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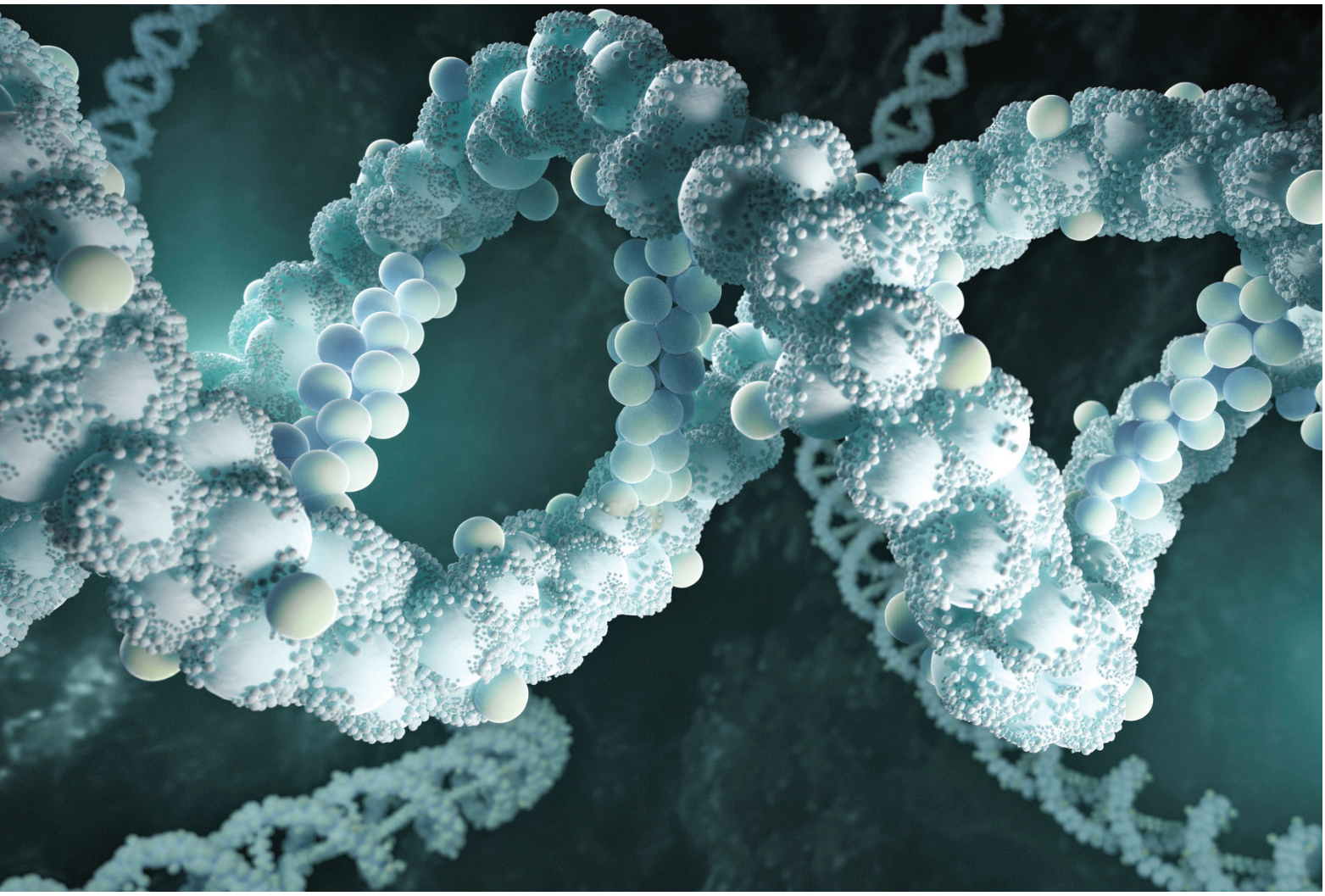

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WHITEPAPER

Evaluating current manufacturing platforms for recombinant AAV production

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Abstract

With the ability of gene therapies to cure a wide range of chronic, rare, and genetic diseases, the opportunities in this space are significant. Viral vectors are a key component of gene therapies and an excellent means to transport nucleic acid cargo into the cell to express the therapeutic protein. A commonly used viral vector in the industry today with great success in gene delivery is adeno-associated virus (AAV).

Recombinant AAV vectors are based on human parvoviruses that are not known to cause serious diseases, so there is no obvious pathogenicity after transduction. There are currently 13 AAV serotypes and greater than 100 variants. This does not include the novel serotypes and the engineered-capsid serotypes that are currently being developed to ensure broader and/or specific tissue tropism. AAV vectors are most desirable because they can infect both dividing and non-dividing cells, and within these cells, they do not integrate into the genome but rather form episomes that have long-lived transgene expression.

Realizing the full potential of viral vector-based therapies requires a successful manufacturing platform for recombinant AAV vectors. As one explores options in this growing and exciting corner of the market, it is important to understand the platforms currently available to properly evaluate their fitness for the unique product needs.

