SARTORIUS

Case Study

Fast-Paced De Novo Cell Culture Medium Development for a HEK293 Suspension Process

Maximizing Efficiency During rAAV Process Development and Production

Customer Profile

Company Type:
Upstream solution provider for cell and gene therapy production

Company Location:
France

Introduction

Cell culture media are crucial to the production of biopharmaceuticals in mammalian cells, including antibodies, vaccines, and viral vectors for in vivo and ex vivo gene therapy applications. These media are meticulously formulated to ensure optimal cellular performance, making them a key component in the production of life-saving medicines.

Sartorius offers a comprehensive portfolio of expertly designed off-the-shelf cell culture media, suitable for a wide range of cell culture processes and products. For clients with specific requirements, we provide customized solutions, with the capability to design and develop bespoke cell culture media that precisely address their unique needs. Coupled with an extensive array of analytical techniques, Sartorius facilitates the establishment of a robust production platform, allowing clients to allocate their resources more efficiently.

The widespread use of recombinant adeno-associated viruses (rAAV) in gene therapy has resulted in the initiation of numerous clinical trials targeting a spectrum of rare and more prevalent diseases. Reducing costs and increasing yields are essential for facilitating broader access; however, production success depends on optimizing cell culture media and key process parameters, considering the genomic variability and specific needs of different HEK293 strains.

Our client was looking for a partner to develop a customized medium formulation optimized for rAAV production with a proprietary transfection reagent. The medium was developed to work with different strains of HEK293 cells and as part of a platform solution in combination with the transfection reagent.

Customer Challenge

The market for protein- and virus-based therapies is undergoing rapid growth and is expected to continue expanding in the coming years. Companies operating in this field must navigate a multitude of complexities when developing new processes and products. One significant initial challenge is the establishment of a robust production platform, which requires high-performing cell culture media solutions that are competitive in terms of pricing, product yield, and quality, and carefully tailored to each process.

For drug producers, maximizing yield without compromising product quality requires considering all critical parameters in process development to analyze and improve cell culture conditions. Media performance is linked to several parameters, including the manufacturing process as well as the source and quality of raw materials. Optimizing a medium formulation is critical to shortening time-to-market and ensuring consistent output. Combining a rational and research-driven approach with statistical methods can enable solutions that are both well-founded and adaptable.

Solution

We employed a rigorous and comprehensive screening process to design a high-performing cell culture medium for rAAV production in a short time frame. The newly developed cell culture medium was optimized for transient transfection with a specific transfection reagent, and its performance was independent of process scale.

Prior to the tech transfer, key process indicators (KPIs), as well as project-specific milestones, were agreed upon in close alignment with our customer in order to assess the project's success. These targets are shown in Table 1.

Table 1: *KPI for the Custom Development Project*

Targets for Medium	Good cell adaptability (less than two weeks)
	Appropriate transfection efficiency
	Higher rAAV titers than reference
	Available as liquid
	Animal-origin free and scalable manufacturing

Results | Conclusion

Technology Transfer
We recognize the critical importance of synchronizing processes between our facility and the client's site. To achieve this, we establish a technology (tech) transfer at the beginning of the project phase. This ensures seamless integration and facilitates the efficient transition of the final product back to the client, paving the way for the next steps, e.g., beta testing.

In this project, tech transfer involved disseminating protocols that should be considered during the development process, including cell line specifications, cultivation parameters, and cell culture methodologies such as transient transfection with plasmid DNA. It was supported by documents provided by Sartorius and close alignment with the client through online meetings.

Two commercially relevant HEK293 derivatives were adapted to grow in off-the-shelf, proprietary cell culture media at Sartorius. Subsequently, research cell banks of both cell lines were prepared. The customer provided the necessary quantities of the proprietary transfection reagent and plasmid DNA for the production of rAAV.

