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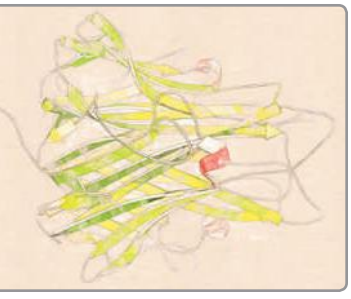

Cost Considerations Drive Lean Technology in Biomanufacturing

Catherine Shaffer

Manufacturing for originator molecules is restricted by regulations, but drug makers can exploit newer technologies for the manufacture of biosimilars.

Four biosimilars have been approved for the market by FDA as of February 2017, and more are in the pipeline. Now that biosimilars are here to stay, manufacturers are developing processes for cost efficiency and reliability using newer technology to compete with innovator products based on processes 10–15 years old. Those technologies include more productive cell lines, single use, and powerful analytical methods.

The problem of copying biologics

Biologic drugs have revolutionized the pharmaceutical industry by attacking disease using the mechanisms of the cell and immune system. These therapies are fragile and require a complex manufacturing process to produce. For the earliest biologics, patents have expired or are near expiration, opening the door for follow-on generic product competitions. FDA approved the first Humira (adalimumab) biosimilar, Amjevita (Amgen), in September 2016, and it was the fourth biosimilar approved in the United States. The European Medicines Agency (EMA) started approving biologics well before the US, and approved 22 biosimilars by the end of 2016.

Unlike a small-molecule drug, however, a biologic drug can't be precisely copied. Biopharmaceuticals are produced in cell cultures, and the final product is much more than a DNA sequence. These products are subject to post-translational modifications such as glycosylation, phosphorylation, methylation, hydroxylation, and sul-

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fation that affect their activity and immunogenicity. Conditions of growth, the type of expression system, the formulation of the final product, and packaging decisions also affect the essential character of the product.

Although the specific growth conditions and formulation of a biopharmaceutical are an intrinsic part of its nature as a drug, the information about processes and formulation are mostly proprietary.

Adam Elhofy, chief science officer for Essential Pharmaceuticals, has worked with innovator biopharmaceutical companies as well as biosimilar companies. "Biosimilars are constrained in that they have to hit certain quality parameters set by the innovator," Elhofy says. Essential Pharmaceuticals provides an animal-component free media that boosts titer and enhances protein quality. It can be difficult to change or improve processes with the strict regulations around biologics and biosimilars. "We're working with several biosimilar companies and also working with innovators," Elhofy says. "In some cases, the protocols are locked down, and it's difficult for them to bring in a new product."

Manufacturing process

Although the specific growth conditions and formulation of a biopharmaceutical are an intrinsic part of its nature as a drug, the information about processes and formulation are mostly proprietary. Biosimilar manufacturers begin the development of a process with a large information gap. So while the technology of manufacturing a biosimilar is largely the same as manufacturing an original biologic drug, the lack of knowledge about the composition of the originator biologic presents a unique challenge.

Establishment of biosimilarity to the original product is a high standard to meet. Biosimilar manufacturers must show that their product performs comparably in analytical and preclinical assays, and must also carry out clinical studies to establish biosimilarity (1, 2).

Monoclonal antibodies (mAbs) are the most common and most well-known class of biopharmaceuticals. As of February 2017, FDA lists 68 approved mAbs, and approvals have been increasing each year since the late 1990s (3). mAbs are comprised of several domains that contribute to their function. The Fab region of an antibody interacts with the target, while the Fc is engaged in cell-mediated cytotoxicity. Under EMA regulations, in-vitro studies must show that the antibody binds to the target antigen, that it is binding to representative isoforms of the three Fc gamma receptors, and that its Fab and Fc domains function as intended.

Functional assays therefore play a critical role in biosimilar manufacturing. *In-vivo* testing may also be required, depending on regulatory concerns. Comparative analysis through clinical evaluation is also required for production of the biosimilar product.

Immunogenicity is another important aspect of biosimilarity. Because it is not possible to predict immunogenicity in humans using animal models,

initial estimates of immunogenicity are based on risk assessment and confirmed through postmarketing vigilance.

Analytic technology is crucial

Because the emphasis in biosimilar manufacturing is on comparability and biosimilarity, analytic technology takes on an even greater importance in process development. Antibody molecules, for example, may have 5 or 10 different functions, requiring 5 or 10 different functional assays. Those may include antibody-dependent cellular toxicity (ADCC), complement-dependent cytotoxicity (CDC) assays, reporter assays, and potency assays. Technologies used typically include ELISA, electrochemiluminescence, and surface plasmon resonance (Biacore, GE Healthcare) analysis.

Biacore, in particular, has become a workhorse of biopharmaceutical production, and plays an even larger role in the manufacture of biosimilars. According to Jason Schuman, a senior product specialist at GE Healthcare, Biacore is used to screen hybridoma lysates for selection of lead candidates, to measure binding properties, and to evaluate safety, quality, and immunogenicity. Schuman says Biacore is used more frequently to test biosimilars than it is to analyze originator molecules. For biosimilars, Schuman says, "There is much more dependence upon bioanalytical assays, as opposed to some other cell-based assays, because that proof has already been accomplished by the originator. Therefore, a biophysical potency measurement plays a much bigger role in the biosimilar market as opposed to [the market for] the original molecule."

Daniel Galebraith, who is chief scientific officer for Sartorius Stedim BioOutsource Ltd., says analytics is emerging as a core technology in process development. One example of how analytics come into play in biosimilar manufacturing is in the development of copies of Humira. "We have a lot of companies trying to make a biosimilar copy of that molecule. One of [Humira's] functions is ADCC activity," notes Galebraith. "Trying to replicate that molecule with its ADCC activity is difficult for biosimilar manufacturers," he notes. "Many companies have used a number of our assays, monitoring whether the process they use is going to get them that biosimilar copy."

Disposable, single-use filtration is an alternative to outdated centrifugation methods that are difficult to scale and complicated to use.

Cost effectiveness

To be price competitive with the corresponding biopharmaceutical product, the cost of biosimilar manufacturing must be kept as low as possible. Price pressure on biosimilars has driven trends toward more cost-effective manufacturing processes, such as single-use technologies, throughout the biopharmaceutical industry. One counterintuitive trend is downsizing or right-sizing the entire process to increase production. Traditional manufacturing processes for biologics make use of large, fed-batch reactors and oversized chromatography columns that end up wasting time or material. Alternatively, upstream process scale-up can make use of perfusion reactors, which use a

constant supply of cell-culture media while removing unwanted byproducts throughout a prolonged production run of typically more than 20 days (4).

Older biopharmaceutical production facilities are burdened with legacy equipment and outdated processes with highly limited flexibility.

Downstream processing goals include multiple stages of purification and concentration. Disposable, single-use filtration is an alternative to outdated centrifugation methods that are difficult to scale and complicated to use. Expanded-bed absorption (EBA) offers an alternative to oversized chromatography columns. EBA combines filtration, centrifugation, and chromatographic separation into a single step and can handle high-density cell feeds directly from the bioreactor.

Nanofiber adsorbants are another resin column alternative. They are able to flow at fast rates, albeit with low binding capacity.

Taken together, upstream and downstream process innovations can be packaged into an automated, continuous, small-footprint antibody production facility that can fit into a single cabinet in approximately 20 square feet of a GMP production facility. In a concept described by Jacquemart *et al.* (2), one cycle of downstream processing was completed in 24 hours.

In contrast, older biopharmaceutical production facilities are burdened with legacy equipment and outdated processes with highly limited flexibility. Those facilities struggle to compete with biosimilar production facilities equipped with small, efficient, highly flexible processes running on single-use technologies. Innovator products are locked into their older process by regulations. Knowing this, many innovators have been developing their own competing biosimilars in order to take advantage of newer technologies.

Cost competition is transforming technology in biopharmaceutical processing, with biosimilar manufacturers leading the way. Smaller equipment, continuous processes, and single-use technologies are replacing large-scale reactors in a movement reminiscent of the lean manufacturing trend. The ultimate beneficiary will be patients, who are likely to see reductions in the currently astronomical price of life-saving biologic therapies.

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Parallel Processing

Agnes Shanley

Samsung BioLogics' aggressive growth strategy begs the question: Are there lessons that US and European pharma might still learn from the electronics industry?

Although some if its manufacturing practices may have, in the words of a now infamous *Wall Street Journal* article, lagged behind those of potato chip makers and soap manufacturers (1), pharmaceutical manufacturing has still been influenced by innovations from other industries. Best practices from the electronics industry have left their mark on facility design and operations. "Cleanroom technologies, and even the air flow and filtration designs in biopharmaceutical facilities advanced largely because of improvements that had been made at electronics plants," says Robert Dream, a consultant who helped the Biomedical Advanced Research and Development Authority (BARDA) design rapidly deployable drug and vaccine facilities as part of the $6-billion US BioShield program, and who has designed facilities in both industries.

Automation advances

Use of sensors and automation in manufacturing; recent application of robotics and prepacked components in sterile filling; modeling for plant construction and process development; and new approaches to data collection and process validation were all influenced by electronics industry practices, says Dream. Even approaches that are now the norm in pharma, such as strategic outsourcing, were first seen in electronics in the 1990s, when the manufacturing of semiconductor chips was outsourced to specialized contract manufacturers.

Today, pharma and electronics continue to intersect in areas such as artificial intelligence, synthetic biology, and the development of biosensors. Multidisciplinary research promises to result in new collaborations in the future.

But, between 2011 and 2012, the two worlds appeared to collide, when the Asian electronics companies, Japan's FUJIFILM Diosynth

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Biotechnologies (a venture between the MSD Group and FUJIFILM) and Korea's Samsung BioLogics (involving two divisions of Samsung and 10% ownership by Quintiles) suddenly entered the biopharmaceutical field (2,3).

An engineer-driven corporate culture

The move surprised many in the United States, prompting questions about how it might change the biopharma industry. Six years later, these questions remain unanswered, but the two companies' business practices might offer a different view of manufacturing for any biopharmaceutical company executive who still sees it as a subsidiary "stepchild" function (4).

First, both companies come from an engineer-led business culture, in which senior executives (all engineers with business training) walk plant and lab floors to better understand development and manufacturing issues first hand. Their CEOs both have chemical engineering degrees.

This culture is common in Japanese and South Korean companies, but rare in the US and Europe, says Dream, and almost nonexistent in pharma or biopharma. Cross-training is also fundamental. "Recruits work in different functions, and may spend say, a year or two in operations, another year in quality, then more time in validation so that they gain an understanding of process and facility from different perspectives," he says.

Instead of entering the biopharmaceutical arena as manufacturers, a potentially suicidal move given the competitive pressures, timelines, and learning curves that would be involved, both companies entered biopharma as contract services companies, a role that would allow them to leverage their engineering and manufacturing strengths.

Both are expanding rapidly. FUJIFILM Diosynth is investing more than $20 million in its $90-million Texas facility, formerly Kalon Therapeutics, originally funded by BARDA to establish innovative, rapidly-deployable vaccine and biomanufacturing technology. The company plans to invest another $110 million in expansions in the US and United Kingdom, where it plans to establish a center of biopharmaceutical manufacturing excellence (5).

Meanwhile, Samsung BioLogics, which has six clients, including Bristol-Myers Squibb and Roche, took its company public with a $7.8-billion initial public offering in November 2016. The company is close to completing construction of a $746-million plant, its third in Korea, bringing its total biopharmaceutical manufacturing capacity to 362,000 L in seven years. Samsung's CEO wants the company to become the world's leading biopharmaceutical contract development and manufacturing organization (CDMO). Currently, Samsung is in discussions with more than 15 pharmaceutical companies, mainly in the US and Europe, about handling their contract development and manufacturing services (6).

The two companies are establishing their own very different identities in biopharmaceuticals. FUJIFILM is focusing on vaccines and the development of platforms for licensing, such as SATURN for monoclonal antibodies. It has strong ties to the University of Texas biotech corridor and its culture of innovation, marked by its acquisition of Kalon Therapeutics in 2014 (7).

Samsung, meanwhile, has been focusing so far on drug substance (i.e., biologic API development and manufacturing) and on biosimilars development through Samsung Bioepis, its five-year-old venture with Biogen Idec, which received approval for its Herceptin biosimilar during the last

quarter of 2016 and for its Remicade biosimilar in April 2017 (8). In 2014, Samsung and AstraZeneca set up the research firm Archigen Biotech, with branches in Cambridge, UK, and Korea, to develop new, lower-cost therapies for patients with unmet medical needs (9).

Samsung has expanded into the contract development and manufacturing of finished biological drug products, and expects to offer clients one-stop "clinic-to-manufacture" capabilities for both drug substance and drug products in the same facility. This capability was built into the company's first facility in Seoul: a multiproduct facility with upstream, downstream, fill finish, prefilled syringes, and liquid filling capacity. Dream pointed to it as an example of next-generation manufacturing in a presentation at the International Society for Pharmaceutical Engineers' 2015 annual meeting (10).

Additional capacity is planned for Samsung's newest plant when it comes online at the end of 2017. The company will offer lyophilization capacity as well as preclinical, clinical, and commercial manufacturing services. In spring 2017, during the Drug, Chemicals, and Allied Trades (DCAT) and INTERPHEX conferences in New York City, James Park, Samsung's vice-president and head of business development, discussed Samsung's history, culture, and plans with *Pharmaceutical Technology*.

Roots in petrochemicals and semiconductors

PharmTech: Why would a company with such an established reputation in electronics move into such a completely different industry, and why did Samsung choose biopharmaceuticals?

Park (Samsung): Our CEO spent years evaluating new business options and considered a number of different possibilities, but, in the end, biopharma was the most compelling. Demand for biopharmaceuticals is growing rapidly, and our corporation has considerable experience building and operating plants, including 23 semiconductor facilities. Each plant is worth around $5 billion, and requires us to use the latest cleanroom technologies and clean utilities practices, which lend themselves so well to biotech. We also run over 50 petrochemical plants, handling engineering and project management, which can also be applied to biopharmaceutical facilities.

