Development of a Comprehensive and Risk-Based Viral Safety Assurance Strategy for the Manufacturing of AAV Gene Therapy

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Tenaya's Manufacturing Capabilities

48K
square foot facility with ~50K square feet for expansion

~45
FTE in-house team conducting Process Development, Analytical Development, Quality Control

Non-GMP thru cGMP Productivity

- IP and know-how to enable scale from starting materials to large (> 5000L) bioreactors
- Maintenance of high potency from small to large volumes
- Consistently high purity vector production

Analytical and Assay Development

- Robust internal development of assay to support DS, DP release
- FDA supports Tenaya CMC strategies (Type B meeting 2021)

Ongoing Optimization Efforts

- Development and validation of proprietary technologies to increase yield in Sf9 and HEK293 systems

Abstract

The risk of viral contamination, arising from either the starting materials or the adventitious agents introduced during the manufacturing process, is an inherent feature in all biological products and can have serious consequences during clinical evaluation. Relative to biologics such as monoclonal antibodies or recombinant proteins, assuring the viral safety of gene therapy products is a complex process, as drug products are also viral vector particles. Therefore, having a comprehensive risk-based strategy to ensure viral safety, without impacting the identity, strength, quality, purity, and potency of the viral vector, is important to ensure patient safety.

Tenaya Therapeutics, Inc. (Tenaya) has developed a consistent and scalable purification process for the production of adeno-associated virus (AAV) gene therapy made using Sf9/recombinant baculovirus for certain genetic heart diseases. Utilizing its purification process, Tenaya is able to demonstrate robust viral clearance, in terms of Log Reduction Values, this approach includes product- and process-relevant specific and non-specific model viruses and provides adequate assurance that AAV drug product is free of viral adventitious agents with an appropriate level of safety margins.

Viral Safety Approach

Viral Safety plays a critical role for complex cell and gene therapy products that require specific approaches. How do we effectively ensure viral safety in our products when the drug is a virus?

- The ICH Q5A and CMC guidelines provides the requirements for viral safety for biotherapeutics. An aspect of AAV process development is viral clearance
- Viral clearance validation is a key regulatory requirement governing all recombinant drug substances and drug products. According to these guidelines, the risks of viral contamination should be assessed by a three-pronged approach or the safety tripod.

Figure 1: The Safety Tripod

How to Develop a Robust Viral Safety Strategy for Gene Therapies (https://www.labiotech.eu/partner/viral-safety-strategy-gene-therapy/)

Figure 1: Safety Tripod

- **Prevention:** Potential sources of virus contamination (cell lines, cell banks, media components, etc.)
- **Detection:** Testing materials and products for the presence of contaminating viruses
- **Removal:** Developing and conducting a phase-appropriate viral clearance study

Viral Clearance Development

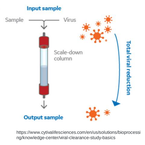
Viral clearance aims to assess process steps that can be considered effective in inactivating/removing viruses and estimate quantitatively the overall level of virus reduction.

- A phase-appropriate study should be developed using a safety by design approach
- As the product advances, the process should demonstrate clearance of a variety of model viruses

Figure 2: Phase Appropriate Viral Clearance Development

A.

B.



https://www.cytivalifesciences.com/en/us/solutions/bioprocessing/knowledge-center/viral-clearance-study-basics

C.

Virus Panel	
Specific	Non-specific
Enveloped	Non-enveloped
DNA viruses	RNA viruses

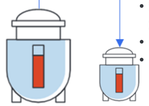
Figure 2: Phase Appropriate Viral Clearance Development. **(A)** Viral clearance timeline **(B)** Example of a viral clearance spiking study **(C)** Considerations for virus panel

Scale-down Model Development

- An important aspect of the viral clearance study is to design a scale-down model that is representative of the clinical or commercial process.
- The performance of the scale-down model should match the large-scale product and process
- Process outputs of the manufacturing scale process needs to be compared to the scale-down

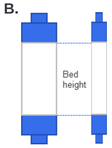
Figure 4: Scale-down model for an upstream and downstream process

A.



Parameters:
- Solvent/detergent (pH, heat)
- Mixing time
- Mass and heat transfer

B.



Column Parameters:
- Same bed height
- Same linear flow rate (cm/hr)
- Residence time
- Loading density

Scale-down model criteria will depend on the process and product

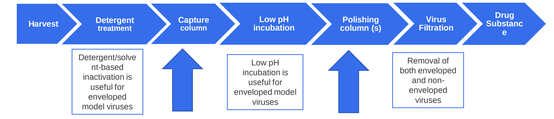
- Product quality attributes
- Key performance indicators
- Other characteristics

Viral Clearance for Gene Therapy

- Viral clearance for AAV's cannot use the same strategies as (MAbs), such as low-pH inactivation and nanofiltration.
- Tenaya has developed a risk-based, chromatography-based process to demonstrate sufficient viral clearance.
- An effective process is composed of either multiple inactivation steps, multiple separation steps, or both (inactivation, chromatography, virus filtration).
- The virus reduction is measured in a logarithmic value (LRV) and the Overall LRV is the sum of individual step LRV.
- A LRV is greater than 4 is considered effective
- A LRV value of <1 is considered negligible and not effective at removing and/or inactivating viruses.

Figure 4: Viral Clearance Strategy for AAV Products

A.



B.

Cumulative Virus Reduction	Model Virus 1	Model Virus 2	Model Virus 3
	>20 LRV	>20 LRV	8 LRV

A. Example of a downstream viral clearance study for AAV gene therapy products.
B. Results of a viral clearance study results using the steps in section A. Adequate viral reduction was observed with all three model viruses that were tested.

Conclusion

Viral safety plays a critical role for complex cell and gene therapy products and requires specific approaches. The risk of adventitious agent contamination should be assessed and mitigated through a safety tripod strategy. Regulatory agencies have guidelines on requirements for viral safety of AAV gene therapy products. For viral clearance, a combination of viral inactivation steps, chromatography resins, and nanofiltration should be utilized to acquire an appropriate safety level in the downstream processing. We have demonstrated that viral clearance of AAV gene therapy products is achievable, risk assessment needs to be performed to ensure the relevant safety factor based on a sponsor's process and dose.