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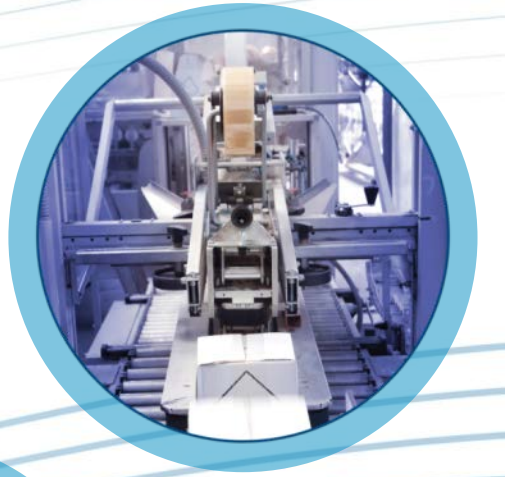
Important Aspects in Environmental Monitoring



By Guenther Gapp

19 Oct 2021

Overview about Presentation

- Introduction: Why is EM (= Environmental Monitoring) required and what does it mean ?
- Rationale of EM Sampling Locations
- Action& Alert Levels / Limits/ Requirements
- “My Best Practices”
- Microbiological Laboratory – points to consider
- How to execute Trend Analyses / Historically Based Alert Levels
- Back-up Slides

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

Important References

- CFR 21 PART 870.70 „Production and process controls“ and 203.32
- PDA Technical Report 13 (2013) / Fundamentals of Environmental Monitoring – under revision !
- FDA Guidance (Sterile Drug Products Produced by Aseptic Processing/ 2004)
- Eudralex Volume 4 - Annex 1 (Draft 2020)
- USP <1116>
- PDA Points to Consider Aseptic I and II (2015/2016)

PDA TR 13 (New Release 2021)

Fundamentals of an Environmental Monitoring Program

Technical Report No. 13 (Revised)

ISBN: 978-0-939459-67-4

PDA TR 13 (New Release 2021)

TECHNICAL REPORT NO. 13 (Revised 2020): Fundamentals of an Environmental Monitoring Program

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PDA Points to Consider 1 & 2

Points to Consider for Aseptic Processing

Part 1
January 2015

ISBN: 978-0-939459-75-9

Points to Consider for Aseptic Processing

Part 2
May 2016

ISBN: 978-0-939459-89-6

PDA TR 13 Revision (Draft version) : Sample Locations Rationale

The following factors should be considered when the team is performing the walk-through of the facility:

1. Adherence to industry and regulatory guidelines, e.g., CFR, ISO 14644, USP, FDA, EU (Note: ISO 14644 only provides a guidance for the classification of cleanrooms, e.g., minimum number of sample locations for total particulates)
2. Sites and locations where microbial contamination would most likely have an adverse effect on product quality and, therefore, have the highest risk, considering—
   - Proximity to open product or product contact surfaces and critical sites (e.g., filling needles, stopper bowls)
   - Activities linked with interventions
   - Areas that are the most inaccessible or difficult areas to clean and disinfect
   - Locations with a high frequency and/or complexity of activities by cleanroom operators, (e.g., touch panel, forceps, door handles)
   - Areas with a large number of personnel and high personnel flow (e.g., floors at the entrance of gowning rooms)
   - Areas with high material flow
3. Uniform geometric pattern or grid-profiling within the cleanrooms, to cover the complete area
4. Assessment and a justification for locations that will not be part of the EM program due to certain restrictions or alternative/worst-case coverage
5. Historical data and/or data obtained during qualification
6. Anaerobic organisms:

PDA TR 13 (Draft): Example about EM Risk Analysis

10.0 Appendix 2: Risk Assessment Examples

Table 10.0-1 shows a real-life example provided by Gapp Associates of a review of an existing EM program at a closed RABS filling operation (vial filling; Grade B background). Note that only 5 risk items - from a total of 19 - are listed in the table below.

