Webinar summary

Integrated upstream and downstream strategies for robust mAb manufacturing

Monoclonal antibodies (mAbs), though well-characterized in contrast to many other emerging biotherapeutic modalities, are nevertheless ripe for additional optimization as demand for these therapeutics surges. Scaling from early clone selection in shake flasks to robust performance in large bioreactors requires more than geometric adjustments; it demands a clear understanding of how cells experience their environment and how parameters such as pH, oxygen transfer, CO_2 stripping, nutrients, and shear force shift across scales and equipment.

Process development (PD) is therefore more than simply the assembly of unit operations; it is the systematic optimization, characterization, and translation of processes across scales and facilities. Effective programs integrate predictive scale-down models, mechanistic and empirical approaches, and structured technology transfer to ensure that cGMP processes are both executable and fully characterized with respect to their impact on critical quality attributes (CQAs). This knowledge enables accelerated troubleshooting, rational optimization, and consistent control strategies throughout the product lifecycle.

The goal in mAb scale-up is to maintain a consistent cellular microenvironment while defining a design space that mitigates stressors impacting growth, productivity, and quality. Hydrodynamic and mass transfer phenomena such as oxygen transfer, CO_2 stripping, and mixing must be balanced alongside media and feed strategies that sustain performance across clones and scales. Neglecting these dynamics risks introducing variability, batch failure, and barriers to technology transfer.

Reliable scale-up ultimately depends on structured, tool-enabled approaches that enhance process robustness. Critical considerations include agitation and sparging for gas-liquid transfer, predictive scale-down models, and media/feed designs that reduce process sensitivity. Expanding the operational design space and implementing data-driven control strategies enables biomanufacturers to minimize variability, accelerate scale-up, and consistently deliver high-quality mAb therapeutics on a commercial scale.

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How bioreactor design and media optimization drive predictable scale-up

Bioreactor platforms such as Thermo Scientific™ HyPerforma™ and DynaDrive™ Single-Use Bioreactors (S.U.B.s) are purpose-built to support scale-up from discovery to production. While early-stage cultures in plates and flasks face minimal stress, their transition into bioreactors introduces new variables, from sparging to impeller mixing and tightly controlled pH, that often represent a clone's first real test. Consequently, scaling strategies must anticipate how cells will respond to these shifts.

Rather than relying on single parameters like power-to-volume ratios, Thermo Fisher Scientific defines robust design spaces that account for oxygen transfer, CO_2 stripping, pH stability, nutrient availability, and shear forces across scales. As an example, sparger-impeller interactions, not agitation alone, often drive the most stressful shear conditions. To address this, engineers must optimize sparger type, pore size, and distribution to vessel geometry, in order to achieve balanced gas exchange and minimize gradients that threaten product quality at scale.

The DynaDrive S.U.B. has undergone this optimization–with low-shear, off-center agitation and customized sparging systems, it delivers efficient mixing while protecting cell health, enabling predictable translation from lab processes to production volumes. Complementing these engineering solutions, the Gibco™ Efficient-Pro™ CHO platform provides basal media and tailored feeds that match clone behavior, whether driving high densities, maximizing specific productivity, or supporting GS knockout lines. Together, these tools allow development teams to scale with confidence, preserving both performance and product quality.

From benchtop to production: a 3 L to 500 L case study in process transfer

Scaling cell culture processes remains one of the central challenges in biologics manufacturing. While benchtop systems allow rapid prototyping and process optimization, the transition to pilot and production scales often exposes unanticipated limitations in oxygen transfer, pH control, nutrient delivery, and automation compatibility. These challenges are magnified as industry moves toward intensified upstream processes, where cell densities exceed 60 x 10^6 cells/mL and oxygen demands increase dramatically. Ensuring that growth, productivity, and product quality remain consistent across scales is therefore a critical test of both process design and bioreactor engineering.

In a recent case study, Thermo Fisher conducted the transfer of a fed-batch process from 3 L benchtop glass reactors to 500 L S.U.B.s, with parallel evaluation of the HyPerforma S.U.B. and advanced DynaDrive S.U.B. designs. By standardizing media, feed strategies, and automation platforms across scales, the study provides a detailed view of how reactor geometry, gas transfer efficiency, and control strategies shape culture performance, as well as what it means for future scale-up to the 5,000 L production level.

