Innovations in Analytical Technology for Cell & Gene Therapy Manufacturing

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Overview

Advancements in bioprocess characterization technologies have progressed rapidly, broadening the range of readily accessible process information. While dissolved gases, pH, and temperature have been measurable in- or on-line for some time, crucial nutrients and metabolites are typically monitored via manual sampling and at- or off-line analysis.

- **PATsmart™ REBEL®**, an at-line metabolite analyzer, has the capability to characterize a comprehensive panel of core metabolites, amino acids, and other media components.
- **PATsmart™ MAVEN® and MAVERICK® provide** on- or in-line monitoring of critical process parameters related to cell proliferation (such as glucose, lactate, biomass). Both devices enable direct feedback control of substrate feeding or media exchange.

These at-, on-, and in-line analytical solutions contribute to an enhanced understanding, characterization, and control of bioprocesses in development and manufacturing of cell and gene therapies.

MAVEN, biosensor-based on-line glucose and lactate monitoring and control, even to very low levels
MAVERICK, a Raman analyzer for in-line glucose, lactate and biomass monitoring; no empirical calibration modeling required
REBEL device for at-line spent media analysis of amino acid, vitamins, and biogenic amines in cell culture media
PATsmart™ ZipChip® CE-ESI separation and ion source for MS analysis of biotherapeutic proteins and small molecules

Figure 1. Repligen upstream PAT solutions for bioprocessing

MAVERICK: Easy to implement PAT for gene therapy applications

The success of the HEK293 triple transfection process relies on optimizing key components of cell culture and refining the feeding strategy. Critical parameters like glucose and lactate, which impact cell culture viability, transfection efficiency, and the resulting viral vector titer and quality, must be meticulously monitored and controlled throughout the entire process. MAVERICK offers a Raman-based analytical solution that eliminates the need for chemometric modeling1, making it easily adaptable for the implementation of Process Analytical Technology (PAT) approaches. Additionally, it supports automated glucose control with plug-and-play in-line monitoring and feedback loop control functionality.

Figure 2. Components of MAVERICK: Optical immersion probe, measurement module and a central monitoring hub; One hub can manage up to 6 modules and bioreactors. Calibration stand and standards.

See more information and data on gene therapy applications from MAVERICK bioprocess monitoring and control on our website: https://www.repligen.com/maverick

REBEL at-line cell culture media analyzer: Actionable information for your bioprocess at the point of need

- Amino acids, vitamins, and biogenic amines analyzed at-line
- Minimal sample required: as low as 10 µL
- Simple sample prep: spin or filter and dilute
- Integrated analyzer includes autosampler, separation, detection, analysis and reporting
- Analysis run-time ~10 min per sample
- Consumable kit optimized for 200 analyses

Figure 3. REBEL System and REBEL Spent Media Analysis Kit

Multi-cytokine backpack – activated CAR T-cell spent media analysis

The nutrient compositions of the final CAR T-cell product spent media were analyzed with REBEL to identify differences in their cellular metabolism. The AA levels of all spent media samples were compared with Dynabead + exogenous IL-2 –activated T-cell spent media as the control and a reference point.

Compounds from the multi-cytokine backpack library were categorized using cluster analysis. Compounds resulting in different phenotypes, with respect to CD8+ and CD4+ levels for less differentiated CAR T-cell phenotypes as were clustered as “high”, “intermediate”, and “low” performance (Ref 1: Lin, Uricoli, et al 2023).

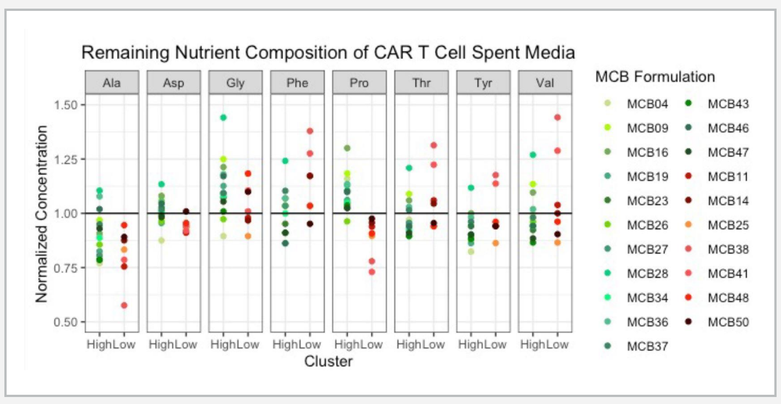


Figure 4. Eight amino acids selected here showed some differences in spent media levels; 1) as compared to the control Dynabead + IL-2 activated T-cell spent media (concentrations used for normalization, black line); 2) between the spent media samples from the “High” and “Low” MCB library compounds. For example, Ala was accumulating less in Dynabead+IL-2 than in the MCB activated T-cells. Pro was accumulating more in the “High” group (less differentiated T-cells) than in the “Low” group. This may indicate different metabolic pathways being active in less differentiated CAR T-cell products.

