halo labs

APPLICATION NOTE 6

Are You Missing the Bigger Picture with Your AAV Analytics?

Get Fast, Low Volume Subvisible Particle Analysis when Characterizing Viral Vectors with the Aura™ System from Halo Labs

- 5 µL Minimum Volume
- 96-Well Automation
- 1 Minute Per Sample
- 1 µm to 5 mm

Sub-Visible Particle Analysis Shouldn't Require Milliliters of Precious Sample

Subvisible particles are particles that are too large for analysis by size exclusion chromatography (SEC) (~ > 0.1 µm), but too small to be visible to the unaided eye (< 100 µm).

Evidence suggests that their presence in parenterally administered drugs can lead to dangerous immunogenic reactions. The FDA tightly monitors particulates in parenteral protein drug formulations, referencing guidelines for subvisible particles >10 µm established by the US Pharmacopeia <788>.

Measuring subvisible particles in viral vector formulations for gene therapy poses challenges, however. Due to the complexity of manufacturing, availability of sample for formulation or quality testing limits the number and type of analytics that can be performed. Standard methods for subvisible particle measurement such as flow microscopy are impractical because they can consume large amounts of sample (> 1 mL).

Measuring Only in the Sub-Micron Range May Not Give You the Whole Picture

Dynamic light scattering (DLS) and SEC can miss aggregates in the subvisible range. Large aggregates can be altered or removed in SEC by the column separation mechanism, and DLS becomes difficult to interpret when samples are polydisperse or if larger aggregates are present. Often the solution to improve data quality is to filter samples before running - which only removes the larger particles so they escape analysis entirely.

SHAKING STRESS				
	SUBVISIBLE PARTICLE COUNT BY FM		% SOLUBLE AGGREGATE BY SEC	
FORMULATION	CONTROL	SHAKE	CONTROL	SHAKE
4	1927	9704	2.78	2.15
6	18632	22699200	2.67	1.90
14	776	2980	3.01	2.35

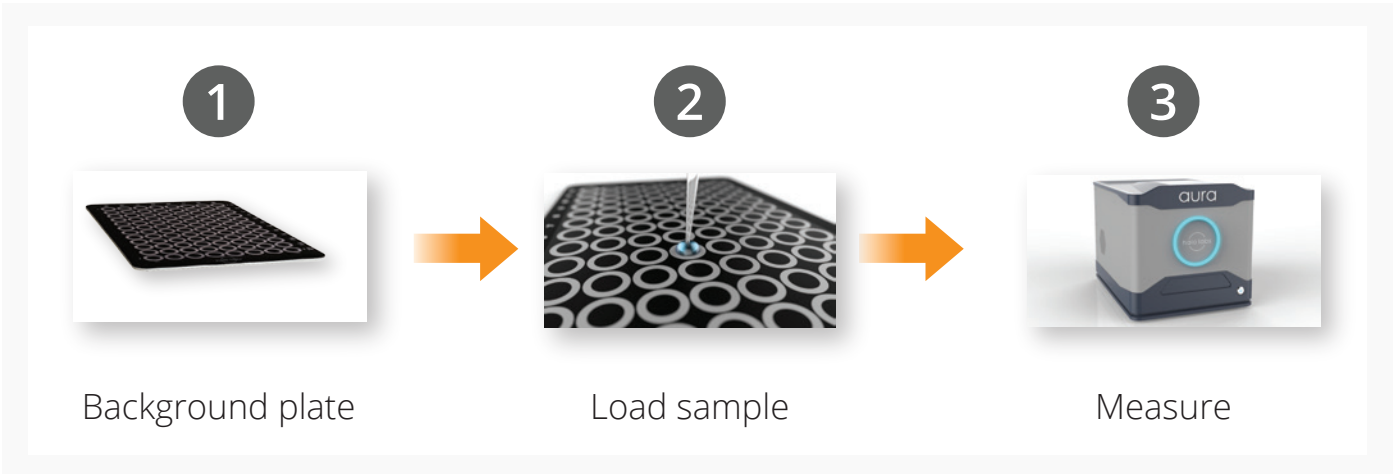
mAb samples subjected to mechanical stress show low counts of soluble aggregates as measured by SEC. However, when aggregates in the subvisible range are measured by flow microscopy (FM), the same samples show high aggregate counts. Adapted from Southall, et al. "Particle analysis as a formulation development tool", Amer. Pharm Rev. (2011).

In other words, subvisible particles can be present even when smaller aggregates are not detected.

The Aura System: Automated Subvisible Particle Analysis

Subvisible particle analysis using the Aura instrument is fast and requires only **5 µL per sample** — making detection of larger, insoluble aggregates accessible for gene therapy analytics.

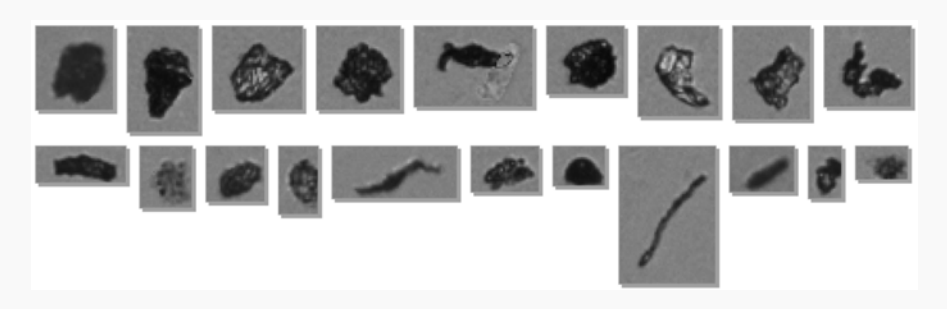
The Aura system uses Backgrounded Membrane Imaging (BMI) technology, a form of automated membrane microscopy that enables particle counting, sizing, and characterization in up to 96 samples in less than two hours.



