

MARCH 2025 | VOLUME 23 | NUMBER 3

BioProcess International

COVERING THE WHOLE DEVELOPMENT PROCESS
FOR THE GLOBAL BIOTECHNOLOGY INDUSTRY

Downstream Processing

- Risk Assessment for Cost Efficiency
- The Importance of Data Literacy
- NIIMBL's Visions for the Future
- Flocculation and Depth Filtration
- Microbial Hold-Time Assessments
- AAV Viral Clearance Strategy
- ONLINE: Vaccine Featured Report
- EBOOK: Process-Related Impurities

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On the Cover Setting up stacked-disc clarification filters from MilliporeSigma (<https://www.sigmaaldrich.com>). *Learn more about this month's BPI contents — both in print and online — on page 2. And find us online at <https://www.bioprocessintl.com>.*



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BPI's March 2025 eBook will discuss product-related impurities. And look for a *BPI Insider* eBook on global biomanufacturing capacity in April.

Featured Report: Vaccines

New vaccines in development could protect against debilitating diseases. Our first digital-exclusive supplement of 2025 will include discussions of personalized cancer vaccines, HIV-1 prophylactics based on inactivated gene-modified virions, and bottlenecks in mRNA delivery (<https://www.bioprocessintl.com/publications/featured-reports/march-2025-featured-report>).

Focus on Risk Management

Risk assessments can drive cost efficiencies in several areas, including streamlining SOPs and change-control processes, refining vendor management, and focusing quality-control and stability-testing efforts based on risk profiles. Olena Chervonenko of PharmaLex (Cencora) highlights the use of risk-based approaches to enhance operations and select CMO partners on **page 6**.

Focus on Human Resources

Biomanufacturers face a conundrum with big data. The challenge lies not in accessing data, but in maximizing their potential value. Organizations that procure technologies without clear goals risk limiting their ability to improve patient care or operational efficiency. There is significant focus on the technical aspects of data, but a gap remains in understanding how to leverage such data for meaningful insights and actions. On **page 9**, Mike King and Philip Johnson emphasize the need for effective data strategies in biomanufacturing.

Focus on Manufacturing

The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), a public-private partnership and one of Manufacturing USA's 18 national institutes, held its annual National Meeting in June 2024 to explore industry barriers. The NIIMBL community represents a cross-section of stakeholders immersed in the transitions facing the biomanufacturing industry. A unifying theme that emerged across presentations was the need for partnerships to encourage innovation at early precompetitive stages. Read more highlights from the meeting on **page 13**.

Improving Harvest Clarification Efficiency Using Flocculation and Filtration

Biotechnology advancements have led to increased cell productivity and density during mAb manufacturing. But high-titer cultures entail greater biomass and more

associated impurities. That factor can diminish harvest-clarification filter capacity and increase the levels of impurities to be removed in downstream operations. On **page 20**, authors from AbbVie examine the pretreatment of cell-culture harvest using precipitation with citric acid and flocculation with pDADMAC to address the bottleneck of high-titer harvest clarification. The writers reported improvements in filter throughput and impurity removal during harvest and protein A capture operations across multiple pretreated high-cell-density feed streams.

Microbial-Control Hold-Time Assessments with Matrix and Bracketing Approaches

On **page 26**, authors from AGC Biologics explore the importance of validating microbial hold times in maintaining drug-product safety and consistency. Because such assessments require robust acceptance criteria for microbial hold times, the writers highlight two key validation approaches — matrix and bracketing strategies. To address regulatory concerns with such approaches, the team proposes a comprehensive risk-assessment framework that includes quantifiable scoring matrices as a function of mixing hydrodynamics, nutrient composition, preparation or hold time, and growth-promoting solution conditions such as pH, temperature, and osmolality.

Exploring Viral Clearance for AAV Products with Detergent Lysis and Chromatography

On **page 33**, teams from Oxford Biomedica and Texcell North America describe a viral-clearance study using a novel platform approach. The teams assessed removal of two model viruses — xenotropic murine leukemia virus (XMuLV) and minute virus of mice (MVM) — through detergent lysis and two chromatography steps, respectively. The study resulted in LRVs of $>4.67 \log_{10}$ for XMuLV and $9.07 \log_{10}$ for MVM.

Alois Jungbauer: A Life in Biotechnology

BPI editorial advisor Alois Jungbauer began his decades-long career in 1982, when he was offered an assistant professor position in downstream processing. Since then, Jungbauer has pioneered work on displacement effects in large-scale chromatography. Read more about his life and career in an interview with *BPI Insider* editor Josh Abbott on **page 48**.

On the Supplier Side

Nagase Viita representatives discuss sucrose as a pharmaceutical excipient, especially for mRNA-based therapies, on **page 38**. An article from BioGenes describes a proprietary CHO-based ELISA for HCP detection on **page 40**. And an article from Sartorius emphasizes the benefits of process intensification on **page 42**.

FROM THE EDITOR

In nearly 30 years of working as an editor covering the biopharmaceutical industry, first with *BioPharm* and later as cofounder of *BioProcess International*, I've copyedited thousands of articles. From business and regulatory discussions to technical reports and reviews, sponsored "advertorial" pieces, and beyond, I've seen it all in relation to the biopharmaceutical industry. Some papers, however, have stuck with me more than others, and one in particular comes to mind often lately.

In the summer of 2000, I worked with consultant Barbara Immel on an article called "A Brief History of the GMPs: The Power of Storytelling," which we published in the August issue of *BioPharm* that year. You can still find it online, and I highly recommend that you give it a read. Immel had a powerful influence on me as I was learning about the industry, particularly in regard to regulations. These days, we hear a lot of griping about such laws from politicians and businessmen who fancy themselves as government watchdogs. But like so many other topics of discussion lately, a historical perspective is crucial to understanding the subject at hand.

Laissez faire was once the motto of the US government when it came to business — even for industries with a direct influence on public health. Over time, company after company demonstrated a clear inability to "self-regulate," and many men, women, and children grew sick and even died as a direct result of well-documented incidents. Industry regulation grew out of public outrage over those events. Now many of us watch, stunned, as self-appointed "efficiency experts" work to abolish the Occupational Safety

and Health Administration (OSHA), as an executive order removes the United States from participating in the World Health Organization (WHO), and as a well-known antivaccine activist is confirmed as head of the US Department of Health and Human Services (HHS).

I suggest that anyone who thinks the pharmaceutical industry can self-regulate should head to the US Food and Drug Administration (FDA) website and peruse the inspection observations and warning letters — while such information still is accessible. My view of the industry, which I've covered closely for nearly three decades, is that there is a perennial tension between the science and technology driving progress and the business interests that seek to profit from them. Regulatory-affairs and quality groups stand between, maintaining the balance. The efforts of all are vital to a company's success, of course, and in particular its ability to help people rather than harm them.

Under my watch, *BioProcess International* will remain firmly on the side of regulation and global harmonization. I encourage anyone in this multinational business to consider history and the long-term effects of decisions that may have short-term appeal. Put science and patients over cost-cutting and expediency every time because you're not making widgets; you're daring to tackle some of the most serious challenges facing humanity — and you can and should be heroes rather than comic-book villains.



Cheryl Scott
Editor in Chief

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The BioProcess Insider portal delivers manufacturing news alongside expert views on the commercialization of biopharmaceuticals. Here are a couple of recent stories edited for print. Visit <https://bioprocessintl.com/category/bioprocess-insider> to find in-depth discussion and sign up for the twice-weekly newsletter.

In the Race To Stop Mpox, GeoVax Prepares for a Marathon by Josh Abbott

After mpox — formerly known as monkeypox — emerged in Western and Central Africa in 2023, medical organizations worldwide sought to mitigate the impact of the disease by fast-tracking modified *Vaccinia ankara* (MVA) vaccines and other treatments to the affected regions facing what has become a deadly epidemic.

Bavarian Nordic arrived as an early leader in supplying vaccines. The company worked with Gavi, the Vaccine Alliance, to send 500,000 doses of its MVA-BN vaccine to Africa in 2024. And in January 2025, KM Biologics announced the distribution of 50,000 mpox-vaccine doses to the Democratic Republic of the Congo.

John Sharkey, vice president (VP) of business development at vaccine maker GeoVax, spoke to *BioProcess Insider* about his company's mission, the challenges presented by the mpox epidemic, and the work his organization is doing to develop, manufacture, and distribute vaccines. GeoVax has two mpox-vaccine programs in development, called GEO-MVA and GEO-CM04S1. Sharkey said that his company acquired rights from the US National Institutes of Health (NIH) to develop and commercialize an MVA-vectored vaccine against both mpox and smallpox. Based on guidance received from regulators and by leveraging the recognized safety of MVA, the company is pursuing an expedited registration pathway to bring GEO-MVA to market.

Sharkey added that GEO-MVA is expected to work for other orthopox viruses as well. MVA vaccines are also preferred by the World Health Organization (WHO) for certain vulnerable populations, such as pregnant and lactating women, children, and people who are immunocompromised.

Although Bavarian Nordic's MVA-BN vaccine fits the WHO's criterion, more companies such as GeoVax seek to step in to meet the needs of the African continent. "African health authorities have requested 20 million doses of vaccines [for mpox], but only 2–5 million are expected to be available by the end of 2025," Sharkey said. "The complicated manufacturing process for MVA remains a significant impediment to manufacture on demand as well as to the establishment of local/regional

manufacturing capacity. GeoVax has initiated efforts to increase the availability of MVA-vaccine supply in the near term with the development of GEO-MVA."

Initially developed as a next-generation COVID-19 vaccine, that company's second vaccine candidate has shown effectiveness toward mpox as well. GEO-CM04S1 incorporates sequences from the SARS-CoV-2 spike (S) and nucleocapsid (N) proteins. "By targeting two major components of the SARS-CoV-2 virus, GEO-CM04S1 appears to elicit a broader, more robust immune response compared to currently approved mRNA vaccines," Sharkey said.

Manufacturing MVA Vaccines: "As a vaccine vector, MVA offers some significant advantages over other platforms," Sharkey said, citing its safety, temperature stability, large coding capacity, and ability to code for multiple antigens of interest.

He expressed hope that the first regulatory dossier submission for GEO-MVA manufactured with a chicken embryo fibroblast (CEF) platform will be achieved by early 2027. With the help of contract development and manufacturing organization (CDMO) Oxford Biomedica (OXB), the company has manufactured materials to support phase 3 clinical trials, which are expected to take place later this year.

But traditional MVA manufacturing processes using CEFs have several drawbacks. The virus does not replicate in mammalian cells, and the specific pathogen-free (SPF) chicken eggs required to obtain MVA cells are not readily available for production processes. Moreover, production requires operators with specialized skills in aseptic techniques. GeoVax recently announced a licensing collaboration with ProBioGen AG (Berlin) to use its AGE.1 continuous duck cell line for manufacturing the company's MVA vaccines, including GEO-MVA and GEO-CM04S1.

"As a suspension cell line, AGE.1 is well suited for the manufacture of MVA," Sharkey said. "It is highly productive, approximately 10-fold better [results] than CEFs are, and as a suspension cell line, [it] can be processed in standard manufacturing equipment. As a continuous cell line, the material can be prepared, characterized, and frozen prior to being needed for a manufacturing run. We believe with the implementation of the AGE.1 manufacturing platform, significantly lower unit costs, increased yield, and manufacturing-site flexibility can be readily achieved."

He added that over the long term, his organization believes that "the transition to a next-generation manufacturing platform using the AGE.1 suspension cell line is the best solution to address MVA manufacturing bottlenecks." In the meantime, the company is working with the WHO, the Africa Centres for Disease Control and Prevention (Africa CDC), and the Coalition for Epidemic Preparedness Innovations (CEPI) to plan for how best to address both current and future viral outbreaks.

Triple Merger: Solarbiotech, GPC Bio, and Eleszto Genetika

by Josh Abbott

Biotechnology companies Solarbiotech, GPC Bio, and Eleszto Genetika (EG) have merged under consolidated ownership and will operate as a unified, vertically integrated organization. Based in Norton, VA, Solarbiotech works in precision fermentation and downstream processing. GPC Bio makes biomanufacturing equipment in La Rochelle, France. And EG genetically modifies microbial platforms for production of target molecules in Budapest, Hungary.

Solarbiotech CEO Alex Berlin told us that the merger will accelerate timelines and lower costs for customers. “We can take an idea or a concept from bench scale to commercial phase,” he said. EG will engineer production strains and perform early bioprocess development; Solarbiotech will scale up processes; and GPC Bio will handle facility engineering, automation, and equipment.

Berlin said that the companies have collaborated and cross-trained to ensure a smooth merger. “Cross-pollination is in the DNA of a vertically integrated organization,” he said. “Just hours before the acquisition of EG in Hungary was complete, we had a team from Virginia closely collaborating in Budapest on two customers’ projects. The same with the engineering team in France: We’ve had personnel from Virginia trained in France on automation and upgrades deployed now in Virginia. Remote troubleshooting is often used to support operations at our plant in Virginia.”

Berlin identified challenges in the biomanufacturing space that this merger is poised to overcome. He said that his company’s “engineered microbial strains are designed with the scale-up challenges in mind, which are often missed in non-integrated operations.” He added that the integrated organization “closes the tech-transfer gap. We work closely with bioprocess developers and

the engineering teams responsible for designing and building the commercial operations. These aspects are crucial for commercial success and are often overlooked in developing new biomanufactured products.”

Recipharm Bolsters Fill-Finish Capacity

by Shreeyashi Ojha

Swedish contract development and manufacturing organization (CDMO) Recipharm has added a sterile filling system for vials and prefilled syringes at its Wasserburg facility in Germany. The system operates within a Grade A isolator for fast and easy modular exchange of small batches of high-value products in clinical development.

The system reduces the risk of contamination, limiting batch rejections, a spokesperson told us. “With both piston and peristaltic pumps, it provides high precision filling [with] minimal overfill and product waste.” The system also allows for high-accuracy adjustments of fill volumes to reduced product loss (<500 mL).

Recipharm divested seven manufacturing and development facilities in April 2024 to focus on higher-margin CDMO services. US-based private-equity firm Blue Wolf Capital Partners acquired five facilities in Sweden, one in France, and one in Spain dedicated to solid/semisolid and liquid oral-dosage products. Recently, Recipharm collaborated with fellow CDMO Exela to produce over 100 million units (vials and prefilled syringes) to support emerging therapeutics such as glucagon-like peptide 1 (GLP-1) drugs and vaccines.

Josh Abbott is editor (josh.abbott@informa.com), and Shreeyashi Ojha (shreeyashi.ojha@informa.com) is a reporter for BioProcess Insider.

Welcome to the Editorial Advisory Board

The BPI editors are pleased to welcome Naveen Ganesh Muralidharan to the publication’s advisory board. A prolific writer as well as a generous and observant reviewer, Muralidharan brings to our board over 18 years of biopharmaceutical-industry experience, including work with conventional recombinant proteins and gene therapies. He specializes in process development, scale-up, and technology transfer to commercial-scale manufacturing. He also possesses deep expertise in manufacturing troubleshooting, late-stage process development, and stage-2 and -3 process validation, all of which help him to ensure excellence across manufacturing support and analytics.

Muralidharan currently works as a senior manager for the manufacturing science and technology (MSAT) team at AGC Biologics in Boulder, CO. Before joining AGC Bio in 2023, he held senior roles at Novartis Gene Therapies

(Chicago, IL), where he managed continued process verification (CPV) and statistical process control (SPC) programs; Lonza (Houston, TX), where he led a team of scientists in developing robust and high-performing processes for adenoassociated virus (AAV) production; and Thermo Fisher Scientific (St. Louis, MO), where he supported transfer and scale-up of upstream activities for recombinant-protein products.

Readers of BPI will be familiar with Muralidharan’s recent articles on process validation and risk management for a breadth of biomanufacturing activities. We are excited to continue working with him as a writer and in this new advisory role.



The Financial Edge

Leveraging Risk Assessment To Drive Cost Efficiencies in the Biopharmaceutical Industry

Olena Chervonenko

In an era marked by heightened competition and financial pressures, managing a biopharmaceutical company has become increasingly challenging. The industry is known for its high business costs, which stem from stringent regulatory requirements, complex manufacturing processes, and the need for rigorous quality control (QC). Companies must strike a delicate balance between maintaining high standards and reducing expenses.

Maintaining high product quality is nonnegotiable, so biopharmaceutical companies must seek out effective strategies to manage and justify their expenditures. Here, risk assessment can be invaluable. By systematically identifying, evaluating, and mitigating risks, companies can optimize their operations and minimize costs without compromising on quality (1).

Risk assessment provides a structured methodology to pinpoint inefficiencies, forecast potential issues, and prioritize corrective actions. Thus, it enables businesses to make informed decisions, allocate resources effectively, and prevent costly problems before they arise. Risk assessments also can help companies to streamline processes, enhance regulatory compliance, and achieve significant cost savings.

Herein, I explore risk assessments as a means to driving cost efficiencies in the following areas: streamlining standard operating procedures (SOPs) and/or change-control processes, refining vendor management, and focusing QC and stability-testing efforts. I also highlight how drug developers can apply



a risk-based approach to selecting a contract manufacturing organization (CMO).

BALANCING QUANTITY WITH QUALITY FOR SOPs

A robust quality-management system (QMS) is the backbone of every successful pharmaceutical operation. The objective is to ensure that every aspect of production, from raw-material sourcing to final product release, meets stringent regulatory standards. Quality-risk management (QRM) activities should be performed using systematic processes that are designed to coordinate, facilitate, and improve science-based decision-making with respect to risk (2).

Central to QMSs are SOPs, which document the processes and guidelines that govern operations. However, maintaining a large number of SOPs can become a significant burden, both financially and logistically (3).

For startups, particularly those involved in technology transfer to CMOs, establishing the correct documentation

from the outset is crucial. By aligning SOP development with a risk-based approach, companies can reduce the possibility of over- or under-documenting processes. That enables creation of a focused, efficient documentation system that directly supports regulatory compliance and operational success. Prioritizing critical processes in the initial development phase can help startups to prevent inefficiencies and allocate resources effectively.

For established companies, the challenge often lies in maintaining and reviewing a large number of SOPs during routine operations. A best practice is to implement periodic reviews as part of a structured risk-assessment approach so that only essential SOPs are maintained and updated regularly. Rather than reviewing all documents with equal frequency, companies can use a risk-based approach to identify which processes require the most attention due to their potential impacts on product quality and compliance. Thus, a risk-based strategy prevents administrative

overload and reduces the likelihood of errors, delays, and excess costs.

By tailoring SOP development and review to a risk-based framework, companies can optimize their QMSs to focus on areas that most directly affect operational integrity, keeping systems lean and efficient. In turn, companies can save time and resources while achieving a high level of compliance and product quality.

RISK-BASED APPROACHES TO CHANGE CONTROL

When introducing proposals or changes — e.g., process modifications, newly adopted technologies, and compliance updates — it is tempting to implement them broadly. However, doing so often leads to overengineering, increasing process complexity, resource requirements, and costs. Change control based on risk assessment can make a significant difference in such cases (4).

By using a risk-based methodology, companies can categorize changes as critical, major, or minor according to their potential impacts on product quality, patient safety, and regulatory compliance. Critical changes might require significant oversight, including extensive validation and regulatory notification, whereas minor changes might involve minimal review and relatively simple documentation. Such stratification helps companies to focus on high-impact changes, while less-risky alterations are processed with reduced complexity.

CONSIDERATIONS FOR VENDOR MANAGEMENT

Effective vendor management requires that biopharmaceutical companies not only select suitable suppliers, but also maintain strong relationships with them to support consistent quality throughout products' life cycles. However, traditional vendor-management practices such as frequent onsite audits can be resource-intensive and costly for both drug developers and vendors. By leveraging a risk-assessment approach, companies can streamline supplier management, reducing unnecessary steps and focusing efforts where they are most needed.

One key strategy is to use risk assessment to reduce the quantity of

onsite audits (5). Not all vendors carry the same level of risk. Some have strong regulatory track records and robust quality systems in place; others might be new to the industry or have less-proven records. Risk assessments enable drug companies to categorize vendors according to their risk profiles and thereby devise targeted auditing schedules. High-risk suppliers might require frequent audits, whereas those with solid reputations and compliance histories might warrant fewer onsite checks. Leveraging such an approach saves developers time and money while helping to allocate resources effectively.

By applying a risk-based approach, companies can tailor the **FREQUENCY** and **DEPTH** of qualification activities to a given process's level of risk.

Implementing a "dock-to-stock" program can enhance supplier management further by streamlining transitions of received materials. Through risk assessments, companies can ensure that suppliers meet predefined quality criteria, allowing for expedited acceptance of incoming goods. That, in turn, reduces the time and costs associated with traditional quality checks while strengthening supply-chain efficiency. Overall, biomanufacturers can implement a targeted, risk-based approach to prevent overburdening their systems with unnecessary checks (6).

Another important consideration is selection of vendors at the product-development stage. Risk assessment can assist in determining the optimal number of vendors to engage, balancing the need for supply-chain security with potential for overcomplicating vendor management.

CMO SELECTION AND QUALIFICATION

Among the most important steps in technology transfer is selection of a

suitable CMO (7). Not all service providers are created equal, and choosing a partner with the right capabilities, experience, and quality standards is vital. Drug companies can use a risk-assessment approach to evaluate potential partners based on factors such as their regulatory track record, quality of products, history of recalls, and inspection outcomes. Drug developers can focus on CMOs that present low risks and high likelihood of success, reducing the chances of costly issues during technology transfer and production.

Once a developer has selected a CMO, it can leverage risk assessment to determine the frequency of qualification activities, such as process validation and equipment qualification. Not all processes require the same level of oversight. Sometimes they are well established and present low risk; other processes might involve higher risk because they are new or based on complex technologies. By applying a risk-based approach, companies can tailor the frequency and depth of qualification activities to a given process's level of risk, helping to focus resources where they are needed and thereby preventing overqualification, which can incur unnecessary costs and delays.

OPTIMIZED QC TESTING

QC is an essential component of biopharmaceutical manufacturing. For instance, QC groups ensure that excipients, active pharmaceutical ingredients (APIs), and intermediate and finished products meet stringent quality standards (8). However, the extensive testing required for each batch can be cost- and resource-intensive. Companies can apply a risk-assessment approach to optimize their QC strategies, reducing unnecessary testing without compromising product safety or efficacy.

In a traditional QC strategy, each product batch undergoes a comprehensive battery of tests, including assays for microbiological purity (9). Although QC tests are critical for ensuring product quality, not all tests may be necessary for every batch of a given product, especially when a drug developer is working with a vendor

that has a proven track record of delivering consistent, high-quality materials from an unchanged process.

Risk assessment can help companies to determine when certain tests can be reduced in frequency or even skipped. Periodic (or skip) testing applies specified tests at release on preselected batches and/or at predetermined intervals, with the understanding that untested batches still must meet all acceptance criteria established for a given product and process (9). For instance, if a vendor consistently manufactures a statistically defensible number of batches of excipients or APIs that pass all quality checks without deviations, the risk of a quality failure is considerably reduced. In such cases, tests for microbiological purity, especially those that are noncritical for a given dosage form, can be scheduled less frequently. For instance, such tests might be performed quarterly or biannually based on the timing of batch production processes and the assessed risk (10).

Similarly, for intermediate and finished products, risk assessment provides information about which quality attributes require routine testing and which might need less-frequent monitoring. If a product has demonstrated stability and purity across multiple batches without variation, the need for exhaustive testing on every batch diminishes. Such an approach to QC saves time and resources while accelerating production timelines.

