Providing Sterility Assurance Between Stainless Steel and Single-Use Systems

John Boehm and Brent Bushnell

As biopharmaceutical manufacturers experience the benefits of single-use systems, many want to expand their use in both upstream and downstream applications. This isn't surprising, considering that the increased volume and diversity of biopharmaceutical products are causing manufacturers to design their facilities based on shorter production runs with multiple changeovers. Meeting such demands requires operational flexibility. Production facilities must be able to add new products easily, rapidly convert processes, and quickly make operational adjustments as needed. This is where single-use systems can deliver significant value, 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 (**1–4**). Recently, single-use bioreactors from suppliers such as Wave Biotech, Sartorius, Applikon, Hyclone and Xcellerex have moved from research and development laboratories into pilot plants and production facilities as integral systems for small- to medium-scale production and seed-train scale-up (**5–7**). However, many downstream process engineers have been reluctant to incorporate single-use systems into their processes (**8**). Their reluctance originates from the increased value of biotherapeutic proteins with every downstream purification and formulation step.

Engineers typically have relied on process equipment made 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 Steam-Thru II connections from Colder Products Company, provide the sterility assurance engineers rely on, along with the flexibility of single-use. The two downstream applications highlighted below clearly illustrate where this technology has played an integral role in incorporating single-use systems with traditional systems.

