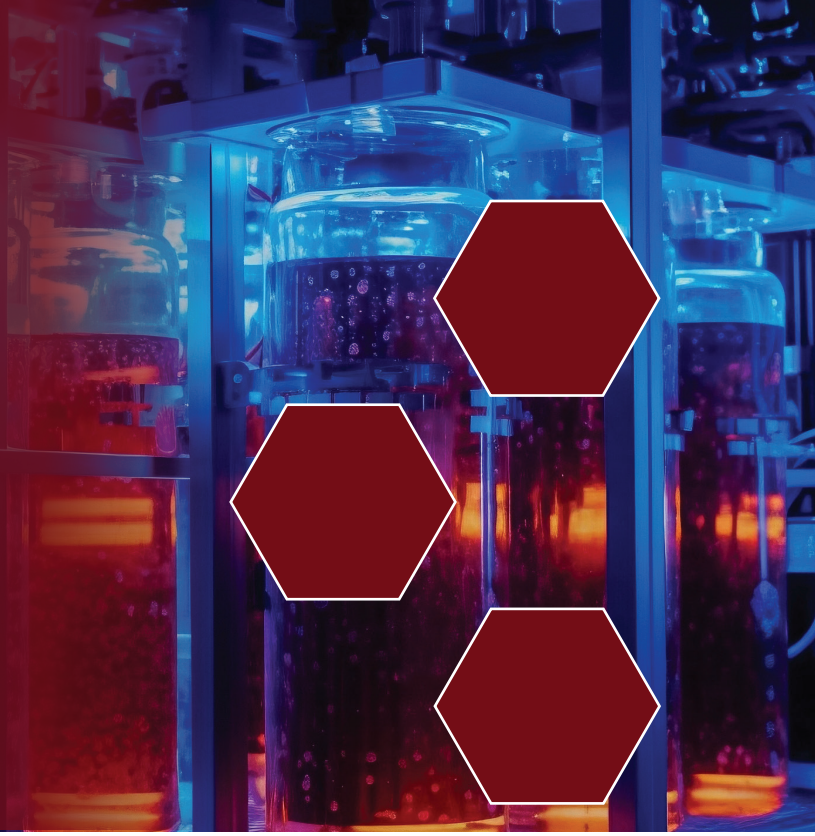
Monoclonal Antibody Manufacturing Trends, Challenges, and Analytical Solutions to Eliminate Bioprocessing Bottlenecks

REDSHIFTBio® | Cell Culture DISH

The Current Landscape of Monoclonal Antibody Production



Bioprocessing technologies to produce therapeutic monoclonal antibodies (mAbs) have evolved tremendously since the first licensed mAb product was introduced to the market in 1986. Early mAb production processes were relatively inefficient and generated titers well below 1 g/L[1].

Over the past three decades, the field has evolved significantly, leading to steady improvements in biomanufacturing efficiency and productivity. Along the way, we have seen improved cell lines, optimized culture media and feeds, intensified processing, and modernized purification platforms have collectively increased mAb titers into the gram-per-liter (g/L) range and beyond[2,3].

As the importance and demand for mAbs continues to grow, innovations and new technologies in this space will be needed to keep pace. Key drivers such as speed to-market, productivity, and cost-efficiency continue to push the boundaries of current mAb production processes. In this article, we will discuss the current landscape of bioprocess technologies and explore industry trends and advancements that have contributed to enhanced efficiency, cost-effectiveness, and accelerated production timelines.

Data-Driven Decision Making

In this era of data-driven decision-making, advanced analytics, particularly through Process Analytical Technology (PAT) as part of Quality by Design (QbD) methodologies, play a crucial role in guiding manufacturing decisions from cell line development to downstream purification. A typical mAb has 15–20 quality attributes that affect its efficacy and stability, including potency, aggregate levels, variant profile, and glycosylation[4]. Monitoring these attributes involves a suite of analytical tools; however, many of them originated in the research laboratory and are not well-suited for the bioprocessing environment.

The need for rapid and precise measurements, particularly during upstream processing, has given rise to innovative PAT tools that leverage established technologies like high-performance liquid chromatography (HPLC) and mass spectrometry (MS) that are designed specifically for the bioprocessing space[4]. Perhaps the most notable shift in mAb manufacturing has been the increased speed of analysis—time-to-data has improved dramatically.

One noteworthy addition to the toolbox is the RedShiftBio HaLCon Protein Analyzer, offering rapid, at-line antibody titer measurements for process monitoring and control that extend across the entire bioprocessing workflow. These next-generation analytical tools can help support PAT initiatives by allowing for real-time adjustments to process variability that help maintain a consistent Critical Quality Attribute (CQA) profile in the final product. Access to rapid and continuous data on critical parameters during various stages of bioprocessing contributes to a more streamlined and data-driven approach to process optimization.