Table 10.0-1: Example: Failure Mode and Effects Analysis (FMEA)

Risk Item	Problem Statement / Requirement	Risk Description / Potential Failure Mode and Impact	Causes of process/ product failure	Current Controls and Preventive Actions/ Comments SEV: assess the risk and impact on sterility and regulatory compliance, in case of a deviation/ deficiency to this statement/ requirement OCC: assess the probability, that the related locations and areas are - in fact- microbiologically contaminated DET: assess the probability, that the implemented EM sampling plan/ frequency/ number of samples/ methods would detect - in fact- a potential contamination	SEV	OCC	DET	RPN	Risk Class	Risk Accepted	Mitigation Measures/CAPAs
1	"High-risk areas" in Grade A are monitored: samples are selected which are -in close proximity to open product and critical surfaces - linked with an increased number of activities and interventions - linked with an extended time of critical interventions - linked with material flow into Grade A. These areas must be properly monitored and sampled in the EM program.	Potential contamination is not detected in high-risk areas, which results in product contamination in Grade A.	Ingress of particles and microbiological contamination into the high-risk areas by many activities and interventions/ extended activities/ material flow.	The high-risk areas in the RABS are a) the filling and stoppering area b) the stopper bowl and c) the turntable, where depyrogenated, open vials are exposed for an extended time. The above areas are well addressed by the surface monitoring program, but not by an active air monitoring/ surface monitoring at the stopper bowl. A settle plate is exposed. This is a deficiency, since high-risk interventions and aseptic activities are performed in the vicinity of the stopper bowl. (Note: active air monitoring is done during the set-up and once in a shift). **SEV: 3** (high impact on product sterility, in case that indirect product contact surfaces = stopper bowl is contaminated) **OCC: 2** (there are several interventions performed, therefore there is a moderate risk) **DET: 2** (no active air monitoring, linked with interventions, therefore reduced detectability and moderate risk; no surface monitoring of critical surfaces, which is a regulatory requirement)	3	2	2	12	MAJOR	N	Introduce an active air monitoring location and surface monitoring at the stopper bowl.

Rationale and Selection of Meaningful Sample (from 2019)

- 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 (2019)

- 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 ... Refer to slide 11

- Oversight expected about EM in case of „self-controls“ by Poduction

Microbiological Requirements of Cleanrooms / FDA 2004 / Action Levels

Clean Area Classification (0.5 um particles/ft^3)	ISO Designation[b]	≥ 0.5 µm particles/m^3	Microbiological Active Air Action Levels[c] (cfu/m^3)	Microbiological Settling Plates Action Levels[c,d] (diam. 90mm; cfu/4 hours)
100	5	3,520	1[e]	1[e]
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

	Recommended limits for microbial contamination (a)			
Grade	air sample cfu/m^3	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“

EU Annex 1 (Draft 2020)

Table 2: Limits for microbial contamination during qualification

Grade	Air sample cfu/m^3	Settle plates (diameter 90 mm) cfu/4 hours [a]	Contact plates (diameter 55 mm) cfu/plate
A[b]	No growth[b]		
B	10	5	5
C	100	50	25
D	200	100	50

(a) Settle plates should be exposed for the duration of operations and changed as required after 4 hours. Exposure time should be based on recovery studies and should not allow desiccation of the media used.

(b) It should be noted that for Grade A, the expected result should be no growth.

Note 1: All methods indicated for a specific Grade in the table should be used for qualifying the area of that specific Grade. If one of the methods is not used, or alternative methods are used, the approach taken should be appropriately justified.

Note 2: Limits are applied using cfu throughout the document. If different or new technologies are used that present results in a manner different from cfu, the manufacturer should scientifically justify the limits applied and where possible correlate them to cfu.

Note 3: For qualification of personnel gowning, the limits given for contact plates and glove prints in Table 7 should apply.

Note 4: Sampling methods should not pose a risk of contamination to the manufacturing operations.

4.34 The requalification of cleanrooms and clean air equipment should be carried out periodically following defined procedures. The requirement for requalification of cleanroom areas is as follows:

EU Annex 1 (Draft 2020)

Table 7: **Maximum action limits for** viable particle **contamination**

Grade	Air sample cfu/m^3	Settle plates (diam. 90 mm) cfu/4 hours [(a)]	Contact plates (diam. 55mm), cfu/ plate [(c)]	Glove print, Including 5 fingers on both hands cfu/ glove
A	No growth[(b)]			
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

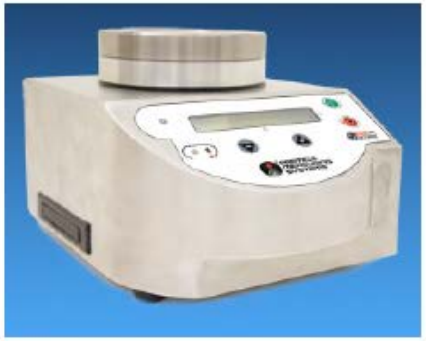
[(a)] Settle plates should be exposed for the duration of operations and changed as required after 4 hours (exposure time should be based on validation including recovery studies and it should not have any negative effect on the suitability of the media used). Individual settle plates may be exposed for less than 4 hours.

