A Disposable Option for Bovine Serum Filtration and Packaging

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Bovine serum is an important component in nutritional media for many cell culture operations. Here we describe HyClone's design and implementation of a modular production line for serum filtration and packaging. For quality and logistical reasons and to maintain manufacturing segregation of the raw serum coming into the modular facility, HyClone has established regional serum processing facilities. The incoming serum is collected and processed using proprietary methods.

CGMP and Quality Considerations: To ensure that the quality and performance of the serum are maintained through the filtration process at remote filtration facilities, it was necessary that reproducible, validated cGMP processes be continued throughout the filtration process. The single-use system components and modular facility described in this article achieve those quality and performance objectives.

Benefits of Single-Use Systems

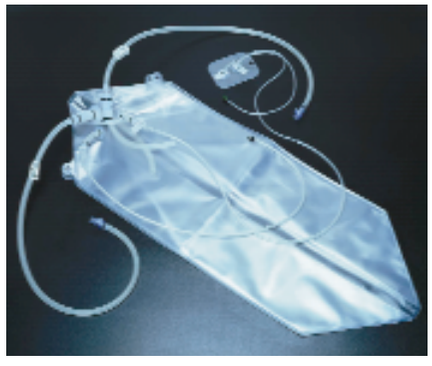
During scale-up processes, single-use disposable systems are particularly beneficial because they are likely to be used only to prepare a limited number of smaller-scale batches. For larger-scale production applications, they are beneficial for a number of reasons:

- Low installation and maintenance costs
- Compressed installation timetable
- No requirement for hazardous cleaning fluids, thus eliminating their handling and disposal
- Reduced water requirements (because cleaning is not required), which can translate to a smaller water system and lower operating costs
- No cleaning validation
- Reduced maintenance, because filter housings and stainless steel tanks are not required
- Reduced time to production.

The single-unit disposable operation described in this paper — filtration of 1000 liters of HyClone bovine serum — demonstrates how production timetables can be shortened with a single-use rather than a hard-piped system. The objective for the facility was to start up as quickly as possible and maintain costs below $300,000.

Facility Design

The facility was designed with five primary modular rooms: an incoming and outgoing material room; a room for thawing frozen product; a filtration room; a clean room with a Class 100 laminar flow hood for filling serum into containers; and a freezer for serum storage (Figure 1).



A 3-D BioProcess Container system (BPC) with attached small face-ported BPC for sampling (HyClone, www.hyclone.com)

The modular design eliminated the need for building permits, which helped reduce production start-up time.

Inside the modular filtration room, serum is received and pooled in a 100-L HyClone tank liner fitted in a holding tank. The tank liner, when filled with raw serum, serves as the feed source for the filtration system. Raw serum can be added to the lined tank as the level is depleted, thereby accommodating batch sizes far beyond the capacity of the tank. Flexible tubing from the bottom of the tank is attached to a filtration train similar to the one shown in Figure 2.

Prefiltration schemes are based on the serum type and batch size. The scheme can consist of two

prefilters as shown in Figure 2. Serum is then filtered through three sequential Pall 0.1-µm filters. As with prefiltration, specific Pall filters depend on serum type. Typical schemes used include combinations of Pall Posidyne NGZ01 and Pall Fluorodyne II DJL filters. Two stages of prefiltration are usually required for serum filtration. Specific prefilter membranes and their required micron ratings depend on the serum.

Filtered material is directed through flexible tubing from the final 0.1-µm filter to a 1000-L HyClone BioProcess Container system (BPC). Filtered serum is pumped out of the BPC though flexible tubing and directed through a wall into a Class 100 laminar flow hood, where the filtered serum is placed into 100-, 500-, or 1000-mL PET bottles. Serum bottles are labeled and then placed in a freezer room for storage until they are packaged for shipment.

Figure 1: Facility design (modular rooms)

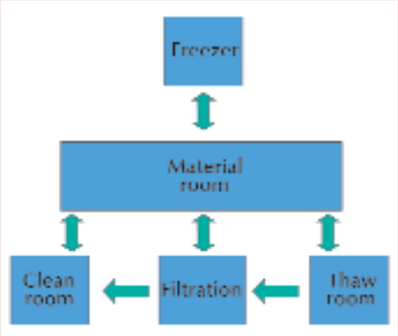


Figure 2: Typical Pall filtration train

Table 1: Pall membrane filters that can be used as prefilters

Type	Membrane	Micron rating
Profile prefilter	Polypropylene depth filter with tapered pore structure	5 µm
Profile Star	Polypropylene with star-shaped pleat structure	5 µm
Ultipor GF Plus prefilter	Bonded glass fiber, with positive Zeta potential	20/2 µm
Preflow UUA prefilter	Resin bonded glass fiber	0.2 µm

Single-use System Equipment

The single-use design incorporates several enabling technologies that make disposable systems possible.