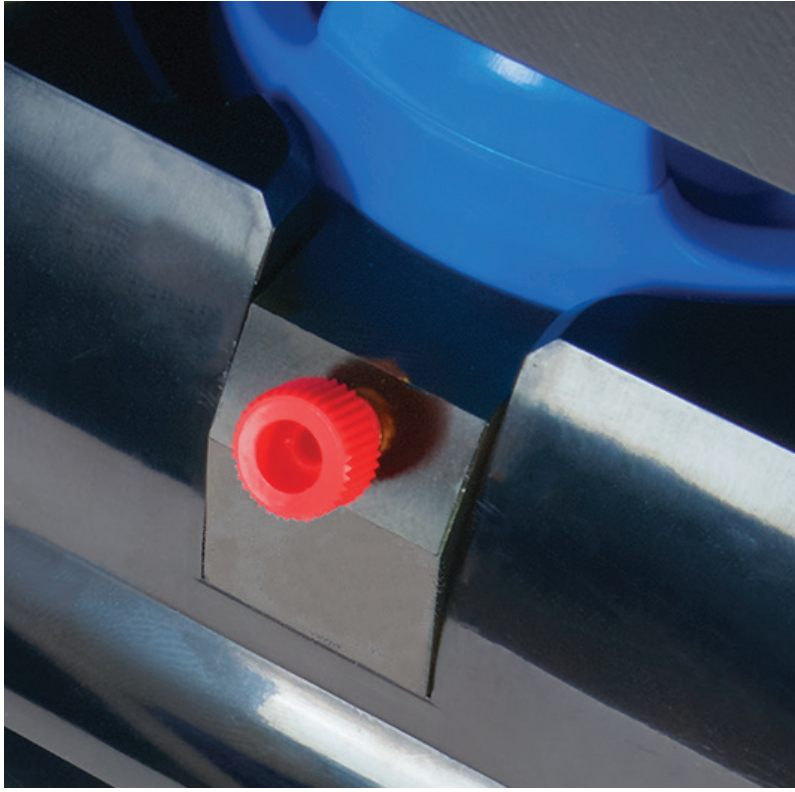
Current State of Upstream Bioprocessing

A variety of mammalian expression systems are utilized for biologics production, but of these, Chinese hamster ovary (CHO) cells dominate the market, producing 70% of biologics and nearly all mAbs[5]. CHO cells are favored for their ease of genetic manipulation, resistance to human viral infection, ability to perform complex human-like post-translational modification, suitability for large-scale industrial culturing, and history of regulatory approval.

Cell Line Development

Developing stable CHO cell lines for efficient therapeutic protein production involves transfecting host cells with a gene for protein expression, amplifying and selecting clones based on stability and productivity, and finally, choosing the most productive clone—a process that is time-consuming and iterative. Advances in genetic engineering and cell line development methods have enabled more targeted approaches to improve cell productivity.

Since the 2010's, the emergence of omics tools and rational design have contributed to the deeper understanding of cellular characteristics and identity profiles that optimize production[5,6].



Once omics analysis identifies targets that influence clone productivity, these characteristics can be engineered into cell lines to create stable, high-performance clones. With the development of CRISPR/Cas9 technology, it is possible to precisely engineer host cell lines that are stable and can produce proteins with the desired quality and productivity.

Another avenue for further development is the potential use of alternative expression systems such as bacteria, insect, yeast, and plant cells that can produce antibodies with even higher productivity while preserving glycosylation patterns that are compatible with the human immune system[3]. These developments have the potential to take the field beyond the current state-of-the-art production in CHO cell culture, yet more work is required to fully evaluate these systems for large-scale biopharmaceutical production processes.

Cell Culture Optimization

To address safety concerns related to prion and viral contamination, serum-free, protein-free, and chemically defined media formulations have replaced serum-containing media in mammalian cell biomanufacturing. Media development, in conjunction with cell line optimization, is a key factor in improving productivity and growth behavior of cells. Media components can have dramatic effects, both positive and negative, on productivity, growth and protein quality. Design of Experiments (DoE) and high-throughput methods are typically employed to assess component concentrations and interactions to identify media components that drive both productivity and desired quality profiles for each monoclonal antibody product[7]. The availability of scaled-down bioreactor systems coupled with at-line and operator-independent analytics platforms like the HaLCon has facilitated efficient testing of various media formulations, enabling more rapid identification of optimal conditions for cell growth and antibody production.

Spent media analysis is a common practice for optimizing formulations and developing feeding strategies in fed-batch systems. Dynamic Flux Balance Analysis (DFBA) is a new approach, which looks at the relationship between media supplementation, feeding strategies, increased product yield, extended growth phase, and higher cell density[7].

Bioreactor Design

Another trend in bioproduction has been the implementation of single-use bioreactor systems, offering benefits like lower capital investment, reduced cost of production, enhanced flexibility, and improved end-product quantity and quality. Improved bioreactor technologies, featuring improved oxygen transfer, mixing, and nutrient distribution, create controlled and efficient environments for cell growth and high-yield antibody production[8].

The biopharmaceutical industry employs various cultivation modes such as batch, fed-batch, and perfusion for biologics manufacturing. In perfusion culture, cells are continuously supplied with fresh culture media while spent media and byproducts are removed from the bioreactor. This process allows cells to remain in the bioreactor for an extended period, in contrast to traditional batch or fed-batch cultures where feed media changes are intermittent. Advantages of perfusion include greater consistency in cell viability, higher cell densities, and volumetric productivity. The integration of real-time or in-line monitoring techniques for critical parameters like cell density, viability, and antibody titer, alongside data analytics and process control strategies, provides ongoing insights into the culture's health, and enable bioprocess engineers to make real-time decisions to optimize the timing for cell harvest to maximize product yield[9].

Downstream Processing Bottlenecks

Upstream process intensification advancements have resulted in higher cell densities and antibody concentrations of 10 g/L and higher. This efficiency has introduced new challenges and bottlenecks in downstream processing due to increased product and contaminant concentrations. Downstream antibody processing is typically a platform approach involving Protein A affinity chromatography, followed by additional chromatography steps for polishing and viral clearance, and final formulation and filling. While many of the workhorse Protein A resins used for capture have dynamic binding capacities (DBC) in the 30-45 g/L range, industry interest has grown in alternative chromatographic methods having higher capacity, throughput, and reduced cost[10].

Alternatives to Protein A

The growing diversity of antibody-based therapies has led to the emergence of new affinity resins designed to bind to non-Fc-containing fragments or resins with better dynamic binding capacity, milder elution conditions, and resistance to NaOH for clean-in-place operations[11]. Bioengineers are also exploring various alternative purification technologies, such as Protein G and Protein L chromatography, in addition to non-affinity methods like cation exchange, hydrophobic interaction, mixed-mode chromatography, as well as precipitation and crystallization[12].

Despite ongoing advancements, Protein A resins are expected to remain the gold standard in monoclonal antibody purification for the foreseeable future. However, the increasing diversification of antibody products and growing cost pressures may introduce new dynamics that warrant a change from the status quo.

Continuous Manufacturing

Continuous biomanufacturing is gaining attention for its potential to improve efficiency, reduce costs, and streamline the production of antibodies. It requires end-to-end integration of all the upstream and downstream unit operations, which occur continuously rather than in distinct batches. This approach offers potential advantages such as increased productivity, reduced footprint, cost-savings, and flexibility in responding to market demands. The benefits of continuous processing have been shown for numerous industries including the small molecule manufacturing sector[7,13].

Continuous operation will amplify the demand for convenient and reliable instrumentation for inline monitoring, and emerging PAT tools can play a catalytic role in accelerating the transition from batch to continuous mAb manufacturing by enabling real-time monitoring and control throughout the entire process[13,14]. Automation and digital strategies could be solutions to overcome the challenges and enable the continuous manufacturing concept, reduce process variability, and lower production costs[8]. Fully integrated end-to-end continuous processes in this space are not yet a reality in commercial manufacturing although research and development of such systems is ongoing and has demonstrated feasibility[7].

