sartorius stedim biotech

Accelerated Development, Manufacturing and Monitoring of Viral Vectors

Viral Vaccines – Gene Therapy – Oncolytic Viruses

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Welcome and Contents

The goals of process intensification are to enhance production while shortening timelines, lessening contamination and environmental risks to products and operators, and reducing operating footprints. Previous publications from Sartorius Stedim Biotech (SSB) have highlighted key elements of such activities. In this report, the authors extend the scope of this discussion to tools and technologies that enable intensification of viral vector manufacturing processes.

The first article summarizes presentations from a 2018 seminar for viral vaccine manufacturers. Three guest presentations highlighted the need to intensify viral vector production: a labor-intensive process for which the high titers and vector quantities needed demand careful process optimization. Building on its platform of single-use technologies, Sartorius offers tools and technologies to reduce capital costs and decrease the consumption of energy and water during vaccine production.

Vaccines, however, are too diverse a product category for a single platform approach to address all of the challenges. In the next article on predefined platform technologies, Amélie Boulais describes how predefined technologies can be integrated with one another to enable development and manufacturing of vaccines. She offers examples of upstream and downstream processing technologies that viral vector manufacturers can use within their processes.

Even established multinational pharmaceutical companies often do not have the capabilities and/or expertise to manufacture viral vectors that are large (>0.2 µm) or replicating or both, especially at industrial-scale batch sizes. A significant endorsement of SSB's platform for viral vector processing and single-use design, the ABL facility described in the third article offers capacity designed to help in the manufacture of viral vectors for a wide range of applications such as oncolytic, vaccines, and gene therapy products.

None of this work can ignore continuing needs for both safety and processing flexibility. The fourth article explores risk factors in viral vector production with single-use components. The authors discuss preventing bag ruptures and filter blockages, along with ways to ensure robustness of processing equipment against long-term working pressures and temperatures. Operator safety should be a significant concern in selection of a single-use technology for vaccine manufacturing. The authors conclude with the importance of challenging single-use bioreactor vendors on their approaches to biosafety risk mitigation to allow a company to make well-founded decisions regarding its future bioreactor platform.

Viral Vector Development, Manufacturing, and Process Intensification

Miriam Monge, Amélie Boulais, and Gerben Zijlstra

On 23 April 2018, Sartorius Stedim Biotech (SSB) hosted a seminar for viral vaccine manufacturers at the Novotel, Amsterdam, the Netherlands. The seminar featured three guest presentations from ABL Europe, Janssen Vaccines, and the Max Plank Institute, Magdeburg. These external presenters spoke alongside experts in vaccine processes and process intensification from SSB. The aim of the seminar was to provide an overview of the tools and technologies available for development of viral vectors and to provide insights into techniques that viral vector manufacturers can use to intensify their production processes to achieve greater productivity.

During her opening remarks, Miriam Monge (Director of Marketing Integrated Solutions, SSB) highlighted the fact that single-use technologies can significantly reduce capital costs and decrease the consumption of energy and water during vaccine production. SSB is focusing on improving the robustness of these single-use technologies so that vaccine manufacturers can apply them during commercial production and benefit from lower costs, greater flexibility, increased speed, and higher quality.

Edwin Janssens from the Janssen Vaccines Process Development group described a design of experiments (DoE) approach for characterizing a viral vaccine purification step using a Sartobind membrane adsorber (Photo 1, next page). He explained that adopting a quality by design (QbD) approach is consistent with regulatory expectations because it decreases reliance on end-product testing but is also a smart way for manufacturers to generate process knowledge. The QbD approach is underpinned by the principle that manufacturers should start by defining the desired quality attributes profile of the product they are developing and then determine the inputs, such as material attributes or processes parameters, which will deliver that output.

Janssen Vaccines applied the QbD approach to the characterization of processes for some of its vector-based products. One of the SSB Sartobind membrane adsorbers is used as part of the purification process. Janssen Vaccines uses it in a bind-and-elute mode because some of the company's vector-based products have a negative charge. The Sartobind family of membranes also come in small-scale formats that facilitate the DoE approach because a large number of experiments can be performed with little starting material.

Janssens explained that manufacturers should start the QbD process with a risk assessment to decrease the number of conditions that must be screened without excluding parameters that can affect the output profile. Successful completion of the risk assessment allows the design of screening experiments and the ranges of the parameters being studied. Manufacturers also must decide what constitutes a critically important parameter based on their control strategy and other factors. The statistical software that scientists use to analyze the results may identify parameters as being critical, but manufacturers should ensure that they can provide a scientific justification for that designation. Those factors deemed noncritical or minimally critical might be removed from the subsequent augmented experimental design that

Photo 1: Using a Sartobind membrane adsorber in a biopharmaceutical application

The Sartobind family of membranes also come in small-scale formats that facilitate the DoE APPROACH because a large number of experiments can be performed with little starting material.

allows all the possible interactions between critical parameters to be understood. From the results of the augmented experimental design, manufacturers can establish proven acceptable ranges for critical process parameters and material attributes. Janssens concluded that the QbD approach demands creativity from an experimenter and requires that person to make choices throughout the process. The amount of knowledge gained about the process makes this approach well worth the effort.

Anthony Da Silva and Morgane Larret from, ABL Europe described development of a flexible, multiproduct, multicustomer unit for viral vector production. ABL Europe is a contract manufacturing organization that is 100% dedicated to viral vector production. At the beginning of 2016, the company had one filling suite, one drug substance suite for adherent cell processes, and a stainless steel drug substance suite initially designed for adenovirus production. The company decided to invest €3 million to expand production capacity by modifying and converting the stainless steel suite to incorporate entirely single-use equipment from vial thaw to drug substance storage. The new suite would allow the production of AAV, adeno, lenti and other viral vectors that can be produced from nonadherent cell lines. ABL Europe needed the suite to accommodate viral vector production up to the 500-L scale. The company wanted to select as many pieces of equipment as possible from a single supplier to reduce vendor management risks, reduce supply chain complexity, facilitate integration of different process steps, and simplify future support requirements from the vendor.

ABL Europe contacted four suppliers initially and ranked them according to criteria such as the

Photo 2: ambr 250 high throughput perfusion, a new multi-parallel bioreactor to fast-track intensified cell culture processes

technical solution they provided, costs, and company expertise. Two suppliers were short-listed to demonstrate their technology at their own facilities. ABL Europe selected SSB as the main supplier at the beginning of 2017. SSB offers compact and flexible equipment that can be shared by different steps. It provides a wide range of suitable processing technology that can be operated as a closed system to allow production of nonfilterable viral vectors. Different pieces of SSB equipment use harmonized software, which minimizes operator training requirements. The company also promotes a 500-L single-use bioreactor that ABL Europe might choose to purchase in the future.

ABL Europe's new single-use production line for viral vectors from suspension cultures comprises orbital shakers, rocking motion, and stirred tank single-use bioreactors from SSB and the XCell ATF6 from Repligen. There are separate production areas for production of cells and viruses. The downstream process uses single-use mixers, two crossflow systems, and a cell harvest system, all from SSB. Chromatography steps are run on an ÄKTA Ready from GE Healthcare. The equipment was delivered, installed, and qualified by the middle of 2017, and the suite was available for use in November of that year.

ABL Europe's state-of-the-art viral vaccine facility is fully compliant with EU good manufacturing processes (GMPs), can produce up to 20 batches of drug substance each year, and can be expanded further by adding a 500-L single-use bioreactor. It allows closed system processing and has a disposable production line. It will support projects that require batches for preclinical studies, clinical phases 1 to 3, and validation.

The need to intensify viral vector production was discussed during the workshop. Felipe Tapia (Max Planck Institute, Magdeburg, Germany) spoke on intensified and continuous vaccine manufacturing processes as part of the scientific activities carried out in the bioprocess engineering group of Prof. Dr.-Ing. Udo Reichl. He believes that the gene therapy industry needs process intensification techniques to realize its full potential regarding the high-dose input required. To achieve very high titers and sufficient vector quantities is labor intensive and demands very careful process optimization for both clinical phase 3 trials and commercial production.

Tapia explained that manufacturers of viral vaccines should consider whether to produce in batch, semicontinuous, or continuous modes. Companies that seek to move away from conventional batch production need to ensure that the cell-specific virus yield, virus quality, and stability are maintained during process intensification.

The upstream processing team at the Max Planck Institute, led by Dr. Yvonne Genzel, have used perfusion to increase the concentration of cells in the bioreactor before infection. An interesting finding was that the filter retention devices with larger membrane cutoffs allowed more virus to be retained within the bioreactor. This possibly could be due to a fouling phenomenon of the hollow-fiber membranes. The team showed that an ATF system could be used as a retention device to intensify cell culture during production of influenza A virus. With this set-up, the increase in cell concentration did not reduce the cell-specific virus productivity and therefore led to a 14-fold increase in titer, a twofold increase in volumetric productivity, and a twofold increase in cell-specific virus productivity.