PharmTech: You built each of your first two biopharma facilities in just over two years, and plan to finish the third, and largest, 180,000-L facility in less than three years. How have you been able to do this?

Park (Samsung): Expertise in engineering and project management is what has allowed us to use concurrent engineering practices to reduce the time required to build a biopharmaceuticals plant. From groundbreaking through validation, we cut the time required from the typical five to six years to less than four years.

After basic design, we handle detailed engineering, procurement, and start basic construction with pilings at the same time, then start the actual building construction a bit later, and utility validation and process equipment validation a bit after that so that the work goes on at the same time, in parallel. Compared with sequential engineering, this approach can reduce the timeline by 40%.

PharmTech: Do you see manufacturing expertise as the main asset you offer clients?

Park (Samsung): Where many pharma ceutical companies might see manufacturing plants as cost centers, we see them as revenue opportunities. Our founder and CEO viewed manufactur-

ing as our key to the biopharma value chain. By offering strength in plant design and construction, validation and operation, and a strong quality culture, we believe that we can help virtual companies focus on innovation and big pharma clients to save capital expenditures, use more of their capacity, and improve flexibility.

Operational excellence and *kaizen* in use

PharmTech: Do you have continuous improvement programs and operational excellence programs in place, and do you use Six Sigma and methods like it in day-to-day work?

Park (Samsung): We use *Kaizen* and cross-functional teams extensively, and offer incentive programs for all employees to reward them for new ideas that lead to improvements. The program has led to a number of successes, e.g., a dramatic reduction in batch release cycle time. Another thing that we do differently from many pharma companies is that we hire top four-year university graduates as entry-level operators. They spend the first few years in engineering and validation, then move into operating equipment. This way, even before they start making product, they already have a good understanding of the facility, process, and equipment.

PharmTech: How about R&D and tech transfer?

Park (Samsung): Since June 2016, we have been doing more to promote our capability in the development space, and in process and product development and process characterization. We have improved technology transfer, which usually takes six to eight months, to a point where we can do it in four to six months. We worked on one product that was approved by FDA in 2015, six that were approved by the European Medicines Agency and FDA in 2016, and one that was approved by Japan's Phamaceuticals and Medical Devices Agency in 2017. Through our work in process characterization, we've been developing the capability to develop our own cell lines, and expect to be able to use them in the near future.

Working on its own pipeline

Although Park wouldn't elaborate, he says that Samsung plans to develop its own biopharmaceuticals in the future. It will be interesting to see whether the company brings the connection between research and manufacturing that it has used in electronics, to inhouse biopharma R&D. The company's attention to manufacturing and engineering suggests the vital role that these functions might play in any biopharma company's strategy.

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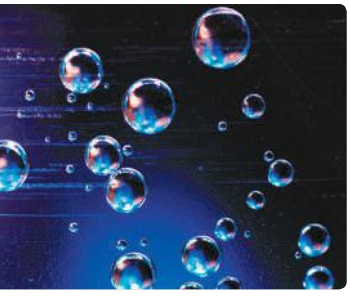

Single-Use Bioreactors Have Reached the Big Time

Cynthia A. Challener

The decision to use disposable bioreactors is now driven by commercial rather than technological considerations.

Single-use bioreactors available from various vendors today are robust and provide the high-performance necessary for commercial manufacturing of biopharmaceuticals. Significant advances in film technologies, bioreactor designs, stirring mechanisms, and sensor systems have contributed to the increasing adoption of disposable reactors from the lab to production scale. Suppliers of single-use bioreactors continue to work closely with customers to address changing needs, such as those for next-generation cell- and gene-therapies and continuous bioprocessing.

"The question of whether to deal with single-use or stainless-steel bioreactors is no longer a technology question, but a commercial one. In other words, does adoption of single-use or stainless-steel bioreactor technology provide greater advantages?" observes Thorsten Adams, director of product management with Sartorius Stedim Biotech. He notes that often for multiproduct facilities with up to 2000 L per bioreactor train, single-use systems are more attractive, while stainless steel or hybrid approaches are typically better suited for large-volume processes for the manufacture of a single product.

Rapid expansion of the single-use bioreactor market supports Adams' statement. Market research firm Markets and Markets projects the US bioreactor market will increase at a compound annual growth rate of 21.6% from $408.4 million in 2016 to $1.09 billion in 2021 (1).

Measurable progress

"Driven by this high demand, the supply chain for disposable or single-use technologies has become more robust, covering more technologies than before and meeting the growing expectations from our customers," asserts Morgan Norris, general manager of upstream and cell culture with GE Healthcare Life Sciences.

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Whereas the first disposable bioreactors were 'plastic' copies of their stainless-steel counterparts, new generations are redesigned while keeping the end-user, their processes, and their final drug products in perspective, according to Annelies Onraedt, director of marketing for cell culture technologies at Pall Life Sciences. "When considering the workflow and objectives of running bioreactors, recent developments have focused on reducing complexity, improving mixing to achieve higher volumetric mass transfer coefficients (kLa) for oxygen, and avoiding current challenges, such as integrity issues," she explains.

Improvements in cell-culture processes have led to higher titers and thus higher cell densities, which have facilitated the adoption of single-use bioreactors through reductions in needed reactor volumes. On the other hand, initial single-use bioreactors lacked the power input and mixing capability observed with stainless steel equivalents, according to Onraedt. Leakage and integrity issues also led to reduced confidence in disposable bioreactor technologies for larger-volume applications.

Newer generations of single-use bioreactors have addressed these issues. Pall, for instance, overcame seal housing challenges with the introduction of a large, bottom-driven, elephant ear impellor that also provides the greater power input that high cell density cultures require, according to Onraedt. She also notes that integrity and leakage are now addressed during bioreactor manufacturing and by improving usability/handling of single-use systems. "For example, Pall's SU bioreactors come with special packaging and an easy-to-implement biocontainer installation method that eliminate the main causes of integrity failures," she says.

Suppliers have also addressed questions about the films used to produce single-use bioreactors, according to Norris. Many suppliers, including GE, Sartorius, Pall, and Thermo Fisher Scientific, have developed new film platforms. GE's technology resulted from a strategic alliance with Sealed Air. Film validation using harmonized methods will, in the future, establish a framework for comparison and validation of product contact films for a process, reducing time and effort for the user, notes Onraedt.

Automation and process control, both of which are essential to enabling consistent and reliable biomanufacturing, have improved greatly for single-use bioreactors as well. "Historically, single-use technologies required manual operation and had standalone automation, which created a challenge to control and monitor the entire process train. We have taken automation to the next level, delivering fully automated process trains," Norris states.

Commercial choice

As such, Norris sees single-use bioreactor processes designed with the entire workflow in mind, delivering a high quality, optimal biologic titer into the downstream unit operation. Adds Adams: "single-use bioreactor technology has matured to the point where suppliers now offer large-scale, robust, well-characterized products that are reliable, scalable, and often preferred over stainless steel."

These improvements mean that the biopharmaceutical industry has a lot more confidence in single-use technologies today, and the use of them is more widespread, according to Norris. "Previous concerns over business continuity and the regulatory requirements relative to implementation have been addressed, and there are now proven solutions available for customers," he comments.

In addition, Norris notes that while early adopters limited use of disposable bioreactors to process development or seed train processes feeding large stainless-steel bioreactors due to the 2000 L peak volume for single-use systems, the increased titers of newer cell culture processes, the growth of biologics targeting smaller patient populations and the expanding demand for biosimilars in emerging markets has meant that total output needs now fit within the capable production range of single-use bioreactors.

In fact, an attractive attribute of single-use bioreactors is their full scalability, according to Adams. Today there are systems available for high throughput R&D through to commercial production. Sartorius, for instance, through its acquisition of TAP Biosystems, now offers AMBR mini single-use bioreactors (15–200 mL) operated with a robotic system for rapid evaluation of critical process parameters using minimal material. "Because this technology is scalable, users can quickly scale up optimal reactions to single-use bioreactors in the production environment for reduced time to market," Adams observes.

Expanding applicability

There are, however, some cases where single-use bioreactors may not be the ideal solution, despite their advantages with respect to reduced setup times and cleaning/cleaning validation requirements. "Sometimes there are unique molecules, or molecules that require such a large output of the biologic that large-volume, stainless-steel bioreactors are more suitable," says Norris. "Examples include traditional vaccines and blockbuster [monoclonal antibody] (mAb) biologics (>2 metric tons of mAb per year); in these cases, single-use bioreactors might not be the most efficient process to use."

Single-use bioreactors have also not yet been widely used for microbial fermentation processes. These reactions have a high demand for oxygen and are typically run under pressure with very high gas flow rates, according to Adams. "Single-use bioreactors on the market today generally meet 70% of the performance of their stainless-steel counterparts and thus do not offer a one-to-one process transfer from stainless-steel to single-use systems; compromises and modifications are necessary," he explains.

The issue: more power is needed to increase the stirring speed and oxygen transfer rate. Suppliers are working on various solutions. Sartorius is developing a new technology and currently is evaluating its performance. GE, meanwhile, recently introduced single-use technologies for microbial fermentation. The system is designed to accommodate the demands of microbial cultures, including mass transfer, mixing, and temperature control, according to Norris.

GE is also developing solutions for vaccine and viral vector manufacturing in single-use bioreactors and is investigating the potential for creating turnkey, biosafety level two (BSL-2) single-use unit operations upstream. The company has also invested $7 million in its single-use manufacturing capabilities in Westborough, MA, shifting the way it develops and manufactures single-use technologies. "This investment highlights our commitment to meet our customer's current and future requirement needs around single-use technologies and drive faster production development processes internally," Norris says.

Sensors are improving

The development of robust single-use sensor technology has been one challenge for suppliers of sin-

gle-use bioreactors. In the past year, however, good single-use sensor technologies have emerged, some of which have become established standards for single-use bioreactors, according to Onraedt. Most common are pH, dissolved oxygen, and carbon dioxide sensors based on fluorescence technology. Others are gaining acceptances, such as capacitance. Newer technologies have more recently been introduced for determination of viable cell mass, glucose, and lactate concentrations.

For instance, data obtained with the Sartorius' Viamass sensor for online viable cell density determination are useful for adjusting feed rates in real time and for defining the point for virus injection during vaccine cell culture, according to Adams.

Meanwhile, glucose and lactate sensors are useful for determining glucose feeds and monitoring cell metabolism, respectively. "With the range of disposable sensors now available, it is possible to obtain key data in real time without the risk of contamination because physical samples no longer need to be taken from the bioreactor," Adams states.

The capabilities of software tools associated with single-use sensors are also advancing. Recipe functions and data acquisition and analysis capabilities, including most recently multivariate data analysis, allow for effective monitoring of bioprocesses and provide greater confidence in the user's ability to determine the optimum operating range, according to Adams.

Perhaps the greatest challenges for single-use sensors are the need to expose them to gamma-radiation during the sterilization process and to extend their lifetimes, particularly for continuous processes, according to Onraedt. Norris agrees that in addition to longer life-times for new processes, the industry is actively seeking single-use sensors with better accuracy and precision. She does note, however, that the state of single-use sensor technology for use with single-use bioreactors in GMP biomanufacturing is constantly improving. "There are several approved biologic manufacturing processes using single-use bioreactors, proving that the current technology meets regulatory requirements," he says.

Single-use and next-generation biologics

The philosophy behind single-use technologies, including their flexibility and ability to produce smaller batches (reducing the scale for more precise therapies), fits well together with the thinking around cell/gene therapies, according to Norris. "GE is taking its experience in single-use bioreactor technologies for monoclonal antibody, recombinant protein, and vaccine processes and applying it in our Xuri line of cell therapy equipment and reagents to support next-generation technologies for new drugs, including scale-up models; regionalizing single-use manufacturing platforms for biologics (increasing local production); and the development of scale-out systems for cell therapy applications," he notes.

For cell-based therapies, Pall has focused on developing single-use adherent cell-culture bioreactors, which it offers under the XPansion and iCELLis brands. "We have asked three key questions: What is the objective of the cell culture? What is the current workflow with traditional technologies? and What is required from a single-use technology to facilitate this process?" observes Onraedt. "It is not only about doing what has been done previously but in a single-use format; it is also important to look at overall productivities and cost-of-goods reduction," she adds.

The iCELLis technology, for instance, can replace roller bottles for major vaccine and gene therapy processes, but also dramatically reduce the footprint required to produce the virus titers, and it requires less pDNA to transfect the cells, according to Onraedt. "Single-use bioreactors such as the iCELLis allow rapid process qualification, implementation, and commercialization, providing the speed-to-market that next-generation biologic manufacturers need," she states.

For the most part, cell- and gene-therapy processes have, to date, been conducted in existing single-use bioreactors. Rocking-motion systems are most widely used in these applications, particularly for autologous cell therapies, according to Adams. "One bioreactor is needed per patient with these patient-specific therapies, which imposes logistics issues that create the need for foolproof tracking systems to prevent material mix-ups. We do expect future developments will address the need for more integrated systems with higher levels of automation. The implementation of Sartorius' integrated non-invasive biomass sensor in our rocking motion bioreactor gives real-time data on cell health and enables early fault detection, and is a major step towards a fully automated cell-therapy production system," Adams says.

Continuous impact

The operating conditions for continuous processes must be more robust, and more rigor is required. "Today, a continuous process can last significantly longer than current fed-batch processes, which means that there are higher expectations and a need for a high-level of sensing analytics, providing a secure production process for a longer period of time," Norris explains. "Understanding how multiple unit operations can be optimized in a continuous setting will also have a major impact on how we develop next generation single-use bioreactors," he adds.

Continuous processes frequently have higher cell densities, which require better mixing and higher power input, according to Onraedt. "Only the newer generation of single-use bioreactors, such as Pall's bottom-driven impeller system, have suitable designs to enable achievement of the necessary oxygen transfer rates and kLas," she says.

GE has invested heavily in its process development capabilities to help optimize cell-line performance, cell-culture media and supplement consumption, and biologic quality in a continuous process. The company plans to go deep into the technical and process details for media consumption and harvest operations to determine how single-use bioreactors can be optimized based on how these operations vary, according to Norris.

