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Reference Review

Spore News

Vaporized Hydrogen Peroxide Isolator Decontamination in a World of Uncertainty: A Modern-Day Field Guide

Introduction

The rise in demand for complex drug products that are not suitable for terminal sterilization has led to an increasing need for advanced aseptic fill and finish capabilities. There are many challenges facing biopharmaceutical manufacturers as they implement aseptic fill processes and technologies. One particularly difficult aspect is the development and validation of the decontamination and/or sterilization methodologies, as these processes are subject to high risk of contamination. Proper decontamination and/or sterilization protocols are critical for manufacturing safe biopharmaceuticals and reducing the risks and associated costs that may result from contaminated product.

Vapor-phase hydrogen peroxide (VH_2O_2) is the primary agent utilized by biopharmaceutical manufacturing to decontaminate isolators and restricted access barrier systems (RABS) as it has significant advantages over other modalities. Organizations such as the United States Pharmacopeia (USP) and the Pharmaceutical Inspection Convention Co-Operation Scheme (PIC/S) publish guidance, but unfortunately there are no widely recognized consensus standards on the methods and acceptance criteria that are affirmed by the regulatory bodies. Complicating matters further, VH_2O_2 exists as both a gas and a liquid during decontamination activities resulting in challenges in controlling and understanding the process. In addition, biological indicators (BIs), which are the most important lethality measurement tool for these processes, are not controlled by standards regarding their manufacturing and performance. Inconsistent and poor quality BI's makes qualification, validation, and revalidation very difficult to perform accurately, consistently, and reliably.

This field guide uses current published resources to provide clarity and direction regarding best practices for validating decontamination processes using VH_2O_2.

Isolator Decontamination Validation

Although many countries have their own guidance and regulatory positions on isolator decontamination, efforts continue to harmonize these guidelines. In the United States, the FDA enforces good manufacturing practices for the pharmaceutical and medical device industries, and the regulations are published in several parts in the Code of Federal Regulation, parts 210 and 211 for pharmaceuticals[1], and Part 820 for medical devices[2]. Concerning the decontamination of isolator systems used for the aseptic manufacturing of drug products, sections 211.671 "Equipment cleaning and maintenance" and section 211.113 "Control of microbiocidal contamination" are relevant. Section 211.67 states "Equipment and utensils shall be cleaned, maintained, and, as appropriate for the nature of the drug, sanitized and/or sterilized at appropriate intervals…". Section 211.113 states "Appropriate written procedures, designed to prevent microbial contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of all aseptic and sterilization processes."[1] Validation activities are to be scheduled and conducted at defined intervals.

Per the literature, qualification of isolators/enclosures used for aseptic manipulations or manufacturing includes the steps outlined below. Following the outline, additional details, including references, are provided on each point with a strong focus on isolator decontamination.

Major Points of an Isolator Validation

1. Isolator location
2. Isolator design
3. Installation Qualification
4. Operational Qualification
5. Performance Qualification
6. **Design and development of decontamination process**
    - **6.1 Bioburden characterization**
    - **6.2 Selection of sporicidal decontamination agent**
    - **6.3 Selection of method for agent delivery (e.g., gas generator)**
    - **6.4 Identification of all surfaces needing decontaminated including critical zone(s)**
    - **6.5 Define lethality quantification and methods (e.g., 6 SLR in critical zones)**
    - **6.6 BI selection and qualification**
    - **6.7 Agent distribution and concentration in system (e.g., CI mapping, electronic sensors)**
    - **6.8 Identification of "worse case" locations (e.g., high surface temp, poor agent penetration, etc.)**
    - **6.9 Cycle verification**
7. **Validation of decontamination process**
    - **7.1 Challenge worse case locations and critical zones with BIs**
    - **7.2 Demonstrate criteria are met (e.g., 6 SLR in replicate cycles)**
    - **7.3 Periodic requalification**

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7.4 Continuous monitoring
7.5 Microbiological monitoring

Major Points of an Isolator Validation, Expanded

1. Isolator location

USP[3] gives guidance on the appropriate location in which an isolator should be placed. Issues such as operator safety and comfort are discussed as well as considerations on the impact the surrounding area will have on isolator decontamination.

