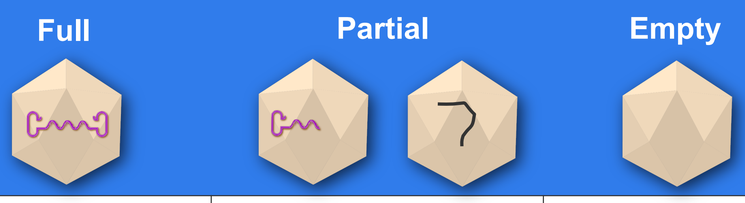
Analytical Characterization of Empty, Partial and Full AAV Capsids

A McColl-Carboni, S Zhou, M MacArthur, R Horton, K Natu, J Gagnon, R Lushi, C Smith, B Burnham, S Dollive, J McGivney

Oxford Biomedica Solutions, Bedford, MA

ABSTRACT

Manufacturing of adeno-associated viral (AAV) vectors is known to produce three types of capsids: empty, partial, and full. While there are different opinions about the impact of empty and partial capsids both on safety and efficacy of AAV gene therapies, they are generally considered impurities because they are not the intended fully intact vector product. The presence of these impurities could impact the efficacy of AAV products due to potential competition with fully packaged AAVs for cellular transduction.



Packaging:	Fully intact VG	Partial VG or host cell DNA	No genome
Therapeutic Effect:	Intended product. Efficacious	Increased risk of immunogenicity? Competition for cellular transduction?	

An rAAV preparation that was known to have a high amount of empty and partial capsids was separated into full (OXBS1-F), partial (OXBS1-P), and empty (OXBS1-E) capsid populations by preparative ultracentrifugation in a cesium chloride density gradient. Full analytical characterization was performed on each population using the methods detailed in the Analytical Toolbox section.

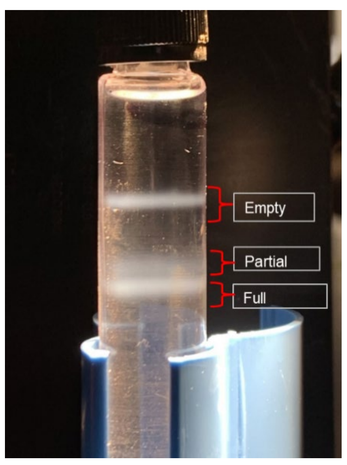
ANALYTICAL TOOLBOX

Oxford Biomedica Solutions has a comprehensive suite of in-house analytical methods to perform full vector characterization.

Attribute Class	Quality Attribute	Method
Content	VG titer	ddPCR
	Capsid titer	ELISA
Product-related impurities	Capsid purity & VP1:VP2:VP3 ratio	CE-SDS
	Percent empty, partial and full	AUC
Process-related impurities	Residual host cell DNA	qPCR
	Residual plasmid DNA	ddPCR
	Residual rep/cap	ddPCR
	Residual E1A	ddPCR
	Residual KanR	ddPCR
	Residual host cell protein	ELISA
	Residual gene expression	mRNA by RT-qPCR (in vitro)
Strength	Infectivity	TCID50 (in vitro)
Potency	Transgene expression	mRNA by RT-qPCR (in vitro)
	Biological Activity / Functional Potency	Product-specific (in vitro and/or in vivo)
Identity	Capsid identity	LC-MS (peptide mapping, intact mass)
	Sequence identity	NGS Sequencing

RESULTS

(1) SEPARATION AND PURIFICATION OF EMPTY, PARTIAL AND FULL AAV CAPSIDS



An rAAV vector lot containing a heterogenous mixture of full (57.4%), partial (26.1%) and empty (16.5%) capsids (**Fig. 2A**) was subjected to bench-scale preparative ultracentrifugation using a cesium chloride gradient to generate distinct bands for each capsid population (**Fig. 1**). The bands corresponding to empty (OXBS1-E), partial (OXBS1-P) and full (OXBS1-F) capsids were extracted individually prior to analytical characterization.

Figure 1. Capsid Fractionation by Preparative Ultracentrifugation. rAAV material was separated into distinct bands corresponding to empty, partial and full AAV capsids by preparative ultracentrifugation using a cesium chloride gradient.

