

## Abstract

In most cases, the titer of an antibody being expressed in a stirred bioreactor is measured offline. The process involves removing an aliquot aseptically from the bioreactor, centrifuging the aliquot and analyzing the resulting supernatant. The supernatant is analyzed either by bio-layer interferometry (BLI), Protein A HPLC or ELISA. Such an approach may limit throughput, workflow and efficiency. Aragen is evaluating a prototype chromatography-based device that is directly connected to a bioreactor. The compact device automatically measures the titer of an antibody being expressed from a stable DG44 CHO clone for 19 days, starting from day 3, post-inoculation. Results of this novel approach to real-time monitoring of antibody titer in a fed-batch stirred bioreactor run are presented below.

## Bioreactor Conditions

### Cells

- DG44 CHO stable clone expressing a human IgG antibody.

### Inoculation

- Inoculum: 300 mL in 2 L flasks with 2–3 X 10<sup>6</sup> cells/mL at 96% to 98% viability
- Inoculation Density: 0.5 X 10<sup>6</sup> cells/mL
- Seed Train Media: CD Opti CHO + 6 mM L-Glutamine + 0.1% F68
- Production Media: BalanCD CHO Growth A

### Feeding

- Balance Feed 3 added to 15% on D3, D6 and D9.
- Glucose feed when needed to prevent depletion.

