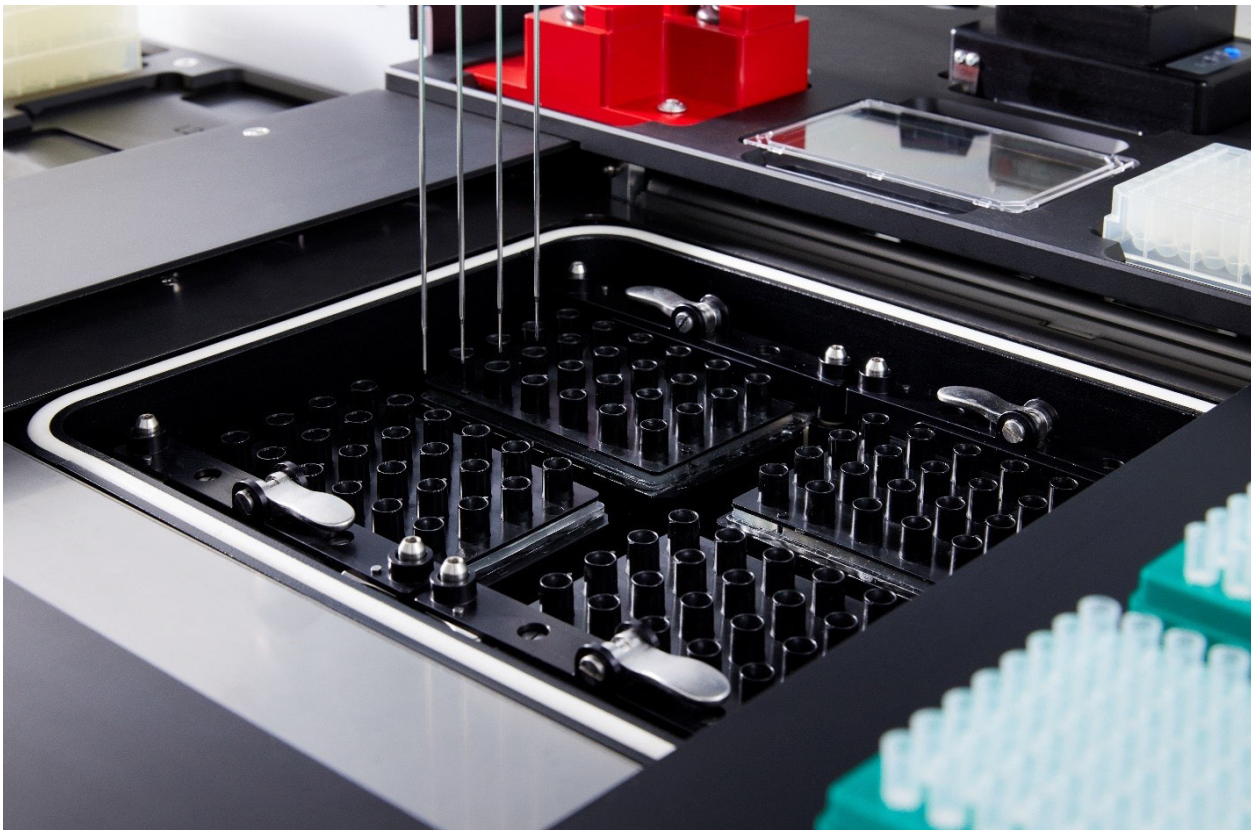
BECKMAN COULTER
Life Sciences

Efficient clone screening with increased process control and integrated cell health and titer measurements with the Cydem VT Automated Clone Screening System

Kathrin Olschok [KOLSCHOK@BECKMAN.COM], Noud Drummen [NDRUMMEN@BECKMAN.COM]
Beckman Coulter Life Sciences



Introduction

The Cydem VT Automated Clone Screening System allows for parallel screening of up to 96 IgG-producing CHO clones. The system combines several trusted Beckman Coulter technologies into a new and innovative integrated solution and provides the data required to make crucial decisions with confidence during cell line development. The system provides individual gassing control with CO_2, O_2 and N_2 for all cultivations, with daily base addition (as required, based on online pH measurements) and feeding events and determination of cell concentration, viability and titer concentration. A prototype of the Cydem VT system was placed at a third-party cell line development laboratory, where it was tested and used by their team of scientists as part of their clone screening process.

