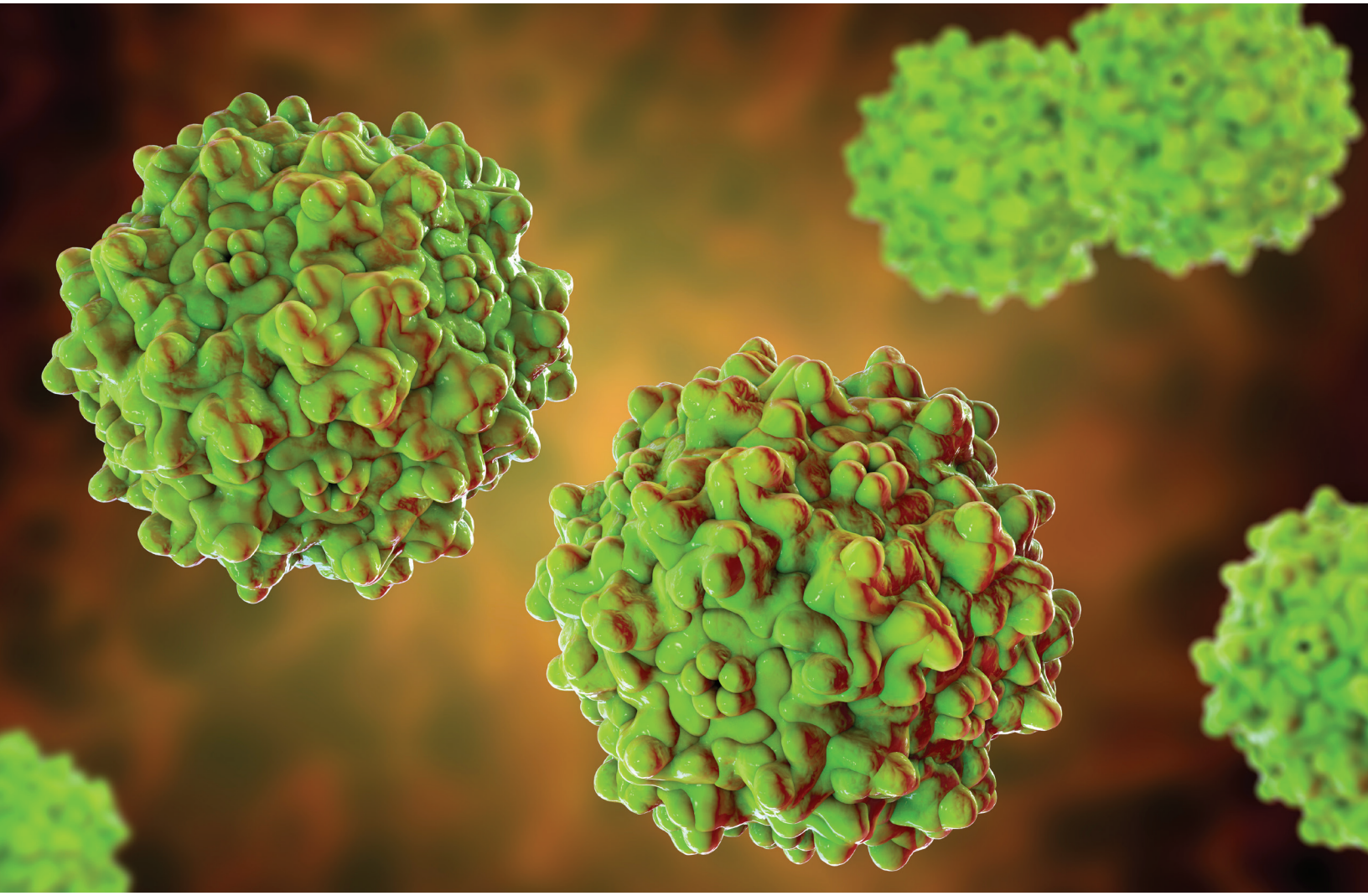
WHITE PAPER

Viral Safety in AAV Production: How Affinity Chromatography Effectively Contributes



A SPONSORED PUBLICATION FROM
GEN Genetic Engineering & Biotechnology News
ThermoFisher SCIENTIFIC

Viral Safety in AAV Production: How Affinity Chromatography Effectively Contributes

An efficient and scalable affinity purification solution, POROS™ CaptureSelect™ AAVX affinity chromatography resin contributes to the overall viral clearance in a process.

