Environmental Monitoring

Purpose of Environmental Monitoring

- Critical process within the pharmaceutical and biotechnology industries.
- Determines the microbial and particulate content of cleanroom air and surfaces.
- Highlights conditions contributing to excessive microbial & particulate levels due to ineffective cleaning, or personnel/equipment issues (Trending).
- Alerts to conditions exceeding classifications
- Pro-active tool for Quality Assurance

Who Does It?

- Quality Control
  - Demonstrate product safety
  - Environmental Monitoring
  - Testing
- Quality Assurance
  - Oversight responsibilities – ensure compliance with GMPs
  - Review and Approve all Records, Reports, written procedures, specifications
  - Audit methods, results, systems and processes

Classifications

Critical Environment Classification		Concentration (particles/meter³) > or = Size Shown					
FS 209E	**ISO 14644-1**	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1.0 µm	5.0 µm
N/A	1	10	2				
N/A	2	100	24	10	4		
1	3	1,000	237	102	35	8	
10	4	10,000	2,370	1,020	352	83	
100	5	100,000	23,700	10,200	3,520	832	29
1,000	6	1,000,000	237,000	102,000	35,200	8,320	293
10,000	7				352,000	83,200	2,930
100,000	8				3,520,000	832,000	29,300
N/A	9				35,200,000	8,320,000	293,000

To be monitored

- Non-viable airborne particulates
- Viable airborn particulates
- Viable surface bound particulates on cleanroom surfaces and personnel

Contamination Sources:

- People ~75%
- Ventilation ~15%
- Room Structure ~5%
- Equipment ~5%

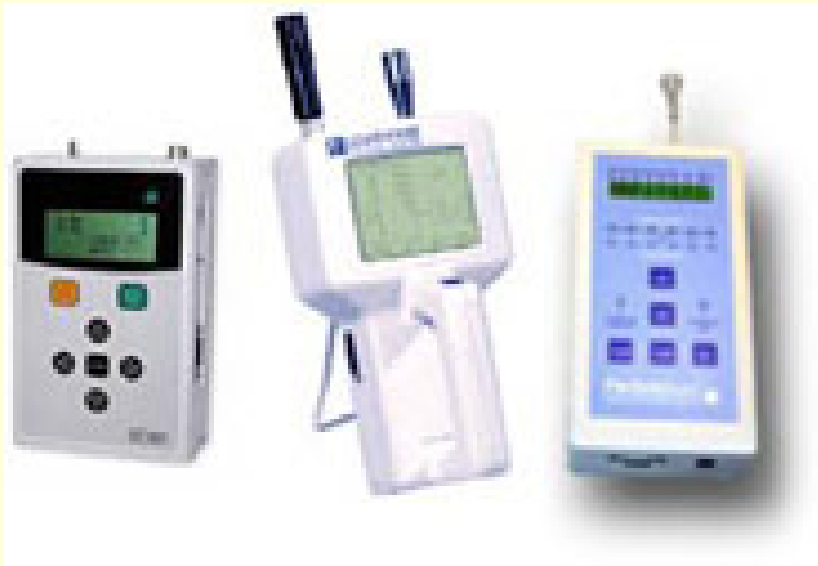
Particle Counter
(Measures non-viable airborne particles)

- **Non-Viable Particulate Monitoring** - *ISO 14644, Fed Std-209E, USP <1116>*

- A calibrated laser particle counter
  - used to sample a defined volume of air.
  - Can measure a variety of particle sizes, most commonly 0.5 and 5.0 micron.
  - Particle counts are recorded as the number of particles per volume of air sampled.
  - Results can be reported per cubic foot, per FDSTD 209E, or **per cubic meter**, per ISO 14644.

Particle Counters

- Handheld are ideal for spot checking.
- Important for tracking down a source of contamination, testing filters, and verifying the cleanroom is working within specified parameters.



http://www.particlecounters.org/cleanroom/#moreinfo

Settling Plates
(Viable airborne particulates)

- **Passive Air Monitoring** - *ISO 14644, Fed Std-209E, USP <1116>* Settling plates filled with media are used to sample the microbial fallout over time.
- The plates are incubated to promote growth
- Microorganisms are counted and results are reported as the number of CFU (colony forming units) per time sampled.

