Utilizing High Density Cell Banking to Drive Process Efficiency

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Alflow Single-use Seminar

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In this session we will go through…

Key words

- Cell Train Based Manufacture
- Technology Trend
- HDCB
- Process Economics
- Aramus™ Single-Use 2D Bags

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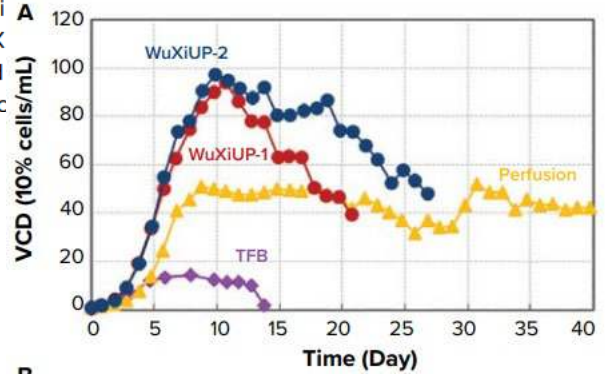
Intensified Process with High Density + Perfusion

Continuous Biomanufacturing Implementation

Using an Intensified and Integrated Bioprocess Platform

Recent world events have demonstrated now more than ever the growing demand for pharmaceutical biologics that can be made rapidly and in high volumes yet somehow remain affordable. Hence, there is an urgent need to develop a next-generation biomanufacturing solution that provides high-yield, high-quality drug products and is highly flexible and cost-effective. Herein we descri
productivity platform (WuX
culture process developed
need. WuXiUP adopts proc

WuXi Biologics
Global Solution Provider



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ecovery, clean-in-place (CIP),
n purposes.

High-density 50 L perfusion cell culture using the XCell ATF 6 Single-Use System

Introduction

Improvements in single-use systems have allowed implementation of high-density cultures in standard workflows. This study shows the integration of the Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) and the Repligen XCell ATF™ 6 Single-Use System to achieve a stable, high-density perfusion cell culture, at 40 x 10^6 cells/mL over 20 days, without modifying the standard systems. The data show that an integrated HyPerforma S.U.B. and XCell ATF 6 system can be used in a high-density seed train or as a production vessel system.

Goal

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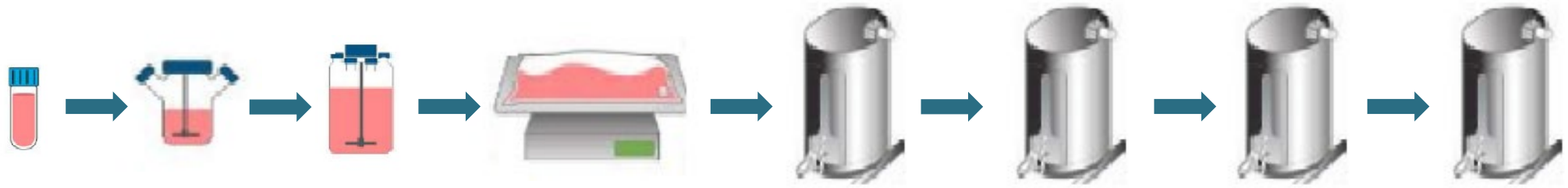
Conventional and Intensified Seed Train Process

Conventional Process

Start from 2ml glass vial, scale up step by step via flask, spinner, wave bioreactor to production bioreactor.



Vial | Cell Expansion Procedure | N-3 Bioreactor | N-2 Bioreactor | N-1 Bioreactor | N Bioreactor

>2 weeks | 1.5-2.5 weeks

Intensified Process

To generate high concentration cell cultures at optimal points during cell expansion , prior to the inoculation of the production bioreactor, so that time and hardware cost can be saved.

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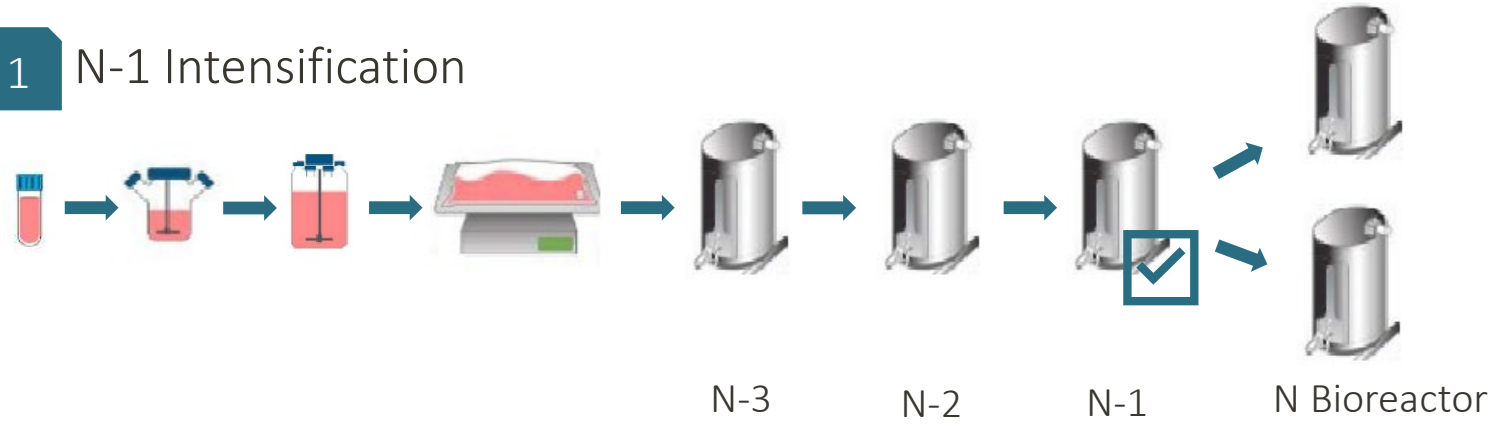
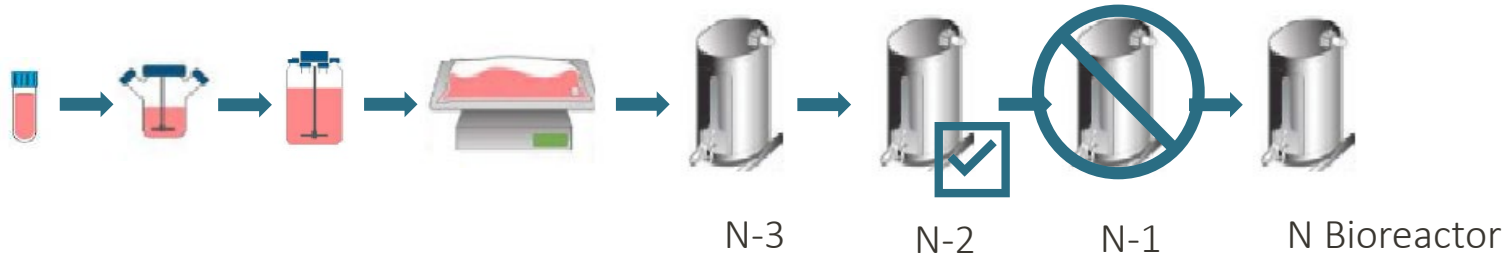
Seed Intensification Methods

