The Bioprocessing Summit: Optimizing Cell Culture Technology Short Course
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Challenges and tradeoffs in developing a scalable mAb process on a constrained budget: A case study

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Overview

- The Challenge
  - Client company/project
- Constraints
  - Equipment and assay limitations
  - Staff
- Progress
  - Equipment sourcing
  - Media and feed studies
  - Implementation in 3L bioreactor
  - Process transfer to CMO and scale up
- Summary

The Challenge: The Client Company

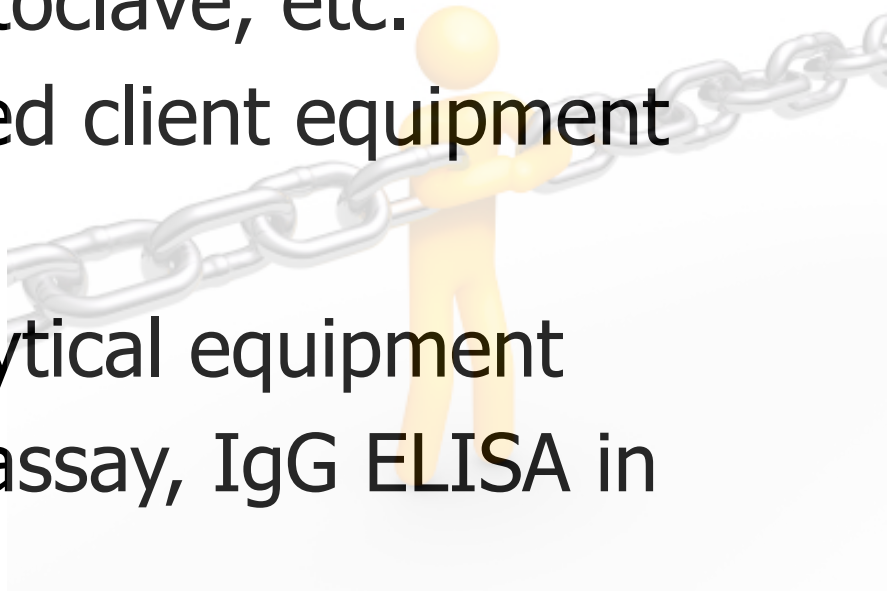
- Small, privately held California biotech company
  - No experience in mAb development
  - Grant-funded
  - Limited flexibility (e.g., minimal capital purchases)
  - Staff unfamiliar with requirements of cell culture process development
- Located in an R&D incubator in which common equipment is shared with other companies
  - No cell culture-dedicated facilities
  - Shared incubator/hood space limited our access
  - Equipment reservations for equipment was necessary weeks in advance

The Challenge: The Project

- Human IgG1 mAb
- Originally obtained from academic collaborator
- Transfected by third party into NS0 cells
  - No codon optimization
  - Believed NS0 line was cholesterol dependent
  - Believed deliverables were single-cell cloned
- Preliminary client efforts
  - Fed-batch expression levels extremely low (<0.1 g/L)
  - Frequent contamination

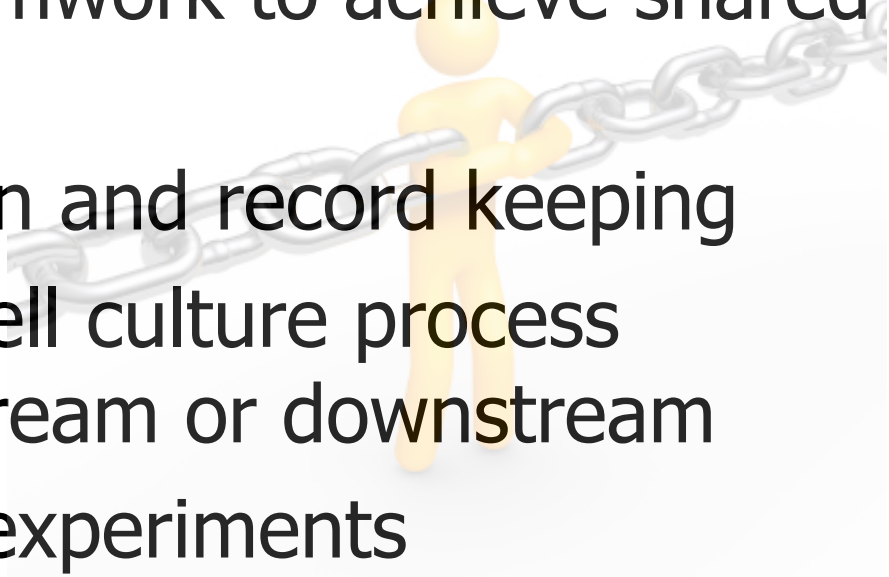
Constraints

- Equipment and Assay Limitations
  - Shared facility equipment including CO_2 incubator, hood, autoclave, etc.
  - Initially, no dedicated client equipment
  - No bioreactor
  - No cell culture analytical equipment
  - No consistent titer assay, IgG ELISA in development
  - No automated cell counter; hemocytometer counts only



Constraints (cont'd.)

- Inexperienced Staff
  - No project-dedicated staff
  - Little managed teamwork to achieve shared goal
  - Poor documentation and record keeping
  - No knowledge of cell culture process development, upstream or downstream
  - Lack of controlled experiments
  - Lack of streamlined communication or management in place



The Goal

- Develop a scalable, reproducible fed-batch process using commercial raw materials (animal component-free)
- Achieve higher titers and good growth profiles
- Use existing staff with minimal additional investment
- Transfer to CMO to scale up for tox and clinical Ph I production
- IND in 2.5 years