2. Isolator design

USP[3] gives guidance on appropriate air handling systems, transfer ports/doors and equipment layout. A PDA technical report[4] discusses the various types of isolator systems and their intended use. The various materials used in the construction of the isolator are also discussed which should be able to withstand repeated exposures to the decontamination agent.

3. Installation Qualification (IQ)

Equipment installation and qualification should be performed per the manufacturer's instructions. A prerequisite to qualifying the equipment is the qualification of any utilities supporting the equipment. USP[3] and PIC/S[5] list items to be completed in the IQ including calibration of instruments, filter certification, operator working instructions, maintenance requirements, and verification that design specifications have been met.

4. Operational Qualification (OQ)

Equipment operational qualification should be performed per the manufacturer's instructions. The OQ demonstrates that the system operates as designed and within defined parameters. USP[3] and PIC/S[5] list additional items to be completed in the OQ including cleaning and leak testing. Decontamination cycle development occurs after the completion of the OQ and is discussed in detail in section 6.

5. Performance Qualification (PQ)

Performance qualification demonstrates the system is functioning within specifications, per procedures, and consistently delivers product meeting specifications. USP[3] states that upon completion of the PQ phase, "the efficacy of the decontamination cycle" is verified and is discussed in section 7.

6. Design and development of decontamination process

The design and development of a decontamination cycle typically occurs upon completion of the OQ. USP[3] again provides guidance on this subject as does PIC/S[6] which published a valuable guidance document detailing the various steps of the process.

6.1 Bioburden characterization

It is the bioburden that is the true target of decontamination activities and as such, it is important to understand its characteristics (identity, quantities, resistance to the process). Kokubo et al.[7] evaluated the resistance of common environmental spores that were recovered from a pharmaceutical plant in Japan. Spore crops were produced from the bioburden isolates and D-value studies were conducted. The resistance of the isolates was compared to the resistance of *G. stearothermophilus*, and the authors found that the D-value of *G. stearothermophilus* spores exceeded the bioburden spore forming organisms by more than a factor of 10. *G. stearothermophilus* is the microorganism recognized for use in BIs challenging VH_2O_2 processes.

6.2 Selection of sporicidal decontamination agent

During the planning phase, a decision on the appropriate decontamination agent needs to be made. ISO 14937[8] provides valuable information for characterizing a sterilizing agent including the development, validation, and routine control of the sterilization process. Although the scope of this document is limited to the sterilization of medical devices, the methods described therein are largely appropriate for decontamination processes. Comprehensive guidance is provided in characterizing the lethal agent including its microbial effectiveness, effects on materials, safety, and the environment.

The most common agent for enclosure decontamination in use today is VH_2O_2 which is the focus of this paper. Other decontamination agents exist such as formaldehyde, peracetic acid and chlorine dioxide. Gordon et. al.[9], summarize numerous literature publications and provide pros and cons on the commonly used agents.

VH_2O_2 continues to gain popularity largely because its by-products, water and oxygen, are friendly to personnel and the working environment, which is not the case with other options. However, VH_2O_2 does have its challenges in that it is a mixed-phase agent, and not a true gas as is the case with formaldehyde and chlorine dioxide.

6.3 Selection of method for agent delivery

The literature discusses hydrogen peroxide being delivered into a system in the form of a vapor, mist, fog, aerosol, or gas. In all cases, the hydrogen peroxide begins in a liquid form and changes its state by one of two methods, vaporization by flash heating the liquid above its boiling point or forcing the liquid through atomizing nozzles. In either case, the temperature of the vapor quickly becomes equivalent to the that of the enclosure.

Unger-Bimczok et. al.[10] utilized flash vaporization and studied the effect of humidity, vapor concentration, and condensation on the inactivation rate of *G. stearothermophilus* spores. Their data support the position that condensation (or micro-condensation) is needed for effective microbial inactivation. Vanhecke et. al.[11] provide details on the delivery of the decontamination

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agent via a fogging method, thus eliminating the introduction of heat into the system that occurs during flash heating vaporization.

