Instant PAT Implementation with Raman-based MAVERICK® System across Cell Lines, Media, and Scales

Application Note

The MAVERICK® System provides an in-line, Raman-based PAT solution for instant monitoring and control of glucose, lactate, and biomass in various CHO and HEK293 cell lines, media types, and bioreactor scales.

Repligen Corp. now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.

Introduction

In this application note, we share results from experiments where the MAVERICK System was used to monitor critical process parameters glucose, lactate, and biomass in real-time, without user refinement or previous bioprocess runs to create measurement chemometric models. Raman spectroscopy traditionally involves complex setup built on process-specific conditions and the combined signals from a complex cell culture. In contrast, the MAVERICK System offers an accessible, plug-and-play solution that is widely applicable across key cell lines used in bioprocess, many cell culture formulations, and bioreactors scales.

Monitoring and control of critical process parameters (CPPs) such as glucose and lactate are essential to maintaining optimal cell culture conditions, ensuring product quality, and maximizing yield. Traditional Raman spectroscopy provides great potential to monitor and control CPPs; however, it requires expert configuration, setup time and modeling iterations that can take months or years, and substantial expense.[1,2] The MAVERICK System offers the advantages of in-line Raman process analytical technology (PAT) without the cost and headaches inherent to the implementation of conventional process spectroscopy-based methods. Unlike conventional Raman analyzers, which rely on empirical modeling of spectral data to off-line reference measurements, the MAVERICK System utilizes a *de novo* algorithm that does not require empirical trial-and-error "training" on bioprocess data.[3,4] The *de novo* model is explicitly built on the generalizable chemistries likely to be encountered in CHO and HEK293 chemically defined cell culture medium, and the detailed performance attributes of the spectrometer and environment directly. Implementation of the MAVERICK System to start measuring glucose, lactate, and biomass in real-time takes about 60 minutes and does not require any modeling expertise.[5]

The MAVERICK System was shown to perform robustly and give readings in accordance with at-line measurements for glucose, lactate, and biomass. We were able to demonstrate PAT deployment across multiple cell lines, cell culture media, and processes.

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Material and Methods

CHO and HEK293 cultures in various cell culture media, bioreactor type and scale were monitored in-line with the MAVERICK System (for glucose, lactate, and biomass) in addition to at-line measurements of Viable Cell Density (VCD), viability, and multiple metabolites as described in Table 1 and Table 2.

Each MAVERICK System measurement module can be connected directly to a feed pump through a simple wired analog connection. The MAVERICK System supports pump control through either current or voltage and uses a simple hysteresis-based, closed-loop control to inform the pump when additions should be made. Configuring the pump control settings is as easy as choosing the analyte to control, choosing a set-point, a dead-band, and the high and low output percentage for the pump.

Setup and Calibration

The immersion probe was attached to the MAVERICK System measurement module. Two-point calibration was performed as per instructions in the user manual, prior to detaching the probe and autoclaving it. After sterilization the probe was re-attached to the module and runs were started. Components of the MAVERICK System are shown in Figure 1.

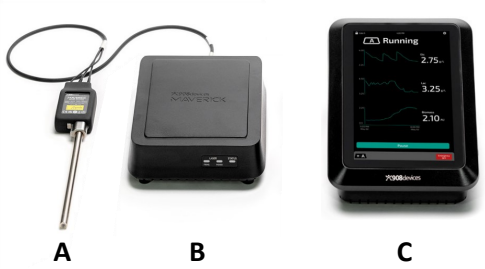

Figure 1. MAVERICK System components: optical immersion probe (A), measurement module (B), and a central monitoring hub (C).