In addition to developing robust single-use bioreactors, Sartorius developed its new kSep technology to ensure optimized power efficiency during continuous operation. The company has also focused on developing single-use bioreactors that can interface with devices, such as cell retention devices, from different manufacturers and that contain sterile connections for integration with both single-use and stainless-steel sensors. Its control system can also be integrated with different user systems. Sartorius has also introduced a new single-use centrifugation system that allows product recovery from the material removed during the cell bleed step. Currently, this product is typically discarded with the used cells.

Reference

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Case Study: Retrofitting Two New High-Purity Water Systems

Brian Lipko, Brian Termine, and Steve Walter

The existing, obsolete high-purity water generation system and water-for-injection generation system were replaced with new, reliable technologies.

GlaxoSmithKline (GSK)'s R&D Biopharmaceutical Pilot Plant in Upper Merion, PA is a R&D clinical trial material (CTM) manufacturing facility with an aggressive processing schedule that requires minimal shutdown interruptions. Utility reliability is paramount to achieve production demands and regulatory quality requirements. To meet the utility demands for increased CTM output, a capital investment project was required to replace the existing, obsolete high-purity water (HPW) generation system and water-for-injection (WFI) generation system with new, reliable technology.

The project drivers were:

- Existing generation systems (HPW and WFI) had insufficient capacity for current operations and were unable to meet the demands of the growing pipeline.
- Spare parts for the existing systems were either not available or becoming more difficult to source.
- Existing systems were costly to maintain, not energy efficient, not reliable, and had insufficient redundancy, increasing the potential for unscheduled production downtime.

The primary objective for the project was to provide water system(s) generation reliability with the following additional requirements:

- Deliver more environmental sustainable systems (i.e., lower water and energy usage)
- Increase supply and storage capacity
- Replace obsolete equipment
- Have no impact on ongoing GMP operations.

GSK engaged Hargrove Life Sciences to complete the design for this project. The conceptual design phase included evaluation of new equipment technologies, a sustainability evaluation including energy and operating cost comparisons, and visiting other recently

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installed GSK water systems at other locations. Also, because the pilot plant was landlocked, with no available space inside the facility for new equipment, investigation and analysis of where to install the new water systems was required.

Selecting the equipment and technology for the HPW and WFI systems was based on evaluations that took into consideration sustainability goals including reduction of energy, water, and carbon footprint. It was also determined that the best way to achieve system generation reliability was to have complete redundancy for all mechanical equipment (i.e., essentially two of everything).

Redesigning the high-purity water system

The existing HPW distribution system provided a continuous flow of 30 gallons per minute (gpm) of purified water at 25 °C. Purified water was constantly circulated to the utility systems in the basement mechanical room and also to the three GMP operating floors using dual, sanitary variable-frequency drive (VFD) pumps rated at 150 gpm. The basement utilities supplied with HPW feedwater included a WFI still and two clean-steam generators.

This existing system produced water with a resistivity greater than 10 Mohm and total organic carbon (TOC) with less than 5 ppb, and GSK wanted to maintain this high quality for the new system while also incorporating a sustainable design.

Criteria for the new HPW system included the following:

- Redundant mechanical equipment (i.e., two of everything). Reliability would be achieved through redundancy, with dual multimedia filters, softeners, carbon filters, reverse osmosis (RO)/continuous deionization (CDI) skids, distribution pumps, and vent filters.
- The existing 34 gpm generation system did not always adequately maintain building operating demands. The new system(s) would require larger capacity (40 gpm) for existing building operating demands, increased WFI generation, and future building expansion or increase in users/processes.
- Start/stop technology was a requirement to reduce electrical energy and water consumption rates.
- The generation system must be designed for hot water sanitization at 80 °C (65 °C minimum).
- A mixed-bed polisher would be required on the new system design to meet resistivity quality requirements.
- A new stainless-steel, HPW storage tank must be provided that would include increased storage capacity to meet larger instantaneous demands of water from expected increases in production. The new tank would also retain a nitrogen blanket that was installed on the existing storage tank, which had proven to be successful in helping to maintain a low bioburden in the system.

Based on these criteria, it was decided that the RO system only needed to be single-pass technology to meet HPW quality requirements. The project team also decided to implement carbon filtration instead of bisulfite injection or ultraviolet light technology as the primary method to remove residual chlorine/chloramines. Although carbon filtration represents the most expensive initial cost, it is the most effective method of removing residual chlorine/chloramines. The downside of using carbon filtration is the carbon filters are an ideal breeding ground for bacteria. However, because the carbon filters would be hot water sanitizable,

the concern of bacteria growth was reduced. Other design considerations included:

- Installing a new bulk brine storage system to eliminate the need for manual material handling of the salt required for softener regeneration
- Installing a RO reject water recovery system used for makeup water to the building's cooling tower
- Supplying HPW to an adjacent biopharm process development facility, thus enabling the decommissioning of a second water purification system serving this adjacent building, which further reduced site operating expense
- Installing a new online microbial detection system.

The online microbial detection technology was released for commercial use in pharmaceutical clean utility systems just prior to the design phase of this project. After various on-site pilot testing scenarios, it was concluded that this online microbial detection technology would be installed on the HPW distribution system, greatly reducing the system's water sampling and analysis work load. The project purchased and installed one of the first commercially available units in the United States. This online microbial detection system complements the online TOC and conductivity systems, which are typical to GSK water system designs.

Redesigning the WFI system

The existing WFI generation system was a 25-year-old multi-effect (ME) still. The unit produced a maximum of 470 gph of WFI at 82 °C. As the facility's GMP processing manufacturing capacity increased, the WFI generation system had inadequate generation capacity, which resulted in rigid planning for various manufacturing users so that the WFI storage tank would not be completely drained during use. WFI manufacturing capacity studies indicated that the maximum WFI usage in the facility could approach 1325 gallons in a two-hour period. With the existing WFI still make-up rate, the WFI storage tank volume would fall dramatically and cause the WFI distribution system to shut down. It was determined that the existing WFI storage tank would not be replaced because of limited head room and access space into the basement utility area.

With maximum site plant steam pressure limited to 90 psig, replacing the existing still with another ME still would require an oversized (de-rated) system, as these are designed to normally operate with plant steam at 115 psig. In addition, the ME still would require an external cooling exchanger to remove excess heat, which would have an impact on the building's process glycol system.

With reduced plant steam pressure and the desire to minimize process glycol loads, the engineering team determined that the replacement WFI stills would be vapor compression (VC) type stills. VC stills are designed to operate with 50 psig plant steam in lieu of 115 psig required for ME stills, which yields energy savings from reduced steam usage. Also, the cooling load could be virtually eliminated, which would reduce approximately 25 tons of process glycol that was required for the ME still. Although VC stills are more expensive from a capital installation cost perspective, they are more economical to operate from a utility demand.

The existing WFI distribution system consisted of three independent supply loops with a dedicated pump for each GMP operating floor of the facility. The new pumping distribution system design consisted of two new VFD-controlled redundant pumps each capable of serving all three floors simultaneously. If one pump were to fail, the remaining pump

Figure 1: Courtyard between two buildings that was chosen as the site of the new, two-story mechanical room.

was designed with sufficient capacity to maintain WFI distribution for the entire facility.

Retrofitting the water systems

At the completion of engineering design, the project team determined that the two new water systems must be delivered using a phased construction and validation approach to minimize shutdown interruptions to the manufacturing areas.

Phase 1 included the construction, installation, and validation of a new HPW generation, storage, and distribution system in a location that would not impact the facility's planned GMP manufacturing schedule. Phase 1A included replacement of the WFI distribution pumps and the main WFI control panel including validation testing. Phase 2 involved the demolition of the existing HPW generation and storage equipment in the building basement to establish the location for the installation of two new VC WFI stills. Phase 2 could only be completed after Phase 1 HPW generation and distribution systems were released for GMP use.

Phase 1. Phase 1 would entail the construction of a strategically placed new mechanical room in an unused courtyard that was isolated between existing facilities, as shown in **Figure 1**. The new mechanical room in the chosen location was required to be two stories, providing a location to install, commission, and validate the new HPW system prior to connecting to the operating pilot plant and decommissioning the existing HPW system.

The redundancy requirement proved to be a challenge because two HPW generation trains would need to reside in the proposed two-story mechanical room, which was constrained in a space that was only 14 ft. wide and 110 ft. long. A thorough and iterative study was done to confirm the equipment could be installed and be serviceable, which included the development of a three-dimensional design model (see **Figure 2**). Once the proposed space design was proved out, the detailed design of the water system and proposed addition began. The GSK/Hargrove team worked closely together to design every aspect of the project to ensure minimal impact to the existing adjacent operating facilities.

Phase 1 construction of the HPW mechanical room in the unused courtyard required the removal of a portion of Building 38's exterior glass façade, potentially exposing the pilot plant to the elements of nature (e.g., weather, insects). Therefore, prior to removing any portions of the building's existing façade, temporary weather-proof interior walls were constructed. As the façade was then being removed, the daily limit of removal was controlled to an area that could be sealed back up in the same day with a temporary facade.

Installation of equipment into the new HPW mechanical room. With the new mechanical room completely surrounded on all sides by existing buildings with no access for bringing in large pieces of equipment, it was necessary to install all the major pieces of equip-

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ment using a hydraulic crane. The challenge with doing this is that the room construction took place prior to equipment delivery. Therefore, the project team staged the construction of the new room to essentially have a section of the mezzanine floor and roof to be constructed after equipment installation. Once the missing building sections were constructed, equipment could then be shifted to its final location, as shown in **Figure 3.**

Phase 1A. Phase 1A included the installation of two new WFI distribution pumps and the new WFI control system. The existing WFI distribution system consisted of three independent loops for each GMP operating floor of the building, each with its own pump. The new distribution system consists of two new VFD-controlled redundant pumps each capable of serving all three floors. This phase was the most critical to ongoing operations because there was no backup plan (i.e., everything had to go right the first time to bring the WFI system back on-line in the shortest possible time frame). Therefore, extensive planning was required including: the development of process operational descriptions for the new control system, which included 86 instruments and 106 input/output points and the development of the WFI system hydraulics using Fathom Modeling for the entire building's distribution system, which turned out to be extremely valuable for vetting the design. The plan to control the distribution system was to use flow control on the return from each parallel floor loop to maintain minimum velocities. Also, the third-floor return pressure, which was the most remote location in the system, would be used to adjust the speed of the pumps to maintain minimum loop pressures.

Once Phase 1 and Phase 1A were completed, the pilot plant would be supplied with high-purity water from the new HPW generation system and supplied with WFI using the existing ME still, new redundant WFI distribution pumps, and new WFI control panel. Phase 2 could then commence, which included the demolition of the existing HPW system in the basement mechanical room and the installation of two new WFI VC Stills.

Figure 2: Three-dimensional design model of two-story mechanical room.

Figure 3: Installation of equipment into the high-purity water mechanical room.

Phase 2. Phase 2 construction activities were conducted similarly to Phase 1 given that the new WFI stills would be installed, commissioned, and validated while the Pilot Plant was fully operational. Due to the challenge with bringing the new equipment into the basement mechanical room through a constrained access door, the new stills were designed to be disassembled and shipped in multiple components that would then be reassembled onsite.

Utility tie-ins for the different project phases. All supporting utility system tie-ins (e.g., nitrogen, air, clean steam) and the connecting of generation systems to the building's existing distribution systems were completed during preplanned windows when the pilot plant was not operating and had no water demand.

Leveraged FAT qualification approach. The project team decided early to use a leveraged factory acceptance test (FAT) qualification approach to reduce the overall project validation schedule.

All GSK equipment FATs are executed and documented in a manner that may allow GSK to "leverage" the FAT execution documentation (FAT turnover package) during future commissioning and validation on-site at their facility by referring back to FAT-executed approved testing and results. The FAT is a GMP activity approved by all stakeholders including the equipment vendor, GSK Engineering and Validation group, Quality Assurance group, and Facilities Operations group to eliminate the need for redundant testing once the system has arrived on site.

Testing that has been successfully completed at the FAT and poses a low risk of being impacted by transport of the system from the factory to the site in a manner that would change the results of the testing will not be repeated. This testing is detailed and leveraged under a later lifecycle qualification document. Testing that has not been successfully completed within the FAT or that poses a higher risk of impact during transport is either repeated or conducted for the first time at the site under a later lifecycle qualification document. This approach reduced the qualification schedule by three weeks.

The total duration of construction was approximately 60 weeks. The majority of this time (44 weeks) was spent on Phase 1, which was the construction of the new HPW mechanical room and installation and validation of the HPW system. Phase 2, which was the removal of the existing HPW equipment in the building's basement and the installation and qualification of the new WFI generation systems, took another 16 weeks to construct and validate.

Project challenges and results

The main challenges included the small area to construct the new HPW mechanical rooms and the limited utility shutdown opportunities available to tie-in two new water systems. Another major concern was installing the new WFI distribution pumps with a new WFI control panel because there was no turning back when the old pumps and control panel were removed. The WFI concern was reduced after full programmable-logic-controller simulations were completed along with hydraulic modeling of the WFI distribution system. In fact, the installation and validation of the WFI pumps and controls went exactly as simulated.

The phased approach for construction, validation, and testing provided for the installation of state-of-the-art water generation systems with minimal plant operation downtime and no impact to ongoing production. Since the release of the project for GMP use, benefits were realized quickly by the business, such as ample supply of water, reliable supply of water, lower energy usage, and lower overall operating costs. The GSK Engineering design team worked with selected equipment vendors to minimize water and electrical usage wherever possible, which has resulted in significant environmental sustainability benefits and operational cost savings. Annual operational expenses were reduced by approximately $170k per year, and annual carbon emissions were reduced by 348 tonnes CO_2.

