

Cell Culture and Upstream
Processing, Berlin, September 2004

Lonza

Mammalian Cell Culture: Current Status, Future Prospects

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Cell Culture: an Industrial Process

- **Much of the early process development driven by vaccines (human and veterinary) – Polio vaccine 1954**
- **FMD vaccine in deep tank suspension culture**
- **Since late 1980s (tPA 1987), large scale use driven particularly by recombinant therapeutic proteins**

Biopharmaceutical Proteins

- **Strong demand – sales of biopharmaceuticals represent 10-30% of all new pharmaceuticals in USA in recent years(1)**
- **Large number (hundreds) of proteins in development**
- **Sales of ca.\$ 30bn in 2003 (2)expected to grow to \$59bn by 2010 (Datamonitor)**
- **Mammalian cell products account for ca. 60% of market (1)**
- **Mabs are the fastest growing category (from 1% of biopharmaceutical market in 1995 to 14% by 2001)(1)**

1. Polastro & Tulcinski, Scrip magazine Sept. 2002

2. Walsh, Nature Biotechnology 2003, 21, 865 – 870

Key Issues in Manufacturing (1)

- Speed in development (especially for early phase clinical material)
 - Rapid cell line creation – avoid amplification and multiple rounds of single cell cloning
 - Use of cell lines (CHO) preadapted to suspension culture
 - Use of generic technology
 - Predictive scale up systems

Key Issues in Manufacturing (2)

- **High dose requirements, particularly for antibodies, leads to large volume demand (10's to 100's kg/year)**
- **Estimated 2004 protein demand > 2000kg (mostly Mabs and fusion proteins)(UBS)**
- **This is a driver for –**
 - **Increased capacity**
 - **Increased reactor size – up to 20000 litres (economy of scale)**
 - **Improved technology to increase cost efficiency (taking note of downstream implications)**

Mammalian Cell Capacity

	Estimated capacity (thousands of litres)	
	Total	Contract Manufacturers
2002	840	190 (23%)
2006	1970	510 (26%)

- Long lead times (3-5 years) and high costs (\$200m - \$500m)
(\$3+ Million / m³)

20,000L Bioreactor & Add Tanks Portsmouth, New Hampshire



Improved Upstream Process Efficiency

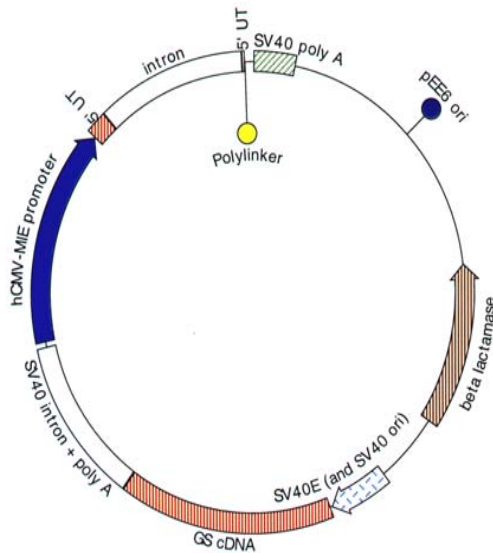
- **Parameters Targeted**
 - **Reactor throughput**
 - **Specific production rate**
 - **Maximum viable cell concentration**
 - **Prolongation of culture at high cell viability**
 - **Growth rate**

- **Routes to optimisation**
 - **Design of gene vector**
 - **Properties of host cell line**
 - **Cell line screening**
 - **Optimisation of fermentation process**

Design of Gene Vector to Maximize Transcription

- **Strong promoter to drive expression of product gene(s)**
- **Amplification of gene copy number**
- **Vectors with elements that create genomic environment for high transcriptional activity (positional independence)**
- **Targeting of expression vector to transcriptionally active site in genome by homologous recombination**

Glutamine synthetase (GS) gene expression system



- Expression vector encoding product gene plus GS gene, allowing glutamine synthesis
- GS is inhibited by methionine sulphoximine (MSX)
- Selection in glutamine-free medium for GS minus cell types (e.g. NS0)
- Selection in the presence of MSX for GS positive cell types (e.g. CHO)
- Only cells with GS gene (and hence product gene) survive
- Increase selection stringency - use weak promoter on GS gene - selects for rare integration into transcriptionally efficient sites in genome
- Expression of linked product gene, driven by strong promoter, enhanced by favourable integration site

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Properties of The Host Cell Line

- **Significant opportunities to create cell lines with improved growth and productivity characteristics**

- **Metabolic engineering**

- **Variant Selection**
 - **Cholesterol independent NS0 variant**
 - **Suspension variants of CHO**

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Cell Line Screening

- **Highly productive transfectants are rare even with a good selection system**

- **Various approaches to improve screening process to find these rare events**
 - **Increase transfection efficiency (larger pool to select from)**
 - **Improve stringency of selectable marker to eliminate low producers**
 - **High throughput methods (FACS + cell surface product capture)**
 - **Early screening needs to predict cell specific production capability AND growth characteristics at production scale**

