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Finding the optimal binding and elution conditions of polyclonal goat IgG. MVS was run on a Nuvia cPrime Cation Exchange Column using ChromLab 6.0 Software on the NGC Chromatography System. Binding buffer was 50 mM MES and elution buffer was 50 mM Tricine. NaCl concentration and pH were varied in both buffers. Good binding and elution were seen in A, binding buffer at pH 5.3 with 30 mM NaCl, elution buffer at pH 8.0 with 300 mM NaCl. Poor binding and elution were seen in both B, binding buffer at pH 6.1 with 110 mM NaCl, elution buffer at pH 8.0 with 300 mM NaCl, and C, binding buffer at pH 6.8 with 200 mM NaCl, elution buffer at pH 8.6 with 0 mM NaCl.

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From the Editor

Innovation and Industry Strength Recognized at CPhI

Using the annual CPhI Worldwide tradeshow as a benchmark, the bio/pharma industry appears to be healthy and growing. Organizers of the 2018 event, held Oct. 9–11 in Madrid, Spain, reported attendance has increased by 9000 attendees compared with the last CPhI event in Madrid in 2015. Nearly 44,500 participants from 164 countries visited the event, which included more than 2500 exhibits, conference sessions, and networking opportunities (1).

Opinions about industry prospects around the globe also were revealed. In an annual study released during the event (2), CPhI reported results of a survey of bio/pharma professionals that ranked India, the United States, and China as the top regions for predicted pharma market growth. The US ranked first of 10 countries evaluated for reputation, followed by Germany, Japan, the United Kingdom, and France. India was ranked sixth; China was ranked last.

CPhI also measured perceptions of the knowledge of bioprocessing professionals, innovation, and quality with the US ranking first, followed again by Germany, Japan, and the UK. In a measure of growth potential, Ireland—home to planned expansions of bioprocessing capacity—ranked third.

Recognizing innovation

In addition to the industry data, innovative technologies and services that support bio/pharma development, manufacturing, and distribution were recognized at the 2018 CPhI Pharma Awards Gala, held on Oct. 9, 2018. The winners are as follows:

**Bioprocessing and Manufacturing**—Merck, Simplification of Fed Batch Processes Using Modified Amino Acids
API Development—Ipca Labs Limited, Atemisinin
Formulation—MiVital AG, Micelle Inside Solubilization
Excipients—Merck, Parteck MXP Excipient
Manufacturing Technology and Equipment—AqVida GmbH, AqVida filling line
Analysis, Testing, and Quality Control—Tornado Spectral Systems, HyperFlux PRO Plus, Tornado Spectral Systems
Drug Delivery Devices—Nemera, e-Novelia, a smart ophthalmic add-on. Highly commended: Stiplastics, Stiplastics
Packaging—Technoflex, Dual-Mix. Highly commended: Aptar Pharma, QuickFlip
Supply Chain, Logistics, and Distribution—Systech International, UniSecure
Contract Services and Outsourcing—CatSci Ltd, Development of a novel biocatalyzed manufacturing route for a generic API. Highly commended: Cambrex, Continuous Flow Centre of Excellence
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Corporate Social Responsibility—West Pharmaceutical Services, Inc., Delivering on the Promise of Good Corporate Citizenship
CEO of the Year—Nanobiotix, Laurent Levy
Pharma Company of the Year—Nanobiotix
OTC—Medical Brands and Vemedia, Excilor 2-in-1 Wart Treatment Device
Patient Centricity—SRS Life Sciences Pte Ltd, SRS Unistraw Delivery System
IT, mHealth, and Digitalization—Qualit-e Cloud GmbH, Qualit-e Cloud

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Survey results and record attendance may show positive signs for established and emerging biopharma regions.

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A main strategy for challenging the high prices of innovator therapies is to support the development and production of more competitive generic drugs and biosimilars, as outlined in FDA’s Drug Competition Action Plan, issued in June 2017 (1). One sign of success is the agency’s approval of a record 971 new generic drugs this past fiscal year, largely due to devising a more streamlined and efficient review process and providing added advice and assistance on product development to manufacturers.

FDA is applying a similar approach to spurring development of more complex generic drugs and combination products, such as injectables, topicals, and patches that act locally on the skin, inhaled drugs affecting the lungs directly, or eye drops that act on the surface of the eye. These all raise challenging issues for production, formulation, and demonstrating equivalence to a brand reference listed drug (RLD). One result is that competition is limited for complex and combination products that involve difficult and expensive product development and production methods, permitting brands to retain high prices even years after losing exclusivity. To achieve a more dynamic marketplace, FDA is issuing more guidance documents and targeted advisories to support R&D on these products, as outlined by FDA Commissioner Scott Gottlieb in a statement on advancing the development of generic copies of complex drugs to improve patient access to medicines (2).

This initiative produced a revised draft guidance advising industry on the design and conduct of studies to evaluate the adhesive performance of proposed generic transdermal and topical delivery systems (TDS) products (3). A second guidance provides recommendations for the design and conduct of studies to evaluate in-vivo skin irritation and sensitization potential of such treatments (4). In addition, FDA issued 235 product-specific guidance documents to help manufacturers identify appropriate methods for developing specific generic TDS products (5).

Anna Abram, FDA deputy commissioner for policy, planning, legislation, and analysis, provided more details on these efforts at an October 2018 workshop on complex generic drug-device combination products sponsored by the Drug Information Association (DIA) (6). She described FDA strategies for spurring the development and review of complex therapies that are difficult to formulate and to manufacture because the drug acts directly on the skin or eye or lungs, producing a therapeutic effect that is hard to measure for bioequivalence through the blood, as with conventional generic drugs. Challenges in identifying the most accurate, sensitive, efficient, and reproducible method for demonstrating “sameness” between the proposed generic and RLD erect notable hurdles in developing complex generic drug-device combination products, Abram pointed out. FDA seeks to engage earlier and more extensively with manufacturers in pre-abbreviated new drug application (ANDA) meetings to discuss new or alternative development strategies for complex drug-device products.

FDA OFFERS ADVICE AND SUPPORT

Complex generics and other combination drug/biologic-device products also can obtain advice and guidance from FDA’s Office of Combination Products (OCP). This office has expanded to better manage a growing number of requests from manufacturers for decisions on designating the review of a combination product to the appropriate lead FDA product center. FDA’s inter-center consult process seeks to ensure consistency and clarity in the combination product review process, explained OCP Director Thinh Nguyen at the subsequent DIA Combination Products conference. A forthcoming Q&A guidance on combination products may provide additional specifics on essential performance requirements.

Jill Wechsler is BioPharm International’s Washington editor, jillwechsler7@gmail.com.
sample sizes for human factors studies, manufacturer consultation with FDA during development, and strategies for documenting same-
ness to an RLD.

FDA officials noted that they suc-
cceeded in addressing some of these issues in the agency’s recent approval of the first generic for EpiPen, the widely prescribed epinephrine auto-
injector that has been in the spot-
light in recent years for its high price, made possible by limited competi-
tion. The new alternative offers a lower-cost option for patients and may help protect against product shortages, Abram commented.

To further advance complex generics, Gottlieb noted that FDA is devising more sensitive and accurate analytical tools and in-
vitro tests to demonstrate same-
ness between a generic and brand. A forthcoming umbrella guidance, moreover, should help generics makers overcome the most chal-
ling regulatory and scientific issues, as will more targeted guid-
ance documents that address legal issues blocking generic competition.

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The US Department of Health and Human Services pub-
lished a proposed rule in October 2018 requiring pharma
companies to list drug prices in TV commercials. Analysts question whether the required price information will be of any use to patients, and legal challenges could delay enforcement of the policy for months, if not years.

The proposal issued by the Centers for Medicare and Medicaid Services (CMS) (1) seeks public comments, which are sure to include multiple objections to the requirement that direct-to-consumer (DTC) drug ads dis-
close the wholesale acquisition cost (WAC) for products that cost more than $35 a month for treatment. Insurers, doctors, and consumer groups applaud the price trans-
parency proposal, while the Pharmaceutical Research and Manufacturers of America (PhRMA) and the advertis-
ing industry strongly oppose it.

A broader issue is whether disclosing WAC prices on drugs in DTC ads would provide any useful information to consumers, particularly those in Medicare or Medicaid, the stated beneficiaries of the rule. Even though FDA regu-
lates prescription drug advertising, the proposal came from CMS, which can claim that price transparency supports its interest in reducing outlays for drugs paid by public health programs. But Medicare and Medicaid patients largely have fixed co-pays that don’t vary with price, and they benefit from negotiated rates instead of paying list prices, as do many individuals in commercial plans. List price disclosure might be of greater benefit to individuals with high-deducti-
ble plans or percentage co-pays, and for consumers without any health insurance who pay cash.

Pharma companies claim that disclosure of WAC rates will only confuse consumers and may inhibit patients from seeking care. And they regard the requirement to disclose proprietary price information as a violation of first amendment protections, particularly if the required information is not rel-

ant to most consumers. The administration counters that court decisions have upheld the right of government agencies to require disclosures that support a public interest.

PhRMA lobbying succeeded in blocking Congressional approval of a similar ad disclosure provision that was added to a big budget bill in September and is ramping up to achieve a similar outcome in this round. As a stop-
gap, PhRMA is pushing an alternative plan for DTC ads to direct viewers to company websites with relevant price information (2). Despite the opposition, the administra-
tion is expected to finalize the rule sometime next year.

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—Jill Wechsler

Politics Drive Direct-to-Consumer Price Disclosure Rule
Single-use systems (SUS)—bioprocessing equipment designed for one-time use or a single product manufacturing campaign—has become the leading paradigm for pre-commercial (preclinical and clinical supplies) manufacturing of biopharmaceutical products (1). An estimated ≥85% of pre-commercial upstream bioprocessing now involves primarily or fully the use of single-use equipment for manufacturing. And the use of SUS for large-scale, commercial manufacturing is just starting.

This article reviews some of the SUS-related results from BioPlan Associates’ 15th annual survey of biopharmaceutical manufacturing professionals worldwide, which included responses from 352 responsible individuals, including 222 working for biopharmaceutical developers/manufacturers, and 130 working for bioprocessing suppliers or vendors. Because SUS operations directly impact bioprocessing capacity and capacity utilization, this survey has long included questions concerning SUS.

SUS CURRENT STATUS AND TRENDS

Much has been written about the benefits and disadvantages of SUS, and the biopharmaceutical industry has accumulated more than a decade of experience with the equipment and materials. Industry consensus now includes recognition that SUS enables lower up-front capital investments and operational costs compared with classic fixed stainless steel-based equipment. SUS also provide more flexibility than stainless systems, including more rapid turnovers and availability of the same process lines for manufacturing multiple products. SUS have lower operational costs due to the elimination of cleaning, sterilizing, and (re)validation of stainless-steel equipment. One downside of SUS, however, is the considerable recurring costs for new devices and equipment purchase for each use or campaign.

Every bioprocessing facility consumes significant amounts of some type of SUS products, if only classic, legacy SUS products, such as tubing and filter membranes. In the past decade-plus, however, newer types of SUS equipment have been brought to market, and nearly all types of bioprocessing equipment are now available in SUS format. The past decade has seen rapid growth in adoption of SUS-based process lines in place of stainless steel-based equipment, to the extent that SUS process lines dominate upstream pre-commercial biopharmaceutical manufacturing.

Downstream bioprocessing has seen less adoption of SUS compared to upstream. It still is difficult, expensive, and rare to implement a fully or even mostly SUS downstream processing stream, as chromatography and other purification technologies to date resist cost-effective adaptation to SUS formats. Due to excessive costs, chromatography columns and separation media/resins continue to be recycled.

SUS has been adopted—nearly exclusively—for mammalian cell-culture applications; microbial bioprocesses have resisted adoption of SUS. Microbial bioprocessing compared with mammalian, requires higher temperatures, pressure, use of special atmospheres, and more energetic mixing and aeration. Some microbial SUS product lines are now marketed, but do not appear to have gained traction in the market. On the other hand, most cellular and gene-therapies bioprocessing involves use of SUS, and despite adoption of plastic systems, a capacity crunch is expected in these sectors (2).

In the early 2000s, a few single-use systems, such as simple WAVE bag bioreactors (now from GE) on rocker platforms, and a few primitive
fixed-wall SUS bioreactors with plastic liners, were available; these systems were limited in size, such as a few hundred liters. Late-stage clinical and commercial manufacturing, however, requires larger capacity. Today, a variety of single-use bioreactors and other products are available, with most product lines going up to 2000-L scale.

Some suppliers are pushing this limit (e.g., ABEC now offers 4000-L SUS bioreactors). Each 1000 L of bioprocessing fluid weighs approximately 2200 lbs., making SUS with capacities above 2000 L unwieldy, difficult to install and move, not practical, or cost-effective for many facilities. Stainless steel still dominates commercial manufacturing, which thoroughly dominates all bioprocessing capacity-wise; pre-commercial manufacturing is only done as needed and involves much smaller quantities than needed to supply world commercial markets.