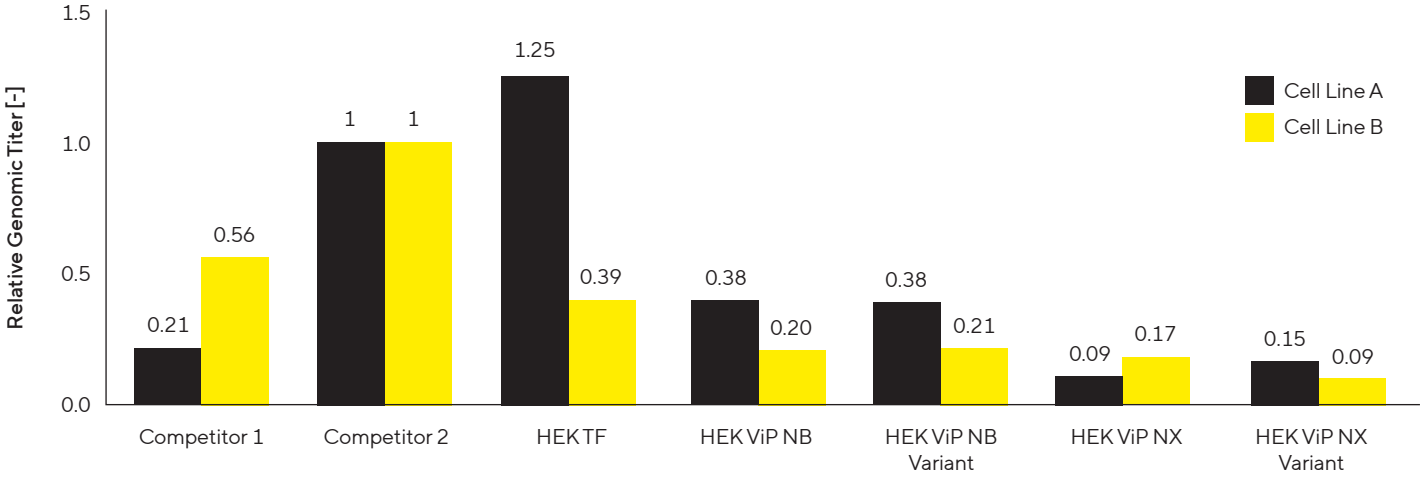
Benchmarking
Benchmarking is a fundamental aspect of every development project. It is imperative to regularly compare the product at the current development stage with at least one internal and one external benchmark. This approach allows us to identify and quantify experimental variability arising from the inherent heterogeneity of biological systems, technical noise during analysis, operator handling differences, and temporal variations. Importantly, it also provides a measure of the success of the cell culture media development project.

In this instance, a benchmark was established using two competitive, commercial HEK293 media that are suitable for the transient production of rAAV. Furthermore, five proprietary and commercially available cell culture media (HEK TF, HEK ViP NB, HEK ViP NX, as well as modified variants of HEK ViP NB and HEK ViP NX) were used with the objective of acquiring comprehensive insights into the behavior of both cell lines, as well as the transfection and rAAV production process in general, which were then employed for de novo development.

The benchmark encompassed seven different cell culture media, with three replicates for each of the two cell lines, resulting in 42 transient rAAV productions in shake flask cultures. The growth performance of each combination of cell line and medium was also evaluated, resulting in 22 shake flask cultures that were not transfected.

The behavior of rAAVs (in this case, rAAV2) varies depending on the serotype. In most cases, these vectors remain internalized within the cell and do not undergo secretion into the extracellular space. Consequently, the comprehensive evaluation of the benchmarking process involved quantitative polymerase chain reaction (qPCR) analysis of 70 samples in triplicates to assess rAAV production in both cell lysate and cell culture supernatant.

Figure 1: *Benchmarking of rAAV2 Production With Two Commercially Relevant HEK293 Cells at Shake Flask-Scale in Seven Different Cell Culture Media*



Note. Genomic titer shown as fold change measured by qPCR 72 h post-transfection in cell lysates. The values presented are the means of n = 3, and they have been normalized to Competitor 2 for each cell line.

Furthermore, 49 ELISA measurements in duplicates were conducted to determine the quantity of intact rAAV capsids present in both the cell lysate and the culture supernatant.

The genomic titer quantification results indicate that the performance is dependent on the cell line in question (Figure 1). It is noteworthy that during the benchmarking process, cell line A in HEK TF demonstrated superior performance to that of Competitors 1 and 2. In contrast, Cell Line B exhibited a 60% reduction in genomic titer when compared to Competitor 2. These findings represent a key step forward in understanding one of the many complex factors during medium development for cell culture processes. HEK293 cells exhibit homologous characteristics when compared to other cell lines, such as CHO cells, but they also demonstrate intra-cell line variability. Even closely related HEK293 strains can perform very differently under the same conditions. This variability extends to the single-cell level, where unique and intriguing characteristics of different subpopulations emerge, adding depth and complexity to these cell culture processes.

To gain further insight into the metabolic profile of each cell line in relation to the tested cell culture media, we conducted a comprehensive examination of spent media samples from selected rAAV production processes. The spent media analysis (SMA) included the quantification of amino acids, vitamins, glucose, and lactate, among others.

