Integration of an automated sampling system for improved understanding and control of an AAV2 production process

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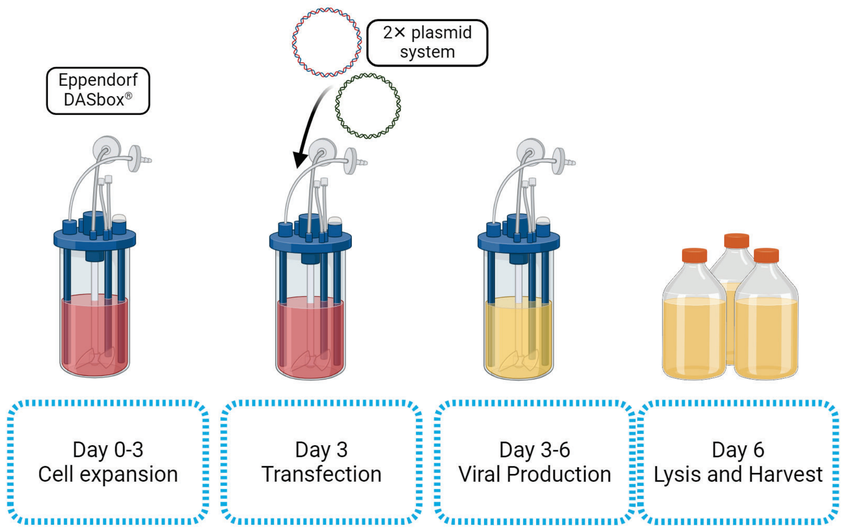
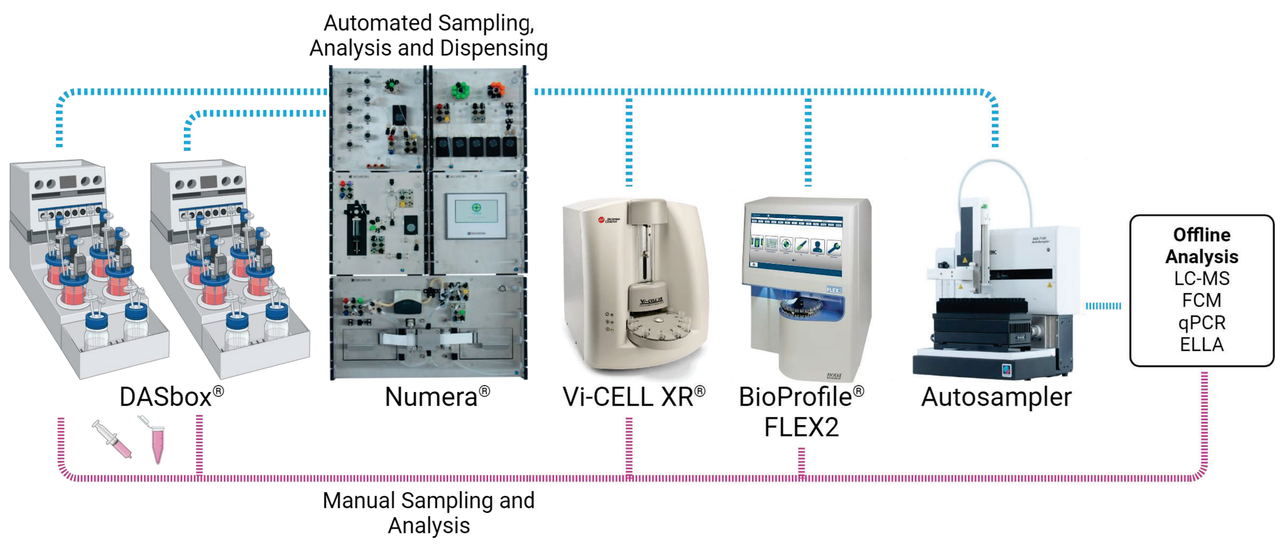
Current practices in AAV process monitoring involve infrequent, manual sampling and analysis. Atline analytics are typically decoupled from bioreactor control systems meaning real-time control of processes is not possible. The Securecell Numera® system enables fluidic integration of atline analytics with bioreactor systems, automated sample scheduling, with atline measurements enabling control of bioreactor operations through the integrated Lucullus software. The Securecell Numera® system was applied to a transient AAV2 production process in an 8-STR DASbox ® system for offline and automated at-line monitoring. Manual and automated sampling yielded similar measurements for cell viability and density, metabolite concentrations, transfection efficiency, and AAV2 titres. This demonstrated the potential of the system as a powerful tool to facilitate improved sampling frequency and in-turn process understanding and data driven process control strategies.

