

## ASK THE EXPERT

# Market Leading Perfusion Medium for CHO Cell-Culture Productivity and Robust Scalability

with Arnav Deshpande and Matthew Stebbins

**F**ounded in 2014, Just Biotherapeutics was created by a team of biotechnology pioneers who were determined to break through the scientific and economic barriers of the development and manufacture of protein therapeutics. Today Just-Evotec Biologics operates as a contract development

and manufacturing organization (CDMO) and wholly owned subsidiary to Evotec SE. Just-Evotec Biologics' mission is to design and apply innovative technologies to dramatically expand global access to biotherapeutics. It leverages artificial intelligence (AI) and machine learning (ML) technologies, world-leading molecular design, cell-line development, process intensification, and continuous manufacturing strategies to advance biotherapeutics from discovery through clinical stages to commercial launch. A key innovation from Just-Evotec Biologics is its proprietary J.Media perfusion cell-culture solution for Chinese hamster ovary (CHO) cell lines, which is designed to enhance operational flexibility and efficiency. In a January 2026 Ask the Expert webinar, Matthew Stebbins (media development group lead) and Arnav Deshpande (senior scientist II) described their groups' benchmarking of the platform in a scale-down model and discussed study results on process scale-up.

### The Presentation

Just-Evotec Biologics's chemically defined and animal-origin-free media formulation can be concentrated fivefold. That capability reduces storage requirements, which is critical for scaled-up perfusion processes that demand large quantities of media. The medium's liquid format is stable for up to 30 days at 1× concentration and up to four months at 5× concentration; the powder format remains stable for up to

24 months at room temperature, surpassing the typical 12-month cold-storage shelf life of other commercially available media. Those features minimize cold-storage needs, reduce costs, and streamline manufacturing operations.

Just-Evotec Biologics's perfusion processes use commercial expansion media for initial batching before transitioning to perfusion media over a three-day ramp-up period. The media's feeding strategy incorporates a cystine, tyrosine, and poloxamer 188 feed, along with a 5× concentrate feed that is diluted with water for injection (WFI) and mixed directly in a bioreactor. Industry-standard feeds such as glucose, antifoam, and base additions also are included to maintain pH control.

High-throughput screening formats, such as 24 deep-well-plate mock perfusion models, are employed to test media additives and optimize formulations. Such models facilitate rapid characterization of media performance and allow for confirmation of changes in small-scale bioreactors during 15- to 25-day perfusion processes. Advanced analytical techniques, such as liquid chromatography with mass spectrometry (LC-MS) and ultrahigh-performance liquid chromatography (UHPLC), can be used to analyze key metabolites and amino acids.

The small-scale mock perfusion model, which operates in deep-well plates (24, 2-mL volume), mimics perfusion through daily centrifugation and replacement of 80% of spent media with fresh media, achieving a perfusion rate of 0.8 vessel volumes per day (VVD). The model requires only one to three hours per day for media exchange and is compatible with automation technologies such as the Tecan Fluent system. It supports complex design-of-experiment studies to address client needs and screen media parameters for down selection in bioreactor studies within four to eight days. Key cell-culture parameters such as viable cell density (VCD), viability, and titer are measured daily. Spent media are stored for routine glucose, lactate, and ammonia monitoring, advanced analysis of media-nutrients exchange

by UHPLC and LC-MS, and assessment of protein product impurities and glycosylation profiles with in-house analytics.

J.Media benchmarking studies were conducted using Just-Evotec Biologics's proprietary J.CHO glutamine synthetase (GS)-knockout cell lines expressing two monoclonal antibodies (mAbs). The medium's performance was tested in side-by-side mock perfusion tests against three commercial perfusion media (A, B, and C). Results demonstrated that the J.Media formulation supports comparable VCDs and maintains >90% viability over eight days. The J.Media solution outperformed in volumetric productivity and achieved 33% and 18% higher cumulative titers for mAb1 and mAb2, respectively, compared with the commercial media. That performance is attributed to higher cell-specific productivity, which measures the amount of protein produced per cell per day.

The medium also performed robustly regarding nutrient use and waste reduction. It consumed comparable amounts of glucose while producing significantly lower levels of lactate and ammonia, two major waste products in CHO cell culture. Reduced lactate production enables efficient use of glucose toward protein production, whereas low ammonia secretion rates improve cell viability. Those attributes improve cell health and specific productivity.

The scalability of the J.Media solution was evaluated in 3-L benchtop reactors and 500-L manufacturing reactors. Results showed consistent performance across scales, with VCDs maintained at a bleed target of 80 million cells/mL and >80% viability for at least 15 days. Cumulative titers were higher for mAb2 than for mAb1, which was consistent with findings from mock perfusion studies. Compared with the small-scale models, the tested bioreactors provided better control over pH, dissolved oxygen, and mixing, resulting in higher viable cell densities and productivity. Additionally, the J.Media formulation maintained low product-impurity levels and consistent glycosylation patterns during scale-up, which are critical for biosimilar programs that require strict glycosylation profiles.

The J.Media formulation offers strong performance, operational flexibility, and scalability. It supports cell-specific productivity, enables high cell densities with sustained viability, and delivers robust scale-up performance with low impurities and consistent glycosylation profiles. Its stability in

both liquid and powder formats eliminates the need for cold storage, reducing costs and enhancing operational efficiency. Additionally, the medium's flexible nature allows for in-house media development services, enabling customers to customize additives or formulations to meet specific needs. With its tunable formulation and advanced analytical capabilities, J.Media formulation is well-suited for diverse customer needs and biosimilar programs.

### Questions and Answers

**What is the daily bleed level during the perfusion process, and is the bleed implemented to control VCD or purge excess volume?** For this study, the daily bleed was 0–15% of the cell-culture volume. We did that to maintain VCDs at a set target. However, bleeds can differ depending on the cell types and processes being used.

**The glycosylation profile data showed a net percentage in the pooled product from days 7 to 15. Can you comment on the temporal dynamics of product quality attributes from those days?** One of the main advantages of perfusion processes is that we can have consistent product quality in terms of glycosylation profiles. We found fairly consistent glycosylation patterns from day 7 to day 15. During testing of our process, we collected the permeate from days 7 to 15, and we found that only beta-galactose has 10–20% lower numbers at day 15 compared with day 7. All other data shown were fairly consistent during that period.

**Has the J.Media formulation been used with a molecule that has passed the investigational new drug (IND) stage? Can you provide regulatory support for that formulation?** Yes, we have a history of first-in-human (FiH) programs with our media-development program. We also have helped clients to address regulatory questions related to formulation.

**What is the cell-specific perfusion rate recommended for use with the J.Media solution?** Typically, we have a set VCD target of 80 million cells/mL, and we perfuse our media at 2 VVD. That translates to roughly 25 pL/cell/day. However, finding an ideal cell-specific perfusion rate can differ depending on processes and cell lines.



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