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White Paper

Seed Train Intensification Using High Cell Density Cryopreservation and Specially-designed Expansion Medium

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Developers and manufacturers of biologics are under intense pressure to improve efficiency and productivity and reduce costs – all while maintaining the highest quality standards. Key imperatives include:

- Managing demand volatility which may require delaying or minimizing new investments
- Adapting to smaller batch sizes which are best served with a smaller footprint, multi-product flexible facility
- Accelerating time to production to overcome competitive challenges

Adoption of next generation approaches for development and manufacturing is heralded as a solution for these imperatives. Strategies such as process intensification have the potential to create shorter lead times, reduce plant footprint, increase flexibility and reduce COGs.

Upstream processes can be intensified using a number of strategies which can deliver significant improvements in speed, flexibility and reliability. In this white-paper, we explore two of these approaches – High cell density cryopreservation (HCDC) and the use of a perfused seed train including a specially-designed expansion medium (Figure 1).

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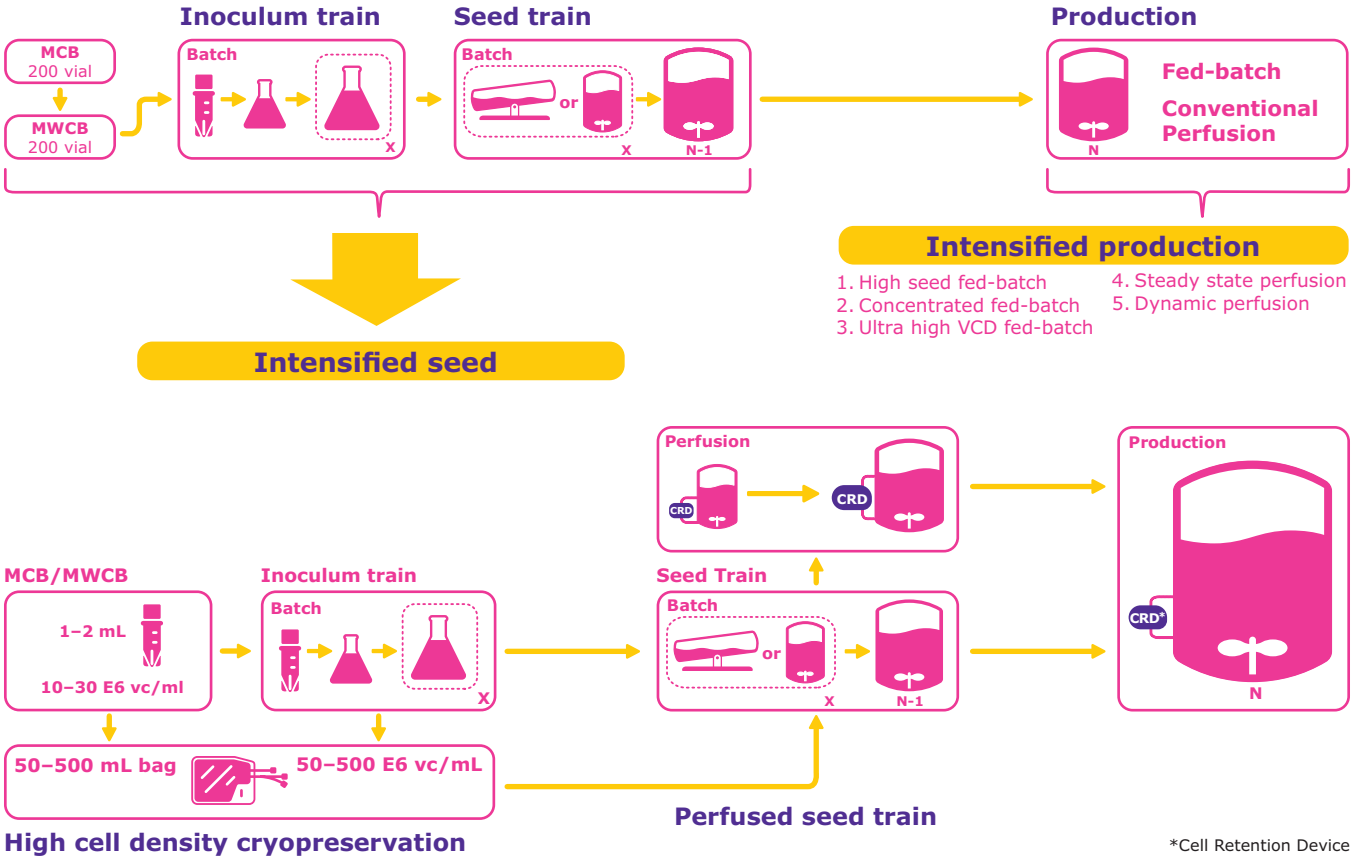


Figure 1. Opportunities for upstream intensification include high cell density cryopreservation and perfused seed train with specially-designed expansion medium.

