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PROCESS DEVELOPMENT FOR MONOCLONAL ANTIBODIES

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- A decorative horizontal bar with a colorful, abstract pattern of green, blue, and purple is located below the title and above the main content area.
- High value market
 - Biopharma sales ca. \$22bn in 2001: mammalian cell products represent ca. 60%
 - MAb market has grown from 1% of biopharma in 1995 to 14% in 2001
 - *Polastro & Tulcinsky, SCRIP magazine Sep 2002.*
 - Fifteen licensed rMabs and large number in development
 - High dose requirement leads to large volume demand (10's to 100's kg/year)
 - Challenge: produce large quantities with cost and time efficiency

- Capacity availability
 - Demand for large number of proteins (hundreds) in development
 - Material supply, up to 100s kg/year
- Cheaper
 - Improved yields of USP and DSP platform processes
 - Process optimisation for Ph III / in-market supply

- Faster entry into clinic and market
 - Reduced USP and DSP development times through use of generic processes to supply PhI / II trials
 - Robust processes minimising risk of failure
 - Streamline regulatory aspects of processes


- Regulatory compliance


Mammalian cell culture: Expected capacity Increases


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	Capacity 2002 (ca. Litres)	Expansions (ca. Litres)	Capacity 2006 (ca. Litres)
In-House	650,000	810,000	1,460,000
Contract Manufacturing Organisations (CMO)	190,000	320,000	510,000
Total Industry	840,000	1,130,000	1,970,000
% CMO	23%	28%	26%

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- A decorative horizontal banner is located below the title bar. It features a collage of images related to biotechnology, including laboratory glassware, microscopes, and cell cultures, with a color palette of blues, greens, and purples.
- A high yielding antibody manufacturing process is the result of:
 - Selecting highly productive cell lines
 - Efficient gene expression and stringent selection
 - Cell culture process supporting high viable cell concentration
 - Optimised process
 - Minimising losses in primary recovery and purification
 - Optimised process

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- Strong promoter to drive expression of product gene(s)
 - Viral, elongation factor
 - Increased copy number of product gene(s) that give proportional increase in gene expression
 - Co-amplification of product and selectable marker genes (e.g. DHFR) in presence of cytotoxic drugs (e.g. methotrexate)
 - Lower cell line stability compared to un-amplified cell lines
 - Vectors with elements (e.g. SAR/MAR) that create genomic environment for high transcriptional activity
 - Targeting of expression vector to genomic hot spot by homologous recombination

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- A decorative banner is located below the title, featuring a horizontal strip of various scientific and medical images, including microscopes, test tubes, and laboratory equipment, in shades of blue, green, and purple.
- Cell line engineering
 - Glutamine independence using GS reduces ammonium accumulation
 - High ammonium levels reduce sialylation
 - Over-expression of anti-apoptosis genes
 - Maintain high viable cell concentrations for extended periods
 - Cell cycle genes
 - Variant Selection
 - Cholesterol independent NS0 variant
 - Suspension variant of CHO

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- A decorative horizontal bar is located below the title. It features a series of colorful, abstract shapes in shades of blue, green, and yellow, resembling a molecular or cellular structure.
- By definition, the transfectants with potentially the highest specific productivities are rare
 - To find these rare events, it is necessary to have:
 - A transfection method that generates large numbers of stable transfectants
 - Maximise the range of productivities
 - Stringent selection to eliminate lower producers
 - High throughput methods e.g. FACS + cell surface product capture