Seed intensified at different points

Option 1 N-1 Intensification



Option 2 N-2 or Earlier Intensification



Benefits

- Less time in production reactor
- More batches per year
- Shorter seed train times
- Reduce plant footprint

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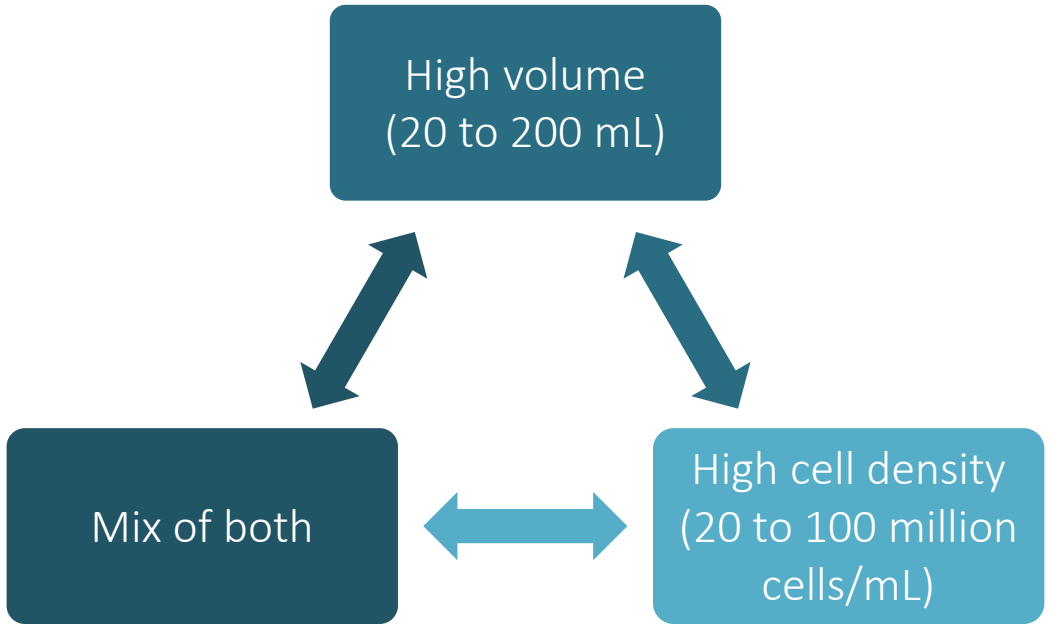
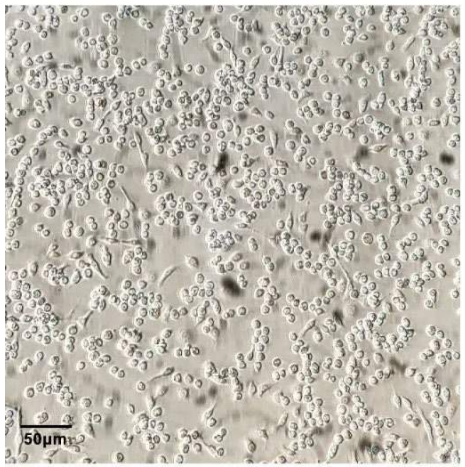
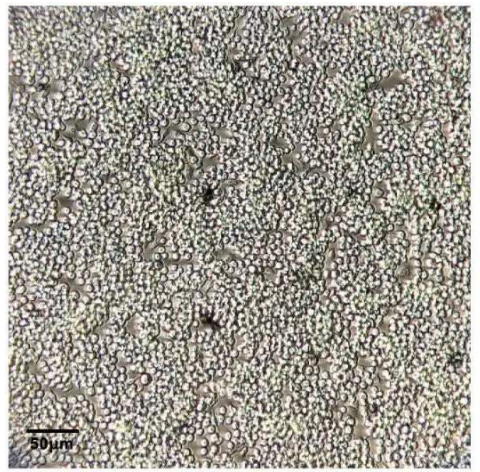
Cell Freezing in Cell-Culture-Based Process

Change of strategy for cell banking

High-density/volume Cell Banking (HDCB)







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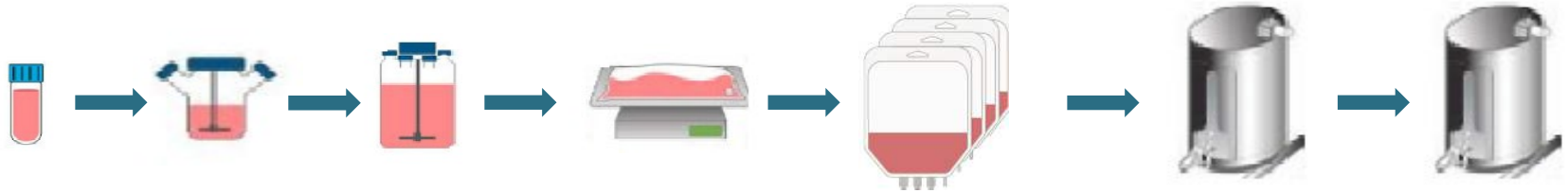
HDCB Intensification Strategy



Steps performs outside production timeline

- Ensure high cell concentration and viability
- Eliminate open operation
- Reduce risk and batch deviation
- Always ready to manufacture
- Distribution easily
- Good and tight schedule

Use Frozen HDBC

- Enable Manufacturing on demand, and a truly flexible facility ready for batch or continuous operation.

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Suppliers of Cell Intensification Solutions Agree on HDCB Efficiency

OPTION 4 | Frozen Process Intermediaries with N-1 Intensification

Combine Frozen Process Intermediaries and N-1 or earlier intensification. This enables Manufacturing on Demand, and a truly flexible facility ready for batch or continuous operation.

- Significantly smaller footprint and less equipment
- Reduced CAPEX for equipment and cleanroom
- Fewer operational steps
- More repeatable output
- Faster turnaround of equipment and more batches per year

Click on the workflow link below to find productivity and throughput solutions you can implement today.

