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Cell Line Development: Accelerating Process Optimization by Combining Ambr[®] 15 Cell Culture with Octet[®] Titer Measurements

Kirby, Jonathan², Rees-Manley, Alison², Apiyo, David³, Klein, Lukas¹, Diem, Eva¹, Dale, Tim²

1. Sartorius Stedim Cellca GmbH, Marie-Goeppert-Mayer-Str. 9, 89081 Ulm-Eselsberg

2. Sartorius, York Way, Royston, SG8 5WY, UK

3. Sartorius BioAnalytical Instruments Inc, 47661 Fremont Boulevard, Fremont, CA 94538, USA

* Corresponding E-Mail: Royston-Info@sartorius.com

Introduction

Cell line development involves the screening of thousands of clones to find those that are stable, produce high yields of the bioproduct and exhibit desired critical quality attributes (CQAs). Typically screening and process optimization activities will be carried out at the small scale in bioreactor cultures to ensure that results translate up to larger bioreactor scales. Performance data is primarily based on cell growth, cell viability, metabolite analysis and product titer, and assessed over the entire culture process duration. Often product titer data will have a longer turn-around time where samples are submitted to analytical groups for analysis. Introducing analytical technology into the cell culture process development workflow can greatly speed up the time to results. The Octet[®] platform is an analytical instrument that can easily be implemented in cell line development labs to facilitate rapid determination of product yield. This capability allows informed decisions directly at the end of the process, for example which samples need to be sent off for quality analysis, reducing overall sample numbers. It also enables the next experiments to be planned based on all of the performance data, rather than waiting days or weeks for product yield results to be available. Here we show the power of using the Ambr[®] 15 Cell Culture system with an integrated Vi-CELL XR and Ambr[®] Analysis Module together with an Octet[®] to identify optimum yield conditions in media screening and process optimization experiments.

Find out more at:

www.sartorius.com/cld

Ambr® 15 Cell Culture

The Ambr® 15 Cell Culture, is an automated, high throughput single-use bioreactor system that provides parallel operation of up to 48 single-use microbioreactors at a time and can be managed by a single operator.

Comparing cultures in identically-sized, multi-parallel bioreactors, allows scientists to screen more clones in a representative bioreactor format early on in the cell line development workflow and obtain meaningful results in a shorter time frame. Multiple experiments can be set-up to evaluate different cell lines or clones, and to investigate the effect of process parameters, such as temperature, feeding, media composition, gassing rates and inoculation densities. Together these features enable cell line selection, media selection, and process optimization to be executed in a quick and efficient way.

Ambr® 15 Cell Culture is the industry standard microbioreactor system, implemented in laboratories world wide. Studies in Ambr® 15 have shown greatly improved results when compared to shake flask or shaking plate cultures due to the high level of automation combined with reliable and independent process control for pH and dissolved oxygen. Flexible operation allows cultures to be run in batch, fed-batch and even perfusion mimic mode. With low working volumes, from 10 - 15 mL, the small-scale stirred bioreactor vessels reduce the cost per experiment by saving substantial amounts on media and feeds.

For ease of product quantification and CQA assessment the Ambr® 15 and Octet Bio-Layer Interferometry can be implemented as platform technologies to manage the cell line development workflow (Figure 1).

Octet® Bio-layer Interferometry (BLI) Platform

Octet® systems operate on the label free, non-fluidic based BLI technology with Dip and Read assay format, that utilizes micro-titer plates and propriety biosensors coated with protein ligands to enable specific binding between the ligand and the relevant binding partner. These systems are commonly used for kinetics and affinity constants analysis for receptors binding to antibodies, viral particles, recombinant proteins and many other biological molecules. For IgG titer determination Protein A or Protein G coated Octet® biosensors can be dipped into IgG samples with the binding expected to occur between the biosensor and the IgG

through the Fc region. BLI measures changes in light interference patterns originating from the tip of the biosensor surface where light wavelengths are made to reflect from two layers; a biocompatible layer at the end of the biosensor surface and an internal reference layer. The spectral pattern of the reflected light changes as a function of the optical thickness of the molecular layer. This spectral shift is monitored at the detector and reported on a sensorgram as a change in wavelength (nm shift).

The key feature for Octet® analysis is that the refractive index changes in the sample do not affect shifts in the interference pattern. This leads to reduced matrix effects enabling the Octet® to quantify IgG concentration from both purified samples and heterogeneous crude lysates. The advantage allows for significant reduction in the analysis time as samples can be simply transferred into micro-titer plates and Protein A or G biosensors dipped in one step for rapid analysis. In addition, the newly launched Sartorius GlyS and GlyM kits for sialic acid and mannose content screening respectively can be used in tandem with the titer measurement biosensors to better understand product quality changes as a function of different media or processing conditions.

