Environmental Monitoring of Aseptic Processing Areas - 2

- ¨*A war against an invisible enemy*¨

Active Air Monitoring (Viables)

Air Sieve Samplers

- Draw air through many holes (the sieve) in a cover plate and directly at the surface of an agar plate.

- In extremely low levels of contamination, at least 1 cubic meter of air should be tested in order to maximize sensitivity (USP 1116).

✦ Estimates the number of microbes in the air

✦ Takes a long time to get results because you must wait for the Incubation time

Active Air Monitoring (Viables)

Centrifugal Samplers

- Agar strips are wrapped around a fan
- When the fan spins it throws air directly at the agar
- When the air gets to the agar it changes direction, and particles hit the agar and grow

BioTest RCS Sampler

Passive Air Monitoring (Viables)

- Settling plates can be kept open for up to **four hours**
- Not related to a fixed volume of air.
- Settling plates are passive, so they do not have a fan to disrupt airflow
- Qualitative Value important as can be placed In Locations with the greatest risk

Surface Monitoring

- Touch Plates
  - Also called RODAC Plates: “Replicate Organism Detection And Counting”
  - The agar is higher than the plastic sides of the plate so it can touch a flat surface
  - Contains Lecithin and Polysorbate-80, which act as surfactants to help lift microbes off the surface and to inactivate quaternary ammonia cleaners

Surface Monitoring

- RODAC plates generally are based on Tryptic Soy Agar (TSA)
- Used for flat surfaces

Surface Monitoring

Swabbing (used for irregular surfaces, corners, etc)

- Moistened swab is rubbed on a defined surface (e.g., 5x5 cm), placed in sterile water or saline, and processed immediately using membrane filtration

Swabs

Filtration

- After sonication, fluid is passed through a 0.45 micron filter which collects microorganisms onto its surface
- Filter is aseptically removed and placed onto growth medium

Preparation of plates: in-house precautions

- Avoid risk of contamination of plates before exposure
- Class A/B (Horizontal UDAF) recommended
- Double door autoclave
- Pre-incubation of plates
- Sterile wrappings
- After pre-incubation, plates are checked in class A/B and re-wrapped
- Peristaltic pump recommended to assure proper volume

USP 1116 - MICROBIOLOGICAL CONTROL & MONITORING OF ASEPTIC PROCESSING ENVIRONMENTS

Selection of Growth Media

- Soybean–casein digest medium (SCDM) supports growth of a wide range of bacteria, yeast, and molds.
- Supplemented with additives to overcome or to minimize the effects of sanitizing agents or of antibiotics. If necessary, Sabouraud's mold agar can be used. In general, monitoring for strict anaerobes is not performed.
- Growth Promotion requirement.

Selection of Culture Conditions

- 62. Environmental monitoring samples should be incubated at a minimum of two temperatures to detect both bacteria and fungi. In practice, the use of 3 to 5 days of incubation at 20 to 25oC followed by incubation 30 to 35oC for an additional 2-3 days has been shown to be sufficient to detect most bacteria and fungi.
- Validate sterilization processes for preparing growth media.
- Sterilize sampling media and their wrappings by moist heat, radiation, or other suitable means.
- For aseptically prepared media, carry out preincubation and visual inspection before introduction into the clean room (*Microbiological Best Laboratory Practices* 1117)

Alert Levels

- ISO 13408 - 3.3. Alert level: “established microbial or particulate monitoring results giving early warning of potential drift from normal operating conditions which are not necessarily grounds for definitive corrective action but which could require follow-up investigation”.
- An established microbial or airborne particle level giving early warning of potential drift from normal operating conditions.
- Triggers appropriate scrutiny and follow-up to address the potential problem
- Alert levels are below the action levels, and action levels below the specification.

Alert Levels

- Alert level definitions are based on historical data and on statistical analysis
  - *In class A/B with very low growth frequency, what level is a statistically significant indication of a change in the environment?*
- Alert levels may take into account the process or activity of the area
  - Different rooms of same classification may have different alert levels because of processing differences
- Repeated excursions (above alert level) may be considered an action level of their own

Alert Level Excursions

- An alert level excursion does not necessarily dictate an investigation
  - Microbial sampling, sanitizations have occurred by time excursion is detected.

- Take Action (Investigate) if:
  - The same isolate is recovered across multiple sampling locations
  - The detected organism is objectionable (e.g. *Salmonella*)
  - The isolate has never been recovered before
  - If same site has repeated excursions

USP 1116 -SIGNIFICANT EM EXCURSIONS

- When contamination recovery rates increase from an established norm, a process and operational investigation should take place, including a review of:
  - - area maintenance documentation;
  - - sanitization/decontamination documentation;
  - - occurrence of nonroutine events;
  - - inherent physical or operational parameters (eg, changes in room temperature and relative humidity;
  - -training status of personnel.
  - - Loss of glove integrity or the accidental introduction of material that has not been decontaminated in RABS.
  - Is this incident isolated or can it be correlated with other recoveries (eg, for at least two weeks before the incident), that might indicate an unusual pattern?

USP 1116 -SIGNIFICANT EM EXCURSIONS

Following the investigation, take actions to correct or eliminate the most probable causes of contamination. They may include:

- reinforcement of training (eg, gowning and aseptic techniques)
- additional microbiological sampling at an increased frequency,
- simulation studies.
- additional sanitization, use of different sanitizing agents,
- identification of the microbial contaminant and its posible source.