High cell density cryopreservation

The traditional process of thawing a single vial of cells to initiate cell expansion for a GMP manufacturing batch is time-consuming and requires open cell culture operations thus increasing the risk of contamination. Use of cells banked at high density and high volume, which feeds into the first seed train bioreactor, can streamline the overall process. Traditional seed train expansion can take up to 20–30 days before inoculation of the production bioreactor. In the example shown in Table 1, a 150 mL bag of 50 x 10^6 cells/mL reduced the standard process by ten days as compared to starting with a 1 mL vial of 10 x 10^6 cells/mL. This time saving is achieved by starting the expansion in N-3.

Specially designed cryo bags eliminate open cell culture operation steps, lead to better reproducibility in seed train expansion, and decouple cell expansion and batch production, allowing for global distribution of cells to production facilities from a central expansion facility, in contrast to only using higher densities in vials.

Cell banks used in manufacturing must meet a number of regulatory requirements and undergo rigorous testing, while process intermediates in process development are relatively free of regulatory constraints. HCDC is ideally suited and easier to implement as process intermediates rather than cell banking. However, the advantages count for both applications.

	[10^6 VC/mL]
Inoculation VCD	0.5
VCD – End of batch	6

	Standard Vial	Bag
VCD [10^6 VC/L]	10,000	50,000
Volume [L]	0.001	0.15
Cell count [10^6 VC]	10	7,500

		Volume [L]	Cell count for inoculation [10^6 VC]	Run time (without lag-phase) [d]
Bioreactors	N (production bioreactor)	15,000	7,500,000	3.5
	N-1	1,250	625,000	6.9
	N-2	105	52,083	10.4
	N-3	8.70	4,340	13.8
	N-4	0.72	361	17.3
	N-5	0.06	30	20.7
	N-6	0.005	2.5	24.2

10.4 days

Table 1. Comparison of process using inoculation with a vial versus a bag. Use of high cell density cryopreserved cells significantly reduce the process time.

In addition to time savings, HCDC offers additional advantages including:

- Closed processing – there are no open cell culture operation steps in manufacturing, which significantly reduces contamination risks.
- Better reproducibility in seed train expansion – bioreactor confirmation runs can start at a comparable, equal starting point.
- Decoupling of cell expansion and batch production – allows global distribution of cells from a central expansion facility to decentralized production facilities (Figure 2).

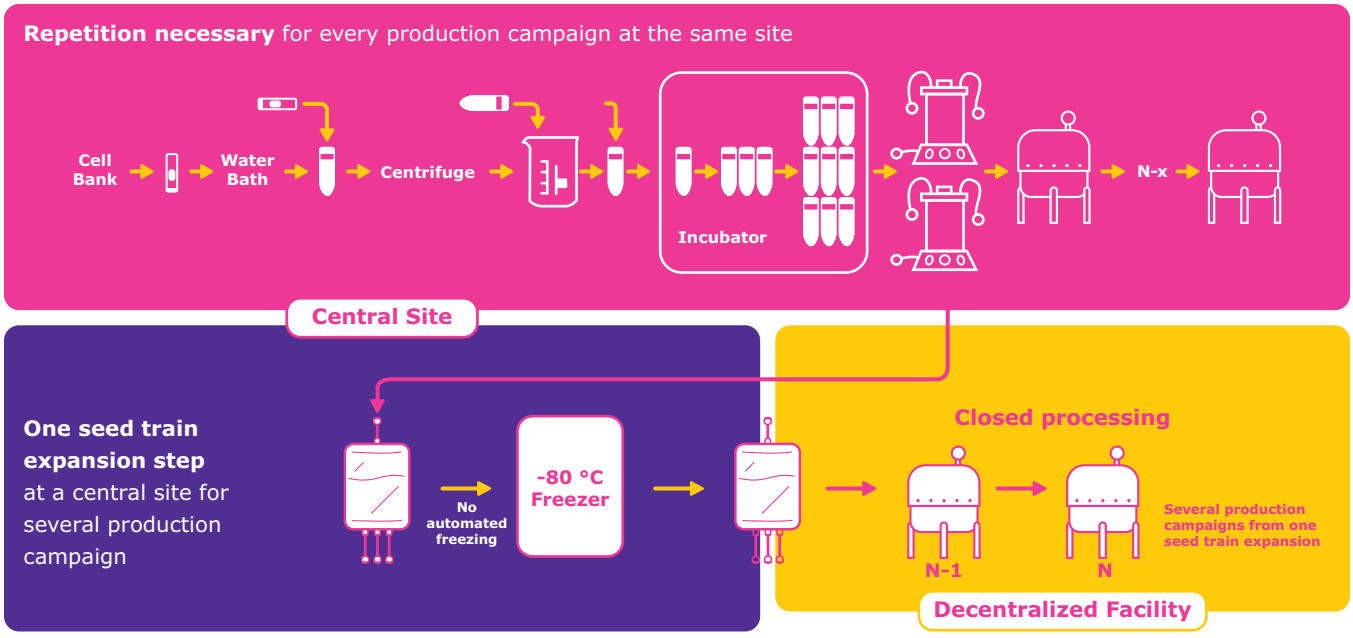


Figure 2. HCDC enables one seed train expansion step at a central site to support several production campaigns, including decentralized manufacturing facilities.