A BPC (Figure 3) is a single-use, flexible, disposable bag made from medical-grade plastic film specifically designed for biopharmaceutical applications. Its applications include media or buffer formulation or filling, bioreactor and fermentation feed and harvest, waste collection, product fractionation, and transport and storage of bulk intermediate and final product. Single-use product containers (BPC) eliminate the cross-contamination risk that may occur in stainless steel tanks, and they require no cleaning or cleaning validation.

HyClone BPCs are made of polymer films formulated to meet process requirements such as gamma-irradiation sterilization. All films pass USP Class IV tests for plastics. Materials that come into contact with liquids are inert; the container walls also act as a gas barrier to help protect liquids. The newest film, HyQ CX5-14, is a five-layer single-web (single sheet of film), 14-mil cast film produced under cGMP guidelines. The outer layer is a durable elastomeric resin skin coextruded with an ethyl vinyl alcohol (EVOH) barrier layer. Product-contact layers are polyethylene.

BPCs have fitments or connection devices for filling, dispensing, and sampling. They come with a variety of flexible tubing sizes and types. Tubing is attached to the BPC and held in place with a cable tie. Plastic shut-off clamps are used on the tubing to allow for isolation of lines as needed.

During filling operations, stainless steel vessels require gas venting to ensure that tanks fill completely. BPCs do not require a vent because the bag is completely empty before filling begins. As fluid fills the bag, the BPC expands to allow filling.

Stainless steel tanks require sterilization by validated steam-in-place (SIP) steps requiring, in turn, a facility equipped with clean steam lines. BPCs are presterilized by gamma irradiation and therefore do not require a facility with SIP capabilities. BPC irradiation procedures are based on the current ANSI/AAMI Guidelines for Gamma Irradiation Sterilization, the industry standard for sterilization by gamma irradiation. BPCs used for the studies in this paper were gamma irradiated at a validated range of 25–38 kGy to achieve 10^6 Sterility Assurance Level (SAL).

Pall Kleenpak Nova T-Style Filter Capsules: Disposable capsule filters have been on the market for more than 25 years, but the available configurations have increased greatly in recent years. A recent development is availability of larger disposable capsule filters. Pall's Kleenpak Nova capsule filters incorporate 10-, 20-, or 30-inch-long standard Pall cartridge filters that were traditionally installed into stainless steel housings. In applications where a particular filter is already specified, users can switch from stainless steel housings to fully disposable Kleenpak assemblies with minimal requalification.

The capsules are either in-line (inlet on top, outlet on bottom) or t-style (inlet and outlet both on one end), which allows processing flexibility. The filters can be manifolded in series so that both pre- and final-filtration stages are

Table 2: Approximate time required for each operation in filtration processes for both single-use and stainless steel systems

Operation	Single-use (hours)	Stainless Steel (hours)
Assembly	1	2
SIP filtered serum tank	0	2
Filter sterilization autoclave (optional)	2	2
Filtration	3	3
Integrity test final filter	0.5	0.5
System disassembly	1	2
CIP filtered serum tank	0	3
Cleaning unfiltered serum tank	0	1
Filter housing cleaning	0	2
Record keeping	0.5	1.5
Total	**8**	**19**

Table 3: Cost and time benefits for the filtration of bovine serum

	Stainless Steel System	Disposable System
Time from design to implementation	6–8 months	<40 days
Facility cost (US dollars)	>$500,000	$250,000
Processing time	19 hours	8 hours

available in single, ready-to-use units. Manifolded systems also can be assembled in a parallel configuration for applications that require a larger total filter surface area than the 30-inch filters provide.

The filtration assembly is illustrated in Figure 2, showing filters installed with pressure gauges on the upstream side at each stage so that filtration pressure can be monitored throughout operation. The capsules are translucent so that the serum being filtered is clearly visible in each one. This feature allows visual confirmation that capsule filters have been properly bled of air so that the entire membrane is used. Completion of filtration also be determined by observing fluid in filters.

Serum can be difficult to filter and requires multiple stages of filtration. Bovine serum requires two stages of prefiltration. Table 1 lists Pall filter membranes that can be used as prefilters. For final serum filtration, three 0.1-µm filters may be used in series for mycoplasma removal. Bovine serum is used in cell culture applications where mycoplasma can grow to high densities (10^7–10^8/mL) without pH or turbidity changes, thus compromising cultures. Small size and lack of a cell wall enable mycoplasma penetration of 0.2/0.22- µm rated filters; typical titer reduction is approximately 10^3–10^5 for 0.2/0.22-µm rated filters whereas 0.1-µm filters typically provide 10^5–10^8 reduction.

