A Comparison Study of Orthoganol Assays, Mass Photometry, SV-AUC, and Absorbance, ELISA and dPCR to estimate empty full AAV particles

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ABSTRACT

Mass photometry is a relatively new analytical technology that can measure the mass of individual AAVs in solution. The method uses light-based interference microscopy to determine quality attributes of AAV samples, including empty/full/partial capsid ratios, sample purity, aggregation, and titer. To date, the gold standard for measuring these attributes has been Sedimentation Velocity – Analytical Unltra-Centrifugation (SV-AUC), a technique which uses the buoyant density between particles of various sizes in a heterogenous mixture. While SV-AUC offers unparalleled resolution of various AAV particles (empty/full/partial/overfilled) in a mixture, it is a laborious technique that requires extensive expertise to execute both from an assay set-up and data interpretation perspective. To ensure that robust process parameters are being met, in process sample testing during vector manufacturing needs to be timely, cost effective, and easily achieved, yet still be reliable in providing accurate information. Towards that end, we compared several commonly employed analytical techniques used in the determination of empty/full capsids: ELISA (dPCR VG / ELISA VP), Stunner (dPCR VG / Stunner VP), Absorbance (A_{260}/A_{280} ratio), SV-AUC, and Mass Photometry. Our results indicate that on purified AAV particle, A_{260}/A_{280}, SV-AUC, and mass photometry equally predict similar % Full AAV particles. On the contrary, we found that ELISA-based methods were inadequate and equally were not accurate in % full estimations. Employing Absorbance and mass photometry methods for particle characterization are simple methods that require very little training yet offer outstanding resolution and accuracy. These metrics for assay performance are highly desirable in large scale manufacturing where test sample quantities and volume require the need for significant speed and cost-savings.

RESULTS

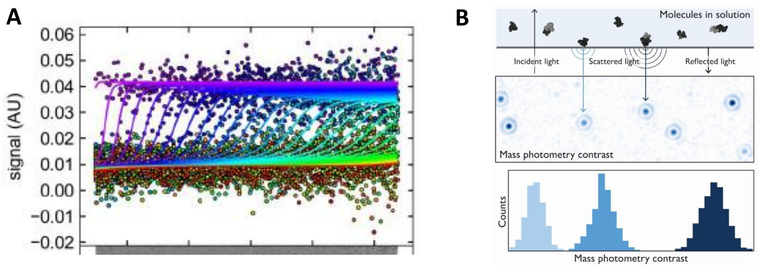
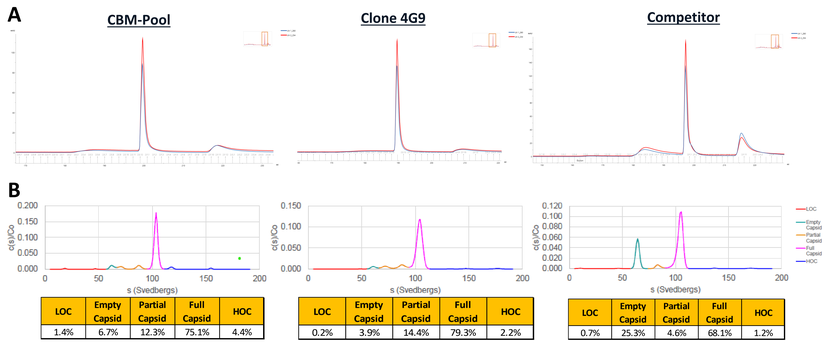


Figure 1. Technology Comparison between SV-AUC and Mass Photometry. A. SV-AUC SV-AUC measures the sedimentation rate at which molecules move in response to the centrifugal force generated in a centrifuge, in solution. B) Mass Photometry measures light scattering of single particles as they adsorb onto a glass microscope slide,