Key benefits of the Octet® platform include:

- Significantly reduce analysis time by using crude samples and ready to use Protein A or Protein G biosensors; product titer can be obtained in less than 5 minutes from as many as 96 samples on the Octet® RH96
- Save costs and complete more projects on the Octet® with minimal analyst time required (Table 1 compares the Octet® to other platforms commonly used for titer determination)

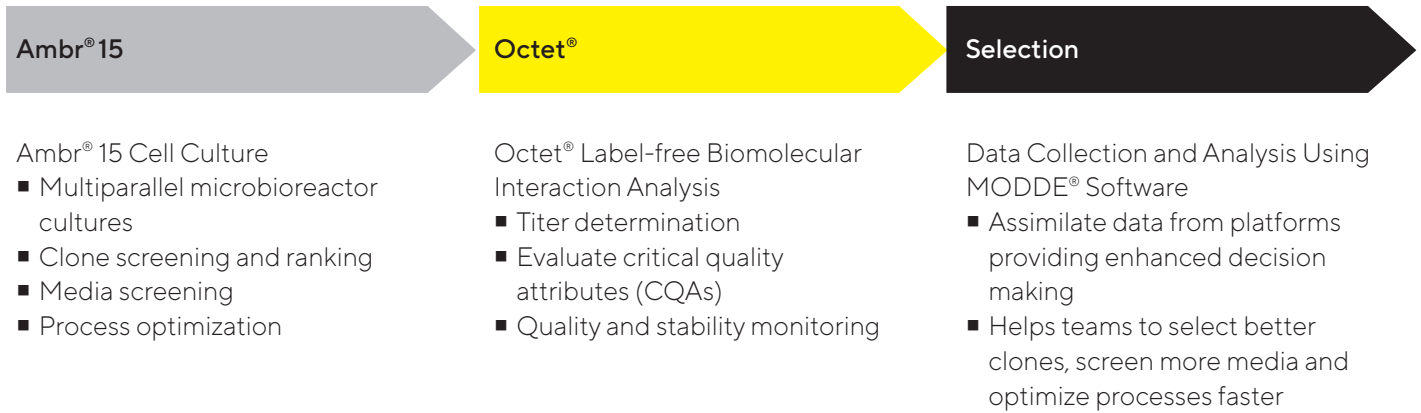
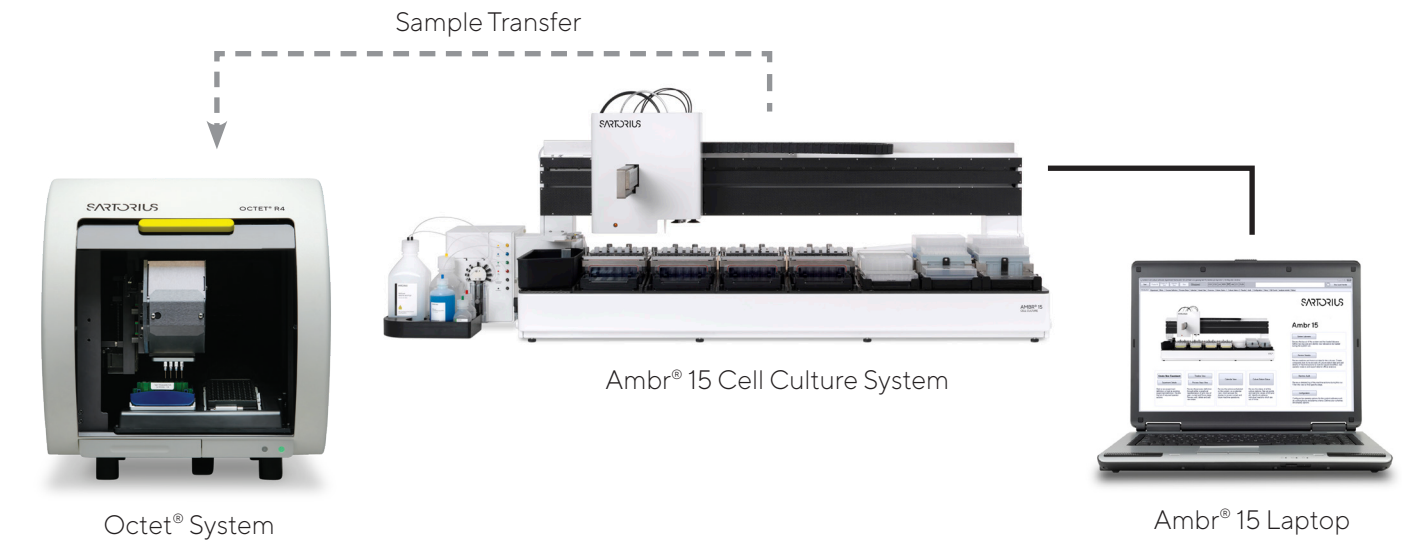
A comparison between the Octet® platform, HPLC and manual ELISA for mAb titer. A project in this example is defined as the titer determination for a total of 10,000 mAbs in a high throughput screening process. The data in the table assumes an analysis labor time of 0.2 hours, 0.5 hours and 3 hours for the Octet® ELISA and HPLC respectively.

Table 1

	Octet®	ELISA	HPLC
FTE labor costs	X	15X	3X
Time to results (hrs)	52	625	1040
# projects/year	40	3	2

Figure 1

The Octet® and Ambr® 15 Cell Culture Setup for Clone and Media Selection



Materials and Methods

Media Screening Experiment in Ambr® 15

Ambr® 15 Cell Culture standard microbioreactors with sparge tube (Figure 2) were inoculated with a density of 3×10^5 cells/mL in a starting volume of 13 mL. Each microbioreactor has individual gassing via sparge tube into the culture. The pH and DO control loops maintained target set-points by applying CO_2 and O_2 as required, along with a fixed ballast gas (air) set at 0.15 mL/min throughout the process. Set-points were as follows; pH 7.0 (upper limit of 7.1), DO 40%, temperature 36.8°C and stirring speed 1300 rpm.