Posidyne NGZ01 filters incorporate charge-modified Nylon 6,6 membranes, which exhibit a positively charged Zeta potential in aqueous solutions. A positively charged filter provides adsorption-enhanced retention of particles smaller than the nominal filter rating. Pall's NGZ01 filters provide high protein recovery from sera and most protein solutions, and their *Acholeplasma laidlawii* mycoplasma titer reduction is rated at >10^6/cartridge.

Fluorodyne II DJLP grade 0.1-µm filters have two layers of PVDF membrane with a built-in 0.2-µm prefilter layer and a final 0.1-µm Layer. The DJLP filters have a flow rate comparable to the flow rate of traditional 0.2-µm filters, which allows for economical 0.1-µm filtration systems. DJLP filters offer high mycoplasma titer reduction (10^8/cartridge).

Comparative Cost and Time Savings

Project Timeline: The time required from start date for design of HyClone's new serum facility and first production filtrations was less than 40 days. By contrast, stainless-steel design would have required six to eight months. Use of disposable systems thus saved the company four to six months in

- Lead time for assembly and installation of components, such as stainless steel tanks and filter housings
- Time required to fabricate and plumb stainless steel systems
- Time required for design and installation of a clean-in-place (CIP) system (not required for the single-use system)
- Time required for cleaning validation.

Facility cost of the single-use system design was approximately $250,000, compared with stainless steel systems estimated to cost $500,000 or more.

Operating Cost: Single-use design positively affects operational overhead by lowering costs of filtration operations. Although filling, freezing, and packaging costs are the same for single-use and stainless steel systems, single-use systems provide overall savings in operation, with lower labor and utility costs. Single-use systems, of course, incur additional consumable costs for filters, tank liners, and BPCs. Capsule filters in polypropylene housings for single-use systems contain the same type of filter as used in stainless steel systems. A number of operational steps are not required for single-use systems, including CIP and SIP of stainless steel tanks and filter housings. Single-use systems therefore reduce utility costs. Net overall savings are estimated at 4–6%, but economies of scale would certainly apply.

Handling steam for CIP and SIP operations is a safety issue for production personnel. In addition to those concerns, steam sterilization can compromise filter operation and performance if not properly controlled. Most filter

damage is due to errors in SIP processes (such as reverse pressurization at the end of a steam cycle if air or nitrogen is not introduced to balance pressure).

Time required to validate cleaning of permanent components used in disposable systems is almost eliminated. At the same time, water demand is greatly reduced. These all translate into added savings.

Table 1 shows that total time required for batch filtration of serum using single-use systems is eight hours, whereas the time required for stainless steel systems is more than double that at 19 hours. This decrease translates into lower labor cost for single-use systems and reduces batch turnaround.

Processing efficiencies are also gained by using disposable systems because they require no assembly of cartridges and housings. In many traditional facilities, housings need to be checked out of a central storage area and parts gathered in preparation for assembly. In addition, housings need to be disassembled and cleaned after use. Several hours can be required for such operations, translating into more efficient use of process time with disposable systems.

Further, checking housing assemblies out from central storage followed by CIP operations usually requires documentation. Filters that do not require assembly or cleaning result in less time spent on record keeping. Multiple-use housings also require maintenance; single-use housings do not. Again, using disposable systems leads to cost savings.

Meeting Increasing Demands

The following benefits of disposable systems are illustrated for this application (Table 3):

- New systems using disposables do not require design and implementation of stainless steel assemblies, thus saving time and expense.
- The time required to clean a disposable system is reduced, and cleaning validation is minimized.
- Disposable systems reduce or eliminate CIP requirements, which in turn reduces water demand translating into smaller water-for-injection (WFI) systems. Additionally, special handling of solvents or steam, required equipment and tanks for CIP procedures, and cleaning validation are unnecessary. These changes all lower costs.

Changing from completely stainless steel systems to completely disposable systems in existing operations may at first appear to be impractical because of capital investment in extant equipment. What's more, validation work that may have been performed on the hard-piped systems can be substantial. It is possible, however, to make gradual changes toward completely disposable processes. For example, as a first step, disposable cartridge filters can replace traditional stainless steel housings.

As more pharmaceutical products are released from R&D and clinical trials, demands on manufacturing facilities will become more intense. Disposable systems provide simple practical, cost-effective solutions to optimizing use of precious space in manufacturing suites.

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