Small samples, big insights: Accelerating AAV stability studies with high-quality, low-volume analytics

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Introduction

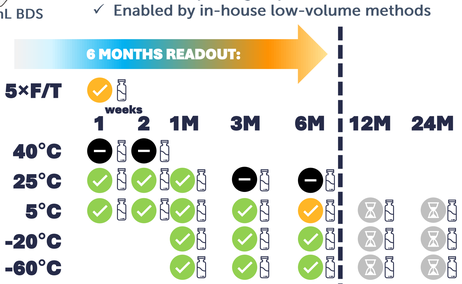
Adeno-associated virus (AAV) products are at the forefront of gene therapy innovation, yet their inherent complexity poses significant challenges for characterization and regulatory compliance. In response, we have developed an extensive portfolio of in-house analytical capabilities tailored to the needs of gene therapy product developers.
To showcase these capabilities, we conducted a stability study that encompasses a comprehensive set of methods and quality parameters.

By employing low-volume techniques such as backgrounded membrane imaging (BMI) and mass photometry, we minimized sample requirements for traditionally resource-intensive assays (e.g., subvisible particles, full-empty characterization). This allowed detailed analysis at earlier time points and extended the study duration to 24 months, using only 25 vials and less than 30 mL of an AAV9 drug product with secreted alkaline phosphatase (SEAP) as the transgene at 2E13 vg/mL stored in industry-standard 2 mL CZ vials with a fill volume of 1.1 mL.

Stability Study Design

< 30 mL BDS

- ✓ < 30 mL AAV9-SEAP bulk drug substance is sufficient to feed a 24-month stability study covering additional time-points < 1 month and encompassing 11 parameters
- ✓ Enabled by in-house low-volume methods

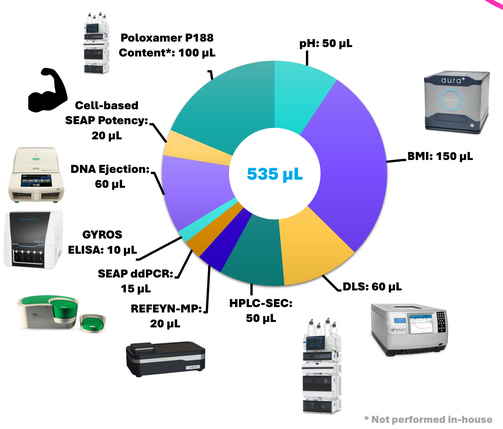


EXTENSIVE STABILITY STUDY ENABLED BY

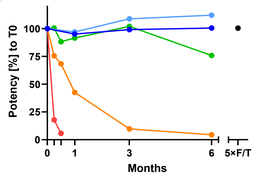
AAV9-SEAP

25 x 2 mL CZ vials
1.1 mL fill volume
2E13 vg/mL

ASCENDS IN-HOUSE LOW-VOLUME METHODS:

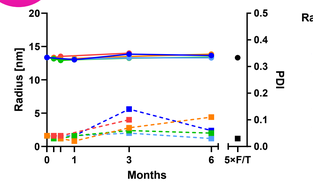


1 Cell-based potency assay (SEAP)



- ✓ Storage at -20°C is sufficient to preserve potency over 6 months
- ✓ Stability at 5 °C for 3 months offers in-use flexibility
- ✓ 5F/T-cycles do not impact potency in vitro
- ✓ Although AAV9 is a poor in-vitro transducer, material requirement still low enough

5 Minimal changes in Size/PDI

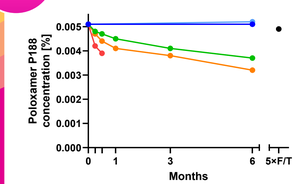


- ✓ Very limited impact of storage conditions on nanoparticle size/dispersity observed
- ✓ Freeze-thaw stress does not impact nanoparticle size