Settling Plates

- In the absence of any kind of influence, airborne microorganisms, typically attached to larger particles, will deposit onto open culture plates.
- Microorganisms are usually found in the air of occupied rooms rafted onto skin cells. Very few present on their own.
- The average size of microbial particle will deposit, by gravity, onto surfaces at a rate of approximately 1 cm/s.

Sample Locations for Settling Plates

- Areas where there is little air movement (i.e. "dead spaces") or where airflows converge or are excessively turbulent. These conditions are most likely to occur:
  - adjacent to doors
  - in pass through hatches
  - at low level return air grilles
  - between HEPA's in clean rooms
  - in corners of rooms
- Areas within the clean room where there is personnel activity or where specific operations are carried out.

Particle Characteristics

- 50 micron particles are visible
- Average human hair is about 100 microns
- Time to fall 1 meter in still air
  - 33 seconds for 10 micron particle
  - 48 minutes for 1 micron particle

Air Sampler
(Viable airborne particulates)

- **Viable Particulate Air Monitoring** - *ISO 14644, Fed Std-209E, USP <1116>*
- *U*sed to sample a defined volume of air, embedding viable particulates onto sterile media strips.
- The media strips are incubated to promote the growth of viable particulates
- The microorganisms are counted and results are reported as the number of CFU (colony forming units) per volume of air sampled.

RODAC Plates
(Viable, Surface-Bound Particles)

- **Surface Monitoring** - *ISO 14644, Fed Std-209E, USP <1116>*
  Contact plates (RODAC Plates) filled with media are used to sample tabletops, walls, benches, floors, garments, and gowned personnel.
- Measure the number of microorganisms per area sampled.
- Plates are incubated to promote growth, the microorganisms are counted and results are reported as the number of CFU (colony forming units) per area sampled.

RODAC:

"Replicate Organism Detection and Counting".

- flat agar surface is above the edges of the dish (so you can press it on flat surfaces) and a grid, allowing counting of cfu per cm^2.

Personnel gown monitoring

- RODAC plates are also used to monitor the contamination level of personnel gowns and Personal Protective Equipment (PPE) before or during manufacturing production.

http://www.microcln.com/html/EnvironMonitoring.htm

Rodac Plates

- One objective of surface sampling is to determine the efficiency of routine cleaning procedures in removing contamination.
- Sampling is done before and after cleaning.
- The medium in the plates contains neutralizing agents, which inactivate residual disinfectants on the surface to be tested, allowing comparative results before and after cleaning.

Viable Particle Testing
(Settling Plates, Air Samplers, RODAC Plates)

- Use a growth medium with low selectivity i.e. capable of supporting a broad spectrum of microorganisms including bacteria, fungi, yeast and molds.
  - TSA (Tryptone Soya Agar) supports general microbial colonies.
  - SDA (Sabouraud Dextrose Agar) supports yeast and fungal colonies.
    - TSA plates are incubated at 30-35°C for 3 days
    - SDA plates are incubated at 20-25°C for 5 days.
- When necessary to detect or search for a particular type of microorganism a selective culture medium should be used.

Positive and Negative Controls

- Typically two positive controls and two negative controls
  - Positive:
    - B. Subitilis (Bacteria)
    - C. albicans (Fungus)
  - Negative:
    - Unopened Plate
    - “Real Time” = Opened and immediately shut

Time of Testing

- Sampling should take place with the facility in the operational condition (personnel present and normal operations being carried out).
- The operational condition for sterile hoods and transfer devices can be considered to be when an operator is working in any part of the clean air device.
- Sampling in the static condition should be performed at an agreed frequency to monitor baseline contamination levels.

Frequency of Testing

For Class 10,000 and Class 100,000 Rooms

Type of Monitoring	Method	Areas to be Monitored	Frequency
Non-Viable Particulates	Particle Counter	Class 10,000	Weekly
		Class 100,000	Weekly
Viable Airborne Particulates	Settling Plates	Class 10,000	Monthly
		Class 100,000	Monthly
Viable Surface Monitoring	Rodac Plates	Class 10,000	Weekly
		Class 100,000	Weekly
Incubator	Rodac Plates	Interior, Class 10,000	Weekly
Centrifuge	Rodac Plate	Interior, Class 10,000	Weekly

Personnel Monitoring (Frequency)

Type of Monitoring	Method	Frequency
Right hand (two fingertips)	Rodac Plates	After all Vialing operations and once/month random
Left hand (two fingertips)	Rodac Plates	After all Vialing operations and once/month random
Right Sleeve	Rodac Plates	After all Vialing operations and once/month random
Left Sleeve	Rodac Plates	After all Vialing operations and once/month random

How many samples?