Currently, 1000-L and 2000-L SUS bioreactors—with multiple bioreactors used in parallel if needed—are on track to become the industry standard for new large- and commercial-scale manufacturing, eventually replacing larger stainless-steel units and facilities. Thus far, there are few such facilities, but a trend for building SUS-commercial manufacturing facilities is starting, and growth in adoption of SUS for commercial manufacturing will be dramatic in coming years, albeit from a very low baseline. However, much commercial manufacturing, particularly at the largest scales, remains more cost-effective when using large, fixed stainless-steel equipment-based process lines/facilities. Most commercial manufacturing, in terms of new facilities and total capacity, will continue to be stainless steel-based for at least the next five years.

A major trend supporting growth in adoption of SUS has been the steady increase in bioprocessing productivity, particularly upstream, in the past three decades since the introduction of recombinant products in the early 1980s (1). Bioprocessing productivity, especially upstream titers, has increased, essentially doubling about every five years, going from just a few tenths of a gram/L to the 3.2 grams/L that is now reported by BioPlan’s survey respondents as the average titer being attained at both clinical and commercial scales. This trend is expected to continue in the near term. Larger volumes of biopharmaceutical products can increasingly be manufactured using smaller or the

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same scale equipment. Only a decade ago, a facility producing several 100 kg/year of monoclonal antibodies would have required multiple dedicated ≥10,000-L stainless steel bioreactors and other comparably-scaled equipment. Today, the same amount can be manufactured with a few or even just one 500–2000-L SUS bioreactor faster and at lower cost.

The survey also found outsourcing of bioprocessing has remained steady, if not slowly increasing, with companies now taking a strategic approach to outsourcing versus seeking to outsource everything they possibly can.

**CMOs AND SUS**

Contract manufacturing organizations (CMOs) use more SUS equipment and use it more frequently compared with developer/manufacturer companies. As shown in **Figure 1**, survey respondents from CMOs reported increased use of SUS products vs. developers/manufacturers in nearly every product category surveyed.

This increased use of SUS by CMOs is fully expected, with CMOs needing the flexibility that SUS offers to rapidly switch over process lines or suites to manufacture different products at different scales. CMOs are highly motivated to get a facility or project up and running as quickly as possible; they do not have time to construct and validate stainless-steel process lines. CMOs, compared to developers/manufacturers, have more products to manufacture for clients and generally at non-commercial scales.

The past 12-year increases in the adoption of different types of SUS as reported by surveyed bioprocessing professionals, in terms of first use at facilities, are shown in **Figure 2**. There have been approximately 50% increases in first use/adoption of major, big-ticket, SUS equipment, including bioreactors, mixing systems, membrane adsorbers, and perfusion devices.

Some problems and issues involving SUS still inhibit its adoption. For example, when asked in the recent annual survey to cite the most important reason for not increasing their use of SUS, 25% of CMO respondents (vs. 17% with developers) cited high cost; 25% said “leachables and extractables are a concern” (vs. only 10% with developers); and 13% of CMO respondents cited a “lack of disposable equipment that meets process requirements”, and “breakage of bags and loss of production material were a concern”, compared to 9% and 8% of developer respondents, respectively. Fully 70% of CMOs cited some factors limiting their adoption of SUS. But
CMOs do what clients want, and clients increasingly want to adopt SUS for commercial manufacturing of products in development. As other parts of the annual survey regularly show, end-users identify improvements in SUS at the top, or among the top areas, where suppliers need to focus their R&D and bring new technologies to market.

**CONCLUSION**

CMOs have extensively adopted SUS-based bioprocessing over the past decade-plus, and CMO bioprocessing—in terms of the number of process lines, facilities, and products manufactured, especially at clinical scale—is now primarily SUS-based. Stainless-steel equipment still dominates in terms of total overall capacity; commercial manufacturing scales often are using legacy stainless-steel facilities or are producing at volumes where SUS is not a viable approach.

CMOs now rarely implement stainless-steel process lines for client product manufacturing. By using single-use vs. stainless steel systems, CMOs can save on facility, campaign and operating costs, and capital investments, while gaining needed flexibility, including rapid process line turnover, enabling manufacture of more products more rapidly at diverse scales. The differences in views and adoption of SUS by CMOs vs. developers reflects the business models of each, with CMOs needing speed and flexibility, and taking a transactional approach. Developers have fewer products to manufacture at larger scales and seek more long-term and cost-effective commercial manufacturing approaches; using large stainless steel-based process lines still generally fits that bill. But with more products in development having smaller, even orphan markets; titers and bioprocessing productivity continuing to increase; and product potency increasing, volumetric needs for commercial manufacturing are decreasing, with this facilitating an increasing percentage of new commercial manufacturing using SUS.

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Biopharmaceutical processes have always been difficult to scale up. Within the past decade, however, the industry has achieved significant improvement by adopting technologies that allow more predictable outcomes, says Alex Chatel, product manager at Univercells. Bioreactor manufacturers now integrate analytical technologies such as sensors, which allow better prediction and control of changes between scales, into their products. Chromatography and filter manufacturers have developed scalable and reproducible low-volume mimics of their industrial-scale equipment, which allow more accurate scale-up work than ever before, he says.

The availability of single-use equipment and platform solutions—such as fed-batch and perfusion bioreactors and multi-column chromatography—has helped achieve faster, more reproducible scale-up results. “Platforms have had a very positive impact on companies’ ability to develop products and manufacture them less expensively,” says Tom Ransohoff, vice-president and principal consultant with BioProcess Technology Consultants. Used almost exclusively for monoclonal antibodies (mAbs), platforms help shorten development times, particularly early in development, Ransohoff says. They also allow manufacturers to leverage performance data across multiple products, reducing the cost of late-stage process characterization and qualification. In addition, they permit some degree of facility design standardization, improving operational flexibility and capital savings, he says.

Developers are exploring how these platforms might be used in novel therapies such as cell and gene therapies. Although these treatments are in early stages of development, gene therapy has already become a reality, with companies such as Spark Therapeutics. The company received “breakthrough therapy” status for retinal dystrophy and hemophilia treatments, and is collaborating with Pfizer on hemophilia therapies, now in Stage III clinical trials.

The key to making gene therapy accessible to patients will be better manufacturing processes, Peter Marks, director of FDA’s Center for Biologics Evaluation and Research, told attendees at the Galien Forum in New York City on Oct. 25, 2018, in a panel discussion on gene therapy. At this point, the FDA has approved one directly-administered and two cell-based gene therapies, Marks said, but the Agency has received over 700 investigational new drug (IND) applications in the gene therapy field. “Sustained, reliable manufacturing will be required to bring gene therapy to patients worldwide,” said...
Mikael Dolsten, president of worldwide R&D at Pfizer.

Great improvements have been seen so far in delivering adeno-associated virus (AAV) vectors. These results demonstrate the inextricable link between clinical work and process development, said Kathy High, cofounder and head of R&D at Spark, who also spoke on the program. Good clinical results drive good process development, she said, in turn driving good manufacturing, which then drives more clinical results. “We’re already up by logs from where we were when we started our research,” she said.

Developers are actively exploring ways in which platform technologies could help improve the yield of AAV and other critical components of personalized therapy development and manufacturing. For example, a new collaboration (1) that involves GE Healthcare, Cobra Biologics, and UK’s Center for Process Innovation, is working on new ways to use fiber-based chromatography to improve AAV scale-up. Other development projects are evaluating different forms of chromatography and centrifugation.

INCREASED PRODUCTIVITY

“Companies are looking for ways to improve productivity because products on the horizon, such as new treatments for Alzheimer’s disease and antibodies for infectious diseases, could require significantly more material than products on the market today,” says Ransohoff. One way they do this, he says, is by “scaling out,” intensifying processes, for example by using fed-batch and perfusion on the upstream side, and multicolumn operations, whether it’s just higher cycling, or more continuous or semicontinuous chromatography (e.g., simulated moving bed (SMB) technologies), downstream, says Ransohoff.

Although use of process intensification is still at an early stage of adoption, more companies are actively studying how it might be used in multicolumn chromatography as well as systems employing membrane absorbers and fiber-based chromatography supports, Ransohoff says.

“Novel affinity ligand development technologies are getting to a point where companies can consider using a platform approach for a broader range of molecules in their pipelines, he says. Viral vectors—for example, AAV serotypes for gene therapies—could benefit from this technology,” he says. “This would allow gene therapy developers to use some of the platform technology thinking that is already being used to develop monoclonal antibodies,” he adds.

“Process intensification will enable much quicker pull through of personalized medicines, especially gene therapies and eventually cell therapies,” says Rick Morris, senior vice-president of product development at Pall Corp. In addition, he says, it will speed up and allow for screening of a much larger number of variants for mAbs, bispecifics, trispecifics, fragments, and recombinant proteins. For some cell therapies, the current process yield is only 30%, says Morris.

In internal testing, Pall scientists optimized process intensification to make 100 g/d of one product, enough to do preclinical and toxicology studies. The approach reduced the time required by six months, compared with traditional processes, says Morris. In addition, the equipment footprint was reduced by four or five fold, and the systems enabled, at large scale, a 40–50% savings in Protein A resin use, he says.

HIGH OPERATIONAL AND CAPITAL COSTS

The cost of current technologies is another challenge, says Chatel. “Many processes rely on the culture of adherent cells for producing a range of biological molecules, including viral vaccines, viral vectors for gene therapy, and oncolytic viruses. Producing these therapies using traditional technology (i.e., static culture-ware, roller bottles or microcarriers in agitated bioreactors) suffers from a number of drawbacks,” he says, “including high operational costs caused by complex processes and the need for manual operations, complex process development, and high facility footprints leading to high capital costs.”

Univercells is applying process intensification and taking a chemical engineering approach to bioprocessing, Chatel says, with its patented scale-X line of bioreactors. The reactors are designed so that a very large surface for cell growth is packed inside a small volume, reducing the number and complexity of operations compared with traditional equipment, he says. This results in a smaller footprint and a more robust process, Chatel adds.

“Direct linear scalability is ensured by applying concepts similar to those found in the scale-up of chromatography columns, whereby the height of the reactor is kept the same with its diameter increased as a function of the scale,” says Chatel. In addition, he says, the physical and chemical conditions are kept the same across scales, ensuring a smooth a risk-free scale-up.

The use of this bioreactor, in turn, led to the development of a “microfacility” concept, the NevoLine, which is designed to produce industrial quantities of viral vaccines (more than 550,000 doses per batch for a polio vaccine) in a near-continuous manner using 6 m² of floor space.

BIOREACTOR SCALE-UP

In more traditional biopharmaceutical development, bioreactors are typically the most difficult pieces of equipment to scale up, says Fredrik Lundström, product manager for bioprocess downstream hardware with GE Healthcare Life Sciences. Use of digital modeling is helping developers understand processes at small scale and assess operations before scaling up, he says.

Bioreactors also present opportunities for scaling out. “The upstream process is where you see major opportunity to intensify the process with
either intensified fed batch or continuous perfusion approaches that can be both scaled up and out,” says Peter Levison, executive director of business development at Pall Corp.

Bioreactor and fermentor technology platforms have had the greatest impact so far, says James Blackwell, principal consultant with the Windshire Group. Cell culture improvements were followed closely by small-scale single-use technologies. As a result, on the bacterial side, developers can now go into production using 100- to 300-L single-use reactors. “You can start working with those technologies at much smaller scales now than was possible in the past,” he says.

Being able to operate 2000-L single-use bioreactors in parallel also means that users can exploit significant improvements in both cell densities and product titer to generate a multifold increase in productivity levels, says Levison. “The key to exploiting this intensified upstream process is having downstream processing solutions that can handle the increase in mass and concentration of product and not become the process bottleneck. The adoption of continuous downstream processing unit operations has been a real enabler in addressing the process requirements of these intensified upstream processes,” he says.

**SCALE-UP CHALLENGES**

Today, scale-up challenges are more associated with the automation and control of the processes, particularly with hybrid approaches, says Levison. Each part of the process must be properly connected and monitored and operating at a production rate aligned to both the upstream and downstream unit operation, he says. Analytics and control systems become critical to delivering reliable, accurate, and precise data for the duration of the entire batch, says Levison. “Not only strong process monitoring and control, but highly relevant assays are needed to ensure that clinically relevant product specifications are met. Where these are on-line or at-line, a high degree of accuracy and precision is a prerequisite,” says Levison.

As development timeframes shorten, developers can focus so intensely on short term goals that they lose sight of the big picture. During initial workflow development, for example, the need to produce material as quickly as possible can override the need to focus on optimizing process simplicity, reliability, and cost-effectiveness. “Often, developers fail to consider the entire workflow and interactions between unit operations, both of which are critical to ensuring a robust, efficient, cost-effective workflow for manufacturing biological drugs,” says Lundström.

“From time to time, we see biomanufacturers entering manufacturing scale with recipes and specifications that are difficult to operate and achieve at large scale,” he says. Prime examples, he says, are recipes with long and shallow linear gradients, as well as pH specifications for process buffers that can only be achieved off-line in the lab. Developers may also use a wide range of buffers to achieve specific results, which is fine in the lab but can create major problems at industrial scale, says Lundström.

Scale-up and scale-down are codependent, says Chatel. “The synergies between the two are essential and must be embraced by equipment manufacturers,” he says. The obvious target for scale-down models that accurately mimic larger scale equipment is in cases where pilot or industrial runs are most expensive (e.g., chromatography process steps or cell culture). However, with novel products such as cell therapy, the costs of production can be so high (e.g., including the cost of reagents for cell adherence and culture), that the whole process itself is essentially of high value and thus a desirable target for scale-down models, says Levison.

