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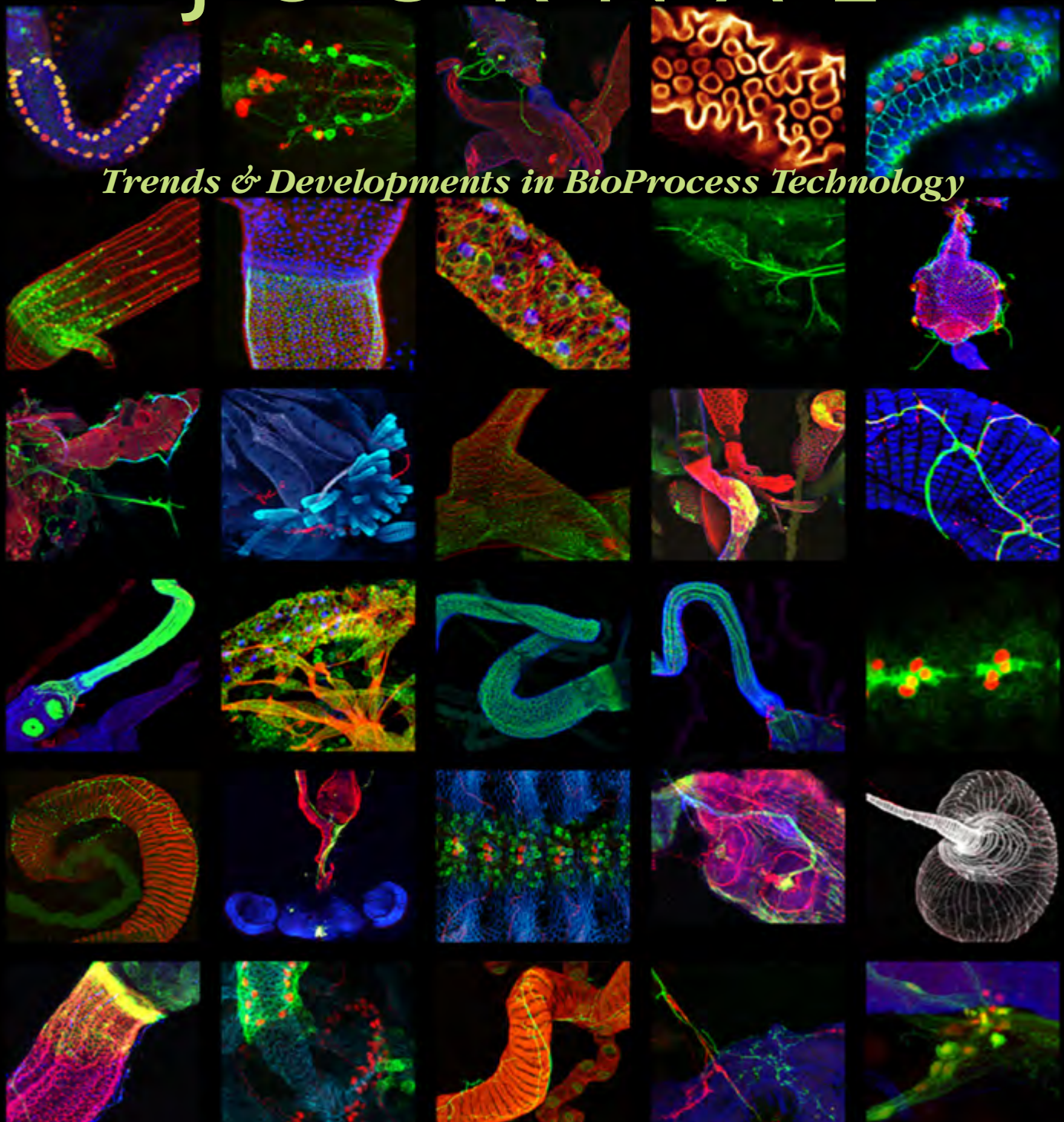

Trends & Developments in BioProcess Technology

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TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

Bio-Burden Control in Bioprocessing Using Hydrogen Peroxide Vapor

By JAMES DRINKWATER and RICHARD LUCAS

Abstract

The emerging use of hydrogen peroxide vapor (HPV) bio-decontamination technology in the late 1990s was initially implemented as part of an alternate strategy for disinfecting rooms, areas, and whole buildings. The technology was developed with rapid cycles and was adopted by barrier isolator users allowing for rapid, effective, and validated bio-decontamination cycles inside critical zones. In the years to follow, the use of HPV technology spread throughout the development and production pipelines of small- and large-molecule biopharmaceutical products.

