GE Healthcare

Cell culture scale-up: Single-use technology and support for solving your biomanufacturing challenges

Achieving the delicate balance of product quantity, quality, cost, and speed is difficult in dynamic circumstances. Add in challenges such as validation of new cell culture media or changes in scale, and this balance can seem just out of reach. Experienced scientists like GE Healthcare's Thomas Falkman and Patrick Guertin know that increasing process flexibility can help evolve the entire biomanufacturing process and mitigate the effects of uncertainty. And sometimes learning from others is the quickest way to resolve issues that could otherwise stall production or waste valuable resources.

Cell culture scale-up

This applications-focused piece is one in a series highlighting the full range of single-use technologies and Fast Trak support services that GE Healthcare offers to help overcome the challenges that you face throughout your bioprocess workflow. Here we will focus on solutions and support services specifically related to cell culture scale-up, including your need for methods that help you to quickly develop an optimized process so you can speed up production.

The ideal cell culture scale-up operation

The objectives of cell culture scale-up are to maximize cell growth and productivity by optimizing the process parameters. Process optimization requires consideration of the cell type, nutrient medium, feeds, and bioreactor settings while minimizing the production of byproducts. It is important to screen for the right medium formulation, because optimization of the media and feeds can enhance cell line productivity and reduce overall cost.

"A typical process duration takes about 3–4 weeks depending on the scale and complexity of the process. Ideally, you are looking to invest 2–4 months in establishing a proven scale-up process that enables you to get a fast and lean transition from small scale to production scale. During this transition it is essential that the cells are kept in conditions that facilitate a similar environment and steady growth."

Thomas Falkman, Scientist, GE Healthcare

White paper, 29214412 AA

GE technical expert spotlight – Thomas Falkman, MSc, chemical engineering

Thomas has been with GE Healthcare since 2010. In his current role as an upstream application expert, he supports in-house projects with extensive knowledge and experience in upstream bioprocessing, including cell cultivation. Activities include conducting process improvements and providing problem-solving support for customers around the world. Thomas holds presentations and courses about cell culture bioprocessing and products for both internal and external personnel and is an inventor on two bioreactor-related patents.

Prior to joining GE, Thomas spent 10 years as an R&D scientist at AstraZeneca. He managed cell culture bioprocessing in both single-use and conventional stirred-tank bioreactors. Large-scale mammalian and insect cell culturing and large-scale microbial fermentation, cross-flow filtration, protein purification, process optimization, and technical improvements are all in Thomas' skill set.

"In our labs at GE we have a lot of expertise in both stirred-tank bioreactors (such as the Xcellerex™ XDR bioreactors) and rocking bioreactors (like the ReadyToProcess WAVE™ 25 bioreactor system). An extensive number of fed-batch and perfusion runs have been completed. We frequently work with fed-batch, but continuous manufacturing is gaining more interest throughout the industry, and we are putting more and more efforts into that area."

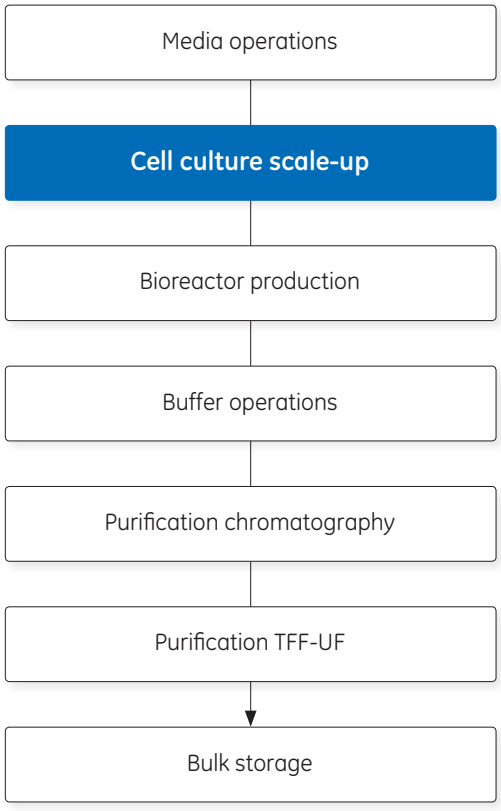

Fig 1. Typical biopharmaceutical manufacturing workflow. TFF-UF is tangential flow filtration-ultrafiltration.