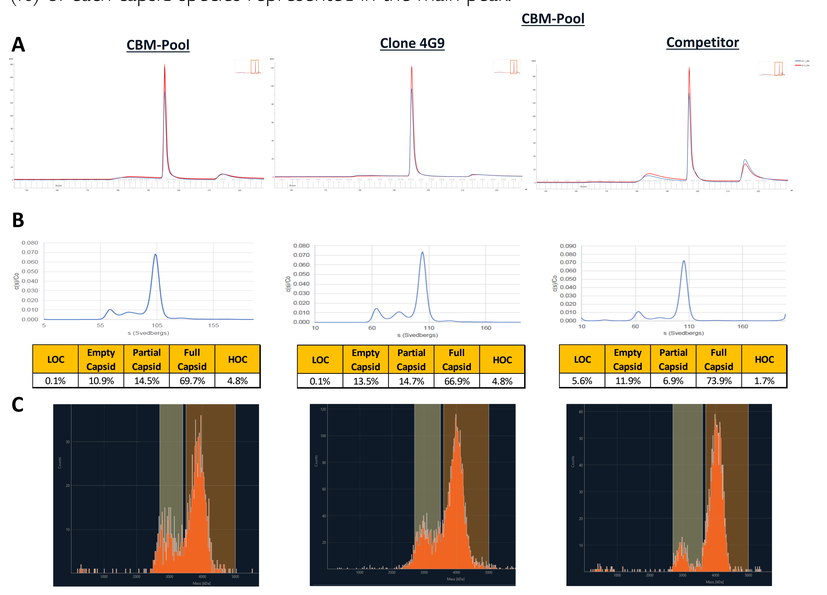


LOC	Empty Capsid	Partial Capsid	Full Capsid	HOC
1.4%	6.7%	12.3%	75.1%	4.4%

LOC	Empty Capsid	Partial Capsid	Full Capsid	HOC
0.2%	3.9%	14.4%	79.3%	2.2%

LOC	Empty Capsid	Partial Capsid	Full Capsid	HOC
0.7%	25.3%	4.6%	68.1%	1.2%

Figure 2. Down-stream processing and SV-AUC analysis. 150mL scale AAV9 produced by triple transfection at molar ratio #1(pHelper:Rep2Cap9:*cis*) was purified by an AAVX affinity resin. A) Affinity eluate material was polished by a 1% increasing salt step-gradient on a Mustang Q column on an AKTA Avant (A_{260}/A_{280} shown). B). Pooled AEX of the predominant peak was subjected to SV-AUC analysis. Table below indicates the (%) of each capsid species represented in the main peak.



LOC	Empty Capsid	Partial Capsid	Full Capsid	HOC
0.1%	10.9%	14.5%	69.7%	4.8%

LOC	Empty Capsid	Partial Capsid	Full Capsid	HOC
0.1%	13.5%	14.7%	66.9%	4.8%

LOC	Empty Capsid	Partial Capsid	Full Capsid	HOC
5.6%	11.9%	6.9%	73.9%	1.7%

Figure 4. Down-stream processing and SV-AUC analysis. Similar to Figure 3, AAV9 was produced by triple transfection at molar ratio #2 (pHelper:Rep2Cap9:*cis*). A) Affinity eluate material was polished on a Mustang Q column using an AKTA Avant (A_{260}/A_{280} shown). B). Pooled AEX of the predominant peak was subjected to SV-AUC analysis. Table below indicates the (%) of each capsid species represented in the main peak. C) Mass photometry histogram for identical pool AEX material used in SV-AUC analysis.

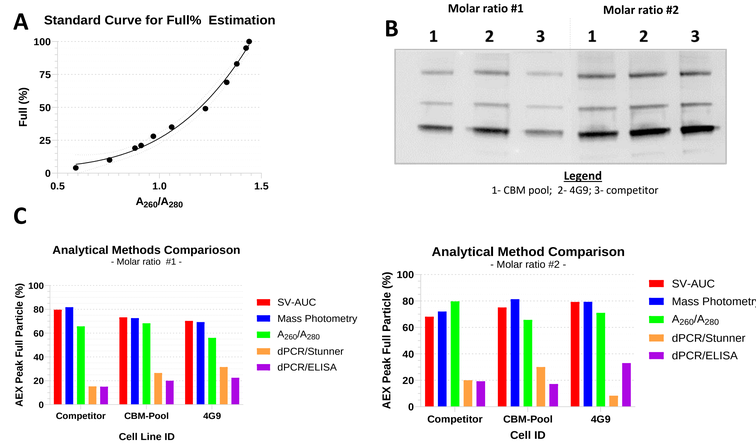


Figure 4. Comparison of Analytical Mehods for % Full Calculation. A) Standard curve used to determine % Full for A260/A280 calculations. B) SDS-PAGE analysis of AEX purified viruses used in this study. C) Orthoganol method comparison to determine % full for ratio #1 (left panel) vs ratio #2 (right panel).

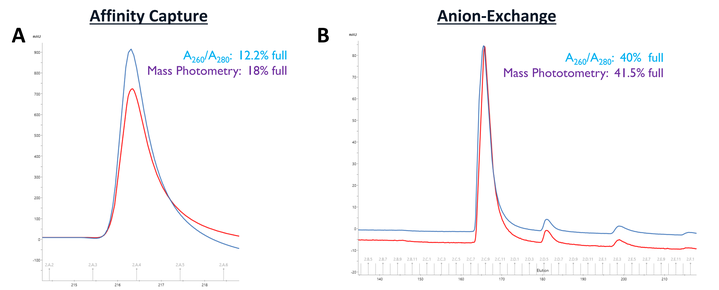


Figure 5. Comparison of Absorbance and Mass Photometry for % Full Calculation. scAAV8 production, under similar conditions as described in Figure 2 and using molar ratio #2 was subjected to A) affinity capture and B) AEX polish and estimation of % full was determined by A_{260}/A_{280} ratio and mass photometry. Asymmetrical A_{280} peak for capture under-estimates the % full. When the peak is symmetrical, Aborbance is highly predictive of accurate % full.

SUMMARY

- Our GMP-ready, internal-derived, serum-free suspension HEK293 clone 4G9 demonstrates comparable performance to commercially available cell lines.
- CBM-HEK293-4G9 is able produce up to 80% full AAV9 particles
- A_{260}/A_{280}, Mass Photometry, and SV-AUC are equally attractive assays for accurate determination of %full. The ease of use for A_{260}/A_{280} offers rapid analytics for in-process testing.