



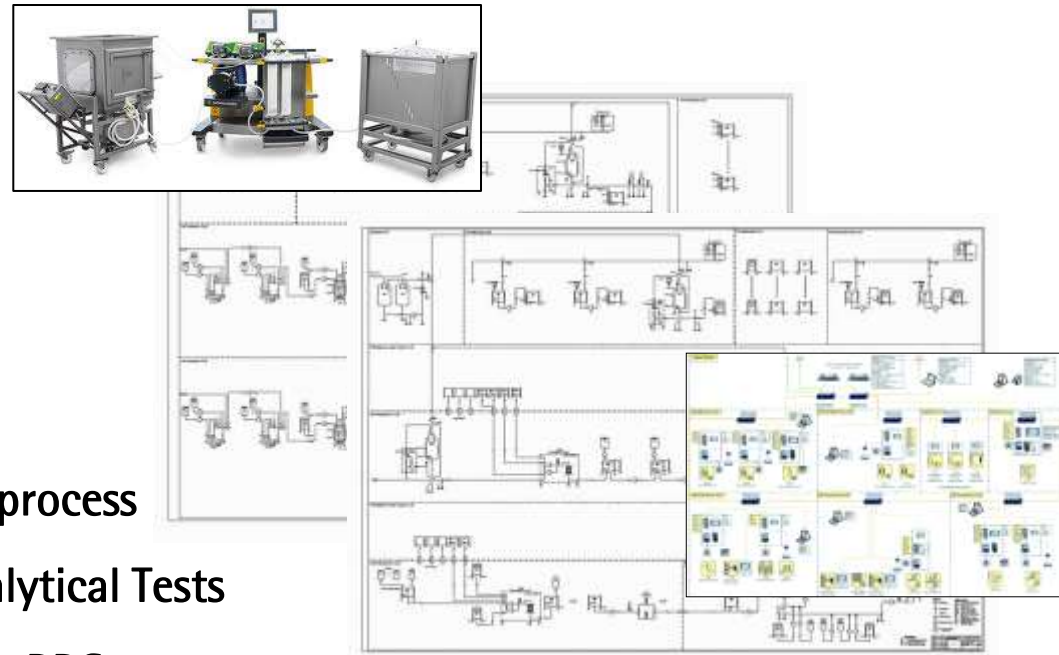
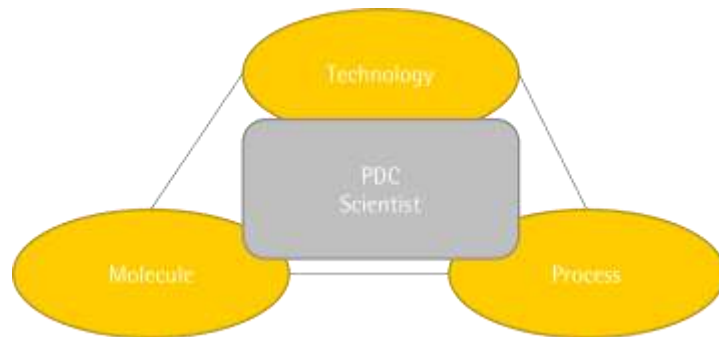
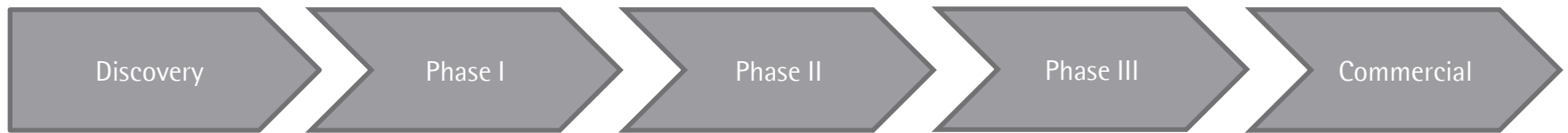
Sartorius early phase mAb Platform  
Process development consultant team introduction



# Agenda

- Introducing the Process Development Consultant team
- mAb Process
- From Concept to Final Product
  - Cellca – Cell Line Development
  - Upstream Process
  - BioOutsource – Analytical Testing
  - Harvest
  - Downstream Process
  - Final Fill and Finish
- Single Use Portfolio
- Process Modeling