Following a comprehensive integration and evaluation of the entire data set, the benchmarking results were presented to the client. Using these insights, we devised a de novo formulation, subsequently be referred to as ViP01, and employed it as the basis for the next development cycle.

Development Cycle 1

The initial development phase involved the assessment of five novel, innovative media variants, which were developed based on the benchmarking data. We adapted the two HEK293 cell lines to all media variants and carried out rAAV production via transient transfection. Eight additional conditions and media supplements were also tested with the objective of acquiring comprehensive process knowledge and evaluating the impact of the pH during pre-complexation, as well as other compounds thought to be crucial during rAAV production in HEK293 cells.

A total of 48 shake flask cultures were transfected with rAAV production plasmids over the course of Development Cycle 1 (Figure 2). The analysis comprised 58 qPCR measurements, conducted in triplicates, in addition to 32 ELISA measurements, performed in duplicates. A number of conditions and compounds that enhanced the performance of the variant ViP01 were identified (Figure 3 - ViP01.1-ViP01.12).

Figure 2: *Overview of the Development Cycles for the Project*

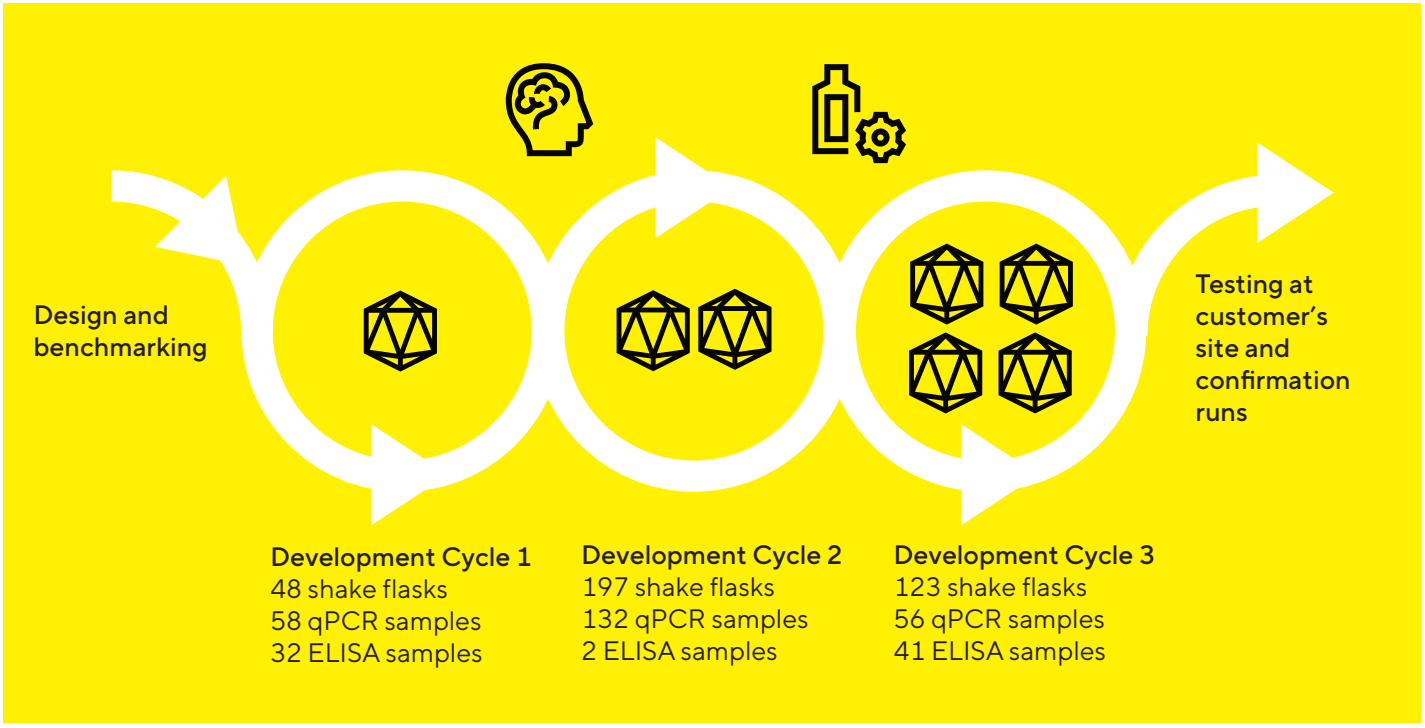
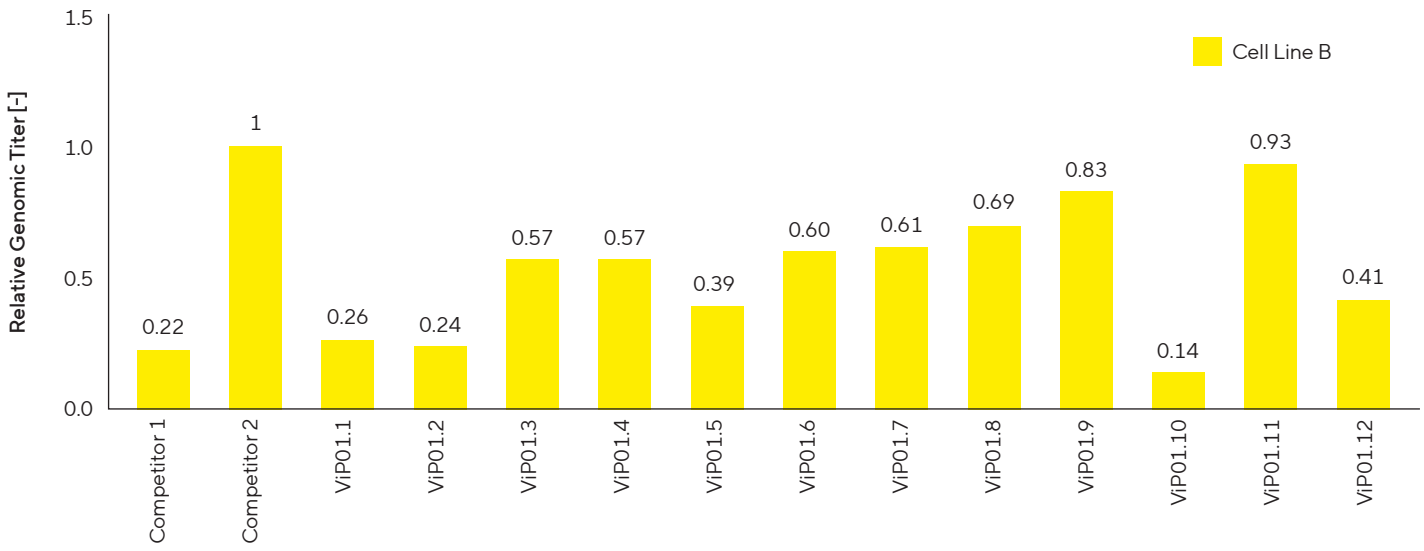