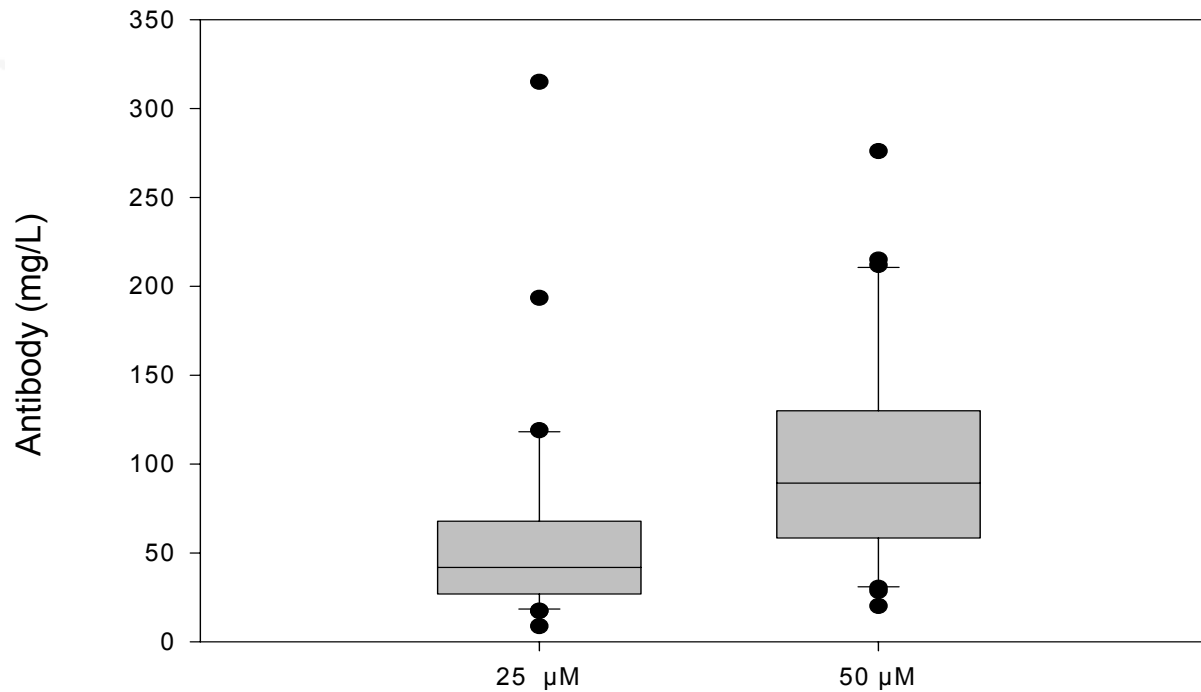
Cell line selection

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

Electroporation condition	Selection condition MSX (μM)	Numbers of stable transfectants
1	25	68
	50	32
2	25	124
	50	57
3	25	197
	50	70

Cell line selection

Influence of selection conditions for GS-CHO cell lines with cB72.3 antibody



Cell lines have not been amplified.

Selection conditions - MSX concentration

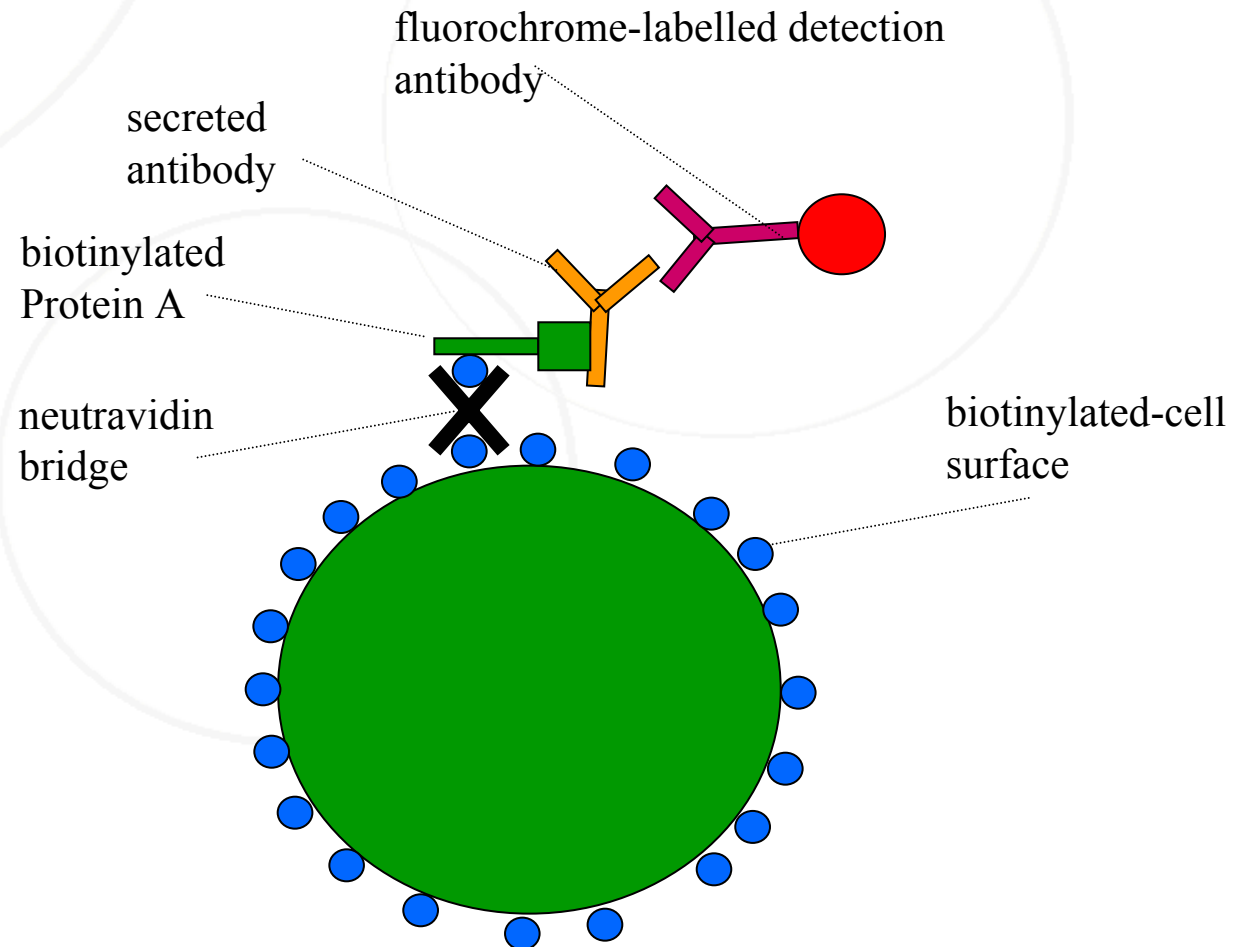


Antibody production by non-amplified GS-CHO cell lines in a shake-flask model of a fed-batch production process

Cell line ID	cB72.3 antibody concentration at harvest (mg/L)
C6	422
C7	514
C11	641
C12	632
C01	417
C18	378
C23	957
LB01	1150