- **3 steps**
- **13 days to product**
- **6 days to N**
- **7 days in N stage**

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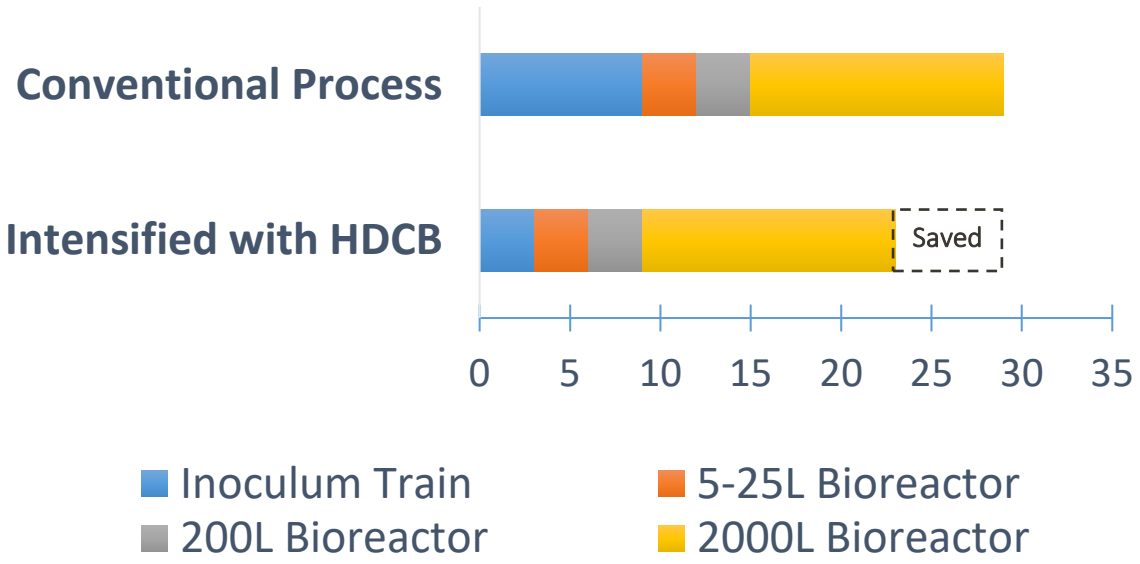
HDCB Process Economics

Aramus™ HDCB strategy

Single-train Process



Consumables
Flasks, pipets reagent

10~25% ↓

Workload
Turn to other missions

~32% ↓

Risks
Contamination cell viability

~35% ↓

Productivity
For more batches per year

~47% ↑

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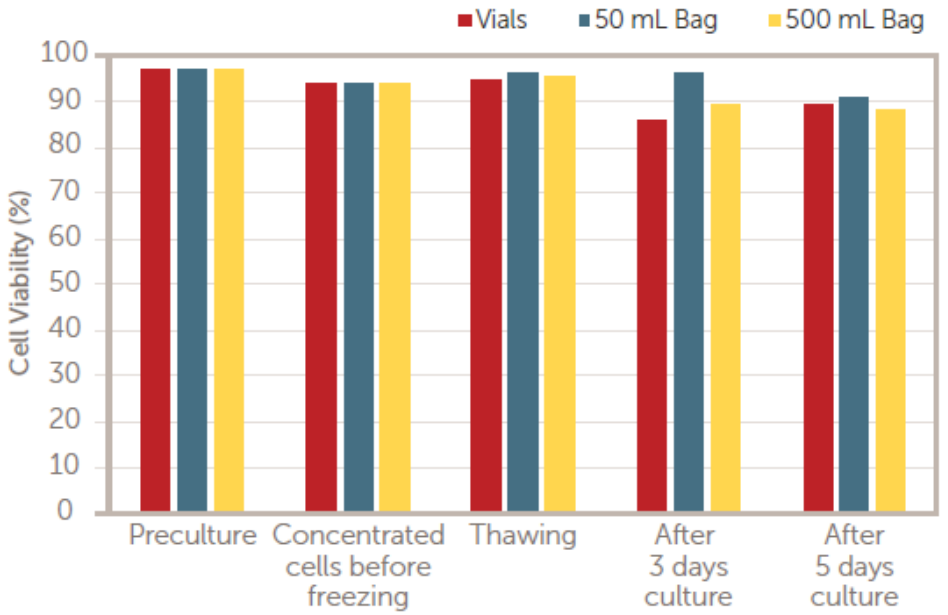
Proof Points - Benefits in Cell banking

- Saves weeks of culture time
- Better cell viability than vials
- Scale sufficient to seed 20L-200L bioreactors
- Completely closed system
- Exceptional cryoprotectant resistance

Figure 4. Thawing data for 50 mL bags.

Measurements of cell growth after thawing show better viability for cells frozen in bags than for vials (Figure 5). Freezing in bags improves homogeneity of the cell culture during freezing and allows for a faster thaw time, both of which are advantageous for cell survival.



Cell viability over experiment steps. Cells were harvested from a bioreactor (preculture) concentrated and resuspended as a single pool before distribution in vials (in Mr. Frosty), 50 mL bag (in Cryobox), and 500 mL (in Cryoshell only). Viability in culture was measured in shake flask cultures.

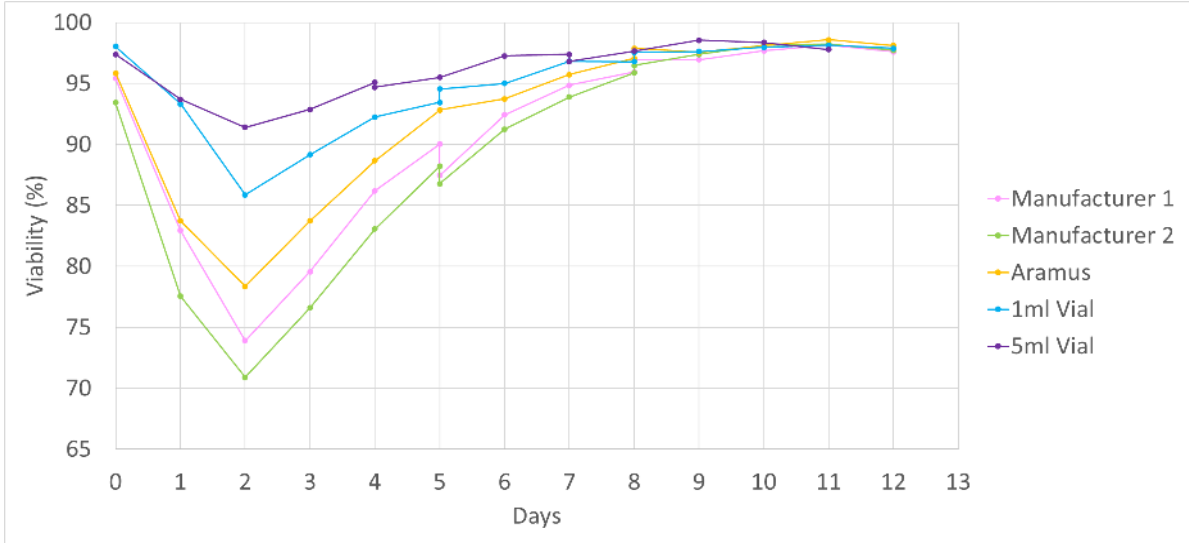
Figure 5. Cell viability before and after freezing for cells frozen in vials, 50 mL bags, and 500 mL bags.