In this application note, the results of an experiment with 96 different clones are shown, where daily cell health and titer data was generated to closely monitor the clones' performance. On day 9 and day 14, offline titer values were determined and compared to the online values to confirm system performance.

Method

Before running the clone screening experiment, a standard curve with the targeted antibody was generated for online titer determination. Therefore, one well of a mixing plate from the Cydem VT system's Titer Kit was filled with 500 µL of IgG standard of a known concentration, and two wells with 800 µL of cell culture media. The Cydem VT system then prepared the dilution series and measured them in triplicates to generate a standard curve.

Since a prototype version was used, the shown methods for standard curve generation deviate slightly from those for the final product. Refer to the Instructions for Use (IFU) manual for information on the current method.

Then the Cydem VT Automated Clone Screening System was decontaminated and prepared for experiment start (see IFU for detailed instructions). The selected clones were grown in a shaken incubator, and the 24-well cell source plates were prepared with a known viable cell density (VCD) of approximately $2*10^6$ cells/mL. The cells were seeded with the known VCD seeding method at a target cell density of $0.33*10^6$ cells/mL at 4.8 mL. The DO setpoint was set at 50% and the following pH setpoints were selected: CO_2: 7.1, base trigger: 6.9, base target: 7.0. For liquid base additions 0.5 M NaOH was added, with a pH control scale factor of 190. The bioreactor was shaken at 800 rpm, 37°C.

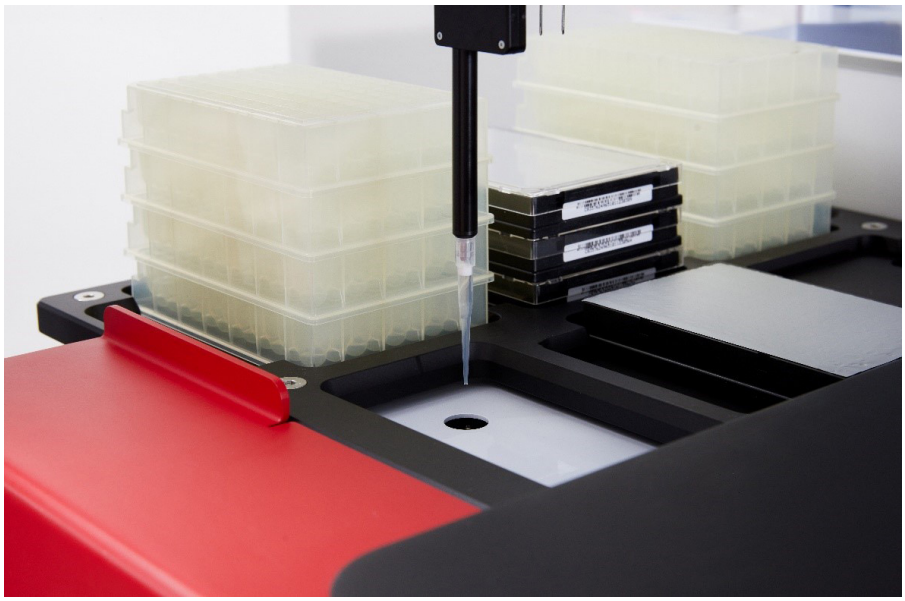


Figure 1. Disposable tip adding a sample to the titer module on the Cydem VT system's deck.

Feeding events were scheduled daily starting on day 3, with both a feed solution and additional glucose feeding (no premixing) based on previously determined feed schedules. Determination of cell concentration and viability was scheduled every day, and titer measurements were run from day 8 onwards. On day 9 and day 14, an output plate was generated for offline titer measurements on a Cedex Bio HT analyzer and checked for correlation.