This article will consider the current and future uses of HPV technology from the perspective of the development through manufacture of a biologically-derived product.

Introduction

There are distinct challenges that biotech firms face in developing and then commercializing biologically-derived products for medical use. Biopharmaceuticals require an intensely demanding manufacturing process (Figure 1). Many of the biologics produced are antibodies and recombinant proteins, and by their nature, can be radically affected by minor changes in their local environment.[1] Their three-dimensional structures are dependent on a number of diverse, often weak, interactions between the amino acids which make up their primary structure.[2] Strict ambient conditions are required for the cells to multiply and prosper.

Simple proteins can be obtained from bacteria. For complicated substances consisting of several proteins, or for substances that have to be modified by the addition of non-protein groups such as sugar chains, mammalian cells are used. To derive products identical to their human equivalents, the appropriate human genes must be inserted into selected cells. The use of well-characterized mammalian cell lines to carry out most of the complex biomanufacturing burden is far more involved than the classical wet chemistry commonly employed in small-molecule manufacture.

Laboratories and manufacturers around the world work with well-characterized cell lines to produce biopharmaceuticals, enzymes, and antibodies. These cell lines are used because they are thoroughly researched and are, as far as is possible with living organisms, amenable

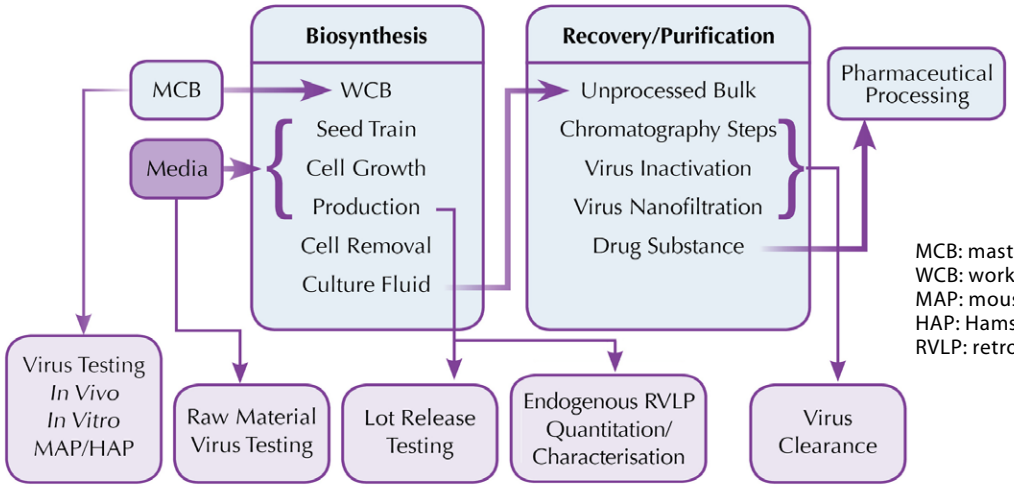

FIGURE 1. Generic biological manufacturing process.

to standardisation. This allows reproducible results to be obtained worldwide. Two well-known organisms used in basic research and the biotech industry include bacteria of the species *Escherichia coli* and eukaryotic Chinese hamster ovary (CHO) cells. The genetically-modified cells are reproduced and stored in a master cell bank at very low temperatures. If prolonged storage is required, cells can be remain viable almost indefinitely in liquid nitrogen at –196 °C (77 Kelvin). It is from cell banks such as these that cells are taken for drug manufacture.

Biological processes often vary greatly, making the development of a robust and reliable process inherently difficult. Couple this with the high intrinsic value of the end-product. A biopharmaceutical firm has to bear an immense degree of risk during its manufacturing process that is unmatched in pharmaceutical manufacturing. Mitigating the manufacturing risks has been a biotech goal for many years, and current approaches correlate with the quality by design (QBD) principles and process analytical technologies (PAT). HPV sterilization plays an important part in the equation, in terms of its ability to be rapidly deployed to an area where cell manipulation is carried out. For example, HPV-enabled, aseptic workstations can supplant laminar flow cabinets as secure and sterile locations for carrying out cell manipulation.