Tapia and his colleagues also experimented with multistage bioreactors for continuous production of influenza viruses. One stirred-tank bioreactor is used for cell growth while another one or two stirred tank bioreactors are used for virus infection and production. Each bioreactor is perfused with fresh medium, and the virus product is collected continuously. The team found that although this could be an efficient production system for genetically stable viruses, the titer could oscillate over extended times if defective interfering particles accumulate. Furthermore, the increase in virus passage number could lead to unwanted viral mutations.

To address these issues, the team developed a plug-flow bioreactor to provide a constant supply of viruses with a defined passage number and with no oscillation in titer. A stirred-tank bioreactor, operated as a chemostat, continuously provides cells. The system infects those cells and injects air bubbles into the medium before the cells enter the plug-flow reactor. The air bubbles provide enough oxygen for the subsequent virus replication phase. Engineers from the Max Planck Institute have used this technique with suspension MDCK cells, which take around 20 hours to pass through the 100-m long reactor. Flow is laminar in this first reactor prototype, which has an internal diameter of 1.5 mm and a flow rate of 12 mL/h. The team expects that virus manufacturers could use a 50-L stirred-tank bioreactor to feed the plug-flow bioreactor with cells at a rate of 1.2 L/h, allowing 1,000 L of harvest to be produced over a 35-day period.

SSB has invested in a range of new technologies to support the biopharmaceutical industry in its attempts to intensify bioprocesses. Many of these technologies are applicable to the viral vector sector. They range from development tools such as the ambr 15 with new centrifuge inserts that allow it to be operated as a perfusion mimic and the ambr 250 high throughput perfusion (Photo 2) that enables development of perfusion processes with up to 24 bioreactors operated in parallel with fully automated liquid handling. Gerben Zijlstra (Continuous BioManufacturing Platform Marketing Manager, SSB), described the company's process-scale technologies including rocking motion bioreactors with integral cell separation filters for $n - 1$ perfusion and the kSep centrifuge for the harvest of high-cell-density cultures. He explained that customers have used rapidly cycled membrane adsorbers to achieve high productivities in a simple and efficient format.

Amélie Boulais (Vaccine Platform Marketing Manager, SSB), believes that the scalability of the portfolio is critical. "Many companies that are in the early stages of developing viral vectors are not thinking about how their processes will scale-up and the process economics of the final process. What works at the preclinical stage may not be suitable for producing sufficient product for phase 3 clinical trials and a subsequent product launch. Sartorius has a range of innovative upstream and downstream processes that are scalable but also can accelerate development of new viral vectors. Crucially, we support customers with a range of development, process modeling, validation, and engineering services that will help customers with viral vectors commercialize their products successfully and that ensure innovative and life-changing medicines are delivered to the patients that need them so urgently."

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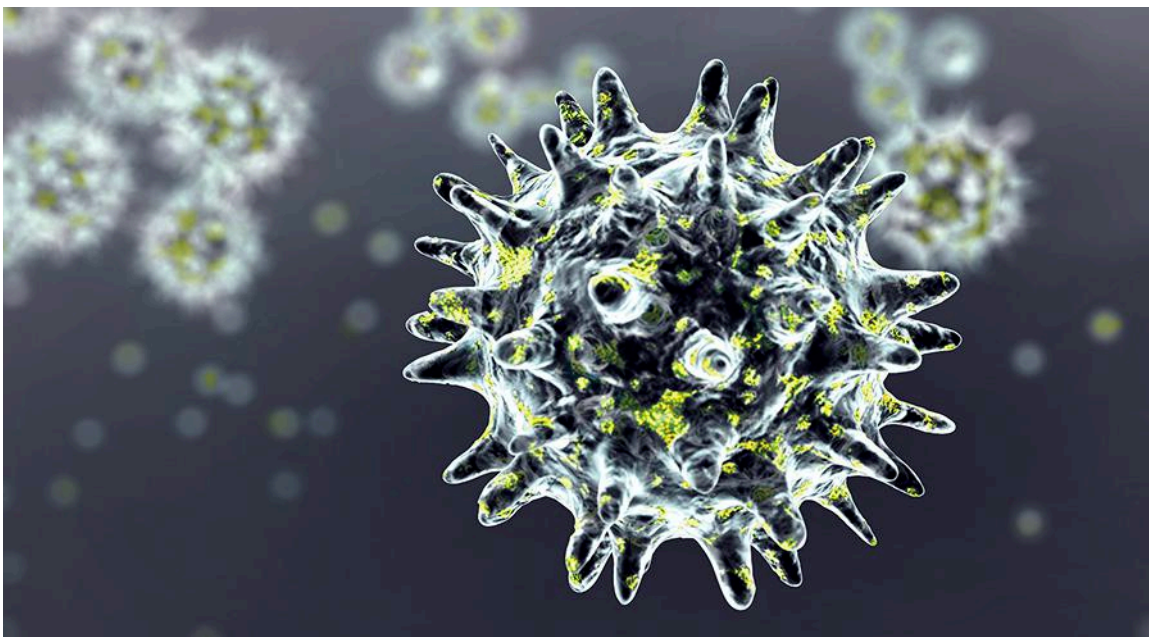
Single-Use Platforms Accelerate Viral Vaccine Development and Manufacturing

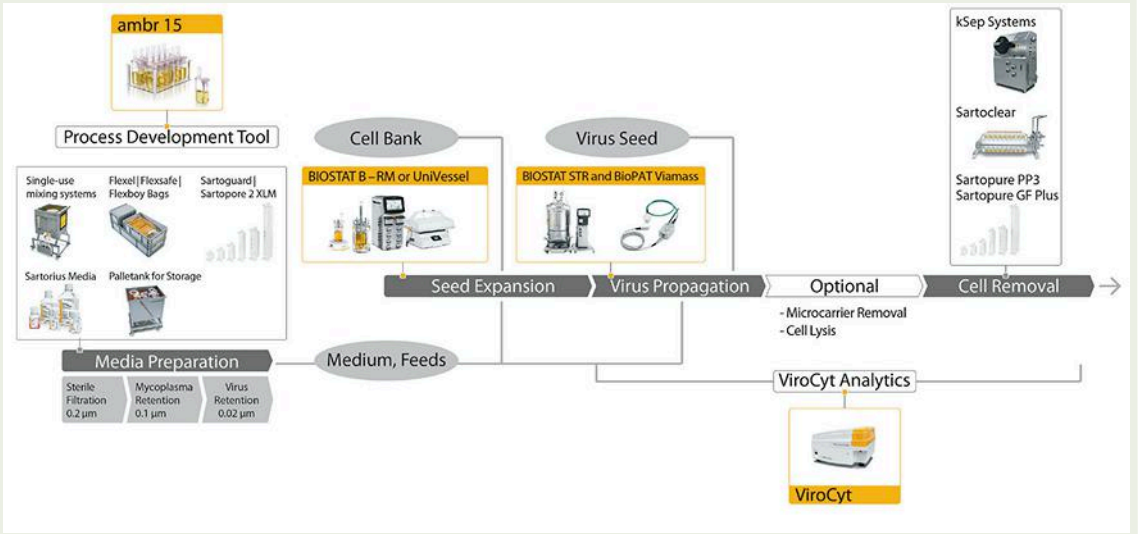
Amélie Boulais



Analyses of the biopharmaceutical industry predict that the market for vaccines could grow at a compound annual growth rate of as much as 10.3% between 2013 and 2024 (**1**). This is faster than growth predicted for recombinant proteins and monoclonal antibodies. Although traditional types of vaccines such as conjugated, inactivated, live attenuated, and toxoid vaccines dominate the market today, new generations of vaccines such as recombinant vector vaccines and subunit vaccine candidates dominate preclinical and clinical development phases. This implies that manufacturers are starting to commercialize a new generation of vaccines based on recombinant technology. Sartorius Stedim Biotech (SSB) is investing resources in understanding the future manufacturing needs of the vaccine industry. We are exploring the best ways of implementing single-use platforms for next-generation vaccines that avoid reinventing the wheel for each candidate, thereby reducing time to market, lowering production costs, lowering risks, and increasing flexibility.

In our experience, every vaccine's process is distinct in one way or another. A vaccine product will have unique characteristics. The cell lines used in manufacturing will introduce their own nuances, and production processes will face individual safety considerations. For this reason, multiple platforms are needed to cover the different vaccine modalities. SSB has begun by

Figure 1: Upstream processing platform for viral vectors



All the vessels in the bioreactor range are geometrically similar to one another to ensure CONSISTENT mixing and gassing strategies during scale-up.

developing a platform for viral vector applications. The platform's predefined technologies can be integrated with one another to allow development and manufacturing of our customers' vaccines. These technologies are complemented by unique services to support the vaccine company's product across its entire life cycle.