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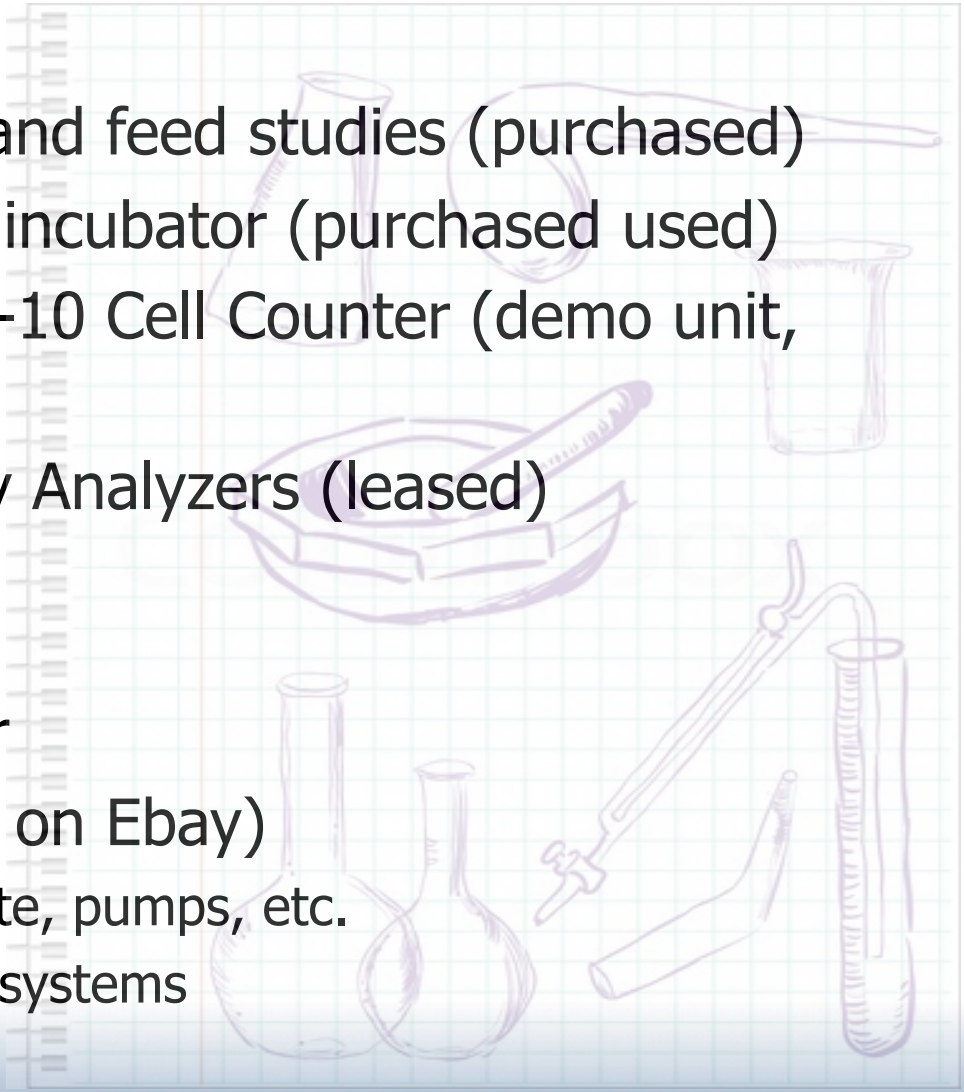
The Approach

- Upgraded equipment
  - Spin tubes for media and feed studies (purchased)
  - Dedicated cell culture incubator (purchased used)
  - BioRad Automated TC-10 Cell Counter (demo unit, purchased)
  - YSI 2700 Biochemistry Analyzers (leased)
    - Glucose/lactate
    - Glutamine/glutamate
  - 3L Applikon Bioreactor (purchased piecemeal on Ebay)
    - Reconditioned headplate, pumps, etc.
    - New probes, sampling systems (purchased)



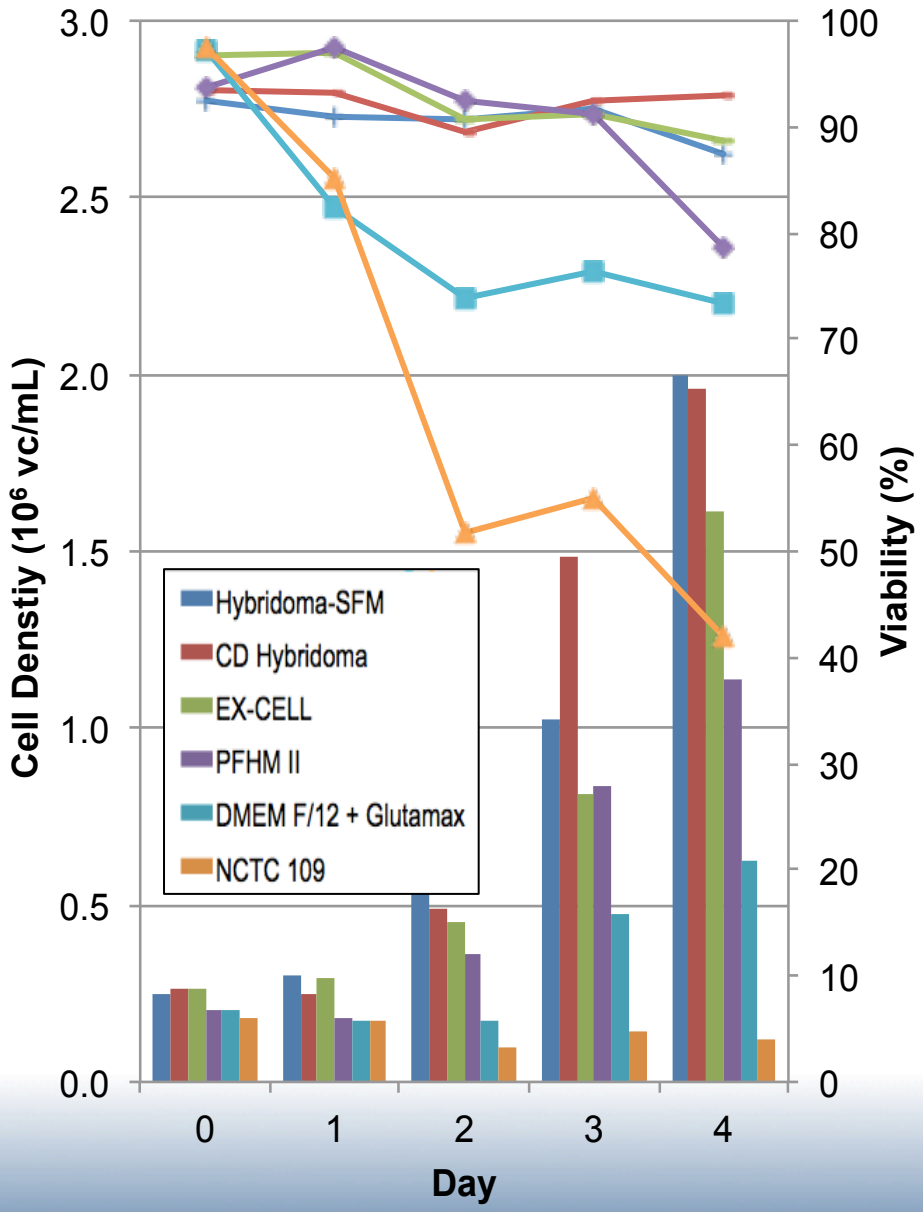
Approach (cont'd.)

