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White Paper

USP <1116> Microbiological Control Of Aseptic Processing Environments And Its Implications

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The recently revised United States Pharmacopoeia (USP) chapter <1116> Microbiological Control and Monitoring of Aseptic Processing Environments includes a thorough description, definitions and guidance on microbiological control and monitoring in aseptic processing environments **(1)**. Chapter <1116> is arguably one of the most comprehensive informational chapters from the USP, and it is particularly challenging due to its proposal regarding measurement of microbial contamination based on Contamination Recovery Rates (CRR) rather than the conventional enumeration of colony forming units (cfu). Instead of using the microbial limits currently endorsed by aseptic guidances **(2–4)** —which are based on cfu—<1116> proposes CRR values expressed in maximum allowed percentage of contaminated samples. The proposal is generating a broad range of discussions among pharmaceutical professionals regarding potential implications of these changes.

It is important to note that <1116> is a “general information” chapter, and as such, it “provides information and recommendations for environments where the risk of microbial contamination is controlled through aseptic processing.” Therefore, the chapter in its current format provides recommendations not yet adopted and not enforceable by the U.S.

FDA or any other government agency. This clarification is important because the recommendation on the adoption of CRR is generating a positive debate that will probably require further discussion and clarification before any enforcement occurs. If adopted, hopefully, a harmonized approach by U.S., European and Japanese authorities will take place to avoid disparity of values for microbial limits.

Main Changes When Compared to Previous

1. Title

The most obvious change concerns the title of the chapter. The previous title of <1116> was *Microbial Control and Monitoring Environments Used for the Manufacture of Healthcare Products* while the revised title is *Microbiological Control and Monitoring of Aseptic Processing Environments*.

2. Scope

The scope of the chapter has been narrowed to apply to the following products manufactured in an aseptic processing environment:

- Pharmaceutical sterile products
- Bulk sterile drug substances
- Sterile intermediates
- Excipients
- Some medical devices

In addition, the types of environments covered in <1116> are:

- Conventional cleanroom with unidirectional airflow
- Blow/fill/seal machines
- Restricted Access Barrier Systems (RABS)
- Isolators

3. Aseptically Filled Product

The emphasis on the word “aseptic” in the introduction implies that the chapter is not applicable to all “sterile” products. This means that terminally sterilized products are outside the scope of the chapter. By “aseptic,” a low level of contamination is acknowledged: “An expectation of zero contamination at all locations during every aseptic

processing operation is technically not possible and thus is unrealistic." Therefore, a low level of contamination—over a given period of time—is a good assumption and it should be accepted as a norm in operations where personnel are present.

4. Room Classes

In the revised <1116>, all old notations (e.g., M3.5) and old FDA 209E classes (e.g., Class 100) were eliminated and replaced by ISO 14644-1 classes in the operational state **(Tables 1–2).**

Table 1 Microbial Limits During Operation, According to European Union Guidelines (Annex 1) (top) and FDA Guidance (2004) (bottom)

Grade	Air Sample cfu/m³	Settle Plates (∅ 90 mm), cfu/4 hours	Contact Plates (∅ 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	–

Clean Area Classification (0.5 μm particles/ft³)	Air Sample cfu/m³	Settle Plates (∅ 90 mm), cfu/4 hours	Glove Print 5 fingers cfu/glove
100	ISO 5	1	1
1000	ISO 6	7	3
10000	ISO 7	10	5
100000	ISO 8	100	50

Table 2 <1116> Suggested Initial Contamination Recovery Rates in Aseptic Environments

Room Classification	Active Air Sample (%)	Settle Plate (9 cm) 4h 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

5. Risk Assessment

The chapter emphasizes that even with a good total particulate monitoring program in place, "It is not possible to clearly distinguish between background particulate contamination generated…by mechanical operations and the total particulates contributed by personnel." Therefore, it is standard routine to implement both total particulate and microbiological monitoring programs. The chapter also discusses the differences between operating in conventional cleanrooms and open RABS, and more controlled environments where personnel interventions have significantly less impact on microbial contamination, such as in closed RABS and isolators. It is clear that the relative risk of microbial quality depends on the different types of aseptic barrier systems; the greater the barrier, then the lower the expected contamination risk.

