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The journey from tech transfer to BLA submission: Case study of a NS0 cell culture process from 2000L stainless steel bioreactor to 2000L disposable bioreactor

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The journey from tech transfer to BLA submission: case study of a NS0 cell culture process from 2000L Stainless steel bioreactor to 2000L disposable bioreactor

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Abstract

A case study of NS0 cell culture process transfer from 2000L stainless steel bioreactor (SST) to 2000L disposable bioreactor (SUB), and through to process validation and BLA submission is reported for production of an antibody therapeutics in this poster. Initial attempts in growing the NS0 cells in the small scale 2D bags yielded non-satisfactory results, as growth was impacted by bag material type as well as by different suppliers of the same bag material type. However, 3D bags of 50L and above proved to be supportive of the NS0 cell line growth.

Process characterization (PC) and process validation (PV) efforts were initiated after successful scale up to the 2000L SUB. Scale down model (3L) was qualified using bench top glass bioreactors, and PC studies identified several critical process parameters (CPPs). Successful process performance qualification (PPQ) campaign followed and BLA was submitted in 2017.

Leachables & extractables on SUBs

- Concern on L&E for cell culture is one of the main challenges for SUB implementation
- Impact of L&E for cell culture
  - ➢ Patient safety: toxic effects on patients
  - ➢ Process impact: cell culture performance impacts

▪Not all bags are the same

- Different bags have different materials & are made with different processes
- Even bags with same contact layer material had different impact on growth
  - Other materials, e.g., additives, could have major impact
  - Ex: Thermo's Aegis 5-14 film vs CX 5-14
- Suppliers might switch films
  - Ex: Sartorius Flexsafe S80 film replacing earlier S40 film

Disposable Bag	Contact Layer
Thermo SUB	**ULDPE**
Thermo Container Bag	**ULDPE**
WAVE Bag	**EVA**
Sartorius RM/Flexboy Bag	**EVA**
Sartorius STR CultiBag	**ULDPE**
Sartorius Flexsafe Bag (RM/STR etc)	**LLDPE**
GE Xcellerex Bag	**ULDPE**
Shake Flask	**PC**
Millipore Container Bag	**ULDPE**

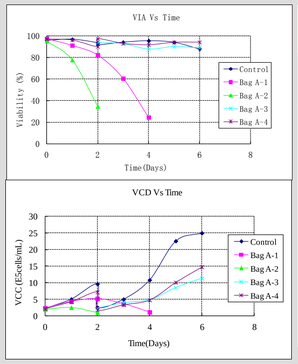
Lack of good scale down models for SUBs

- None of the major suppliers of SUBs offer representative scale-down models of the larger scale SUBs
- 50L SUBs appear to be the most appropriate models to represent 2000L scales. But it is too expensive to be an economical model. Surface/volume ratio worse than 2000L
- Benchtop glass bioreactors are still being widely used as scale-down models for large scale SUBs.
- However, leachables & extratables can not be tested with glass bioreactors. Product quality impact from SUBs also can not be evaluated with glass bioreactors

Growth challenges in 2D bags during process transfer (from 2000L stainless steel bioreactor to 2000L SUBs)

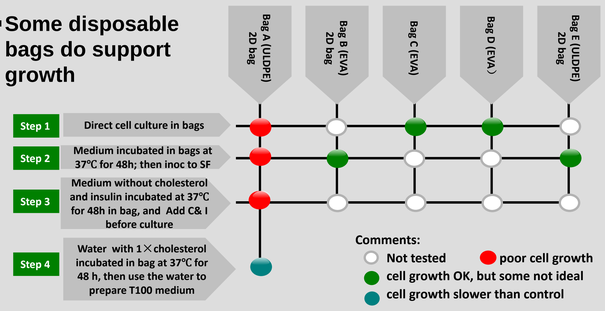
▪Background: NS0 cell line with chemically-defined medium
  ▪Medium contains insulin & cholesterol
  ▪Robust process demonstrated by 2000L SST GMP runs
➢ Objective: transfer & scale-up to 2000L SUB for PhIII trials

▪Challenges in growing NS0 cells in disposable bags



Study name	Study conditions
Bag A- 1	Direct culture in Hyclone 2D bag
Bag A- 2	Media incubation @ 37C, 48hr; then inoc to SF
Bag A- 3	Media w/o cholesterol & insulin incubated @ 37C, 48hr; then add chol & insulin back; inoc to SF
Bag A- 4	Water w/ 1x chol incubated @ 37C, 48hr; then used to prep medium and inoc to SF

▪Some disposable bags do support growth



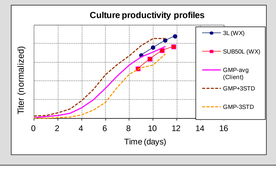
➢ **Various attempts in trying to grow the cells with the 2D Bag A did not succeed**

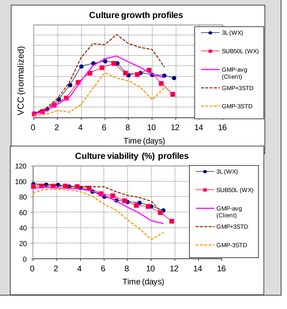
Satisfactory results from 50L SUB

- Various attempts using vendor A 2D bags did not lead to satisfactory results
- The fact that other bags w/ the same ULDPE material supported growth was encouraging
- 2D bag might not be a good scale-down model of 3D & large volume bags
  - Surface to volume ratio much larger
  - If there are leachables, 2D bag would be worst case scenario
- Two options
  - Try vendor A 50L SUB to see if growth is OK
  - Try vendor B SUB

