Evaluating the Performance of the Causeway Sensors TITAN System for Monoclonal Antibody Quantification in Bioprocessing

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Executive Summary

In collaboration with CPI, the following case study presents an evaluation of the **Causeway Sensors TITAN platform** for the rapid quantification of therapeutic monoclonal antibody trastuzumab during its production in CHO culture. Quantitative measurements of trastuzumab titre by TITAN in 34 minimally processed bioreactor samples were in excellent agreement with those produced for the same samples by industry-standard biolayer interferometry (FortéBio Octet® RED384, Protein A), yielding a highly positive correlation coefficient with no statistically significant measurement bias. Further comparison with Protein A HPLC also demonstrated highly positive correlation. These results, achievable in less than 20 minutes from sample preparation, position the TITAN system as a significant advancement in process analytical technology that offers high measurement accuracy in combination with speed, compactness and at-line functionality for real-time critical quality attribute analysis directly within the bioproduction space.

Introduction

Protein-based therapeutics, particularly immunoglobulin G (IgG) monoclonal antibodies manufactured through mammalian cell culture, have demonstrated exceptional success in the treatment of numerous disorders including cancer and infectious disease. As global demand for biopharmaceutical treatments increases, innovation in process analytical technology (PAT) is essential to achieving regulatory and economic targets by supporting the real-time analysis of multivariate critical quality attributes (CQAs) during the manufacturing process. In particular, real-time, at-line quantification of IgG during cell culture is of paramount importance in ensuring the sustainability of biologics biomanufacture by supporting the development of higher productivity processes. By providing actionable insight into the relationship between bioreactor productivity and controllable critical processes during drug production, at-line IgG quantification technology enables accelerated process-specific decision-making to produce greener, more predicable cell culture processes that yield higher quality biopharmaceuticals.

In this report, we examine the performance of the first generation of Causeway Sensors TITAN nanobiotechnology for the quantification of biologic titre in a simple chip-based form factor, designed for straight-forward and effective operation directly on the manufacturing floor. Pilot deployment of TITAN in an active biomanufacturing environment was facilitated through collaboration with CPI (Darlington, UK), where the TITAN platform was applied to the quantification of IgG biologic trastuzumab. The encouraging results obtained in this study demonstrate the ability of the TITAN instrument platform to measure antibody titre with competitive accuracies in concentration ranges relevant to process-scale operations. We believe that the development direction of the TITAN system represents a highly viable technological solution to the cost, accuracy and production speed bottlenecks that hinder sustainable scale-up of therapeutic monoclonal antibody production to the levels required for transformative healthcare innovation.

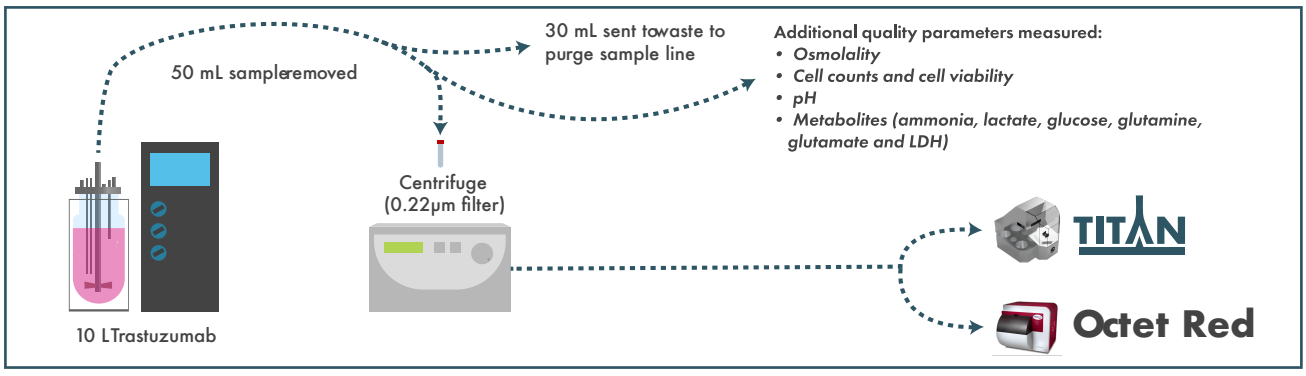

Figure 1: *Methodology for bioreactor cell culture sample extraction, processing and analysis employed in this study.*

Materials and Methods

Cell culture

CHOK1 Glutamine Synthetase (GS) knockout host HD-BIOP3-trastuzumab cells were cultured in a 10 L glass vessel (Sartorius) at 37°C in CD FortiCHO™ medium (Gibco™) over a 14-day batch process. Both a glucose and a commercially available feed were added to the culture over the 14-day period as per CPI's standard process. Target set-points for pH and dissolved oxygen (DO) were maintained by control loops applying CO_2, O_2 and 1M sodium hydroxide as required.