“There is a significant body of literature on how to scale up and scale down the unit operations that we work on most commonly for cell culture, fermentation processes, tangential flow filtration (TFF), and chromatography,” says Ransohoff. “Challenges tend to come with scale-dependent parameters that are more difficult to mimic, such as mixing, heat transfer, and mass transfer,” he says.

**HIGH THROUGHPUT**

There is still a limitation in scale-down models at the sub-milliliter scale that would enable high-throughput screening for process development to work at the earliest stages of drug development, says Chatel. “As interfacial science and data analytics evolve, however, within the next 10 years, process development work may routinely be done on a bench-top,” he says.

Some high-throughput tools are already used early on and in process characterization, allowing users to run many experiments in parallel at smaller scale, with less material and in less time. One example Ransohoff cites is plate-based chromatography resin screening, enabled by technologies such as GE Predictor plates, that allow users to consider binding properties of resins and solutions in more multiplexed formats.

Beyond process development, on the drug product delivery side, personalized medicine will be much more challenging to scale up, says Blackwell. With autologous therapies, he says, scale-up involves a single unit and operations around the flows of a single product. “Scale-up becomes about achieving efficiencies on the operating floor, so you can scale up those processes efficiently in parallel. A new shift in thinking has to happen within the industry,” says Blackwell.

**REFERENCE**

Within the past three years alone, FDA has approved 20 novel antibody therapeutics for oncological treatment, and more than 300 therapeutic antibodies are currently in oncology-based clinical trials. Despite this exponential growth, however, most therapeutic antibodies rely on a monomeric immunotherapy-based mechanism. Therefore, there is an urgent demand for new therapeutic strategies such as combinatorial therapy and novel modalities such as bispecific antibodies.

**KEY LEARNING OBJECTIVES**

In this webcast, we will cover:

- An overview of therapeutic antibodies, focusing on the opportunities and challenges of current monotherapy.
- The major benefits of bispecific antibodies and the current formats used in clinical trials.
- An in-depth, integrated single-domain antibody fused to monoclonal antibody (SMAB) case study from rational design to preclinical development.

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__PRESENTERS__

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For questions contact Kristen Moore at Kristen.Moore@ubm.com
Although Chinese hamster ovary (CHO) cells are the standard in antibody-based drug production, there are limits to their use (1). Market pressures to develop more affordable biologic drugs quickly are also driving the need for optimizing and/or updating current methods of protein expression. Innovations in cell bank sources are attempting to address the needs in biopharma manufacturing for which a CHO cell line may be insufficient.

In an interview with BioPharm International, Dr. Nicole Faust, CEO and chief scientific officer of Cevec Pharmaceuticals, and Mark Emalfarb, CEO of Dyadic International, spoke about the cell-line innovations their respective companies have taken on to bridge that gap in biopharma manufacturing for which a CHO cell line may be insufficient.

In contrast to the Chinese hamster ovary-derived cells, our Cevec amniocyte production (CAP) cell lines are of human origin. This has several advantages when producing recombinant proteins: the cells only add human post-translational modifications, thus avoiding any immunogenic structures, like non-human sugar structures attached to the protein. Moreover, any residual host cell proteins in the recombinantly produced product will be of human origin. The presence of non-human host cell proteins is a concern, in particular since there have been some cases (e.g., CHO derived blood coagulation factor IX) in which residual CHO host cell proteins have caused an immune response in the recipient.

A second differentiation criterion to CHO cells is the ability to produce complex glycosylated recombinant proteins. While the CHO-platform has been continuously optimized for the production of antibodies, CAP cells are particularly well-suited for new protein classes like therapeutic proteins that require complex glycosylation.

DIFFERENT FROM CHO

BioPharm: What about your innovative cell line source differentiates it from the industry standard CHO cells?

Faust (Cevec): In contrast to the Chinese hamster ovary-derived cells, our Cevec amniocyte production (CAP) cell lines are of human origin. This has several advantages when producing recombinant proteins: the cells only add human post-translational modifications, thus avoiding any immunogenic structures, like non-human sugar structures attached to the protein. Moreover, any residual host cell proteins in the recombinantly produced product will be of human origin. The presence of non-human host cell proteins is a concern, in particular since there have been some cases (e.g., CHO derived blood coagulation factor IX) in which residual CHO host cell proteins have caused an immune response in the recipient.

A second differentiation criterion to CHO cells is the ability to produce complex glycosylated recombinant proteins. While the CHO-platform has been continuously optimized for the production of antibodies, CAP cells are particularly well-suited...
to express more complex proteins, such as, for example, blood coagulation factors or high molecular weight molecules. We have further engineered the CAP-Go platform to generate specific cell lines allowing the production of glycoproteins with tailor-made glycosylation patterns.

**Emalfarb (Dyadic):** CHO cells have a 20-hour doubling time, whereas our Eukaryota-engineered C1 cell line has a doubling time of only two hours, two times to 10 times higher productivity, and a production cycle of one-third to one-half the fermentation time with one-tenth the media cost compared to CHO. In addition, with our cell line, there is no need for viral inactivation. C1 is a better cell line than CHO because it is a natural enzyme (protein) producer that is more stable and can tolerate a wide range of growth conditions. In addition, C1 can express, secrete, and produce proteins such as antibodies at high levels (i.e., 2.4 grams per liter per day) and can easily grow in fermenters. In addition, C1 has a more stable genome than CHO since it is smaller and is haploidic. Therefore, it inherently has the potential benefits for becoming a successful protein production platform for biologics. In comparison, the CHO cell line was developed for many years before it became suitable as an industrial production platform.

**Biopharm:** How and why did you choose this source from which to develop a technology platform to produce biotherapeutics?

**Emalfarb (Dyadic):** Fungi in general, and C1, in particular have a natural efficient system for protein expression. C1 however, also has the following benefits:

- **Can tolerate high glucose concentration**
- **Easy scale up** (was scaled up and used commercially to produce enzymes at greater than 100m³)
- **Advanced genetic tools** (efficient transformation).

**Faust (Cevec):** When developing the CAP platform, we were pursuing two goals: establishing a cell line from human origin that was not derived from tumors or from aborted embryos like the then-existing human cell lines and ensuring that the cells’ history was carefully documented. For this reason, we chose amniocytes (i.e., fetal cells obtained for prenatal diagnostics) as the primary source of cells. They were immortalized using defined DNA segments of the Ad5 genome, and every single step of generating the cell lines was documented in detail. Thus, the full history of the cell line is known as are all materials the cells have been in contact with. All this is included in a biologics master file deposited with FDA, which our clients can reference.

**MEDIA/SUPPLEMENT OPTIMIZATION**

**BioPharm:** What are the media and/or supplement requirements for growing and maintaining the population of these cells? Do these requirements differ from that used to grow and maintain CHO cells?

**Faust (Cevec):** The cells grow as single-cell suspensions to very high cell densities in serum-free, chemically defined media. The cells’ requirements are very similar to other suspension cell lines growing in serum-free cultures. For optimal process yields, fine-tuning and adjustment to the specific metabolic needs of the CAP cells are beneficial.

One remarkable feature of the CAP cells is that they are robust in their growth behavior. For example, changes in media composition do not appear to result in changes to the pattern of post-translational modifications.

**Emalfarb (Dyadic):** This is a complete synthetic and defined media that is based on glucose as carbon source, salts to provide macro- and micro-elements, with a few free amino acids to supply organic nitrogen. It is very simple, robust, and low-cost media. Not many other organisms can grow and produce so well on such simple media.

The control of the fermentation is also robust, based on a simple fed-batch strategy, requiring just control standard variables such as pH, dissolved oxygen, or foaming, which ensure scalability and provide versatility to the bio-manufacturing process. Additionally, the system is energy efficient and requires only little oxygen.

**BioPharm:** Is there a need for newer/innovative media and supplements in the market for emerging cell sources, such as yours?

**Emalfarb (Dyadic):** Since we are using simple defined already low cost media that is based on glucose, we don’t think that there is a need for newer/innovative media and supplements in the market.

**Faust (Cevec):** For producer cell lines like CHO or CAP-Go, there are already excellent media available. However, when you produce very diverse products like we do with the CAP-Go platform, ranging from small growth factors to highly complex multimeric proteins, it becomes apparent that the optimal media/supplement composition depends very strongly on the particular product.

With a growing number of complex recombinant proteins being manufactured, it would be very desirable to have ‘media optimization kits’ to be used to establish the optimal media composition during process development with the option to then obtain exactly this composition in GMP quality from the media manufacturer.

**REFERENCE**

Regulators require that data used during biopharmaceutical processes, including drug development and manufacturing, be available to support biologics license applications (1). According to Billy Sisk, life-sciences industry manager in EMEA at Rockwell Automation, manufacturing data such as pressures, feed flow flux, and pH are generated during harvest, filtration, and purification processes. And these data must be collected and controlled in accordance with regulations. “Biopharmaceutical producers need to ensure that data are recorded, processed, retained, and used for a complete, consistent, and accurate record throughout the data lifecycle. [Companies] need to maintain formal management of the records and data throughout the company and control all phases of the data lifecycle—from initial data creation to destruction,” says Sisk.

Regulations state that data must be secure, reliable, and available for review, but they don’t necessarily tell companies how to do this. In the past few years, FDA has made data integrity a focus. The agency has issued guidance on data integrity requirements (2) and has cited a number of companies for lack of proper data safety procedures (3). It appears as if data integrity has suddenly become a problem that was not there before, but is that truly the case?

According to Els Poff, executive director—Data Integrity Center of Excellence at Merck, data integrity may not have been well understood until multiple guidance documents were published. It has been a few years since the publication of these guidance documents, and therefore, most companies now have programs in place that ensure the appropriate prevention and detection controls are applied to secure their data.

ARE ALL DATA EQUALLY CRITICAL?
There is some concern and debate in the industry, however, about how stringent companies need to be when securing data. The Parenteral Drug Association (PDA) is working with members of industry and regulators to develop a technical report that industry can use to determine what companies need to do to stay compliant with data integrity regulations and requirements.

According to Poff, a member of the working group writing the report, PDA is trying to provide industry with clarity on what should be done regarding data that aren’t directly
linked to the quality of the batch. “From a data integrity perspective, [industry is] not 100% sure how they should treat that data. Do the controls for [generating, using, and reporting these data] have to be the same as we would use with the data in our laboratories or for inline testing elements of a manufacturing process?” Poff asks.

The PDA working group is looking to develop levels of criticality for certain types of data. “We as a team are challenging ourselves to see if there are places where the controls are in line with the criticality of that data point. The high critical [data points] are easy [to define] … your critical process parameters (CPPs), your critical quality attributes (CQAs), in-line testing results … but what about everything else? Are there levels of criticality you can assign to [other types of data] that are less critical? … We are trying to identify a number of data integrity controls … based on [the] vulnerability [and] complexity of [the] process … to provide better understanding on what’s really needed based on a risk-based approach,” Poff says.

GOING TOO FAR?

“Industry struggles with defining what [criticality] is. [Companies may be afraid] that the way they interpret [criticality] may not satisfy the regulator who is going to come and visit them next, so by being overly conservative, they are safeguarding themselves from observations, and not be in any position where anything gets called into question,” Poff says.

But all this work might be unnecessary. For example, says Poff, human-machine interfaces (HMIs) on the shop floor may simply turn an asset off or on, but they allow data, such as speed and time, to be entered. Older HMIs may not be capable of having individual logins and passwords, but regulations indicate that any data entered must be attributable to a specific individual. “So, what do we do with that asset? Do we need to rip it out and put something else there?” asks Poff. “Or can you look at what it’s controlling, define the data it’s controlling or generating, and make a rational decision to say this HMI only starts and stops the machine. That’s all it does. So, why are we calling that into the data integrity realm? Or is it setting a line speed that doesn’t really reflect the quality of the batch because it’s only controlling the speed of the line? So, although there are data there, [these data are] not relevant to the quality of the batch. And therefore, controls should be applied that are commensurate with the risk to product quality and/or patient safety. It’s that kind of logic that we are trying to outline for [industry] and give them a framework to evaluate their system, process, and people to determine if everything is needed, or is there a level of control that can be different for this situation?”

Poff hopes the new PDA report will help companies make some of these choices. “The intent of the tech report is to make sense of some of the black and white rules … Manufacturing is different than the lab, and if we apply a black-and-white rule, we might be putting the business at a disadvantage.”

ONE SIZE DOES NOT FIT ALL

Poff states that the fact that different companies have different data systems complicates the development of guidelines that suit every process or every company. “Some [biopharma companies] are highly automated, highly technology enabled; some are paper based, and the majority are a hybrid where some elements of the manufacturing process are automated and other elements are paper based.”

“There is not a one-size-fits-all solution to ensuring data integrity. In many cases, there will need to be point applications or manual processes, such as second-person verification, to ensure data integrity,” says Melissa Seymour, vice-president, Global Quality Control at Biogen. “Some commercial solutions, such as ComplianceBuilder by Xybion or LDAP [Lightweight Directory Access Protocol], can help manage access control. Additionally, alternative Windows shells can be configured to block users from deleting data on personal computer-connected instruments.”

