Practical Application of Rapid the USP<1116> Contamination

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The past year has seen a change in the way pharmaceutical manufacturers monitor their aseptic environments with the most recent revision to the guidance chapter <1116> in USP 35/NF 30, now entitled "Microbiological Control and Monitoring of Aseptic Processing Environments."[1] One of the major changes within this chapter is guidance for the assessment of contamination recovery rates (CRR) for environmental monitoring (EM) data, moving away from the Alert and Action level concept. The USP defines contamination recovery rate as "the rate at which environmental samples are found to contain any level of contamination."[2] Use of the CRR recognizes the limitations of traditional microbiological tests, creates a paradigm shift away from long-standing guidance in environmental monitoring, and places a focus on the evaluation of trends versus comparison against the standard colony forming unit count approach. The CRR concept also moves the industry closer to utilizing a strategy that does not rely on a single test methodology, and instead focuses on the incidence of contamination occurrences, making this USP revision highly conducive to implementation of more cost-effective and sensitive microbiological methods that speed the time-to-result and reduce sampling bias.

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
All operators are aseptically gowned in these environments (with the exception of background environments for isolators). These recommendations do not apply to production areas for nonsterile products or other classified environments in which fully aseptic gowns are not donned.				

Figure 1: Suggested initial Contamination Recovery Rates in aseptic environments (excerpted from USP <1116>, Table 3).[2]

Traditional EM methodology and limitations

The method of bacterial detection typically used by the pharmaceutical microbiologist has been the Heterotrophic Plate Count method (HPC), which is defined by the detection of individual bacterial colonies from an environmental sample (air, water, surface, etc.) that may grow on a Petri dish, also known as colony forming units (CFUs). Interestingly, the CFU is a derivative quantity; the individual CFU from the HPC method represents merely a subset of the target measurement (microbes in the environment), rather than directly counting that quantity. There are two factors for this disparity: first, the HPC incubation conditions do not support recovery of all organisms (this concept has been extensively treated in literature; refer to citations 3, 4, 5, and 6 for a sampling of sources), and second, the probabilistic nature of a single finite sample, which cannot represent an entire environment in space or time.

It is tempting to believe that the CFU is an actual quantity that can be measured reproducibly. After all, the test yields a whole-number count, and tests over time in the same location using the same techniques typically give similar results. As volume and/or microbial concentration decrease, however, it becomes harder and harder to detect low levels of organisms using the HPC method. Broadly speaking, the number of samples needed to accurately quantify the microorganisms in an environment is inversely related to the concentration of microbes. Yet, these tests are valuable in that a detection of trend is possible, though the fidelity of that trend depends on the reproducibility of the sampling and test regime being employed.

Yielding to the temptation of the CFU

Trend detection is the rationale given for the use of this traditional approach, even in light of the limitations. In the USP 23/NF 18 revision of <1116>, it was deemed critical that "…an environmental monitoring program be capable

Microbiological Methods to Recovery Rate Approach

of detecting an adverse drift in microbiological conditions in a timely manner that would allow for meaningful and effective corrective actions." The problem arises when one tries to summarize the state of quality of an environment with a particular CFU count. Nevertheless, there still remains a temptation to assign a critical weight to the CFU values generated from these samples, and to ascribe a level of quality to the data generated. This represents one of the fallacies of microbiological monitoring: that the quality of the product being manufactured is represented by the measured environmental quality. An HPC result of "zero" does not represent an environment that is sterile or free of objectionable organisms. Rather, it indicates that trends otherwise detected at higher cleanroom grades or ISO levels cannot be detected at the low concentrations encountered in that environment.

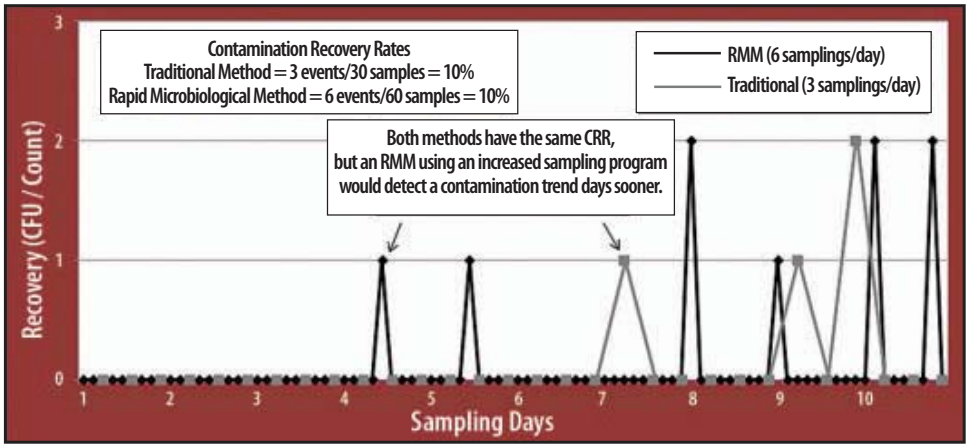

Figure 2: An illustration of sample results in counts vs. days sampled. With increased quantity and frequency of samples collected using rapid microbiological methods, as compared to traditional methods, a CRR may be compiled more quickly and precisely.

