

# “Successful transfer and scale up of a mammalian cell culture process producing a monoclonal antibody from a stirred tank reactor to an air-lift fermenter”

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## Abstract

Large scale mammalian cell culture is typically performed in suspension culture. Two configurations that are used in industry are stirred tank and air-lift fermenters. The debate about the advantages and disadvantages of these configurations is one that has received attention in recent years. This poster examines how a fed-batch transfectoma process operated in a stirred tank reactor was successfully transferred to an air-lift fermenter. Cell growth and metabolism, productivity and product quality are compared. Optimisation of both modes of operation to ensure a similar physicochemical environment for the cells maximises the ability to maintain equivalent product quality and yield. The process of transferring technology from customer to contract manufacturer and between development and manufacturing is described with particular reference to areas that require careful consideration to ensure seamless process transfer to manufacturing scale.

## Introduction & Aim

- Objectives :
  - To transfer and scale-up a fed-batch mammalian cell culture process from 1200 L Stirred Tank Reactor (STR, ImClone) to 5000 L Air-Lift Fermenter (ALF, Lonza)
  - Maintain process and product equivalence
- Key determinants of equivalence :
  - Product quality
  - Productivity (volumetric, specific)
  - Cell growth
  - Cell metabolism
- Strategy :
  - Minimise changes to the process
  - Evaluate impact of any process modifications
  - Control critical raw materials during scale-up

## Comparison of STR vs. ALF

- |   |  |
|---|--|
| <b>Stirred Tank Reactor</b> <ul style="list-style-type: none"> <li>Low aspect ratio (typically 1:1)</li> <li>Greater flexibility in terms of operating volume</li> <li>CO<sub>2</sub> accumulation may be an issue</li> <li>Mixing time : 3 minutes (Ref 1)</li> <li>k<sub>L</sub>a typically up to 10 hr<sup>-1</sup> (Ref 1)</li> <li>Tip speed 0.5m.sec<sup>-1</sup></li> <li>Impeller requires mechanical seals, maintenance</li> </ul> | <b>Air-Lift Fermenter</b> <ul style="list-style-type: none"> <li>High aspect ratio (typically 5:1)</li> <li>Restricted flexibility in operating volume</li> <li>Ballast gas ensures CO<sub>2</sub> does not accumulate to toxic levels</li> <li>Mixing time : 5 min</li> <li>k<sub>L</sub>a typically up to 20 hr<sup>-1</sup></li> <li>Liquid velocity 0.1m.sec<sup>-1</sup></li> <li>No moving parts or mechanical seals for impeller</li> </ul> |
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## Key Areas for Consideration

- Raw Materials
  - Identify Critical Raw Materials
  - Vendor Qualification
- Inoculum Methodology
  - Static vs. shake flask culture
  - Method of gas exchange
- Subculture Ratio
  - 1 in 6 (STR) vs 1 in 9 (ALF) subculture ratio
- Gas Flow Rate Differences
  - 0.02 vvm (STR) or 0.04 vvm (ALF)
  - (NB : vvm = maximum total gas flow rate per unit liquid volume)
  - potential differences in pCO<sub>2</sub>
- Foaming : Antifoam usage
  - Total sparge gas flow rate higher for ALF than STR
  - Effect of antifoam usage on Fermentation and Downstream Operations

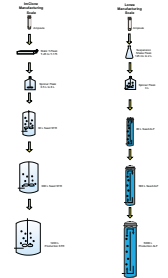
## Program

- Process review
- Raw materials qualification
- Laboratory evaluation in flask culture and 10 L ALF
- Scale process to 130 L ALF
- Demonstrate equivalence of product derived from laboratory and pilot scale ALF to 1200 L STR
- Process scale-up to 5000 L ALF pilot lot
- Demonstrate equivalence and consistency of 5000 L ALF manufacturing scale to 1200 L STR

