

Vibalogics

The Vaccine Manufacturer

5th VPM Development Days
Hannover
1-2 September, 2011
Dr. Kai Lipinski, PhD

VIRUSES

BACTERIA

BIOLOGICS

Implementation of Disposable Technologies for the Manufacture of
Biologicals – Live Virus & Microbial Case Studies



Company



Service



Facility



Philosophy



Project

BioPharma stake holders: challenges & strategies

Industry Growth

New Technologies

Smaller Markets

Cost Pressures



Multiple Customers

Multiple Processes

Multiple Products



Adaptable Process Scales

Product segregation

Dedicated Process Equipment

Control Capital investment



DISPOSABLES

Applications of Single-Use-Equipment

- Bags (storage, mixing, media preparation, etc.)
- Fermentation
- Filtration technologies
- Rotors/Centrifugation
- Virus Reduction (Nanofiltration)
- Chromatography (Membrane adsorbers)
- Vials, pumps and vials filling systems
- Sensors (Lactate, Glucose, pressure)
- Sampling system
- Sterile tubing connectors
- Sterile tube welding

Pros:

- Reduced cleaning and sterilization (and validation)
- Reduced engineering costs
- Reduced equipment lead times
- Easier and quicker process set-up
- Reduced cross-contamination risk
- Reduced investment

Cons:

- Higher risk of material failure
- Limited scalability
- Increased running costs/waste
- Restricted compatibilities (e.g. sensors, sampling)
- Leachables/extractables risk
- Supply chain dependency
- Regulatory acceptance

Case Study I

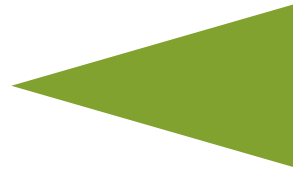
**Virus production in disposable,
fixed-bed bioreactor (iCELLis)**

Development towards commercial scale

Development scale
(bed: 40mL – 400mL)



18cm



Commercial scale
(bed: 7.5L – 37.5L)



25L iCELLis™ bioreactor mounted on process control skid

Current developments:

- Stainless steel lid version: ± non-disposable biomass probe
 - Fully disposable PD version: + disposable biomass probe
- 5-10m² scale: (117x 850cm² RBs)

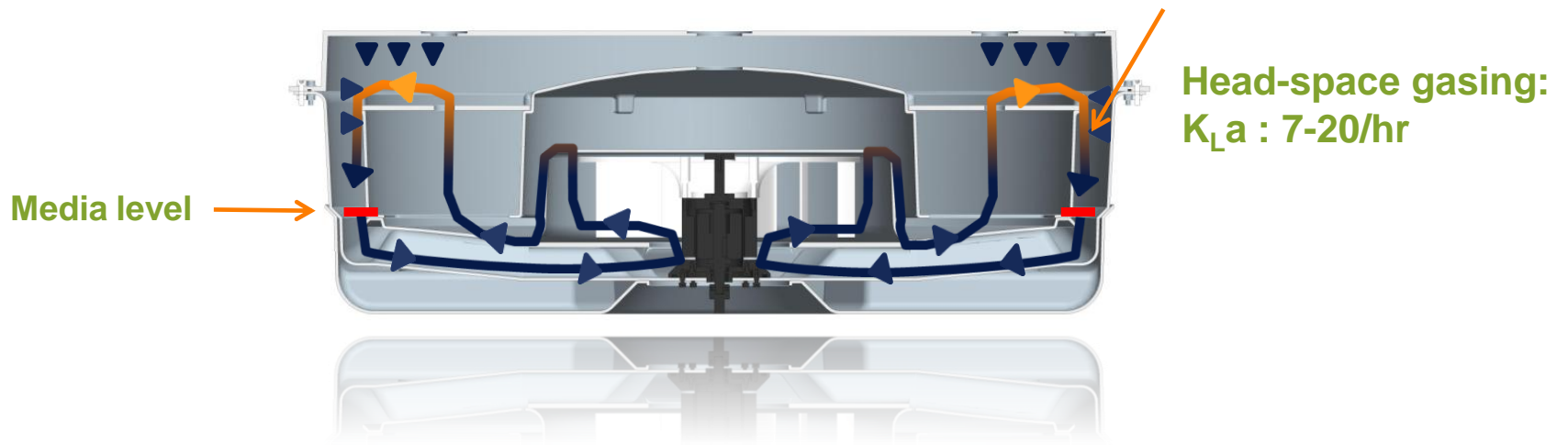
Principle: Fixed Bed & Waterfall oxygenation

