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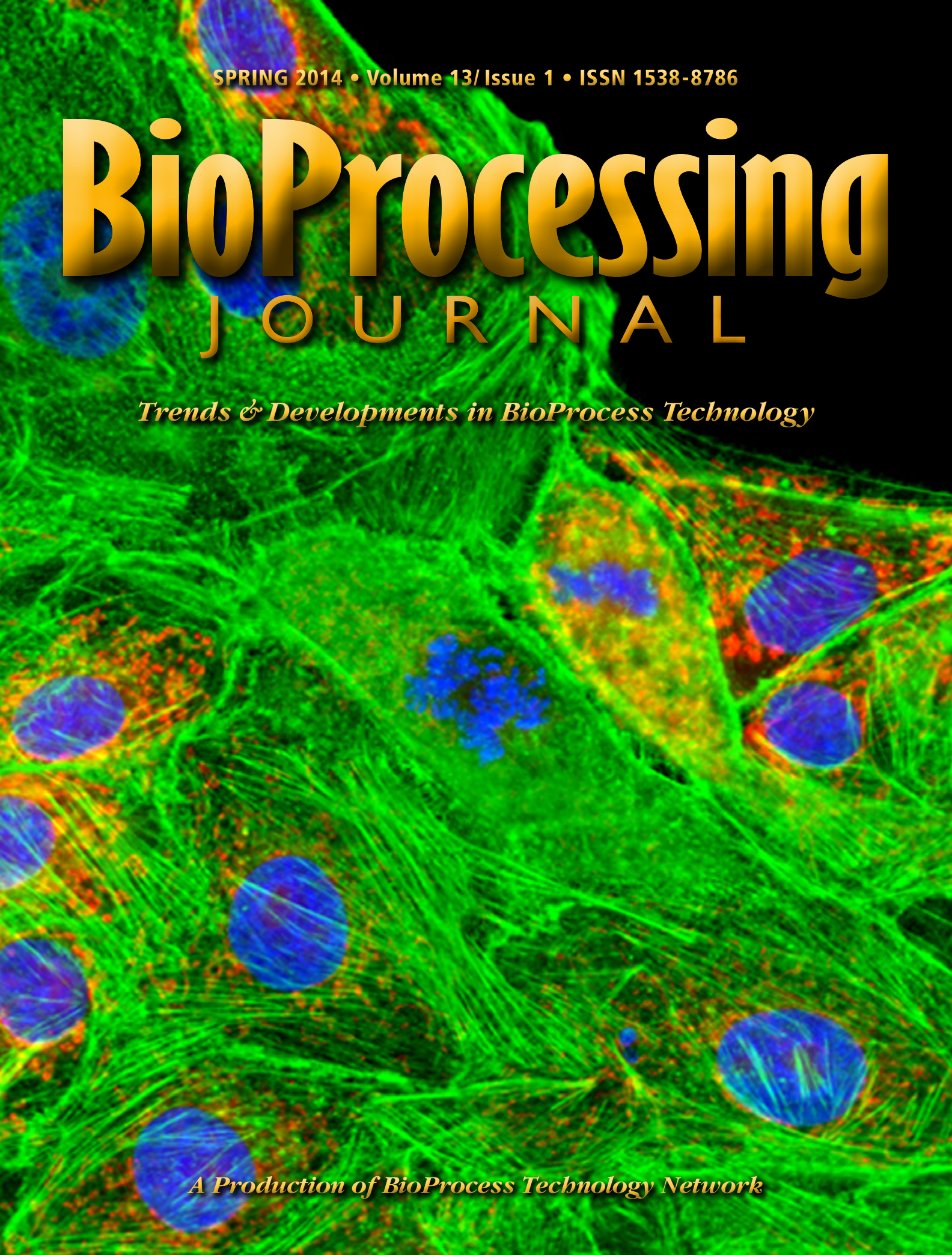
BioProcessing JOURNAL

Trends & Developments in BioProcess Technology



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TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

Connection Technology Advances Closed System Processing:

Single-Use Connectors Create Improved Flexibility and Reliability, and Drive Cost Savings for Biopharmaceutical Manufacturers

By JOHN BOEHM

Introduction

The global demand for new biologics and vaccines, combined with the growing emergence of biosimiliars, is challenging drugmakers to re-evaluate their processes and seek ways to make them more flexible, reliable, and cost-effective. Increasingly, manufacturers are turning to closed, single-use processing systems to meet aggressive campaign turnaround times, reduce risks, and control costs.