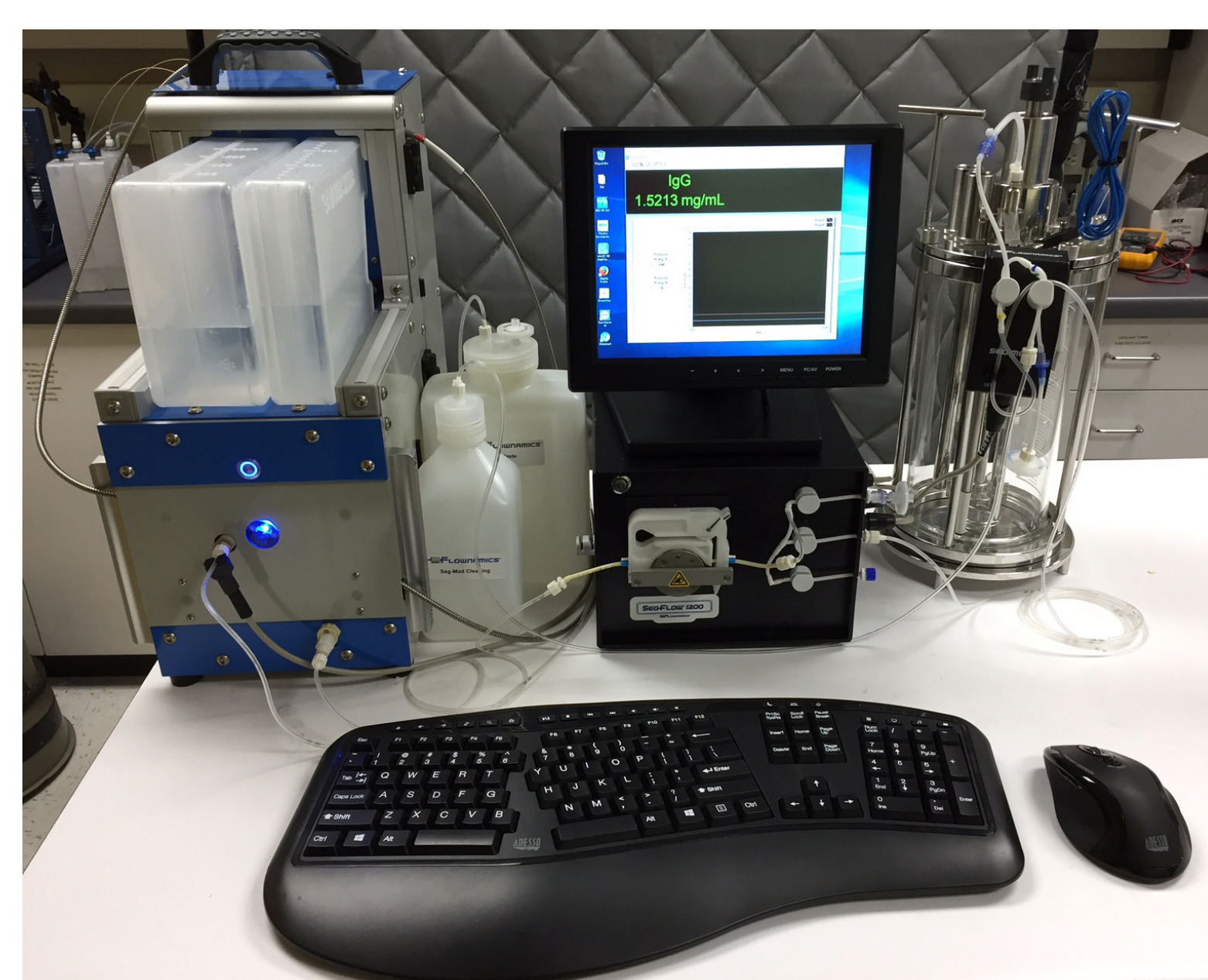