Digital Approaches to Process Development

Advancements in PAT marked by the introduction of new technologies along with the development of comprehensive data libraries, deep learning algorithms, and artificial intelligence (AI) are paving the way for the integration of digital approaches into bioprocessing[8]. For example, in-silico process modeling provides opportunities to accelerate cell line development allowing for rapid optimization of attributes from potency and selectivity to manufacturability[6]. Digital twins are another approach that holds promise for reducing costs, accelerating speed-to-market, and enhancing post-approval optimization. A digital twin is a dynamic, real-time digital replica of the physical production process. It incorporates PAT and other monitoring tools to pinpoint process bottlenecks, define crucial engineering goals, and identify operational strategies that enhance the reliability and productivity of their physical counterparts[7].

Into The Future

The current landscape of antibody production continues to evolve alongside industry growth, with progress seen in upstream processes such as cell line development and media optimization, as well as innovative downstream purification strategies and the integration of cutting-edge analytical tools like RedShiftBio's HaLCon Protein Analyzer. These innovations enhance productivity, reduce costs, and help accelerate speed-to-market of the next generation of antibody therapeutics.

With their targeted specificity and therapeutic potential, mAbs are offering new therapeutic solutions across various medical conditions from cancer treatment to infectious diseases to autoimmune disorders, making them an important part of the future of healthcare. Continued improvements, like the ones we have discussed here, to the current performance of antibody production processes will be critical to increase patient accessibility, affordability, and sustainability.

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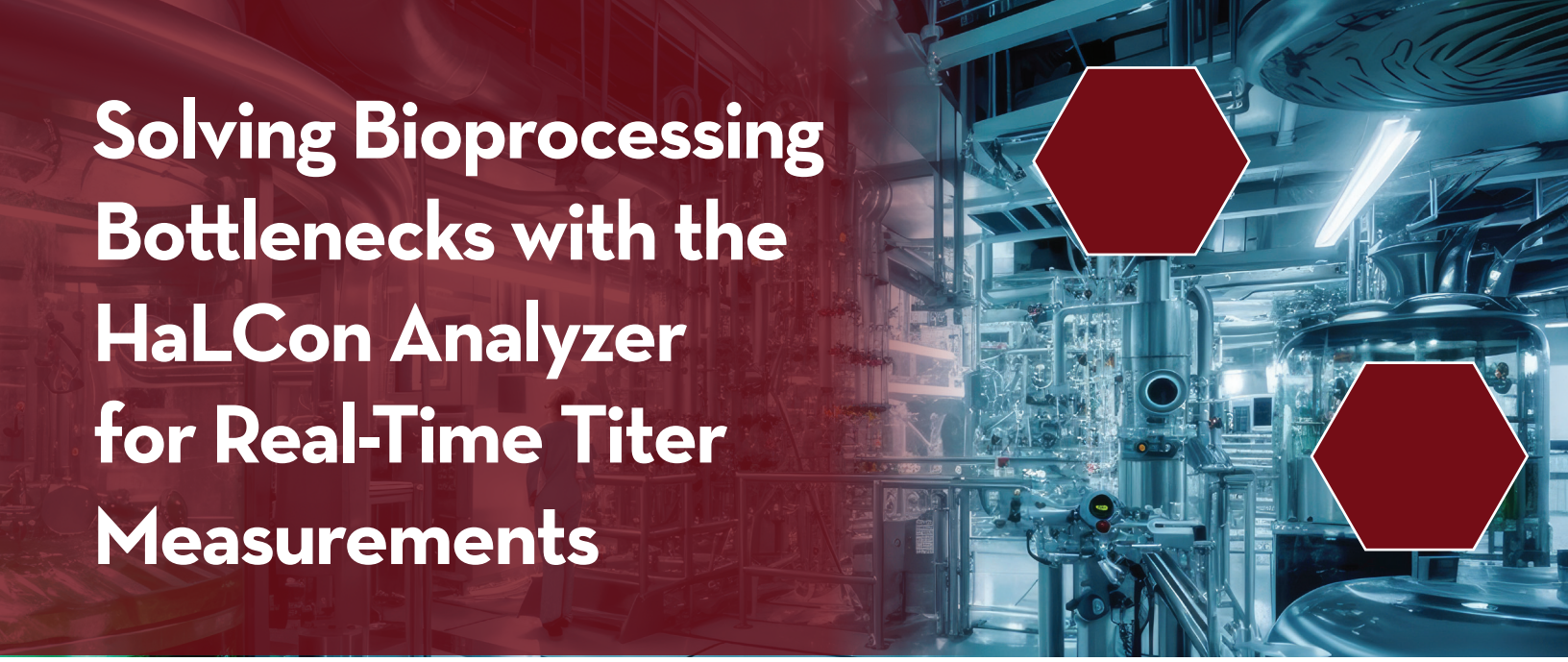
Solving Bioprocessing Bottlenecks with the HaLCon Analyzer for Real-Time Titer Measurements



Biologics, including monoclonal antibodies (mAbs) and therapeutic proteins, have had a profound impact on the therapeutic landscape by offering treatment solutions for a wide range of diseases. Their importance will continue to escalate in the years ahead, driven by the emergence of new targeted and personalized therapies. To meet the demand, ongoing innovation within the biomanufacturing sector is essential, with a focus on enhancing productivity and cost-efficiency to ensure broader patient access to these therapeutics.

In response to this, regulatory agencies have encouraged biomanufacturers to embrace the concepts of Quality by Design (QbD) and Process Analytical Technology (PAT)[1] for process optimization efforts, such as upstream bioprocess intensification and continuous bioprocessing. This systematic approach focuses on the identification and control of critical process parameters (CPPs) that influence the critical quality attributes (CQAs) aimed at increasing process knowledge to enhance productivity and achieve high product titers while maintaining quality standards.

Successful PAT relies on the analysis and monitoring of CQAs within the bioprocess, such as protein titer. Protein titer serves as both a benchmark for yield estimation and a quality control metric, helping to guide the production process from early cell line development, process development, through to manufacturing. However, measuring protein titer in a bioprocessing environment has been a persistent bottleneck, especially when using instruments originally designed for research in a production setting.