Complex, critical raw materials required for viral vector production

Gene therapy manufacturing requires critical raw materials. Many of these still include animal-derived components, specifically serum, especially for adherent cells that require transient transfection. In some cases, media components or feeds contain reagents like transferrin made from animal sources. In addition, there are complex starting material requirements, including mammalian or insect cells as well as plasmids that must be produced at the right GMP grade and of the highest quality for transfection. For some platforms, viral banks may also be required. Finally, a secure supply of single-use technology, which have characteristics ideal for vector manufacturing, such as smaller batch sizes, improved scalability, process economy, and increased flexibility, must also be available. The variability and demand of these materials may impact:

- viral clearance
- impurity profile
- analytics
- process robustness/consistency
- supply chain
- yield

Analytics

Analytics in viral vector production is often referred to as measuring or monitoring a needle in a haystack, and this is due to their complexity as well as a focus on analytes that are similar to the impurities one is trying to discern against. Viral vector analytics can be divided into several buckets (Figure 1): nucleic acid based, protein based, cell based (bioassays), and analytical chemistry. These buckets are also further divided into subcategories based on assay qualification and requirements as described in the ICH guidelines for bioanalytical method validation. These include strength- and potency-based assays, impurities/purity-based assays, safety-based assays, and several other assays that can be used for characterization of these materials.

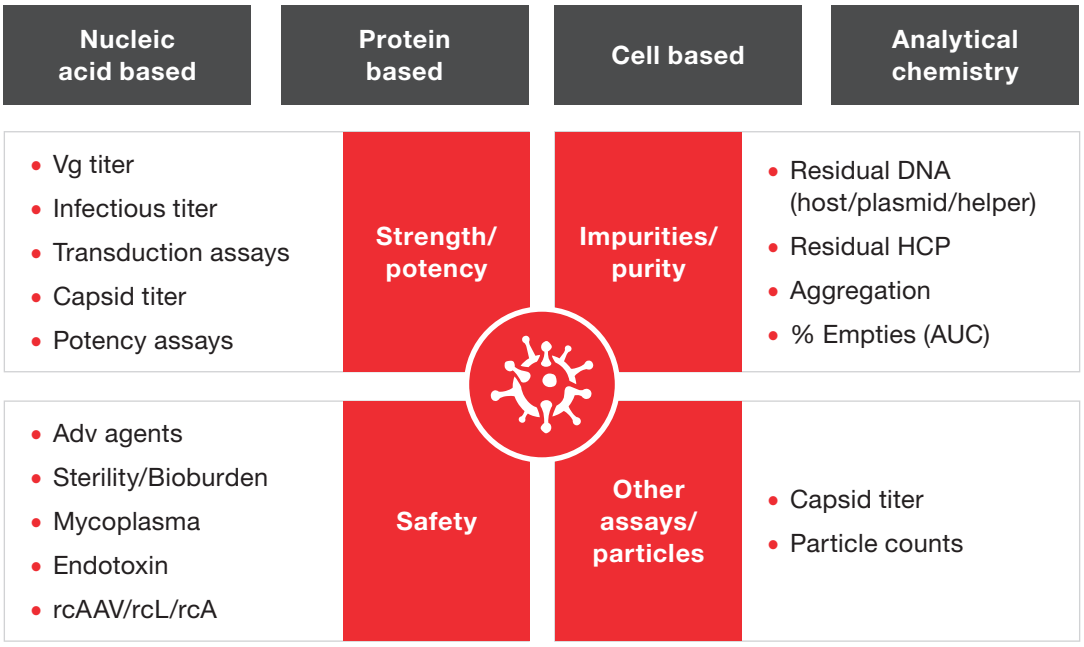


Figure 1: Analytical method categories and assay types frequently used during the characterization process of viral vectors and based on ICH M10 Bioanalytical Method Validation guidelines.

The most critical of these assays within the strength and potency category is the vector genome titer (Vg), which is used to provide the therapeutic dose. It is important that these assays are qualified and fit for use to inform the critical quality attributes (CQAs) of the material being produced. It is also important that these assays are robust and qualified early during clinical development and that suitable/representative reference standards are used to avoid comparability issues as the process matures.

Generic process flow diagram for viral vector production

Figure 2 outlines a generic process flow diagram for viral vector production, which begins with thawing a master/working cell bank (MCB/WCB). The host cells are expanded through a series of passages up to the final cell mass in the terminal reactor. At the terminal reactor, given appropriate cell density/confluency, cells are infected or transfected, depending on the choice of platform (transient transfection, helper virus [Baculovirus or HSV] systems, or stable transfected [producer/packaging cells]) to initiate vector production.

