QUANTIFICATION OF AAV CAPSID LOADING FRACTIONS: A COMPARATIVE STUDY

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Introduction

The Therapeutic Context:

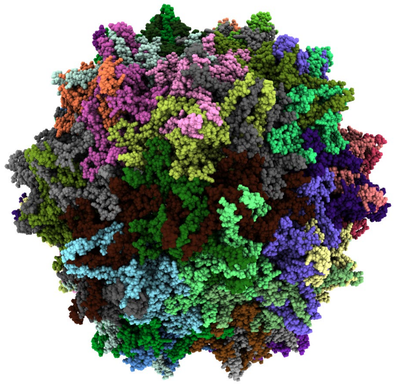
Gene therapy has the potential to treat a wide range of diseases from cancer to cardiovascular disorders. Disease caused by mutated or defective genes can be treated by therapeutic genes which have to be packaged and delivered to the target cells. Recombinant Adeno Associated Virus (rAAV) vectors are one of the most promising mechanisms for therapeutic gene delivery

The Viral Vector Production / Quality Control Challenge:

The production of large quantities of bioactive rAAV particles is fraught with production challenges such as maintaining integrity and maximizing the packaging efficiency of the delivery vehicle. A typical rAAV prep will include bioactive particles/capsids which are fully loaded with the therapeutic gene, as well as undesirable but partially loaded and empty capsids. Identifying and quantifying these different species is vital from a quality control standpoint, since improperly loaded viral capsids do not produce the desired therapeutic effect, but still elicit an immune response.

Solving the Challenge with AUC:

Analytical Ultracentrifugation (AUC) is a native state, solution phase, first-principle technique used to quantitate the amounts of empty, full and partially loaded AAV capsids. Here we present the results of a panel of blind tests on AAVs which were conducted to compare and contrast the empty/full ratio readout from AUC vs. that from Transmission Electron Microscopy (TEM). The AAV samples used are the AAV reference standard materials from Vigene Biosciences. We observe that AUC can potentially have more resolution to distinguish partially vs. fully packaged capsids.



20 nm diameter

Adeno Associated Virus as a Gene Therapy Vector

The Delivery Vehicle:
20 nm icosahedral capsid, mass ~ 3.9 MDa

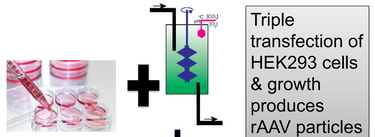
Capsid Composition
60 monomers of proteins
VP1:VP2:VP3 in ratio 1:1:10

Genome Highlights:
WT Genome is 4.7 kbase ssDNA
145 base ITR at each end & 2 ORFs – *rep* & *cap*
In rAAV: Promoter & transgene spliced between ITRs, *rep* & *cap* provided by helper plasmid

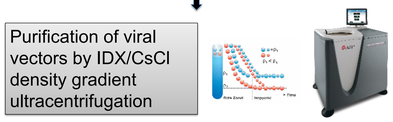
AAV Packing Fraction

AAV production: a general protocol

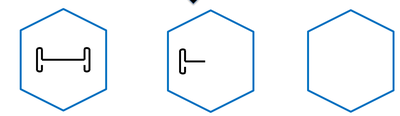
rAAV vector with the Gene of Interest (GOI) produced by co-transfection



Triple transfection of HEK293 cells & growth produces rAAV particles



Purification of viral vectors by IDX/CsCl density gradient ultracentrifugation



Therapeutically effective

Therapeutically ineffective & undesirable

"…viral particles that do not contain the therapeutic gene are unlikely to have therapeutic activity. However, these particles themselves might produce adverse reactions, such as an allergic response…"

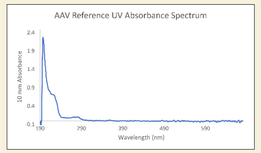
Source: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products Guidance for Industry June 2017

Issued by: Center for Biologics Evaluation and Research, US FDA

Docket Number: FDA-2013-D-0576 →

AUC Workflow

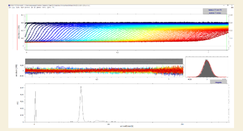
Record AAV UV-Vis Absorption spectrum



Buffer exchange AAV sample by centrifugal concentration if needed

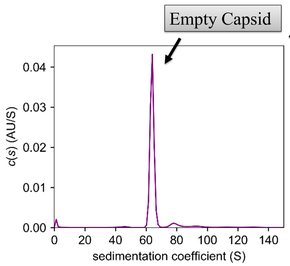
Prepare & load sample. Design expt & view live data via browser

Analyze data to obtain AAV Empty/Partial/Full Ratios



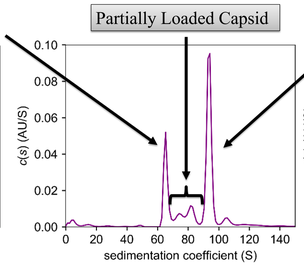
AUC can quantitate AAV Empty/Partial/Full Ratios

Reference Standard # 1



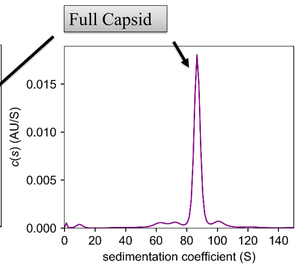
%E = 92.9 ; % P = 4.9; % F = 2.2

AAV8 – 3 kb insert



%E = 24.3 ; % P = 15.9; % F = 55.4

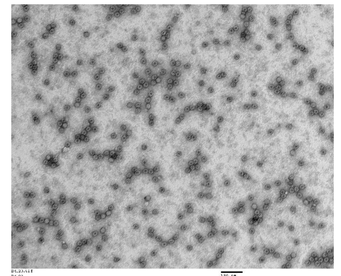
Reference Standard # 11



%E = 6.2 ; % P = 4.9; % F = 88.9

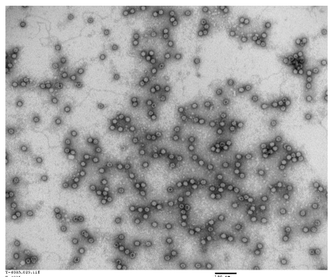
TEM can quantitate AAV Empty/Full Ratios but cannot detect partially loaded capsids

Reference Standard # 1



Manual %E = 86
Image Sorting %E = 87.6

Reference Standard # 11



Manual %F = 60
Image Sorting %F = 74.8

Conclusions:

- AUC experiments provided a native-state, serotype-independent quantification of Empty/Full ratios for the Vigene AAV Reference Standards in a single blind test.
- Analyzed data from AUC is in agreement with orthogonal data from TEM.
- Data from AUC experiments can be analyzed to quantify partially loaded capsids, which TEM cannot discriminate.

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