Applications data support - solutions to overcome your cell culture scale-up challenges

GE is dedicated to developing resources to help address cell culture scale-up challenges. Support for several of these challenges is provided in GE application notes (see references 1–3). One of these documents is highlighted here, demonstrating how the use of perfusion in the seed culture expansion process, in combination with the use of high-density cell banks, can drastically reduce processing time, simplify operations, and maximize equipment utilization (Fig 2).

Overview

"There is a lot of interest in high cell density culture. Perfusion cultivation allows cells to reach steady state and stay in production phase longer than in batch or fed-batch culturing. This enables much higher cell densities to be achieved and, in turn, leads to higher productivity. Successful perfusion culturing requires an optimized nutrient medium formulation and a robust perfusion bioreactor that can maintain the culture parameter set points even at high cell densities. This application note and the underlying study describe the use of GE's ReadyToProcess WAVE 25 rocking bioreactor in perfusion mode to support a one-step seed culture process sufficient to seed a 2000 L production bioreactor. The seed bioreactor was inoculated with one vial from a high-density cell bank generated without centrifugation, also using WAVE 25 and perfusion culture (Fig 2).

Thomas Falkman, Scientist, GE Healthcare

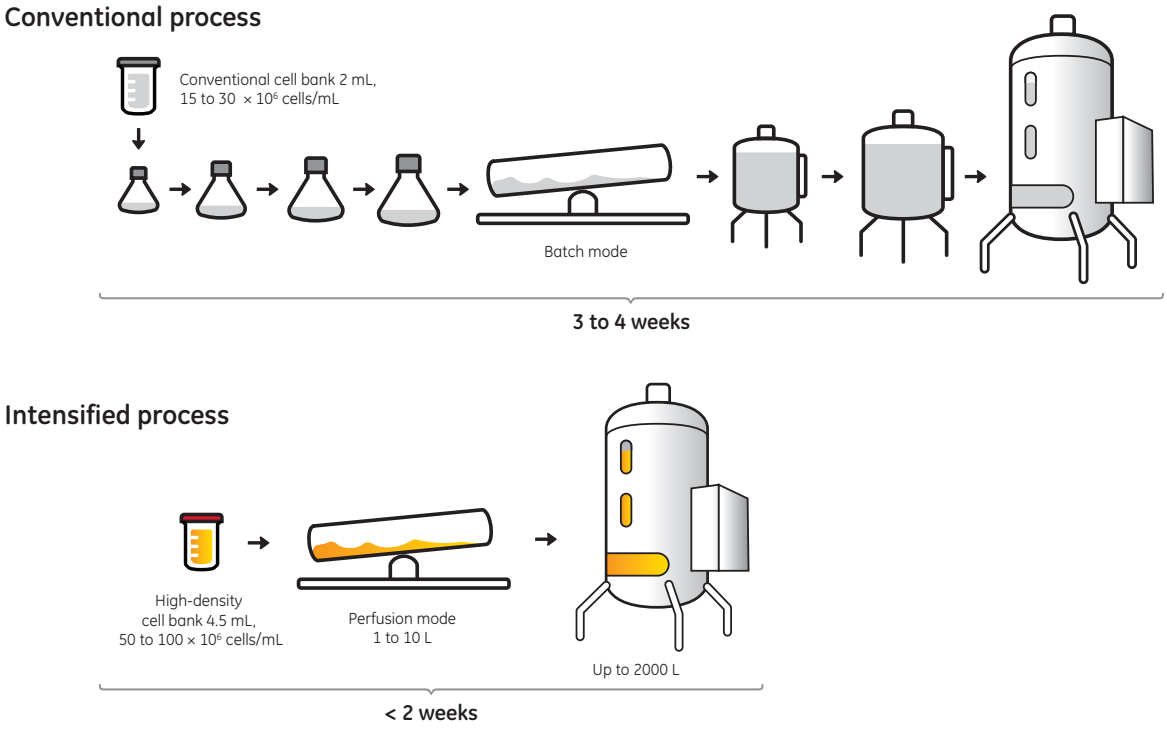

Fig 2. A high-density cell bank can be used for direct seeding of a small bioreactor culture, eliminating the need for shake flask operations. The use of perfusion in the production of seed enables cells to stay in exponential growth phase throughout the entire culture, resulting in higher cell densities for a one-step bioreactor seed culture.