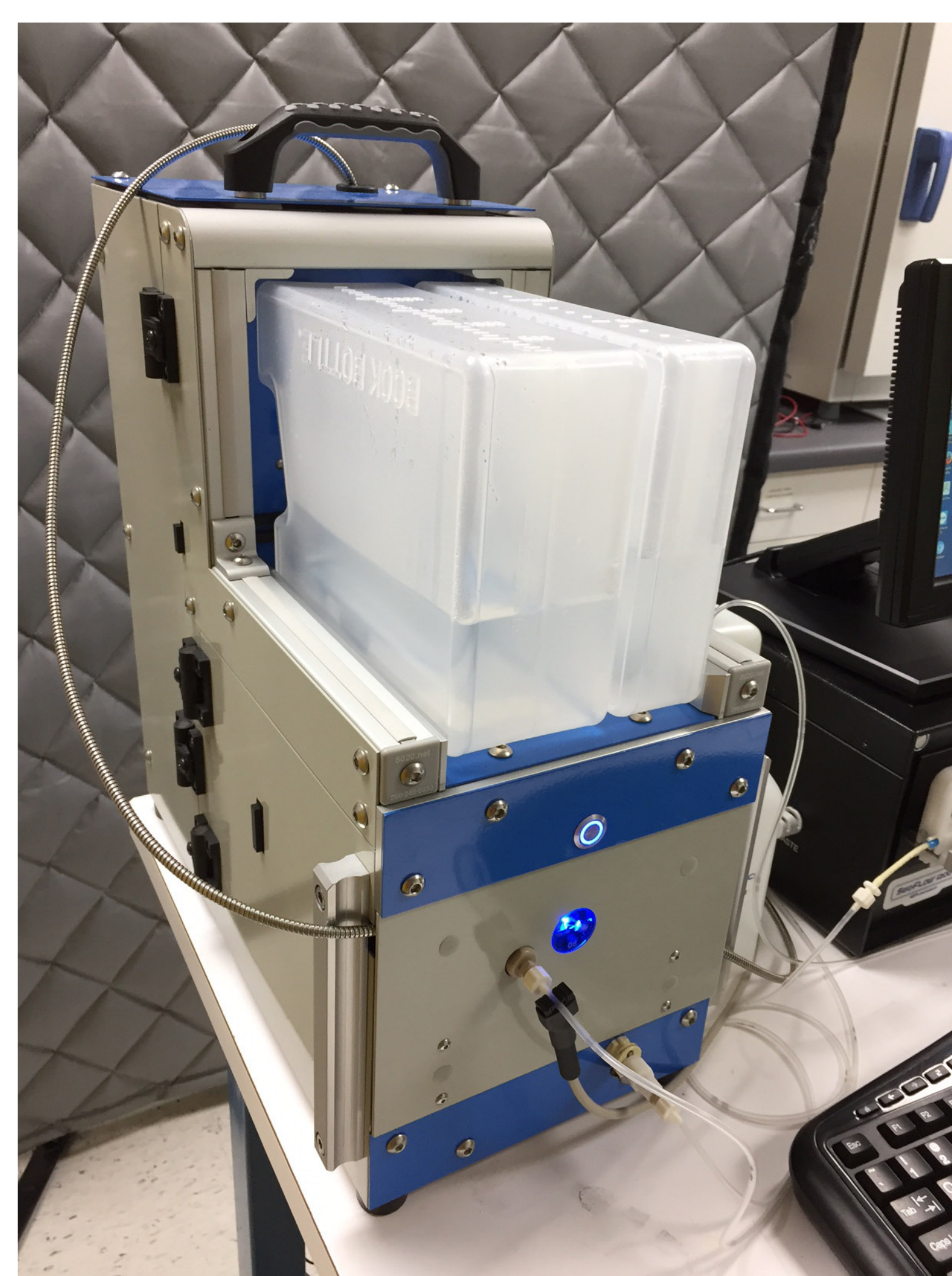
### Analysis