Results

The standard curve result **(Figure 2)** shows that not all three quality criteria were fully matched; in this case the upper limit of the protein range was a little lower than the recommended 1000 mg/L. This is likely due to a relatively high binding affinity of the antibody to the probe, causing the curve to saturate before reaching 1000 mg/L. A maximum measurement range up to 8.9 g/L was deemed sufficient, since maximum titer values were not expected to reach higher during the clone screening experiment. The quality criteria for the delta shift and replicate CV showed excellent results, thus the standard curve calibration results were used for the clone screening experiment.

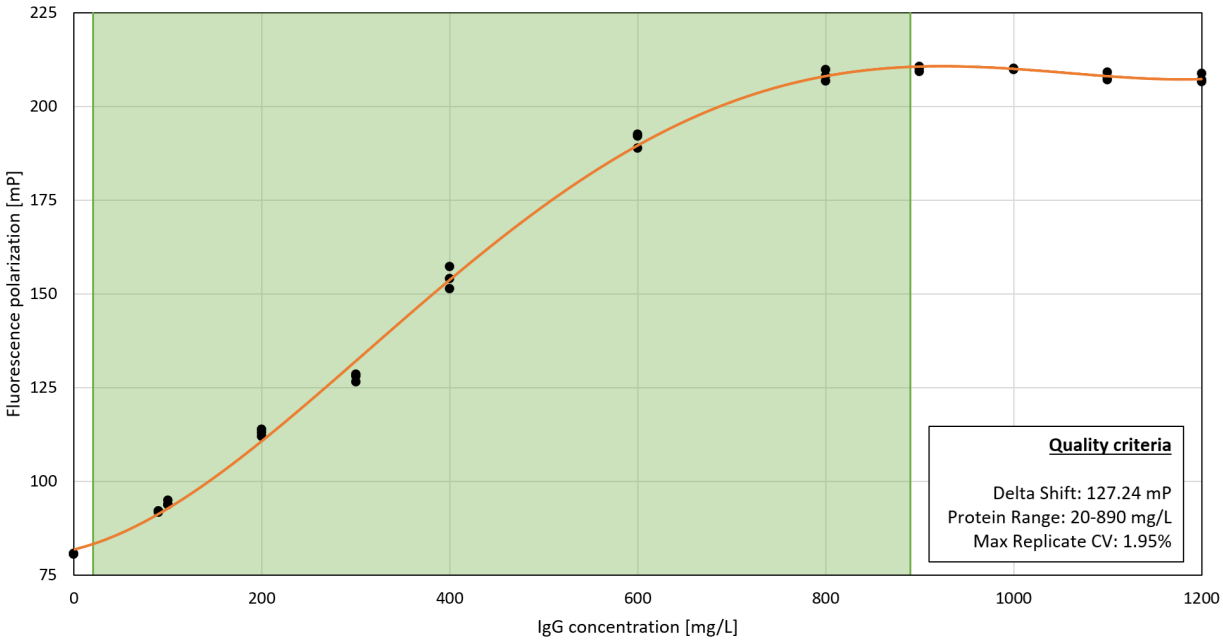


Figure 2. Standard curve calibration results, including quality criteria values.

After the experiment was completed, all data was collected and analyzed. The cell health measurements on day 6 and day 7 were missed. For visualization purposes, just 10 clones were selected for deeper analysis in this application note, with different growth and titer production profiles throughout the experiment. The selection consisted of two non-producers, some low- and mid-range producers, as well as the highest producing clone.