Cell line selection

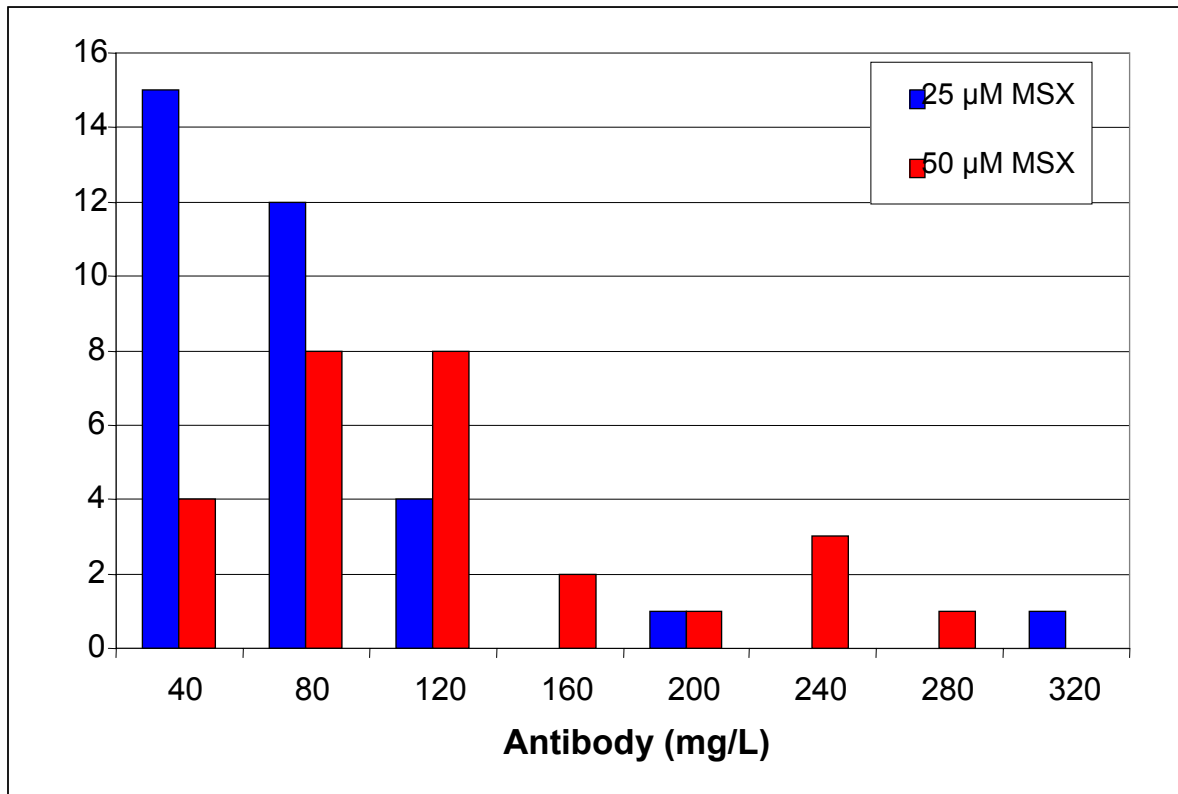
Transfection and selection conditions for GS-CHO cell lines expressing cB72.3 antibody

