

Understanding Molecule Sensitivity in Aseptic Fill/Finish

Aseptic fill/finish of biopharmaceuticals requires an understanding of the structure and limitations of each molecule.

BioPharm
FOR PERSONAL NON-COMMERCIAL USE

FELIZA MIRASOL

Biologics and other complex biomolecule modalities present challenges to fill/finish steps. Being able to ensure a sterile environment during aseptic processing is one of them.

The aseptic/fill finish process for biologics can be challenging because of the sensitivity of biologic products. In addition, where the product is located in the manufacturing process at a given time makes aseptic fill/finish difficult, says Michael Francis, senior product manager, CPC Biopharma.

According to Francis, two primary challenges are maintaining sterility and minimizing contaminants, and, because the fill/finish steps for complex modalities such as cell therapies, for example, are the last processing steps before a product is delivered to a patient, biomanufacturers must carefully consider patient safety along with the high costs—in time and money—if a batch is lost and has to be reproduced.

"Biologics are often more sensitive to contamination with bacteria, viruses, or particulates, so maintaining sterility is paramount. Even with advanced automation, human intervention can still be the source of contamination risks, so ensuring operators can easily and stringently adhere to aseptic techniques is critical," Francis states.

ENSURING MOLECULAR STABILITY

In addition, one of the most critical responsibilities in biopharma aseptic fill/finish is ensuring the integrity and viability of the molecule, explains Jackie Klecker, executive vice-president and general manager at Lifecore Biomedical, a US-based contract development and manufacturing organization. Ensuring molecular integrity and viability requires careful and accurate characterization of the key properties of these highly complex, sensitive, and expensive products. "Each molecule differs from the next and any number of forces can negatively impact the product, including light, temperature, shear, oxygen, and product contact, among others," Klecker says.

Pirkko Kortteinen, PhD, chief operating officer, 3PBIOVIAN, agrees that product stability is one of the most significant challenges for the aseptic fill/finish of biologics and complex biotherapeutics. "The stability of large

Aleksandr Matveev-Stock.Adobe.com

complex molecules such as proteins, living virus particles, or viable cells can be compromised during aseptic fill/finish by environmental factors such as temperature, high pressure, metal ions, adsorption agitation, or shear stress," she adds.

"The aseptic fill/finish process must be designed to preserve molecule structures, biological activity, and cell viability and avoid degradation, aggregation, or other damage. Many products cannot withstand holding at room temperature," Kortteinen further emphasizes.

"Understanding the limitations of each molecule is essential to establishing and maintaining expert control of the optimal environment, across all aspects and timepoints of the fill/finish process," says Klecker. "This can include strict temperature control requirements/timing during production steps, such as controlled thawing timing, as well as use of low-shear peristatic pumps for filling."

Meanwhile, Kortteinen explains that, due to the nature of these biopharma products, total processing time must be kept short—in hours rather than days, in some cases—to complete the operations. Therefore, all processing steps, including melting, sterile filtration (if applicable), filling, closing, visual inspection, sample withdrawal, and secondary packaging must be carried out within a tight time frame.

The sensitive nature of biotherapeutics limits choices of sterilization methods, so, although heat sterilization in the final container is recommended, it is most often impossible, Kortteinen points out. Sterile filtration of the final bulk solution for many biological products must instead be performed before aseptic fill/finish. In contrast, a final cell therapy product, which consists of viable cells or cell-derived matrices that are not amenable to final sterilization or filtration, may instead require aseptic techniques throughout the manufacturing process, Kortteinen remarks.

ESTABLISHING AN ASEPTIC FILL/FINISH STRATEGY

Francis also notes that, because many aseptic fill/finish operations are performed in an isolator, aseptic fill/finish for biopharmaceuticals can be extremely expensive, and the equipment challenging to use. "Open manipulations within the isolator require good aseptic handling procedures to be successful. Incorporating single-use systems (SUS) into these applications has helped reduce contamination and particulate risks; however, these SUS designs are often very complex, sometimes resulting in 'tubing spaghetti'," Francis states. "These overly complex systems can be very difficult for an operator to handle—and any difficulty in assembly can introduce unintended risks of operator error."

> "Biologics are often more sensitive to contamination with bacteria, viruses, or particulates, so maintaining sterility is paramount."
>
> —*Michael Francis, CPC Biopharma*

Furthermore, minimizing product loss at the aseptic fill/finish stage is critical because the starting materials, and therefore the drug substance, for biotherapeutic products are extremely valuable, Kortteinen points out. "Minimizing the loss of volume during processing is an important target for fill process designers," she states.