Optimizing Cell Cultivation Includes Bio-Burden Control

Scientists have been studying the composition, evolution, and behaviors of cells—some millions of years old—for several centuries. There is plenty of material to study and take advantage of. However, the difficulty in early stage development work still centres around finding optimum environmental conditions for the cultivation of these cells.[3]

To begin with, selected cells are transferred from the cryogenic cell bank to a liquid nutrient medium where they are allowed to reproduce. The length of time required depends on the type of cell used. Under favorable conditions, bacterial cells such as *E. coli* usually divide once every 20 minutes, thus one cell gives rise to 4.7×10^{21} cells within 24 hours.[4] By contrast, mammalian cells such as the CHO divide about once every 24 hours, and it takes correspondingly longer to obtain a sufficient number of cells to work with. Typically, during the growth phase, the cell culture is transferred to progressively larger culture vessels. Transfers are normally carried out through aseptic connections to reduce contamination risks (*e.g.*, with any species or material present in the environment around the process vessels). Along with sampling or process interventions into the closed system, these connections can present opportunities for material transfer from the clean room into the process train. Thus, environmental conditions surrounding the process also need bio-burden, viral, and possible biological product residual control with reduction or clearance as necessary.

The challenge of decontaminating complex surfaces manually (outside of process equipment or within the surrounding bioprocess environment) presents a very real obstacle to cleaning validation. Manual decontamination leaves room for operator inconsistencies, and residuals management is a key issue with any disinfection agent that has an oxidizing potential, no matter how it is applied. In contrast, HPV decontamination is a fully-automated bio-decontamination process with controlled delivery and removal of the decontamination agent and its subsequent residuals. Coupled with the ability of HPV to repeatedly decontaminate complex geometry due to its vaporous nature, HPV is increasingly being deployed in clean rooms to remove any detected species or material as well as part of a routine bio-burden reduction program.

Before implementation, risk assessments should be undertaken to verify that HPV, at the decontaminated, saturated vapour conditions, has no potential for adverse effects due to direct contact with biological products. HPV gas distribution through the complex structures and surfaces of the closed process equipment should be studied with chemical indicators and then optimised as necessary. Hot surfaces from any associated process equipment heat sterilization procedure should be allowed to cool in order for the HPV process to be effective.

Mobile or fully-integrated HPV generators can be specified but both need engineering integration, in terms of controlling the room status (sealed and secured) prior to the HPV cycle vapor delivery, together with distribution and controlled removal of decontamination agent residuals. Highly-efficient catalyst technology may be used to speed up aeration phases if the process room HVAC systems do not have sufficient exchange rates to achieve rapid dilution.

Taking Measures to Reduce Cell Line Cross-Contamination

Studies suggest that cells used in experiments have been misidentified or contaminated with another cell line 15–20 % of the time.[5] Problems with cell line cross-contamination have even been detected in lines from the NCI-60 panel which are used routinely for drug-screening studies.[6] Major cell line repositories, including the American Type Culture Collection (ATCC) and the German Collection of Microorganisms and Cell Cultures (DSMZ), have received misidentified cell line submissions from researchers.[7] Such contamination poses a problem for the quality of investigations using cell culture lines,

and the major repositories are now authenticating all cell line submissions. One significant mammalian cell-line, the immortal HeLa, has been implicated in numerous cross-contamination events.[8]

Common manipulations carried out on culture cells are media changes, passages, and transfections. They are generally performed using sterile culture techniques. Such aseptic protocols are aimed at avoiding contamination with viruses, bacteria, fungi, or other cell lines. Operations are typically carried out in biosafety hoods or laminar flow cabinets to exclude contaminating micro-organisms.

The biocidal ability of HPV is well-documented, acting rapidly on mammalian and bacterial cells, ensuring their destruction through a combination of damage to cell membranes, intracellular components, and DNA (in the nucleus itself). According to Linley *et al.*[9] the use of HPV removes the potential for cell cross-contamination.