STABILITY ASSESSMENT

In a typical stability-study program, all product batches undergo a full range of tests at multiple time points under different storage conditions (11). Although such tests are vital, especially during product development and early commercialization stages, they may not be necessary for every subsequent batch, particularly for products with a well-documented stability profile and a consistent manufacturing process.

Companies can perform risk assessment to evaluate the need for continuous stability testing of a given product. For example, if a drug consistently exhibits stability over several batches and years, and if its manufacturing process remains

unchanged, then the frequency of stability tests could be reduced. Tests that monitor noncritical attributes — e.g., for certain chemical or physical properties that are unlikely to degrade over time — can be scheduled less frequently than normal or even skipped for specific batches.

A risk-based approach also can be applied to evaluate different packaging configurations and storage conditions. If stability studies have demonstrated that a product remains stable under multiple conditions, then future testing could be tailored to focus on critical scenarios, reducing the overall testing burden (12).

Effective risk management requires **ONGOING** risk assessment to ensure that proposals and control strategies remain **ALIGNED** with an evolving risk landscape.

Risk-based predictive stability (RBPS) tools such as the accelerated stability-assessment program (ASAP) are used routinely within pharmaceutical development for quick evaluation of a drug's stability characteristics, especially to understand mechanisms of degradation. Such models provide stability insights within weeks, whereas analysts might need months or years to understand a drug product's characteristics using long-term stability conditions only (13).

PROPOSAL REVIEW AND CONTROL

Effective risk management requires ongoing risk assessment to ensure that proposals and control strategies remain aligned with an evolving risk landscape. Risk assessment is not a static process, but rather a dynamic one that needs to be integrated continuously into proposal review and adaptation. Hence, a quality team should consider revisiting a risk assessment to identify and evaluate possible changes in risk after

implementation of a risk-reduction process (14).

Continuous risk assessment involves identifying risks to product quality, compliance, safety, and operations. Based on those results, companies can establish an appropriate review frequency, with high-risk proposals requiring frequent attention and low-risk ones needing fewer reviews. Control strategies must be monitored continuously and adjusted as conditions evolve, ensuring that they remain effective over time. Regular updates to proposals based on shifting risk levels help to maintain the relevance and efficiency of risk-management measures.

ENSURING COMPLIANCE, QUALITY, AND COST-EFFECTIVENESS

Continuous risk assessment enables drug companies to streamline processes, reduce unnecessary steps, and allocate resources effectively. For instance, it enables developers to optimize SOPs, minimize audits, tailor CMO qualifications, focus QC testing, and adapt to needs for continuous risk control. The latter benefit ensures that cost-saving measures align with evolving industry standards and operational realities. Moreover, continuous risk assessment can provide substantial financial savings by minimizing unnecessary expenditures and optimizing resource allocation.

Integrating a risk-based perspective into biopharmaceutical operations helps drug companies to navigate complexity effectively. Thus, they can maintain high standards of product quality while realizing significant cost reductions, enhancing their competitiveness in an increasingly demanding market.

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The Paradox of Data Overabundance in Biomanufacturing

Data Literacy Is Key to Unlocking Value

Mike King and Philip Johnson

Biopharmaceutical manufacturers today face a conundrum with big data. Valuable information is abundant across product and process development, so the challenge lies not in accessing data, but in maximizing their potential value. Mismanaged data can impede outcomes, and organizations that procure technology without clear purpose risk being overwhelmed and limited in their ability to improve patient care or operational efficiency.

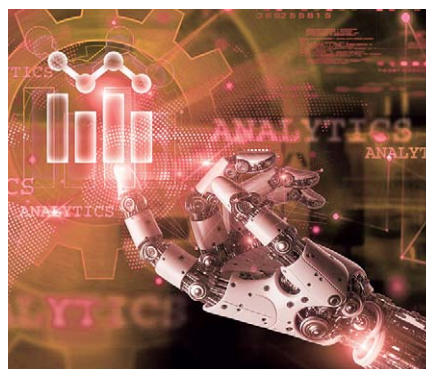
Even with significant focus on the technical aspects of data — securing, managing, structuring, and collecting them — a gap remains in the industry's understanding of how to leverage the resulting information for meaningful insights and actions. That imbalance results in organizations accumulating data, in many cases across multiple systems, without fully realizing the potential value. Emphasizing data collection and storage often overshadows the critical need for an effective data strategy that includes a focus on interpretation and application.

DATA LITERACY IS LACKING

The reasons for the imbalance between data collection and effective interpretation are multifaceted. Below are some problems that can contribute to that knowledge gap.

Emphasis on Collection and Security:

Because of privacy concerns and laws such as the European General Data Protection Regulation (GDPR) (1), organizations often focus on gathering vast amounts of data and ensuring their security. However, that emphasis



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sometimes overshadows the ultimate purpose of the data.

Data-Hoarding Mentality: Many companies collect data without a clear plan for their use, believing that more data inherently equals more value.

Complexity of Data Understanding: Effective data interpretation requires specialized skills in statistics and data science. In the biopharmaceutical industry, those often are in short supply, creating a gap between data collection and data comprehension (2, 3).

Tools Over Insights: Organizations often place greater emphasis on data-management tools and platforms than on the critical thinking skills needed to derive meaningful insights from available information.

Short-Term Over Long-Term Thinking: Managing data provides immediate results; using data for strategic decision-making is a longer-term process with less immediate results.

Actionable Insights Are Challenging: Translating data into actionable insights that drive business value is a complex endeavor. Bridging technical analyses to practical applications requires expertise in both.

Data-Quality Issues: Poor data quality can hinder effective analysis. Some companies are prioritizing quantity over quality, complicating the extraction of reliable insights.

Siloed Data and Lack of Integration: Data often exist in “silos” across different departments and systems. That complicates efforts to gain a holistic understanding and use information effectively.

Ethical Considerations: Using data ethically requires careful consideration. Some organizations avoid ethical issues due to such complexities and potential limitations on data use.

Data Literacy Becomes Essential: Many decision-makers lack the ability to interpret and apply data effectively, leading to missed opportunities or misinterpretations. Technical skills in data management are relatively easy to teach; developing nuanced interpretation skills presents a challenge. The resulting gap can be exacerbated by rapid technological advancements, the pace of which often outstrips people's ability to make sense of collected information.

Data literacy, the ability to analyze data, ask the right questions, and communicate insights effectively, has become crucial in today's biomanufacturing landscape. Those skills transform raw data into valuable insights that drive decision-making and innovation. Improving data literacy requires both structured training and practical experience to help stakeholders differentiate high-quality insights from poor ones.

A significant difficulty lies in overcoming the tendency to present

Key Drivers for Data Literacy in Biomanufacturing

The growing importance of data literacy in the biopharmaceutical industry is driven by several compelling needs.

Informed Decision-Making: Vast amounts of data are generated in drug development, clinical trials, and postmarket surveillance. To make critical decisions, industry professionals need to interpret and use data effectively.

Regulatory Compliance: Proper analysis is essential for understanding data, meeting regulatory requirements, and submitting comprehensive reports to authorities such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMA).

Personalized Medicine: Data literacy would support the development of targeted therapies based on patient-specific information, ultimately advancing the field of precision medicine.

Drug Discovery: Analyzing large datasets can help companies identify new drug targets and/or repurpose existing drugs. Data literacy thus offers potential for accelerating drug-discovery processes.

Clinical-Trial Optimization: Excellence in data interpretation would help to improve clinical-study design, patient selection, and

outcome analysis. That should increase the efficiency and cost-effectiveness of future clinical trials.

Pharmacovigilance: Effective monitoring and analysis of postmarket data are crucial to identifying and addressing safety concerns promptly.

Operational Efficiency: Data-driven insights can optimize many aspects of biopharmaceutical operations, from supply-chain management to bioprocess development to marketing strategies.

Collaboration: As interdisciplinary teams and outsourcing become more common, a shared understanding of data facilitates communication and collaboration across different departments and organizations.

Artificial Intelligence and Machine Learning: The biopharmaceutical industry is seeking to use advanced information technologies, which will require professionals to understand and work with complex data sets (2).

Patient Engagement: Data literacy can enhance patient education and engagement strategies, helping to improve health outcomes and adherence to treatment regimens.

selective data that favor an organization. True data literacy demands comprehensive and unbiased information gathering and reporting, which are essential for enhancing overall product quality and driving both commercial and operational performance. As detailed in the “Key Drivers” box, improving data literacy throughout the industry is becoming a nonnegotiable proposition.

WHY IMPLEMENT A DATA-LITERACY PROGRAM?

To address existing challenges, the industry must develop comprehensive data-literacy programs that emphasize not only technical skills, but also critical thinking, statistical understanding, and effective communication of data-driven insights. Companies should foster a culture in which evaluating data is as natural as collecting them.

Interdisciplinary approaches that combine technical knowledge with

domain expertise can bring more meaningful and contextual interpretations of data than disparate groups can provide alone, ultimately enhancing the depths of insight that can be gained. Consider potential generational differences in data literacy: Younger professionals might excel in data gathering, handling, and manipulation but lack their more experienced counterparts’ deep domain knowledge, training, and regulatory understanding that give needed context to develop focused insights. Blending both skill sets — data savviness with clinical and regulatory expertise — will be crucial to future success.

Implemented correctly, data literacy can provide biomanufacturers with valuable, verifiable intelligence that translates into operational business value. Below we elucidate several operational areas in which that can produce tangible results.

Quality Assurance and Control (QA/QC): Data literacy will play a

pivotal role in upholding stringent QA/QC standards in pharmaceutical manufacturing. It empowers real-time monitoring of critical process parameters (CPPs) and critical quality attributes (CQAs), helping to ensure that processes perform within specified limits. Statistical process control enables rapid detection and response to process variations while process analytical technology (PAT) facilitates continuous quality verification (CQV) throughout manufacturing.

Risk-based decision-making will improve as data-literate teams effectively interpret complex information. Trend analysis can help them to predict and prevent quality issues, and improved root-cause analysis for out-of-specification (OoS) results will enhance problem solving. Such data-driven approaches will create a robust QA/QC framework that meets regulatory requirements and drives continuous improvement in both product quality and manufacturing efficiency.

Regulatory Compliance: In the highly regulated pharmaceutical industry, data literacy will be instrumental for maintaining compliance with stringent regulatory requirements. It can enable organizations to adhere to good manufacturing practices (GMPs) and other regulatory standards by ensuring accurate interpretation and application of related guidelines. Data-literate teams can produce timely reports for regulatory agencies to meet critical submission deadlines.

Implementation of data-integrity principles is strengthened when staff members understand the significance of each element of the ALCOA+ principle: making data *attributable, legible, contemporaneous, original, accurate, complete, consistent, enduring, and available* (4–7). Data literacy also can improve the preparation and management of complex regulatory submissions — e.g., biologics license applications (BLAs) and new drug applications (NDAs) — while ensuring that all required information is compiled and presented accurately. During regulatory inspections and audits, data-literate employees can

navigate and explain data-related processes more effectively.

Furthermore, maintaining electronic records and signatures in compliance with 21 CFR Part 11 becomes more manageable when staff understand the principles of data security and traceability (8). Ultimately, a data-literate workforce is essential to regulatory-compliant biopharmaceutical operations, reducing risks and fostering trust with regulatory authorities.

Process Optimization: Data literacy will catalyze continuous process improvement in biopharmaceutical manufacturing by enabling sophisticated optimization strategies. It underpins the successful application of quality by design (QbD) principles to help ensure that quality is integrated into processes from the start. Data-literate teams can design and analyze design of experiments (DoE) strategies to optimize manufacturing processes.

Real-time process optimization becomes achievable through advanced process control techniques, allowing for immediate adjustments based on data insights. Multivariate data analysis helps employees to understand complex process interactions, ultimately improving the effectiveness of controls. Data literacy also enhances lean/six-sigma methodologies, reducing process variability and eliminating waste (9).

Moreover, data-driven insights are crucial for technology transfer and scale-up optimization, ensuring smooth transitions from laboratory to full-scale production. Biopharmaceutical companies that foster a data-literate workforce can create a culture of continuous improvement, leading to improved product quality and operational efficiencies.

Cost Reduction: Improved data literacy can contribute significantly to cost reduction in biomanufacturing through a number of interconnected mechanisms. Deepened process understanding helps to reduce batch failures and product recalls. That can prevent costly setbacks and reputational damage. Data-driven insights help to optimize raw-material use, minimizing waste and associated costs. And predictive-maintenance strategies informed by data analyses can minimize

Data-Literacy Training

Data-literacy programs help organizations collect, store, read, and understand information to drive efficient and conscious decision making, bringing transparency to risk and clarity to how decisions support broader organizational goals. Training on data structure, data capture, data cleansing, data representation, data use, and data management are key tenets of such programs.

A simple example would be the capture and reading of the date “03-07-25.” In some global regions, that would be read as the 7th of March; in others it would be the 3rd of July. Without a common understanding, the four-month gap between those two dates could significantly affect activities such as delivery and stock-reorder dates, equipment recalibration timing, regulator response deadlines, and so on. A number of excellent data-literacy courses are available online.

Further Reading

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unplanned downtime, which smooths operations and can prevent the need for expensive repairs.

Data-driven facility management can enhance energy efficiency and thus reduce utility costs. Workforce productivity improves as employees make informed decisions based on data insights, streamlining operations and increasing output. Additionally, data literacy can facilitate inventory optimization, enabling companies to reduce carrying costs while maintaining adequate supplies. Substantial cost savings can result from all these improvements to enhance the overall financial performance of biopharmaceutical manufacturing operations.

Research and Development (R&D):

Data literacy drives innovation and efficiency in biopharmaceutical R&D. It can accelerate drug discovery through high-throughput screening and data mining, enabling researchers to identify promising product candidates rapidly. Enhanced data-interpretation skills improve the design and analysis of preclinical and clinical trials so that robust studies can produce meaningful results.

Data literacy will deepen researchers' understanding of structure–activity relationships during drug design, enabling such experts to make informed decisions about molecular modifications. Insights gained will enhance the effectiveness of adaptive clinical-trial designs by helping development teams to interpret and respond to interim data.

Integrating real-world evidence will enrich drug development, providing valuable knowledge that complements traditional clinical trial data. Furthermore, data literacy can help formulators use predictive modeling to optimize drug-delivery systems. Collectively, such data-driven approaches will streamline biopharmaceutical R&D, potentially reducing time to market while increasing the success rate of drug-development projects.

Supply-Chain Management: Data literacy can enhance biopharmaceutical supply-chain operations significantly by improving the sophistication and efficiency of related management practices. Demand forecasting and production planning will improve, enabling companies to align their manufacturing operations with market needs. Real-time tracking of materials and products will enhance inventory control, reducing stockouts and overstock situations.

The implementation of blockchain technology will be facilitated by data literacy, improving transparency and security across the biopharmaceutical ecosystem. Data-literate teams can conduct comprehensive risk assessments and develop effective mitigation strategies, enhancing overall supply-chain resilience. Increasing robustness of vendor quality management and performance analyses will support data-driven supplier selection and continuous improvement in supplier relationships.

Widespread data literacy would help to optimize logistics and distribution networks, improving the efficiency of routing, reducing transportation costs, and improving delivery times. Collectively, such enhancements will provide for an agile, transparent, and cost-effective pharmaceutical supply chain that is well equipped to meet the demands of global healthcare markets.

Continuous Improvement: A data-literate organization cultivates a culture of continuous improvement by leveraging information across operations. That is exemplified through effective implementation of corrective and preventive action (CAPA) systems that rely on accurate data interpretation to address and prevent quality issues. Management-review meetings with data-driven key performance indicators will enable leaders to make informed decisions.

Knowledge management and best-practice sharing will flourish as with effective dissemination of insights from data analysis. Goal setting and performance management become more precise when they are backed by concrete data, and change-management initiatives gain traction through data-supported rationales. A data-centric approach fosters an organization culture of learning in which evidence-based decision-making is the norm, encouraging all employees to apply insights from data to their daily work. A commitment to data literacy transforms an organization into an agile entity capable of continuous adaptation and improvement in response to both internal and external challenges.

Technology Integration: In pharmaceutical manufacturing, integration of new technologies hinges on data literacy. It is crucial for effective leveraging of advanced technologies, including implementation of manufacturing execution systems (MES) and laboratory information-management systems (LIMS) to streamline operations and ensure compliance. Adopting internet of things (IoT) devices can enable real-time data collection, providing valuable insights into manufacturing processes.

Artificial intelligence (AI) and machine learning (ML) enhance efficiency and QC through process optimization and predictive analytics. Augmented-reality and virtual-reality technologies can enrich training and maintenance procedures with immersive learning experiences. Digital twins allow for risk-reduced testing and refinement of manufacturing processes. Finally, cloud computing and big-data analytics enable large-scale data processing and

analysis, driving informed decision-making across an organization.

Collectively, those technological advancements could transform biopharmaceutical manufacturing, but their full potential will be realized only by a data-literate workforce capable of interpreting and acting on the insights they provide.

Cross-Functional Collaboration: Data literacy serves as a powerful catalyst for enhanced collaboration both within and among organizations. By establishing a common language for discussing performance and quality metrics, teams from different departments can communicate effectively about key indicators and outcomes. Shared understanding then will facilitate data-driven, cross-functional problem-solving drawn from diverse perspectives.

Creating integrated dashboards provides a holistic view of operations, enabling decision-makers to gain insights that span multiple areas of a business. The resulting perspective supports knowledge transfer across critical functions such as R&D, manufacturing, and QA/QC, ensuring that innovations and best practices will be disseminated quickly throughout an organization.

Improved data literacy also enhances communication with external stakeholders, including regulators, suppliers, and customers. By presenting clear, data-backed information, biopharmaceutical companies can build trust, demonstrate regulatory compliance, and strengthen partnerships.

Most important, fostering data literacy cultivates a data-driven decision-making culture across all levels, empowering employees to base their choices on empirical evidence rather than intuition. That should lead to more informed and effective outcomes.

CONVERTING RAW DATA TO INSIGHTFUL KNOWLEDGE

Data literacy is increasingly important in today's data-driven world, empowering individuals to make informed choices and engage in meaningful discussions across varied fields. The expanded view herein underscores how critical data literacy is

becoming across all aspects of the biopharmaceutical industry, from early stage research to postmarket activities. As the industry evolves with new technologies and data sources, the importance of data literacy will continued to grow.

Biomanufacturers must balance data collection and management with the ability to understand and use information effectively. Such a balance is essential to harnessing the power of data for driving progress and innovation. To manage data overabundance, organizations should align comprehensive data collection with focused analysis. Successful companies will set clear objectives, identify relevant data sources, and implement efficient filtering mechanisms — which are vital for meeting pharmacovigilance obligations, for example.

When implementing modern technologies, biomanufacturers should refrain from merely replicating old processes in new formats, which can increase risk when deploying global solutions takes too much time. Embracing new methodologies and understanding how evolving technologies can enhance data analysis are key to effective decision-making. By shifting focus from merely possessing data to truly understanding and leveraging information, organizations can unlock the full potential of their data assets, leading to more meaningful outcomes. Pharmaceutical professionals must develop skills to filter noise and identify meaningful patterns, which are fundamental for effective decision-making in healthcare.

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Vision and Challenges for the Future of Biopharmaceutical Manufacturing

Stefanie Pluschkell, Barry Buckland, Emily Carson, and Kelvin H. Lee

In the development and manufacturing of innovative biotherapeutics and vaccines, many US-based and globally acting companies can look back on decades of experience that stress the importance of optimizing three pillars that are foundational to success: quality, speed, and cost. Such optimization has become challenging in today's biopharmaceutical landscape as the industry anticipates future epidemic and pandemic scares, leaps into novel treatment modalities, reacts to infrastructure and supply-chain disruptions, and troubleshoots dynamic shifts in workforce demands.

The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), a public-private partnership and one of Manufacturing USA's 18 national institutes, held its annual National Meeting in June 2024 to explore those issues. Comprising companies that develop and manufacture biotherapeutics and vaccines as well as organizations that provide specialized manufacturing equipment, supplies, services, and facility designs, the NIIMBL community represents a cross-section of stakeholders immersed in the transitions facing the biomanufacturing industry.

Three sessions at the National Meeting convened senior industry leaders to enable deep dialogue on the current visions and challenges to the future of biopharmaceutical manufacturing. Speakers shared concerns in relation to cost of goods (CoG), risk management, continuous



The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) held its annual National Meeting in June 2024 to explore key issues in the manufacture of novel biotherapeutics and vaccines.

operations, shifting roles of platform manufacturing, gaps between data and process sciences, changing demands for workforce skills, and silos that obstruct much-desired progress. A unifying theme that emerged across those diverse topics was the opportunity to address widespread challenges with novel partnerships established at early, precompetitive stages. Herein, we highlight key points and pressing issues from the sessions to share solutions that depend on collaboration across the industry.

THE CHANGING CULTURE OF CMC DEVELOPMENT

The trajectory of chemistry, manufacturing, and controls (CMC) development in biopharmaceutical manufacturing is one of high speed and complexity. The importance of speed unquestionably is driven by the need to keep CMC deliverables as much as possible off the critical path of any clinical program. Rapid vaccine

development during the COVID-19 pandemic also demonstrated the possibility of expediency, whether or not such speed was sustainable.

Complexity continues to rise with the expanding variety of molecular modalities that require changes to otherwise heavily platformed unit operations. Bioprocess technology and synthetic chemistry have converged to the extent that traditionally defined lines separating biologics from small molecules — and their respective research and development (R&D) departments — increasingly are blurred. We anticipate an expansive future in which any modality and combination thereof is theoretically possible, bringing opportunities but also challenges. “The desire to treat disease is strong,” explained Paul Collins (vice president at Eli Lilly and Company). “That hasn’t changed, but the expectation going forward is that we will use any modality of treatment possible.” Tom Piombino (managing



Jon Coffman (executive director at AstraZeneca) discussed the possibility of decreasing the cost of goods (CoG) for monoclonal antibody (mAb) manufacturing to US\$10 per gram in the age of high inflation.

director of Integrated Project Services, LLC) shared a similar perspective from his experiences in process architecture: "I've been in the business for 30 years. I probably work on 60 to 70 [facility] layouts a year. I see lots of different modalities from gene therapies to antibody–drug conjugates (ADCs) to biologics, and I've never seen such a fusion of all those modalities coming together." Collins noted that novel modality combinations ultimately "[are changing] the culture around how CMC should be done."

Such shifts have escalated the challenge to developing flexible manufacturing solutions, product-agnostic unit operations, and analytical methods: "Many of these modalities did not even exist 10–15 years ago in the drug-development space," explained Ranjeet Patil (senior director of strategy and transformation at Waters Technology Corporation). "Now, the existing techniques have limitations on their ability to detect and accurately analyze these entities. So, it's not just a matter of having the tools, integrating them, and making sense of the data. For in at least several cases, the technologies themselves are not suitable." That trend will influence the entire ecosystem, including manufacturers, suppliers, analytical-service providers, and regulators. As the industry maintains the drive toward ever-faster CMC development, it is imperative that it happens without loss of effective end-to-

end control strategies and operational integrity. Speed, quality, agility, and value need to be maintained and optimized in lockstep; and quality is a *sine qua non* — the license to operate.

CoG is an urgent pressure point, particularly given the recent extended period of high inflation. "Our industry is extremely capital intensive," noted Patil. Jon Coffman (executive director at AstraZeneca) devoted his talk to antibody manufacturing: "One of the things I think about a lot is how we can get CoG down to US\$10 a gram," he explained. The entire ecosystem has experienced higher costs of materials and equipment, low appetite for capital investments, and a period of reduced funding opportunities for new entrants into the biotechnology industry.