- Beckman Coulter Vi-Cell XR for cell density and cell viability.
- Nova Biochemical BioProfile FLEX2 for pH and metabolites.

### Bioreactor Parameters

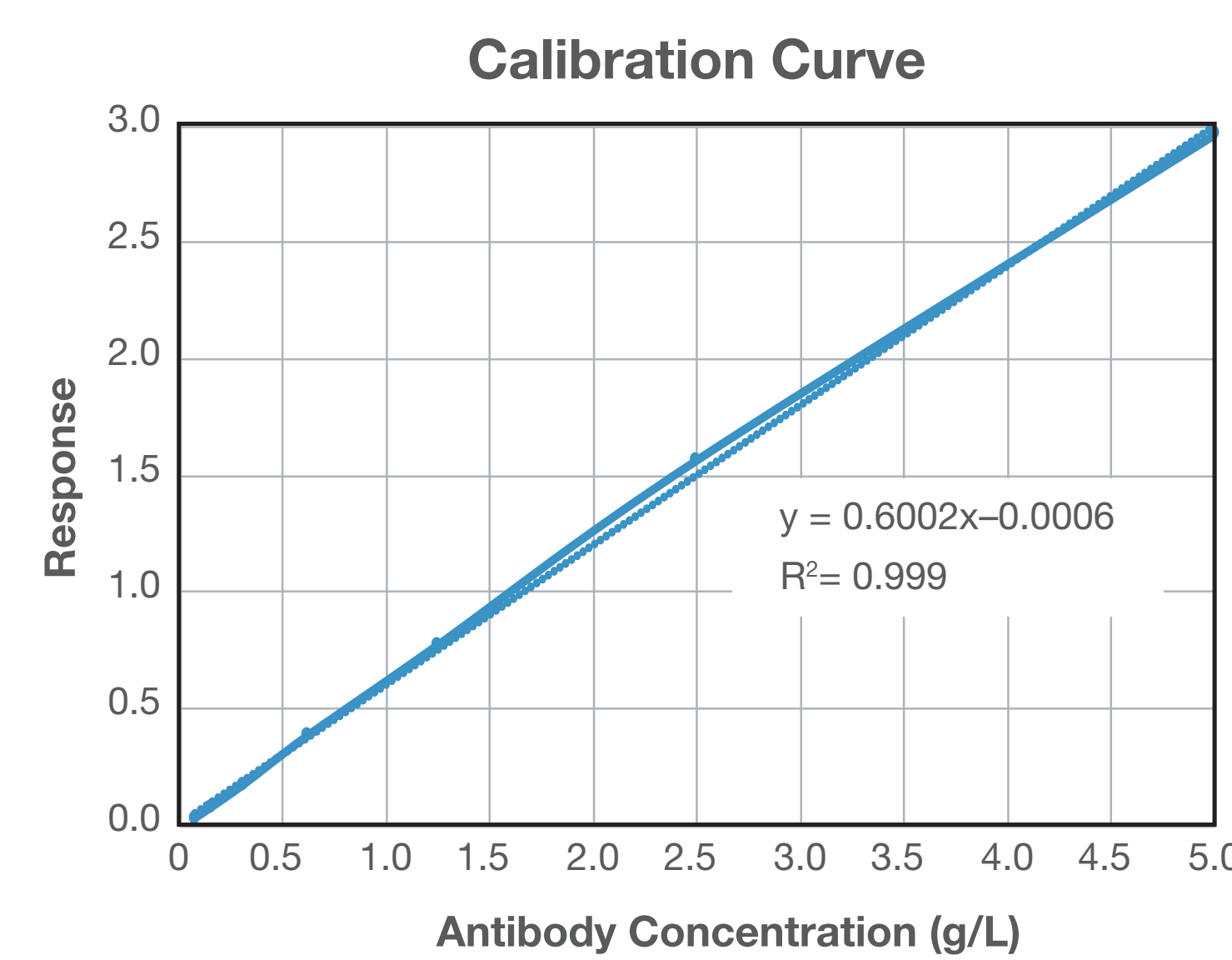
- Working Volume: 1.0 L
- Temperature: 37°C
- Dissolved Oxygen: 30%
- Constant Air Sweep: 0.1 L/min
- pH: 7 ± 0.2
- pH Control: CO<sub>2</sub> and 0.5 N NaOH
- Agitation: 250 rpm