Affinity-matrix surface capture

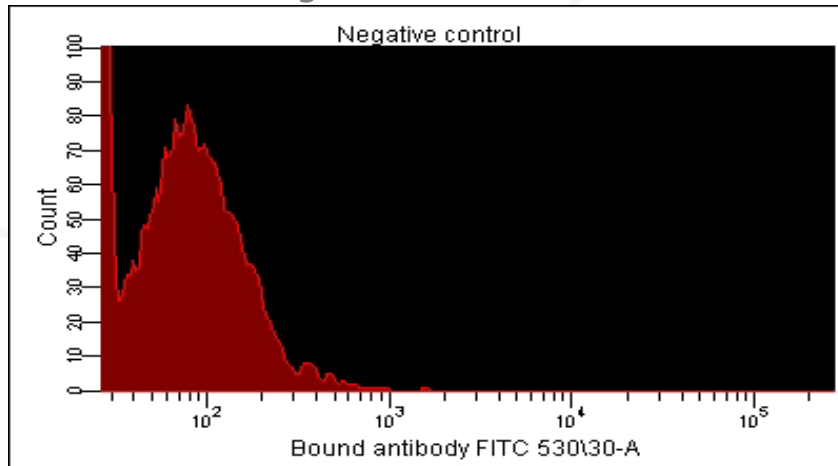
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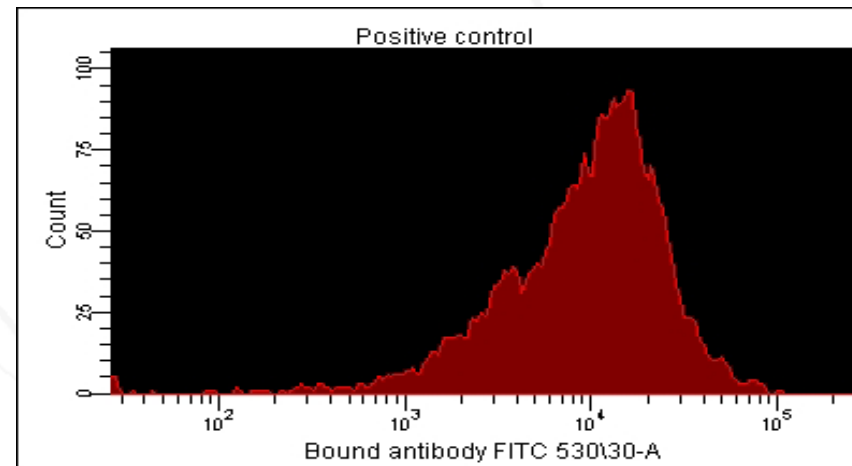
Flow cytometric analysis

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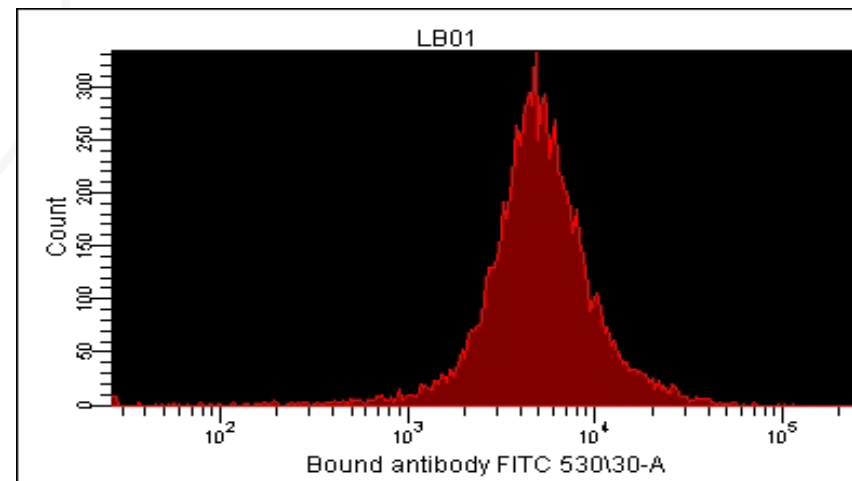
Negative control



Positive control



GS-CHO cell line, LB01



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Summary

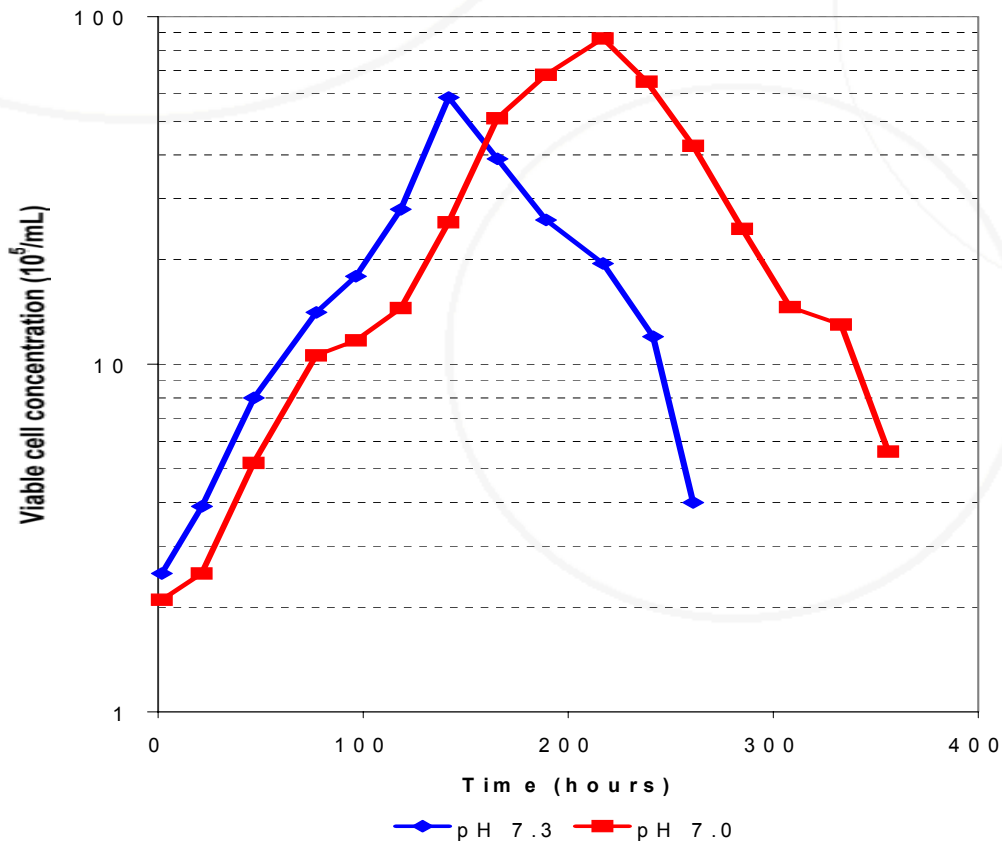
- Manipulation of the transfection conditions results in a substantial increase in the number of transfectants
- Increasing the stringency of the selection conditions substantially increases the median antibody productivity