6.4 Identification of all surfaces needing decontaminated including critical zone(s)

Critical zones are locations within the isolator that have a high likelihood of contaminating the product if viable microorganisms are present. Complex parts on filling/packaging equipment may contain features that impede vapor penetration (dead legs, lumens, etc.) and are candidates. Sigwarth et. al.[12] evaluated the resistance of *G. stearothermophilus* spores inoculated on numerous materials-of-construction typically found in isolator systems. They found that spores deposited on certain materials (e.g. anodized aluminum) resulted in increased resistance and noted these materials may not be suitable for use in systems where VH_2O_2 decontamination is utilized.

6.5 Define lethality quantification and methods

The "intended degree of inactivation or lethality"[4] needs to be specified. Although USP[3] states that "greater than a three-log reduction against highly resistant biological indicators" is appropriate (PDA[4] has a similar position), the current expectation of the regulatory authorities is to demonstrate a 6-log reduction. Sigwarth and Moirandat[13] published a method for the "quantification of H_2O_2 decontamination", including methods for BI qualification (to be used not only for the initial validation but also for subsequent validations). PIC/S[6] provides four options for the evaluation of the spore log reduction (SLR). The first two involve the removal of surviving spores from the carrier and either directly enumerating, or culturing aliquots in a liquid medium for a Most Probable Number (MPN) estimation. The other two include a 2-BI method where one unit is cultured and the other held in reserve (the held BI is directly enumerated only if the cultured BI is positive for growth) and lastly, the use of triplicate BIs for a MPN estimation. The first two methods are quite labor intensive and are rarely used.

Case studies have noted the targeted use of a 4 SLR in non-critical areas and a 6 SLR in areas judged to be critical.[11]

6.6 BI selection and qualification

As VH_2O_2 has poor penetration abilities, it is crucial to select a BI that has been designed for use in surface decontamination processes. Currently there are no standards for the manufacture and qualification of these BIs however, ISO 11138-114 provides general requirements for BIs used "in the validation and routine monitoring of sterilization processes", and its guidance on manufacturing controls are relevant for BIs used in decontamination processes. Additionally, a PDA Technical Report[15] provides guidance on "Specification, Manufacture, Control, and Use" of BIs for vapor-phase decontamination processes.

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The cleanliness of the spores is especially important as cellular debris and media components can protect the spores during the decontamination process. Moreover, the spores should be well-characterized and have traceability to a recognized culture collection. The most widely used organism for VH_2O_2 processes is *G. stearothermophilus*, which includes two strains, ATCC #12980 and ATCC #7953. The ATCC # 12980 strain has historically been the predominant organism used in VH_2O_2 BIs, and data routinely collected by Mesa Labs on Apex BIs demonstrate that the ATCC # 12980 strain exhibits higher resistance to the VH_2O_2 process than does the ATCC # 7953 strain.

Stainless-steel is the most common spore carrier utilized in VH_2O_2 BIs as it does not absorb or catalyze the hydrogen peroxide, and it is a material that is heavily represented in most isolator systems. The inoculum is deposited on the carrier in a manner that promotes the formation of a monolayer of spores. A perfect monolayer across the entire surface is not possible, especially in BIs containing >1.0 x 10^6 spores/carrier, however, minimizing spore piling promotes uniform exposure of the spores to the VH_2O_2.

Inoculated carriers are typically placed into primary packaging that is permeable to the decontamination agent and like the carrier, does not absorb or catalyze the hydrogen peroxide. (Tyvek® is commonly used in primary packaging.) The primary packaging must be robust such that it can withstand transport and any manipulation needed for BI placement into the isolator system. Its main purpose is to protect the spore carrier from outside contamination making it a key component in preventing post exposure contamination, which can lead to false positive BI results. The packaging can impact the resistance of the BIs which the user should consider if the planned use is to expose the spore carrier naked (unpackaged). It has been reported[10] that the primary packaging can increase the resistance of the BI as it impedes VH_2O_2 penetration.