PAT initiatives have paved the way for a new generation of analytical technologies specifically designed to provide timely and actionable protein titer data in bioproduction environments. New analytical platforms, such as RedShiftBio's HaLCon Protein Analyzer fills a critical technology gap, providing researchers with the means to overcome industry bottlenecks head-on.

Challenges of HPLC in Protein Titer Measurement for Bioprocessing

High-Performance Liquid Chromatography (HPLC) with Protein A affinity capture is one of the most widely used methods for protein titer measurement and is considered the gold standard for protein quantification. However, its application in a production environment is hindered by the size and complexity of HPLC instruments. This makes HPLC accessibility at-line rare, limiting opportunities for real-time monitoring of product yields and the ability to implement real-time corrective action during a bioreactor run.

Typically, HPLC samples are processed in centralized Quality Control (QC) laboratories situated apart from the production suite. These core labs are often fielding measurement requests from multiple departments on multiple quality attributes, which can lead to HPLC backlogs. Furthermore, the time-intensive sample preparation process, the specialized training for HPLC operation, and instrument maintenance pose additional challenges.

This delay in time-to-data can holdup downstream purification processes that require protein titer information, such as scaling Protein A capacity, column selection, or determining the number of columns and cycles for simulated moving bed systems. Relying on retrospective data that can take days to generate is not conducive to the needs of bioproduction workflows, so alternative, automated methods are increasingly being considered as a replacement for HPLC.

Fit for Purpose Instrumentation

RedShiftBio's HaLCon Protein Analyzer (Figure 1) is a compact and easy-to-use protein A liquid chromatography system purpose-built to meet the demands of the bioproduction environment[2-4]. Measuring less than 10 inches in width, the small footprint of the device allows it to be conveniently placed in a laboratory setting where bench space is at a premium or directly within a production suite, facilitating real-time data acquisition without disruption to existing equipment and workflows.

The instrument can be used at-line for protein titer measurement, with sample-to-data turnaround in under 5 minutes or integrated with an online automated sampler for seamless and hands-free sample analysis. Samples require minimal manipulation and can be prepared by filtering them through a 0.2 µm or 0.45 µm filter or by centrifuging and subsequently sampling the supernatant using a syringe. Operating the instrument is a simple process, involving just four steps: entering sample information, loading the sample, initiating the analysis, and swiftly obtaining results.

The plug-and-play system requires minimal (if any) maintenance and is designed with efficiency and user convenience in mind, consisting of two consumable parts, the reagent pack and the analysis module. This simplifies routine analysis by providing up to 1,000 samples with 90-day on-board reagent stability for each set of consumables. Optimized analytical conditions and pre-formulated reagents eliminate the need for method

Figure 1. *The HaLCon Analyzer is a self-contained instrument with pumps, valves, analysis module, detector, and reagent pack, built into a small footprint (20.5 x 9.75 x 16 in). The integrated computer interface makes for a simple, user-friendly experience.*

development, reagent preparation, specialized chromatography expertise and extensive operator training. This not only saves valuable time, but it also helps minimize user error to ensure consistent results. The sample flow path is fully enclosed within the analysis module, aligning with the requirements of single-use bioprocessing.

Chromatography Without Complexity

The HaLCon uses a proprietary analysis module containing a Protein A affinity column to quantify the antibody titer in a bioreactor over a dynamic range of 0.1–10 g/L in a cell-free sample with minimal sample manipulation and no dilutions required.

Users can actively monitor titer and acquire accurate at-line protein titer measurements, which correlate well with off-line HPLC and other protein measurement techniques like bio-layer interferometry (BLI). The instrument effectively eliminates bottlenecks associated with HPLC setup time, training, and the need to send samples to a core lab for testing, providing a more streamlined and efficient solution for titer measurement in the bioproduction environment.

A series of experiments designed to evaluate different performance aspects of the HaLCon Analyzer are outlined below.

Assay Linearity

In one study, the analyzer's assay linearity and accuracy were compared with a Protein A HPLC system (Agilent 1100 HPLC system) across a measurement range of 0.1 to 6.5 g/L[2]. Figure 2 shows a calibration curve generated using a human IgG isotype control standard at concentrations of 0.1, 1.0, 2.5, and 5.0 g/L. The results demonstrate a wide dynamic range with an upper limit of 10g/L and robust linearity, with a linear regression (R^2) value of 0.99973 within the specified measurement range.

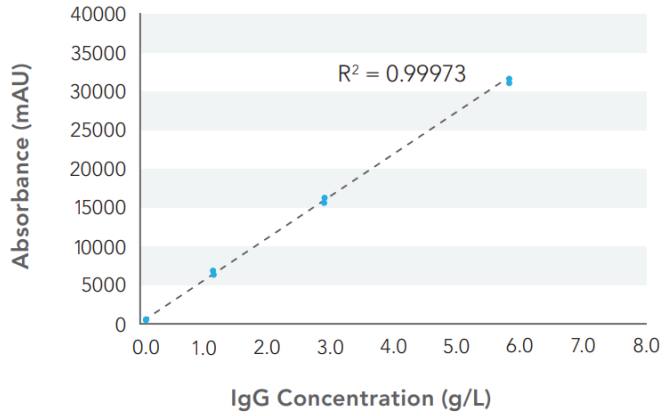


Figure 2. *Linear calibration curve for the off-line HaLCon Analyzer titer measurements.*

The extended dynamic range allows for extrapolation of values to higher titers, which is especially useful since current fed batch bioprocesses can yield protein titers >10 g/L.

The HaLCon can store multiple standard curves. This provides users with the flexibility to generate a specific standard curve using the antibody of interest or to select a more general calibrant for standard curve generation for use with multiple antibody products. Additionally, the standard curve remains valid for the entirety of the analysis module's operational life, which spans 3 months or 1,000 samples (whichever comes first). This greatly streamlines the workflow and enables rapid sample analysis, while retaining high accuracy and precision for reported antibody concentrations.

