Improving Cell and Gene Therapy Manufacturing Processes by Automated On-line and In-line Bioprocess Analytical Technologies

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Overview

Advancements in technologies for near real-time bioprocess characterization have progressed rapidly, broadening the range of readily accessible process and product attributes. While fundamental factors like dissolved gases, pH, and temperature have been measurable in or online for some time, other crucial nutrients and metabolites are often infrequently measured using offline analytics.

The PATsmart™ REBEL® System, an at-line metabolite analyzer, has the capability to characterize a comprehensive panel of core metabolites, amino acids, and other media components. Moreover, essential metabolites, waste components, and attributes related to bioprocess proliferation (such as glucose, lactate, biomass, and protein productivity) now have *in-situ* possibilities with various solid-state, membrane, and optical transducers like the PATsmart™ MAVEN® and MAVERICK® Systems.

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

Figure 1. Repligen solutions for bioprocess

Results

Cell therapy manufacturing needs efficiency and robustness to consistently produce products with improved treatment outcomes in patients. The cell therapy manufacturing process is dependent on multiple stages of cell manipulation, throughout which cell growth and health are imperative. Continuous, automated (on-line) measurement of process key components, lactate and glucose, enable data-driven decisions to be made for an effective cell product manufacturing process. However, this has been challenging due to the low level of analyte measurements required, small batches, and processing bioreactors and methods currently used. We discuss an automated on-line, biosensor-based analytical solution for various cell therapy bioprocess scales and processes.

MAVERICK: Easy implementation of PAT for cell and 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 modeling, 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 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.

Figure 3A

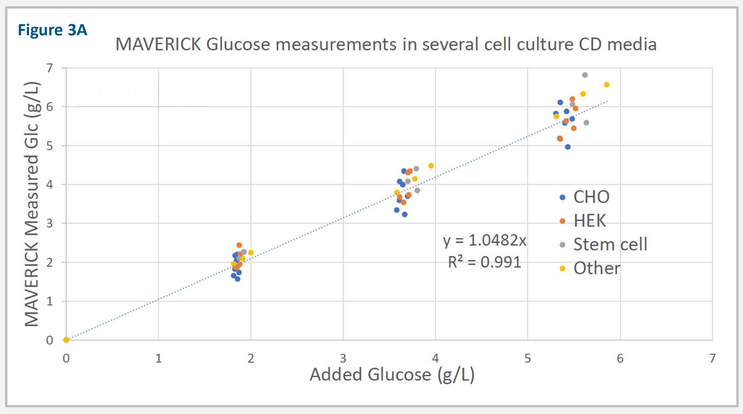


Figure 3B

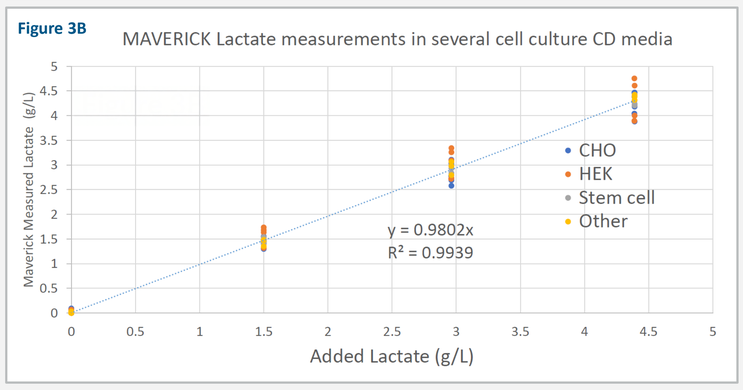
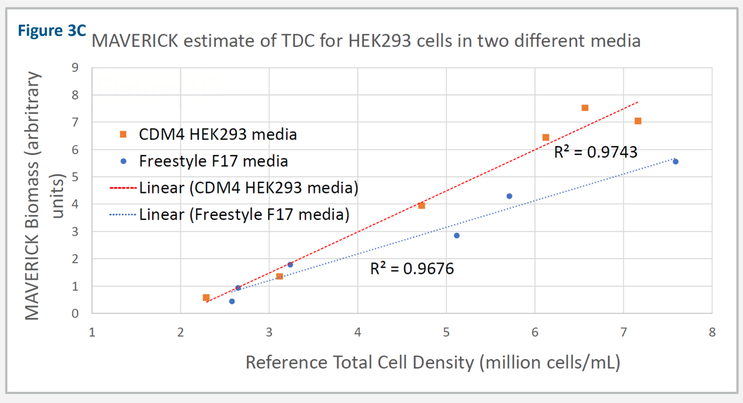


Figure 3C



Evaluation of consistency, linearity, and selectivity of MAVERICK measurements

- by spiking in **glucose and lactate**
- in mammalian cell culture media samples[1]
- linearity and correlation to off-line reference measurements shown here (**Figure 3A and 3B**).
- In addition: MAVERICK measurements typically will provide a precision of <0.1 g/L for both glucose and lactate: MAVERICK measurements in fresh CD HEK293 medium under stable conditions showed a standard deviation of 0.09g/L for glucose and 0.04g/L for lactate.

