

Developing new perfusion capabilities for Ambr® bioreactors

Barney Zoro^{1*}, Alison Rees-Manley¹, Ellen Lam², Juan Bastidas², Asma Ahmad, James Edwards¹

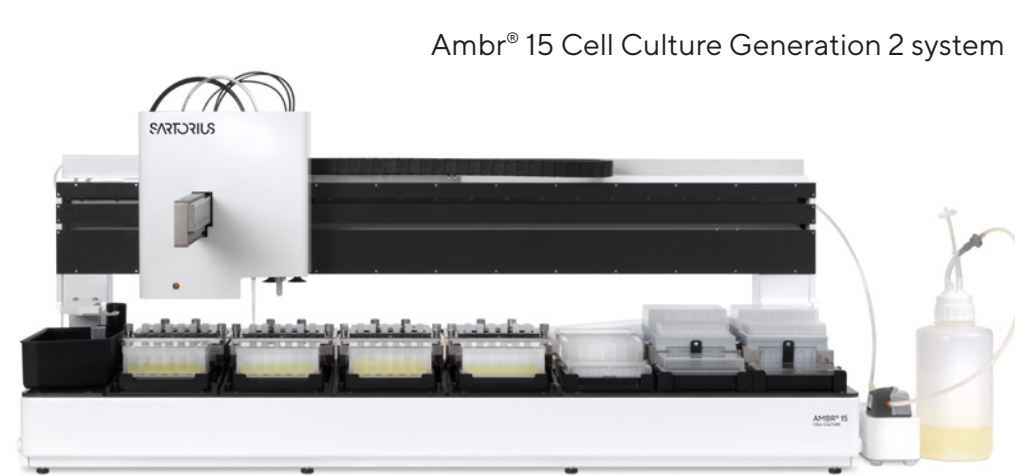
¹Sartorius Royston, UK. ²Sartorius Bohemia, USA.
*barney.zoro@sartorius.com

1. Aims

- Test perfusion mimic processes in Ambr® 15 Cell Culture Generation 2
- Investigate process capability and scalability of Ambr® 250 High Throughput Perfusion

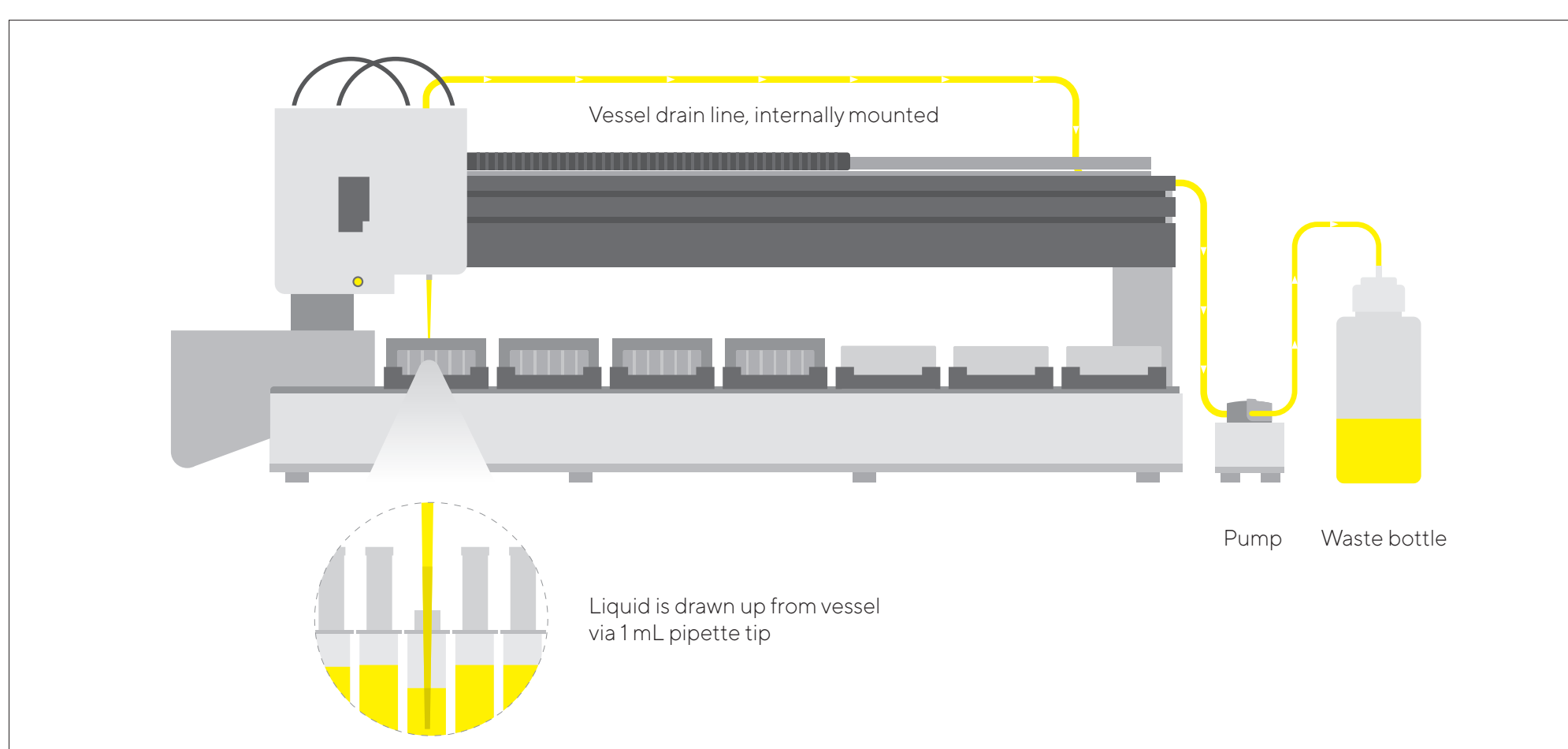
2. New Features of the Ambr® 15 Cell Culture Generation 2