Sample Preparation

Samples were manually removed from the cell culture vessel at 11:00 am, 2:00 pm and 5:00 pm on days 3-13 of the batch process, and at 11:00 am on day 14 prior to harvest (Figure 1). Samples destined for trastuzumab titre quantification by Causeway Sensors TITAN or Sartorius Octet Red systems were centrifuged, filtered (0.22 µm) and stored at -20 °C prior to analysis.

Titre Measurement – TITAN

Measurement of trastuzumab titre in the prepared bioreactor cell culture samples was performed on the Causeway Sensors TITAN system, a chip-based, label-free localised surface plasmon resonance (LSPR) ligand-binding assay platform. All microfluidic assay steps were performed automatically *via* an integrated sequential microfluidic dispenser, controlled by the operator using the TITAN Loki data acquisition and analysis interface (Figure 2). Regeneration of the Protein G sensor chip was performed in duplicate using 0.1 M glycine hydrochloride buffer (pH 2.2) after each antibody binding event. Following regeneration, the flow cell was returned to HBS-EP running buffer (20 mM Hepes [pH 7.4], 150 mM NaCl, 3.4 mM EDTA, 0.05% Tween 20).

TITAN – Ligand Immobilisation

For specific detection of the antibody of interest, Protein G was immobilised onto the nanostructured sensing surfaces of dual-channel TITAN microfluidic chips *via* standard carbodiimide conjugation chemistry. Once immobilised, TITAN chips were found to retain activity after storage in phosphate buffered saline (PBS) at 4 °C for several weeks.

TITAN – Calibration Curve

For interpolation of trastuzumab titre, a calibration curve comprising seven triplicate non-zero calibrator standards was performed on each TITAN chip prior to use (Figure 2). The analytical calibration standards utilised in the generation of standard curves were prepared by successive dilution of trastuzumab (MedChemExpress LLC) in fresh CD FortiCHO™ media. Fitting of the titre-response relationship to a typical four-parameter logistic (4PL) model enabled interpolation of unknown antibody titre for multiple successive samples within a linear dynamic range of approximately 18 – 200 µg/mL.

Titre Measurement – FortéBio Octet® RED384

Quantification of trastuzumab titre in the same bioreactor samples was performed using the FortéBio (Sartorius) Octet® RED384 biolayer interferometry (BLI) system according to the standard instrument methodology for Protein A biosensor tips [1]. For the purposes of direct comparison, identical calibrator reagents were used for the construction of calibration curves on both the Octet system and the TITAN system.

Titre Measurement – Protein A HPLC

Additional quantification of trastuzumab titre in the same bioreactor samples was performed using a ThermoFisher UltiMate 3000 HPLC equipped with an Applied Biosystems™ POROS™ Prepacked Protein A Affinity Column (20 µm, 2.1 x 30 mm, 0.1 mL, Thermo Scientific™) according to the standard instrument methodology.