iCELLis 1000

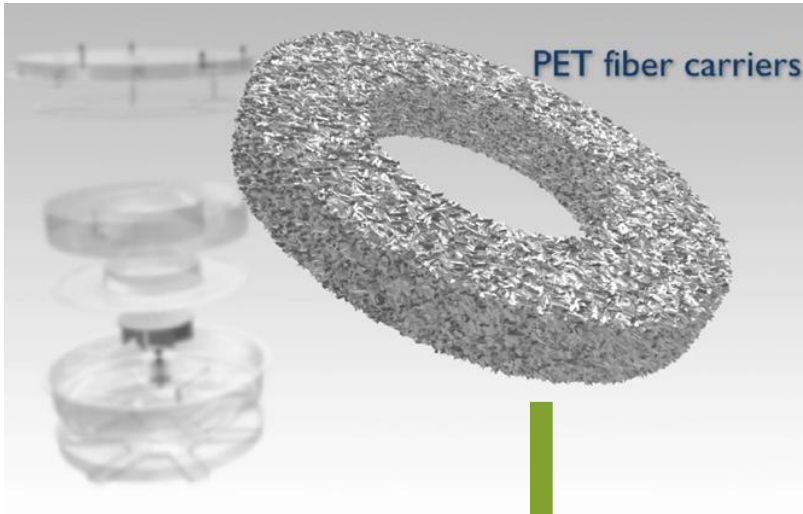
Average media fill = 50-60L

Waterfall Media

Oxygenation



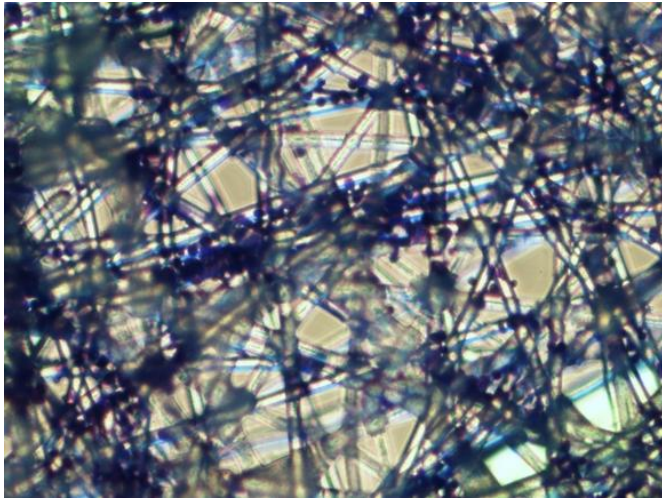
PET Carriers



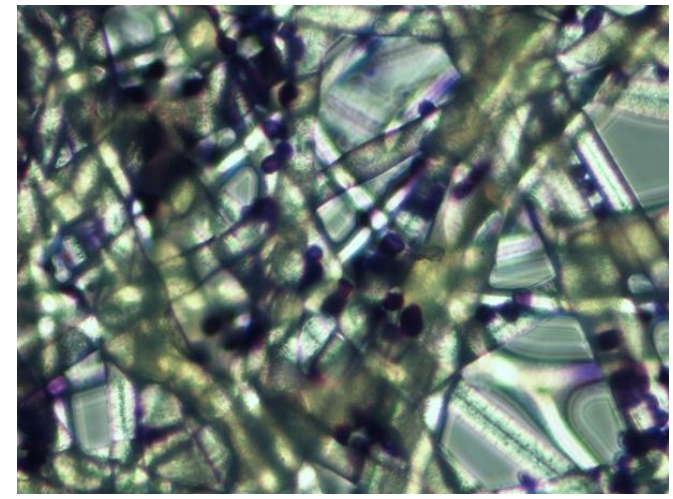
- In-house carrier production
- Microfibers of PolyEthyleneTerephthalate (PET)
- Medical-grade, non-woven
- Compressed into doughnut-shaped basket
- Carriers for adherent & suspension (entrapment) cell culture