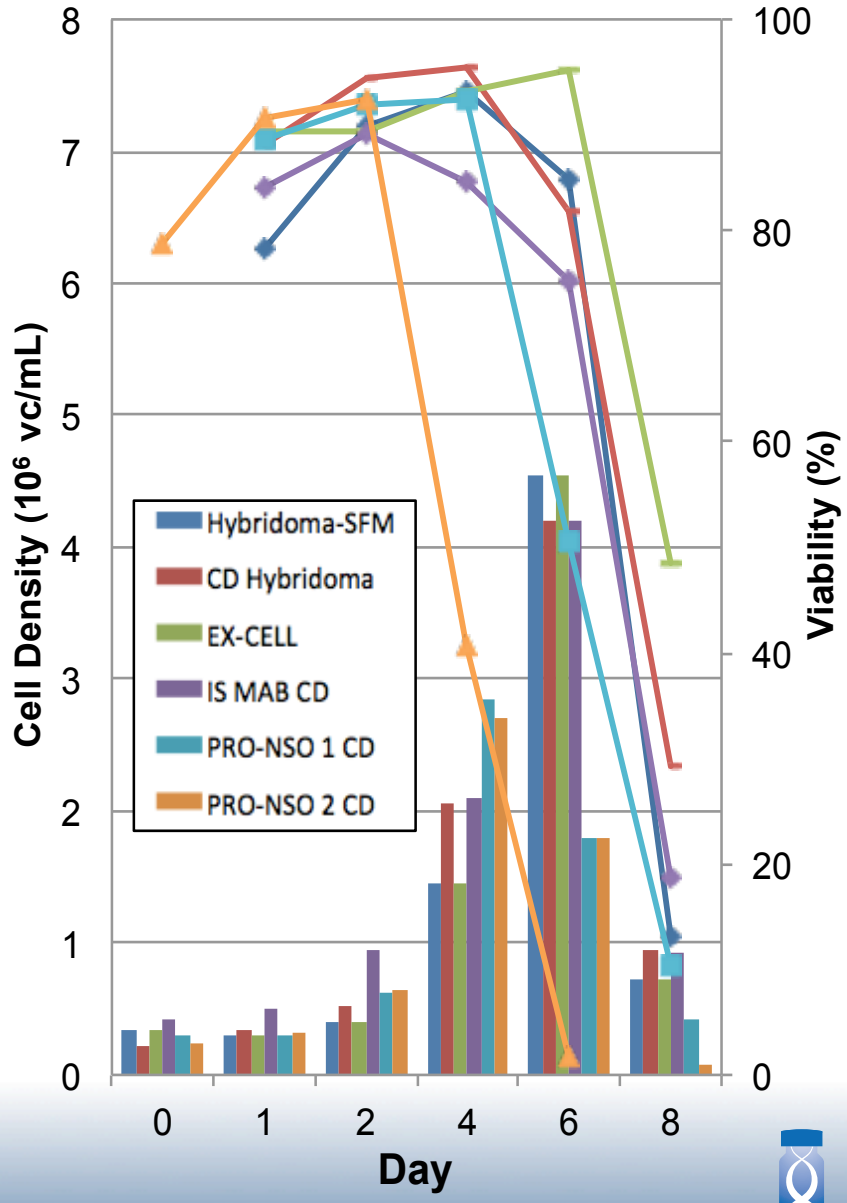
- Organized lab, cell culture workspace
- Organized workflow
  - Established a "project team"
  - Project management
  - Restructured personnel, hired dedicated personnel
  - Weekly meetings and staff training
- Design and supervision of experiments
  - Basal media and feed screens in 50 mL spin tubes
  - Fed-batch 3 L bioreactor
    - VCD, IgG titer, glucose/lactose, glutamine/glutamate, pH, CO_2, DO
    - Demonstrated reproducibility
    - Demonstrated scalability

Basal Media Studies

- Screened 9 commercially available media
  - Serum-free, protein-free, most were chemically defined
  - Limited time frame/funding for first few months (client wanted to complete basal media selection in 2-3 weeks)
    - No titer assay available
    - No ability to measure metabolites or feed components
    - All facility-shared equipment needed to be reserved, resulting in early termination of experiments
  - Two studies performed in spin-tubes in duplicate
    - Measured cell density and viability via hemocytometer

Media Screens





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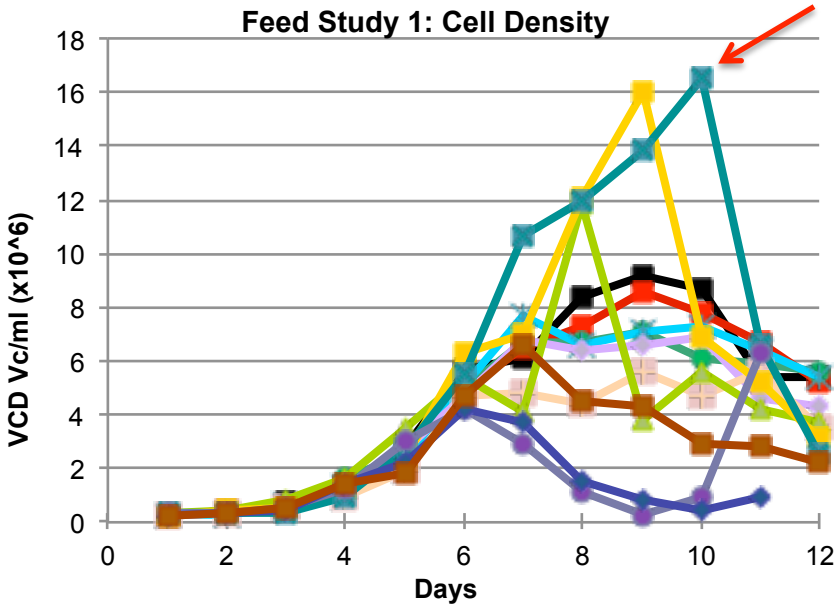
Media Screen Results