Electroporation condition	Numbers of stable transfectants
1	68
2	124
3	197

2.5×10^6 cells electroporated

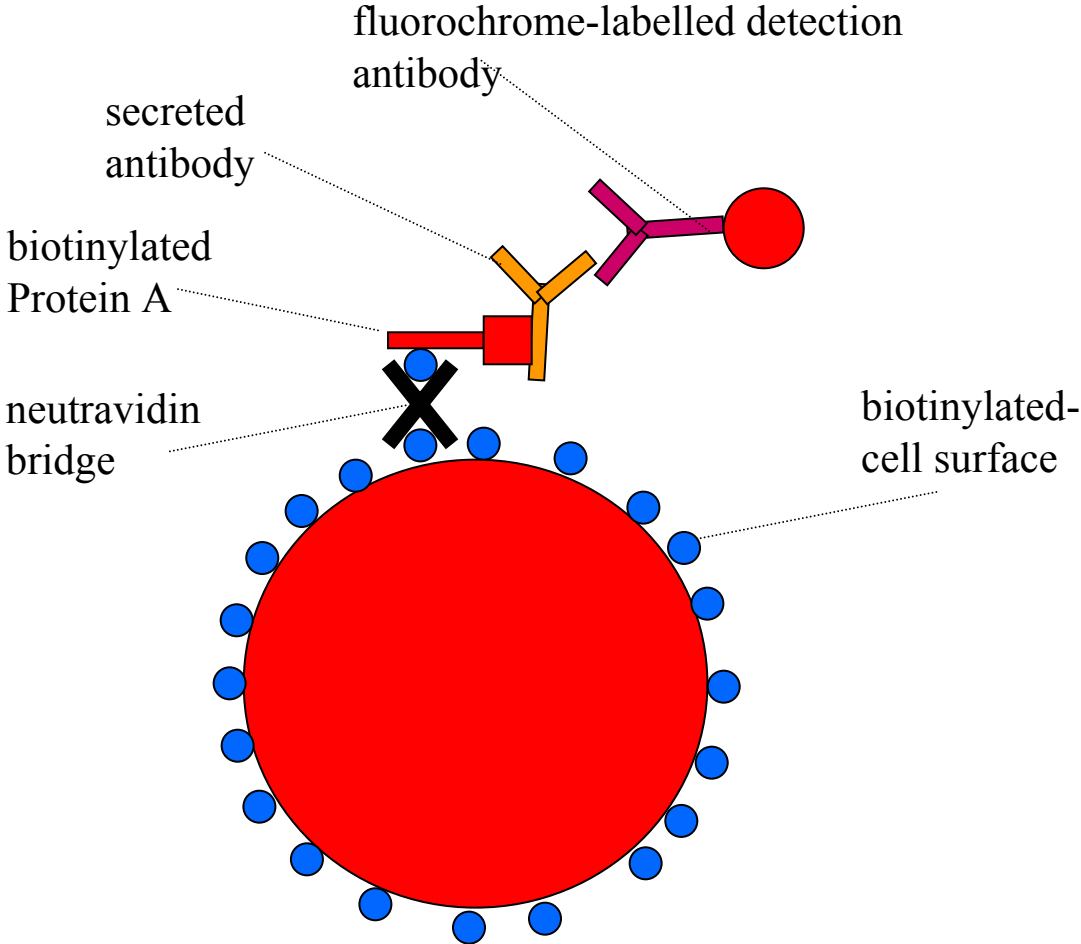
Cell Line Selection

Influence of Selection Conditions for GS-CHO Cell Lines making cB72.3 Antibody

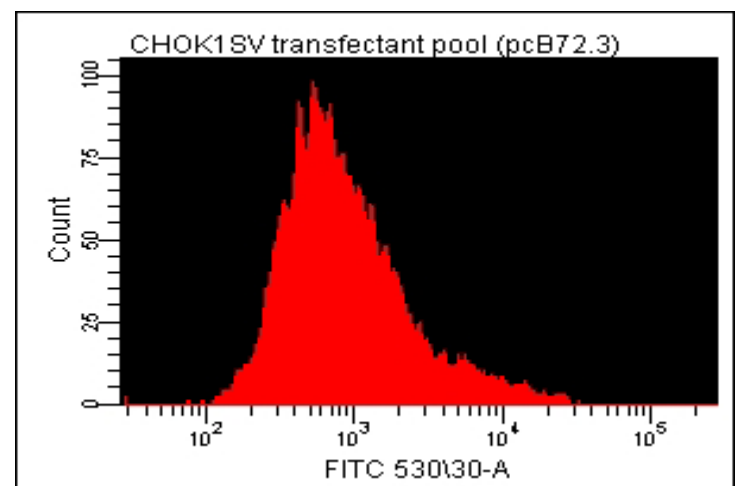
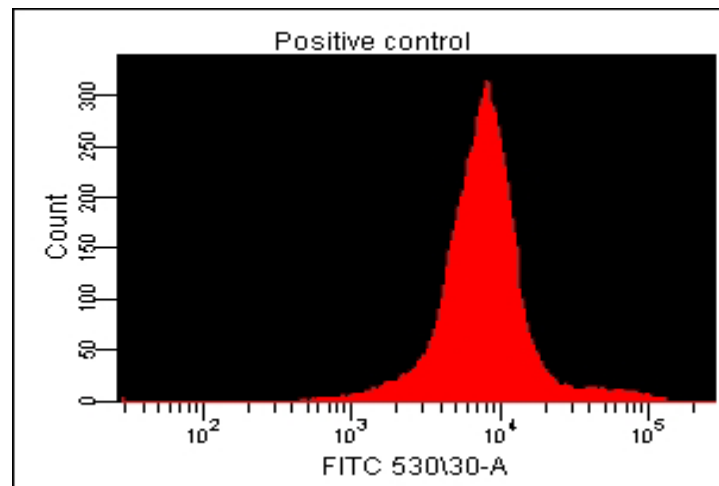
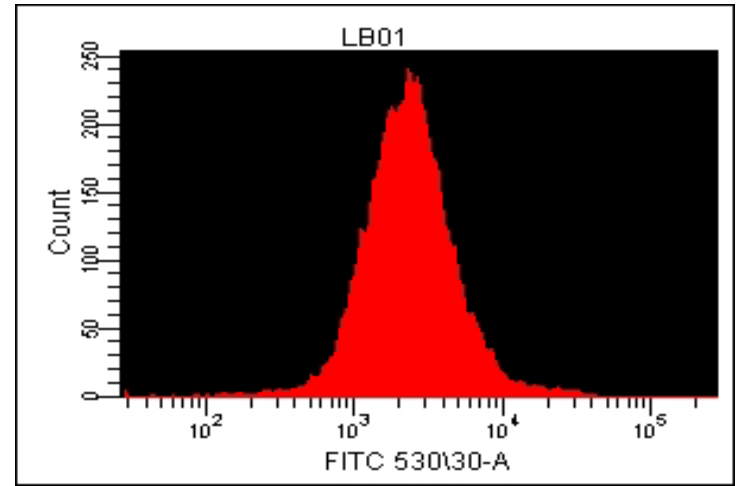
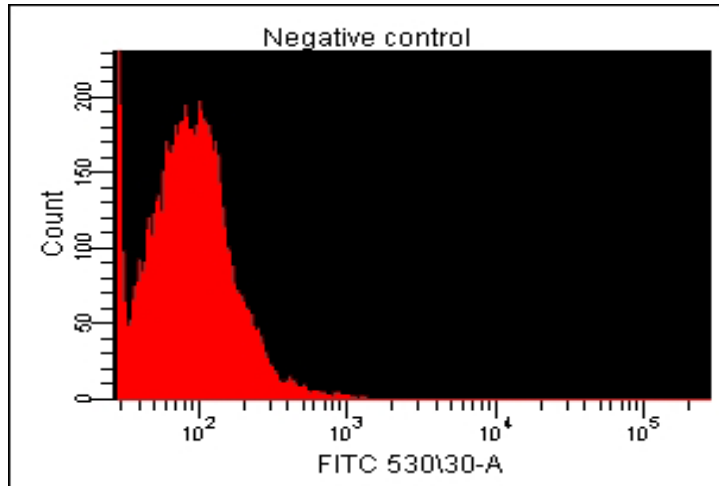