1 carrier = 11.2cm² growth surface

Growth study development scale: VERO cells



< Post-Inoculation >



	Top	Middle	Bottom
1	1,368E+06	1,641E+06	1,816E+06
2	1,175E+06	1,566E+06	2,002E+06
3	1,023E+06	1,521E+06	1,947E+06
4	1,034E+06	1,616E+06	1,914E+06
5	1,304E+06	1,657E+06	1,440E+06
Average	1,181E+06	1,600E+06	1,824E+06
Total cells/mL fixed bed	2,789E+07	3,779E+07	4,307E+07
Total cells/cm²	2,090E+05	2,832E+05	3,228E+05
Total cells/carrier	2,362E+06	3,201E+06	3,648E+06

- Serum-free medium cultivation

- 9 days growth to exhaustion

-> high surface density

Virus Production in iCELLis bioreactor

- Paramyxovirus model (enveloped) virus
- Comparison T-flask versus iCELLis bioreactor
- Analysis with:
 - Infectious particle titer (TCID₅₀ and EID₅₀)
 - Genomic particle titer

(based on double-staining of DNA/RNA and protein; InDevR)

Virus production T-flask versus iCELLis bioreactor

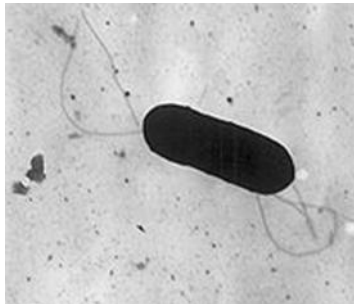
Normalization: Growth surface			
	Assay Type		
Vessel	TCID ₅₀	EID ₅₀	Virus Counter
T-flask	1	1	1
Nano200	11.2	5.5	11.2

Normalization: Cell number			
	Assay Type		
Vessel	TCID ₅₀	EID ₅₀	Virus Counter
T-flask	1	1	1
Nano200	7.9	3.9	7.9

Case Study II

Production of live bacterial vaccine

and foam-drying technology



Advaxis Inc.: The Therapeutic Live Listeria Biotech Company

- *Listeria monocytogenes*: facultative pathogen for human (Listeriosis; food poisoning)
- *Listeria m.* is highly immunogenic:
 - infects APC to induce innate and adaptive immune responses (MHC class I and II)
 - reduces T_{reg} & MDSC (Myeloid-Derived-Suppr-Cells) selectively in tumours
 - increases synthesis of new myeloid cells
 - causes maturation of immature myeloid cells
 - Many salutary, non-classical immune effects
- Multi-deletion mutants are safe and well tolerated (auxotrophy and reduced virulence; **S1-level**)
- Expression of truncated LLO (lysteriolysin O)-Ag fusion proteins enables efficient proteolysis & MHC presentation
- Current pipeline:
 - HPV
 - Prostate cancer (PSA)
 - HER2/neu-positive cancer types



