Lysis and clarification strategies for AAV suspension processes

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Abstract 748

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Introduction

- The need for high-yield and high-quality adeno-associated virus (AAV) manufacturing is expected to increase as demand for AAV gene therapies grows.
- We are developing a high-yield suspension cell manufacturing platform for AAV vectors that is completely free of animal-derived components.
- Scalable and robust strategies for lysis and clarification are required for our AAV suspension cell manufacturing platform as these are key determinants of vector yield and quality.

Objective

- To evaluate lysis and clarification methods for our suspension cell manufacturing platform to identify approaches that enable production of high-yield, high-quality AAV

Methods

Investigation of different lysis and clarification methods

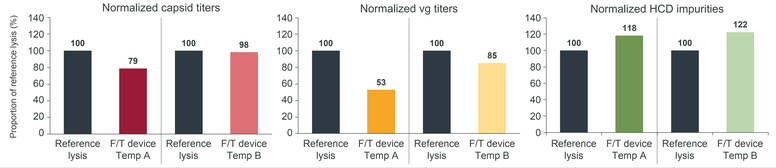
- Different methods for cell lysis were assessed: 1) a freeze and thaw (F/T) device; 2) detergent-induced lysis; 3) salt-induced lysis; and 4) mechanical lysis.
- Capsid titers, viral genome (vg) titers, and encapsidated host-cell-derived DNA (HCD) impurities were analyzed for each method above and were normalized against a “reference method” of three manual F/T cycles at −80°C.
- Synthetic and organic depth filters with different pore sizes were evaluated for impact on recovery and turbidity.

Results

Freeze and thaw device

- The F/T device was tested at two different freezing temperatures using three controlled F/T cycles (**Figure 1**).
- At both temperatures, capsid and vg titers were decreased and there were minor increases in HCD impurities compared to the reference method.

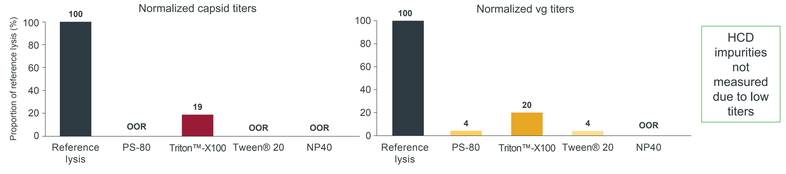
Figure 1: F/T device at two freezing temperatures compared with reference lysis, normalized at 100%



Detergent-induced lysis

- Results from a one-hour incubation with a 1% concentration with each of four different detergents are shown (**Figure 2**).
- With all four detergents, there were substantial decreases in capsid and vg titers relative to the reference lysis method.

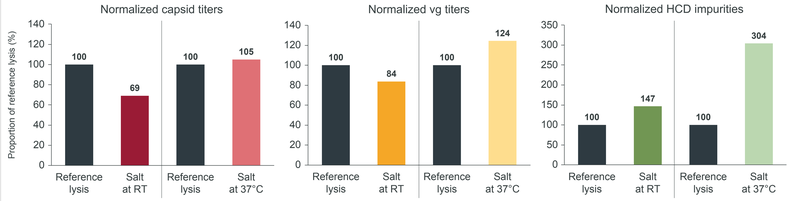
Figure 2: Detergent-induced lysis with four different detergents compared with reference lysis, normalized at 100%



Salt-induced lysis

- High salt concentration was investigated at room temperature (RT) and at 37°C (**Figure 3**).
- At RT, capsid and vg titers were decreased, and HCD impurities increased versus the reference lysis method.
- At 37°C, capsid and vg titers were increased, but HCD impurities were also increased by approximately three-fold versus reference lysis.

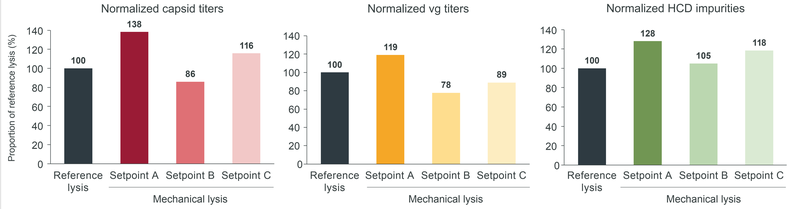
Figure 3: Salt-induced cell lysis at RT or 37°C compared to the reference lysis, normalized at 100%



Mechanical lysis

- Three setpoints were investigated using mechanical lysis (**Figure 4**).
- The highest titers were achieved using setpoint A, though minor increases in HCD impurities were also seen.