In developing the process of HCDC, a cryomedium to support freeze/thaw, bag assembly and process for testing the medium and assembly were developed (Figure 3). The cryomedium had to support freezing and thawing of cells without cell damage. Additionally, it needed to be compatible with the medium for previous and subsequent expansion to ensure:

- No cell damage during freezing and thaw
- Fast growth with minimum or zero lag phase after thaw
- A constant growth rate and specific productivity over thaw, expansion and production

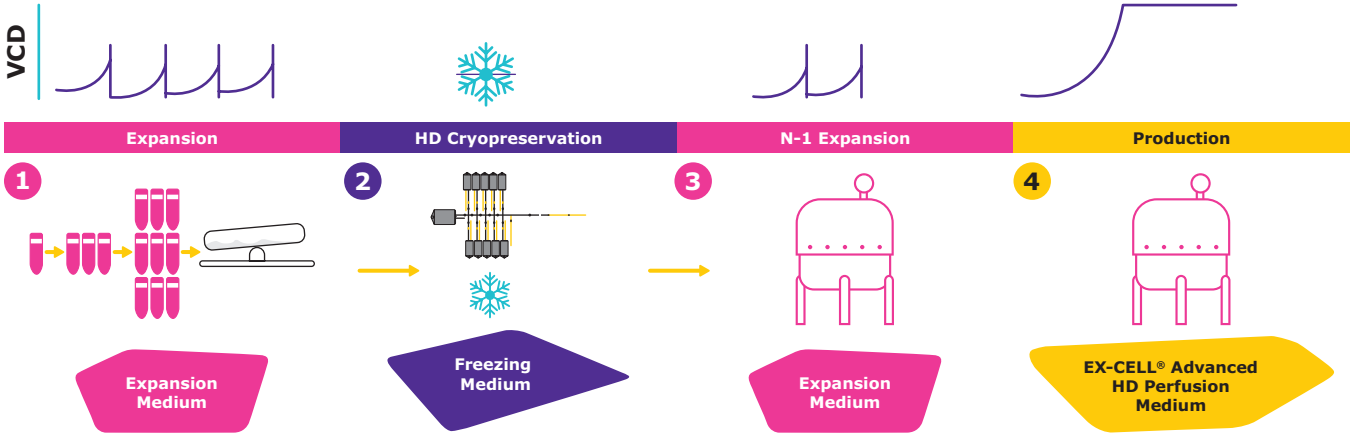


Figure 3. The HCDC cryomedium is integrated into the overall upstream process.

Figure 4 shows the main components of the bag assembly (A) and the filling process (B). In this example, there are ten 250 mL cryobags. As soon as one bag is filled, it can be disconnected via a sterile process with a NovaSeal™ crimping tool; a line on each cryobag allows it to be connected to a bioreactor after thaw for inoculation.

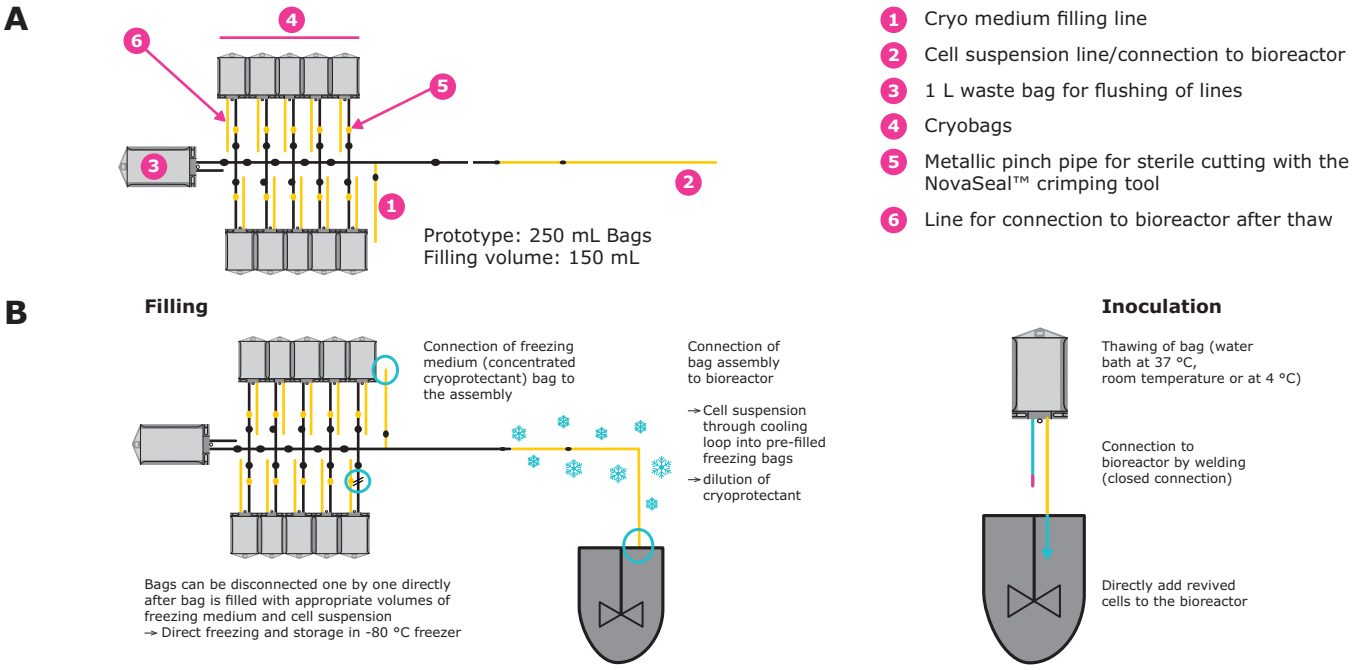


Figure 4. Main components of the HCDC bag assembly (A) and process for filling and inoculation.