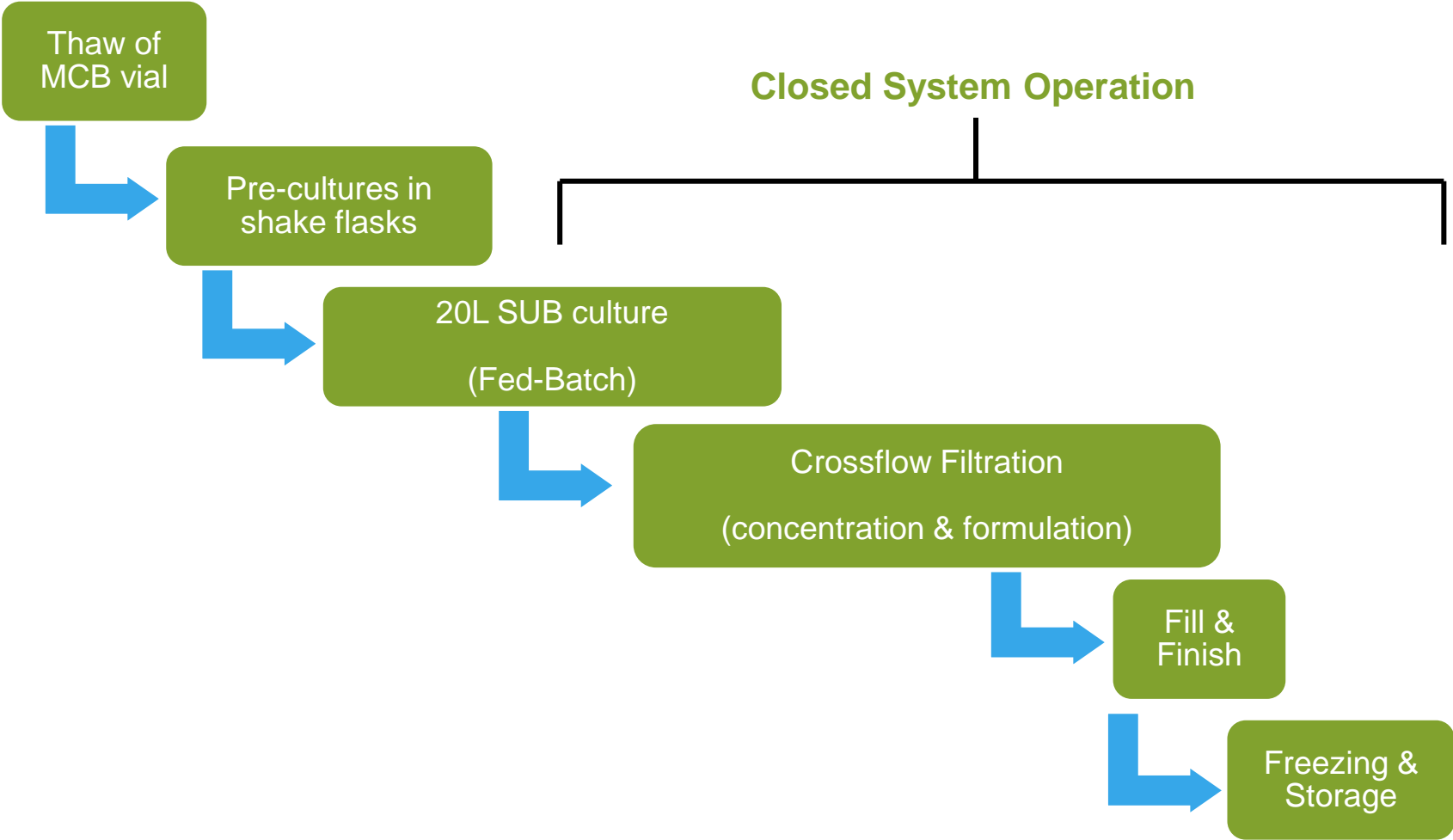
ADVAXIS

Sartorius SUB Technology



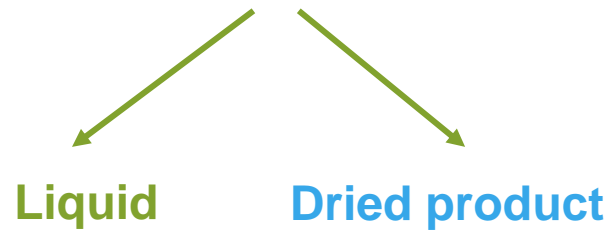
- Range: 12.5L - 1000L
- „Plug & Play“ BioPAT MFCS/win cGMP-compliant software (Multi-Fermenter-Control-System)
- Option for perfusion