The process began in a 3 L Thermo Scientific™ HyPerforma™ Glass Bioreactor, where a CHO-K1 IgG-producing cell line was seeded at 0.3 x 10^6 viable cells/mL. From the outset, the goal was to design a scale-up path that minimized variables. For that reason, all reactors, from benchtop through 500 L, were operated on the Thermo Scientific™ TruBio™ automation platform, ensuring that data structures, control logic, and operator interfaces remained constant.

As the cultures were transferred upward through 50 L and 500 L volumes, two reactor geometries were compared: the cylindrical HyPerforma S.U.B.s and the cuboidal DynaDrive S.U.B.s. Although both designs supported high-density cultures, their differences became clear in oxygen transfer. The DynaDrive S.U.B.s consistently achieved target DO levels with lower agitation rates, tip speeds, and sparged O_2 requirements, a reflection of their optimized gas transfer design. This characteristic proved especially advantageous in anticipating future intensified processes, where oxygen demand would be the limiting factor during scale-up.

Table 1. Production scale-up study parameters comparing HyPerforma Glass Bioreactor with DynaDrive S.U.B. technology across 3 L to 500 L scales.

Parameter	HyPerforma Glass Bioreactor 3 L	DynaDrive S.U.B. 50 L	DynaDrive S.U.B. 500 L	HyPerforma S.U.B. 50 L	HyPerforma S.U.B. 500 L
Controllers	Thermo Scientific™ HyPerforma™ G3Lab™ Bioprocess Controller with TruBio software	Thermo Scientific™ HyPerforma™ Bioprocess Controller with TruBio software			
Start working volume	1.7 L	36 L	360 L	36 L	360 L
Maximum working volume	2 L	50 L	500 L	36 L	360 L
Temperature set point	37°C				
pH set point	7.15				
Dissolve oxygen set point	40%				
Agitation	350 rpm	150 rpm	69 rpm	183 rpm	101 rpm
Sparger type	Drilled pipe	Laser-drilled hole			
Foam control	Gibco™ Irradiated AOF (animal origin–free) Antifoaming Agent delivered as needed via foam probe and pump by controller software controller software				
Cells	CHO-K1 IgG mAb producing				
Seeding density	0.3 x 10^6 viable cells/mL				
Medium and supplementation	Efficient-Pro Medium with 6 mM L-glutamine				
Feed and feeding strategy	Efficient-Pro Feed 1, daily at 2.25% beginning day 3				
Glucose supplementation	2 M glucose solution supplemented as needed to maintain 3 g/L				

Feeding was managed through a continuous nutrient delivery strategy rather than bolus additions, ensuring that glucose and amino acid availability remained consistent across volumes. This approach eliminated oscillations in nutrient levels that often complicate scale-up and aligned well with the tighter control needed in large-scale reactors. Glucose supplementation was applied as needed to maintain ~3 g/L, while Efficient-Pro Feed 1 supported steady nutrient delivery beginning on day 3.

Overcoming scale-up challenges: case study key takeaways

At the 50 L scale, one replicate experienced lower initial viability, likely due to the reactor being used as a scale-up vessel and then reused 3 days after drain as a production-stage vessel, highlighting the sensitivity of scale-up work to seemingly minor operational choices. Similarly, while pH was well-controlled without base addition, slight deviations were observed in the 500 L DynaDrive S.U.B. due to relatively lower pCO_2 stripping, a factor easily corrected by refining gas delivery strategies.

Perhaps most significant was the reactor response to metabolic byproducts. Across all scales, lactate followed a characteristic pattern: an early accumulation phase peaking below 1.2 g/L, depletion by mid-culture, and a modest secondary rise (~1.3 g/L). Ammonium levels remained within manageable limits, underscoring the suitability of the feeding strategy for both growth and downstream compatibility.

The outcomes of this case study demonstrate the robustness of the Thermo Fisher process and approach: cell growth routinely exceeded 60 x 10^6 viable cells/mL and final titers averaged 3.6 g/L across all replicates, with less than 0.1 g/L variation. These outcomes held steady regardless of scale, providing strong evidence of process transferability. Importantly, the DynaDrive reactors delivered equivalent performance with lower power input and superior oxygen transfer, offering a clear pathway for handling intensified cultures at larger scales.

The consistency of results across 3 L, 50 L, and 500 L scales likewise demonstrates a scalable engineering framework.

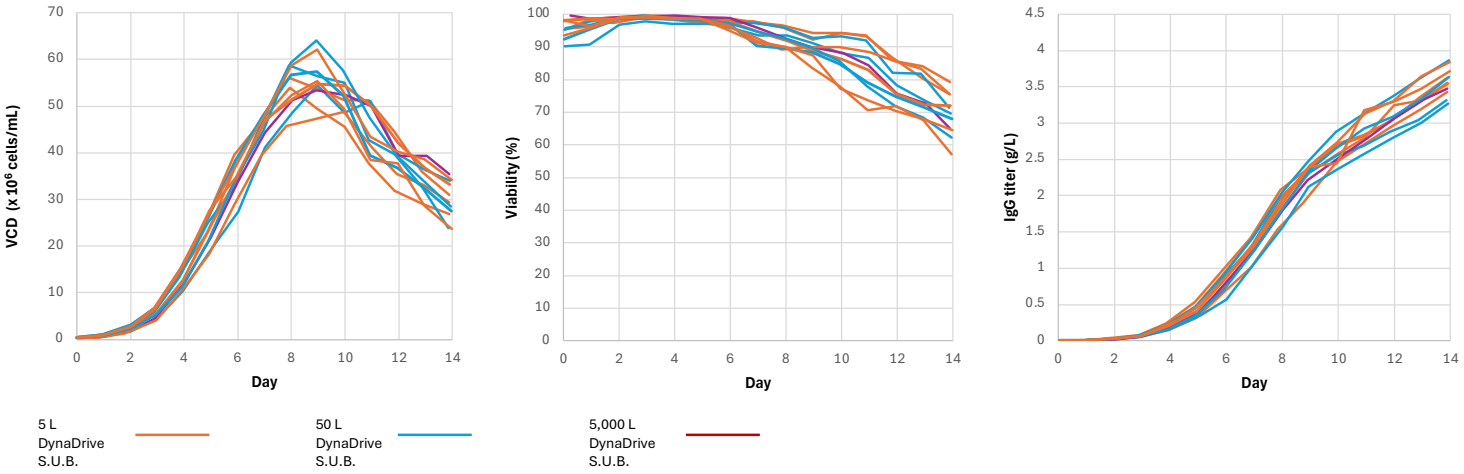

Figure 1. Benchmark cell culture performance across the DynaDrive S.U.B. platform. CHO-K1 cells were grown in Gibco™ Efficient-Pro™ AGT Medium. Data are derived from 6 replicates of the 5 L culture, 4 replicates of the 50 L culture, and one replicate of the 5,000 L culture.

In subsequent evaluations, the same process was scaled down to 5 L and up to 5,000 L using DynaDrive reactors, with remarkable alignment in growth, viability, and productivity across a 1,000-fold range (Figure 1). For developers navigating the complexities of modern biologics manufacturing, this case study illustrates two critical points:

- Automation continuity can be as important as biological consistency in successful scale-up.
- Bioreactor geometry and mass transfer efficiency can fundamentally shift what is possible in high-density culture.

By uniting these principles, the study highlights a model for scaling processes that is not only reproducible, but also future-ready for intensified upstream manufacturing.

Multistep purification and quality analysis of mAbs

If the upstream work defines the potential of a biomanufacturing process, the downstream purification strategy determines whether that promise translates into a therapeutic product that meets the industry's highest quality standards. For mAbs, achieving consistency across scales is not only a matter of achieving sufficient yield but also ensuring that purity, safety, and product quality attributes are not compromised when moving from bench-scale to commercial-scale production. Thermo Fisher addressed this challenge by creating a simple, two-step purification strategy that is supported by rigorous analytics, proving the process is robust and reliable across bioreactor scales.

The process begins with clarified cell culture harvest, which is captured on Thermo Scientific™ MabCaptureC™ Protein A affinity matrix. This engineered ligand offers high dynamic binding capacities, as demonstrated by IgG subclass-specific data showing greater than 60 g/L at standard residence times. Importantly, the resin's narrow particle size distribution (averaging

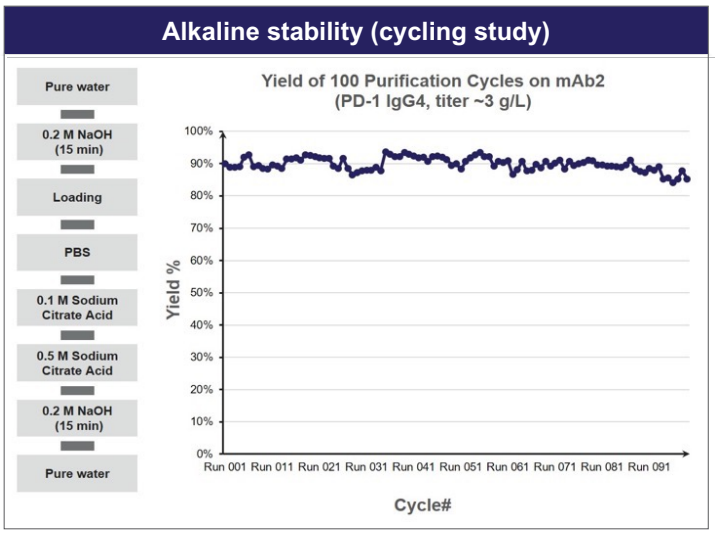

Figure 2. Performance characteristics of MabCaptureC affinity matrix for monoclonal antibody purification.

75 µm) enables excellent pressure-flow performance and ease of column packing. These resin characteristics help to ensure comparable performance when scaling up.

The MabCaptureC matrix maintained performance across 100 cycles with caustic cleaning, reflecting its ability to be reused in a manufacturing setting (Figure 2).

Following Protein A capture, eluates were neutralized, diluted, and subjected to a polishing step using Thermo Scientific™ POROS™ HQ anion exchange resin in a flow-through configuration. Unlike soft gel matrices that exhibit nonlinear pressure profiles at high flow rates, POROS resins are built on a rigid polystyrene-divinylbenzene backbone with relatively large through-pores. This allows for fast flow rates, low mass transfer resistance, and high resolution without compromising pressure-flow performance. The HQ resin is particularly effective at removing impurities at fast flow rates, cementing it as a crucial component of the broader anion-exchange portfolio offered by Thermo Fisher.

Across multiple bioreactor scales, the purification process consistently achieved recoveries in the 90% to 100% range, demonstrating both the robustness of the upstream process as well as the consistency in the downstream process. In addition to yields, host cell DNA and protein levels were reduced after the Protein A step to acceptable levels following POROS HQ resin polishing. Glycan analysis showed stable profiles across scales, with consistent species predominating and other minor species tightly controlled. Charge variant distribution remained consistent across scales, reflecting upstream stability.

These results illustrate how a two-step purification platform can be used to verify upstream scale-up performance and meet sufficient purity requirements, depending on the complexity of the mAb. By pairing advanced resin chemistries with comprehensive analytics, Thermo Fisher demonstrated that robust upstream performance can be matched downstream, yielding a process that is reliable, scalable, and quality-driven.

Conclusion

Upstream results from this Thermo Fisher case study demonstrated reliable performance across scales, with consistently high cell growth, viability, and titers, alongside low levels of inhibitory metabolites and stable specific productivity. Downstream, the simplified two-step purification delivered both high recovery and high purity, while preserving critical quality attributes such as glycosylation, charge variants, and monomer content.

For bioproduction, the impact is significant: these technologies streamline process development, reduce the time and resources required to identify optimal conditions, and enable efficient, predictable scale-up. Coupled with expert support, each solution provides end users with practical, reliable tools that translate scientific consistency into manufacturing confidence. Ultimately, this work highlights how alignment between upstream and downstream processes, supported by robust technologies, creates a clear pathway from early development to commercial production without compromising quality.

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