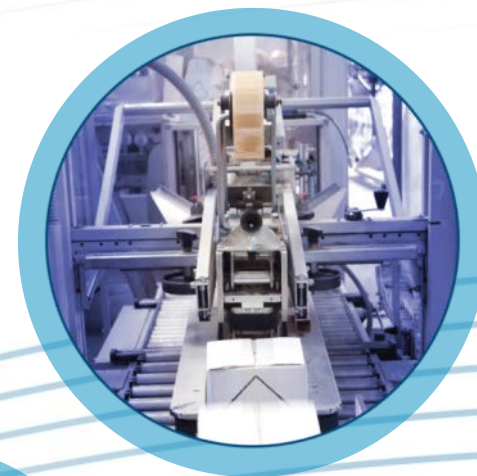




Connecting People, Science and Regulation®

# Important Aspects in Environmental Monitoring

By Guenther Gapp





# Overview about Presentation

- Introduction: Why is EM (= Environmental Monitoring) required and what does it mean ?
- Important References and excerpts of FDA Guidance/ Annex 1 and PDA TR13
- Regulatory Requirements and current Best Practices
- Microbiological Laboratory – points to consider
- Rationale of EM Sampling Locations
- Examples of EM programs (Conventional Filling Line/ Isolator)
- How to execute Trend Analyses / Historically Based Alert Levels
- Regulatory Citations from WL / for 483



# Important References

- PDA Technical Report 13 (2013) / Fundamentals of Environmental Monitoring
- FDA Guidance (Sterile Drug Products Produced by Aseptic Processing/ 2004)
- Eudralex Volume 4 - Annex 1 (2008 and Rev. 2017)
- USP <1116>
- PDA Points to Consider Aseptic I and II (2015/2016)



# PDA TR 13

## Fundamentals of an Environmental Monitoring Program

Technical Report No. 13 (Revised)

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# PDA Points to Consider 1 & 2

## Points to Consider for Aseptic Processing

Part 1  
January 2015

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## Points to Consider for Aseptic Processing

Part 2  
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## *FDA Guidance 2004*

### **A. Environmental Monitoring**

#### *1. General Written Program*

In aseptic processing, one of the most important laboratory controls is the environmental monitoring program. This program provides meaningful information on the quality of the aseptic processing environment (e.g., when a given batch is being manufactured) as well as environmental trends of ancillary clean areas. Environmental monitoring should promptly identify potential routes of contamination, allowing for implementation of corrections before product contamination occurs (211.42 and 211.113).

*... meaningful information about the quality of the environment*



## FDA Guidance 2004

### 4. Monitoring Methods

Acceptable methods for monitoring the microbiological quality of the environment include:

#### a. Surface Monitoring

Environmental monitoring involves sampling various surfaces for microbiological quality. For example, product contact surfaces, floors, walls, and equipment should be tested on a regular basis. Touch plates, swabs, and contact plates can be used for such tests.

#### b. Active Air Monitoring

Assessing microbial quality of air should involve the use of *active* devices including but not limited to impaction, centrifugal, and membrane (or gelatin) samplers. Each device has certain advantages and disadvantages, although all allow testing of the number of organisms per volume of air sampled. We recommend that such devices be used during each production shift to evaluate aseptic processing areas at carefully chosen locations. Manufacturers should be aware of a device's air monitoring capabilities, and the air sampler should be evaluated for its suitability for use in an aseptic environment based on collection efficiency, cleanability, ability to be sterilized, and disruption of unidirectional airflow.<sup>20</sup> Because devices vary, the user should assess the overall suitability of a monitoring device before it is placed into service. Manufacturers should ensure that such devices are calibrated and used according to appropriate procedures.



c. **Passive Air Monitoring (Settling Plates)**

Another method is the use of passive air samplers, such as settling plates (petri dishes containing nutrient growth medium exposed to the environment). Because only microorganisms that settle onto the agar surface are detected, settling plates can be used as qualitative, or semi-quantitative, air monitors. Their value in critical areas will be enhanced by ensuring that plates are positioned in locations posing the greatest risk of product contamination. As part of methods validation, the quality control laboratory should evaluate what media exposure conditions optimize recovery of low levels of environmental isolates. Exposure conditions should preclude desiccation (e.g., caused by lengthy sampling periods and/or high airflows), which inhibits recovery of microorganisms. The data generated by passive air sampling can be useful when considered in combination with results from other types of air samples.





## *FDA Guidance 2004*

Environmental monitoring methods do not always recover microorganisms present in the sampled area. In particular, low-level contamination can be particularly difficult to detect. Because false negatives can occur, consecutive growth results are only one type of adverse trend. Increased incidence of contamination over a given period is an equal or more significant trend to be tracked. In the absence of any adverse trend, a single result above an action level should trigger an evaluation and a determination about whether remedial measures may be appropriate. In all room classes, remedial measures should be taken in response to unfavorable trends.

*False negatives occur*

*Adverse trends ... consecutive growth results*



*FDA Guidance 2004:*

*....at the conclusion*

*...lead not a batch rejection*

interventions. Critical surface sampling should be performed at the conclusion of the aseptic processing operation to avoid direct contact with sterile surfaces during processing. Detection of microbial contamination on a critical site would not necessarily result in batch rejection. The



Maximum Limit ?