Adeno-associated virus (AAV) vectors, the workhorse for gene therapy delivery, have been administered in an estimated 250-300 clinical trials.[1] To date, two gene therapy medications are approved–Luxturna for patients with confirmed biallelic *RPE65* mutation-associated retinal dystrophy and Zolgensma for children less than 2 years old with spinal muscular atrophy (SMA). An anticipated surge in AAV-enabled gene therapy clinical trials is expected to drive increased demand for vectors along with the need for reliable and scalable manufacturing platforms.

Affinity resins, such as POROS™ CaptureSelect™ AAV resins, represent some of the more efficient and scalable purification tools available to purify AAV vectors. These resins reduce the number of purification steps, maximize productivity, and offer scalability and process consistency at commercial scale.

POROS™ CaptureSelect™ AAV8 resin and POROS™ CaptureSelect™ AAV9 resin are serotype-specific to AAV8 and AAV9, respectively, while the POROS CaptureSelect AAVX resin is a universal capsid affinity resin covering all naturally occurring serotypes known to date, as well as engineered and chimeric versions tested.

"The POROS CaptureSelect AAVX resin enables a paradigm shift in viral vector downstream purification," said Nicolas Laroudie, Senior Field Application Specialist, Purification, Thermo Fisher Scientific. "The versatility of this chromatography resin opens opportunities for its use as a platform product." In addition, free conjugated ligands in biotinylated and HRP formats are available for the development of in-house total capsid quantification ELISAs.

Decomplexifying Downstream Purification

The process of disrupting production cells for vector release, combined with the multiplicity of upstream production systems, results in a large range of impurity profiles in the initial feedstock and increases purification complexity. AAV capsids are less than 0.1% of the total proteins generated by the cells.

To make AAV purification more straightforward, affinity chromatography is often used as the first chromatography step in the downstream process. POROS CaptureSelect AAVX resin is a robust, scalable tool that provides high capacity, high specificity, and high throughput to obtain product at high yield and purity.

By immobilizing a proprietary recombinant V_HH fragment, derived from a single-domain antibody, onto the highly

permeable POROS™ backbone, the resin achieves a fine-tuned specificity for AAV recognition with an increased surface area and high capacity for binding.[2]

"The physical properties of the resin result in a fully linear pressure flow curve making scaleup predictable in terms of back pressure," said Laroudie. "Different flow rates have no significant impact on binding capacity or elution, enabling process flexibility and a reduction in processing times." Greater than 80% vector recovery has been reported on scales from 10-2000L.

The robustness of POROS CaptureSelect AAVX resin was demonstrated by purifying AAV6 from two production systems, HEK293 and Sf9 cells. The resin showed high dynamic binding capacity for both feed streams resulting in high yields independent of the production system. Similar to other chromatography resins, the affinity resin can be used multiple times for the same serotypes with negligible AAV carryover.

Because of its high specificity and its affinity towards the broadest range of serotypes, the use of POROS CaptureSelect AAVX resin for platform manufacturing simplifies the downstream process. The resin binds greater than 10^{13} vg/mL of resin allowing the use of smaller columns and reducing the manufacturing footprint.

"Optimization produces the most robust process possible," said Laroudie. "Specialists are available to assist in optimizing parameters to achieve easy, predictable scaleup."

Ensuring a Safe Product

One elusive aspect of AAV process development is viral clearance. According to the ICH Q5A guidance all biotechnology products derived from cell lines of human or animal origin must demonstrate that the products are safe by implementing appropriate testing and demonstrating that the manufacturing process is capable of clearing any endogenous or adventitious viruses.

"Manufacturers need to demonstrate that they are providing a safe product through a robust process," said Alejandro Becerra, PhD, Principal Applications Scientist, Global Purification Technical Lead, Thermo Fisher Scientific.