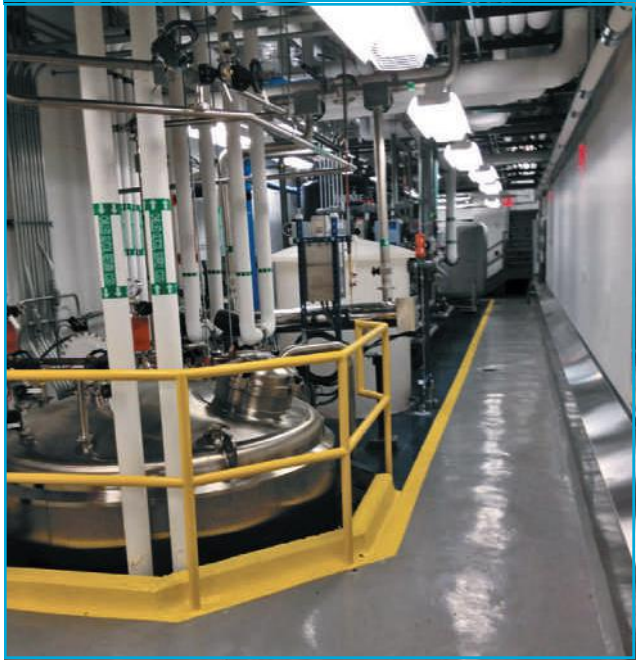
The two new redundant HPW generation systems (see **Figures 4 and 5**) represent the latest technology for energy and water efficiency in the production of *United States Pharmacopeia* grade water. They are designed using commercially available technology that enables one of the systems to automatically be brought off-line to be sanitized and put back on-line when required. This control technology also consumes significantly less water and energy and produces significantly less waste-water compared to conventional systems. This system translates into operating savings, with more environmentally responsible, energy-efficient, purified water generation processes. In addition, customized software programming requirements were implemented to reduce potable water usage during the softener regeneration cycles, and the RO membrane cleaning is now determined on normalized differential pressure software monitoring in lieu of a traditional totalized flow rate approach.

Major benefits included in the implementation of the new HPW generation systems included the following.

- Water make-up and sewer savings were approximately 11,000 gallons per day (or 4M gallons per year).
- Electrical costs were reduced by 88% because the HPW generation system shuts down if there is not a demand for HPW storage tank make-up.
- The on-line microbial detection system has reduced manual quality grab samples by 20%.
- The bulk brine tank system reduced site labor costs because the manual salt replenishment process has been eliminated.
- Additional operational savings were realized by having the new HPW system supply the biopharmaceutical development pilot plant, thus eliminating a costly vendor service ion-exchange contract.

Figure 4: First floor of the new water treatment room with the new high-purity water generation system (reverse osmosis and continuous deionizer skids).

Figure 5: Second floor of the new water treatment room showing the top of the high purity water storage tank with multimedia, softeners, and carbon filters in the background.



The two, new, redundant VC stills provide additional capacity and ensure reliability. The VC stills were also designed and validated to operate using variable compressor speeds to reduce electric demands when providing WFI to the storage and distribution system. For example, if the required

WFI fill rate is minimized, the still compressor can operate on a slower speed to lower electrical operating costs.

Major benefits included in the implementation of the new WFI generation systems include:

- Electrical costs reduced by 66% (VC does not have a feed water pump)
- Plant steam consumption reduced by 65%
- Chilled water consumption reduced by 97% (not required by VC for cooling)
- Blow down of high purity water to drain reduced by 91% (VC does not blow down in standby mode).

The water systems were installed with minimal planned downtime, and the two water-generation systems were constructed and validated in approximately one year. All project objectives were exceeded. **PT**

UNDERSTANDING *USP* <1231> WATER FOR PHARMACEUTICAL USE

Manufacturing of pharmaceutical products, medical devices, biologics, cell- and tissue-based products, and many other medical products requires significant volumes of water. Water is more complicated than what most people think. The two major categories are bulk water (i.e., produced on-site where used from an internal water system) and packaged water (i.e., produced elsewhere, packaged, sterilized to preserve microbial quality throughout the packaged shelf life, and purchased). Regardless of whether it's bulk water or packaged water, the type of water is then determined by the testing performed, as defined by *United States Pharmacopeia (USP)* <1231> (1). The following definitions can help navigate the complexities of the different types of water and provide a better understanding of their appropriate usages.

Purified water. Purified water is most commonly used as a diluent in the production of non-sterile products for injection, infusion or implantation, cleaning equipment, and cleaning non-sterile product-contact components. Purified water systems must be validated to consistently produce and distribute water of acceptable chemical and microbiological quality. However, they may be susceptible to biofilms, undesirable levels of viable microorganisms, or endotoxins, which means frequent sanitization and monitoring to ensure appropriate quality at the points of use.

Water for injection (WFI). WFI is most often used as an excipient in the production of sterile products and other preparations when endotoxin content must be controlled. Examples are pharmaceutical applications such as cleaning of certain equipment and sterile product-contact components. WFI must meet all the same chemical requirements of purified water with added bacterial endotoxin specifications, because endotoxins are produced by microorganisms that are prone to inhabit water. As with a water system producing purified water, WFI systems also must be validated to reliably and consistently produce and distribute water of acceptable chemical and microbiological quality.

Pure steam. Pure steam is intended for use in steam-sterilizing porous loads and equipment and in other processes, such as cleaning, where condensate would directly contact official articles, containers for these articles, process surfaces that would in turn contact these articles, or materials which are used in analyzing such articles. Pure steam is prepared from suitably pretreated source water, analogous to the pretreatment used for purified water or WFI, vaporized with a suitable mist elimination, and distributed under pressure.

Water for hemodialysis. This type of water is specifically for hemodialysis applications and primarily for the dilution of hemodialysis concentrate solutions. Water for hemodialysis is typically produced and used on site as bulk water. This water contains no added antimicrobials and is not intended for injection.

Sterile purified water. This water has been packaged and rendered sterile. It is used for preparation of sterile products or in analytical applications requiring purified water when access to a validated system is not practical and only a small quantity is needed. It is also used when bulk packaged purified water is not suitably microbiologically controlled.

Sterile water for injection. This water has been packaged and rendered sterile. This water is for the processing of sterile products intended to be used intravenously. Additionally, it is used for other applications where bulk WFI or purified water is indicated but access to a validated water system is either not practical or only a relatively small quantity is needed. Sterile WFI is typically packaged in single-dose containers that are typically less than 1 L in size.

Sterile water for irrigation. This water has been packaged and rendered sterile. This water is commonly used when sterile water is required, but when the application does not have particulate matter specifications. Sterile water for irrigation is often packaged in containers that are typically greater than 1 L in size.

Sterile water for inhalation. This water has been packaged and rendered sterile. This water is usually intended for use with inhalators and in preparation of inhalation solutions. It carries a less stringent specification for bacterial endotoxins than sterile WFI and, therefore, is not suitable for parenteral applications.

Bacteriostatic water for injection. This water is sterile WFI to which one or more suitable antimicrobial preservatives have been added. This water is typically intended for use as a diluent in the preparation of sterile products, mostly for multi-dose products that require repeated content withdrawals, such as liquid pharmaceuticals. It may be packaged in single-dose or multiple-dose containers, usually less than 30 mL.

With nine different types of water, each with specific testing requirements and applications, it is crucial to understand how they can impact products. Using a less stringent type of water for a product based on its intended use could be a costly mistake. Similarly, using a more stringent type of water, when not required, could result in increased costs. Add in the increased scrutiny of the ever-changing regulatory landscape, it becomes even more critical to have a complete understanding of the water a process requires.

Reference

1. *USP* <1231> Water for Pharmaceutical Purposes. (Rockville, MD, March 8, 2017).

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Developing an Injectable Compound for a Dual-Chamber Delivery System

Joerg Zimmermann

Prefilled dual-chamber cartridges offer several advantages. Several steps should be taken to determine if a dual-chamber system is viable for a lyophilized injectable drug product.

As global demand for injectable systems grows, so too does the demand for innovative delivery options beyond the traditional system of syringe and vial. For lyophilized forms, dual-chamber systems offer advantages. The prefilled dual-chamber system or cartridge is self-contained, holding both the lyophilized product and diluent in separate chambers. As such, there are fewer reconstitution steps. And there is reduced overfill, which results in API savings. The predefined dosing also means greater safety for the patient and caregiver as well as ease of self-administration.

When considering whether to use a dual-chamber system, it is important to understand the necessary development process, which is summarized in **Figure 1**. The following sections describe five steps that can help determine whether lyophilization in a dual-chamber system is a viable option for an injectable drug.

Step one: Lyo cycle feasibility studies

Step one of the process involves lyophilization cycle feasibility studies, which include freeze-drying microscope and differential scanning calorimetry studies. Dual chamber trials based on existing vial lyophilization development are performed. Cycle options to test the viability of a product in a dual chamber and concentration and fill volume studies for multi-dose products are also performed.

Step two: Process characterization studies

In step two, process characterization studies that help assess the current upstream process and any studies needed for the development in a dual-chamber system are completed. Compounding/mixing studies

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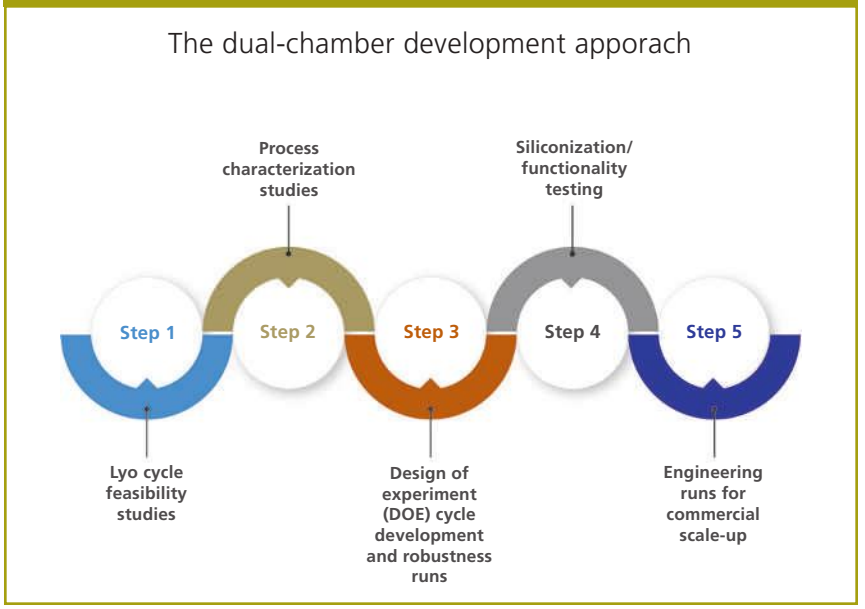
to determine mixing parameters and excipient matrix and tracer studies with minimum and maximum compounding volumes are undertaken. Filtration studies are used to determine the necessary filter sizes and flush volumes. Finally, pumping and dosing studies are undertaken to develop pump settings and filling needle movement for precise dosing.

Figure 1: Dual-chamber process development summary.



Step three: Design of experiment cycle development and robustness runs

Step three includes design-of-experiment (DOE) cycle development and robustness runs to test the limits of the design space for both primary and secondary drying. The DOE approach is used with different temperature and pressure combinations. Target cycle parameters are selected to determine robustness for scale-up. Visual lyo cake appearance, product critical quality attributes (CQAs), residual moisture, and reconstitution times are analyzed.

Step four: Siliconization/functionality testing

Step four involves siliconization and functionality testing. Different levels of silicone used to lubricate the dual-chamber system are tested for their impact on the drug product and the delivery device, such as the break-loose and gliding forces and the lowest and highest silicone spray rates achieved with different silicone emulsion concentrations. Samples of the formulation are filled into the dual-chamber system and tested for silicone level. Stability testing is also undertaken for the drug product—the same CQAs as in the lyo cycle development are assessed, and this completes the functionality testing step.

Step five: Engineering runs for commercial scale-up

The final step entails engineering runs for scale-up to commercial production to ensure the process is scalable. Here, there are two stages. The first is non-GMP commercial scale-up consisting of general feasibility at production scale, fill volumes, product concentration testing, product temperature mapping, and sample analysis. The second step is lyo-cycle adaptation and testing including trials performed under "seeded run conditions" (i.e., several different test samples of the product are positioned in lyophilization storage units) and testing of multiple concentrations.

Process qualification/validation in the form of robustness runs to challenge the design space and extremes in temperature and pressure are undertaken. The minimum requirement is usually analysis of samples from two runs: high energy/high pressure and low energy/low pressure. Process qualification is performed at nominal conditions and a bracketing

FIGURE IS COURTESY OF THE AUTHOR

approach is used to cover several lyophilizers, product strengths, and minimum and maximum loads.

Conclusion

The steps outlined are essential in a typical product development approach used to assess if the dual-chamber system is suitable for delivering a specific drug formulation. If the lyophilization feasibility studies show that the dual-chamber system is a viable option for the drug product, characterization studies are required to optimize the process and develop a robust lyophilization cycle. Siliconization and functionality testing are important for determining the optimal silicone level and assessing its impact on the drug product and dual-chamber system. Finally, engineering runs are carried out to enable a scalable process. **PT**

Improving Air Quality from Vacuum Pumps

Compressed air used in pharmaceuticals manufacturing is held to the highest possible standards. However, there is currently a risk that sites may be overlooking another potential source of contamination—the exhaust air emitted by vacuum pumps.

Air quality

Few major industries in the world place a greater level of importance on hygiene and avoiding contamination than pharmaceuticals manufacturing. Stringent standards regulate the quality and specification of the compressed air used throughout manufacturing sites, most notably ISO 8573 (1). The nine parts of this ISO standard detail the amount of contamination allowed in each cubic metre of compressed air and specify the methods of testing for a range of contaminants, including oil and viable microbial contaminants.

As well as making sure that they are compliant with ISO 8573, an increasing number of pharmaceutical sites follow the principles of the Hazard Analysis Critical Control Point (HACCP). Originally designed for use in the food manufacturing industry, these principles ensure that sites are complying with hygiene legislation and either eliminating any potential hazards or reducing them to an acceptable level.

Yet, while most manufacturers spend countless hours making sure that their direct production processes are scrutinized in great detail, ancillary processes and utilities can often be skimmed over or even omitted entirely. Despite the comprehensive standards for the quality of the compressed air, there are no matching standards covering the exhaust air being emitted by the system's vacuum pumps. As these vacuum systems will generally be located around the production environment, a contaminated pump exhaust can cause hygiene issues that completely undermine the time and effort spent ensuring that the compressed air itself is pure.