Run # and Location	CHO Strain and Seeding	Cell Culture Basal and Feed Media	Bioreactor Type and Scale	Feeding Strategy	At-line Analytics
1. 908 Devices	CHO-S (Thermo Fisher Scientific) Seeding at 1.41 x 10^6 cell/mL	**Basal medium:** HyClone ActiPro (Cytiva) **Feed medium:** Cell Boost 7A/7B (Cytiva)	Sartorius Biostat A plus; 2 L	**Fed-batch** Feeding at 2% v/v daily **Glucose feeding** to 6 g/L with trigger point at 4 g/L	**VCD/viability:** ViCELL XR (Beckman Coulter) **At-line glucose/lactate:** BioProfile FLEX2 Analyzer (Nova Biomedical)
2. Culture Biosciences (South San Francisco)	NIST CHO-mAb Seeding at 1 x 10^6 cell/mL	**Basal medium:** EX-CELL Advanced CHO Medium (SAFC) **Feed medium:** EX-CELL Advanced CHO Feed 1 with glucose (SAFC)	Culture bioreactor; 250 ml	**Fed-batch** Feeding at 3.8% v/v daily starting at day 2 **Glucose feeding:** bolus feeding to 5 g/L with trigger point at 4 g/L	**VCD/viability:** ViCELL XR (Beckman Coulter) **At-line glucose/lactate:** BioProfile FLEX2 Analyzer (Nova Biomedical) **Titer:** Cedex Bio Analyzer (Roche)
3. CPI (UK)	GS-CHO-mAb Seeding at 0.3 x 10^6 cell/mL	**Basal medium:** CHO basal media Panel medium 6 (Thermo Fisher Scientific) **Feed medium:** CD Efficient Feed C (Thermo Fisher Scientific)	Sartorius Biostat B-DCU II; 10 L	**Fed-batch** Feeding every other day beginning on day 3 with pyramid schedule (2%, 2%, 4%, 4%, 2%, 1%). **Glucose feeding:** continuous feeding at 2 g/L	**VCD/viability:** ViCELL XR (Beckman Coulter) **At-line glucose/lactate:** Cedex Bio HT Analyzer (Roche) **Titer:** Octet (Sartorius)
4. 908 Devices	NIST CHO-mAb Seeding at 0.5x 10^6 cell/mL	**Basal medium:** EX-CELL Advanced CHO Medium (SAFC) **Feed medium:** EX-CELL Advanced CHO Feed 1 without glucose (SAFC)	Distek BIOne 1250 dual; 2 L	**Fed-batch** Feeding every other day 5% v/v beginning on day 3. **Glucose feeding:** bolus feeding to 6 g/L with trigger point at 4 g/L	**VCD/viability:** ViCELL XR (Beckman Coulter) **At-line glucose/lactate:** BioProfile FLEX2 Analyzer (Nova Biomedical) **Titer:** HalCo (RedShiftBio)

Table 1. Various CHO runs monitored with the MAVERICK System for in-line continuous glucose, lactate, and biomass measurements.

Run # and Location	HEK293 Strain and Seeding Density	Cell Culture Basal and Feed Media	Bioreactor Type and Scale	Feeding Strategy	At-line Analytics
5. U MASS Lowell (Prof. Yoon)	HEK293 CRL-1573 (ATCC) Seeding at 0.5 x 10^6 cell/mL	**Basal medium:** FreeStyle F17 **Expression medium:** (Thermo Fisher Scientific)	Yokogawa BR1000; 3L	Media were supplemented with 4 mM L-glutamine. Glucose was fed as a bolus to 4 g/L with trigger point at 2 g/L	**VCD/viability:** Cellometer cell counter (Nexcelom) **At-line Glucose/lactate:** BioProfile FLEX2 Analyzer (Nova Biomedical) and YSI 2900 Series Biochemistry Analyzer
6. U MASS Lowell (Prof. Yoon)	HEK293 CRL-1573 (ATCC) Seeding at 0.5 x 10^6 cell/mL	**Basal medium:** CDM4HEK293 medium (Cytiva)			
7. 908 Devices	HEK293F Seeding at 0.5 x 10^6 cell/mL	**Basal medium:** Gibco LVMAX (Thermo Fisher Scientific)	Distek BIOne 1250 dual; 2L	Glucose control set point at 5 g/L, feed includes GlutaMAX with an action limit <1 mM & a supplement target of 2 mM	**VCD/viability:** ViCELL XR (Beckman Coulter) **At-line Glucose/lactate:** BioProfile FLEX2 Analyzer (Nova Biomedical)

Table 2. HEK293 runs monitored with MAVERICK for in-line continuous glucose, lactate and biomass measurements.

Results: Monitoring of Process Parameters in Various CHO and HEK293 Cell Lines and Media With MAVERICK Systems

MAVERICK Systems were installed in all runs described in Table 1 and Table 2. No training occurred for any of these bioprocesses or cell culture media; instead, the MAVERICK System *de novo* model works right out of the box,[4] taking into account both static and dynamic parameters measured during the actual bioprocess runs. Static parameters include spectrometer specifications, probe properties, and media components. Dynamic parameters include the light environment, increasing fluorescence as the cell mass increases, and changing composition of the cell culture media. Real-time, in-line monitoring of glucose, lactate, and biomass was started immediately at the start of each culture. At-line glucose and lactate concentrations and total cell densities were also collected as described in Table 1 and Table 2.