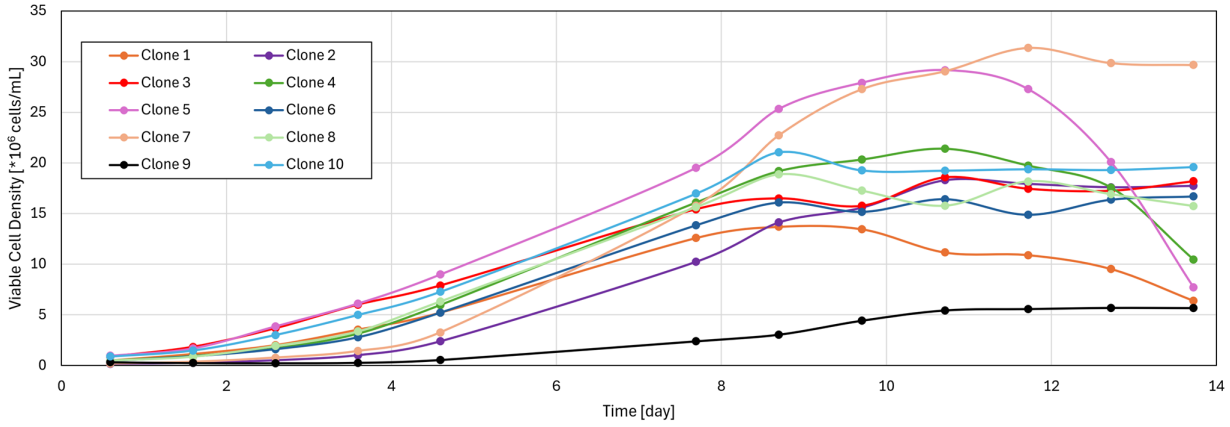


Figure 3. Viable cell density during the 14-day fed-batch cultivation.

The viable cell density and viability **(Figure 3** and **Figure 4)** show that all cultures had increasing viable cell counts during the first 9 days, and all reached viabilities >95% during the cultivation. However, large differences were observed between the growth rates and maximum cell densities reached. On day 14, clone 9 reached a VCD of barely $6*10^6$ cells/mL, whereas clone 7 reached over $30*10^6$ cells/mL. Most selected clones showed good viability at the end of the 14-day experiment, but clones 1, 4 and 5 declined earlier – starting on day 9 – and dropped below 50% viability on the last day.

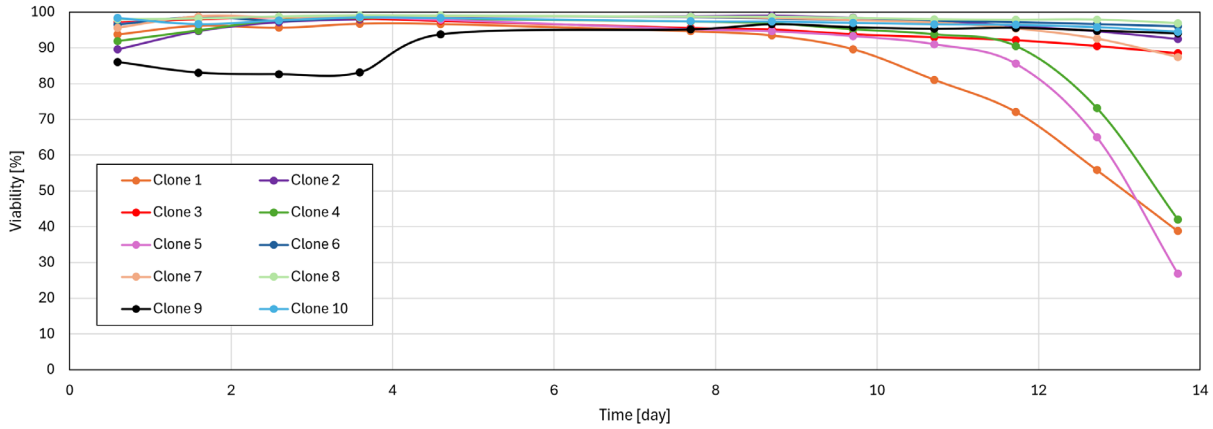


Figure 4. Viability during the 14-day fed-batch cultivation.

The online titer measurements **(Figure 5)** also showed very distinct differences. Some non-producers (clones 5 and 7) were seen where titer values consequently remained 0. The highest producing clone reached 3.6 g/L IgG, which is over 50% more compared to clone 3, which had the second highest concentration on day 14. Furthermore, it is interesting to notice that titer production accelerated at different timepoints, e.g., clone 6 showed a significant increase between day 10 and day 11, whereas clone 2 shows a similar increase between day 12 and day 13. These effects would not have been observed without the daily titer determination.

