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WHITEPAPER

Application of mass photometry in GMP-regulated environments for characterization of AAV samples

The Samux^{MP} and Samux^{MP} Auto can be used for characterization of AAV samples in GMP-regulated environments such as manufacturing facilities. Refeyn's offer includes a software package that supports compliance with FDA 21 CFR 11 (US) and EU GMP Annex 11 regarding features like user management, audit trails and electronic signatures. The Refeyn service teams also provide installation and operational qualifications, as well as documentation and on-site training.

Mass photometry for AAV sample characterization

Adeno-associated viruses (AAVs) are a widely used vector for recombinant genome delivery into cells, and promising vehicles for novel gene therapies. However, sample characterization represents a major challenge for commercial, large-scale manufacturing of AAV-based gene therapies[1]. Current gold standard AAV characterization methods such as analytical ultracentrifugation (AUC), electron microscopy (EM) or qPCR/ELISA present drawbacks such as long turnaround times, high sample consumption, need for highly specialized staff or outsourcing.

Mass photometry can overcome many of the bottlenecks while providing reliable data aligned with the traditional characterization methods. Mass photometry is a bioanalytical technique that measures mass of AAVs and biomolecules[2, 3] in solution and at the single-particle level. The analysis requires small amounts of sample – 10-20 µl of AAV sample at a final concentration of 10^{11} particles/mL – and takes less than five minutes.

The output of the mass photometer is a mass distribution histogram reporting the mass of each counted particle. From mass photometry histograms one can obtain information about empty/full AAV ratios as well as presence of partially filled and overfilled AAV capsids. Refeyn provides mass photometry instruments tailored for AAV analytics: The Samux^{MP} and Samux^{MP} Auto, as well as support for their implementation in GMP-regulated environments.

GMP regulatory requirements

For processes involved in drug manufacturing, regulatory agencies such as the US Food and Drug Administration (FDA), and the European Union established a series of regulations known as good manufacturing practices (GMP).

These regulations demand that manufacturing processes are adequately designed, established and controlled (Fig. 1). The aim is to ensure the quality of the final product and to be able to trace back and solve any manufacturing quality issues. In order to use laboratory equipment – such as the Samux^{MP} – in GMP-regulated environments, some requirements need to be met to ensure validation of the process, qualification of the instrument, and compliance with 21 CFR 11 for the US market (or the equivalent for other countries).

In this whitepaper we define how the Samux^{MP} and Samux^{MP} Auto can be applied in a GMP-regulated environment and describe the key required features of the Samux^{MP} software package.

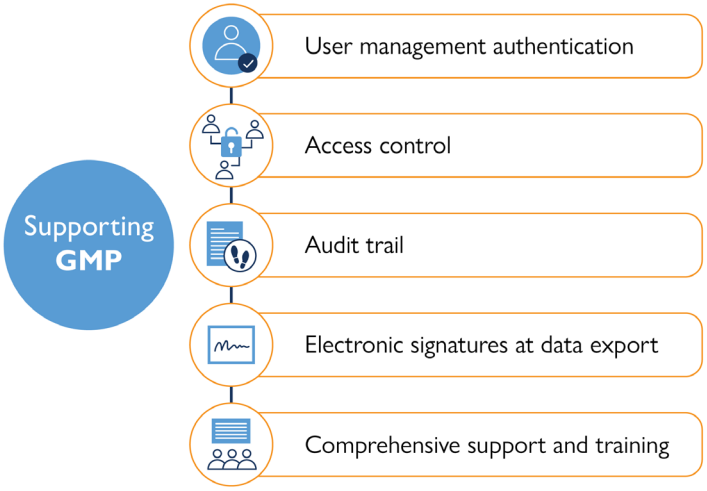

Fig. 1. Refeyn supports the use of the Samux^{MP} and the Samux^{MP} Auto in GMP-regulated environments. The GMP software package for the Samux^{MP} and Samux^{MP} Auto includes all the necessary features to comply with FDA 21 CFR 11 (US) and EU GMP Annex 11. Furthermore, Refeyn's service teams provide support and training to fulfill the necessary Installation Qualification and Operational Qualification.

System qualification

Mass photometers destined for GMP-regulated environments go through Factory Acceptance Tests before leaving Refeyn, making sure the system fulfills Design Qualification (DQ) requirements. Once delivered to the customer, the Refeyn service team perform the Installation and Operational Qualifications (IQ, OQ).

The OQ includes the use of a standard calibrant and sample to validate the device specifications as well as the qualification of the software features essential to ensure 21 CFR 11 compliance.