Prevent, detect, and remove are the three tenets of viral safety programs. Virus-free cell lines, control of raw materials, and the use of closed-process steps and single-use systems minimize the entry of adventitious viruses into the process. As it is impossible to guarantee prevention of adventitious viruses from entering the process, detection tests monitor critical points of the production process in cell banks, harvest, and bulk drug substances. However, assays have detection limits, so it is crucial that the process is capable of removing residual viruses.

"In the AAV field there is limited information in the public domain on the subject of viral clearance specifically focusing on the purification processes," said Becerra. "This was an opportunity for us to contribute knowledge."

Four companies, REGENXBIO, Texcell NA, MockV Solutions, and Thermo Fisher Scientific, joined forces to assess the adventitious virus removal capability of a two-step chromatography process that began with affinity capture using POROS CaptureSelect AAVX followed by anion-exchange polishing step.[3,4]

Designing the Viral Clearance Study

The viral clearance study employed REGENXBIO's production process for AAV8 using HEK293 cells adapted for suspension culture and a triple transfection process without helper viruses. The purification work was performed at the Texcell NA specialized facility.

As stated earlier, AAV capsids are less than 0.1% of the proteins generated by the cells. The main product impurities are the host cell proteins, DNA/RNA, plasmid DNA, cell culture medium components and supplements. POROS CaptureSelect AAVX resin was used to remove these impurities.

Viral spiking studies evaluate removal of viruses by

downstream steps such as chromatography. For these studies, concentrated stock virus solutions are spiked into the load, and the purification step is executed under scaled-down conditions representative of the manufacturing scale.

Both the load and the step product are evaluated for viral titers to determine the LRV (log reduction value)–Log 10 of the ratio of viral concentration in the load compared to the viral concentration in the product. Due to assay variability, LRV <1 is considered negligible and cannot be counted towards the overall viral clearance. LRVs 1-3 are considered contributing and LRVs ≥4 reflect a robust viral clearance step.

Spiking viruses were propagated and purified by Texcell NA according to standard protocols. For DNA viruses, the study used enveloped pseudorabies virus (PRV) and non-enveloped minute virus of mice (MVM); for RNA viruses the enveloped xenotropic murine leukemia virus-related virus (XMuLV) and non-enveloped reovirus type 3 (Reo-3) were used. Since a HEK293 human-derived producer cell line was used for upstream production, two human viruses, hepatitis A (HAV) and herpes simplex virus 1 (HSV-1), were added based on the risk assessment of possible operator sources of contamination.[3]

To determine viral clearance across the affinity purification step, viral concentration in the load and elution were measured by infectivity for non-enveloped viruses or by qPCR for enveloped viruses. AAV8 elutes at a low pH, and low pH elution inactivates the enveloped viruses; therefore, an infectivity assay is not suitable for demonstrating that the chromatography step removes enveloped viruses.

The chromatography step evaluated performance at the target manufacturing parameters as well as worst case conditions. The worst-case conditions were selected as high load ratio, challenging the column with a higher viral load, and high load and elution residence time to allow viruses more time to bind to the resin during load or to dissociate from the resin into the product fractions during elution. Worst case conditions were evaluated separately and in combination.

Although, theoretically, the spiked viruses should not bind to the affinity ligand and would flow through the column, mass balance testing of all fractions determined where the viruses were going during the chromatography step.

Another set of experiments evaluated product-virus interactions by using an AAV null load that was generated by collecting the non-bound fraction of the affinity column run, basically the same matrix and impurity composition as an affinity load but without product. The AAV null load was then spiked with viruses and purified, and the LRV compared to the control run with AAV capsids.

To assess nonspecific interactions between the viruses and camelid V_HH ligand or POROS™ beads, control POROS™ resins were custom made by Thermo Fisher with identical base beads to the affinity resin but incorporating either an alternative V_HH ligand specificity (nonbinding for AAVs) or no V_HH functionalized ligand. For this experiment, due to capacity constraints, only the most commonly-used viruses in viral clearance studies, XMuLV and MVM, were used. XMuLV represents the large enveloped retro viruses and MVM the small, hardy, non-enveloped Parvo viruses.