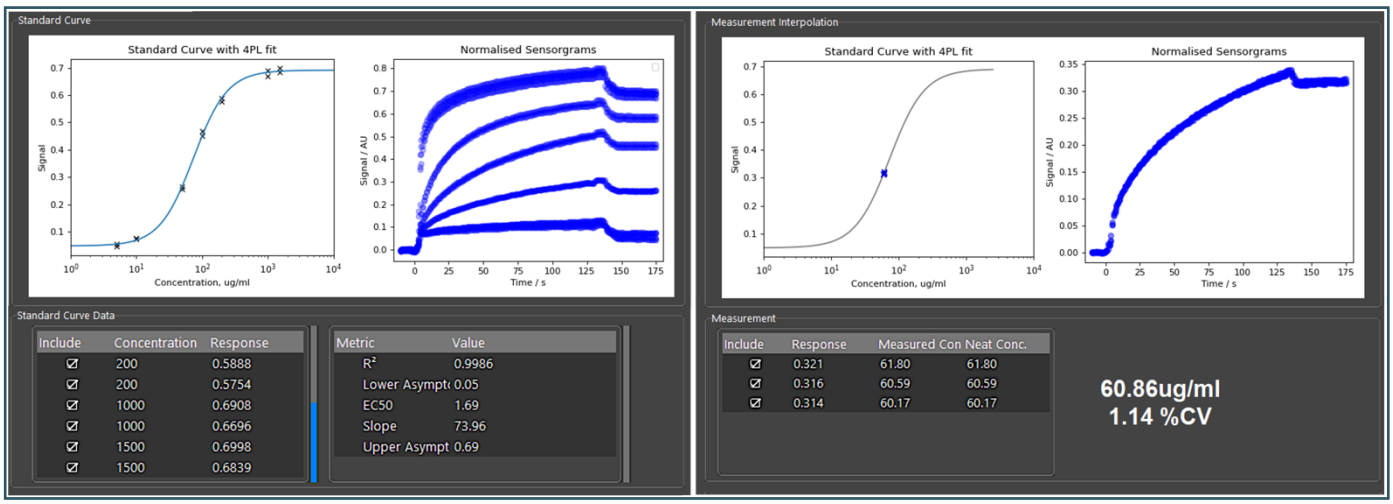

Figure 2: *Extracts from the Loki data acquisition and analysis interface showing real antibody quantification data produced on a TITAN Protein G sensor chip.* **(Left)** *Generation of a seven-point standard curve in duplicate, with associated 4PL metrics shown.* **(Right)** *Subsequent interpolation of antibody titre in an unknown bioreactor sample in triplicate, calculated automatically from the previously generated standard curve.*

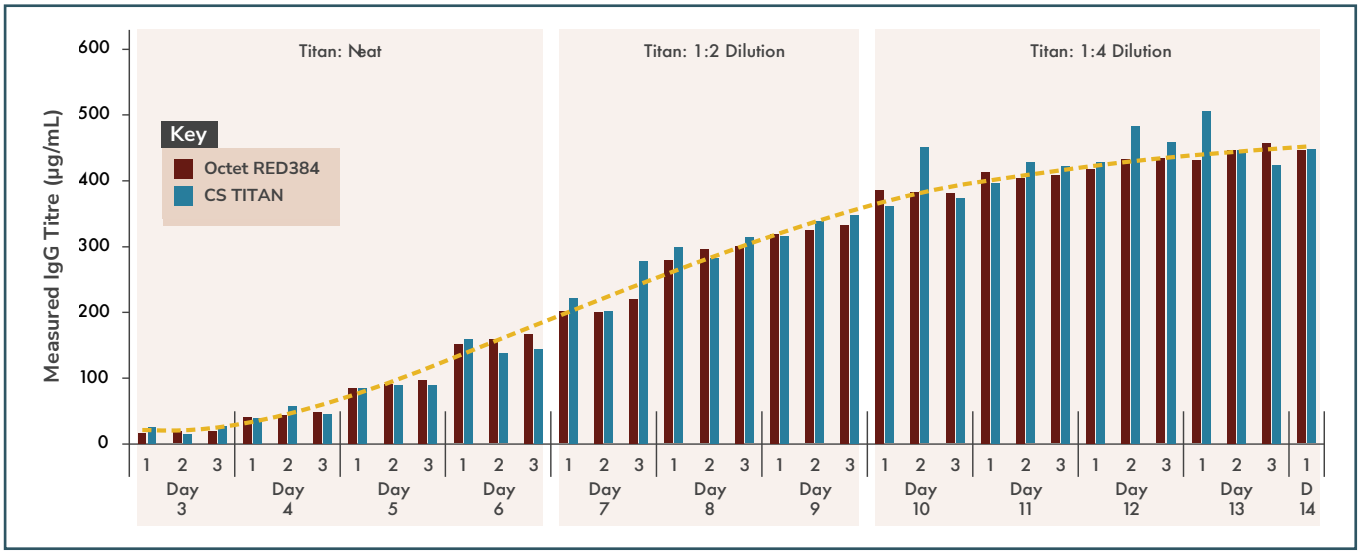

Figure 3. *Bioreactor cell growth profile generated using results achieved by TITAN and FortéBio Octet for the quantification of trastuzumab from cell culture samples drawn during a 2-week production process. Sample dilution is performed using CD FortiCHO medium where indicated.*

Results Summary

The measurement accuracy achieved by the Causeway Sensors TITAN platform for the quantification of trastuzumab titre produced in CHO cell culture was evaluated by analysis of 34 minimally processed bioreactor samples drawn over a 14-day batch process.