More information on the calibrant and standard, as well as information on IQ/OQ templates, can be requested from Refeyn.

Upon request, Refeyn can facilitate Performance Qualification (PQ) by providing additional support to help the customer validate the correct operation of the instrument and associated software using the customer's own standards and samples. Refeyn also offers maintenance services to guarantee the instrument performs to the highest standards.

Refeyn software applications for GMP-regulated environments

Refeyn has developed software solutions for use with Samux^{MP} instruments in GMP-regulated environments, such as AAV manufacturing facilities. The software package includes three applications:

- **Manage^{MP}:** For system configuration, user management, access control and audit trail storage
- **Acquire^{MP}:** For instrument operation, data acquisition and processing
- **Evaluate^{MP}S:** To use the data generated by Acquire^{MP} to determine empty/full AAV ratios, as well as quantify partially filled capsids

Both Acquire^{MP} and Evaluate^{MP}S are desktop applications. Acquire^{MP} is installed on the computer connected to the instrument, usually provided by Refeyn. Evaluate^{MP}S can be installed on any computer. Manage^{MP} is a web server application linked to an SQL database where user management, access control, audit trail and licensing information are stored. All three applications are designed to complement each other, ensuring compliance with the requirements of 21 CRF 11 and Annex 11.

User management and access control

In order to access any software applications in this package, user authorization is required. Each user has unique login information to be used across all applications. Currently, the system supports both Open ID Connect and password-based authentication provided by Refeyn.

In Manage^{MP}, administrators can create new users and define different roles with selective access to the functionalities of Refeyn's software applications (Fig. 2).

Roles and permissions include:

- User management and settings – ability to define system configuration and manage users that can access the system as well as the permission levels for each. This role is referred to as "administrator" in this document
- Audit trail access – Users can visualize and export the audit trail in human-readable form
- Acquire^{MP} basic – Users can perform measurements in Acquire^{MP}
- Acquire^{MP} advanced – Same as Acquire^{MP} basic plus access to settings
- Evaluate^{MP}S basic – Users can access the semi-automated and automated workflows for AAV ratios determination
- Evaluate^{MP}S advanced – Users can access both automated, semi-automated and manual workflows for AAV ratios determination

Users cannot be deleted from the system, as their information is required for the audit trails. However, user accounts can be deactivated, releasing their licenses for use by others. Administrators can also lock users outside of the system temporarily.