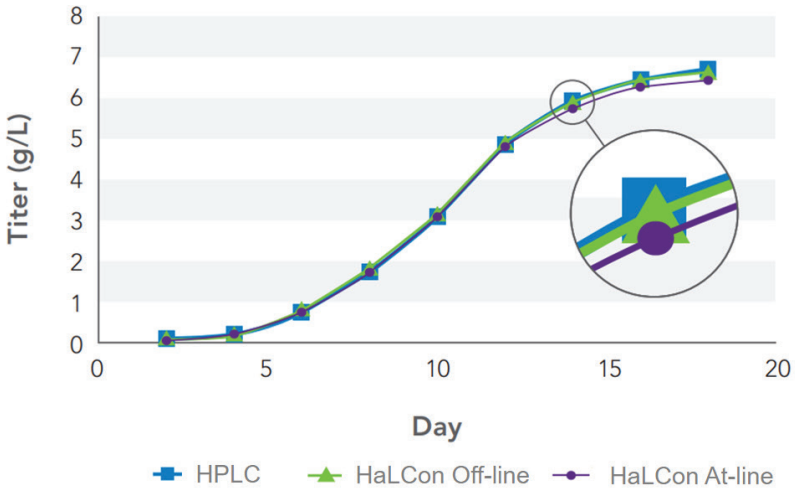
Instrument Accuracy

Manual and automated aseptic sampling measurements of a bioreactor run performed with the HaLCon demonstrated strong correlation with the off-line HPLC system. The automated aseptic at-line sampling was performed using the Flownamics Seg-Flow® 1200 with the F-Series FISP probe. Both the HaLCon Analyzer at-line and on-line titer values fell within 10% of the off-line HPLC titer values. Additionally, excellent intra-assay precision was evident, with a coefficient of variation (CV) below 3% observed for both off-line and at-line titer measurements (Table 1).

Table 1. Relative errors for at-line and off-line HaLCon measurements relative to Protein A HPLC titer values demonstrate the accuracy of the HaLCon analyzer.

Day	HPLC Titer (g/L)	HaLCon Off-Line Error (%)	HaLCon At-Line Error (%)
2	0.10	1.99	9.21
4	0.21	2.79	3.08
6	0.76	7.31	4.31
8	1.75	5.90	1.54
10	3.09	2.70	1.30
12	4.86	1.86	0.62
14	5.92	0.21	2.62
16	6.44	0.37	2.41
18	6.68	0.56	3.74

The analyzer yielded accurate at-line and off-line titer measurements compared to HPLC for titers up to 6.5 g/L without the need for sample dilution (Figure 3). Antibody titer at each sampling point was calculated using the average of three measurements for all data sets. The measurement results were also unaffected by changing BSA levels up to 2.5% (v/v).



Day	IgG (g/L)	% BSA
2	0.10	0.10
4	0.20	0.15
6	0.75	0.23
8	1.75	0.34
10	3.00	0.51
12	4.75	0.76
14	5.75	1.14
16	6.25	1.71
18	6.50	2.56

Figure 3. *Accurate titer measurements from simulated cell culture from HaLCon at-line and off-line instruments compared to off-line HPLC.*

The titer measurements exhibited strong correlation with HPLC titer measurements throughout the titer range, as demonstrated by an R^2 value of 0.99974 (Figure 4).

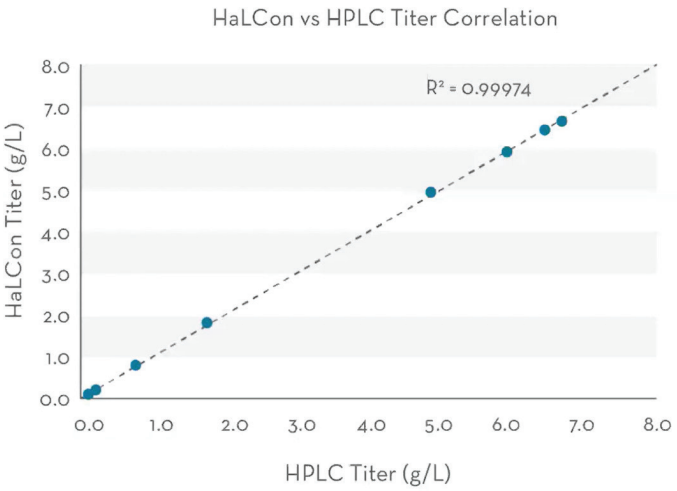


Figure 4. *HaLCon vs. HPLC titer values show excellent agreement across the titer range.*

Comparability to HPLC and BLI

In a separate study, the performance of the HaLCon was assessed by comparing it to traditional off-line HPLC (Agilent 1100 HPLC) and BLI (ForteBio Octet RED96) methods for monitoring protein titer during a bioreactor manufacturing run using a stable CHO cell line expressing a humanized IgG antibody, Adalimumab[4]. The antibody titer was measured throughout the 19-day culture period, with the average of three daily measurements plotted in Figure 5. Protein titer at harvest was subsequently back-calculated following Protein A purification.

The titer measurements for Adalimumab obtained using the HaLCon and HPLC instruments exhibited a high degree of comparability throughout the entire bioreactor run, with values falling within of +/-5% of each other. Although the BLI data also displayed overall correlation, larger differences were observed in comparison to the other two instruments. The larger discrepancies could be attributed to the need for multiple sample dilutions prior to BLI analysis.

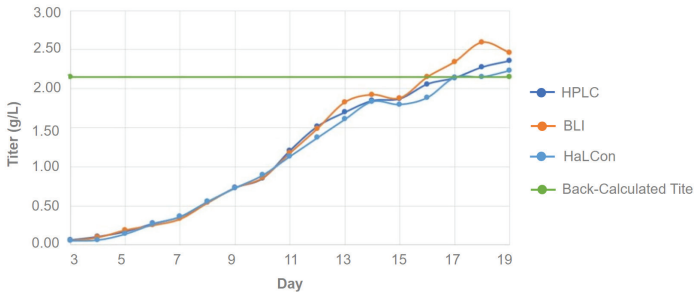


Figure 5. *Comparison of HaLCon real-time antibody titer with off-line HPLC and BLI antibody titer measurements.*

The data derived from these studies show the capability of both traditional HPLC instruments and the HaLCon Analyzer to generate exceptional titer data. However, the differentiating factor lies in the HaLCon Analyzer's purposeful design for application within the manufacturing suite. Optimized to provide simple, real-time, and dependable HPLC-level titer data with unparalleled efficiency, it is a suitable alternative to traditional HPLC in bioprocessing settings, providing the industry with a precise tool for titer measurement.