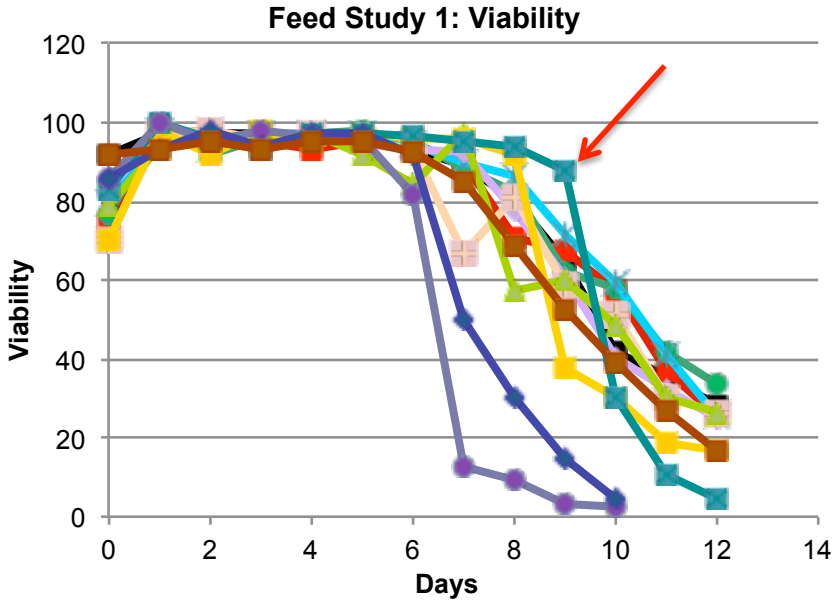
- Hybridoma–SFM and EX-CELL® NS0 media had the best overall growth profiles
  - Highest resident peak cell density
  - High viabilities
- EX-CELL NS0 had the slowest drop in viability and was selected as our basal media for feed screening
  - Commercially available
  - Discounted pricing for small companies
  - Animal-component free
  - Chemically defined

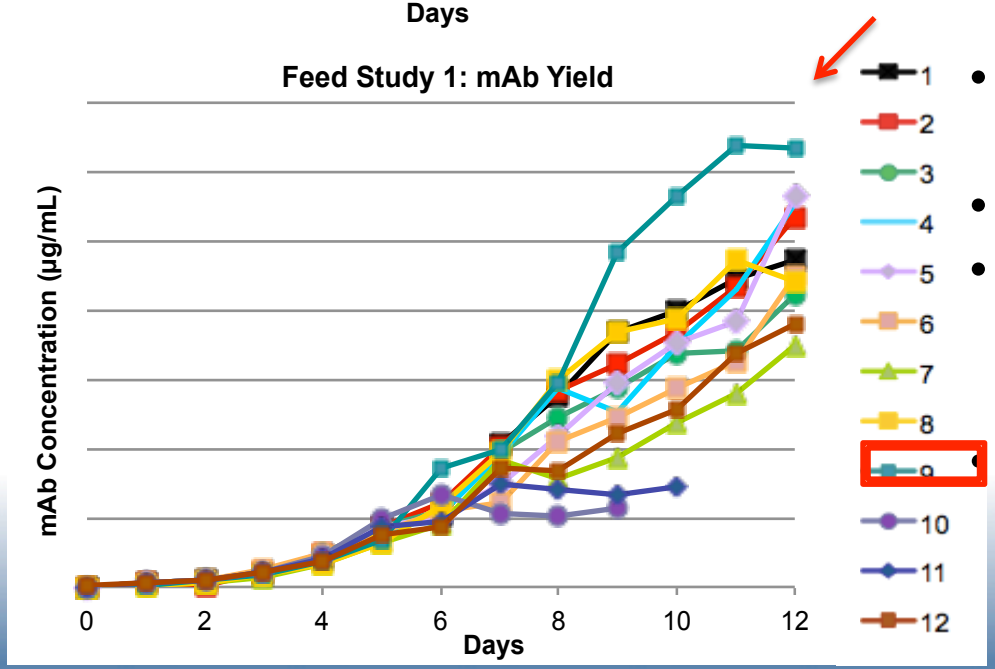
Feed Study 1

- 12 varied conditions in spin-tubes - 12 days in EX-CELL NS0 basal media
- 3 different feed conditions + 2 soy hydrolysates
- 3 different daily feeding schedules
- 250X Cholesterol Lipid Concentrate (Gibco) was added to media
- Glucose, lactose, glutamine, and glutamate were measured by YSI (Rochelle Scientific)
- Feed was added once daily
- Samples were taken daily for cell count and titer

Feed Study 1 Results







- Tube 8 and 9 had the best growth profiles
- Tube 9 had highest titer
- Tube 9 saw a steep drop in viability on Day 9 from 90% to 30 % on Day 10
- **Tubes 8 and 9 were best performers**

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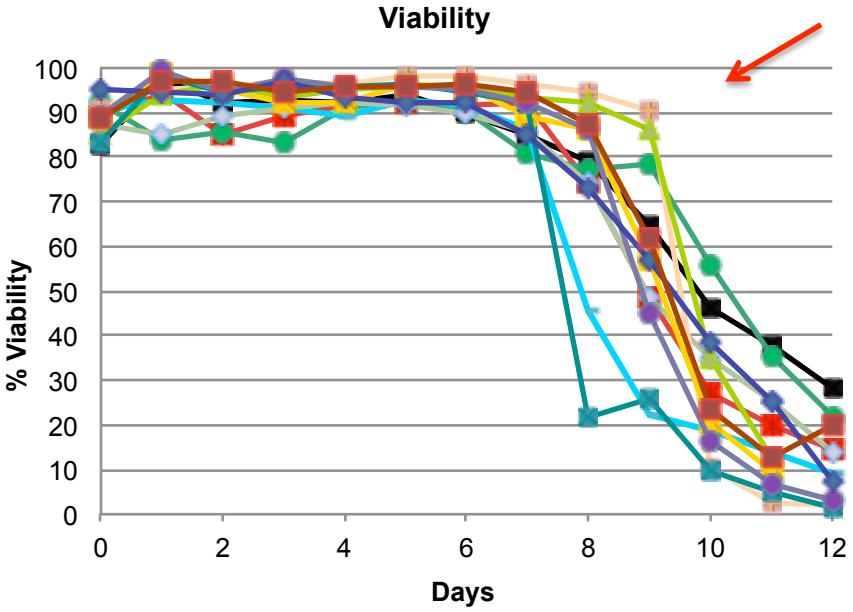
Progress Update!