HDCB Competitive Benchmarking

Post Cryopreservation Recovery of High-Density CHO Cell Culture in Cryobags

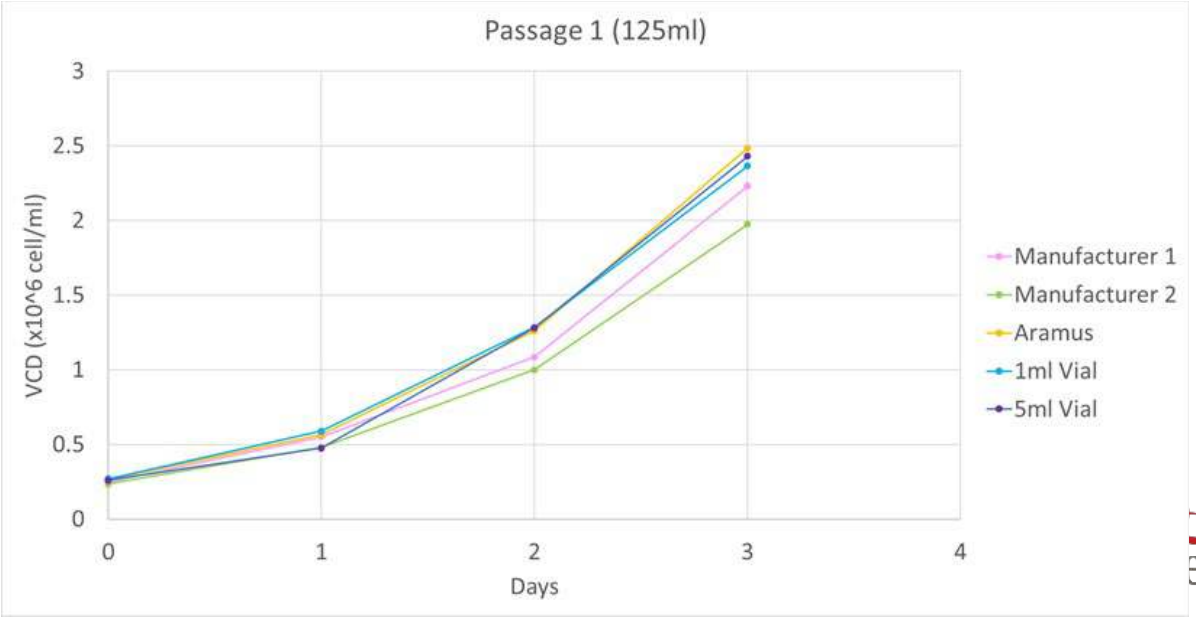
Scope of Work:

- High Cell Density of 20 x 10^6 Cells/mL
- Saint Gobain & Origen Competitor Bags
  - 1mL & 5mL Cryovial Controls
- Frozen in LN2 for 2 weeks, thawed and cultured for 2 passages up to 13 days



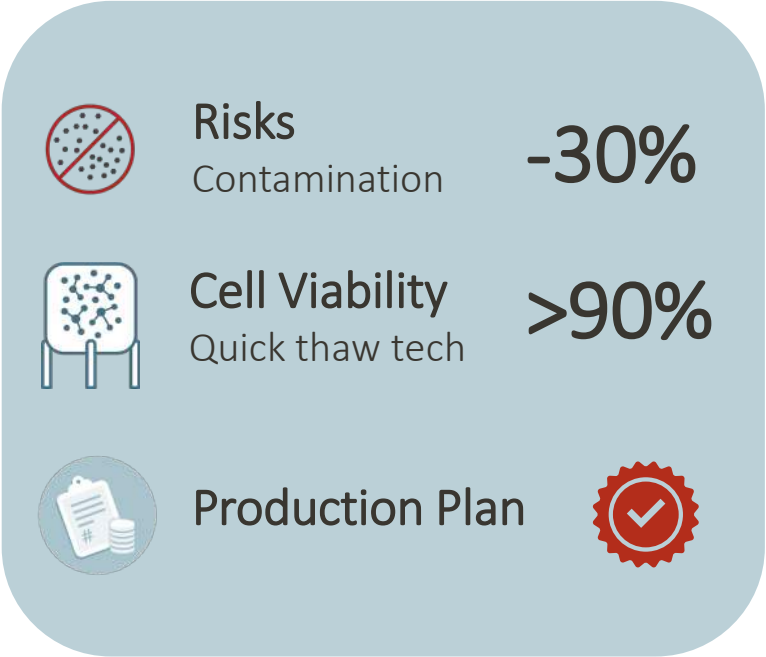
Results:

- *Aramus had quickest recovery among cryobags and retuned to >95% viability*
- *Aramus had quicker doubling times (growth) by reaching comparable rates to vials in Passage 1 vs. Passage 2 for other cryobags*

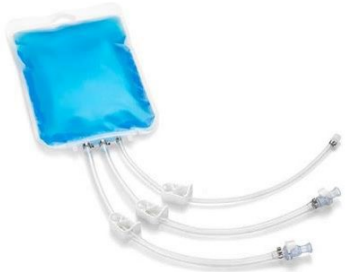


Alternating Bioreactor Train Process Using HDCB Reduces Risk to Save Time and Cost

- HDCB strategy ensures
  - reducing risks by eliminating manual operation
  - maintaining homogeneity of the cell, avoiding deviation
  - production stick strictly to the schedule



Aramus™ HDCB strategy – ensure upstream and downstream production trains do not de-couple and maximum batches are achieved per year



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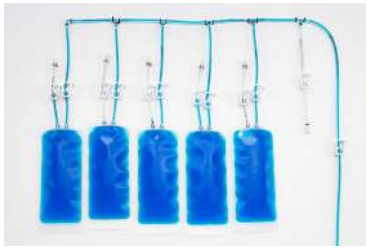
Set of tools to explore, practice and develop HDCB process

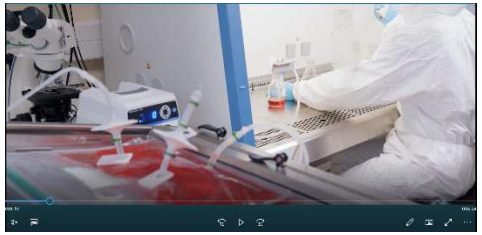
High Density Culture



Filled into multiple seed cultures

Freeze & Store

Thaw as needed



Skip smaller inoculum steps

x5-10 Batches, or more

HDCB Starter Kits

- 2 Purpose-Built kits (manifold, overwrap)
- LN2 Immersability Claim
- Overwrap Study & Sealing Settings
- LN2 Compatible Design Space
- Quick Start Guide