As the biopharmaceutical industry continues to face the growing demands for faster drug development and cost-efficiency, the importance of robust analytical methods is taking center stage. The ability to generate real-time, actionable data in minutes provides invaluable insights that enhance process control, support the effective design of experiments (DOE) as part of PAT, and facilitate the development of predictive models. Users can make critical decisions earlier to maximize bioreactor output and address operational bottlenecks to save time and money. Innovations like the HaLCon Analyzer mark the evolution of fit-for-purpose analytical tools, designed to meet the challenges of modern-day bioprocessing applications.

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REDSHIFTBio®

HaLCon Protein Analyzer: Purpose-built Liquid Chromatography System for Bioprocessing

KEY FEATURES

- Compact design
- High correlation with HPLC
- At-line and on-line capability
- No HPLC expertise or method development needed
- Sample flow path is completely contained
- Manual or Autosampler compatible
- Plug-and-play consumables
- Low maintenance instrument

BENEFITS

- Enables frequent & routine titer measurements
- No sample dilution needed
- Harvest on time
- Save time & money
- Stable calibration curves
- Make critical decisions faster during process development and manufacturing

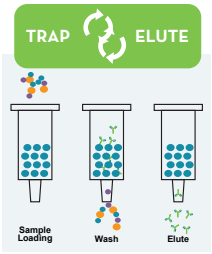
Chromatography without Complexity

Real-Time Protein Titer Measurements in 5 Minutes

A Cell-free sample from bioreactor

B Antibodies bind to Protein A resin, while other materials flow through to waste

C Antibodies are eluted with reagent B and quantified by HalCon (OD280)

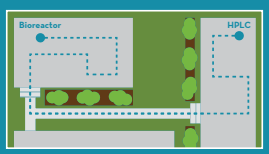


HaLCon in Action

Streamlining CDMO Processes for Biologics Development and Manufacturing

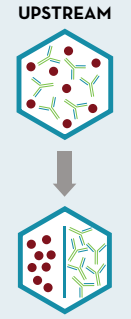
CHALLENGES

- QC turnaround time 24+ hours
- Retrospective data only
- Multiple projects with differing impacts on titer production
- Different and/or new molecules requiring individual HPLC calibration
- Off-line HPLC bottlenecks:



HaLCon SOLUTIONS

- ✓ Reduce delays in moving batch to downstream purification
- ✓ Trend monitoring during process development & optimization (DOE)
- ✓ Wide dynamic range of 0.1 - 10 g/L
- ✓ Generic calibration curve can be applied to different molecules with standard Fc regions
- ✓ At-line monitoring of product concentration to optimize bioreactor use
- ✓ Rapidly identify adverse conditions enabling real-time corrective action



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How Real Time Titer Measurement And Monitoring Is Advancing Bioproduction Across Multiple Applications

A panel discussion

Panel Members:

- **Carrie Mason** - Associate Director, R&D at Lonza Biologics
- **Laura Madia** - Independent Industry Consultant
- **Alan Opper** – Director of HaLCon Sales at RedShiftBio
- **David Sloan, PhD** – Senior Vice President, Life Sciences at RedShiftBio
- **Brandy Sargent**, Editor in-chief, Cell Culture Dish and Downstream Column (Moderator)

Where is the industry at today with titer expectations and what are the best practices for measuring titer?

Laura Madia

With respect to expectations regarding titer over the years, what we've seen is a need for increased titer within the upstream development of a drug. As an industry, we have moved from the 80s where titers were closer to .2 to .5 grams per liter to the early 2000s where concentrations of titer production rose to 3 to 5 grams per liter. What we see today is a continued increase in titer concentrations, which creates a challenge to make sure that you have technologies that can accurately measure titer concentration without introducing any errors.

The other thing that we have seen within the industry is the need for more data to not only understand what is happening in the tank, but also to be able to make decisions about the product as the process is running or shortly after.

Lastly, it is important to consider people and resources. It has been exacerbated by COVID, but it is difficult to find people to work within the industry and there are fewer people within a production suite. This has helped to drive the need for online and remote monitoring and automation to make it easier to get the necessary measurements.

David Sloan

To follow up on the lack of workers, one of the things that we constantly hear from the customers we are working with is that training employees can be a real challenge and a very time-intensive process. Technologies that are easier to use and require less expertise help get people up and running and minimize errors amongst new users of a technology.

Laura Madia

As for the current best practices for measuring titer, HPLC is the gold standard. But HPLC presents some challenges including training and HPLC requires a highly skilled person to get accurate results. There is a need for something that is simple and easy to use when it comes to measuring titer. You will still need HPLC results for approval and decisions at the end, but to be able to monitor titer throughout the process is important.

What are the challenges associated with the way that titer is measured today and what can we do as an industry to improve?

Laura Madia

One of the challenges is that most of the assays available today are batch processes, so that lends itself to providing a retrospective look and means that most people don't run samples throughout the process. This is because most people save these tests until the end when they can run a batch and make it more cost effective, and it is typically a long time to result so running it during the process isn't helpful. Systems today are more for batch process and are not set up for at-line measurement, unless you are lucky enough to be able to have an HPLC that's dedicated to that tank.

Another challenge is speed and accuracy. Many of the techniques that are offline today are longer assays because they're running as a batch. You must wait for the entire batch, which is a long time to first result. That is where having a system like the HaLCon, which you can move online and do a simple one or two injections to get the concentration is a nice thing. To be able to move measurements online and closer to the tank with an easy to use system is critical.

David Sloan

One of the challenges with some technologies is that they just don't have the dynamic range that's required to cover from the low concentrations you see earlier in the tank to those high concentrations that you're getting later or at the end of the production run. With a technology like HaLCon, you can measure from the low concentrations of the .1 grams per liter all the way up to the higher concentrations of 8-10 grams per liter without needing to do any sort of dilutions or serial dilutions.

What it really comes down to is accuracy and how closely does it agree with HPLC and do you have the dynamic range that's required to be able to cover the whole concentration range without needing to do multiple dilutions. As soon as you start bringing dilutions into the equation, you're increasing the chance of error and the variability sample to sample, run to run, and user to user. If you have a technique that is user friendly and even better if it's automated to some extent, you minimize the human aspect of it and the potential error that the lab analyst brings to the assay, thus providing a more reliable and reproducible result.