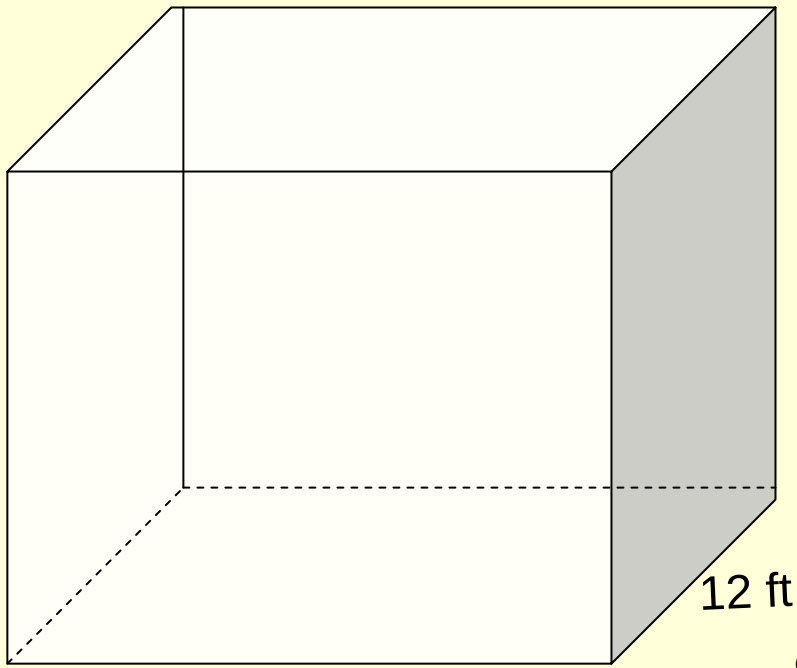
- The number of sampling locations is based on the size of the room.
- Determining the number of sampling locations is based on the formula:

$$nL = A$$

"nL" is the minimum number of sampling locations (round up to the next whole number), and "A" is the floor area of the cleanroom in m^2.

How Many Samples?

- The sample locations should be evenly distributed within the area under test.
- A site plan should be prepared indicating sampling locations.



nL = A

15 ft x 12 ft = 180 ft^2

10.69 ft^2 = 1m^2

180 ft^2/10.69ft^2/m^2 = 16.83 m^2

= 17 sample locations

Out of Specification?

- **Alert Level** – alert levels are quality levels that, when exceeded, signal a possible deviation from normal operating conditions and may not require action, but may need to be monitored more closely.
- **Action Level** – action levels are quality levels that, when exceeded, signal an apparent deviation from normal operating conditions and requires immediate action.

Alert & Action Levels

Air Classification Alert Level	Non Viable Particulate Count (0.5 μm particles/ft^3)	Rodac Plate Surface Counts (cfu/plate)	Settling Plates (cfu/14cm plate)
100	50 (at rest) 80 (operational)	2	1
10,000	8000	4	3
100,000	80,000	5	5

Air Classification Action Level	Non Viable Particulate Count (0.5 μm particles/ft^3)	Rodac Plate Surface Counts (cfu/plate)	Settling Plates (cfu/14cm plate)
100	99 (at rest)	3	2
10,000	9999	5 10 for floors 20 floors dirty side	5
100,000	99999	30 for floors	20

Personnel Monitoring

Sample site	Alert	Action
Hand	1/plate	3/plate
Sleeve	1/plate	3/plate

Actions to take when levels are breached

- Identify
  - Possible cause
  - Contaminating microorganisms
- Investigate
  - Whether isolated sample or whole area involved
  - Personnel - operator status (grade), level of training, health, technique, wash up
  - Cleaning procedures
  - Changing procedure
  - HEPA filter integrity of room/clean air device
  - Processes carried out
  - Previous test results for trends or other identified problems.
- Liase with
  - Aseptic personnel
  - Microbiology personnel
  - QA/QC personnel

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