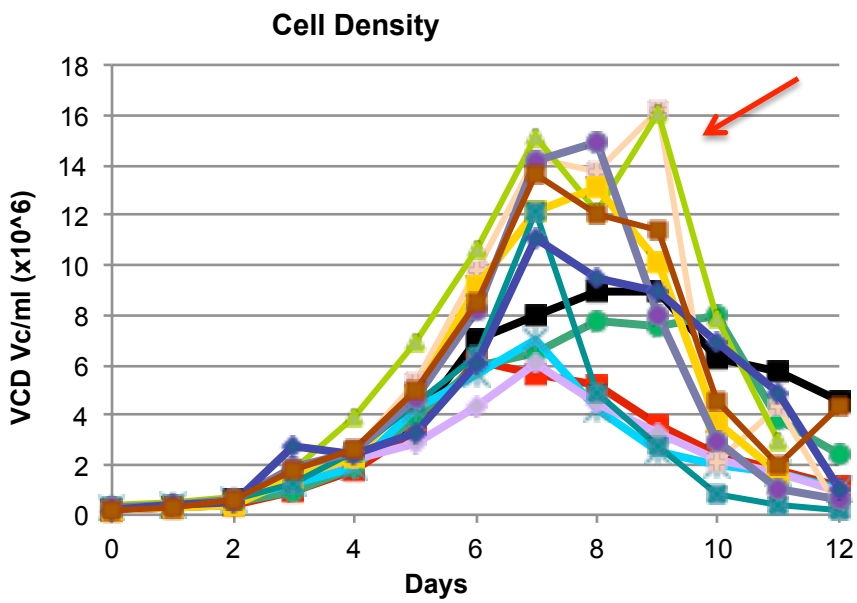
- Purchased dedicated cell culture incubator (used)
  - Ability to maintain experiments for extended periods and w/o scheduling conflicts with other companies
  - Ability to test temperature shifts
- Purchased dedicated cell culture hood for client lab
- Purchased automated cell counter (demo)
- Leased metabolite analyzer to measure glucose, lactose, glutamine, and glutamate

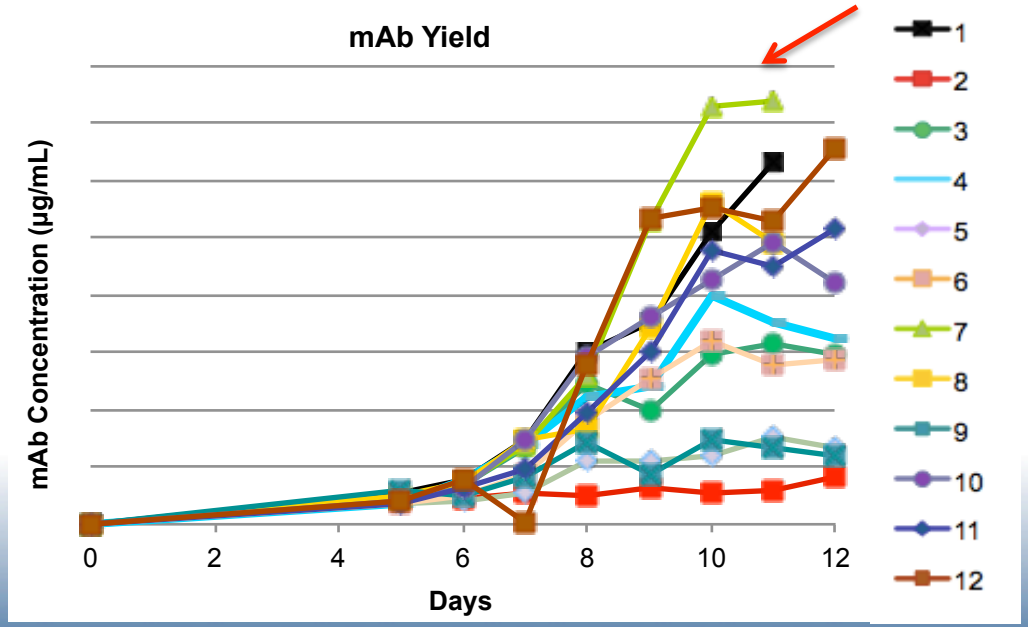
Feed Study 2

- 12 feed conditions in spin-tubes carried out for 12 days in EX-CELL basal media
- 5 different feed conditions
- 3 different hydrolysates
- 3 feeding schedules
- Temperature shifts
- 1 low glucose and low glutamine condition, all others held at 5 g/L and 1 g/L, respectively
- Lipid vs. no lipid

Feed Study 2 Results







- Temperature shift adversely affected cell growth/production
- Tube 7 - best grower/highest titer (Tube 9 from FS1)
  - Hyclone Cell Boost 5
  - No additional supplements
  - No lipids
  - Split day feeding schedule

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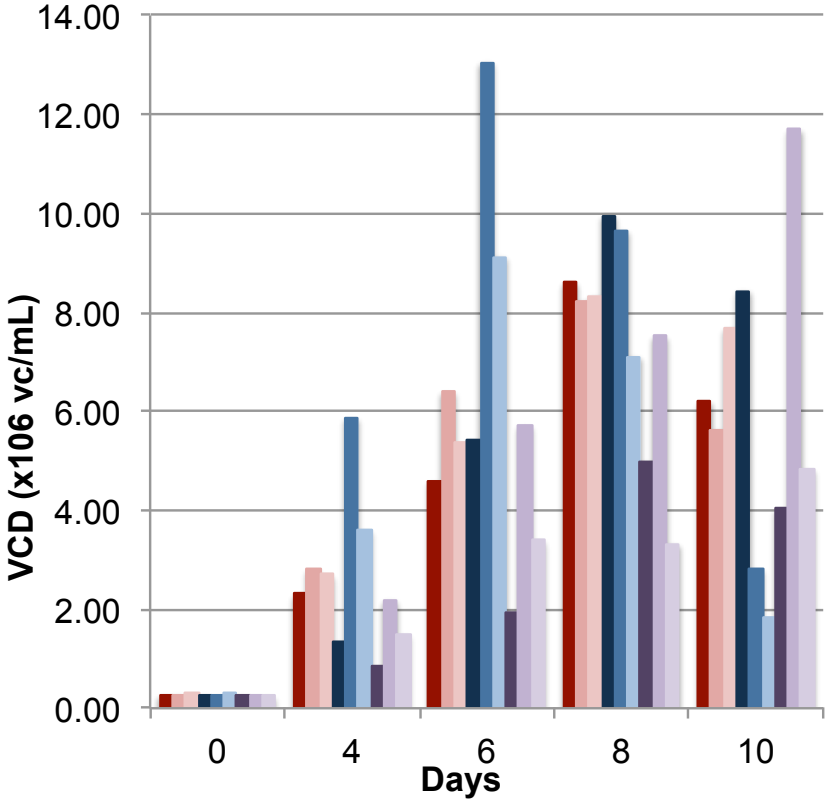
Progress Update!