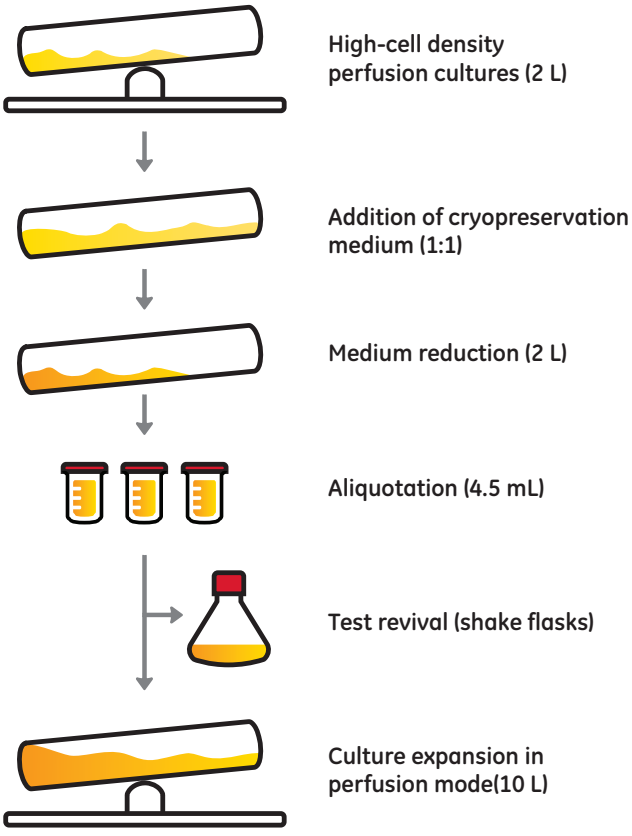

Fig 3. Overall experimental set-up.

Experimental set-up

The overall experimental set-up, including both cell bank and seed culture generation, is provided in Figure 2 and Figure 3. High-density cell banks were created from Chinese hamster ovary (CHO) DG44 cells* in ActiCHO™ P medium. CHO cells were grown in a disposable Cellbag™ using the ReadyToProcess WAVE 25 system in perfusion mode (1). Cells from one vial of the cryopreserved cell bank were recovered and directly transferred to a new 20 L Cellbag bioreactor at 1 L. The volume was stepwise expanded to a final working volume of 10 L, whereupon the culture was continued in perfusion mode. The target for the seed culture was 10 L with > 50 × 10^6 viable cells/mL and > 95% viability.

* licensed from Cellca GmbH

Results

The described method generated a large vial stock of cryopreserved high-density cell bank at 50 × 10^6 cells/mL and > 95% viability. Cells were successfully revived following cryopreservation at > 90% viability, which is similar to the performance of the originator conventional cell bank (Fig 4). One vial of the high-density cell bank was used to inoculate a 20 L Cellbag perfusion culture chamber at a starting volume of 1 L. The culture volume was further expanded to 10 L, after which perfusion was initiated. A final density of > 50 × 10^6 viable cells/mL and > 95% viability, with cells in full exponential growth phase, was achieved in 10 days (Fig 5). As shown in Figure 6, dissolved oxygen (DO) was well controlled even at high cell density and 10 L volume.

"This study demonstrates a method that generates enough cells to seed a 2000 L bioreactor without the need for intermediate seed bioreactors. Using a combination of perfusion cell culturing and a high density cell bank, the process took just 10 days from cryovial to N-1 bioreactor. In addition to saving time in the seed culture process, a higher level of process control was achieved due to the use of a robust bioreactor automation system with stringent control features. The described method can potentially be used to decrease capital and operational expenditures and to reduce equipment footprint."

Thomas Falkman, Scientist, GE Healthcare

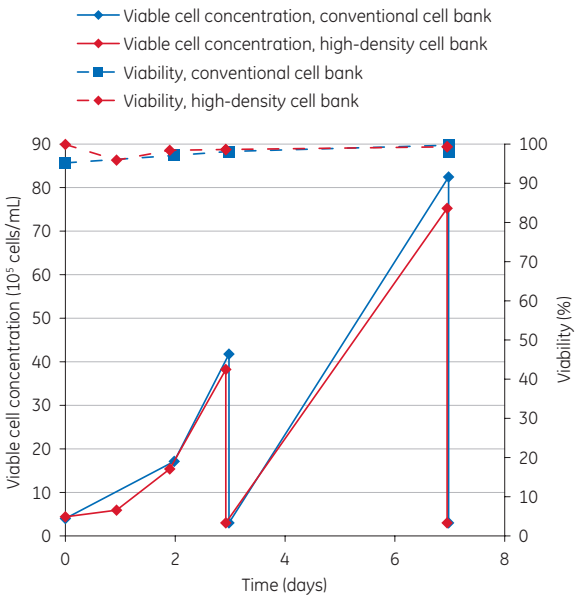

Fig 4. Cells from the high-density cell bank were revived with a viability of > 95%. Growth after revival was similar to growth from a conventional cell bank.