To fill the bag assembly, a container with cryomedium containing a concentrated cyroprotectant is connected to the assembly; the cryoprotectant will be diluted with addition of the cell suspension to the bags. As soon as one bag is filled, it is easily disconnected and placed in the freezer. For inoculation, the bag is simply removed from the freezer, thawed and connected to the bioreactor. Figure 5 shows the cell growth and titer of a simulated experiment comparing a bioreactor inoculated with an HCDC bag and another bioreactor seeded with inoculum originating from a standard expansion from a vial; both bioreactors were run in perfusion. Subsequently, two additional bioreactors were inoculated with cells from the first two bioreactors, ran in perfusion again, followed by a steady state at 50 Million VC/mL. Results were comparable, confirming that the HCDC application can be implemented without any negative effects on cell performance.

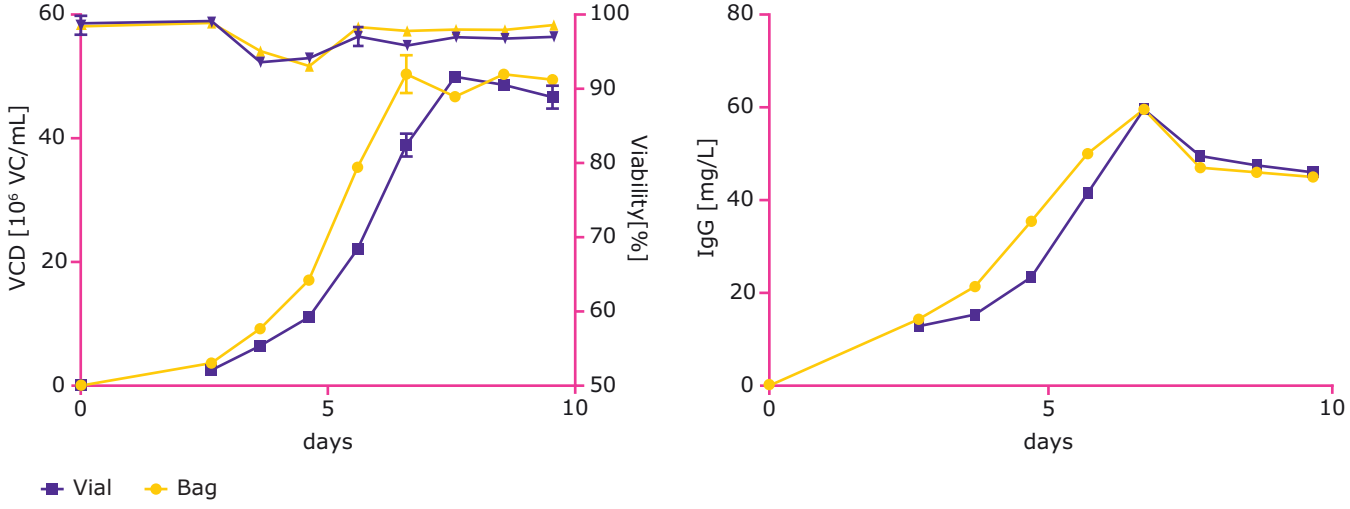


Figure 5. Growth and titer in simulated production bioreactor for CHO-K1. Growth and titer were comparable for both bioreactors during steady state.

Seed train expansion

Seed train expansion is usually performed in batch mode scaling from lab to production bioreactors up to N-1. With the use of batch mode, however, the maximum seeding cell concentration is lower than what can be achieved in a perfused seed train, due to the need to maintain a reproducible healthy cell growth to inoculate the next stage. Introduction of perfusion culture within the seed train to increase cell density can reduce the number of bioreactors needed or increase the biomass used for the inoculation of the final production bioreactor. Seed train expansion is usually performed in the same medium that is used in production phase. However, when applying perfusion, a medium is required that can support these higher cell densities and controls the metabolic profile of the cells, both to minimize toxic byproducts and to ensure adequate nutrient levels for high-cell densities. Expanded cells should be directly and without adaptation be transferrable into the production bioreactor, so these media need to be compatible to avoid any lag phases.

Based on these requirements, a model to screen different media prototypes was developed. Cells were cultured in tissue culture tubes, adapted and expanded in the media prototypes. Cells were then frozen in these media by adding dimethyl sulfoxide (DMSO) as cryoprotectant. After thawing, cells were cultured for two passages, again in the prototype media. A batch run was then started in EX-CELL® Advanced HD Perfusion Medium to simulate the production phase.