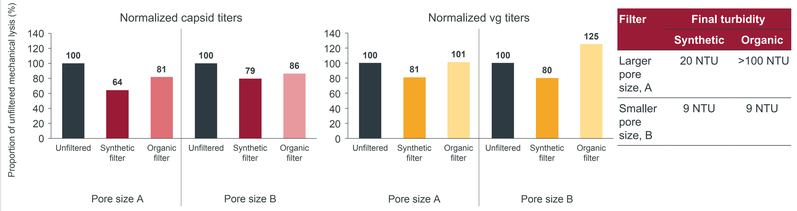
Figure 4: Mechanical lysis at three different setpoints compared with reference lysis, normalized at 100%



Clarification via depth filtration

- Synthetic and organic depth filters with different pore sizes were tested for impact on clarification (**Figure 5**).
- The highest titers were achieved using the organic depth filter and pore size B relative to the control of unfiltered mechanical lysis.

Figure 5: Synthetic and organic depth filters with different micron rating sizes compared with unfiltered lysed material as a control, normalized at 100% and including final turbidity values



Filter	Final turbidity	
	Synthetic	Organic
Larger pore size, A	20 NTU	>100 NTU
Smaller pore size, B	9 NTU	9 NTU

Main advantages and disadvantages of the methods tested and the future research direction

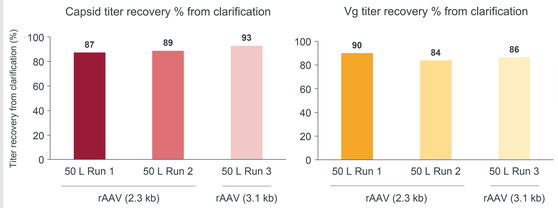
Table 1 Advantages and disadvantages of each lysis method

	Advantages	Disadvantages
F/T device	• Single use • No need for downstream processing (DSP) to remove chemical components	• Lower capsid and vg titers • Substantial time is required to prepare and run the lysis • Limited scalability
Detergent-induced lysis	• Rapid • Scalable	• Very low capsid and vg titers • Requires DSP to remove chemical components • Additional assays necessary to prove detergents have been removed
Salt-induced lysis	• High capsid and vg titers at 37°C • Rapid • Scalable	• High HCD impurities • Requires DSP to remove chemical components • Additional assays necessary to prove salts have been removed
Mechanical lysis	• Higher capsid and vg titers than reference method • No need for DSP to remove chemical components • Rapid • Scalable • High repeatability	• Slight increase in HCD impurities • Not single use, but good manufacturing practice (GMP) compliance can be achieved with adaptations such as cleaning in place (CIP) or steam in place (SIP)

Scalability of mechanical lysis and clarification

- Mechanical lysis followed by a depth filtration step was successfully scaled up to 50 L for two rAAVs with different viral genome lengths (**Figure 6**).
- Recoveries ranging from 80% to 90% were achieved, as well as final turbidity values ranging from 1 nephelometric turbidity unit (NTU) to 5 NTU.

Figure 6: Capsid and vg recoveries from clarification, including final turbidity values



50 L run	#1	#2	#3
Final turbidity	1 NTU	5 NTU	3 NTU

Conclusions

- Our results demonstrate that mechanical lysis followed by depth filtration may enable production of high-yield, high-quality AAV in a suspension cell manufacturing platform.
- Mechanical lysis can generate higher capsid and vg titers compared to a reference F/T lysis method.
  - A minor relative increase in HCD impurities was observed, but absolute levels were low.
  - Mechanical lysis is a scalable and rapid method with high repeatability and no need for the removal of chemical components.
  - Although it is not single use, GMP compliance can be achieved through adaptations to the process via CIP/SIP.
- The best recoveries, assessed by vg titer, were achieved using an organic depth filter with a smaller pore size, which also showed low final turbidity values.
- Scalability of mechanical lysis followed by a depth filtration step was successfully shown for 50 L runs, with recoveries ranging from 80% to 90% and low final turbidity values ranging from 1 NTU to 5 NTUs.

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Disclosures: CW, JB, BA, JW, MH, KH, and AY are employees of Freeline Therapeutics; SH and LH were employees of Freeline Therapeutics at the time the study was conducted.

Abbreviations: AAV, adeno-associated virus; CIP, cleaning in place; DSP, downstream processing; F/T, freeze and thaw; GMP, good manufacturing practice; HCD, host-cell-derived DNA; kb, kilobases; NTU, nephelometric turbidity unit; OOR, out of range; RT, room temperature; SIP, steam in place; vg, viral genome

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