

Automated, Low-Volume Subvisible AAV Aggregate Analysis with the HORIZON® System

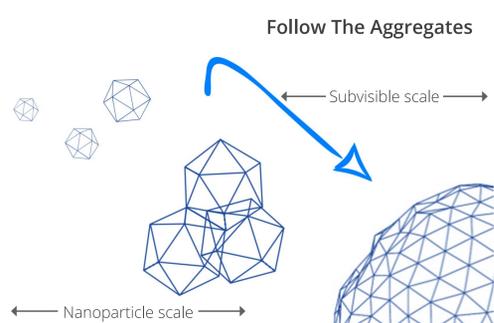


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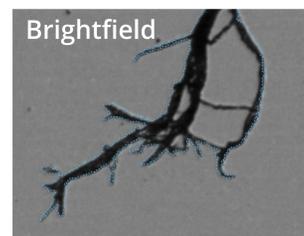
INTRODUCTION

- The presence or absence of subvisible viral aggregates is used as an indicator of the purity, stability and the quality of viral vector samples.
- That is why the US and European Pharmacopeias established the current Good Manufacturing Practices (Chapter <788> and 2.9.19 respectively), which mandate quantification of subvisible particles as a release compliance requirement for all injectable therapeutic protein products; since aggregates are known to exert undesirable effects upon the vector's efficiency, biodistribution and immunogenicity.
- However, assessing vector particle aggregates at the subvisible range (10^0 – 10^4 μm) is especially challenging where only limited sample is available.



OBJECTIVES

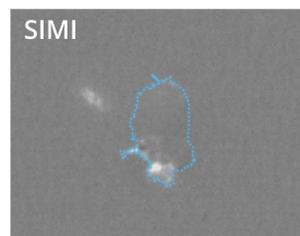
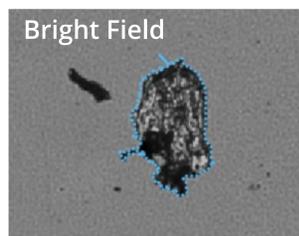
- Validate the use of Backgrounded Membrane Imaging (BMI) with the HORIZON® system to assess viral sample purity.
- Assess the usefulness of BMI as a quality control, release compliance and formulation tool for AAV development.



- AAV particles aggregate as filamentous precipitates
- Aggregate viral source confirmed by offline FL

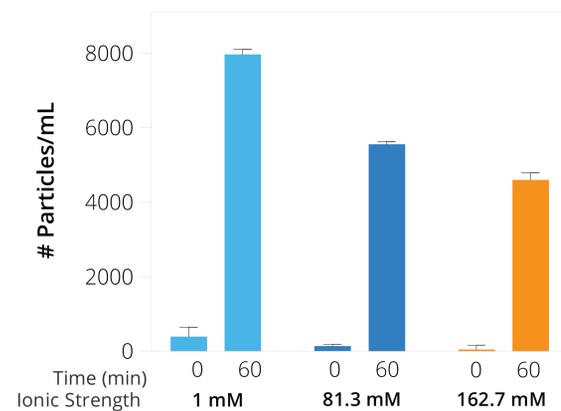
RESULTS

- Figure 1. The HORIZON® System Accurately Measures and Characterizes Ionic Strength AAV Aggregation Propensities.** AAV samples (at concentrations as low as 10^{11} GC/mL) were artificially aged by incubating them in PBS based solutions with different ionic strength magnitudes (orange bars represent the PBS 1X standard). 30 μL aliquots analyzed by BMI show a significant ($P < 0.05$) inverse dependence between AAV aggregation and the medium's ionic strength. This observation is equivalent to what has previously been determined utilizing DLS techniques (J. Fraser Wright, et. al.; *Molecular Therapy*; 2005; Volume 12(1); 171–178).
- Figure 2. The HORIZON® System Correctly Determines AAV Vector Freeze-Thaw Dependent Quality.** The AAV vector was subjected to a stability study to determine whether aggregation occurs following multiple freeze-thaw cycles (-20 to 25°C in PBS 1X). As expected, lower stabilities were observed after AAV vector samples were subjected to more than one freeze-thaw cycle.
- Figure 3. The HORIZON® System is Useful for AAV Formulation Screening.** Initial additive screening was performed to identify additives that affect aggregation with respect to the PBS standard (orange bar). Pluronic detergent significantly improved AAV stability to accelerated aging, while PBS pH 6.5 or PBS + 5% Glycerol did not significantly change the vector's stability; whereas the 1% Tween 20 or Tween 80 conditions seemed to significantly destabilize the viral vectors.

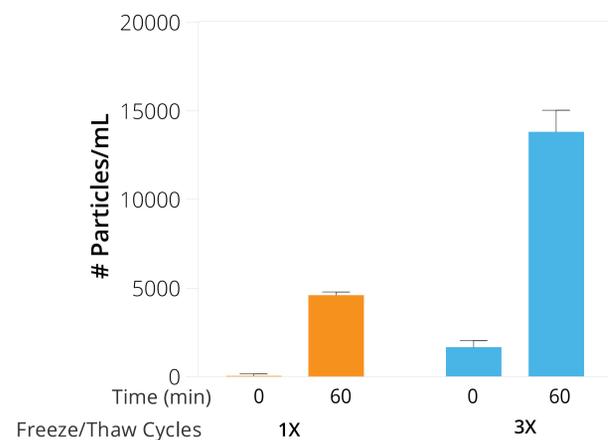


- AAV solid aggregates can be distinguished from gel particles (from additive sources) using the side illumination (SIMI) feature on the HORIZON® system.

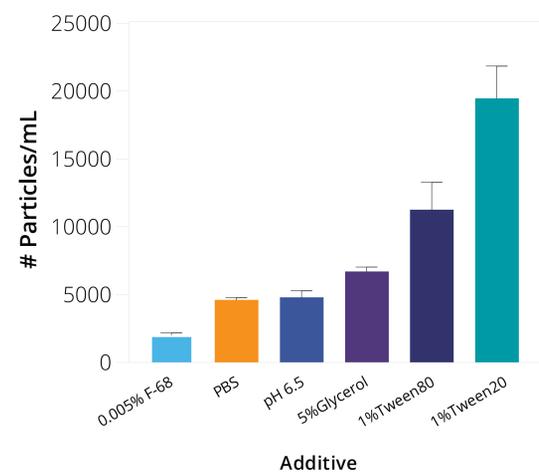
1. AAV Aggregate Formation (60 °C)



2. AAV Aggregate Formation (60 °C)



3. AAV Aggregate Formation (60 °C, 60 min)



BMI OVERVIEW

- In Backgrounded Membrane Imaging (BMI) samples are vacuumed through a filter plate and particles are captured on a flat membrane where they are counted by automated light microscopy.
- The HORIZON® system from Halo Labs employs (BMI) technology to measure and characterize subvisible particles in a high-throughput manner, using as little as 25 μL sample.
- Samples are simply pipetted onto disposable 96-well membrane plates then the automated imaging software quickly generates information on counts, size, and distribution of particles captured on the membrane.



- Here we present data that demonstrates how viral aggregate counting can be used to assess the purity, stability and quality of AAV viral vector samples.

CONCLUSIONS

- Here we have demonstrated how high throughput membrane imaging with Backgrounded Membrane Imaging on the HORIZON® system offers reliable purity, stability and quality assessment of AAV viral vector samples.
- Whether used as a primary solubility screening method, process compliance technique, or as a release compliance routine test, BMI technology can accelerate AAV drug development by providing more complete information with less time and less material than traditional methods.