Q: Mass Photometry: Does this technique rely on the Brownian motion to catch the AAV particles? Do you need to offset buffer viscosity and temperature?

Mass photometry method is based on interference reflection microscopy and Raleigh scattering. Measurement requires the AAV particle to interact with the glass surface and remain (momentarily) static. Refeyn is the leading manufacturer of mass photometry instruments and has produced technical notes that explain the scientific principles. https://www.refeyn.com/about-mass-photometry

---

Q: USP AAV standards: Are they serotype specific? How many sertotypes do you have?

The first AAV standards for empty:full characterization are the AAV8 serotype. Our intention is to add additional serotypes. This serotype is being developed first due to its thorough characterization in a recent USP-NIIMBL-NIST round robin study of empty:full analytical methods. The USP standard is intended for use as a system suitability control, to confirm instrument and assay performance. It is not intended to be a capsid- or product-specific comparator. It is best practice to establish product-specific comparators as an in-house reference using material that is representative of a manufacturer's product and process.

Q: Thanks for the excellent talks and discussion so far. One major roadblock NGS/HTS adopters face is the avaialblility of a validated data analysis and bioinformatics pipeline. Any suggestions.?

There was a very good discussion of this topic in Jarrod Dean's presentation and in the discussion session that followed. Briefly, it was acknowledged that validation of data analysis and bioinformatics pipelines is one of the most difficult parts of implementing an NGS test in a quality environment. A starting point is considering the regulatory expectations in "Guidance for Industry and FDA Staff: General Principles of Software Validation". Colette Cote also recommended considering ISPE GAMP 5: A Risk-Based Approach to Compliant GxP Computerized Systems (2nd Edition).

Validation requires strong support at several levels of an organization and sufficiently modern IT infrastructure to support the storage and processing of large amounts of NGS data. Support must be present for running software/scripts that are customized and updated in an environment with semantic version control and traceable internal code review.

Validation should include a comprehensive risk assessment and be documented by a full description of the process, the functionality of all aspects, the environment and architecture, user acceptance testing, and performance with dry-lab simulations and wet-lab results.

It was also noted that the validation requirements for identity tests from ICH Q2(R2) are easier to accommodate than are the requirements for impurity or assay tests.

Q: Current Ph.Eur 2.6.1 and UCP 71 addresses the sterility testing requiremenst for final products with a % of a batch.. Is there a thinking to accommodate starting materails and intermediated with reduced volume testing.

USP is developing compendial chapters to support reduced volume sterility testing. See the draft chapters: <72> Respiration-Based Rapid Microbial Methods for the Detection of Contamination in Short Shelf-Life Products and <73> ATP Bioluminescence-Based Rapid Microbial Methods for Detection of Contamination in Short Shelf-Life Products. Additionally, USP has an informational chapter in development, <1114> Microbial Control Strategies for Cell Therapy Products. There are more chapters to come but they are still works in progress.

More generally, when it is possible to assure the sterility of starting materials and intermediates by compendial methods, it is strongly recommended. If the compendial methods are not fit for purpose, an equivalent or better performing method can be fully validated as an alternative as described in ⟨1223⟩ Validation of Alternative Microbiological Methods. In the cases where sterility cannot be assured through testing, a comprehensive risk-based control strategy is required to provide sterility assurance.

21 CFR 610.12 (Sterility), in-process testing using rapid methods may be used in lieu of final product testing when fully justified and in accordance with the regulatory agency. Another method to evaluate the probability of contamination and to help define an adequate sampling volume may be using a contamination probability calculation in the chapter.