Bioburden Contamination Control: A Holistic Overview

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Introduction

Bioburden contamination control is a critical aspect of pharmaceutical, medical device and personal care product manufacturing and one that is a primary focus of cGMP[1]. This article will attempt to provide an overview of contamination control from a holistic perspective, discussing this control system as a process with different aspects of validation, control and monitoring, each contributing to the final state of control.

The process has various aspects to it. While there might be many different ways to look at these subtopics, we will present the following as a logical breakdown of the concept:

- Buildings and Facilities
- Equipment
- Personnel
- Process
- The Importance of Solid Microbiological Data
- Sterile vs. Non-sterile Manufacturing Concerns

A second important breakdown in understanding these concepts is to clearly differentiate between validation (or qualification), monitoring activities and specific control activities (see Table 1). The need for clear design, planning and validation of processes (or qualification of equipment functions) is evident. However, we should explore the differences between monitoring activities and specific control activities.

As we are dealing with microbiological concerns, we have no direct measure of the level of contamination in our process, facility or equipment. All measures are indirect whether we are counting colony forming units (CFU), most probable number (MPN) or relative light units (RLU). None of these measurements, nor the activities used to generate them, can be viewed as changing the counts – they only provide a measurement. Similarly, treating the area with a sanitizer or a sporicide may be useful in changing the level of contamination, but does not provide a measure of that level. These two different aspects can be thought of as "Gauges" (to measure) or "Dials" (to change amounts) in bioburden contamination control.

Finally, a critical concern in all aspects of bioburden contamination control is a well-structured SOP and Quality system. The golden rule of GMP is "Do what you say, say what you do" and nowhere is this more important than in contamination control. As we will see, the complexity of maintaining a state of control in the process and facility bioburden is beyond the ability of any one individual or even

Table 1. Matrix Overview of Contamination Control

	Validation	Control	Monitoring
Facility	Qualification of the Clean Room area and HVAC System	Maintenance of Facilities Sanitization; Revision of Barriers, Traffic Patterns, or Air Balance	Environmental Monitoring (EM)
HVAC	Qualification of the Clean Room area and HVAC System	Certification and Preventative Maintenance (PM) of System; Repair of HEPA Filters	EM
Water	Qualification of Water System	Certification and PM Regular Sanitization of System	Bioburden Monitoring of Water System
Equipment	Qualification of the Equipment as Suitable for its Intended Use	Certification and PM Regular Sanitization	EM Finished Product Release Testing
Sanitization	Validation of Cleaning, sanitization and sporicidal treaments	Regular cleaning and sanitization of facilities and equipment	EM
Personnel	Proficiency Criteria Participation in Media Fills Trending Data by Operator	Training Discipline	Personnel Monitoring Trending Data by Operator
Process	Process Validation	Acceptance Testing of Raw Materials and Containers	In-process Bioburden Monitoring Finished Product Release Testing

This matrix is not intended to be a comprehensive listing of all validation, control and monitoring activities of importance but rather to underscore the point that these are separate and distinct activities.