- Significant potential to increase volumetric productivity of process
 - Maintain high viable cell concentration for extended period
 - Physicochemical environment (pH, temperature)
 - Medium design (including use of chemically defined media)
 - Feeding strategies

- Control pH, temperature, dissolved oxygen concentration
- Small changes in pH can have a profound effect upon cell growth and productivity
 - Responses are cell line specific and can impact:
 - Maximum cell concentration
 - Time integral of viable cell concentration
 - Specific production rate

Effect of culture pH

Model GS-NS0 producing a recombinant antibody in a CDACF & PF bioreactor process



Increased specific production rate

**0.59 pg/(cell·h)
compared with 0.47
pg/(cell·h)**

**Increased productivity
590 mg/L compared
with 240 mg/L**

Medium design and feeding strategies

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- Optimise basal medium
- Optimise feeds
- Maintain nutrient sufficiency
- Minimise waste product formation

Chemically-defined, animal component free and protein-free media (CDACF & PF)

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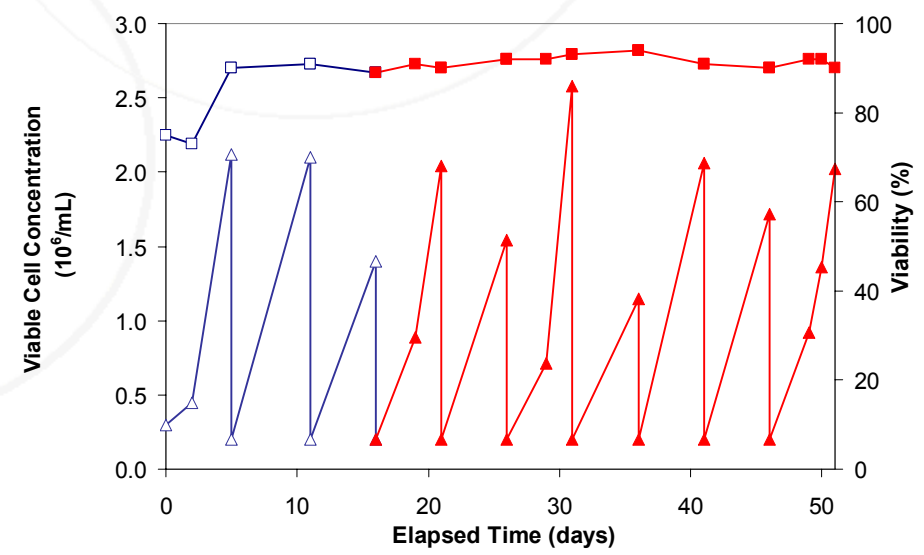
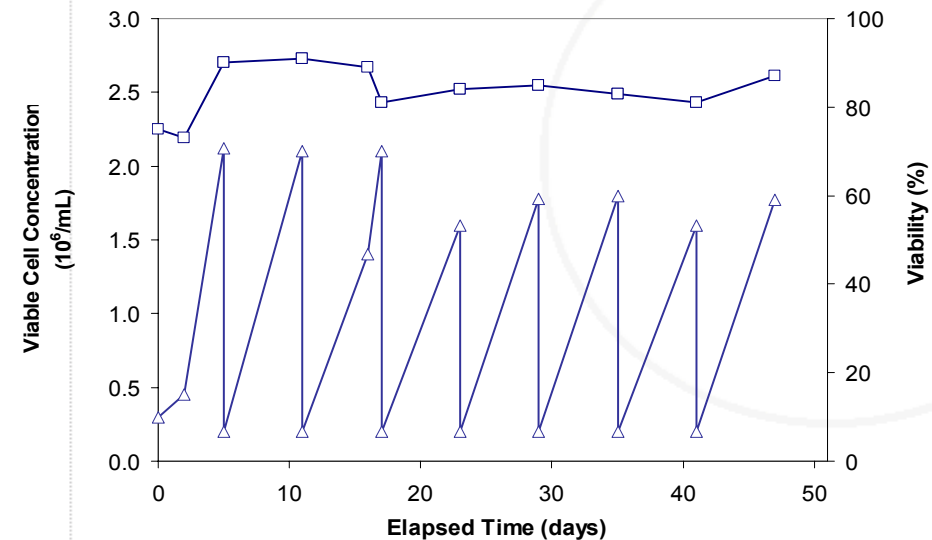