6. Air Changes

As specifications for air changes per hour and air velocities were not included in ISO 16444 ***(5),*** nor in Federal Standard 209E, chapter <1116> provides the following guidance: ISO class 8 (minimum 20 air changes per hour [ac/hr]), ISO class 7 (>50 ac/hr), and ISO class 5 (>100 ac/hr). In isolators and cRABS, lower air changes and air velocities can be justified. USP <1116> emphasizes that these specifications should be used only as a general guide due to the numerous variations on designs and operational use of cleanrooms.

7. The Case for CRR

Chapter <1116> emphasizes that if human operators are present, microbial contamination at some level is inevitable. The following points on the conventional way to evaluate microbial contamination are discussed:

- Real-time active monitoring of Total Particulate, even if run continuously, does not provide direct information on the microbiological content of the environment.
- Airborne microorganisms are enumerated as cfu, but a great diversity of physical states (single cells, aggregates associated to particles, microbial cells associated to inert particles, etc.) make the counts subject to significant variability.
- A microbial monitoring sample represents only the microorganisms captured during a narrow length of time at a particular location.
- The absence of growth on a microbiological sample means only that growth was not discovered; it does not mean that the environment is free of contamination.
- Numerical differences between Alert and Action Levels have become quite small in ISO 5 and other areas.
- Those differences are not significant considering the large variability in microbiological assay recovery ($\pm 0.5 \log_{10}$) ***(1).***

Based in part to the above points, <1116> proposes a new perspective on environmental control relying on incident rates rather than Action/Alert Levels. Under this proposal, all contamination events (≥1 cfu, including events that exceed and events that do not exceed the level mandated by current aseptic guidance) will be considered for the trending analysis. Could this trending help to improve data analysis and help to maintain a continuous state of control? The answer will need to be tested by comparative analyses of one method versus the new alternative one.

The proposal emphasizes than *"rather than isolated events, analysis of data upon time would detect changes in the contamination recovery rate (CRR) that may be indicative of changes in the state of control within the environment."* Because of the inherent variability of microbial sampling methods and the cfu values, <1116> recommends the use of CRR as a more useful measure of trending results than the number of colonies recovered from a given sample (Tables 1–2). The incident rate is the rate at which environmental samples are found to contain microbial contamination (≥ 1 cfu). For example, an incident rate of 1% would mean that only 1% of the samples taken have any contamination regardless of colony number. In other words, 99% of the samples taken are completely free of contamination.

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

Case Study: Garment Contamination Rates

Garment samples at a large European manufacturing facility were tabulated and trended on an annual basis. There were approximately 100 samples collected per quarter (horizontal axis of **Figures 1** and **2).** Two annual evaluations are shown in Figure 1 (2013 and 2014). In this example, noncontaminated samples were assigned a value of 1, and the samples that were contaminated with cfu values lower than the action limit were assigned a value of 2 (vertical axis of Figures 1 and 2). Samples with values equal or above the Action Level were not observed. In this study, the rate of contaminated samples for 2013 and 2014 were 4% each (Figure 1). It is important to consider that in terms of garment limits, for EU GMP Grade B/ISO class 7 areas, the industry understanding is often to adopt the same limits as per the limits applied to finger plates. Following this common understanding, the Action Level for gowns is ordinarily 5 cfu/25cm2, and the facility complied based on cfu results (all positives were <5 cfu/sample) (as shown in Table 1, top, for the European microbial limit). In addition, this facility also complied based on CRR (Table 2). All positives analyzed on an annual basis presented an incidence rate of 4% —which is a value complying with the <1116> recommendation of <5% limit (Table 2) for grade B.

If the annual CRR is updated on a quarterly basis (see Figure 2), however, then three of the updated trend analyses show noncompliance to the <1116> recommendations. As seen in Figure 2, CRR of 5% is observed for the analysis ending in Q1 of 2014, CRR of 6% is observed for the trend ending on Q2 of that year, and a 5% CRR is again observed now for the trend ending in Q3. In this case, it appears that the rolling quarterly CRR analyses brought a closer and more continuous look at the trending data and it seems to be useful to identifying some loss of control in a more sensitive way than following the more conventional data analysis approach.