**Process Development Consultant (PDC) :** Taking mAb development support to the next level through an integrated network of experts and services



- Choosing the Right Clone
- Perform HTS and scale up of upstream process
- Guide and Execute complete list of Analytical Tests
- Complete Downstream Know how up to BDS

# A Typical Mab Process

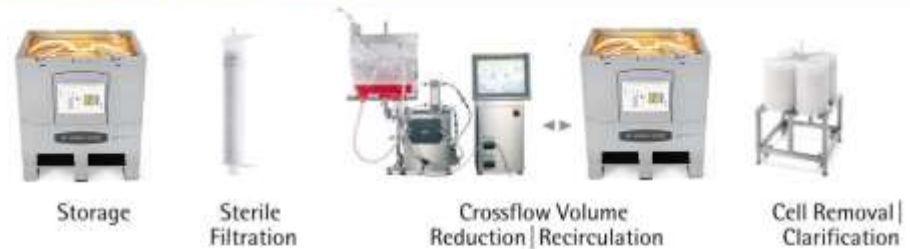
Development of clone and  
Research Cell Bank

Clone and Product testing



## Media Preparation

## Fermentation | Cell Culture



## Buffer Preparation

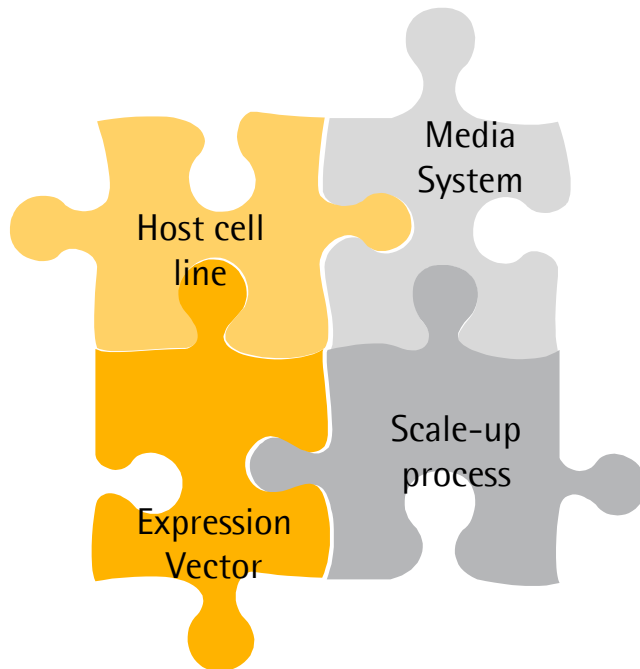
## Cell Harvesting



## Purification

Product Testing

# Technology Platform For Industrial Protein Production



**Host Cell line:** CHODG44 licensed from Prof Chasin, growth in suspension, 18h doubling time, long term stability, predictability



**Expression Vector:** DHFR selection system, low DHFR expression, S/MAR elements, optimal signal peptide



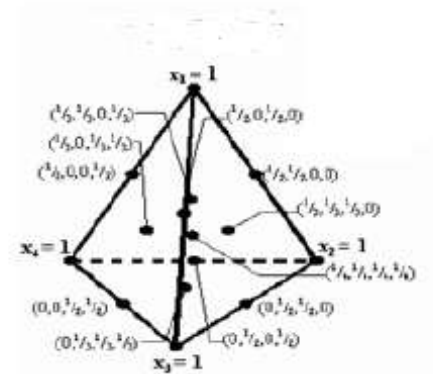
**Media System:** Optimized, chemically defined, free of animal components, proteins & peptones, high cell densities, viabilities & productivity



**Scale-up process:** Robust process, scalable upto 50, 200 or 1000L, transferrable to stainless steel, glass or single use bioreactors, no loss of performance

# The "CHOptimizer"

- Flexible in-house service using the CHOptimizer media optimization package:
  - modular structure
  - choice of support level up to full package provided by a world-wide team of media application specialists
  - choice of supply agreements
- **module 1: media mixtures for Design of Experiment optimization approach**
- module 2: process parameters, spent media analysis, optional CQAs
- module 3: complete fed-batch process, optional CQAs