Five different media were investigated as part of this experiment with two different clones.

Process Optimization Experiment in Ambr® 15

Ambr® 15 Cell Culture standard microbioreactors with sparge tube were inoculated with a density of 3×10^5 cells/mL at three different starting volumes ranging from 12 to 14 mL. Each microbioreactor has individual gassing via sparge tube into the culture. The pH and DO control loops maintained target set-points by applying CO_2 and O_2 as required, along with a fixed ballast gas (air). In this experiment the ballast gas (air) flow rates were between 0.05 to 0.25 mL/min. The process set-points were as follows; pH 7.0 (upper limit of 7.1), DO 40% and temperature 36.8°C .

Four different stirring speeds were investigated as part of the DOE experiment, these ranged from 1050 to 1650 rpm.

Media and Reagent Additions

Proprietary basal and feed media were used for the experiments. Daily feed and antifoam solutions were added to each vessel automatically for both experiments.

Sampling and Analysis

Viable cell concentration (VCC) counts were obtained via a coupled Vi-CELL XR (Beckman Coulter, USA). pH measurements were obtained via an integrated Ambr® Analysis Module. Glucose and lactate measurements were performed off-line with an EKF BIOSEN S-Line device (EKF-Diagnostic GmbH, Germany) according to manufacturer's protocol. Cell viability and cell count were determined daily with a cut-off for titer measurement set at 70% viability i.e. when the viability fell below 70%, titer was not measured. For titer measurement daily samples were taken automatically by the Ambr® 15 Cell Culture. These samples were centrifuged for 5 min at $6600 \times g$, the cell-free supernatant was removed and retained for titer measurement.

Titer Measurement in Octet®

The cell-free supernatant samples were diluted in the required 96-well plate. The plate was then vortexed for at least 1 minute at a moderate level. An Octet® QKe was used with Protein-A biosensors. The system is suitable for measurement of protein concentration between 1 and $500 \mu\text{g/mL}$, for higher protein titers the samples were diluted in media. Calibrator samples with known concentration were used for the generation of the standard curve (Figure 3). Biosensors were regenerated for re-use by dipping into regeneration buffer followed by neutralization buffer for 5 seconds each. Three cycles of regeneration were used. Quantitation assay time was set to 120 seconds with the sample plate temperature set to 30°C .