(2) CAPSID CONTENT BY AUC

The first step in analytical characterization was to determine the capsid content purity of each population by analytical ultracentrifugation (AUC). AUC was chosen as it provides sufficient resolution to distinguish between empty, partial and full capsids. The first fraction (OXBS1-F) was enriched for full capsids (91.1%), with a low level of partial capsids (8.6%) (**Fig. 2B**). The second fraction (OXBS1-P) was a mixture of partial (55.8%) and full (43.8%) capsids (**Fig. 2C**). The third fraction (OXBS1-E) was enriched for empty capsids (83.0%), with a low level of partial capsids (17.0%) (**Fig. 2D**).

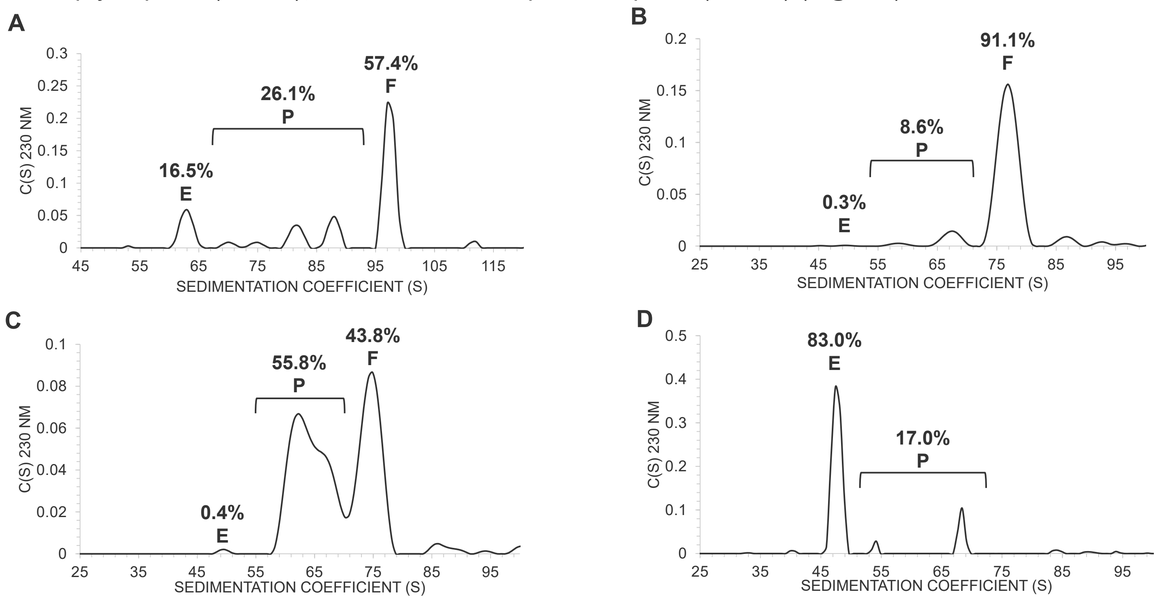


Figure 2. Capsid Content Purity Determined by Analytical Ultracentrifugation. AUC was used to quantify empty, partial and full capsids using an Optima AUC (Beckman Coulter, Brea, CA). A) Unfractionated AAV; B) OXBS1-F enriched for full capsids; C) OXBS1-P enriched for partial capsids; D) OXBS1-E enriched for empty capsids.

Summary: Preparative ultracentrifugation successfully generated capsid populations that were enriched for either full, partial or empty capsids. Therefore, we moved forward with full analytical characterization of the capsid populations.

(3) VECTOR GENOME TITER AND CAPSID TITER

OXBS1-F, OXBS1-P and OXBS1-E samples were formulated at target 3E+13 capsids/mL prior to determination of VG titer and capsid titer (**Fig. 3**). The VG titer for OXBS1-P (43.8% full, 55.8% partial) was comparable to that for OXBS1-F (91.1% full, 8.6% partial), suggesting that partial genomes for this construct contribute to the vector genome of the product.

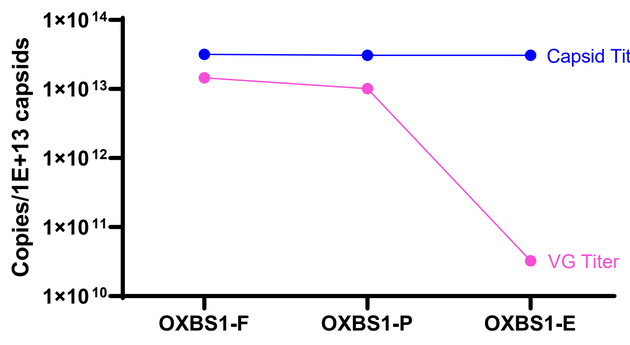
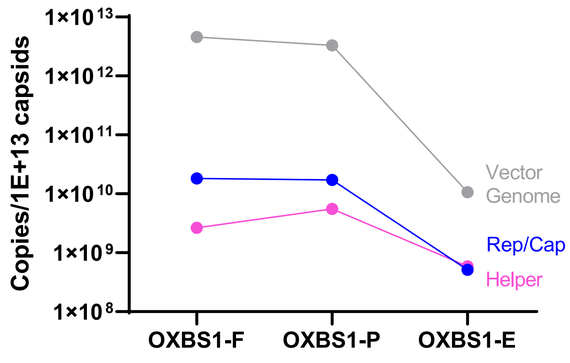


