

Providing Sterility Assurance between Single-Use and Stainless Systems

By John Boehm and Brent Bushnell

As numerous biopharmaceutical manufacturers have gained firsthand experience with the benefits of incorporating single-use systems into their facilities, they are looking to expand use in both up-stream and down-stream applications. This is no surprise as increased volume and diversity of pharmaceutical products is causing manufacturers to design their facilities based on shorter production runs with multiple changeovers. Meeting these demands requires operational flexibility. Production facilities must be able to easily add new products, rapidly convert processes, and quickly make operational adjustments as needed. This is where single-use systems can deliver significant value. These systems offer many benefits including rapid implementation and cost savings.

Single-use systems first gained acceptance in large-scale bioprocessing facilities for sterile cell culture media and process buffer storage applications. More recently, single-use bioreactors from suppliers such as Wave, Sartorius, Thermo Fisher, ATMI and Xcellerex have moved from R & D labs into pilot plants and production facilities as integral systems for small to medium scale production and seed-train scale-up. However, down-stream process engineers have been more reluctant to incorporate single-use systems into their processes. This reluctance originates from the increased value of biotherapeutic proteins with every down-stream purification and formulation step. Engineers have typically relied on process equipment of stainless steel and Hastelloy connected with fixed pipes or reusable flexible transfer lines, all of which require validated clean-in-place (CIP) and steam-in-place (SIP) processes to ensure sterility and product safety. Single-use systems that incorporate components such as Colder Product's Steam-Thru[®] II Connection are capable of providing the sterility assurance engineers rely on with the proven flexibility advantages of single-use. Listed below are two down-stream applications where Steam-Thru Connection technology has played an integral role in incorporating single-use systems with traditional systems.

Adapting Facilities for New Products

Alice Wang is a process engineer working in a down-stream processing group at a major biopharmaceutical company. Although their facility was designed around CIP/SIP equipment and fixed stainless steel piping, adapting this facility for new processes has shown the advantages of single-use-system. Alice's group is implementing a new process which requires flushing of a critical filter element prior to filtering their final drug formulation. This single-use, SIP filter is encased in a stainless steel housing that was not piped for a filter-flush step. Their solution to this problem was to design a single-use bag system with a Steam-Thru[®] II Connection inlet to capture the flush solution. This bag system is received pre-sterilized and routed through their cGMP raw material inspection and release process. When preparing the filter for SIP sterilization, the bag is connected via the Steam-Thru II to the filter housing's bleed port. During SIP, steam passes through the filter housing, out the bleed port, into the Steam-Thru II middle port and out the lower port to a steam trap. Once SIP is complete, the Steam-Thru II's valve is transitioned to the flow position creating a sterile flow path for collecting the flush solution. After the filter flush is complete, the Steam-Thru II's valve is transitioned back to the steam position isolating the bag system and flush solution. Alice indicates that sterility assurance is the primary reason for selecting a single-use bag system with the Steam-Thru II as an inlet. "When filtering during drug formulation steps, maintaining sterility is an absolute priority. The combination of pre-sterile bags and the Steam-Thru II provides the assurance we need without additional process equipment." Another factor considered was Steam-Thru technology's track record. "We've used Steam-Thru Connections for several points up-stream in other processes over past few years, so when we identified this new problem down-stream, we knew that Steam-Thru would meet our requirements."

Creating a Completely Sterile TFF Loop

Chris Heynes of Point Biomedical Corporation also attests that sterility assurance was the main reason they selected Steam-Thru as part of their down-stream tangential flow filtration (TFF) system. Point Biomedical's bioSphere[™] technology consists of two-layer microspheres composed of biopolymers that are about the same size as red blood cells. This size creates a serious problem for down-stream processing; unlike therapeutic proteins, once the bioSpheres