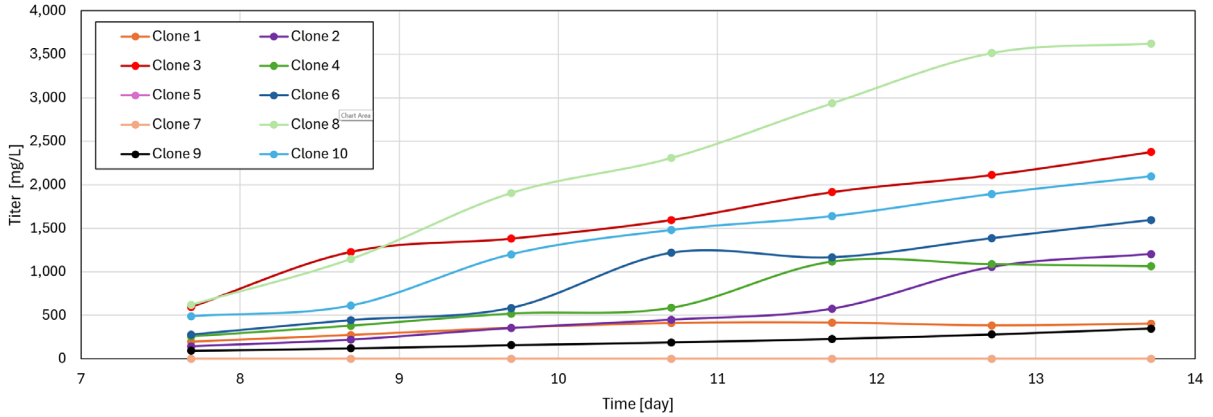


Figure 5. Online titer during the 14-day fed-batch cultivation.

The titer values on days 9 and 14 measured with the Cedex Bio HT analyzer were correlated against the online Cydem VT system values **(Figure 6)**. The observed correlation of $R^2 = 0.98$ demonstrates the accuracy of the online titer measurement across the entire range. A correlation of $R^2 = 0.97$ was seen when all 96 clones were considered.