Potential risks

The majority of vacuum pumps currently in use throughout the pharmaceuticals industry are lubricated with oil. These pumps have been the standard for many years and most will be perfectly reliable. Nevertheless, poor maintenance practices or minor equipment faults can create the risk of oil discharging from the exhaust. In addition, if the system is operating at high temperatures with an open-ended inlet port, oil could carry over from the pump. A separator element, which removes any oil particles remaining in the air, may also fail due to misuse or through the use of non-genuine spare parts.

Solutions

If a system is well maintained, then the chance of any contamination is already low, but eliminating the potential risk of leaks from an oil-lubricated vacuum pump can be achieved through a range of measures. These include using a specialist food-grade lubricant to reduce the impact of any potential contamination, fitting a downstream exhaust filter, or remotely piping the exhaust air.

Although reducing the risks associated with oil-lubricated vacuum pumps is comparatively straightforward, the sensitive nature of pharmaceuticals production means that for some companies it may make sense to implement an oil-free model instead.

Oil-free vacuum pumps have been developed specifically to meet the needs of manufacturers that require only the highest air purity environments. They generally require a slightly higher up-front investment. However, there is no need to replace the oil or filters because they do not require the same level of maintenance as oil-lubricated models, which may generate savings over the course of a pump's lifetime. In addition, an oil-free vacuum pump does not have to be removed to carry out essential maintenance servicing, so there is no equipment downtime and no associated costs from oil, waste oil disposal, or labor.

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—Gareth Topping is sales manager at Gardner Denver.

Ensuring the Biological Integrity of Raw Materials

Catherine Shaffer

A multi-pronged approach to raw materials testing can help mitigate the risk of future contamination events.

Contamination with microbes, mycoplasma, viruses, and other adventitious agents can be a significant problem in biopharmaceutical manufacturing. Although contamination can occur from the cell culture itself or from labware and the laboratory environment, raw materials are the most significant source of contamination. That can lead to false research results and a serious health risk to patients receiving the product.

Contamination of biologic drugs and vaccines by adventitious agents is extremely rare. However, when contamination incidents do occur, they can be costly in terms of time and resources.

In 2010, Eric Delwart, PhD, researcher and adjunct professor of laboratory medicine in the Blood Systems Research Institute at the University of California San Francisco, tested eight viral vaccines using polymerase chain reaction (PCR) and DNA sequencing, and found that three of the vaccines contained unexpected viral sequences (1). One affected vaccine was Rotarix, a rotavirus vaccine manufactured by GlaxoSmithKline. Porcine circovirus was detected in the vaccine, a discovery that led to a halt in the use of Rotarix, which is given to babies at two, four, and six months of age. The contamination traced back to the use of raw materials originating from animals in the production of the vaccine, and highlighted the need for better procedures to eliminate viral contamination and for better tests to detect adventitious agents.

Protocols for control of contamination in raw materials rely on cleaning and decontamination procedures combined with rigorous testing. These procedures are effective for most agents, but some organisms, particularly viruses and prions, have evaded standard prevention and testing methods. New technologies in biosafety testing target those previously undetectable contaminants.

Catherine Shaffer is a contributing writer to *Pharmaceutical Technology*.

Types of adventitious agents

Raw materials can be contaminated with a variety of adventitious agents. Those include bacteria, yeast, molds, viruses, and sometimes prions.

Mycoplasmas are the smallest of free-living organisms, and are frequent contaminants of mammalian cell cultures. They can alter the metabolism and properties of cells and change product yield, cause false assay results, and generally wreak havoc in the culture.

Viruses are some of the simplest of all organisms. They are very small and are generally comprised of a small amount of DNA or RNA surrounded by a lipid envelope. They rely on the host for reproduction, and sometimes incorporate their genetic material into the host cell's genome. Viral contamination is generally the greatest contamination risk because of the ability of viruses to evade detection and cause silent infections in cell cultures. There is no universal, one-size-fits-all method for treating materials that will eliminate all viruses.

Stopping contamination

The most common type of contamination incident happens when a media component is contaminated. For example, bovine serum can be contaminated with reovirus, epizootic hemorrhagic disease virus, Cache valley virus, or *Vesivirus* 2117. Porcine circovirus is sometimes found in porcine trypsin. Minute virus of mice (MVM) is a common source of raw material contamination of various media components due to infestations of mice in facilities where products are manufactured (2, 3).

Global regulations, including those from the United States Department of Agriculture, the European Medicines Agency, and the FDA's Center for Biologics Evaluation and Research (CBER) set standards for minimizing viral contamination, particularly spongiform encephalopathies (4).

Standard procedures for inactivation of adventitious agents include the use of heat, filtration, pH, and gamma irradiation. Thorough cleaning of equipment, testing, and review of material sources are also important steps to take. Bovine serum, for example, should be sourced from a country with a negligible risk of bovine spongiform encephalopathy. Animals should be less than 30 months old, designated for human consumption, and test free of all forms of transmissible spongiform encephalopathy. And there should be a quality assurance system in place with a system for delineation of specific batches. The supplier should have a regular audit routine (2).

It is impractical to test all raw materials for every possible adventitious agent. Two testing approaches may be used to screen materials for most types of viruses and other contaminants. One is based on identifying the characteristics of the contaminant, such as cytopathic effects of viruses. Another option is to test using immunoassays or PCR for a panel of viral antigens or sequences.

Archie Lovatt, biosafety scientific director of SGS Vitrology, advocates an active risk mitigation strategy incorporating multiple strategies and approaches for managing contamination risk. "Essentially, it's about knowing your manufacturing process, knowing your raw materials, and going deep. Understand exactly what the risks are, then try and mitigate the risk," Lovatt says. That would include preliminary testing of materials and process monitoring. "If there is a contamination, you catch it early—before you send the batch for purification."

Trending strategies for testing raw materials are included in GMP practices, quality by design, and process analytical technology. Single-use manufacturing devices, disposable consumables, and ready-

to-use reagents and media are also reducing rates of contamination in the industry.

Faster, more accurate tests are being introduced to the market to address the problem of contamination. "Traditional test methods require up to seven days for reliable results," Theresa S. Creasey, MilliporeSigma's head of applied solutions strategic marketing and innovation tells *Pharmaceutical Technology*. To reduce testing delays, MilliporeSigma offers its Milliflex Quantum system, a fluorescence-based test method that gives results in one-third of the time of traditional media methods.

Negative test results do not guarantee that there is no contaminant in the material, according to Mark Plavsic, chief technology officer of Lysogene. "Assuming that all sourcing of raw materials has taken place in a controlled manner, assuming the components are well selected and examined, assuming that all of the testing has been done by the letter of the law, what is left is treatment for viral inactivation and removal. Not every company is doing this. Not every supplier is doing this," says Plavsic.

Ray Nims, a consultant at RMC Pharma, advocates a multi-pronged mitigation strategy. Nims explains, "Where testing fails is this. You typically test one bottle. And out of the bottle, you test a small amount, maybe 100 mLs. These lots of serum can be 3000–5000 bottles, so the serum company may test from one or two bottles. The company procuring the serum typically will test 100 mL from another bottle. If the testing passes, the lot is declared released and then used. The assumption that if you tested it clean the entire lot is clean fails sometimes."

Disinfection approaches

Barrier technologies complement testing. The most common barrier disinfection method is gamma irradiation, according to Nims. Gamma irradiation is standard for manufacturers of bovine serum, however; it's not normally an option for other raw materials such as media. Two alternatives are an in-line treatment called high-temperature short-time processing (HTST) and ultraviolet irradiation, a technology that has a great deal of potential applicability, but is has not yet been taken up by the industry.

Ultraviolet disinfection is a powerful technique for neutralizing living microorganisms. Exposure to the UV light causes the formation of dimers between neighboring nucleic acids in the genome, which prevents the organism from reproducing. Ultraviolet (UV) disinfection is commonly used to treat waste water and drinking water in the United States. UV disinfection has some support from the EMA, which recommends it as one of two complementary virus reduction steps. Combining inline UV disinfection with other barrier methods for preventing contamination, such as filtration, is a "belt and suspenders" approach that would be more effective than either method alone.

Non-animal-based materials may not be the solution

There is a trend in the industry away from animal-based sources of raw materials. Use of serum-free media can instantly eliminate the most common contaminants, including virus risk and most mycoplasma risk. Animal serums are considered rather old-fashioned in the production of biological drugs and vaccines. Most processes can be adapted to use serum-free media. However, many legacy processes currently still make use of animal-derived materials, particularly fetal bovine serum.

Non-animal sources are not a panacea. Plant source materials can be exposed to soil, animals such as field mice, bird feces, human contact, and other

environmental contaminants. Human handling can also introduce adventitious agents to source material.

Adam Elhofy is chief scientific officer at Essential Pharmaceuticals, which manufactures an animal-free media supplement for cell culture called Cell-Ess. He points out that reliance on Chinese hamster ovary (CHO) and other non-human cells is more of a problem. "Cross-species contamination for a virus is fairly low," Elhofy said. "There's still the risk. The problem is people are using cells that are not human cells. Those cells can be infected by animal origin viruses."

Strategies for preventing contamination include upfront testing of materials, barrier disinfection methods, and adhering to best practices in processing and sanitation. Avoidance of animal-based raw materials eliminates the most common and problematic sources of contamination, as well as careful sourcing of any materials used. Contamination risk can never be fully eliminated, but with vigilance, it can be minimized.

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SUPPLIERS LAUNCH SERVICES AND PRODUCTS FOR BIOLOGIC DRUG DEVELOPMENT

Sartorius Stedim Biotech Launches Chemistry Testing Services

BioOutsouce, a subsidiary of Sartorius Stedim Biotech (SSB), announced on May 4, 2017 the expansion of chemistry testing services for the characterization of the physicochemical properties and structural attributes of therapeutic monoclonal antibodies (mAbs). The company has expanded laboratory space by 340 square meters at its facility in Glasgow, UK, and added scientific staff with chemistry testing experience.

The service platform methods have been developed to ensure rapid sample analysis and reporting for mAbs and biosimilars and comply with International Council for Harmonisation (ICH) Q6B scientific guidelines for pharmaceuticals for human use, and includes methods to characterize protein structure, carbohydrate profile, post-translational modifications and impurities utilizing ultra-high-performance liquid chromatography (UHPLC) and LC–mass spectrometry instruments, according to a company statement (1).

Charter Medical and INCELL Announce Distribution Agreement

Charter Medical Ltd., a manufacturer of products for the regenerative medicine and bioprocessing industries announced a partnership with INCELL Corporation that provides Charter Medical the exclusive rights to market, sell, and distribute INCELL media products on a global basis. INCELL develops and manufactures specialty medias and formulated solutions for tissue and cell collection, transport, processing, and storage.

The partnership will enable both organizations to meet the growing customer demand for more comprehensive solutions in cell culture, cell expansion, and cryopreservation, Charter Media reported in a press release (2).

Cell Culture Media Polymer Designed for Lot-to-Lot Consistency

Poloxamer 188 EMPROVE EXPERT, a surface-active nonionic polymer from MilliporeSigma, is used in cell culture media as a shear protectant and increases robustness of mammalian cells to shear from sparging, resulting in increased viability of cells in the bioreactor, the company reports (3).

The polymer, which was developed to help ensure lot-to-lot consistency and reliable performance, has been cell-culture tested and optimized. The product comes with polymer dossiers to help manufacturers meet regulatory requirements for risk assessment.

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—*The editors of* Pharmaceutical Technology

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Getting Biopharmaceutical Tech Transfer Right the First Time

Agnes Shanley

Good project management, budgeting, planning, and clear documentation are the only ways to prevent overruns and project failure.

Technology transfer is essential to any innovator's success, but even the best science or the most innovative technology can be derailed by simple human failures (e.g., inadequate or infrequent communication, or reliance on approaches that reflect individual preferences or corporate cultures, rather than a clear focus on what is needed to advance the project). The most effective tech transfers eliminate personalities and focus on data and clear communication, so that the right people are in touch about the right issues at the right time. In this article, Stephen Perry, CEO of Kymanox, James Blackwell, principal of The Windshire Group, and Michiel Ultee, president of Ulteemit Bioconsulting, share some of the lessons they've learned to help prevent overruns and wasted time, and ensure that biopharmaceutical technology transfers succeed.

Mistakes in tech transfer

PharmTech: From what you've seen, what are the biggest mistakes that companies make when working on tech transfer for biologics?

Blackwell (Windshire Group): Sometimes a tech transfer team will have to deal with a poorly characterized process, in which process and product parameters are not defined in terms of their criticality. In these cases, they will need to understand and furnish what is missing in order to characterize the process sufficiently so that it will be robust enough for transfer. Just what will be needed will depend on the phase of development, and the clinical stage that the process would support. Early on, patient safety issues are most critical, but later, process characterization becomes crucial.

Poor project management is also a problem, and situations where responsibilities are unclear for either or both parties, or communica-

tion is poor. One doesn't want to be too dependent on personalities, or on requirements that are not defined clearly up front.

Another problem is not having easy and ready access to previous process data. It is much better to have that in a technical report than not to have it at all. Ideally, the information should be in a form that will make it easier to find, analyze, and manipulate specific data. Years ago, data systems had to be built, in house, around specific manufacturing platforms. Now one can use a cloud-based systems and the process is much easier.

"One of the biggest problems is poor planning, including poor budgeting."
—Stephen Perry, Kymanox

Ensuring data integrity is essential, and depends on clear policies and procedures (e.g., a good lifecycle policy and a good approach to documentation) to define the tech transfer process. In order to get to this point, the team must determine what studies and technical documents will be needed at various process stages.

For more complex processes, Earned Value Analysis (1) is a method that can help get financial people, tech people, scientists, and project managers together and on the same page. More companies should consider using this method to monitor and study their tech transfer processes.

Most teams designate a clear point of contact on the sponsor and the contract partner side. It is crucial to have the right people on the checkup calls so that the they provide necessary information at the right time.