Figure 6 shows the VCD (Viable Cell Density) for the final batch, which was performed in EX-CELL® Advanced HD Perfusion Medium for both conditions. The control, shown in yellow, was expanded and cultured in EX-CELL® Advanced HD Perfusion Medium. The blue condition was expanded in the expansion medium prototype and transferred to the EX-CELL® Advanced HD Perfusion Medium for the production. While growth could be increased to some extent for the four CHO cell lines tested, results indicated that using one single medium for the whole production campaign might be suboptimal.

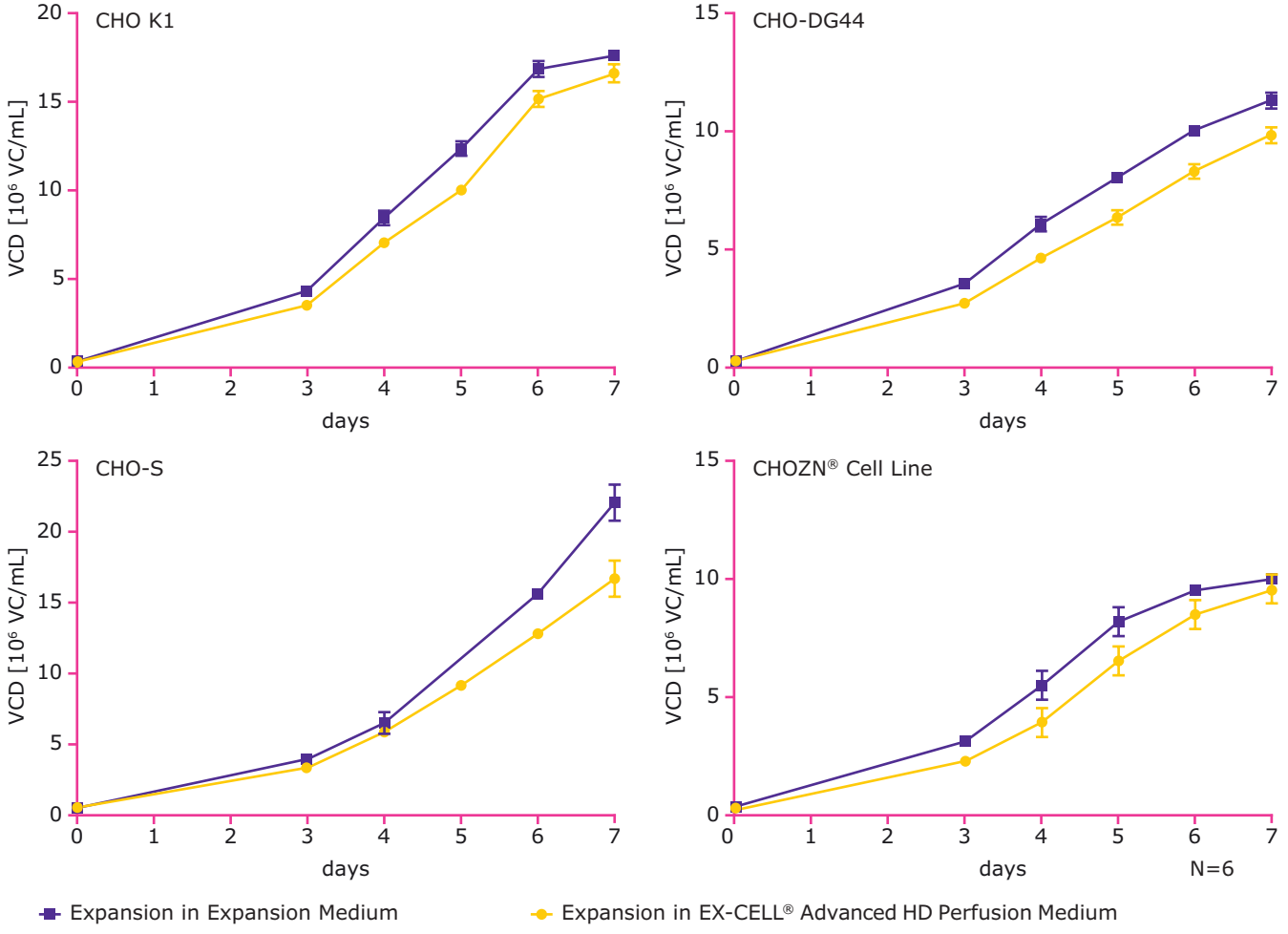


Figure 6. Growth monitored in simulated production batch. Use of a single medium for the whole production campaign is suboptimal.

As shown in Figure 7, the expansion medium had an impact on both growth and productivity; up to a 54% increase in product concentration was observed for one CHO cell line when the expansion medium was used as a companion medium to the EX-CELL® Advanced HD Perfusion Medium.