are assembled, they can not undergo any sterile filtration steps at 0.1 to 0.2 μm . This has required Point Biomedical's down-stream engineers to design a sterilely robust process system. For example, one critical purification step is microfiltration TFF. To meet their sterile assurance requirements, Point Biomedical has developed a sterile TFF loop consisting of both reusable stainless steel and single-use components. As Chris points out, "Using Steam-Thru, we can connect our single-use waste collection bag in advance and sterilize the connection point when we SIP our stainless steel retentate vessel. After actuating the Steam-Thru valve, we've created a completely sterile TFF loop with both reusable and single-use components." Ease-of-use and storage issues are other benefits of Point Biomedical's system. "Steam-Thru Connections are light-weight compared with steamable valve assemblies. Our 200 L single-use bag system with the Steam-Thru doesn't require much storage space allowing us to keep 1000 L or more of waste collection capacity on a couple of shelves. Cleaning one vessel instead of two for our TFF is also a big plus."

Process Steps

Colder Products Company's patented Steam-Thru[®] Connection technology creates a sterile link between pre-sterilized bag systems or tube sets and stainless process equipment. The product's innovative three-port design maximizes sterility assurance by eliminating "dead legs" and allowing steam to pass directly through the connection to "steam on" to stainless equipment. Sterility assurance is further expanded with the introduction of Steam-Thru II, which offers "steam on" and "steam off" capability, allowing both a sterile connection and sterile disconnection without the need for a laminar flow hood. This reduces the potential of microbial contamination of the process flow path, as well as environmental contamination within facilities.

The Steam-Thru product line includes several valve and termination options that provide the flexibility needed to meet today's mounting and flow requirements. The connection is attached to single-use bag systems or tubing, then pre-sterilized by gamma irradiation up to 50 KiloGrays or autoclaved up to 128°C for 30 minutes, depending on the product configuration.

For mounting to the processing equipment, engineers may specify the connection with either a ¾" or 1 ½" sanitary termination on the middle port. Tri-clover clamps are used to secure the middle sanitary to the equipment and the lower sanitary to the steam trap. Once attached, these

two lower ports allow a true steam through SIP process that eliminates potential “dead legs” that trap contaminants. With Steam-Thru[®] II, an SIP cycle of up to 135°C or 35 PSI can be performed for up to 60 minutes to “steam on” the connector to the equipment. Effectiveness of the “steam on” cycle was confirmed through bacterial challenge testing using *Bacillus stearotheromphilus* performed at the University of Minnesota’s Biotechnology Research Center.

Once the SIP cycle is complete, the operator depresses the thumb latch to allow valve transition from the steam position to the flow position. This creates a sterile flow path between the stainless equipment and the single-use system, allowing aseptic fluid transfer to occur. A benefit of the Steam-Thru II valve design is a maximum Cv value of 8.2, which translates to a flow rate of 43.9 liters per minute at 2 PSI, which makes it appropriate for high volume applications.

After fluid transfer is complete, depressing the thumb latch again allows repositioning of the valve back to the steam position for a second SIP or “steam off” cycle. This eliminates any biologic residual remaining between the process equipment and the single-use system, minimizing the potential of environmental contamination at disconnection.

In addition to the bacterial challenge testing completed on the “steam on” cycle, bacterial challenge tests were performed following media transfer on the “steam off” cycle. Bacterial ingress tests using *Brevendimonas diminuta* were conducted by Northview Labs to verify post sterilization seal performance. Other performance tests, including helium/steam leak, tensile, and maximum burst, were completed to assure design and process integrity. The Steam-Thru II is comprised of non-animal origin USP Class VI polysulfone and platinum-cured silicone seals, which were tested for biocompatibility.

Realizing the Rewards

Biopharmaceutical manufacturers have proven the advantages of single-use systems in many up-stream processes and sterile buffer hold applications. The challenges of capitalizing on these advantages for critical down-stream processing and formulation steps has been in maintaining sterility assurance, especially when integrating single-use systems with traditional stainless equipment. Single-use technologies like Steam-Thru[®] II Connections allow large and small

companies alike to incorporate disposable systems into their most critical process steps. Companies that gain experience with and embrace single-use technology will be well positioned to establish a competitive advantage in the years to come.

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