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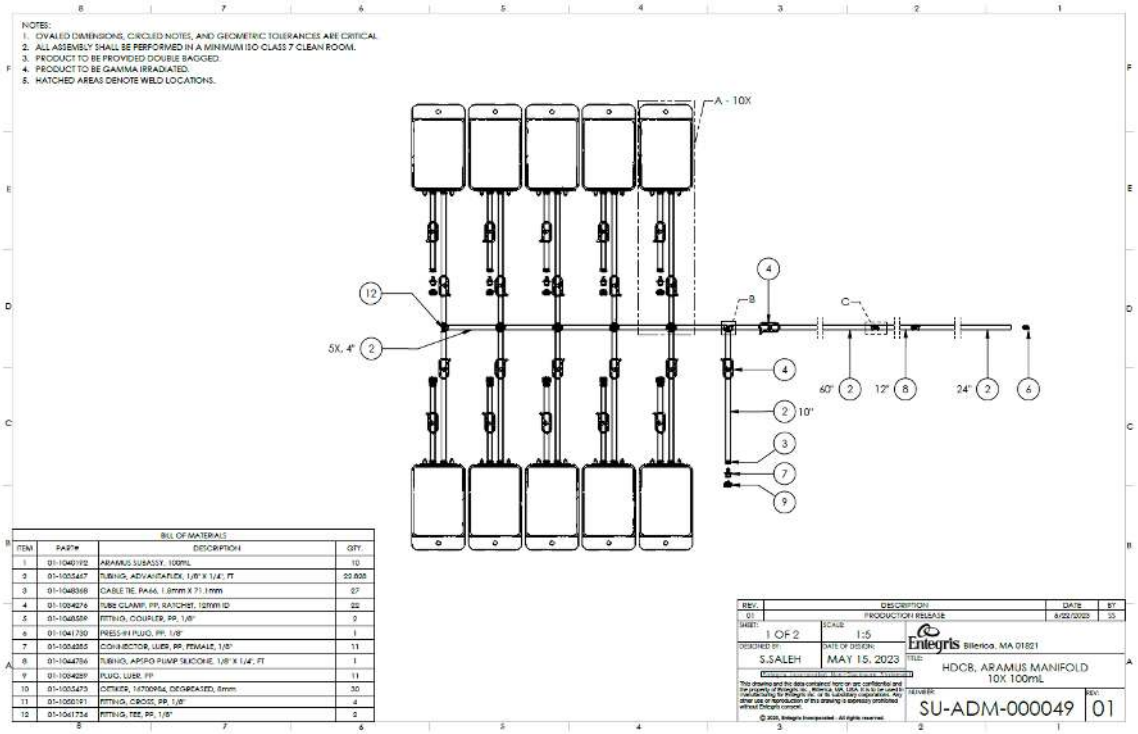
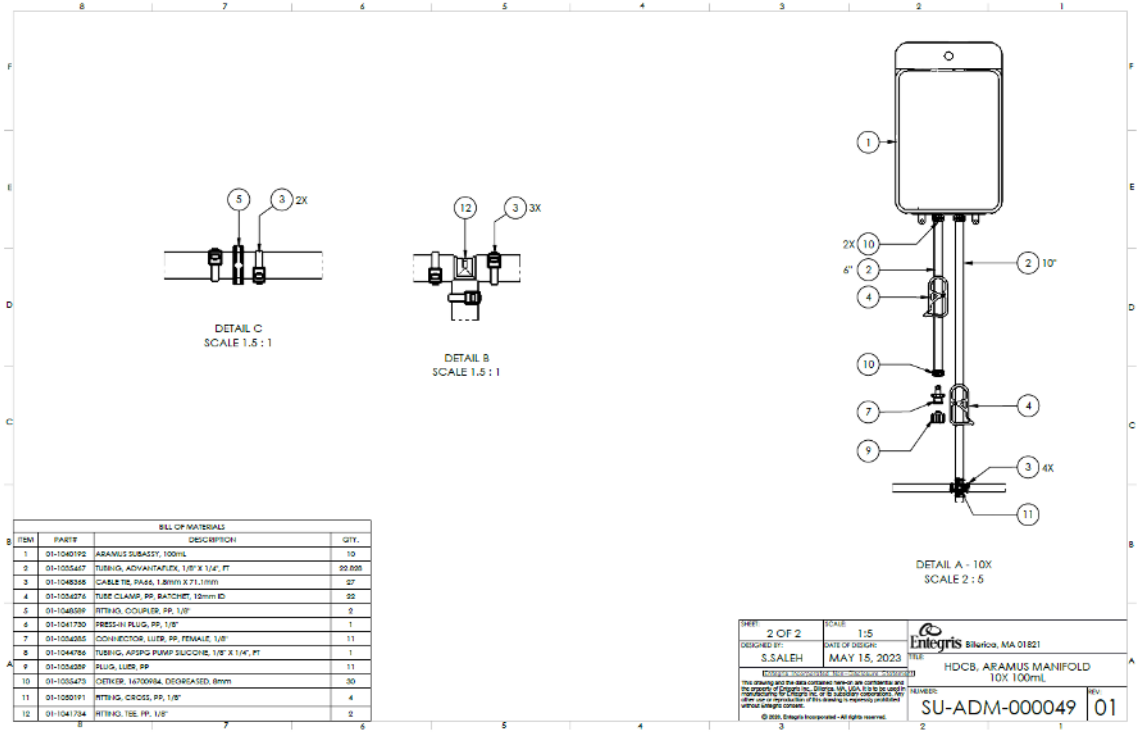
PN & Drawings