After an appropriate amount of incubation time, post-infection or post-transfection to produce viral vector, the reactor is harvested under the appropriate conditions and the harvested material clarified to filter out gross impurities. The clarified harvest is then purified through a sequential number of downstream steps to make the bulk drug substance (BDS). There are several ways to achieve this. One approach is to buffer exchange and concentrate the clarified harvest or go directly to capture chromatography by utilizing an affinity step. The eluate from capture affinity is then applied to a polishing step to further improve the purity profile for both process and product impurities. Usually, this polishing step is anion exchange (AEX) chromatography to separate empty from full particles. However, alternative techniques, including cesium chloride or iodixanol gradient ultracentrifugation, may also be used depending on the quality target product profile (QTPP) desired.

Additionally, this process may include viral clearance steps, such as nanofiltration and detergent inactivation. The final step of the process is diafiltration into the final formulation buffer and ultrafiltration to the target concentration of the drug substance. The BDS is then compounded to the final concentration and filtered through a 0.2-micron filter and filled into the vial configuration required for the product.

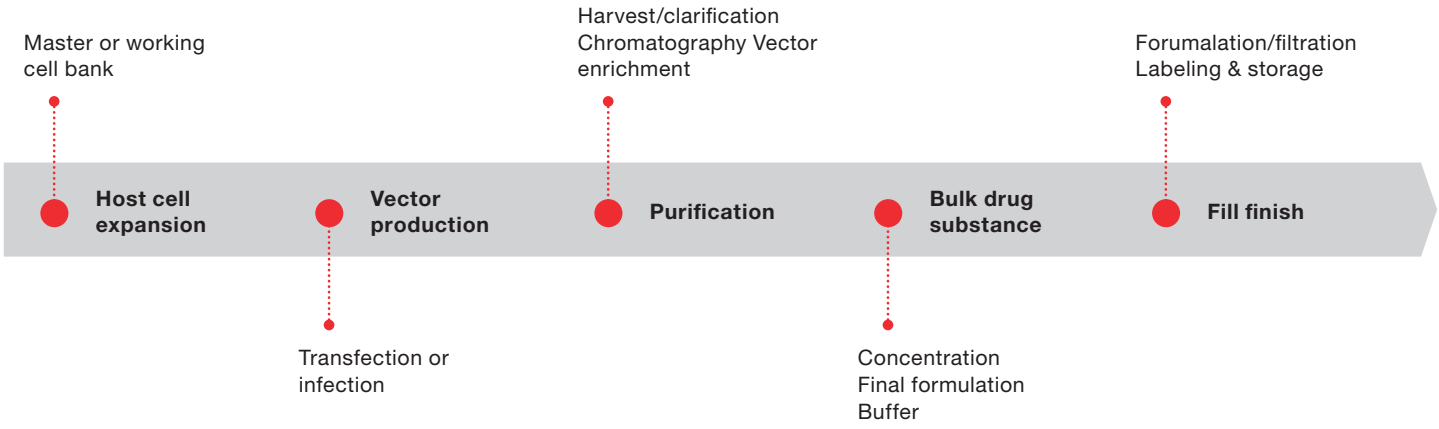


Figure 2: The standard process flow for viral vector production involves host cell expansion, vector production, purification, bulk drug substance generation and fill finish steps. Each stage further divides into the accompanying technologies, methods and biological components required to support processing of the viral vector material. Some examples of these process components include cell banks, plasmids, filtration and affinity chromatography skids, AEX and ultracentrifugation for capsid enrichment, UF/DF, formulation and fill of product.

Complex manufacturing platforms for viral vectors

The following platforms have been used to manufacture AAV vectors:

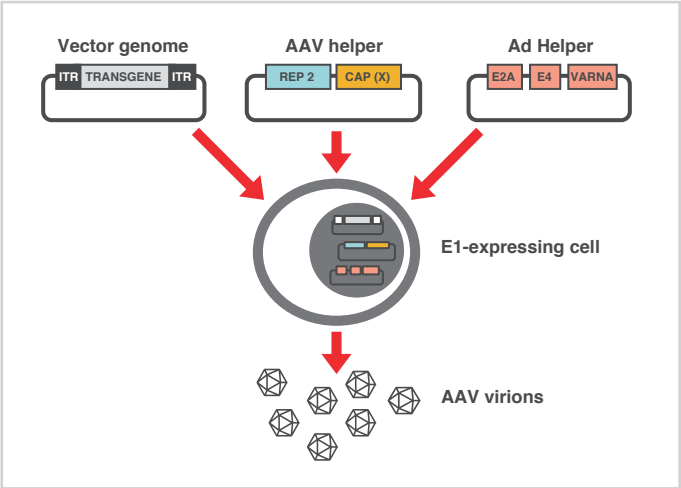
- Transient Transfection of adherent/suspension HEK293 cells
- Baculovirus expression vector system utilizing insect, Sf9 cells
- Packaging or producer cell lines (stable transfection systems)
- Herpesvirus helper processes utilizing mammalian cells

The transient transfection and producer cell line platforms are based on the same premise. In the transient transfection platform (Figure 3A), two or three different plasmids are cotransfected into a permissive cell to produce the AAV vector. In this case, the adenovirus helper genes are localized on the Ad-helper plasmid; AAV rep/cap genes are on the AAV helper plasmid (packaging plasmid), or the genes for AAV and Ad can be together on a single plasmid. A third plasmid, transfer plasmid, would contain the transgene or the gene of interest (GOI) straddled between the left and right AAV inverted terminal repeats (ITRs).