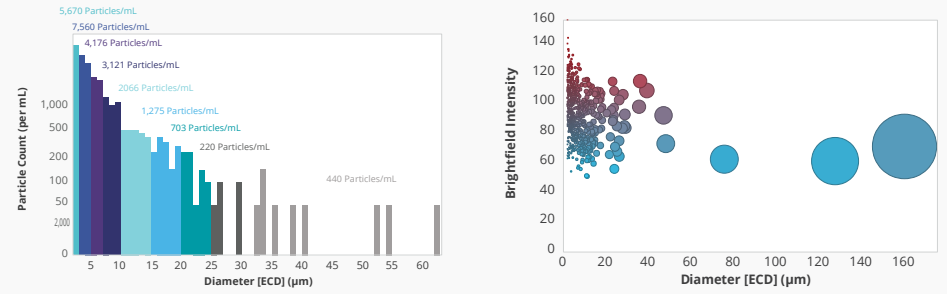
Simple and flexible workflow on the Aura system.

The Aura System Delivers AAV Aggregate Particle Images, Count, and Size Distribution Information

Highly resolved particle images, count and size distribution are provided. Optional fluorescence enables differentiation between protein-containing capsid particles and extrinsic particles such as glass, metal, or plastic.



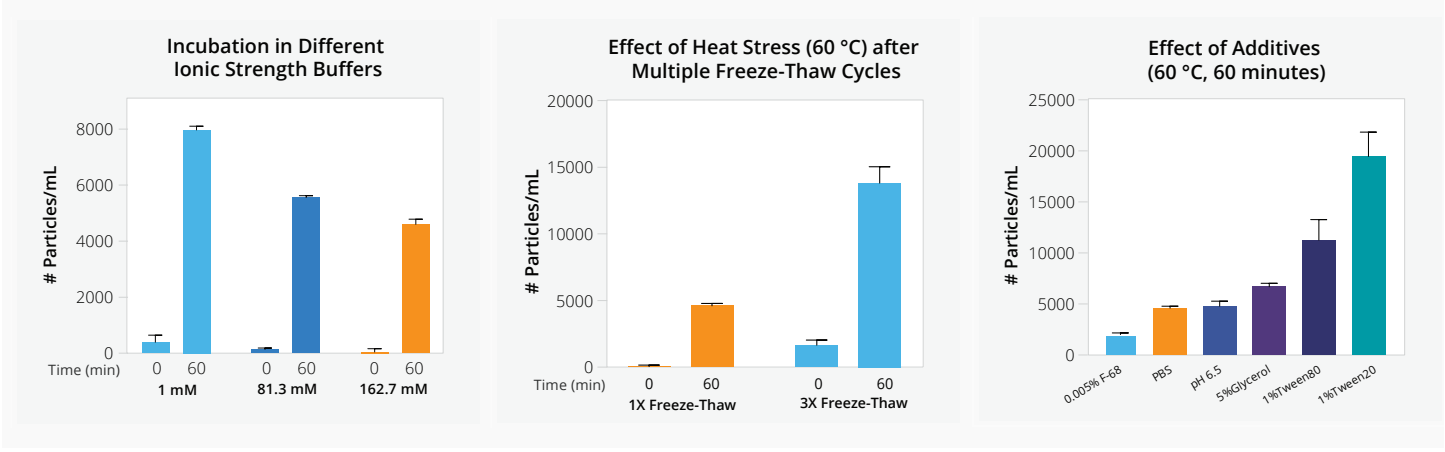
Individual AAV aggregate particles >5 µm, imaged using BMI technology.



User-friendly software enables comprehensive data visualization providing size and count distribution analysis and detailed information on each particle.

Subvisible Particle Counts Are an Indicator of Stability

Measurement of subvisible particles helps to complete the stability picture – which helps to minimize risk and enable more informed decisions about viral vector formulations and quality assessment.



Effects of freeze-thaw cycles, ionic strength, and different additives on insoluble aggregate formation in AAV samples. Lower ionic strength of buffer is known to have a de-stabilizing effect on AAV, and is shown here by increased subvisible particle counts. Similarly, particle count increase corresponds to higher number of freeze-thaw cycles when virus is heated to 60 °C.

In conclusion, the Aura system detects and characterizes 1 µm to 5 mm particles in your gene therapy samples - particles that are often missed by DLS or SEC. It only requires as little as 5 µL of sample per test to provide detailed information on particle size, morphology, counts, and distribution. It's 96-well format makes it possible to test many conditions in one experiment and it takes about 1 minute per sample for analysis.

You'll get high quality images for each particles without any worry about interference from buffers or different matrices. The Aura also has the sensitivity required to help you detect changes as a function of stress and solution conditions. Couple that with 21 CFR Part 11 software, and you have an ideal tool for viral vector quality assessment.

Why Use the Aura for Viral Vector Quality Assessment?

- Detects and characterizes particles not measured by DLS or SEC
- Low sample consumption (as little as 5 µL per test)
- 96-well format for testing lots of conditions
- Provides detailed information on particles — size, morphology, counts, distribution, identification
- Rapid analysis time of about 1 minute per sample
- Wide working range: Measures particles from 1 µm to 5 mm with high reproducibility
- Particles are imaged without the interference of buffer or matrix for higher sensitivity
- Quality images of particles
- Sensitivity to detect changes as a function of stress and solution conditions
- 21 CFR Part 11 software available

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