Innovative single-use technologies provide biopharmaceutical manufacturers greater flexibility for replacing traditional stainless piping, valves, equipment, or even entire process suites with polymer-based solutions. The benefits of converting to pre-sterilized, single-use systems have been documented in numerous articles and case studies, and these benefits would be lost if manufacturers could not safely and securely connect a variety of systems and components together to create a complete aseptic process.

Connectology may appear to be a small part of an overall system design; however, connection and disconnection of tubing for process fluid transfer is a critical aspect of single-use processing. Manufacturers must carefully consider the available options because the right connector not only affects the operator's convenience, but can be the deciding factor in maintaining process sterility and product quality.

Connection Options

There are many ways by which biopharmaceutical manufacturers can create a sterile connection within or between their unit operations. The right selection is highly dependent on the needs and preferences of each facility.

Quick-disconnect couplings or sanitary and Luer fittings are commonly used with a variety of tubing types and sizes. These connectors provide quick, easy, and secure connections when used in conjunction with laminar flow hoods. Tube welders are an alternative for creating sterile connections with thermo plastic elastomeric tubing. These systems use heated, replaceable blades to maintain sterility while welding two separate tubes together. Single-use steam-in-place (SIP) connectors create sterile connections between a variety of single-use systems and stainless steel processing equipment. These solutions require a steam supply at the point of connection. There are a number of single-use, sterile connectors that allow a tubing-to-tubing sterile connection to be completed without the need for a laminar flow hood or tube welder. This capability opens up the option to create sterile fluid transfer between separate single-use systems, even in gray space. In addition to sterile connectors, there are aseptic disconnects that maintain the fluid sterility during or after disconnection of single-use tubing, even in an uncontrolled environment.

Deciding which solution is best suited for a particular application depends on a number of factors such as ease-of-use, product robustness, flow requirements, preference for gendered or genderless connector designs, and space availability for laminar flow hood, SIP, or tube welding equipment. Additional factors to consider include biocompatibility/extractables data, material compatibility, validation approval, product availability/supply chain assurance, and supplier technical support.

Best Practice Test Matrices

Due to the range of solutions available, it can be difficult for manufacturers to determine the best approach to evaluate or qualify connection technology for a particular process. The BioProcess Systems Alliance (BPSA) Technical Committee formed subcommittees to address best practices for major categories of single-use technologies including films and containers, filter capsules, tubing, and connectors and fittings. Because plastic connectors have different characteristics when compared with traditional stainless valves, a team of connection experts from various companies developed consensus guidelines on quality test methods used to address key performance attributes of single-use connection technology.[1, 2]

The result of this collaborative effort is a matrix that outlines tests common to connectors and fittings, test frequency, and a summary of available test references. Bursts, integrity leaks, bacterial challenge/soiling, particulates, flow rate/pressure drop, biocompatibility, physicochemical, and sterilization process compatibility are tested. This matrix is an excellent reference for drug manufacturers when developing an approach for selecting, qualifying, and validating connection solutions. The connector subcommittee is presently updating the matrix and adding new areas including hose barb retainers. Representatives from BPSA member companies can provide valuable support and assistance to users during technical evaluation.

Application Examples

Sterile connector options have expanded to include a full range of 1/8″ to 1″ flow options, helping to facilitate implementation from upstream to downstream applications. Genderless configurations have further simplified system design to enable standard building blocks of single-use technology that can be easily combined to provide a wide range of complex solutions.

Fermentation Seed Trains with Stainless Reactors:

Modern bioprocessing facilities often feature production bioreactors with capacities of 1,000 to 10,000 liters. Scaling up inoculum from a few million cells in several milliliters of culture to these high production volumes is a challenge that requires aseptic transfer at each point along the seed train. Traditional bioprocessing facilities accomplish scale-up using a dedicated series of stainless steel bioreactors linked together with valves and rigid piping. To prevent contamination between production runs, a clean-in-place (CIP) system is designed into each bioreactor, vessel, and piping line to remove any residual materials. Sterility assurance at the start of each culture is provided via a SIP system consisting of steam pipes, temperature sensors, and condensate collection piping. These CIP and SIP systems require extensive validation testing, and the valves and piping contained in these systems can create additional validation challenges. In addition, CIP and SIP systems must be revalidated following significant maintenance or changes to connecting piping and valve networks.