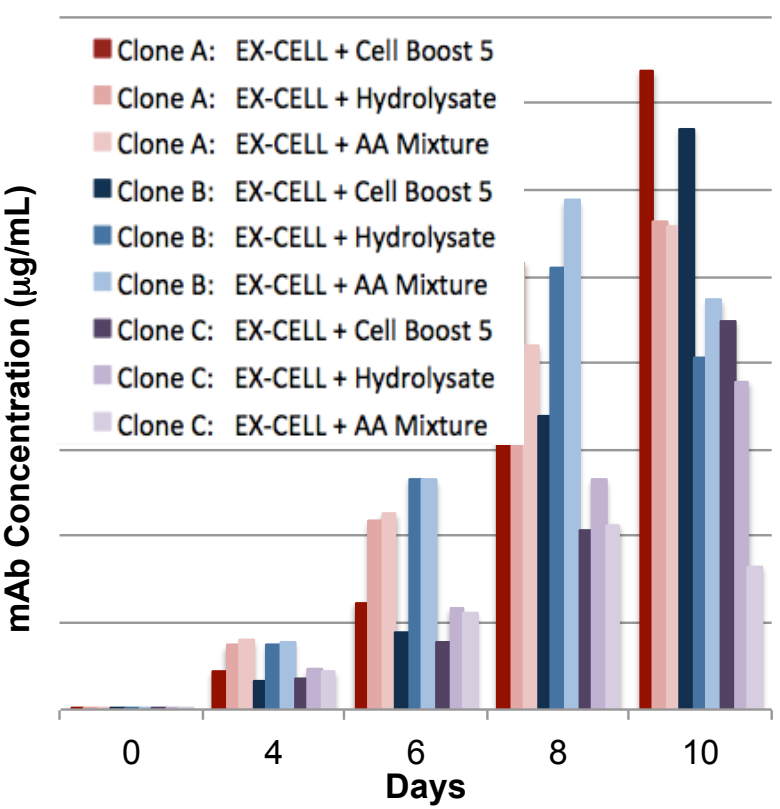
- Culture samples (3 time pts.) analyzed for metabolite composition/consumption (outsourced)
  - Tested selected amino acids
- Discovered non-clonal line was modified to be cholesterol independent
  - Eliminated cholesterol additions
  - To counter steep decline in viability, feed doubled on Day 5PM+
- Discovered current cell line had *not* been single cell cloned
  - Quickly/cost-effectively produced clonal RCB (outsourced)
  - Streamlined clone selection
  - Delay of 2-3 months

Single Cell Cloning Screens

- Compared top 3 clones
  - Quick comparative fed-batch study
  - 50mL spin-tubes
- Folded in 2 test feeds
  - Hydrolysate
  - Amino Acid Mixture
- Assessed parameters
  - VCD/VIA
  - Titer
  - Glucose/lactate
  - Glutamine/glutamate

Clonal Screen Feed Study





Day 10 Clonal Feed Study Summary

- **Clone A** produced 1.2x more mAb than Clone B and 1.6x more than Clone C
- Cells fed with CB5 produced 1.5x more mAb compared to Hydrolysate Feed & AA Mixture Feed
- Clone C was the top "grower" but the poorest "producer"
- Alternative feed studies performed with Clone C
  - No increase in productivity

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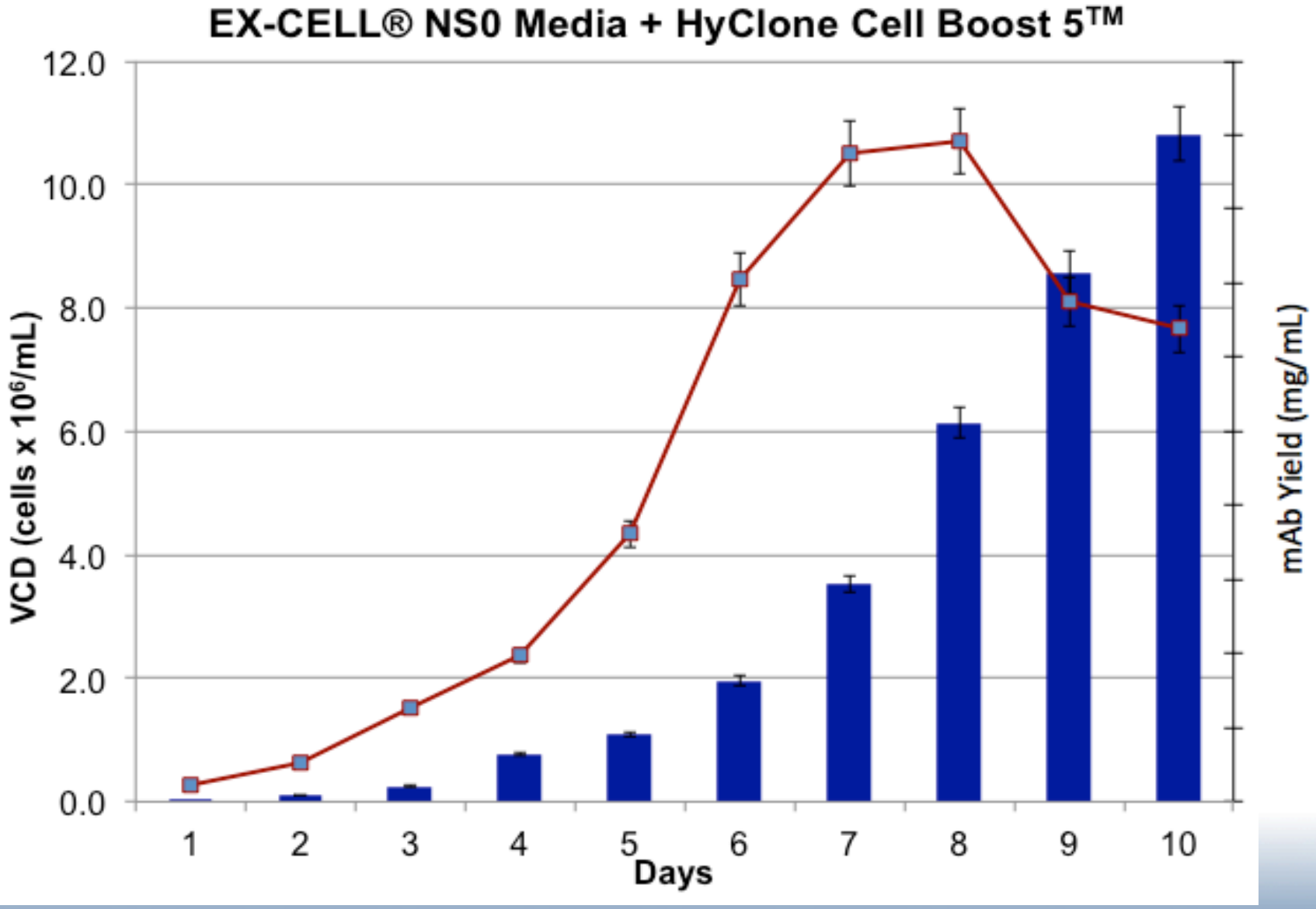
Progress Update!

