Recommendations from *USP* <1116> on "Contamination Recovery Rates"

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United States Pharmacopeia (USP) <1116> "Microbiological Control and Monitoring of Aseptic Processing Environments" approaches analysis of environmental monitoring data in the aseptic core from a perspective of "contamination recovery rate." This is a more accurate and useful approach when the data consist mainly of "zero". *USP* <1116> suggests using "percent contamination recovery rate" as the measure, but other options are available. USP also suggests the use of quality control (QC) control charts. The use of most probable number (MPN) has been suggested for analysis. This approach may be a more appropriate method given the Poisson distribution of the data and its very low numbers. Limitations of this approach should also be considered. There is a need to track magnitude of excursions as well as trending of microorganism identity throughout the facility.

INTRODUCTION

The *USP* <1116> "Microbiological Control and Monitoring of Aseptic Processing Environments" (1) marks a significant shift in regulatory thinking regarding microbiological monitoring of aseptic areas. This shift leads away from arbitrary numerical levels in these extremely clean environments to a more qualitative trending methodology. In addition to the important information in this chapter on new ways to set alert and action levels for environmental monitoring (EM) programs, this chapter also stresses the separate and important task of control of these environments. The following describe its contents:

- Introduction
- Clean Room Classification for Aseptic Processing Environments
- Importance of a Microbiological Evaluation Program for Controlled Environments
- Physical Evaluation of Contamination Control Effectiveness
- Training of Personnel
- Critical Factors in the Design and Implementation of a Microbiological Environmental Monitoring Program
  - Selection of Growth Media
  - Selection of Culture Conditions
- Establishment of Sampling Plan and Sites
- Selection of Sample Sites Within Clean Rooms and Aseptic Processing Areas
- Microbiological Control Parameters in Clean Rooms, Isolators, and RABS
- Significant Excursions
- Further Considerations About Data Interpretation
- Sampling Airborne Microorganisms
- Surface Sampling
- Culture Media and Diluents
- Identification of Microbial Isolates
- Conclusion
- Appendix/Glossary

USP <1116>

USP <1116> was first proposed in 1991 to add a new general information chapter on the evaluation and classification of clean rooms and clean zones for

aseptic processing (2). This *Pharmacopeial Preview* was extensively reviewed and expanded in 1995 (3) and moved to an *In-Process Revision*. This version drew a good deal of commentary, and another *In-Process Revision* was published (4) with the following note:

"Following the USP Open Conference on Microbiological Compendial Issues in January 1995, the Microbiology Subcommittee has made substantive changes to the proposed information chapter: The scope of the chapter has been clarified, and the suggested frequency of sampling controlled environments has been modified. The Subcommittee has reviewed the arguments for the deletion and retention of the various action levels and has decided to include them as information in this chapter."

This version generated a great deal of commentary as well, including a thoughtful commentary by PDA (5). As noted by USP:

"The number and scope of comments indicate a strong interest in this issue and a need for this type of information in the USP. The Subcommittee has reviewed all comments, and the changes that they felt were appropriate were made. These proposals are slated for implementation in the Eighth Supplement to USP 23-NF 18, with an official date of May 15, 1998" (6).

USP proposed an additional revision to this chapter in 1999 with an expanded scope to include isolator environments and the use of controlled environments for aseptically manufactured sterile drugs. The proposed revisions introduced guidelines for product contact surfaces and critical zone surfaces and expand the discussion on surface monitoring (7). Parenteral Drug Association (PDA) remained concerned about this informational chapter, and immediately published a lengthy list of comments, along with a plea for USP to host an open conference in collaboration with PDA to discuss these issues (8). The next open conference was held in Sanibel Harbor, Florida and dealt extensively with harmonization–a topic that was to consume a very large amount of resources for the next five or six years. This chapter remained unchanged with no *In-process Revisions* published until 2005 (9).

A major development in this area occurred with the publication of the US Food and Drug Administation's *Aseptic Processing Guidance 2004* (10). In addition, USP's Microbiology Subcommittee underwent some changes with a new chairman elected. A new proposal for *USP* <1116> was released with the following justification: "On the basis of comments received, elimination of Federal Standard 209 E, and advances in the field, it is proposed to revise and clarify this general information chapter. To reflect these changes, the title of the chapter has been changed to Microbiological Control and Monitoring Environments Used for the Manufacture of Healthcare Products."

Little happened with this proposed revision, and a totally new revision was proposed in 2010 that laid the groundwork for a complete revision of the chapter. Among other changes, the position was taken that trending in the aseptic core (as well as surrounding areas) might better be performed by analyzing for "contamination recovery rates" (e.g., samples that returned microbial counts greater than zero) rather than looking at specific numbers (11).

Contamination Recovery Rates versus Numerical Levels

USP <1116> defines contamination recovery rate as the percentage of plates that show any microbial recovery irrespective of number of cfu. The glossary in *USP* <1116> defines this term:

"The contamination recovery rate is the 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."

The alert and action levels are then defined relative to these percentages. The user is encouraged to

collect data and set these averages for the specific facility and sample site .

This is in sharp contrast to currently accepted levels of contamination listed in the FDA Guidance (see Table I) as well as other guidance documents (10). The FDA *Aseptic Processing Guide* is used only for illustrative purposes without any intention of singling this document out for special mention. All current regulatory guidance in aseptic processing from Europe and the United States, as well as trade industry technical reports, repeat these, or very similar action levels. This break with accepted dogma may be the single greatest contribution of this chapter revision. A grade B (Class 1000, International Organization for Standardization [ISO] [6]) great significance is placed on a result of 6 cfu versus 7 cfu (pass/fail) in active air monitoring, or 2 cfu versus 3 cfu in settling plates. All of these numbers are well within the noise level of the plate count method.