Table 6: Recommended maximum limits for microbial contamination

<b>Grade</b>	<b>Air sample cfu/m<sup>3</sup></b>	<b>Settle plates (diam. 90 mm) cfu/4 hours <sup>(a)</sup></b>	<b>Contact plates (diam. 55mm), cfu/ plate</b>	<b>Glove print 5 fingers on both hands cfu/ glove</b>
A <sup>(b)</sup>	1	1	1	1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

<sup>(a)</sup> Individual settle plates may be exposed for less than 4 hours. Where settle plates are exposed for less than 4 hours the limits in the table should still be used. Settle plates should be exposed for the duration of critical operations and changed as required after 4 hours.

<sup>(b)</sup> It should be noted that for grade A the expected result should be 0 cfu recovered; any recovery of 1 cfu or greater should result in an investigation.



Alert Level - An established microbial or airborne particle level giving early warning of potential drift from normal operating conditions and triggers appropriate scrutiny and follow-up to address the potential problem. Alert levels are always lower than action levels and are established based on historical and qualification trend data and periodically reviewed.

Action Level - An established microbial or airborne particle level that, when exceeded, should trigger appropriate investigation and corrective action based on the investigation.



The data regarding environmental contaminants should be collected in conformance with current Good Manufacturing Practices (cGMP), which states that the personnel supervising the environmental monitoring program should be competent in the scientific discipline and have appropriate training and authority. Equipment used should be calibrated, systems should be appropriately validated, media should be properly qualified, prepared, and tested, and all operational procedures should be written and followed with appropriate controls to support their use. The methods selected should be justified for use as appropriate.

*Personnel supervising the EM -*

*Production and/or QC ?*



## **4.2 Sample Site Selection**

Suitable sample sites vary widely depending on the cleanroom design and manufacturing process. Careful evaluation of each process should be made in selecting sites. A documented risk assessment for the selection of the sites should be performed. Some examples of risk factors to consider in selecting sites for routine surveillance are as follows:

1. Sites or processes in which microbial contamination would most likely have an adverse effect on product quality
2. Sites that would most likely demonstrate the heaviest microbial proliferation during actual production
3. Whether site selection should involve a statistical design or should be made on the basis of grid profiling
4. Whether routine monitoring sites should be rotated
5. Sites that represent the most inaccessible or difficult areas to clean and disinfect
6. Modes of microbe dispersal in the environment
7. Sampling at a given site that may disturb the environment sufficiently to cause erroneous data to be collected or to contaminate product



**Table 4.2-1** Examples of Sampling Sites

System	Site
Environmental air (filling line)	Near open or filled containers
Room air	Proximal to work area
Water	Point of use
Surface (facility)	Door handles, walls, curtains
Surface (equipment)	Filling line, control panels, stopper bowl, filling needles (post fill)
Compressed air	Point-of-use site in the system farthest from compressor
Operator on filling line or operator glove in an isolator	Finger (glove) impressions, at a minimum of five fingers of both hands
Laminar airflow (e.g., hood)	Near high-activity areas, finger (glove) impressions



## **4.5 Data Management (Data Collection, Analysis, Approach, and Interpretation)**

Routine review and analysis of environmental monitoring data for trends at an appropriate frequency is essential to aid in the interpretation of process stability and assess overall environmental control performance. Management must be kept abreast of trends and the subsequent state of operations within facilities with review of quarterly and yearly monitoring reports.

Based on the large number of samples tested by a given facility, a computer-based data-tracking system may be useful. Before implementation, all database applications used should be validated or qualified for specific software applications.

### **4.5.1 Collection**

Routine data are aligned into a source in a consistent record format. The record format should include (at a minimum) monitoring date and time, specific sampling locations, sampling methods including media used, incubation conditions, colony-forming units (CFU) or nonviable count results, identifications performed, product lot information, and current alert or action levels, signed and verified





by the appropriate person, depending on the type of system used. Some alternative microbiological methods can use different measurements than CFUs, provided that they have been properly validated before use, for example, relative light units, cells, and so forth. A manual data entry or image scanner system with advantages of speed and accuracy can be used to populate tables. Regardless of the type of system used, data integrity must be verified prior to analysis.

## 4.5.2 Analysis

Trending is expected by regulatory agencies. Histograms or tables characterized by a number of data points that fall within a common frequency are valuable tools. Different room classifications with defined requirements will produce different histograms. For example, the CFU spread obtained across an ISO 8 data set will not be observed in a data set from an ISO 5 area. Therefore, each area (or area type) and accompanying data set must be viewed as distinct. A mathematical model could be applied with not only the objective but also the type of data to be analyzed in mind. Examples of statistical methods and control charts can be found in *PDA Technical Report 59: Utilization of Statistical Methods for Production Monitoring* and in the article by Husong and Madsen, *Analysis of Environmental Microbiology Data from Cleanroom Samples (17,18)*.



