Live Webinar Q&A Sheet:

Real-Time Monitoring of AAV CQAs in a Chromatographic Polishing Step

The recorded webinar may be viewed from the RT-MALS webinars page. These questions were submitted by live viewers. Additional information on RT-MALS may be found on the Wyatt web Library under Webinars, Application Notes, Featured Publications and Bibliography, as well as on the corresponding Product page and Solutions page of our web site.

The responses to the questions below were prepared with the assistance of the webinar speaker, Dr. F. Michael Haller of Lonza, to whom Wyatt extends sincere appreciation for his support. Please contact info@wyatt.com with any additional questions.

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Questions & Answers

Q: *Could you estimate the total time saved per run when you implement RT-MALS, or how much it would effectively speed up the development of a method for a new AAV?*

A: It depends on the goal of the experiment. From my experience, maybe about 25 to 50% of time can be saved. Let's say your buffer pH and column are set, and you want to optimize full, empty, and aggregate separation. In our experience, we would need about five separate runs, and maybe up to ten if you have a challenging construct. The time saved will depend a lot on your offline measurement.

Having said that, the RT-MALs can be used effectively to quickly compare the quality of the AAV construct. A good construct will show a clear separation between empty and full. If you have a bad construct with a lot of partials, you get a heavy overlay between empty and full with little to no separation. The RT-MALS system gives you an upfront look at the quality of your construct without too much effort.

Q: *How much effort and down time were involved in adding RT-MALS to your chromatography system?*

A: It's actually very fast. The downtime to install the system was about half a day. We have it permanently connected to our AKTA, but it is easy to take off-and-on. About half a day of installation and a day of training, so about 2 days in total.

Q: *Are there any adverse effects on the chromatography system's performance from adding RT-MALS?*

A: Not really. There is some band broadening because of the additional tubing and flowcell but it's not a lot.

Q: *Can I still use RT-MALS if I don't know the extinction coefficients for my AAV?*

A: Extinction coefficients for different AAV constructs can differ by up to roughly 10%. Even if you do not yet have accurate values for the specific constructs, the default values provided by Wyatt are sufficient to get

approximate results that clearly indicate trends in empty/full ratios and titers in the course of a process run, and will help to quickly optimize the process. Once you have aliquots of clean empty capsids and at least ~30% full, you can use a SEC-MALS system with ASTRA™ software's Viral Vector Analysis module to obtain more accurate extinction coefficients.

Q: *Can RT-MALS be used for UF/DF or other downstream processing of AAVs?*

A: The most straightforward implementation would be on-line with the TFF, pulling a small slipstream to measure the capsid size and titer. Changes in capsid size could be indicative of aggregation or other degradation, while titer can be used to determine the UF endpoint. Note that titer by MALS alone may not be accurate if aggregation occurs. If you add a UV detector, you can also monitor empty/full and full capsid titer which are not impacted by aggregation.

Q: *Can RT-MALS be used for the AAV affinity capture step?*

A: Yes. Adding an ultraDAWN inline with affinity capture will enable monitoring capsid and genome titer as well as size, to give you an indication of the total capsid load, the full capsid ratio and potentially aggregation. Note that co-elution of impurities like proteins or nucleic acids may impact accuracy of the results when using MALS-UV for capsid and genome titer. Using just MALS for total capsid titer will not see much of an impact by such impurities other than aggregates, but the OBSERVER™ software workflow that provides capsid titer and size by MALS alone precludes empty-full analysis.

Q: *Can RT-MALS be useful for clarified harvest fluid analysis?*

A: In clarified harvest, it would not be feasible to assess empty-full. However, if the clarification is sufficient to remove most cellular debris and other nano-particulates, you can use the size and particle concentration measurement to get some idea of the titer. Feasibility would depend on the specific process.

Q: *What is the minimum detectable concentration of nanoparticles / viruses by RT-MALS?*

A: It depends on the size of the virus. For AAV, with a diameter of 25 nm it would be in the range of 10^{10}/mL. For larger viruses such as adenovirus and lentivirus, it would be around 10^{6} to 10^{7}.

Q: *What is the AAV concentration range needed to get accurate empty/full reading on RT-MALS?*

A: Similar to the detection limit for SEC-MALS, mid- to upper- 10^{10} particle/mL. This mostly depends on UV sensitivity so if elution titer is low, it is preferable to install a 10 mm UV flow cell rather than rely on the standard 2 mm cell.

Q: *How long would the measurement of aggregation, molecular weight, etc take if connected to an ÄKTA? How often can we take samples?*

A: The RT-MALS instrument is connected inline with lab-scale systems such as ÄKTA pure or avant, so no samples are removed. The eluting product simply flows through the ultraDAWN just like it does for the FPLC's UV detector, and you get results every second or so. For larger scale chromatography such as ÄKTA process, RT-MALS is configured online, meaning that a pump draws a low-flow slipstream, on the order of 0.5 – 1 mL/min, from the main flow. Measurements are made continuously, every second or so, but there may be on offset of 10

– 60 seconds in time relative to the main flow path.

Q: *Can we use RT-MALS for any other viruses apart from AAV?*

A: Yes, as shown in the webinar RT-MALS is also effective for larger viruses like adenovirus or lentivirus. However, It cannot determine attributes like empty/full ratio or genome titer for these larger viruses because scattering of UV light from viruses prevents accurate measurements of protein and nucleic acid content. Only size and particle concentration (physical titer) are monitored. Other small viruses or virus-like particles with size similar to AAV can be monitored with the full set of attributes – Vg/Cp, empty and full titer, capsid and genome molecular weight, etc.

Q: *Is the RT-MALS system suitable for method qualification and validation?*

A: Since RT-MALS is applied as an inline PAT tool during process development and production, it probably does not need the same level of qualification and validation that one would expect for a release test. However, it is straightforward to qualify and validate the method by comparing real-time results with measurements performed on fractions by reference offline methods, as the primary attributes it measures can all be determined directly by other methods like ddPCR, ELISA, SEC-MALS, mass photometry, etc.

Q: *What causes the lagging elution of empty capsids in the separation and how do you mitigate this?*

A: Lagging elution of species that appear to be empty capsids could be aggregates of empty capsids, damaged capsids that have lost their genome, or other impurities. Further identification is assisted by observing additional attributes provided by RT-MALS such as size and molecular weight. However, since RT-MALS averages the contents of eluting fractions, these are only hints. Absolute identification should be performed with in-depth offline methods such as SEC-MALS, cryo-EM, etc. An abundance of damaged capsids probably indicates a poor construct.