Gaining control of rAAV production: real-time monitoring with Raman Spectroscopy

B L CARNIO[1], J WAGNER[1], M BOSCHER[1], J BABIC[1], D BITNEL[1], T FISCHER[1], S NDONWI[1], H EL RADI[2], L SAVARY[2], C ZACH[1], B HAGER[1], S RITTER[1], M GORA[1], T KLOETZLER[1], M WEIDLER[1], A SCHOBERTH[1], M LANGHAUSER[1], M HOERER[1], K HELLER[1], A YOUSSEF[1]

[1] *Ascend Advanced Therapies GmbH, Germany*
[2] *Merck KGaA, Darmstadt, Germany*

Poster 1941

ascend

ABL inc. ADVANCED BIOSCIENCE LABORATORIES

MERCK

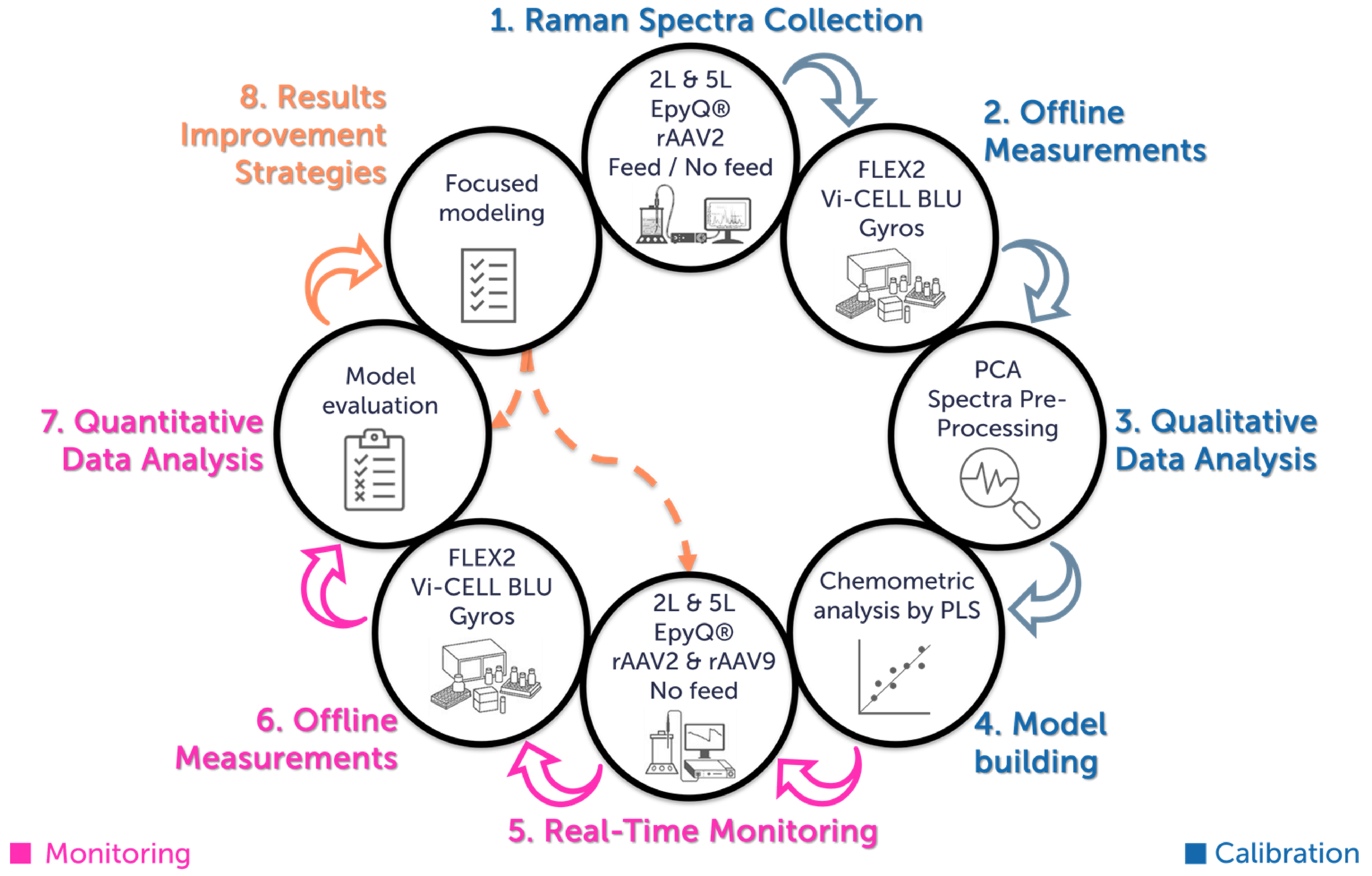
Introduction

Raman spectroscopy is a label-free analytical technique that enables molecular identification based on the inelastic scattering of monochromatic light, typically from a laser source. When incident photons interact with molecular vibrations, the resulting energy shifts —known as Raman— generate spectral patterns. They are highly specific to the chemical bonds and molecular composition of the sample, forming a unique chemical fingerprint.

Due to its capacity to provide real-time insights into complex biological systems, Raman spectroscopy has emerged as a promising tool for advanced bioprocess monitoring. In the context of recombinant adeno-associated virus (rAAV) vector manufacturing, where minor fluctuations in cell metabolism can significantly impact viral yield and quality, the integration of in-line analytical technologies, such as Raman spectroscopy, can offer substantial advantages.

In this study, we evaluated the performance of the ProCellics™ Raman Analyzer, developed by Merck, to monitor key bioprocess parameters during rAAV production. The aim was to build a predictive model based on spectral data capable of quantifying, in real-time, the concentrations of critical nutrients and metabolites (glucose, lactate, glutamine and ammonia), viable cell density (VCD), and viral capsid titers.