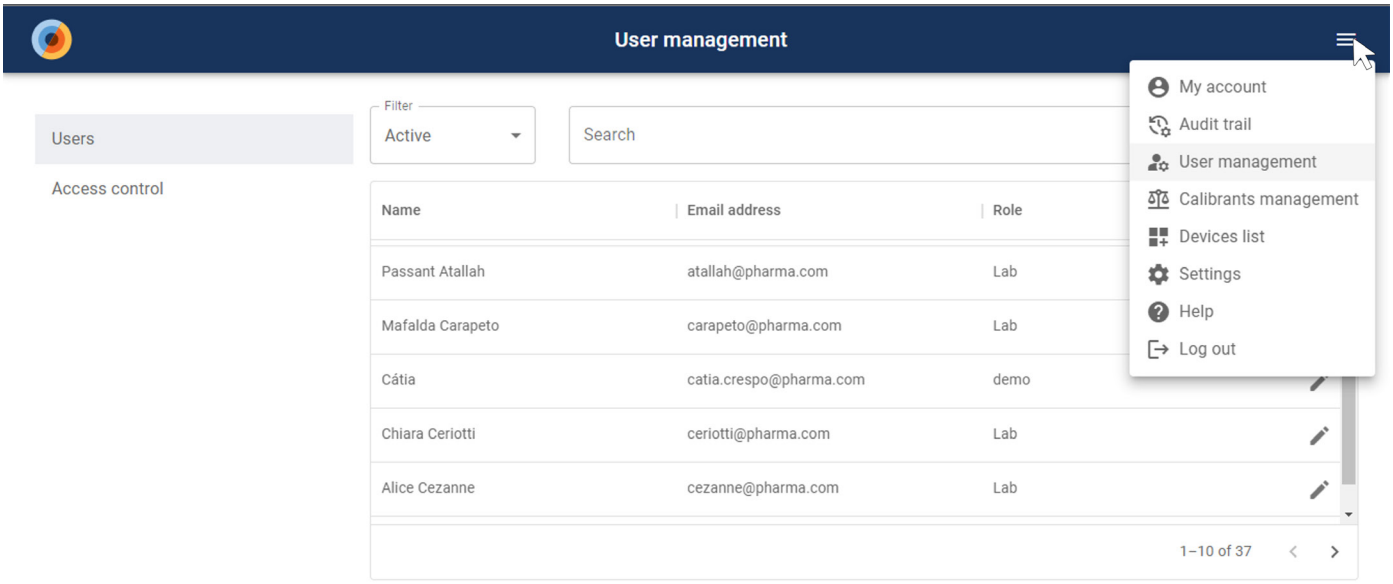

Figure 2. User interface in the Manage^{MP} software. This web server application contains all the tools needed to successfully manage one or more Samux^{MP} or Samux^{MP} Auto instruments installed in a GMP-regulated environment. From here, administrators can manage users, devices and calibrants, as well as user access and audit trail export.

Security measures have been put in place to ensure password complexity and privacy. Additional security measures have been defined to ensure only authorized users can access Refeyn system, such as:

- Locking of user accounts after the use of the wrong password three consecutive times
- Enforced periodic password updates (time period to be configured by administrators)

Data acquisition, management and analysis

Acquisition of raw data and instrument operation is controlled by Acquire^{MP}. The user records a movie that is processed by the application in order to obtain the mass photometry contrast values of the particle landing events, which are translated to molecular mass upon calibration.

The raw data generated is saved as an .mpr file in a directory chosen by the user. Once saved, further data management and backup is handled by the IT infrastructure of the customer organization. When saving acquired data, the user must input the expected molecular mass of the empty AAV capsids as well as the size of the transgene. This step is required when using the automated definition of mass ranges for empty, partial and full AAVs.

The next step in the workflow is loading the .mpr files generated by Acquire^{MP} into Evaluate^{MP}S. Multiple files can be loaded at the same time.

As a first step before analysis, the system requires the user to select a calibration measurement from the loaded files in order to apply it to all other measurements. When selecting the calibration measurement, the calibrant type can be defined from a limited preselected list using a dropdown menu, ensuring no mass errors are introduced. The calibrant list can be managed by administrators and Evaluate^{MP}S advanced users. The empty, partial and full AAV populations are then automatically determined (Fig. 3, Table 1).

Mass ranges are automatically defined based on the user inputs for the expected mass of the empty capsids and the size of the genomic payload. These estimates are refined using the measurement data and fitted to a model of two Gaussians and an additional distribution of events outside the central region of the empty- and full-capsid peaks. This fit defines the count integration region limits for empty and full capsids as a configurable sigma value around the found peak position corresponding to each population. Partials are integrated over the interval ranging from the upper limit of empty capsids to the lower limit of full capsids. The fitting for each measurement is displayed and approved individually by the user.

Once all measurements have been accepted, the results – including mass histograms, empty/full AAV ratios, mass calibration used and data quality metrics – can be visualized and exported to any desired directory as a single report in .pdf or as a .zip including .csv for tables and .png for histograms.

A semiautomated mode is also available. In this mode, the user manually indicates where the empty and full peaks are on the histogram and the limits are automatically applied for empty, partials, full and overfilled (optional).