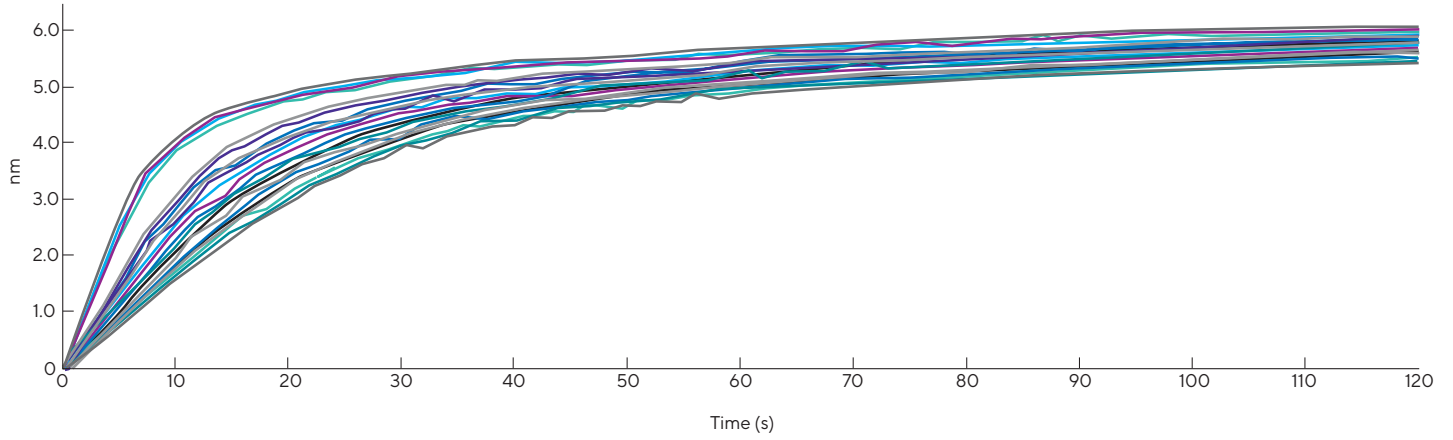
Figure 2

*Ambr® 15 Cell Culture
Microbioreactor
(With Sparge Tube)*

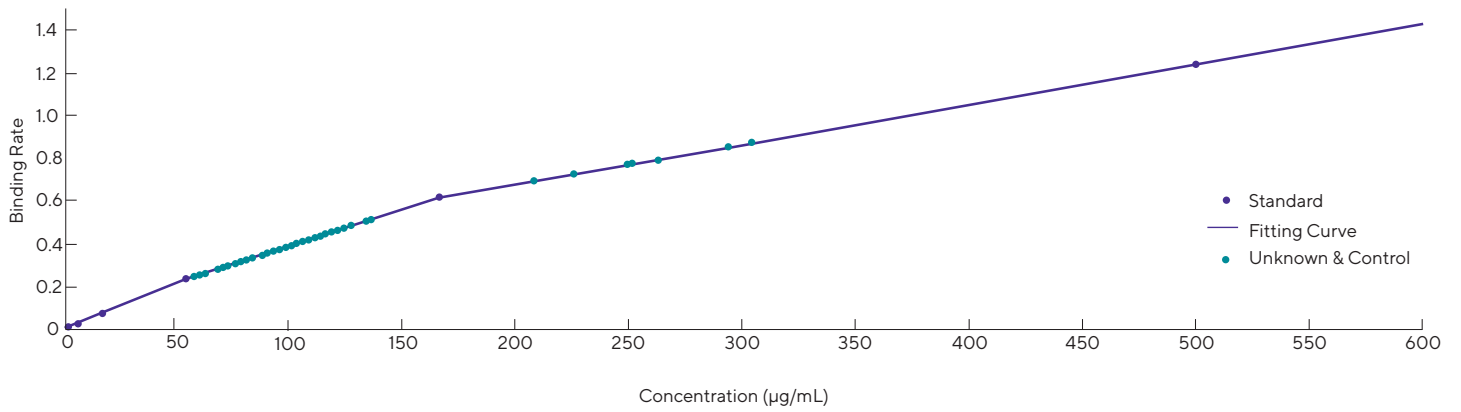
Figure 3

IgG Titers. (A) Protein a Biosensor Binding Curves of IgG Molecules Generated From Two Replicate in Each of the Five Media Growth Conditions. (B) All 96 Sample IgG Concentrations (Orange) Plotted on the Calibrator Curve (Blue, 0-500 ($\mu\text{g}/\text{mL}$))

A



B



Results and Discussion

Media Screening Experiment

Implementation of the Octet® platform alongside the Ambr® 15 Cell Culture allows cell line developers to identify the best path forward for the choice of the top clone and the best media combination during the early stages of development. This study demonstrated the ease, simplicity and speed of performing multiple conditions in one experiment using Ambr® 15 Cell Culture by assessing five different media compositions on two different clones.

One clone (Figure 4 - Clone 1) shows all five media types resulted in similar viable cell count profiles and cell viabilities, with slightly higher peak cell densities for Media 3 and Media 5. The product titer results (Figure 4B) clearly highlight Media 5 as the best performing media for mAb production, and this is shown in the cell specific productivity (Qp) graph (Figure 4D).

Results from a second clone (Figure 5 - Clone 2) shows comparable cell growth in all five media types up until day 6, after which both Media 1 and Media 2 outperform the other media in terms of peak viable cell densities and also cell viabilities. For this clone the cell viabilities in Media 3, 4 and 5 start to decline fairly early on, from day 8 onwards. In the titer graph (Figure 5B) we can see higher product titers in both Media 1 and Media 2, but overall Media 2 has higher productivity after day 8 and for the remainder of the process resulting in higher Qp (cell specific productivity) values (Figure 5D).

Furthermore comparison of both clones, shows that overall Clone 2 achieved a maximum product titer of more than double the best results for Clone 1. However had Media 5 been chosen as the best media then the results would have indicated Clone 2 to have a lower performance, since Media 5 gave lower productivity in combination with this clone. Therefore it should not be underestimated the power of screening multiple clones and different media types together since performance can vary significantly.

Figure 4

Time-Course Cell Count and Titer Analysis for Clone 1 in Different Media Types; Cell Count Was Performed Using Vi-CELL XR While the Octet® Was Used for Titer Determination