- Flexible deck layout for tips and plates
- Expanded tip bin increases walk-away time
- Lower stirrer speed for sensitive cells
- Media mixing steps facilitate media optimization studies
- Rapid vessel drain accelerates culture passaging, media exchanges and perfusion mimic processes
- Ambr® Clone Selection software applies simplified multivariate data analysis to improve cell line ranking

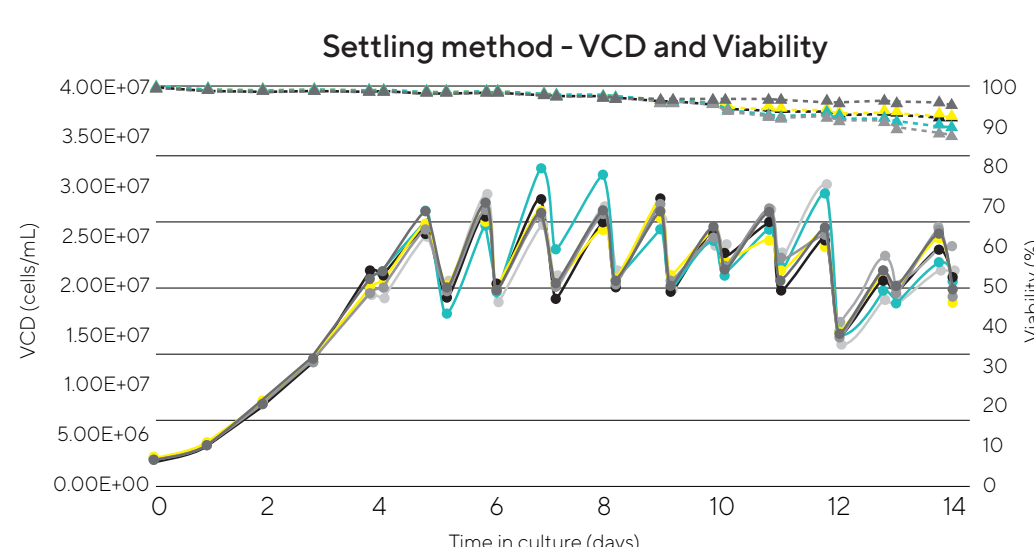
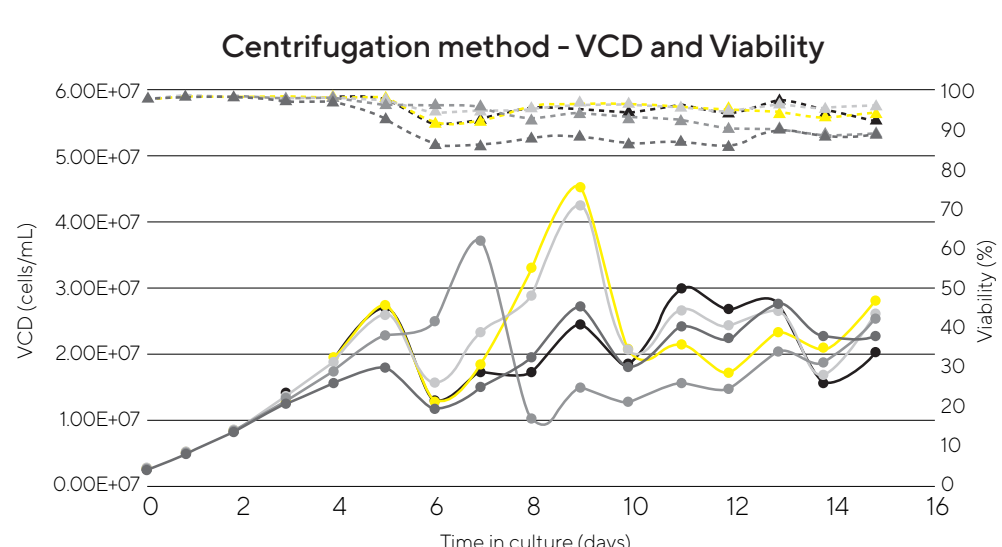
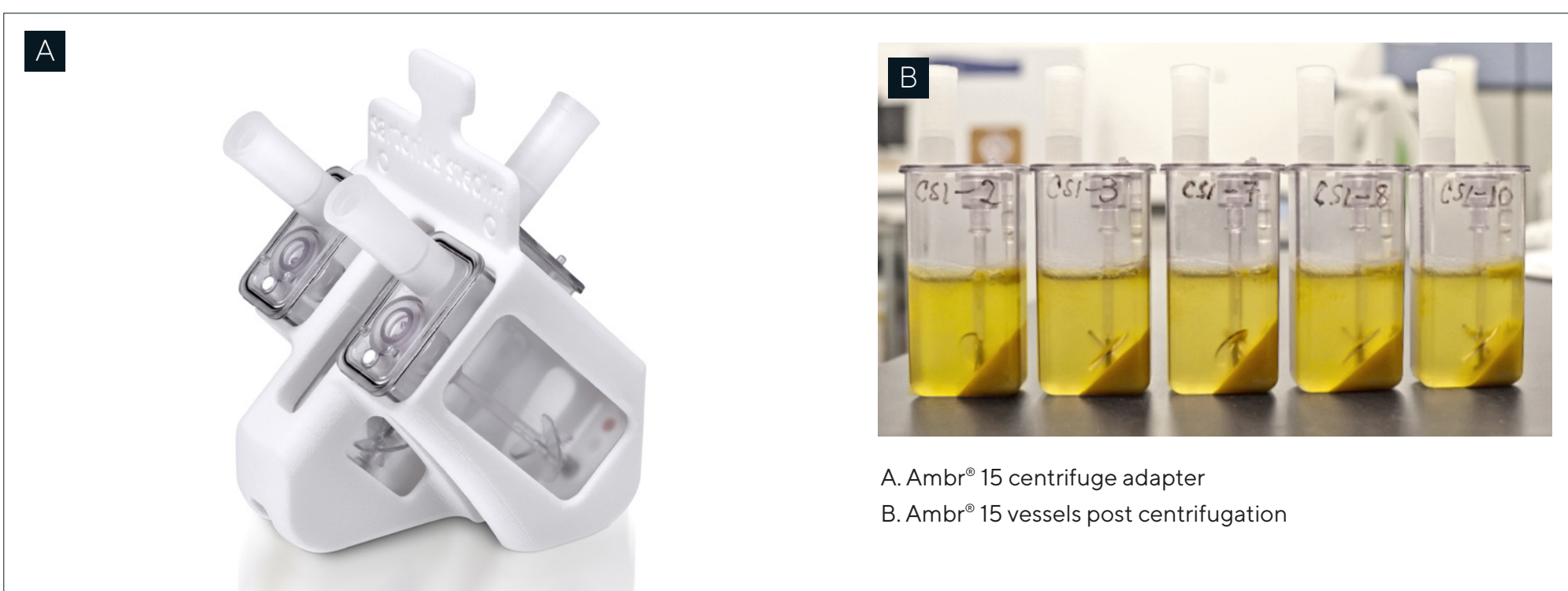