Viral Clearance Results

"The study achieved a clearance of greater than 3 LRVs for most of the viruses tested, particularly for the center-point condition," said Becerra. "To put this into context, in the mAb field using Protein A affinity chromatography resins the viral clearances are usually lower than what we observed, typically 1–3 logs. The POROS CaptureSelect AAVX resin had a viral clearance higher than the most commonly used affinity resin in bioproduction under the process conditions tested."

The POROS™ CaptureSelect™ AAVX columns showed robust clearance at target manufacturing parameters.

Reo-3 and HSV-1 were the only viruses with observed clearances below 4 LRV. However, they still showed significant viral reduction by the affinity resin (*Table 1*).

For the worst case conditions the level of viral clearance remained the same when the flow rate was increased. When 33% more was loaded onto the column, the clearances were reduced slightly but even at conditions of combined higher load and reduced flow rate the observed viral clearances were essentially the same, within 1 LRV except for XMuLV which was 1.8 LRV, of the clearances achieved under the control run (*Table 1*).

The AAV null run containing the spiked virus showed similar clearances compared to the control run indicating minimal, if any, interactions between the capsid and the viruses. The non-significant effect of the presence of AAV capsids on the LRVs suggests the possibility of bridging viral clearance results generated with one AAV8-based product to other AAV8-based projects (*Figure 1*).

The experiments testing the nonspecific interactions to the V_HH ligand and the POROS bead showed clearances similar to the control run. No nonspecific interactions were found. The mass balance results also were in line with expectations. The majority of the viruses were in the flow through, some were detected in the first wash, and the amount of the virus actually binding and remaining on the resin was less than 1%.

"The findings of the viral clearance study suggest that the POROS CaptureSelect AAVX resin contributes to and can be used for the overall viral clearance in a process," said Becerra. The study demonstrated that robust clearance of XMuLV, MVM, HAV and PRV viruses could be achieved at REGENXBIO's target manufacturing conditions for AAV8.

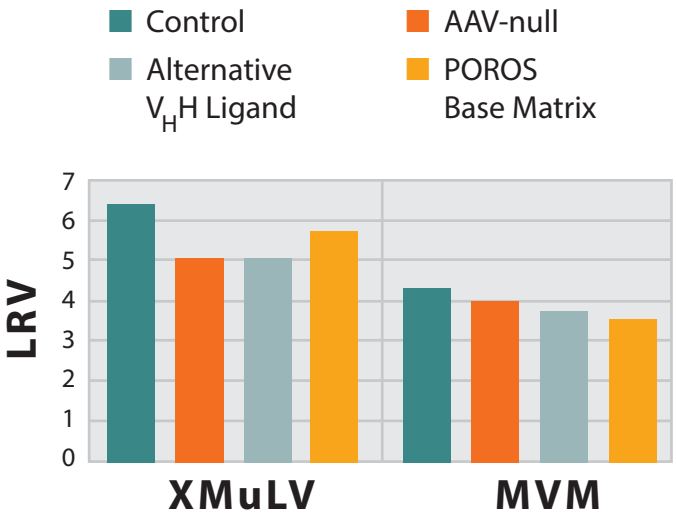


Figure 1. Characterization of nonspecific interactions based on log reduction values (LRVs) for xenomorphic murine leukemia virus (XMuLV) and minute virus of mice (MVM).[3]

Virus name	xMuLV	MVM	Reo-3	HAV	PRV	HSV-1
Virus type	RNA env	DNA non env	RNA non-enveloped		DNA enveloped	
Centerpoint process conditions	>6.4	4.4	2.7	>4.9	4.0	3.1
Worst case conditions (high load ratio and residence time)	4.6	3.6	2.5	5.0	3.8	3.6
Clearance level	Effective	Effective	Contributing	Effective	Effective	Contributing

Table 1. Viral Clearance Results using six various model viruses. Clearance was tested at standard manufacturing conditions and under worst case conditions.