- Increasing use of chemically defined media free of animal derived raw materials
 - Reduced risk of introducing adventitious agents
 - Improved process consistency and robustness (avoids potential variability of raw materials such as hydrolysates)
 - Benefits purification (reduced contaminant load)

- Traditionally a lengthy procedure, often taking up to 16 weeks
- Often accompanied by transient poor growth and viability
- Potentially less productive than serum-free processes

Adaptation of a model GS-NS0 cell line to CDACF & PF medium

- Three process development iterations required
- First two failed either because too long or success rate too low
- Third iteration: 60 / 60 cell lines adapted within 4-7 weeks



—△— Growth in Serum-free Medium —□— Viability in Serum-free Medium

—△— Growth in CDACF & PF Medium —□— Viability CDACF & PF Medium

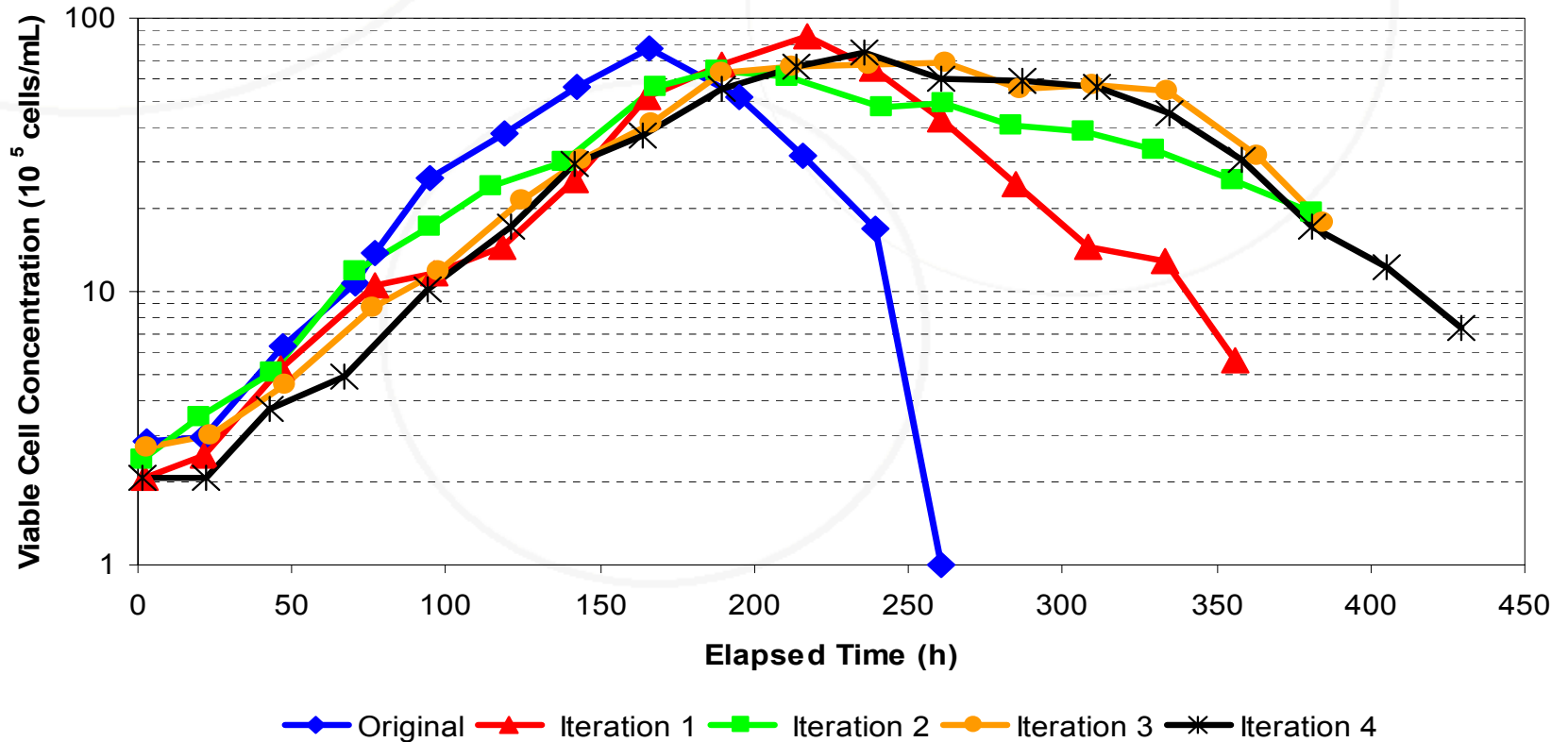
- Removal of serum or BSA (and any other animal-derived component) from the cryopreservation mixture is highly desirable
 - Potential sources of adventitious agents
- CDACF & PF-adapted NS0 cell lines often showed poor viability and growth upon revival of cryopreserved cell stocks
 - Loss of process robustness