CHO Cultures

Four CHO fed-batch culture runs using three different CHO cell lines, three different CHO chemically defined media, and three different bioreactor scales (from 250 mL to 10 L) were performed as described in Table 1.

MAVERICK devices were installed in each bioreactor. Maximum VCD reached between 20–35 million cells/mL (Figure 2). The CHO cultures reached 60%–84% viability on last day of the cultures. As shown in Figure 3, the MAVERICK System predicted values align closely to the at-line glucose and lactate measurements. In addition, biomass measurements were compared to total cell densities measured at-line as shown in Figure 4 and showed close alignment.

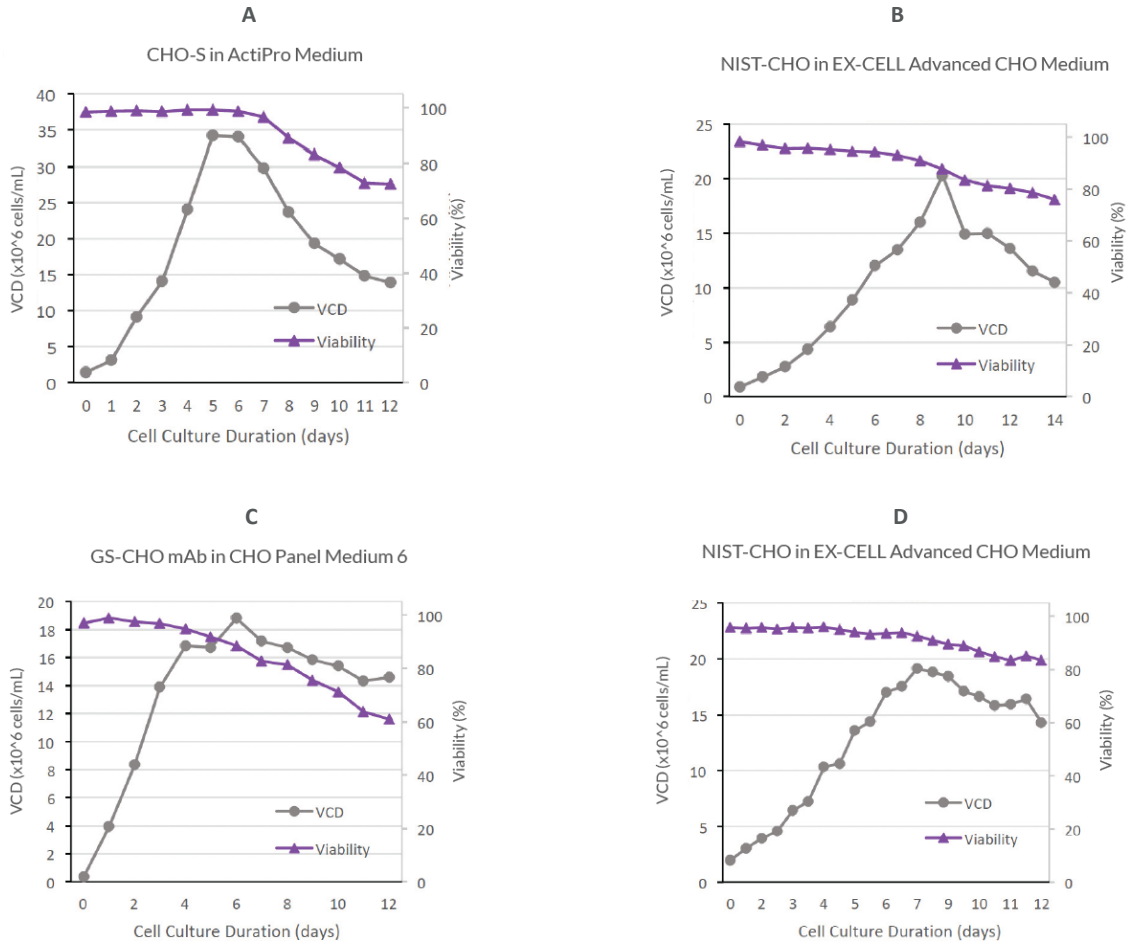

Figure 2. At-line viable cell density and viability measurements for the CHO cultures as described in Table 1.