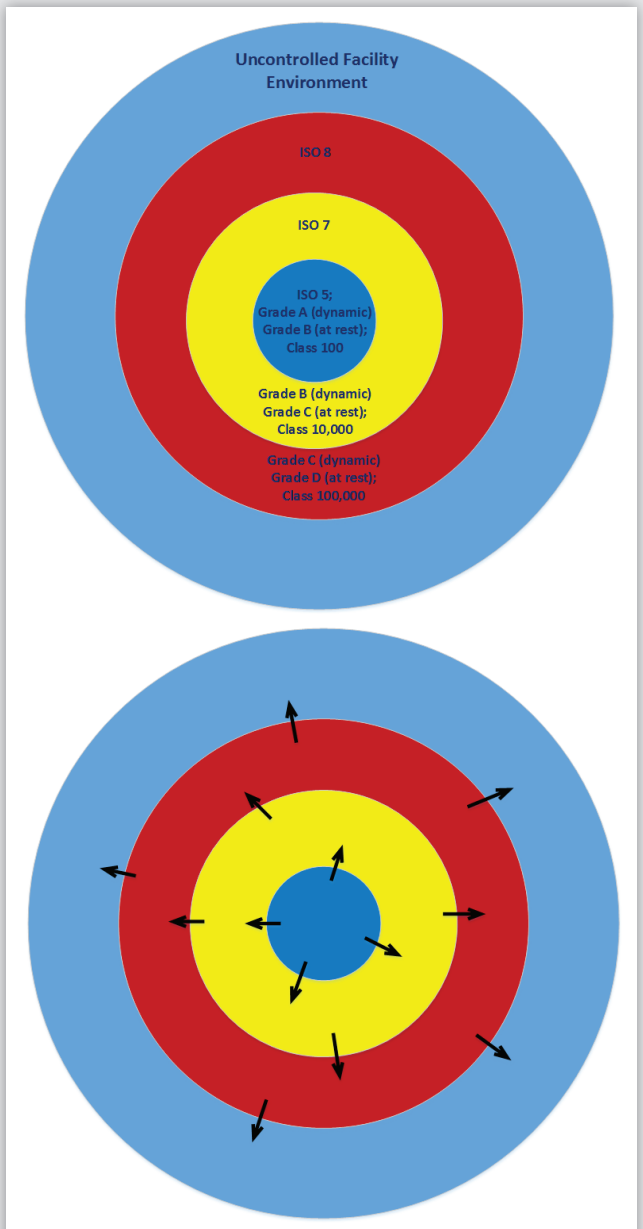

Figure 1. Top. "Target" Depiction of Room Classification as a Control Feature Figure 2. Bottom. Air Balance Cascade Within Target Depiction

one functional group in the facility to simultaneously monitor and control, and so well-established procedures and disciplined adherence to them is critical to the success of the organization.

Buildings and Facilities

Exclusion of microorganisms from areas of risk

The qualification of the facility for its ability to maintain a state of control in terms of bioburden should be established from the design phase forward. Generally these designs rely on a "target" concept. In this concept the areas of greater facility control are surrounded by areas of lesser control (see Figure 1). Critical to this design is a facility air balance arrangement where HEPA filtered air is supplied to each area so that a pressure differential of 0.03-0.05 inches of water exists between adjacent areas, "sweeping" aseptic air outward (See Figure 2). In addition, traffic patterns in this facility are designed to minimize the likelihood of cross-contamination by providing separate routes for personnel and material ingress and egress. Separation of the different areas of classification during this flow is accomplished by the design and use of air-locks and pass-through chambers to preserve the barriers to bioburden contamination while allowing personnel and material traffic amongst the rooms (for more information on facility design see references 2 and 3).

One system that deserves particular attention in this design is the HVAC system that provides not only the air pressure central to the establishment and maintenance of the air balance throughout the facility, but also the source of HEPA filtered air to the entire facility (see reference 4 for an excellent review). Some thought should also be given to the location of the HEPA filters as some evidence suggests that terminal location (e.g. just before entering the clean room as opposed to placing yards of ductwork between the HEPA filter and the clean room) is preferable in terms of air cleanliness. However, this advantage may be offset by issues in monitoring and maintenance.

A second system of special importance to the control of bioburden contamination is the water system. The water system is unique in that it

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frequently serves to provide both a utility (water for cleaning and rinsing) and a raw material (water as an ingredient). In addition, by its nature water encourages microbial contamination. Qualification of the water system, regular PM and frequent monitoring to ensure maintenance of water quality are vital to maintaining the state of control[5,6].

Continuing on with the theme of microbial exclusion from the aseptic core, where open product will be filled and therefore can be assumed to be at its most vulnerable, further protection can be provided by isolator technology providing an opportunity to render the immediate environment as aseptic as is physically possible.

Equipment

The facility design is focused on excluding as many microorganisms from the aseptic core as possible. Within the controlled environment equipment is selected not only for its utility in the manufacturing process, but also for its ability to be cleaned and sanitized between batches, or even completely replaced in the case of single-use manufacturing design[5]. Designs that enhance the effectiveness of the cleaning and sanitization programs are to be pursued.

The fill lines in a facility should be laid out in a manner to minimize confusion, mix-ups or the potential for cross-contamination. I have observed several non-sterile facilities, for example, where fill-lines were laid out in alternating orientations to maximize the number of lines that could fit in a given space. An unfortunate outcome of this arrangement is that it resulted in finished product being boxed on every other line while raw materials and containers were being loaded on the lines immediately next to them. Clearly this is a sub-optimal arrangement, encouraging mix-ups and increasing the risk of contamination.