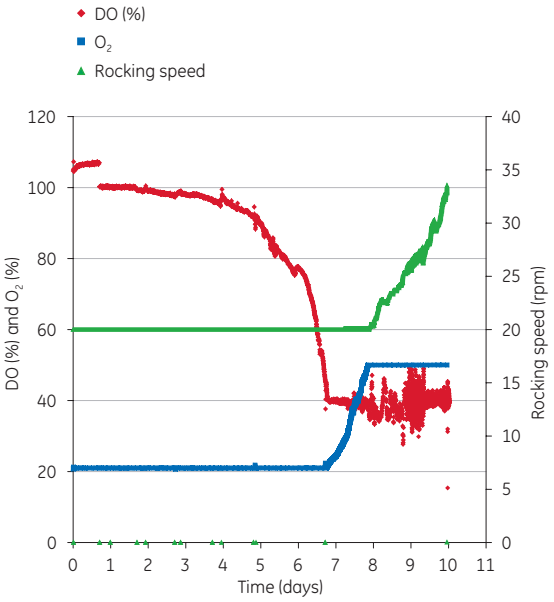

Fig 6. The bioreactor system allowed real-time monitoring and control of culture parameters. For example, dissolved oxygen was shown to be stable at process set points, even at high cell density and large working volume.

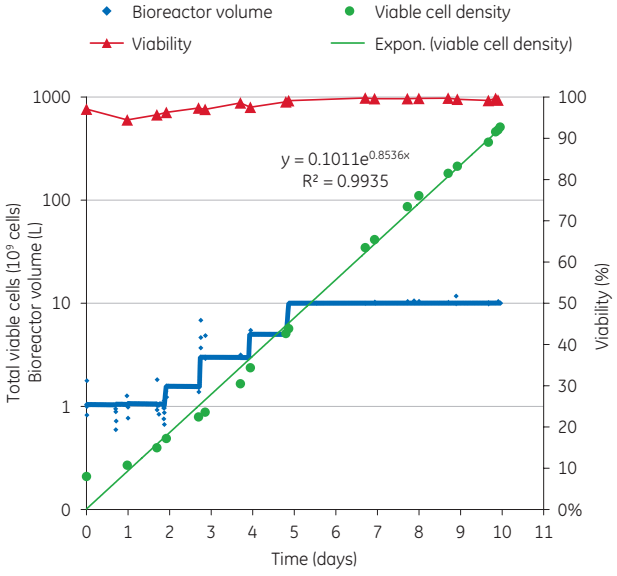

Fig 5. One-step seed culture process. Cells were maintained in rapid exponential growth throughout the culture. Average specific growth rate was 0.853 day^{-1} (doubling time ~ 19.5 h). Cell-specific perfusion rate was 140 pL/cell/d, starting from Day 5, and culture duration was 10 days. At harvest: 51.2 × 10^6 viable cells/mL, cell viability > 95%.

Conclusions

The cell culture process described in this application note enabled:

- A simple method for production of high-density cell banks in a closed system, without the need for a separate centrifugation step.
- Direct inoculation of a disposable bioreactor culture from one vial of the cryopreserved high-density cell bank, without prior cell expansion in shake flasks.
- Efficient one-step seed culture process, generating high cell densities at a culture volume that eliminates the need for intermediate seed bioreactors.

Evaluating applications data is one way to learn about products and technologies to support cell culture scale-up. Another option is to collaborate with experts who work with biopharmaceutical developers and manufacturers like you around the world to solve their bioprocessing challenges by providing Fast Trak manufacturing services or training.

Manufacturing and training services – solutions to overcome your cell culture scale-up challenges

"We don't just supply equipment; we support customers along the whole biomanufacturing process. Our Fast Trak Process Development and Bridge Manufacturing Services can assist customers in solving specific cell culture scale-up challenges, for instance, converting from traditional stainless steel to single-use bioreactors while keeping the cell densities, productivities, and shear sensitivities in mind. Or we can deliver training targeted to the needs of operators, so they gain knowledge in specialist techniques and can help themselves."