"Facilities with the flexibility to implement creative solutions, whether it be utilizing state-of-the-art equipment with advanced control features or optimizing aspects of the filling process, are best equipped to minimize product waste," Klecker adds.

Meanwhile, product freezing and shipment, which are more common with biotherapeutic products, also present challenges, Francis explains. Kortteinen points out that many biotherapeutics, especially in their early product life cycle, are stored at low temperatures, such as -80 °C or cryopreserved in liquid nitrogen. These low storage temperatures challenge container integrity during cryopreservation, she specifies.

"System designers need to consider component materials that can handle the wide temperature ranges associated with cryopreservation and subsequent thaw cycles that many biologic products undergo," Francis adds. "Component suppliers should be able to provide leak, burst, microbial ingress, and other testing that demonstrates how their products perform throughout the entire process and associated activities [such as] sterilization, shipping, and freeze-thaw."

Overall, notes Klecker, the complexities and specific requirements of biologics manufacturing necessitate a high level of expertise and meticulous process management to address these challenges effectively.

MANUFACTURERS' SOLUTIONS

Solving the problems encountered during aseptic fill/finish of biopharmaceuticals was greatly helped by the advent of single-use technology (SUT).

SUTs have seen vast development in the past two decades, emphasizes Kortteinen, who explains that single-use supplies, typically sterilized

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

plastic components or assemblies, have changed aseptic fill/finishing processes by adding operational efficiencies.

"Using single-use filling lines for fluid transfer can shorten overall manufacturing time," Kortteinen says. "By using ready-made and sterilized assemblies, one can avoid time-consuming and laborious campaign changeover procedures of equipment. The need for sterilization in place for the fill line disappears when the suppliers sterilize the disposable fill line. Also, using high-quality, high-capacity disposable sterilizing grade filters shortens the manufacturing time."

A significant benefit to using single-use materials in aseptic fill/finish is the minimization of cross-contamination risks, which is a crucial concern, especially for multi-product facilities and contract manufacturing organizations, Kortteinen remarks.

However, Francis explains that, although SUTs such as disposable filling lines and single-use bags help reduce cross-contamination risks and simplify cleaning and validation processes, manufacturers still need to ensure that the SUS components perform as intended within their processing parameters. "For example," Francis says, "newer polymer SUS must be shown to withstand the applicable processing temperature and pressure levels that traditionally posed no issues for stainless-steel equipment."

"Previously," explains Kortteinen, "fill/finish manufacturers washed and sterilized primary packaging materials in-house. The processes needed heavy equipment and facility investments. Validation of such quality critical processes and qualification and maintenance of equipment and systems was labor-intensive." In comparison, today, high-quality ready-to-process vials, stoppers, and other packages are available from suppliers.

"Outsourcing the preparation and sterilization of primary packages has simplified the fill and finishing operations. Validation activities when using ready-to-process components focus on material transfer and loading sterile components into the filling line to demonstrate that all components are kept sterile throughout the processing," Kortteinen states.

Francis further notes that, to create optimal systems, manufacturers should ask component suppliers for key information on chemical compatibility, flow performance, life cycle testing, extractable data, and product integrity in freeze-thaw cycles, among other information. "Many new therapies are being cryopreserved at temperatures beyond -150 °C, for example, so the components in the fill/finish stage need to deal with these ultralow temperatures," he says.

> "Understanding the limitations of each molecule is essential to establishing and maintaining expert control of the optimal environment."
>
> —*Jackie Klecker, Lifecore Biomedical*

Relying on the latest disposable systems, utilizing low shear peristatic pumps within fillers, and streamlining the isolator filling line setup to maximize yield and minimize opportunities for contamination are also important solutions, says Klecker. "Equally important as these in-process solutions are the extensive testing protocols that we develop with our customers to incorporate into the fill/finish process. These play a key role in not only characterizing the molecule and identifying its vulnerability to external factors, but also in monitoring for any unwanted impact during the aseptic fill/finish work," she adds.

Klecker also emphasizes the point of "keeping the end in mind" for each product's commercial fill/finish process. "By working to learn about and address any unique, product-specific behaviors early in our work with a molecule," she stresses, "we can ensure that our final process is optimized for the valuable commercial lots we will produce."