Summary

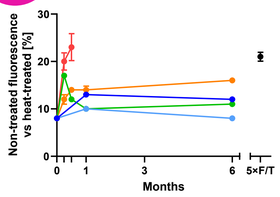
- Storage at –20 °C maintains AAV stability; ≤ –60 °C not required.
- Potency unaffected by freeze-thaw stress although particle formation apparent
- Functional loss only after prolonged/high temp liquid storage (40 °C; 3 months at 25 °C; 6 months at 5 °C).
- No capsid or genome titer loss in all conditions except 40°C.
- DLS: low particle dispersity across all temps, even after 6 months at RT and 5 freeze-thaw cycles.
- Visual inspection: no visible particulates after storage at -20°C
- BMI: detected subvisible particles after 1 and 5 freeze-thaw cycles to ≤ –60 °C;
- HPLC-SEC and Free DNA: small aggregates and elevated % free DA after freeze-thaw ≤ –60 °C, not at –20 °C.

2 Poloxamer 188 degradation



- ➢ Liquid storage leads to slow and steady Poloxamer 188 degradation
- ✓ No impact after frozen storage or repeated freeze-thaw cycles
- ➢ Poloxamer 188 concentration below 0.005% is not recommended

6 Free DNA using SYBR GOLD

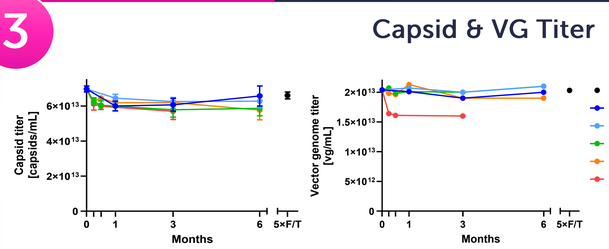


- ➢ Repeated freeze-thaw stress leads to DNA ejection similar to 40°C-stress
- ➢ Storage at 25°C leads to less DNA ejection compared to 40°C.
- ✓ Frozen & refrigerated temperatures exhibited lowest DNA ejection after 6 months

9 Visual Inspection

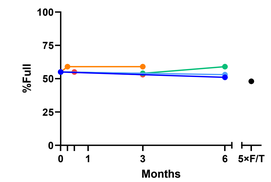
- ✓ No visible particulates observed after storage at -20°C for 6 months
- ➢ Low number of replicate vials per conditions limits statistical strength

3 Capsid & VG Titer



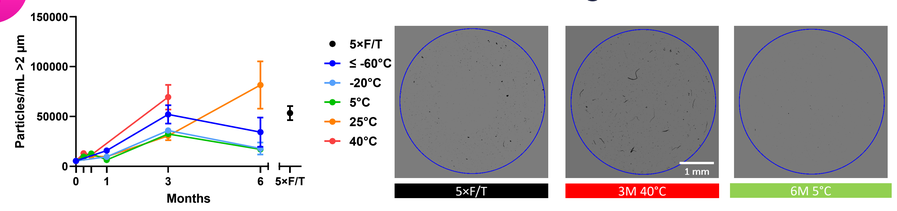
- ✓ No impact of storage conditions & freeze-thaw stress on capsid titer or transgene-specific ddPCR vg titer
- ➢ 40°C-condition leads to vector genome loss, but no impact on capsid titer observed

7 Stable %Full particles via Refeyn-MP



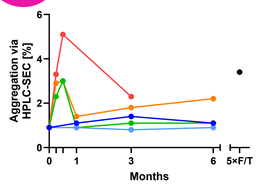
- ➢ Conditions that lead to DNA ejection do not exhibit lower %Full
- ✓ %Full remains relatively constant, indicating stability of filled AAV

4 Subvisible Particles via Backgrounded Membrane Imaging



- ✓ Storage at and below 5°C prevents formation of subvisible particles
- ➢ Repeated freeze-thaw stress leads to elevated particle content. Every freeze-thaw step adds to the particle load.

8 Aggregates via HPLC-SEC



- ➢ High impact of repeated freeze-thaw stress on low-molecular aggregates
- ✓ -20°C or ≤-60°C storage prevent formation of aggregates

Aim higher

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