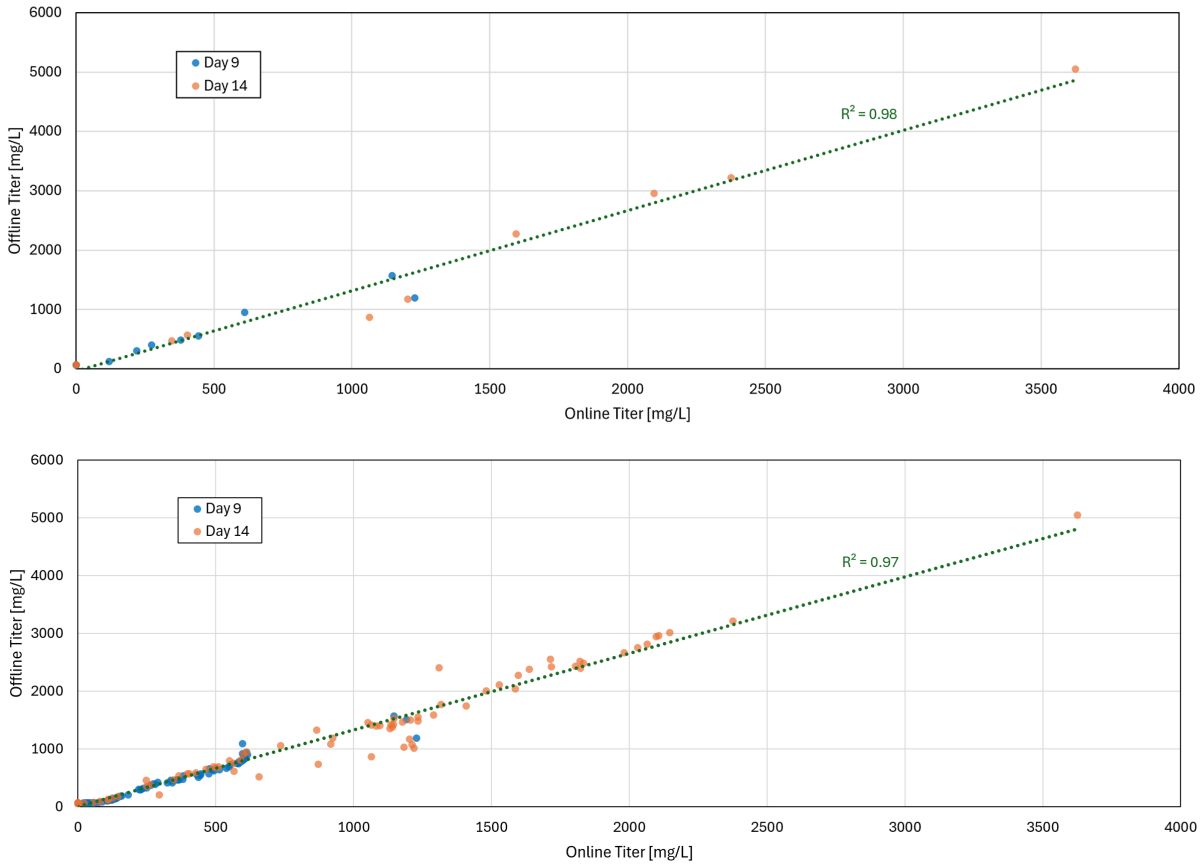


Figure 6. Correlation between online and offline titer measurements for selected **(upper graph)** and all clones **(lower graph)**.

Finally, the available experiment data from the Cydem VT system export was used to calculate the productivity of the clones throughout the experiment with the following formula:

$$Productivity \left[\frac{pg}{cell} \right] = \frac{IgG\ titer}{total\ cell\ density}$$

The resulting graph **(Figure 7)** shows similar differences between the selected clones as observed in Figure 5. The most remarkable change is the relatively high ranking of clone 9 in the early days, when productivity is considered. Despite the slow growth behavior, titer productivity was higher compared to most of the other clones.

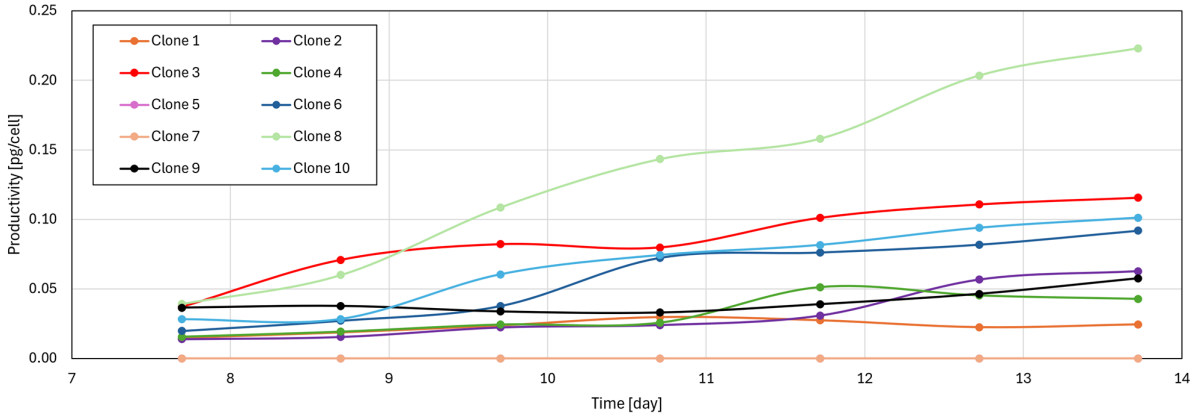


Figure 7. Productivity during the 14-day fed-batch cultivation.

Discussion

Altogether, the clone screening experiment showed how distinctly different clones perform during a typical clone screening experiment. All clones showed high viability, but this did not necessarily translate into high growth rates. Also, titer production and productivity showed that the selected clones behave differently, where clone 8 showed higher titer results.

The excellent correlation to the offline titer values provides confidence in the observed values, and the high data density – made possible by the automated, daily measurements – provides additional information and detail which might be crucial for identifying top clones during screening experiments.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

2024-GBL-EN-105878-v2