## Media Screening Diversified Choice

Media for CHO (GS, DG44, CHO-S, CHO K1)	Non-Animal Origin	Protein-Free	Chemically Defined
Base Media			
PowerCHO™ 1	Yes	Yes	Yes
PowerCHO™ 2	Yes	Yes	Yes
PowerCHO™ 3	Yes	Yes	Yes
PowerCHO™ GS	Yes	Yes	Yes
ProCHO4™	Yes	Yes	No
ProCHO5™	Yes	Yes	No
V8 (license required)	Yes	Yes	Yes
Feeds			
PowerFeed A	Yes	Yes	Yes
PowerFeed A w lipid	Yes	Yes	Yes
XtreemFeed	Yes	Yes	Yes

# Upstream Production from Lab to Production: A radically novel approach to upstream mAb development



ambr

15 up to 250 ml



UniVessel SU

0.6 up to 2 liters



BIostat® RM

0.1 up to 300 liters



BIostat® STR

12.5 up to 2,000 liters



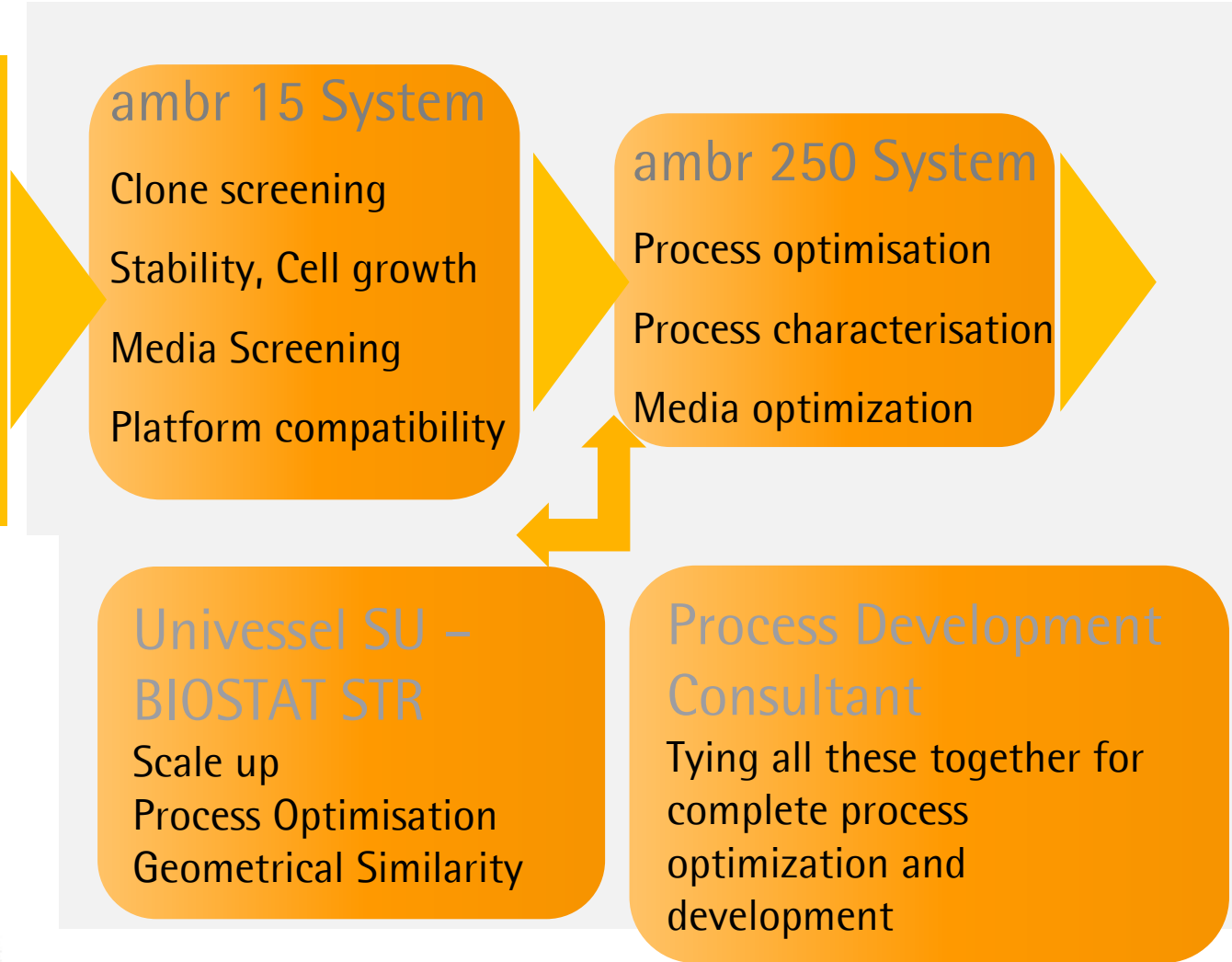




# Process Knowhow (PDC)- Upstream Process Development



High-throughput screening,  
automation, robotics, small scale



## Biosafety Testing Services

Health authorities, including the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), require all biopharmaceuticals to undergo stringent safety testing.

### Testing Capabilities

- Bacterial and fungal contaminants
- Adventitious (unknown) virus
  - Retrovirus
  - Species specific viruses
- Genetic Characterisation
- Analytical methods

### Analytical methods (Mainly on BDS)

- pH and appearance
- Endotoxin Testing
- Determination of BSA by ELISA
- Residual Host Cell Protein by ELISA
- Residual DNA by PCR



# Cell Harvesting: Finding the perfect solution

## Sartoclear Depth Filtration



- » Fully disposable solution
- » Plug and Play Technology

## Sartoclear Dynamics (Body-Feed Filtration)



- » Replaces the centrifuge and Depth filtration in high cell density processes
- » Enables fast flow rates and does not need pre-flushing
- » Primary and secondary clarification in one
- » Single use processing creates flexibility
- » Small footprint compared to centrifuge.

# Centrifuge-free single-use harvesting becomes a reality with Sartoclear Dynamics

1 – 2000 L



- New SU technology
- Based on body feed filtration
- Eliminates the centrifuge
- Up to 2000 L in a single step
- No pre-flushing