Perry (Kymanox): One of the biggest problems is poor planning, including poor budgeting. People forget about the regulatory implications of their tech transfer and FDA gets notified late, rather than early. If FDA can be brought in during the very early planning stages, regulators can be a huge asset. The opposite may be true if they are the last ones to know, or they feel that they are being force fed a design that they may not understand, late in the process. All of this can usually be traced back to planning.

Another project management mistake is de-emphasizing project monitoring in the early stages so that it becomes overemphasized in late stages. Confidentiality, supply, and quality agreements are all on the project's critical path, but project managers often let these items slip until later (e.g., when the project reaches the validation stages). Then they have to focus on critical path issues as well as validation, and much of their energy goes to monitoring issues that should have been under control from the start. Good project managers will focus on these issues at the beginning of the project so that they don't become problems later on, when they might jeopardize the project's end date.

Another problem is that analytical methods are sometimes an afterthought. At Kymanox, we break up our transfers into three parallel tracks: materials, analytical methods, and process. They're done in that order. With materials, a process may require a reference standard or specialty reagent for which there is only one supplier. There may be very limited quantities, and if materials are not requested as early as possible in the process, progress will be stalled.

Often, people start focusing on the process too soon, diving into risk management and questions

like: What's going to stay the same with the process, and what's going to scaleup or be diffrent with regard to equipment? People zero in on these questions and leave analytical methods behind. Analytical method transfer can require a 30-week block of time, but the tech transfer cannot move forward without it, because a process cannot progress unless it can be measured. Making analytical methods an afterthought is a big mistake, but one that is surprisingly common.

"It is extremely important that the people on the receiving end of the tech transfer, whether a CDMO or another team within the company, truly understand the molecule and its properties."

—Michiel Ultee, Ulteemit Bioconsulting

Ultee (Ulteemit Bioconsulting): First is providing incomplete information about the nature of the biopharmaceutical or protein molecule such as its properties, its activities, and its stability under different conditions. Often, companies know this information, but don't pass it on to, say, the CDMO that will do the work. It is extremely important that the people on the receiving end of the tech transfer, whether a CDMO or another team within the company, truly understand the molecule and its properties.

Another mistake is not allowing enough time. With a standard mammalian cell process, for example, it can take five weeks or so just to scale up the cells and then run the bioreactor. In addition, transferring the subsequent downstream processes or protein purification processes will take a couple of weeks. And, they must be run multiple times to ensure consistency. Some of this work can be done in parallel, but don't try to rush into manufacturing. Transfer the process in first.

The third mistake is not arranging for scientist-to-scientist interaction during the transfer process. Scientists from similar departments at both the transferring company and the receiving company need to get acquainted, understand the transfer process, and then work side by side at the bench or in the plant. Without that personal interaction, your transfer is risky. I've seen many paper transfers fail because what was written wasn't clear to the individual on the receiving end. You can supplement the paper transfer with email and phone calls, but there's no substitute for person-to-person face time.

The fourth area is not defining trouble spots in the process where extra attention to the procedure is needed. Related to this is not defining the design space or flexibility around each process. For instance, you may have run a process at a pH of 6.5, but what would happen if the pH was higher or lower? Without knowing this information, you don't know how to respond should this happen in manufacturing. Knowing this information may also optimize your process; it may work better at a different pH, for instance. You need to define the design space for each of your critical process parameters.

The last area is not identifying the hold steps where the process may be safely paused downstream in the event of unforeseen occurrences like power failures and absences of people.

Sharing information

PharmTech: What information should be shared with clients, how and when?

Ultee (Ulteemit Bioconsulting): Define the molecule of interest as fully as possible and share the knowledge gained during early research and development with the receiving scientists so they are prepared to deal with your protein molecules. These are complicated molecules. The receiving people must know as much as possible about that protein so they can make proper judgments during the production and purification of material.

"It is essential that the people developing the process understand the needs of those receiving it."

— James Blackwell, The Windshire Group

Perry (Kymanox): At Kymanox, we use a detailed product and process description template. There can be a sender's version and a receiver's version of the document, but it is essential to something like this on hand because people often under document their process and don't provide enough detailed information. In other cases, they may edit out crucial information.

Miscalculations and misrepresentation can result in extremely expensive problems. On both sponsor and partner sides, people often overestimate or underestimate their teammates, partners, and suppliers.

Sponsors can be so proud of their baby (i.e., their product and the process that goes with it) that they highlight the past successes of their manufacturing campaign rather than the problems or failures that they may have seen along the way. But those misrepresentations will statistically rear their ugly heads later on in the transfer. It's better to bring your product and process to the transfer team, warts and all, early on and to bring up past failures with process or product concepts.

A good product and process description has an introduction section and describes what state the project is at currently (e.g., clinical or commercial). It also provides source documents, references that can be extremely useful to the team.

On a fundamental level, information is needed on what the product is and who it is for. I find it so sad when I go onto a pharmaceutical company's manufacturing floor and ask an operator what they're making, and they don't know what the product is or what it is used for. Everyone on the tech-transfer team needs to know this information, not just the critical quality attributes and process parameters.

Ideally the document should include a professionally rendered process flow diagram and details on operating parameters, temperatures, and cleanroom levels during manufacturing. In addition, all analytical methods, not just final release methods but all of the in-process controls and tests required for the product, should be listed. The same holds for process and analytical equipment, and all materials required to make a batch of the product.

Sampling (see **Image**) should also be included, with a list of all sampling steps required, how samples are to be taken; how much material should be used; storage conditions for samples;materials of construction and sizes required for sample vials; and an explanation

Setting clear requirements for sampling is crucial to tech transfer success.

of why the samples are being taken at that specific time (e.g., whether they are simple process checks or whether they will be used by quality staff to run a very specific analysis).

Blackwell (Windshire): One important, but overlooked question to ask when working with a CDMO is: Who controls the intellectual property? Recently, I worked with a company that had a commercial product that had been developed by a leading CMO. Even though the process and product were theirs, they never received and did not have access to all the technical reports associated with the development of that product.

Not only was that an obvious problem for the technical people supporting that process, but it became a real issue when Inspection came to inspect the sponsor's facility and they didn't have all the details and reports pertaining to the product and process. If you're paying for the development of a process, you need to have the process history documented and the rights to all the reports and raw data that went into those reports so that you have the complete process history.

This needs to be spelled out in writing, in a formal supply or quality agreement, or it can pose potentially serious problems with regulators.

Ensuring reproducibility

PharmTech: What is the key to ensuring the reproducibility of procedures and processes? What should the receiving end demand, and what should the sponsor provide?

Ultee (Ulteemit Bioconsulting): Sponsors should demand and expect to work with competent technical staff with experience in the types of proteins being developed. The best collaborator will have a track record for the technical capabilities that the project requires.

"In order to have reproducibility in the future, it is important to know what has been done in the past."
—Stephen Perry, Kymanox

Another necessity is clearly written descriptive batch records and test procedures because they are communication vehicles that are used in manufacturing. Without them being clearly written and descriptive, mistakes will happen.

Finally, clear and frequent communication is required between the contracting partner and the CDMO, as well as internally at the sponsor and at the CDMO. A company may have transferred their process over to a process development scientist, but if their communication with manufacturing is tenuous or incomplete, then the tech transfer may fail.

Perry (Kymanox): In order to have reproducibility in the future, it is important to know what has been done in the past. Along with the product and process data sheet, a run history is needed, going all the way back to earlier generations of the process. Ideally, it should be put in a timeline format,

and link back to data to summarize what each run was all about.

Three suggestions for ensuring robust and reproducible processes [are as follows].

Aim low. Everyone talks about setting the bar high, but there are times when the bar needs to be low, so that it can always be cleared.

Some people look at a high yielding run and set the process up so that is the bar to clear. It may be that that high yield results will be very difficult to reproduce consistently, so the team may discover that it is actually designing an entire process around a statistical outlier. Rather, keep expectations in check, and the value proposition should still be strong if the overall drug development program is good.

Do things the same way. Sometimes (and people with a QC background may relate to this), the best way to ensure reproducibility is to handle a process or procedure the same way every time. It doesn't even necessarily have to be the best way, just the same way.

For example, there are a half dozen different ways to pipette, and one can argue about which way is the best way. In the end, laboratory staff should pipette materials the same way, consistently.

With manufacturing, the same thing holds. People need to agree on the same way of doing something, so that reproducibility can be established. Then, when something is off target, because the precision level is high, the process can be moved and still aim for the bullseye. If precision is off, results will be scattered.

Finally, get raw data and be sure you analyze that data using the latest advanced statistics. We use specialty software for multivariable analysis, but no software will be useful without raw data. Using filtered or truncated data can skew analyses. Process engineers should be demanding access to raw data files (e.g., temperature profil during a process step) and using the proper tools (e.g., Excel, Mintab, JMP, and SIMCA) whenever possible. Process engineering teams should use advanced statistical analysis whenever possible.

"Sponsors should demand and expect to work with competent technical staff with experience in the types of proteins being developed."
—Michiel Ultee, Ulteemit Bioconsulting

Blackwell (Windshire): It is essential that the people developing the process understand the needs of those receiving it. Near the end of the development process, they should document data in a form that will be useful to receiving unit, but those receiving the data should take part in developing that document, and should review it before it can be finalized.

Organizations should make data integrity and developing the right chain of custody of data a priority, and review to ensure that there are no gaps and that those issues are incorporated into procedures going forward.

Stage gates can be useful

PharmTech: Do you use the stage-gate approach? Where is it useful and how many staged gates should be used?

Perry (Kymanox): At Kymanox, we use four gates, and control the entry to and exit from each. At

the initial stage, we look at the target product profile and decide whether or not to transfer once we reach the 'go/no go' stage. Sometimes the team realizes that it doesn't want to transfer a process.

"Initially, one does assessments and reviews and gets a plan in place, then develops process and analytical methods."

— James Blackwell, The Windshire Group

Successful tech transfer typically has a bookend on the back end that defines success. Once manufacturing people say the process is stable, you're in continuous improvement mode.

Blackwell (Windshire): Part and parcel of the product lifecycle approach is a stage gate tied to the needs of various clinical stages. Going through the International Council for Harmonization guidelines and meeting requirements is part of the overall process, which includes risk management and making sure that you are assessing risk at the appropriate phases of tech transfer.

The best number typically ranges from six to nine stage gates. Initially, one does assessments and reviews and gets a plan in place, then develops process and analytical methods.

From lab to the real world

PharmTech: How do you ensure that procedures and analytical methods are correctly translated from labs to real-world environments?

Ultee (Ulteemit Bioconsulting): A detailed tech transfer protocol is needed, one that's been agreed on by the two parties. Spell out the key assay parameters, the expected results, the flexibility at different steps, the time ranges allowed, whether you need triplicate determinations to enhance the accuracy and reproducibility, and so forth.

A second best practice is face-to-face meetings and side-by-side transfer of the process between the transferring and receiving analysts. At one of my previous companies, we had a three-step approach, where for transferring an assay between the analytical development group and the quality control group, we would have the two analysts (one from analytical development, one from quality control) do the assay together.

First, the transferring analyst would do the assay with the receiving analyst observing. And then, the receiving analyst would do the assay with the transferring analyst observing. Finally, the receiving analyst would do it alone. If the results were comparable, the receiving analyst could do it reproducibly on his or her own, and the process was shown to be effectively transferred.

Another best practice is to use comparable analytical instrumentation and mechanisms to address any functional differences between instruments. Look at what's available at the receiving lab. If it's a different instrument, be sure that tthe process is compatible with the instrumentation.

And finally, carefully review any transfer results and troubleshoot any differences so that you can repeat transfers, if necessary, and change the areas that are weakening the transfer protocol.

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Cold Chain Logistics for Personalized Medicine: Dealing With Complexity

Kirk Randall

The complex packaging and logistics required for personalized medicine pose significant challenges, but proactive planning can help ensure success.

The pharmaceutical industry's intense focus on personalized medicine and novel treatments such as cell, tissue, and gene therapies is creating new and evolving challenges for transportation and logistics providers. The complex packaging and logistics needed to support clinical trials in these areas set new standards for timing and control. All too often, specific requirements are only considered late in the planning stages for clinical trials or even commercialization.

Movement of sensitive biomarkers, patient samples, and the therapies themselves, all require exact, time-limited logistics support, and they cannot be considered independently, because they are inextricably linked to packaging and logistics. It is not atypical to see three or four different temperature requirements per supply chain for a regenerative therapy. This creates the need for multiple packaging modalities within the same trial or treatment regimen, further complicating logistics. Coordinating these new and complex supply chain issues requires both forethought and dynamic flexibility.

Some regenerative therapy transportation requirements must be met within hours, not the days or weeks seen with more traditional treatments. In addition, the samples being transported are often patient-specific, so they require distinct, independent identification codes unique to each patient, but also compliant with the Health Insurance Portability and Accountability Act (HIPAA) and other global regulatory standards.

A successful, well-run trial and commercial distribution strategy assumes that logistics service providers can deliver integrated solutions to clinics and manufacturing sites. The stakes are extremely

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high. Given the number of regenerative therapies currently being developed, aphaeresis, cell manufacturing, and patient pretreatment protocols and logistics must be timed perfectly at each step of the program, and executed impeccably. Doing anything less risks not only treatment failure, but even patient death.

Clinical trial, and commercial, success depends on achieving and maintaining optimal conditions for temperature-sensitive biomarkers, patient samples, and therapies throughout the trial. Careful planning of all packaging, transport, storage, and handling steps, as well as strict adherence to-planned processes, is needed to ensure that delays and temperature excursions do not jeopardize the quality of any of the materials transported in support of the program. This article outlines the most important points to consider, as early as possible during product development, when seeking a cold chain logistics partner.

Initial validation and requalification

Dewars are commodities, in that anyone can purchase and use them. No special expertise or skill is required to handle them. Transporting regenerative and personalized therapies, however, entails more than just purchasing the dewar and sending it with traditional carriers.

Although dewar manufacturers do validate basic performance characteristics of their vessels, they do not perform full validation to International Air Transport Association (IATA) and other global standards. This work should be done by the logistics service provider. Shippers should be validated to meet all applicable logistics quality standards as well as requirements set by new global good distribution practices (GDPs) regulations (1).