Advances in single-use system technology allow bioprocess engineers to replace most storage vessels and fixed piping networks with single-use storage systems and transfer lines. Single-use eliminates the need for CIP validation for many components and reduces maintenance and capital expense by eliminating expensive vessels, valve, and sanitary piping assemblies. Figure 1 depicts a production suite

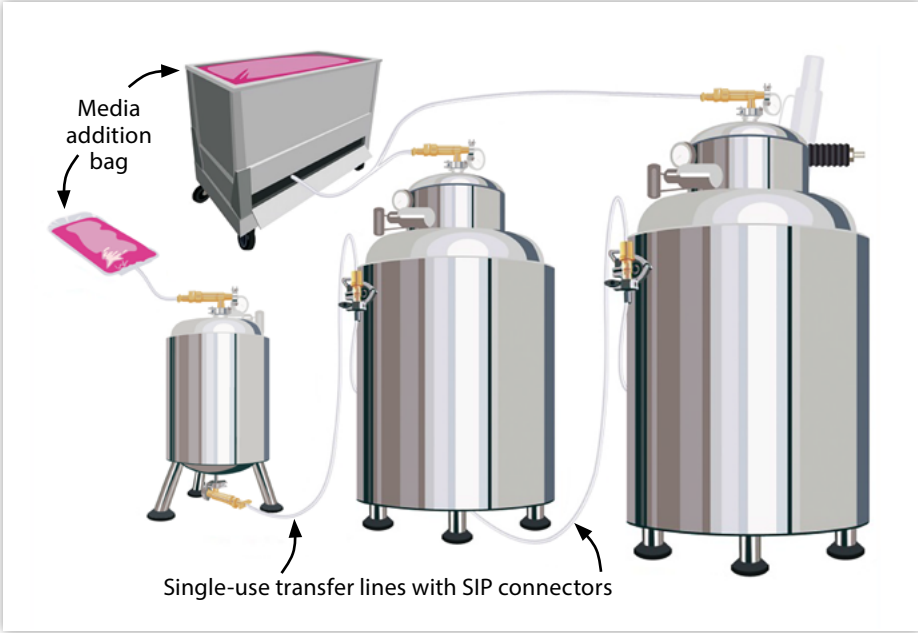

FIGURE 1. Fermentation seed train with stainless bioreactors.

which relies on stainless steel bioreactors but integrates single-use technology for cell culture media storage and key transfer lines. Media storage systems arrive at the bioprocess facility pre-sterilized by gamma irradiation and are often fitted with integrated filters, sampling systems, and connectors. Using a SIP connector allows operators to make sterile connections between these pre-sterilized, single-use systems and stainless steel bioreactors for aseptic transfer of media. Similarly, single-use transfer lines may be used to transfer inoculum between bioreactors using either a peristaltic pump or headspace pressure. Such transfer lines can reduce the number of reusable valves required for transfer as well as eliminate problem areas for CIP and SIP validation. Terminating each pre-sterilized transfer line with a single-use SIP connector closes the system and provides sterility assurance equal to that of traditional fixed piping, at lower capital costs.

With the increasing acceptance and availability of single-use bioreactor (SUB) technology, users can create flexible seed train systems consisting entirely of various volume SUBs, or a combination of SUBs and traditional stainless bioreactors when high volumes are required. It is not practical to have a train of SUBs assembled, sterilized, and installed as one complete system, so single-use connections can be used to provide a secure aseptic process by joining the various components together once they are installed within the process suite. As outlined above, this can be done with quick connectors, tube welding, or sterile connection devices. In the event that smaller volume SUBs are connected with larger volume, traditional stainless reactors, quick connects or sanitary fittings can be used in conjunction with laminar flow hoods, or SIP connectors can be used to establish sterile media transfer. Figure 2 shows a model facility that incorporates single-use bioreactors in combination with stainless steel bioreactors for the two largest vessels.

Buffer/Media Application:

In-house media/buffer preparation requires inline sterile filtration after mixing and prior to storage. Many manufacturers are using filter integrity testing to ensure product purity and minimize product loss. Filter integrity testing verifies that the filters used are functioning properly and are capable of removing a minimum particle size from filtrate. These tests can be conducted pre-filtration, post-filtration, or both, to identify potential breaches in sterility and whether the re-run of a batch process is necessary.

