

# USP <1116> and Contamination Recovery Rates

Scott Sutton

## ABSTRACT

*United States Pharmacopeia (USP) <1116> "Microbiological Control and Monitoring of Aseptic Processing Environments"* approaches analysis of environmental monitoring (EM) data in the aseptic core from a perspective of "contamination recovery rates" while noting a need to improve EM data analysis.

There are two difficulties in using EM data from the aseptic core:

- Most of the plate counts should be "zero"
- The actual numerical plate counts seen are far below the limit of quantification (LOQ) for the plate count method.

USP <1116> suggests using "percent contamination recovery rate" as the measure, but other options are available:

- The use of quality control (QC) control charts has been suggested for these data
- The use of most probable number (MPN) analysis has been suggested for analysis of these data—this 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; some tracking of magnitude of excursions as well as trending of microorganism identity throughout the facility is needed.

## INTRODUCTION

The *United States Pharmacopeia (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 controlling these environments. The following outlines this chapter:

- 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

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- Identification of Microbial Isolates
- Conclusion
- Appendix/Glossary.

### **USP <1116> MICROBIOLOGICAL CONTROL AND MONITORING OF ASEPTIC PROCESSING ENVIRONMENTS**

The concern about reliable alert and action levels for the aseptic core hinge on two considerations:

- The limit of quantification for the plate count method
- The prevalence of "zero" in the data set.

This paper will first look at the issues in the limit of quantification.

### **LIMIT OF DETECTION VERSUS LIMIT OF QUANTIFICATION**

The general ranges in common acceptance for countable numbers of colonies on a plate are 30–300 or 25–250 colony forming units (cfu). Breed and Dotterer noted that, "the kind of bacteria in the material under examination will have an influence on the size of the colonies, and consequently, on the number that can develop on a plate." They also noted that food supply can be an issue, that colonies close to each other on the plate may merge, and that neighbor colonies may inhibit growth or conversely stimulate growth.

"Because of these and other difficulties certain plates in any series made from a given sample are more satisfactory for use in computing a total than are others. The matter of selecting plates to be used in computing a count becomes therefore a matter requiring considerable judgment (2)."

This study determined that plates with more than 400 cfu were unsatisfactory, as were those of less than 30 cfu, with best results in the range of 50–200 cfu/plate (2). From this study originated the 30–300 rule for the "countable" range of colonies on a plate.

Tomasiewicz et al reevaluated the question of countable range on a plate, again taking data from colony counts of raw milk. This study used data from three different experiments (each dilution plated in triplicate) to determine a mean-squared-error of the estimate for all plates. Their recommendation, at the end of the study, was for a countable range of 25–250 cfu/plate in triplicate. This study is also notable for *excellent cautionary advice*.

"The data presented herein are not necessarily applicable to other systems. For automated equipment, the optimum range may well vary with the instrument...Furthermore, even if automation is not used appropriate numbers of colonies that should be on a countable plate can vary widely, depending on many other variables. With soil fungi for example...(3)"

USP has recently harmonized a microbial enumeration test (4). This test recommends, "Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for [total aerobic microbial count] TAMC and 50 for [total combined yeast and mold] TYMC. In determination of the resistance of biological indicators, USP recommends a range of "20 to 300 colonies, but not less than 6 (5)." However, the most complete description of the countable range is found in the informational chapter <1227> (6).

"The accepted range for countable colonies on a standard agar plate is between 25 and 250 for most bacteria and *Candida albicans*. This range was established in the food industry for counting coliform bacteria in milk. The range is acceptable for compendial organisms, except for fungi. It is not optimal for counting all environmental monitoring isolates. The recommended range for *Aspergillus niger* is between 8 to 80 cfu per plate. The use of membrane filtration to recover challenge organisms, or the use of environmental isolates as challenge organisms in the antimicrobial effectiveness testing, requires validation of the countable range (6)."

ASTM provides countable ranges of 20–80 cfu/membrane, 20–200 for spread plates, and 30–300 for pour plates (7). The US Food and Drug Administration's *Bacterial Analytical Manual* (BAM) recommends 25–250 cfu/plate as a countable range (8).

The general consensus of these documents is that, at best, the limit of quantification (LOQ) for the plate count method is no less than 20 cfu/plate. This leads to direct problems with the currently accepted regulatory levels that are set near the limit of detection (1 cfu/plate) rather than the LOQ (9).

### **CONTAMINATION RECOVERY RATES VERSUS NUMERICAL LEVELS**

Contamination recovery rates in USP <1116> are defined as the percentage of plates that show any microbial recovery irrespective of number of cfu. This term is defined in the glossary of USP <1116>.

**Table: Suggested initial contamination recovery rates in aseptic environments.**

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

This table is reproduced from Table 3 of *USP <1116>* (1)

"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 (see Table for suggested contamination rates).

This is in sharp contrast to currently accepted levels of contamination listed in the 2004 FDA guidance (10) as well as other guidance documents where, in a grade B (class 1000, International Organization for Standardization [ISO] cleanroom six), 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. Note, 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.

#### WHY THE MAJOR CHANGE IN FOCUS?

The problem with looking at numerical limits for microbiological tests is that the levels have to be reasonable in terms of the capability of the method. *USP <1227>* (1) relies heavily on the established scientific literature in its discussion of this range of countable colonies on a plate (2, 3) to note that colonies have a lower LOQ 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 (1), and these contamination recovery rates appear in tables of suggested levels for different classes.

The table on suggested initial contamination recovery rates in aseptic environments (reproduced above) 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, a general input on methodology, and 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; it is the first regulatory document to address this question from a valid estimation of the plate count method's 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." In specifically considering environmental monitoring data, one can look at two publications in the recent decade.

## OTHER METHODS OF TRENDING "NON-ZEROES"

### Caputo and Huffman

Caputo and Huffman propose two methods to trend EM data from highly 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 well-known quality control (QC) graphing techniques, 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 (n=100) and eight-day intervals (n=85) intervals of "non-zero" readings. As both of the graphing methods are appropriate for normally distributed data, both methods worked admirably with this data set.

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 (11).

The difficulty with this method is that, while it is admirably suited for used with data that follow a "normal" distribution, it may not be appropriate for data that follow a Poisson distribution (such as EM data) (12). A more appropriate model might be that recommended by Sun et al (13).

### Sun et al

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 (14); this forms the basis for their data analysis. From this base, they develop a compelling argument for the use of this MPN method:

- 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) that included active air monitoring data and surface (floor) samples; EM data from a laminar flow hood in a test lab that was collected passive monitoring (settle plates) collected. All data sets met the chi-squared 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 (12)."

The MPN equation used here is:

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

Or, 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. Now, 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.

### Other 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 (e.g., 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.

### CONCLUSIONS

The common regulatory method of setting microbiological alert and action levels for the environmental monitoring program is not scientifically defensible as the arbitrary levels are set well within the noise level of the plate count assay. The recent revision of *USP* <1116> "Microbiological Control and Monitoring of Aseptic Processing Environments" addresses this with

the strong recommendation that these questionable levels be replaced by trending "zero" and "non-zero" in these cases. The trending tool recommended is the use of "recovery frequency" measures.

Two other methods of analysis are discussed in this review that might be useful in trending data from the aseptic core and in qualifying the alert and action levels. One method uses QC charts (11) and the other a variant of the MPN method (13). The MPN method was validated using actual EM data, and it would be this paper's recommendation for a useful method of qualifying alert and action levels and trending microbiological EM data in the aseptic core.

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**JVT**