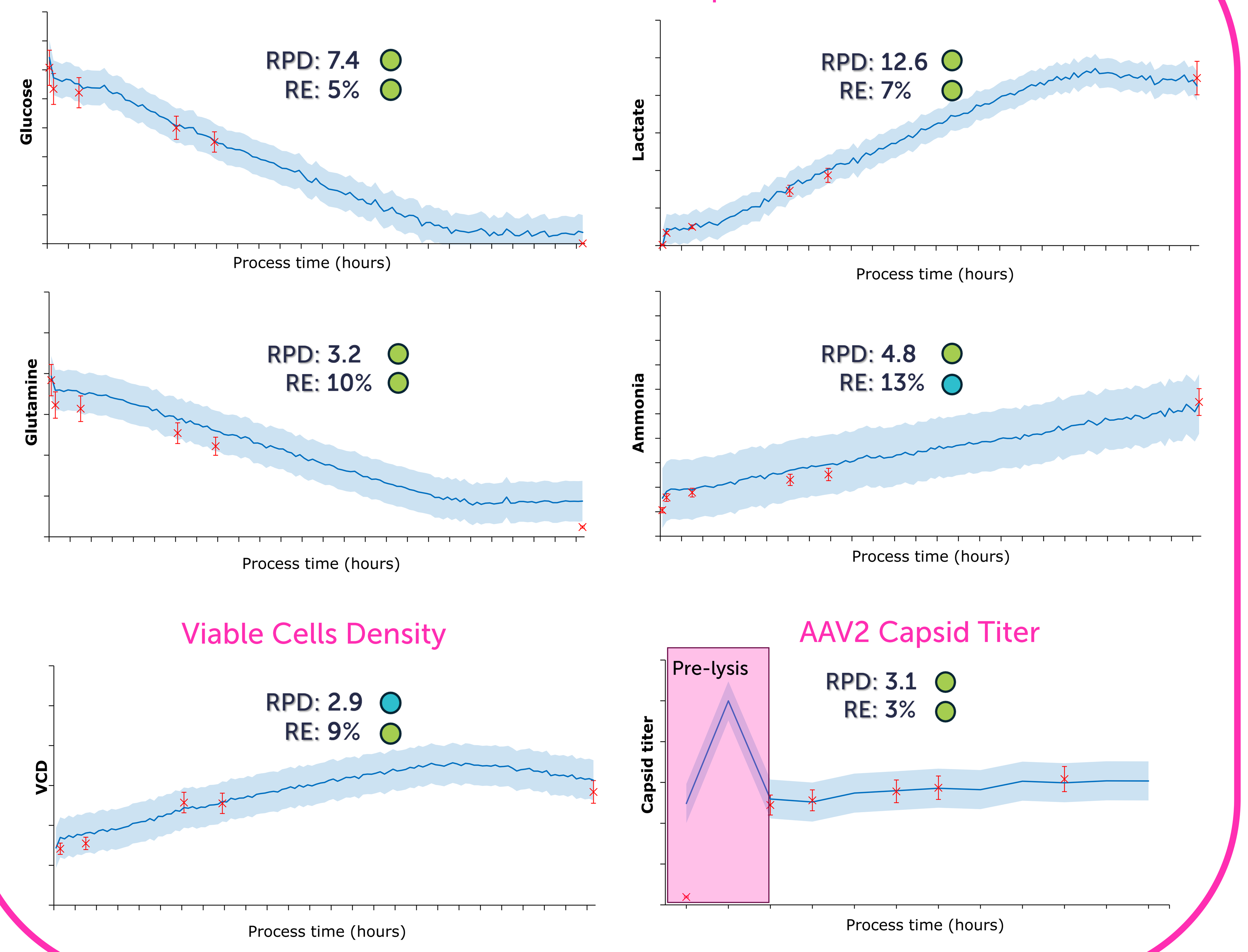
Experimental design



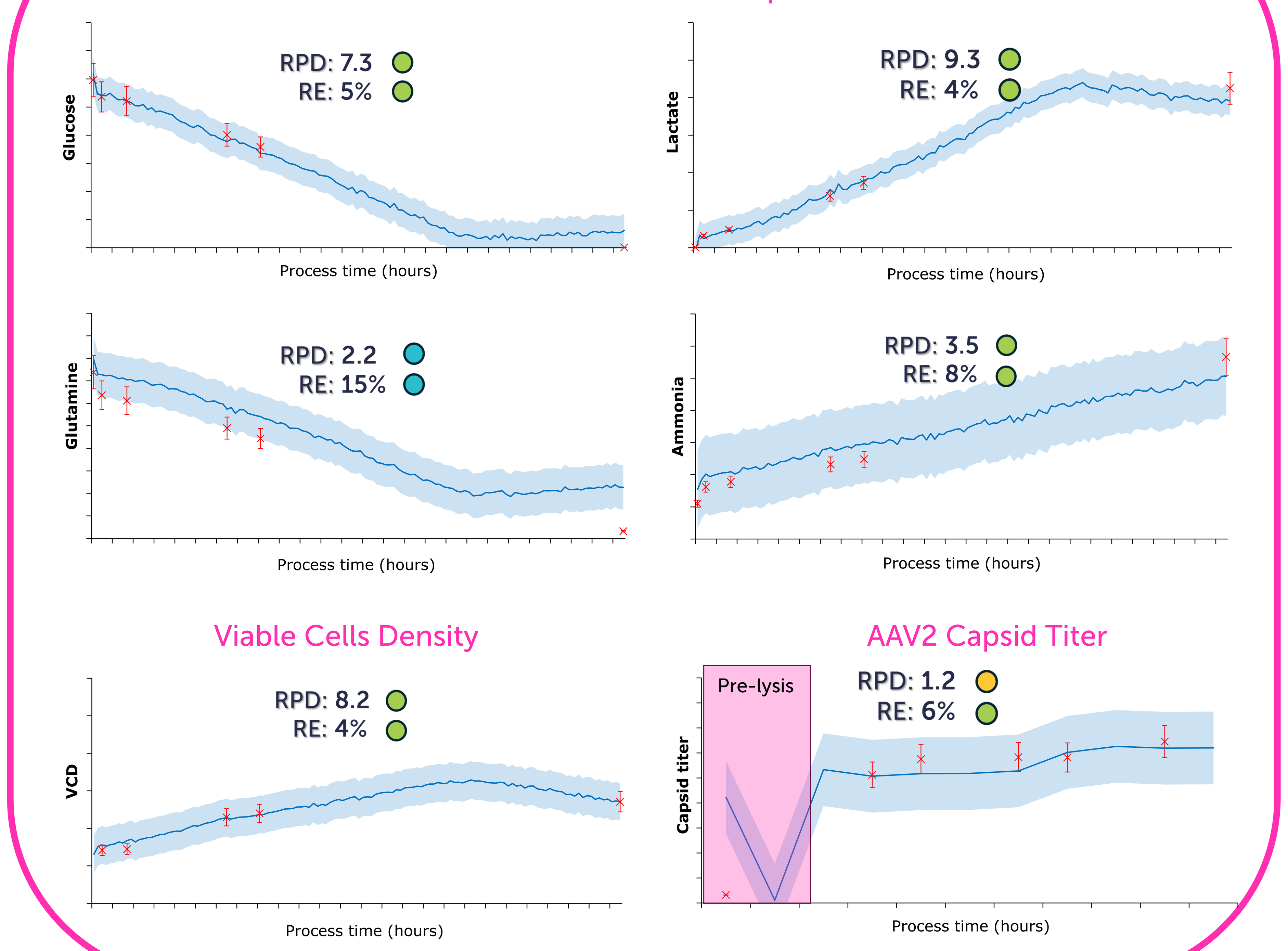
Data Analysis

- All runs used our EpyQ® split-2 plasmid system designed for Enhanced Performance regarding Yield and Quality, combined with PEI transfection to produce rAAV2 and rAAV9 vectors encoding a 3kb SEAP reporter gene.
- The experiments were operated under different feeding strategies to enhance model robustness.
- Raman spectra pre-processing followed by Principal Component Analysis (PCA) were performed to identify and handle outliers. Normalization algorithms were applied to reduce process variabilities.
- Time-specific spectral windows were used to enhance model robustness by restricting analyte quantification to biologically relevant process phases —pre-lysis for metabolic profiling and post-lysis for capsid