Patrick Guertin, Fast Trak Global Technical Manager, GE Healthcare

GE's portfolio of services is designed to provide end-to-end process support to help take you from molecule to market in the shortest time possible. For instance, with Fast Trak Bridge Manufacturing Services we can manage your process development through scale-up cGMP manufacturing to deployment and start-up. But our goal at Fast Trak is not just to solve your biomanufacturing challenges. It is to provide full transparency to our scientist engineers and processes so we can empower you to solve them, too. Within Fast Trak, you can also access hands-on training to strengthen your skills in, for example, in cell culture scale-up.

Fast Trak Bridge Manufacturing—an end-to-end solution for your bioprocessing needs

If you want to add the flexibility, efficiency, and convenience of single-use technologies to a new or existing production line, we can manage this, or parts of it, for you. Our Fast Trak Bridge Manufacturing Services offer a cost-effective solution to decrease time-to-market. For example, the end-to-end process development of a product can be performed in 12 to 24 months at our facilities, and then transferred to your site for clinical and commercial manufacturing (Fig 7). Or we can support certain parts of your process in a much shorter time frame. We also offer operational training and on-going technical support post-installation. Table 1 lists some of our extensive biomanufacturing experience.

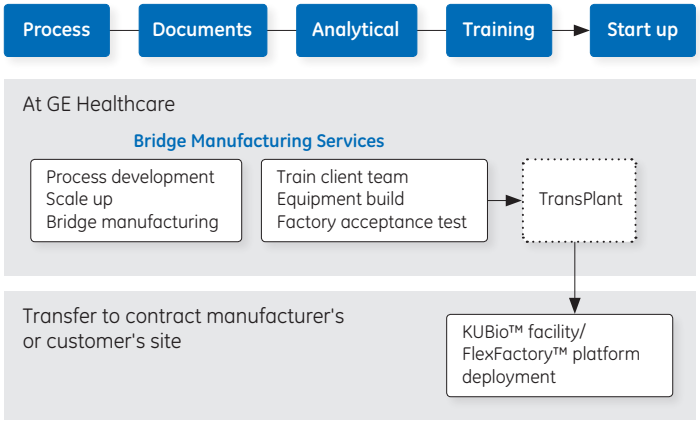

Fig 7. Fast Trak Bridge Manufacturing Services gives you access to process experts, enabling technologies in single-use, and provides you additional manufacturing capacity while you are waiting for your facility to be built. Upon completion we transplant your process back to your teams with complete transparency and training. This not only ensures your process timeline continuity but also facilitates speed to market.

Table 1. Examples of the biomanufacturing experience within our Fast Trak Bridge Manufacturing team

Product	Cell line	Xcellerex XDR bioreactor scale	Highlights
mAb and mAb fusions	CHO cells Hybridoma cells PerC.6™ cells	Scales from 10 L to 2000 L	Manufactured clinical mAb material in our Fast Trak biomanufacturing cGMP suite for clinical trials, as an alternative to using a contract manufacturing organization (CMO) Successful EU QP phase III audit First Investigational New Drug (IND) Application that used a single-use stirred-tank bioreactor
Recombinant proteins, including therapeutic enzymes	CHO cells Human HEK 293 cells	Scales from 10 L to 1000 L	First 1000 L perfusion culture that was performed in a single-use bioreactor
Vaccines: subunit, inactivated, and virus-like particle (VLP)	Insect S2 cells Vero cells Bacteria	Scales from 10 L to 1000 L, including 50 L on microcarriers	Enabled rapid technology transfer First IND Application that utilized microcarriers in a single-use stirred-tank bioreactor

GE technical expert spotlight – Patrick Guertin, MSc, biochemistry

Patrick Guertin has been with GE Healthcare since 2006. He is currently a Fast Trak Global Technical Manager (GTEM) who works closely with customers from biopharmaceutical companies, CMOs, and government labs to solve their specific upstream biomanufacturing challenges. Patrick brings more than 25 years of industry experience and substantial expertise in upstream process development, pilot plant operations, and cGMP manufacturing for mAbs, recombinant therapeutics, and vaccines.