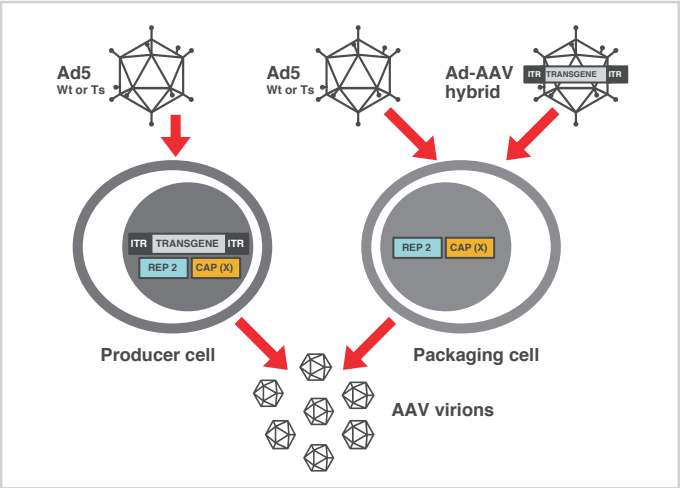
The producer cell line system (Figure 3B) is similar to the transient transfection except that these genes are stably transfected into the permissive cell. Once the stably transfected cells are grown to an appropriate cell mass, expression of the essential genes can be turned on by infection with adenovirus and allow production of the AAV vector.

There are two other platforms often used for AAV production, and those are the helper systems. The baculovirus helper system (Figure 3C) uses permissive SF9 insect cells, and the mammalian herpesvirus (HSV) helper system (Figure 3D) uses permissive HEK293 or BHK cells for AAV production.

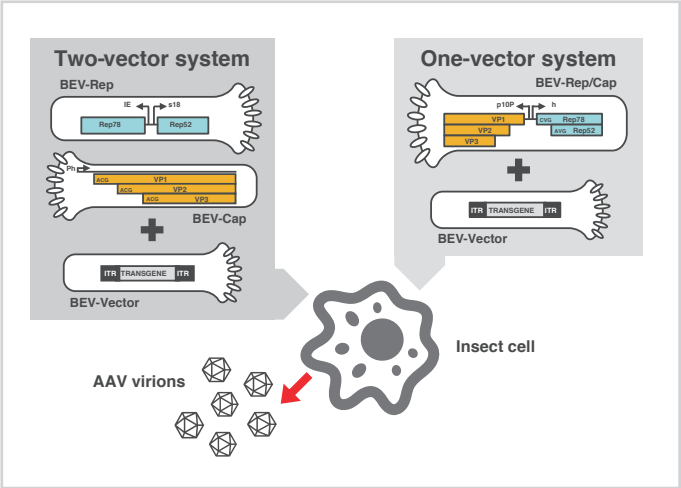
In both systems, a two-virus infection is used to coinfect the permissive cell and produce AAV vectors. In the case of the two-infection system, there is a helper virus and a vector virus. The baculovirus helper or the HSV helper virus contains the replicase and capsid genes for AAV production (helper functions), while the baculovirus vector virus or HSV vector virus include the transgene/GOI straddled by AAV left and right ITRs. Coinfection with these two viruses will allow for AAV vector production. It is important to note that the baculovirus system uses replication-competent viruses and the herpesvirus system uses replication-deficient viruses. Therefore, in the AAV production system, there is no further replication of the herpesvirus helper and vector starting material. However, the herpesvirus system requires a much higher multiplicity of infection (MOI) of the helper and vector starting material to allow for maximal productivity of AAV in the process. Alternatively, the baculovirus system uses a very low MOI and, therefore, a significantly smaller volume of starting material for maximal productivity of AAV in the process.



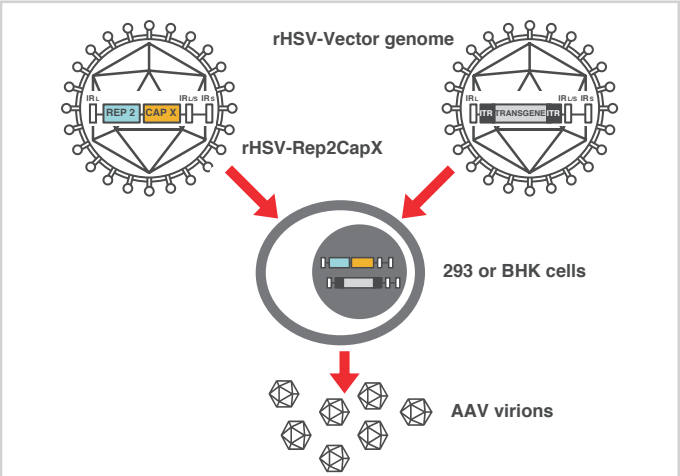
A. Transfection manufacturing platform



B. Producer cell line manufacturing platform



C. Baculovirus helper system



D. HSV helper system

Figure 3. Diagrams of different manufacturing platforms available for AAV vectors. Ayuso et al. Current Gene Therapy, 2010, Vol. 10, No. 6