Cryopreservation of CDACF & PF-adapted cells

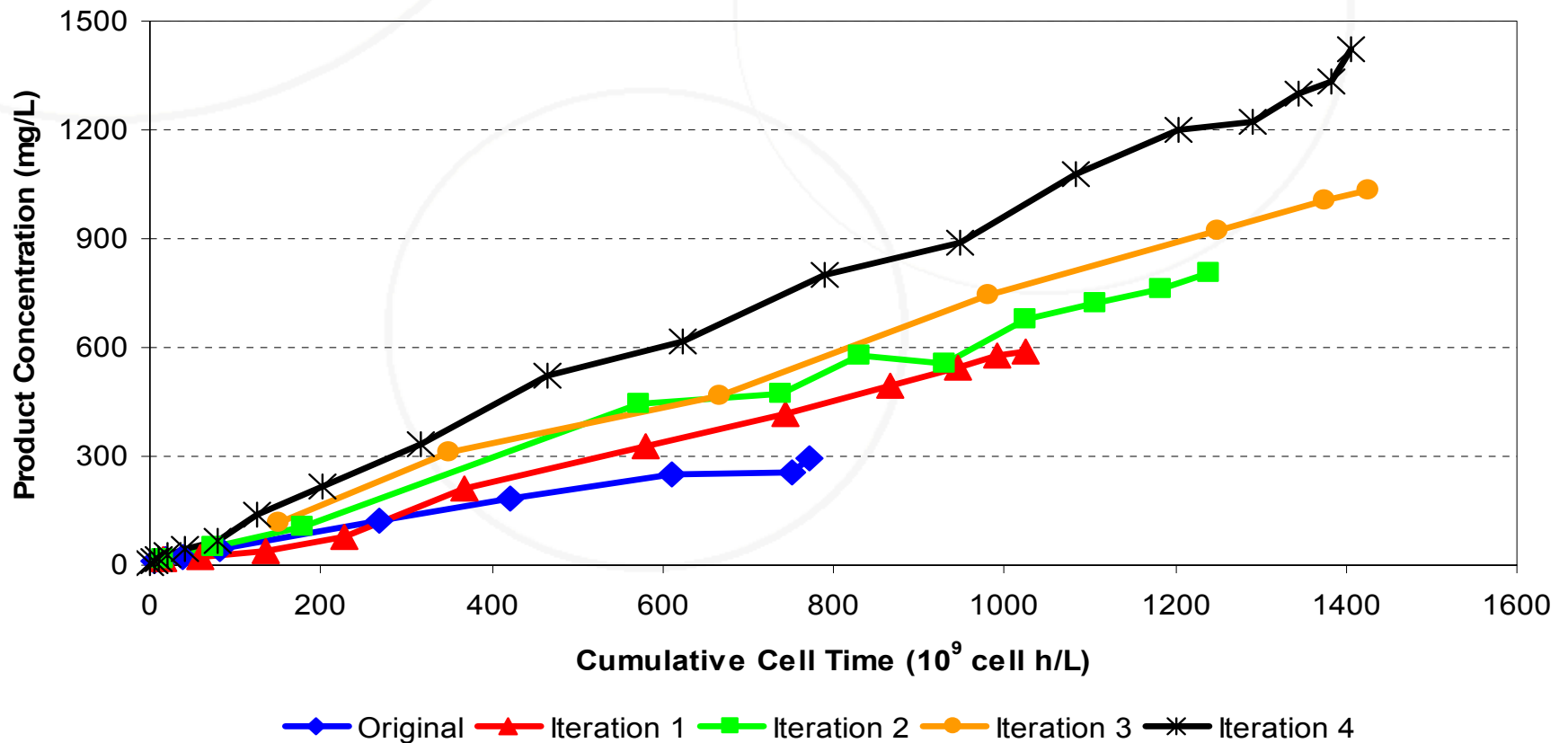
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CDACF & PF medium	Serum in cryopreservation mixture	Culture viability prior to cryopreservation (%)	Culture viability upon recovery (%)
Round 1	Yes	≥ 90	≤ 10
Round 3	Yes	≥ 90	≥ 90
	No	≥ 90	≥ 90

Growth kinetics in a CDACF & PF bioreactor process



Product kinetics in a CDACF & PF bioreactor process



Process optimisation for a model GS-NS0

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CDACF & PF bioreactor process