USP 1116 -SIGNIFICANT EM EXCURSIONS

- Establishing a definitive cause probably will not be possible, and only general corrective measures can be considered.
- The investigation and the rationale for the actions chosen must be carefully and comprehensively documented (including the immediate Corrections taken).

- **TRAINING OF PERSONNEL**

Rigorous discipline and strict supervision of personnel, essential. Equally important for personnel responsible for the monitoring program.

USP 1116 -IDENTIFICATION OF MICROBIAL ISOLATES

- Microbial monitoring cannot and need not identify and quantify **all** microbial contaminants. **Appropriate level** of identification is acceptable.

- Identification of isolates from critical and immediately adjacent areas should take precedence over identification of microorganisms from noncritical areas.

Trending

- Trend Analysis done periodically (USP recommends “monthly”)
- Trending reports should be published periodically as a Quality Indicator of the state of control of the aseptic process
  - Report by sample location, by operator, by other identifiers that help quantify useful information
- Year-to-year variations addressed

USP 1116 -TREND ANALYSIS

- A trend analysis is used to facilitate **decision-making** for requalification of a controlled environment or for maintenance and sanitization schedules".

- EM detects changes in the Contamination Recovery Rate (CRR) that may indicate changes in the state of control within the environment.

- Assessment of **risks** associated with manufacturing environments must be made over a significant period; How is CRR **metrics** established ?

USP 1116 -Contamination Recovery Rate (CRR)

- **CRR:** “rate at which environmental samples are found to contain any level of contamination. For example, an incident rate of 1% would mean that only 1% of the samples taken have any contamination regardless of colony number”.

- Use contamination recovery rates (CRR) to **track ongoing performance** and to **refine** the microbiological control program to foster improvements.

- Practical Example CRR Tool

3.2. Clean room classification based on airborne particulates

3.2.1. WHO requirements

Table 2. Maximum permitted airborne particulate concentration per air grade[2]

Grade	At rest		In operation	
	Max. permitted particles / m^3		Max. permitted particles / m^3	
	≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

Combined GMP / ISO Table

Classiffication in accordance to ISO 14644-1

	Maximum permitted number of particles per m^3 ≥ the tabulated size			
	At Rest		In Operation	
Grade	0.5 µm	5.0 µm	0.5 µm	5.0 µm
A	3,520 ISO 5	20	3,520 ISO 5	20
B	3,520 ISO 5	29	352,000 ISO 7	2,900
C	352,000 ISO 7	2,900	3.520,000 ISO 8	29,000
D	3.520,000 ISO 8	29,000	Not defined	Not defined

WHO 961 Annex 6

E.M. of cleanrooms in Vaccine Manufacturing Facilities

Microbial limits during operation (EU Annex 1, FDA Guidance 2004, WHO 961 for aseptic environments

Room classification	Active air sample (CFU/m^3)	Settle plate (9cm) 4 hr. exposure (CFU)	Contact plate (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	--

Changes in USP <1116> "Microbiological control and Monitoring of aseptic processing environments"

- More specific to aseptic process; addresses RABS, isolators

Table 2 "Suggested initial Contamination Recovery Rate (CRR) in aseptic environments"

Room classification	Active air sample (%)	Settle plate (9cm) 4 hr. exposure (%)	Contact plate or swab	Glove or garment
Isolator / closed RABS (ISO 5 or better)	<0.1	<0.1	<0.1	<0.1
ISO 5	<1	<1	<1	<1
ISO 6	<3	<3	<3	<3
ISO 7	<5	<5	<5	<5
ISO 8	<10	<10	<10	<10

CRR in aseptic environments

- Calculate two examples of Active Air sampling in Class B cleanroom in operation, in one month: a. 64 plates exposed/2 positives; b. 64 plates exposed/4 positives

$$CRR = \frac{\text{Number of plates with growth}}{\text{Number of plates exposed}} \times 100$$

a. CRR = __ X 100 = ___% (maximum is <__) **PASS / FAIL ?:_____**

b. CRR = __ X 100 = ___% (maximum is <__) **PASS / FAIL ?: _____**

Changes in USP <1116> **Recommendations when using CRR**

- Use frequency of contamination instead of absolute numbers detected in a sample
- Determine recovery rates for each cleanroom environment
- Detection frequency should at least be retabulated monthly
- If CRR are adopted, any single ISO 5 excursion of > 15 CFU should prompt an investigation, even if CRR is < 1%
- Investigate if the incident was isolated or can be correlated with other recoveries including events of 1 – 5 CFU that might indicate an unusual pattern.

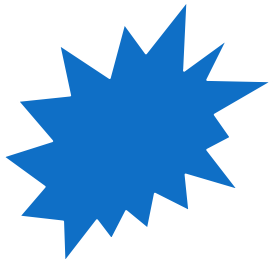
Changes in USP <1116> **Conclusions when using new USP 1116 approach (CRR)**

- A compliant system using the individual plate microbial limits may become noncompliant using the CRR method
- At very low recovery levels there is no way to establish Alert or Action levels as statistically significant.
- Incident rates in percentage values force us to look historically to many samples.
- Helps to identify trends

Cleanroom monitoring program

- Routine monitoring program as part of quality assurance
- Additional monitoring and triggers, e.g.
  1. Shutdown
  2. Replacement of filter elements
  3. Maintenance of air-handling systems
  4. Exceeding of established limits

Setting Alert & Action Microbiological Levels based on CRR



- Exercise tool

READY TO MONITOR ?