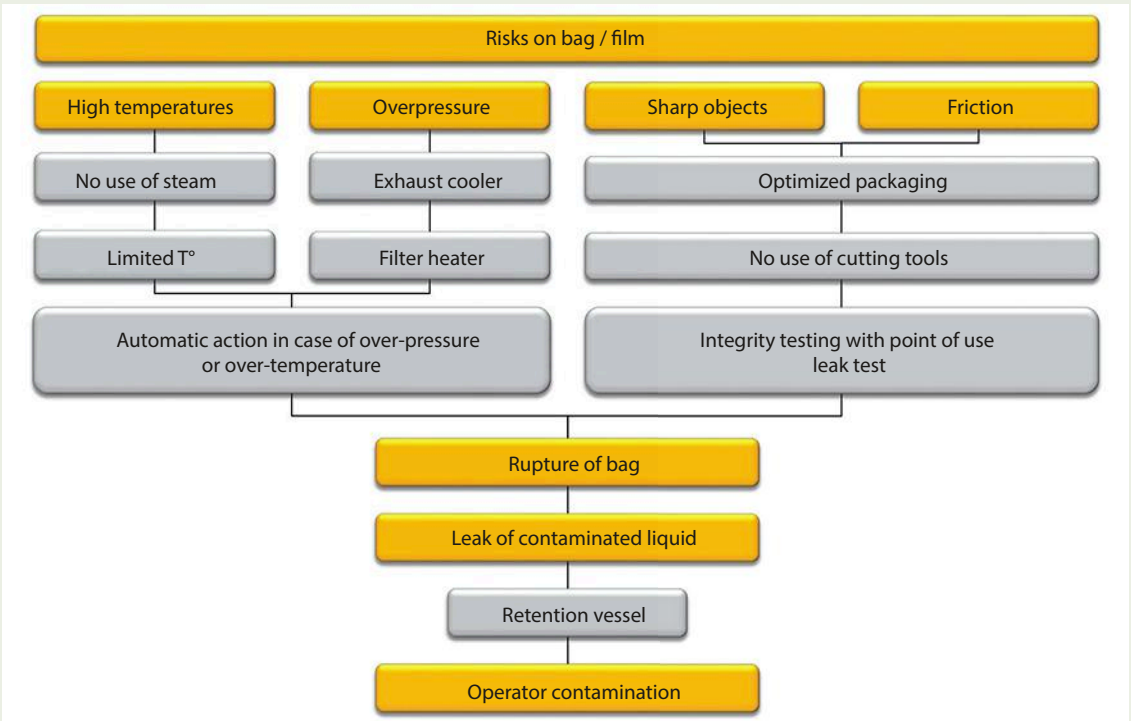
An Upstream Toolbox for Vaccine Processing

Figure 1 shows the upstream processing technologies that viral vector manufacturers can use within their processes. The life-cycle approach is evident if we consider SSB's portfolio of single-use bioreactors, which are readily scalable from the 250-mL ambr 250 high-throughput bioreactor through to the 2,000-L BIOSTAT STR 2000 product. All of the vessels in the bioreactor range are geometrically similar to one another to ensure consistent mixing and gassing strategies during scale-up. This allows customers to simplify scale-up and scale-down studies, easily switch between conventional and single-use bioreactors, and reduce risk during process transfers.

The ambr 250 high-throughput and modular systems are fully automated and allow large design-of-experiments (DoE) studies to be run quickly and easily during process development and optimization activities. To address development of adherent cell culture processes, SSB has developed a new generation of ambr vessels to support the growth of cells on microcarriers. SSB has recently published work describing the physical, computational fluid dynamics, and biological analysis of a microcarrier process run in the ambr 250 high-throughput bioreactor (**2**). Vero cells were grown on microcarriers in an ambr 250 vessel with a modified design. During the next phase of development work, engineers will verify the new vessel design by culturing human mesenchymal stem cells (hMSCs) and will try to demonstrate the scalability of microcarrier cultures from the ambr 250 high-throughput through to SSB's large-scale single-use bioreactors.

Studies already performed with mammalian cells in suspension show that cell viability, cell concentration, and product concentration are highly reproducible from the ambr 250 modular unit to the 5-L BIOSTAT B, the 50-L BIOSTAT, and finally to the 1,000-L BIOSTAT bioreactors. Two vaccine manufacturer customers have verified the scalability of the SSB single-use bioreactor portfolio. Zoetis reported the

Figure 2: BIOSTAT STR and Flexsafe STR single-use bags provide safety by design. (Modified after Chaubard et al., *BioPharm International*, 2 November 2010)



successful scale-up of its upstream viral vaccine process from 2 L to the BIOSTAT STR 50 and then to the BIOSTAT STR 200 vessels. The company cultured BHK21 cells on microcarriers and found that the cell concentrations and virus titers were consistent between scales. Similarly, GSK described the successful scale-up of its BSL2+ culture from 10-L glass bioreactors to the single-use BIOSTAT STR 200 and then the BIOSTAT STR 1000 vessels. The single-use bioreactors gave equivalent performance to the 10-L glass bioreactor with regard to cell density and antigen titer.

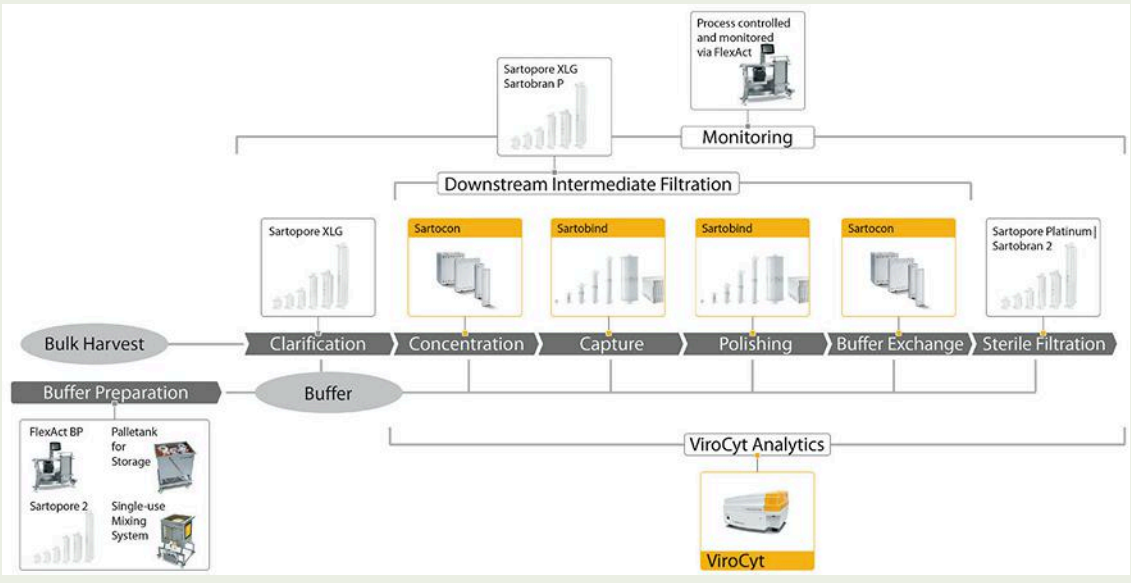
Vaccine manufacturers often process live pathogens and, therefore, take safety considerations very seriously. By adding unique design features, SSB focuses heavily on ensuring that its bioreactors provide a high level of containment. It has adopted a systematic approach to preventing leaks from bioreactors by adapting a risk assessment that a vaccine manufacturing organization has published previously (**3**). Figure 2 summarizes the modified risk assessment (**4**).

BIOSTAT STR bioreactors operate with bags constructed from the robust, multilayer Flexsafe film. SSB delivers the consumable portion in protective cleanroom-compliant packaging. Manufacturing operators can use a specifically designed installation device to place the 2,000-L bag into the holder. The bags are fitted with noninvasive sensors and with back-up lines for gas inlets and outlets. BIOSTAT STR bioreactors feature a containment tray that manufacturers can connect to a kill tank. This ensures that if a leak occurs, the contaminated material can be treated safely. The bioreactor automation includes numerous safety interlocks and shutdown mechanisms designed to protect the bags from excessive pressures and temperatures.

SSB has developed the Sartocheck bag tester for qualified pressure INTEGRITY TESTING of bioreactor bags.

SSB has developed the Sartocheck bag tester for qualified pressure integrity testing of

Figure 3: Downstream processing platform for viral vectors



Design engineers have taken care to maintain the flow path principles throughout the range, ENSURING that the membrane adsorbers are suitable for use at any stage in the lifecycle of a vaccine product.

bioreactor bags. It detects leaks that may have been introduced before installation. The integrity test takes 24–60 minutes, including inflation and deflation, and tests the consumable up to the first clamp on the tubing. A fleece on the bag holder prevents defects from being masked by the bag holder itself.