Another consideration is how the company tests and verifies that the dewar will perform for each individual shipment. To ensure performance, a cryogenic shipper should be requalified after each use. Most service companies only retest performance of dewars quarterly or semi-annually, however, and some do not retest them at all after original service, so their customers will not be able to predict, much less ensure, whether their valuable payload is safe and whether it has been maintained at the required temperatures of 150 °C or below.

To ensure transparency, a service provider should serialize its dewar fleet so that customers can track individual shipper use over time. This way, problematic shippers can be repaired or retired if needed to protect shipments whose catalog value can easily reach the tens of thousands of dollars, but whose value is really priceless, measured in a patient's life, or opportunity for a better life.

Traditional integrators (e.g., FedEx, UPS, DHL, etc.) and specialty couriers offer "white glove" transport services that generally provide web-based tracking of point-to-point shipments. This is certainly key to monitoring a product's shipment location, but it does not tell the critical story: the dewar's condition and that of its precious payload.

Therapeutic developers would be wise to choose a logistics partner that has strong IT and data logging capabilities. Ideally, the company's IT should be integrated with that of its courier. That way, the partner can offer clients one dashboard allowing them to view and monitor, in real time, the complete chain of condition and custody of the treatment. This capability requires a total IT and data-logging solution that tracks a dewar's position, internal and external temperature, orientation (critical for maintaining hold times), as well

as shock events, allowing these data to be viewed in real time.

Real-time data access allows alarms and notifications to be sent to the logistics partner and customer whenever any temperature measurement or other data point moves beyond acceptable levels, so that they can intervene quickly before product is lost. Without this access, an adverse shipping event will only be learned of after the fact, when treatment effectiveness has been lost.

Detailed, long-term performance monitoring

In choosing a logistics provider, it is important to work with someone who is independent and carrier-agnostic. The solution provider should be able to track and use each carrier's performance data, to ensure performance for their client's, and ultimately, the patient's benefit.

No carrier will perform at peak levels in all shipping lanes and at all times. Therapeutics manufacturers should use an IT solution that tracks carriers by shipping lane, cost, on-time performance, and other criteria. This tool will allow them to select the best carrier for each leg of a shipment's journey to ensure optimal delivery to the final destination, the treatment or clinical trial location.

Logistics solution providers should offer this capability as a key part of the IT solution they provide to manufacturers. The ability to make educated shipping decisions will ensure that a company has selected the best partner, and applied best practices for containing costs and ensuring delivery to patients.

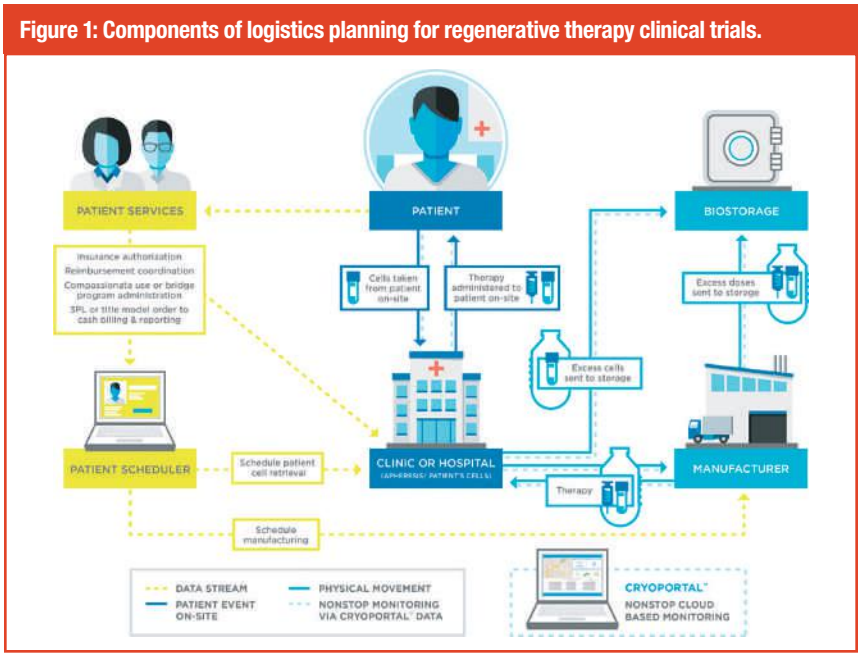
Recently, additional complexity was added to cold-chain logistics for cell-based therapies: the need to track individual patients, from initial cell harvesting (aphaeresis) to the manufacturing site and back to the patient. This introduces not only patient identification challenges, but the risk of introducing other cold-chain temperature band issues. In particular, many manufacturers transport the initial aphaeresis cell harvest at refrigerated (2–8 °C) vs. cryogenic (- 150 °C) temperatures. This creates the need for additional transport packaging considerations that require the same ability to track chain of custody and condition as cryogenic transport. While this is a different temperature band, it remains just as critical to maintain that temperature throughout the transport cycle to ensure that the cells can be processed to meet the patient's treatment needs.

Another point to consider is the ability to integrate IT solutions for initial patient cell harvest, logistics, manufacturing scheduling, and return shipment. While it might not be critical to patient treatment and packaging, *per se*, it could simplify a complex chain of events (see **Figure 1**). Patient treatment and cleanroom manufacturing scheduling programs such as TrackCell and iCAN can be integrated into a logistics partner's IT chain-of-condition and chain-of-custody solution.

These capabilities would permit customers to have a full one-stop view of primary patient aphaeresis, manufacturing cleanroom scheduling, ordering of appropriate shipper(s), scheduling of carriers, as well as the transportation to and from the manufacturer and back to the patient. In the past, this was done in separate silos. Fortunately, the technology is now available to combine these processes, and, ideally, a cold-chain services provider should offer this capability.

Another question to ask is whether the service provider understands the science behind the therapy, and can bring that understanding to its service packages. When choosing solution providers for cold chain management, most manufacturers

Figure 1: Components of logistics planning for regenerative therapy clinical trials.



look only at the transportation logistics involved. For a cold chain logistics provider to provide a truly comprehensive, innovative, and powerful solution, they must understand the therapy, the patient, and how logistics and packaging choices intertwine.

Return logistics

Historically, manufacturers of cell-based products have purchased and maintained their own fleet of cryogenic shippers. These fleets come with high capital costs, because dewars can cost up to $5000 each. For large-scale manufacturers, the fleet size required to distribute products worldwide can number in the thousands, resulting in tens of millions of dollars in upfront capital expenditures. If the return logistics of these costly shippers is not managed well, even larger fleets will be required, due to low return rates.

In addition, manufacturers typically lack the resources and IT required for managing return logistics, resulting in more lost dewars and higher dewar replacement rates, which can add millions of dollars in capital expenditures every year. Without predictable return of shippers for re-use, manufacturers cannot budget reliably for this additional capital cost.

An experienced cryogenic logistics solutions provider, which has a robust IT and data-tracking system, can more effectively manage and minimize these costs, requiring smaller fleets and lower upfront capital expenditures. The improved return logistics solution will result in lower annual capital-fleet replacement costs as well. A logistics provider that can serialize and assess dewars and requalify them after each use, and repair or replace shippers as needed, can more effectively manage costs. The best partners can also use their IT platforms to choose the most efficient and cost-effective carriers for return shipping segments.

Shipping cell-based products involves many challenges and issues that do not come into play in traditional pharmaceutical distribution. It is up to the manufacturer to make sure that they work with partners that offer the best technologies and practices. This effort will enable vast improvement in capital cost management and healthcare provider satisfaction, but, more importantly, successful patient treatment and therapeutic adoption.

Reference

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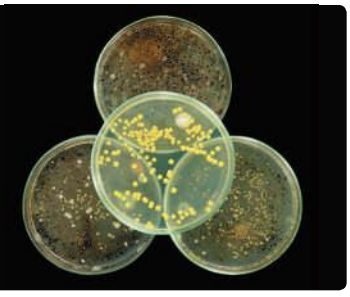
FIGURE COURTESY OF THE AUTHOR



Kill the Bioburden, Not the Biological Indicator

James Agalloco

Understanding the purpose of the biological indicator can guide the development of an effective sterilization process.

Sterilization processes are used to ensure the safety of patients treated with products and materials expected to be sterile at time of use. The objective is to eliminate microorganisms in and on products that are introduced into the body in a manner that defeats the ordinary protections of skin, intestines, and other safeguards present. In considering patient safety with respect to sterility, a minimum requirement of one contaminated unit in a million units is considered acceptable for sterilized materials (1). The original term for this value, sterility assurance level (SAL), is non-intuitive and defining it usually entails the use of the word 'probability'. Increasingly, this value is being called the probability of a non-sterile unit (PNSU). In routine practice, additional precautions are taken so that this minimum expectation is substantially exceeded.

The calculation of PNSU uses **Equation 1**, in which the lethality delivered, D-value, and initial population of the microorganism are inserted.

$$\log N_u = \frac{-F_0}{D} + \log N_0$$

where:

N_u = Probability of a non-sterile unit (PNSU)
D = D-value of the microorganism
F_0 = Equivalent time, in minutes at 121 °C (lethality)
N_0 = Initial population

[Eq. 1]

The equation is simple enough; however, there is a common misconception in its use. The problem lies in the incorrect use of values for population and resistance from the biological indicator rather than for the bioburden. The safety expectation relates to the routine use of a sterilizer where the bioburden is present, rather than the initial or periodic validation of the sterilization process when

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a biological indicator is employed. In the majority of instances, materials sterilized in conjunction with the validation exercise are not intended for patient use. The minimum PNSU as derived from the bioburden present is the critical concern. **Equation 2** estimates the PNSU for a 3-minute process at 100 °C with a starting population of 100 CFU/unit and an estimated D100 of 0.0003 minutes (2).

$$\log N_u = \frac{-3}{0.0003} + 2 = -9{,}998 \qquad \text{[Eq. 2]}$$

It should be immediately evident that this extremely short and low-temperature sterilization process provides an overwhelming margin of safety that is nearly 10,000 times greater than the minimum expectation. The moist heat resistance of the bioburden is so minimal at these conditions that there is essentially no chance for its survival (3). This is true even though the process is 3 minutes at 100 °C, not the more commonly (and wrongly expected) process performed in excess of 121 °C. The lethality of this low temperature process cannot be established with the conventional biological indicator of *Geobacillus stearothermophilus*, whose resistance is such that the assumed process would have no meaningful impact on its population.

Requiring destruction of a 106 population of *G. stearothermophilus* to the minimum PNSU expectation of 6 would require a process at 121 °C and an F_0 >10 minutes. Such a process offers no benefit to the patient because the bioburden will already have been killed well beyond minimum expectations at the lesser condition. If a 121 °C process delivering an F_0=10 minutes were utilized instead, the PNSU would be as shown in **Equation 3**.

$$\log N_u = \frac{-10}{0.000003} + 2 = -3{,}333{,}331 \qquad \text{[Eq. 3]}$$

The estimated PNSU in this example would be extreme: not more than one positive in more than three million times the minimum requirement. The only justification for using such a cycle is to destroy a bioindicator that has no resemblance to the native bioburden, is present at a concentration that exceeds any reasonable real-world situation, and has extreme moist heat resistance. Killing the bioindicator is certainly safe, but this approach arbitrarily increases the adverse process impact on the product. The real target in sterilization is always the bioburden, which is generally far easier to kill. Therefore, the sterilization process should be developed with that as the objective.

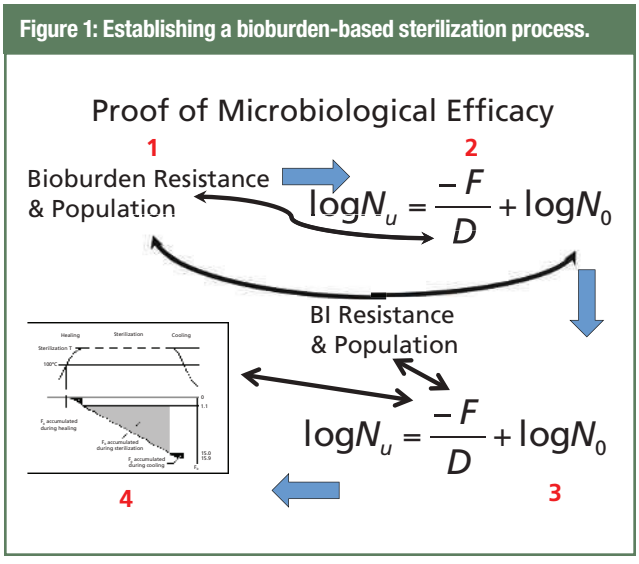
The purpose of the biological indicator in sterilization is not to define the process, but rather to measure it. The steps involved in sterilization process development are outlined in **Figure 1**.

Define and Validate

The activities needed to define and validate a sterilization process focused on reliable destruction of the bioburden follow a simple sequence.

Selection of a bioburden model. The resistance of the bioburden can be obtained from experimental data collected on materials prior to sterilization or based on assumptions regarding the expected bioburden. Resistance information can be obtained from the literature or experimentally determined. The *United States Pharmacopeia* includes a boil test that can be used to estimate microbial resistance (1,3). The boil test can be adapted to estimate bioburden D-values at the appropriate temperature if a temperature other than 121 °C is

Figure 1: Establishing a bioburden-based sterilization process.



used. The population determination or estimation is straightforward.

Calculation of process duration. Inserting the population and resistance information for the assumed bioburden along with the desired minimum PNSU into **Equation 1**, the minimum process dwell time (F) can be determined.

Selection of the biological indicator. With the process duration established, a biological indicator with appropriate population and resistance can be identified that is appropriate for the determined process duration. The biological indicator should not be so resistant as to completely survive the process, but it should represent a meaningful challenge to confirm the required process conditions have been achieved. Partial kill of the biological indicator is most definitive as it confirms that the biological indicator possesses adequate resistance to support the process condition. Surprising as it may seem, complete destruction of the biological indicator does not provide that confirmation. Appropriate biological indicator options could include mesophilic sporeformers such as *Bacillus megaterium* or *Bacillus oleronius* (3,4).