Figure 1 Garment: Microbial Monitoring, Annual Evaluation (2013 and 2014)

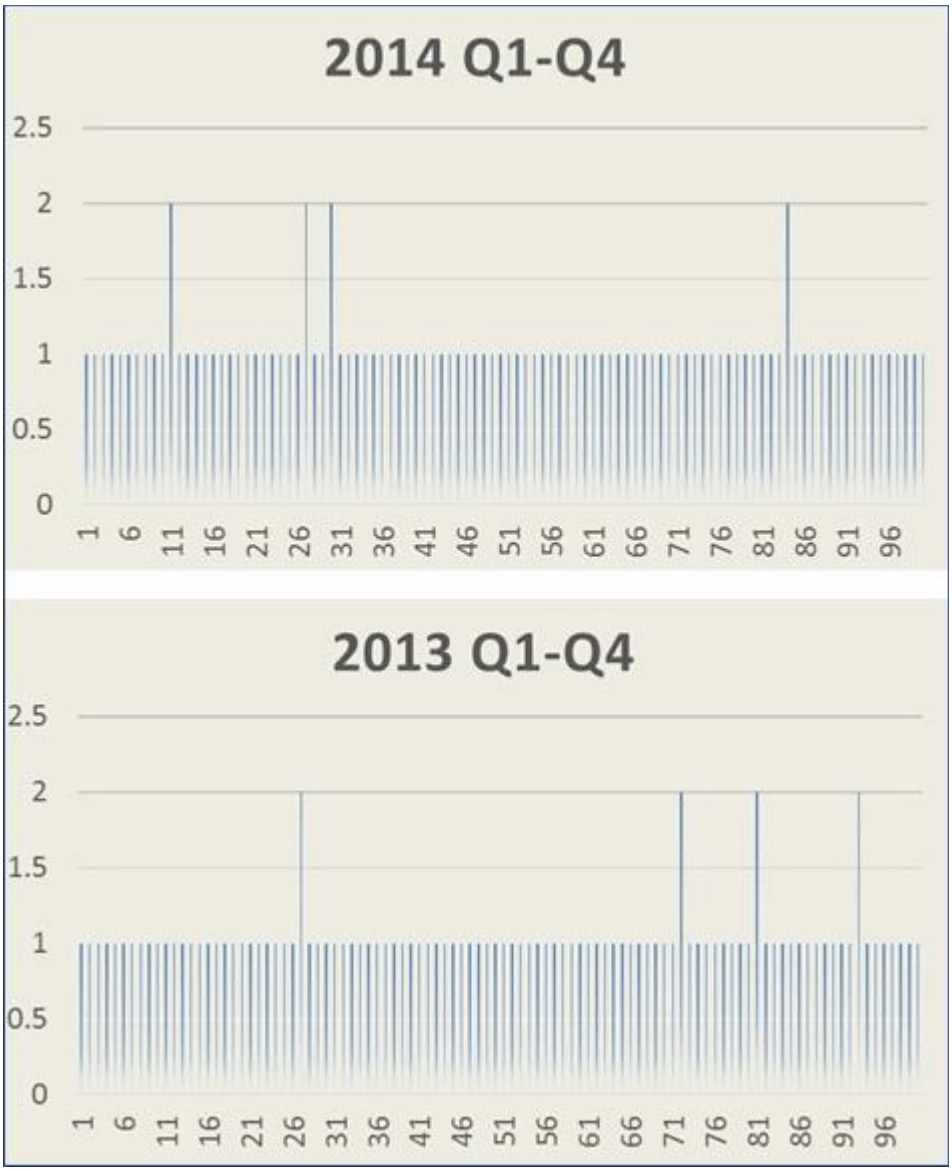
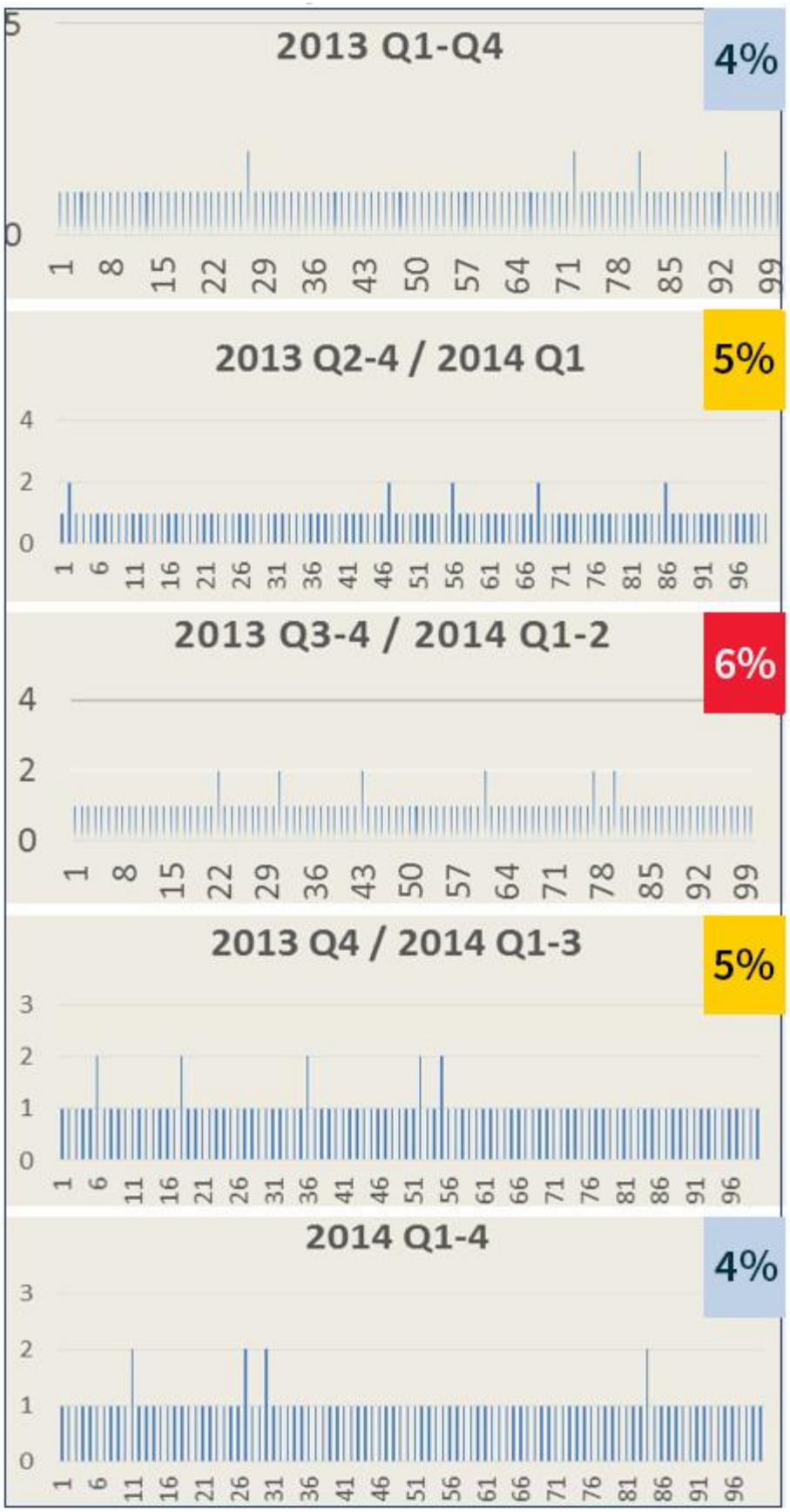


Figure 2 Rolling Contamination Rates (2013–2014, Quarterly)



Conclusions on CRR

- Current guidance on microbial limits for aseptic processing environments is based on cfu.
- Chapter <1116> proposes a new way to look at microbiological data by adopting CRR as percentage value of maximum allowed contaminated samples (those with a number of cfu equal or larger to one).
- The case study illustrates that depending on how the data is looked at (either through the current cfu-based limits or through the proposed CRR-based limits) an environment that was compliant under the first, could become noncompliant under the new limits.
- At very low recovery levels there is no way to establish Alert or Action Levels as statistically significant. Instead, emphasis should be on incidents, even those having just 1 cfu.
- Incident rates in percentage values force us to look historically at least 100 samples back, instead of focusing on just a single current incident, or only on samples showing contamination above Action Levels.
- It also helps to focus on all samples that have any contamination regardless of colony number. There could be a trend indicative of loss of control.
- Even if CRR are adopted as a way to analyze microbial contamination, <1116> emphasizes that for an ISO 5 cleanroom, any excursion of >15 cfu should also be investigated.

Note: This article originally appeared in the PDA Letter, a publication produced by the Parenteral Drug Association. August 27, 2015

References

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