Speakers posited partnership as key to offsetting budget and other constraints. Daria Fedyukina (project manager at the Advanced Research Projects Agency for Health, ARPA-H) discussed how her agency focuses on advancing biomedical and health research and targets funding support toward "areas where there is too much risk for any given stakeholder." Speakers agreed on a number of beneficial scenarios: small companies with bespoke capabilities partnering with large ones, experts in biologics and synthetic-molecule development learning from each other, companies bringing in customers and academics, and organizations reducing CoG not

merely for business and profit but also for equity to bring global access to vaccines.

NEW DEVELOPMENTS IN PLATFORM AND FLEXIBLE MANUFACTURING

Speed: The urgent response to the COVID-19 pandemic redefined the industry's role in making effective vaccines and drugs available rapidly. Within hours of the SARS-CoV-2 sequence's publication in January 2020, several groups realized (based on prior experience with coronaviruses) that the surface spike protein was an excellent target for creating a vaccine; a protein-based vaccine became the logical path forward. In an unexpected outcome, two alternate approaches proved to have shorter timelines than making the protein antigen. The first approach used mRNA delivery (Pfizer–BioNTech and, independently, Moderna). The second approach was based on replication-deficient adenoviruses, which were developed by an Oxford University team collaborating with AstraZeneca and by Johnson & Johnson. Collectively, those vaccines were provided at a scale of billions of doses; now, both approaches can be considered platforms.

Manufacturers achieved flexibility in their facilities by making extensive use of single-use equipment. Unit operations with closed connections are protected from external contamination, resulting in facility layouts resembling ballrooms with multiple unit operations running in the same room (1). Piombino noted that his customers valued having a single point of contact for communication with government regulatory agencies: "[We're] trying to convert to a nonclassified environment" for knowledge-sharing that leads to "a discussion with the FDA [US Food and Drug Administration]" as a means to get feedback. "NIIMBL is uniquely positioned to have that conversation," Piombino added.

Emerging Technologies: Many NIIMBL member companies represented at the National Meeting develop and manufacture monoclonal antibodies (mAbs) at large scales. In nearly every case, mAbs are made by fed-batch cell cultures based on Chinese hamster

“We’re not competing with each other. We’re competing with the **DISEASE.**”

— H. Chen

ovary (CHO)–cell platforms using several standard unit operations (e.g., protein A affinity chromatography for primary purification). Switching from fed-batch processing to continuous operations can help to reduce facility sizes and CoG. Thus, several NIIMBL member companies are looking to make such a switch in antibody manufacturing.

Although many of the member companies that design and make equipment have interest in process patents, those companies still share knowledge about technology developments, thereby building platforms that perform better and allowing new products to get licensed and introduced more rapidly. As Coffman noted, “I 100% agree on the value — certainly to [biopharmaceutical] companies — of allowing broad use of innovative technology.”

New developments in platform manufacturing will include revisions to how regulatory agencies view the role of prior knowledge and commercial experience. The FDA has issued proposed guidance on platform technologies (2). The proposed document “Guidance for Advanced Manufacturing Technologies Designation” would create a pathway for review of new technologies that is independent of biologics license applications (BLAs) (3).

Environmental Concerns: Extensive application of single-use equipment leads to the common problem of how to handle remaining plastic waste. “We’re all driving toward carbon-neutral bioprocessing,” said David Pollard (head of advanced bioprocessing at Sartorius Corporate Research), “but obviously an element of this needs to come from plastics recycling. Corporate research is passionate about making this successful.” Pollard shared Sartorius’s



Tom Piombino (managing director of Integrated Project Services, LLC) discussed fusion modalities from a chemistry, controls, and manufacturing (CMC) perspective.

recent successes with recycling polycarbonate from automated bioreactor systems and mechanically breaking down film plastics. Pollard also used the opportunity at the meeting to solicit partners: “I really need help from the community here . . . to put a pilot plastics collection and sorting process in place . . . on the East Coast and West Coast of the United States.”

Jerome Bill (director at Genetech) emphasized the widespread investment in sustainability: “Whether it’s academia, vendors, [or] industry, everyone’s talking about sustainability right now. With that industry-wide focus and legislation driving changes, it’s going to push sustainability.” Addressing the challenge of recycling plastics is an ideal project for a consortium approach. Through its process-intensification program and funded projects, NIIMBL has focused on sustainability, establishing partnerships to develop models for process developers. Speakers discussed NIIMBL’s ability to bring together multiple biopharmaceutical companies, equipment-supply companies, and regulatory stakeholders to accelerate technology development of single-use equipment that can be recycled fully. Industry-wide process-intensification efforts aim to address sustainability in parallel with demands for higher productivity, lower CoG, and increased support for pipeline complexities. Bill noted the opportunity in recent advances: “We have new tools, processes, and metrics to measure and manage our impact on sustainability. That allows vendors to develop better direct materials. It allows our development organizations to develop

better processes with sustainability in mind. And as we’ve already seen from next-generation facility designs, we have the ability to cater our manufacturing facilities towards the significant contributors to sustainability such as saving water or saving energy.”

TECHNOLOGY TRANSFER, COMPARABILITY, AND QUALITY

Advances in vaccines, gene therapies, cell therapies, and therapeutic proteins raise the stakes for quality control. Those modalities differ in complexity. For example, if the product is a peptide, then its developer probably can claim that the product is well characterized so long as typical analytical technologies are used appropriately. Therefore, bridging a technology transfer can be based primarily on analysis of final products.

For an inactivated or attenuated viral vaccine, that claim is far more difficult: “What I have always wanted from PAT [process analytical technology] is that kind of rapid ability to understand if a product is under control based on a quality-indicating parameter. That’s what’s been hard in the biologics space — having tools that get at those parameters really well,” noted Collins. Analytical tools play key roles in rapid process development and high-quality vaccine manufacture (4).

“We really believe automation is going to drive a lot of the success toward low-cost, new modalities, and we’re working toward self-driving processes, autonomous processes,” Pollard shared. Fedyukina described her agency’s enthusiasm to fund out-of-the-box ideas that would speed up control processes, asking, for example, “What if release



From left to right: Joey Studts, Rick Morris, Michael Phillips, David Pollard, and Eric Fallon

testing could be done in one hour — release testing for gene therapy — to make it quick, make it efficient?”

An industry-led steering committee established through NIIMBL is identifying high-priority projects for funding around analytical characterization of vaccines. A new vaccine analytical-characterization laboratory at NIIMBL headquarters in Newark, DE serves as a key resource for partnership through that vaccine initiative.

NONTRADITIONAL INVESTMENTS IN PANDEMIC PREPAREDNESS

Organizations such as the Biomedical Advanced Research and Development Authority (BARDA) in the United States have identified pandemic preparedness as a key goal. After a declaration of a pandemic by the World Health Organization (WHO), the race is on to develop and manufacture safe, effective vaccines at huge quantities. Continuing concerns over harmful pathogens have sparked diversity of investment and approach for pandemic preparedness: “Now you have not just big pharma, but also [small] biotech companies engaging in vaccines, and certainly there are alternatives, not just the classical prophylactic vaccines,” explained Hao Chen (assistant vice president of vaccines and advanced biotechnologies process R&D at Merck).

To respond efficiently to health crises, organizations require several key capabilities. Agile discovery groups are needed to create product candidates and generate critical reagents, such as antibodies needed for developing potency assays. Ideally, vaccine candidates are developed by leveraging

platform technology that already has been developed and tested at scale in a large, good manufacturing practice (GMP)-compliant facility. For instance, developers now are leveraging mRNA-based vaccine platforms such as those established by Pfizer-BioNTech and Moderna during the COVID-19 pandemic. In those cases, the timeline from antigen discovery to production of tens of millions of doses per month was less than a year. The Coalition for Epidemic Preparedness Innovations (CEPI) provides funding to help biomanufacturers generate tools for vaccine-based solutions that take less than 100 days from declaration of a pandemic to vaccine availability (based on emergency-use authorization, as appropriate) (5).

Health authorities can respond rapidly to future pandemics caused by influenza viruses or coronaviruses. However, if a novel pathogen suddenly circulates, then timelines would become more variable. Human immunodeficiency virus (HIV) provides a sobering reminder; patients still do not have an effective vaccine after 20 years of investment. Patients rely on lifetime treatments with chemically synthesized antiviral drugs for disease control. Work is being done by VIR Biotechnology in that domain, but as Eric Fallon (vice president of bioprocess development at VIR Biotechnology) noted, partnership is key: “partnerships for development and manufacturing, but also many nontraditional partnerships in the CMC space.” Chen likewise characterized the race for vaccines as a partnership: “We’re not competing with each other. We’re competing with the disease.”

HOLISTIC APPROACHES TO WORKFORCE DEVELOPMENT

Speakers at the meeting addressed the urgency of attracting, training, and retaining a workforce with skills relevant to address the ever-increasing complexity of biomanufacturing. Many organizations have initiated new recruiting methods — e.g., targeting community colleges, broadening the number of universities where companies recruit, and expanding R&D footprints geographically to locations with large talent pools such as San Diego, CA and Boston, MA. Having a breadth of perspectives is a shared goal to lead to stronger solutions for technical challenges. Speakers reported having recruiters work aggressively to get resumés of applicants with varying backgrounds and experiences before interviewing. Recruiting more experienced and/or postgraduate talent is also a difficult and lengthy process in the current environment, but it is important for organizational success and ensuring good role-modeling for junior staff.

Several industry leaders are looking to Germany, where an established apprenticeship and work-study system is a model for creating a similar approach in the United States. Joey Studts (director of downstream development at Boehringer Ingelheim) noted that because internships are integrated firmly into the training pipeline in his German company, he must limit the number of privately recruited interns on his teams. Building dynamic teams in growing areas, such as microfluidics and process automation, calls for collaboration among industry and academics to establish ways to increase capability in educational programs that have such key areas in mind, noted Pollard. Instituting educational tracks that specifically train for apprentice technical roles such as qualified laboratory technicians, would resolve challenges building this sector, he continued. Such tracks would ease filling those roles by sourcing well-trained candidates from a broad, diverse base.

Just as important is talent retention. As noted by Fallon, this calls for “having a culture where people can bring their whole selves to work, an environment where people feel they can

“I’m excited about this time in history because, at this intersection of science and technology, it’s never been as **DISRUPTIVE** as it is now.”

— M. Adkins

really be themselves.” Retention, many speakers emphasized, is about supplying opportunities to move within an organization, providing tools for professional development, and instilling a learning environment for people. Creating organizational cultures that keep people throughout their career ensures that role models are sustained, especially when there is conscious emphasis on and support for experienced professionals staying current with next-generation approaches and technologies.

Practically speaking, companies seek to extract knowledge and decision power from a rapidly increasing wealth of data. To facilitate that, people need to develop skills for working across different areas and levels of experience. Some organizations have instituted rotational development programs, particularly so that managers can receive hands-on experience in different groups across R&D sectors and learn how different areas connect.

Increased consistency in biomanufacturing training programs could be developed through nationally accessible training centers for the current and future workforce, paired with the ability to rely on standardized competency and skills assessments, including a reliable system of credentials to provide transparent evidence of workforce-readiness. “Workforce is a high priority for [implementing Sartorius’ sustainability initiatives],” noted Pollard, “and we’re very active in the NIIMBL workforce team.” Likewise, NIIMBL’s training database, funded by the Gates Foundation, allows members to

exchange resources around professional development, academic programs, and career services (6).

CONNECTED FLOWS IN DATA SCIENCE AND DIGITAL SOLUTIONS

Discussions on the future of biopharmaceutical manufacturing inevitably include the recognition of the importance and promise of data science and digital solutions. A great opportunity lies in closing the gap between data and process science, from digitization to expansive use of artificial intelligence (AI) and machine learning (ML). Pulling in mechanistic and statistical modeling is just as relevant as continuing to focus on data analytics, process and plant automation, and robotics. Studts shared ways that data are driving technology development for his company: “We’re trying to take these two technologies — mechanistic modeling so that we can provide more predictive power to our data and PAT with Raman spectroscopy to have more data from an experiment — to understand the data that we’re getting and be able to use live, on-line data to make better models.”

Although PATs are not new, industry attendees expressed the need for more efficient PATs and noted that legacy infrastructure can raise frustrating obstacles toward implementing such improvements. The vision of the future includes “just-in-time technical wisdom” enabled by connected tools, integrated workflows, and simple user experiences.

A consequence of the interest in continuous manufacturing is the need to link data in real-time from one unit operation to the next. That leads to the

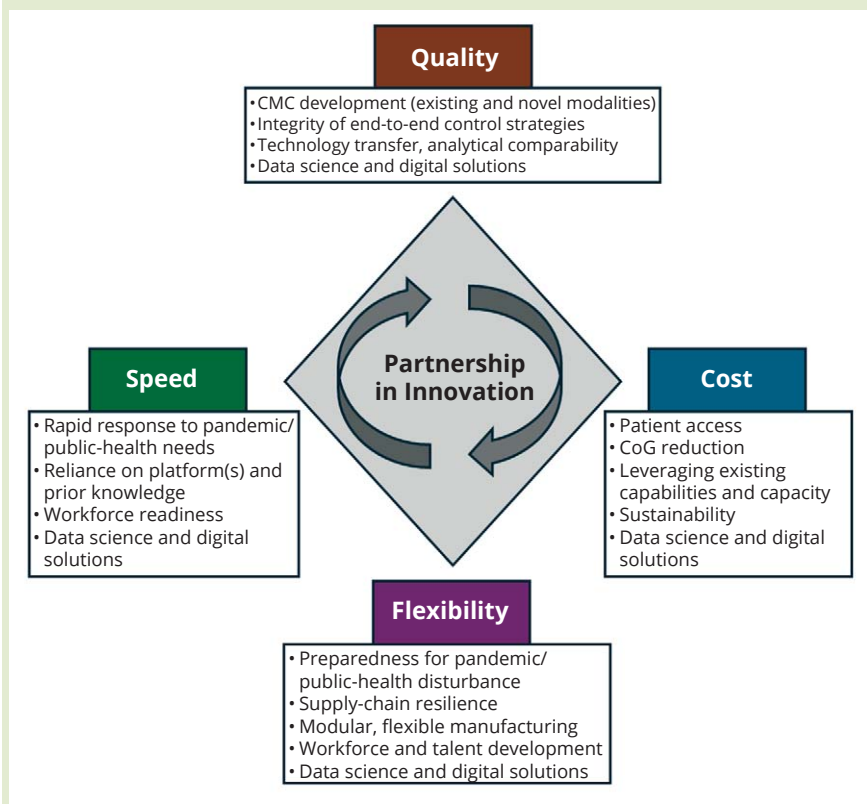
emphasis on standardization and the importance of compatible data-handling platforms. “I think it’s definitely the standardization and the openness in terms of sharing these learnings [that will be critical], so forums like NIIMBL meetings are definitely a part of the solution,” said Patil. The discussions at this particular meeting highlighted the desire to achieve efficiencies by driving toward practical standards and consistent structure in data ontologies, ultimately with an eye toward enabling “one-click” technology transfers, reduced cycle times, and regulatory convergence.

Michalle Adkins (director of life sciences strategy at Emerson) remarked on the common interest in “the ability to share data anonymously. That’s going to be helpful for the whole industry and will ultimately benefit patients.” Piombino agreed, highlighting the importance of NIIMBL’s big-data program and its efforts to establish consistent data ontologies: “We need to be able to get that data with context in some kind of a common format, with some commonalities that you can make sense out of it.” That too relies on a commitment to partnership. As Fallon summarized, “If everybody says we’re going to do this in a common way with a common vernacular and common tools, everybody wins.” Studts described an aggressive publishing strategy to share the models that his company developed with NIIMBL support. His company also included those models in a BLA, with the understanding that “Boehringer going



From left to right: Wayne Heacock, Michalle Adkins, Tom Piombino, and Ranjeet Patil

Figure 1: The four pillars of the current biopharmaceutical landscape; CMC = chemistry, manufacturing, and controls; CoG = cost of goods



it alone would have been a much more difficult path.”

The “facility of the future” must continue to mature from “digital islands” to connected, predictive, and ultimately adaptive plants. A significant breakthrough for the industry could come from a future state of flexible manufacturing networks, in which investments are decoupled from technology transfer (e.g., success milestones of specific products in a pipeline). Patil shared such a vision for transforming the industry: “In the ideal world, we will be able to deconvolute the process and product. And if we are able to overcome that dependency [on inflexible infrastructure], we can be bolder in our process choices. We can be more ambitious in our process design and more efficient in the productivity. Analytics are what we believe are the key enablers of that transformation.” The translation of factory operations into digital twins and the desire to leverage more extensive mechanistic and/or empirical modeling also present new opportunities to engage with academia.

Speakers weighed the challenge their companies faced to make this major transition on their own or work together: “It’s a big lift for tool providers to be able to adapt to those standards because of limited resources,” noted Wayne Heacock (biopharmaceutical market specialist at Agilent). “Organizations such as NIIMBL are incredibly valuable this way. We need to figure out a way to collaborate a little more closely or, though it might seem radical, consider cost sharing to raise the boat for everybody.” The current atmosphere of accelerated manufacturing brings risk, noted Collins, “so you have to free people up through central groups that make it a little safer for us to try things together.” Using a consortium can reduce risk.

Pollard asked, “Can there be more pressure on regulatory agencies to come up with some incentives to drive end users to be those first adopters of new technologies and workflow solutions?” A consortium can enable companies to work together to develop pilot-scale prototypes, as was the case with NIIMBL’s process-intensification initiative.

“[A proof of concept] helps derisk implementation. It helps demonstrate value. But most importantly, it helps **CREATE ALLIES.**”
— M. Phillips

Rapid prototyping is essential in this environment. “An innovation sandbox is critical,” explained Michael Phillips (technical director for innovation at MilliporeSigma/EMD Serono). He said that NIIMBL provides valuable support as a precompetitive consortium that connects allies to use test beds and derisk innovation to achieve proof of concept. As Studts remarked, “What drove us to come to NIIMBL was the extensive collaboration network of industry and academic colleagues to help drive innovative ideals. We need more data faster to understand how our models are working, why they are working, and when they’re not working.” And Phillips explained, “What you’re doing is changing something that’s theoretical into something that’s experiential. A proof of concept is really worth a thousand words in so many different ways. It not only supports technical feasibility, but also helps you to identify gaps and opportunities. It helps derisk implementation. It helps demonstrate value. But most importantly, it helps create allies.”

PARTNERSHIP IN INNOVATION

Although the “arrows” of speed, quality, and cost do not necessarily point in opposing directions, flexibility is needed in carefully managing constraints. In today’s world, flexibility may best be seen as a rightful “fourth pillar” that can address the urgencies and innovations in the field to deliver concrete societal advantages. This is certainly the case in an environment where systemic disturbances, such as a pandemic, are more a question of

“when” than “if.” Shown by examples in Figure 1, each of the four pillars comprises several factors that underscore both the needed solutions and means to deliver on them, as were explored by the speakers at the NIIMBL National Meeting.

The complexity and interconnectedness of all system elements across the four pillars of the current biopharmaceutical landscape point to the importance of innovation. The industry needs models for reducing the risk of failure and increasing the likelihood of regions, countries, and societies improving their responses to the next (or yet unresolved) public health crisis.

The rising promise of data sciences and digital solutions (e.g., data analytics, AI, and ML) plays a central role: “We have data, and we have knowledge, and whoever converts those tons of data into knowledge, they win,” noted Studts. As Studts described it, the range of perspectives presented by the National Meeting attendees all centered around “communication, taking a holistic view and starting from the data — understanding the data flows that will have an impact — and then building around that information.” This suggests an inescapable demand for integration and increased literacy across functional expertise: how to have bioprocess specialists communicate with data scientists, how to convey cost–benefit rationales to upper management, how to build dynamic teams in new areas, how to connect industry and academics, and how to improve literacy at all levels of the organization to deliver innovative solutions quickly through integrating and leveraging knowledge areas.

Rick Morris (distinguished fellow of technology and innovation strategy and chief technical officer at Cytiva) noted NIIMBL’s role as connector across such areas: “They have the test bed. They can pull in the SMEs [subject-matter experts] and drive the convergence and then the value proposition.” Fallon reinforced the importance of focusing on strategic collaboration: “That means building relationships. It means building trust. It means having unified goals, having an arrangement where

our company advances. And we do that together. NIIMBL has existed since day one with this thought of ‘us-based innovation.’” Adkins described the potential in harnessing the complexity of large-scale changes across the ecosystem: “What if we could use beneficial, disruptive technologies for our processes — manufacturing plants, products and people — in ways that also help the planet? What if we could get products to market faster, by being flexible in how we manufacture them, operating plants efficiently to cost effectively get therapies to patients as soon as possible once they’re released to the market, and do all of this in a sustainable way? What an amazing future to look forward to as part of the life-science and technology industries working with NIIMBL. I’m excited about this time in history because, at this intersection of science and technology, it’s never been as disruptive as it is now.”

Although NIIMBL is a key dot-connector, the field is ripe for increasingly interconnected innovations forged across the biopharmaceutical landscape. James Pierce (executive director at Amgen) captured this sentiment in a concluding moment at the meeting: “The big surprise was that we’re not actually that different, that there are common threads that run through all of this . . . and there’s opportunity to collaborate on driving the sciences forward.”

ACKNOWLEDGMENTS

NIIMBL is a public-private partnership whose mission is to accelerate biopharmaceutical innovation, support the development of standards that enable more efficient and rapid manufacturing capabilities, and educate and train a world-leading biopharmaceutical manufacturing workforce, fundamentally advancing US competitiveness in the industry. NIIMBL is part of Manufacturing USA, a diverse network of federally sponsored manufacturing innovation institutes, and is funded through a cooperative agreement (#70NANB21H086) from the National Institute of Standards and Technology (NIST) in the US Department of Commerce, with significant additional support from its members.

We gratefully acknowledge the thoughtful participation of our conference speakers, who generously shared their expertise and

insights to generate illuminating dialogue around the future of biopharmaceutical manufacturing at NIIMBL’s June 2024 National Meeting: Michalle Adkins, Jerome Bill, Hao Chen, Jon Coffman, Paul Collins, Eric Fallon, Daria Fedyukina, Wayne Heacock, Rick Morris, Ranjeet Patil, Michael Phillips, James Pierce, Tom Piombino, David Pollard, and Joey Studts.

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Flocculation and Filtration

Improving mAb Harvest Clarification Efficiency from High-Density Cell Cultures

Johanna Gervais and Chen Wang

Downstream processing of recombinant therapeutic antibodies from mammalian cell culture typically requires an initial clarification step to remove cells, cell debris, and other insoluble impurities from the product stream. In response to increased demand for therapeutic antibodies, upstream-production technology has made considerable progress. High productivities and cell densities have become the new norm in today's bioprocessing (1, 2). That development poses significant challenges to downstream operations starting from harvest clarification.

Traditional harvest-clarification operations rely on depth filtration, either alone or in combination with continuous disk-stack centrifugation. Depth filters typically have a cellulose fiber bed, an ionic charged-resin binder, and a porous filter aid such as diatomaceous earth (DE) (3). Because of those construction materials and device design, depth filters not only retain insoluble matter based on size exclusion, but also can exploit ionic, hydrophobic, and other chemical



[HTTPS://WWW.EMDMILLIPORE.COM](https://www.emdmillipore.com)

interactions (3, 4) to remove soluble impurities such as host-cell proteins (HCPs) and DNA (hcDNA), as well as other charged particles (5, 6). The pore sizes of depth filters cover a range of particle sizes. Many harvest processes use two stages of depth filters: a primary or coarse clarification stage (e.g., using a Millistak+ DOHC filter from MilliporeSigma) to retain intact cells followed by a secondary or finer clarification stage (e.g., using a Millistak+ XOHC filter) to retain cellular debris (7).