Physical and microbiological confirmation of sterilization process. Use a combination of physical measurements and microbiological challenges to confirm that the required lethality is delivered.

Throughout this exercise, worst-case assumptions can be made to increase the confidence in the sterilization process. The typical assumptions include:

- Assuming a higher initial bioburden population
- Assuming a higher bioburden resistance
- Increasing the required minimum PNSU
- Arbitrarily increasing the minimum process dwell time
- Increasing the temperature setpoint for the process.

All worst-case assumptions need not be utilized, because doing so can result in a final process that is overly harsh to the quality attributes of the materials being sterilized.

There are many reasons why the bioburden should be understood as the focus of the sterilization and the bioindicator relegated to a supportive role in the validation of the process:

- The bioburden is present during routine processing and its destruction to a safe level must be understood as the intent of the sterilization process.
- Controls over the bioburden are an essential consideration in GMP operations producing sterile products. Attention must be directed to its removal to safe levels.
- The biological indicator is used only during the validation exercise, and in the majority of instances, the materials from the validation cycle are never used with patients.
- Determining the sterilization process based

upon biological indicator destruction extends the process duration unnecessarily, with negative impact on the sterilized materials (1).

- Changes in the biological indicator resistance can create problems in periodic revalidation activities.

The validation of sterilization processes must balance the often competing considerations of increased process safety and the negative impact of over-processing. The biological indicator should be chosen to support a sterilization process that provides a reliably stable and efficacious product with an adequate margin of safety. Extending process dwell and increasing temperature merely to kill biological indicators beyond what is necessary for patient safety is never appropriate. The correct use of a biological indicator is as a measurement tool confirming sterilizing conditions have been attained within the load items sufficient to render the process sufficiently safe. Sterilization and sterility assurance need to consider bioburden destruction to safe levels as the only true objective.

References

1. USP, *USP–NF* 39, General Chapter <1229>, "Sterilization of Compendial Articles."
2. J. Agalloco, "Increasing Patient Safety by Closing the Sterile Production Gap–Part 1–Introduction," accepted for publication in the *PDA J Pharm Sci and Tech*.
3. I. Pflug, *Microbiology and Engineering of Sterilization Processes* (Environmental Sterilization Laboratory, Otterbein, IN, 14th ed. 2010), Table 13.7, p. 13.18.
4. M. Izumi, et al., *PDA J Pharm Sci and Tech*, 70 (1) 30-38 (2016). **PT**

FDA CITES PHARMA FIRM AND COMPOUNDING PHARMACY

In recent inspections, FDA noted violations in sterile manufacturing practices, cleaning methods and cleaning validation practices, and unacceptable levels of biodurden.

FDA cites API manufacturer for cleaning validation failures

In a Feb. 3, 2017 warning letter (1), FDA noted that Resonance Laboratories Pvt. Ltd. did not provide sufficient information about how it planned to improve validation procedure deficiencies discovered during a May 2016 inspection of the company's Bangalore, India facility.

During that inspection, FDA officials found that the company failed to demonstrate that distilled water used to clean equipment downstream of the purification steps was suitable for use. The distilled water used for cleaning equipment in the cleanrooms, after passing through a micrometer filter, had an unacceptable level of bioburden.

In addition, FDA found that the cleaning procedures were ineffective. The FDA investigator discovered that 105 cleaning verification samples taken between 2015 and the May 2016 inspection failed the firm's specification for residual drugs. The company repeated cleaning until it obtained passing verification results; however, it failed to investigate recurring cleaning procedure ineffectiveness and did not remediate the deficient procedures, FDA reported.

The agency recommended that the firm hire a consultant to assist with meeting cGMP requirements and noted that the firm's executive management is responsible for resolving all deficiencies and for ensuring ongoing CGMP compliance.

Compounding pharmacy cited for unlicensed biologics

FDA also sent a warning letter dated May 3, 2017 to Pharmaceutic Labs, LLC (2), citing the company for violations of the Federal Food, Drug, and Cosmetic Act (FDCA). FDA personnel inspected the company's Albany, NY facility from Aug. 31, 2015–Sept. 23, 2015 and found inadequate sterile processing procedures and that the company was not meeting FDCA and Public Health Service Act (PHS Act) requirements for drugs produced by an outsourcing facility.

According to the warning letter, the company was producing biologic products without a biologics license and not under GMPs and had not properly disinfected aseptic processing areas. "Your firm failed to demonstrate through appropriate studies that your aseptic processing areas are able to provide adequate protection of the ISO 5 areas in which sterile products are processed. Therefore, your products may be produced in an environment that poses a significant contamination risk," FDA stated in the letter. Other violations included failure to establish written procedures for the prevention of microbiological contamination, failure to establish an adequate cleaning and disinfecting system, failure to determine conformance specifications, and failure to establish laboratory controls.

References

1. FDA, Resonance Laboratories Private Limited 2/3/17, Warning Letter (Feb. 3, 2017)
2. FDA, Pharmaceutic Labs, LLC. 5/3/17, Warning Letter (May3, 2017)

—*The editors of* Pharmaceutical Technology

Clean, Disinfect, and Validate

Axel Wehrmann

Effective cleanroom disinfection programs require extensive testing and evaluation processes. A three-step process can help ensure that the cleanroom environment will satisfy regulatory requirements and be safe for biopharmaceutical manufacturing.

Effective disinfection of equipment and surfaces in bio/pharmaceutical cleanrooms, where drug manufacturing is conducted, is crucial. To satisfy regulations that the drug manufacturing environment is safe, a formal cleaning validation process is required. Disinfectant products must be specifically designed for the contaminant. Tests must be carried out to demonstrate that the disinfecting products—and the way in which they are used—adequately clean and disinfect. Validating a cleaning process involves three steps described in the following sections.

Step one: Select appropriate disinfectants

Choosing the correct disinfectant product, or products, is generally straightforward, given the nature of the potential contamination. The best disinfectant type to use is dependent on the nature of the contamination that is present or possible.

Gram-positive bacteria, which are carried through the air, will alight on surfaces and pose a source of potential risk to the pharmaceutical product that is being manufactured. In the majority of cases, vegetative forms of these bacteria, however, are straightforward to eradicate; a disinfectant product from the quaternary ammonium family usually proves sufficient to kill them. These positively charged ions bind to and disrupt the negatively charged exterior surfaces of the bacteria, affecting the cell membrane and leading to cell death.

Gram-negative bacteria, which are more likely to be deposited by human operators working within the cleanroom, are more problematic to eradicate. The negative charge on the surface of these bacteria is less pronounced; quaternary ammonium disinfectants do not bind as well to the cell surface, greatly reducing their efficacy. The effectiveness can be boosted by adding glucoprotamin or guanidine-based

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products into the disinfectant mix, which will also have the benefit of being more effective against fungal contamination.

Gram-positive bacteria also form spores, which are difficult to remove completely using disinfectant products; more drastic strategies are required if spores are present. One chemical that can be used, peracetic acid, oxidizes the proteins and phospholipids in the bacterial membrane, and then damages the cell's contents, including the ribosome. Unfortunately, peracetic acid is both acidic and corrosive and causes non-discriminate damage, including to the surfaces being cleaned. It is, therefore, not often part of the routine disinfection regime and is only used when other strategies have failed.

Chlorine-based products, which are much more popular in the United States than they are in Europe, are another alternative. Sometimes disinfectants based on alcohol provide an alternative, though they are less useful for treating very large surface areas. Alcohol-based products reduce the solubility of membrane proteins, resulting in a breakdown in the membrane potential. They are non-specific, but are fast-acting, safer for operators to use, and cause no damage to surfaces.

The disinfectant chosen must be qualified, and the vendor must supply documented certification that shows it is suitable for use in the facility.

Step two: Verify the disinfection procedure

While the disinfectants should have been rigorously tested by the suppliers, these tests are only likely to have demonstrated that the disinfectants kill bacteria on an ideal standard surface, usually stainless steel. Removing bacterial contamination is straightforward when the surfaces are smooth and rigid, as is the case with stainless steel and glass. Other surfaces in a typical cleanroom such as the walls, floors and curtains, however, are more challenging to disinfect. Therefore, verification tests must be carried out using the chosen disinfectant products on every material and surface within the cleanroom, to show that they are fit for purpose under the defined cleaning conditions (contact time, temperature) for the specific cleanroom area.

Although there are many different standardized tests for testing the effectiveness of disinfectants under ideal conditions, general requirements for a practice-oriented approach are hard to find. The preferences and requirements of different regulators mean there is no single standard that meets all the guidelines of all the regulators. In the design of a verification of a given disinfection procedure, it is often most effective to combine the best elements of several different standards in a way that will meet all the relevant regulatory demands.

United States Pharmacopeia (*USP*) General Chapter <1072> is the only guideline to define disinfection effectiveness specifications for the pharmaceutical environment; such specifications are absent from European guidelines. The *USP* chapter calls for a 3-log reduction of bacterial contamination under normal circumstances which, with care, can be done. It also calls for a 2-log reduction for spores, which is difficult to achieve on each material. Killing 99 out of 100 spores sometimes requires the use of harsh disinfectants such as peracetic acid for prolonged contact, which may damage equipment with little benefit. Keeping in mind that in pharmaceutical cleanroom areas a high-number contamination with spores should be a rare event, the general need for a 2-log reduction can be questioned. Unless there is a recurrent problem with spores, a 1-log reduction can be reasonable in low-risk areas.

Table I: Summary of validation protocol for disinfection study in a vaccine manufacturing plant.

Disinfectant	Contact time	Test microorganisms	Surfaces	#Tests (including controls)
#1 Spray disinfection (Peroxide 1%, Peracetic acid 0.08%)	10 min 60 min	*Staphylococcus aureus* ATCC 6583 *Pseudomonas aeruginosa* ATCC 15442 *Escherichia coli* ATCC 11229 *Bacillus subtilis* ATCC 6633 *Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404 *Micrococcus luteus,* Isolate 1 *Bacillus thuringiensis,* Isolate 2 *Stenotrophomonas maltophilia,* Isolate 3	1. Wall 2. Bench 3. Floor (Pharma-Terazzo) 4. Stainless steel 5. Glass	270 (432)
#2 Spray disinfection (Peracetic acid 0.07%)	10 min 60 min			270 (432)
#3 Wiping Disinfection (Peracetic acid 3%)	10 min 60 min			270 (432)

To test the effectiveness of disinfectants, 5 cm x 5 cm tiles of the surface material are contaminated with standard reference strains of microorganisms and isolates from the cleanroom itself. Up to a dozen tests for each disinfectant product against different microorganisms must be carried out. The effectiveness of different contact times also must be assessed. Three control tiles should be tested for each microorganism. One is a positive control, with microorganisms and no disinfectant, which allows the effectiveness of the disinfectant at killing the bacteria to be assessed and the reduction log factor to be calculated. A second control is used to assess whether the neutralization solution affects the viability of any residual bacteria. The third control tile is used to validate the recovery method.

All tests must be run in triplicate for each material used within the cleanroom; therefore, dozens of test tiles must be run for each disinfectant product. More than 1000 samples may require evaluation as part of a validation qualification process. Such an undertaking increases the potential for error; it is important that experienced analytical scientists conduct the tests.

Table I demonstrates the work needed to test and validate three sporicidal disinfectants in three independent replicates for a vaccine manufacturing plant. Approximately 1300 sample tiles were tested.

With the requirements for methodical procedures and the need to adhere to current regulatory guidelines, cleanroom facilities may choose to work with external contractors to carry out the validation.

Testing practicalities

During testing, a disinfectant must be applied using the same concentration that will be employed in the cleanroom and should be left on the test tile for the correct time. After the time has elapsed, the tile is placed in a vessel containing a neutralizing solution, which removes surviving microorganisms. The rinsing liquid is studied for the presence of microorganisms on an agar plate, or by using membrane filtration, followed by the incubation of the filter on an agar plate.

While reference standard bacterial isolates are likely to behave in a reproducible manner in the tests, this is not always the case for the isolates from the cleanroom itself. In the test, unusual microbial growth of isolates from the cleanroom environment is especially a problem for the positive control, because if the positive control does not grow properly, then it is impossible to get an accurate assessment of how well the disinfectant worked.

Various substrates behave differently during testing. Some substrates are more wettable than others,

which makes testing a challenge. On silicone-based substrates, an aqueous solution containing the microorganisms tends to pool on the surface rather than spread evenly across it. *Pseudomonas* species prefer to remain in an aqueous environment and can easily die once spread on a surface, even in the absence of disinfection. Conducting tests in a humid environment can reduce their propensity to die without disinfection.

A large supply of test plates will be needed for the number of tests. Substrates that are not absorbent, such as glass and stainless steel, can be re-used. Absorbent substrates such as PVC, which are damaged during decontamination in the first test, cannot be reused.

Experience is important when conducting disinfection validation studies. The range of materials, layout, and environmental conditions means that there is no standard way of running the tests. Rather, a suitable protocol must be established for each facility and its conditions.

Step three: Monitoring

Regular monitoring of the success of a disinfection procedure completes the validation process. The frequency (e.g., every shift, day, week, month) has to be individually defined by the manufacturer based on a risk assessment. Alert limits and action levels, based on data collected from testing and a statistical comparison, need to be set low enough to trigger warnings and ensure safety. When a warning levels is exceeded, remedial work must be implemented immediately. There are no precise specifications for these levels in any of the guidelines; the levels must be determined for each facility on the basis of the data collected during the validation process.

Conclusion

The validation of surface disinfection programs in a pharmaceutical environment requires the selection of appropriate disinfectants, qualified for the intended use and the verification of the disinfection method by reproducing the already established procedure in the lab. Different disinfectant concentrations, surface materials, contact times, and test microorganisms have to be tested in parallel with the appropriate controls, resulting in a high number of tests to be conducted. The execution details and requirements have to be defined in a protocol based on the recommendation of *USP* <1072> considering the specific conditions in the facility. In addition, routine monitoring has to be defined to ensure disinfectant efficacy on a regular basis, and allow data selection for trending purposes.

By following the three-step process detailed herein, one can be confident that the cleanroom environment will satisfy regulatory requirements and be safe for bio/pharmaceutical manufacturing. **PT**

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