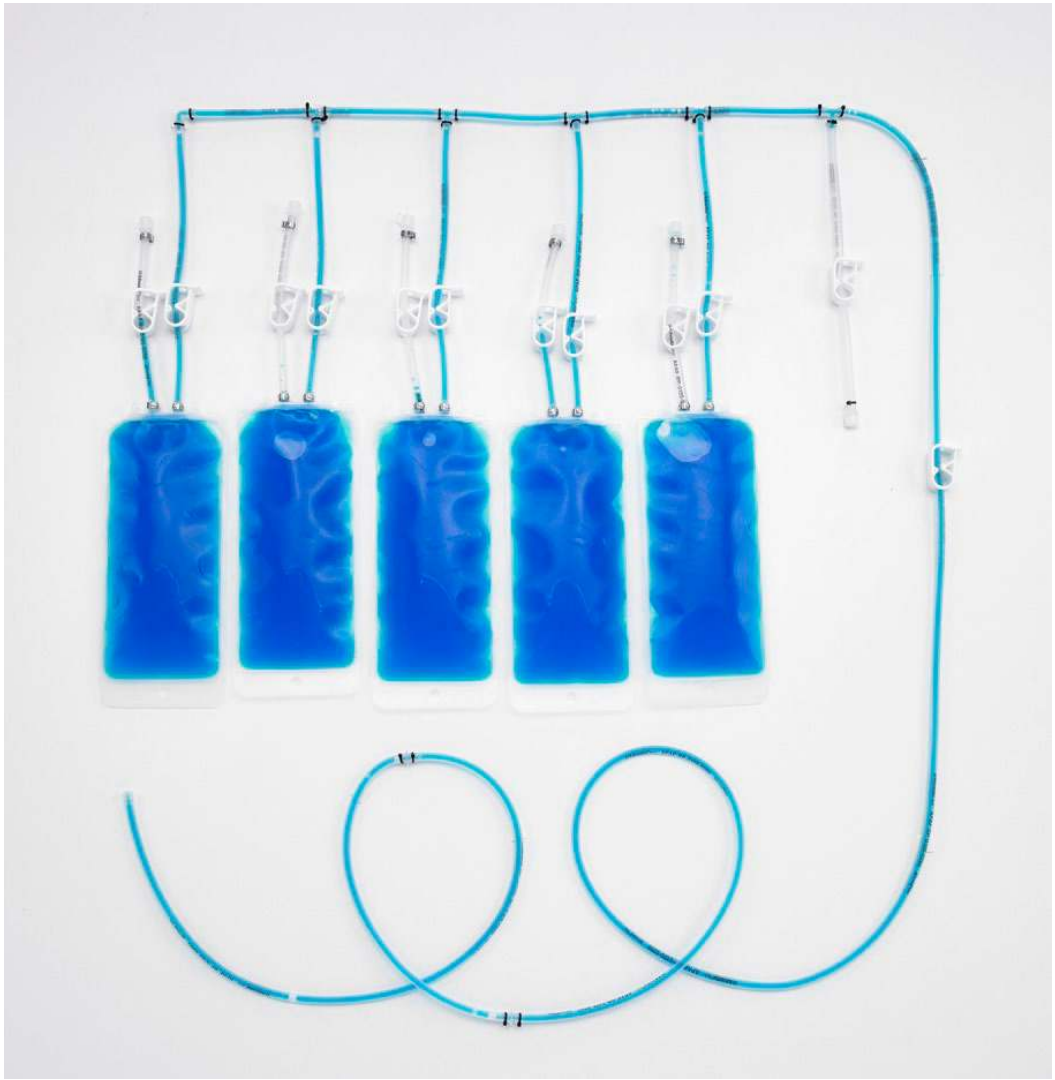
Standard Assemblies

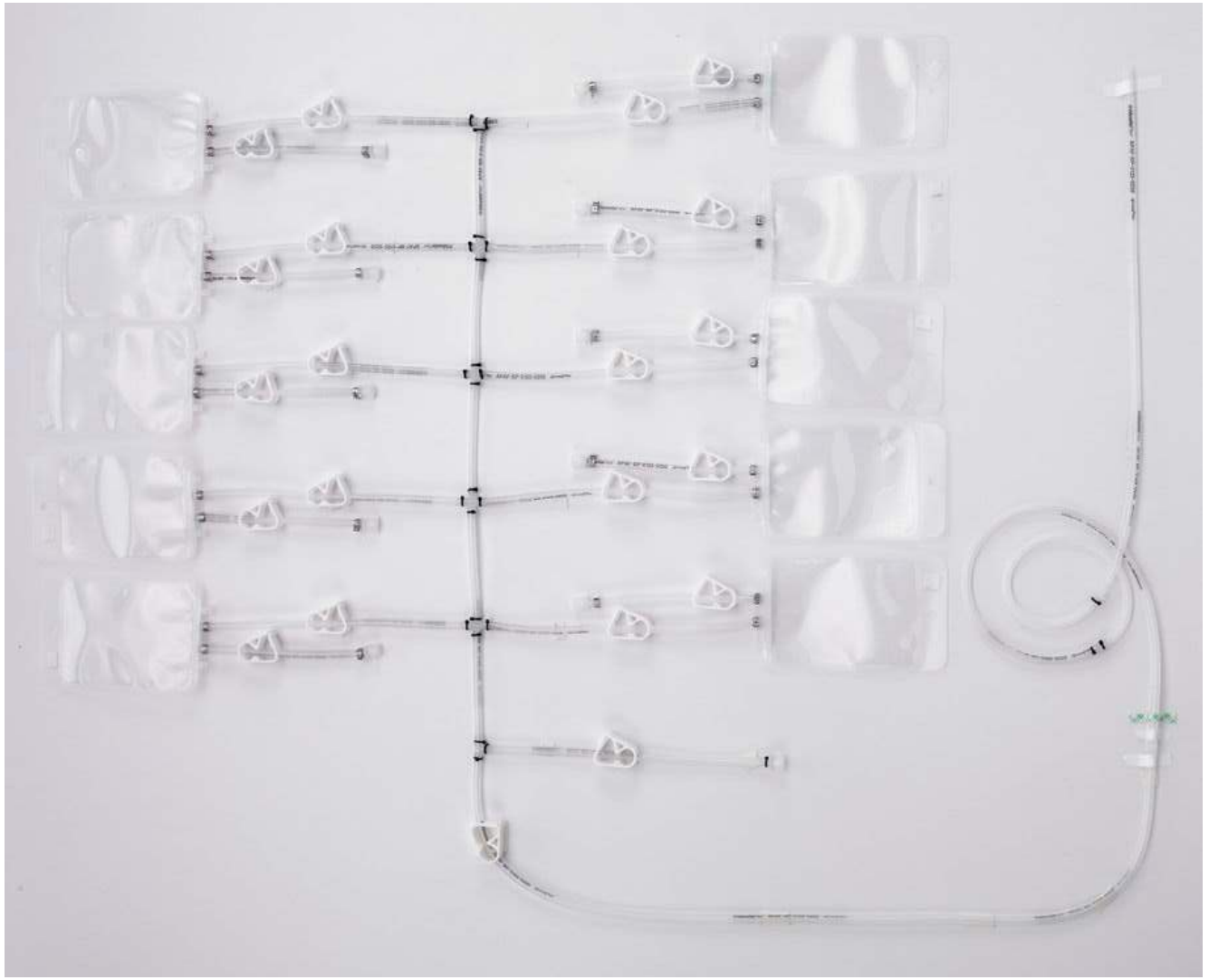
SU-ADM-000048	ADVANCED CRYO SOLUTION KIT, ARAMUS, 5X 100ML
SU-ADM-000049	ADVANCED CRYO SOLUTION KIT, ARAMUS, 10X 100ML
SU-ADM-000050	ADVANCED CRYO SOLUTION KIT, ARAMUS, 5X 250ML
SU-ADM-000051	ADVANCED CRYO SOLUTION KIT, ARAMUS, 10X 250ML