6.7 Agent distribution and concentration in system

A true gas will expand to fill an enclosure but as VH_2O_2 is not a true gas, it must be distributed through the space by mechanical means (e.g., fans or blowers). Humidity and temperature mapping, along with the use of chemical indicators (CIs) and/or electronic sensors can provide an indication of VH_2O_2 distribution throughout the enclosure.

6.8 Identification of “worse case” locations

VH_2O_2 is most effective when some level of condensation on surfaces occurs; therefore, areas of low vapor concentration and/or hot spots (which do not readily promote condensation) may prove difficult to decontaminate. Temperature mapping and areas of low vapor concentration should have been identified in step 6.7. Challenging these locations with BIs will identify areas of low lethality. In the event of positive BIs in one or more location, adjustments to the cycle parameters and/or equipment layout are needed until subsequent cycles provide satisfactory results.

6.9 Cycle verification

Cycle verification is simply gaining confidence that the newly developed decontamination cycle

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will routinely meet the acceptance criteria (e.g. 6 SLR). Performing replicate cycles with acceptable results reduces the risk of failure during the validation activities.

7. Validation of decontamination process

7.1 Challenge worse case locations and critical zones with BIs

The decontamination cycle should be well characterized prior to the initiation of the validation. Validating the efficacy of the decontamination process is demonstrated by the successful execution of three replicate cycles[3]. A successful cycle is one that demonstrates the acceptance criterion, a defined SLR, has been met. The only tool capable of demonstrating a Spore Log Reduction is a biological indicator containing well characterized spores. Validation requires a quantifiable output, and the use of a single BI/location carries some risk as BIs are generally cultured for growth; the outputs of which are binary A BI (10^6 spores/unit) that is negative for growth meets a 6 SLR criterion, however the SLR cannot be determined from a BI unit that is positive for growth. The use of replicate BIs (e.g., the 2 or 3 replicate BI methods described in 6.5) is a potential risk reduction option. These options do not necessarily require the use of additional BIs per cycle, rather BIs located in non-critical areas during cycle development/verification are reduced while adding replicate BIs into the critical areas.

7.2 Demonstrate criteria are met

Per USP[3], "The ability of the process to reproducibly deliver a greater than three-log kill is confirmed in three consecutive validation studies." It is here where there seems to be a disconnect between the USP (along with other organizations) and the regulatory authorities regarding the acceptance criteria. The FDA's default position appears to be that a six-log kill is the expectation, however other values will be considered provided the firm can justify and defend their position.

7.3 Periodic requalification

PDA Tech Report No. 34[4] states, "The isolator and its contents are decontaminated on a regularly scheduled basis…or until a maintenance operation requires the aseptic environment within the isolator to be broken." Typically, re-validation of the decontamination process is performed on a defined cadence (e.g., annually) or upon completion of significant maintenance or repair activities.

7.3.1 BI selection and qualification

BIs used for the re-validation of a system should be selected in the same manner as was done during the initial validation. To minimize the chances of unexpected positive BIs, care should be taken not to select BIs that have significantly higher resistance to the process.

7.4 Continuous monitoring

Continuous real-time monitoring during routine decontamination cycles provides some

assurance that the process remains in control. These variables typically include temperature, humidity, pressure, and particle monitoring.

7.5 Microbiological monitoring

Microbiological monitoring includes using settling plates/swabs upon completion of a decontamination cycle and then periodically until the next decontamination cycle is scheduled. Other monitoring methods include sterility testing of final product, and media fills. PIC/S[16] provides detailed guidance on these methods.

Conclusions

The information presented here is an overview of existing literature on isolator decontamination, and there is no doubt the "state of the art" will continue to evolve with improvements in technology and methods. Biological indicator design and manufacturing techniques have also evolved making the BI a valuable tool in cycle development activities and ultimately demonstrating the lethality of the decontamination process during validation.

References/Resources

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