## Real Time Antibody Titer Prototype Device



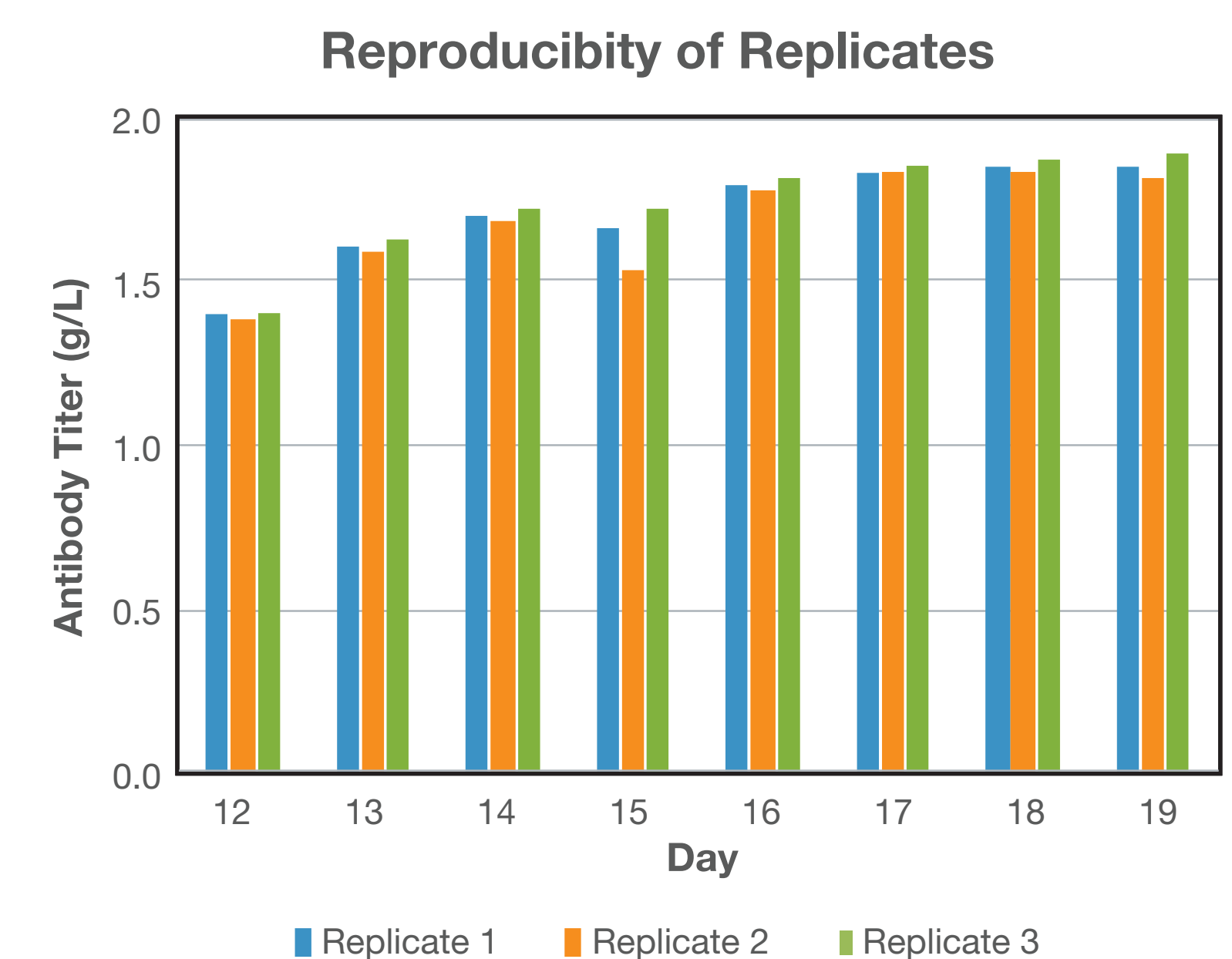
- The chromatographic device contains pumps, valve, detector, analytical module and buffer reservoirs, all built into a small footprint:
  - Height: 17" (43.2 cm), with handle: 18 ¼" (46 cm)
  - Width: 8" (20 cm)
  - Depth: 16" (40.6 cm)
  - Weight: 19.5 kg (full buffer reservoirs)
- A separate online sampling instrument withdraws a user-defined volume of cell-free sample directly from the bioreactor and delivers it to the chromatographic device.
- The antibody in the cell-free sample is analyzed chromatographically and the internal PC displays the titer results of the antibody.
- The device measures the titer without any dilution or re-calibration during the bioreactor run.
- The sampling rate and the analysis of the cell-free sample are programmed into the internal PC, creating an automated, hands-free real time measuring of antibody titer from the bioreactor.
- The device can be accessed remotely by Wi-Fi so it can be monitored while running unattended.