Cell lines have not been amplified.

Affinity-matrix surface capture



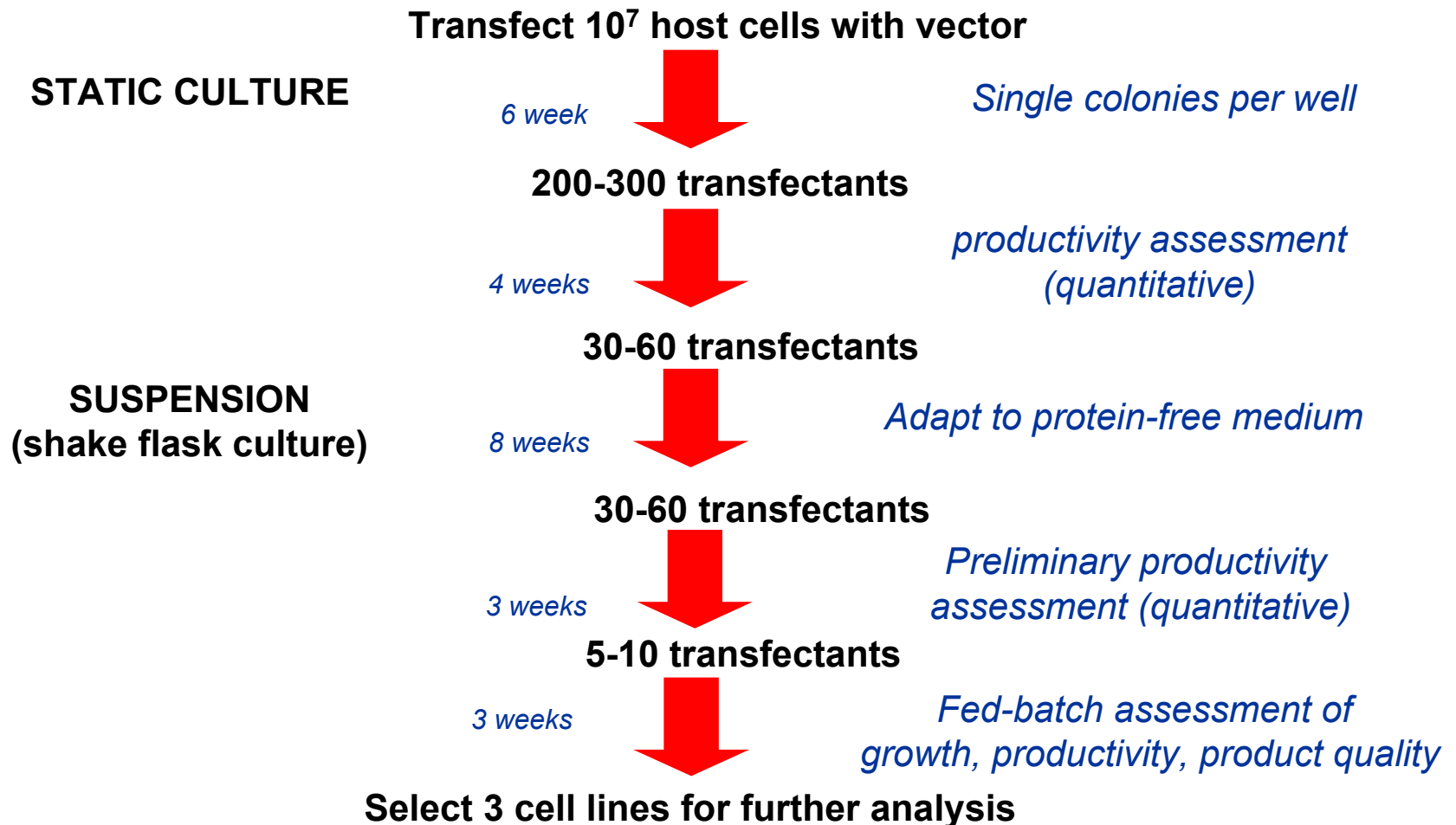
Flow cytometric analysis of AMSC-labelled GS-CHO cells