A: CHO-S, 2 L bioreactor, Hyclone ActiPro medium

B: NIST-CHO, 250 mL bioreactor, EX-CELL Advanced CHO Medium

C: GS-CHO mAb, 10 L bioreactor, Thermo Fisher Scientific CHO basal media Panel medium 6

D: NIST-CHO, 2 L bioreactor, EX-CELL Advanced CHO Medium

HEK293 Cultures

Two different HEK293 cell types, three different chemically defined media, and two bioreactor scales were performed as shown in Table 2. MAVERICK probes were installed in each bioreactor.

Maximum VCD reached between 5–7 million cells/mL (data not shown). As shown in Figure 5 and Figure 6, the MAVERICK System measurements align closely with the at-line glucose and lactate measurements. Biomass also correlated nicely with the total cell density measurements from at-line devices (described in Table 2).

Conclusions

The MAVERICK System provides an in-line, Raman-based PAT solution for monitoring and control of glucose, lactate, and biomass in up to 6 bioreactors right out of the box. Data presented in this application note demonstrates how the MAVERICK System can be used across several CHO and HEK cell lines, eight chemically defined media, and bioreactor scales from 250 mL to 10 L. The MAVERICK System eliminates the implementation hurdles inherent to traditional in-line Raman-based PAT process controls. Contact Repligen to learn how MAVERICK Systems can accelerate your next bioprocess.