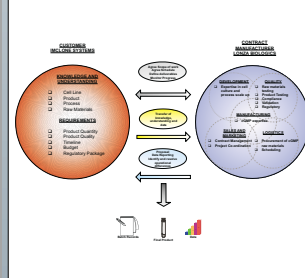
## Materials & Methods

- Cell line : Mouse SP2/0 AG14 Transfectoma
- Product : Murine/Human Chimeric IgG<sub>1</sub>
- Serum-free medium with several concentrated nutrient feeds
- Control parameters : Temp = 36.7°C, pH 7.1 to 6.8, DO 25% saturation with air
- Product concentration determined by Protein A HPLC
- Glucose, glutamine and lactate concentrations determined by autoanalyser
- Ammonia determined by colorimetric assay
- pCO<sub>2</sub> determined by Blood Gas Analyser
- Product quality : Isoelectric Focusing (IEF) and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis (Data shown)
- More extensive product characterisation performed (Data not shown)

## Process Flow

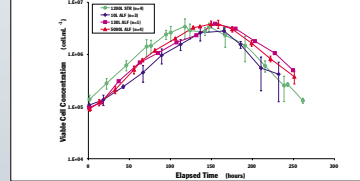


## Process Transfer

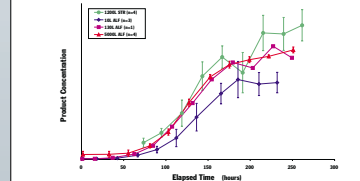


## Results

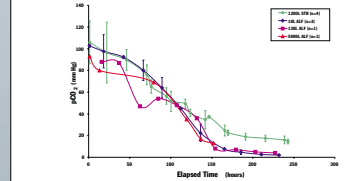
### Cell Growth



### Product Accumulation



### Carbon Dioxide Partial Pressure



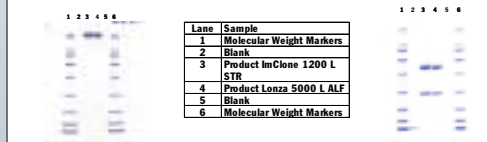
### Product and Metabolite Summary

	ImClone Manufacturing Scale (n=4)	Lonza Lab Scale (n=3)	Lonza Pilot Scale (n=1)	Lonza Manufacturing Scale (n=4)
μ <sub>max</sub> (d <sub>opt</sub> ) <sup>-1</sup> (10 <sup>6</sup> cells/L) <sup>-1</sup>	100 18	90 9	79	82 4
q <sub>max</sub> (d <sub>opt</sub> ) <sup>-1</sup> (g/L cells/L) <sup>-1</sup>	10.7 3.0	19.6 4.1	14.2	14.8 0.7
q <sub>glu</sub> (d <sub>opt</sub> ) <sup>-1</sup> (g/L cells/L) <sup>-1</sup>	6.0 0.7	5.9 3.7	8.0	6.5 0.5
q <sub>lac</sub> (d <sub>opt</sub> ) <sup>-1</sup> (g/L cells/L) <sup>-1</sup>	8.0 1.5	10.2 1.0	7.8	8.0 0.8
q <sub>am</sub> (d <sub>opt</sub> ) <sup>-1</sup> (g/L cells/L) <sup>-1</sup>	0.7 0.1	0.7 0.0	0.7	0.6 0.1

### IEF Analysis



### Non-Reduced SDS-PAGE Analysis



## Conclusions

- Successfully transferred fed-batch mammalian cell culture process from STR to ALF
- Process shows high degree of equivalence and consistency
- Cell specific productivity and nutrient metabolism were statistically equivalent between 1200 L STR and 5000 L ALF
- Product quality was equivalent between STR and ALF
- Process Import and Transfer performed in approximately six months

## Acknowledgements

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- Lonza US Manufacturing Fermentation Suite 3 Staff
- Lonza Quality Services Laboratories
- Lonza Purification & Assay Development Staff
- ImClone Systems Manufacturing, Process Development and Analytical QS Method Development Groups

## References

<sup>1</sup> Nisow, A.W, et al (1996) Cytotechnology 22, 87  
 “Homogenisation and oxygen transfer rates in large agitated and sparged animal cell bioreactors : Some applications for growth and production”