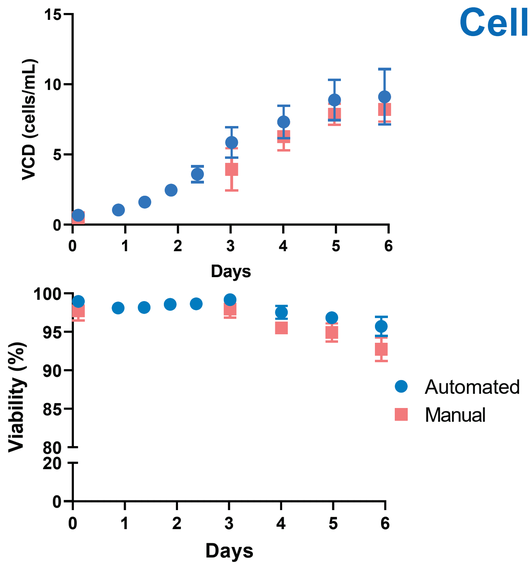
Process Overview



Sampling and Analytical Overview



Cell Counting

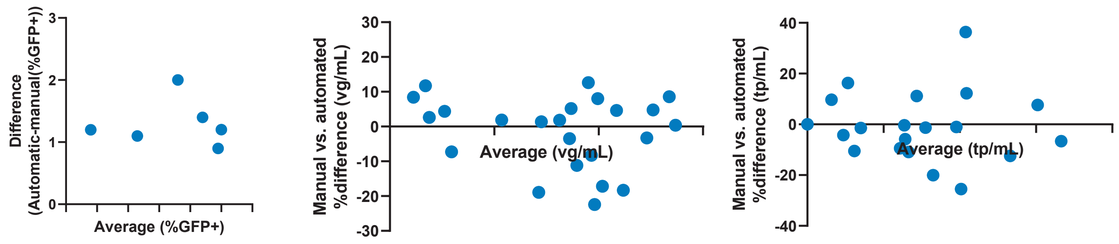


Cell counts were performed on automated and manually taken samples using the Vi-CELL XR®

Similar viable **cell density** and **viability measurements** were observed across the experiment, with a drop in cell viability observed following transfection on day 3

The Numera® enabled **additional sampling** to be performed between days 0 and 3 **without the need for an operator**

Transfection and AAV2 Quantification

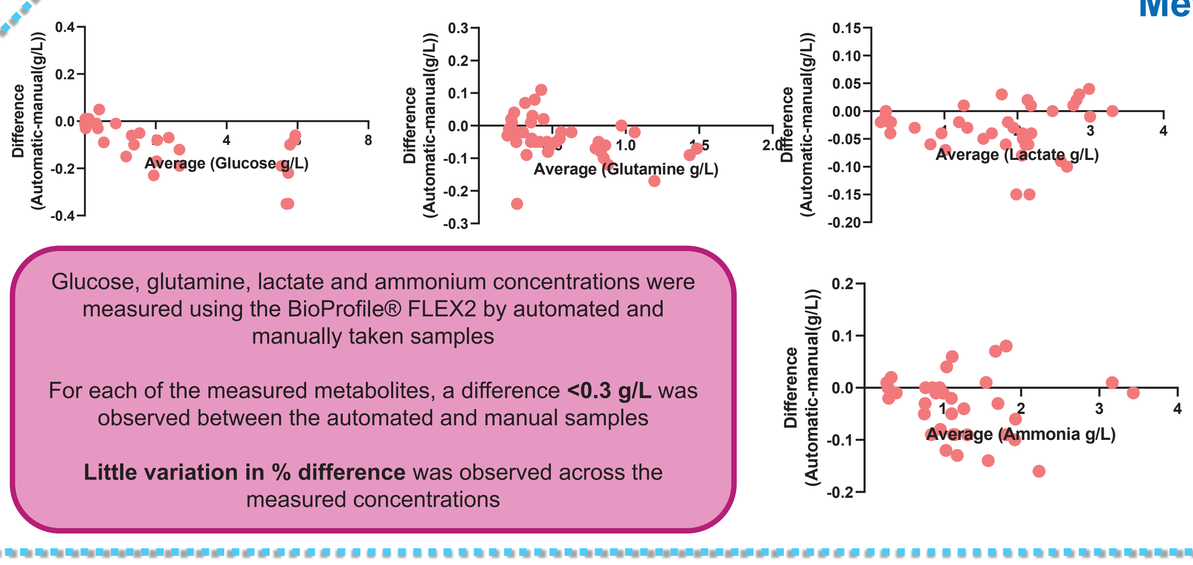


Transfection efficiency, vg/mL and tp/mL were determined using FCM, ddPCR and automated ELISA respectively