## Product Highlights

### Single-Use

For high cell density fed-batch cultures

### Robust

Independent on cell viability changes

### Fast

Very high Flow rates and no need for pre-flushing

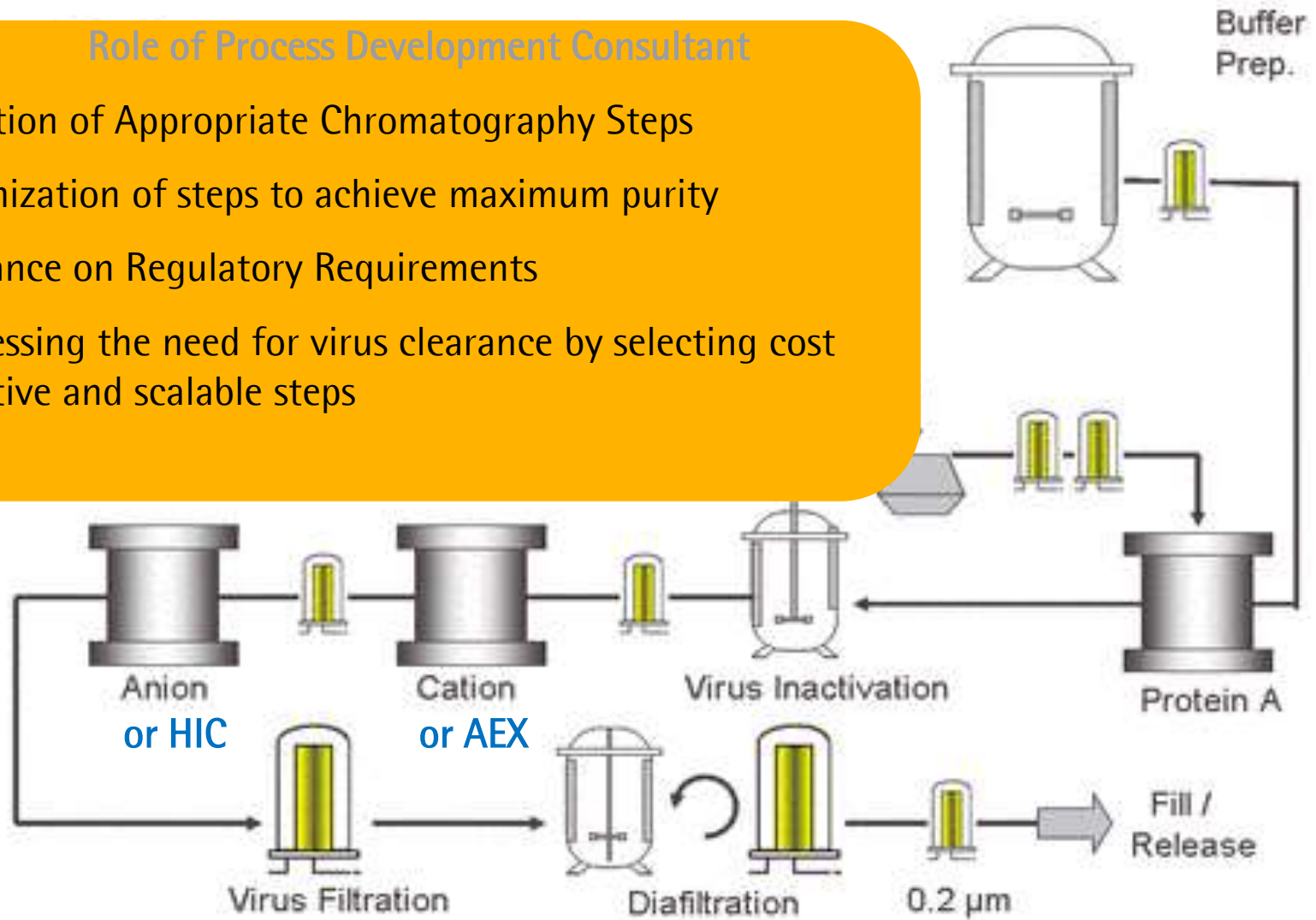
### Single Step

Primary and secondary clarification in one step

# Downstream Process Flow for a Mab Process

## Role of Process Development Consultant

- Selection of Appropriate Chromatography Steps
- Optimization of steps to achieve maximum purity
- Guidance on Regulatory Requirements
- Addressing the need for virus clearance by selecting cost effective and scalable steps



# Chromatography for a Mab Process

Process step	Products Available	Features /Product positioning
<p><b>Polishing</b></p> <ul style="list-style-type: none"> <li>▪ Q (AEX)</li> <li>▪ STIC (AEX)</li> <li>▪ HIC</li> <li>▪ S (CEX)</li> </ul>	 <p>4 mm bed height</p>	<p>Contaminant removal: flow-through mode to remove DNA, Host cell proteins, endotoxins, viruses</p> <p>Single-use</p>