Figure 3: *rAAV2 Production With a Commercially Relevant HEK293 Cell Line at Shake Flask-Scale in 12 Different Cell Culture Media Variants Based on the De Novo Formulation ViP01*



Note. Genomic titer shown as fold change measured by qPCR 72 h post-transfection in cell lysates. The values represent n = 1, and they have been normalized to Competitor 2.

The results showed a considerable increase in viral vector productivity, reaching levels comparable to those observed in Competitor 2 for Cell Line B. Remarkably, Cell Line B exhibited suboptimal performance compared to Cell Line A during the benchmarking phase. This outcome emphasizes the intricate nature of optimizing a specific process and cell culture medium to attain a desired outcome. However, it also demonstrates that the meticulous effort invested in this instance was justified, validating the significant impact of precisely designing new media variants.

Upon the conclusion of the initial development cycle, one of the newly designed media variants was identified as comparable to the competitor benchmark. In view of the knowledge acquired and in line with the client's feedback following the presentation of the initial cycle's outcomes, the recently devised, de novo cell culture media formulation was further developed. This involved combining well-known compounds with experimental ones identified in the latest experiments. This new iteration of the de novo formulation, subsequently be referred to as ViP02, was then used as a basis for the next development cycles.

Development Cycle 2

The substantial performance boost we achieved in Development Cycle 1 paved the way for the second phase of the development process. This phase involved the rigorous assessment of multiple surfactant molecules during the production of rAAV. Surfactants are typically employed to reduce shear stress within the culture environment, a practice that is widely regarded as advantageous for both growth and recombinant protein production. We also postulated that a reduction in shear stress would be beneficial for virus stability and reduce adherence between the cell surface and rAAV capsid, leading to a higher recovery of produced rAAVs in the supernatant.

Five distinct surfactants were evaluated by adding each to the recently devised de novo formulation, ViP02, and compared to the five customized media variants that constituted part of the screening process during the initial development cycle. ViP02 was also augmented with different well-known or newly identified compounds, targeting cellular processes including the citric acid cycle, redox balance, nucleotide metabolism, and processes dependent on trace elements, such as DNA synthesis. This resulted in eleven medium formulations, designated ViP03 to ViP12. In order to account for the potential impact of biological variability during handling, each transfection experiment included reference processes in competitor media as well as the best-performing internal off-the-shelf cell culture medium (data not shown).