Alan Opper

I typically see that the instruments out there, except for HPLC, are not fit for purpose. They are used for a lot of different things. For example, there are some instruments that can only measure titer after it's purified, not prior to purification or not in the upstream lab. There are other instruments that analyze dozens of metabolites, and they can measure titer, however, they're using methods such as turbidity, which is not as accurate throughout the whole entire production run. Results could be anywhere from 10 to 30% off the expected value.

Second, retrospective analysis. Why are we doing retrospective analysis? Why are people pulling samples at the end of the run to send it to an analytical lab? Well, it's because the current methods besides HPLC, which takes a long time, are not very accurate. End users don't trust the results or making process controlled decisions based on the results.

Lastly, HPLC is a wonderful test, but it is complex to run. It's often in a separate analytical lab which can take, depending on the company and whether it's production or development, anywhere from hours to days to weeks.

HPLC also requires a very well trained and well versed operator. With the HaLCon, it is a small instrument that sits directly in the lab, it takes 5 minutes to run using liquid chromatography Protein A. It is very accurate, and you don't need to do batch methods of the cells because you could take daily samples and rest assured, you're going to know that those are very accurate and reproducible.

What benefits can be gained from real time titer measurement?

Carrie Mason

In the development laboratory, a lot of time you'll do a batch study for bioreactors. You're running small scale bioreactors to optimize the process and taking samples. The process may run 10-15 days and then you batch the samples all together and send them to get results.

One of the exciting aspects of real time titer measurement with the HaLCon is seeing that it really simplifies the end use, and it allows the bioreactor operator to run a test on the HaLCon, as easy as injecting any of the other types of methodologies, they use for monitoring a bioreactor. It allows you to get titer results within the day, so now your researcher has an actual snapshot of what's happening. They can start charting that information and see what their titer looks like in relation to all the other bioreactor parameters. That's very powerful, because now instead of having to wait until the end when you gather up all your results and see if bioreactor one was better than bioreactor two conditions, you can now start understanding what's happening and speed up the time to decide on what conditions are the best.

In a perfect world you can use this information to start making changes in your bioreactors, to start tweaking media feeds or other conditions to get optimal titer. In the past you were just extrapolating cell growth as you know best cell growth gave you the best titer, but that's not always what we see in a bioreactor. So, in the development lab, this gives a lot of power to the end user and now allows them to see insights in their process and make faster decisions.

Looking at continuous manufacturing, you are not going to have the liberty of waiting for an HPLC result because your process is running continuously. We are going to have to have controls within that process to ensure that we're running within the set points that we want the process to execute in. With technology like the HaLCon and it's fast results, you could have this sitting next to your continuous process and be taking small samples across a day. This would ensure that what you're putting into your downstream process that is coming out of your continuous upstream process is exactly what you think it is. This would ensure good process control when it comes to continuous manufacturing.

Alan Opper

Real time monitoring and real time data can lead to much more accurate process adjustments or real time process adjustments, which will also increase your process understanding and that will enable you in the development scheme to save a tremendous amount of time, resources and money in bringing the product to market.

On the flip side, when you get into CGMP production, typically they'll close down the bioreactor and then take the sample and measure titer. Based on the titer, they will

properly load their columns, because Protein A can be quite expensive. With the HaLCon, you can take a sample in real time and load your column immediately, which can save you hours or even a production shift. This can save a tremendous amount of time because every hour lost in the production environment is quite costly.

David Sloan

One last thought, it is all about changing bioprocess from a black box or a big unknown until the retrospective analysis occurs to a data rich process where you understand what's occurring in the process while it's still going on. There's just so much additional information you can add to your understanding when you are able to add titer measurement to all the other measurements that you are taking during the bioreactor run.

What does real time titer measurement mean for bioproduction moving forward?

Carrie Mason

If you look at several of the directives that are coming out from regulatory agencies and the push for more information, it's real time release. In my mind this is one step towards that pathway. All of this is really building momentum to be able to understand what your process is doing and have much better process control, which is what every regulatory agency wants to see. If we can say that we are taking these steps, even if there are small steps along the way that we are increasing our process vision or process knowledge, making much better process decisions and being able to verify that we're running within our control strategy from the beginning, this is significant.

In my world of looking at PAT and automation, this feels like a great place to be because it gives us information that we can make decisions with and can be used for automation purposes. If we are looking at feed streams as being continuous manufacturing, you could potentially look at this as a way to collect this data and then use this data to determine time to shift to the next column or time to do another operation. In the past we would not have had the ability to make on demand changes to processing.

I think that this is a key tool when we talk about what's going to enable manufacture in the future. I see this as being a really strong partner for driving a lot of that forward and giving us the tools we need to make our products for our patients in a much more controlled manner.

Laura Madia

Historically the HaLCon is unique because now you can put real time titer measurement inside the manufacturing suite, and you can put the titer measurement in the hands of the people that are manufacturing the product, not sending it out to QC. That's a huge paradigm shift in how the process works and where the equipment goes within the traditional workflow.

Another thing to add is that with the HaLCon, titer can now be a data point that you monitor just like pH and DO. This gives you a better picture of where everything is happening because you're measuring the titer and the cell concentration at the time it's being made, not after it's been frozen for a few days and then analyzed so that's really a much better snapshot of what's happening in the tank.

How does HaLCon compare to HPLC in both conducting the testing and results?

David Sloan

We get this question a lot from customers that we're working with and we also get customers who present us with samples that they'd like to test on the HaLCon. Frequently they already have HPLC data for those samples from their bioanalytical core, so we get to do a head-to-head analysis quite regularly, and the HaLCon compares favorably with HPLC. We presented some of the data back to the bioanalytical core itself and they are always overwhelmed and happy that the HaLCon agrees so well with their HPLC results because in many cases the bioanalytical core is also extraordinarily happy not to have to run titer samples, not to have to be presented with stat titers that need to be done.

We see very similar absolute concentration and agreement between the concentration numbers, the gram per liter numbers that HaLCon is reporting. HaLCon agrees well from a data perspective and from a repeatability and reliability perspective when compared to HPLC.

As far as conducting the test is concerned, it's a different story. HPLC is complicated and requires expertise, a lot of setup including buffers to be prepared. HaLCon couldn't be farther from that scenario. It is super straightforward to run and the system comes with a set of buffers, so there's nothing for the end user to prepare. The buffers are ready and optimized for the single purpose of measuring antibody titers. The Protein A column is loaded on the system and it is always ready to run. When you get a sample, it is as simple as injecting that sample and getting your concentration about 5 minutes later.