We also have designed a single-use exhaust cooler to prevent filter blockages at high airflow rates. A blockage on the exhaust filter could lead to a pressure build-up that could damage the consumable and lead to a containment loss. Condensate is returned to the bioreactor bag to prevent product losses.

A Downstream Toolbox for Vaccine Processing

Figure 3 shows downstream processing technologies that viral vector manufacturers can use within their processes. SSB recommends that viral vector manufacturers use a membrane adsorber technology during viral vector purification. Membrane adsorbers have significantly larger pore sizes than conventional bead chromatography resins. This gives them more than tenfold greater binding capacity than columns for viral vaccines, virus-like particles, and large proteins. Vaccine producers also can operate membrane adsorbers at 10-fold higher flowrates. SSB has developed a unique cassette format to allow large-scale processing and provide higher flexibility. It will enable manufacturers of viral vectors to implement the technology during commercial production without encountering the scale limitation of 5-L membrane volumes — the largest size available in a classical capsule format. SSB design engineers have taken care to maintain the flow path principles throughout the range, ensuring that the membrane adsorbers are suitable for use at any stage in the lifecycle of a vaccine product.

Demonstrating the Platform Approach in Downstream Processing

SSB collaborated with IBET (Lisbon, Portugal) to develop a complete single-use downstream process for production of adenovirus (Ad5) with commercially available technologies (**4**). The unit operations used standard SSB technologies from the vaccine platform. The Ad5 vector was produced in HEK293 cells. Initial experiments were performed at the 2-L scale, and then the process transferred to the 20-L scale. Adenovirus

Vaccines are too DIVERSE a product category for a single platform approach to address all of the challenges.

was captured during downstream processing using a Sartobind Q anion-exchange membrane adsorber. Polishing was performed using the Sartobind STIC membrane adsorber. This is a salt-tolerant anion-exchange membrane, which was demonstrated to be highly effective for removing residual DNA impurities. The process delivered a product with a final host-cell protein concentration of less than 20 µg/mL, less than 10 ng/dose of host cell DNA, less than 5 ng/mL of benzonase, and a yield of 55%. The platform was operated "out-of-the-box" with little development work, and IBET scientists believe that the yield could be increased to 65% easily.

The case study shows how manufacturers can use the SSB viral vaccine toolbox to implement a completely single-use and scalable process for adenovirus purification. The use of membrane adsorbers allows for faster, simpler, and more economical processing. The large-scale run achieved recovery yields that were comparable with those reported in the literature and met product specifications and guidelines for preclinical and phase 1 studies.

New Toolboxes for Vaccine Development

The vaccine industry requires new tools to help bring its pipeline of new products to the market quickly, safely, and economically. Vaccines are too diverse a product category for a single platform approach to address all of the challenges. Instead, SSB has initially assembled a toolbox of technologies that are suitable for recombinant viral vector production and is gathering an increasingly large set of data to demonstrate the effectiveness of those technologies in viral vector processing applications. This toolbox includes consumables, systems, analytics, and automation. Further toolboxes will be developed to meet the needs of the vaccine industry. The company is commercializing breakthrough single-use technologies to meet the specific needs of vaccine producers. SSB vaccine platforms include associated services such as process development consultants, analytical testing, validation services, and application specialists that will accelerate product development and allow new vaccine concepts to become a reality.

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GMP Manufacturing Facility for Viral Vector Production at ABL Europe

New Production Capacity for Viral Vectors

Amélie Boulais and Nick Hutchinson

A new production facility for the GMP manufacture of viral vectors from nonadherent cell cultures is opening in Strasbourg, France. This will alleviate growing concerns that a lack of suitable manufacturing capacity is restricting the speed with which this nascent industry is able to bring viral vector products from the laboratory to the patient.

Fully Integrated Service Offering

Patrick Mahieux, General Manager of ABL (Advanced Bioscience Laboratories) Europe, the contract manufacturing organization responsible for the site expansion, said that "The introduction of nonadherent cell-culture capacity complements our existing adherent cell-culture viral-vector production technologies. It is the only pure-play, dedicated viral vector production site capable of manufacturing drug substance and drug product materials for toxicological studies, all clinical phases, and even commercial launch. Everything from process development, manufacturing, and QC release testing is performed under one roof."

This "all under one roof" concept is key to Mahieux's vision. Clients face considerable costs and avoidable risks when having to transfer processes between CMOs and production facilities during clinical development. "Many of our customers are at a very early stage with little more than a concept for a product. We can support them throughout their product's life cycle with process development, clinical batch production, and then through process validation for commercial launch — all from within the Strasbourg facility. I also firmly believe that the development of processes and analytical testing must go hand-in-hand. Other manufacturers outsource QC testing or perform it at different locations within their group, but that gets more complicated and risky. Lead times increase as a consequence," Mahieux said. He believes that CEOs of companies developing viral vectors are coming under increasing pressure to bring their products to market since Amgen's successful registration of the T-VEC oncolytic viral vector product showed that such products could receive regulatory approval. This is increasing demand for a long-term production strategy.

Crucially, the French regulatory authorities (ANSM) have inspected ABL's facility, and it is licensed in accordance with European Medicines Agency GMP (good manufacturing practice) regulations. Many US contract manufacturers producing early stage clinical trial materials are not GMP certified because it is not an FDA requirement until product approval. However, producing clinical lots from ABL's Strasbourg site will assure current GMP (cGMP) compliance throughout all phases of development, allowing customers to perform worldwide clinical trials.

The new capacity will help the company manufacture viral vectors for a wide range of applications such as oncolytic, vaccines, and gene therapy products. Even established multinational pharmaceutical companies often do not have the capabilities and/or expertise to manufacture viral vectors that are large (>0.2 µm) or replicating or both, especially at industrial-scale batch sizes. ABL Europe has the virology know-how and quality systems management that allows it to perform aseptic production of drug substance and drug product, developing the necessary analytical tests and preparation of the required documentation for the health authorities that justify the adopted approach. Although the company believes that most clients' products will require only Biological Safety Levels 1 and 2, they have designed the facility to handle products requiring Biological Safety Level 3 containment.

Mahieux considers that the biggest demand will come from those companies developing oncolytic and immunotherapy virus products for cancer treatments. "Customers expect us to be flexible and agile. Their top priority is reaching the clinic as quickly as possible. We have created a facility that will allow them to meet this objective. Our new state-of-the-art viral vector productions suite contains a single-use, fully disposable production line and can produce 15 to 20 batches per year," he said.

Single-Use Platforms Bring Flexibility

ABL Europe considered single-use technologies to be the obvious choice when designing the facility. "Customers don't want to pay for cleaning validation. It adds no value. We could never have gotten the new facility up and running in 11 months with stainless steel equipment. We never even prepared a business case for a stainless steel plant," said Mahieux.

The new facility has separate suites for cell production, virus propagation, and downstream processing. The company has installed single-use bioreactors and cell cultivation technologies from Sartorius Stedim Biotech (SSB), including a BIOSTAT STR 200 bioreactor. It has equipped this 200-L bioreactor with an alternating tangential-flow filtration system from Repligen to allow upstream process intensification and even greater productivity. The suite has space and utilities in place for rapid installation of a 500-L single-use bioreactor. The downstream processing of viral vectors will be performed using a combination of SSB's FlexAct 2.0 platform with associated mixing systems and GE's ÄKTA chromatography systems.

Anthony Da Silva, Process Development Specialist, explained. "We took the decision to work with a very limited number of suppliers, with Sartorius providing a large proportion of the consumables. This simplified our supply chain and allowed us to accelerate construction of the suites. Using a supplier's entire platform for viral vector production allowed us to integrate the single-use automation into our MES platform easily. The Sartorius downstream processing systems were

selected because of their flexibility and their ability to handle a wide variety of process volumes with a small footprint."

Mahieux said, "Sartorius delivered the project exactly on-time and according to our expectations. It all ran very smoothly, and we received good support during qualification. There were a small number of minor nonconformances, but those were rectified very quickly."