**Table 4.5.4-1** USP Chapter <1116> Suggested Contamination Recovery Rates

Room Classification	Suggested Initial Contamination Recovery Rates (%)			
	Active Air Sample	Settle Plate (9 cm) 4-Hour Exposure	Contact Plate or Swab	Glove or Garment
Isolator/closed RABS or 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



# Microbiological Requirements of Cleanrooms / FDA 2004 / Action Levels

Clean Area Classification (0.5 um particles/ft <sup>3</sup> )	ISO Designation <sup>b</sup>	≥ 0.5 μm particles/m <sup>3</sup>	Microbiological Active Air Action Levels <sup>c</sup> (cfu/m <sup>3</sup> )	Microbiological Settling Plates Action Levels <sup>c,d</sup> (diam. 90mm; cfu/4 hours)
100	5	3,520	1 <sup>e</sup>	1 <sup>e</sup>
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

Comments: .....

Levels/ Definition of Action Level .... / No gloves  
„1 cfu“ would be accepted



# Microbiological Requirements of Cleanrooms / EU 2008/ Action Limits

<b>Recommended limits for microbial contamination (a)</b>				
<b>Grade</b>	air sample cfu/m <sup>3</sup>	settle plates (diameter 90 mm) cfu/4 hours (b)	contact plates (diameter 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	-

## Notes

(a) These are average values.

(b) Individual settle plates may be exposed for less than 4 hours.

Comments: limits / average values ... interpretation by industry as a requirement of 0 cfu

Current industry standard: within grade A / Iso 5 ... requirement „0“



## *What is EM ?*

Control, that environment of open product/ containers is not contaminated

- EM is related to **Clean Rooms** (including isolators) / **Water**/ Process Air/ Nitrogen/ ...
- EM is an Indirect Control and Monitoring of product quality , and no direct quality parameter (no specification as Sterility, Endotoxins/ ...)



## *Which methods are used ? Elements of Environmental Monitoring (Clean Rooms)*

- Viable air monitoring (Active and Passive)
- Total airborne particulate monitoring
- Surface monitoring
- Personnel monitoring
- Temperature and relative humidity monitoring
- Room air pressure differential monitoring



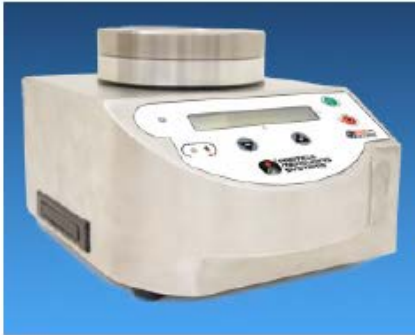
## *Is a control of ....*

- **Viable air monitoring (Active and Passive) :** HVAC control / material and operators particulates (and microorganisms) shedding/ airflow conditions ...
- **Total airborne particulate monitoring:** HVAC control/ airflow conditions/ Nonviable and viable particulates
- **Surface monitoring\_** Cleaning & Disinfection control, personnel behaviors
- **Personnel monitoring:** aseptic practices/ training
- **Temperature and relative humidity monitoring** (to control acceptable working conditions and product )
- **Room air pressure differential monitoring :** prevent ingress from outside



# Environmental Monitoring

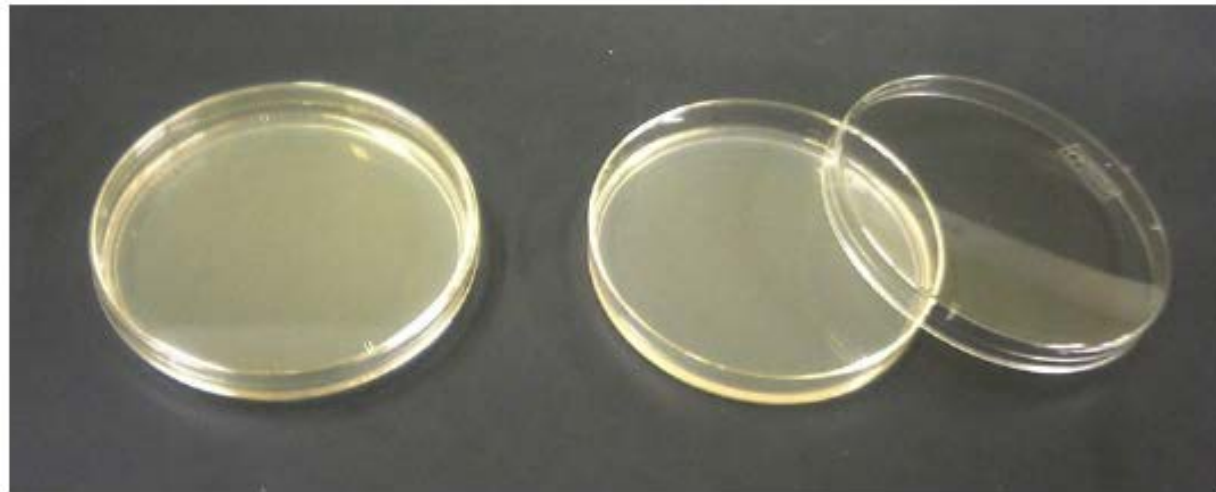
## *Viable Air Monitoring*







# Microbiology Test Methods: Settle Plates



# Surface Monitoring

Lower  
Recovery

?