3. Rapid Vessel Drain

The Rapid Vessel Drain provides capability to quickly remove spent media or culture from each Ambr® 15 microbioreactor in a single step.



4. Ambr® 15 Cell Culture Generation 2 Perfusion Mimic Case Studies



Centrifugation method

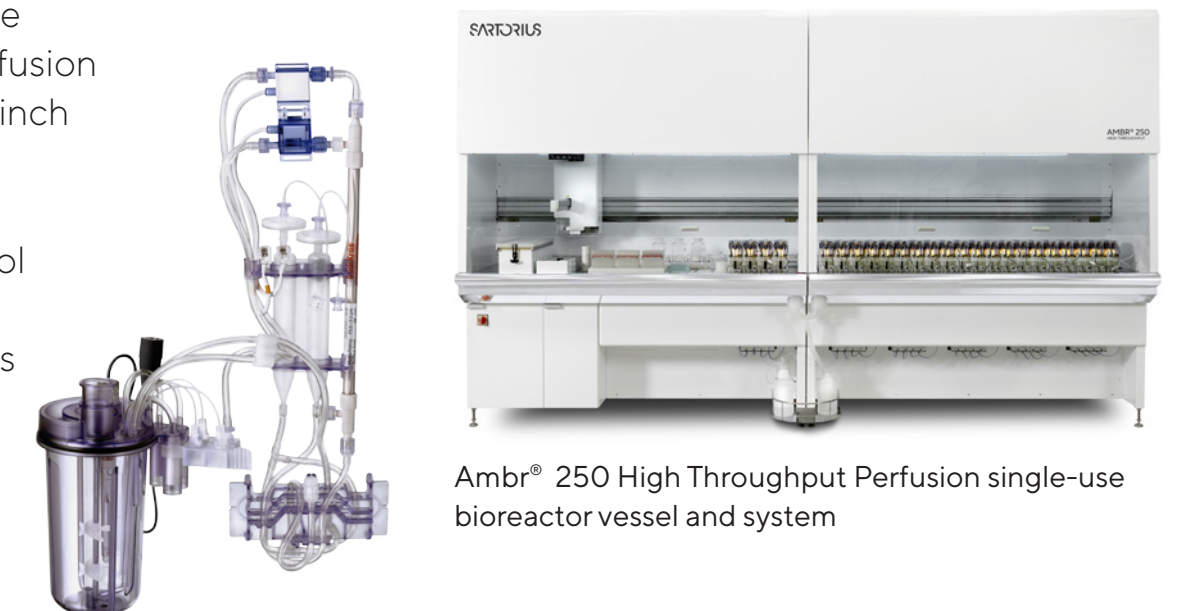
- Semi-automated process 1VVD exchange 1x daily by angled centrifugation rapid vessel drain steps; media refill
- Triggered bleed step after cell count when VCD > 20Mcells/mL
- Consistent VCDs once protocol adjusted to manage cell concentration at target level
- Average daily product titer of 1.0 g/L from day 5 onwards and cumulative product titer reaching 14g/L on day 15 (data not shown)
- Centrifugation method involved manual steps to centrifuge cultures, however only required to perform once per day to achieve 1VVD