5 & 10 Bag Assemblies





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THANK YOU

Backup Slides

Study on HDCB Application with Aramus™

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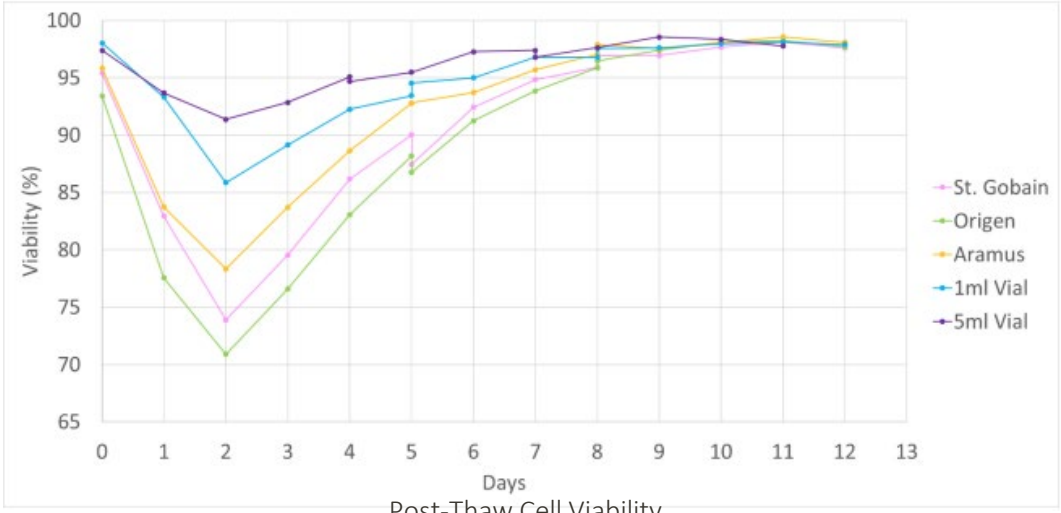
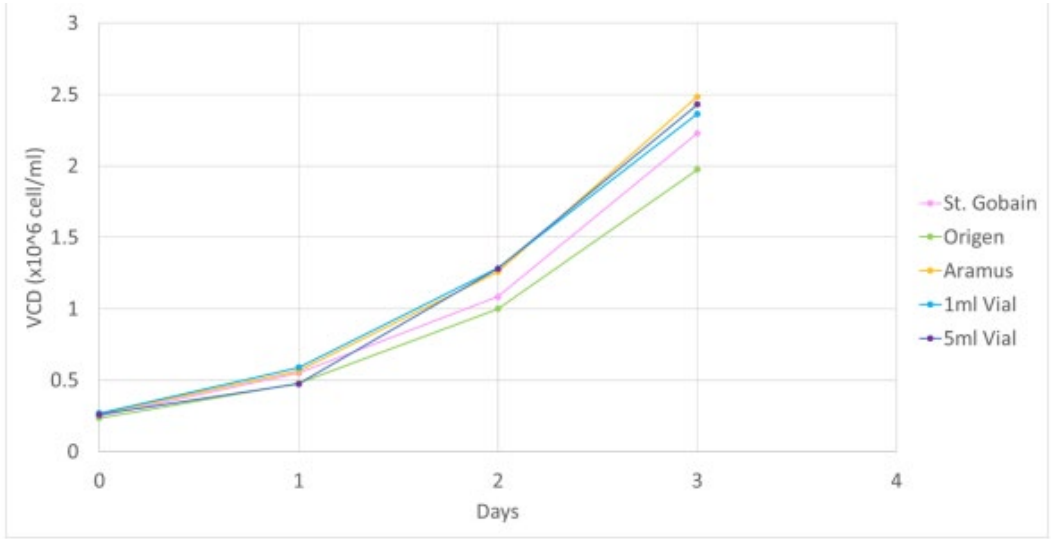
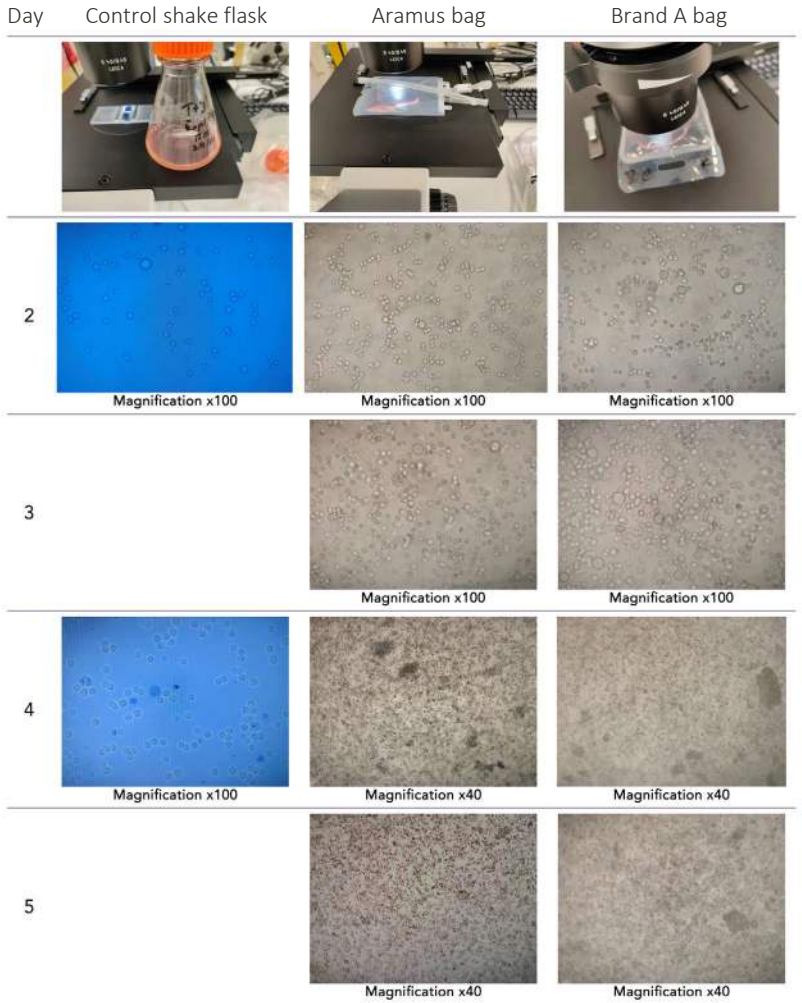