A considerable difference in cellular behavior was observed between cell culture media variants, mirroring the results from the benchmarking phase. For instance, compared to competitor 2, Cell Line A demonstrated superior performance when cultured in ViP08, whereas Cell Line

B exhibited higher titers in ViP04 (Figure 4). Again, this emphasizes the significant distinction in cellular behavior and requirements for each HEK293 derivative and other subclones. Additionally, it underscores the necessity for comprehensive optimization of media formulations and process parameters for each HEK293 clone.

To understand the impact of a pH-controlled environment on the new medium formulation, HEK293 cultures were also cultivated and transfected in a 2 L benchtop-scale system. This system was used to compare growth and rAAV production in the newly devised medium variant, ViP02, with our proprietary off-the-shelf media (data not shown). With extensive experience in optimizing cell culture processes and designing customized media across various modalities, we recognize that performance variations observed in uncontrolled small-scale systems, such as shake flasks, can be even more pronounced in pH-controlled systems, including benchtop bioreactors or the Ambr® 15 and Ambr® 250 systems.

During Development Cycle 2, 127 shake flasks and two 2 L bioreactors were transfected. In total, 197 shake flask cultures were handled, including the collection of data on growth profiles in each tested variant. To evaluate rAAV production in each newly devised variant, 132 qPCR samples were measured in triplicate (Figure 2).

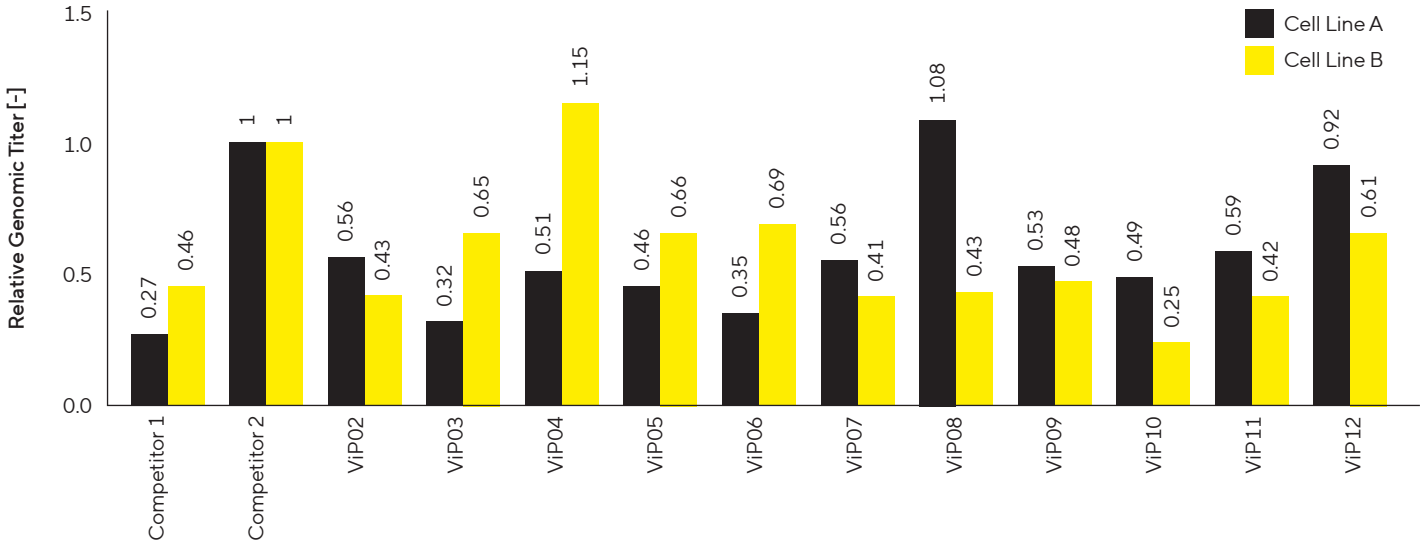
Development Cycle 3

As the development project neared its scheduled completion, we began the third and final development cycle. In this cycle, we tested 15 more media variants, designated ViP13 to ViP27, which were generated based on the valuable insights gained during the previous cycles. These variants included additional compounds proposed to facilitate the citric acid cycle, maintain cellular redox balance, and regulate lipid and polyamine metabolism.