[(b)] It should be noted that for Grade A, any growth should result in an investigation.

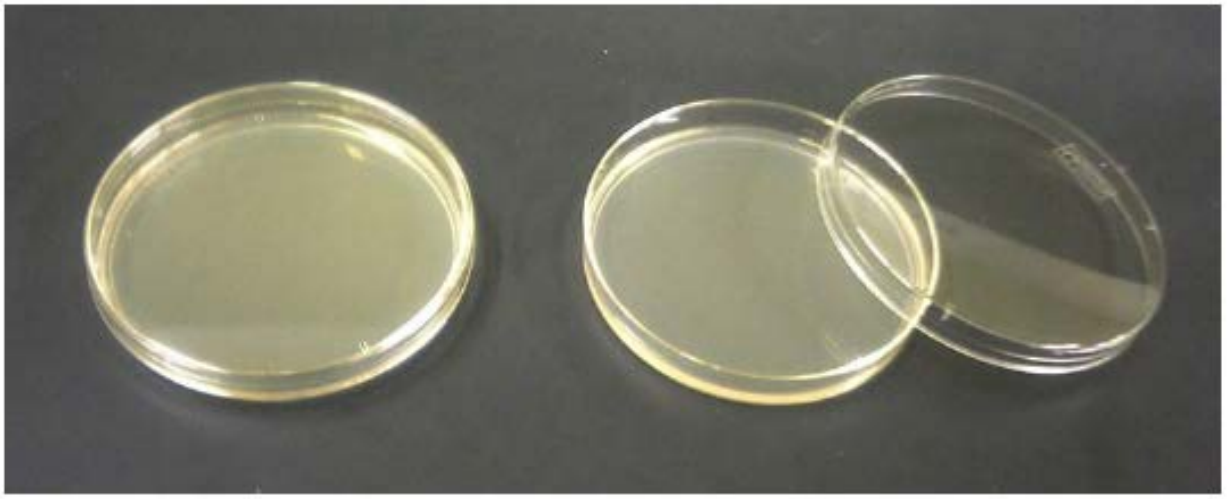
[(c)] Contact plate limits apply to equipment room and gown surfaces within the Grade A zone and Grade B area. Routine gown monitoring is not normally required for Grade C and D areas, depending on their function.

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^2

- Contact plates (Rodacs) / 25 cm^2

Nutrient residues

Video Surface Monitoring/ Wall

Video

Glove Monitoring

Video

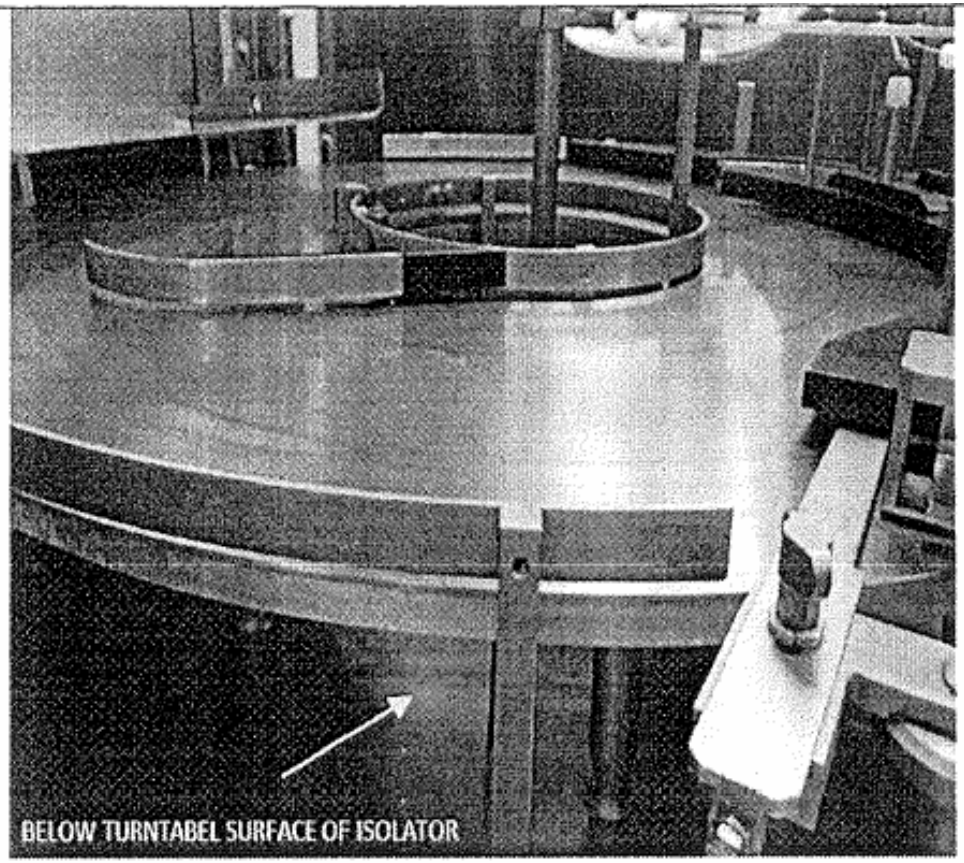
Current Best Practices in Microbiological EM I