- Purchased 3L Applikon Bioreactor on Ebay
  - Refurbished controllers (purchased separately)
  - Reconditioned headplate, pumps, and electronics
  - Purchased new probes, sampling systems

- Developed a robust scalable fed-batch process
  - Consistent VCD/viability,
  - IgG titer, glucose/lactose
  - Glutamine/glutamate
  - pH, CO2
  - Run length
  - Demonstrated reproducibility
  - Achieved consistent cell growth and higher titers

3L Fed-Batch Runs (Ave. of 3 runs)



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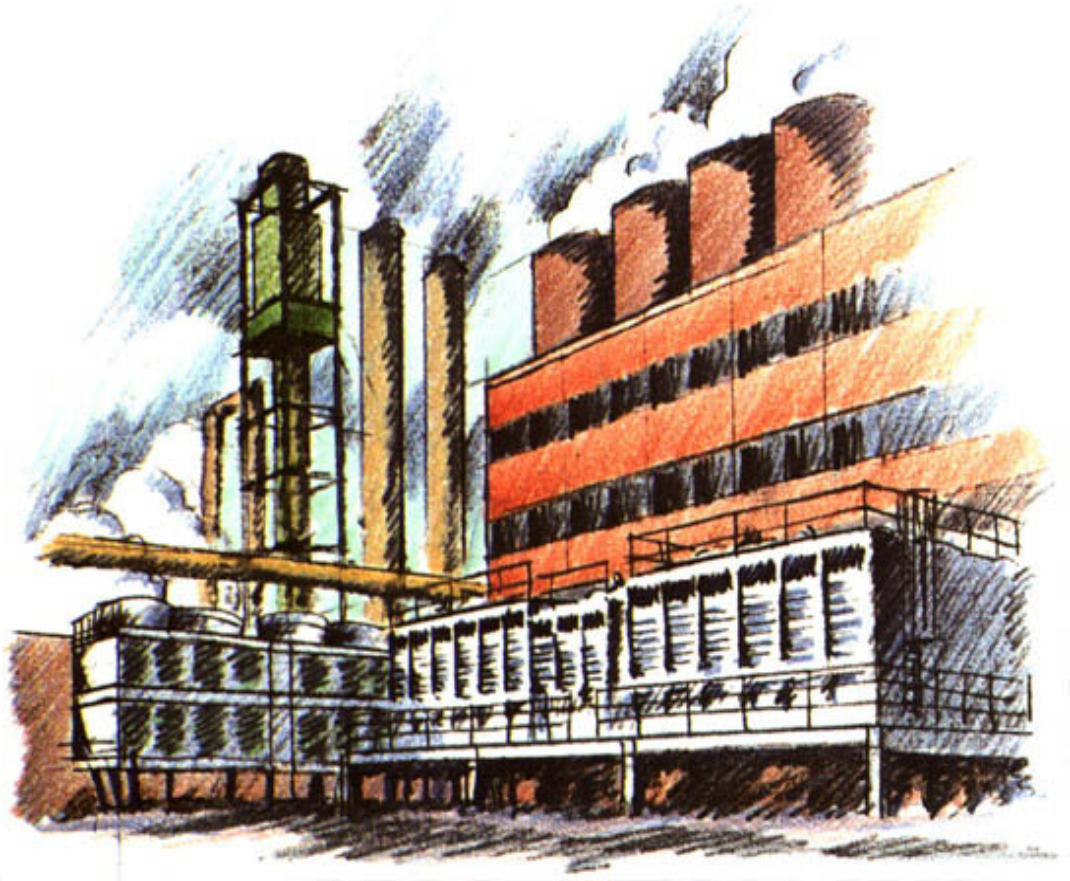
Progress Update!

- Technology transfer to CMO
  - 3L fed-batch process transferred to CMO
  - Easily scalable, predictable growth and productivity profiles