Cell settling method

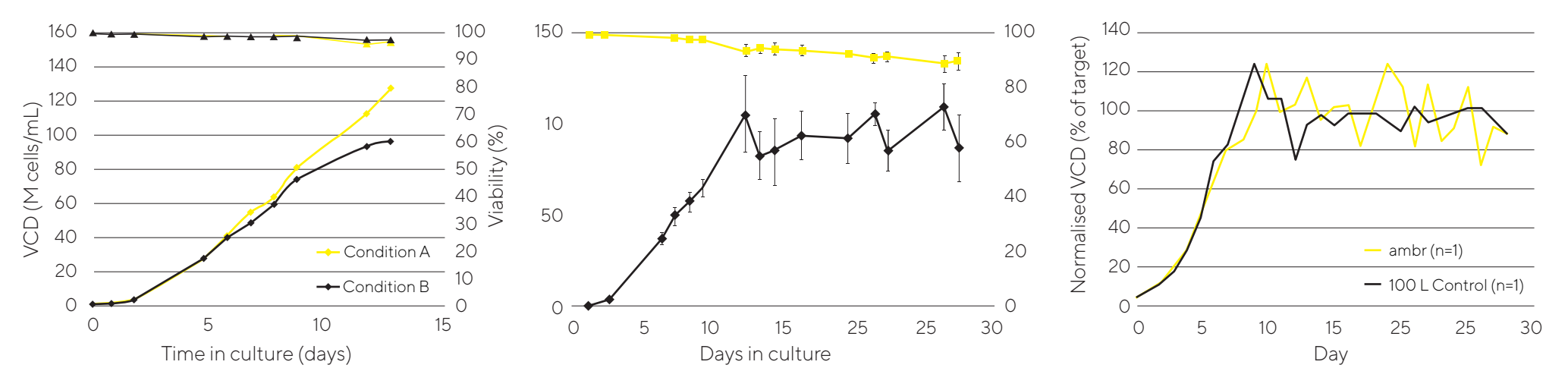
- Fully automated process 1/3 VVD exchange 3x daily by cell settling (every 8h); rapid drain; media refill
- Triggered bleed step after cell count when VCD > 20M cells/mL
- Consistent VCDs throughout culture duration
- Average daily product titer of 0.8 g/L from day 5 onwards and cumulative product titer reaching 9g/L on day 13 (data not shown)
- Cell settling events were programmed to occur automatically (3x daily), these steps did not require an operator to be present
- Full automation of all steps including cell counting can be achieved using this method with an integrated cell counter analyzer

5. Key Features of the Ambr® 250 High Throughput Perfusion

- Operates in either fed-batch or perfusion mode
- Integrated single-use bioreactor including perfusion filter, pump chambers, pressure sensors, and pinch valve cassette
- High kLa sparger (40h⁻¹; 3.5x kLa vs. open pipe)
- Automated bleed system for cell density control
- Medium exchange rate of 0.5-4 VVD
- Single-use bleed and permeate collection bags



6. Ambr® 250 High Throughput Perfusion Case Studies



N=24 perfusion bioreactors

- 100-125 Mcell/mL
- N=12 for each of 2 media conditions
- CHO-S; max. 4 VVD, 9.5 g/L glucose
- Media A ~125 Mcells/mL at day 13 (glucose limited > 13 days)
- System distinguished between media A and B