- Dynamic Monitoring (DURING) Routine Aseptic Operations (Air Monitoring) / 1 -3 times a 1 m^3
- Settle plates: continuous exposure; alternating; maximum 4 hours
- Set- Up of Filling line (risky) included in EM program
- Surface and Personnel Monitoring: at the end or at the exit, 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 : shift-wise
    - Grade B: daily
    - Grade C: weekly/ monthly (depends on operation)
    - Grade D: monthly/ quarterly (depends on operation)

Current Best Practices in Microbiological EM II

- Prevent contamination of sterile products by EM execution
- Training /Qualification of EM sampling 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

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.

Photo: Isolator Filling Operations with an exposed settle plate

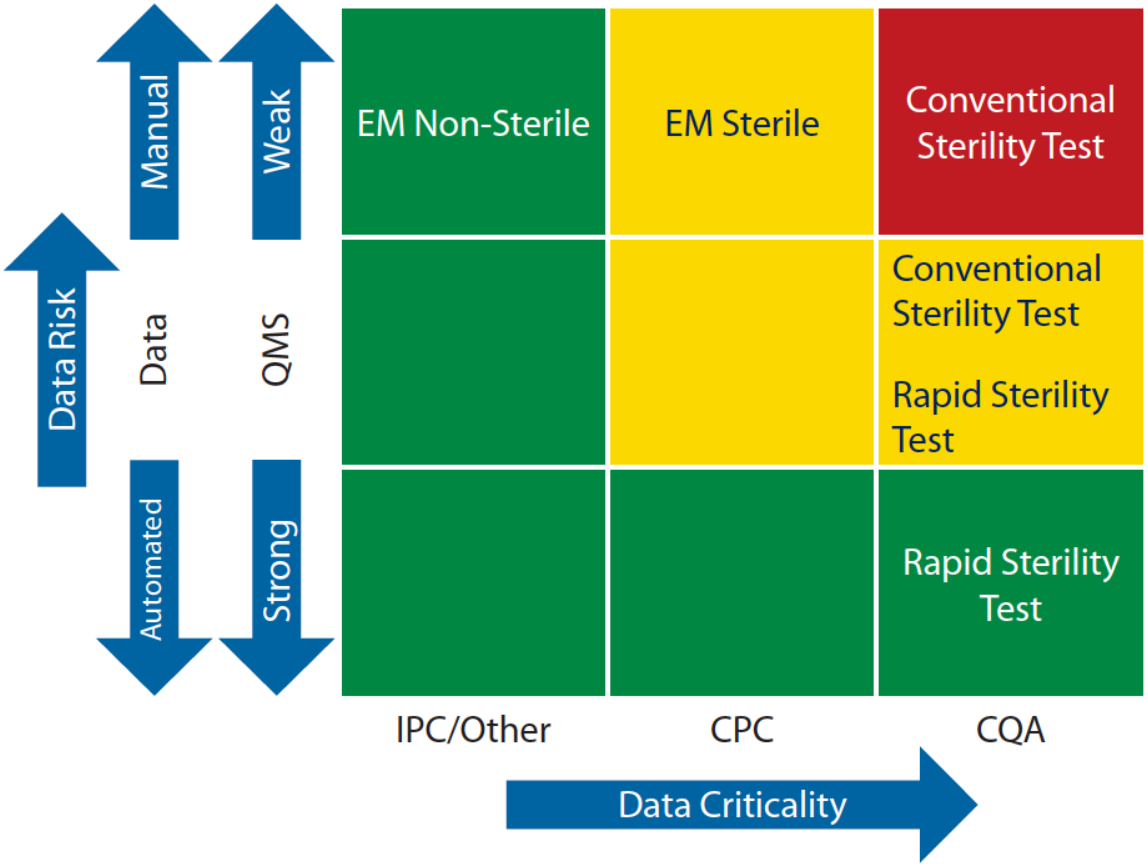