<p><b>Bind and Elute</b></p> <ul style="list-style-type: none"> <li>▪ Q (AEX)</li> <li>▪ HIC</li> <li>▪ S (CEX)</li> </ul>	 <p>8 mm bed height</p>	<p>Purification: bind &amp; elute of viruses and virus like particles, large proteins</p> <p>Single-use / intra-batch use / re-use</p>

## Gel versus Membrane when polishing of a monoclonal antibody- Decreased Operation Time

	Q Resin	Q Membrane
Flux / flow rate	100 – 150 cm/h	450 – 600 cm/h
Capacity	50 – 100 g/L	> 3 kg/m <sup>2</sup> or > 10.9 kg/L
Buffer used	100 %	5 %
Operation time	8 – 9 h	2 – 2.5 h
Cleaning validation	Yes	Single use

Zhou J X, Tressel T, AMGEN Inc.: Membrane chromatography as a robust purification system for large-scale antibody production. *Bioprocess International*, Sept. 2005, p. 32-37

# Orthogonal Virus Clearance – A Regulatory Requirement

Inactivation

UVivatec®



Inactivates most Viruses  
> 4 logs Inactivation of  
PPV/MVM

Adsorption

Sartobind® Q Membrane Adsober



Adsorbs all Viruses  
> 4 logs Adsorption of  
PPV/MVM  
> 5 logs Adsorption of  
xMuLv



Size Exclusion

Virosart® CPV/HF Nanofiltration



Robust Filtration of all  
Viruses  
> 4 logs Removal of  
PPV/MVM  
> 6 logs Removal of xMuLv