N=12 perfusion bioreactors

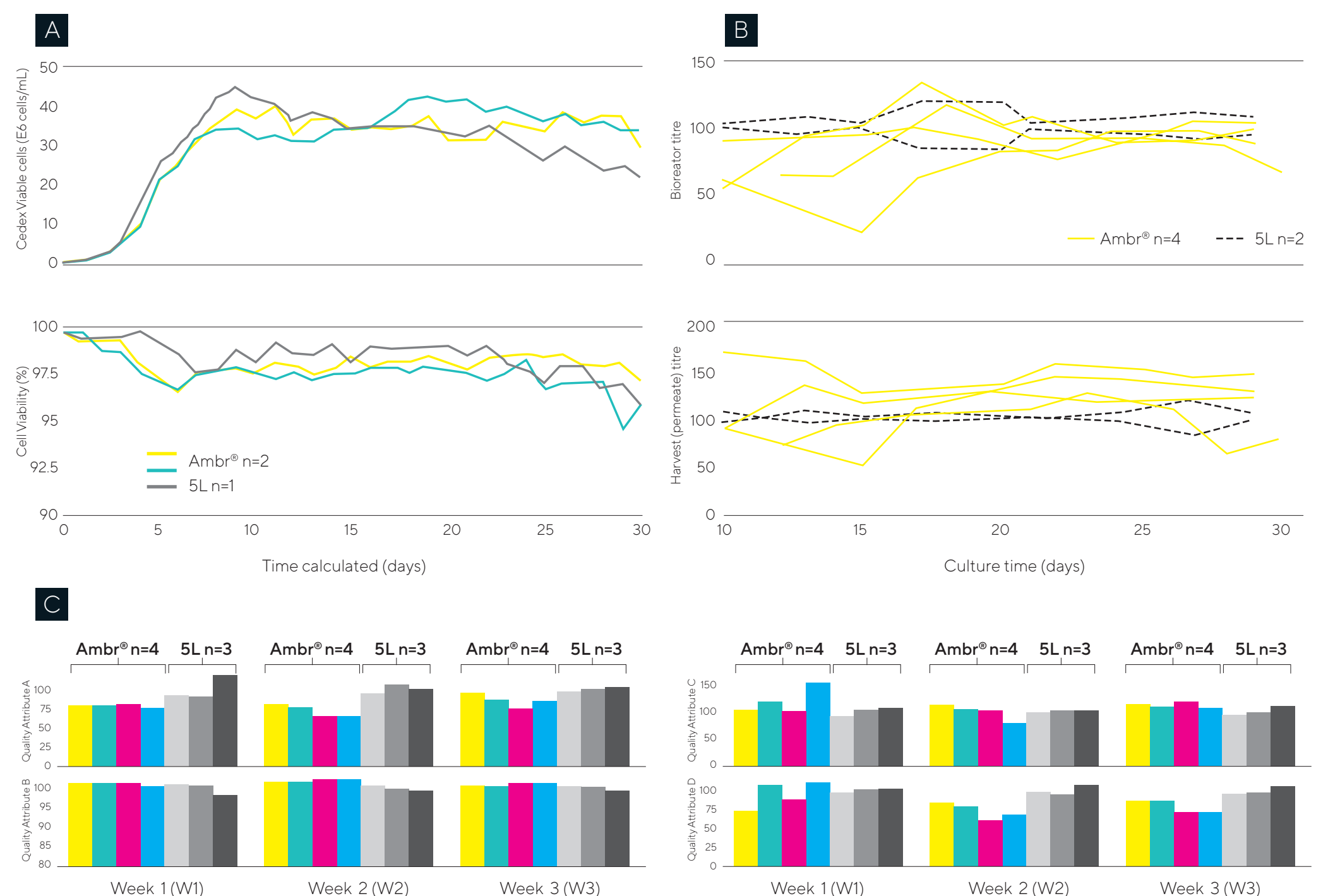
- 100 Mcell/mL >25 days
- CHO-S; automated VCD control (online Cedex)
- High cell viability throughout (90.2% on day 27)
- External media supply issue ended run on day 27
- System supported > day 27 perfusion at 100M cell/mL

Industry data:

- Ambr® 250 High Throughput Perfusion vs. 100L
- Comparable VCD profiles between the Ambr® 250 High Throughput Perfusion and a 100L bioreactor
- Comparable metabolite and titer also (not shown)
- Automated bleed system controlled VCD to day 28 based on offline VCD and growth rate

7. Ambr® 250 Industry Case Study: Good Match to 5L for VCD, Titer and Quality

- Coagulation factor, 30 day process, ATF mode, parallel 5L runs
- Automated bleeding to match reference process VCD
- Equivalent performance to 5L bioreactor for VCD (A), viability (A), normalised product titre (B), product quality (C)



8. Conclusions

- Ambr® 15 Cell Culture Generation 2 supports perfusion mimic processes, showing consistent VCD, viability, and titre, using two different methods
- Ambr® 250 High Throughput Perfusion supported:
 - N=12 reached >125 Mcells/mL in a short test
 - Sustained N=12 at 100M > 25 days (automated VCD control)
 - Sustained 40 Mcells/mL for 30 days industry process, equal to 5L VCD, titer, product quality
 - Equivalent to 100L 28 days industry process for VCD, metabolites, product titer
- Ambr® systems can facilitate and accelerate biopharm perfusion process development