To assess the linearity between **biomass measurements** using MAVERICK and offline total cell densities, two cell lines (a CHO and a HEK293 cell line) were cultured for 14 days and 6 days respectively.[2]

We saw great correlation between MAVERICK biomass data and offline total cell counts for CHO (not shown) and HEK293 (see **Figure 3C**).

MAVEN: On-line automated analysis and data processing with *in-situ* probe sampling

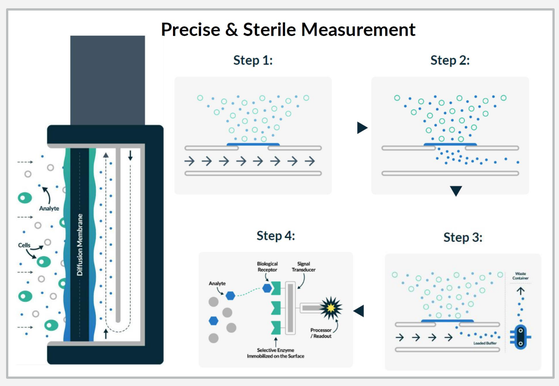


Figure 4.

Step 1: A small amount of clean buffer is delivered from the buffer bag into the diffusion probe

Step 2: Buffer flow is paused and analyte molecules diffuse through the membrane into the buffer solution

Step 3: Flow is resumed, and the loaded buffer is delivered to the measuring cell

Step 4: Analyte concentrations are measured by the biosensor and the used buffer is disposed into the waste container

Automatic, on-line glucose and lactate monitoring

- Diffusion probe installed in bioreactor connects bioreactor content to biosensor
- Enzyme-based biosensor detects glucose to 0.01g/L and lactate to 0.05g/L
- Small molecules diffuse through the semipermeable membrane into the buffer solution
- No loss of bioreactor volume
- Significantly reduced risk of contamination as compared to sample pulls
- Enables on-line analytics and process control

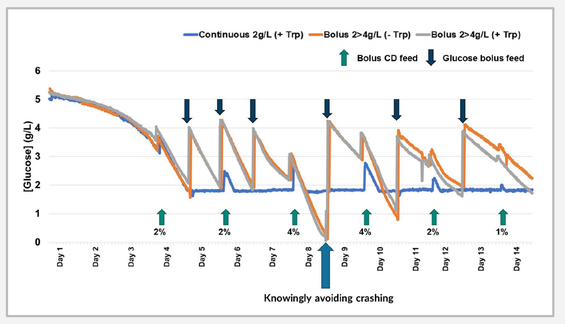


Figure 5A. Glucose measurements using the MAVEN in a CHO 10L bioreactor run: bioreactor 1 (continuous 2g/L glucose controlled by MAVEN), bioreactor 2 (bolus 2 to 4 g/L glucose (-Trp) and bioreactor 3 (bolus 2 to 4 g/L glucose (+Trp).

On day 8 the glucose levels dropped close to zero in bioreactors 2 and 3. A potential cell culture crash was avoided by monitoring the glucose levels closely with the MAVEN realtime measurements and adjustment of glucose feeding time.

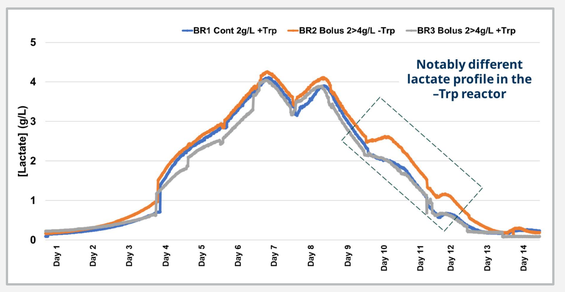


Figure 5B. Lactate profiles in run 1 for all three bioreactors. The real-time measurements showed a shift in the days 9-13 for the bioreactor that was not fed extra tryptophan in the amino acid mix.

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

- Minimal sample requirement 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 6. REBEL Spent Media Analysis Kit

Conclusions

Recent advances in these cell and gene therapy bioprocess analytical technology regimes, approaches to de novo analytical modeling/calibration of these systems, and how their informing power can be leveraged with the increasing sophistication of bioprocess metabolic models for predictive bioprocess optimization

References

1. 908 Devices: Instant Implementation of Raman-based PAT with MAVERICK for Monitoring Glucose and Lactate
2. 908 Devices: Determining Total Cell Count with MAVERICK Biomass Measurements
3. Continuous Monitoring and Control of Glucose and Lactate with MAVEN: Impact on Cell Culture Performance and Protein Quality

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