Process step	Products Available	Features /Product positioning
<p>UF/DF Re-usable</p>	 <p>Hydrosart</p>	<ul style="list-style-type: none"> <li>• Crosslinked Regenerated Cellulose</li> <li>• &gt; 95% Recovery</li> <li>• Low Protein Binding</li> <li>• Easy Cleanability</li> <li>• 1N NaOH stable</li> </ul>
<p>UF/DF SU</p>		<ul style="list-style-type: none"> <li>• Pre flushed and sterilized disposable units</li> <li>• Single Use design eliminate cleaning in place efforts</li> <li>• Chances of adoption higher in CMO environments</li> </ul>

Guiding from small lab scale to large scale manufacturing with complete process optimization know how



# Sartoflow Crossflow Equipment

Bench, Pilot, and Production Scale UFDF



Slice 200



Advanced



Alpha Plus



Beta Plus

SU Production Scale UFDF



FlexAct UD



Sartoflow Alpha Plus SU

## Your Filtration Needs

- Broad Spectrum of Filters for both prefiltration and sterile filtration
- Helping you choose the right filter is the key
- With small scale filtration trials performed by our state of the art Zero T system

### Small Scale



### Large Scale



### Integrity Tester Sartocheck



## Bioburden Control/Sterility Assurance in mAb/Rec. Prot. Manufacturing

### Sartopore® Filter Platform

Buffer Prep

Media Prep

Harvest, DS  
Intermediates

Bulk Drug  
Substance

#### Sartopore 2 (HF)

- High flow rate
- Single layer 0.2  $\mu\text{m}$  for large-scale Buffer Prep.
- Double layer configuration 0.45/0.2  $\mu\text{m}$

#### Sartopore 2 XL\*

- High capacity 0.2 or 0.1  $\mu\text{m}$  filters
- Heterogeneous Double layer configuration
- Validated mycoplasma removal with 0.1

#### Sartopore 2 XLG

- High capacity 0.2  $\mu\text{m}$  rated filter
- Double layer configuration 0.8/0.2  $\mu\text{m}$

#### Sartopore Platinum

- Highly hydrophilic
- Easy wetting
- Most reliable integrity testing
- Largest surface area per filter element

## Bringing it all together - Robust Single Use Processing for All Scales

### One Film For All – No Matter What Size or Process Step



Flexsafe® enables single-use bioprocessing throughout all process steps and all applications – all using just one film

With a unique material throughout all process steps, Flexsafe® significantly reduces the validation effort and costs

- Extractables and leachables studies,
- Toxicological assessment and stability studies

#### Bags for all bioprocessing steps



Raw material and process controls provide a well-characterized and consistent extractables profile

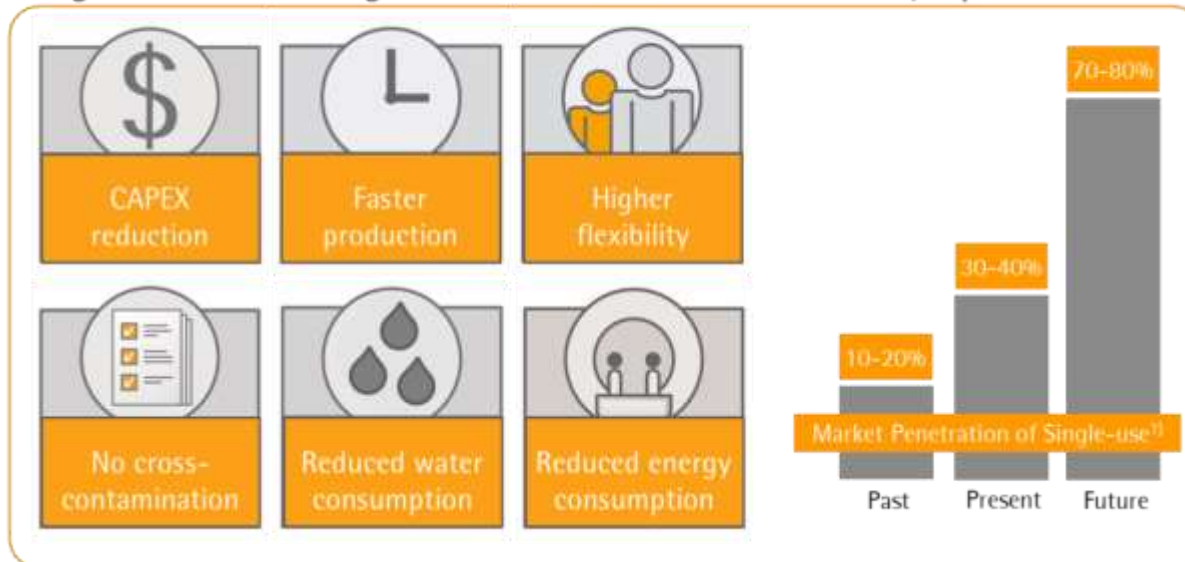
- Facilitate leachables validation & toxicological assessment
- Sustainable, reproducible & reliable process validation data