Comparison of the titre results achieved by TITAN with those obtained by the FortéBio Octet® RED384 BLI system revealed excellent quantitative agreement, generating an appropriate bioreactor growth profile and final antibody yield of 448.1 µg/mL on day 14 (Octet = 446.4 µg/mL) (Figure 3). Statistical evaluation of the agreement between two methods through ordinary linear regression analysis (Figure 4) yielded a highly positive correlation coefficient and a significant linear relationship (Pearson's r = 0.989, t = 37.20, p<.001) with no statistically significant constant (slope = 1.038, 95% CI = [0.954, 1.122]) or proportional (intercept = -3.232, 95% CI = [-28.746, 22.282]) bias. These results positively demonstrate that the two methods behave consistently in response to variations in measured titre without significant quantitative measurement bias across the 2-week production cycle presented in this study.

Excellent correlation was also demonstrated between titre results achieved by TITAN and those obtained by Protein A column chromatography (Vanquish HPLC), an industry-standard analytical techniques utilised by CPI for the quantification of IgG titre in addition to BLI. Ordinary regression analysis (Figure 5) revealed highly positive correlation and a significant linear relationship (Pearson's r = 0.991, t = 42.32, p<.001) between TITAN and Protein A HPLC estimations of trastuzumab titre across 34 bioreactor samples. It can be seen from the regression plot that the linear regression line lies above the line of identity, implying the presence of positive bias; statistical analysis indicates that this significant bias is both proportional (slope = 2.458, 95% CI = [2.322, 2.595]) as well as constant (intercept = -42.264, 95% CI = [-61.672, -22.856]) in nature. However, the highly positive correlation between the results produced by TITAN and those estimated by Protein A HPLC highlights the consistency of the measurement relationship between the two methods across multiple TITAN assay chips.

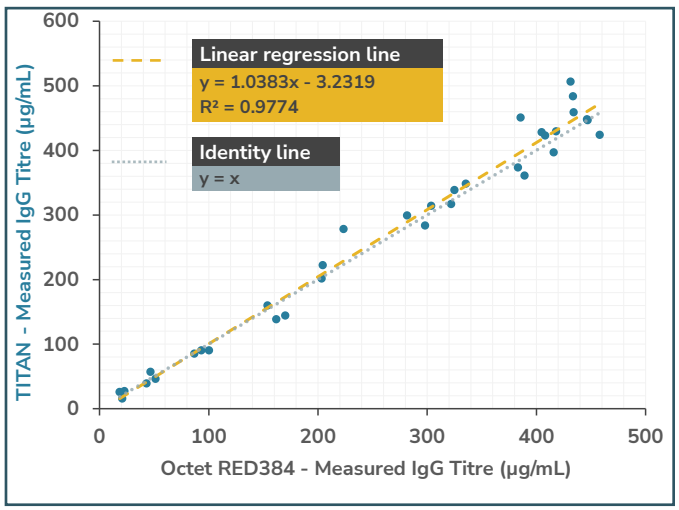

Figure 4. *Statistical evaluation of the agreement between the results produced by TITAN and Octet RED384 through ordinary linear regression analysis.*

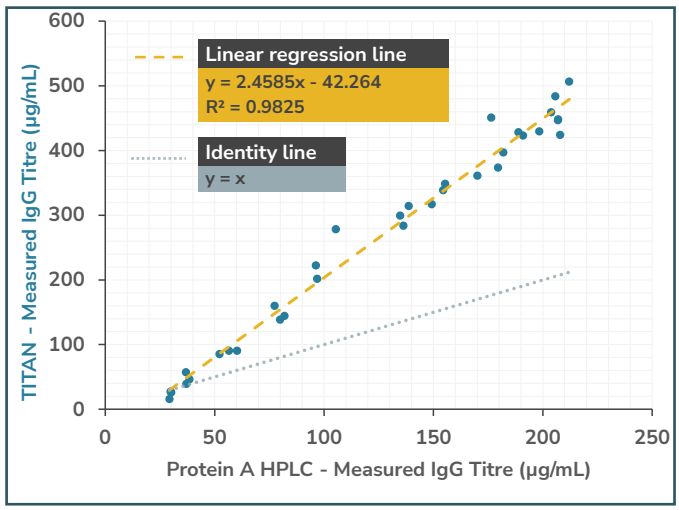

Figure 5. *Statistical evaluation of the agreement between the results produced by TITAN and Protein A HPLC through ordinary linear regression analysis.*