Depth filtration can be costly, often requiring substantial filter area, and thus represents a significant bottleneck during processing of large-scale, high-density ($>20 \times 10^6$ cells/mL) cell-culture harvest material (8). Not only do harvest titer and cell biomass (cells and cell debris) increase in high-density cell cultures, but elevated levels of soluble particles such as hcDNA, colloids, and HCPs can be generated (9, 10). The increased biomass of high-density harvest reduces efficiency in traditional harvest-recovery operations. Centrifugation of that increased biomass

Table 1: Millistak+ (DOHC and/or XOHC) and Clarisolve depth filters (MilliporeSigma) used for the control sample and after treatment with acid or polymer flocculant

Pre-Treatment	Depth Filter Types Used	
	Centrifuge-Free Method	Centrifuge-Based Method
None (Control)	DOHC or DOHC:XOHC	XOHC
Citric Acid	Clarisolve 20 MS	XOHC
pDADMAC	Clarisolve 60HX	XOHC

requires frequent discharge intervals, leading to product loss and limited product recovery. Additionally, the elevated soluble particles in high-density harvest reduce depth-filter throughput, requiring increased filter area that often will be constrained by the realities of equipment size and facility footprint. The problems compound when a centrifuge is unavailable and only a filtration train is used to clarify a high-density culture — as typically is the case in manufacturing suites based on single-use technology.

Alternative methods of enhancing clarification efficiency are needed to enable a next-generation, high-productivity manufacturing platform that fits within existing facilities rather than expanding the manufacturing space. Clarifying agents such as charged flocculants and precipitants could help alleviate such constraints. Positively charged polymeric flocculants (11, 12) — e.g., polyethylenimine (PEI), polydiallyldimethylammonium chloride (pDADMAC) (Figure 1), chitosan, polyethylene glycol (PEG), and modified polyallylamine (mPAA, such as in Clarisolve filters from MilliporeSigma) —

PRODUCT FOCUS: Monoclonal antibodies and other proteins

PROCESS FOCUS: Downstream processing

AUDIENCE: Process development, manufacturing, QA/QC

KEYWORDS: Depth filtration, pDADMAC, citric acid, centrifugation, process optimization

LEVEL: Intermediate

Table 2: Area ratio of depth filters used in the centrifuge-free control condition

Molecule	DOHC:XOHC Ratio
Molecule A	1:0
Molecule B	3:1
Molecule C	1:1
Molecule D	1:1

have been evaluated to facilitate harvest clarification (4, 7, 13, 14). In addition to agglomerating cells and cell debris, such clarifying agents bind negatively charged molecules such as hcDNA, HCPs, and colloids, creating insoluble complexes that will settle out of solution readily (15). Harvest acidification, notably with citric acid and caprylic acid, neutralizes the surface charge of molecules with lower isoelectric points under acidic conditions and precipitates those impurities from solution (4). Clarifying agents not only improve harvest filterability, but they also can alleviate the burden on downstream polishing steps to remove HCPs and hcDNA, improving product quality and stability (9). Moreover, removal of hcDNA and colloids from cell-culture harvest has been shown to extend the lifetime of chromatography resins, particularly expensive protein A affinity-based media often used for mAb capture (16–18).

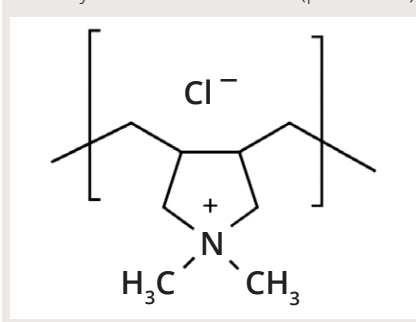
Traditional depth filters can clog with the large complexes created by flocculation and acidification. EMD Millipore's Clarisolve filters were designed to handle large particles and are made with similar materials of construction as the same company's established DOHC and XOHC filters. With nominal pore sizes of 0.6–20 µm, Clarisolve 20 MS filters were designed for particulates formed by acid precipitation. Clarisolve 40 MS (0.6–40 µm) and 60HX (8–60 µm) filters were designed for larger particles created by polymer flocculation. The filters are made in the same Pod cartridge format as that of Millistak+ filters, which makes them easy to set up for large-scale manufacturing.

Use of clarifying agents to aid in Chinese hamster ovary (CHO) cell-culture harvest filtration has been published elsewhere. Kang et al. demonstrated effective reduction of HCPs, hcDNA, and aggregates using polyacrylic acid (PAA) flocculant

Table 3: Harvest information for one bispecific and three monoclonal antibodies (mAbs)

Molecule	Structure	Peak Cell Density	Harvest Viability	Solids	Strategy Tested
A	Bispecific	20×10^6	70%	4%	Both methods
B	mAb	15×10^6	80%	5%	Both methods
C	mAb	24×10^6	76%	7%	Centrifuge-free method
D	mAb	11×10^6	80%	3%	Centrifuge-free method

Figure 1: Structure of polydiallyl-dimethylammonium chloride (pDADMAC)



followed by depth filtration (11). Mc Nerney et al. used two flocculants — pDADMAC and PEG — to increase filter throughput (12). Singh et al. found that acidification and flocculation with chitosan or a flocculant polymer improved filtration throughput and clarification efficiency (4). Tomic et al. demonstrated improved filter performance and DNA reduction using pDADMAC flocculation in combination with Clarisolve filters on cell densities up to 16×10^6 cells/mL (7). No publications yet have reported a thorough study, however, evaluating both flocculant and acidification in combination with Clarisolve filters for cell densities $>16 \times 10^6$ cells/mL in either a centrifuge-free process or with traditional depth filters in a centrifugation-based process.

We evaluated pDADMAC flocculation and techniques based on citric-acid precipitation combined with state-of-the-art depth filtration for their ability to enhance clarification efficiency in CHO cell harvests of $\leq 24 \times 10^6$ cells/mL. And we compared the results with those of a traditional depth-filtration approach, using methods both with and without centrifugation to explore different suite scenarios for implementation. Our results show that pDADMAC and citric-acid treatment improve filter efficiency and DNA removal when benchmarked against the performance of traditional depth filtration.

MATERIALS AND METHODS

Flocculation of Harvest and Filtration

Experiments: For this study, cell broth was either flocculated with 0.05–0.08% pDADMAC (10% solution) from Millipore or adjusted to pH 5.0 with 1 M citric acid from JT Baker (Avantor), then mixed for an hour before harvest operations started at ambient temperature. We maintained the filtration flow rate at a target of 150 liters/m²/hour (LMH).

For the centrifuge-free process, treated cell broth was filtered through a Clarisolve filter as described in Table 1. The control sample (no pretreatment) was filtered with a DOHC filter (for Molecule A) or a DOHC:XOHC filter train (for all other molecules) to simulate a platform centrifuge-free filtration strategy. Table 2 shows the ratio of DOHC and XOHC filters used for the control conditions. Harvests were stirred continuously during the filtration process to prevent settling of cells, precipitants, and flocculants.

For the centrifugation-based method, pretreated cell broth was centrifuged at 2500g for six minutes to simulate the platform process. Centrates with and without treatment were filtered at ambient temperature with an XOHC filter according to the current platform centrifugation harvest strategy. We determined product titers using a POROS A cartridge (Thermo Fisher Scientific) run on a high-performance liquid chromatography (HPLC) skid. And we calculated the filtration yield using the following equation:

$$\% \text{Yield} = 100 \times (C_F \times V_F) \div (C_H \times V_H)$$

where C_F = product titer in filtrate, V_F = filtrate volume, C_H = product titer in harvest supernatant, and V_H = harvest volume. Portions of the harvest filtrates were purified over a protein A affinity column. HCP and DNA levels were analyzed for both the harvest filtrates and protein A eluates.

Figure 2: Harvest pretreatment study design

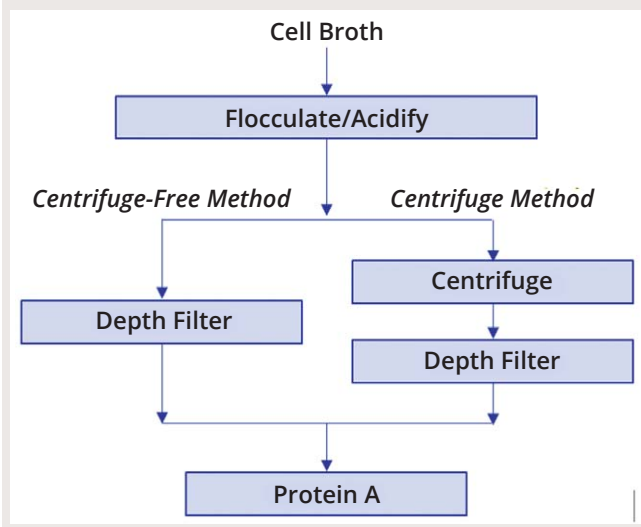


Table 4: Harvest yield and throughput (L/m² at 15 psi) improvement with acid or polymer flocculant treatment in centrifuge-free process

Molecule	Pretreatment	Yield	Improvement*
A	Untreated	103%	—
	Citric Acid	99%	2 fold
	pDADMAC	99%	4 fold
B	Untreated	93%	—
	Citric Acid	94%	2 fold
	pDADMAC	92%	5 fold
C	Untreated	100%	—
	Citric Acid	100%	5 fold
	pDADMAC	100%	5 fold
D	Untreated	97%	—
	Citric Acid	96%	2 fold
	pDADMAC	95%	4 fold

* Defined as the ratio of depth-filter loading when filter-inlet pressure reaches 15 psi in the treated condition divided by the throughput of the control condition

Table 5: Product-quality improvement over control conditions with acid or polymer flocculant treatment in a centrifuge-free process; ND = none detected, ProA = protein A affinity chromatography, pDADMAC = polydiallyldimethylammonium chloride

Molecule	Pretreatment	Host-Cell Protein Improvement		Host-Cell DNA Improvement	
		Harvest Filtrate	ProA Eluate	Harvest Filtrate	ProA Eluate
A (bsAb)	Citric Acid	0.3 fold	3.0 fold	4.0 log	2.6 log
	pDADMAC	2.0 fold	12.0 fold	6.8 log	2.9 log
B (mAb)	Citric Acid	1.1 fold	1.0 fold	1.4 log	1.6 log
	pDADMAC	0.8 fold	12.7 fold	3.9 log	1.7 log
C (mAb)	Citric Acid	2.2 fold	1.6 fold	1.3 log	1.5 log
	pDADMAC	3.6 fold	2.0 fold	2.8 log	1.6 log
D (mAb)	Citric Acid	2.4 fold	2.9 fold	ND	ND
	pDADMAC	0.7 fold	7.8 fold	ND	ND

Analytical Assays: We evaluated HCP levels using an enzyme-linked immunosorbent assay (ELISA) with proprietary anti-CHO HCP antibodies and a standard, calculating the improvement as the HCP level in the test condition (ng/mg) divided by that in the control condition (ng/mg). Host-cell DNA levels were measured by quantitative polymerase chain reaction (qPCR), with improvement calculated as the logarithm (log) of the DNA level in the test condition (pg/mg) divided by the DNA level in the control condition (pg/mg).

RESULTS AND DISCUSSION

Processing suites for monoclonal antibody manufacturing may or may not include a centrifuge. Both strategies, depicted in Figure 2, along with two settling methods, flocculation and acidification, in combination with the traditional and Clarisolve depth filters, were evaluated for cell culture harvest

from multiple molecules, described in Table 3.

Centrifuge-Free Method: As summarized in Table 4, acid treatment and flocculation of cell-culture broth demonstrated improved depth filter efficiency, up to fivefold greater throughput than with untreated harvest, in removal of cells and cell debris without impacting harvest yield. Figure 3 depicts the pressure profiles obtained with Molecule A, as a representative case. In most cases, the flocculation treatment combined with Clarisolve 60HX filtration had greater throughput than acidification with Clarisolve 20 MS filtration. Filter pressure begins to rise at a lower loading and increases more rapidly in the acidified material than the flocculated harvest, likely due to the smaller pore size of the Clarisolve 20 MS filter.

Generally, the pDADMAC and acid treatment also improved clearance of soluble contaminants such as DNA and HCPs, see Table 5. The level of HCP

reduction in flocculated harvest was molecule dependent, with some molecules showing no improvement and others obtaining up to a 12.7-fold improvement in HCP removal in protein A affinity chromatography eluate compared to the untreated condition. Of note, the protein A purification process for Molecule C included an optimized wash to remove HCPs that nonselectively bind to protein A resin, whereas the protein A purification process of the other three molecules did not include this optimized wash condition. The lower HCP reduction in Molecule C and the significantly higher improvement in flocculation compared to acidification treatment in the other three molecules suggests that flocculation also may selectively remove populations of HCPs that nonselectively bind to the protein A resin (data available on request). In all cases studied, the DNA reduction in pDADMAC and acid-treated harvests was significant, with at least 1.6 log reduction in DNA over the untreated harvest.

Centrifugation Method: As summarized in Table 6, when centrifugation was used to remove cells and flocculants prior to filtration, pretreatment with acid or polymer flocculant improved the harvest filter throughput by at least twofold and threefold, respectively. Figure 4 depicts the pressure profiles obtained with Molecule A, as a representative case. In all treated harvests, except Molecule B acidification, the volume of centrate prepared for these studies was depleted prior to observation of pressure



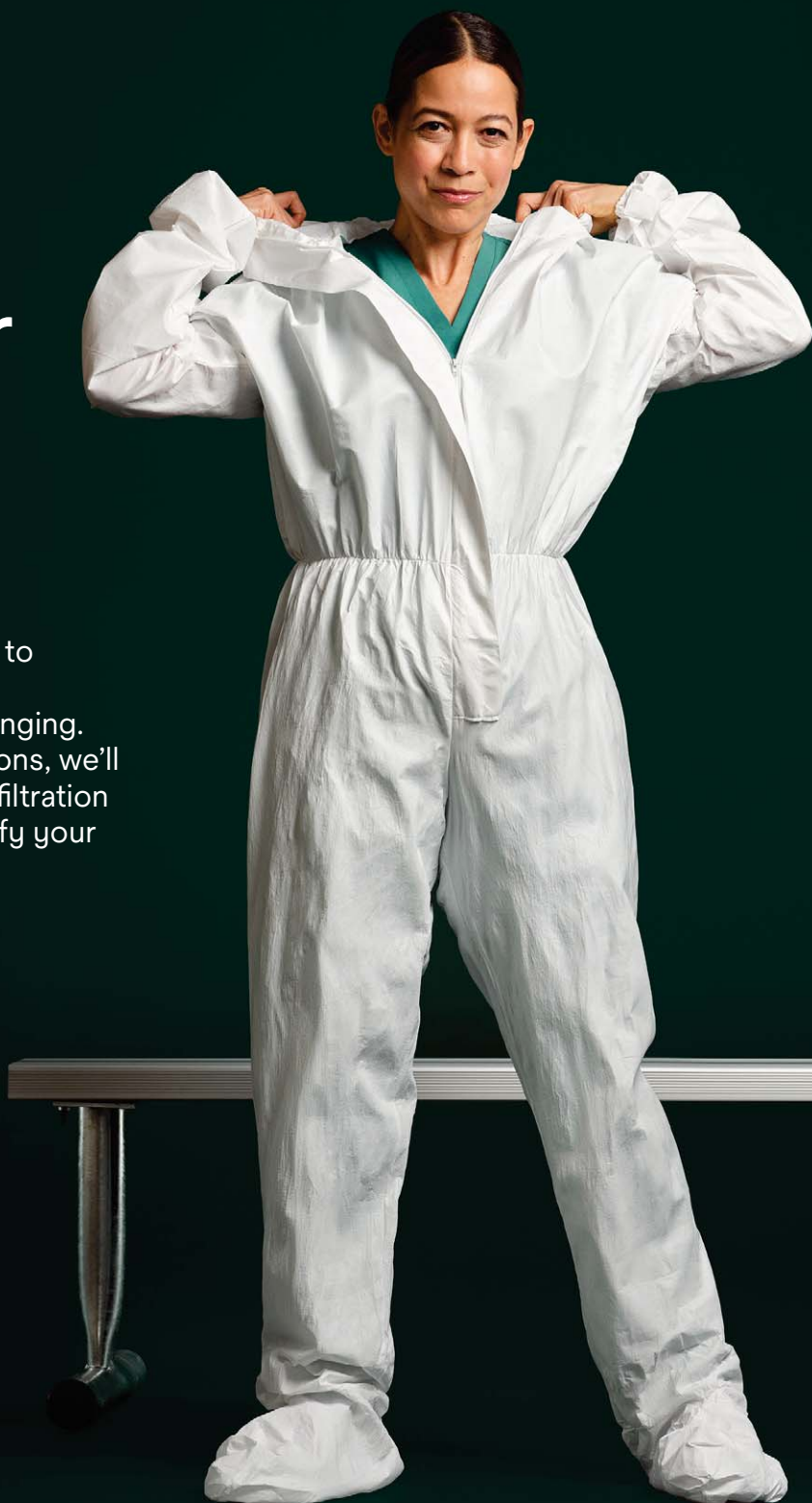
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Figure 3: Molecule A depth-filter pressure profiles with untreated (blue), acid-treated (orange), and pDADMAC-treated (dark blue) harvest. The untreated harvest was filtered through a D0HC cellulose filter, the acid-treated harvest through a Clarisolve 20 MS filter, and the pDADMAC-treated harvest through a Clarisolve 60 MS filter. Filter throughput was defined by loading to a pressure of 15 psi.

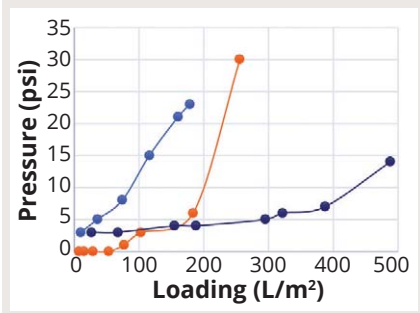


Figure 4: Molecule A X0HC depth-filter pressure profiles with untreated (blue), acid-treated (orange), and pDADMAC-treated (dark blue) harvest; filter throughput was defined by loading to a pressure of 15 psi. In most cases with acid-treated and pDADMAC-treated harvest materials, the prepared concentrate volume was insufficient to achieve loading levels in which a 15-psi pressure was observed.

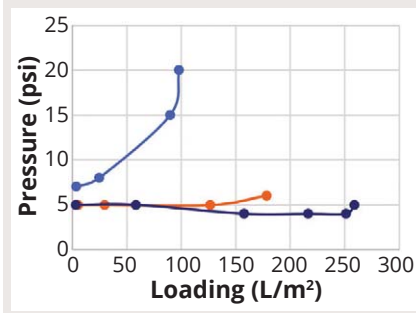


Table 7: Product-quality improvement (over control conditions) with acid or polymer flocculant treatment in centrifugation-based process; ProA = protein A affinity

Molecule	Pretreatment	Host-Cell Protein Improvement		DNA Improvement	
		Harvest Filtrate	ProA Eluate	Harvest Filtrate	ProA Eluate
Molecule A	Citric Acid	2.5 fold	1.0 fold	5.4 log	3.0 log
	pDADMAC	2.3 fold	14.9 fold	5.9 log	3.1 log
Molecule B	Citric Acid	1.3 fold	1.3 fold	2.9 log	1.5 log
	pDADMAC	0.9 fold	12.6 fold	3.8 log	2.2 log

increase. Because filtration ended prior to reaching the pressure target in the treated harvests, the total filter throughput improvement was underestimated in this study.

As Table 7 shows, pDADMAC and acid pretreatment improved the clearance of DNA and HCPs. Acid treatment performed similarly to polymer-flocculant treatment in harvest filtrate. However, like the centrifuge-free method, the polymer flocculation is significantly better for HCP reduction in protein A eluates than is acidification, again suggesting that flocculation may remove populations of HCPs that nonselectively bind to the protein A resin.

DNA reduction was significant with pDADMAC and acid-treated harvests. DNA levels in protein A affinity eluate from treated harvest were between 1.5 and 3.1 log lower than in eluates from untreated harvest.

In addition to improving filter efficiency by agglomerating cells and cell debris, pDADMAC binds negatively charged soluble molecules, such as DNA and HCPs, and citric acid neutralizes the surface charge of such soluble impurities. In both cases, DNA and HCP aggregate

with cells and cell debris and settle out of solution, thereby facilitating the reduction of those molecules in treated harvest.

CONCLUSIONS

This study demonstrated significant yet feed-stream-dependent filter throughput improvement by polymer flocculation and acid-precipitation-based methods, providing an effective toolbox to address challenges associated with harvest clarification. Flocculation with pDADMAC or citric acid was effective both with and without the use of a centrifuge. The combination of flocculants and state-of-the-art filtration devices can improve the harvest filtration step by reducing the cost and/or footprint of the harvest filter train with 2–7× greater filter throughput.

The combination of pDADMAC or acid pretreatment with Clarisolve filters reduces harvest filtration to a single stage, thereby reducing the complexity of the typically two-stage filter train. Moreover, polymer flocculation and citric-acid precipitation increased impurity clearance with markedly lower DNA levels (1–450 pg/mg) in all cases

Table 6: Bispecific (A) and mAb (B) harvest yield and throughput fold improvement of filter throughput (L/m² at 15 psi) with acid or polymer flocculant pretreatment in centrifugation-based processes

Mol.	Treatment	Yield	Improvement*
A	None	103%	—
	Citric Acid	92%	>2
	pDADMAC	99%	>3
B	None	94%	n/a
	Citric Acid	95%	2
	pDADMAC	95%	>6

* Defined as the ratio of depth-filter loading when filter inlet pressure reaches 15 psi in the treated condition divided by the throughput of the control condition. Feed material was depleted prior to reaching the 15-psi target for pDADMAC treated harvests and citric-acid-treated Molecule A harvest.

and significantly lower HCP levels (1–150 ng/mg) in protein A eluate with some molecules. In most cases, pDADMAC exhibited better performance improvement than citric acid. High-molecular-weight levels, monitored by HPLC size-exclusion chromatography (data not shown), remained unchanged by either treatment, suggesting that flocculation and acidification are not effective pretreatments for removing antibody aggregates. In conclusion, pDADMAC flocculation and citric-acid precipitation alone (for centrifuge processes) or in combination with Clarisolve filters (for centrifuge-free processes) can provide an alternative to traditional harvest methods for high-density cell-culture challenges with higher filter throughput and improved HCP and DNA clearance.

The ability to scale up a flocculation or precipitation pretreatment was not addressed in this study. Further evaluation is needed to ensure that parameters such as treatment concentration, treatment addition rate, treatment temperature, and tank mixing rate are defined properly for larger scale conditions. Additionally, tank cleaning studies are needed to ensure proper manufacturing hygiene. More importantly, pDADMAC is cytotoxic, and the clearance of this polymer through the downstream process must be demonstrated. McNerney et al. used a sensitive DNA inhibition assay to confirm efficient clearance of pDADMAC from flocculated harvest — to 3 logs below the acceptable daily exposure limit for IV or subcutaneous dose — in depth filtered neutralized Protein A

eluate (14). Additional chromatography steps in the typical mAb process could provide additional safety margin.