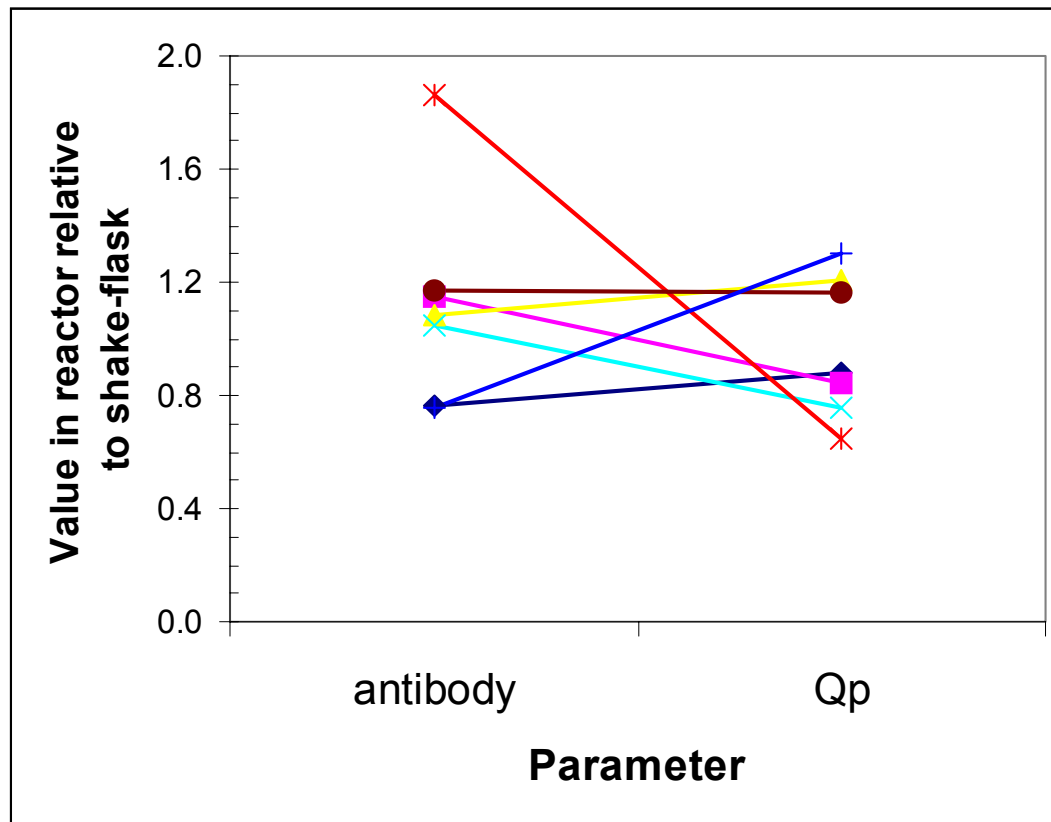
Clonal Variation – GS-NS0 (non-optimised culture)

Specific prod.rate (pg/cell/day)	Max.viable cell concn. (10^5 /ml)	Cumulative cell time (10^9 cell·h/L)	Product concn. (mg/l)
17	17	379	260
19	8	209	165
19	19	212	170
24	22	518	535
31	19	573	750
60	14	320	790