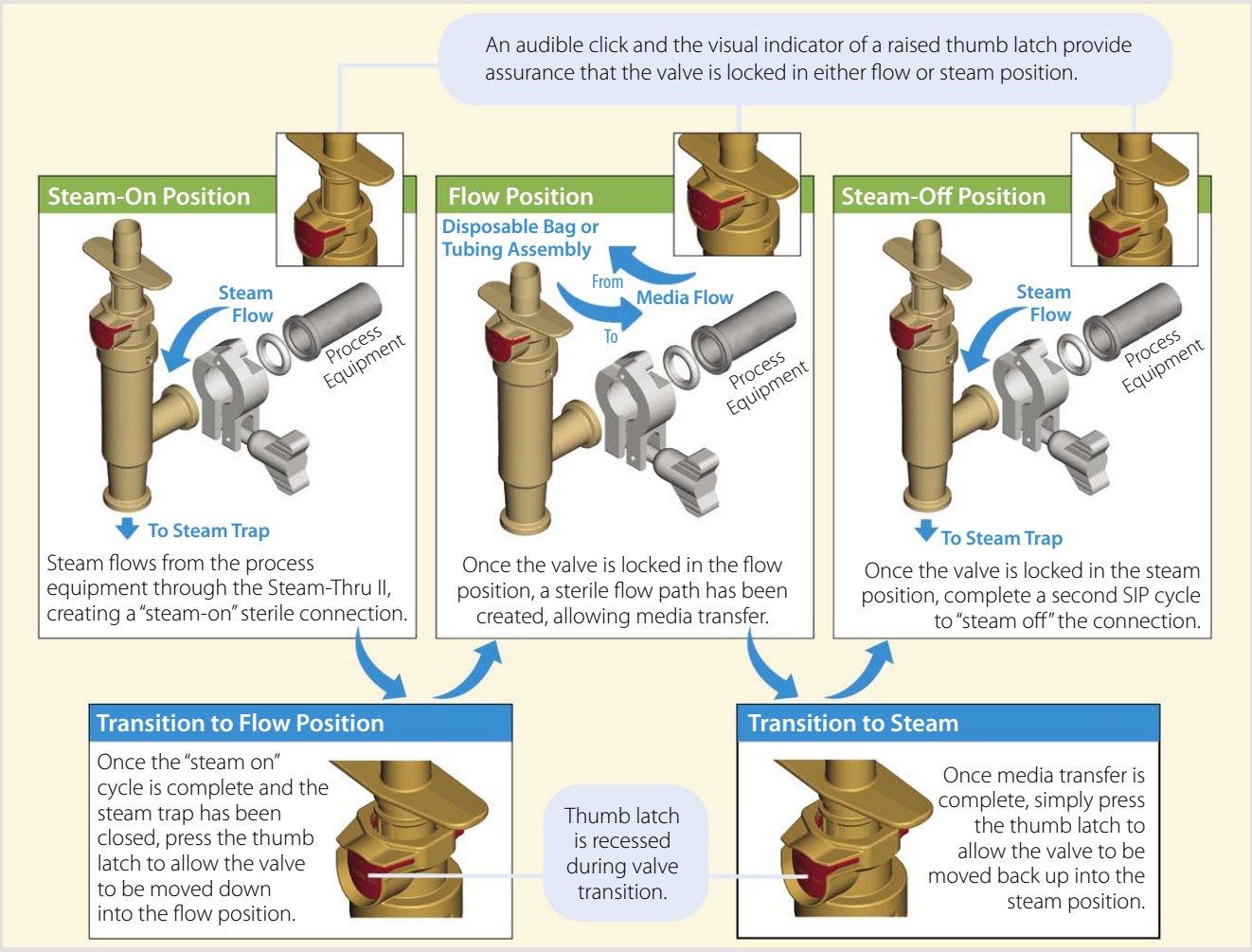
Adapting Facilities for New Products

Alice Wang is a process engineer working in a downstream processing group at a major biopharmaceutical company. Although the facility she works in was designed around CIP/SIP equipment and fixed stainless-steel piping, its adaptation for new processes has revealed the advantages of single-use-systems.

Wang's group is implementing a new process that requires flushing of a critical filter element before filtering the final drug formulation. A single-use SIP filter is encased in a stainless-steel housing that was not piped for a filter-flush step. The company's solution to this problem was to design a single-use bag system with a Steam-Thru II connection inlet to capture the flush solution. The downstream process group receives that bag system

Photo 1: As a critical link between stainless-steel equipment and single-use systems (e.g., bags or tubing sets), Steam-Thru technology creates a contamination-free connection. Made of non-animal–origin, USP Class VI polysulfone with platinum-cured silicone seals, these products can be safely connected to either bioreactors or process skids.

Figure 1: A Steam-Thru II connector creates a sterile flow path between stainless-steel equipment and single-use systems. It can be used as either an inlet or outlet point for bag systems.



presterilized and routes it through their CGMP raw materials inspection and release process.

To prepare the filter for SIP sterilization, the bag is connected by the Steam-Thru II connector to the filter housing's bleed port (Photo 1). During SIP, steam passes through the filter housing, out the bleed port, into the connections middle port and out the lower port to a steam trap. Once SIP is complete, the valve is switched to its flow position, creating a sterile flow path for collecting the flush solution (Figure 1). After the filter flush is complete, the valve is switched back to its steam position, isolating the bag system and flush solution.

According to Wang, sterility assurance was the primary reason for selecting a single-use bag system with the Steam-Thru II connection as an inlet. "When filtering during drug formulation steps, maintaining sterility is an absolute priority," she said. "The combination of presterile bags and Steam-Thru connections provide the assurance we need without additional process quipment." Another factor considered was the proven track record of the technology. "We've used Steam-Thru connections for several points upstream in other processes over the past few years, so when we identified this new problem downstream, we knew that it would meet our requirements."

Creating a Completely Sterile TFF Loop

Chris Heynes of Point Biomedical Corporation (www.pointbio.com) of San Carlos, CA, attests that sterility assurance also was the main reason his company selected Steam-Thru connectors as part of its downstream tangential flow filtration (TFF) system.

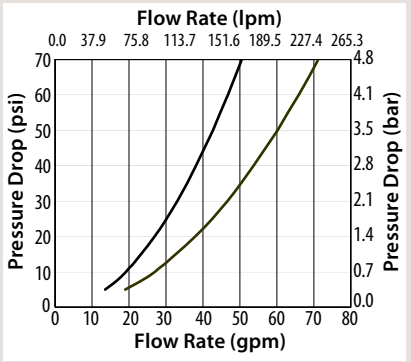
Point Biomedical's bioSphere technology consists of two-layer microspheres composed of biopolymers that are about the same size as red blood cells. Their size creates a serious problem for downstream processing because, unlike therapeutic proteins, once the microspheres are assembled, they cannot undergo sterile filtration steps at 0.1–0.2 µm. This has required Point Biomedical's downstream engineers to design a robustly sterile process system. To meet its requirements, they developed a sterile TFF loop consisting of both reusable stainless steel and single-use components.

As Heynes pointed out, "Using Steam-Thru [connectors], 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 connection's 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," Heynes said. "Our 200-L single-use bag system doesn't require much storage space, allowing us to keep 1,000 L or more of waste collection capacity on a couple of shelves. Cleaning one vessel for our TFF instead of two also is a big plus."