Process flow

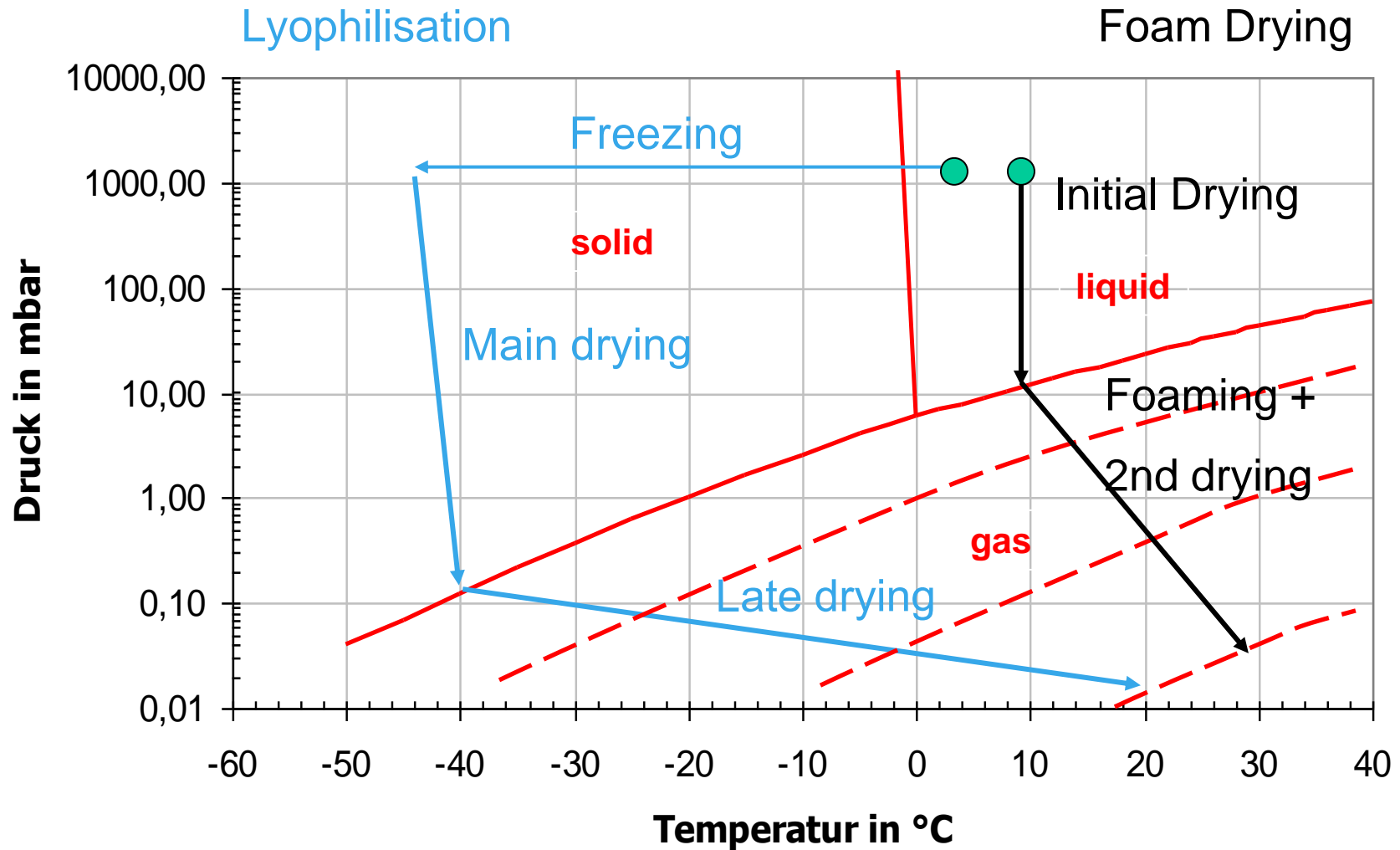


Listeria: SUB Production

Sample	OD ₆₀₀	VCC [cfu/mL]	Weight [kg]
Fermentation broth	5.2	7.6x10 ⁹	20.92
Drug substance	37.0	9.4x10 ¹⁰	1.54
Concentration factor	7.1	12.4	13.6