Selecting high producing cell lines



Prediction of bioreactor behaviour from shake-flask model



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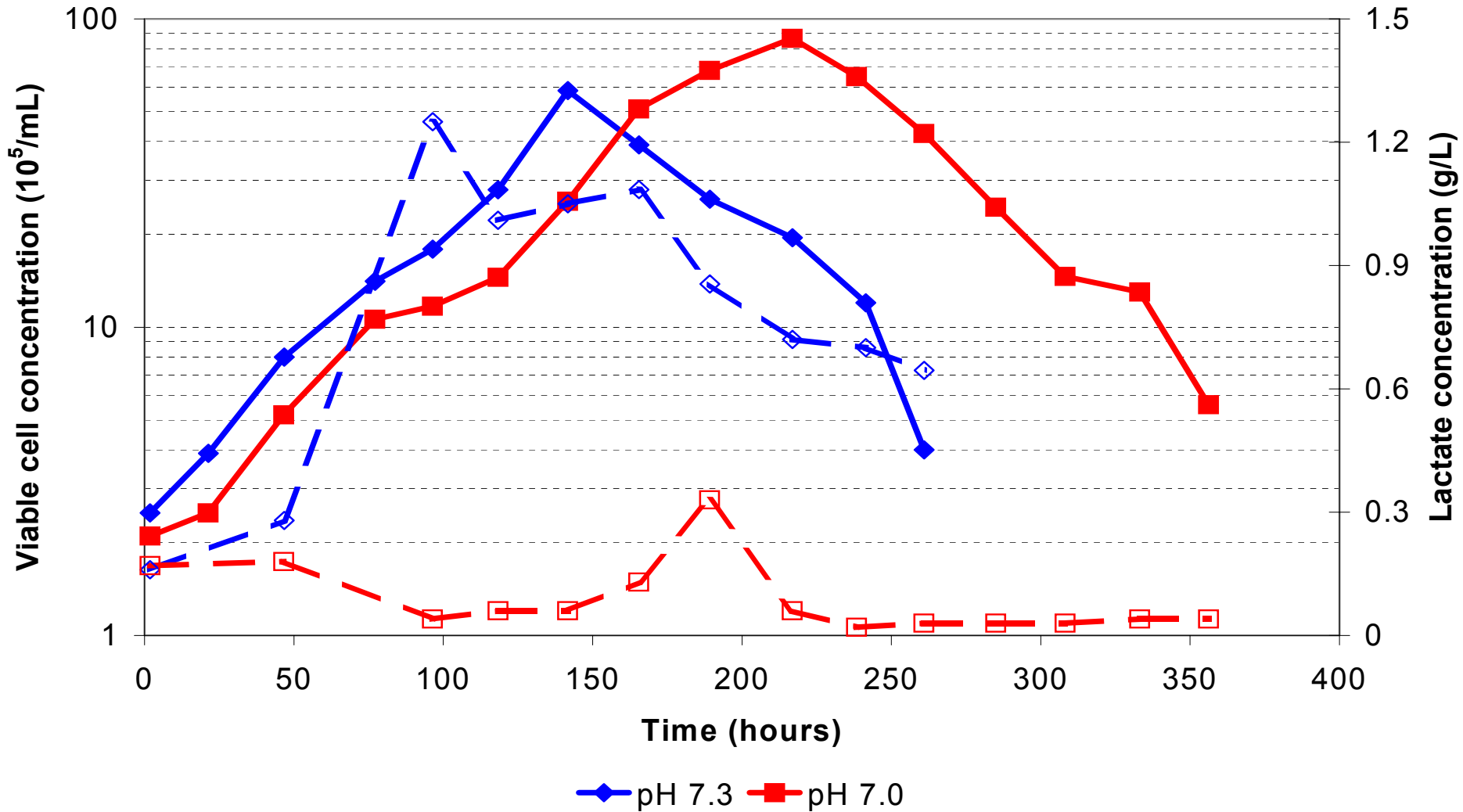
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Improving the Fermentation Process

- Significant potential to improve processes
 - Physicochemical environment
 - Medium design and feeding strategies (most processes are fed-batch, some perfusion culture)
 - Use of chemically defined media

The Physicochemical Environment

- Control pH, temperature, dissolved oxygen concentration
- Small changes in pH can have profound effect on cell growth and productivity
 - Responses are cell line specific and can impact:
 - Maximum cell concentration
 - Integral viable cell hours
 - Specific production rate
 - Metabolism: lactate accumulation



Chemically Defined Media

- Increasing use of chemically defined media free of animal derived raw materials
 - Reduced risk of introducing adventitious agents
 - Improved process consistency and robustness (avoids potential variability of raw materials such as serum proteins and hydrolysates)
 - Chemical definition assists process optimisation
 - Benefits purification (reduced contaminant load)

Downstream Benefits of Chemically Defined Medium for GS-NS0 Cell Line

Purity of MAb at harvest

Optimised Protein containing culture

<30%

Optimised protein free culture

62%-76%

Medium Design and Feeding Strategies

- Optimise basal medium
- Optimise feeds
- Maintain nutrient sufficiency
- Minimise waste metabolite formation
 - Use of GS system avoids accumulation of ammonium ions from metabolism of glutamine