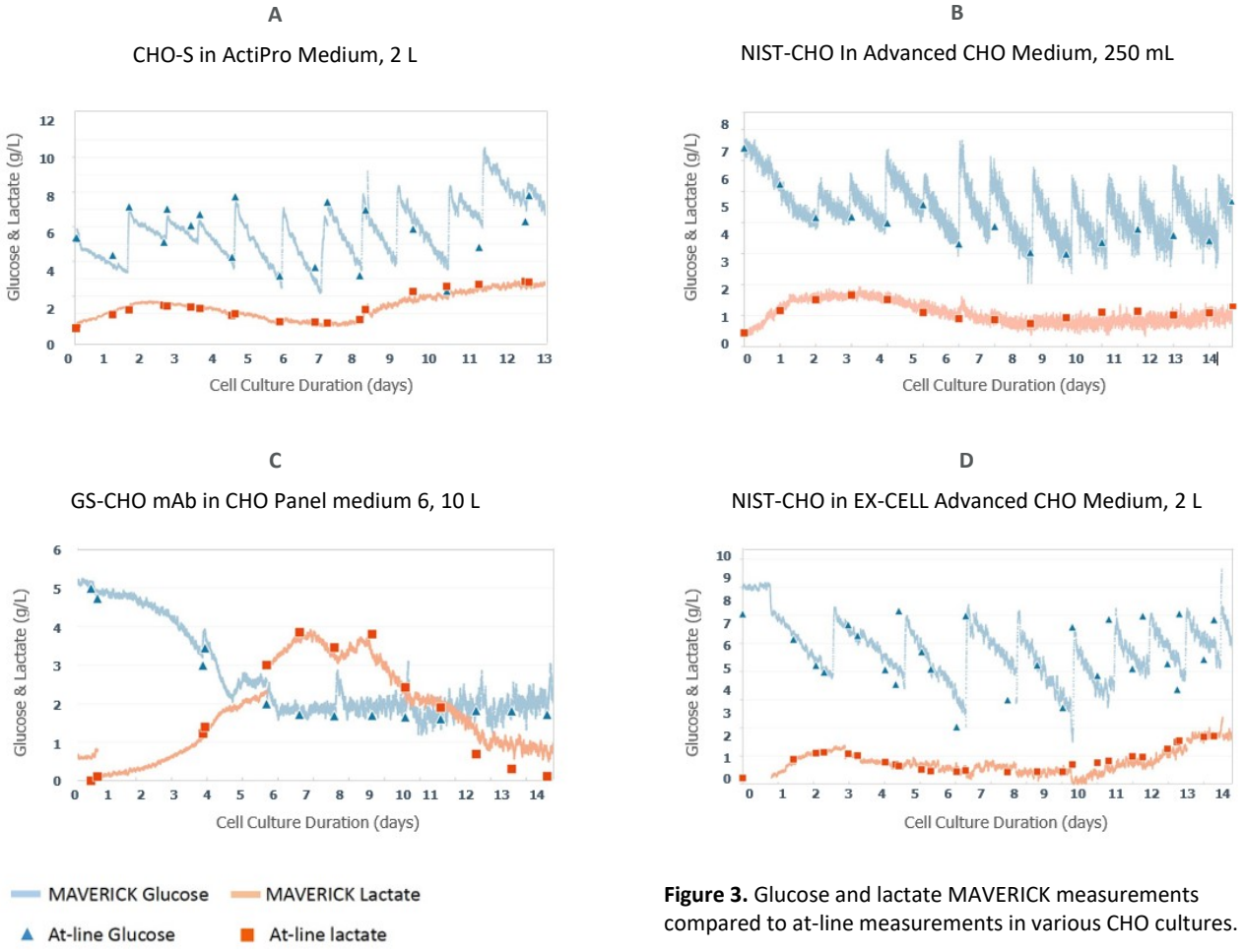

Figure 3. Glucose and lactate MAVERICK measurements compared to at-line measurements in various CHO cultures.

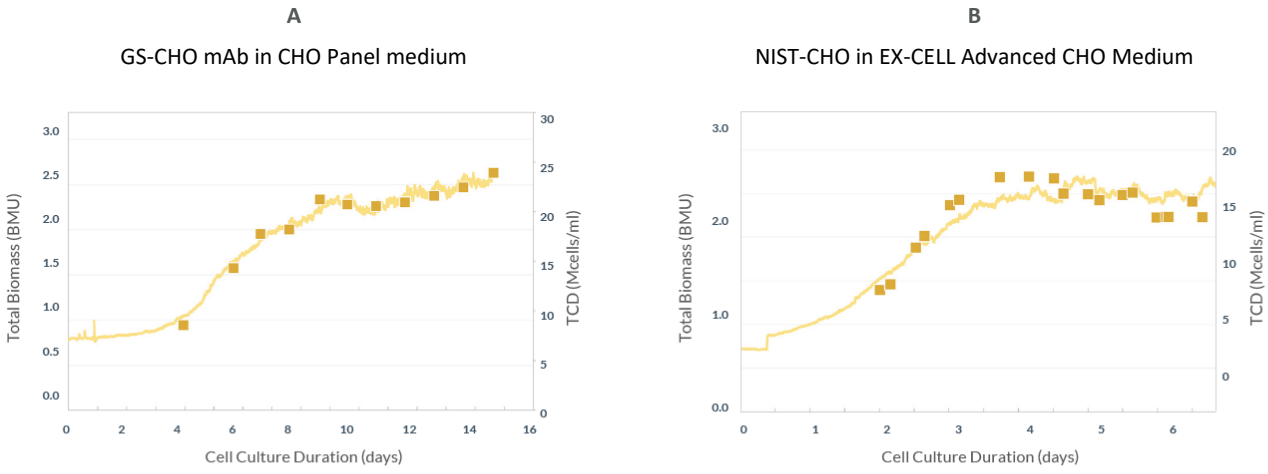

Figure 4. MAVERICK biomass measurement and at-line total cell density for CHO runs 3 and 4. Total cell density (TCD) were measured as shown in Table 1.

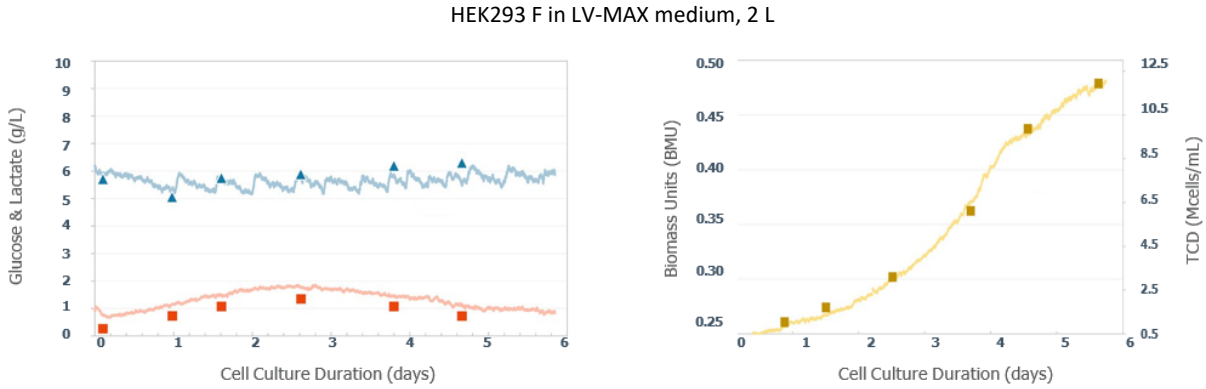

Figure 5. Glucose and lactate MAVERICK measurements and comparison to at-line measurements for HEK293 F in LV-MAX medium in a 2 L Distek bioreactor.