Introduction: Lyophilisation versus Foam Drying



Freeze-Drying versus Foam-Drying

Cake

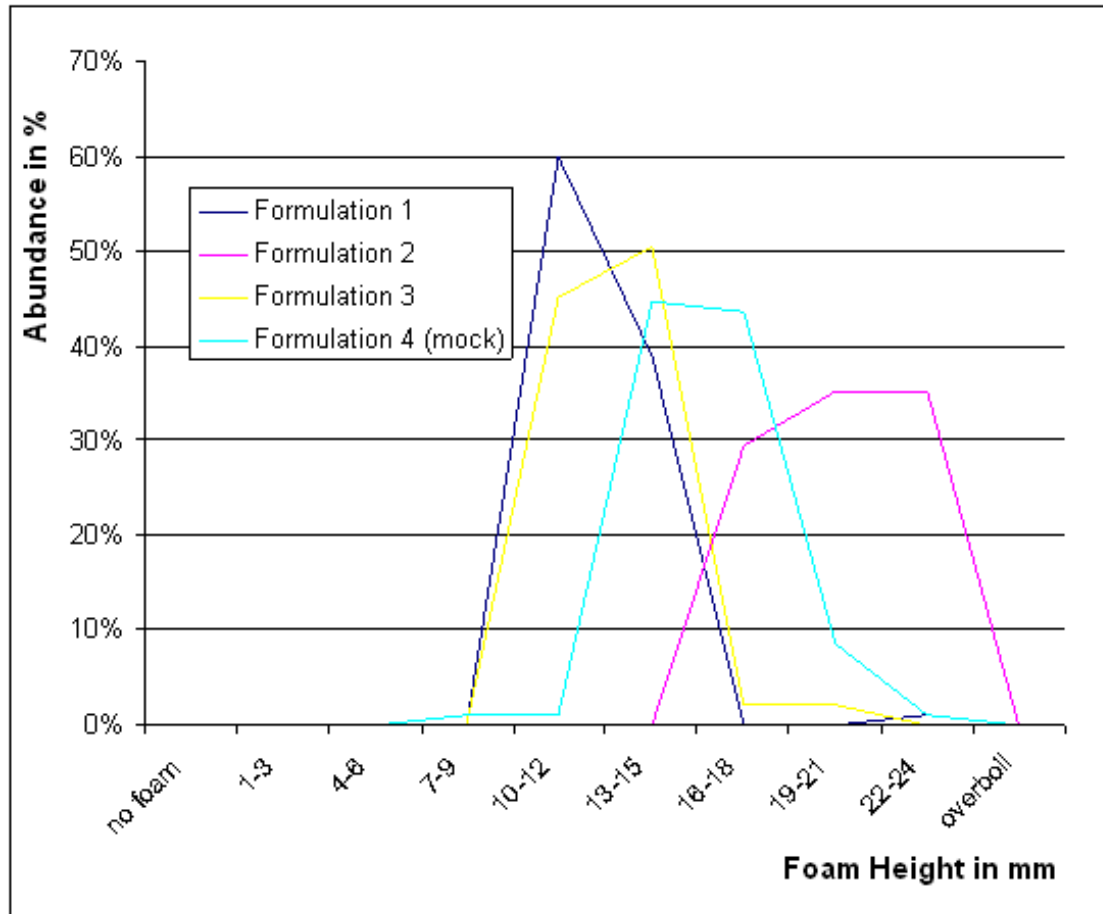


foam



- Control of formulation (foam matrix), temperature and vacuum programs are crucial parameters
- QC:
 - appearance
 - residual moisture
 - foam height
 - live titer / stability