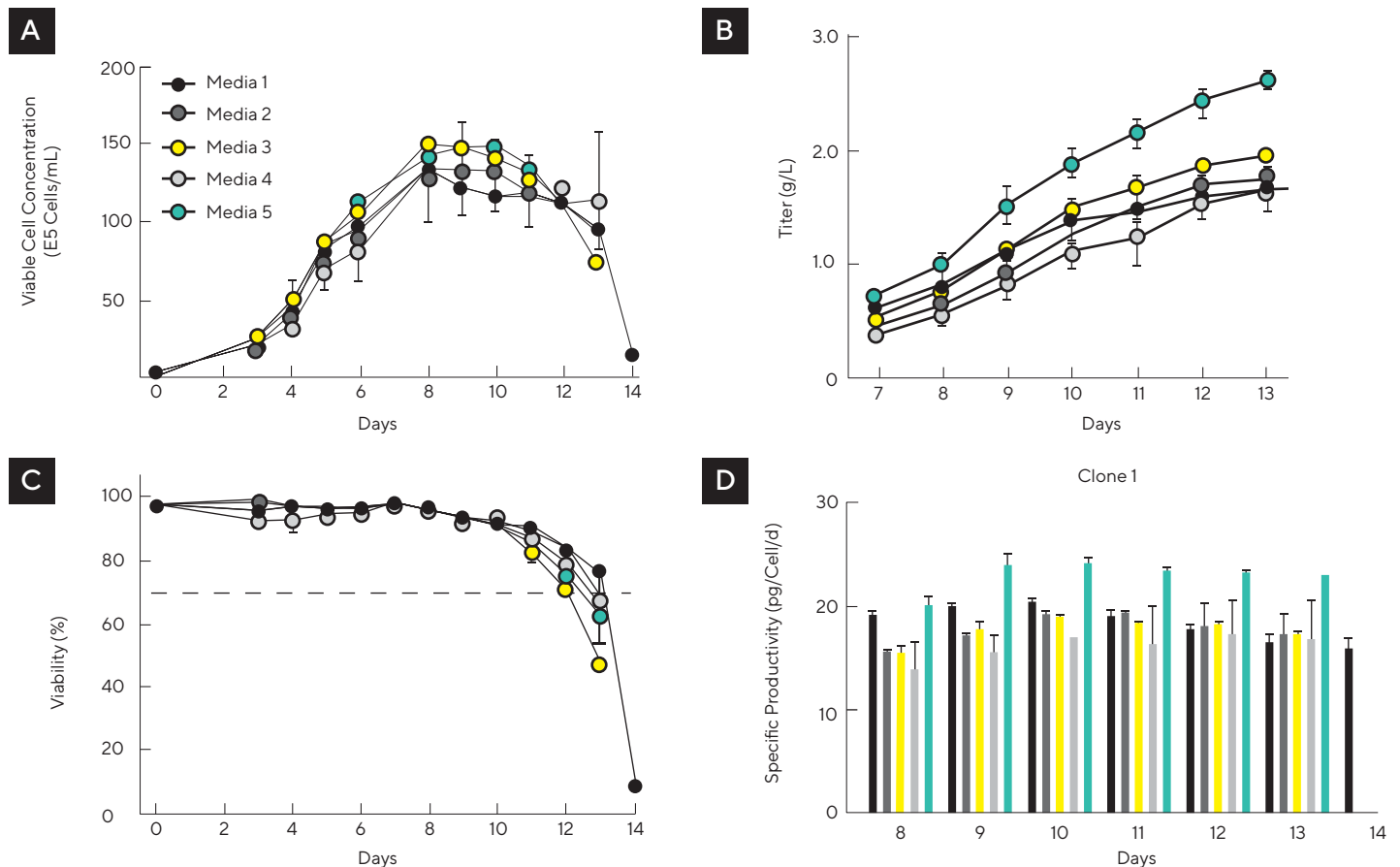
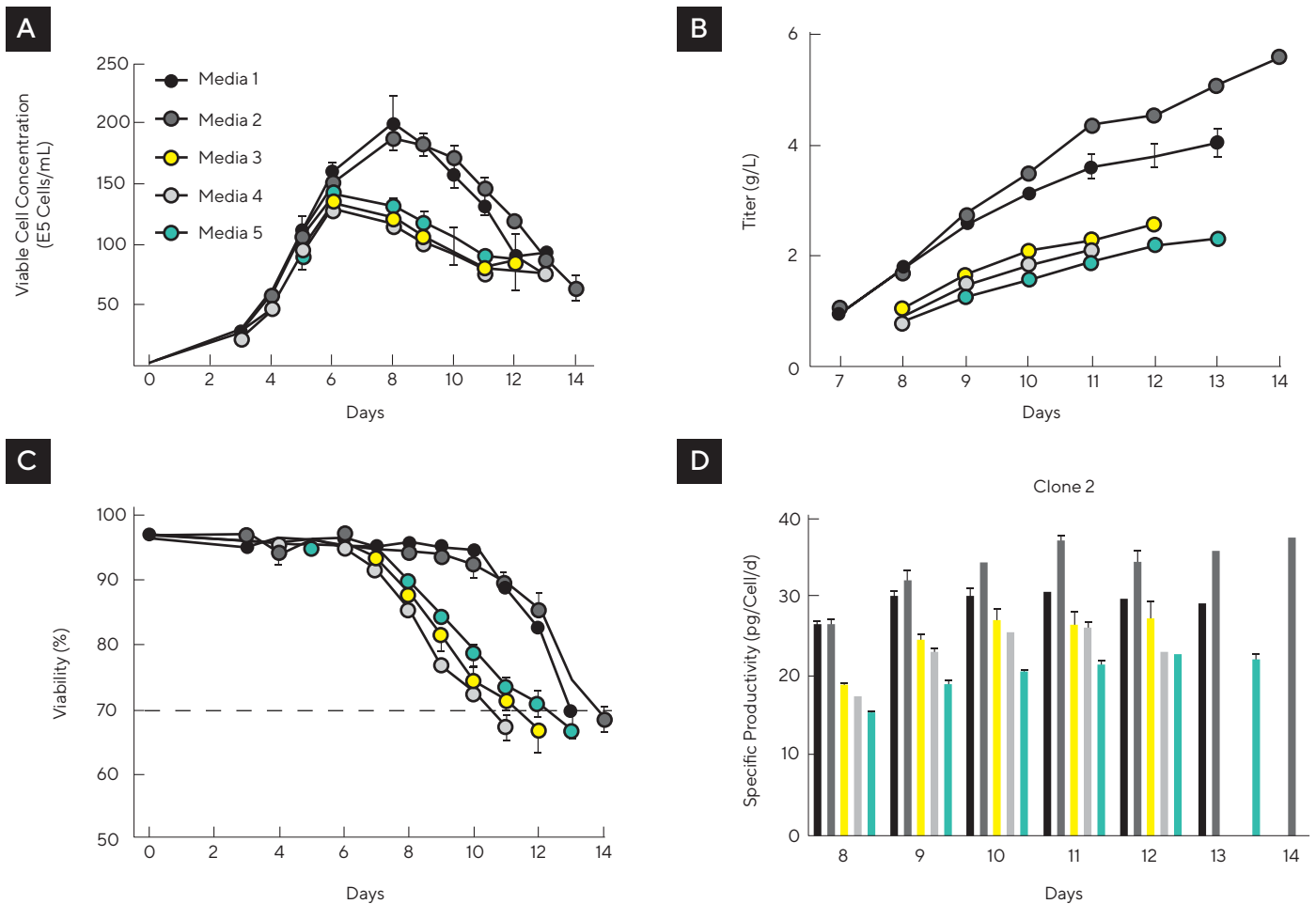