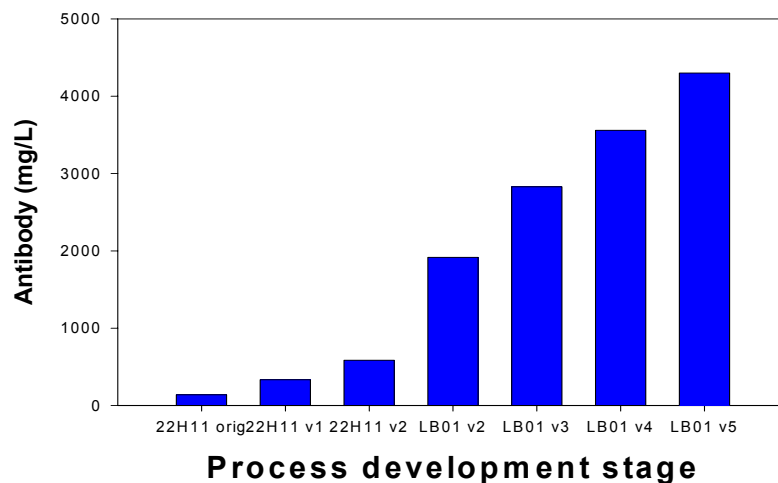
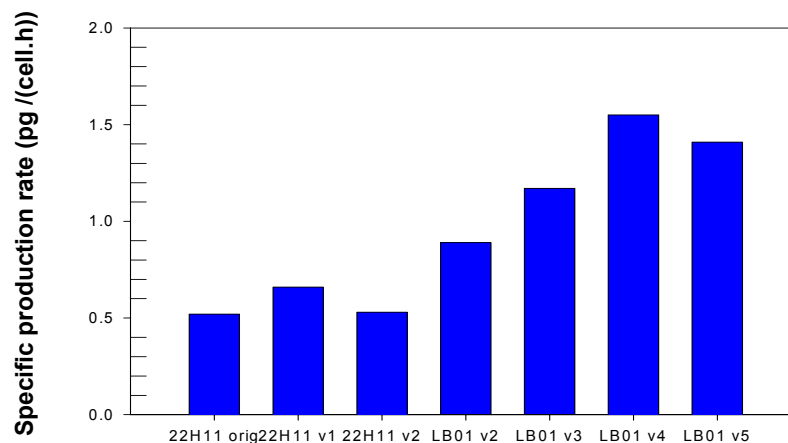
Optimisation of a GS-CHO Process

- Culture conditions for a GS-CHO making cB72.3 antibody were optimised
- Suspension variant of CHO-K1 isolated:
 - grows in chemically defined medium without need for adaptation (can take several months)
- Efficiency and stringency of transfection conditions increased to improve selection of highly productive clones
- Growth conditions further optimised

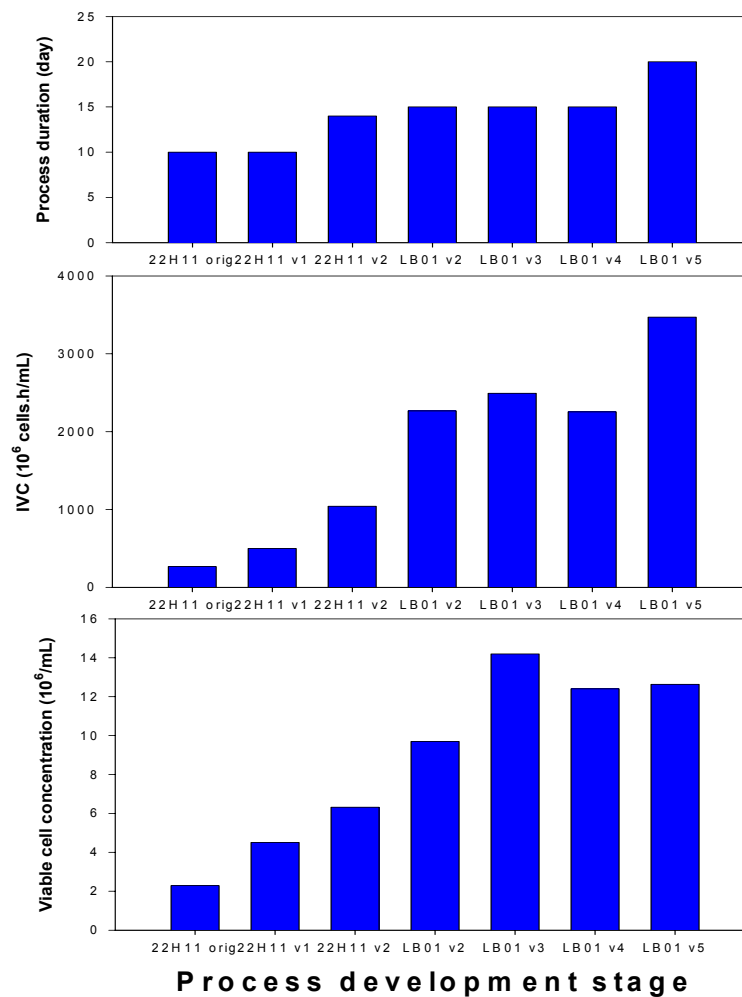
Process optimisation for a GS-CHO cell line

