

Qualification of an Environmental Monitoring Program

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"Microbiology Topics" discusses various topics in microbiology of practical use in validation and compliance. We intend this column to be a useful resource for daily work applications.

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KEY POINTS

The following key points are discussed in this article:

- The number of sites to be used in qualifying clean rooms for non-viable particulate measurements can be found in International Organization for Standardization (ISO) 14644-1. However, there are no recognized standards for determination of this number for viable air (passive and active) nor for surface monitoring. A method is suggested in this article
- Recommendations for the selection of sample sites to be used in the qualification program are provided. These recommendations are directed at providing data to allow creation of a program useful in determination of the state of control of the facility
- The frequency of sampling during a qualification study of this type should minimally be at least the rate of the eventual routine monitoring program for each area

- The qualification study should provide data to allow determination of meaningful alert and action levels for that facility. It must be noted that there are significant technical and scientific issues with the regulatory guidelines for the areas of an aseptic core region. A suggestion consistent with the proposed revisions to *United States Pharmacopeia* chapter <1116> "Microbiological Control and Monitoring Environments Used for the Manufacture of Healthcare Products" is provided
- The qualification program is an excellent opportunity to begin the study of the microbial flora in your facility.

INTRODUCTION

The qualification, or requalification, of an aseptic manufacturing facility depends in large part on the demonstration of controlled microbial conditions. The following are several areas where this is especially true:

- Cleaning studies
- Contamination control planning (1)
- Equipment hold-time studies (i.e., establishment of clean and dirty hold times—process hold times are process-specific)
- Selection of sample sites for environmental monitoring
- Establishment of facility-relevant alert and action levels for controlled environments.

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This article focuses on a method to qualify and justify the selection of the sample sites within a facility used for routine environmental monitoring. The discussion presented by the author is not meant to describe the only possible approach to this selection but rather one that the author has used in the past with success. Due to the limitations of space, this discussion does not include sampling of the water system, gasses, or personnel that have distinct considerations.

NUMBER OF SITES FOR QUALIFICATION STUDIES

ISO 14644-1 describes a method to determine the number of sampling sites for site qualification. Annex B states that we should determine the minimum number of sample sites by the following equation (2):

$$N_L = \sqrt{A}$$

Where, N_L is the minimum number of sampling locations (rounded up to a whole number).

A is the area of the clean room or zone in meters².

This might work well enough for non-viable particulate measures (which is the intent and scope of ISO 14644-1), but we also wish to consider viable air sampling (both passive and active) and viable surface monitoring. Frequently, the sample site study is worked into the facility HVAC performance qualification study for ease of documentation and logistic considerations. Let's make this a bit easier and argue that for the initial facility HVAC qualification protocol, both viable and non-viable active air sampling sites should be done at the same locations (or as close as practical to avoid compromising the other measure or the product integrity). This leaves determination of the number of sites for passive air sampling and surface sampling.

Passive air sampling (i.e., settle plates) is a frequently-used measure of clean room (or controlled zone) monitoring. Settle plates have several advantages in this regard, chief among them the ability to remain in continuous exposure for up to four hours. Four hours is cited in the European Union (EU) guidance (3)—extended exposure times must be demonstrated via demonstration of the growth promoting capabilities of the aged and exposed media. In addition, passive viable monitoring (settle plates) is not disruptive to the immediate environment and may possibly sample sites very near product exposure points (4). In addition, settle plates are not as prone to variation among

different vendors as are active samplers (5). However, it is not clear whether all the advantages cited for passive sampling apply in areas of laminar air flow at the rates used for modern clean rooms. In addition, settle plates may be particularly susceptible to handling, transport, and lab contamination. However you view their usefulness, current regulatory expectation for air monitoring includes the use of settle plates and the justification of sampling sites. A prudent measure is to use the same number of sampling sites for settle plates as used for the active viable and non-viable sampling programs. These will not be the same sites, but similar in number.

This leaves us with determination of the number of surface sampling sites for the qualification study. There is no regulatory guidance directed to this point for the international pharmaceutical industry. Even the Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S), which generally can be counted on to provide details on almost everything microbiological, is silent on this point (6). Oddly enough, even the Parenteral Drug Association (PDA) *Technical Report #13* (7) offers no help here. We are left to our own devices. One approach to determination of the number of sites would be to address it in a manner similar to that of ISO 14644-1 for the walls and floors (as relevant). Each surface would then be treated as a separate item and the minimum number of sites determined for each. While this might work for walls and floors, the number of surface sampling sites for equipment remains unanswered and is not noticeably amenable to this approach. This, quite frankly, may well be something that must be left to determination at each individual site—the numbers could be driven by the nature of the equipment and the associated manufacturing process.