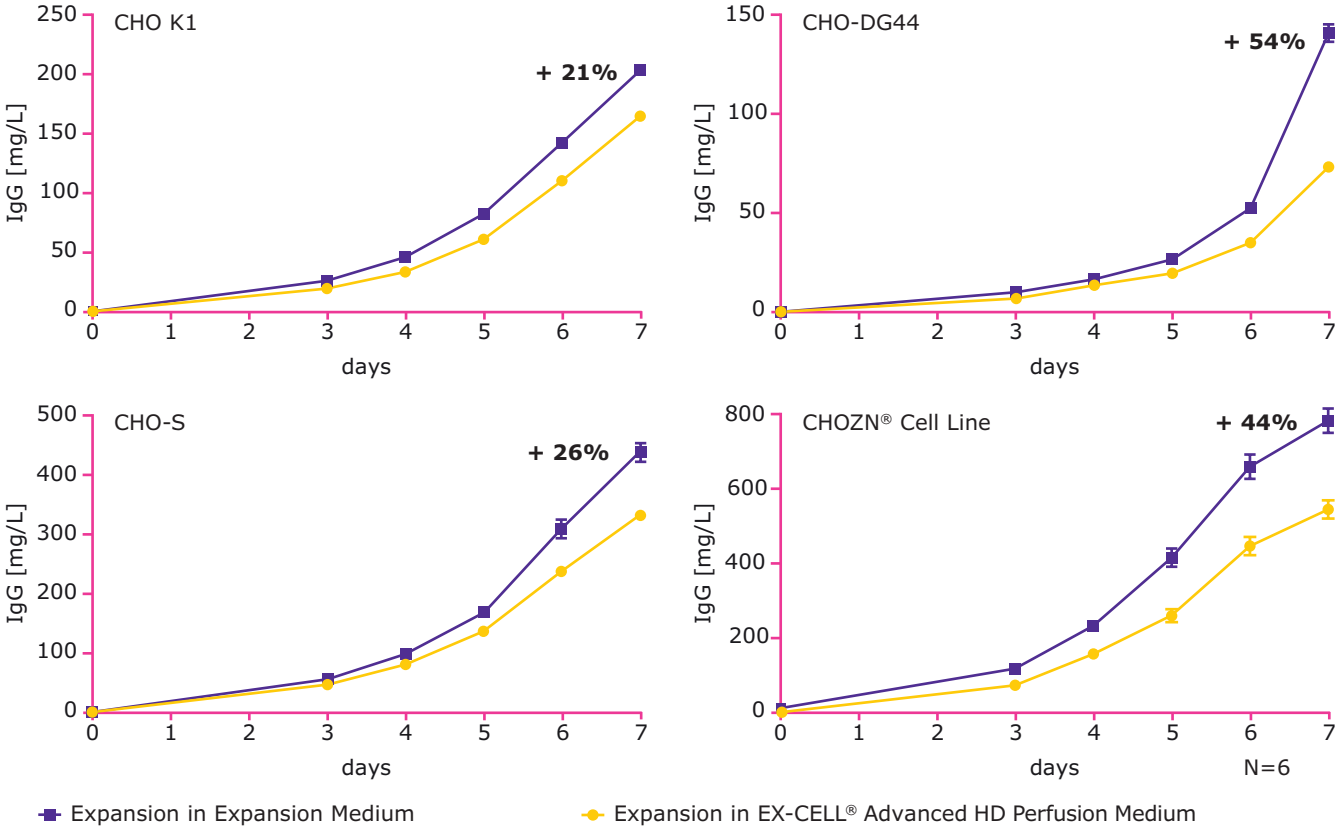
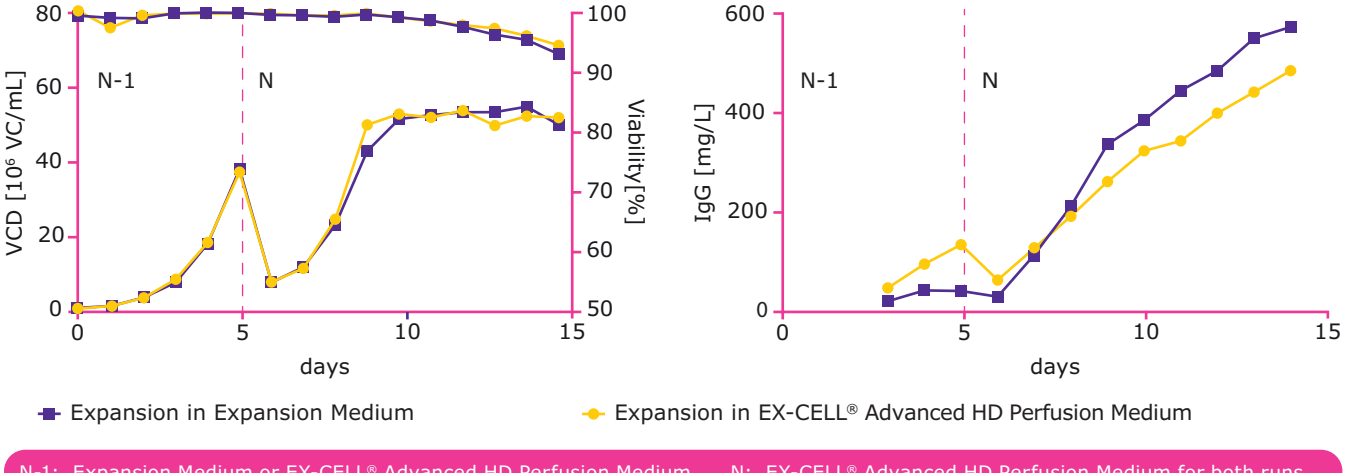


Figure 7. Increased IgG concentrations were reached when expansion medium was used as a companion to EX-CELL® Advanced HD Perfusion Medium.