## Swabs

Employed for equipment and irregular surfaces

Sample area is usually 25 cm<sup>2</sup>

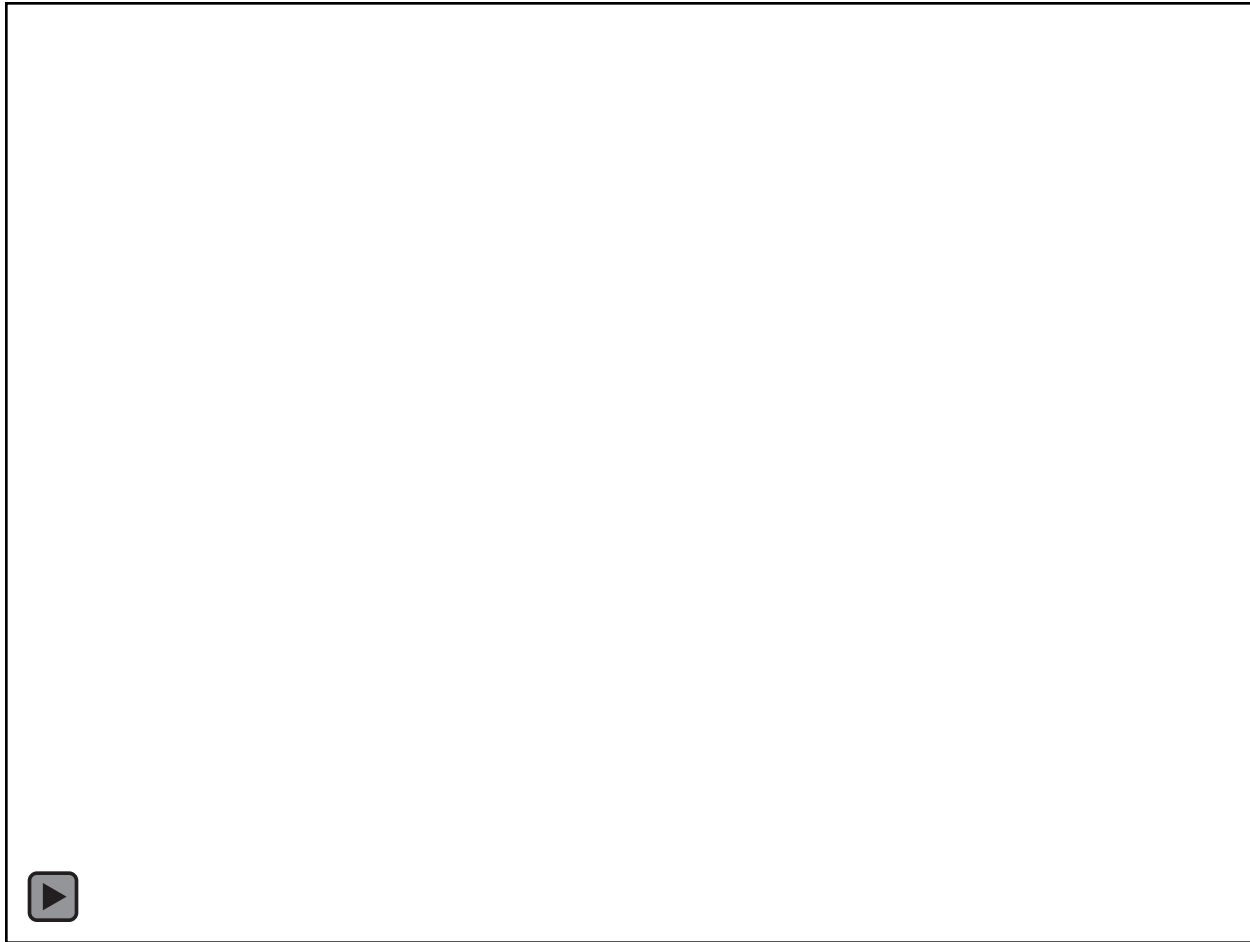
➤ Contact plates (Rodacs) / 25 cm<sup>2</sup>

Nutrient  
residues





# Video Surface Monitoring/ Wall





# ***Current Best Practices in Microbiological EM I***

- Dynamic Monitoring (DURING) Routine Aseptic Operations (Air Monitoring) / 1 -3 times a 1 m<sup>3</sup>
- Settle plates: continuous exposure; alternating; maximum 4 hours
- Set- Up of Filling line (may be risky) included in EM program
- Surface and Personnel Monitoring : at the end, or even after operations ; cleaning afterwards or glove- removal
- Glove Monitoring after Set- up and after “risky“ interventions
- Have a written rationale for Sampling Locations (e.g. worst case locations, see also below) and for number of samples
- Frequency
  - Grade A : shiftwise
  - Grade B: daily
  - Grade C: weekly
  - Grade D: monthly



## *Current Best Practices in Microbiological EM II*

- Prevent contamination of sterile products by EM execution !
- Training /Qualification of personnel (by QC or Production)
- QA oversight during EM is very important
- Valid growth conditions & prevent secondary contamination
- Good documentation practices (Data Integrity)
- Good Deviations Procedures according to adequate Action / Alert Level requirements
- Good Trending Methods