Pros and cons of viral vector production platforms

There are several factors that should be considered when selecting a platform, such as the targeted patient population of the product, the amount of desired yield, and potency requirements. Table 1 below outlines the pros and cons for these AAV production platforms.

Cell Lines: All the mammalian systems utilize human or rodent cells that are potentially contaminated with adventitious viruses that could be detrimental when injected into a patient. Insect Sf9 cells, while they do contain insect adventitious agents, do not need rigorous testing like the human virus panel to confirm adventitious viruses are being removed.

Starting Materials: Plasmid DNA is needed as starting material for the transient transfection platforms, both adherent and suspension. Virus seed stock is needed for the rest of the platforms. A master and/or working viral bank (MVB/WVB) is also needed to ensure consistency in the process.

Animal-Derived Components: The transient transfection adherent HEK293 system primarily utilizes animal-derived components like FBS (fetal bovine serum). The media for all other platforms are primarily animal-component free or can be easily made animal-component free.

Resources Needed: Processes for the transient transfection adherent system as well as the HSV helper system are laborious and require the most resources, as they include several steps requiring multiple flat stock vessels that require extensive manipulation.

Scale: All processes can be scaled up to the volume desired, but the transient transfection adherent system must also be scaled out. Because of the multiple vessels needed, at Thermo Fisher these platforms can be scaled up to 48 hyperstack or 40 cell stacks. Given the amount of viral vector needed, the process needs to be scaled out significantly. In addition, for the HSV helper platform, the HSV virus seed is scaled up using adherent cells in multiple vessels and then the scale-up for AAV is a straightforward process using stirred tank bioreactors similar to other suspension processes. The transient- and stably-transfected cell systems and the baculovirus systems can all be linearly scaled up (or via perfusion systems) to yield large volumes of material. The Thermo Fisher transient transfection suspension process is currently scaled up to 200L and then scaled out to multiple 200L to allow for a combined harvest processed downstream.

Facility Needs and Cleanroom Space: The adherent processes have a larger footprint need due to the large number of incubators needed for incubation. Additionally, the HSV helper system also requires additional cleanroom space for production of the HSV virus seed stock at a regular cadence to AAV vector production. Unlike the adherent processes, the suspension processes do not require a large footprint.

	Transient Transfection: Adherent HEK	Transient Transfection: Suspension HEK	HSV Helper Suspension	Producer Cell Suspension	SF9/ Bac Suspension
Cell Line	Human: HEK293 (-)	Human: HEK293 (-)	Rodent: BHK21 (-)	Human: HeLa/ HEK293 (-)	Insect: SF9 (+)
Starting Material	Plasmid DNA	Plasmid DNA	Virus Seed Stock HSV Helper and Vector	Virus Seed Stock: Adenovirus	Virus Seed Stock: Baculovirus Helper and Vector
Animal Component	Serum (-)	NA (+)	NA (+)	NA (+)	NA (+)
Resources Needed	Laborious (-)	Nominal (+)	HSV seed stock is adherent process and laborious (-)	Nominal (+)	Nominal (+)
Scale	Scale out (+)	Scale up to 200L Scale out to 4x200L (+) (-)	Scale up: AAV Production Scale out for HSV seed (+) (-)	Scale up to 2000L (potential)/Perfusion process (+)	Scale up to 2000L (+)
Facility needs	Large footprint (incubators); Fixed bed (-)	Single Use Bioreactors (+)	HSV production incubators, Single use Bioreactors (-) (+)	Single use Bioreactors (+)	Single use Bioreactors (+)
Cleanroom space/ Lot	1- 2 suites (+)	1- 2 suites (+)	2-3 suites (-)	1-2 suites (+)	1-2 suites (+)
COGs	High: Serum, Plasmids, vessels, labor (-)	High: Plasmids (-)	Very High (production of HSV seed stock for every batch of AAV produced) (-)	Low (+)	Low (+)
Time to clinic	16-22 months (+)	16-22 months (+)	8-24 months (-)	18-24 months (-)	18-24 months (-)
Product Quality	No impact to potency based on PTM, methylation patterns, E:F, P:I (+)				Differences in PTM, methylation, P:I, E:F may impact potency (-)
Yield/Liter	1E+13 to 1E+14 vg/L		1E+14 vg/L (+)	1E+13 to 1E+14 vg/L	5E+13 to 5E+14 vg/L (+)

Table 1: Pros (+) and Cons (-) for AAV production platforms. Having the flexibility to assess multiple platforms and overcome associated challenges allows for an efficient approach to achieving the viral vector product profile objectives. In these cases, Thermo Fisher Scientific can provide the essential support across all components involved in the platform evaluation to overcome constraints as they relate to process material, manufacturing capacity requirements, readily available technologies, timeline to execute, and product quality.