To confirm if the effects in the bioreactor shake tube model were reproducible in perfusion mode, an N-1 bioreactor in perfusion was run with one condition in the expansion medium and the other in EX-CELL® Advanced HD Perfusion Medium. The cell suspension was bled down, to simulate the inoculation of the final manufacturing stage and continued running in perfusion but with EX-CELL® Advanced HD Perfusion Medium in both bioreactors. Expanding cells in the expansion medium improved productivity by 18% compared to using one single medium for the whole process (Figure 8).



N-1: Expansion Medium or EX-CELL® Advanced HD Perfusion Medium N: EX-CELL® Advanced HD Perfusion Medium for both runs

Figure 8. Bioreactor confirmation of compatibility with EX-CELL® Advanced HD Perfusion Medium.

Use of N-1 perfusion can also be beneficial when fed-batch is used as the final end-stage; as such, the compatibility of this medium with fed-batch platforms Cellvento® 4CHO/4Feed and EX-CELL® Advanced fed-batch was explored. In order to determine if the positive effects can be also seen when the expansion medium is used as a companion for these two platforms, two CHO lines were expanded either in the typical medium for these platforms or in the new developed expansion medium before the fed-batch was run (Figure 9).

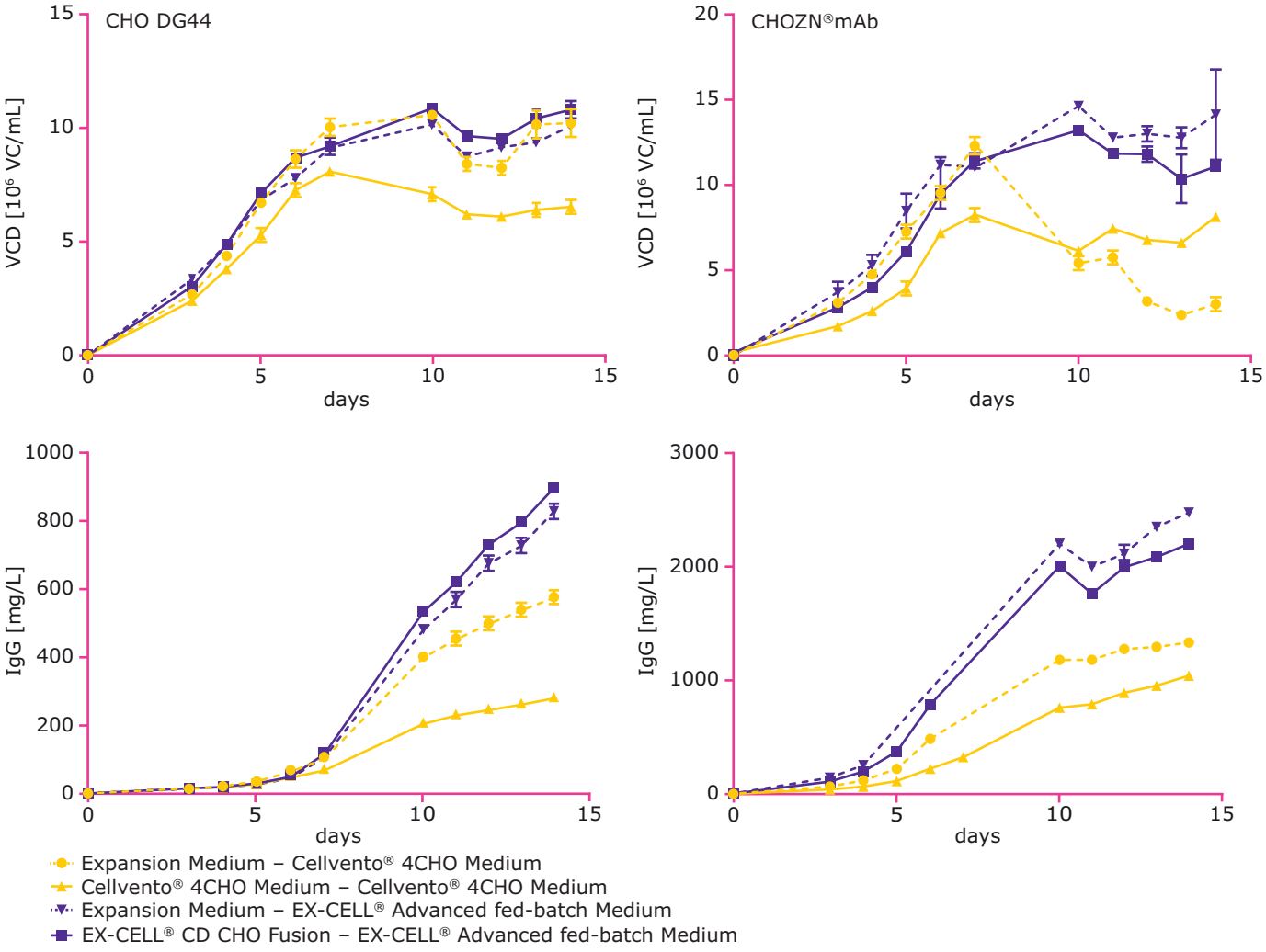


Figure 9. Compatibility of expansion medium with fed-batch Cellvento® 4CHO & EX-CELL® Advanced CHO fed-batch Media.