Having determined the number of sites for each room, we now need to determine their location. It should be obvious that using the "square root of the area" method will frequently yield a larger number of sample sites than is needed for routine monitoring, but that is appropriate for a validation study. Use the data collected from this "over sampling" to determine the appropriate sample sites.

SELECTION OF SAMPLE SITES FOR THE QUALIFICATION STUDY

The selection of sample sites should be designed to provide useful information for eventual selection of routine sample sites.

PDA. PDA *Technical Report #13* (7) provides the following useful guidance in this regard:

"Factors to consider in selecting sites for routine surveillance are:

1. At which sites would microbial contamination most likely have an adverse effect on product quality?
2. What sites would most likely demonstrate heaviest microbial proliferation during actual production?
3. Should site selection involve a statistical design (e.g., following the calculations in Federal Standard 209E) or should site selection be made on the basis of grid profiling? Should some sites for routine monitoring be rotated? [Note from author: As 209e has been withdrawn in favor of ISO 14644, the answer is "No"]
4. What sites would represent the most inaccessible or difficult areas to clean, sanitize, or disinfect?
5. What activities in the area contribute to the spread of contamination?
6. Would the act of sampling at a given site disturb the environment sufficiently to cause erroneous data to be collected or contaminate product?"

FDA. The US Food and Drug Administration's aseptic processing guidance document (8) also provides some guidance in section IVA:

"Air in the immediate proximity of exposed sterilized containers/closures and filling/closing operations would be of appropriate particle quality when it has a per-cubic-meter particle count of no more than 3520 in a size range of 0.5 μm and larger when counted at representative locations normally not more than one foot away from the work site, within the airflow, and during filling/closing operations. This level of air cleanliness is also known as Class 100 (ISO 5).

"We recommend that measurements to confirm air cleanliness in critical areas be taken at sites where there is most potential risk to the exposed sterilized product, containers, and closures. The particle counting probe should be placed in an orientation demonstrated to obtain a meaningful sample. Regular monitoring should be performed during each production shift. We recommend conducting nonviable particle monitoring with a remote counting system. These systems are capable of collecting more comprehensive data and are generally less invasive than portable particle counters. See Section X.E. for additional guidance on particle monitoring.

"Some operations can generate high levels of product (e.g., powder) particles that, by their nature, do

not pose a risk of product contamination. It may not, in these cases, be feasible to measure air quality within the one-foot distance and still differentiate background levels of particles from air contaminants. In these instances, air can be sampled in a manner that, to the extent possible, characterizes the true level of extrinsic particle contamination to which the product is exposed. Initial qualification of the area under dynamic conditions without the actual filling function provides some baseline information on the non-product particle generation of the operation."

Further, Section XA states:

"Sample timing, frequency, and location should be carefully selected based upon their relationship to the operation performed..."

"It is important that locations posing the most microbiological risk to the product be a key part of the program. It is especially important to monitor the microbiological quality of the critical area to determine whether or not aseptic conditions are maintained during filling and closing activities. Air and surface samples should be taken at the locations where significant activity or product exposure occurs during production. Critical surfaces that come in contact with the sterile product should remain sterile throughout an operation. When identifying critical sites to be sampled, consideration should be given to the points of contamination risk in a process, including factors such as difficulty of setup, length of processing time, and impact of interventions."

EU. The EU guidance document "Manufacture of Sterile Medicinal Products" (3) provides the following site selection guidance:

"18. Where aseptic operations are performed monitoring should be frequent using methods such as settle plates, volumetric air, and surface sampling (e.g., swabs and contact plates). Sampling methods used in operation should not interfere with zone protection."

USP. Similarly, the following guidance in the proposed revision to *United States Pharmacopeia* (USP) chapter <1116> (9) is of general interest:

"Microbiological sampling sites are best selected when human activity during manufacturing operations are considered. Careful observation and mapping of a clean room during the qualification phase can provide information concerning the movement and positioning of personnel within these rooms. Such observation can also yield important information about the most frequently conducted manipulations and interventions.

“Other areas of concern relative to introduction of contamination into clean rooms are at entry points where equipment and materials move from areas of lower classification to those of higher classification. Therefore, areas within and around doors and airlocks should be included in the monitoring scheme.”

I would like to distill from these different sources some specific considerations for sample site selection in the qualification study. After the minimal number of sites in a room is determined, their most useful location must be determined. This determination should be documented in a written justification and should consider the following:

- Contamination vectors (e.g., handles, control panels, doors, etc.)
- High traffic areas
- Personnel flow
- Material flow
- Waste flow
- Surfaces that are difficult to disinfect
- HVAC returns
- Product risk
- Extent of product exposure
- The type of activity performed near that site
- Interventions and manipulations
- Surfaces that are difficult to disinfect.