Cost of Goods: The cost of goods for each system varies, based on raw materials. For example, the transient transfection system may need serum as well as plasmids, which can be expensive, especially at high volumes. The HSV helper system requires seed stock at the same cadence of AAV production. All of the platforms utilize single-use technology, which adds to the cost of goods. However, the highest cost of goods would be for those platforms that need serum, and/or plasmids, and/or scale out instead of scale-up for production.

Time to Clinic: The time to clinic for all systems is between 16 to 24 months from the inception of process development to the release of the first batch of clinical material. Although the virus helper systems (HSV and baculovirus) and the packaging/producer cell line systems need initial tools dev-eloped upfront that can take up to 6 months of development time, the overall increase in time is only two to three months, given the ability to start process develop-ment in parallel using population virus and/or transiently transfected cells.

Product Quality: For all platforms that employ production of AAV vectors in mammalian systems, the product quality is comparable to that observed for AAV, as there should be no untoward differences in post-translational modifica-tion, methylation patterns, empty:full virions, and particle: infectivity ratios. However, all of these can impact product quality in the insect cells utilizing baculovirus. Therefore, it is important to understand and solve for these differences early during development if the baculovirus production system is the platform of choice.

Productivity and Yield: The yield for the transient transfection systems range between 1E+13 to 1E+14 vector genomes per liter (vg/L). This range depends on the cell type, the serotype, and the transgene being produced. The antici-pated yield for all other systems is greater than 1E+14 vg/L at harvest. Yield for the producer cell line is dependent on the transgene used. The highest productivity is observed for the Sf9/baculovirus system with the production being between 5E+13 to 5E+14 vg/L. However, productivity in the baculovirus system is highly influenced by the transgene and whether the AAV genome is single-stranded or self-complementary.

AAV production case studies

The first case study, which is intended to demonstrate AAV production at VVS, Thermo Fisher, is based on the baculovirus helper system. In the first study, the VVS team used a scaled-down model utilizing the ambr® 15 system to develop higher production for the Sf9/baculovirus system followed by linear scale-up studies up to 2,000 L and showed equivalent yields and a comparable quality target product profile (QTPP) regardless of scale. The team used the scale-down model to optimize production parameters as compared with baseline data established by the team for the baculovirus system.

The ambr® 15 system is approximately 7,000 times smaller than a 100 L STR v/v. To identify the optimal conditions that promote increased AAV production compared to the current process, several aspects were examined, specifically cell density at infection and the impact of cell lysis on productivity (Figure 7). Based on the data, the highest increase in vector genomes was observed when the cells were infected at a cell density of 2E+06 vc/ml. There was no significant increase in titer as a result of cell lysis for this particular serotype and under these conditions. However, there are other serotypes where an increase in titer of less than 30% was observed as a result of detergent lysis.

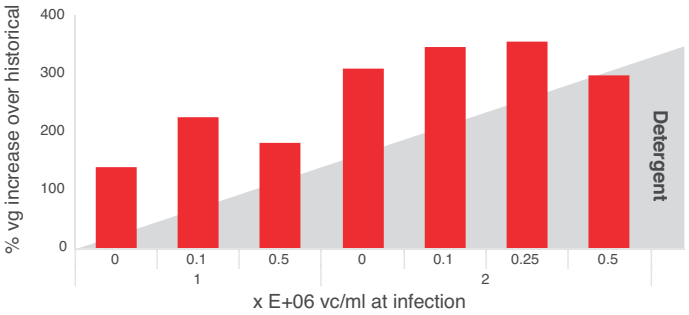


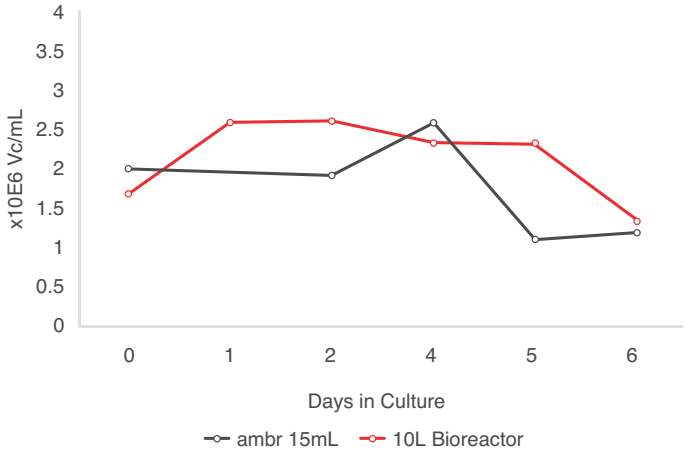
Figure 7: The impact on AAV production was evaluated by varying viable cell density targets at the infection step along with addition of cell lysis inducing detergent at different concentrations. Although the higher viable cell density condition evaluated during this experiment showed increased AAV viral genome titer, using a cell lysis method did not significantly change AAV production across all conditions for this particular serotype. Outlined here are a few process conditions that can be assessed during optimization for AAV vector yield, but results can vary depending on serotype and platform used.

