weekly for high-risk compounding. Sampling may be conducted using media filled plates or commercial media fills. At least two plate samples need to be collected at opposite ends of the cabinet, 6 inches from the interior wall. Settling plates or active air sampling can be used for the assessment. Settling plates should be exposed for the entire compounding procedure (1-3 hours) and are an acceptable alternative due to the laminar downflow within the cabinet.

(1) It is also recommended that finger touch plates be conducted monthly. This procedure requires the technician to contact a media plate during compounding with the gloved fingertips.

(2) Swabbing of interior cabinet surfaces also need to be conducted using pre-filled media tubes with a swab insert or a commercial media fill (Rodac plates).

(3) Media fills need to contain a neutralizing agent (i.e., polysorbate 80) to inactivate residual cleaning and disinfection solutions.

(4) Following incubation, colonies need to be counted and reported as cfus. Samples from ISO Class 5 environments normally yield no microbiological colonies.

4. REFERENCES

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

b. United States Pharmacopeia, USP29-NF24, Chapter 1116, Microbiological Evaluation of Cleanrooms and Other Controlled Environments.

c. Hussong and Madsen, Analysis of Environmental Microbiological Data from Cleanroom Samples, Pharmaceutical Technology, Aseptic Processing 2004. http://vaww.ceosh.med.va.gov/PharmacySafety/Final%20-%20Env%20Monitoring.pdf

d. FDA, Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practices. http://www.fda.gov/cder/guidance/5882fnl.pdf

e. American Conference of Governmental Industrial Hygienists, Guidelines for the Assessment of Bioaerosols in the Indoor Environment, 1989.

f. American Society of Health-System Pharmacists, Compounding Sterile Preparations(2nd edition), Chapter 18: Environmental Monitoring, 2005.

5. Inquiries. Questions regarding this information letter may be addressed to Network Program Support (10NB) at (202) 273-5870.

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