Fast process development and process set up through scalable, single-use solutions

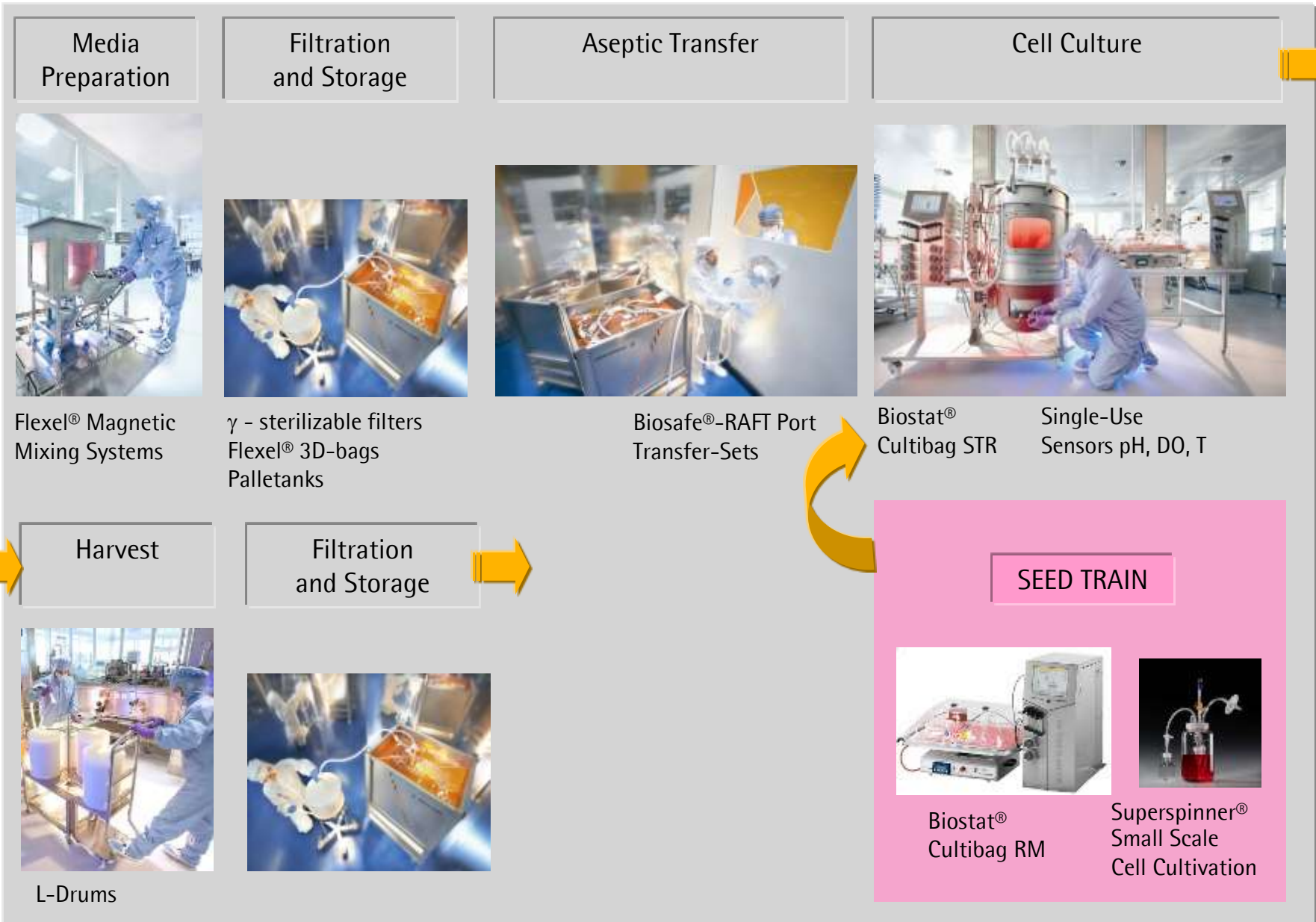
Products in Market :