Correlation between amino acid consumption and capsid titer in AAV production (HEK293)

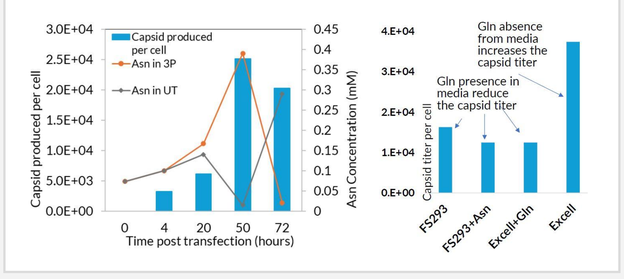
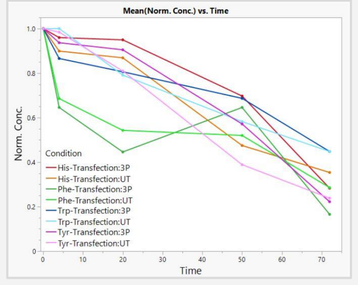


Figure 5.a Capsids produced per cell correlates with the endogenous Asn production (FreeStyle 293 medium)

Figure 5.b Capsid titer per cell increase when Gln is deprived from the culture media



Data by Prasanna Srinivasan, Paul W. Barone et al., MIT-CBI, Ref 2

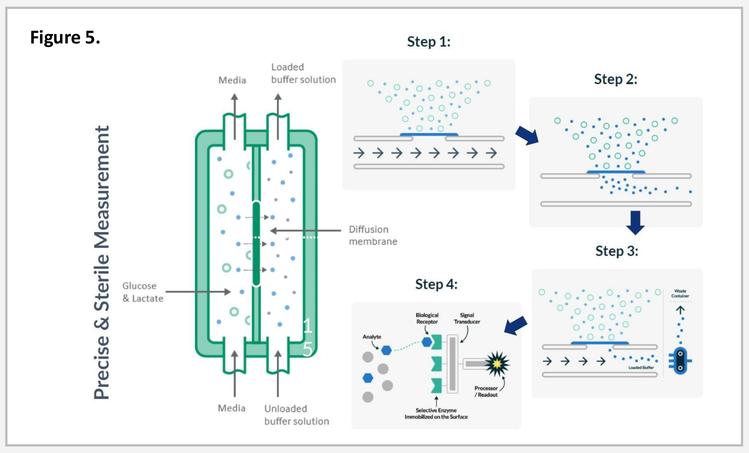
Figure 5.c - His, Phe Trp and Tyr profiles in FreeStyle 293 medium. All 4 AA showed progressive depletion post transfection similarly between 3P and UT samples. Graph generated in JMP Statistical software, 908 Devices add-on.

MAVEN: On-line monitoring in closed-system processing of cell therapies

Automatic, on-line glucose & lactate monitoring and process control (Ref 3, 4, 5)

- Flow cell provides a sterile process interface to the bioreactor
- Small molecules diffuse through the semipermeable membrane into the buffer solution
- Enzyme-based biosensor measures glucose to 0.01g/L and lactate to 0.05g/L
- No loss of bioreactor volume with diffusion-based sampling
- Significantly reduced risk of contamination as compared to manual sampling

Figure 5.



Step 1: Small amount of clean buffer from the buffer bag delivered to diffusion flow cell
Step 2: Buffer flow paused, small molecules diffuse through membrane to buffer solution
Step 3: Flow is resumed, loaded buffer delivered to measuring cell
Step 4: Analyte concentrations measured by biosensor, used buffer disposed into waste container

MAVEN integration with Terumo BCT Quantum Flex Cell Expansion System

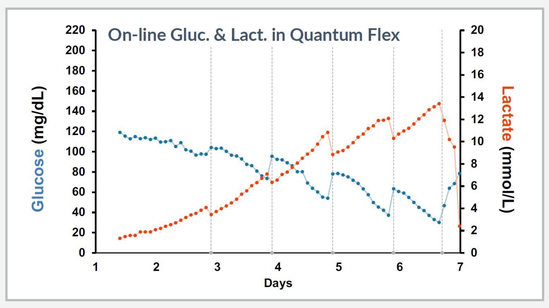


Figure 6. Quantum Flex Cell Expansion System (Terumo Blood and Cell Technologies): Jurkat Cell Expansion MAVEN flow cell integrated into the extra-capillary (EC) loop MAVEN detects culture changes in real-time, enabling fine control of cell expansion conditions and reducing hands on time for measurements.

(Ref 3, 4)

Results

Gene and cell therapy manufacturing needs efficiency and robustness to consistently produce products with improved treatment outcomes in patients.

- Gene therapy process development can be improved with easy implementation of Raman based real-time monitoring of glucose, lactate and biomass using MAVERICK.
- Cell therapy manufacturing process is dependent on multiple stages of cell manipulation, throughout which cell growth and health monitoring are imperative. With MAVEN integration into cell therapy expansion systems, such as Terumo BCT Quantum Flex, continuous, automated (on-line) measurement of lactate and glucose, enables data-driven decisions for optimization of cell therapy manufacturing.

Conclusion

The instant PAT implementation with MAVERICK, versatility, and sensitivity of MAVEN and point of need availability of amino acids analysis with REBEL, enable deeper knowledge and actionable information of cell and gene therapy bioprocesses.

Further, the devices informing power can be leveraged with the increasing sophistication of bioprocess metabolic models for predictive bioprocess optimization and control.

References

- Ref 1: Lin H and Uricoli B et al. Adv Healthc Mater. 2024 Jan 21:e2302425
- Ref 2: Moving Towards a Scalable AAV Vector Production at High Volumetric Efficiency
- Ref 3: Terumo BCT Brochure – MAVEN connection – 2024
- Ref 4. Webinar: Improving Cell Therapy Manufacturing with On-line Monitoring
- Ref 5: 908 Devices: Continuous Monitoring and Control of Glucose and Lactate with MAVEN: Impact on Cell Culture Performance and Protein Quality

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