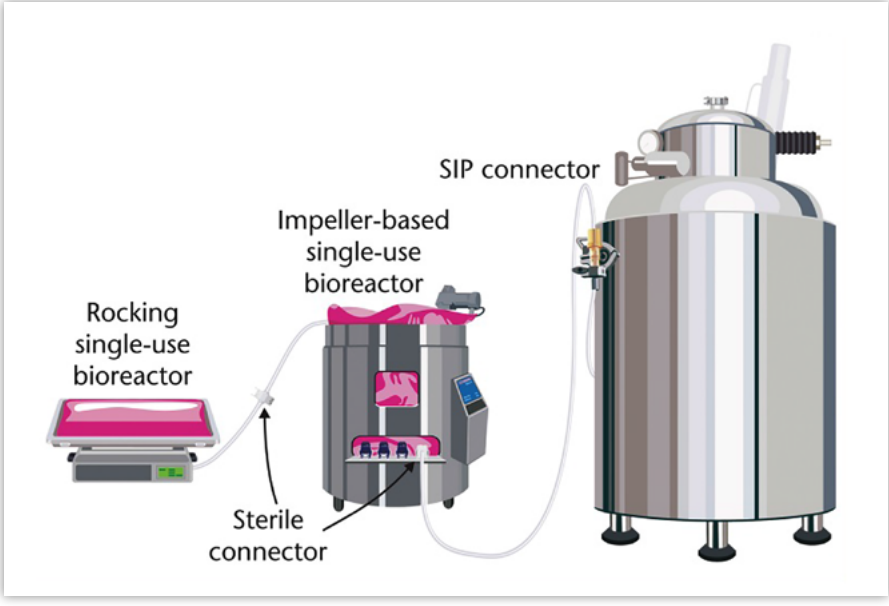

FIGURE 2. Fermentation seed train with single-use bioreactors.

Historically, bioprocessing facilities used hard-plumbed systems with replaceable filter elements in stainless steel filter housings, which required time-consuming set-up, validation, and post-production cleaning. Introducing single-use components into these filter integrity test systems reduces the risk of contamination in addition to improving process flexibility. Single-use components used in filtration and filter integrity testing include disposable capsule filters, bags, tubing, clamps, and connectors. Connectors are a vitally important interface between the components within these filter systems and also provide quick and easy integration of the subsystem into the larger production process.

Single-use components and systems can replace some or all of the stainless steel equipment used for culture media and buffer preparation (Figure 3, on the follow page). Single-use tank liners or specialty mixing bag systems substitute for fixed placed mixing tanks. Bag systems with integrated capsule filters replace both stainless filter housings and sterile hold tanks. After the process fluid has been filtered into the sterile hold bag, the filter can be quickly and easily detached for post-filtration integrity testing using valved, sterile disconnect couplings. The resulting sterile disconnection enables technicians to confidently remove the filter without any contamination risk to the contents stored in the holding bag while also keeping the filter wetted for integrity testing. After removal, filters may be tested using automated equipment by bubble-point, gas diffusion

or pressure decay methods. Once testing confirms filter integrity, the stored cell culture media may be released for continued processing. Extending the use of single-use components to filter integrity testing is an additional way manufacturers can streamline the bioprocess and get product to market faster.

Single-Use for Suite-To-Suite Transfer:

After completion of the upstream production, the protein-containing medium must be aseptically transferred to a different location in the production facility to be prepared for the final filling operation. This suite could be in a room next door or much further away. Traditional bioprocessing facilities accomplish this transfer by using stainless steel manifolds with piping or reusable hoses as transfer lines. This equipment requires validated CIP and/or washing procedures prior to use and sterilization before each media transfer.

With the acceptance of single-use components, process engineers are incorporating single-use transfer lines between the bioreactor in the process suite and the transfer vessel to simplify this process. Using pre-sterilized connectors and tubing, the medium can be moved from the production suite to the preparation suite without the need to sterilize stainless steel piping or equipment. In addition to faster production, these single-use systems also give the manufacturer greater flexibility when determining which process to run in each production suite. The enhanced mobility eliminates many of the restrictions that hard-plumbed piping can place on the manufacturing facility. The risk of cross-contamination in the suite-to-suite transfer process can be high, especially in multi-product facilities. Therefore, utilizing sterile connectors as an interface between key processes can reduce these risks and improve the speed and safety of drug development and delivery.

Formulation and Fill:

Product safety concerns and overall product value are highest during final formulation and dose filling. Like the previous applications, traditional filling processes consist of stainless steel equipment connected via reusable valves, rigid tubing, and steel pipes. Once again, single-use technology, in the form of bulk storage containers, filters, tubing, connectors, and even disposable filling manifolds, can be integrated to minimize contamination risks and reduce operational downtime.