Figure 2: The Steam-Thru II valve design makes it ideal for high-volume applications. Test results show a maximum relative flow capacity (*Cv*) value of 8.2 and a flow rate of 43.9 L per minute at 2 psi.



Process Steps

As shown above, Colder Products Company's patented Steam-Thru connection technology creates a sterile link between presterilized bag systems or tube sets and stainless-steel 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 the stainless-steel equipment. Sterility assurance is further expanded with the introduction of Steam-Thru II connectors, which offers "steam on" and "steam off" capability that allows both a sterile connection and sterile disconnection without the need for a laminar-flow hood. So it 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 help users meet flexible mounting and flow requirements. The connection is attached to a single-use bag system or tubing, then pre-sterilized by gamma irradiation up to 50 kGy or autoclaved up to 128° C for 30 minutes, depending on the product configuration.

For mounting to the processing equipment, engineers may specify their connections with either a ¾-in. or 1½-in. sanitary termination on the middle port. Triclover clamps are used to secure the middle sanitary ports to the equipment and the lower sanitary ports to the steam trap. Once attached, those two lower ports allow a true steam-through SIP process that eliminates potential dead legs that can trap contaminants. With Steam-Thru II, an SIP cycle of up to 135° C or 35 psi can be performed for up to 60 min. to "steam on" the connector to the equipment. Effectiveness of the "steam on" cycle was confirmed through bacterial challenge testing, as shown in Table 1, using *Bacillus stearotheromphilus* performed at the University of Minnesota's Biotechnology Research Center (**9**).

Once the SIP cycle is complete, the operator depresses the connector's thumb latch to switch the valve from steam position to flow position (Figure 1). This creates a sterile flow path between the stainless-steel equipment and the single-use system, allowing for aseptic fluid transfer. A benefit of the Steam-Thru II valve design is its maximum relative flow capacity (*Cv*) value of 8.2, which translates to a flow rate of 43.9 L per minute at 2 psi, which makes it ideal for high-volume applications (Figure 2).

After fluid transfer, depressing the thumb latch again allows repositioning of the valve back to its steam position for a second SIP or "steam off" cycle. This eliminates biologic residuals that might remain 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 Laboratories (www.northviewabs.com) in Northbrook, IL, to verify post sterilization seal performance (**9**). Other performance tests — including helium/steam leak, tensile, and maximum burst — were completed to assure design and process integrity. The Steam-Thru II is made of non-animal origin, USP Class VI polysulfone with platinum-cured silicone seals, which were tested for biocompatibility (Table 1).

Table 1: Effectiveness of the "steam on" and "steam off" cycles were confirmed through bacterial challenge testing using *Bacillus stearothermophilus* and *Brevendimonas diminuta*. Other performance tests listed were completed to ensure design and process integrity.

Test Type Description	Steam-Thru II Connections	Steam-Thru Connections
Bacterial challenge	Passed	Passed
Microbial ingress	Passed	Passed
Steam leak	Passed	Passed
Helium leak	Passed	Passed
Dye leak	Passed	Passed
Burst	Passed	Passed
Media flow	*Cv* = 5.3 to 8.2	*Cv* = 4.2 to 4.6
Biological reactivity test in vivo (polysulfone)	Passed	Passed
Physicochemical test (polysulfone)	Passed	Passed
Physicochemical test ising alternative extract (polysulfone)	Passed	Passed
Cytotoxicity study using the ISO elution method (polysulfone)	Passed	Passed
In vitro hemolysis study (polysulfone)	Passed	Passed
Biological reactivity test in vivo (silicone)	Passed	Passed
Physicochemical elastomeric closures (silicone)	Passed	Passed
Cytotoxicity study using the MEM elution method (silicone)	Passed	Passed
Cytotoxicity study using the agarose overlay method (silicone)	Passed	Passed
In vitro hemolysis study (silicone)	Passed	Passed

Realizing the Rewards

Biopharmaceutical manufacturers are proving the advantages of single-use systems in many upstream processes and sterile buffer-hold applications. A primary barrier preventing companies from capitalizing on such advantages for critical downstream processing and formulation steps has been that of maintaining sterility assurance, especially when integrating single-use systems together with traditional stainless-steel equipment. New connection technologies address that issue, allowing large and small companies alike, to incorporate single-use systems into their most critical process steps. Companies that embrace and gain experience with single-use technology, will be well-positioned to establish a competitive advantage in the years to come.

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