Carrie Mason

Looking at PAT and automation, one of the biggest challenges that I face is trying to use a different methodology for a test. If people are very comfortable with doing one methodology for their test, you know that's what they file. That's what they understand and that's what their process is characterized on. So, if I was to say, I would like to introduce a spectroscopic method for modeling your titer in a process, we may get a little bit more pushback, because now there's a lot more work that must be put into validating that system.

What really attracted me to this technology is the fact that it's like for like. I can look at this and say, it's a miniaturized Protein A, HPLC assay. The modality is the same, so I don't

have to prove that this is a new novel way of measuring, because in essence really this isn't a new novel way of measuring. It's measuring the same way with affinity chromatography and detection very similar to what we do on our HPLC. But the fact that it's been so focused and purpose built for this one process and that it takes all the complexity out of it is what makes it great. It allows me to share that with colleagues and with customers, that it looks different, but fundamentally the science is the same. That is a great advantage of this technology.

When talking about ease of use, if you look at beyond just the batching, but if you go into having to do sample handling and sample analysis, you're really relying on humans to look at that information to do those manipulations. So really what I want to do with PAT and automation is look at ways to remove human error and human deviations from our processes and control as much as we can in a manner that is more reproducible and more robust. The fact that the dynamic range of the HaLCon is such that you don't have to do dilutions is extremely powerful. To be able to just take a sample and inject it instead of having to do dilution not only saves time, but also saves potential error.

How does the HaLCon fit into a traditional bioprocess workflow?

Laura Madia

The HaLCon is based on chromatography, so it will correlate directly to HPLC data that's already been generated and we know that it aligns with results within the QC lab. The way it fits within the bioprocess workflow is because it is small and compact. It can fit on any bench top very easily. It comes self-contained with the consumables, the reagents and columns that are needed. It has a very small footprint, simple interface, and can connect right beside your bioreactors or on a bench space. You can put it beside the tank and anytime you want to check what the titer is within the tank; you just load a small sample and later you'll have the results. The other benefit is that it's so simple and easy to use. You don't need the skills required that you would for an HPLC, anybody can get a result quickly and easily.

Alan Opper

Typically, the current workflow of the laboratory, involves taking daily samples and measuring them, but it's more of a trend because the inaccuracies can be quite great. Therefore, samples are usually pulled at the end of the run, sent to an analytical lab for measurement on HPLC and it takes hours to days or even weeks in some cases to obtain results.

With the HaLCon, the proposed workflow is to take daily samples with accurate, real time results. As a result, process control decisions can be made based on titer and cell productivity and there's no need to pull all the samples at the end of the run and wait days for the analytical lab to get back with results.

In the CGMP environment, the typical workflow is to pull samples at the end of the bioproduction run, obtain measurements and then load the chromatography column. With the HaLCon, results are delivered right away, so the column can be loaded immediately saving valuable time and effort.

Is the HaLCon compatible with automated sampling systems and how do you see it fitting into PAT initiatives?

David Sloan

Yes, HaLCon is compatible with automated sampling systems. It works with online systems, such as the MAST® system as well as the Seg-Flow® system from Flownamics. For groups that hook it up to an online sampling system, they can, in an automated fashion, grab samples from the bioreactor. Those samples are pushed directly to HaLCon and this triggers HaLCon to take the measurement and then record that measurement and make it available within the designated Laboratory Information Management System (LIMS), online notebooks, or automated data collection system. This permits collecting, collating, and making all the data available in real time to the development and manufacturing scientists.

For me, PAT is all about data and utilizing the analytical tools that are available to generate the data that's required to create a high quality, high yield product as quickly and easily as possible. PAT is about putting analytical power into the hands of the scientists so they can ensure processes are going smoothly and will result in the product and ultimately the drug that the patients need.

Carrie Mason

Interconnectivity of all these systems is critical. Looking at PAT tools, giving another lab bench tool to an operator has its value, but where I really see the most value is if this can be integrated into the entire ecosystem. So, not only are you looking at integrating and liquid handling for samples coming in, but then also the integration of the data coming out. We don't want to have another source of paper. With devices like the HaLCon it is so critical for us to be moving forward and connecting the system to put the data into our distributed control systems, that's critical for new PAT tools. From what I've seen, the technology is moving in that direction and has shown what it can do when it comes to being connected with liquid handling systems for automated sampling. If I was to just say, here's one more tool, I don't think that it would be as well received as if I say, this fits into our ecosystem.

What do you find or what do your customers find most exciting after using the HaLCon?

Alan Opper

What customers really like about the HaLCon is that it's fast, it's accurate, and it's very easy to use compared to other methods. They also like the fact that it uses the same Protein A liquid chromatography method that is used with HPLC. They don't have to reinvent the wheel trying to validate another type of technology. However, the difference between this and typical HPLC is that the HaLCon doesn't require the method development, the preparatory work or the specialized training required with HPLC.

Also, with the HaLCon, as we've discussed, there's no sample dilution needed or purified samples needed with this instrument. Because it runs through a range of .1 to 10 grams per liter, that's quite a wide range. We don't need to have any human intervention. The system doesn't need frequent recalibration either.

Lastly, it eliminates reliance on other labs, but also allows you to make critical decisions sooner which will ultimately save a lot of time and money for the customer or the company.

Carrie Mason

What I like about it is the fact that it is equivalent to an HPLC method. In a company where you have various groups all over the world that are all trying to measure titer, it is important to be able to recognize that this is a slightly different system, but I get a comparable result. It is critical, especially if you look at facilities that are going from small scale R&D through the development process, scale up, clinical and commercial, we want to know that we're basing our decisions and making our process control agreement strategies based on data that is equivalent. We don't want to say that there's something different between one facility and the other. So, by being able to have that equivalency across between HPLC and this system is very critical because it allows us to then implement it in a controlled manner and to have confidence that this system will provide the results that we need.

For customers and even development scientists to be able to have titer information rapidly available and to be able to have the ability to intervene or to make a process decision there's a lot of value in that.

If we can do real time analysis within the day, it allows us to speed up our development iterations and be able to go through that process much quicker, which in the end ultimately gets the product to the market quicker.