ACKNOWLEDGMENTS

The authors are grateful to Karen Hurkmans (for DNA analysis), Heidi Althouse, and Martyna Pawlikowska.

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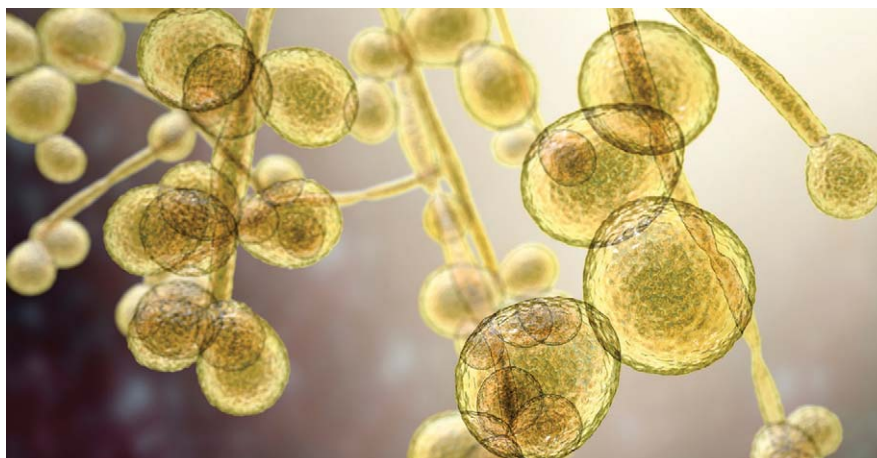
Microbial-Control Hold-Time Assessments

Guidance on Matrix and Bracketing Validation Approaches

Naveen Ganesh Muralidharan, Leya S. Rose, Tyler Younger, Manisha Walia, Alexander Elkin, Grace Chan, Shandilyn Ball, and Emma Bolduc

In biopharmaceutical manufacturing, maintaining product safety, consistency, and efficacy is paramount. Assessments of microbial growth during process hold times are critical to ensuring that such requirements are met. As regulatory agencies such as the US Food and Drug Administration (FDA) impose stringent validation rules, quality assurance and control (QA/QC) teams must adopt robust methodologies that adequately assess microbial risks associated with hold times. Here, we focus on microbial hold-time validation, exploring the use of bracketing and matrix approaches to ensure comprehensive evaluation. By addressing key parameters such as nutrient composition, hold-time duration, and growth-promoting conditions, we offer insights into developing reliable process-validation strategies.

To facilitate our evaluation, we use the “cardinal values” microbial-growth model to assess growth-promoting conditions (including pH, temperature,



Artist rendering of *Candida albicans*, one of six representative microorganisms used to assess microbial growth during validation of bioprocess hold times
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and osmolarity) under different process scenarios. That model provides a scientific basis for understanding risks associated with microbial proliferation during hold times. We also introduce a risk-assessment framework that uses objective scoring matrices, which are developed as a function of mixing hydrodynamics, nutrient conditions, and the specific hold-time characteristics of a given process. By integrating those factors, we present a more precise and data-driven method for evaluating microbial risks than can be achieved with a conventional validation strategy to ensure that biopharmaceutical processes remain compliant with regulatory standards while maintaining high product quality.

MICROBIAL HOLD-TIME STUDIES

In biopharmaceutical process validation, microbial hold-time studies are critical

for ensuring drug quality and sterility throughout a product’s life cycle (1, 2). The primary objective is to ascertain how long a solution can remain in a tank or other storage container without generating significant microbial growth. Such information is essential for maintaining product sterility during manufacturing and preventing contamination during storage and processing delays. By understanding microbial risks, manufacturers can implement effective control measures to safeguard product integrity — e.g., to ensure that no harmful levels of bioburden will compromise a product’s safety before it reaches a patient.

Establishing Acceptance Criteria for Microbial-Control Samples:

Managing hold times is critical for microbial control and product integrity. Storage intervals between processing steps must be defined and validated

PRODUCT FOCUS: Proteins, antibodies, parenterals

PROCESS FOCUS: QA/QC, downstream processing

AUDIENCE: Process development, manufacturing, analytical, QA/QC

KEYWORDS: Microbial control, hold-time studies, mixing vessels, risk assessment, cardinal microbial-growth model, grouping criteria

LEVEL: Intermediate

rigorously based on risk assessment, with tailored strategies to address unique microbial risks. Both bioburden and endotoxin limits are somewhat relaxed during early production stages to accommodate expected microbial loads. As a process advances, limits are tightened to align with regulatory guidelines.

Defined as storage periods between processing steps, **hold times** must be managed carefully to prevent microbial contamination. Each biomanufacturing stage, from cell culture to final formulation, presents unique risks that necessitate validated hold times based on comprehensive risk assessments. Early processing steps such as culture harvest by depth filtration or centrifugation followed by depth filtration typically take five to six hours (3). Muralidharan et al. demonstrated that a 12-hour prefiltration hold time for nutrient-rich media is feasible for cell-free harvest broth from bioreactors when supported by robust microbial-control strategies to prevent bioburden increases (1). As a process advances to intermediate stages such as harvest clarification and chromatography, hold times generally are reduced to 12–24 hours (4). In later downstream steps, especially before sterile filtration, hold times often are limited to 6–12 hours because of heightened contamination risk (5).

Biomanufacturers also need to manage hold times for growth-promoting cell-culture media and downstream buffers that are applied before sterile filtration. For culture media, industry best practice generally recommends a microbial hold time of ≤12 hours before filtration (1). That timeframe balances the need for sterility assurance with practical processing considerations. Similarly, for downstream buffers, microbial hold time before filtration typically is assessed within 24 hours (4). Validation and adherence to established practices are essential for upholding the efficacy and safety of both culture media and downstream buffers.

Bioburden and endotoxin limits also are critical for maintaining microbial control throughout biopharmaceutical production and processing. In the early upstream stages of sterile cell-culture

operations, bioburden limits typically are set at no more than 1 colony-forming unit for every 10 milliliters of media (1 CFU/10 mL), and endotoxin limits are set to 10 endotoxin units per milliliter of culture (10 EU/mL) (1). As a process advances to intermediate stages (e.g., clarification and capture chromatography), bioburden limits are increased to 100 CFU/10 mL, then are reduced gradually from 30 to 10 CFU/10 mL as the process approaches the drug-substance (DS) stage. Similarly, endotoxin limits decrease from 5 EU/mL to as low as 1 EU/mL depending on proximity to the DS stage (6). During final purification stages (up to sterile filtration), bioburden limits are tightened to <10 CFU/100 mL and to <1 CFU/10 mL for final products. Endotoxin limits ultimately are decreased to 0.25–1 EU/mL to ensure effective sterilization and product safety (7). For nonsterile products, including DSs and drug products (DPs) before sterile filtration, a bioburden limit of <10 CFU/100 mL generally is acceptable for regulatory agencies such as the FDA and European Medicines Agency (EMA), provided that such a limit is justified through process validation (8).

MATRIX AND BRACKETING VALIDATION APPROACHES

Quality teams use matrix and bracketing approaches during process validation to reduce the number of validation tests required while ensuring comprehensive process understanding. The *matrix approach* involves testing a subset of variable combinations, such as different product compositions, batch sizes, or equipment settings, assuming that untested combinations will behave similarly to the tested ones. Conversely, the *bracketing approach* focuses on testing extremes of such variables — e.g., the smallest and largest batch sizes or highest and lowest risk conditions — assuming that intermediate conditions will be covered by those extremes. Although a matrix approach provides flexibility and broad coverage across varying conditions, bracketing is more efficient when a given process behaves predictably between extreme points (9, 10).

For microbial hold-time studies, both approaches are used frequently to

optimize the validation process while ensuring effective microbial control. The matrix approach would involve testing a subset of conditions across variables such as solution type, storage temperature, and hold duration rather than testing every possible combination of factors (10). Again, the assumption is that untested combinations will behave similarly to tested ones. By contrast, the bracketing approach validates the extremes of key variables under the assumption that those extremes would cover intermediate conditions.

Regulatory Concerns: Using matrix and bracketing approaches during process validation presents several risks associated primarily with potential for incomplete evaluation of critical process variables. Although such approaches reduce the number of validation studies to perform, they might not fully capture all key variations if the grouping of products or conditions is not justified scientifically. Gaps in data can lead to insufficient evidence for ensuring product consistency and safety across operational conditions. Moreover, underestimating worst-case scenarios can limit analysts' understanding of interactions between parameters. Ultimately, product failures or quality deviations could occur during commercial manufacturing. To mitigate such risks, thorough risk assessments and scientific rationale must be applied to support an intended grouping strategy (10).

Preliminary Assessment and Grouping Criteria: Before applying a matrix or bracketing approach in microbial hold-time studies, quality teams first must establish key criteria for grouping conditions. Table 1 provides some sample criteria for studies of microbial hold times: material-dispensing location, method of raw-material introduction, source of water for injection (WFI), cleaning and sanitization procedures, raw-material storage conditions, and operational procedures. Those criteria ensure that conditions within each group are suitably comparable across preparation tanks, enabling a robust process validation.

Understanding Limitations of Matrix Approaches: In microbial-control hold-time validation, analysts must test all tanks used in a given

Equations 1–4: Assessing mixing hydrodynamics relevant to microbial hold time

Equation 1: $\frac{P}{V} = \frac{N_p \rho N^3 D_i^5}{V}$

Equation 2: $Fr = \frac{N^2 D_i}{g}$

Equation 3: $t_{blend} = 4 \times t_{cir} = 4 \times (V \div Q_{imp}) = \frac{4V}{N_F N d_i^3}$

Equation 4: $RS_{Mix,dynamics,i} = (w_{P/V} \times \frac{P/V_i}{\sum_{i=1}^n P/V}) + (w_{Fr} \times \frac{Fr_i}{\sum_{i=1}^n Fr}) + (w_{Mix,T} \times \frac{\sum_{i=1}^n t_{blend}}{t_{blend,i}})$

Table 1: Preliminary criteria for grouping conditions in microbial hold-time validation (WFI = water for injection)

Criterion	Grouping Description	Purpose
Material-dispensing location	Materials dispensed from the same or similar locations	Dispensing methods and locations can influence contamination risks and should be consistent across tanks to ensure comparability of microbial-control study.
Method of raw-material introduction	Raw materials introduced using the same or similar means	Method of introduction can influence contamination potential. Thus, it is an essential factor for ensuring consistent validation results.
WFI source	WFI from the same source or sources of equivalent quality	WFI quality can affect microbial growth and contamination risk. Using the same source ensures consistency during testing.
Cleaning and sanitization procedures	Raw-material-dispensing containers cleaned and sanitized according to the same procedures	Variations in cleaning and sanitization methods can increase microbial-contamination risks and should be consistent across tanks.
Raw-material storage conditions	The same or similar environmental conditions for raw-material storage (e.g., temperature, humidity, and cleanroom classification)	Environmental conditions can affect microbial growth and thus have implications for hold times.
Operational procedures	Consistently applied operational procedures (e.g., for filling and handling steps)	Variations in procedures can influence contamination risks and microbial behavior.

manufacturing process under at least one condition to assess potential risks. Doing so is consistent with both FDA and EMA guidelines (8, 11).

To identify the worst-case condition from grouped conditions in a matrix approach, analysts should focus on tank mixing hydrodynamics because mixing and oxygenation influence microbial growth (12). Moreover, a matrix approach also must account for growth-promoting solution properties — e.g., pH, temperature, osmolarity, and hold time — in combination with mixing hydrodynamics. Thus, a comprehensive, risk-based, and quantifiable approach should be used to evaluate such factors thoroughly, ensuring effective grouping of conditions and identification of the worst-case scenario. A comprehensive approach begins with identification of the tanks involved, followed by grouping of solutions by tank and performance of risk assessment:

Identify All Tanks: List all tanks used throughout a manufacturing process.

Group Solutions by Tank: Organize solutions prepared in each tank based on tank label. Each preparation is referred to as a “condition” within the group.

Conduct Risk Assessment: Perform a detailed risk evaluation for each condition within a group to identify the worst-case scenario, focusing on microbial hold time. Assessment occurs over four stages:

- Evaluate mixing hydrodynamics — analyze mixing dynamics for the grouped conditions, then assign a risk score in relation to microbial-control hold time
- Assess solution properties — evaluate the intrinsic properties of each solution and assign a risk score, accounting for factors such as pH, osmolarity, temperature, hold time, and nutrient composition
- Calculate overall risk — derive an overall risk score by combining these

In a matrix approach for microbial hold-time validation, it is crucial to account for **VARIABILITY** in mixing hydrodynamics arising from differences in mixing conditions within a group.

three factors: (risk from mixing hydrodynamics) × (risk from solution nutritional content) × (risk from solution preparation/storage conditions).

- Test critical conditions — validate the most critical conditions to ensure that microbial risks are controlled effectively across all tank sizes and configurations.

The above process provides for a structured and thorough evaluation, enabling identification of worst-case scenarios and maintenance of control over microbial growth throughout a process.

MIXING HYDRODYNAMICS FOR GROUPED CONDITIONS

In a matrix approach for microbial hold-time validation, it is crucial to account for variability in mixing hydrodynamics that arises from differences in mixing conditions within a group, such as variations in preparation volume, mixing speed, viscosity, and density. Such differences affect a tank's aspect ratio, which in turn influences critical factors such as average shear, vortex formation, mass-transfer rates at the air–liquid interface, and overall mixing-tank blend time. Thus, a comprehensive, risk-based, and quantifiable approach should be implemented to evaluate those factors thoroughly, ensuring effective grouping of conditions and identification of the worst-case scenario:

- Identify normalized engineering parameters to assess mixing dynamics across different operating conditions and operating scales
- Assign weight to each normalized engineering parameter according to its

influence on mixing and microbial growth

- Consolidate the risk scores and weights for each parameter and condition assessed.

Identifying Normalized Engineering Parameters: Key parameters used to assess mixing performance and mass-transfer efficiency in stirred-tank bioreactors include power per unit volume (P/V), Froude's number (Fr), and blend time (t_{blend}). Those parameters all play critical roles in ensuring optimal mixing dynamics and minimal microbial growth.

Average Shear Assessment: The P/V ratio is a critical metric for assessing average shear across the entire volume of a preparation. It normalizes the effects of different impeller types, numbers, and configurations, facilitating consistent comparisons across tanks. The P/V ratio is calculated using Equation 1 (13, 14).

Vortex-Formation Assessment: Froude's number evaluates vortex formation by comparing inertial and gravitational forces (Equation 2) (13, 14). In nonsparged stirred-tank systems, mass transfer across an air-liquid interface is influenced by the depth of the vortex and the turbulence created by the impeller. Therefore, Fr values are used as surrogate measures for surface mass transfer. In Equation 2, N denotes the impeller rotational speed, D is the impeller diameter, and g is acceleration due to gravity (9.81 m/s^2).

Blend Time in Impeller-Agitated Systems: Differences in impeller design, scale, and speed as well as tank height to impeller diameter (H/D) ratios all significantly influence discharge rate per unit volume and overall mixing efficiency. Variations in such values can be normalized across scales using t_{blend} values, which represent the time needed for a solution to achieve homogeneity in a tank. Using t_{blend} values allows for consistent comparison of impeller performance during scale-up or scale-down (13). The impeller discharge rate (Q_{imp}) is calculated using the impeller flow number (N_F), agitation rate (N), and impeller diameter (D). Circulation time (t_{cir}) is the ratio of tank volume to discharge rate. *Mixing time* — the time needed for a solution to reach 95% homogeneity — is about $4 \times t_{cir}$ (13).

Table 2: Weights for normalized engineering parameters according to their influence on mixing and microbial growth

Parameter	Weight (%)	Rationale
Power per volume (P/V)	30%	High P/V enhances mixing efficiency, ensuring consistent nutrient distribution and minimizing stagnant zones where microbial growth could thrive.
Froude's number (Fr)	40%	High Fr values indicate enhanced oxygen transfer from vortices and waves at the gas-liquid interface. That factor supports microbial-growth conditions.
Blend time (t_{blend})	30%	Prolonged mixing times can elevate the likelihood of microbial contamination due to extended exposure.

Table 3: Risk-scoring guidance for grouping nutrient-rich cell-culture growth media and feeds (15, 16)

Risk Level (Score)	Description
High (5)	Solutions containing all key nutrients (e.g., carbohydrates, amino acids, vitamins, minerals, and lipids), thus providing an ideal environment for microbial growth
Moderate (3)	Solutions containing carbohydrates, hormones, and sera but only two essential nutrients (amino acids, vitamins, or minerals); moderate potential for microbial growth
Low (1)	Solutions containing only one basic nutrient (e.g., amino acids, vitamins, or minerals), with no carbohydrates or growth promoters; low potential for microbial growth
No risk (0)	Solutions containing no growth-promoting components (e.g., only salts or buffers), posing minimal risk for microbial growth

N_F values = 0.7–0.9 for radial impellers and 0.3–0.5 for axial-flow impellers (14). Equation 3 demonstrates how to calculate t_{blend} values.

Assigning Weight to Each Normalized Engineering Parameter According to Its Influence on Mixing and Microbial Growth: Table 2 outlines key parameters used in assessing vessel mixing dynamics. Analysts must consider each parameter's influence on processes such as turbulence, shear forces, mass transfer, and oxygen supply based on its importance to overall risk for microbial proliferation. In Table 2, the assigned weights reflect the relative risk of each parameter in controlling microbial loads, with references provided to support our rationales.

Final Risk-Score Calculation for Mixing Hydrodynamics: The consolidated risk score for microbial control from mixing hydrodynamics for each condition will be a weighted average of individual risk scores for each condition within a group (Equation 4).

MICROBIAL-CONTROL HOLD-TIME TESTING
Assessing Worst-Case Conditions for Microbial-Growth Promotion Within a Group: After assessment of physical conditions related to mixing

hydrodynamics, the next step is to evaluate the worst-case growth-promoting solution within the group. Doing so is essential for identifying and mitigating the highest microbial risks in a biomanufacturing process. The worst-case solution can be determined based on parameters such as nutritional composition, preparation-vessel contact surface area, pH, temperature, osmolarity, and time, all of which significantly influence microbial growth.

In our analysis, nutritional composition is scored independently from preparation-vessel contact surface area. However, risks for temperature, pH, osmolarity, and time are scored comprehensively using a cardinal-values microbial-growth model. Finally, risk scores from mixing hydrodynamics, nutritional content, and solution growth-promoting properties can be consolidated, yielding a comprehensive risk score.

Nutritional Composition: Table 3 categorizes different risk levels associated with media compositions based on their potential to support microbial growth. The table also assigns a score to each risk level and provides a description of the media components that contribute to that classification. Such a system helps in evaluating and

Table 4: Cardinal growth parameters for temperature, pH, and osmolarity, with supporting references (Ref.); min = minimum, opt = optimum, max = maximum

Microorganism	Temperature (°C)				pH				Osmolarity (Osm/kg)			
	min	opt	max	Ref.	min	opt	max	Ref.	min	opt	max	Ref.
<i>Bacillus cereus</i>	4	30–37	55	17	4.5	6.5	9.0	22, 23	≈0.2	≈0.5 to 0.9	≈1.5	28, 29
<i>Bacillus subtilis</i>	4	30–37	50	18	4.5	7	9.0	24, 25	≈0.2	≈0.5 to 0.8	≈1.2	28
<i>Candida albicans</i>	20	37	42	19	2.5	4.0–6.5	8.0	19	≈0.2	≈0.6 to 0.8	≈1.0	19
<i>Escherichia coli</i>	7.0	37	50	20	4.0	7.0	9.0	20	≈0.1	≈0.3 to 0.6	≈0.9	30, 31
<i>Pseudomonas aeruginosa</i>	4	37	42	19	5.5	7.5	9.0	19	≈0.2	≈0.5 to 0.8	≈1.5	19
<i>Staphylococcus aureus</i>	7.0	30–37	48	21	4.0	6.0–7.5	10	26, 27	≈0.2	≈0.5 to 0.7	≈1.0	26, 27

Equations 5–12: Assessment of microbial-control hold-time testing

Equation 5: $A_{\text{Vessel,contact}} = (\pi \times (D^2 \div 4)) + (\pi \times D \times H)$

Equation 6: $RS_{\text{Vessel,contact}} = \frac{A_{\text{Vessel,contact},i}}{\sum_{i=1}^n A_{\text{Vessel,contact}}}$

Equation 7: $\mu_{R(T,pH,Osm,Time)} = (\mu \div \mu_{\text{opt}}) = f(T) \times f(pH) \times f(Osm) \times (Time)$

Equation 8: $f(T) = \begin{cases} 0 & , T \leq T_{\min} \text{ OR } T \geq T_{\max} \\ \left(\frac{T - T_{\min}}{T_{\text{opt}} - T_{\min}} \right) \left(\frac{T_{\max} - T}{T_{\max} - T_{\text{opt}}} \right) & , T_{\min} \leq T \leq T_{\max} \end{cases}$

Equation 9: $f(pH) = \begin{cases} 0 & , pH \leq pH_{\min} \text{ OR } pH \geq pH_{\max} \\ \left(\frac{pH - pH_{\min}}{pH_{\text{opt}} - pH_{\min}} \right) \left(\frac{pH_{\max} - pH}{pH_{\max} - pH_{\text{opt}}} \right) & , pH_{\min} \leq pH \leq pH_{\max} \end{cases}$

Equation 10: $f(Osm) = \begin{cases} 0 & , Osm \leq Osm_{\min} \text{ OR } Osm \geq Osm_{\max} \\ \left(\frac{Osm - Osm_{\min}}{Osm_{\text{opt}} - Osm_{\min}} \right) \left(\frac{Osm_{\max} - Osm}{Osm_{\max} - Osm_{\text{opt}}} \right) & , Osm_{\min} \leq Osm \leq Osm_{\max} \end{cases}$

Equation 11: $RS_{(T,pH,Osm,Time)} = \text{Max}\{\mu_{R(T,pH,Osm,Time),i=BC,BS,CA,EC,PA,SA}\}$

Equation 12: $RS_{\text{Micro,Total}} = (w_{\text{mix,dyn}} + RS_{\text{mix,dyn}}) + (w_{\text{vc}} + RS_{\text{vc}}) + (w_{\text{Nutr}} + RS_{\text{Nutr}}) + (w_{T,pH,Osm,Time} + RS_{T,pH,Osm,Time})$

grouping media based on their nutrient profiles and growth-promoting characteristics.

Vessel surface contact area refers to the total internal surface area of a tank that interacts with a solution. Tanks with larger surface areas can harbor more microorganisms than those with smaller surface areas can, thus posing higher contamination risks. Equation 5 shows how to calculate a vessel's approximate surface contact area, and Equation 6 enables determination of individual risk scores for each condition within a group.

Combined Effects of Solution Conditions on Microbial

Proliferation: The *cardinal microbial-growth model* plays a critical role in predicting microbial behavior across

different conditions by integrating key factors such as temperature, pH, and osmolarity. Using equations developed by Rosso et al. (32), the model quantitatively simulates how different environmental factors can influence microbial proliferation, supporting determination of effective control measures. Combining hold-time assessment with the cardinal pH, temperature, and osmolarity models increases prediction accuracy, helping to assess worst-case scenarios for microbial risk.