The results once more highlighted the importance of developing a tailored cell culture medium for each cell line in order to achieve optimal process performance and viral vector productivity. Several media variants, including ViP17, ViP20, ViP21, and ViP22, were identified as demonstrating superior performance compared to Competitor 2 when using Cell Line A (Figure 5). However, our objective was to identify a formulation that would support high viral vector productivity in a range of cells. Accordingly, ViP23 and ViP27 were selected as the most promising candidates for the final confirmation runs, given that both cell lines exhibited relatively high performance in both media.

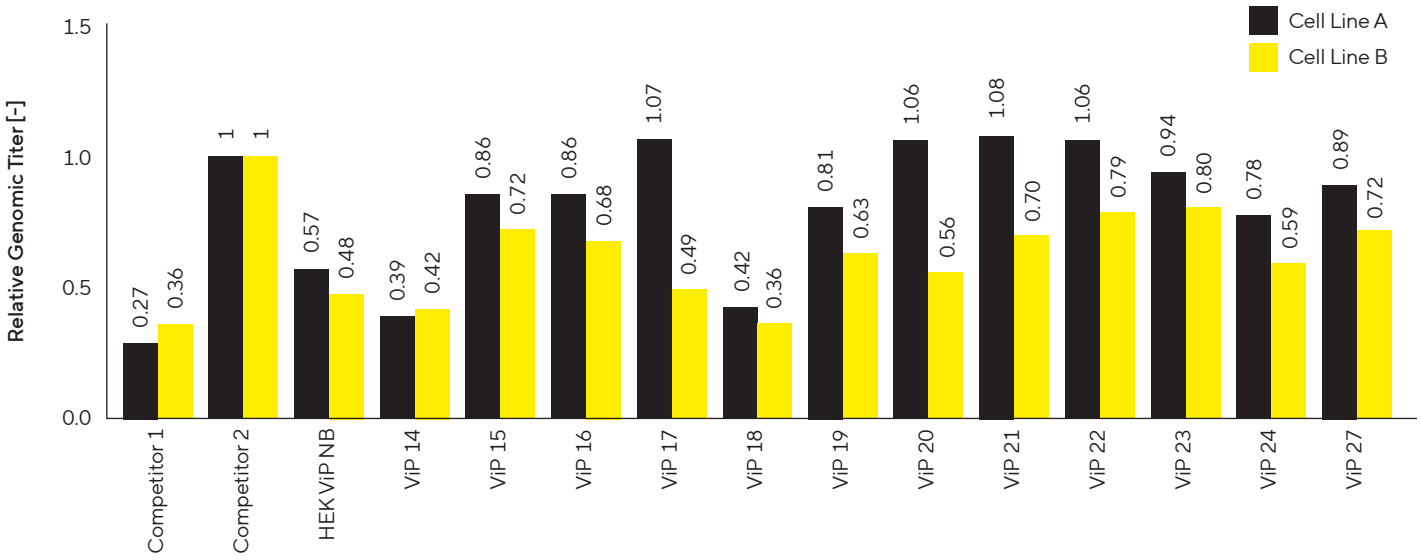
A total of 96 shake flask cultures were transfected with rAAV2 production plasmids during Development Cycle 3. In total, 123 shake flask cultures were handled during the third cycle, including the evaluation of cellular growth in each

Figure 4: *rAAV2 Production With Two Commercially Relevant HEK293 Cell Lines at Shake Flask-Scale in 11 Different Cell Culture Media Variants (ViP02 – ViP12), Based on the Newly Iterated De Novo Formulation ViP02.*



Note. Genomic titer shown as fold change measured by qPCR 72 h post-transfection in cell lysates. The values represent n = 1, and they have been normalized to Competitor 2.

Figure 5: *rAAV2 Production With Two Commercially Relevant HEK293 Cell Lines at Shake Flask-Scale in 12 Different Cell Culture Media Variants (ViP14 – ViP27), Based on the Newly Iterated De Novo Formulation ViP02 and in Comparison to a Reference Process in HEK ViP NB.*



Note. Genomic titer shown as fold change measured by qPCR 72 h post-transfection in cell lysates. The values represent n = 1, and they have been normalized to Competitor 2.