The conditions that were expanded in the new expansion medium are shown with a dashed line; conditions expanded in the typical basal medium are shown as solid lines. With the Cellvento® 4CHO platform, the expansion medium had a significant titer-increasing effect; with EX-CELL® Advanced fed-batch Media platform, the medium also worked as companion but with comparable titers. These results demonstrate that the expansion medium can be also used when using fed-batch in final production step.

The same results were then confirmed in the bioreactor (Figure 10). The Cellvento® 4CHO platform was tested for compatibility, with two replicates each. The purple condition was expanded in the Cellvento® 4CHO basal medium; the yellow condition expanded in the expansion medium. Following expansion, the cell cultures were used to inoculate the bioreactors, both run in fed-batch, using the Cellvento® 4CHO fed-batch platform. Use of the expansion medium during cell expansion at bioreactor scale confirmed productivity-increasing effects, even in fed-batch when using it as the companion for the Cellvento® 4CHO platform.

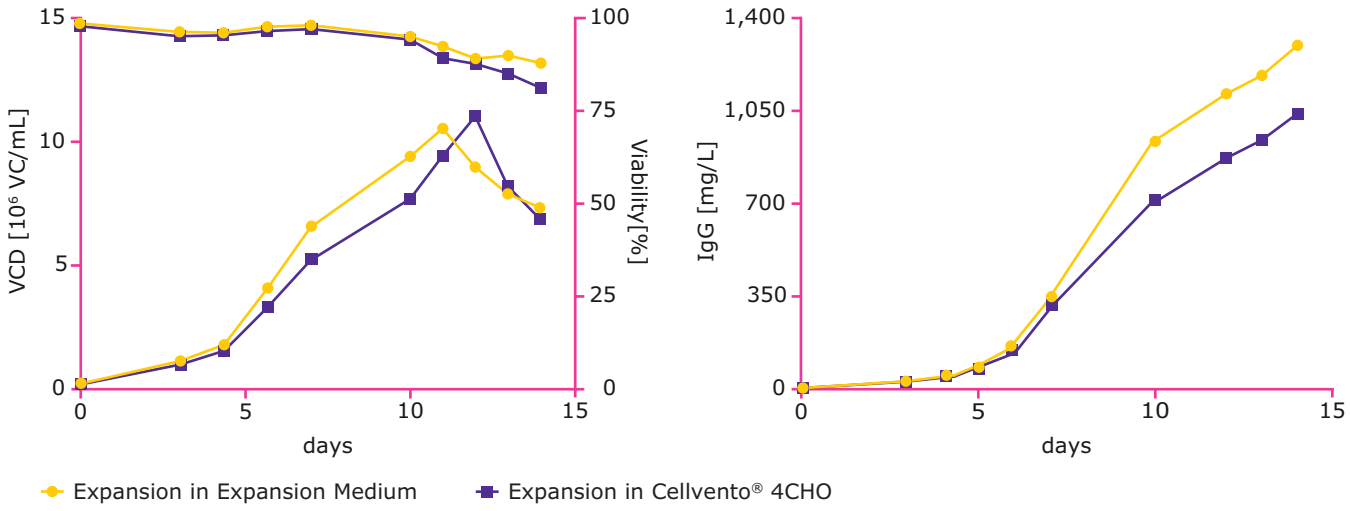


Figure 10. Bioreactor confirmation of compatibility with Cellvento® 4CHO fed-batch platform.

As drug manufacturers seek new ways to improve efficiency and productivity and reduce costs, adoption of intensification strategies continues to increase. As described in this white paper, HCDC and specially-designed expansion medium are two approaches which can have a significant impact on upstream processes. Use of specially-designed expansion medium can improve both cell growth and productivity while HCDC can:

- Shorten the seed train and time required to get to the production bioreactor
- Provide greater facility flexibility through rapid initiation of each run and in the case of a process failure, enable a more rapid recovery
- Reduce cell laboratory infrastructure with one central site managing the Master Working Cell Bank
- Prevent contamination through use of a closed system as compared to transferring cells from one container to another

Conclusion

A specially-designed expansion medium can improve both cell growth and productivity. Cellvento® 4CHO-X Expansion medium has been specifically developed for a perfused seed train for Chinese Hamster Ovary (CHO) cell lines. It is formulated with a high nutrient concentration to achieve low cell specific perfusion rates (CSPRs) at high cell densities for greater seed train process efficiencies including lower manufacturing CoGs, improved plant flexibility, increased capacity, and reduced manufacturing footprint. It is compatible with perfusion and fed-batch production processes.

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