TOOLS FOR CONTROLLING DATA INTEGRITY

“Producers should understand the data lifecycle, manage data quality, manage reference and review data, and manage quality risks,” says Sisk. Risk assessment, behavioral steps (e.g., culture and training), procedural steps (e.g., audit trail review, control of access, supervision of manual entry), and technical steps (system validation, user access control, rights management, segregation of duties, etc.) are all tools that can be used to ensure data integrity, according to Sisk.

The technology is developing, and industry has been looking for ways to control the integrity of data, such as automation. Automation creates its own complications, however, and can’t be completely relied upon.

“In recent years, the collection of data during manufacturing has become more automated which, while providing benefits from a trending and integration perspective, [also] increases risk with respect to data loss and/or data manipulation,” says Seymour. “Additionally, the move towards more on-line and at-line testing to determine chromatographic performance creates more system integrations resulting in more complex data flows and subsequently more complex data mapping. Data [are] becoming less siloed and more integrated. For example, an evaporating light scattering detection (ELSD) system may be coupled with high-performance liquid chromatography (HPLC) and integrated into the manufacturing execution system (MES) at a chromatography step. Compounding the complexity, the MES may be integrated to a laboratory execution system (LES) and one or more enterprise systems for devia-
tions or business management processes. As data become more electronic and more accessible, this provides the industry with tools for better product process and quality understanding. At the same time the quality of the data then becomes of utmost importance,” says Seymour.

“A validated electronic batch recording (EBR) solution, combined with a single data source, promotes a high level of data integrity,” says Sisk. “EBR supports data integrity through a multitude of validation measures, including monitoring interface communication, integrating the automation layer with standardized interfaces for automated data acquisition, checking consistency during recipe design and execution, and automatically recording changes to electronic records. In addition, fully integrated exception management in EBR supports tracking anomalies during process execution, limiting violation exceptions, overriding exceptions, recording canceled exceptions, providing real-time availability of exception review, and controlling user authorization during review and closure of exceptions.”

CULTURE OF QUALITY
A culture of quality that puts emphasis on data integrity is also essential. Quality culture and data integrity are linked, according to Susan Schniepp of Regulatory Compliance Associates (4).

“It is clear that regulatory authorities consider quality culture an important element in establishing the veracity and integrity of the data being generated by companies that support the products they manufacture,” said Schniepp. “Auditing a company to determine if their culture is conducive to generating data that meets the attributable, legible, contemporaneous, original, and accurate (ALCOA) concepts is on the horizon and may become a part of routine audits performed by regulators or industry auditors when evaluating the suitability of a manufacturer, potential partner, or service provider,” she added.

“First and foremost, people are the most critical element in any data integrity initiative,” agrees Seymour. “Organizations must create awareness and educational programs to achieve the appropriate quality culture. Additionally, close collaboration between information technology, quality, and business units is critical in defining the overall structure of a data integrity program. Robust data governance policies that define and standardize processes across the organization are also critical to instilling integrity of data. Key in the development of data integrity programs is the utilization of business process mapping in defining the flow of data and identification of quality information as an asset. This includes the need for data mapping from initial capture through the complete lifecycle, ensuring that systems integrations and electronic transcriptions are well understood, risk assessed, and remediated. Clarity of definitions and procedural controls are also necessary,” says Seymour.

CONSIDERATIONS WHEN OUTSOURCING
The sharing of data becomes equally important in the global pharma environment. Integrity and quality are important when transferring and sharing data with contract manufacturing or research organizations (CMOs and CROs) (1). Biopharmaceutical development and manufacturing data can be complex and can have specific language and formats (1). Sponsor companies must be sure the CMOs or CROs they work with can access and understand these data, and regulatory data integrity requirements make this task imperative.

“Getting the right data landscape in place is now an even more critical business objective,” according to Paul Denny-Gouldson of IDBS. Companies should use a common lexicon or catalogue of terms. “This is perhaps the simplest of things, but it causes the most issues in peer-to-peer data exchange,” stated Denny-Gouldson (1).

Sponsor companies should consider if their partners have their own data systems. And the sponsor’s definition of quality might be different than their outsourcing partner (1). Specifics about each data point, such as equipment and software used, lab conditions, buffers and media used, and the training of personnel should be known (1).

“The utopian view would be a network of software solutions—an ecosystem—that all work seamlessly together to deliver the data collaboration services required,” according to Denny-Gouldson (1). Having a system to ensure quality data are secure and available should be a critical objective in the sponsor/contractor relationship.

EVOLVING TECHNOLOGY AND INDUSTRY
With the ever-evolving nature of technology, how does the bio/pharmaceutical industry ensure the quality and integrity of its data in the years and decades to come? Poff thinks this is a question that is still unanswered.

“Our retention periods are so long. How do you ensure that data remain accessible over [their] lifespan? I think in the short term, we have somewhat found a way to make this possible. But with the rapid change of technologies and more automation/data capture on the shop floor, companies will need to ensure they look at long-term data retention strategies,” Poff says.

REFERENCES
Single-Use Mixing Technology for Virus Clearance (Part 2: Compatibility, Leachables, and Scale-Up Studies)

RONAN MCCARTHY, NICK HUTCHINSON, ISABELLE UETTWILLER, AMÉLIE BOULAIS, FRANÇOIS COUTARD, EVE DEPAUW, AND LUDOVIC BOUCHEZ

ABSTRACT

The potency of recombinant proteins such as monoclonal antibodies remains typically unaffected by detergent-based virus inactivation treatments because detergents tend to target lipids and lipid derivatives rather than proteins. Virus inactivation treatments that use detergents are robust with respect to temperature, which allows these steps to be performed at room temperature without thermoregulation. In this two-part article, the authors describe the testing performed to demonstrate the applicability of single-use mixing technology for a virus inactivation step performed using detergent.

As the authors described in Part 1 (1) of this paper, solvent or detergent treatments for virus inactivation were originally developed for use in the manufacture of blood products (2, 3). They do not require expensive reagents or expensive equipment. The method inactivates enveloped viruses by solubilizing the viral envelope’s lipid membrane structure, which prevents the virus from binding to or infecting cells. The potency of recombinant proteins such as monoclonal antibodies (mAbs) is typically unaffected because the detergent targets lipids and lipid derivatives rather than proteins (4). Virus inactivation treatments that use detergents such as Triton X-100 (MilliporeSigma) are robust with respect to temperature, which allows these steps to be performed at room temperature without thermoregulation. The solvent that has been added must be removed after the step, often by chromatography, to prevent it entering the final product.

Biomanufacturers must be confident that virus inactivation steps using detergents are compatible with single-use technologies that may be used for this step. In this two-part article, the authors describe the testing performed to demonstrate the applicability of single-use mixing technology for a virus inactivation step performed using detergent (Triton X-100, MilliporeSigma).

The study was performed in a series of steps. Part one of this paper (1) described the study of the inactivation of enveloped viruses (xenotropic murine leukemia virus [X-MuLV], bovine viral diarrhea virus [BVDV], and pseudorabies virus [PRV]) by detergent treatment (Triton X-100, MilliporeSigma) within a single-use bag (Flexel, Sartorius Stedim Biotech). Testing showed that no significant adsorption of the detergent to the bioprocess container occurred. Enveloped virus spiking studies showed that a minimum reduction factor of 3.84 log_{10} could be expected in this single-use step and that, in most cases, the reduction factor would be greater than ≥ 5.01 log_{10}.

Ronan McCarthy is senior downstream process scientist, François Coutard was bioengineering unit manager, and Eve DePauw and Ludovic Bouchez are downstream process technicians; all at the bioengineering unit at LFB Biomanufacturing. Nick Hutchinson was technical content marketing manager, Isabelle Uettwiller is head of validation lab, confidence validation service, and Amélie Bouais is process development consultant, integrated solutions marketing, all at Sartorius Stedim Biotech.

Peer-Reviewed

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Accepted: April 18, 2018.
In Part 2 of this paper, the authors will describe chemical compatibility and leachable studies performed on the single-use bags (Flexel, Sartorius Stedim Biotech) for the magnetic mixer in the presence of detergent (Triton X-100, MilliporeSigma). Finally, the authors will describe the evaluation of film adsorption and detergent homogeneity in a 50-L magnetic mixer to verify that the results generated at the small scale can be translated to an industrial scale operation.

STUDY DESIGN

At the production-scale, the virus inactivation of a neutralized Protein A mAb-eluate is performed using detergent (Triton X-100, MilliporeSigma). The inactivation is performed at a final concentration of 1% (w/v) detergent for one hour at room temperature (20 ±5 °C) in a magnetic mixer (Sartorius Stedim Biotech). A typical processing volume of 85 L of mAb is treated in a 200-L single-use bag (Flexel, Sartorius Stedim Biotech), constructed from S40 film (Sartorius Stedim Biotech). The S40 film is a multi-layer film which includes an ethylene vinyl alcohol (EVOH) gas barrier and a polyethylene (PE) fluid contact layer.

The critical parameters for the inactivation step are the detergent concentration, product-detergent contact time, the film surface area-to-product volume ratio, and the temperature. The adsorption, chemical compatibilities, and leachable studies were performed under worst-case contact time conditions (see Table I).

Experiments were performed by LFB Biomanufacturing in collaboration with Sartorius Stedim Biotech Confidence Validation Services.

METHODS

Chemical compatibility

The mechanical resistance of the single-use bags (Flexel, Sartorius Stedim Biotech) to be used with the magnetic mixer was evaluated following exposure to 20% detergent (w/v) (Triton X-100, MilliporeSigma) for 72 hours at 5 °C ±3 °C.

The test mimics the concentrated detergent (Triton X-100, MilliporeSigma) solution storage conditions that would be applied during a manufacturing campaign. At process scale, the solution is stored in concentrated form and then subsequently diluted to provide the 1% (w/v) detergent solution used for virus inactivation. The inactivation step is performed with 1% detergent at 20 ±5 °C for one hour. Although processing occurred at a higher temperature than the storage mimic, the storage time in process conditions was 72-fold shorter and the concentration of the detergent was 20-fold less. According to the ASTM F1980 Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices (5), 72 hours at 5 °C correspond to 25.7 hours at 20 °C. Thus, it was judged that the storage conditions were worst-case conditions for the chemical compatibility study.

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<td>Worst case</td>
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<tr>
<td>Temperature (°C)</td>
<td>20 ± 5</td>
<td>20 ± 5</td>
<td>14±1</td>
<td>5±3</td>
<td>30±5</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>Process condition</td>
<td>Process condition</td>
<td>Process condition</td>
<td>Lower limit</td>
<td>Storage condition</td>
<td>Upper limit</td>
<td>Process condition</td>
</tr>
<tr>
<td>Continuous mixing</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

All figures courtesy of the authors

**Table I. Rationale for the parameters selected during the studies.**
The test was performed by adapting the standard test method for resistance of plastic to chemical reagents (ASTM D543) (6). The test articles were a 500-mL single-use bag (Flexel, Sartorius Stedim Biotech) with PE port, silicone tubing, and PE impeller parts. The contact surface-to-volume ratio of the scale-down mimic was 1.2 cm²/mL compared to the 0.2 cm²/mL ratio for the 200-L bag that would be used at the large scale. The scale-down experiment was, therefore, considered to represent worst-case processing conditions. The 500-mL single-use bag was gamma-irradiated prior to the experiment at 25–45 kGy, which was also representative of the large scale. A control was prepared by replicating the small-scale experiment but replacing the 20% detergent (Triton X-100, MilliporeSigma) solution with purified water.

To demonstrate the chemical compatibility, a series of tests were performed following incubation with the detergent: a visual inspection allows evaluation of the impact of the solution on the plastic, and criteria such as transparency; film brittleness; cracking; presence of bubbles; and surface damage, such as decomposition and delamination, were also evaluated. Additionally, a weight-loss

<table>
<thead>
<tr>
<th>Material description</th>
<th>200-L bag for magnetic mixer</th>
<th>50-L bag for magnetic mixer</th>
<th>500-mL scale-down bag</th>
<th>75-mL scale-down bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>In process</td>
<td>Detergent-homogeneity during mixing and adsorption</td>
<td>Chemical compatibility and leachables</td>
<td>Detergent film adsorption, virus inactivation studies</td>
</tr>
<tr>
<td>Film material</td>
<td>S40 film</td>
<td>S40 film</td>
<td>S40 film</td>
<td>S40 film</td>
</tr>
<tr>
<td>Configuration</td>
<td>3D</td>
<td>3D</td>
<td>2D</td>
<td>2D</td>
</tr>
<tr>
<td>Sterilization</td>
<td>Irradiation at 25-45 kGy</td>
<td>Irradiation at 25-45 kGy</td>
<td>Irradiation at 25-45 kGy</td>
<td>Irradiation at 25-45 kGy</td>
</tr>
<tr>
<td>Agitation method</td>
<td>Magnetic mixer</td>
<td>Magnetic mixer</td>
<td>Static</td>
<td>Orbital shaker</td>
</tr>
<tr>
<td>Surface-volume ratio</td>
<td>0.2 cm²/mL</td>
<td>0.2 cm²/mL</td>
<td>1.2 cm²/mL</td>
<td>3.5 cm²/mL</td>
</tr>
<tr>
<td>Impeller material</td>
<td>Yes</td>
<td>Yes</td>
<td>Fragment</td>
<td>Fragment</td>
</tr>
</tbody>
</table>

Table II. Characteristics of commercial and scaled-down single-use bags (Flexel, Sartorius Stedim Biotech). Detergent used was Triton X-100 (MilliporeSigma).
analysis was performed to measure any potential reduction of bag barrier properties. Mechanical properties were tested by tensile-strength testing the film material on the sealing parts and on the connections. The results were then compared to specifications. Stress-test conditions were applied using a drop test to evaluate the mechanical resistance of the container after the interaction with the 20% (w/v) detergent solution.