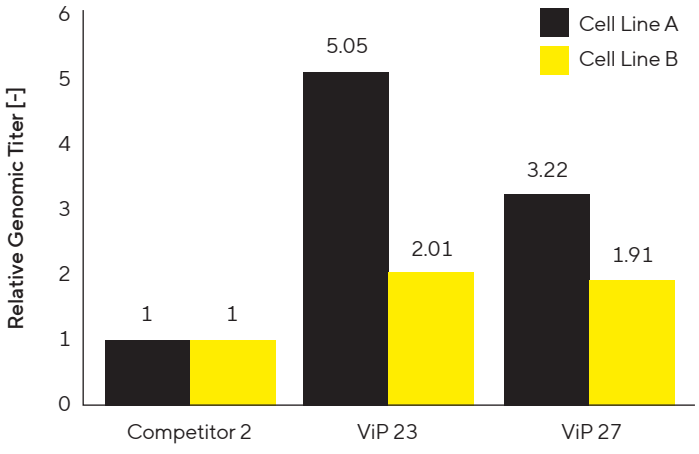
individual medium variant. Cycle 3 also comprised a total of eight bioreactors at 2 L benchtop-scale. In this instance, 56 qPCR measurements were conducted in triplicate to assess rAAV production capabilities in each newly devised medium variant. This holistic approach also included 41 ELISA measurements in duplicate to quantify the capsid concentration (Figure 2).

Final Confirmation Runs

To conclude the wet lab work, we performed final confirmation runs. This step was part of the evaluation of the final variants, as agreed upon during the project's drafting phase. These confirmation experiments involved eight 2 L benchtop bioreactor runs. Our aim was to confirm the efficacy of variants ViP23 and ViP27 at larger scales, particularly in pH-controlled systems. These systems provided an opportunity to further assess their performance at the uncontrolled shake flask-scale. Cell culture samples from the bioreactor cultures were subjected to a range of analytical assays, including those designed to assess the concentration of amino acids, vitamins, and other compounds that have been identified as having a significant impact on cell culture performance.

In addition to ViP23 and ViP27, Competitor 2 medium was evaluated to enable a direct comparison of the performance of all media under investigation. Both media variants demonstrated superior performance compared to the competitor medium in the two HEK293 cell lines at the 2 L benchtop-scale, which suggests that the pH-controlled environment exerts a profound influence on the overall process performance (Figure 6). Ultimately, ViP27 was deemed the optimal variant, given its favorable characteristics in terms of manufacturability, stability, and cost-effectiveness.

Figure 6: *rAAV2 Production in Two Commercially Relevant HEK293 Cell Lines at 2 L Benchtop-Scale With Two Media Variants From the Final Development Cycle.*



Note. Genomic titer shown as fold change measured by qPCR 72 h post-transfection in cell lysates. The values represent n = 1, and they have been normalized to Competitor 2.

Conclusion

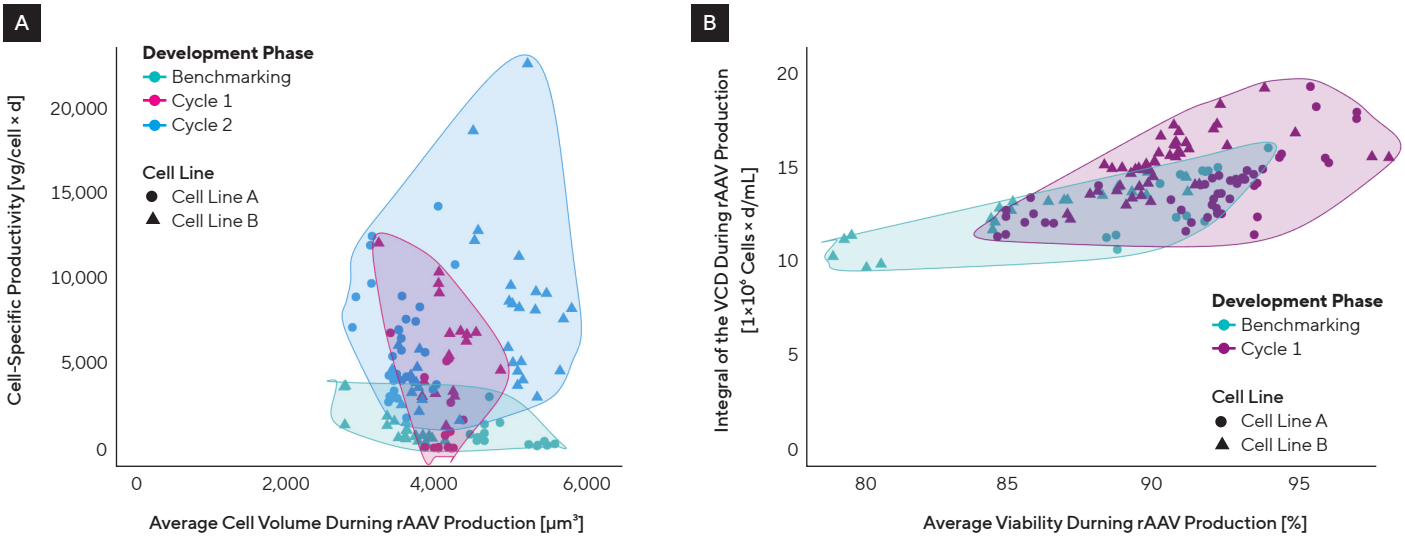
A meta-analysis of all gathered data points demonstrates that cell-specific productivity was enhanced throughout the development phase (Figure 7A). Comparing the results from the benchmarking phase with those from Development Cycle 3 shows that the latter medium variants enhance the integral of the viable cell density and the average viability during rAAV production (Figure 7B). Both are essential process variables, as low viabilities frequently result in the accumulation of undesirable cell debris, including host cell DNA.