# PDA TR 13

**Table 3.0-1** Cleanroom Standards—Airborne Particulate Limits (particles/m<sup>3</sup>)

Particle Size	U.S. FDA (Aseptic Processing Guidance)		USP <1116>	EU Annex 1 and WHO Annex 4
	ISO 14644			
	ISO 5	Class 100 <sup>1,2</sup>	ISO 5/Class 100	Grade A Grade B (at rest)
≥0.5 μm	3,520	3,520 <sup>3</sup>	3,520	3,500
≥5 μm	29	Not specified	Not specified	20 <sup>4</sup>
	ISO 6	Class 1000	ISO 6/Class 1000	NA
≥0.5 μm	35,200	35,200	35,200	NA
≥5 μm	290	Not specified	Not specified	NA
	ISO 7	Class 10,000	ISO 7/Class 10,000	Grade B (in operation) Grade C (at rest)
≥0.5 μm	352,000	352,000	352,000	350,000
≥5 μm	2,900	Not specified	Not specified	2,900
	ISO 8	Class 100,000	ISO 8/Class 100,000	Grade C (in operation) Grade D (at rest) <sup>5</sup>
≥0.5 μm	3,520,000	3,520,000	3,520,000	3,500,000
≥5 μm	29,000	Not specified	Not specified	29,000



# Annex 1 / 2017 Rev

**Table 5: Recommended limits for airborne particle concentration for the monitoring of non-viable contamination**

Grade	Recommended maximum limits for particles $\geq 0.5 \mu\text{m}/\text{m}^3$		Recommended maximum limits for particles $\geq 5 \mu\text{m}/\text{m}^3$	
	in operation	at rest	in operation	at rest
A	3 520	3 520	20	20
B	352 000	3 520	2 900	29
C	3 520 000	352 000	29 000	2 900
D	Set a limit based on the risk assessment	3 520 000	Set a limit based on the risk assessment	29 000



## *Microbiological Lab : Best practices*

- Growth Promotion Testing (of each nutrient batch) , including house-isolates
- One nutrient should be enough (TSA)
- Evaluate elevated temperatures incubation : recovery of molds ?
- For grade A : usage of purchased, gamma irradiated nutrients
- Inactivators are added (of disinfectants or antibiotics)
- Incubation temperatures: should be able to recover mold
- Negative controls
- Incubator temperature control monitoring / Alarm Management/  
Cleaning and disinfection
- Validated Identification methods of isolates (All isolates from grade A should be identified to species level, and a representative number of lower classes)
- Good Documentation practices – independent review by a second person
- Good investigational procedures in case of OOL (out of Level) deviations





## ***Rationale and Selection of Meaningful Sample***

- Following Factors to consider for the selection of meaningful locations/ areas
  - Locations close to open product, or close to product contact surfaces
  - Locations which are product contact surfaces/ indirect product surfaces
  - Locations with a lot of activities by the cleanroom operators, frequently passed /touched locations
  - Sampling locations should represent “worst case positions” e.g.,
    - Floors in grade B, chairs, benches in gowning rooms, door knobs, touchscreens
    - Sampling locations most likely having heaviest microbial proliferation, e.g drains
    - Sites that represent the most inaccessible or difficult to clean and sanitize location
    - Locations with extended storage times of product and product contact surfaces
    - Air exit locations
- Personnel Monitoring: gloves (= fingertips) and forearms of gloves of the cleanroom operators after critical interventions and at exit
- Locations where smoke studies show turbulences or stagnant air
- Important: Active Air Monitoring devices & settle plates: „at working level“



## ***Additional Criteria for Selection of EM locations***

- Areas / rooms with higher temperatures (reason: may support microorganism proliferation/ increase operators perspiration/ wet gowning and furthermore increased shedding of particulates)
- Wet areas (water based environments in the vicinity of sinks, drains)
- Extended duration of activities (additionally to the item above “a lot of activities”)
- Low cleaning / disinfection frequency - inclusion of mobile equipment (e.g., trolleys/ mobile vessels)
- EM program may be assessed by a Risk Assessment (FMEA) ....  
**SEVERITY/ OCCURANCE / DETECTABILITY**



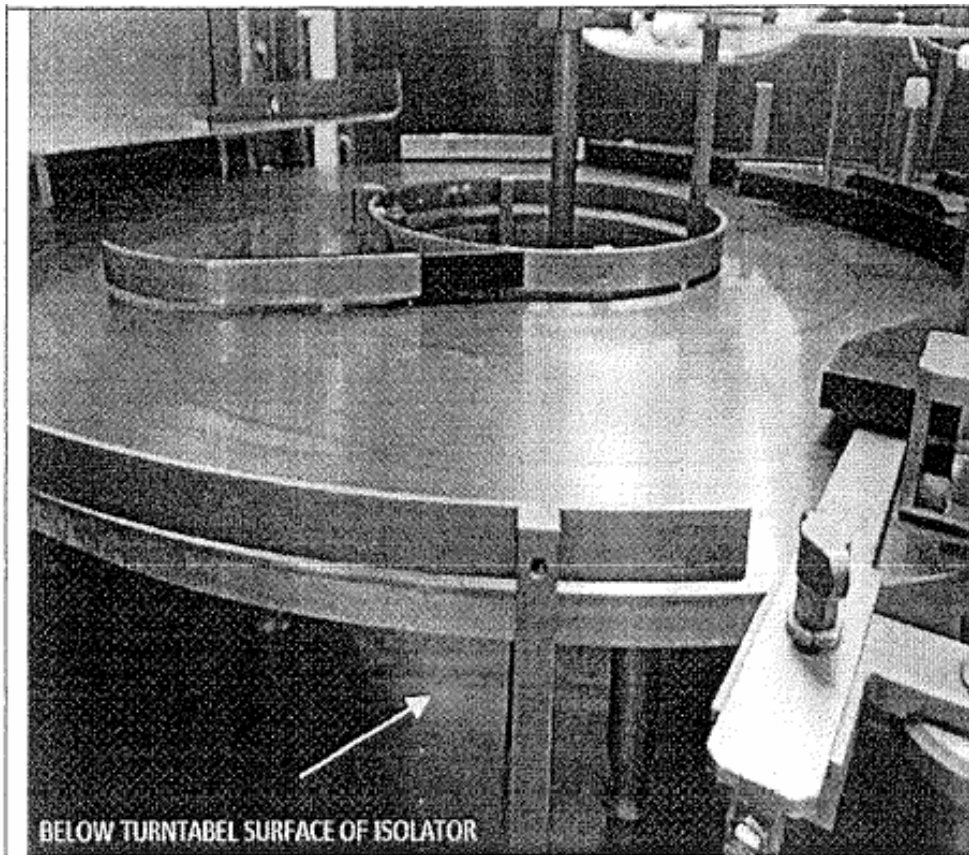
## *Isolator Filling Operations: Settle plate*



