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HYBRID

You don't need affinity to purify rAAV Part 2 & beyond empty/full separation

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Improved analytical methods allow us to better understand the capsid heterogeneity in AAV therapeutic preparations. This better understanding allows us to develop manufacturing processes that produce AAV therapeutics with fewer non-functional capsids. Four different two-column processes at 50-L scale will be compared to our 200-L ultracentrifugation (UC) process for yield, purity, and potency. All five runs were derived from the same triple-transfected, 500-L, HEK-293 suspension-culture. We consider the UC process the gold-standard for functional-capsid purification. Simplified process descriptions for the capture and empty/full (E/F) separation steps are listed below along with the analytical methods used to characterize these drug substance lots.

Process Descriptions:

1. **Capture:** CEX monolith (NaCl gradient), AEX filter (flow-through), **E/F:** UC
2. **Capture:** CEX monolith (NaCl gradient), **E/F:** AEX monolith (NaCl gradient)
3. **Capture:** CEX monolith (pH gradient), **E/F:** AEX monolith (NaCl gradient)
4. **Capture:** CEX monolith (pH gradient), **E/F:** 2-cycle AEX (NaCl Gradient)
5. **Capture:** Affinity resin (pH step gradient), **E/F:** AEX monolith (NaCl gradient)

Analytical comparability of Drug Substances:

1. **Vector yields:** total vg by ddPCR and total capsids by ELISA
2. **Process Impurities:** Host-cell protein, plasmid-DNA, and host-cell DNA
3. **Product Impurities:** Empty, partial, & full capsid content by SV-AUC, CDMS, and Mass Photometry, aggregation by size exclusion chromatography, and capsid protein charge heterogeneity by icIEF
4. **Vector protein purity:** CE-SDS (capillary electrophoresis);
5. **Vector DNA purity:** DNA gel electrophoresis, long-read and short-read NGS
6. **Potency:** *In vitro* eGFP expression

Sedimentation-velocity AUC analysis showed that the UC method and three of four two-column processes produced AAV9 Drug Substances with > 90% full capsids.