Figure 3. Determination of Vector Genome Titer and Capsid Titer. Vector genome (VG) titer was measured by standard droplet digital polymerase chain reaction (ddPCR). All test samples were treated with RNAse-free DNase I and proteinase K prior to analysis. Processed samples were amplified in ddPCR Supermix (BioRad, Hercules, CA, USA) using primers/probe specific for the gene of interest (GOI). Following the droplet generation via an oil:water emulsification, samples were amplified to the endpoint in a thermal cycler and subsequently scanned on a QX200 droplet reader (BioRad). Data were analyzed with QuantaSoft™ software (BioRad). Capsid titer was determined by commercially-available AAV9-specific ELISA (Progen, Germany) and performed according to the manufacturer's instructions.

(4) PROCESS-RELATED IMPURITIES

The results for rep/cap and helper plasmid process-related impurities are shown in **Fig. 4**. Samples were normalized to capsid titer to generate residuals per 1E+13 capsids. OXBS1-P and OXBS1-F samples had a higher level of residuals when compared to OXBS1-E (OXBS1-P > OXBS1-F >> OXBS1-E).

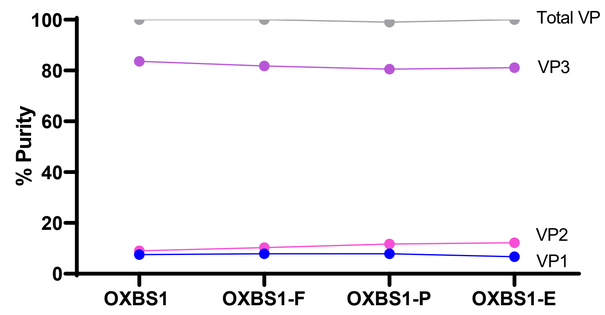


Summary: Process-related impurities were present in both the OXBS1-P and OXBS1-F fractions at 2-3 logs lower than vector genome.

Figure 4. Measurement of Process-Related Impurities by ddPCR. Residual encapsidated Rep/Cap and Helper plasmid impurities, as well as vector genome, were measured by ddPCR. Samples were prepared and analyzed using primers/probes specific to the target sequence. The reported impurities are normalized to capsid titer (copies/1E+13 capsids).

(5) CAPSID PROTEIN CHARACTERIZATION

The AAV capsid is composed of three viral proteins (VP; VP1/VP2/VP3) assembled into an icosahedron at a molar ratio of approximately 1:1:10. Characterization of the VP proteins can offer insights on capsid heterogeneity. The capsid VP components of each fraction were characterized using CE-SDS for capsid purity and % VP1, VP2 and VP3 (**Fig. 5**).



Summary: Full (OXBS1-F) and partial (OXBS1-P) capsid enriched populations had comparable capsid VP purity (100%) and VP1:VP2:VP3 ratio (1:1:10). Empty capsid (OXBS1-E) pool had slightly lower VP1 and slightly higher VP2 resulting in a 1:2:12 ratio.

Figure 5. Capsid Purity Determined by CE-SDS. Vector purity and VP ratio were determined by CE-SDS. Samples, reference materials, and standards were heat-denatured in SDS-containing buffer and then separated on a PA800 Plus (Beckman Coulter, Brea, CA, USA). Proteins were detected spectrophotometrically by absorbance at 220 nm.

CONCLUSIONS

- OXB Solutions has a comprehensive analytical toolbox to perform full vector characterization of AAV gene therapy products.
- Partials contribute to the vector genome titer of the rAAV preparation presented here.
- Full and partial capsids have similar capsid properties and are difficult to separate. Highlights the need for good construct design and purification process in order to achieve a high % full vector profile and the most efficacious AAV product.

CONTACT

Partner with us:
Visit www.oxbsolutions.com to learn more or scan the QR code

Presented at ESGCT, October 2022, Edinburgh UK