Figure 5

Time-Course Cell Count and Titer Analysis for Clone 2 in Different Media Types; Cell Count Was Performed Using Vi-CELL XR While the Octet® Was Used for Titer Determination



Process Optimization Experiment

Design of Experiments (DOE) is a rational and cost-effective approach to practical experimentation that can provide a great deal of information about the effect on a response variable due to one or more factors. DOE can also identify important interactions that may be missed when experimenting with one factor at a time.

The Ambr® 15 software includes a license for MODDE® DOE which has been integrated to allow planning of DOE experiments and already factors in the set up of the Ambr® 15 system. Process parameters that are included in the DOE study can be tagged in the Ambr® 15; this facilitates running of the experiment and allows easy transfer and analysis of the results in MODDE® on completion. The DOE MODDE® software integrated in the Ambr® 15 software enables scientists to quickly establish a Design Space where relevant bioprocessing conditions are varied simultaneously. Analysis of product titer using the Octet® through the process allows data analysis and further experiment planning to occur without delay and can have a positive impact on project scheduling.

In this experiment, stirring speed, ballast gas flow rates and starting volume were all factors that were varied in one experiment (Table 2). A reduced combinatorial design was used to strike a good balance between the factors investigated with limited number of experiments and replicates. Cell counts and product titer results were used to calculate Qp as a response.