Manufacturers these days are also looking for ways to simplify SUS that make their way into the isolator, according to Francis. For instance, sterile connectors allow SUS to be used outside of the isolator, where the connectors function as a passthrough on the beta port. "Removing the surge bag and even a portion of the tubing manifold, for example, can reduce the 'tubing spaghetti' that otherwise complicates handling by an operator. And as part of a closed system, aseptic connectors help stop particulates and contamination from entering the flow path when connecting filling equipment," he explains. "These connectors also make modular systems possible by connecting components within the systems such as tubing, bags, filters, and manifolds. The integration of closed processing and modular systems enables efficient, flexible workflows while providing the aseptic processing biologics manufacturers need to minimize contamination risks."

THE FUTURE OF BIOPHARMA FILL/FINISH

Filling line manufacturers have already developed innovative new designs and technologies for aseptic fill/finish that are suitable for biotherapeutics,

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

points out Kortteinen. For instance, small batch sizes used in gene therapy and cell therapy have created a need for small automatic filling lines suitable for multi-product fill/finish operations in cell and gene therapy manufacturing facilities. Meanwhile, automatic filling lines improve quality by avoiding human interventions, and automation adds operational efficiencies and enables effective scaling up of fill/finish operations, she notes.

"[T]he industry will continue to optimize facilities and equipment to improve the ability to monitor for and address external factors that may jeopardize the viability of complex biotherapeutic products," remarks Klecker. These innovations may include: the addition of continuous temperature controls throughout the entire fill/finish process and more low-temperature storage capacity; greater adoption of disposable components to reduce burden of cleaning and cleaning validation; and facilities that are designed with specific light spectrum ranges.

Meanwhile, Francis expects that there will continue to be greater use of closed SUS, which he points out are adaptable to any scale or system depending on a unique biologic or therapy needed. "SUS allow smaller, modular segments to process specific batches of product, so modularity will be more broadly deployed. Modularity helps simplify operator SOPs [standard operating procedures] and workflow, too, because all personnel use the same or similar systems.," he says.

Francis also notes that the ability to continuously filter in upstream processing via perfusion is starting to create a continuous flow of product to the filling stage. This continuous processing, along with modular SUTs, is creating a future of faster manufacturing. However, while there is a need for more automation, Francis points out that increased automation as a solution may be difficult to implement because there is no single solution. "With more process data collection and quick analytics through AI [artificial intelligence], there's an expectation that future filling will be tailored and precise. Automation can provide greater reliability, faster processes, and less risk of operator error, but current automation designs are often very complex so you can lose some of the benefits automation is intended to deliver. Also, there's a need to standardize on those designs. The industry recognizes the benefits of standardization, but it's tough to implement for a variety of reasons," he explains.

> "Using single-use filling lines for fluid transfer can shorten overall manufacturing time."
>
> —*Pirkko Kortteinen, 3PBIOVIAN*

Furthermore, Francis anticipates that biologics manufacturers will seek more efficient processes earlier in drug development to allow for more streamlined scaling because thinking ahead to the commercial manufacturing process while still in the drug discovery and efficacy testing stages saves time later on. "For example, the more manual and operator-dependent aseptic techniques such as tube welding and the use of biosafety cabinets might work fine in small-scale processes, but that doesn't translate well when it comes to commercialization," he says.

"Finally," Francis adds, "many hurdles and challenges occur when researchers and developers, especially universities and start-ups in the [cell and gene therapy] space, look to transfer their technology or product from lab scale to commercial scale. As start-ups gain a broader understanding of how their products might eventually be produced—and if they begin incorporating those approaches into their own early-stage processes—the more seamless tech transfer can be."

Kortteinen also highlights newer regulatory considerations that may affect the future of aseptic fill/finish. She brings up that the new Annex 1 guideline (1) introduced in Europe, for example, has a broader scope to include new technologies (e.g., isolators and restricted access barrier systems [RABS]), especially for new facilities or installations. Kortteinen explains that isolators can be completely closed and decontaminated with hydrogen peroxide, which minimizes contamination or cross-contamination risks. "Automatic filling in an isolator is a choice for viral products that require containment at biosafety level 2, as it protects operators from exposure to the products. In the future, aseptic [fill/finish] in either isolator or RABS will be an expectation," she states.

"Annex 1 requires a unidirectional flow for the transfer of materials, equipment, and components. Separate change rooms for entering and leaving the filling are desirable," Kortteinen summarizes. "These requirements will influence the cleanroom design of new facilities and may induce facility renewals in existing cleanroom setups."

REFERENCE

1. EC. Annex 1: Manufacture of Sterile Medicinal Products. *EudraLex* Volume 4, Brussels, Aug. 22, 2022. ◆

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE