A new paradigm in process development

To accelerate biologic manufacturing of mAbs, vaccines, and novel biotherapies

gelifesciences.com

First published as a special report in
BioProcess International, November 2017

Introduction

Fast Trak services accelerate global biopharmaceutical development

By Cheryl Scott, BioProcess International's senior technical editor

As the need to accelerate biopharmaceutical development around the world continues to grow, biomanufacturers face a host of challenges so they, too, can grow. Increasing process productivity, reducing cost, mitigating risk, and bringing products to market faster are just a few of the issues frequently addressed. But with support in process development, cGMP manufacturing and training, accelerating bioprocess development can become less challenging.

Several biomanufacturers have successfully navigated these issues in collaboration with GE Healthcare's Fast Trak Services. Whether it was through process and analytical development, process scale-up, or manufacturing of drug material for use in toxicology studies, recent collaborations have yielded remarkable results. The following case studies describe projects in which collaborative efforts resulted in fast resolutions to common biomanufacturing challenges.

Janssen's vaccine development

As the pharmaceutical industry continues to look for new and innovative ways to treat disease, some companies are putting more focus on how to prevent them. Vaccine development, while a long and complex process, has the potential to save millions of lives globally, especially in low-income countries where some of the world's most at-risk populations exist. Securing a partner that could serve as an extension of the development team became essential to reducing both risk and the time needed for delivery of the clinical supplies.

Roivant Sciences in Switzerland

Farber disease is a rare lysosomal storage disorder caused by a defect or deficiency in the enzyme acid ceramidase. The deficiency causes intracellular accumulation of ceramide that leads to inflammation and tissue damage. Today, there is no specific treatment for Farber disease, but bone marrow transplantation can alleviate the symptoms. In this case study, Roivant Sciences worked with GE's Fast Trak Services to develop a cGMP biomanufacturing process for their Farber disease treatment to provide material for toxicity studies.

mAbxience in Spain

A Spanish biosimilars company, mAbxience engaged GE's Fast Trak Services to improve a first-generation process established by a third-party contract manufacturer. To achieve manufacturability and purity required for phase 1 clinical trial materials, Fast Trak scientists focused on improving the affinity chromatography (AF) capture step and intermediate purification and polishing steps while keeping the same process materials. They also developed a purification scheme more suitable for manufacturing scale.

A flavivirus project

Flavivirus vaccine development and production constitute many challenges and can be both space-and resource-consuming. In this final case study, an overview of modern tools and solutions is discussed, highlighting how they add flexibility and speed to both upstream and downstream operations in flavivirus vaccine production. Single-use production bioreactors and chromatography purification columns mitigate cross-contamination risk and support increased operator safety, while reducing time to market by eliminating costly and time-consuming cleaning operations. For downstream processing, modern chromatography resins offer high selectivity and excellent pressure and flow properties for high productivity in manufacturing-scale purifications. The use of such resins can increase purity and yield of a flavivirus vaccine manufacturing process.

Utilizing external collaboration to accelerate vaccine development

By Chris Rode, Scientific Director, Janssen Biotherapeutics Development

As the pharmaceutical industry continues to look for new and innovative ways to treat disease, some companies are putting more focus on how to prevent them. Vaccine development, while a long and complex process, has the potential to save millions of lives globally, especially in low-income countries where some of the world's most at-risk populations exist. In 2017, Janssen Vaccines & Prevention B.V., one of The Janssen Pharmaceutical Companies of Johnson & Johnson announced promising results for an HIV prophylactic vaccine candidate containing immunogens delivered through a combination of an initial recombinant viral vector priming dose followed by a purified protein booster dose. The prime-boost regimen aims to produce stronger and longer-lasting immunity to HIV.1 As exciting as this prospect is, development of the production process for the purified protein booster presented several challenges to Janssen's Biotherapeutics Development API-Large Molecule team. Securing a partner that could serve as an extension of the API development team became essential to reducing both risk and the time needed for delivery of the clinical supplies.

A strategy driven by speed

One of the most consistent demands for any project is development speed. For Janssen's vaccine, the pressure for faster speed to clinic was driven by two factors. The first is the need for the vaccine itself. For HIV, an epidemic tracing back to the mid- to late 1970s, the United States has seen considerable progress with prevention and treatment. Yet, for areas like sub-Saharan Africa, where 66 percent of new HIV infections occur,2 efforts to develop effective protection against a wide range of viral strains continue. With so many patients in South Africa being 15 years old or younger, developing a vaccine is critical.

The second driver for speed involves the strategy of a prime-boost application. The process development strategy and clinical evaluation of the vaccine relies on concurrent availability of the prime and the booster. If the booster injection is not administered to a clinical study participant within a specific calendar time frame after the prime injection, the study participant may not achieve the desired immune response. Therefore, parallel development of the prime and boost must be achieved and streamlined, so the final vaccine product can be produced, labeled, packaged, and delivered.

One option for drug and vaccine developers to achieve the fastest and most efficient path to clinic and market is to build strategic partnerships with contract development or manufacturing organizations that offer unique capabilities, specialized products or services. This approach can provide rapid resource scale-up, including the ability to run multiple activities in parallel, as well as access to novel technology and established expertise. This was the approach Janssen chose to accelerate development of the manufacturing process for its HIV vaccine booster.

A purification problem

For this HIV booster, Janssen's development team was facing a completely new class of therapeutic protein, which also came with new challenges. "Historically, my team worked on monoclonal antibody process development. In this case, our product was a recombinant construct of a trimeric cell surface protein from the HIV virus, so it is an extremely complex, highly-glycosylated protein," explains Chris Rode, Scientific Director at Janssen Biotherapeutics Development. "While it is produced on the upstream side by mammalian cell culture, no convenient affinity purification step, such as Protein A resin used for monoclonal antibodies, exists." The team needed to identify potential chromatography resins that would ultimately form the downstream purification process.

The conventional pathway to finding a resolution to this is to hypothesize a mechanism of bind/release through a chromatography resin matrix, try classes of resins until one is found that provides suitable separation, and then repeat the process in order to incrementally improve the purification. It is a lengthy, step-by-step process of trial and error. "To facilitate the speed of clinical process development, we had to assess how this approach would affect our project timeline and then look at strategies to shorten the R&D phase," says Rode. After detailed planning and evaluation, Janssen chose to look externally for options to accelerate high-volume resin screening.

Efficiency and reliability on a large scale

When bringing a product with potential for worldwide application into the market, the scale of manufacturing can be immense. The process developed for Janssen's protein booster had to be manufacturing-friendly and robust to avoid any downtime due to batch failure or delay. Cost of goods was also a concern, as were consistent purity and safety. The absence of a Protein A capture step presented a major challenge for this project when it came to these needs. "We had to figure out how we would design a primary capture process that sets us up for success throughout the entire downstream," explains Rode. "That would dictate the overall process yield, which ultimately drives the cost-of-goods. We also had to ensure a consistently high product purity and safety regarding removal of process-related impurities." Janssen decided that the best option to facilitate speed of development and maximize the potential of finding viable chromatography resin systems was to enlist the assistance of the GE Healthcare Life Sciences Fast Trak team (with whom Rode had worked previously to troubleshoot downstream purification problems).

Achieving success through "One Team"

GE's Fast Trak Centers replicate a real-life industrial setting where biopharmaceutical manufacturers have access to industry expertise encompassing process and analytical development, process scale-up, and manufacture of drug substances for use in toxicology studies or Phase 1 and 2 clinical testing. Based on past experience, Rode knew Fast Trak had a 96-well format resin system in which his team could complete robotic high-throughput resin screenings. In addition, the Fast Trak offers customers on-site access and transparency during all production batches, so Rode and his team could be actively involved during the screenings.

"My personal philosophy is not to establish a traditional vendor/customer relationship, but, in any and every way possible, establish a one-team strategic relationship, which is what we were able to do with Fast Trak," he explains. "With Fast Trak, we did not operate in a scenario where I ship a sample to their lab, they perform an experiment, and then they return samples back, so we can perform analyses. Instead, we sat down together and agreed to the project goals and what was feasible, including analytical support. Then, we managed the project as one common group. The analytics were done on-site and side-by-side with the corresponding experiments. This allowed us to map out a potential manufacturing process that my team could then pick up and work with in-house to finish the actual application

and final development." Along with the Fast Trak team, Rode and his team completed a rapid screening of the GE portfolio of resins to look for quick hits of what could be a feasible purification strategy. Through this collaboration, the two organizations were able to work together to identify high-potential resins utilizing GE's high-throughput process development (HTPD) techniques to optimize for improved product recovery. At the same time, the team knew the resins they chose would be robust for large-scale operation.

Effective collaboration calls for open communication and transparency

Ultimately, Janssen Biotherapeutics Development successfully utilized an external collaboration approach to accelerate the HIV vaccine booster process development and production of clinical supplies in a very aggressive timeline. Rode credits the building of effective partnerships and a "one team" approach. "Working with GE to facilitate process development, the Fast Trak team worked as an extension of my development team," he says.

Regardless of how many external service providers a company works with, Rode says developing any partnership requires each team to take the time to get to know each other. "Relationship building is critical, and it is a learning experience. If you are going to develop an external collaboration, it is more than just picking up the phone, placing an order, and coming back in three months," he explains. "Both sides must be engaged and observant to build and foster that relationship and to be able to intercede if you see anything that could be detrimental to the relationship."

The Fast Trak team's transparency made it possible for Janssen and GE to have open communication and open sharing between colleagues regarding project status and challenges, which is what Rode credits for driving the project's success. "If Fast Trak or our team became aware of a challenge or an issue and didn't share that with each other, there would have been a lot of time spent dealing with it without realizing the other had helpful insight. That leads to a breakdown in communication and trust, which kills efficiency," says Rode. "Bringing two organizations together means combining cultures and modes of operation into one common team. If either one views the relationship as nothing more than transactional, the effort will not be successful."

References

1. Johnson & Johnson Announces Encouraging First-in-Human Clinical Data for Investigational HIV Preventive Vaccine — http://www.janssen.com/johnson-johnson-announces-encouraging-first-human-clinical-data-investigational-hiv-preventive.
2. HIV.gov, The Global HIV/AIDS Epidemic — https://www.hiv.gov/federal-response/pepfar-global-aids/global-hiv-aids-overview.

Roivant Sciences accelerates process development to speed orphan therapy to market

Although the demographic of orphan therapies is small, making therapies for rare diseases available has a huge impact for the affected patients. Cooperation to expand capacity and expertise during process development and manufacturing for preclinical and clinical phase studies is one way to increase speed to market. This case study shares the work of GE's Fast Trak Services team to help accelerate development of a process for cGMP production of material for toxicology studies. Frequent communication between the Fast Trak team and the client ensured transparency while protecting customer's intellectual properties. GE scientists worked closely with Roivant Sciences to facilitate tech transfer, and a cGMP manufacturing process was developed. As a result, 400 g of RVT-801 was produced for toxicology studies.

Background

Acid ceramidase is coded by the *ASAH1* gene, of which two mutant copies lead to Farber disease. Roivant Sciences, a biotech company focusing on such rare conditions, has suggested recombinant human acid ceramidase (rhAC) as an enzyme replacement therapy. In preclinical models, cells take up rhAC, which thereafter breaks down ceramide stored in their lysosomes. The rhAC enzyme replacement therapy is intended to be a better option than bone-marrow transplant, which carries risk of toxicity with no guaranteed outcome.

To reduce risk in biomanufacturing and increase speed to market, Roivant Sciences initiated a collaboration with GE Healthcare's Fast Trak Services to develop a process for production of rhAC. The goal of the project was to develop a cGMP production process that would provide sufficient material for toxicity studies. To improve manufacturability, a new process was developed from the original process, which had consisted of rhAC production in hollow-fiber bioreactor systems and purification in a three-step chromatography process using Con A Sepharose™ affinity, Blue Sepharose affinity, and Superose™ size exclusion chromatography resins. The process at GE included upstream production in single-use Xcellerex™ stirred-tank bioreactor systems and downstream purification in three consecutive steps using modern Capto™ S ImpAct cation-exchange, Capto Butyl hydrophobic-interaction, and Capto Q anion-exchange chromatography resins.

Selection of best-performing clone

Clone selection was performed in 125 mL shake-flask cultures. Three different Chinese hamster ovary (CHO) cell clones (47, 09, 77) expressing rhAC were screened based on viable cell density and cell productivity, as determined by SDS-PAGE analysis. The cells were cultured in BalanCD™ CHO Grow A basal medium (Irvine Scientific) supplemented with 5.0% HyClone™ Cell Boost™ 7a and 0.5% Cell Boost 7b on a specified schedule. Culturing of the best performing clone (47) was repeated with feed ratio Cell Boost 7a to 7b of 4.0%/0.4%. Culturing was performed in 37°C at an agitation rate of 120 rpm over 12 days. The results are shown in Figure 1.

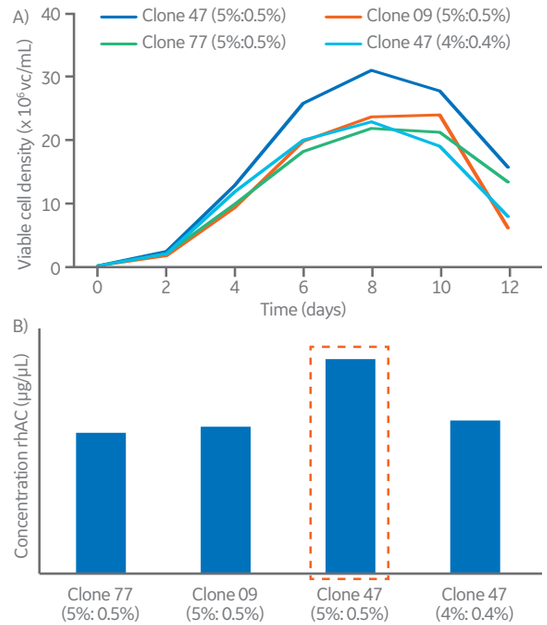

Fig 1. (A) Viable cell density over the culture period and (B) cell productivity on Day 12 of the tested clones in shake-flask cultures.

Optimizing process performance

Optimization of feed conditions for the selected clone (47) was performed in 125 mL shake-flask cultures. Using BalanCD CHO Grow A as basal medium, the following fed-batch conditions were evaluated:

- Condition 1 — once-daily bolus additions of 5.0% Cell Boost 7a and 0.5% Cell Boost 7b.
- Condition 2 — once-daily bolus additions of 5.0% EfficientFeed™ B (Thermo Fisher Scientific).
- Condition 3 — once-daily bolus additions of 5.0% Cell Boost 5.
- Condition 4 — once-daily bolus additions of 5.0% Cell Boost 6.
- Condition 5 — once-daily bolus additions of 5.0% Cell Boost 7a and 0.5% Cell Boost 7b with a temperature reduction on Day 4.
- Condition 6 — once-daily bolus additions of 4.0% Cell Boost 7a and 0.4% Cell Boost 7b.

Culturing was performed in 37°C (with a temperature shift on Day 4 for Condition 5) at an agitation rate of 120 rpm over 12 days. A sampling schedule was followed for analysis of viable cell density and cell productivity to support the decision on best-performing fed-batch condition. Ammonium levels were monitored over the culture period.

The results are shown in Figure 2. An outlying variable that separates Condition 5 from the other conditions is the temperature reduction on Day 4. Because of a slower metabolic rate, Condition 5 consumed less glutamine than the other conditions, and therefore showed lower ammonium concentrations, which might have contributed to the prolonged viable cell density of Condition 5. In addition to the improved viable cell density observed later in culture for Condition 5, the rhAC titer was significantly improved.

Best performance was achieved with Cell Boost 7a and 7b under Condition 5, with a peak viable cell density of 3×10^7 viable cells/mL (<2.5×10^7 viable cells/mL for all other conditions) and a productivity of ≥1.2 µg/µL (≤0.8 µg/µL for all other conditions).

Process scale-up

For optimization of bioreactor culture parameters, the selected clone (47) was cultured in BalanCD CHO Grow A supplemented with once-daily bolus additions of 5.0% Cell Boost 7a and 0.5% Cell Boost 7b using the Xcellerex XDR-10 bioreactor system. Process conditions are listed in Table 1. Parameters monitored over the culture period were viable cell density, cell productivity, culture pH and partial CO_2 pressure (pCO_2), as well as concentrations of glucose, ammonium, and glutamine. Cell growth and productivity are shown in Figure 3. The best results were achieved with a 20 µm sparge configuration that provided a higher mass transfer (k_La) than the 0.5 mm drilled-hole configuration. As cells showed low sensitivity to shear forces, agitation could be increased at the end of the run to increase titers.

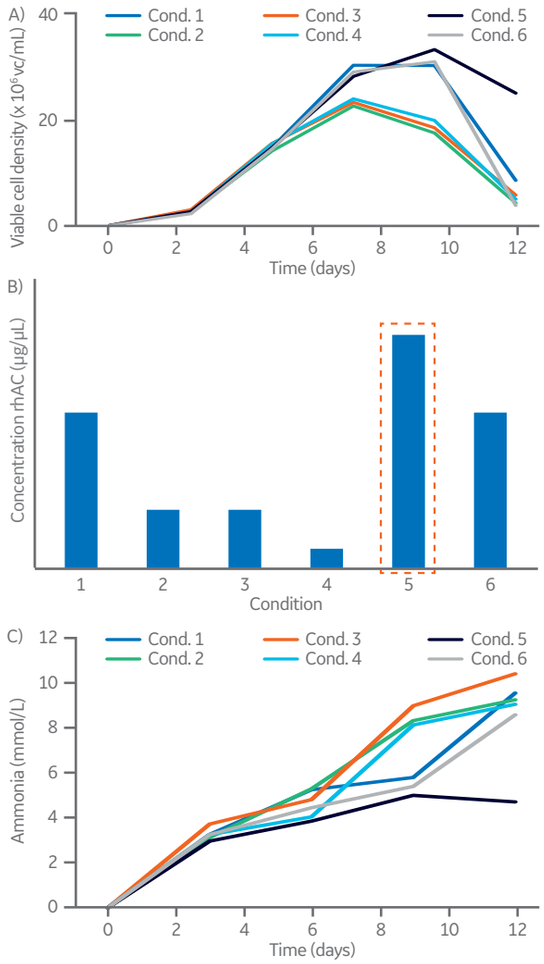

Fig 2. (A) Viable cell density over the culture period, (B) cell productivity on Day 12, and (c) ammonium levels for the selected clone (47) in shake-flask cultures.

Table 1. Process conditions for optimization of bioreactor culture parameters

Parameter	Run 1	Run 2	Run 3
Starting volume	7.5 L	7.5 L	7.5 L
Temperature	37°C with a temperature shift on Day 4	37°C with a temperature shift on Day 4	37°C with a temperature shift on Day 4
Dissolved oxygen	40%	40%	40%
pH	7	7	7
Agitation rate	At 7.5 L: 100 rpm	At 7.5 L: 100 rpm	At 7.5 L: 100 rpm
	At 10 L: 100 rpm	At 10 L: 100 rpm	At 10 L: 120 rpm
Sparge porosity	20 µm	0.5 mm drilled hole	20 µm
Air sparge high limit	0.05 sL/min	0.05 sL/min	0.05 sL/min

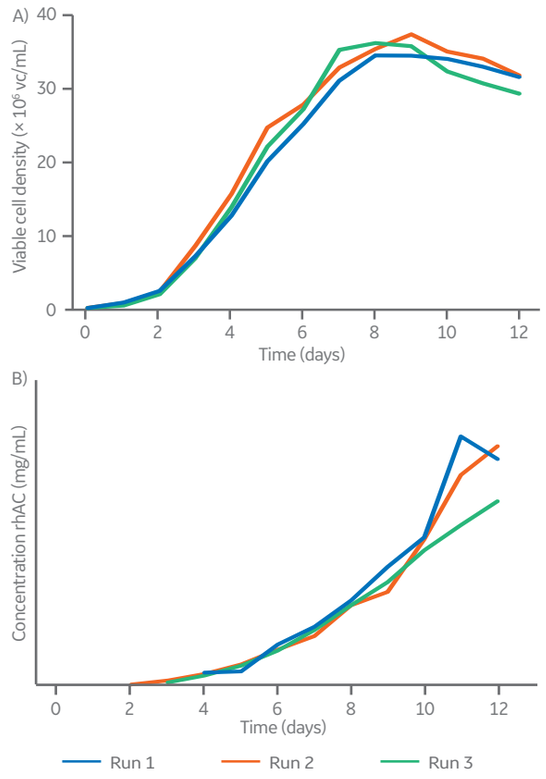

Fig 3. (A) Viable cell density and (B) cell productivity over the culture period for the selected clone (47) cultured in XDR-10 bioreactor system.

Small-scale cGMP production: Using bioreactor parameters from Run 3 in 10 L scale, the process was scaled to 200 L using the XDR-200 bioreactor system. To demonstrate equivalence between scales, the results were compared with the XDR-10 bioreactor run with regards to viable cell density, cell productivity, culture pH and pCO_2, as well as concentrations of glucose, ammonium, and glutamine. Cell growth and productivity from two engineering runs (XDR-10 and XDR-200) and two cGMP runs (XDR-200) are shown in Figure 4.

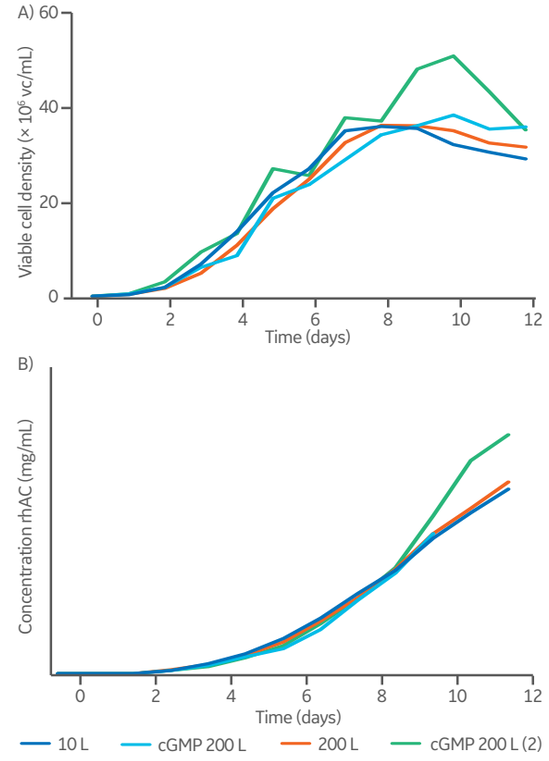

Fig 4. (A) Viable cell density and (B) cell productivity over the culture period for the selected clone (47) cultured in XDR-10 and XDR-200 bioreactor systems.

Downstream purification and final formulation

To improve manufacturability, purity, and recovery, downstream purification of rhAC was optimized from the original academic laboratory-scale process based on Con A Sepharose affinity, Blue Sepharose affinity, and Superose size exclusion chromatography (1). It was also important that the developed downstream process was scalable and robust to allow for execution of a 200 L toxicology run.

Table 2. Results from downstream purification of rhAC (NA = not applicable; ND = not detected)

Process step	Volume	Concentration	Total rhAC	Step recovery	Purity
Bioreactor day 12	201.0 L	3.89 mg/mL	782.0 g	NA	ND
Harvest clarification (depth filtration)	537.0 L	1.03 mg/mL	553.5 g	70.80%	~85%
Capture (Capto S ImpAct eluate)	135.2 L	3.16 mg/mL	427.2 g	76.40%	95%
Intermediate polishing (Capto Butyl eluate)	179.1 L	1.90 mg/mL	340.3 g	79.60%	99%
Final polishing (Capto Q flow-through)	191.0 L	1.74 mg/mL	332.3 g	97.70%	>99%
Viral filtration	198.0 L	1.70 mg/mL	336.6 g	101.30%	NA
Ultrafiltration (M_r 10,000 NMWC)	28.4 L	11.00 mg/mL	312.4 g	92.80%	NA

The optimized process comprises the following steps:

- Harvest clarification by depth filtration.
- Virus inactivation by low pH.
- Capture using Capto S ImpAct cation exchange chromatography resin.
- Intermediate polishing using Capto Butyl hydrophobic interaction chromatography resin.
- Final polishing using Capto Q anion exchange chromatography resin.
- Reduction of remaining virus particles by nanofiltration (20 nm).

The final product was concentrated by ultrafiltration through a filter with a M_r 10,000 nominal molecular weight cut-off (NMWC) to reach a target concentration in formulation agreed with the Roivant team. Results are shown in Table 2. Overall process recovery was ~44%, as determined spectrophotometrically at 280 nm, at a purity of >99%, as determined by SDS-PAGE analysis. Host cell DNA was reduced from initial 33×10^6 ng/L to <6.0 ng/L over the process.

Process summary and tech transfer

To maximize rhAC production, the CHO cell clone that exhibited the highest cell growth and productivity was selected and upstream culture conditions were optimized. The optimized upstream process was successfully scaled from 125 mL shake flasks to 10 L and 200 L bioreactor cultures. To improve manufacturability, purity, and recovery of the downstream process, a purification process comprising clarification by depth filtration and purification in three consecutive chromatography steps was developed. Using this process, rhAC was produced in a 200 L process according to cGMP, generating sufficient material for toxicology studies. After completion of the project, a final report was delivered in accordance with set timelines, and the documentation required for technology transfer was prepared by the Fast Trak Services team.

Conclusions

Enzyme replacement using rhAC is an orphan therapy with an identified need to get to market fast to treat patients. This case study demonstrates development and optimization of a process for the production of rhAC, as a collaboration between Roivant Sciences and GE Healthcare's Fast Trak Services team. The project helped to ease Roivant's risk and cost burdens, while increasing speed to market. Through clone selection as well as optimization of upstream production and downstream purification processes, sufficient amount of product could be produced in accordance with cGMP to be used in toxicology studies. Because technology transfer can be a challenge, Fast Trak scientists worked in close collaboration with the Roivant team to facilitate this process.

Acknowledgment

We thank Roivant Sciences for kindly providing us with permission for use of this body of work as a demonstration of our Fast Trak Services.

"Roivant greatly appreciates the way the GE team took this project on as if RVT-801 was their own program. They recognized the need for Farber's patients and have gotten us in a position where we are successfully executing toxicology studies and are getting ready to begin clinical trials. Everyone has been focused on the patients and that is what made this relationship work so well."

— Alex Tracy (Vice President of Pharmaceutical Development, Roivant Sciences)

Reference

1. He X. *et al.* Purification and characterization of recombinant, human acid ceramidase. *JBC* **278**, 32978–32986 (2003).

mAbxience optimizes and accelerates downstream biosimilar process development

Biosimilars represent an innovative solution that can benefit both patients and healthcare systems by reducing the burden of rising treatment costs. To improve the availability, price, and access of medicines, many countries are implementing strategies to establish their own production capacity. To support such development, mAbxience (a Spanish biotechnology company specialized in research, development, and manufacture of biosimilar drugs) is committed to provide the manufacturing of high-quality products and processes that meet regulatory and technical requirements in all countries where it operates, using cutting edge single-use technology. Currently, mAbxience has sales contracts in more than 70 countries.

One of the biosimilar specialties present in mAbxience pipeline constitutes an Fc-fusion protein, with a molecular weight of M_r 150,000 and an isoelectric point of <5, for which a first-generation process was established by a third-party contract manufacturing organization (CMO). The molecule exhibits monoclonal antibody (mAb) behavior, but with a challenging glycosylation profile and complex tertiary and quaternary structures. Consequently, low product recovery and purity were obtained in the first-generation purification process. In addition, the purification protocols were poorly suited for manufacturing scale.

With the aim of improving the first-generation process to reach the manufacturability and purity required to produce material for phase 1 clinical trials, mAbxience contacted GE Healthcare's Fast Trak Services team to initiate a collaborative project. This case study demonstrates the optimization of the downstream purification process to improve product purity and recovery of the biosimilar Fc-fusion protein. The optimization work was conducted by Fast Trak scientists. Process optimization focused on improving the affinity chromatography (AF) capture step as well as the intermediate purification and polishing steps — using hydrophobic interaction chromatography (HIC) and anion exchange chromatography (AIEX), respectively — keeping the same process materials. In addition, a purification scheme more suitable for manufacturing scale was to be established.

Optimization of the capture step

The goal was to improve dynamic binding capacity (DBC) of the chromatography resin used in the capture step while maintaining or improving yield. A comparison of several protein A affinity resins was conducted, from which the MabSelect SuRe™ resin was selected. MabSelect SuRe resin was developed for process-scale mAb capture. The resin is designed with an alkali- and protease stabilized recombinant protein A ligand coupled to a rigid, high-flow agarose base matrix. The stability of the protein A ligand min imizes ligand leakage and allows for the use of rigorous and cost-effective cleaning procedures that include sodium hydroxide (NaOH). The highly cross-linked agarose base matrix of the resin enables the use of high flow velocities at manufacturing scale.

The DBC of the MabSelect SuRe resin was tested under the optimized conditions, and the results showed a 10% breakthrough at 24 mg mAb/L resin (Fig 1), a 100% improvement from the first-generation process.

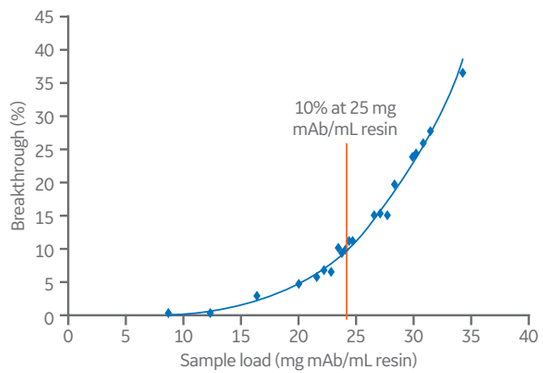

Fig 1. Results from testing of DBC of MAbSelect SuRe resin.

Optimization of the intermediate purification step

The aim of this step is to remove misfolded versions of the target molecule. However, the first-generation process offered poor resolution between correctly folded and misfolded target (Fig 2A). The optimization goal for the intermediate purification step was to improve not only manufacturability, but also product purity. Although a preliminary screening of alternative resins from GE found two candidate resins that offered significantly better resolution, implementing a new resin was not possible with the limited time before project delivery. Hence, the work focused on optimizing process conditions for the current resin.

Optimization of loading conditions and changing from gradient to stepwise elution resulted in improved resolution, generating increased product recovery from 30–40% to ~50% and improved product purity (Fig 2B). However, the resin resolution was still too poor to remove impurities eluting at the front of the peak. Consequently, a partial gradient was still needed at the elution start to obtain the required product purity. Analysis of the purified protein by surface plasmon resonance (SPR) using a Biacore™ instrument verified a similar behavior of the target molecule to that of the originator molecule.

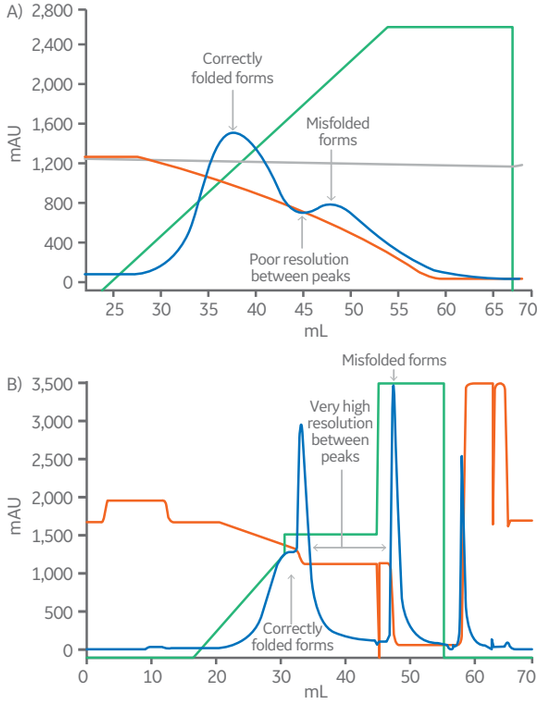

Fig 2. Chromatograms from intermediate purification step of (A) the original process and (B) the optimized process.

Optimization of the polishing step

Q Sepharose Fast Flow anion-exchange resin was used in the polishing step. The resin was developed for industrial downstream processes and exhibits a high chemical stability, allowing for the use of well-proven cleaning-in-place (CIP) and sanitization protocols. The hydrophilic nature of the base matrix ensures low levels of nonspecific binding, leading to low levels of host-cell–derived impurities in the elution pool.

The aim of this step was to reduce charge variants to match those of the originator molecule. The first-generation process offered poor resolution, resulting in undesired basic charge variants in the product peak (Fig 3A). By optimizing loading conditions and changing from gradient to step-wise elution, resolution and product purity could be improved (Fig 3B). Also, the step yield could be increased from 60–70% to ~90% using the optimized protocol. Analysis of the purified protein by SPR using a Biacore instrument verified a similar behavior of the target molecule to the originator molecule.

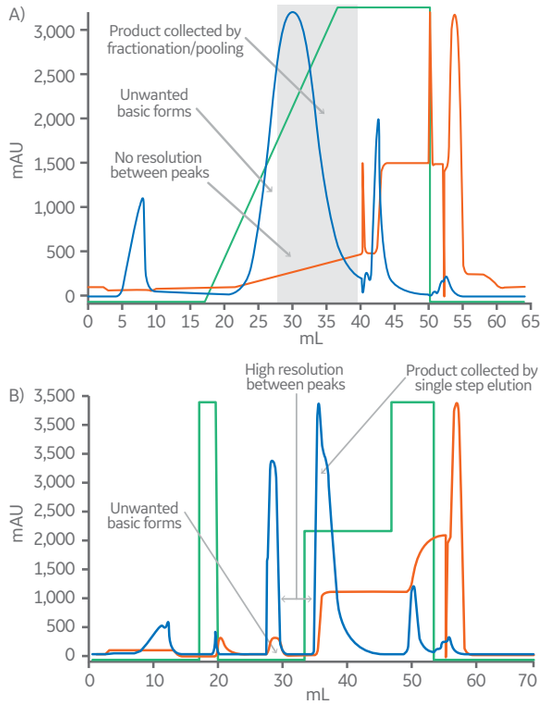

Fig 3. Chromatograms from the polishing step of (A) the original process and (B) the optimized process.

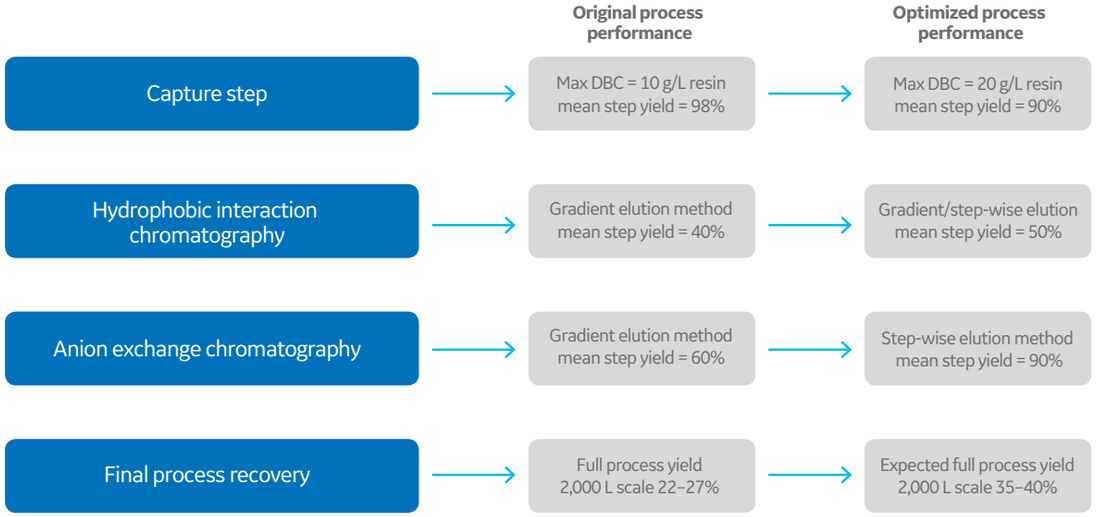

Fig 4. Improvements from the original process, using the optimized process

Confirmation runs

The final optimized process conditions were confirmed in three consistency batches at 0.5 L scale. Manufacturing batch record and solution record documents were prepared by the Fast Trak Services team, and the results showed reproducibility in yield and purity between the runs. Based on these results, technology transfer documentation was prepared.

Process summary and discussion

Process improvements from the original process using the optimized process are listed in Figure 4. A final report was delivered in accordance with set timelines, and the documentation required for technology transfer was prepared by the Fast Trak Services team. To facilitate technology transfer, Fast Trak scientists worked directly with a third-party CMO. By defining process-critical parameters in a simulation of conditions of the scaled-up process, the optimized process was successfully scaled to 500 L and 2,000 L for cGMP clinical manufacturing.

Conclusion

Collaborative projects can help ease risk and cost burdens while increasing speed to market. This case study demonstrates the optimization of a downstream purification of a mAb biosimilar as a collaboration between mAbxience and GE Healthcare's Fast Trak Services team. Through resin selection and optimization of loading and elution conditions, product recovery, manufacturability, and purity were greatly improved. DBC of the resin used in the initial capture step was doubled, and recovery was significantly increased over the subsequent intermediate purification and polishing steps.

Acknowledgment

We thank mAbxience in Spain for kindly providing us with permission to use this body of work as a demonstration of our Fast Trak Services.

"To speed up the process optimization of the described biosimilar product, collaboration with GE Healthcare's Fast Trak Services team was crucial for mAbxience. Thanks to the strong expertise and commitment of the Fast Trak team to protein downstream processing, the work was carried out smoothly to achieve the expected results according to the agreed timeline. Due to the valuable outcomes of this synergy, mAbxience will continue to collaborate with GE Healthcare's Fast Trak Services team on other biosimilar projects."

— Vincenzo Rivieccio (R&D Specialist, mAbxience, Spain)

Flavivirus vaccine production accelerates with modern bioprocess tools and solutions

As with all viral vaccines, the complex nature of flaviviruses makes process development technically challenging. In addition, vaccine production can be both costly and difficult to scale to meet market demands. In egg-based vaccine production, for example, 100–300 vaccine doses can be produced from one fertilized hen's egg. However, the eggs used for production need to be supplied from special pathogen-free chicken flocks, limiting availability of eggs and making vaccine production difficult to scale up. To meet the needs of preventive campaigns, including routine immunization and emergency response stockpiling, millions of vaccine doses would be required, making production both space- and resource-consuming.

For a more efficient response to market needs, cell-based vaccine production can be an alternative to egg-based production. However, cell-based vaccine production is traditionally performed in stainless steel bioreactors that require extensive cleaning and sterilization preparation time. Alternative single-use equipment min imizes the need for costly and time-consuming cleaning operations, as manufacturing components that have been in contact with the process material can be disposed of after use. Single-use equipment also min imizes cross-contamination risk and contributes to increased operator safety by eliminating the need for open handling of products. The reduced need for cleaning and cleaning validation allows for quick start-up and changeover between production campaigns. Because less cleanroom space is required, single-use technologies help reduce manufacturing footprint as well as costs for utilities, heating, ventilation, and air conditioning (Fig 1).

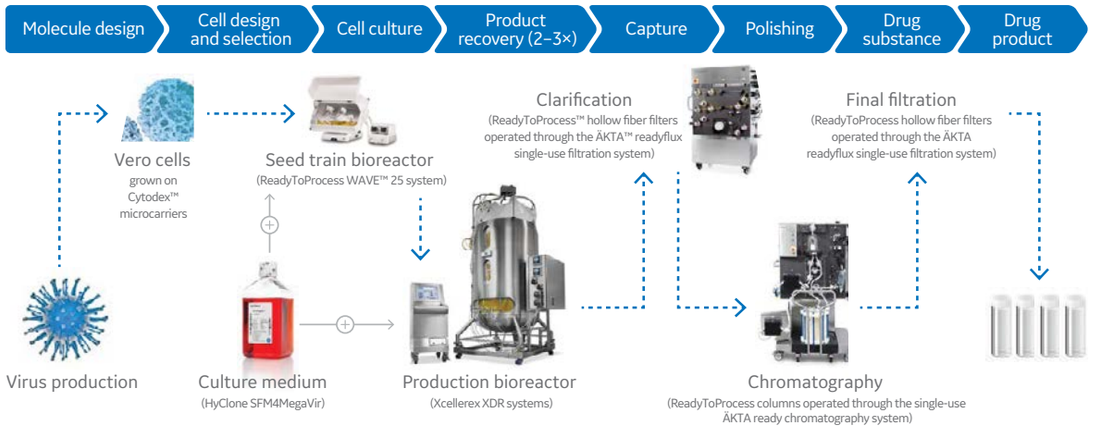

Fig 1. Process train comprises single-use equipment from GE Healthcare that help accelerate flavivirus vaccine manufacturing. Included systems are suitable for biomanufacturing of regulated products under various quality management systems. The systems are controlled through either GE Healthcare's UNICORN™ or Schneider Electric's Wonderware® system control software. To enable use of the systems in regulated environments, both programs are configured for use in a 21 CFR Part 11 and GAMP 5 compliant manner. All records are stored in a single, unalterable database, including results and extended run documentation. Specially trained and certified engineers perform on-site IQ/OQs and CCPs in accordance with cGMP, as well as provide on-site training for relevant personnel.

Cells commonly used for virus propagation, such as Vero cells, are anchorage dependent and can only proliferate when provided a suitable surface. To meet that need in bioreactor cultures, microcarriers are used. Compared with traditional shake-flask systems and roller-bottles, microcarriers provide a larger ratio of surface area to volume, enabling production of higher titers in a reduced footprint. Increasing upstream titers, however, puts pressure on capacity in downstream purification processes. Chromatography provides a highly selective and scalable alternative to purification techniques such as precipitation and ultracentrifugation. Compared with legacy products, modern chromatography resins offer improved pressure and flow properties that increase productivity. With such features, more product can be produced within a shorter period, making modern resins more suited for manufacturing applications than legacy products. In vaccine production, a short time to market is not only beneficial for the manufacturer, but for patients too.

Addressing shear sensitivity in adherent cultures

Adherent cells are sensitive to shear stress. A rocking bioreactor system provides gentle agitation of the culture to better control shear stress while providing sufficient aeration of the culture. Single-use rocking bioreactor systems are available for applications such as process development, seed culturing, and small-scale productions. Although rocking systems have a different vessel geometry, studies have shown that they can give a representative reflection of processes performed in a stirred-tank bioreactor (1). Hence, rocking bioreactor systems also can be used as scale-down bioreactors from a stirred-tank system.

Single-use stirred-tank bioreactor and fermentor systems are based on the same principles as conventional stainless steel bioreactors. Traditional scaling methodology, based on measures such as shear, tip speed, power per unit volume, $k_L a$, and specific process sensitivities, can be used during scale-up. With stirred-tank system platforms, technology transfer is straightforward, min imizing the need for costly and time-consuming process redesign (Fig 2).

Fig 2. Designed for scalability and robustness, the Xcellerex XDR bioreactor system platform provides the performance and flexibility needed from process development to large-scale biopharmaceutical manufacturing. The complete range of XDR bioreactor systems are available with maximum working volumes ranging from 10 L to 2,000 L, from the smallest XDR-10 to the largest XDR-2000 system.

In bioreactor cultures, microcarriers are used to provide a suitable growth surface for the adherent cells commonly used in virus production (Fig 3). Microcarriers based on low-density dextran beads enable easy mixing and low shear (2). Bead size and density are optimized to support high cell growth rate and yield. The biologically inert polysaccharide products are supplied dry and shrunken to save storage space and facilitate transportation. To simplify transfer to cell culture vessels, the microcarrier container is equipped with flexible connection options.

Fig 3. Cytodex Gamma microcarriers are delivered gamma sterilized and ready for use for quick culture startup. In addition, Cytodex Gamma matrix provides a stable but nonrigid, tissue-like substrate for stirred cultures. Dextran-based microcarriers are translucent, allowing for easy microscopic examination of attached cells.

Whereas many cell lines employed in vaccine production are obligate attachment cells, the EB66® cell line (Valneva), derived from duck embryonic stem cells, grows in serum-free suspension culture at high cell density, allowing for easy and efficient scale-up (Fig 4). EB66 cells form loose aggregate structures that facilitate infection of nonsecreted, cell-to-cell transmitted viruses (3). To increase cell density and virus titer, both microcarrier-based adherent and suspension cell cultures can be run in perfusion mode using bioreactors equipped with cell-retention filters (4, 5).

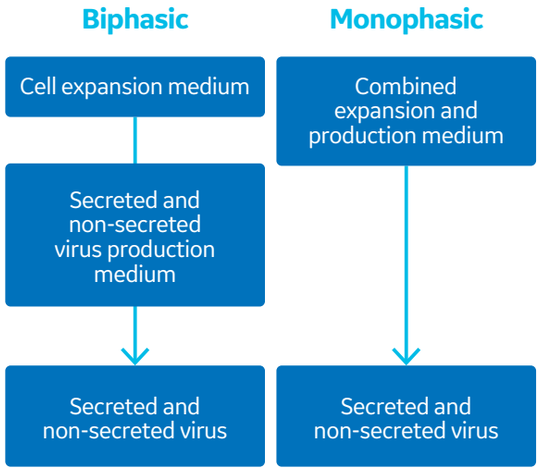

Fig 4. While traditional virus production in EB66 cells is biphasic, requiring two or more media and multiple additives, CDM4Avian medium is designed to support the simpler monophasic approach, requiring fewer additives.

Increasing productivity in upstream operations

Selection of the right cell culture medium is important to enhance process yields in the manufacture of viral vaccines. For regulatory readiness, a cell culture medium free of animal-derived components is recommended. Modern culture media are developed to provide optimized conditions for high cell growth and productivity. However, the cell culture medium and feed strategy should be selected with respect to the nutritional requirements of the specific cell clone used.

Nutrient concentrations need to be kept within a certain range, as concentrations that too high or too low can be detrimental to the cells. Design of experiment (DoE) methodologies can be used to identify component groups in the medium that have the greatest effect on cell growth and productivity. This approach produces maximum amount of data with min imum number of experiments and meets the demands from regulatory authorities for better process understanding, one of the cornerstones of the quality by design (QbD) initiative.

Achieving efficiency in downstream purification

DoE methodology also can be used for identifying parameters affecting purity and yield in downstream processes. Once the chromatography resins are selected, conditions for optimal hcDNA and HCP reduction at maximal product recovery are determined.

Both cation exchange and anion exchange chromatography resins commonly are used to reduce impurity levels in virus vaccine purification processes. There are also examples of affinity chromatography resins with ligands that exhibit affinity for specific viruses such as the adenoassociated virus. For more challenging separations, multimodal resins with multiple modes of actions (ion exchange, hydrophobic interaction, and hydrogen bonding) can be used. In recent years, a new class of multimodal resins has been developed. In those resins, dual layers have been introduced in the bead design, combining size exclusion properties from an inactive outer layer with adsorption chromatography from a ligand-activated core (Fig 5). Small molecules enter the core, where they are captured; viruses and other large entities are excluded and can be collected in the flowthrough.Modern resins are designed for large-scale chromatographic processes, where high throughput and process economy are essential. Their base matrices have exceptional mechanical stability and optimized pore size to enable efficient capture under high-flow conditions. The improved mechanical stability also increases flexibility in terms of bed height and the ability to process highly viscous feeds. The chemical stability of these resins ensures a long lifetime even when harsh cleaning procedures are used. By offering a combination of high volume throughput and capacity, modern resins provide a powerful solution for fast and efficient processing of large amounts of protein. When high throughput is of utmost importance, membrane chromatography is an alternative option. Chromatography membranes exhibit a high porosity suitable for virus purification while providing the opportunity for using high flow rates.

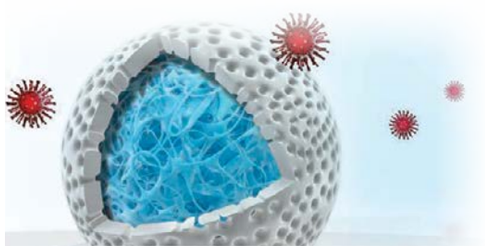

Fig 5. Schematic representation of Capto Core 700 shows a bead with the inactive, porous shell and the ligand-containing core. Proteins and impurities penetrate the core, while target viruses and larger biomolecules (>M_r 700,000) are excluded from the resin and pass in the flowthrough.

Filtration of delicate targets: Cross-flow filtration (CFF), also known as tangential flow filtration (TFF), is a technique extensively used in vaccine production. In contrast to normal flow filtration (NFF), the feed is recirculated over a permeable membrane surface. In CFF, liquid and compounds with molecular weights less than the membrane cut-off can pass through the membrane, whereas larger molecules or particulates are retained and concentrated. For delicate targets, such as the flavivirus, hollow-fiber filters are commonly used for the CFF step. Because of the open channel structure, a hollow-fiber filter usually causes less damage to the target product than does a filter cassette (Fig 6). For virus particles expressed in low titers that need to be concentrated as much as 200- to 500-times before further processing, single-use tubing assemblies can be used in the design of circuits with low working volumes to enable high concentration factors (7).

Fig 6. GE Healthcare's 750 C hollow-fiber filter, with a M_r 750,000 nominal molecular weight cutoff (NMWC), is designed for use in virus-purification workflows. It effectively removes ovalbumin and other proteins in allantoic fluid from egg-based virus production as well as host-cell–derived impurities from production in cells. When compared with a 500 C hollow-fiber filter — with the same 0.5 mm lumen diameter but a M_r 500,000 NMWC — in a concentration and diafiltration process, the more open structure of the 750 C filter gave a 1.5–2.0 orders of magnitude higher host cell DNA (hcDNA) removal at similar host cell protein (HCP) removal and virus yield (6).

Gaining insights with versatile analysis technology

The complex nature of viruses also presents challenges for process analytics. Ideally, analytical methods for vaccine characterization are developed in parallel with process development to aid in gaining regulatory approval and for further manufacturing.

Vaccine design depends on structural and functional interactions with the host immune system. Label-free molecular interaction analysis based on surface plasmon resonance (SPR) is extensively used in vaccine development and production in areas such as design and characterization, immune response studies, vaccine quantitation, and in analyses during production and quality control. As has been shown with Zika virus, for example, interaction data can be used to gain insights into the binding of neutralizing antibodies to viral epitopes (8). Using SPR, detailed information also can be obtained from analyses of binding kinetics, specificity, immune responses, epitope mapping, and concentration (9).

Case study on improving purity of flavivirus

To meet the concerns with live, attenuated vaccines, a client process for production of inactivated whole-virus yellow fever vaccine was developed by GE's Fast Trak Services team (10). Virus was produced in adherent Vero cells grown on Cytodex 1 microcarriers in medium that was free of animal-derived components and supplemented with recombinant human albumin, using the XDR-50 bioreactor system. The system was selected because it features many properties that address the requirements of the shear-sensitive culture. The impeller is designed with an optimized profile, angle, and number of impeller blades to provide good mixing while min imizing shear forces. A broad and adjustable agitation speed range also supports a well-mixed tank without undue shear effects on the cells. The gas-sparging discs provide both micro- and macrosparging capabilities for effective mass transfer. The proximity of the gas-sparging discs relative to the impeller shear zone ensures excellent gas dispersion, especially at low gas flow rates.

Downstream purification was optimized as summarized in Figure 7. Initially, the purification process was based on affinity chromatography using Cellufine™ sulfate resin (Chisso Corp.) (Generation 1). Because that purification approach resulted in insufficient HCP removal, a sucrose-gradient ultracentrifugation step was included to increase purity of the virus (Generation 2). However, that step was found to be cumbersome and costly, and not all manufacturing facilities have access to the required equipment. Therefore, the purification process was further optimized by replacing it with two simple chromatography steps (Generation 3). Capto DeVirS exhibits an affinity-like behavior for several virus types and was selected for the initial capture step. Capto Core 700 was chosen for its efficient removal of remaining impurities in the subsequent polishing step.

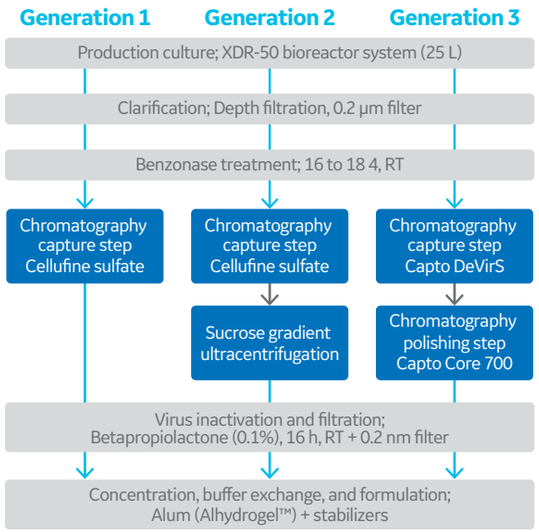

Fig 7. The initial virus production process, including purification on Cellufine sulfate resin, was complemented with an ultrafiltration step to improve HCP reduction. In the final optimized process, Cellufine sulfate and ultracentrifugation steps were replaced by two chromatography steps based on Capto resins.

Compared with the legacy process (Generation 1), HCP was significantly reduced at a similar or even slightly higher recovery in half the process time (Table 1). With the optimized downstream purification process, manufacturability was significantly approved. In addition to removing the ultracentrifugation step, the optimized process could be conducted at room temperature (RT), in contrast to the legacy process performed at 2–8°C. The developed process is easily scaled and compatible with both single-use and conventional technologies, and all process materials meet stringent regulatory requirements.

Table 1. Results from the optimized process compared with those from the initially developed process

		Generations		
Metric	**Method**	**1**	**2**	**3**
Residual HCP (µg/mL)	Vero cell ELISA	45	<0.2	<0.2
Residual DNA (pg/mL)	PCR assay	10	10	NA
Residual virus activity	Plaque assay	ND	ND	ND
Harvest titer (virus/mL)	2×10^{10} epitope ELISA	10^8	10^8	10^8
Alum-bound titer	2×10^{10} epitope ELISA	8.6 log10	8.6 log10	8.6 log10
Process temperature		2–8°C	RT	RT
Process time		40 h	40 h	20 h
Virus recovery	2×10^{10} epitope ELISA	20–35%	20–30%	25–35%

NA = not analyzed ND = not detected RT = room temperature

Conclusion

Technological challenges can dominate vaccine production. This case study gives an overview of modern products and services that can help solve many challenges in flavivirus vaccine production. Bioreactor systems based on single-use technologies support significant time savings while increasing process and operator safety in cell-based vaccine production. Microcarriers provide the cell surface required for high volumetric productivity of adherent cells in bioreactor cultures. With modern chromatography resins, more product can be purified within a given time frame.

Label-free molecular interaction analysis based on SPR technology, can be used for reliable quantification and characterization of the end product. Regulatory-friendly system-control software allows equipment to be used in a cGMP-compliant manner. Modern vaccine production platforms support reduced process time and cost to help accelerate your flavivirus vaccine production.

References

1. Application note: Efficient, high-titer monoclonal antibody production in a fedbatch process using single-use stirred-tank and rocking bioreactor systems, GE Healthcare, 29119376, Edition AA (2014).
2. Application note: Validation of the production of influenza virus in ReadyToProcess WAVE 25 bioreactor system, comparing Cytodex and Cytodex Gamma microcarriers, GE Healthcare, 29209415, Edition AA (2016).
3. Application note: Development of a chemically defined medium for virus production in suspension-adapted avian cells, GE Healthcare, 29235842, Edition AA (2017).
4. Nikolay, A. *et al.* Evaluation of producer cell lines in yellow fever virus production in up to 1 L bioreactor scale, Poster at Vaccine Technology VI, June 12–17 (2016).
5. Nikolay, A. *et al.* Propagation of Brazilian Zika virus strains in static, microcarrier-based and suspension cultures using BHK and Vero cells. Poster at Vaccine Technology VI, June 12–17 (2016).
6. Application note: Concentration and diafiltration of cell-derived, live influenza virus using 750 C hollow fiber filter cartridge, GE Healthcare, 29092826, Edition AA (2014).
7. Application note: Efficient concentration of a low-titer bovine IgG with high recovery in low volume, GE Healthcare, 29228378, Edition AA (2016).
8. Dai L. *et al.* Structures of the Zika virus envelope protein and its complex with a flavivirus broadly protective antibody. *Cell Host & Microbe* **19**, 696–704 (2016).
9. White paper: Biacore systems in vaccine development and production, GE Healthcare, 28987028, Edition AB (2016).
10. Monath *et al.* Inactivated yellow fever 17D vaccine: development and nonclinical safety, immunogenicity and protective activity. *Vaccine* **28**, 3827–3840 (2010).

gelifesciences.com

GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752, USA
GE Healthcare Japan Corporation, Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan
GE Healthcare Bio-Sciences AB Björkgatan 30SE-75184 Uppsala, Sweden

KA2216071218EB