This is especially important for MVM because chromatography is one of the few options to clear this virus. MVM belongs to the same virus family as AAV. It is approximately the same size, and it is highly resistant to inactivation steps such as incubation, detergent, or low pH conditions.

The MockV MVM Kit

"Most companies do not have the capability to work with viruses since they require special containment laboratories, such as those at Texcell NA," said Becerra. The MockV™ MVM kit uses a non-infectious MVM surrogate (MVM-MVP) that mimics MVM's physiochemical properties and can be used in any BSL-1 laboratory. The LRVs are quantitated using immuno-qPCR.

The kit is a useful tool to make an assessment of the viral clearance in a process before a CRO performs the enabling studies. "Using it for an AAV process was novel but the mock particles have demonstrated similar correlations with multiple types of unit operations in mAb purification processes," said Becerra.

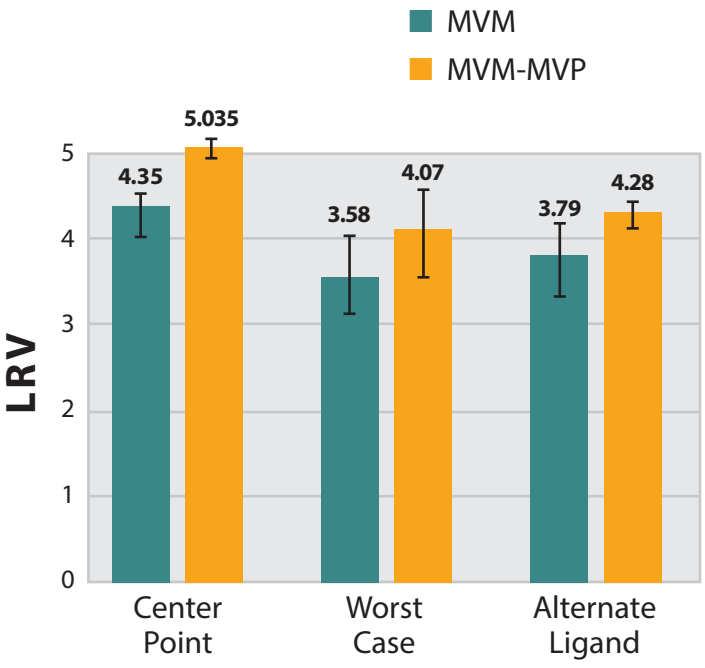


Figure 2. Log reduction value (LRV) determinations for affinity resin runs spiked with minute virus of mice (MVM) and noninfectious MVM mock virus particles (MVM-MVPs).[3]

The study showed comparable LRVs using MVM and MVM-MVP under center-point conditions, worst-case run conditions and alternate ligand runs (*Figure 2*). This demonstrated the utility of MVM-MVP as an analysis tool for AAV process development and characterization.

Conclusion

The results of the viral clearance study demonstrate the contributions of the POROS CaptureSelect AAVX resin to the clearance of adventitious viruses during AAV downstream purification and its applicability as a platform purification product. The universal capsid affinity resin showed robust viral clearance at target manufacturing parameters, particularly for the center-point condition, and no interactions between the viruses and the AAV capsids or nonspecific resin interactions.

References

1. Brooks PJ, Ottinger EA, Portero D, Lomash RM, Alimardanov A, et al. *Human Gene Therapy*. Oct 2020, 1034-1042.

2. Terova O, Hermans P, de Rooij J, Detmers F, *Cell Gene Therapy Insights* 2018; 4(2), 101-111.

3. Winkler M, Goldfarb M, Weng S, Smith J, Wexelblat S, et al. Viral Clearance in a Downstream AAV Process: Case Study Using a Model Virus Panel and a Noninfectious Surrogate. *BioProcess International*. April 2021.

4. Viral Safety in AAV Production: How Affinity Chromatography Effectively Contributes, GEN webinar, May 2021, https://www.genengnews.com/resources/webinars/viral-safety-in-aav-production-how-affinity-chromatography-effectively-contributes/