Alert and Action levels, as indicated by the previous iteration of USP <1116>, reflect microbiological recoveries for which a certain activity (such as investigation) needs to occur. The temptation here is the belief that the lower the CFU counts are, the fewer remediation activities need to be performed. Taking this to its logical conclusion, CFU counts of zero would then be considered ideal. If one looks closer at the statistics involved with using the HPC method in these clean environments, however, the low counts begin to have a high rate of standard deviation, ranging from 20% at a mean CFU count of 25, to 100% at a mean CFU count of one.[7,8] In accordance, counts generated in cleanrooms over long periods of time begin to resemble not a standard quantification of organisms, but rather a series of positive or negative incidence data, similar to tests performed using the most probable number (MPN) method. Probabilistic effects are apparent, and while collecting more samples or greater sample volumes may alleviate some of these statistical issues, it is very resource-intensive, to the point of impracticality.

Contamination recovery rates: Refocusing on science

With the USP 35 version of <1116>, a new paradigm of trend analysis has been proposed, called the Contamination Recovery Rate (CRR) approach. The paradigm is simple—in a given data set of samples over a monthly time period, a certain percentage of those samples can be expected to exhibit non-zero recoveries of contamination; the percentage of samples presenting contamination is then defined as the Contamination Recovery Rate. These percentages are listed in Figure 1.

Additionally, there is guidance within <1116> for significant excursions which considers that CFU counts in excess of 15 from a single ISO 5 environmental sample generally indicate that a significant change has occurred in that environment, as the occurrence of counts of that magnitude should generally be infrequent. Nonetheless, the chapter again stresses that the number, in and of itself, is not important (the chapter illustrates this by stating that a CFU count of 14, or even 25, is not scientifically different from a count of 15), but rather environmental changes of a significant nature are important.

In discussing the reason for change in <1116>, the chapter describes how "growth and recovery in microbiological assays have normal variability in the range of ±0.5 $\log_{10}$," explicitly highlighting traditional microbiological method limitations as they relate to statistical error.[2] The CRR approach takes into account these limitations by removing the CFU concept, and instead relies

on the simple presence or absence of contamination. In this way, the CRR becomes very similar to the MPN method, but rather than evaluating a single sample with multiple replicates, it suggests looking at repeated samples in a room or sample site over a longer period of time. In this way, a contamination rate is more accurately approximated by using greater quantities of samples.

The CRR approach, however, still accommodates existing EM data collected through traditional means, and therefore, has limitations; in this case, the statistical variability due to the nature of the traditional microbiological methods. Additionally, the CRR approach may utilize the same sampling frequencies as before, so the underlying and often incorrect assumptions of a consistent and heterogeneous environment between samples still exist.

The application of rapid microbiological methods to the contamination recovery rate paradigm

For over a decade, proposed alternate or rapid microbiological methods (RMMs) have yielded innovative ways to reduce the time it takes to collect a sample, perform a test, and analyze data. Traditional methods rely on microbial growth, so RMMs like Azbil BioVigilant's IMD-A instantaneous microbial detection system create a genuine opportunity to provide a faster time-to-result.[9]

When a faster time-to-result is considered as the sole benefit of RMMs, however, routine RMM use may not seem justified, especially in light of traditional paradigms that are benchmarked against CFU analysis.

Shifting from Alert and Action Levels to the CRR moves the focus from magnitude to frequency, which is independent of test methodology. It also favors aggregating large data sets and frequent sampling. More frequent EM sampling may reveal one of two scenarios: first, if the CRR stays the same after the sampling frequency increases, then this demonstrates the fitness of the existing sampling plan. If the CRR increases, however, then the increased frequency may indicate that the previous sampling plan contained bias that artificially kept the recovery rate low. The implementation of RMMs facilitates the collection and processing of samples, not for strictly time-to-result reasons, but instead to gather samples more frequently and reduce bias in a more cost-effective way.

For example, during a typical day of pharmaceutical manufacturing, a site within a cleanroom may be sampled once per shift. For that location, three data points each day are generated with an associated CFU result. An issue arises, however, when it is assumed that these three data points represent the totality of microbiological quality for that site throughout the day. This sampling regime only creates three individual 'snapshots in time' of the microbiological activity in that location. A month's worth of data in this location would yield about 90 individual sample points from which a rate could be derived. Yet, because aggregation of data is required to determine a true rate, accurate conclusions can only be drawn about the localized trend after long periods of time. A key benefit of utilizing RMMs then becomes one of increasing this sampling frequency, so that the contamination rate can be determined more quickly and precisely. New RMMs are uniquely suited to this task, as they can sample at faster rates than traditional methods, often at lower cost-per-test, with some having the capability to sample remotely. Ultimately, by developing a way to assess a contamination rate daily, or even continuously using a rolling CRR analysis approach (enabled by continuous RMM data), manufacturers are empowered to maintain the manufacturing environment with a higher degree of control, detection, and responsiveness. Such an implementation allows the industry to meet the goals of an EM program guided by USP<1116>, "to demonstrate that that the aseptic monitoring environment is operating in an adequate state of control." [2]

Contamination recovery rates support process understanding

As pharmaceutical manufacturing processes continue to improve, the tools and statistics by which pharmaceutical manufacturers monitor and analyze those processes must also improve. Ready RMM tools exist with which to monitor these processes in an enhanced way, but their implementation has been limited by long-existing sampling and trending paradigms, which ultimately hamper process knowledge and control.

The revised USP <1116> guidance has refocused the industry on the goal of gaining knowledge and understanding of processes, to achieve a higher state of control, and ultimately a higher state of quality assurance.

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