Microbiological Lab: Best practices

- Growth PromotionTesting (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

Currently, a high percentage of the tests conducted in microbiology laboratories are observational, that is, the results (such as a colony count) are viewed and manually recorded on a paper document or in a computer record. Absent an easy, reliable method to verify the recorded data, some laboratories require microbiologists to use second-person verification (e.g., supervisor) by physical examination of the test plates. Further, the second-person verification could be performed as a discreet step prior to approval of the data or combined with the data-approval step.

second review verification ...if no reliable method to verify recorded data

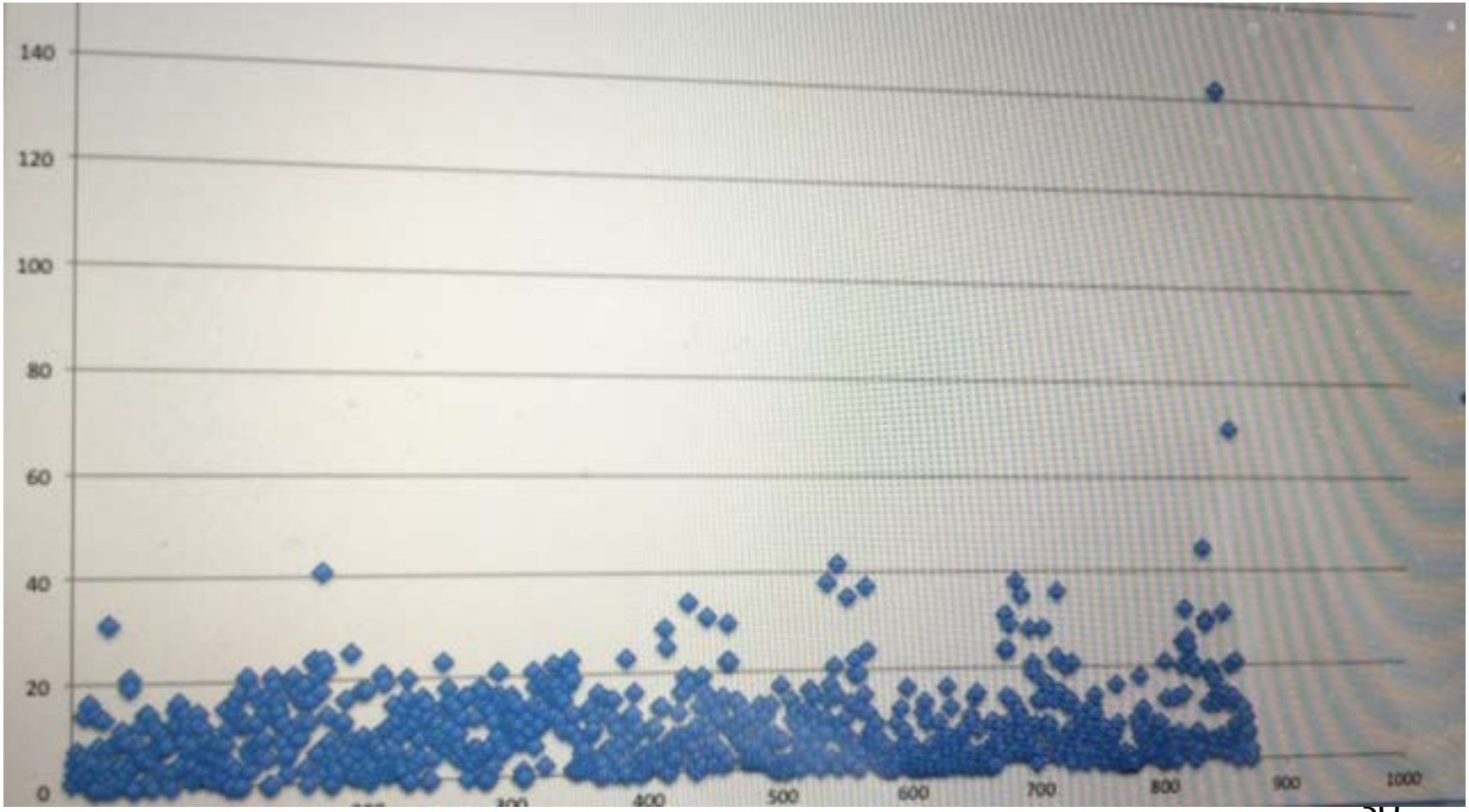
Potential Data Integrity Risk Matrix for Microbiological Testing