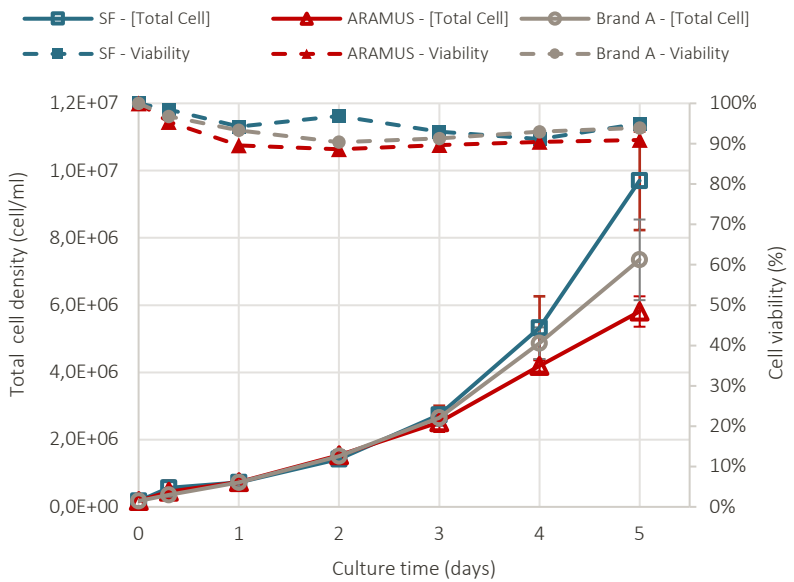
Post-Thaw Cell Viability



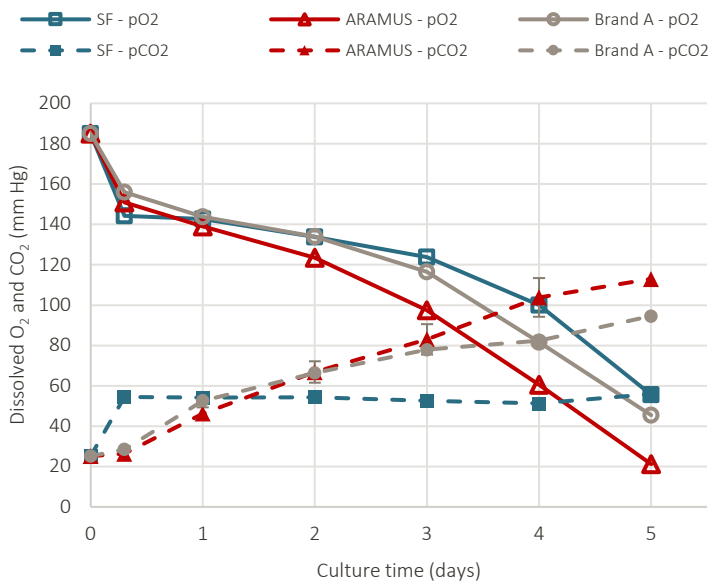
Passage 1 Viable Cell Density

Fluoropolymer Aramus Bags are Suitable for Cultivation of Gene Therapy HEK293 Cells Up to 5 Days





Cell density and cell viability in cultures. Average and standard deviation from 3 to 5 replicates.

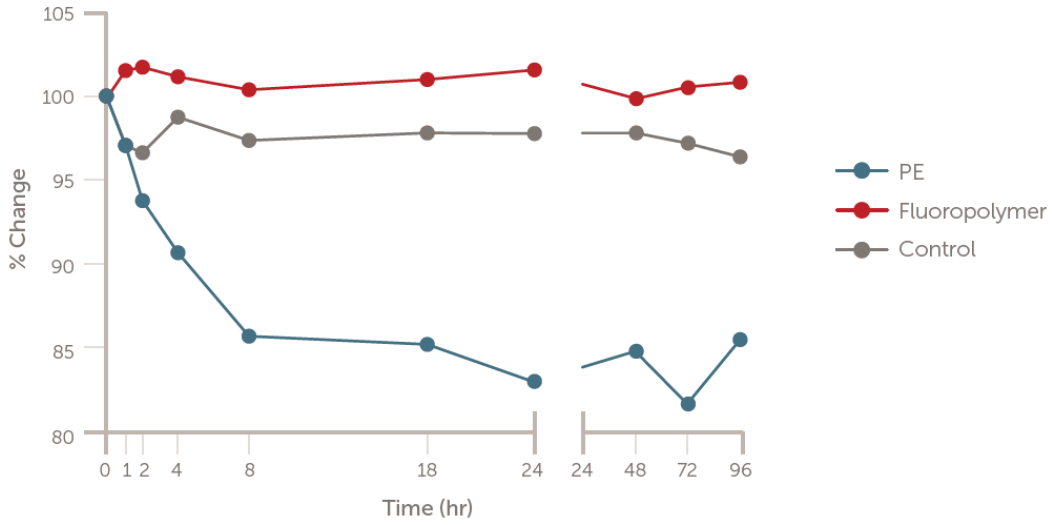


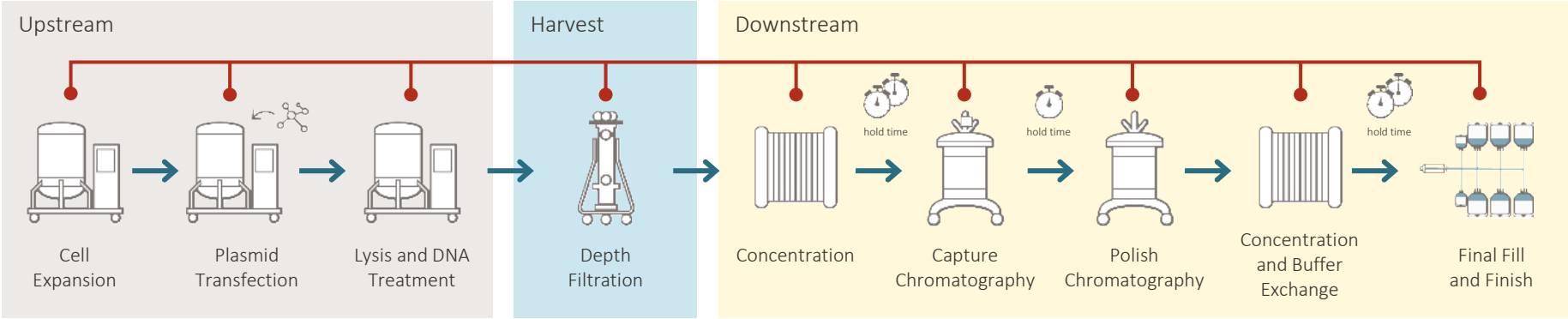
Partial pressure (p) of oxygen and carbon dioxide in cell culture media for all conditions over time. Data are the average of 3 to 5 replicates.

Left: Microscopic observations of cell suspension along the culture. Cells from shake flash were sampled, diluted in Trypan Blue and spread on hemocytometer before observation. Cells from bags were directly observed in the bags.

Credit: Julien Muzard (Entegris), Mathieu Cesari (InnobioVir, CPE, France)

Fluoropolymer Bags Demonstrate Minimal AAV Viral Vector Adsorption Compared to PE Bags Held at Room Temperature for 4 Days





- Possible hold steps within manufacturing process
- No extractables during hold steps
- Minimal product loss due to interaction with hold containers

Credit: Fujifilm Diosynth Biotechnologies Team (Cari Sadowski, Sara Mata, Ashoka Maddur, Steve Pincus (now at Neurogene)

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