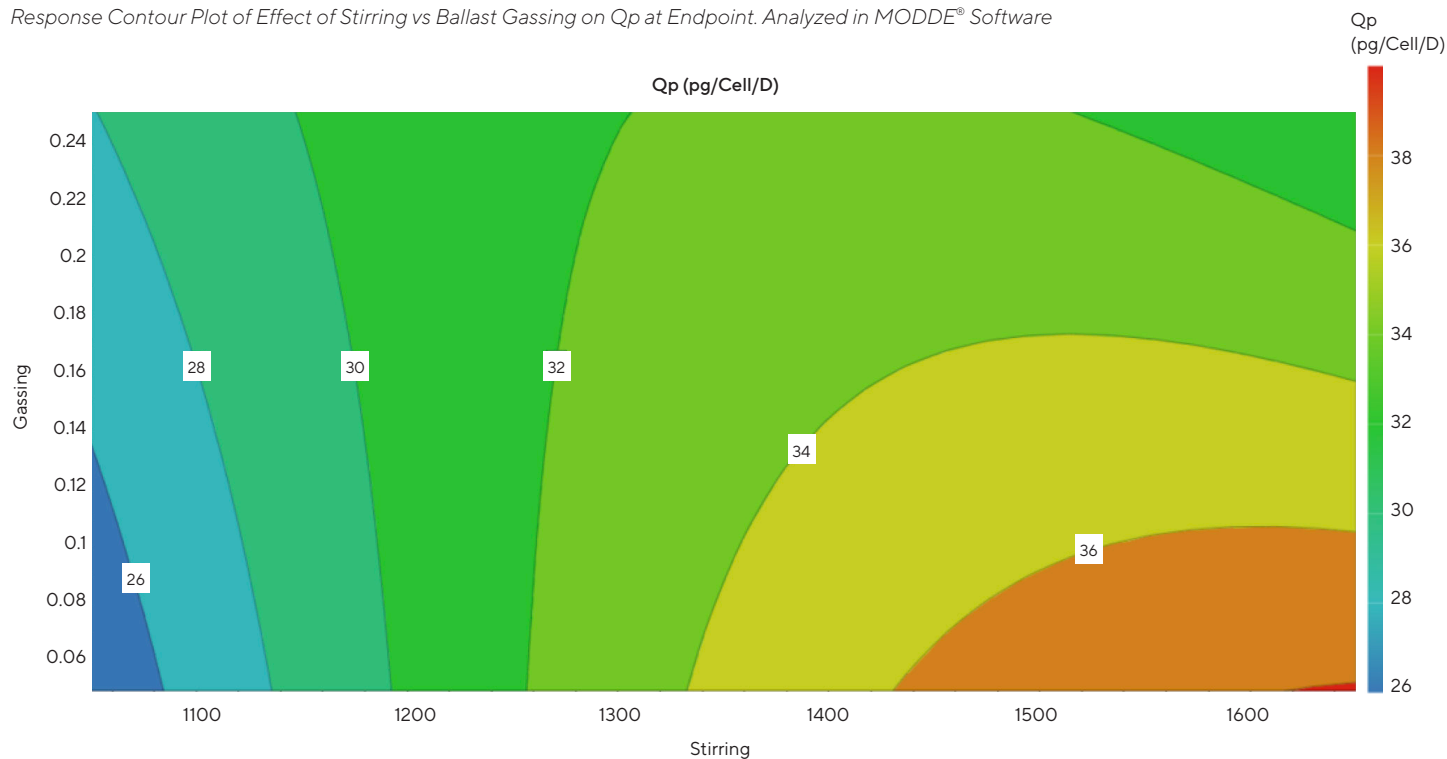
Table 2

Overview of Process Parameters and Responses of the DOE Study

Process Parameters	Range
Stirring speed	1050 - 1650 rpm
Ballast gassing (air)	0.05 - 0.25 mL/ min
Starting volume	12 - 14 mL
Responses	Qp (cell specific productivity)

Figure 6

Response Contour Plot of Effect of Stirring vs Ballast Gassing on Q_p at Endpoint. Analyzed in MODDE® Software



The DOE software MODDE® provides an easy-to-use, user-friendly interface for experimental design as well as statistical data analysis and visualization. Together with the design Wizard and analysis Wizard, MODDE® provides the user with guidance through their process investigations.

The response contour plot (Figure 6) shows how Q_p is impacted by the combination of stirring speed and ballast gassing. At low stirring speeds increasing the ballast (air) gas flow rate has a small but positive effect, as the stirring speed increases to 1200 rpm there appears to be no effect from the ballast gas flow rate. At the highest stirring speeds however, it actually has a detrimental effect to Q_p and lower ballast gas flow rates are desirable. Overall, the highest specific productivity values are seen at the higher stirring speeds combined with lower ballast gas flow rates.

In general, as stirring speeds increase so does the k_La - the mass transfer coefficient; a measurement of the capacity of the bioreactor to transfer oxygen into the culture. This is due to the energy input of the stirrer providing better distribution and reduction in size of the gas bubbles. Smaller bubbles improve oxygen transfer since they have a larger gas-liquid interface per unit of liquid volume and they have longer residence time in the medium, when compared with larger bubbles. Together with an increase in the amount of gas sparged into the cultures, this will further increase k_La , however higher shear stress exerted on cells may impact cell viability, which in turn will decrease cell productivity.

The main effect plot is a plot of the mean response values at each level of a single process variable. The stirring speed main effect plot (Figure 7A) shows only the relationship between stirring speed and cell specific productivity Q_p . The productivity of the cells improves with stirring speeds up to approximately 1500 rpm, after which the cell productivity plateaus and at the highest stirring speeds tested there is a very slight decrease in Q_p . Cell viability profiles (data not shown) displayed a slightly faster decline over time under the higher gassing and higher stirring conditions. Whilst the maximum Q_p was obtained at the higher stirring speeds tested, for this particular clone it appears that some shear sensitivity was observed.

The starting volume main effect plot in this study (Figure 7B) suggests a lower fill volume provides a higher Q_p (higher yield per cell). Changing the volume inside a bioreactor changes the total surface area for gas exchange. In Ambr® 15 Cell Culture the interfacial area between headspace volume and liquid volume is a higher contributor to the overall vessel k_La than might be the case in a larger bioreactor. Additionally at lower working volumes, for the same stirring speed the power input per unit volume rises, which means that the energetic input going directly into disrupting and encouraging suspension of gas bubbles in the liquid is greater, which will also make an important contribution to the k_La . Thus, the relationship of fill volume with other parameters must be considered carefully during process optimization.

