Environmental Monitoring: How Much is Enough? A U.S. Perspective

Lynn O’Donnell, PhD
Director – Cell Therapy Laboratory
What is required? FDA
21 CFR Part 1271 – Human Cells, Tissues, and Cellular and Tissue-Based Products

Sec. 1271.190 Facilities.
(a) General. Any facility used in the manufacture of HCT/Ps must be of suitable size, construction, and location to prevent contamination of HCT/Ps ... and to ensure orderly handling of HCT/Ps without mix-ups. You must maintain the facility in a good state of repair. You must provide lighting, ventilation, plumbing, drainage, and access to sinks and toilets that are adequate to prevent the introduction, transmission, or spread of communicable disease.

(b) Facility cleaning and sanitation.
   (1) You must maintain any facility used in the manufacture of HCT/Ps in a clean, sanitary, and orderly manner, to prevent the introduction, transmission, or spread of communicable disease.
What is required? FDA
21 CFR Part 1271 – Human Cells, Tissues, and Cellular and Tissue-Based Products

Sec. 1271.190 Facilities.
(c) Operations. You must divide a facility used in the manufacture of HCT/Ps into separate or defined areas of adequate size for each operation that takes place in the facility, or you must establish and maintain other control systems to prevent improper labeling, mix-ups, contamination, cross-contamination, and accidental exposure of HCT/Ps to communicable disease agents.
What is required? FDA
21 CFR Part 1271 – Human Cells, Tissues, and Cellular and Tissue-Based Products

Sec. 1271.195 Environmental control and monitoring.
(a) Environmental control. Where environmental conditions could reasonably be expected to cause contamination or cross-contamination of HCT/Ps or equipment, or accidental exposure of HCT/Ps to communicable disease agents, you must adequately control environmental conditions. Where appropriate, you must provide for the following control activities or systems:

1. Temperature and humidity controls;
2. Ventilation and air filtration;
3. Cleaning and disinfecting of rooms and equipment to ensure aseptic processing operations; and
4. Maintenance of equipment used to control conditions necessary for aseptic processing operations.
Sec. 1271.195 Environmental control and monitoring.

(c) Environmental monitoring. You must monitor environmental conditions where environmental conditions could reasonably be expected to cause contamination or cross-contamination of HCT/Ps or equipment, or accidental exposure of HCT/Ps to communicable disease agents. Where appropriate, you must provide environmental monitoring for microorganisms.
The James Cell Therapy Lab

- Hospital-based processing laboratory
- Rapid growth, 25%-50% last year
- 500 products processed per year, 400 other procedures (infusions, research samples, cell sorts for chimerism)
- Staff size – currently 4 technologists, 1 Manager, shared Quality Manager
The James Cell Therapy Lab

- Mostly HPC, Apheresis and HPC, Marrow with minimal manipulation
- CD34 enrichments on IDE and IND
- Manipulated products manufactured by companies (DCs, MSCs) – store, thaw, wash only
- Pancreatic islets under IND
The James Cell Therapy Lab

- 500 sq ft, semi-divided, U-shape
- Typical hospital lab facility
Environmental Control

General - Existing
• Key card added to existing key access
• Acoustic ceiling tiles
• Supply storage loosely controlled – wherever space could be found

General - Improvements
• Removed master key access – only Security has master
• Installed vinyl clean room ceiling tiles
• No cardboard boxes beyond the blue areas
Environmental Control

HVAC - Existing
- 100% fresh air
- HEPA filtered at air handler (entire hospital)
- Pneumatic controls
- Facilities checks & changes filters as needed

HVAC - Improvements
- Set to positive pressure and air exchanges at 10-15/hr
- Digital controls & new mixing box – better temp control
- Facilities contracts out quarterly measurement of air exchanges & positive pressure
Ready to Begin Environmental Monitoring Program – but How?