The use of Bioquell's HPV technology provides full assurance that treated areas are free from previously worked-with materials. In addition, the Bioquell QUBE (Figure 3) rapid-gassing system creates an aseptic Grade A/ISO 5 environment for the safe manipulation of cells. Its rapid gas cycling minimises facility downtime while protecting valuable pure cell lines.[10]

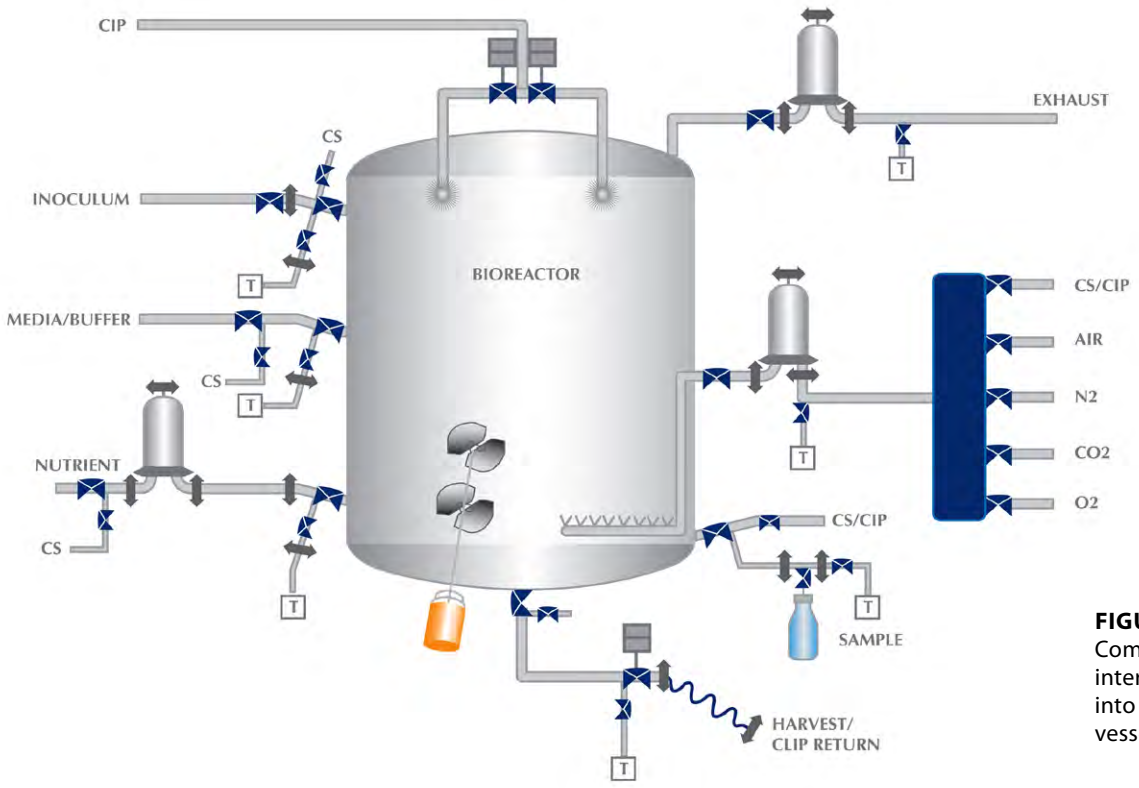

FIGURE 2. Common interventions into process vessels.

Maximising Production and Sterility During Fermentation

The actual production of the biopharmaceutical product occurs during the fermentation or culture phase. The culture medium contains substances needed for the synthesis of the desired therapeutic product. In total, the medium can contain somewhere in the range of 80 different constituents in combinations unique to the particular process and manufacturer. Numerous industrial-scale steel fermentation vessels in use have capacities of 10,000 litres or more. There are not only technological but also biological constraints on the capacity size of the reactor vessel: the bigger a fermentor is, the more difficult it becomes to create uniform conditions around all the cells within it. There has been a shift toward smaller, single-use systems to maximize production flexibility while reducing labor and capital equipment costs.

Monitoring all that is happening during the fermentation process is vital to ensuring healthy progression of the culture batch. Processes to watch for and understand include: (a) the conversion rate of nutrients to product; (b) any formation of unwanted byproducts; and in extreme cases, (c) the onset of contamination. Growth kinetics and substrate consumption are key indicators of the progress. Again, at this stage there will commonly be multiple ports in process vessels for: (a) sampling; (b) the addition or removal of components; (c) vents; and (d) harvest points (Figure 2). Although the greatest care is

typically taken with all cGMP process steps, the possibility still exists that viruses, mycoplasma, or foreign cells can make their way into these sealed processes. Insufficient sanitization of bioprocess material vessels and container surfaces prior to entering the production suite can result in contamination.