United States Pharmacopeia <111>

“Microbiological Examination of Non-Sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use”

Our proposed methodology offers a **SYSTEMATIC, DATA-DRIVEN** approach, helping to enhance process validation, ensure regulatory compliance, and support production of safe and high-quality biopharmaceuticals.

identifies six microorganisms as key models for pharmaceutical-quality assessments: *Bacillus cereus* and *subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (33). In Equation 11, those species are abbreviated as BC, BS, CA, EC, PA, and SA, respectively. Those microorganisms can be used to represent a breadth of microbial populations encountered in biopharmaceutical manufacturing environments because they collectively account for characteristics such as high resistance, the ability to form spores, rapid growth kinetics, and pathogenicity (33). They can be assessed through the cardinal model to identify worst-case growth conditions, ensuring a comprehensive microbial risk evaluation in pharmaceutical production.

Mathematical Models: The combined relative growth rate (μ_R) as a function of the pH, temperature, osmolarity, and time models can be calculated using Equation 7, in which μ_{opt} is the maximum

growth rate under optimal conditions, and $f(T)$, $f(pH)$, and $f(Osmo)$ are the individual component functions for temperature, pH, and osmolarity, respectively. Equations 8, 9, and 10 model temperature, pH, and osmolarity, respectively. Table 4 lists cardinal growth parameters for those factors.

Comprehensive Scoring for Severity of Microorganisms Assessed: The maximum relative growth rate of six evaluated microorganisms can be used to calculate a consolidated severity score for a growth-promoting condition for each solution condition grouped (Equation 11).

Combined Comprehensive Risk Score: The combined comprehensive risk score for microbial-control hold-time validation hinges on these key factors:

- fluid-mixing hydrodynamics, including average shear, intensity of vortex formation, and tank blend time
- solution nutritional composition, based on combinations of carbon sources, amino acids, growth promoters, trace elements, and salts
- preparation conditions, such as temperature, pH, osmolarity, and preparation time.

Equation 12 demonstrates how to calculate each parameter's overall impact on microbial growth during hold time. Table 5 explains the weighting for those parameters.

STRUCTURED, OBJECTIVE MICROBIAL-RISK ASSESSMENT

Validation of microbial hold time is essential for maintaining biopharmaceutical safety, consistency, and efficacy. We have demonstrated the utility of the cardinal microbial-growth model in thoroughly evaluating growth-promoting conditions, including pH, temperature, osmolarity, nutrient composition, and mixing hydrodynamics. We show that incorporating those factors into a structured, objective scoring matrix yields a more precise assessment of microbial risks and better identification of worst-case scenarios than can be achieved by traditional means. Furthermore, application of bracketing and matrix approaches, combined with a comprehensive risk-assessment framework, effectively addresses regulatory concerns raised by agencies such as the FDA regarding

Table 5: Weighting parameters affecting microbial growth during hold times

Parameter	Weight (%)	Rationale
Mixing dynamics	25%	Inadequate mixing can create localized pockets of poor homogenization, which in turn can encourage microbial growth. Effective mixing enhances microbial safety by providing oxygenation and uniform conditions.
Vessel contact-surface area	10%	Larger surface areas, especially rough or poorly sanitized surfaces, provide more opportunities for microbial adhesion and biofilm formation than do smaller ones, increasing contamination risks during hold times. However, validated cleaning and sanitization processes provide assurance against contamination.
Solution nutrient value	35%	High nutrient content (e.g., sugars and amino acids) in culture media significantly increases the risk of microbial proliferation if contamination has occurred.
Preparation condition	30%	Temperature, pH, and osmolarity conditions are highly influential in promoting or inhibiting microbial activity. Time is a critical factor as well because microbial load increases with prolonged exposure.

microbial hold-time validation. Our proposed methodology offers a systematic, data-driven approach, helping to enhance process validation, ensure regulatory compliance, and support production of safe and high-quality biopharmaceuticals.

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Exploring Viral Clearance for AAV Products

A Study of Detergent Lysis and Chromatography Steps

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Akunna Iheanacho, Guang Yang, Iraj Ghazi, and Qi Zhang

In 1999, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) introduced the ICH Q5A(R1) guideline, which covers the viral safety of biotechnology products derived from cell lines of human and/or animal origins. The recommendations focus on biologics derived from in vitro cell cultures such as interferons, monoclonal antibodies (mAbs), and other recombinant-DNA-derived therapeutics. Recommendations for *inactivated vaccines* — live vaccines containing self-replicating agents — and genetically engineered live vectors were excluded from the document's scope (1). Over the subsequent decades, the number of platforms using viral vectors to deliver gene therapies for treating a range of clinical indications has multiplied. A few examples of approved and clinical-stage viral-based therapies include Gendicine (recombinant human p53 adenovirus) for non-small-cell lung cancer, Roctavian (valoctocogene roxaparvovec) for hemophilia A,

PRODUCT FOCUS: Gene therapies, viral vectors

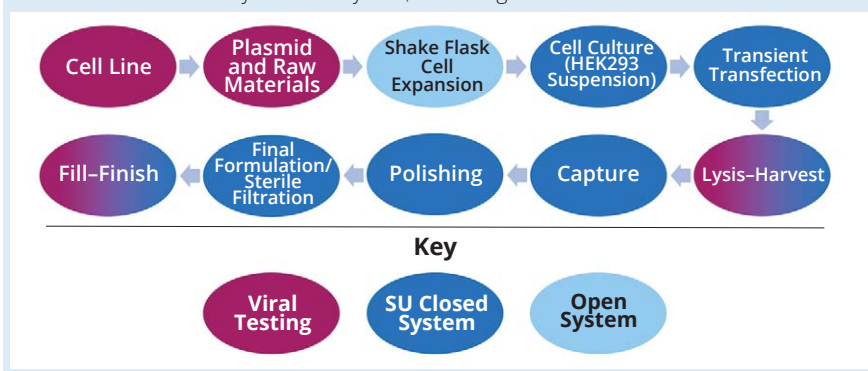
PROCESS FOCUS: Downstream processing

AUDIENCE: Process development, analytical, and manufacturing

KEYWORDS: Viral clearance, capture and polishing chromatography, viral-inactivation detergents, viral filtration

LEVEL: Intermediate

Figure 1: Process steps of the InAAVate viral-safety and manufacturing platform; HEK293 = human embryonic kidney 293, SU = single use



Zolgensma (onasemnogene abeparvovec) for spinal muscular atrophy, and Luxturna (voretigene neparvovec) for retinal dystrophy. The latter three products all use adenoassociated-virus (AAV) delivery systems: serotypes 5, 9, and 2, respectively. Viral vectors also have been used for some COVID-19 vaccines, such as AstraZeneca's ChAdOx1 nCoV-19 and Ad26.COV2.S vaccines (2–5).

Made effective in June 2024, ICH Q5A(R2) clarifies viral-safety guidelines for genetically engineered viral vectors by adding a section devoted to viral-vector-derived products. The guideline calls for comprehensive virus testing and assessments of virus removal and inactivation during biomanufacturing. Risk-based approaches should be followed to demonstrate viral safety of final products, and associated viral safety assessments should be part of the initial process development through investigational new drug (IND) and subsequent marketing applications (6).

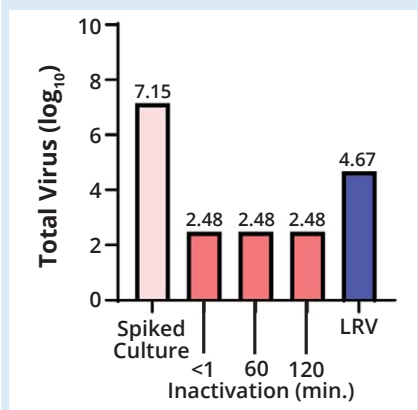
Abstract

Herein, we describe the viral-safety components used in the Oxford Biomedica (OXB) InAAVate platform and discuss an adenoassociated virus 9 (AAV9) viral-clearance study performed in partnership with Texcell North America to assess the removal of model adventitious viruses through lysis and chromatography steps. The study resulted in a log reduction value (LRV) of $>4.67 \log_{10}$ for enveloped xenotropic murine leukemia virus (XMuLV) through detergent treatment and $9.07 \log_{10}$ for nonenveloped minute virus of mice (MVM) for two chromatography process steps — affinity and anion exchange (AEX) — when operated at center-point conditions. LRV results for MVM were within the range of values in previous studies that used affinity capture and AEX polishing with different AAV serotypes and resins. We expect that our data can help drug developers in the early stages of platform development to form expectations about the level of viral clearance obtainable for a plasmid-based production system in the absence of large-pore (35–70 nm) virus filters.

Table 1: Model viruses used in the viral-clearance study

Spiked Viruses	Family	Genome	Type	Size	Physicochemical Resistance
Xenotropic murine leukemia virus (XMuLV)	<i>Retroviridae</i>	RNA	Enveloped	80–110 nm	Low
Minute virus of mice (MVM)	<i>Parvoviridae</i>	DNA	Nonenveloped	18–24 nm	High

Figure 2: Xenotropic murine leukemia virus (XMuLV) inactivation by detergent treatment; LRV = log-reduction value



Viral-risk mitigation in biomanufacturing relies on three pillars: prevention, detection, and removal. Developers can prevent adventitious viruses from entering viral-vector manufacturing processes through careful selection and testing of raw materials, such as cell lines and media. Physical barriers and closed systems also reduce risks of viral contamination. Testing at different process steps for viral contaminants is vital to ensuring the safety of final products. The type and extent of such tests vary depending on the production mode of a given viral vector and initial risk assessment but should include, at minimum, characterization of a master and working cell bank (MCB, WCB), virus stocks, bulk harvest, and drug substance (DS). The most important risk-mitigation tool is the cumulative viral-clearance capability of upstream inactivation kinetics and the downstream purification process, as determined by viral-clearance studies (6–12).

THE VIRAL-SAFETY PLATFORM

Figure 1 shows an overview of the Oxford Biomedica (OXB) InAAVate platform and its viral-control elements. The platform uses a transient

transfection production system with human embryonic kidney 293 (HEK293) cells and helper plasmids (13). Control strategies address the prevention of viral entry through cell lines and raw-material control, as well as viral testing.

Cell-Bank Viral Safety Testing: The teams performed several adventitious-virus tests on samples from a HEK293 cell bank. In vitro tests were performed in HEK293, Vero, and MRC-5 cell lines; in vivo assays used adult mice and embryonated hen eggs. Samples were screened for standard pathogens such as hepatitis viruses A, B, and C; human immunodeficiency virus 1 and 2; (HIV-1, HIV-2); human T-lymphotropic viruses 1 and 2 (HTLV-1, HTLV-2); and bovine and porcine viral agents. Test results were documented as part of the HEK293 MCB specification. Release depended on confirmation that materials were free of viral contaminants.

Raw-Material Screen: Transfection plasmids were grown in *Escherichia coli* and tested for mycoplasma contamination. Media used for growth and transfection of suspension-adapted HEK293 cells were chemically defined and free of proteins and sera.

Plasmid-Based Transient Transfection: Drug developers use different methods to produce recombinant AAV (rAAV) vectors; such vectors bring unique considerations regarding initial viral-risk assessments (14–16). For example, AAV processes that use stable producer cell lines with adenovirus 5 (Ad 5) will be required to show clearance of those helper viruses (17, 18). Production platforms that use transient plasmid-transfection systems without helper viruses involve less risk but still must be assessed for clearance with model viruses that represent a breadth of relevant biophysical traits, such as size, genome type, and envelope (7, 8, 19–21).

Operational Controls: Single-use closed systems throughout manufacturing provide barriers to lessen viral-contamination risks by reducing batch-to-batch virus carryover from inadequate cleaning. Closed systems — such as single-use mixers, manifolded filling assemblies, and molded sample collection — provide physical barriers against the entry of viral contaminants.

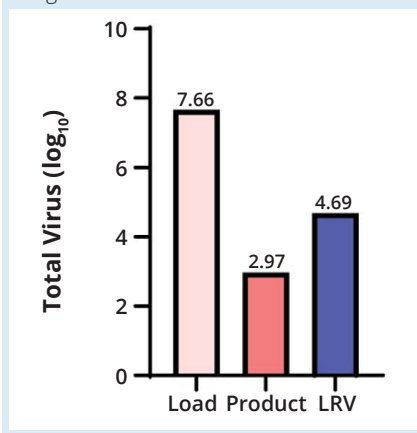
In-Process Testing and Release Specification: Routine bioburden samples were taken from preinoculation media, postinoculation cell cultures, posttransfection, prelysis, postclarification, affinity capture, anion-exchange (AEX) polishing and drug-substance steps. Prelysis and preharvest samples were taken from bioreactors and assayed for mycoplasma and adventitious agents. The DS release panel tested for residual host-cell DNA (hcDNA), plasmid DNA (pDNA), helper-plasmid DNA (hpDNA), and adventitious agents.

VIRAL-CLEARANCE STUDY

OXB partnered with Texcell North America to evaluate the InAAVate platform's ability to remove and/or inactivate two model viruses (Table 1). The team assessed three unit operations and spiked either xenotropic murine leukemia virus (XMuLV) or minute virus of mice (MVM) into load material at the start of each process step, then quantified remaining amounts. For inactivation, XMuLV was spiked into prelysed culture harvest followed by treatment with a detergent-based lysis buffer. For capture, MVM was spiked into an affinity-chromatography load. MVM also was spiked into the load of an AEX-chromatography process, which served as a polishing step. All load material used for spiking came from aliquots of process intermediates generated from a 50-L pilot-scale run.

XMuLV was chosen as a model RNA virus for several reasons: It is larger than AAV and, as an enveloped virus, is more susceptible to inactivation by commonly used cell-lysis detergents (e.g., Tween 20, Triton X-100, and Deviron C16 products). MVM was chosen because it belongs to the same family as AAV and thus shares similar physicochemical characteristics, such as resistance to detergent treatment. And

Figure 3: Minute virus of mice (MVM) removal by affinity chromatography; LRV = log-reduction value



because MVM's size is similar than that of AAV, use of small-pore retentive viral filtration could result in product-recovery issues.

MATERIALS AND METHODS

Generation of AAV9 Material: AAV9 process intermediates used in our study were produced in a 50-L bioreactor and purified using OXB's InAAVate platform for that serotype. Scaled-down versions of each production-scale unit operation were used during the viral-clearance study.

Chromatography Resins: Prepacked Repligen columns were used for both chromatography steps in this study, along with POROS CaptureSelect AAV9 resin for the affinity capture and POROS 50 HQ resin for the AEX polishing unit operation (Thermo Fisher Scientific).

In a **preliminary evaluation of test materials**, we determined potential effects of viral-clearance samples on the test systems used to quantify the model viruses. Toxicity, interference, and hold-control testing assessed effects of process materials on infectivity assays; potential effects to test systems were mitigated by sample dilution as necessary. For virus inactivation by detergent treatment, we performed an additional evaluation (threshold of inactivation). The threshold of inactivation enabled us to determine the dilution of detergent-treated material required to terminate virus inactivation.

Experimental Design: Centerpoint conditions were selected as operating parameters to help us understand the viral-clearance capability of the

Table 2: Percentage of minute virus of mice (MVM) in affinity product compared with spiked affinity load; TCID₅₀ = 50% tissue culture infectious dose

Sample Description	Total MVM (TCID ₅₀)	%MVM in Fraction Compared with Load
Affinity load	4.57×10^7	—
Affinity product	9.22×10^2	0.00

InAAVate platform. For chromatography steps, our centerpoint settings included column:load ratios, residence times, elution pH, and conductivities, all of which can affect viral clearance. Although the use of worst-case conditions on operating parameters was not in the scope of this study, note that both chromatography steps were run at cycle 2 (cycle 1 being a control run with an AAV load without a virus spike).

Virus Quantitation: Texcell personnel used validated infectivity assays to quantify virus titers for XMuLV and MVM. Virus titers of samples spiked with XMuLV were determined with a plaque-forming infectivity assay based on direct quantitation of plaque-forming units (PFUs). MVM process samples were evaluated using a 50% tissue culture infectious dose (TCID₅₀) assay and determined in a quantal assay, in which measured virus titers were based on detection of infected cells by observation of cytopathic effects. Log-reduction values (LRVs) were calculated using one of the following equations:

$$\text{LRV} = (\text{lesser of } \log_{10} \text{ total virus in load and hold control}) - (\log_{10} \text{ total virus in timepoint sample})$$

$$\text{LRV} = (\text{lesser of } \log_{10} \text{ total virus in load and hold control}) - (\log_{10} \text{ total virus in product sample})$$

Detergent Treatment: Cell cultures were spiked with XMuLV and treated with lysis-buffer-containing detergent to test enveloped-virus inactivation. A spiked-culture sample was assayed to determine virus titer before detergent treatment. An aliquot of the load was held for the duration of the inactivation process and assayed. The remaining volume of the spiked test article was treated with lysis-buffer-containing detergent and held at 37.0 ± 1.0 °C for

Table 3: Percentage of minute virus of mice (MVM) in anion-exchange (AEX) eluant compared with spiked AEX load; TCID₅₀ = 50% tissue culture infectious dose

Sample Description	Total MVM (TCID ₅₀)	%MVM in Fraction Compared with Load
AEX load	2.03×10^8	—
AEX eluant	8.54×10^3	0.00

120 minutes. Samples of the spiked inactivation load were taken at three timepoints to demonstrate inactivation kinetics over time.

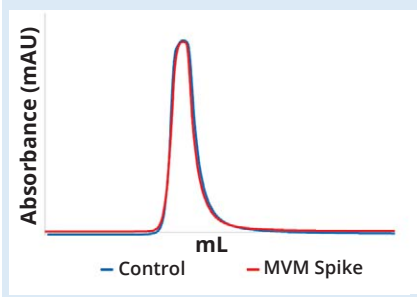
Affinity and AEX Chromatography:

Load materials for affinity and AEX columns were spiked with MVM to evaluate nonenveloped-virus removal during each chromatography step. Load samples were assayed to determine virus titer at the start of chromatography. An aliquot of the load, designated as the hold control, was held for the duration of the chromatography process and assayed for virus titer. Affinity and AEX eluates also were sampled and assayed to determine virus titers at the end of each chromatography step. No process intermediates (e.g., flow-through, wash, or strip) were analyzed for virus removal.

RESULTS AND DISCUSSION

Among the three strategies for mitigating viral risk, virus removal has the most significant role in reducing potential viral contamination of final therapeutic products. A viral-clearance capability demonstration evaluates the effectiveness of upstream and downstream unit operations to inactivate or remove spiked-in model viruses. Traditional approaches used for mAbs and other recombinant proteins — e.g., heat application, low-pH hold, and detergent exposure — have minimal effects on nonenveloped viral vectors that share characteristics with AAVs. Small-pore (20-nm) viral filters used in antibody production processes remove parvoviruses but also target AAVs and therefore decrease yield. Large-pore (35–70-nm) viral filters can remove larger viruses while allowing AAVs to pass but cannot filter small adventitious agents. Thus, adding chromatography for surface-charge modulation to separate AAVs from similar-sized adventitious viral agents could be the

Figure 4: Affinity elution chromatogram comparison with and without minute virus of mice (MVM) spike



key to demonstrating robust viral clearance (11, 22, 23). Based on data from previous experiments, we designed our study to assess the effects of detergent treatment and inactivation kinetics (for XMuLV) with cumulative chromatography clearance (for MVM).

Detergent treatment with lysis buffer resulted in an LRV of $>4.67 \log_{10}$ for XMuLV with near-immediate inactivation. Along with calculated LRVs, Figure 2 shows $\log_{10}$ values of total virus present in samples as a function of time. In under one minute and throughout the time course investigated in the study, virus titer in the treated sample was <1.50 PFU/mL (assay detection limit). That corresponded to $<2.48 \log_{10}$ of total virus for XMuLV and indicated rapid viral inactivation with an LRV of $>4.67 \log_{10}$ that persisted over the two-hour timeframe.

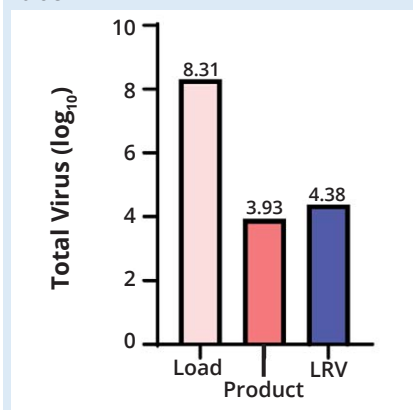
Chromatographic Clearance: Figure 3 illustrates the $\log_{10}$ value of total virus present in the affinity load and product samples and LRV obtained for the **affinity unit operation**. Affinity chromatography was an effective virus-removal step for MVM as demonstrated by an LRV of $4.6 \log_{10}$ using manufacturing centerpoint conditions. We did not expect the resin beads to bind to MVM due to their ligands' high selectivity to AAV9 capsids. Because MVM is resistant to detergent inactivation, it is important to demonstrate effective removal through affinity-based chromatography (23, 24). The percentage of spiked MVM particles present in the affinity product compared with the spiked affinity load was negligible (Table 2).

Figure 4 compares elution peaks from the blank and spike runs, showing no extra peaks or substantial differences in chromatographic profiles. Elution and

Table 4: Total log-reduction values (LRVs) for nonenveloped xenotropic murine leukemia virus (XMuLV) and enveloped minute virus of mice (MVM); NA = not applicable

Virus Type	Detergent Inactivation (120 Min.)	Affinity Chromatography	AEX Chromatography	Cumulative Reduction
XMuLV	>4.67	NA	NA	>4.67
MVM	NA	4.69	4.38	9.07

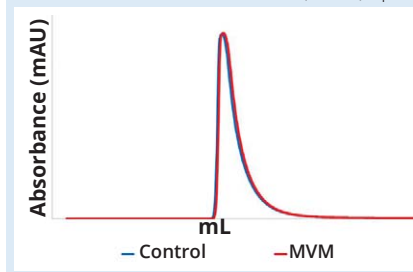
Figure 5: Minute virus of mice (MVM) removal by anion-exchange (AEX) chromatography; LRV = log-reduction value



strip peak areas were comparable, as A260:A280 ratios for blank and spiked runs. Such results accord with previous studies. For instance, Winkler et al. observed that model viruses (MVM and XMuLV specifically) showed no affinity toward resin base beads or functionalized ligands (POROS CaptureSelect AAVX); the ability of AAV serotypes to bind to the functionalized ligand was uncompromised in the presence of model viruses, and the majority of MVM particles were present in affinity capture flow-through (25).

Figure 5 shows the $\log_{10}$ value of total virus present in the AEX load and product samples, with the LRV for the **AEX step**. AEX chromatography was also an effective virus removal step for MVM, as demonstrated by an LRV of $4.38 \log_{10}$ at manufacturing centerpoint conditions. That bind-elute AEX chromatography step provided DNA-containing capsid enrichment by exploiting small charge differences between empty and full AAV capsids. The resolution between capsid populations partially depends on the concentration of process-related impurities in load materials. An LRV of $>4 \log_{10}$ for MVM, without modifications to platform process parameters, demonstrates a lack of interference on resin performance from MVM at the tested concentration. In the affinity step, the percentage of spiked MVM particles

Figure 6: Anion-exchange (AEX) elution chromatogram comparison with and without minute virus of mice (MVM) spike



present in the AEX product compared with the spiked AEX load was negligible (Table 3).