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

Downstream benefits of CDACF & PF medium for GS-NSO Cell Line

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Purity of MAb at harvest

Optimised protein containing culture

<30%

Optimised CDACF & PF culture

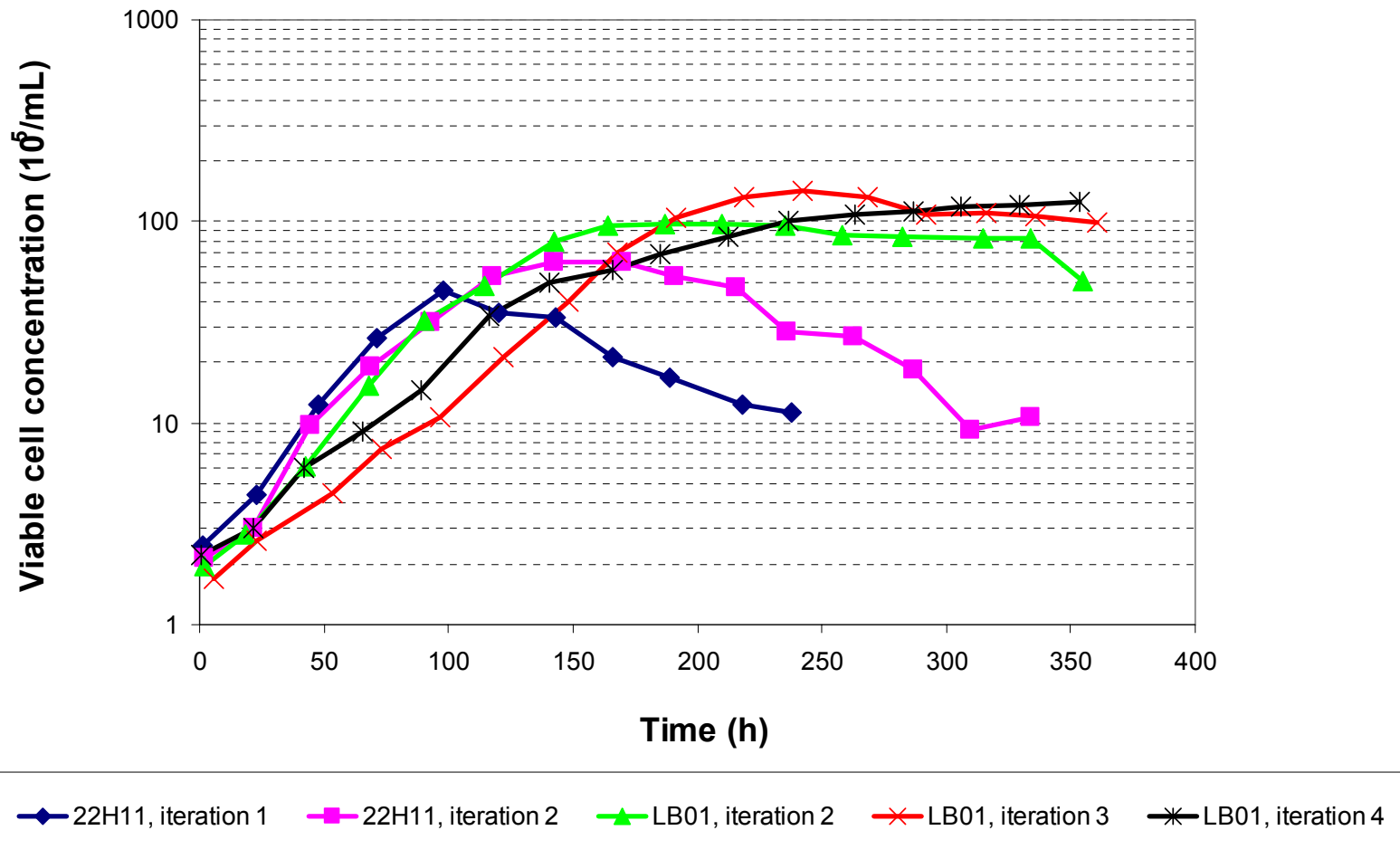
62%-76%

Optimisation of a GS-CHO Process

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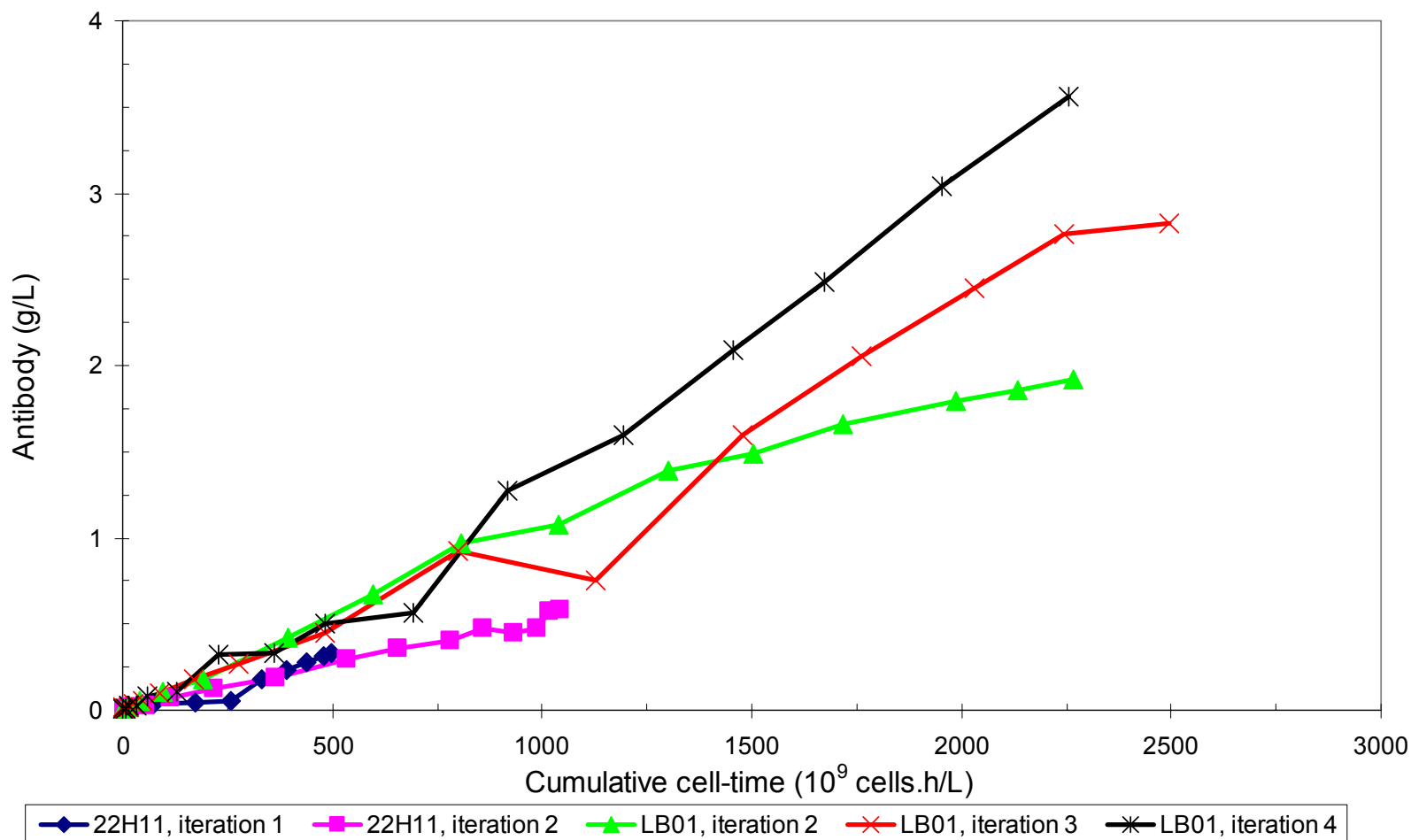
- Similar approach taken as with GS-NS0 cell lines
 - Fed-batch culture, initially using the same feed as the GS-NS0 process
- Suspension variant of CHO-K1 which grows in CDACF & PF medium without need for adaptation (can take several months)
- Improved selection of highly productive cell lines