It is a good practice to decontaminate production materials as they exit the process areas. The need for strong microbiological monitoring programs, both inside and outside of the process vessels, are included in production protocols. Special HPV decontamination transfer chambers are available for material/load decontamination transfers at walk-in or smaller scale with transfer HPV cycle times in the range of 20–60 minutes. Such transfer chambers have reversible interlock prioritization assuring that HPV cycle steps are completed on material entry to a process area or material/waste exit.

FIGURE 3. Bioquell QUBE, a cost-effective modular aseptic workstation system with built- in rapid bio-decontamination.

Bio-Safety Preparation for Effective Downstream Processing

In technical terms, the production of biopharmaceutical products in microbial or mammalian cells is a one-step process, and the product can be purified immediately after fermentation. In the simplest case, the product is secreted into the ambient solution by the cultured cells, then the cells are separated from the culture medium (*e.g.*, by centrifugation or filtration) and finally, the desired product is then isolated via several purification steps. If, on the other hand, the product remains in the cells following biosynthesis, the cells are first isolated and digested (*e.g.*, destroyed), and then the cellular debris is separated from the solution together with the product and then purified.

The yield from bioproduction processes is usually much lower than from chemical synthesis. For example, a 10,000 litre mammalian cell fermentor typically yields only a few kilograms of a therapeutic antibody. The production and purification steps can take several weeks. Several more weeks are needed for purity testing to avoid quality fluctuations. A 99.9 % purity level is required for regulatory approval. Only then can the finished product be further processed and shipped.

Cell lines have the potential for in-process viral contamination within the closed primary manufacturing environment as some continuous cell lines produce large quantities of endogenous retrovirus-like particles. Continuous cell lines therefore support the replication and amplification of some viruses that can potentially contaminate cell culture processes. Even if these viruses present no risk to human health, they can have a detrimental effect on primary cell health to the point that batches are lost as efficiency falls to unacceptable levels.

Typically, in-process (within the closed cell line) viral inactivation or clearance steps use one of the following processes: γ-irradiation, UV-C, high-temperature/short-time (HTST), nanofiltration, pH extremes, chemical treatment solvent/detergent (tri[n-butyl]phosphate [TNBP]/Tween 80), or chaotropes (urea, guanidine HCl). These processes are not appropriate for the secondary manufacturing room environments surrounding the cell line. In this case, the process of HPV decontamination can be applied to manage the requirement for environmental contamination control, reducing both bio-burden and clearance of environmental viral contamination.

When HPV is the decontamination method of choice[11,12] for control of: (a) bio-burden in downstream aseptic processing; and (b) clearance of potential environmental virus residuals on complex outer surfaces (in clean rooms and on closed process trains); then characterization studies are recommended at early process development stages so baselines are set for scale-up. The optimum HPV sanitizing process requires that saturated vapour conditions are achieved. Such settings are created by sealing the room or enclosure and injecting the decontaminating vapour via flash evaporation followed by even distribution of the H_2O_2 agent ensuring contact with all exposed surfaces. The final bio-decontamination step is aeration, a process of removing the hydrogen peroxide by re-evaporation, outgassing, and then dilution — in some cases with the use of carbon catalysts — such that the residuals meet a target (and validated) cycle endpoint.

From Drug to Drug Product: Aseptic Conditions for Fill-and-Finish

The final steps in the production of biopharmaceuticals are also demanding. The sensitive proteins are converted to a stable pharmaceutical form and must be safely

packaged, stored, transported, and finally administered. Throughout each step, the structural integrity of the molecule has to be safeguarded to maintain efficacy.

The fill and finish process presents a common opportunity for contamination of the final product. Modern biopharmaceutical production facilities operate their fill and finish processes in complex isolator systems while separating personnel from the process as much as possible. Here, HPV is commonly employed, ensuring aseptic conditions before vital final processes occur.