Information for clean rooms for pharmaceutical manufacturing

• Federal Standard 209E - Airborne Particulate Cleanliness Classes in Clean Rooms and Clean Zones
  – Long been the only classification definitions
  – Per cubic foot
  – “Class 100”, “Class 10,000”

• International Organization for Standardization produced 10 standards documents related to clean rooms – ISO 14644
  – Switch to metric – per cubic meter
  – “Class 5”, “Class 7”
  – 3 new classes – 2 cleaner and 1 dirtier
## Compare Classifications for Particles > 0.5 μm

<table>
<thead>
<tr>
<th></th>
<th>FS 209E (per ft³)</th>
<th>ISO 14544-1 (per m³)</th>
<th>Metric (per m³)</th>
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<tbody>
<tr>
<td>Class 100</td>
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Other Sources

United States Pharmacopeia Chapter <797>
Pharmaceutical Compounding – Sterile Preparations

- Processing CT products in functionally closed systems is much like sterile compounding by pharmacies
- Fairly specific guidelines for facility design, environmental controls, ISO class, operations and environmental monitoring
Environmental Monitoring – Getting Started

- Purchased particle counter for nonviable particles – APC Plus from Biotest
- Purchased air sampling device for viable particles – RCS Plus from Biotest – impaction onto agar strip
Environmental Monitoring – Getting Started

• Wrote validation plan to gather data
  – Weekly nonviable particles > 0.5 μm
    • BSCs at rest (static) and during use (dynamic)
    • Facility in processing, storage and testing areas
    • 1-2 minute sampling time
  – 2-4 times per month viable particles
    • BSCs at rest (static) and during use (dynamic)
    • Facility in processing, storage and testing areas
    • 1 m³ (= 1000L) sampled
    • Incubate strips at 30-35°C for 3 days
Environmental Monitoring – Getting Started

• Gathered validation data May 2006 – April 2007
• Evaluated validation data quarterly
• Graphed particle counts vs. time
  – Look for trends, exceeds classification levels
• Graphed particle count distribution
  – Look for normal or negative exponential distribution
  – Alert and action levels are set differently depending on the distribution
Environmental Monitoring – Validation Results

Weekly Particle Counts

Support Areas

Processing Areas

Weekly Particle Counts - CTL NON-Processing Areas

Mean=37,475, Max=221,000

NEVER exceeded ISO 7 (CI 10,000)

Weekly Particle Counts - CTL Processing Areas

Mean=23,700, Max=159,000

NEVER exceeded ISO 7 (CI 10,000)
Environmental Monitoring – Validation Results

• Set alert and action limits - distribution of data points matters

• If Normal Distribution
  – Alert Level = mean + 2SD
  – Action Level = mean + 3SD

• If Negative Exponential Distribution
  – Alert Level = mean x 3.0
  – Action Level = mean x 4.6

Ref. – Booth, A. Environmental Monitoring Practices and Regulations for the Sterile Manufacturer. www.samedanltd.com
Environmental Monitoring – Validation Results

Processing Areas

Nonviable Particles

Viable Particles

Negative Exponential

BSCs – both viable & nonviable – VERY low counts - Negative Exponential

closer to Normal
# Alert & Action Limits

## NONViable PARTICLES / m³

<table>
<thead>
<tr>
<th>Area</th>
<th>Alert</th>
<th>Action</th>
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<tbody>
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<td>Processing Areas</td>
<td>&gt; 71,000</td>
<td>&gt; 109,000</td>
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<tr>
<td>BSC</td>
<td>&gt; 1000</td>
<td>&gt; 3000</td>
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</table>

## VIABLE PARTICLES - CFU/ m³

<table>
<thead>
<tr>
<th>Area</th>
<th>Alert</th>
<th>Action</th>
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<tr>
<td>BSC</td>
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Alert & Action Limits – Action if Exceeded

• If between Alert and Action Limit
  – Repeat count
    • if repeat is below alert – done
    • If repeat is between alert & action – repeat daily x3, notify QA
    • If repeat is above action – notify QA

• If over Action Limit
  – Repeat daily x3, notify QA
    • If ALL repeats are below alert – done
    • If ANY repeats are between alert & action – notify QA
    • If ANY repeats are above action – notify QA – hood out of service
Continuing EM Program

Weekly Particle Counts - CTL Processing Areas

- Processing Areas
- ISO Class 5 (100)
- ISO Class 6 (1000)
- ISO Class 7 (10,000)

Nonviable Particles (>0.5µm/m³)

Date

Continuing EM Program

Weekly Particle Counts - CTL Biological Safety Cabinets (hoods)

- ISO Class 5 (100)
- ISO Class 6 (1000)
- ISO Class 7 (10,000)

Hood 1

Hood 2

Date

Nonviable Particles (>0.5um/m3)

Repeat count 8000, 10000

Hood out of service, call repair

Motor failing, replaced
CONCLUSIONS

• A robust EM program is capable of detecting failing environmental control
• Our EM program was not terribly challenging to establish
• It takes time to get sufficient data points
• If you don’t have alert and action limits set, what’s the point?
• We have not done surface monitoring except for in-process for islet isolations