Figure 6 overlays the blank and spiked AEX elution peaks. As with the affinity step, no extra peaks or differences in chromatographic profiles were observed. The AEX polishing step's ability to remove MVM may be tied to the specific pH levels and conductivities used to target full AAV9 capsids based on charge profiles. Platform conditions for the separation of empty and full capsids did not coelute the majority of MVM particles despite the similar physicochemical properties of the two viruses. Those observations support findings published by Winkler et al., in which the majority of MVM particles were present in the AEX strip (CIMmultus QA column, Sartorius) (25).

EVOLVING PLATFORM AND FUTURE REGULATORY OUTLOOK

Table 4 lists viral-clearance values obtained with the InAAVate platform using virus inactivation and removal. Typically, LRVs below $1 \log_{10}$ fall within assay variability and therefore are considered negligible with no contribution to overall clearance. LRVs of $1-3 \log_{10}$ are considered supportive steps and contribute acceptably, but LRVs $>4 \log_{10}$ demonstrate robust viral clearance across the particular unit operation being evaluated (26, 27).

Virus inactivation by detergent treatment was shown to be effective, as evidenced by an LRV of $>4.67 \log_{10}$ for XMuLV. An overall reduction factor of

9.07 log₁₀ of MVM for the manufacturing process highlights the effectiveness of our platform at manufacturing centerpoint conditions for an AAV9 serotype. A similar LRV of >4 log₁₀ was obtained for each chromatographic step in the Winker et al. study for the MVM model virus at platform centerpoint conditions across POROS CaptureSelect AAVX affinity resin and CIMmultus QA monolith AEX column for an AAV8 serotype (25). OXB's platform control strategy, viral-clearance data, and overall viral-risk assessment highlight a built-in viral safety factor greater than the industry-accepted target of 6 log₁₀ LRV for processes without endogenous viruses (24).

ACKNOWLEDGMENTS

We thank everyone who assisted with this study. Guang Yang, Iraj Ghazi, Qi Zhang, and Richard Gilmore contributed through planning and material generation. Ashish Sharma, Luke Mustich, Thomas Thiers, Bill Kerns, and Akunna Iheanacho contributed to the manuscript.

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Expanding the Toolbox of Biopharmaceutical Excipients

Sustainably Manufactured, Injectable-Grade Sucrose Solutions

Brian Gazaille with Toshio Ariyasu and Kanso Iwaki

Sucrose has a long and successful history as a pharmaceutical excipient. As I learned from Toshio Ariyasu (senior scientist) and Kanso Iwaki (pharmaceutical business development advisor) of Nagase Viita, that saccharide has been used not only in oral formulations of small-molecule drugs, but also in biopharmaceuticals formulated for parenteral administration. They noted, “Sucrose serves as an excellent stabilizer for monoclonal antibody (mAb) products, peptide-based drugs, and traditional vaccines. It even has been used in mRNA-based vaccines against viral infections.” Indeed, Pfizer–BioNTech’s Comirnaty and Moderna’s Spikevax COVID-19 vaccines leveraged high concentrations of sucrose as a cryoprotectant — a nontrivial factor considering those products’ distinctive requirements for cold storage (1).

As biopharmaceutical products proliferate, industry demand for saccharide excipients will grow. Thus, biomanufacturers will benefit from an expanded array of tools for stabilizing the quality of drug products. Ariyasu and Iwaki explained to me that their company is leveraging 140 years of expertise in glycoscience and saccharide production to address such needs. Nagase Viita already has developed the Solbiote family of saccharide excipients — including high-purity, low-endotoxin Trehalose SG and Maltose PH products — to help biomanufacturers stabilize their drug products. In 2025, the company will expand the portfolio by introducing the injectable-grade Sucrose SG saccharide excipient.



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During our discussion, Ariyasu and Iwaki explained how sucrose stabilizes biopharmaceutical quality and how addition of the Sucrose SG solution to the Solbiote portfolio “will increase the breadth of solutions available to the industry.” They also highlighted their company’s commitment to sustainable saccharide manufacturing.

ADDRESSING AN INDUSTRY NEED Why did your company develop this saccharide product, and how is it manufactured?

We developed it to address the critical issue of stability in parenteral biopharmaceuticals. Manufactured and refined in Japan, the highly purified sucrose provides guaranteed safety and reliable quality.

Endotoxin removal is critical to the Sucrose SG saccharide’s manufacture. The purification process is controlled strictly by advanced refining technologies that our company has developed through decades of experience. We also conduct endotoxin analysis according to internationally standardized methods in major pharmacopoeias, including the *United States Pharmacopeia–National Formulary* (USP–NF), the *European Pharmacopoeia* (EP), and the *Japanese Pharmacopoeia* (JP). By complying with stringent global

standards for parenteral dosage forms, our Sucrose SG excipient helps biopharmaceutical developers to ensure exceptional quality in their products.

A BREADTH OF APPLICATIONS How does sucrose stabilize antibody formulations? For which kinds of antibody products might it be effective as an excipient?

Sucrose is a disaccharide comprising glucose and fructose molecules linked by α -1,2 glycosidic bonds. Because of its structure, it is nonoxidizing, nonreducing, and highly water-soluble, presenting as colorless crystals.

Sucrose stabilizes antibody formulations by interacting with both water molecules and protein structures. Through hydrogen bonding with such molecules, sucrose helps to maintain native protein conformations, reducing risks for denaturation (2).

In freeze-dried formulations, sucrose replaces water molecules and inhibits protein unfolding during drying. It also prevents antibody aggregation and degradation during freezing by suppressing ice-crystal formation and reducing molecular motion. Furthermore, sucrose is known to minimize surface adsorption, reducing protein loss or denaturation caused by adsorption to final-container surfaces. Because of such properties, sucrose is particularly effective for antibody formulations in freeze-dried and highly concentrated solutions as well as for antibody–drug conjugates (ADCs).

In addition to its role as lyoprotectant, sucrose enhances protein stability in liquid formulations,

making it an essential excipient for preserving antibodies under a breadth of long-term storage conditions.

Can sucrose also work for lipid nanoparticles (LNPs) containing nucleic acids? It indeed stabilizes LNP formulations by preserving particles' structural integrity during freezing and storage (1). Sucrose prevents LNP fusion and aggregation by reducing water activity, promoting formation of a "glassy" state that immobilizes the nanoparticles. Thus, sucrose effectively minimizes LNP damage during freezing by inhibiting ice-crystal formation. It also helps to maintain stable microenvironments within LNPs, preventing nucleic-acid degradation. Such properties make sucrose particularly effective for LNP formulations that require long-term stability, such as mRNA-based therapeutics and vaccines.

To validate general use of sucrose as a stabilizer for LNPs, our scientists performed experiments with LNP-preparation kits from Cayman Chemical. LNP compositions were prepared by replacing the sucrose used in Comirnaty vaccine formulations with other carbohydrates, such as our Maltose PH, Trehalose SG, and Sucrose SG products. Then, samples were subjected to freeze-thaw cycles, with storage at -20°C for one hour followed by storage at room temperature for 30 minutes.

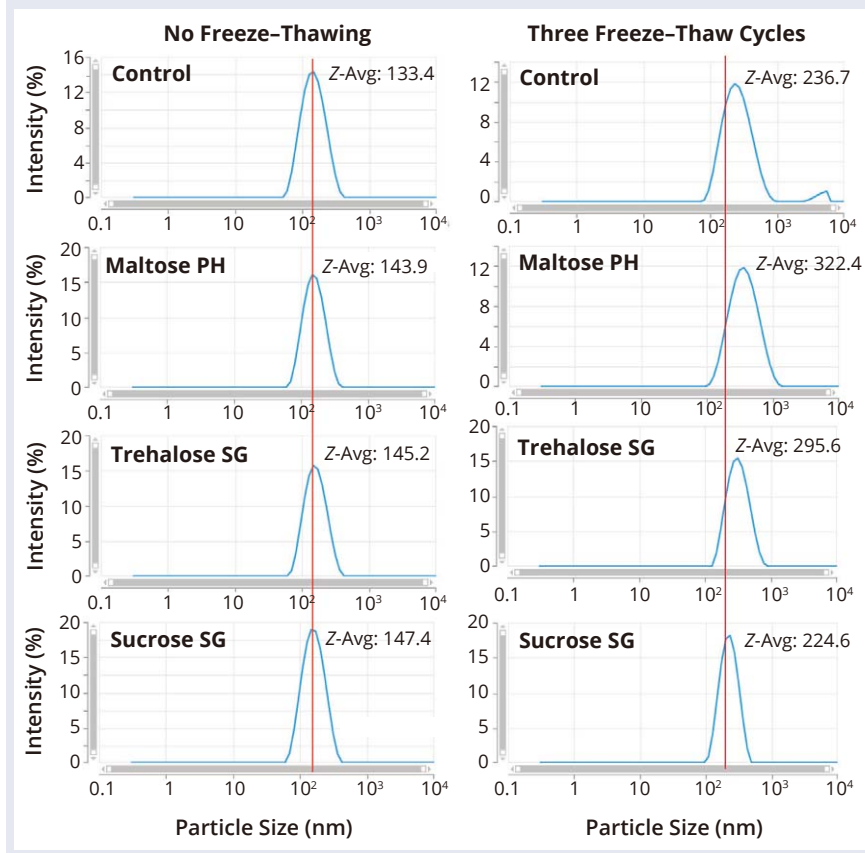
Addition of the Sucrose SG excipient resulted in the least change in particle size among the tested carbohydrates. Notably, our scientists observed a trend toward larger LNP sizes with the other tested molecules (Figure 1).

MOVING GLYSCIENCE FORWARD

How does the Sucrose SG product's manufacturing process fit into your company's strong sustainability commitments?

Our proprietary manufacturing system operates in an environmentally responsible and resource-efficient way. All products in the Solbiote portfolio are manufactured using water throughout the process instead of ICH Q3C class 1, 2, or 3 solvents, which are used often during saccharide purification and recrystallization. Thus, our products are manufactured at high purity with

Figure 1: Results from dynamic light scattering (DLS) analysis of lipid-nanoparticle (LNP) stabilization after zero (LEFT) and three (RIGHT) freeze-thaw cycles; control samples contained no saccharide excipient, whereas other samples contained Maltose PH, Trehalose SG, or Sucrose SG saccharides from Nagase Viita.



low endotoxin levels for injectable applications without relying on organic solvents. Our manufacturing processes are optimized through strategic inventory management and meticulous planning to preserve water and minimize waste and CO_2 emissions.

Our sustainability approach prioritizes environmental and social impacts along with maintenance of product quality. By offering sustainably manufactured, high-quality excipients to biopharmaceutical developers and manufacturers, we are helping to advance sustainability in the industry.

What is next for your company?

Since our founding, our advanced R&D capabilities in glycoscience have driven us to explore new possibilities for saccharides. For example, we are investigating applications of Solbiote solutions as processing aids during both upstream and downstream bioprocess development.

Our goal is to be an essential partner in the sustainable manufacture and equitable distribution of

biopharmaceuticals. We are eager to continue developing saccharide excipients and providing thought-leadership in the glycoscience field.

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Monitoring PLBL2 Levels in CHO-Based Biopharmaceuticals

Comparative Study of ELISA Kit Performance

Stefan Sommerschuh and Charlotte Coenders

Development of Chinese hamster ovary (CHO) cell-based biopharmaceuticals requires stringent monitoring of host-cell protein (HCP) impurities to ensure product safety and quality. In particular, many studies have reported phospholipase B-like 2 (PLBL2) as a high-risk, difficult-to-remove protein because of its immunogenicity and tendency to copurify with recombinant proteins (1, 2). The protein shares ≈80% of its amino-acid sequence identity with a human ortholog, but key surface-exposed differences make it likely to be immunogenic in humans (3). That immunogenic potential has been observed in clinical studies, such as those involving lebrikizumab, in which ≈90% of patients developed antibodies specifically recognizing PLBL2 (4).

Furthermore, PLBL2 often copurifies with therapeutic monoclonal antibodies (mAbs) due to protein-protein interactions in recombinant CHO-based processes (5). Because PLBL2 is difficult to remove completely during downstream processing, precise monitoring is needed to ensure its removal from final drug substances. Such a process requires a specific enzyme-linked immunosorbent assay (ELISA) kit with high sensitivity to native PLBL2 in CHO-expressed therapeutics, thus ensuring reliable detection of trace amounts of PLBL2 even in complex process samples.

PLBL2 ELISA COMPARISON STUDY

To support risk assessment, the team at BioGenes developed a PLBL2-



specific ELISA and ran a comparative study to evaluate the company's newly developed CHO-PLBL2 ELISA kit and two CHO-PLBL2 ELISA kits available from other suppliers (named Competitors 1 and 2). Performance characteristics of the three PLBL2 kits are demonstrated by two parameters — limit of detection (LoD) and working range:

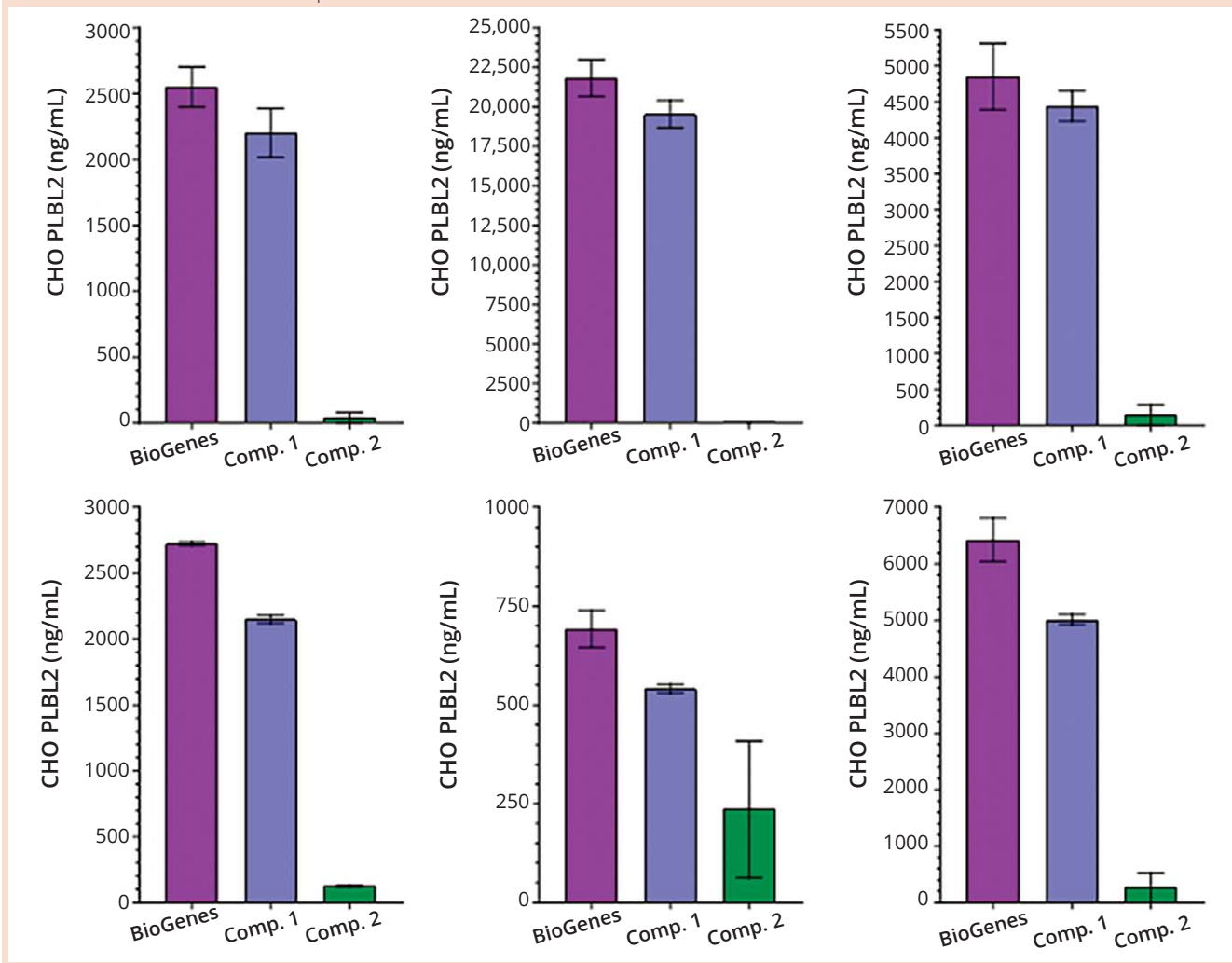
- BioGenes: LoD of 0.015 ng/mL (calculated according to ICH Q2(R2) using the final standard curve from ELISA development), working range of 0.078–5 ng/mL
- Competitor 1: LoD of 0.035 ng/mL (calculated as a PLBL2 concentration corresponding to a twofold ELISA blank-well absorbance value), working range of 0.31–20 ng/mL
- Competitor 2: LoD of 0.017 ng/mL, working range of 0.39–25 ng/mL; data taken from instruction leaflet.

To assess kit applicability, CHO-derived process samples from two distinct manufacturing processes (Processes A and B) were analyzed twice each using the three PLBL2 ELISA kits. Early and middle downstream

process (DSP) samples and final drug substances were provided by industry partners. Figure 1 depicts the results.

Two of the three kits — BioGenes and Competitor 1 — adequately detected CHO PLBL2, whereas the Competitor 2 kit significantly underestimated all tested DSP samples, as verified by spiking experiments with native CHO PLBL2 (data not shown). The discrepancy probably is due to the mAb pair used in the Competitor 2 kit: Because mAbs are epitope-specific, they can fail to recognize native CHO PLBL2 if protein structures differ from that of the PLBL2 antigen used for antibody generation. By contrast, the BioGenes and Competitor 1 kits are based on polyclonal antibodies (pAbs), specifically chosen for their superior versatility to mAbs. The Competitor 1 kit consistently yielded lower values compared with the BioGenes kit. Overall, the BioGenes ELISA kit demonstrated higher analytical sensitivity and consistent reliability in quantifying native CHO PLBL2 in DSP than competitor kits could provide.

Figure 1: Phospholipase B-like 2 (PLBL2) levels in three Chinese hamster ovary (CHO)-based process samples — early downstream (LEFT), middle downstream (CENTER), and drug substance (RIGHT) — from Processes A (TOP) and B (BOTTOM), represented as mean and standard deviations for each sample



PROCESS OPTIMIZATION AND RISK ASSESSMENT

Specific ELISA kits for native CHO PLBL2 are critical to regulatory compliance and process optimization for CHO-based biomanufacturing. Regulatory authorities require robust data on impurities, especially high-risk HCPs such as PLBL2, to ensure patient safety. PLBL2-specific ELISAs support the optimization of purification processes by providing valuable insights for identifying immunogenic risks before clinical testing. BioGenes's CHO-PLBL2 ELISA kit is a powerful tool for monitoring native CHO PLBL2, providing accurate quantification, process optimization, and regulatory adherence and contributing to the development of safe, effective therapeutics.

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Streamlining Purification

Proof of Concept for a Fully Connected Downstream Process

Benoît Mothes, Célia Valentim, Thomas Prouzeau, Frédéric Thiebaut, Geoffrey Pressac, and Kevin Brower

Protein-based therapeutics remain the largest biopharmaceutical market — dominated mainly by monoclonal antibodies (mAbs) and their derivatives (1). Through advancing technologies over the past 10 years, companies making such products have experienced a shift in the challenges involved, with mAbs now produced at high expression titers (≥ 5 g/L), resulting in constraints on the downstream side. Complex engineered constructs such as fusion proteins, bispecifics, and trispecifics usually are produced at lower titers; however, they often are less stable and/or more prone to aggregation than traditional mAb molecules. With large numbers of therapeutic candidates having passed through early clinical trials in the past decade, demand is increasing for production of materials for phase 3 studies and commercial needs.

Those combined phenomena are driving a trend in process intensification and (semi)continuous manufacturing, which have been discussed since the early 2000s but are now finally gaining traction. A number of biomanufacturers are implementing such technologies to help solve challenges brought on by high expression titers, unstable molecules, and growing demands for manufacturing capacity (2, 3).

Process intensification encompasses an array of solutions that can make bioprocessing faster, cheaper, and more sustainable than is possible with legacy processes. In downstream processing, such technologies include sequential and batch multicolumn



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chromatography (S-MCC and B-MCC), rapid-cycling chromatography (RCC), single-pass tangential-flow filtration (SPTFF), and connected processes. Table 1 lists key process intensification technologies and summarizes their expected benefits.

Among those strategies, RCC and S-MCC especially enable strong productivity increases at the step level. However, the real bottleneck of the downstream process lies in the batch approach itself, which requires that each step begins after the previous one ends. One way to increase the efficiency of biomanufacturing would be the connection of process steps combined with use of membrane chromatography (4–6). Below, we showcase a proof-of-concept study of a connected, membrane-based downstream process that increases productivity at the facility level.

MATERIAL AND METHODS

Process Design and Buffer Screening:

We defined conditions for each chromatography unit operation through laboratory-scale tests using

an Äkta pure 25 purification system (Cytiva). Consumables included Sartobind nano membranes (1.2 mL for Sartobind Rapid A with 4-mm bed height, 1 mL for Sartobind S and STIC with 4-mm bed heights, all from Sartorius).

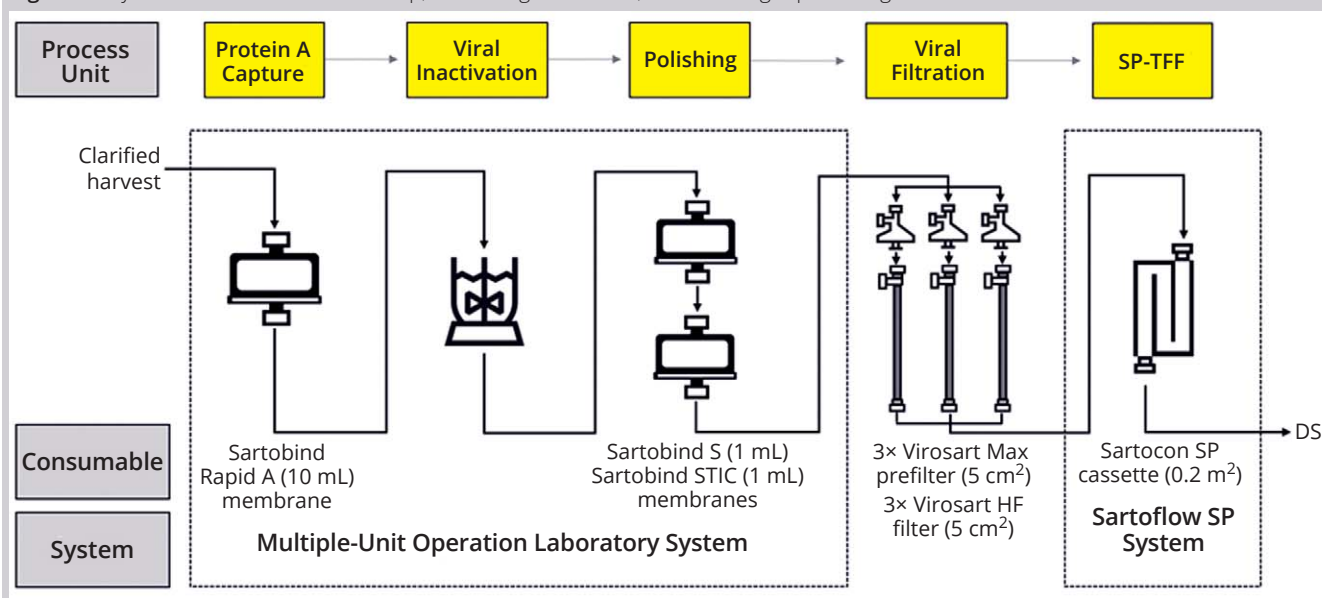
Proof of Concept for the Connected Membrane Process: For testing purposes, we used a standard immunoglobulin G class 1 (IgG1) antibody at a harvest concentration of 3.0 g/L with 6.3% high-molecular-weight (HMW) content.