One of the strengths of HPV is its innocuous byproducts: water and oxygen. Most disinfecting chemicals have the potential to damage both the product and the materials used. Obviously, material and biocompatability studies need to be conducted on all disinfection options prior to their inclusion into the process. For HPV, there are two principle analytical methods: (1) to detect residuals in solutions; and (2) in the environmental gas phase. To specify a compatibility study plan, a risk assessment is required first to define the potential routes of chemical agent contamination to the biological product. With HPV, these can be summarised as:

- Gaseous environmental residuals of HPV agents transferred to biological product solutions (*e.g.*, open, filled vials.) After a vapour-phase decontamination cycle, such gaseous residuals may come from plastics used in the process equipment or the HEPA filters that form part of the aseptic barrier. New developments in PTFE (hydrophobic) HEPA and ULPA filters may further mitigate process and product risks resulting from HPV residuals in HVAC systems.
- Absorption into materials and subsequent outgassing of decontamination agent residuals into product solutions (*e.g.*, exposed product containers and stoppers/caps that come into contact with biological products).
- Direct exposure of product contact parts or biological products to the HPV decontamination cycle that would typically be protected by an impermeable barrier (packaging or closed-process equipment) when hydrogen peroxide permeates and has direct contact with critical surfaces or products.

Additional considerations include:

- When filling biologic products in the form of liquid fills or lyophilized solid products, the impact of H_2O_2 residuals would be on the product in solution. Direct exposure or contact of the product with parts that have been exposed to H_2O_2 residuals would need to be studied.
- Filling lines typically have unidirectional airflow barrier systems, isolators, or restricted access barriers (RABS) so they have a significant surface area-to-volume relationship, particularly with the extent of HEPA filtration.
- If clean-in-place and sterilize-in-place (CIP/SIP) systems are used for the product path, including silicon tubing to filling needles, the impact on H_2O_2 residual absorption into tubing and subsequent diffusion transfer needs more study.
- If the filling line is for multiple biological products, consideration needs to be given to any potential for cross-contamination between product batches or campaigns. In this case, there are two product conditions that require risk mitigation in the isolated filling line system design. As the biological product is in solution, potential cross-contamination is primarily from product aerosols that are generated in filling, accumulating in the isolator system, potentially passing through HEPA filters, and eventually being re-introduced into another product batch. In this case, the inactivation of biological products in the barrier system environment and on related surfaces can be achieved via the HPV cycle. It is essential to remove hydrogen peroxide gaseous or biological product molecule residuals that may pass through HEPA filters and contaminate other products. Such residuals can be removed by airflow dilution with endpoint studies that confirm a lock of residual that would impact another product.
- At the freeze-drying stage, the powder being formed can be contained by terminal filtration at the air exhaust from the barrier HVAC system. These filters would be the "bag-in/bag-out" type of safe change. The duct connection from the barrier process zone to the terminal exhaust HEPA or ULPA filter should be capable of CIP.

Pertinent Guidance Documents

There are a number of guidance documents that relate to the primary manufacturing environment within particular cell lines providing information on characterization, virus validation studies, viral safety evaluation, and other biological contaminants, including but not restricted to:

- Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, 1987 and 1993, FDA;[13]
- Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses, 1996, CPMP/BWP/268/95;[14]
- Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, 1999, ICH Q5A;[15]
- Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products, 2008; EMEA/CHMP/BWP/398498/2005;[16] and

- Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications, 2010, FDA.[17]

Summary

HPV bio-decontamination processes fully support the requirements for aseptic processing and cross-contamination control in processing biological products.

The oxidizing potential of HPV may have an impact on biological product efficacy. Therefore, residual impact studies are required. Analytical methods and monitoring technologies are well-developed to facilitate management of these issues.

In order to facilitate faster removal, it is essential to manage the condensable vapor of H_2O_2 for optimum delivery, distribution, and rapid rise to process lethal conditions, since residuals of low hydrogen peroxide concentration, down to the parts per billion level, may impact biological products. The less H_2O_2 input, the less to remove. Rapid cycles mean reduced contact or exposure time for absorption reducing any subsequent out-gassing times.

The application of HPV decontamination cycles extends through the biologics product development life cycle, from early development phases through to cell line storage and culture as well as process trains, product filling through to pharmacy preparation in the clinic.

The strengths of HPV decontamination far outweigh the compatibility challenges in biologics applications and such challenges are fully manageable with well-defined analytical and validation studies.

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