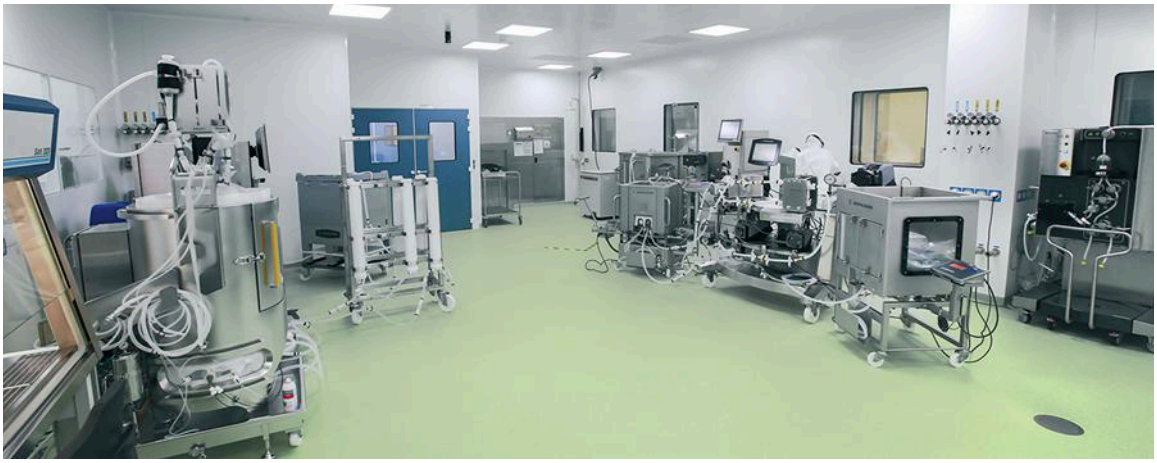
Installing the new capacity within the existing building has allowed ABL Europe to bring it online very quickly. The company commissioned the €3 million investment in the fourth quarter of 2016, and the facility was qualified and GMP-ready in Q4 2017. Amélie Boulais Raveneau, Vaccine Platform Marketing Manager at SSB, said of the project, "We're very proud to have been selected by ABL Europe to supply upstream and downstream processing technologies. It is a significant endorsement of SSB's platform for viral vector processing and the single-use design capabilities of the Sartorius Integrated Solutions team. Our process development experts can teach clients how to make the best use of our technologies to overcome challenges in viral vector applications."

"Producing viral vectors in nonadherent cell culture allows for greater scalability and the ability to produce economically at the largest scales, globally," said Da Silva. "Certain viruses, such as poxviruses, are easier to produce in adherent cell culture; however, there are benefits to propagating viruses in suspension cell cultures. The production capacity of 60 m^2 of adherent cell culture capacity is approximately equivalent to that of a 100-L bioreactor. There are companies producing viral vectors in 2,000-L single-use bioreactors," he continued.

Empowered Employees with Unique Expertise

Mahieux has been keen to instill lean principles into the organization, which has become highly focussed on continually improving operations to deliver greater value to its customers with less waste. "Empowered employees underpin ABL Europe's lean enterprise, and lean green belt training is being rolled out across the organization. We try to maintain a very flat structure at ABL Europe. The business has a hierarchy with only three layers. In future, as the company expands, we may need more, but even then five layers should be enough for a large but lean organization."

"We are fortunate to have access to a great pool of talented people," said Valenteen Sterling, Human Resources Manager. "We are located in the Alsace BioValley, a hotbed for European



pharma and biotech, yet our location is very accessible and only two hours away from Paris. We are located right next door to both the Faculty of Pharmacy of Strasbourg University and the École Supérieure de Biotechnologie de Strasbourg, one of the leading schools for biotechnology teaching and research in France. Getting and motivating the people with the right skills is critical to our operations. It is our people's know-how that is the real value of the company," she continued.

The company also has recognized the importance of breaking down many of the "silos" that often exist across organizations. Project owners take hands-on responsibility for successful development, transfer, and operation within the GMP environment of customer processes. "It cuts out a lot of the waste that can occur during process transfer activities," explained Mahieux. "Engineers that are developing processes have first-hand experience of what works at the production scale and can feed this back into development activities. It is an excellent opportunity for our staff to develop and hone new skills. They get to experience all aspects of viral vector bioprocessing," he said.

Mahieux already is seeing strong demand from customers for the company's services. He is focussed on executing projects for its existing clients while finding new clients for the site's available capacity. "We believe the service offering we have put together here is very compelling. We have a state-of-the-art single-use facility and a dedicated team of fantastic people. I'm sure the model is going to be very successful. In the future I expect we will need to expand by buying new sites to operate according to the same principles."

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Biosafety Considerations for Single-Use Bioreactors

Davy De Wilde, Joerg Weyand, Ute Husemann, Bernward Husemann, and Gerhard Greller

Single-use bioreactors are widely accepted in the pharmaceutical industry and are increasingly being used to perform mammalian cell cultures in commercial manufacturing applications. They address some key challenges the industry faces by decreasing time-to-market, reducing validation efforts, increasing flexibility, reducing investment costs, and optimizing cost of goods.

Vaccine manufactures require a high level of flexibility from a bioreactor platform because of the large variety of cell lines they use and the number of different vaccines they produce within one facility. The flexibility offered by single-use bioreactors means that they are becoming the preferred tool for cell culture in the vaccine industry. Vaccine manufacturers must consider various design aspects when selecting the proper single-use bioreactor technology. Single-use bioreactors have a much lower degree of automation compared with stainless steel solutions. Operators make a large number of manual connections instead of working with transfer lines and automated valves. The transport of the single-use bag into the cleanroom and installation in the bag holder also require a high degree of manual interaction. If not performed properly, those activities may result in damage to the bioreactor or incorrect connections that lead to a break in the sterile barrier. Ensuring bag integrity is of critical importance during cell cultivation. A production batch must be discarded if the sterile barrier is broken, resulting in financial loss, a negative influence on production planning, and even product shortages in the market.

Critically, when infectious viruses are present within the bioreactor, a break in the sterile barrier poses a biosafety risk to an operator and the environment. Such processes are typically classified at biosafety risk level 2 and 3. From a biosafety perspective, the cell culture vessel is the first barrier protecting the environment against pathogenic organisms, whereas the room in which the system is installed is described as the secondary containment. An elevated risk is associated with failures of the first barrier when using single-use bioreactors because the materials of construction are inherently more fragile than stainless steel.

Manufacturers should challenge single-use bioreactor suppliers on how they mitigate potential risks of bag rupture during transportation, storage, installation, and use. Furthermore, they should understand how ruptures can be detected and how proper containment can be ensured in the event of a bag failure.

Biosafety Risk Assessment of BIOSTAT STR and Flexsafe STR Technology

Sartorius Stedim Biotech (SSB) focused heavily on robustness and risk mitigation measures during the development of the single-use, stirred bioreactor BIOSTAT STR and Flexsafe STR bags. The sources of bag ruptures can have a number of root causes. Appropriate safety measures were put in place to mitigate the risk of a rupture depending on the specific source of risk. During product development, four critical questions were considered when evaluating the risk of bag ruptures:

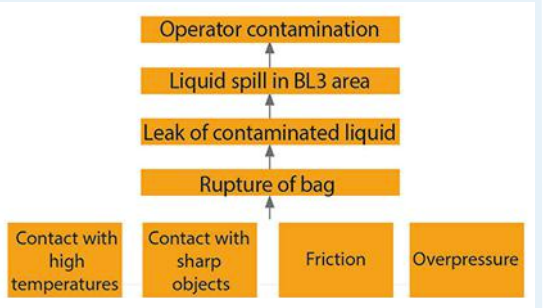
- What could cause damage to the single-use bioreactor?
- How could the risk of damage be mitigated?
- How could potential damage be detected?
- How can the hazardous liquid be contained in the event a rupture occurs?

FMEA Methodology: A Failure Mode and Effect Analysis (FMEA) methodology allowed a thorough evaluation of risks and areas on which to focus. The criticality of a risk was assessed by

Table 1: Temperature safety shut-off levels for the exhaust filter heater system of the BIOSTAT STR

	Temperature Limit of Control Loop (°C)	First Safety Shutoff (°C)	Second Safety Shutoff (°C)
BIOSTAT STR 50	50	58	94
BIOSTAT STR 200	50	58	94
BIOSTAT STR 500	50	58	94
BIOSTAT STR 1000	50	58	94
BIOSTAT STR 2000	65	85	94

Figure 1: Theoretical risk factors for bag rupture (Source: modified graph based on Chaubard et al., *BioPharm Int.*, 2 November 2010)



multiplying four factors that had to be quantified:

I: Impact (1–3)
O: Occurrence (1–3)
D: Detectability (1–3)
A: Action response time (1–3)

Products can be classified according to the quantified risk as follows:

- Low risk: 1–3
- Medium risk: 4–6
- High risk: 7 and above

Risk Analysis BIOSTAT STR with Single-Use Flexsafe STR Bags: Figure 1 describes sources of potential bag damage. Proper protection of the plastic material is required against high temperatures, cutting objects, friction and overpressure.

Biosafety Risk Mitigation Approach with BIOSTAT STR and Flexsafe STR Technology: Preventing Bag Ruptures

Temperature Protection: Two heat sources must be considered when a single-use bioreactor is operated: the filter heater used for filter protection against filter blockage and the temperature control system used to heat up the bioreactor through the double wall bag holder. The filter heater is in direct contact with the exhaust filter whereas the heated bag holder is in direct contact with the Flexsafe film. Safety measures are required based on the temperature resistance of the filter and Flexsafe film.