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SOP's : add pictures for detailed location, and rationale for choosing this location

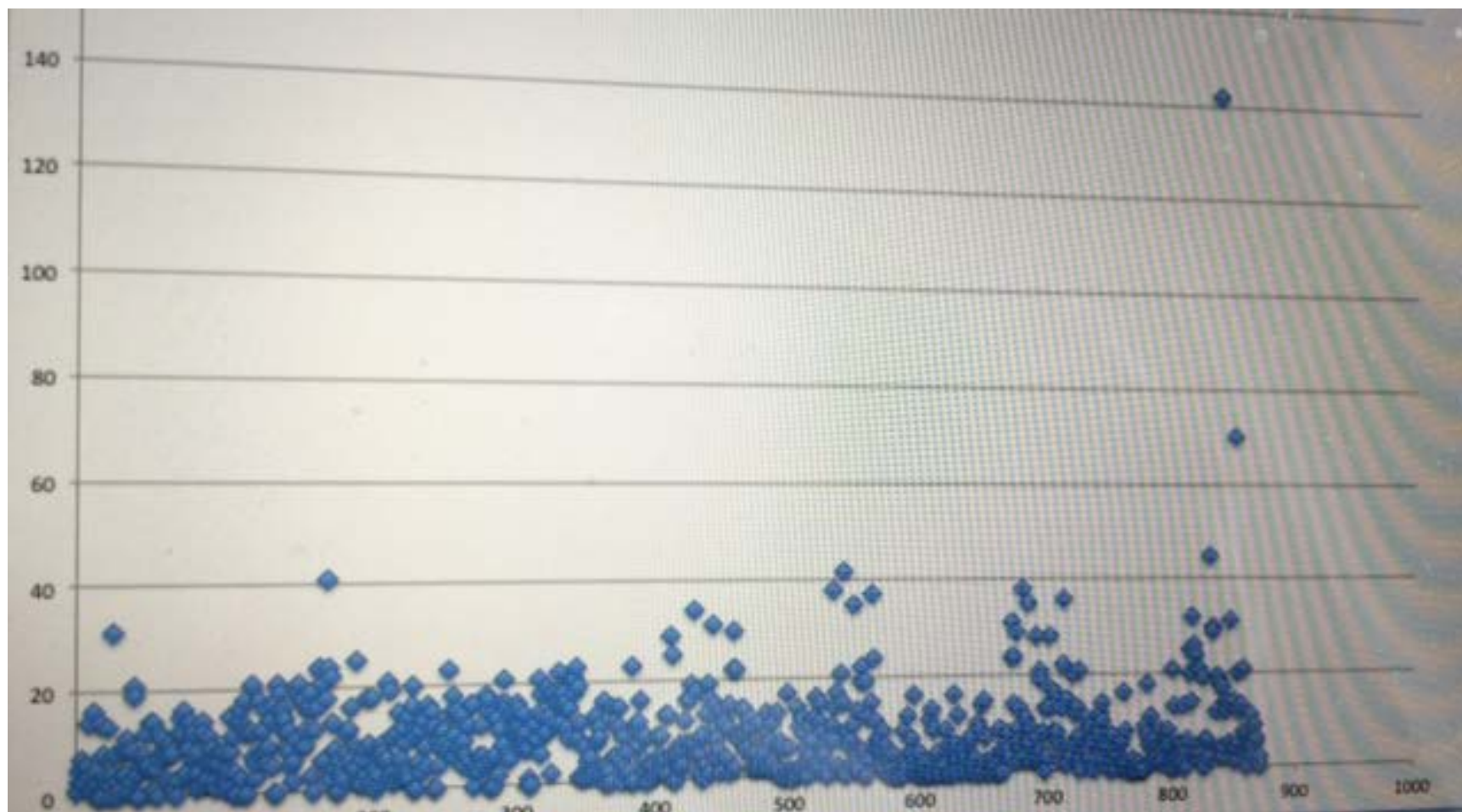


**ISM26**

To determine effectiveness of cleaning and decontamination process for difficult to clean areas. Site, which if contaminated has adverse effect on product sterility.