In the second case study, scale-up conditions from ambr® 15 up to a 100 L process were examined. The differences in productivity for population virus seed versus clonal virus seed stocks as starting material were also evaluated. Figure 8 shows comparable viable cell densities as well as the percent viability of Sf9 cells in culture over six days post infection.

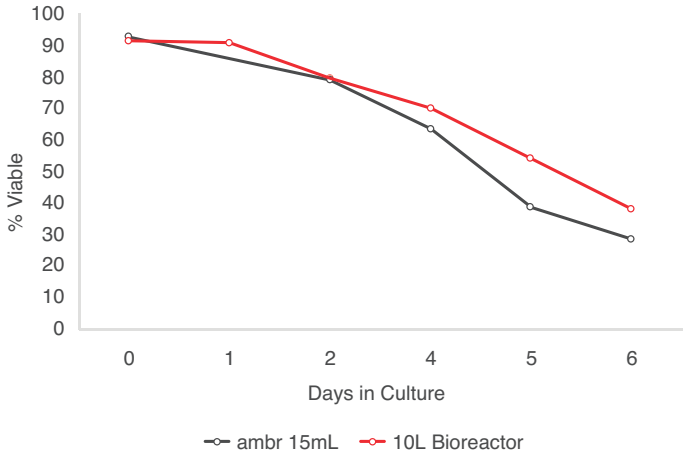
Additionally, both the ambr® 15 and the 100 L processes showed comparable productivity at harvest. As expected, a comparable five-fold increase in titer at both scales (40 L and 100 L) tested was observed when a clonal virus seed was used to infect the Sf9 cells (Figure 9).

For commercial manufacturing, the baculovirus process was scaled up to 2,000 L. Figure 10 is representative data from a single run at 2,000 L and trended to compare data from 2 L, 200 L, and 400 L scales. The viable cell densities and percent viability post infection were comparable to each other regardless of scale.

Additionally, the productivity at harvest was comparable at all scales and overall yields for the process were similar (as shown in Figure 10). Other critical quality attributes, including infectivity and process residuals (residual host cell DNA, baculovirus DNA and host cell protein), were also evaluated and shown to be comparable regardless of scale.

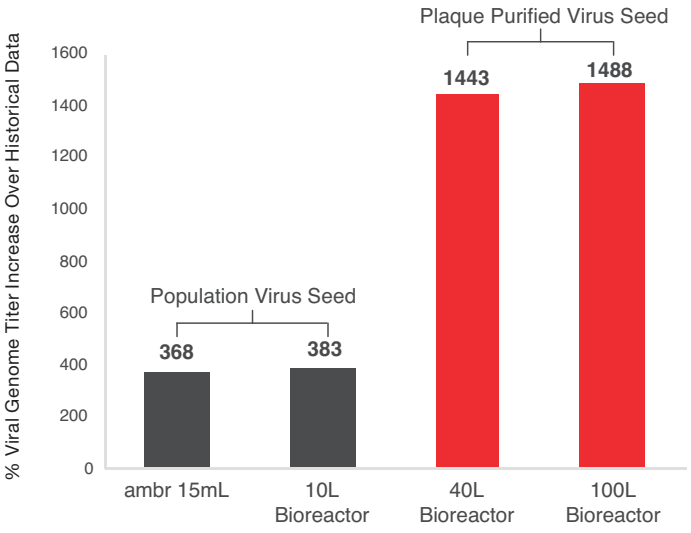


a. sf9 Viable Cell Density Comparison: ambr(R)15 and 10L Scale



b. sf9 Cell Viability Comparison: ambr(R)15 and 10L Scale

Figure 8: Comparison of Sf9 viable cell densities and percent viabilities at different scales. a) Cell growth trends observed in the ambr 15mL scale using Sf9 cells were replicated using a 10L stirred tank bioreactor with comparable results, indicating robustness of the process at micro and bench scale. b) Cell viability profiles of Sf9 cells were nearly identical at both scales further supporting the scalability of this process along with the application of micro scale systems such as the ambr®15 technology for process development.



Viral Genome Titer Comparison: Scale up and Viral Cloning

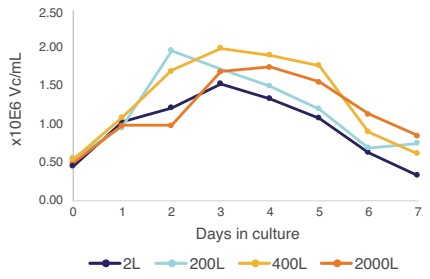
Figure 9: Viral genome titer comparison across various production volumes indicates robust scalability of the same Sf9 process from bench to large manufacturing scale. Higher AAV vector productivity can be achieved upon clonal purification of the Baculorvirus seed stock.