Exhaust Filter: The filter heater is installed around the exhaust filter to protect against filter blockage due to excessive humidity. Standard Sartofluor filters are used with the Flexsafe STR that withstand sterilization cycles of up to 134 °C for 30 minutes. However, resistance to long-term heat exposure at temperatures above 65 °C has yet to be demonstrated. Therefore, to protect against overheating, a safety shut down mechanism should be installed that is activated at the appropriate temperature. In the BIOSTAT STR technology, a three-staged safety mechanism is in place, ensuring that the filter heater control loop is limited to a defined maximum temperature. Table 1 shows the temperature shut-off levels. An automatic and reversible filter heater shut-off is activated at the first safety shut-off temperature limit. An independent and nonreversible shut-off at the second safety temperature limit occurs if the first limit is breached. The final mechanical shut off ensures safe use of the filter heater even in the event of a malfunction of the first two safety levels.

Flexsafe Film: During development of the Flexsafe STR bags, various tests were performed to evaluate both short-term and long-term stability against temperatures that can occur during cell culture processes. The long-term stability was evaluated to ensure safe usage of the Flexsafe STR bags under normal cell culture conditions. Testing confirmed that the Flexsafe STR bags can be used safely for a minimum of 21 days at a temperature range between 16–40 °C.

The short-term stability was determined to ensure that the single-use bag could withstand higher temperatures for a limited time during the initial heating phase of the medium in the bioreactor. Tests have been performed to help define the temperature safety shutdown limits in case of malfunction of the temperature control system. These tests confirmed the resistance of the film material to temperatures of 2–43 °C for 24 hours. Further tests confirmed the film is resistant to short-term temperature peaks of up to 60 °C for two hours.

Table 2: Burst test values of Flexsafe STR bags, measured in the bag holder when filled with water to the maximum filling volume

	Average Pressure at Burst [mbar]
BIOSTAT STR 50	830–859
BIOSTAT STR 200	371–388
BIOSTAT STR 500	191–198
BIOSTAT STR 1000	159
BIOSTAT STR 2000	ND

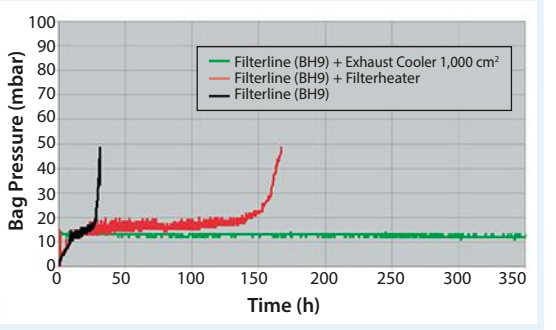
During the heating phase, a temperature overshoot beyond the set point has been limited to ±1 °C inside of the bag. The jacket, however, is allowed to reach higher temperatures, below the determined limits that the film can withstand, for a defined period of time.

Temperature safety measures have been established to ensure immediate correction and stabilization of permissive temperatures in case of unexpected overheating. The critical temperature based on these tests is 60 °C across the film material. However, in the event of overheating of the double wall, there is a time lapse to be considered because it takes some time for the temperature to transfer from the temperature circulation system through the stainless steel of the bag holder to the film material of the bag. Safety measures have been designed in such a way that they automatically switch off the heating elements of the electrical heater. The temperature linked to the activation of these safety interlocks is measured directly in the jacket loop by a standard pT100 sensor. When a temperature of 60 °C is reached, a first shut-off is initiated. In case of malfunctioning of the first shut-off, an alarm signal is generated by a mechanical switch inside the heating element at a temperature of 70 °C (± 5 °C). A second, independent safety temperature limiter measures the temperature inside the electrical heater and turns the heater off if 90 °C (± 8 °C) is reached. This mechanical limiter can be reset only manually.

These multistage cascaded safety measures offer full protection against overheating, not only in case a temperature control failure occurs, but also in case the first safety measure fails. This back-up safety measure has been established to offer maximum reliability and risk mitigation.

Overpressure: The three main reasons a pressure increase may occur are linked to gas flow, specific feeding strategies, or filter blockage. Both short-term and long-term

Figure 2: Pressure increase evolution in case of filter blockage caused during a BIOSTAT STR 200 run with deionized water at 37 °C; the red line shows the exponential increase over time when using the filter with filter heater; the green line shows the pressure evolution using an exhaust cooler.



stability tests were performed to evaluate the robustness of the Flexsafe film against high pressures. The long-term film stability was evaluated to ensure safe usage of the Flexsafe STR bag during normal cell culture conditions. The short-term resistance defines the worst-case scenario in the event of an abnormal overpressure event.

Long-term Pressure Resistance: Worst-case robustness trials were performed during the qualification of the Flexsafe STR bags. Bags of all sizes were tested in a standard STR bag holder under the following test conditions:

- Maximum working volume
- Maximum stirrer speed
- Temperature: 40 °C
- 30 mbar pressure
- Duration: 21 days

All Flexsafe STR bags passed these tests successfully. At the end of the robustness test, the pressure was increased in 5 mbar steps to 50 mbar. The pressure was maintained for one hour at each 5 mbar increment. The results are documented in the Flexsafe STR Validation Guide and are available on request. These robustness trials confirm that Flexsafe STR bags can be used safely at operating pressures below 30 mbar.

Short-term Pressure Resistance: Safety measures are required to avoid abnormal pressure increases beyond critical values during cell culture processes. Exhaust filter blockages often cause such pressure increases. In such an event, the pressure will increase rapidly and exponentially as shown in Figure 2. Therefore, short-term pressure resistance tests are sufficient to define critical pressure values. To define the

Figure 3: Pressure resistance test in Flexsafe STR 2,000-L bags

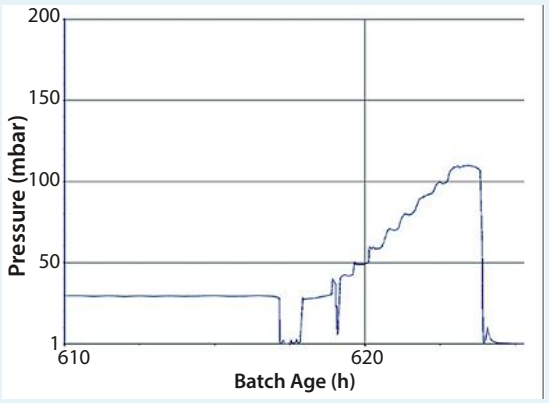
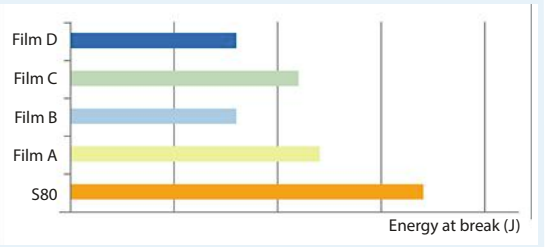


Figure 4: Energy-at-break data for Flexsafe film (S80) compared with other PE films. (Vachette et al. Robust and Convenient Single-Use Processing. *BioProcess Int.* 12(8)s 2014: 38–42.)



short-term pressure resistance of the film, further tests were performed on selected bags from the successfully completed, worst-case robustness tests described above. After the 21-day robustness test, the pressure was increased gradually to 110 mbar and maintained at 110 mbar for 1 hour. Tests performed on the Flexsafe STR 2,000-L bag can be considered worst-case conditions because pressure resistance increases as bag sizes decrease. Figure 3 describes the test for a Flexsafe STR 2,000-L bag. The test is successful because the bag does not leak or burst. Similar tests have been performed on Flexsafe STR bags, and the results are shown in Table 2. To ensure operator safety, no burst test was performed on a Flexsafe STR 2,000-L bag. The tests show the pressure resistance of the bags up to 110 mbar, which is above that at which the last safety measure is activated (70 mbar).

The Flexsafe film shows superior performances in comparison to other PE films (Figure 4). Bag bursting pressure are far above the pressure at which safety systems are activated. Also, S80 film robustness has been compared with other PE films and demonstrate superior mechanical characteristics. Results are shown in Figure 4. Based on this data it was decided to implement a three-stage cascaded pressure safety approach. An electrical safety switch will shut down gassing when a pressure of 50 mbar is reached inside the bag. Gassing is automatically reactivated in case the pressure returns below 30 mbar. This reactivation will avoid cells becoming short of oxygen in the event of short-term pressure peaks.

The pressure could also increase due to the action of pumps that are adding fresh media or other feeds to the single-use bag. Therefore, in the event that the pressure continues to rise after the first shut-off, a second electrical safety switch is activated at 60 mbar that will deactivate all connected pumps. The pumps will automatically be reactivated once the pressure recovers to below 60 mbar. As a final safety measure, a pressure relief valve is installed in the system, it will open at 70 mbar.