For the connected process experiment, we used a custom Resolute BioSC Lab chromatography system (Sartorius) designed to operate three different steps (three modules), adding a manifold to the system outlet for virus filtration and a Sartoflow SP single-pass tangential-flow filtration (SPTFF) system from Sartorius for final concentration and buffer exchange. Figure 1 shows the system and consumable setup; Figure 2 shows how all steps were orchestrated.

All consumables described herein come from Sartorius. We used a

Table 1: Summary of downstream process-intensification technologies and their benefits

Technology	Principle	Benefits
Batch multicolumn chromatography (B-MCC)	Scaling out: Use two or three smaller columns rather than one large column.	Relative ease of handling; enables continuous loading and processing
Sequential multicolumn chromatography (S-MCC)	Using multiple columns in parallel, including two in series, allows overloading of a column without yield loss.	Enables continuous loading; reduces resin volume (by up to 80%); lowers buffer consumption and potentially processing time
Membrane-based polishing	Use convective sorbents to perform flow-through steps (with or without cycling).	Eliminates packing efforts, column capital expenditure, and maintenance; speeds up both processing and validation; reduces consumables sizing and buffer consumption, facilitates handling, and lowers costs; reduces bioburden risks
Rapid-cycling chromatography (RCC)	Use smaller chromatography matrices with convective mass transfer to make >40 cycles within a batch.	Eliminates packing efforts, column capital expenditure, and maintenance; speeds up both processing and validation; reduces consumable sizing and buffer consumption, facilitates handling and lowers costs; reduces bioburden risks
Single-pass tangential-flow filtration (SPTFF)	Users can change buffer and/or concentrate the product without recirculation in a tank.	Enables buffer exchange and/or product concentration between steps
Connected processing	Orchestrate operations to process material through each step as soon as possible (before the previous one finishes). This can combine a traditional batch approach with RCC or MCC, cutting one batch into small subpools that get processed by fully automated cycling chromatography media.	Uses one central automation platform to reduce the number of systems, column sizes, and intermediate tank sizes, as well as their related footprints and costs; reduces processing time by up to 40% and consequently increases the number of batches that can be processed per year

Figure 1: System and consumables setup; DS = drug substance, SPTFF = single-pass tangential-flow filtration

Sartobind Rapid A Mini membrane (10 mL) for mAb capture on the first module. Two Rapid A elutions were pooled and sent to the second module for automatic virus inactivation (VI). In that process, the system automatically lowers the solution pH to a target of 3.5 using 5 M acetic acid (AcOH), performs 30 minutes incubation, and then neutralizes the solution to a target of 7.0 using 2 M tris. Meanwhile, protein A elution continues in a dedicated tank.

Post-VI product was pumped into the third module for a double flow-through combining Sartobind S and STIC Nano membranes (1 mL with a 4-mm bed height) into a single step. Table 2 describes detailed recipes for the chromatography steps based on screening studies that we had performed up front.

For nanofiltration, we directly combined three Virosart Max prefilters (5 cm²) with three Virosart HF filters (5 cm²) using a filtration

manifold. Filters were vented and flushed with buffer according to manufacturer recommendations.

Finally, we used a Sartoflow SP filtration system with a Sartocoon SP cassette (0.2 m²) for SPTFF. The cassette was equilibrated in diafiltration buffer before product loading. First, product was recirculated on the SPTFF cassette for 10 minutes to build a polarization gel so that we could identify working parameters. Because the cassette is

Figure 2: Recipe scheme for detailed orchestration of all steps

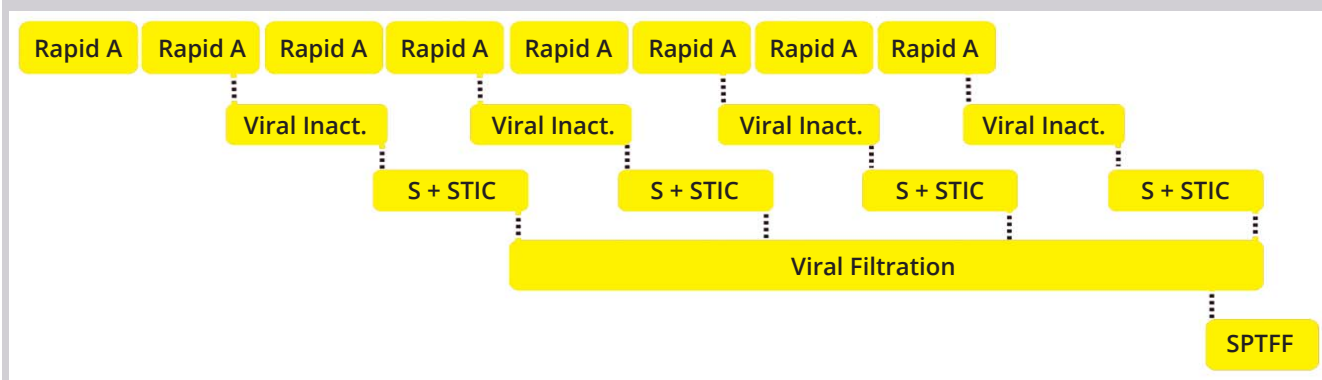


Table 2: Detailed recipe for chromatography steps; flow rate on the Sartobind Rapid A membrane was limited by pump capability of the custom Resolute BioSC Lab control system; mAb = monoclonal antibody, MV = membrane volume, NA = not applicable.

	Sartobind Rapid A Mini Membrane (10 mL, 2.8 MV/min)	Sartobind S and STIC Membranes (1 mL, 5 MV/min for both types)
Equilibration	2 MV of 50 mM tris and 20 mM NaCl at pH 7.5	70 MV of 50 mM tris at pH 7.0
Load	10 MV of mAb solution at 3.0 g/L	110 MV from previous step
Wash	2 MV of 50 mM tris and 20 mM NaCl at pH 7.5 + 4 MV of 50 mM tris and 1 M NaCl at pH 7.5 + 10 MV of 50 mM tris and 20 mM NaCl at pH 7.5	50 MV of 50 mM tris at pH 7.0
Elution (with peak cut)	12 MV of 25 mM NaOAc at pH 3.7	NA
Strip	3 MV of 0.5 M AcOH	35 MV of 50 mM tris and 1 M NaCl at pH 7.5
Equilibration	18 MV of 50mM tris and 20mM NaCl at pH 7.5	35 MV of 50 mM tris at pH 7.0
Clean in place	Only the day before the test using 0.2 M NaOH with a 10-minute contact time	35 MV of 0.2 M NaOH

relatively large (no smaller model was available), we initiated SPTFF only toward the end of the connected process sequence when a sufficient pool of Sartobind eluate was gathered in the feed vessel for the Sartocon SP cassette. That was a design limitation of the small-scale skids available. At larger scales, the goal would be to start SPTFF once enough product has gone through the virus-retentive filters (ideally, soon after product exits the filter).

Analytcs: Only the input material (clarified bulk harvest) and process-output material (post SPTFF) were tested by our quality control (QC) group. No intermediate samples were taken between process steps, with one exception: Product exiting the polishing train was tested for mAb concentration to confirm a

concentration factor for the SPTFF step. We tested mAb concentration using size-exclusion high-performance liquid chromatography (SEC-HPLC) and determined total host-cell protein (HCP) content using a disc-based immunoassay with the Gyrolab CHO-HCP E3G kit on a Gyrolab xP workstation (all from Gyros Protein Technologies). HMW content was assessed using SEC-HPLC.

RESULTS AND DISCUSSION

Connected Process Execution: The test was successful. The custom Resolute BioSC Lab system carried out all chromatography steps and tank-based VI. Including all subcycles and steps (Figure 2), four cycles were completed within five hours.

We did encounter a minor issue: Flow-through material exiting the

double polishing step at 10 mL/min ran through the virus-retentive filters with a rising pressure — up to 3.5 bar in the first cycle — which caused leaks from the single-use tubing. With the Virosart Max and HF filters positioned on the polishing outlet, they were loaded directly by the chromatography system pumps, so the pressure on the Sartobind S + STIC membranes went higher than 4 bar, which is the maximum pressure allowed for those capsules.

Consequently, after the first cycle's completion, we decided to change the nanofiltration installation by incorporating an independent pump to prevent backpressure on the polishing operation. That pump was unable to deliver flow rates <10 mL/min, however, so we could not carry on using the filter train as set up. We believe that using a smaller independent pump would solve this problem easily in the future. Our experiment highlighted the benefits of using a constant flow instead of connecting a virus-retentive filter directly at the polishing outlet. Therefore, we do not consider this event to be an indication that the connected process would not work, only that a minor adjustment would be required.

Once a sufficient pool of polished product was collected, the SPTFF started. After testing various settings, we targeted a concentration factor of 5.5. The transmembrane pressure (TMP) remained stable at 0.2 bar.

Simultaneously, the Sartocon SP cassette also provided in-line diafiltration of the feed with three diavolumes. The system automatically

We have shown that a **FULLY CONNECTED** membrane-based process can produce purified product for clinical trials.

measured conductivities on the feed, buffer, and retentate lines. Input conductivity of the feed was 10.0 mS/cm, and it was diafiltered against phosphate-buffered saline (PBS) with a conductivity of 16.5 mS/cm. The retentate conductivity value stabilized at 15.2 mS/cm, giving an estimated diafiltration efficiency of 80%. Without further optimization, that value is admittedly below the expected performance of a final TFF step used for formulation of drug substance. However, it is an interesting performance for a continuous TFF step that could run for long periods as part of a connected process. The final buffer adjustment for formulation could be improved either by optimizing the method further (by using two SPTFF cassettes in series) or by adding a final standard TFF to concentrate product further at the end of the process. Sizing of that extra step would be greatly reduced by the preconcentration and buffer exchange already performed by the SPTFF.

In total, the system operated in single-pass mode for 31 minutes. The Sartoclon SP cassette processed an incoming feed titrated at 2.3 g/L. After pooling with a buffer flush, the system recovered 176 mL of mAb concentrated at 9.0 g/L from the retentate of the cassette. Therefore, the observed in-line concentration factor was 3.9. So this entire connected process allowed us to produce 1.6 g of drug substance, starting from the clarified harvest, within 5 hours and 20 minutes. That translates to an overall process performance of 25 g/h/L membrane, including VI, virus filtration, and SPTFF. Note that flow rates were nonoptimal,

Table 3: Analytical results; HCP = host-cell protein, HMW = high-molecular-weight species, mAb = monoclonal antibody, SPTFF = single-pass tangential-flow filtration

Fraction	mAb Titer	HMW	HCP	Yield
Load Rapid A (clarified bulk harvest)	3.0 g/L	6.3%	64,315 ppm	—
SPTFF pool (including buffer flushes)	9.8 g/L	4.0%	35 ppm	67%

Table 4: Scale-up projections for steps of a 200-L clinical batch; SPTFF = single-pass tangential-flow filtration

Step Consumables	Cycles	Time	
		Step Starts	Step Ends
Capture: Sartobind Rapid A membrane (200 mL)	90	0 hours	14.5 hours
Virus Inactivation: Flexsafe bag in Pro Mixer system (20 L)	15	1.5 hours	15.6 hours
Polishing: Sartobind S membrane (75 mL) + Sartobind STIC membrane (75 mL)	15	2.1 hours	15.8 hours
Nanofiltration: Virosart Max filter (0.2 m ²) + Virosart HF filter (0.2 m ²)	1	2.3 hours	16.0 hours
SPTFF: Sartoclon SP filter (1.7 m ²)	1	2.5 hours	16.2 hours

especially for the Rapid A membrane, due to system limitations, so this value could be increased further. The setup outperforms the standard resin-based process for this mAb, which culminates at 2.5 g/h/L resin.

Analytical Results: Table 3 gives our analytical results. Note that those data match these targets for a phase 1 process: <5% of HMW species in pool SPTFF and HCP levels of <100 ppm. Also, the HMW concentration in the load was high only because the product had been stored for some time; the purity would be better in real process conditions. Adding a depth filter after VI could provide further purity regarding HMWs and HCPs.

Considering the results above, a sample taken during the third cycle after the polishing steps showed a total yield of 78% for all chromatography steps and VI combined. That result is comparable to the batch process that this continuous setup would replace. However, a yield gap manifests with the SPTFF, which reduced that total to 67%. We needed a high buffer flush to recover the mAb, given the large size of the SPTFF cassette. As a

consequence, it is likely that some product remained present on the membrane. Further time spent on process optimization using more adequately sized SPTFF consumables would increase both the concentration and yield further. Thus, we consider our unoptimized test results to be very promising.

Large-Scale Implementation:

Based on the small-scale process in this study, we calculated process projections for a clinical batch of 200 L at 3 g/L. The entire batch could be processed within two work shifts using a Resolute BioSC Pilot system with a 200-mL Rapid A capsule together with Sartobind S and STIC capsules of 75 mL connected. Rapid A elutions would be pooled for VI, and each post-VI product would correspond to a polishing cycle.

The virus filter size would require confirmation through a sizing study. Based on standard performance and available filter sizes, however, the Virosart HF filter (0.2 m²) would be a good fit with enough of a safety margin. As mentioned above, that filter should be operated by an independent pump. Finally, the SPTFF

Ultimately, this connected process strategy should generate **DRUG PRODUCT** from clarified harvest within a day, using only very small and relatively inexpensive consumables.

could be carried out with a 1.7-m² cassette running continuously. Certainly, further optimization would be required; in our experiment, we were limited by low flow of product exiting the polishing train relative to the cassette that we used for SPTFF.

Ultimately, this connected process strategy should generate drug product from clarified harvest within a day, using only very small and relatively inexpensive consumables. Indeed, the specific overall productivity for the entire chromatography train would be 72 g/h/L at a cost of 35 €/g (about US\$37/g) of mAb produced (considering only the chromatography membranes for a single-use process). This process outperforms not only the current resin-based batch process, but even its connected version. Indeed, connecting the three current resins using the same system would result in costs of €65/g (\$68/g) to produce three clinical batches. The productivity of this connected, resin-based process would be 2.5 g/L/h, as mentioned above (29-fold lower than with membranes).

CONCLUSION

This first proof of concept for a fully connected downstream process — including VI, virus-retentive filtration, and SPTFF — has been a success. For follow-on studies, minor design adjustments would be required to enable full inclusion of the virus-retentive filtration step and improve the SPTFF performance. However, we have

shown that a fully connected membrane-based process can produce purified product for clinical trials. Membranes enable purification of large amounts of product with small consumables. They are a perfect fit with connected skids such as the Resolute BioSC system for leveraging a rapid cycling approach, not only for capture, but also for the polishing — which substantially reduces overall costs.

The footprint of the entire process is reduced compared with that of a batch operation. First, only one system (rather than two or three) can be used to control all the chromatography steps. Second, smaller chromatography consumables can be applied, with an entire 200-L harvest processed using only 0.35 L of membrane. Finally, the intermediate tanks between steps and for VI are also reduced in size. In this case, just four mixing tanks of 10–20 L would be required for our 200-L process. This connected, membrane-based downstream process therefore offers major advantages first for production of clinical materials and later for commercial manufacturing.

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FURTHER READING

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ACKNOWLEDGMENTS

We thank Camille Bailly, Paul Humbrecht, and Olivier Huot for their contribution to this experimental work. 🌐

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An Innovative Funding Model for Biotechnology Companies

with Joe Payne and Jeff Wolf

Many early stage biotechnology companies face challenges in securing funding. The biotechnology sector is highly competitive, and capital has been scarce in the last few years, leading some companies to seek alternative collaboration opportunities. In a December 2024 Ask the Expert webinar, two representatives from Scorpius BioManufacturing — Joe Payne (president and chief operating officer, COO) and Jeff Wolf (founder and chief executive officer, CEO) — introduced their company's innovative hybrid funding model, Scorpius Ventures, for early stage biotechnology companies in need of financial assistance.

THE PRESENTATION

Funding Challenges in Biotechnology:

Early stage biotechnology companies often struggle to attract investments because of demanding expectations from funding sources. Investors increasingly require substantial preclinical data, including information about cell-line stability, process scalability, and toxicology results. However, emerging companies often lack the capital necessary to generate such data, creating a “chicken-and-egg” dilemma — funding is needed to produce meaningful data, which in turn are necessary to secure funding. This problem is compounded by the competitive and capital-intensive environment of the biotechnology sector, particularly for companies working to develop biologics domestically in the United States.

Scorpius BioManufacturing, with its current good manufacturing practice (CGMP)-grade facility in San Antonio, TX, seeks to bridge the gap between innovation and funding for biotechnology companies. Scorpius recognized that

many promising scientific endeavors fail to progress because of the prohibitive costs associated with biologic development. Scorpius Ventures was established as a response to that challenge, aiming to support early stage companies by offering an alternative funding pathway that retains scientific development within the United States.

Scorpius Ventures uses a hybrid funding model, which is a partnership-based approach rather than a standalone investment fund. Scorpius provides contract manufacturing services and covers costs related to clinical manufacturing in exchange for small equity stakes in partner companies. Biotechnology partners, in turn, fund essential components such as consumables, cell-line development (CLD), and storage. That model reduces risk for both parties while fostering collaboration. By aligning interests, Scorpius enables biotechnology companies to access capabilities without needing large, upfront capital investments.

Current Progress and Future

Outlooks: The response to Scorpius Ventures has been positive since its launch, with several biotechnology companies expressing interest. The company is preparing for a busy 2025, having already expanded capabilities and booked microbial fermentation projects through the year. Scorpius anticipates that economic conditions in the United States will improve, making 2025 a milestone year for the biopharmaceutical industry. Through partnerships, the hybrid model is expected to drive growth while helping biotechnology companies to achieve their scientific goals.

Although the Biosecure Act has yet to pass through the US Congress as of

January 2025, its potential impact is already being felt across the industry. The legislation encourages biopharmaceutical development and production in the United States to reduce risks associated with overseas manufacturing, such as quality concerns, supply-chain vulnerabilities, and logistical complexities. Those factors have further incentivized US biotechnology companies to partner with domestic manufacturers such as Scorpius. Payne and Wolf said that, by eliminating the challenges of overseas production, companies can help to ensure product quality and patient safety.

QUESTIONS AND ANSWERS

How has the potential passage of the Biosecure Act affected biotechnology companies? Drug sponsors are trying to be proactive. Some of them plan to shift operations from China to India or Europe; however, quality concerns would still be a challenge because the US Food and Drug Administration (FDA) represents the gold standard in quality and patient safety. Partnering with suppliers overseas also comes with supply-chain risks and time-zone differences.

How can a biotechnology company learn more about Scorpius Ventures?

Please visit our website at <https://www.scorpiusbiologics.com/scorpius-ventures>. There you can fill in an interest form and send us your nonconfidential pitch deck. In 2025, we plan to start enacting confidentiality agreements and having preliminary conversations with applicants. 🌐

Alois Jungbauer

A Life in Biotechnology

Josh Abbott

BPI editorial advisor Alois Jungbauer serves as a professor teaching downstream processing, protein technology, and bioprocess engineering at the University of Natural Resources and Life Sciences (BOKU) in Vienna, Austria. He has trained about 90 PhD students in bioprocess engineering and has written hundreds of papers, with chromatography being a favorite topic.

"I stumbled into this field," Jungbauer told me at BPI Europe in 2024, recalling the moment in 1982 when Professor Hermann Katinger offered him an assistant professor position in downstream processing. Katinger, a prolific writer and a pioneer who foresaw the rise of recombinant-DNA technology, would go on to found contract development and manufacturing organization (CDMO) Polymun Scientific in 1992. "It was a lucky incident in my life," he said. "I could have said no to that offer."

In the 1990s, Jungbauer pioneered work on displacement effects in large-scale chromatography. "Nowadays, these type of things are important for the purification of gene-therapy vectors." He followed those with studies on scaling up chromatography. "From academia, we knew then how to scale up and scale down chromatography, which is really an engineering [pursuit]."

And because of his expertise, Jungbauer is a fixture at industry events, including but not limited to those hosted by *BioProcess International*. In July 2024, for example, he discussed sustainability at the 28th European Society for Animal Cell Technology (ESACT) meeting in Edinburgh, Scotland, sharing insights that will help the industry survive into the future.

"We have always overlooked the emission intensity of the biopharmaceutical industry," Jungbauer said at the conference before revealing data detailing the CO₂ emissions of many major biopharmaceutical companies.

From Humble Beginnings

Jungbauer told me that his early interest in science led to an education in food technology, which he described as a combination of biology and engineering, not unlike bioprocessing. He earned his PhD in food technology and biotechnology from BOKU. That interest predated the era of recombinant DNA and was nurtured by a general fondness for science.

His interest in food science started when he was a child growing up on a farm two hours south of Vienna in a village with about 1500 people. His family grew crops and raised animals, selling products such as barley, wheat, eggs, and milk. He worked long summer hours, performing whatever chores were needed, whether it was harvesting grain, baling

hay, or milking cows. At the end of each summer, he went back to school.

"I enjoy academia because you don't have to work so hard," he said with a laugh. He received a broad education free from the early specialization that modern curricula often prioritize.

Jungbauer developed an affinity for science and math and began focusing on those subjects. At university, his relationship with the world shifted. On the farm, nature was a resource that humans could harvest or extract. But Professor Uwe B. Sleytr, who ran a center for microscopy, showed him nature under the lens of a microscope.

When he first examined images of bacteria and other microorganisms, Jungbauer's place in the world took a turn. He was fascinated by the beauty of nature. The city offered opportunities away from the farm, enhanced by the newfound autonomy of a life away from home. Jungbauer savored the opportunity to explore new facets of the environment. He developed a love for museums, such as Vienna's Museum of Natural History, which is prized for its extensive collection of gemstones. He spoke fondly of the Kunsthistorisches Museum, which houses paintings from some of history's most renowned artists, including Dutch painter Pieter Bruegel. Jungbauer expressed a particular interest in Bruegel's work, which shares commentary about society and human nature.

Since moving from a family farm in a quiet village to a city overflowing with culture and professional science, Jungbauer has established himself as one of the most important voices in the industry. "When I went to university and came to Vienna, it was for me..." He paused to find the right word. "Freedom. Freedom. Freedom." 🌍

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BioProcess International Europe 2025 is coming to Hamburg, Germany, May 12-15; <https://informaconnect.com/bpieurope>.





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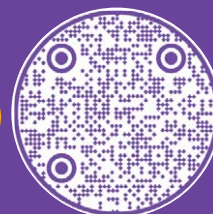
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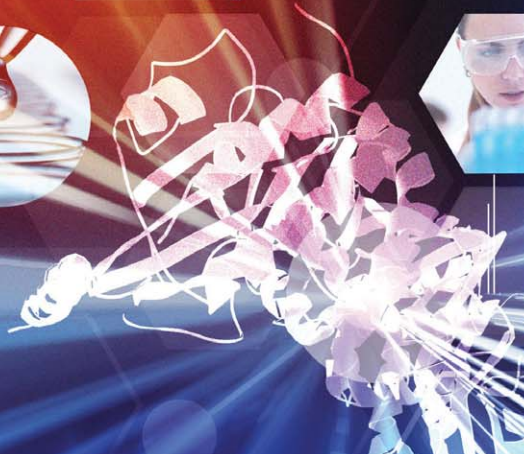
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