Interactions between bag components and the detergent were also evaluated by verifying that the dimensions of major components were still within the required specifications following contact with the test solution. Finally, spectral properties of the film were verified and compared to the control sample in contact with water.

### Leachable study

The leachables from the single-use bags (Flexel, Sartorius Stedim Biotech) for the magnetic mixer constructed from S40 film were evaluated by performing an extraction with a 1% (w/v) detergent (Triton X-100, MilliporeSigma) solution. Biomanufacturers can also be concerned about particulates generated by mixing operations, which can ultimately lead to aggregate formation. The proportion of monoclonal antibody monomer in the final product of this process, however, exceeded 99%, and an analysis of particulates was outside the scope of this study.

The 500-mL scale-down, irradiated bag with the same design as that used in the chemical compatibility study was used. A 24-hour incubation of the solution was performed.

### Table III. Chemical comparability testing results of the 500-mL scale-down mimic of the 200-L single-use bags (Flexel, Sartorius Stedim Biotech) with 20% detergent (Triton X-100, MilliporeSigma) solution at 5 °C ±3 °C.

<table>
<thead>
<tr>
<th>Test category</th>
<th>Test description</th>
<th>Specification</th>
<th>Meets specification?</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>Weight-loss analysis on bags and accessories</td>
<td>Weight loss is less than 1%</td>
<td>Pass</td>
</tr>
<tr>
<td>Category 1</td>
<td>Visual inspection of bag and impeller: Transparency/opacity</td>
<td>No transparency/opacity modification</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Visual inspection of bag and impeller: color modification</td>
<td>No color modification</td>
<td>Pass</td>
</tr>
<tr>
<td>Category 2</td>
<td>Film thickness</td>
<td>175–225 μm</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Polyethylene seat dimensions</td>
<td>External diameter: 110.49–111.55 μm, Internal diameter: 8.46–8.72 μm</td>
<td>Pass</td>
</tr>
<tr>
<td>Category 3</td>
<td>Film tensile strength</td>
<td>Machine direction tensile strength &gt; 40 N, Traverse direction tensile strength &gt; 40 N</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Tensile strength of the film seals</td>
<td>Tensile strength &gt; 80 N</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Tensile strength of the film/port seals</td>
<td>Tensile strength &gt; 80 N</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Tensile strength of the port/tubing connections</td>
<td>Tensile strength &gt; 80 N</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Tensile strength of the tubing/connector connections</td>
<td>Tensile strength &gt; 80 N</td>
<td>Pass</td>
</tr>
<tr>
<td>Category 4</td>
<td>Visual inspection of the bag and impeller: cracking/bubbles</td>
<td>No cracking/no bubble</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Visual inspection of the bag: delamination</td>
<td>No delamination</td>
<td>Pass</td>
</tr>
<tr>
<td>Category 5</td>
<td>Drop test</td>
<td>Checking for leaks on component connections</td>
<td>No leaks on component connections</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Checking for leaks on the bag seals</td>
<td>No leaks on bag seals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Checking for leaks on the film/port seals</td>
<td>No leaks on film/port seals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Checking for leaks on the port/tubing connections</td>
<td>No leaks on the port/tubing connections</td>
</tr>
<tr>
<td></td>
<td>Visual inspection of the bag and impeller: decomposition</td>
<td>No decomposition by dissolution</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Visual inspection of the bag: brittleness</td>
<td>No brittleness</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Visual inspection of the bag and impeller: crater/hole</td>
<td>No crater/hole</td>
<td>Pass</td>
</tr>
</tbody>
</table>
at 30 ± 5 °C, under static conditions, thereby ensuring that the upper end of the production-scale temperature specification of 20 ± 5 °C was covered. A control was performed by replicating the small-scale experiment but replacing the 500-mL bag with a glass bottle and a polytetrafluoroethylene (PTFE) bottle incubated under the same conditions.

Process solution and control samples were analyzed by inductively coupled plasma–mass spectrometry (ICP–MS)/optical emission spectrometer (OES), reversed phase ultra-high-performance liquid chromatography with ultraviolet detection (RP–UHPLC–UV), and gas chromatography–mass spectrometry (GC–MS). Element impurities analysis was performed by ICP–MS to evaluate the migration of inorganic compounds from the plastic materials (35 elements were evaluated according to the ICH Q3D, EMA, and USP <232> requirements) (7, 8, 9). The GC–MS analysis was used to detect volatile and semi-volatile organic leachables, and the UHPLC–UV provides additional information by screening the UV-active organic compounds independently of their molecular weight. The combination of these three analytical methods provides a comprehensive leachable profile.

**Translation to industrial scale**

A study was performed to verify that the detergent (Triton X-100, MilliporeSigma) concentration was homogeneous throughout a 50-L single-use 3D bag (Flexel, Sartorius Stedim Biotech) once agitated by the magnetic mixer. Both adsorption and effective mixing were verified under process conditions at a scale that was 25% of the final production scale volume. The findings are not generalizable for different agitation rates or mixer working volumes. The study was performed with purified water instead of Protein A eluate, which was considered legitimate under the assumption that differences in the respective viscosities of water and the protein solution would be negligible. The protein solution had a concentration of less than 10 g/L.

A 50-L single-use bag was filled with 50 L of purified water representing a ‘worst-case scenario’ for the film surface area in contact with product. Detergent (Triton X-100, MilliporeSigma) was added to the single-use bag (Flexel, Sartorius Stedim Biotech) to a final concentration of 1% (w/v) via the top port of the bag using a peristaltic pump and a calibrated scale to ensure that the required quantity of detergent was added. For GMP operations under aseptic conditions, detergent addition can be performed using a dedicated sample port at the bottom of the bag. The mixing rate was 40 rpm. This gave efficient mixing but did not create vortices or foam. Individual samples were taken after 0, 30, 60, and 120 minutes at a point that was 30 mm below the liquid surface and from a sample port at the bottom of the bag. In this way, the detergent concentration could be assessed at the top and bottom of the container. Once mixing was established and the solution appeared homogeneous, an initial sample was taken at time = 0 minutes.

A control experiment was performed in parallel in a 5-L borosilicate glass bottle with 2105 g of 1% (w/v) detergent (Triton X-100, MilliporeSigma) in purified water. A magnetic mixing plate and stirrer bar was used to mix the solution. The protocol was the same in all other respects. Samples were taken after 0 and 120 minutes.

Sampling was performed using 30-mL sterile glass vials, which were immediately stored at 2–8 °C. The detergent concentration was measured by RP–UHPLC.

**RESULTS**

**Chemical compatibility**

The results of a chemical compatibility study between the single-use bags (Flexel, Sartorius Stedim Biotech) used in conjunction with the magnetic mixer and a 20% (w/v) detergent (Triton X-100, MilliporeSigma) solution are shown in Table III. A category number indicating the criticality of the test is attributed to each test. Higher category numbers indicate a higher level of criticality. The values for all the tests are combined within an overall chemical compatibility calculation score. This scoring system is based on previous experience and product-use knowledge. The results from the tests show that the scale-down mimics combining single-use bags and impeller parts demonstrate excellent chemical compatibility when exposed to 20% detergent solution for 72 hours at 5 °C ± 3 °C. This provides further confidence that the single-use bags for the magnetic mixer will provide a suitable container for the large-scale virus inactivation step.

**Table IV. Results from the extraction with 1% detergent (Triton X-100, MilliporeSigma) solution on a single-use bag (Flexel, Sartorius Stedim Biotech) with impeller parts.**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element impurities</td>
<td>ICP–MS/OES</td>
<td>Potassium and zinc detected at quantifiable and equivalent levels in the blank and bag extract</td>
</tr>
<tr>
<td>Organic leachables</td>
<td>RP–UHPLC-UV</td>
<td>No leachables detected</td>
</tr>
<tr>
<td>Organic leachables</td>
<td>GC–MS</td>
<td>Two hydrocarbon leachables detected at an estimated concentration of 1 ppm each</td>
</tr>
</tbody>
</table>

ICP–MS/OES is inductively coupled plasma mass spectrometry/optical emission spectrometer.
RP–UHPLC–UV is reversed phase ultra-high-performance liquid chromatography with ultraviolet detection.
GC–MS is gas chromatography–mass spectrometry.
Leachable study

Table IV shows the results of the leachables study between the single-use bag (Flexel, Sartorius Stedim Biotech) and 1% detergent (Triton X-100, MilliporeSigma) solution. Although potassium and zinc were detected in the extract solution, they were also detected at the same level in the blank control sample and are considered matrix artifacts. No leachables were detected by the UHPLC–UV method of analysis. Under scale-down conditions, two organic leachables were detected by GC–MS, each with an estimated concentration of 1 ppm which, when combined, give a total leachable concentration of 2 ppm.

These results were extrapolated to the manufacturing process conditions by multiplying the scale-down leachable concentration by the film surface area/volume ratio expected at process scale and dividing by the film surface area/volume ratio encountered during scale-down. This extrapolation gives an anticipated total leachable concentration at the production-scale of approximately 0.3 ppm. The hydrocarbons detected by GC–MS at retention times of 14.2 and 17.1 minutes are most probably intermediate molecular weight C13 and C14 compounds. These would most likely pass through the permeate of subsequent ultrafiltration/diafiltration steps or be removed in the flow-through of subsequent bind and elute chromatography steps.

Translation to industrial scale

Figure 1 shows the results from the detergent (Triton X-100, MilliporeSigma) homogeneity study. All data points were close to the target specification of 1% detergent solution (w/v) and within the specified limits of 9000–12,000 mg detergent.

The difference in detergent concentration between the upper and lower sampling locations within the 50-L bag was 2.7% and therefore did not exceed a maximum specified limit of a 10% difference in concentration. The ability of the magnetic mixer to ensure a homogeneous environment within the bag, therefore, was demonstrated. The concentration of detergent was deemed stable over time in the single-use bag, as the greatest difference between lower and higher measured concentrations was 7.90%, which was within the specified limits of 0 to 10%. The average detergent concentration obtained in the 50-L bag did not exceed a 2.86% difference between that observed in the glass bottle control, thus it is compliant with the ≤10% difference limit.

This study demonstrated that the distribution of detergent (Triton X-100, MilliporeSigma) in the mixing bag is homogeneous even under the worst-case conditions for adsorption of high film contact surface area/volume ratio. To consolidate the results and optimize the set-point for mixing rate, the detergent concentration was measured at 0 minutes and 60 minutes, at the lower and upper part of the bag, during the production of the first mAb batch performed at industrial scale.

CONCLUSION

In this study, the use of a single-use mixing technology was evaluated and characterized for the performance of a detergent-based virus inactivation step during a mAb production process. Testing showed that no significant adsorption of detergent (Triton X-100, MilliporeSigma) to the bioprocess container occurred. Enveloped virus-spiking studies demonstrated that a minimum reduction factor of 3.8 log$_{10}$ could be expected in this single-use step and that, in most cases, the reduction factor would be greater than ≥5.0 log$_{10}$. Further tests showed that the single-use bags (Flexel, Sartorius Stedim Biotech) for the magnetic mixer had excellent chemical compatibility with a 20% solution of the detergent and that leachables were negligible when extractions were performed with 1% detergent solution. Finally, a scale-up study showed that the magnetic mixer provided sufficient agitation to ensure detergent homogeneity within the single-use bag.

The data obtained during this study was considered compelling, and LFB
Fast-Track Clone Selection and Cell Line Optimization Workflows: Automating Sample Prep to Screen Hundreds of N-Glycan Samples per Day

ON-DEMAND WEBCAST  Aired November 2, 2018

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EVENT OVERVIEW:
New high-throughput N-glycan screening technologies can rapidly analyze large sample sets—providing you with the necessary results to make decisions on clone selection and cell line optimization in real time.

Learn a new automated sample preparation protocol that can decrease your analysis time by as much as 40% using the Biomek i5 Liquid Handler Platform. This workflow greatly simplifies the process by minimizing human intervention and variability in results.

Key Learning Objectives
- Learn how to screen hundreds of N-glycans per day
- Reduce sample prep time for a 96-well plate to less than 2 hours using automation
- Discover tips and tricks to reproducibly prepare glycan samples
- See how the SCIEX C100HT software can:
  - Identify glycans
  - Separate samples by your N-glycan profile’s pass/fail criteria

Who Should Attend
- Bioprocessing laboratory managers and scientists at biopharmaceutical companies and contract research labs performing clone selection and cell culture optimization
- LC and CE users looking for increased screening capabilities

 Presenter
Tingting Li
Senior Biopharma Application Development Scientist, Biologics
SCIEX

 Moderator
Kaylynn Chiarello-Ebner
Managing Editor, Special Projects
LCGC

For questions contact Kristen Moore at kristen.moore@ubm.com
Advanced therapy medicinal products (ATMPs), based on genes, cells, or tissues, are targeted therapies that deliver a therapeutic benefit to a patient-specific population and often treat rare diseases or improve upon existing therapies. Because these products contrast with current biomanufacturing processes for compounds that are synthetically derived (i.e., small molecule) or proteins or peptides expressed by cellular systems (i.e., large-molecule biopharmaceutical), they are often faced with unique manufacturing challenges that must be supported by appropriate facility designs.