Both cell lines demonstrated robust adaptability to new culture conditions, exhibiting minimal effects on cell growth and viability following transfer to a novel medium variant. The transfection efficiency was consistently high, indicating that the intermediate variants did not exert any deleterious effects on the pre-complexation of plasmid DNA and transfection reagent, as well as the cellular uptake of the complexes. At the shake flask-scale, genomic titers were found to be competitive with the benchmark medium Competitor 2, while outperforming Competitor 1 in almost all cases. It is noteworthy that when scaled up to a pH-controlled system such as the 2 L benchtop bioreactors, the medium variants ViP23 and ViP27 led to a substantial, several-fold increase in genomic titers when compared to Competitor 2. This emphasizes the impact of the overall process conditions on performance. Furthermore, both variants were available in liquid form as chemically defined and animal-origin-free media.

Depending on the customer's needs, successful media development is often followed by a transfer from liquid to powder medium. This is advantageous, as the storing and shipping of powder is often easier and more cost-efficient. Thanks to many years of experience in this area, a possible powder production is already considered during the development phase of the liquid medium. This includes, for example, the selection of raw materials (production, origin, quality) and production technologies. These are essential factors that have a decisive influence on the quality of the end products. At our dedicated powder manufacturing site in Schloss-Holte (Germany), highly experienced engineers and scientists work on the realization and production of the powder medium. The production of the final powder product can be scaled up on different scales, as needed.

In order to be able to make statements regarding the stability of the media (powder and liquid medium), shelf-life studies are often carried out on the products in question. Here,

Figure 7: ***(A)*** *Cell-Specific Productivity Plotted Against the Average Cell Volume During rAAV Production.* ***(B)*** *The Integral of the VCD Plotted Against the Average Viability During rAAV Production.*



Note. **(A)** Throughout the development project, we were able to consistently increase the cellular productivity as well as the average cell volume in comparison to the results collected during benchmarking. The cell volume is generally considered to be an important indicator for cellular recombinant protein as well as viral vector production capacity. **(B)** Our holistic approach towards the design of a tailored cell culture medium enabled us to increase the average cell growth during AAV production, as well as the viability. It is critical to achieve high viabilities during AAV production to minimize the accumulation of cell debris, such as host cell proteins, which might cause problems during downstream processing.

chemical parameters, as well as cell growth, are tested at previously defined intervals. Depending on the study design, the tests can be defined and extended as required. Storage conditions such as cold storage, room temperature, or any temperature excursions can also be simulated.

In this media development project, we successfully achieved all KPIs defined with the client (Table 1). Continuous communication throughout the entire project, coupled with our 15 years of expertise in designing and evaluating tailored cell culture media, ensured successful delivery of the final product within six months.

As previously noted, our comprehensive range of services extends beyond the initial stages. The de novo design and optimization of the cell culture medium, including final confirmation experiments to conclude the wet lab work, represent the initial phase towards industrial-scale production and commercialization. We are committed to supporting you throughout this process and will strive to ensure a successful outcome.

At a Glance

Comprehensive Tests Performed Across Three Development Cycles*

52
Different media

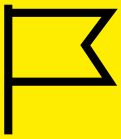
197
qPCR samples

121
Growth tests

87
ELISA samples

324
Transfections

Before



- rAAV titer was ~70% of competitors'
- Required fast solution
- Needed a custom media designed and tested

6 Months

After

- rAAV titer 100 – 400% of competitors'
- Good cell adaptability
- Available as liquid and powder
- Animal-origin free
- Scalable manufacturing

* Total Project Duration: 6 Months

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
sartorius.com

Status: 4 | 2025