GS-CHO growth characteristics



GS-CHO product accumulation

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Process optimisation for a model GS-CHO

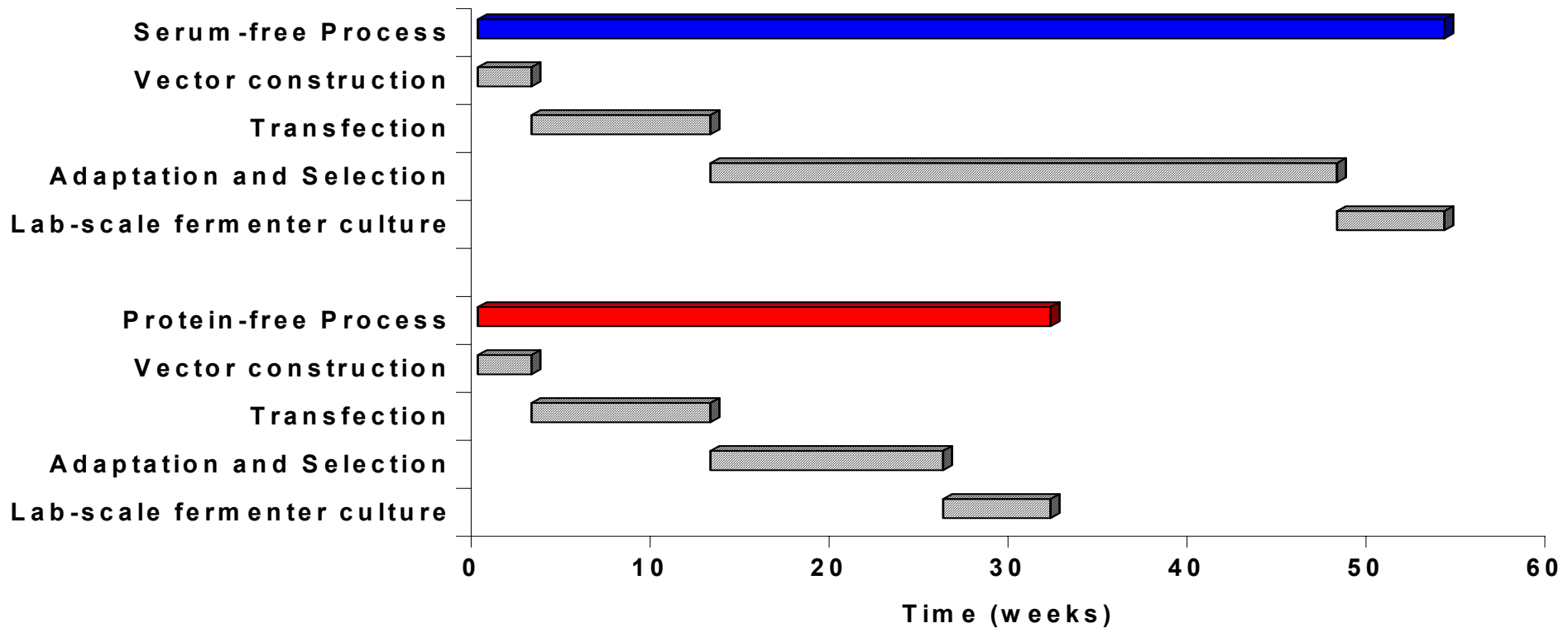
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CDACF & PF Bioreactor Process

Cell line	Process	Cumulative cell time (10^9 cell·h/L)	Antibody (mg/L)	Q_p pg/(cell·h)
22H11	Original protein-free	267	139	0.52
22H11	Iteration 1	498	334	0.66
22H11	Iteration 2	1041	585	0.53
LB01	Iteration 2	2266	1917	0.89
LB01	Iteration 3	2493	2829	1.17
LB01	Iteration 4	2254	3560	1.55

GS-CHO process development timelines

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- A horizontal decorative bar with a collage of green and blue microscopic images, possibly showing cells or laboratory equipment.
- Number of approaches to achieving substantial process improvements
 - Cell line construction, process, potentially metabolic engineering
 - Each approach gives increases, but synergistic improvements are possible
 - Combination of efficient expression system (GS) and process optimisation gives high productivity for non-amplified NS0 and CHO cell lines
 - Use of CDACF & PF media simplifies process optimisation and product purification
 - Significant potential for further improvements based on process optimisation and cell line improvements