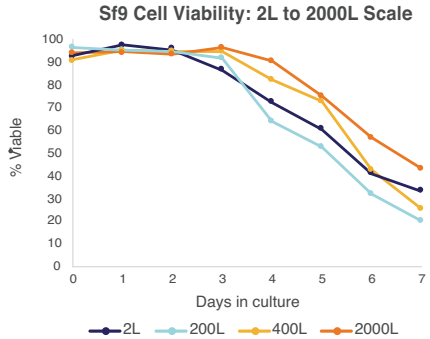
Summary

Although multiple manufacturing platforms are available for AAV production, the critical quality attributes of a product, its clinical needs, the cost of goods, and targeted speed to market ultimately determine the most appropriate choice for a customer's project. Patheon Viral Vector Services utilizes both mammalian and insect cell systems to manufacture AAV. Our team continues to improve our upstream and downstream processes and technologies, as driven by market need.

In addition, while we recognize analytics are still limited, our focus is to develop more sensitive, accurate, and precise methods. These approaches must continue to be QC friendly so that we can quickly look at our specification and out-of-trend aspects in order to address analytical needs and release criteria. The case study presented in this paper shows not only our ability to demonstrate consistency in productivity and CQAs during scaleup to a commercial process but also how Thermo Fisher can leverage its experience, technology, cell lines, equipment, products, and logistics to address complex customer needs.

a.





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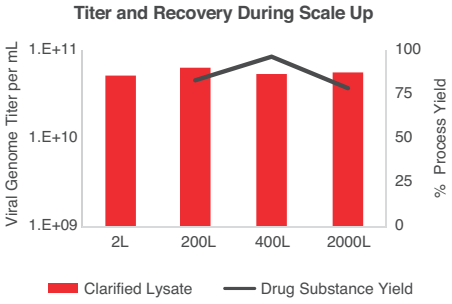

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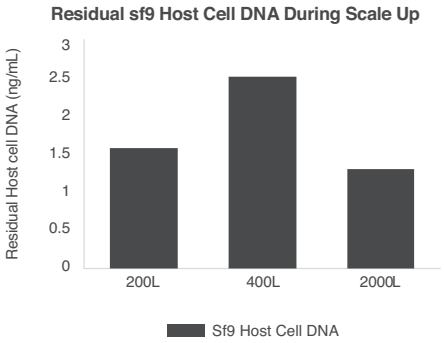

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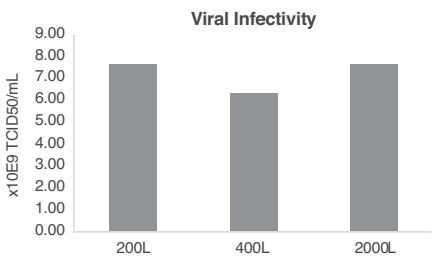

e.

Production Scale	Drug Product Sf9 Host Cell Protein Levels (ng/mL)
200L	<LOQ
400L	<LOQ
2000L	<LOQ

LOQ = limit of quantification

Figure 10: Consistent performance observed during scale-up of an Sf9-based AAV production process from 2 L to 2,000 L. a) Sf9 cell density and viability data showed comparable trends across various scales while maintaining target process ranges b) High and consistent AAV vector production and process yields achieved throughout all variables c,e) Vector purification steps involved in removing process impurities such as host cell DNA and protein were not impacted by scale. d) Comparable TCID50 levels obtained up to 2000L scale suggesting that AAV vector produced maintains infectivity attributes.

About us

Thermo Fisher Scientific provides industry-leading pharma services solutions for drug development, clinical trial logistics and commercial manufacturing to customers through our Patheon brand. With more than 55 locations around the world, we provide integrated, end-to-end capabilities across all phases of development, including API, biologics, viral vectors, cGMP plasmids, formulation, clinical trials solutions, logistics services and commercial manufacturing and packaging. We give pharma and biotech companies of all sizes instant access to a global network of facilities and technical experts across the Americas, Europe, Asia and Australia. Our global leadership is built on a reputation for scientific and technical excellence. We offer integrated drug development and clinical services tailored to fit your drug development journey through our Quick to Care™ program. As a leading pharma services provider, we deliver unrivaled quality, reliability and compliance. Together with our customers, we're rapidly turning pharmaceutical possibilities into realities.

Samira Shore

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Samira Shore is the Director of Technical Program Design at Patheon Viral Vector Services North America (VVS-NA), part of Thermo Fisher Scientific. In this role she oversees the team involved in providing technical solutions and options that assist clients in achieving their viral vector development, production requirements, clinical needs, regulatory filing strategy and business goals for all of the different classes of cell and gene therapies and different viral vector types.

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