Aseptic facilities have different concerns in equipment design and maintenance. Ease of cleaning and sanitization are even more important, as is efficacy in the exclusion of microorganisms from product contact. One example of this is the preservation of laminar air flow under dynamic conditions in this sensitive area. Perhaps it is to be expected that this issue is a frequent source of regulatory attention (it is, after all, an important consideration that lends itself to easy audit review) with smoke studies taken as the most compelling evidence of compliance.

Finally, the operators are the greatest source of contamination in an aseptic clean room core. One attractive method of minimizing contamination is in the complete automation of the filling process, completely eliminating this concern. Other changes being evaluated in the industry include completely closed systems[7] and single use systems (see below).

Sanitization of The Areas of Potential Product Risk

The efficacy of the sanitizers and sporicides used in the program must be demonstrated in a study designed to test their efficacy on the materials of construction and against resident microorganisms found in the facilities governed by the Contamination Control Plan[8]. This can be done optimally in a four-step process:

1. Suspension test of efficacy – eliminate inappropriate sanitizer candidates
   This is a screening effort designed to evaluate your candidate sanitizers against lab strains of indicator organisms as well as a variety of the microorganism species found in your facility. The goal of this assay is both to eliminate poor sanitizers and to determine the "most resistant" microorganism(s) for the next step.
2. Coupon study
   Using the representative organisms (gram positive, gram negative, spore former, yeast and mold) and robust organism(s) identified in the previous study, test the efficacy of the sanitizing agents on coupons of materials found in the facility. The purpose of this test is to demonstrate efficacy on these materials using the appropriate application procedures.
3. "Mock" sanitization study
   This study provides real-world evidence of efficacy. Let a representative room go untouched for a period of time to become "contaminated". Take bioburden samples throughout the room, then sanitize the surfaces and repeat the bioburden sampling. The samples taken after cleaning should be far less contaminated than the first set.
4. Confirm efficacy from Environmental Monitoring
   The final step in validating the sanitization program will be ongoing evidence that the program allows the facility to operate in a state of control. This evidence is usually provided by the annual environmental monitoring trending report showing maintenance of a state of control in your facility.
5. The sanitization program will ideally consist of a qualified disinfectant/sanitizing agent, used appropriately (concentration and contact dwell time observed) as the primary agent. The use of this sanitizer will then be periodically rotated with the use of a sporicidal agent (also validated for effectiveness)[9,10].

Personnel

Personnel are the primary source of bioburden contamination in a well-designed and operated clean room[11]. By some estimates, an individual at complete rest sheds about 10,000 particles per cubic meter if completely

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at rest[12]. Moderate to brisk activity increases this contamination by orders of magnitude.

Gowning methods and materials are of critical importance to the minimization of contamination as a barrier between the contamination source (personnel) and the at-risk process. This is true for both sterile and non-sterile manufacturing facilities. The need for complete coverage only exists for aseptic processes, but minimizing contamination from personnel through proper gowning is a key factor in contamination control for all manufacturing environments.

A major handicap of most control programs is the relative inability to “validate” the human operator. The closest cGMP gets is the requirement for participation in successful media fill simulations before working on an aseptically manufactured batch. Proficiency testing and constant monitoring of the operators and the process product comprises the major monitoring capabilities. This monitoring can be aided by trending of EM results and finished product test results by the operator. Control mechanisms are likewise limited, the principle tools available being additional training and disciplinary measures. Strong training programs in cleanroom behavior are essential.

The most direct way to minimize personnel effects on the process is to remove personnel from the process. Isolators are one mechanism for accomplishing this separation. A second is the use of isolators coupled with significant automation of the process, removing the need for operators in the immediate environment. In general, process changes that minimize personnel interaction with the process decrease the likelihood of microbial contamination.

Process

Process design characteristics are another important aspect of contamination control. There are obvious considerations, such as streamlining the process to minimize opportunities for mix-ups or cross-contamination and minimizing the need for human interventions. There are also less obvious process opportunities such as the incorporation of bioburden reduction steps or establishing opportunities within the process flow to allow for in-process bioburden monitoring. Contamination control begins at the design stage here as well and should be one of the process design considerations.