▪1st 50L SUB showed good performance

- Culture performance in 50L SUB was comparable to 3L glass vessel and historical GMP data
- Indeed 2D bag was not a good scale-down model





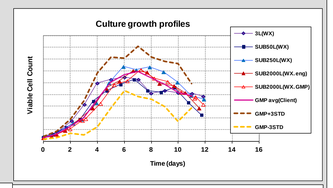
Successful scale-up to 2000L SUB

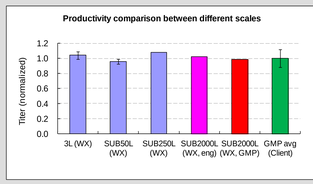
▪Process confirmation at 250L SUB

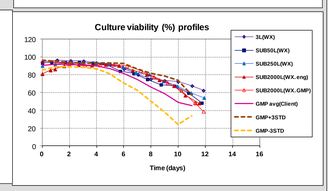
- 250L SUB as last step before scaling up to 2000L SUB
- Process designed to mimic 2000L operation as much as possible
- Good performance at 250L SUB, with full analytical comparability assessment
- Cleared to scale-up to 2000L SUB

➢ **Successful scale-up to 2000L SUB**

- Growth & productivity at 2000L SUB (eng run & GMP run) matched very well with historical GMP data

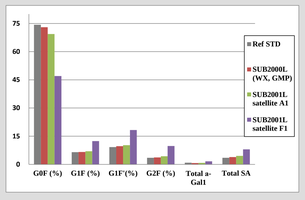






Scale down models for 2000L SUBs

▪Unexpected challenge in glycosylation profiles

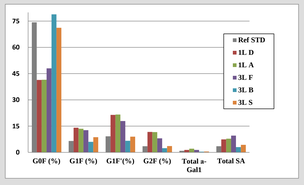


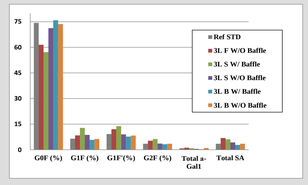
- Satellite cultures of 2000L SUB had dramatic difference in glycosylation profiles
  - One matched 2000L SUB well
  - The other had significant differences
- Other performance indicators were comparable, e.g., titer, growth etc

▪Unexpected challenge in scale-down model transfer

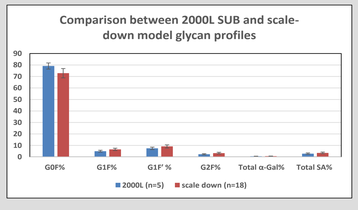
- Significant differences in glycan profiles among different small scale cultures
  - Difference between 1L vs 3L model
- Even among 3L bioreactors, difference remained
  - Glass vessel had same dimensions
  - Agitator diameter different
  - Sparger different
  - Baffle presence or not also made a difference

➢ **Need to be careful in picking the right scale-down model!**



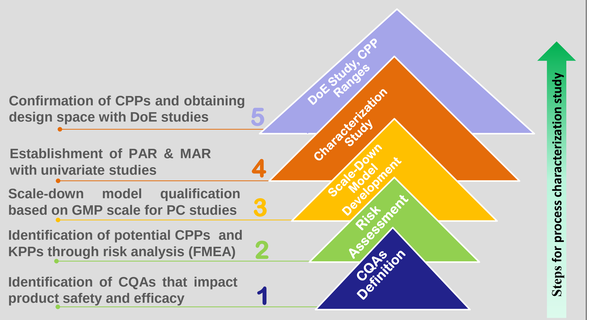


▪Successful scale down model verification

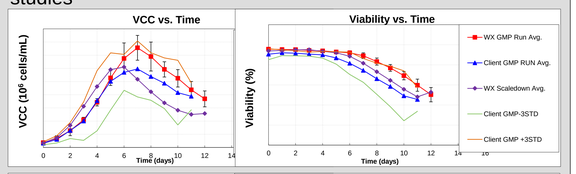


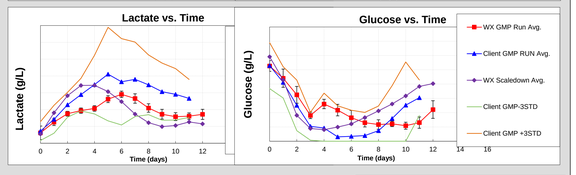
- Picked the BR model that's closest to 2000L SUB data, and also most consistent product quality data

Process Characterization & PPQ campaign



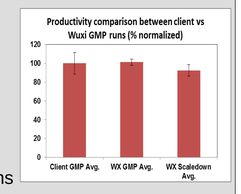
- Four CPPs identified: N-1 culture & production culture medium concentration; culture temp; culture pH; 1st feed glucose & VCC level
- NOR (normal operation range) of the CPPs defined through DoE studies; MOR (max operation range) defined through univariate studies
- Control strategies defined based on development & PC studies





PPQ runs executed after completion of process characterization studies

- High level strategy shared with the FDA
- Protocols drafted for upstream & downstream
- Batch records drafted based on PPQ protocols
- Detail sampling & in-process testing plans designed to support validation



▪BLA submission

- Rolling BLA submission (with Orphan & Breakthrough status)
- Module 3 submission in parallel with Phase III trial
- Limited stability data from PPQ runs at time of submission
- Complete BLA submission once Phase III data was in. Additional stability data submitted to help set shelf life

▪Pre-License Inspection (PLI), BLA approval

- PLI conducted ~10 weeks after BLA submission
- Five inspectors, thirteen days total for the inspection
  - Including two CMC reviewers
- PPQ run batch records, development reports, CPP & control strategies reviewed (NOR/MOR, IPCs etc)
- Continuous Process Verification (CPV) post BLA

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