Legend	
CQA	Critical Quality Attribute
CPC	Critical Process Control
IPC/ Other	In-Process Control
Auto/ Elec	Automated/ Electronic
QMS	Quality Management System

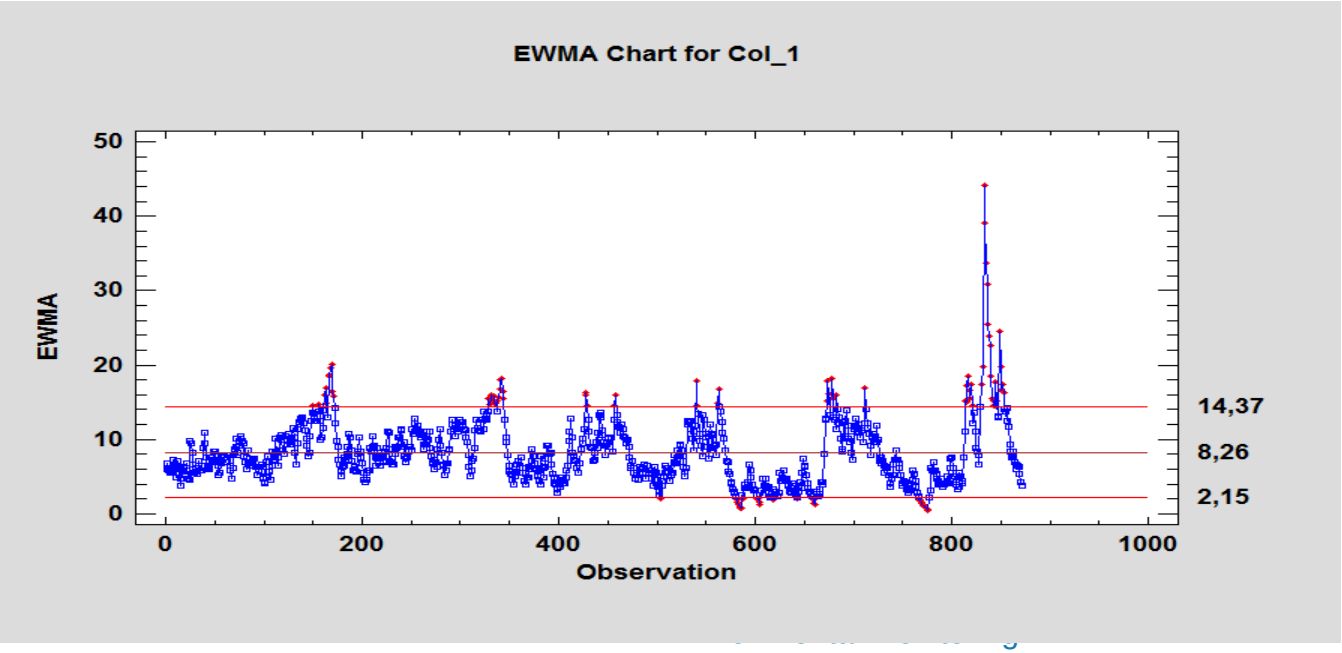
Figure 7.2-1 Risk Matrix Example for Microbiological Testing