## *Trend Analysis: This is no Trend Analysis*

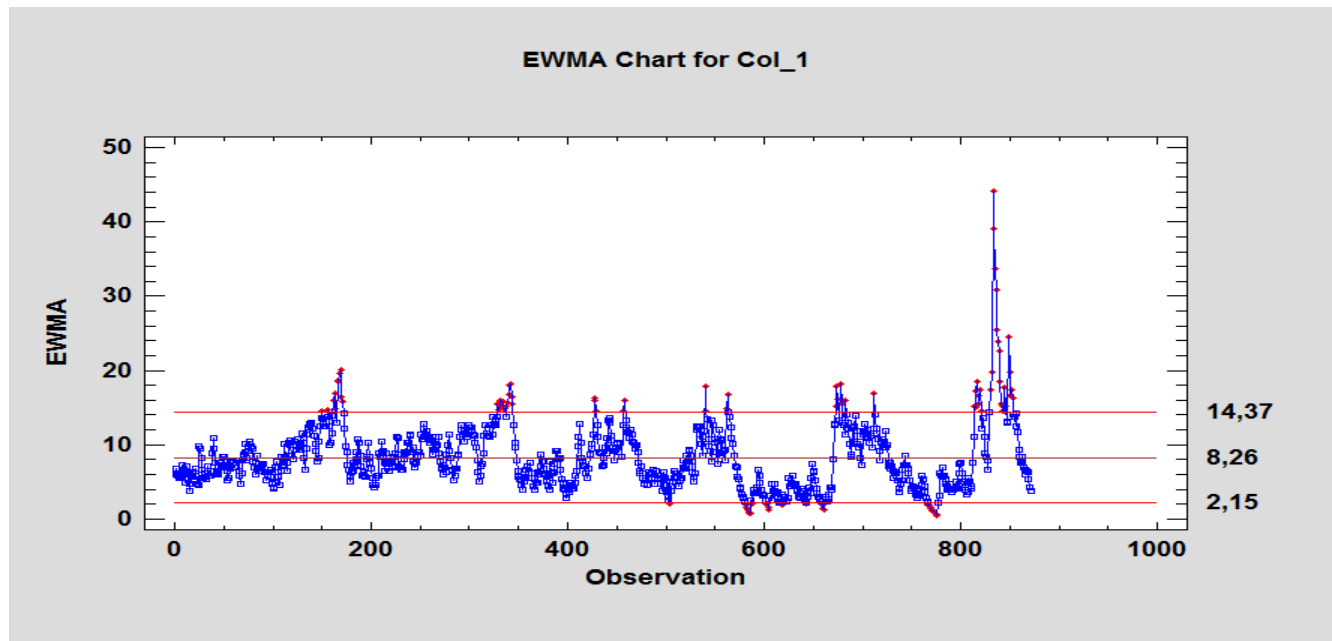


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## Trend Analysis: Points to consider

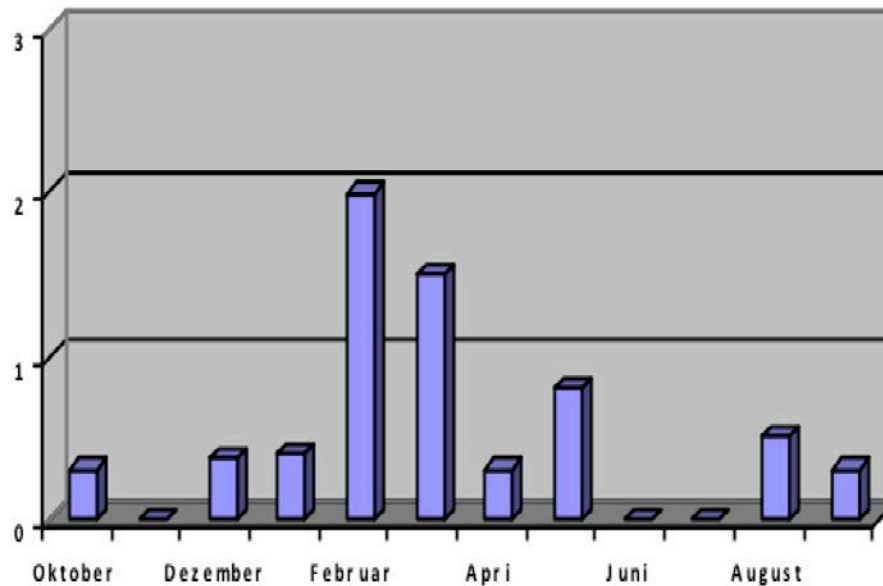
- Historically based Alert Levels: between „95th- 99th Percentile“
- Shifts in trends should be detectable in the graphics
- Recommend to perform this Quarterly and Annually; Written Report
- How to assess „adverse trends“ ? Usage of Statistical Control Charts – use an applicable tool, e.g. „Moving Average analysis“





# EM Trending – Quarterly reports

Positive Recovery Results  
in Class B (surfaces):