SAMPLING FREQUENCY FOR THE QUALIFICATION STUDY

The sampling frequency for the qualification of a specific controlled environment should, in general, follow that of the regulatory recommendations for that level of control. This is a matter of some discussion as the recommendations in Europe (EU Annex 1), ISO, and the US (USP) are not in complete agreement. The qualification study may benefit from more frequent sampling and under more conditions (e.g., sampling under both dynamic and at-rest conditions) than are planned for the routine monitoring program.

The environmental monitoring (EM) qualification protocol should allow for the sampling frequency to be described in detail and justified from the perspective of generating sufficient information under relevant conditions to allow selection of meaningful routine sample sites.

DURATION OF QUALIFICATION STUDY

The duration of the qualification study should be determined by the need to acquire sufficient data and the frequency of testing for that sample site. A site tested on a weekly basis may require three months of

data (approximately 12-14 data points) before enough data are available. The qualification protocol should justify the duration of the study on this basis (and not what best fits the mandated timeline for facility qualification).

SELECTION OF ROUTINE SITES

The qualification study should include sufficient replicates under conditions both “at rest” and “dynamic” to allow identification of sites that provide useful information. It should be clarified that the term “useful information” is not meant to describe those sites that give the most desirable counts but rather those sites that either give the highest counts (i.e., serve as the most sensitive measure of the state of control of the room) or were shown to be appropriately placed to herald a problem in the room. The number of sites in a room or zone should similarly be driven by data generated during this study. Both the number and location of sites or each clean room or zone should be justified in the report from this qualification study.

The following section (X.1.A) from the FDA guidance (8) is relevant for consideration:

“All environmental monitoring locations should be described in SOPs with sufficient detail to allow for reproducible sampling of a given location surveyed. Written SOPs should also address elements such as 1. frequency of sampling, 2. when the samples are taken (i.e., during or at the conclusion of operations), 3. duration of sampling, 4. sample size (e.g., surface area, air volume), 5. specific sampling equipment and techniques, 6. alert and action levels, and 7. appropriate response to deviations from alert or action levels.”

ESTABLISHMENT OF ALERT AND ACTION LEVELS

Data from the qualification study should be used to set the initial operating alert and action levels for the routine environmental monitoring program. A good rule of thumb is that the Alert Level should be at the 95th percentile of observed readings for a given period of time, the Action Level at the 99th percentile (see the PDA *Technical Report #13* for an excellent discussion of setting alert and action levels). While common industry practice is to uncritically accept regulatory recommendations for predefined clean zones, this practice is discouraged in the US (8). There is controversy over the regulatory guidance for highly controlled areas as concern with control levels set so far below the level of quantification for plate count

assays (generally 25-30 CFU per plate, compared with regulatory guidance setting alert and action levels as low as single digits). This concern led USP to suggest a frequency distribution approach for these areas (9). An interesting discussion of this approach can be found in Caputo and Huffman (10).

Whichever approach is chosen to the determination of the initial alert and action levels, they should be one of the deliverables from the EM qualification program.

A NOTE ON THE "MICROORGANISM CATALOG"

FDA has clearly recommended establishment of a listing of common microorganisms found in the aseptic manufacturing environment. This expectation is laid out in section X.B. (8), as follows:

"Characterization of recovered microorganisms provides vital information for the environmental monitoring program. Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for an investigation. Monitoring critical and immediately surrounding clean areas as well as personnel should include routine identification of microorganisms to the species (or, where appropriate, genus) level. In some cases, environmental trending data have revealed migration of microorganisms into the aseptic processing room from either uncontrolled or lesser controlled areas. Establishing an adequate program for differentiating microorganisms in the lesser-controlled environments, such as Class 100,000 (ISO 8), can often be instrumental in detecting such trends. At minimum, the program should require species (or, where appropriate, genus) identification of microorganisms in these ancillary environments at frequent intervals to establish a valid, current database of contaminants present in the facility during processing (and to demonstrate that cleaning and sanitization procedures continue to be effective)."

The EM qualification study is an excellent opportunity to start this catalog and to generate information on the effectiveness of the cleaning and sanitization program from a microbiological perspective. Make sure that the EM qualification program includes relevant evaluations of all organisms isolated from air and surface samples, to the species level.

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ARTICLE ACRONYM LISTING

EM	Environmental Monitoring
EU	European Union
FDA	US Food and Drug Administration
ISO	International Organization on Standardization
PDA	Parenteral Drug Association
PIC/S	Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-Operation Scheme
USP	United States Pharmacopeia