Cell therapy products, either autologous or allogenic, are manipulated whole living cells that act at the cellular level to treat disease or injury. Gene therapy consists of recombinant nucleic acid as the active substance that will regulate, repair, replace, add, or delete a genetic sequence in the patient.

Preparation of an autologous cell-therapy presents a significant departure from typical biopharmaceutical manufacturing in that it is manufactured from cells obtained from the patient. Collected by apheresis, the cells are modified, expanded, and returned to the patient. This process presents a difficult challenge to scalability. One batch will treat one individual patient throughout the treatment cycle, so the ATMP volumes are currently small scale, and the presence of whole cells prevents typical bioburden reduction steps, such as filtration through sterilizing grade filters. All processes must be aseptic to prevent the introduction of any contaminant/adulterant.

Batch identity and tracking must be flawless when concurrently processing treatments for multiple patients. Starting material becomes part of the manufacturing process and requires the collection site to be qualified in the specific apheresis and tissue-collection methods along with the shipping preparation process. Control of storage conditions and time during transport of the raw material and finished product is critical to maintain cell viability.

In contrast, some allogeneic cell therapies can be used to treat multiple patients, enabling larger manufacturing process scales, but the contamination control challenges remain the same. It also eliminates the challenge of patient cell harvest and transport.

ROBERT DREAM AND JEFFERY ODUM

Advanced therapy medicinal products pose unique manufacturing challenges that will require appropriate and thoughtful facility design and equipment.
In gene therapy where a viral vector is used to genetically modify the patient’s cells, the preparation of the viral vector presents a second manufacturing challenge. A gene that is inserted directly into a cell will, under most circumstances, not function. The vector becomes the carrier of the gene to the infected cell (1). Scale can vary from traditional lab scale up to 2000-L scale because a single lot can be used to transduce cells from a single patient or many different patients.

These processes are similar to typical biopharmaceutical manufacturing platforms that expand a frozen cell line, but the twist is that a virus is purposely introduced into the culture to manufacture viral particles.

Cell-therapy manufacturing, like all current fed batch-based manufacturing processes, is generally segmented into a series of discrete unit operations that may differ between cell types according to the specific needs of the product. But one important difference between these two different manufacturing platforms is in the source of the cells that are used. Traditional monoclonal antibody (mAb)-based protein products use genetically modified cells that have been characterized and tested. Cell therapies come direct from patient donors and therefore have limited potential for the exponential expansion seen in protein-based products, thus the challenge of scale-up.

A typical good manufacturing practices (GMP) process for cell-based allogenic or autologous products follow the general steps identified in Figures 1 and 2. In these processes, closed systems, aseptic operations, and automated solutions will play key roles in defining the manufacturing operations.

Gene-therapy manufacturing is also a GMP-focused process that follows a similar unit-operations manufacturing approach, but generally involves fewer and often simpler steps.

Some critical aspects of the manufacture of ATMPs for both cell and gene therapies include:

- Ensuring the integrity and chain-of-custody of the initial cellular material
- Standardization of procedures, protocols, and testing of materials
- Logistical control, especially in the areas of temperature control and storage
- Equipment and process validation.

**FUTURE CHALLENGES**

The current baseline model defining the majority of biomanufacturing operations for human therapeutics (proteins) is batch driven. Here, the “batch” is based on a paradigm where the target protein is well characterized, screened, banked, and optimized and is focused on a large, well-defined patient population where the goal is a “one-size-fits-all” drug. This model implements a basic flow of well-defined and robust unit operations, well-characterized product and process attributes, and a focus on product quality and risk that has been determined over four decades of regulatory GMP oversight.

ATMPs are personalized, targeting either specific groups of patients or individual patients, so efficient commercial production will not be achieved with the large process volumes and higher titers of traditional biopharmaceuticals. Although many unit operations are similar (cell culture, concentration, freeze/thaw, etc.) industrialization of these operations at small scale through robotics and novel equipment will provide the needed scalability.

The sum of intrinsic characteristics associated with ATMPs results in manufacturing-specific requirements that significantly deviate from current “typical” biologic products (e.g., mAb). The patient-centric nature of autologous cell therapies requires small volumes and a limited number of batches (e.g., single patient) for an entire treatment duration. Additionally, the presence of cells prevents the use of sterile filtration technology, so all manufacturing steps should be aseptic by both definition and operation.

This scale of manufacturing and the specific 1:1 treatment-to-patient nature of autologous therapies do often mimic hospital laboratory or compounding pharmacy operations, but the need to produce these therapies for larger patient populations in a safe, pure, and effective manner will require GMP-regulated facilities (2).
MANUFACTURING FOCUS

A manufacturing process can be either open or closed. If the primary goal is to protect the product during the manufacturing operations and transfer, closed processing presents less of a risk to the product. This is a risk that primarily comes from the ability to control the immediate manufacturing environment. The GMP goal is to ensure the product is never exposed to the environment unless the environment is constantly maintained as bioburden-free.

Aseptic operations, “[p]rocesses that are devoid of measurable (detectable) bioburden” (3), generally require sterilization of the environment, equipment, and process solutions to achieve the desired state prior to use (4). Current FDA guidance (5) is often associated with the manufacture of sterile injectable drug product(s) and applies to the manufacture of all cell-based ATMPs to prevent contamination by foreign cells that would pose an unacceptable risk to the patient.

RISK MITIGATION

In Europe, GMP requirements for ATMPs were adopted at the end of 2017 by the European Commission (EC). As a result, “other documents developing GMP requirements for medicinal products which are contained in Volume 4 are not applicable to ATMPs, unless specific reference thereto is made in these Guidelines” (2).

Despite this specific GMP ruling for ATMPs, the GMP principles are the same as for traditional biologic products. ATMP production, therefore, must be designed to meet the most stringent GMP requirements to guarantee quality and avoid contamination or cross-contamination. If open processes are implemented, then manufacturing must be conducted under Grade A conditions with the appropriate surrounding background environment (Grade B for open systems and Grade C or D for isolator-based systems) with dedicated areas or some form of time-based segregation for each patient-specific batch to avoid environmental contamination and/or cross-contamination between each batch.

A closed system is preferred in ATMP manufacturing to mitigate risk and achieve optimized production. This has been long discussed (6) and adopted in global GMP regulations. Closure can be claimed at the primary packaging level (i.e., equipment, system) or at the enclosure level (i.e., an isolator). Both scenarios are also supported by the full implementation of single-use systems to minimize exposure, cleaning, and sterilization of product contact surfaces.

Proof-of-closure is a challenge. It must be demonstrated that the system is designed and operated as closed. A structured closure analysis (7) as well as a strong risk assessment (3) can support such a claim. This is not limited to normal production and should address non-routine operations such as breaches or maintenance incursions.

Requirements for ATMP production can be even more rigorous, considering the nature of an aseptic drug product manufacturing process. The use of large numbers of manual single-use sterile connections and the place where they occur have been questioned as a risk by global regulators (8). The demand to cover (aseptic) lines under Class 100 (ISO 5) when they require intensive manual intervention could become a prescriptive requirement applicable to the aseptic processing of ATMPs. It will be important that the industry continue applying risk management and validation/verification studies for system closure before adapting easier prescriptive GMPs.

NEED FOR AUTOMATION

ATMP clinical development generally focuses on a small number of patients where the source material from individuals comes from a single site equipped to carry out the required collection operations. Testing of the cells, while time consuming, is manageable for these small volumes/donors.

Scale-out challenges moving into commercial manufacturing, however, will drive the demand and development of new, specialized tooling and instrumentation to automate what are now manual manipulations. This will further enhance the robustness of the commercial process and should notably reduce the risk of contamination. It is a well-documented fact that human presence, intervention, and touch-points are the most significant risk factors and sources of contamination (9). Aggressive implementation of system closure and automation will likely define the success for future lean ATMP manufacturing.

INNOVATION AND EXPANSION

Manufacturing techniques and equipment are evolving rapidly in the ATMP commercial space, and adoption of improvements requires verification that outcomes are comparable to the previous clinical results. This is achieved through process validation and product comparability studies, both of which rely heavily on strong quality by design implementation and well-developed manufacturing control strategies.

The ability of the process to meet quality and reproducibility requirements requires early stage clinical development of product and therapy-specific critical quality attributes (CQAs) that will address both in vitro behavior during cell transduction and expansion, as well as in vivo function and performance (potency, efficacy, etc.) (10). For example, current process analytical technology and automation sensor capabilities may require advancement of in-line/on-line technology to adequately control defined critical process parameters (CPPs) through the ATMP manufacturing process.

Patient-specific starting materials (autologous) and the collection of that material at a clinical site introduce a significant variability at the beginning of the manufacturing process. Often, this activity occurs in a hospital or clinic that is controlled not by GMP but by current good tissue practices. In many cases, this is seen not as a manufacturing operation but more of an extension of patient therapy.
It is crucial to standardize these operations across multiple sites/operators to minimize patient-to-patient variability and ensure GMP compliance under a defined quality management system. Rapid and robust collection, handling, processing, and testing are critical to patient safety and effective therapy. This aspect of material supply/control also necessitates well-defined supply chain control, from track-and-trace sourcing and temperature control through manufacturing and cryopreservation of finished product. This time-critical, circular supply chain model is at the heart of the manufacturing and quality infrastructure for ATMP therapies and is markedly different than the deliberate, methodical linear supply chain of typical mAb production. For some ATMPs, the timely, successful completion of the manufacturing process is a CPP. Commercially paced quality/integrity, batch record reviews, and release testing will again require highly automated platforms. The need for real-time visibility and control of manufacturing and supply chains will drive many Industry 4.0 solutions into implementation.

Manufacturing and supply of autologous products must address chain-of-identity through well-documented steps. It is recognized that the implementation of higher levels of automation will facilitate scale-out of manufacturing and logistics of these types of products. In addition, compliant change-control management and sound comparability practices are critical to perform development and manufacturing lifecycle activities.

ATMP development will require an integrated approach with end-to-end data management and analysis. Understanding CQAs and CPPs will be key to a successful development and product commercialization. This should start from the initial material drawn *ex vivo* from patients through to the clinical response to ensure the quality of commercial ATMP products over their entire lifecycle.

**MANUFACTURING FLEXIBILITY**

Capacity planning for ATMPs and traditional biologic therapies share the same challenging landscape of unknown regulatory timing, uncertain demand, and highly variable reimbursement decisions. A flexible facility design will contribute to reduced financial risk as products progress from Phase I to Phase III clinical and ultimately commercial launch (or patient use). Just as with traditional biologics, changes in product demand, process improvements, or increases in automation implementation, and continued process improvements during the clinical phases will challenge the facility attributes around flexibility.

ATMPs can be manufactured in a multi-product-based facility that is designed to address the potential risk factors normally associated with this type of manufacturing flexibility. Risk factors include product contamination, product mix-ups, human, and documentation errors.

Current regulatory focus around flexibility remains on product protection. As an example, the European Union (EU) clearly defines expectations around concurrent manufacturing and batch/product protection in its guidance (2). Other examples of flexible attributes that are acceptable in the case of investigational ATMPs by European Medicines Agency (EMA) regulators (Figure 3) that do mirror FDA requirements include:

- For first-in-man clinical trials, production in an open environment may be performed in a critical clean area of grade A in a background clean area of grade C if appropriate controls of microbiological contamination, separation of processing procedures, and validated cleaning and disinfection are put in place. A risk-analysis study should be conducted, and it should be demonstrated that the implemented control measures are adequate to ensure aseptic manufacturing.
- In early phases of clinical research (clinical trial Phases I/II), when the manufacturing activity is very low, annual calibration, inspection, or checking can be limited to the

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*Figure 3. Decision tree for advanced therapy medicinal products (ATMPs).*

Source: European Medicines Agency and author analysis.
facility, cabinets, incubators, isolators, freezers, air sampler, and particle counters, unless a lower frequency is justified due to periodicity of use. The rest of the equipment could be tested less frequently based on a risk analysis and the production activity. The suitability for use of all equipment should be verified before it is used.

**QUALIFICATION OF FACILITY AND EQUIPMENT**

Many of the fundamental GMP requirements for equipment and facility qualification and validation are similar to those implemented for traditional biologic therapeutics manufactured in fed-batch process platforms. Again, the EU is clear regarding the principles of qualification for ATMPs (2). Some of the key qualification attributes outlined in the EU guidance include:

- Clean rooms should be qualified in accordance with ISO 14644-1 and re-qualified at appropriate intervals in accordance with ISO 14644-2.
- If computerized systems are used, their validation should be proportionate to the impact thereof on the quality of the product.
- For investigational ATMPs, it is expected that at least the suitability of the air quality system (in accordance with ISO 14644) and the suitability of the premises to adequately control the risk of microbial and non-viable particle contamination is verified. Any other aspect of the premises that is critical having regard to the specific risks of the intended manufacturing process should be qualified (e.g., containment measures when viral replicating vectors are used).
- Critical equipment should also be qualified.
- It is also recommended that a traditional design qualification/installation qualification/operation qualification/performance qualification approach for the facility design and qualification be conducted to ensure compliance with user requirements and supplier specifications.