Filter Blockage: Various solutions to mitigate the risk of filter blockage on Flexsafe STR bags are available. The most commonly used solution is the use of a heater around the exhaust filter. The filter heater heats up the condensate in the exhaust gas entering the filter and hence reduces

Table 3: Vessel dimensions of Flexsafe STR bags

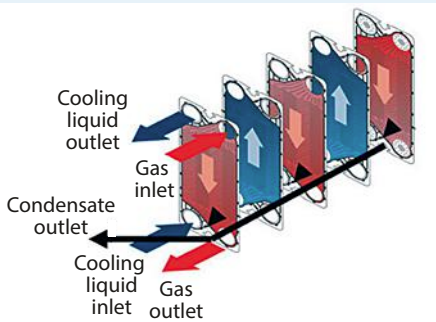
Vessel	STR Flexsafe 50 L	STR Flexsafe 200 L	STR Flexsafe 500 L	STR Flexsafe 1,000 L	Flexsafe STR 2,000 L
Total volume (L)	70	280	700	1,300	2,800
Max. working volume (L)	50	200	500	1,000	2,000
Min. working volume (L)	12.5	50	125	250	500
Vessel diameter (mm)	370	585	815	997	1,295
Vessel height (mm)	666	1,055	1,467	1,800	2,330
Liquid height at max working volume (mm)	470	783	1,005	1,360	1,670

Photo 1: Single-use exhaust cooler

Table 4: Detectable leak sizes for the Flexsafe STR family

Min. detectable leak size @ 20 min test time	50 L	200 L	500 L	1,000 L	2,000 L
Flexsafe STR (S80)	100 µm	200 µm	200 µm	400 µm	600 µm

Figure 5: Design principle, single-use exhaust cooler



the relative humidity. This allows the gas to pass through the filter without causing blockage. The use of such a filter heater has proven successful for the vast majority of cell culture processes. However, a traditional filter heater might not be sufficient for highly demanding processes for which high flow rates are used for a long period of time (e.g., continuous processing). To illustrate this, Figure 2 shows the speed of filter blockage when continuously applying a gas flow of 0.1 vvm in a STR 200-L bag filled with deionized water and heated to 37 °C.

To offer a second option of risk mitigation for more demanding processes, SSB has developed a single-use exhaust cooler (Photo 1), which is installed upstream of the exhaust filter. The single-use exhaust cooler is a plate heat exchanger in which cooling water circulates in the opposite direction to the exhaust gas flow and cools the gas leading to vapor condensation (Figure 5). The condensate is collected in a condensate trap from which it can either be pumped back to the bag chamber or alternatively to a waste bag. Figure 2 describes the enhanced efficiency of the single-use exhaust cooler when protecting the filter against humidity in the exhaust gas at normal to medium flow rates. Gas flow rates of 0.4 vvm were used to fully test the capacity of the device. This is twice as high as the maximum possible flow rate in a BIOSTAT STR unit.Figure 6 shows that even under such extreme conditions, the single-use exhaust cooler offers a unique solution against filter blockage caused by high humidity in the exhaust gas. The protection offered by a traditional filter heater under these conditions is not efficient.

Finally, excessive foaming can cause filter blockages. To mitigate the risk of foam reaching the exhaust filter, the Flexsafe STR bags were designed with a large headspace representing 23–29% of the total bag volume (Table 3). This results in a single-use bag design with one of the largest headspaces of all single-use bioreactors on the market. This provides maximum protection to the filter from foam.

In the event that filter blockage does occur, every Flexsafe STR exhaust line is designed so that a new, second exhaust filter line can be manually connected to the single-use bag. This avoids a premature end to the cell culture. SSB also offers an option where a valve automatically opens a preinstalled, second exhaust line without the need for human intervention. This is triggered at a specific pressure limit in the exhaust line. It further mitigates the risk of bag rupture in the event that no operators are present in the production facility when filter blockage occurs.

We conclude that the sum of these safety measures, in combination with the pressure resistance of the Flexsafe STR film, ensures proper protection against overpressure that could occur, especially during high–cell-density cell culture processes.

Friction and Cutting Objects

Contact of bags with cutting objects should be avoided at all times during manufacturing,

transportation, and handling. The use of sharp objects is not permitted in the manufacturing area of SSB to avoid accidental damage to the bags. Furthermore, SSB has developed a transportation box for the STR bags with a specially designed support frame that not only ensures overall protection during transport and storage, but also ensures that no sharp objects can accidentally damage the bag (Photo 2). During transport, single-use bags are exposed to various vibrations and shocks. This could result in friction where tubing and connectors are in direct contact with the bag chamber, leading to damage over time. The specifically designed packaging frame includes special compartments to hold the different feeding and harvesting lines on the top and bottom. In this way, contact between parts is prevented, and no friction can occur during transport. During unpacking, special markers indicate the exact location where the inner plastic protection can be opened without risk of bag damage (Figure 7). No specific measures are required to protect the bag chamber against friction during use because no moving parts contact the bag chamber, and hence, no bag rupture due to friction can occur.

Finally, to ensure full protection against cutting objects, we recommend users to avoid having sharp objects in close proximity of the bioreactor. This helps prevent accidental damage during bioreactor operation once the bag is installed.

Risk Mitigation Concept in Case of Bag Rupture

Bag Damage Before the Start of the Cell Culture Process: The quality assurance programs of bag manufacturers are designed to ensure leak-free single-use bags upon delivery. After delivery, however, various risk factors still exist that can affect the integrity of a single-use bag. In contrast to a stainless steel bioreactor, a high degree of manual operation is required to transport a single-use bioreactor bag from storage to the cleanroom and prepare the single-use bag for installation and use. Various manual connections must be made to connect feeds to the bioreactor bag. This implies the need for stringent operator training and the implementation of suitable standard operating procedures (SOPs). Additional safety measures may be useful to consider along with clear instructions and regular training.

Figure 6: Pressure increase in time at 0.4 vvm gas flow rate using a BIOSTAT STR 500-L bioreactors filled with deionized water at 37 °C

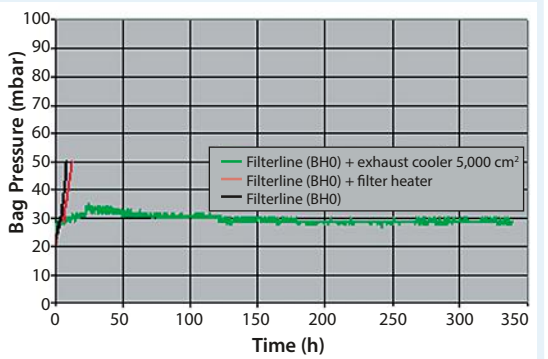


Figure 7: Unpacking instructions with clear indication where it is allowed to cut the protective packaging safely

Photo 2: A protective packaging frame prevents contact of sharp objects with the bag film and tubing during transport. Special compartments at the bottom and top separate tubing and connectors from the bag chamber.

SSB has developed a unique, automated tool that allows the testing of Flexsafe STR bioreactor bags for leaks after installation but before use. This helps detect a damaged bag or bad connection. The pressure-decay test is performed using the

Figure 8: Risk mitigation approach implemented in the BIOSTAT STR and Flexsafe STR technology to allow safe usage in Biosafety Level 2 and 3 environment. (Source: modified graph based on Chaubard et al. *BioPharm International,* 2 November 2010).