- High throughput bench scale devices
- SU Solutions at bench, pilot, and production scale
- Scalable devices







Single-use Technologies Reduce Production Costs by up to 40%



# UPSTREAM Single-Use Portfolio



# DOWNSTREAM Single-Use Portfolio

 Diafiltration Concentration	Aseptic Transfer	Filtration and Storage	Polishing and Storage	
				
Sartoflow® Alpha plus-SU	Biosafe®-RAFT Port Transfer-Sets	Flexboy® bag with $\gamma$ - sterilizable filters	Sartobind® Membrane chromatography	

 Virus Inactivation	Storage	Virus Filtration	Formulation and Transfer	Aseptic Transfer
				
UVivatec	Flexboy® bag	Sartopore 2 gamma 0,1 $\mu$ m Virosart CPV	Certomat RM Flexboy® bag	Biosafe®-RAFT Port



## SSB Overview – Sterile Connections / Disconnections



**Opta® SFT Connector**  
Mechanical connection  
Si(Pt) tubing, TPE tubing  
others tubings (if qualified)



**BioWelder®**  
Thermal connection  
TPE tubing



**BioWelder® TC**  
Thermal connection  
Liquid filled TPE tubing

Sterile connections

Sterile Disconnections



**Clipster® Aseptic Disconnecter**  
Mechanical disconnection  
Si(Pt) and TPE tubing



**BioSealer®**  
Thermal disconnection  
TPE tubing



**Vante™ Sealer®**  
Disconnection by RF  
EVA, PVC tubing

# From Reusable to Single-use Technologies: How to get to a meaningful assessment?



# BioSolve Process Configurable Process Model - Process Modelling provided by the Process development consultant team

Rapid process configuration

Rapid option evaluation

Technologies

- Processes
- Scalable mass balance
- Standard format
- Consumables estimates

Scalable equipment list

- Based on standard costs, sizes
- Basis for capital cost estimate

Insight into utilities, environment

- PW & WFI
- Waste streams

Capacity and capital estimates

Process Definition		MAb Typical
Product Titre		5.00 g/L
Capacity Utilisation		80%
Production Bioreactor Volume		5000 L

Single-Use Systems	
Bioreactors (up to 2000L)	No
Media Preparation (up to 2000L)	Yes
Buffer Preparation (up to 2000L)	No

Process Sequence	Conc (g/L)	Vol (L)	Mass (g)	Yield (%)	Time (h)
1 N-2 Seed	0.0	50	0		58.0
2 N-1 Seed	0.0	500	0		59.0
3 Production	5.0	5,000	25,000		210.0
4 Centrifugation	5.0	4,250	21,250	85%	9.0
5 Depth Filtration	4.3	4,250	18,063	85%	9.2
6 UF/DF #1	40.4	425	17,159	95%	12.4
7 Protein A	3.1	4,926	15,443	90%	12.9
8 Virus Inactivation	3.0	5,025	15,135	98%	2.5
9 Capture IEX	7.8	1,847	14,378	95%	11.4
10 Flow Through IEX	7.4	1,847	13,659	95%	6.2
11 Viral Filtration	7.2	1,847	13,386	98%	9.3
12 UF/DF #2	35.5	369	13,118	98%	12.4
13 Sterile Filtration	34.8	369	12,856	98%	9.1

# Process Development through Commercial Scale BioProcess Solutions – We are there every Step of the Way

Fully scalable, robust, single-use upstream portfolio (250ml-2000L).

Downstream single use solutions for all processing scales.

FlexSafe PE film for robust, consistent, single use processing.

Process modeling capabilities to drive single use technology selection and process optimization.

Full complement of single use technologies and services to facilitate rapid and efficient process development.

# Complete Product Portfolio to Allow for Single-Use mAb Process Development