**CONCLUSION**

One of the early key challenges facing the development-to-commercialization of ATMPs is how to ensure these products are meeting current GMP guidelines, regardless of whether they are being developed in an academic or commercial environment. ATMP manufacturing design spaces are subject to GMP protocols and regulatory guidance; regulatory mandates enforced by national agencies but internationally harmonized to ensure production of high quality non-adulterated drug products that pose no risk to patients and or the public. ATMP manufacturing requires a stringent and carefully controlled bioprocess to control the intrinsically complex and variable nature of cell and gene therapy drug substances and products.

The value chain for ATMPs places notable emphasis on novel manufacturing solutions. Manufacturers are now limited by the usefulness and scale of available manufacturing solutions. Innovation of scalable bioprocessing solutions is crucial for the commercial success of advanced therapies over the next 5–10 years.

Current bioprocessing solutions are largely adopted from biopharmaceutical manufacturing supply chains and are usable but sub-optimal for long-term commercial sustainability because of the potential for high failure risks, high manufacturing costs, and inflexibility for optimization. Advanced therapies are currently manufactured through manual, labor intensive processes that limit the supply, demand high production costs, and ultimately hinder return on investment (ROI). The long-term unsustainability of this model is becoming increasingly apparent as technology developers realize the importance of innovative manufacturing solutions.

Designing and implementing advanced manufacturing solutions early in ATMP product development is crucial to controlling risk. Upfront process development and manufacturing optimization before the major value inflections offered by clinical trial results is an undeniably high-risk investment, compounded by a relatively long time to ROI. Historical and ongoing case studies demonstrate that manufacturing remains central to controlling therapy cost and, therefore, facility design and that flexibility concepts remain central to the commercial success of ATMPs.

**ACKNOWLEDGEMENTS**

The authors would like to thank Andre Walker of Andre Walker Consulting, David Estape, technology manager Biotechnology at CRB, and Diane Dream, associate partner at Infosys Consulting, for their contributions.

**REFERENCES**

Timely, Accurate Analytical Purity Data Helps to Avoid Late Surprises When Scaling Up
Automated TEM Analysis Data of Gene Therapy Vectors and VLPs

ON-DEMAND WEBCAST

Register for free at http://www.biopharminternational.com/bp_p/puritydata
Can’t make the live webcast? Register now and view it on-demand after the air date.

While 77% of current trials for gene therapies are still in Phase I or Phase II, more and more manufacturers are moving into commercial phase to meet challenges with scale up and regulatory requirements. As a result, more developers are focusing on purity and how the purity profile of the sample varies under different process conditions and scales.

Robust, accurate analytical solutions are required to monitor process change to avoid late surprises during scale up. Transmission electron microscopy (TEM) can provide unique insights in the characterization of viral gene delivery platforms such as adeno-associated viruses, adenoviruses, and retroviruses such as lentiviruses.

**KEY LEARNING OBJECTIVES**

In this webcast, learn how a TEM system with automated imaging and image analysis can be used to:

- Compare metrics on the purity profile of the viral particle sample
- Automatically differentiate intact viral particles from process related impurities
- Develop a correlation between potency and viral particle morphology

**WHO SHOULD ATTEND:**

Scientists, senior scientists, and department heads in analytics, formulation, process development, and biophysics involved in vaccine and gene therapy process development.

**PRESENTERS**

Josefina Nilsson
Head of Business Unit
EM Service Vironova

Martin Ryner
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For questions contact Kristen Moore at Kristen.Moore@ubm.com
Almost all drug products, whether they are small molecules or biologic in nature, will require some level of comparability studies to be performed. Whether they are called 'sameness' for generic drugs, 'comparability' for biologics, or 'analytical similarity' for biosimilars, these tests are crucial for any regulatory submission, which is vital to the entire product development process.

Standard testing routines comprise a selection of experimental techniques chosen because of their ability to monitor various quality attributes of the molecule. These are typically among those listed in the International Council for Harmonization (ICH) Q6B guidelines (1), although it is also important to note that complex generic APIs generally call for more creative analytical solutions. The quality attributes that are assessed include higher order structure for macromolecules, which involves the characterization and comparison of secondary, tertiary, and quaternary structures.

There are several standardized techniques used for the elucidation of secondary, tertiary, and quaternary characteristics of macromolecules. For secondary structural characterization, for example, common industry-standard techniques are far-ultraviolet circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR). While these two techniques are orthogonal, they are also complementary in that they are biased counter to one another: CD is biased toward reporting on α-helical content; and FTIR is biased toward reporting on β-sheet content. Similarly, for reporting on quaternary structure, analytical ultracentrifugation (AUC) and size-exclusion chromatography with multi-angle static light scattering (SEC-MALS) are commonly used orthogonal techniques, with respective over-reporting and under-reporting of oligomeric species. Instrumental techniques are often selected based on familiarity, commercialization, and efficiency. While these are all good criterion for routine method selection, the techniques themselves are not necessarily aptly suited to specific molecules or systems.

**Using Multiple Techniques in Biosimilar Analysis**

Application of multiple techniques at different conditions presents a more complete picture of a dynamic situation.

**REBECCA STRAWN**

REBECCA STRAWN, PhD, is senior biophysical scientist at SGS Life Sciences, West Chester, PA.
Each molecule and/or formulation system can present unique obstacles. For example, the sequence of the molecule may preclude certain techniques, or make other techniques difficult because of the size or molecular weight of the product. Similarly, the formulation buffer may include excipients that cause some techniques to fail, or the formulation concentration of the API may be too low to detect using certain techniques. These occasions may lead to the inability to truly build any orthogonality into the high order structure analytical comparability. When these issues are encountered, it is key to empirically define the critical parameters of the available techniques in order to use each of them to the fullest advantage.

One criterion that is crucial for demonstrating suitability of a given technique is its ability to exhibit being “fit for purpose”; specifically, the method should be able to demonstrate specificity, or the ability to distinguish between non-degraded and degraded samples. However, despite this recommendation appearing in both EU and US guidelines for comparability studies, it is key to empirically define the critical parameters of the available techniques in order to use each of them to the fullest advantage.

One criterion that is crucial for demonstrating suitability of a given technique is its ability to exhibit being “fit for purpose”. Proteins are dynamic molecules that do not exist in a single conformation, but as a complex distribution of an ensemble of different conformations. This dynamic conformational landscape will commonly have a complex system of local energy minima under different conditions. As a result, while several different production batches of a protein may have a similar conformation when assessed at the same temperature, if the temperature changes, the conformational populations may structurally diverge from each other as they explore their independent dynamic landscapes. Therefore, if macromolecules are characterized and compared at different temperatures, the comparability assessment will be more reliable. Comparability should at the very least be evaluated at both the room temperature industry standard, and also at 37 °C, which is more biologically relevant.

**CASE STUDY: PRELIMINARY PEPTIDE ANALYSIS**

In this example, a medium-sized peptide was investigated. This system presented challenges due to the small size and limited structure of the peptide. Two orthogonal, industry-standard structural characterization techniques were explored and assessed for suitability.

The first technique employed was CD. Samples were prepared to approximately 0.2 mg/mL in an appropriate buffer, and CD measurements were made using a Jasco J-815 CD spectropolarimeter; the spectra were analyzed for secondary structural composition using the Cdsstr (2) program. While the small size of the peptide likely precludes structural relevance to the fitting values, they can serve as a comparative metric between samples. The spectra suggest that, while the samples show...
The general similarity above about 25 °C, the alpha-helix and beta-sheet content changes below this temperature (Figure 1). This may suggest that CD can report on sample differences as a function of temperature, thereby demonstrating specificity.

Experiments were also conducted on a Bruker Tensor 37 FTIR instrument with a photovoltaic MCT detector, using an AquaSpec cell; structural fitting was done via the vendor-specific software Opus. Buffer-subtracted spectra were generated by acquiring a background measurement of the formulation buffer immediately prior to spectrum acquisition for each sample. At all temperatures investigated, the structural components of the FTIR samples were similar (Figure 2). These results are in contrast to those observed with CD, which may highlight the potential limitation of this experimental technique, and may indicate that FTIR is less sensitive at discerning structural differences.

While a full method qualification of specific quality attributes requires much more statistical rigor, this preliminary case study demonstrates that exploring a broader landscape may add valuable comparative power. It also illustrates that exploring orthogonal techniques, and indeed, letting them 'compete' with one another, can enhance experimental design, especially for products challenging the resilience of a given methodology and/or technique.

THE IMPORTANCE OF MULTIPLE CONDITIONS
It is clear from these studies of the same peptide—at different temperatures, using different techniques—that there was divergence in higher order structure. These results highlight the inherent dynamic nature of peptides and proteins and illustrate the need for increasing the experimental scope of comparability studies. In fact, the regulatory agencies are continuously looking for more rigorous analytical strategies to have greater confidence in the obtained results, and to ensure accurate and reliable conclusions can be drawn from comparability/same-ness/biosimilarity studies.

Testing biologic molecules under multiple conditions greatly benefits study design. It gives multiple testing points within a single experimental technique, thus expanding its comparative power. This is especially beneficial in those instances when a system cannot be tested using multiple orthogonal techniques because of experimental limitations. Using appropriate conditions would also lead to the generation of representative forced degraded forms, which are necessary to establish the specificity of any analytical methodology.

It also gives an insight into the dynamic landscape of the material and allows a more complete characterization to be made at various temperatures, or a range of other conditions. This can aid in understanding the different degradation pathways that might be in play, another factor that is a key element of a comparability study.

Overall, the application of multiple techniques at different conditions will give a far better understanding of the molecule. The application of multiple techniques at different conditions will give a far better understanding of the molecule.

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Overall, the application of multiple techniques at different conditions will give a far better understanding of the molecule and its biological activity and will mitigate the risks of obtaining restricted information when taking a snapshot at a single set of conditions. This approach may give a more complete picture of a dynamic situation.

REFERENCES
Over the past several months, manufacturers have introduced new and updated products to improve biopharmaceutical operations. The following is a sampling of some of these new product options.

**SINGLE-USE SYSTEM ENABLES PROCESS AND PRODUCT PROTECTION**

Parker Bioscience’s SciLog SciPure FD System is an automated single-use system for the filtration and dispensing of products into either bottles or bags (1).

The new system offers process protection at the bulk pack-off stage of API production by combining the fully automated bulk filtration and dispensing of biopharmaceutical products with a shipping solution that ensures product integrity. Additionally, the system’s hardware and consumables have been developed in tandem to minimize product losses and maximize yield.

The company’s fully validated shipping solution complements and extends the capabilities of the SciLog SciPure FD System. The company states that it has created a patent-pending bottle design that offers manufacturers the confidence that bulk drug products will arrive at their final destinations without contamination. The bottle integrity has been validated down to -89 °C while undergoing a validated ASTM D4169 shipping study for performance testing of shipping containers and systems.

**NEW PERFUSION-ENABLED BIOREACTORS**

On Sept. 4, 2018, Sartorius Stedim Biotech (SSB) and Repligen Corporation announced a collaboration agreement to integrate Repligen’s XCell ATF cell-retention control technology into SSB’s BIOSTAT STR large-scale, single-use bioreactors to create next-generation perfusion-enabled bioreactors (2).

A single control system for 50–2000 L bioreactors used in perfusion cell-culture applications is designed to control cell growth, fluid management, and cell retention in continuous and intensified bioprocessing and simplify the development and cGMP manufacture of biological drugs.

Additionally, the companies will also collaborate to equip SSB’s recently launched ambr 250 high throughput (ht) perfusion single-use mini bioreactor system with Repligen’s...
KrosFlo hollow-fiber filter technology. The bioreactor system will be sold by SSB as a complete single-use assembly. This design conserves hollow-fiber filter technology across scales, enabling users to fast-track development and scale up their cell-culture perfusion processes, SSB reports. The bioreactor system will be sold by SSB as a complete single-use assembly. This design conserves hollow-fiber filter technology across scales, enabling users to fast-track development and scale up their cell-culture perfusion processes, SSB reports.

MINI MICROCARRESTER FOR CULTURING ADHERENT CELLS
Also from SSB comes a new mini bioreactor vessel for its ambr 250 ht system (3). The new single-use vessel is based on cell-culture bioreactors in the ambr 250 ht system and designed for optimal growth of adherent cells on microcarriers, enabling the rapid, scalable cell-culture process development of vaccines, the company reports.

The mini bioreactor has a working volume of 100–250 mL and features a single Elephant Ear impeller. This impeller type generates improved mixing and suspension of microcarriers, allowing adherent cells to grow over the entire microcarrier surface. Using this bioreactor on the ambr 250 ht system allows rapid scale-up of optimized adherent cell culture processes to the company’s BIOSTAT STR range of pilot- and manufacturing-scale stirred bioreactors.

The company claims that this results in shorter process development timelines than would be achieved by scientists using benchtop bioreactors and spinner flasks. The mini bioreactor reduces set-up and turnaround time and is suitable for design-of-experiments studies, as stated by SSB.

UPDATED PURIFIER PRODUCT FOR CLARIFICATION
3M added new features to its Emphaze AEX Hybrid Purifier that include sterilization/sanitization compatibility and additional sizes (4).