The first opportunity for process bioburden contamination is, of course, the incoming raw materials. All incoming materials (chemicals, water and containers) should be tested for bioburden against documented acceptance criteria for both sterile and non-sterile manufacturing operations. In addition, regular in-process bioburden should be performed for both sterile and non-sterile operations at relevant control points identified in the design phase. The interested reader is recommended to several recent reviews[13-15] for more information on this topic.

The Importance of Solid Data

As has been apparent in this discussion, all monitoring activities for bioburden contamination control are indirect in nature. That is to say that we cannot see or directly measure microbial contamination. Samples will be taken, sent to the Microbiology Lab, assayed, and then after days of incubation, data will be available to review and hopefully from which to draw information. Lab Operations are a critical concern as we need to have confidence in these monitoring data which will direct control activities. USP has recently released an informational chapter on this topic that can be useful in establishing defensible laboratory practices[16,17]. This chapter, USP chapter <1117>, is important to consider in designing your laboratory quality system as it breaks down the laboratory operations into a series of different, interconnected systems.

While on the topic of microbiology laboratory GMP, there are a variety of other chapters in USP that may be useful in developing or defending your laboratory procedures. A guide to identifying chapters of use can be found immediately inside the General Chapters section of Volume 1 (USP 2015) where a collection of “Chapter Charts” can be found. Microbiology-related USP chapters are listed in Chart 10 of this collection. Chapter Chart 10a is presented as appropriate for non-sterile products, Chapter Chart 10b as appropriate for sterile products. These charts are invaluable in locating most chapters of interest but are not complete and the microbiology lab management would be rewarded with time spent reviewing the USP Table of Contents for other chapters (both referee and informational) that have relevance for their work.

In addition to having confidence in the laboratory data, it is critical to have a proceduralized investigation process that meets the expectations of FDA’s Investigation of OOS guidance document[18,19]. While this OOS guidance explicitly states that it is not meant to apply to biological data, the general approach described in the guidance should observed. This investigation is split into two separate phases:

- Phase I Investigation: Designed to determine the validity of the data. In other words, an investigation of the laboratory testing and results to determine if the apparent OOS is valid or the result of lab error.
- Phase II Investigation: Designed to determine the root cause of the Out of Specification test result.

Note that this approach assumes completion of the lab investigation (Phase I) to determine the need for the Phase II investigation.

A version of the full OOS investigation should also be applied to environmental monitoring (EM) excursions or “Out of Trend” results. These are more common and less concerning, however, as it is expected that correctly set Alert Levels and Action Levels are at a level that predict excursions (5% and 1% excursion rates respectively)[20]. Clearly we should not be investigating expected EM excursions as if they denote product safety issues.

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Trending of Data

A comprehensive bioburden contamination control plan will rely on the trending of data for early indications of problems. There are a variety of reasons for this reliance.

The first, and overwhelming, reason for reliance on trending is that the measurement tools available to us are limited and imprecise. Estimation of bioburden by recovering and counting colony forming units (CFU) is highly variable even in the linear range for plate counts (25 – 250 CFU/plate for E. coli)[21]. As many of the regulatory EM levels are well in the noise range of this assay, individual measurements are relatively meaningless on their own. (USP <1227>[21] has a good review of the lack of accuracy in measurements dependent on less than 20 CFU per plate.) Only by trending these data can reliable information be extracted[14].

The second reason that trending is critical to monitoring the state of bioburden contamination control is that our analytical instruments are a major portion of the assay. An example of this is in air monitoring, where the effect of the sampling device on the number of CFU recovered is so extreme that changing this instrument in a facility requires a cross-over study[22]. The EM readings are relatively meaningless individually but provide information when taken in context of other data[14].

Sterile vs. Non-Sterile Concerns

Non-sterile manufacture has the same concerns of facility and equipment control, process control, personnel control and quality of monitoring data as do the sterile manufacturing facilities. The level and direction of concerns are different however, as it is expected that the amount of bioburden contamination in non-sterile products should not be too great, nor that it pose neither a health threat to the patient nor a challenge to the integrity of the product[23].

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