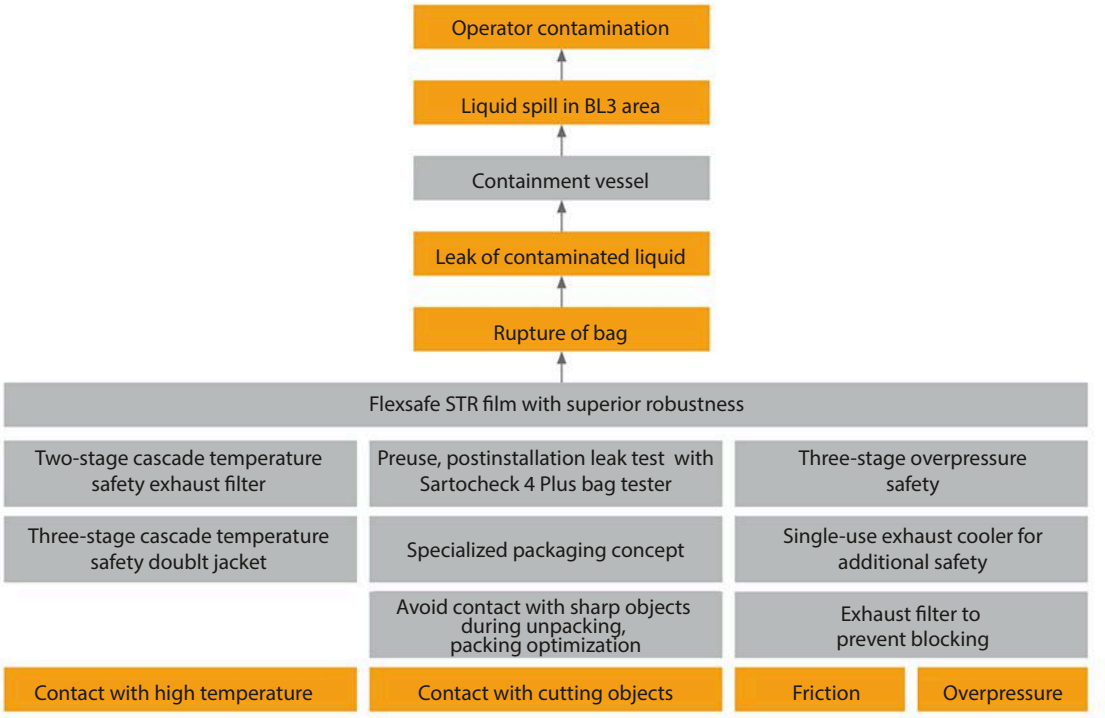


Photo 3: Sartocheck 4 Plus bag tester experimental set-up with the BIOSTAT STR 2000

Sartocheck 4 Plus bag tester after installation of the Flexsafe STR bag in the corresponding bag holder and once all feed lines have been connected (Photo 3). This device can measure a pressure drop over time and enables detection of potential leaks that might have been introduced during storage, unpacking, and installation. This device detects damage until the point at which tubes are clamped. That allows for detection of improper manual connections that could result in a loss of integrity. It helps to detect potential damages accidentally introduced through human failure and offers a unique solution to increase the risk mitigation level when working in biosafety level 2 environments.

A patented fleece is available from SSB that can be installed inside the bag holder during the test. The fleece is made of a porous material that allows gas to pass through even when the fleece is pushed against the stainless steel surface. Hence, masking caused by the bag being pushed against the stainless steel bag holder wall during the pressure decay test is prevented, and damage to the bag can be detected regardless of its location. The minimum detectable leak sizes are shown in Table 4. Further details on this have been published (**1**).

Bag Damage Occurring During the Cell Culture Process: To ensure full risk mitigation and offer a

maximum level of operator safety, a worst-case scenario where bag rupture occurs during the cell culture process must also be considered. In such cases, it is important that the contamination be contained as efficiently as possible with limited or no exposure to operators and the environment. The BIOSTAT STR has been designed so that in the event of a bag rupture, the liquid can leave the surrounding bag holder either only through the bottom opening or through the sensor windows at the side of the bag holder. In such cases, a downward flow of liquid is most likely to occur with liquid leaving the bag holder through the bottom port.

Finally, it is critical that the spilled cell culture liquids do not spread across the cleanroom area but instead are collected and inactivated appropriately. SSB has developed a containment tray that can be placed directly under the bag holder (Photo 4). This containment tray can collect up to 25% of the maximum working volume of the bioreactor. This is usually enough to cover the volume of cell culture broth that could leak out of a single-use bioreactor before detection. Furthermore, the containment tray has been designed such that the spilled liquid is brought into a groove in the tray from where the contaminated culture can be evacuated through a tri-clamp connection to a kill tank. This ensures that no spillage can occur in the clean room area, and collected liquid can be safely removed for further processing.

Photo 4: Containment tray directly installed under a BIOSTAT STR 2000 bag holder

Ensuring Operator Safety

Figure 8 summarizes the most important safety measures established in the Flexsafe STR and BIOSTAT STR technology for safe use of single-use bioreactors in a Biosafety Level 2 or 3 environment. Considering the biosafety risks that are inherent to a vaccine manufacturing process, the proper selection of a single-use bioreactor vendor is critical. Operator safety should be a significant concern is selection of a single-use bioreactor technology for use the vaccine manufacturing. It is extremely important for users to challenge single-use bioreactor vendors on their approaches to biosafety risk mitigation to allow a company to make well-founded decisions regarding its future bioreactor platform.

The different safety measures offer a holistic approach covering the different identified risks when working in a biohazardous environment. Irrespective of whether problems are linked to human interactions, system failures, or process parameters, the BIOSTAT STR and Flexsafe STR technology is designed to mitigate these risks. Moreover, the fact that safety measures are incorporated through a staged and complementary approach provides full protection in a worst-case situation when biohazardous material leaks from the primary containment of the bioreactor. This holistic approach makes the BIOSTAT STR and Flexsafe STR bag technology one of the most secure solutions for the vaccine industry and the preferred solution for many vaccine manufacturers around the world.

Reference

1 Stering M, et al. Pressure Decay Method for Post-Installation Single-Use Bioreactor Bag Testing. *BioProcess Int.* 12 (Supplement 5): 58–61.

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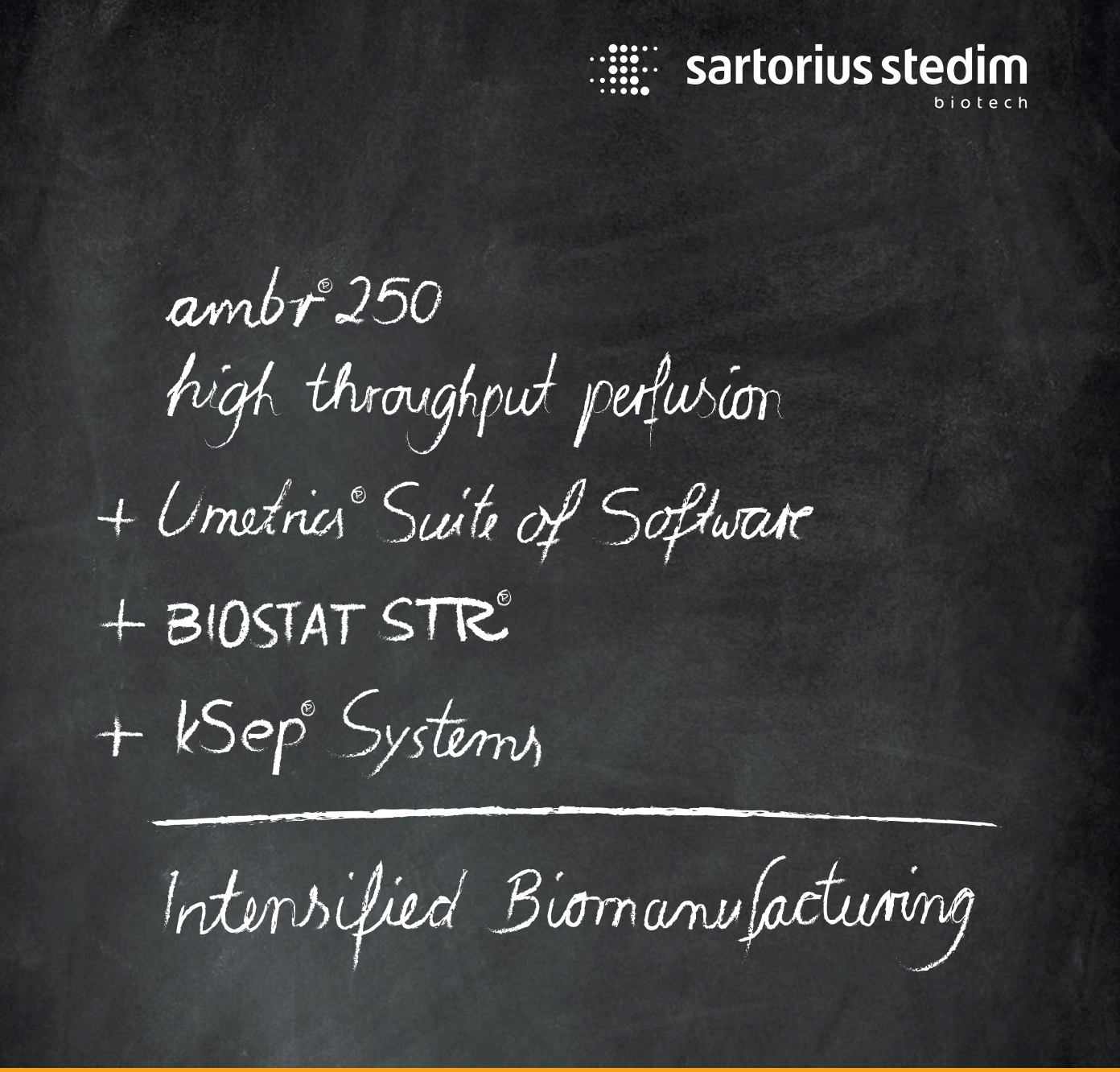

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