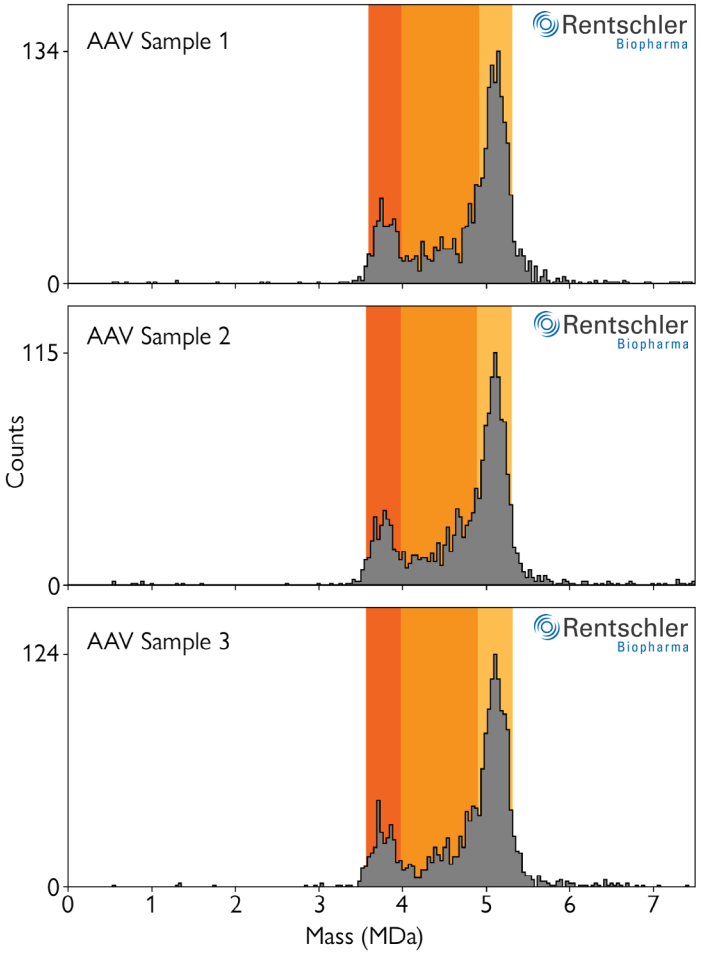

Figure 3. Evaluate^{MP}S automatically determines empty, partial and full AAV populations. The plot shows three mass histograms corresponding to independent measurements of the same sample. The mass ranges corresponding to empty (dark orange), full (light orange) and partially filled (mid orange) capsids are automatically determined by the program, and the corresponding particle counts are analyzed to calculate the relative proportion of each population (Table 1). Data acquired by Rentschler Biopharma SE with a Samux^{MP} and analyzed using the automated empty/full AAV detection in Evaluate^{MP}S.

Table 1. Empty, partial and full capsid percentages: Automatically calculated for each of the three measurements shown in Figure 3.

Name	% Empty	% Partial	% Full
AAV sample 1	17.4	29.8	52.8
AAV sample 2	16.5	30.8	52.7
AAV sample 3	15.8	27.5	56.7

The size of the interval is defined by a configurable sigma value of a Gaussian fitted at the location selected. Just like for automated mode, partials are considered to be all counts between the empty and full regions. The overfilled region is defined as everything above the full region. Finally, advanced users of Evaluate^{MP}S are able to manually adjust the mass ranges for empty, partial and full capsids, as well as add mass ranges corresponding to overfilled capsids.

Audit trails and data integrity

The applications in the Samux^{MP} GMP software package do not allow deleting any data. The audit trail tracks every action related to the generation and modification of data while using all applications that are part of this software package. For every action, relevant information such as time, user or empty/full AAV ratio analysis results (when relevant) are added to the audit trail.

Individual measurements are associated to a unique identifier so the audit trail can keep track of the activity related to any given measurement – even if an advanced user of Evaluate^{MP}S modifies analysis parameters or runs multiple analyses on the same dataset. The audit trail also stores information related to user creation and tracks user access to the different applications.

To ensure that all AAV ratios determined by Refeyn applications cannot be altered, Evaluate^{MP}S can only open files generated in Acquire^{MP}. Any possible changes to .mpr files outside of Evaluate^{MP}S will be detected, prevent data loading and result in a user warning.

It is not possible to delete, deactivate or edit the audit trail within the applications, including Manage^{MP} itself. The database is searchable based on multiple criteria such as date, user or measurement ID. Finally, filtered results can be exported in a human-readable form (.pdf file). This allows for visibility of all users' activities.

References

[1] *Gimpel et al., Mol. Ther. Methods Clin. Dev. 2021*

[2] *Young et al., Science. 2018*

[3] *Wu et al., Gene Ther. 2022*

Electronic signatures

Results from Evaluate^{MP}S are exported as a .pdf file that can be signed during the export process by re-entering the unique ID and password of the user determining the AAV ratio in Evaluate^{MP}S.

The signature applied to the document is unique and displays the signer's name, unique ID, date, meaning of signature and additional comments added during export.

Signatures are displayed in human-readable form in the footer of every page of the .pdf report. Only one signature can be applied per export; additional signatures required – for example for data approval – need to be applied using appropriate systems. Electronic signature settings are configured using Manage^{MP}.

Signatures can be inactivated, made optional at data export or made mandatory for all data exports. Information related to the electronic signature is also stored in the audit trail as part of the 'Summary report export' action details.

Documentation and training

As part of instrument installation and qualification, Refeyn provides on-site training and clear and comprehensive documentation – including user manuals for the main acquisition options and software functions. If needed, additional training can be provided upon request.

Customer feedback

The data presented in this whitepaper was acquired by Rentschler Biopharma SE. A Rentschler representative commented:

"The GMP software is simple, easy-to-use and all aspects of the workflow are captured in an audit trail. The automated analysis algorithm eliminates user bias and generates very repeatable data."

-Lewis Wharram, Project Lead at Rentschler

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