## Evaluation Results



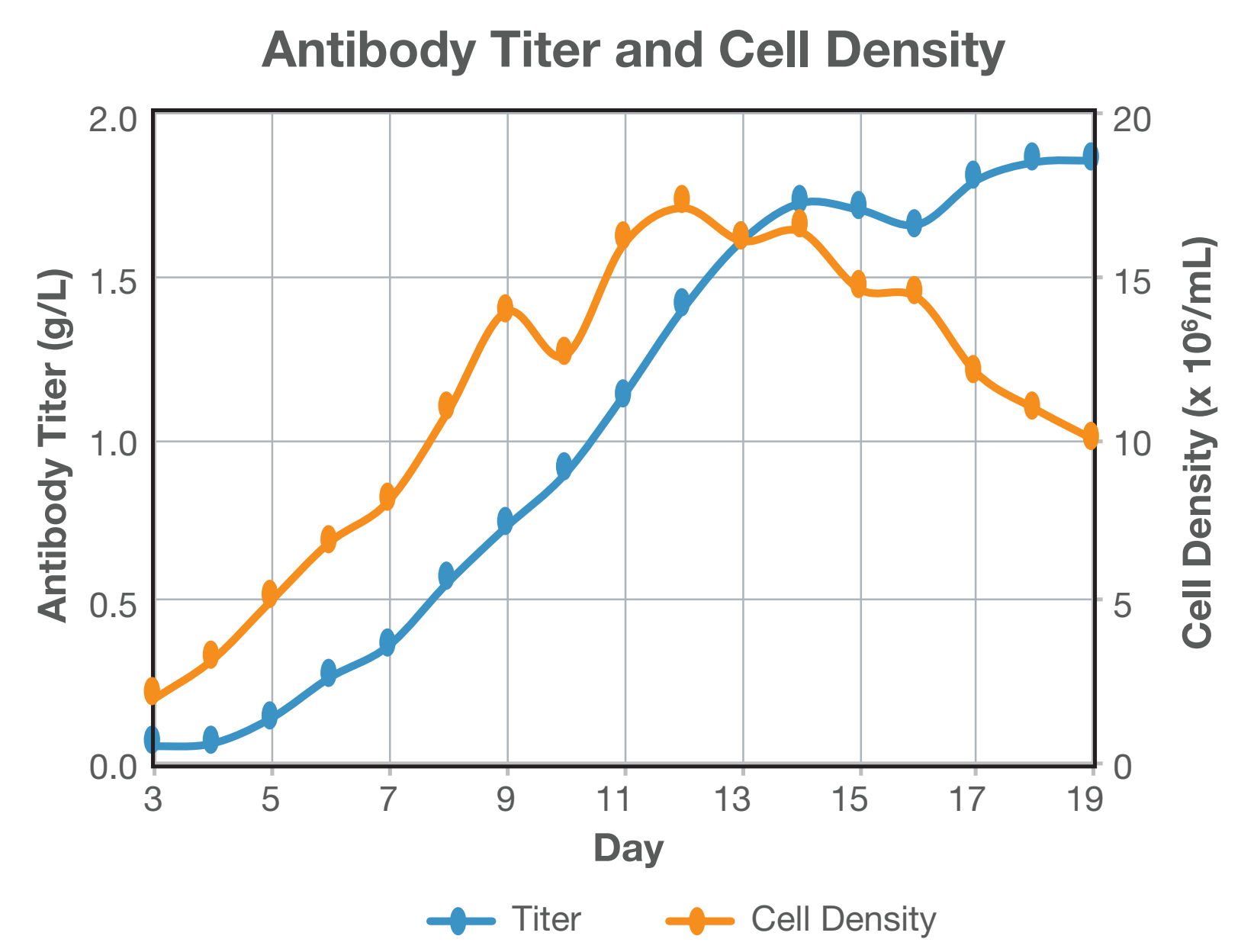
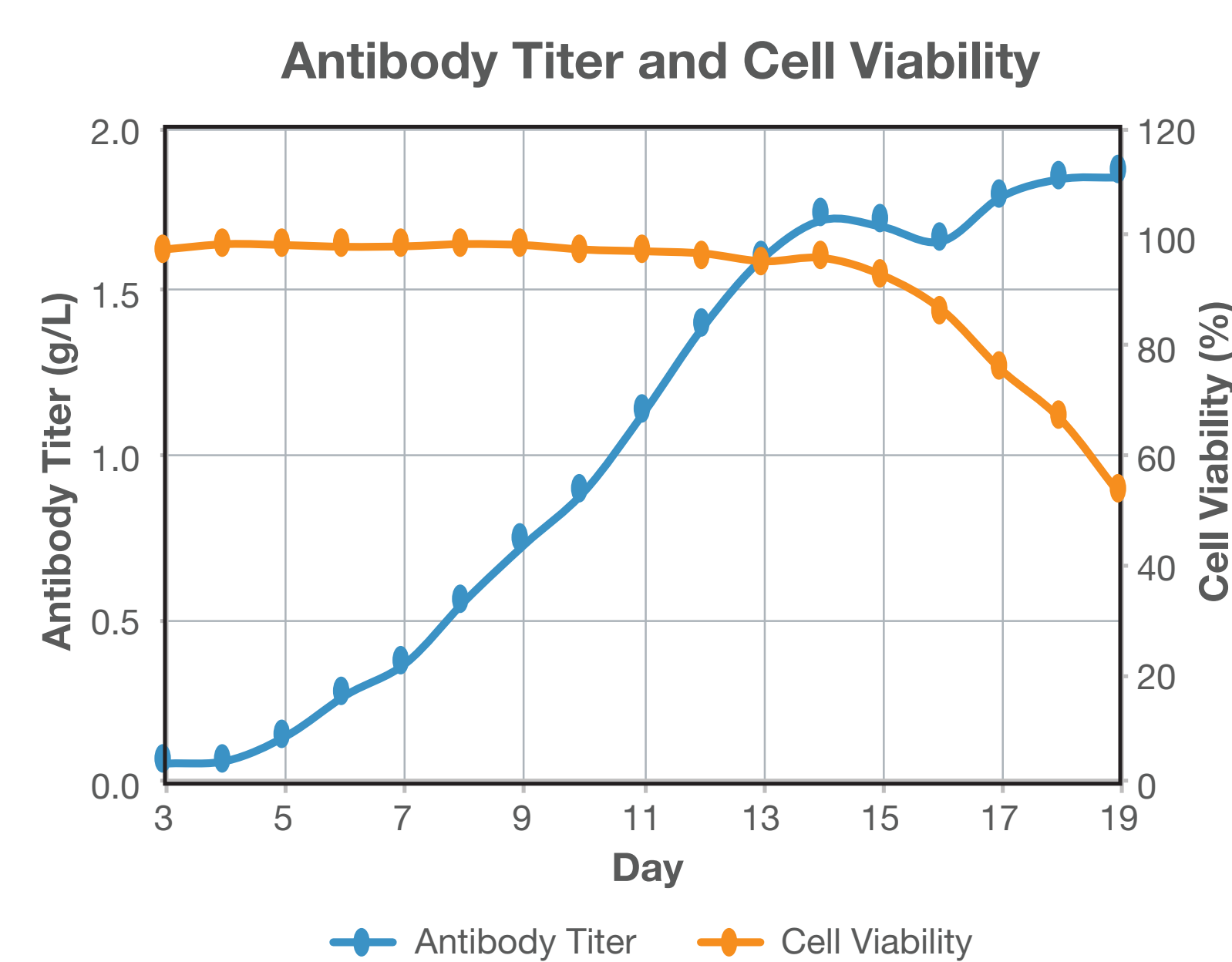
### Calibration Curve Graph