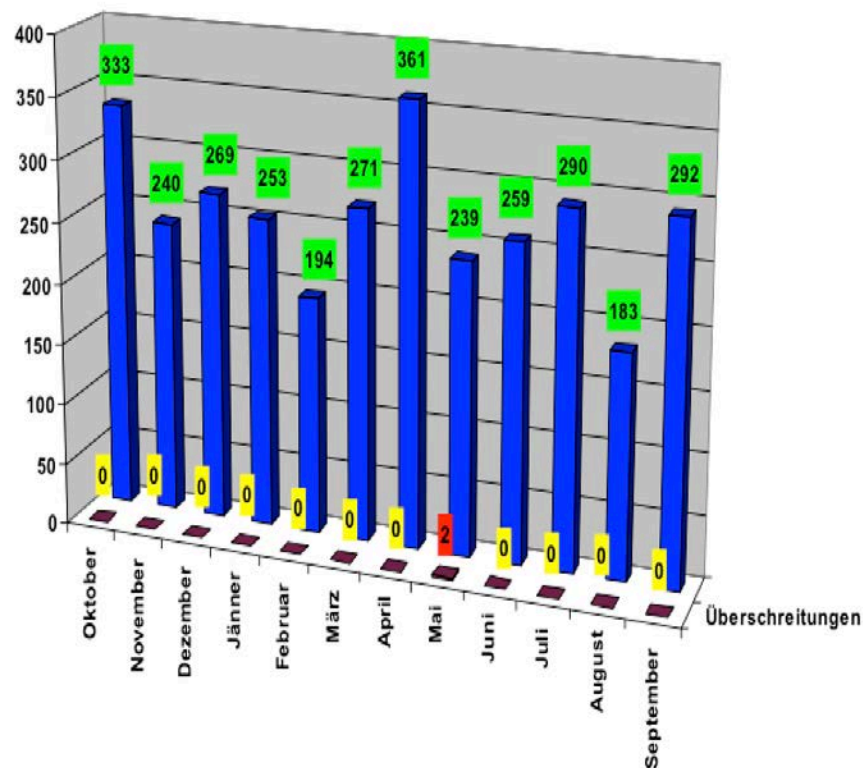




# EM Trending – Quarterly reports – Feedback to Personnel

Number samples  
OOL results

Illustrate in  
production area  
!







## *What initiates an Investigation ?*

- Action Level(s) is exceeded
- Alert Level has been exceeded for 2 or 3 times
- Trend worsening detected
- Recovery of objectionable (pathogenic) microorganism
- Recovery of bacterial „sporeformers“
- Higher Percentage of molds detection in the cleanrooms grade C/D
- Missing sample(s) in the routine EM program



*How to proceed in case of a OOL*

*(OUT OF LEVEL) ?*



## *WL Examples and Form 483 observations : HOT TOPIC !*

- Your environmental monitoring data is **not reliable**. This is a serious deviation, as your ability to detect microbial contamination in the manufacturing environment during aseptic processing is fundamentally compromised. (WL, June, 2012)
- Approximately **846 environmental monitoring (EM) samples were not collected in the Class 100 (Grade A) and the Class 10,000 (Grade C) areas** from March 2010 to February 2012 during the manufacture of sterile products. This substantial number of missed samples suggests a pattern that **raises concerns** regarding your environmental program. Your response mentions that some of the “missed” samples were actually samples for which the exposure time for the plate had been exceeded or delayed, which suggests that you may have **rejected or invalidated** some results. (WL, December, 2012)



## *WL Examples and Form 483 observations : HOT TOPIC*

- We are concerned that you may have underestimated the number and type of bacterial species that are present because you have no data to support the equivalent sensitivity and efficiency of bacterial recovery on the media. **FDA expects that microbial culture media used for environmental monitoring be validated as capable of recovering fungi (i.e., yeast and molds), as well as bacteria.** Appropriate trending of environmental monitoring data depends on consistent methods to provide an indication of the amount and type of microbiological organisms present. (WL, September, 2014)
- You did not utilize environmental monitoring data to identify environmental control issues and identify appropriate follow-up actions. **You did not examine the data for trends or take appropriate follow-up action.** We observed an operator entering the RABS and in contact with gloves without sanitizing his gloved hands. Additionally, on video and in person, we observed employees with (b)(4) on their hands before EM checks. **Sanitizing gloved hands just before sampling is unacceptable because it can prevent recovery of microorganisms.** This undermines the reliability of personnel monitoring data. We are also concerned about your failure to review the results of microbial tests to identify possible trending problems in environmental control in aseptic processing areas. (WL, August, 2015)



## *WL Examples and Form 483 observations : HOT TOPIC*

- **Our investigators observed dried media plates** you used for surface and personnel monitoring in the facility incubators. We documented that 36 plates inside the Plant incubator showed signs of dryness and desiccation. **Your EM data for the filling areas did not specify the sampling location of the RABS used during filling and operations.** *SOP Procedure of Surface Monitoring by Swab* does not require sampling from **predetermined locations** identified as critical risk points of your filling and operations. Instead, the procedure permits individual operators to determine the location to be sampled. Additionally, you only collected a swab sample from xxx, and failed to sample other locations used in daily aseptic operations. **Your firm lacked personnel monitoring data for aseptic operations on line.** Documents generated in the laboratory for personnel monitoring did not identify specific employees involved in filling operations. Despite your claim that your operators were appropriately trained, video recordings of your manufacturing operations clearly showed that your employees were **not following proper aseptic techniques.** (WL, March, 2016)

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# Team Work

Prepare a routine EM program for a conventional Filling Line and adjacent grade B rooms



## *Prepare an EM program for a Conventional Filling line*



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## 3 TAKE HOME Messages