Trend Analysis: This is no Trend Analysis



Trend Analysis: Points to consider

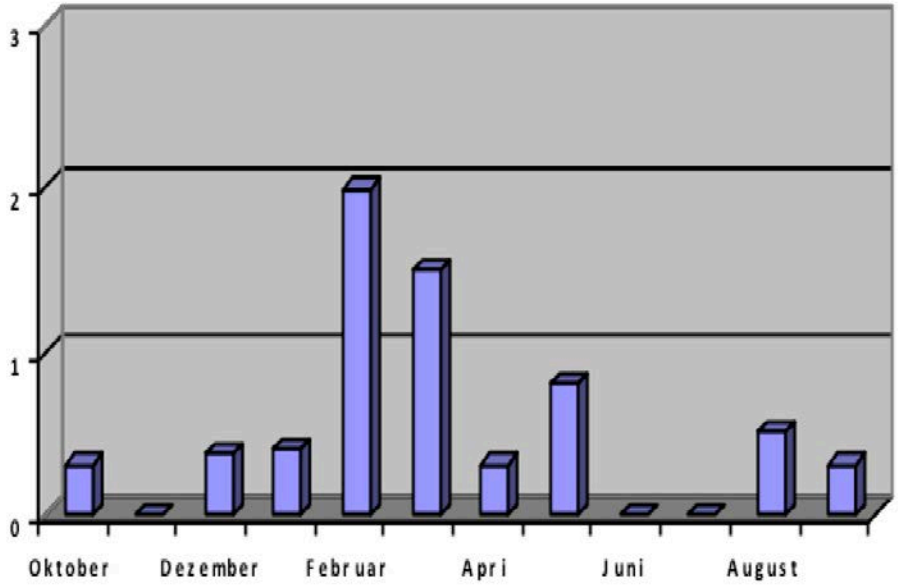
- Historically based Alert Levels: between „95th- 99th Percentile“
- Shifts in trends should be detectable in the graphics
- Recommend to perform this Quarterly and Annually; Written Report
- How to assess „adverse trends“ ? Usage of Statistical Control Charts – use an applicable tool, e.g. „Moving Average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



EM Trending – Quarterly reports

Positive Recovery Results

in Class B (surfaces):

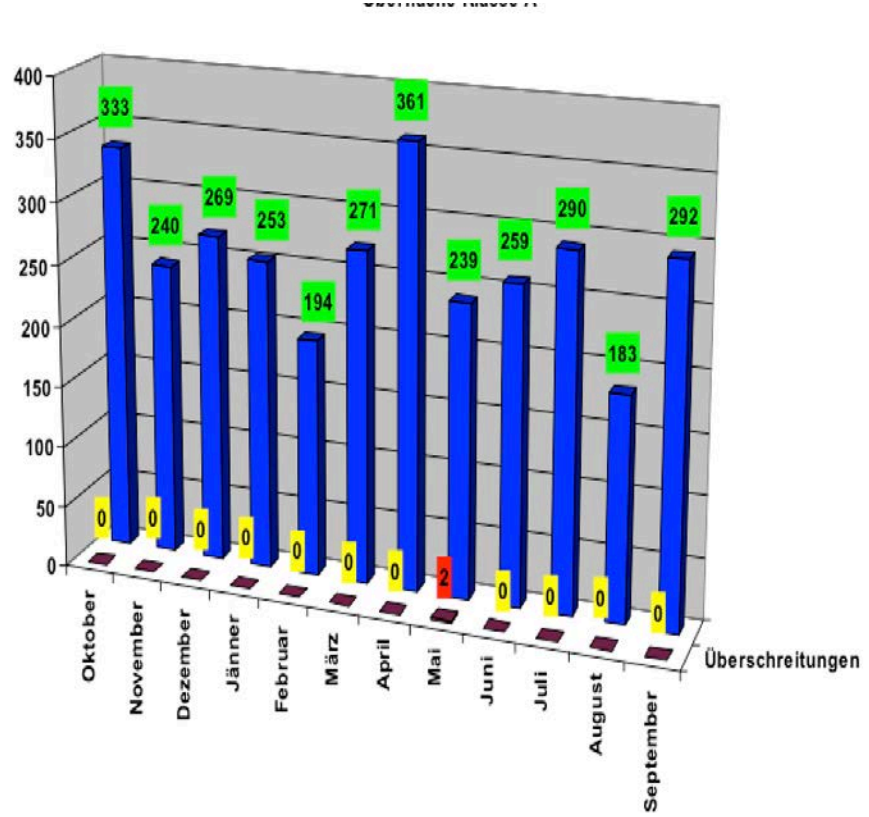


EM Trending – Quarterly reports – Feedback to Personnel

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) micoorganism
- Recovery of bacterial „sporeformers“
- Higher Percentage of molds detection in the cleanrooms grade C/D
- Missing sample(s) in the routine EM program

Back – Up Slides

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.[20] 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

END and Questions