- A well-characterized human IgG was used as a calibrant.
- Starting from 5 g/L down to 0.07 g/L, an R<sup>2</sup> of 0.999 was achieved with this device.
- CHO cell lines whose titers go up to 5 g/L in a bioreactor run can be monitored in real time.



### Reproducibility of Replicates

- Starting on day 12, while the device was on manual sampling mode, three consecutive measurements were performed.
- After the 3rd measurement was taken, the device was returned to automatic sampling mode.



### Antibody Titer Graphs

- Antibody titer was measured in real time every 6 hours and the average of three measurements per day are plotted.
- The relationship of antibody titer to cell density and viability is shown above.

## Summary & Conclusions

- The prototype device automatically and directly measured the titer of the human IgG antibody being expressed from a stable CHO cell line during the fed batch bioreactor run.
- The device measured the titer from 15 µL of cell-free sample.
- The device eliminated the tedious need to withdraw and to prepare a sample from the bioreactor for titer analysis by alternate methods.
- Along with an online sampling instrument, the device can be programmed to perform numerous titer measurements automatically during the course of a bioreactor run.
- If an online sampling instrument is capable of withdrawing cell-free samples from multiple bioreactors, the device can automatically and efficiently measure the titers from those bioreactors.
- The device can accurately measure titers of CHO cell lines whose titers goes up to 5 g/L during a bioreactor run.
- The device can be an invaluable tool for measuring critical process parameter (CPP) in real time during the manufacturing of an antibody for therapeutic use.