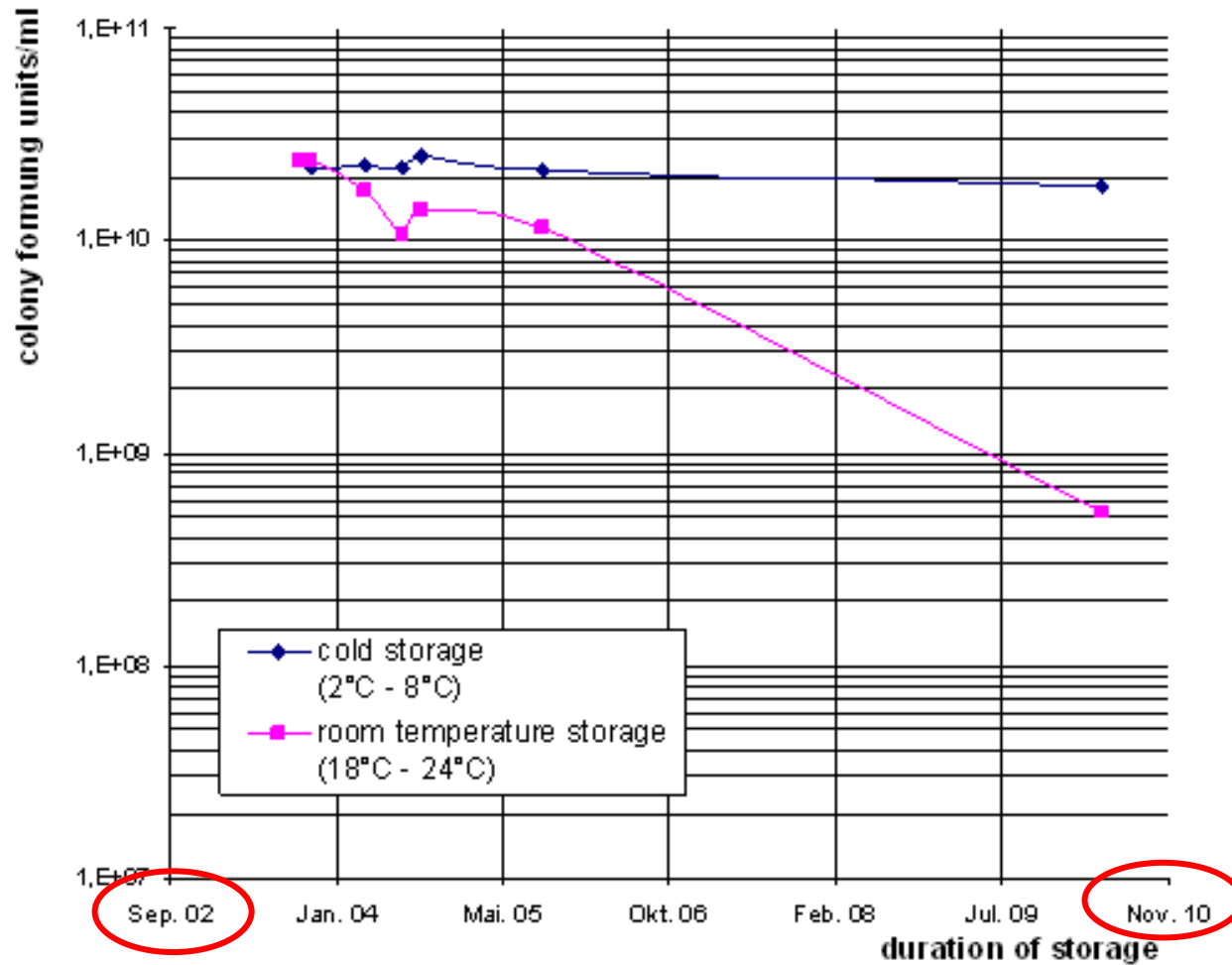
Formulation versus foam height distribution (a)



Recovery of foam-dried live *Listeria*

	VCC				
	before drying		in foam		Residual Moisture in %
	in 1 ml	absolut per vial	per vial	Recovery in %	
Formulation 1	5.4E+10	2.3E+10	8.7E+09	38	
Formulation 2	4.6E+10	1.9E+10	8.6E+09	44	
Formulation 3	5.4E+10	2.3E+10	7.4E+09	33	
Formulation 4 (mock)	n.a	n.a	n.a	n.a	

Stability data: *Salmonella* sp.



Summary

- Disposables: offer many strategic solutions relevant to CDMOs
- Disposables: help to meet client's expectations
- iCELLis: attractive system for adherent culture and virus production
(and might substitute egg systems on case-by-case evaluation)
- SUB: applicable to cell culture and microbial fermentation
-> extend to other important species (e.g. *Salmonella*, *Mycobacterium*)
- Foam Drying: proof-of-principle for *Salmonella*, *E.coli* and *Listeria*
-> virus feasibility studies will follow

Thank you

Vibalogics GmbH

Zeppelinstraße 2 | 27472 Cuxhaven |
www.vibalogics.com