Why the Major Change in Focus?

When looking at numerical limits for microbiological tests, the problem is that the levels have to be reasonable in terms of the capability of the method. This leads directly to the question of the linear range of plate counts. *USP* <1227> (1) relies heavily on the established scientific literature in its discussion of this range of countable colonies on a plate (12, 13) to note that colonies have a lower limit of quantification of approximately 25 colonies per plate. This is opposed to the limit of detection of one colony per plate. EM alert and action levels in the 1–10 cfu range are therefore of questionable accuracy.

There is a real need for better quality tools, and this need has led to the shift to "contamination recovery rates" rather than arbitrary cfu numbers as proposed levels. This chapter is now official (14), and contamination recovery rates appear in tables of suggested levels for different classes.

The table "Suggested Initial Contamination Recovery Rates in Aseptic Environments" suggests initial rates (percent contamination–non-zero–samples) in different areas. The obvious method of implementation for these rates is on a rolling average, but it is left to the operator to determine the appropriate interval for this average.

In addition to the contamination recovery percentage, the role of "significant excursions" (i.e., excursions of approximately 15 cfu on a plate) is discussed. The chapter provides a good discussion of how to evaluate these events for significance, as well as general input on methodology, finishing with a glossary of terms.

While there may be some difficulty with using this particular measure (contamination recovery rates) as a trending tool (see discussion below on EM data as normally distributed or in a Poisson distribution), the great contribution of this chapter has to be the recognition that current EM criteria in the aseptic core is completely arbitrary and contrary to good science. Making critical decisions on the state of control of a facility based on numbers well into the noise range of the assay is unwise. A different method of analysis for these data should be developed, and *USP* <1116> describes one such method–and is the first regulatory document to ad-

TABLE: Suggested initial contamination recovery rates in aseptic environments (Table 3 of *USP* <1116> [1]).

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

dress this question from a valid estimation of the plate count method capabilities. In addition, this analysis fits in well with the FDA recommendation that "Increased incidence of contamination over a given period is an equal or more significant trend to be tracked (10)."

There are other recommendations in the literature on how to address EM data from aseptic core areas where the predominant result will be "zero cfu." Two recent publications are relevant considering environmental monitoring data.

OTHER METHODS OF TRENDING "NON-ZEROES"

Caputo and Huffman

In 2004, Caputo and Huffman proposed two methods to trend EM data from aseptic areas. Like *USP* <1116>, they note that most data are "zero" from these areas, and this makes any type of data analysis difficult. They also stress that in many cases, the magnitude of an individual excursion is less informative than the frequency with which contamination occurs.

Both of the methods proposed use the individual value/moving range (I-MR) control chart and the exponentially weighted moving average (EWMA) control chart. To test their proposed methods, Caputo and Huffman generated a normally distributed data set of values around 10 day intervals (n=100) and around eight day intervals (n=85) of "non-zero" readings. As both of the graphing methods are appropriate for normally distributed data, both methods worked admirably with this data set (14).

This study is noteworthy as it is the first formal treatment of the use of contamination rate (e.g., the frequency of "non-zero" readings) to trend EM data. In this, it is a great step forward beyond the use of arbitrary numbers located deep in the noise range of the plate count method.

The difficulty with this method is that while it is admirably suited for use with data that follow a "normal" distribution, it may not be appropriate for data that follow a Poisson distribution (such as EM data [15]). Sun et al might have recommended a more appropriate model (16).

Sun et al–MPN

In 2006, Sun's group described the use of most probable number (MPN) technology for trending bacterial counts in EM data. Their discussion begins with an excellent introduction to the MPN method, with specific emphasis on the Halverson and Ziegler equation, as this forms the basis for their data analysis (17, 18). From this base, they develop a compelling argument for the use of this MPN method as follows:

- It is appropriate for data following a Poisson distribution.
- It is computationally straightforward.
- It yields numerical estimates more accurate (and more sensitive) than averaging when the contamination rate is <0.2%.
- It allows trending of "numerical" data.

They tested this method using data generated from two main areas. EM data from an aseptic manufacturing suite (grade B) included active air monitoring data and surface (floor) samples. Passive monitoring (settle plates) collected EM data from a laminar flow hood in a test lab. All data sets met the χ^2 test for goodness-of-fit with a Poisson distribution.

The method is used "...to analyze the trend of the environmental monitoring data. The raw ... are grouped choosing the minimum total sampling number n, and at least including one positive sample. This calculation will result a maximum MPN when a positive count is observed, thus increasing the sensitivity of the monitoring (17)."

The MPN equation used here is:

$$MPN = 2.303 * \log_{(10)} \frac{Total.in.Group}{Zeroes.in.Group}$$

The MPN estimate is equal to 2.303 times the $\log_{10}$ value of the ratio of the total number of readings in the group divided by the number of readings in the group of no recovery. While this method is described in the text as a method to trend by dates, this can also be used to trend by operators, locations, and other factors.

TRENDING REQUIREMENTS IN ASEPTIC CORE

It is important to remember that this method is restricted to trending and alert/action levels for "quantitative"

EM only. While it will admirably suit FDA expectations for trending of "data generated by location, shift, room, operator, or other parameters (10)", it will not meet expectations for trending programs of microorganisms (by identity or by characteristic such as trending spore forming microorganisms) as a check on the sanitization program. In addition, as pointed out in *USP* <1116>, it is also important to track and trend significant excursions as part of the EM program.

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