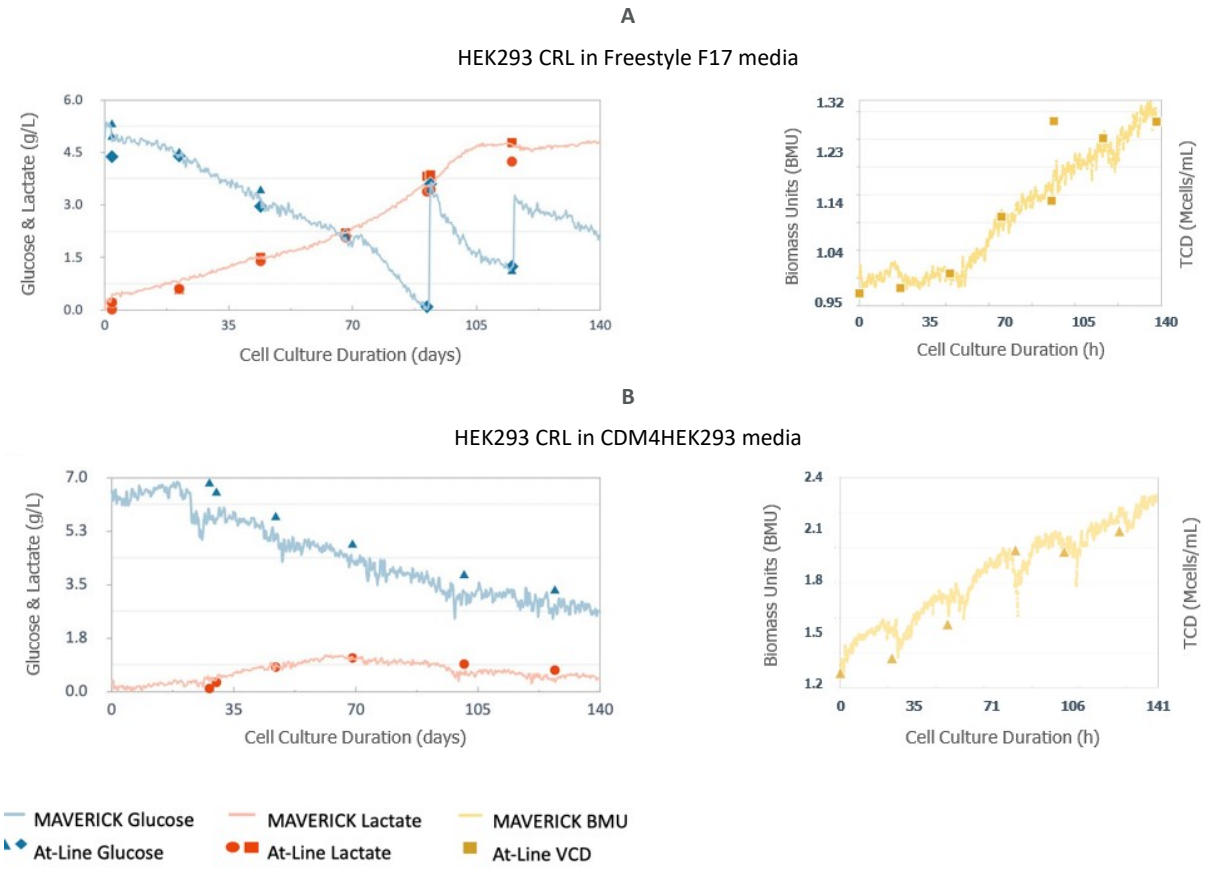

Figure 6. Glucose and lactate MAVERICK measurements and comparison to at-line measurements for HEK293 CRL in two different media in 3 L Yokogawa bioreactor.

References

This work was partly performed in Professor Seongkyu Yoon's laboratory at the University of Massachusetts Lowell and at Culture Biosciences, South San Francisco.

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