A difference of **1-2% GFP+** cells was measured for samples taken manually and automatically

For titration measurements of vg/mL and tp/mL, a difference between **0 and 20%** was observed, which is within the expected ddPCR and ELLA assay variance

Metabolites



Glucose, glutamine, lactate and ammonium concentrations were measured using the BioProfile® FLEX2 by automated and manually taken samples

For each of the measured metabolites, a difference **<0.3 g/L** was observed between the automated and manual samples

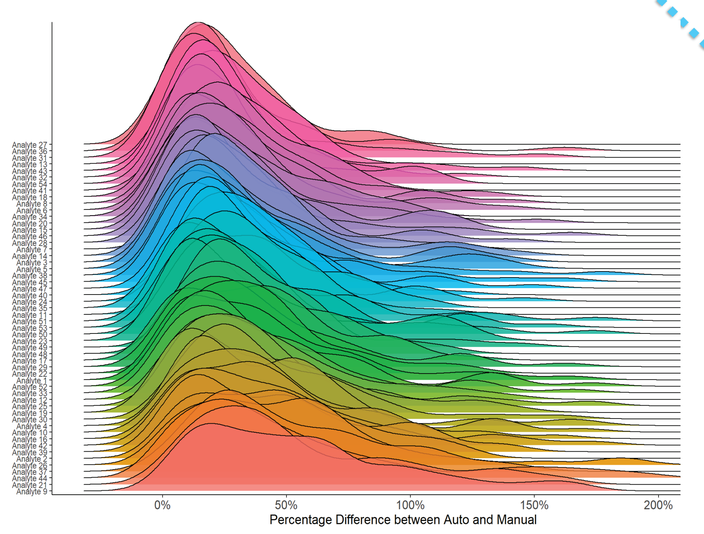
Little variation in % difference was observed across the measured concentrations

Daily automated and manual samples were taken prior to offline LC-MS analysis

A range of metabolites were measured, and the similarity of manual and automated samples was evaluated

The median difference of **28%** was measured

The difference was within the expected variance of the assay, with those measurements with greater variance close to the limit of detection

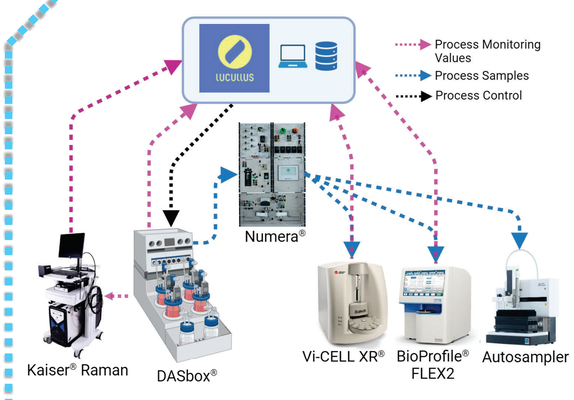


Conclusions

The Numera® autosampling system was used to monitor an AAV2 production process

Comparable measurements for **cell counting**, **metabolite profiling** and **AAV2 quantification** were shown for samples taken manually and automatically

The system has now been integrated with a data management and capture system (**Lucullus**) to allow centralised monitoring and control of AAV2 production experiments



Cell and Gene Therapy Catapult Process Analytical Technologies (PAT) Laboratory

This work was performed at the new Cell and Gene Therapy Catapult PAT Laboratory. This laboratory is an advanced bioprocessing laboratory allied to fully automated sampling and real time sample analysis. The PAT Laboratory is equipped with innovative in-line and at-line monitoring equipment which together with multi-omics capabilities, allows collaborators to identify and assess critical process parameters in real-time, while state-of-the-art automation and digitalisation facilitate rapid process control.

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