The new Emphaze capsules are sterilization/sanitization compatible and can be used across various aqueous-based biopharmaceutical processes, including vaccine purification and now include two new laboratory-scale capsules and a scale-up capsule, allowing for evaluations at laboratory and scale-up volumes.

Emphaze AEX Hybrid Purifier products are synthetic multi-mechanism single-use purifier products used for biopharmaceutical clarification. They deliver consistent, high-purity clarified process fluid by removing soluble and insoluble impurities, such as DNA, host cell protein (HCP), and cell debris, through a combination of chromatographic and size-exclusion mechanisms. The product combines three 3M technologies—advanced polymer materials, fine fiber nonwovens, and membranes—to deliver a synthetic clarifying product line containing a novel anion exchange nonwoven media and a fine particle, reduction membrane.

“Customer input is critical in evaluating the performance and effectiveness of any product to maximize performance,” said Himanshu Nivsarkar, global marketing manager, 3M Separation and Purification Sciences Division, in a company press release. “With the Emphaze AEX Hybrid Purifier, customers experience benefits in typical monoclonal antibody purification processes, including nominal 30% HCP and greater-than-4-log DNA reduction. It has a consistent output turbidity (<5 nephelometric turbidity unit) leading to potential downsizing of the sterilizing grade membrane and increased product purity post-protein A. This also reduces turbidity post viral inactivation/neutralization step leading to a lower impurities load on the downstream AEX column, thus allowing customers to optimize their processes.”

DETACHABLE AODD PUMP
Wilden, part of PSG, a Dover company, released its new 6 mm (1/4”) Velocity Series air-operated double-diaphragm (AODD) pump (5). The V2550 Velocity Series pump features a detachable mounting foot, providing adaptable mounting capabilities suitable for all small-dosing applications.

The company reports that the pump can be reoriented by loosening a single screw. The design provides the user with multiple mounting options while allowing the pump to fit into restricted spaces, a critical consideration for original equipment manufacturer systems and skids.

The pump’s design also eliminates the effects of torque decay and delivers improved dry suction lift at all operating parameters for better priming under a variety of system conditions. Additionally, the pumps feature an interchangeable footprint with competitor and previous company designs. This pump series comes equipped with a simple air distribution system (ADS) with two moving parts that reduce the risk of downtime, according to the company. Wilden Velocity Series pumps can handle suction lifts from 10–14 feet. These pumps are available in polypropylene and PVDF construction, as well as an Accu-Flo (solenoid) option (5).

REFERENCES
The increasing complexity of biologic drugs in development is in part driving the need for innovative delivery mechanisms. Innovation in syringe technology is one example of how drugmakers strive to address the challenges of administering these newer therapeutics.

The rapid growth of newer and targeted molecular entities, such as monoclonal antibodies (mAbs), interferons, peptides, vaccines, and ophthalmics; the increasing prevalence of chronic diseases and cancers; and the growth of biosimilars are driving the growth of prefilled syringes. Another factor that contributes to this growth is the need for improved safety and ease of administration for patients and healthcare workers. Prefilled syringes are known to be easy to use and to need only a few administrative steps, which contributes to improved safety (1). In talking with BioPharm International, Dr. Nicolas Eon, global product manager of syriQ at SCHOTT, and Claudia Roth, PhD, vice-president Innovation Management at Vetter, discussed key challenges, required standards, and the potential future impact of biologic drug development on the prefilled syringes market.

**BIOLOGIC DRUG CHALLENGES**

**BioPharm:** What are the main challenges or deficits in prefilled syringe technology today, specifically challenges meeting the needs of biologic drugs?

**Eon (SCHOTT):** Over two-thirds of the drugs in the development pipeline of pharmaceutical companies are biologics. These drugs offer new treatment options for some of the hardest-to-treat diseases, such as cancer, and chronic diseases. Yet their highly complex molecule structures are more sensitive to temperature and environmental conditions and are prone to interactions with the pharmaceutical container. Hence, biologics require innovative packaging to ensure drug stability over the entire shelf life.

With prefilled glass syringes, the drug comes into contact with up to seven materials, with each presenting a potential risk of interaction. Subsequently, it is of high importance to reduce this risk by using components made of high quality as well as optimized processes. For example, when it comes to gluing the needle, a process optimization of the gluing step can reduce drug/container interactions by minimizing the quantity of residuals glue monomer.
Moreover, biologic drugs tend to be highly viscous, which means that more force is needed during administration. In addition, over 75% of biologics are used in combination with an auto-injector for self-administration. Subsequently, the prefilled syringe must meet highest accurate dimensions as well as ensure that the gliding performance remains consistent from one injection to the next. The mechanical strength of the glass container must also withstand the pressure applied during the administration process.

**BioPharm**: What specifications are required for packaging biologic drugs in prefilled syringes?  

**Roth (Vetter)**: As is often the case, creating easy-to-apply systems means a more complicated production process at the outset. As we know, a prefilled syringe system is a complex construct because it has many individual components. That is why it is critical to achieve the correct individual conjunction and interaction of its parts in order to enable both functionality and usability. For example, when administering a drug with a syringe, you need to have a plunger rod that operates in concert with the other components, especially the glass barrel. To achieve this, a slight coating of lubricant is placed on the inner surface of the glass barrel. Therefore, a key challenge is to define the appropriate coating process and to control the correct amount of lubricant needed to enable the best possible movement of the plunger rod. This must be achieved in a manner that avoids any form of interaction between the lubricant and the drug substance.

In the biologics sector where the active ingredients of compounds are large and very complex molecules, their manufacture is equally complex and challenging. Thus, a vast degree of experience and a substantial level of process expertise is required. Biologics react with far greater sensitivity to environmental influences such as heat or light than other substances do. That is why it is important to design, develop, and implement the correct product-related production processes. For example, using a suitable dosing method and proper pumps in the filling process is critical.

Another example is the fact that biologics can react negatively to the chemical composition of the stopper within the syringe. For manufacturers, this means it is important to have significant knowledge of packaging materials and components.

**Eon (SCHOTT)**: The Parenteral Drug Association’s (PDA) Technical Report (TR) No. 73, Prefilled Syringe User Requirements for Biotechnology Applications (2), published in 2015, can be used as a baseline to design syringes for biological drugs. Depending on the drug and the intended use, the detailed specification must be agreed upon between the biopharma company and the prefilled syringe supplier.

**INNOVATIONS AND FUTURE IMPACT**

**BioPharm**: What innovations in prefilled syringes are currently on the market, or in development, to address the challenges that biologic drugs face?  

**Eon (SCHOTT)**: SCHOTT recently launched syriQ_BioPure, prefillable glass syringes that are specifically designed for self-administered biologics. These syringes ensure drug stability during shelf life and ease-of-administration for patients. The syringes are made of highly inert Fiolax borosilicate glass and are manufactured under improved processes to lower tungsten oxide and adhesive residuals. As more and more biologics are administered in combination with a device, the syringes work with leading safety and auto-injector devices, thus enabling patients to move from hospital to homecare. A uniform silicone layer ensures that the flow rate of the injection process is as smooth as possible, thus enhancing the patient experience.

**Roth (Vetter)**: In our opinion, despite all the innovative activities being considered today, quality will always be the leading criteria of achievement. After all, we are in an industry where patients literally get injected with the products we manufacture for pharma and biotech companies. It is for our customer, as well as the patient, that we make every effort to be as innovative as possible while striving to achieve the highest-possible manufacturing and process quality on a day-by-day basis.

Another central target is achieving compatibility between the active ingredients of a drug and its method of administration. That means it has to be as ‘end-user friendly’ as possible. It is a well-accepted principal that the higher the usability of a drug-delivery system, the greater the acceptance among patients and caregivers. The reasons for this include the homecare sector where a patient-friendly system fits well and contributes to cost avoidance and time-intensive visit to doctors’ offices and hospitals. Also, patient adherence itself is strongly dependent on the convenience level of the drug-delivery system.

To put it simply, whatever protects or increases the safety of a drug-delivery system while increasing its usability is an enhancement and an innovative advance for the market and, ultimately, for the patient.

**BioPharm**: What trends in biologic drug development do you think will have an impact on the future development of prefilled syringe technology?  

**Eon (SCHOTT)**: One trend we see is the higher complexity of biologics as well as the higher sensitivity of the drugs. This results in the need for a superior extractables and
leachables profile of the pharmaceutical primary packaging. More specifically, this means that the amount of tungsten and glue residual must be minimized and the selected rubber formulations carefully considered. In addition, the options for lubrication, such as the use of silicone, must be evaluated.

Another trend is the move from hospital to homecare to allow patients to self-administer the drugs in an environment where they feel most comfortable. To ensure that this is as easy and safe for the patient as possible, the self-injection is handled in combination with a device, such as auto-injectors. For the development of prefilled syringes this means that the container must be compatible with a number of devices and meet the specific requirements and dimensions.

The rise in personalized medicine, including biologics, targeting small target groups also effects the development of prefilled syringes. Due to the smaller batch sizes that are required, a new supply chain concept and a flexible platform approach are needed. This flexibility can be introduced early on in the drug development process by using standard secondary packaging for the primary packaging. For example, SCHOTT recently launched its iQ platform concept in which syringes, including syriQ BioPure, are packaged in a standardized tub, which simplifies the changeover from, for example, vials to prefilled syringes to run on one filling line. Subsequently, the syringe development needs to fit in with a broader picture in order to meet market demands.

Lastly, the industry is confronted with more stringent regulations, which require the packaging to provide 'design history file-ready' documentation and be 'essentially free of particle'. SCHOTT continuously improves its process and online inspection technology to ensure compliance with current and future regulatory requirements.

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2. PDA, TR No. 73, Prefilled Syringe User Requirements for Biotechnology Applications, 2015, https://store.pda.org/TableOfContents/TR73_TOC.pdf.
Biomanufacturing plans to implement the virus inactivation step using single-use technology as part of their mAb production platform.

**Note:** A recent amendment of Annex XIV in the European Union (EU) REACH regulation (10) will lead to the substance 4-(1,1,3,3-tetramethylbuthyl)phenol, ethoxylated being prohibited from Jan. 4, 2021 unless an authorization is granted. The Triton X-100 (MilliporeSigma) family is included in this new amendment based on the fact that it degrades to the substance in this new amendment based on the impact of the compound’s degradation due to concerns over the environmental impact of the compound’s degradation products. For this reason, European Union biomanufacturers must seek alternatives to Triton X-100 with equivalent efficacy and safety. The research in this article is valuable for biomanufacturers not affected by the EU REACH regulation, and the approach is informative for biopharmaceutical companies inside of the EU considering using other detergents for virus inactivation steps.

**ACKNOWLEDGEMENTS**

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**REFERENCES**

Ask the Expert — Contin. from page 46

qualification). This is where it becomes essential that the URS is compliant with the regulations. To give an unacceptable example would be to write in the URS: ‘the screen refresh must be fast.’ This cannot be tested as it is a wholly ambiguous requirement. Another example would be to require ‘an easy-to-read screen.’ But how do you describe this in testable terms if you are not the software expert? This is where your IT team will have to support you and provide appropriate language. For example, they could suggest a refresh rate of 100 milliseconds or recommend a certain screen display quality that will meet your needs.

Despite its age, one of the best guidance documents for writing a URS is FDA’s General Principles of Software Validation; Final Guidance for Industry and FDA Staff from January 2002 (1). Other agency guidance documents and industry best practices are available online (e.g., European Directorate for the Quality of Medicines [EDQM] [2], the European Union [3], or GAMP [4], to name but a few). As the use of automated systems will surely increase in the future, it is sensible to build up relevant expertise in-house, so that in future you will be as comfortable writing spec sheets for a mechanical system as for a piece of software.

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You may be at the mercy of the supplier with regard to functionality and performance, but mostly you will have a choice.

Ad Index
Q. We are a well-established pharmaceutical company, and part of our procurement process requires us to prepare a ‘spec sheet’ (i.e., a document listing our requirements for either the service or the equipment we want to acquire). That has served its purpose well; however, we are finding it difficult to use when purchasing automated systems or software products. As we often have very little expertise or understanding of the software component, we struggle to write sensible specifications. We are tempted to simply accept the vendors’ system descriptions. Do you have any advice for us as to how we can overcome this challenge?

A. Your situation is not unusual, and the reason, as you already mentioned, often is a certain lack of expertise with software products. Whereas there is a lot of process and product expertise in companies, including processing equipment, the same cannot be said for automation. Let us first look at the parties/departments involved in preparing spec sheets for equipment. This will involve the operations group (e.g., manufacturing department, laboratories, utilities group), engineering, procurement, and the quality unit. When it comes to automated systems or pure software applications, the information technology (IT) department must be involved too. In a few cases, you may be at the mercy of the supplier with regard to functionality and performance, but mostly you will have a choice. For example, if you are looking for software to support a quality management system, there are systems available that offer you everything you may wish for. But, you may only want to use certain functionalities (e.g., workflow) and specific modules (e.g., deviations and complaints management modules, but not the change management module). In this case, it would be wrong to merely copy/paste the full system description by the vendor into your URS; instead the URS has to be specific to your needs (i.e., requirements).

In computerized systems validation (CSV) terms, the URS forms the basis for user acceptance testing (also referred to as performance building up relevant expertise in-house will make writing spec sheets for software easier, according to Siegfried Schmitt, principal consultant at PAREXEL.

When it comes to automated systems or pure software applications, the information technology department must be involved too.
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