Process	Antibody (mg/L)	Fold increase
Original cell line	139	
Iteration 1	334	2
Iteration 2	585	4
New cell line	1917	14
Iteration 3	2829	20
Iteration 4	3560	26
Iteration 5	4301	31

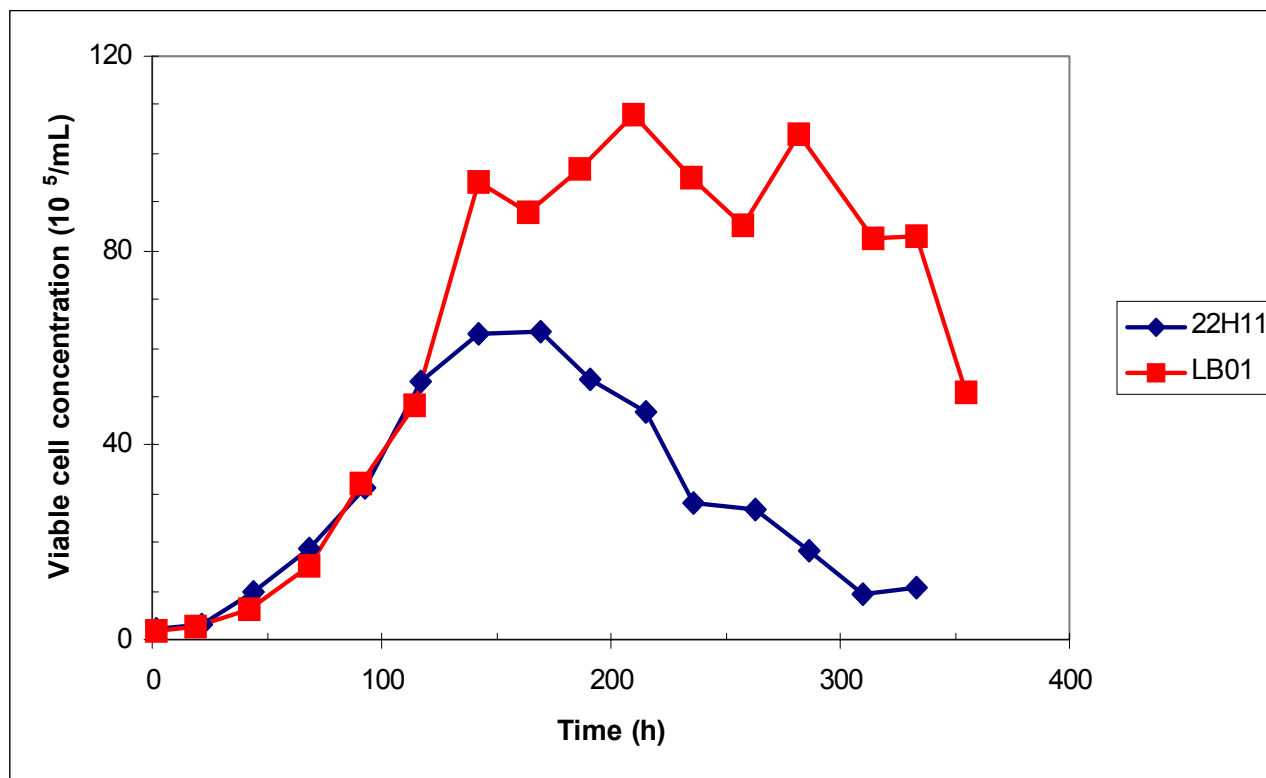
GS-CHO: antibody production



GS-CHO growth



Growth comparison: “old” vs. “new” GS-CHO cell lines



Process optimisation for a model GS-NS0

Process	cB72.3 antibody (mg/L)	Cumulative cell time (10^9 cell·h/L)	Q_p pg/(cell·h)
Serum-free	476	640	0.74
Chemically defined	293	772	0.36
Iteration 1	589	1026	0.60
Iteration 2	807	1239	0.64
Iteration 3	1035	1427	0.71
Iteration 4	1422	1405	0.97

Future Opportunities (1)

- Continued process improvements (10g/l achievable ?)
- Continued improvements in selection procedures for finding highly productive clones
- Improved cell lines e.g. engineered for improved growth, product synthesis, energy metabolism , glycosylation characteristics
- Use of genomic and proteomic approaches to increase our knowledge of cell physiology and to inform design of cells and processes

Future Opportunities (2)

- Improved product potency – impact on quantities required
- Alternative manufacturing routes – microbial production of e.g. antibody fragments or whole antibodies, transgenic production for very large quantities ?
- Mammalian cell culture likely to be key technology for foreseeable future

Summary

- Increasing number of mammalian cell products in development is a driver for improved and faster process development technologies
- High volume demands are driving both capacity and improvements in process efficiency
- Great potential to use advances in basic science to inform process improvements