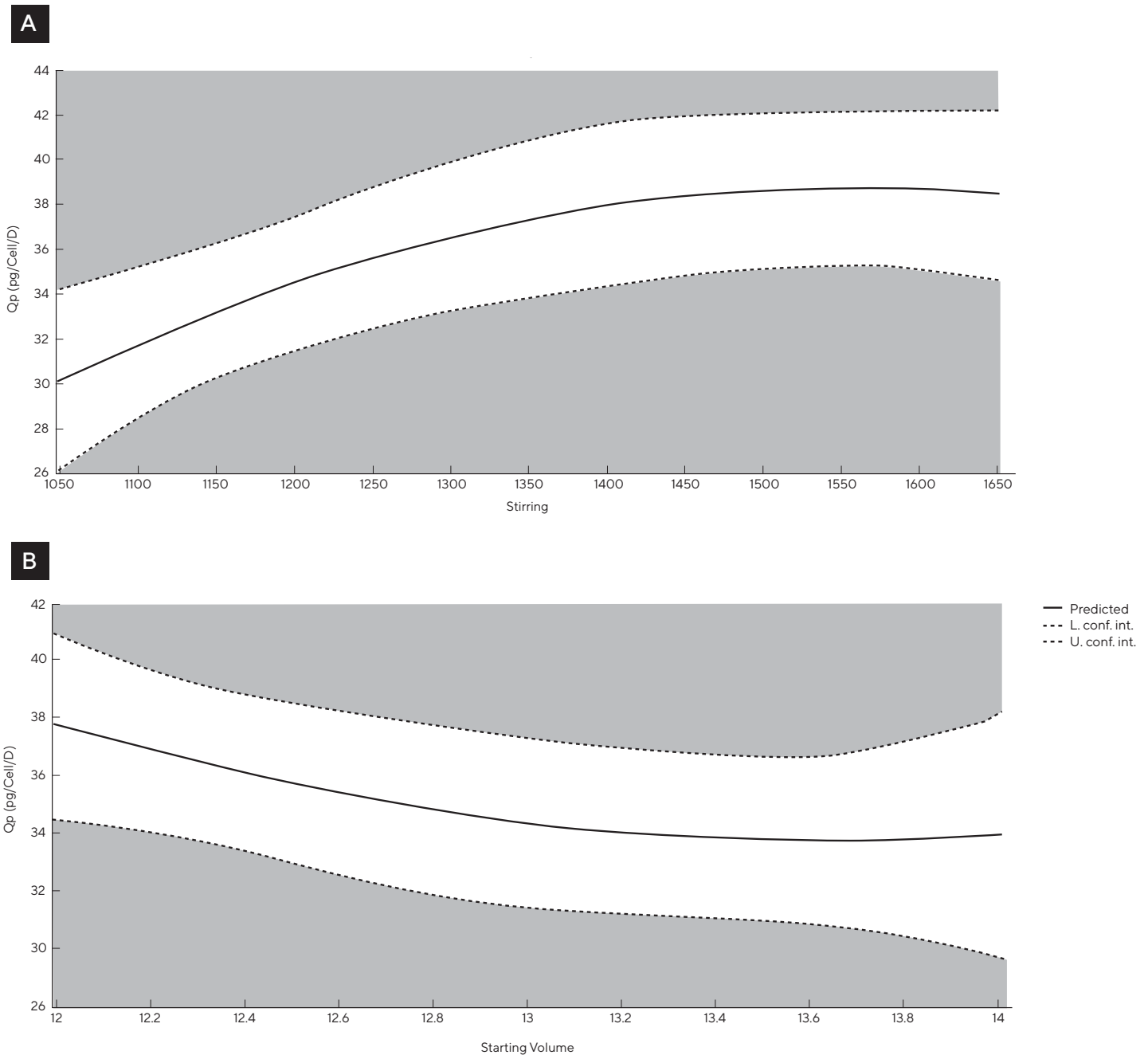
The DOE experiment provides great insights into how product yield is affected by the different process parameters, and can be viewed individually (main effects plot), in combination (contour plot), or in other formats which may help to identify which variables are critical to a particular cell line and/or process, and this knowledge can be applied to support further optimization work. The results from this study showed that for the tested clone, higher stirring speed, lower ballast gassing and lower starting volume resulted in the highest specific productivity, however care must be taken

when defining the optimum setpoints, to ensure that other important factors, such as cell viability, are also taken into account.

The powerful features in MODDE® embedded within Ambr® 15 allows high throughput DOE studies to be performed together with product titer analysis using the Octet®, the results of which assist with understanding the relationships between the process parameters and the cell specific productivity of a particular clone(s).

Figure 7

Main Effect Plots for Specific Productivity (Q_p) for (A) Stirring (B) Starting Volume. Analyzed in MODDE® Software





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List of Abbreviations

BLI	Bio-Layer Interferometry
CQA	Critical Quality Attribute
DO	Dissolved Oxygen
DOE	Design of Experiment
ELISA	Enzyme-Linked Immunosorbent Assay
HPLC	High-Performance Liquid Chromatography
IgG	Immunoglobulin G
Qp	Cell Specific Productivity (pg Protein/Cell/Day)

Summary

Implementation of the Octet® platform alongside the Ambr® 15 Cell Culture system within the cell line development workflow allows easy and rapid product quantification, and could also enable assessment of critical quality attributes when combined with the newly launched Sartorius Octet® GlyS and GlyM kits.

The Ambr® 15 Cell Culture automated microbioreactor system for mammalian cell culture, is able to run up to 48 × 15 mL cultures per experiment, thus offering considerable advantages for screening of multiple cell lines or clones in parallel, and reducing the experimental costs associated with media and feeds, especially for fed-batch or intensified bioreactor processes. The Octet® on the other hand can perform process analytics on crude samples with minimal sample processing. Compared to traditional titer determination techniques such as ELISA or HPLC, the Octet® enables a faster turnaround of yield analysis with minimal analyst involvement.

In these studies, we've demonstrated the power of combining an Ambr® 15 Cell Culture with the Octet® platform by comparing cultures in identically-sized, multi-parallel bioreactors for the rapid identification of the best combinations of media and clones based on the product titers and cell specific productivity. A DOE experiment was then used to examine the role of different physical process parameters; starting volume, stirring and ballast gassing rates on product yield.

References


1. Biolayer Interferometry as an Alternative to HPLC for Measuring Product Concentration in Fermentation Broth, Anurag S. et al., LCGC, Volume 35, Issue 12, 870–877.

Germany

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA

Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

 For further contacts, visit
www.sartorius.com