detection— thereby minimizing spectral noise and improving predictive reliability. This was performed at stage 8 according to the experimental design.

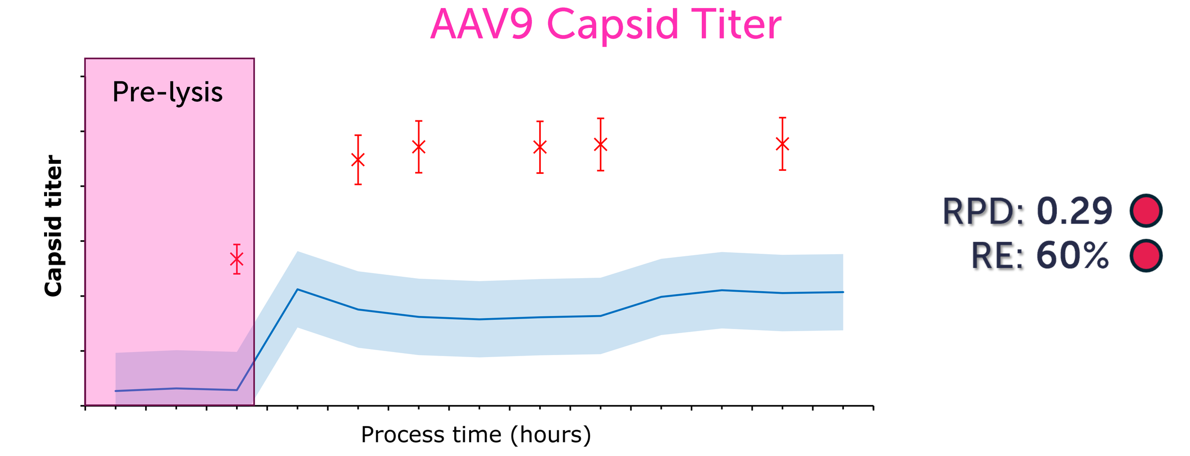
2L stirred-tank bioreactor Real-time monitoring



5L stirred-tank bioreactor Real-time monitoring



2L stirred-tank bioreactor Real-time monitoring



Legend: — Raman monitoring ■ Confidence interval × Offline value + Offline error bar (10%)

Ratio of Prediction to Deviation: poor | 1 | acceptable | 2 | good | 3 | excellent

Relative Error (%): excellent | 10 | good | 15 | acceptable | 20 | poor

Summary

- By introducing in-line capsid quantification, DSP can be directly started, avoiding the need for in-process sample analysis using standard analytical assays.
- Despite having metabolite and cell density measurements applicable to both presented serotypes and bioreactor scales, the rAAV2-based capsid titer model could not predict rAAV9 capsid titer, demonstrating its serotype specificity.
- Our collaborative innovation, leveraging ProCellics™ Raman Analyzer data, yielded a representative real-time monitoring model for scalable rAAV production. While the models for glucose, lactate, glutamine, ammonia and VCD are broadly applicable across serotypes, the capsid titer model requires serotype-specific adaptation.
- This innovative process monitoring method enables seamless platform development and process validation, providing robust and scalable solutions for a smooth transition to next-generation technologies, enhancing efficiency and confidence.

Aim higher

Ascend Advanced Therapies, 9800 Medical Center Dr D, Rockville, MD 20850

www.ascend-adv.com
ahmed.youssef@ascend-adv.com