Among his skill set are:

- scale-up and scale-down procedures in microcarrier, fed-batch, and perfusion modes
- process optimization in traditional and single-use mammalian and microbial bioreactors
- development of animal-derived component free (ADCF) media and feed
- technology transfer, process design, and data analysis

"One of the things customers value most when working with us is our collaborative nature. We look at customers' data carefully and collaborate with them to formulate conditions that optimize their cells and final molecules. We are attentive to the process sensitivity, whether they are using stainless steel or single-use technology. Something that we do really well is scalability. For example, when converting from stainless steel to single-use technology, we can scale down a customer's 10 000 L run to 10 L for process development, then scale back up in one of our larger single-use bioreactors."

In Fast Trak Bridge Manufacturing, process development and manufacturing scale-up are performed at our facility equipped with similar equipment, instrumentation, and automation that will eventually be deployed to your facility. We offer complete transparency. You are welcome to visit the facility to witness cGMP processing and receive training during the non-cGMP work. This approach saves time by enabling equipment testing and process verification to be performed pre-deployment and reduces costs and risk by minimizing the number of process changes required post-installation.

Solutions for process development

With over 300 years' collective experience among the Fast Trak process development leadership team, we know that quality, reliability, and cost are major considerations for your project. Early collaboration with our experts provides you access to the in-depth practical skills of our highly trained bioprocess technicians, as well as the broader experience and insight essential for developing a strong and sustainable biomanufacturing strategy for your facility. For instance, we perform bioreactor process optimization to help maximize cell line yield.

Solutions for upstream

Our upstream manufacturing teams have significant experience in scaling up single-use biopharmaceutical processes for clinical manufacturing. With expertise in batch, fed-batch, perfusion, suspension, and adherent techniques for mammalian, microbial, and vaccine manufacturing projects, we can work with you to help optimize conditions for even very challenging cell expansions, such as adherent cells on microcarriers. As an example, we have successfully scaled up the process for vaccine-containing Vero cells from flasks to an Xcellerex XDR stirred-tank bioreactor platform.

Solutions for downstream

Our specialist scientists and engineers are qualified in chromatography process development supporting mammalian, bacterial, virus, virus-like particles (VLP), and recombinant protein purification. They have extensive experience with purification platforms and filtration systems for bulk formulation of drug substances.

Comprehensive training for your specific needs

To further empower you, we provide detailed hands-on training and education, both on-site at our facility and off-site at your facility, whether for training existing staff or new equipment operators. Fast Trak training and educational courses can also be held at Fast Trak regional centers around the globe. Our standard courses cover areas such as cell culture, filtration, chromatography, design of experiments (DoE), and high-throughput process development (HTPD). We also offer customized courses to support you in the optimization and troubleshooting of existing unit operations specific to your process needs, for example.

Spotlight on a customer success story

Customer objectives

- Convert process from conventional stainless to single-use workflow
- Fit process to already designed manufacturing platform under construction
- Recover clinical timeline
- Optimize facility ramp up

Project challenges

- Maintain product efficacy
- Poorly defined existing process
- Timeline challenges
- Customer new to biotherapeutics market

Outcome of working with GE

- Produced cGMP material for two molecules
- Accelerated bulk drug substance release
- Two new molecules added to the project for development

These courses will help you build specialist bioprocessing skills and give you access to our experts, our knowledge, and our experience on specific technologies and practices. When asked what they like best about our courses, participants often answer that the blend of lecture- and lab-based training is just right, instructors are very knowledgeable of many different industrially relevant processes, are patient, and easy to understand, and what they learn is highly relevant to their work at biomanufacturing organizations around the world.

If you are interested in learning more about process development and evaluation, scale-up, and bioengineering in animal cell culture, we recommend the following course:

→ Fast Trak Advanced bioreactor cultivation technology (CELL1)

Summary

Biomanufacturers interested in maximizing productivity and minimizing costs can benefit from GE's single-use bioprocess solutions and Fast Trak support services that span the entire process. Whether you need one piece of single-use equipment, an integrated platform, an entire single-use facility, or technical expertise and support for a specific manufacturing challenge, GE can deliver. Our technical experts are on hand to provide Fast Trak Process Development, Bridge Manufacturing and training courses that will help propel your organization to success.

References

1. Application note: One-step seed culture expansion from one vial of high-density cell bank to 2000 L production bioreactor, GE Healthcare, 29160932, Edition AA (2015).
2. Application note: Efficient, high-titer monoclonal antibody production in a fed-batch process using single use stirred-tank and rocking bioreactor systems, GE Healthcare, 29119376, Edition AA (2014).
3. Application note: Scale-up of adherent Vero cells grown on Cytodex™ microcarriers using ReadyToProcess™ equipment, 29043548, Edition AA (2013).

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