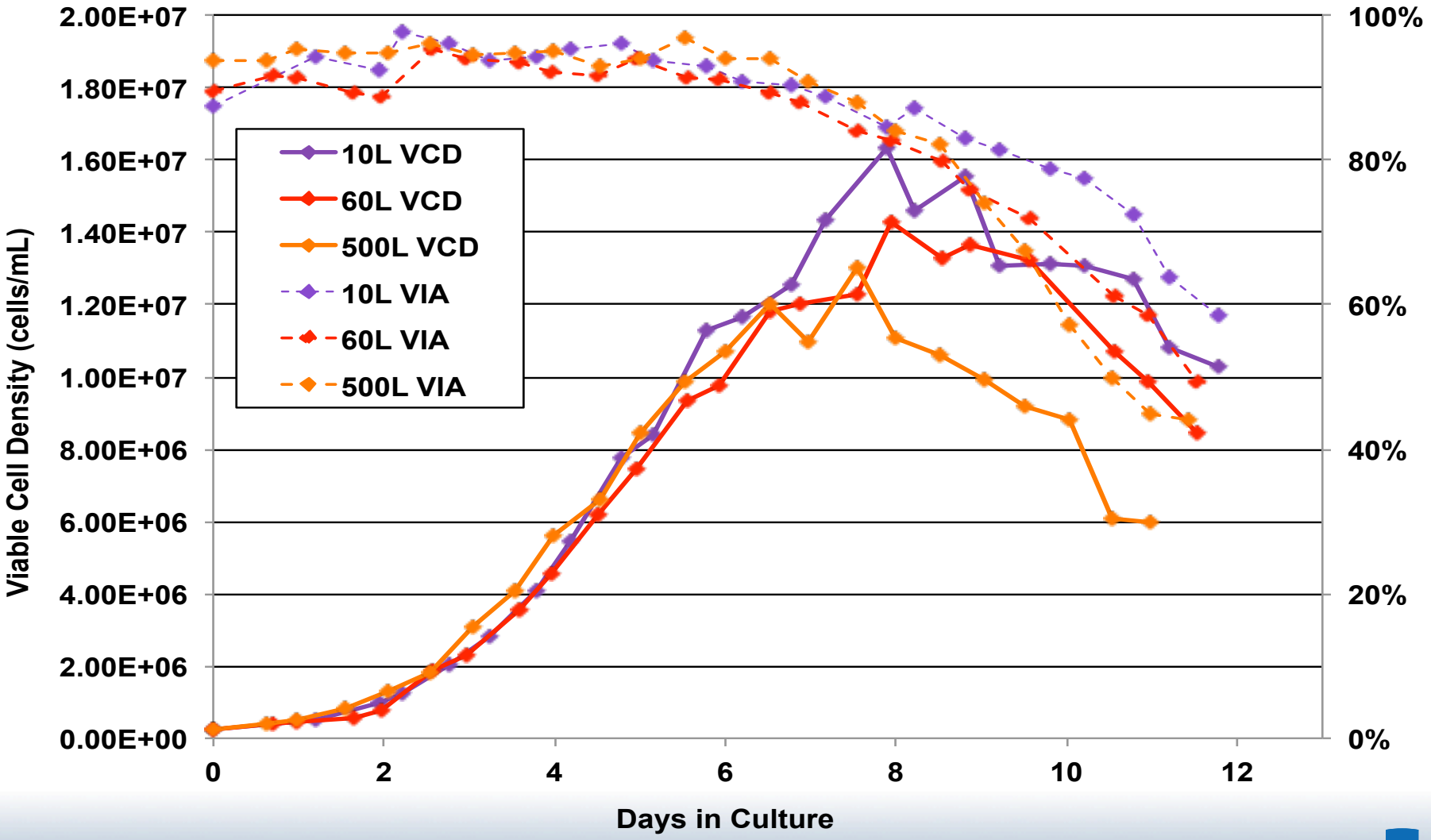


CMO Transfer, Scale-up, and Production Run

- Transferred process
  - RCB
  - Process description
- CMO duplicated our run at lab scale
- Scaled production

  - Tox
  - Clinical Phase I

CMO Scale-up and Production Runs



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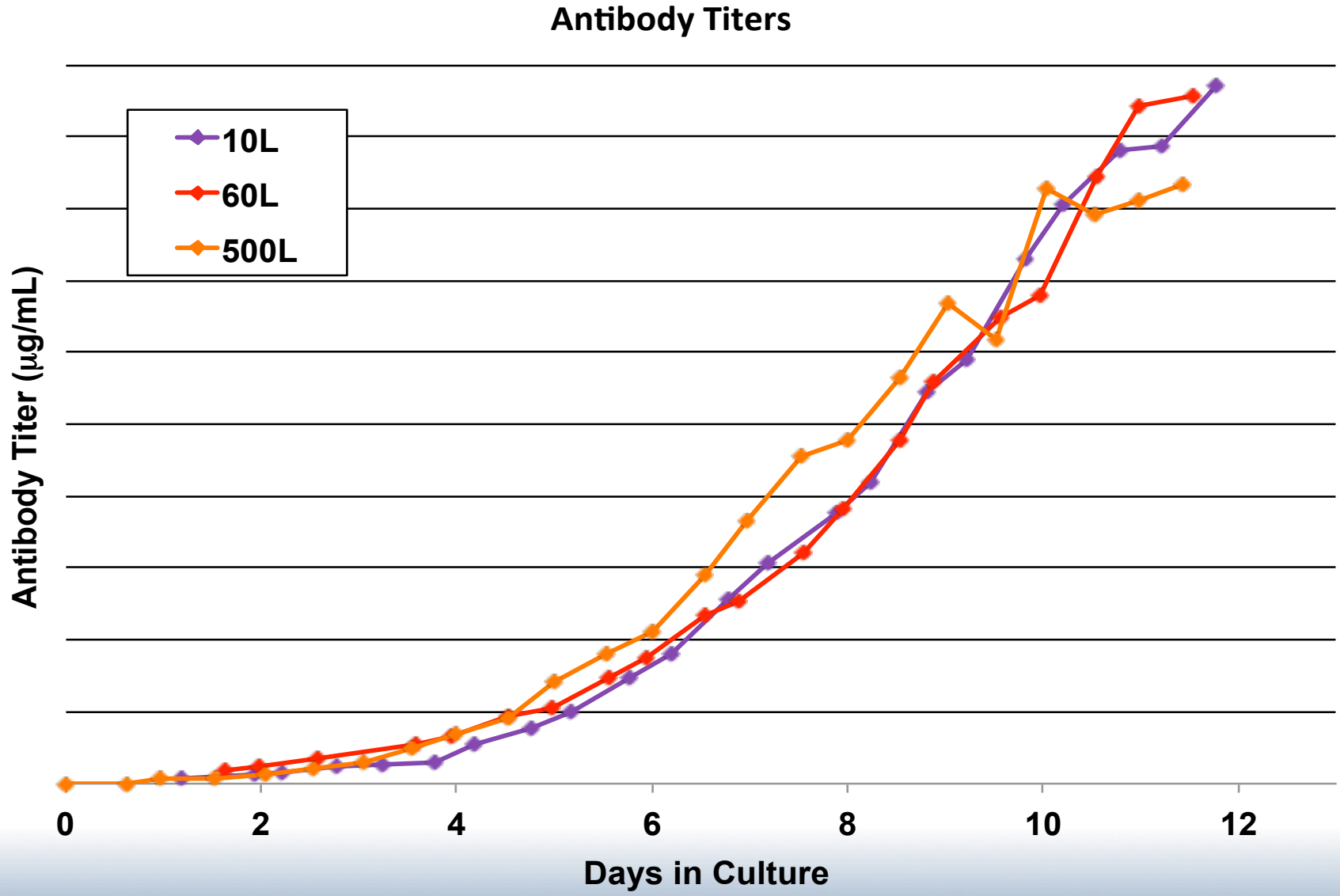
CMO Scale-up and Production Runs (cont'd.)



GOAL ACHIEVED!!

- Despite obstacles and on a limited grant, developed robust upstream and downstream process
- Trained a small client company in mAb development
- Successfully transferred process to CMO
  - CMO generated MCB
  - CMO scaled process from 3L to 500L, produced tox and clinical material
- Achieved success on a constrained budget

Where You Really Should Not Cut Corners (as we learned the hard way!)

- Importance of proper due diligence in evaluating cell line to verify client's information—don't take anything for granted
- Ensure proper money is spent on upstream development
  - Efficient gene vectors
  - Cell line development/screening
- Importance of experienced staff
  - Hire project-dedicated staff if possible with strong communication and data analysis skills
  - Handy staff can help with refurbishing equipment
- Importance of focusing the team on a common goal
- Importance of a competent and flexible CMO
  - With constrained budget for development/tools, flexible CMO means extra work without additional charges

Where You Can Cut Corners (and save money)

- Tradeoff: time vs. money
  - On a constrained budget, allow more time to complete program
- Money can be saved!
  - Efficient media/feed screen programs
    - Quickly screen commercial media/feeds
    - No analytical equipment doesn't mean you can't have effectively designed experiments
  - Get multiple bids from 3rd party service providers
    - Deals are out there, vendors will discount
  - Used or leased equipment as an alternative to new capital equipment
    - Bidding sites, refurbished equip. sites, liquidating companies
    - Ask vendors what used/leased equipment is available