Discussion

A core design principle of TITAN technology is the seamless, real-time delivery of advanced bioprocess CQA insights, typically associated with labour-intensive analytical techniques, to the near-bioreactor setting. This approach significantly enhances the process intelligence available to bioreactor operators during a live production process to enable more efficient procedural interventions, increased process productivity and greener, more scalable biologic drug production. The results of this study, achieved using genuine CHO cell culture bioprocess samples, clearly demonstrate the ability of TITAN technology to meet this aim, generating quantitative measurements using semi-automated, user-friendly and cost-effective instrumentation that confidently competes with commercial platforms currently deployed in active biomanufacturing environments.

In contrast to FortéBio Octet® RED384 and Protein A HPLC platforms, the compact design of the TITAN system addresses specific at-line testing restrictions such as limited bench space (TITAN: 0.01 m^3 instrument volume; Octet: 0.49 m^3; HPLC: 0.27 m^3), changing production area configurations requiring manual system portability (TITAN: 9 lbs instrument weight; Octet: 65 lbs; HPLC: 198 lbs) and a limited number of highly-trained analytical personnel (Figure 6). The chip-based format of the TITAN system negates the need for extensive manual serial dilutions and well-plate loading as required for the Octet and is therefore distinctly suitable for implementation on the manufacturing floor, with sample and calibrant dilution performed internally when directed by the operator through the Loki data acquisition interface. Further integration with autosampler technologies is also feasible in this form factor. Following calibration of the TITAN sensor chip, antibody titre quantification can be performed in technical triplicate within 20 minutes from sample acquisition, immediately delivering quantitative results to the operator on-screen. As no post-measurement sensor recalibration is necessary between sample measurements, a sampling frequency of 3 times per hour is theoretically achievable on the TITAN platform, with a streamlined workflow that only requires the operator to add a new sample to the load port when desired and restart the automated measurement procedure.

The small size and mechanical simplicity of the TITAN platform is also anticipated to minimise the capital investment required to purchase, operate, maintain and service the system compared to comparator technologies, preventing analytical bottlenecks or pauses in production due to instrument unavailability.

Conclusion

The first external pilot trial of Causeway Sensors TITAN technology, conducted in collaboration with CPI, has demonstrated the excellent performance of the platform for the interpolation of unknown biologic antibody titre from upstream process samples in CHO cell media. Tight and highly positive correlation without significant measurement bias was found between the titre values yielded by TITAN analysis and those estimated for the same samples by FortéBio Octet® RED384, an industry-standard, commercial analytical instrument in bioprocess monitoring and control. These results highlight the potential of the Causeway Sensors TITAN system as a significant technological advancement in process analytical technology, combining fast and reliable sensing performance with portable, low cost, intuitive and user-friendly instrumentation. Future developments of the TITAN platform towards real-time, at-line operation are anticipated to offer expanded potential for rapid and high-throughput screening of critical product quality attributes on the manufacturing floor, enabling time-sensitive process-specific parameter adjustments to be made with more speed, frequency, intelligence and confidence.

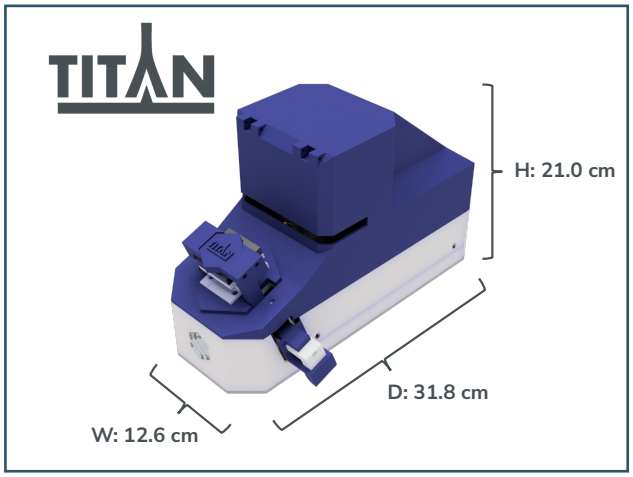

Figure 6. *Physical dimensions of the Causeway Sensors TITAN system.*

References

[1] *https://www.sartorius.com.cn/download/585680/octet-kinetics-assay-method-development-guideline-technical-note-en-sartorius-data.pdf*

To learn more or discuss how our solutions can meet your specific needs, contact our team at contact@causewaysensors.com.

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