Figure 4 depicts a filling operation utilizing a single-use manifold system with a sterile filter to transfer the drug product between the bulk storage bag and the filling equipment in the isolator. To address product quality and reduce waste, both pre- and post-filtration integrity testing may be

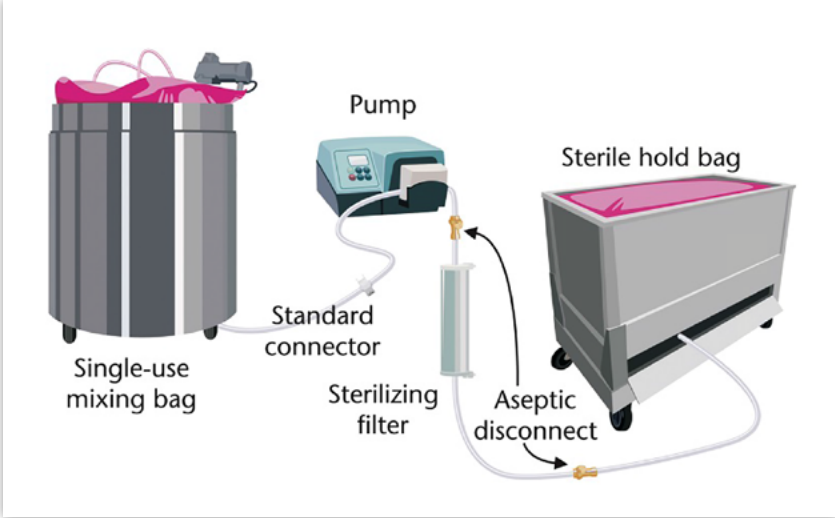

FIGURE 3. Buffer/media preparation.

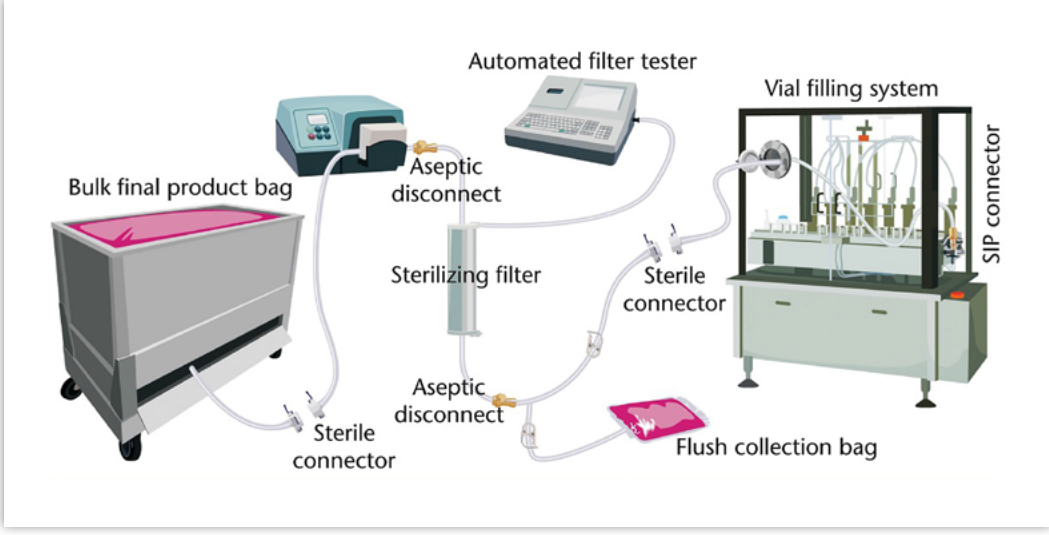

FIGURE 4. Final fill operation.

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combined during final production stages. To conduct pre-filtration testing, the filter assembly is connected to the sterile hold tank using a sterile connector. Once connected, the filter is wetted using product formulation and then pre-filtration testing can be performed. After filter integrity is confirmed, the line to the flush bag is clamped off and the filter assembly is aseptically connected to the isolator transfer line. The isolator line can be pre-installed to the vial filling system using an SIP connection, minimizing downtime. The flow clamp is then opened for filtration to begin. Once filtration is complete, the filter can be removed from the assembly using sterile disconnect couplings to maintain the sterility of the filter until post-filtration integrity testing can be performed. By conducting both pre- and post-filter integrity tests, operators are assured that product purity has been maintained in the final drug formulation and is ready for release.

Future Growth for Single-Use Connections

Connection and disconnection of tubing for fluid transfer is critical to aseptic processing with single-use systems or in combination with traditional stainless steel equipment. A wide range of 1/8" to 1" sterile connection technology enables different subsystems or processes to be combined to create increased flexibility and efficiency from upstream fermentation through downstream filling. Because the connector can be the deciding factor in maintaining process sterility, users need to carefully select their connection solutions.

Continued collaboration within organizations like the BPSA, along with evolving product development from suppliers, will further address aseptic processing needs of faster product changeover for small batch production. Larger throughput systems and tubing ranges will create even more efficient operations and give manufacturers the flexibility they need to get their products to market faster. As a result, the advancements in connection technology are helping address industry demands for rapid drug development, process reliability, and improved manufacturing efficiencies.

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About the Author

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