Vibalogics

The Vaccine Manufacturer

5th VPM Development Days
Hannover
1-2 September, 2011
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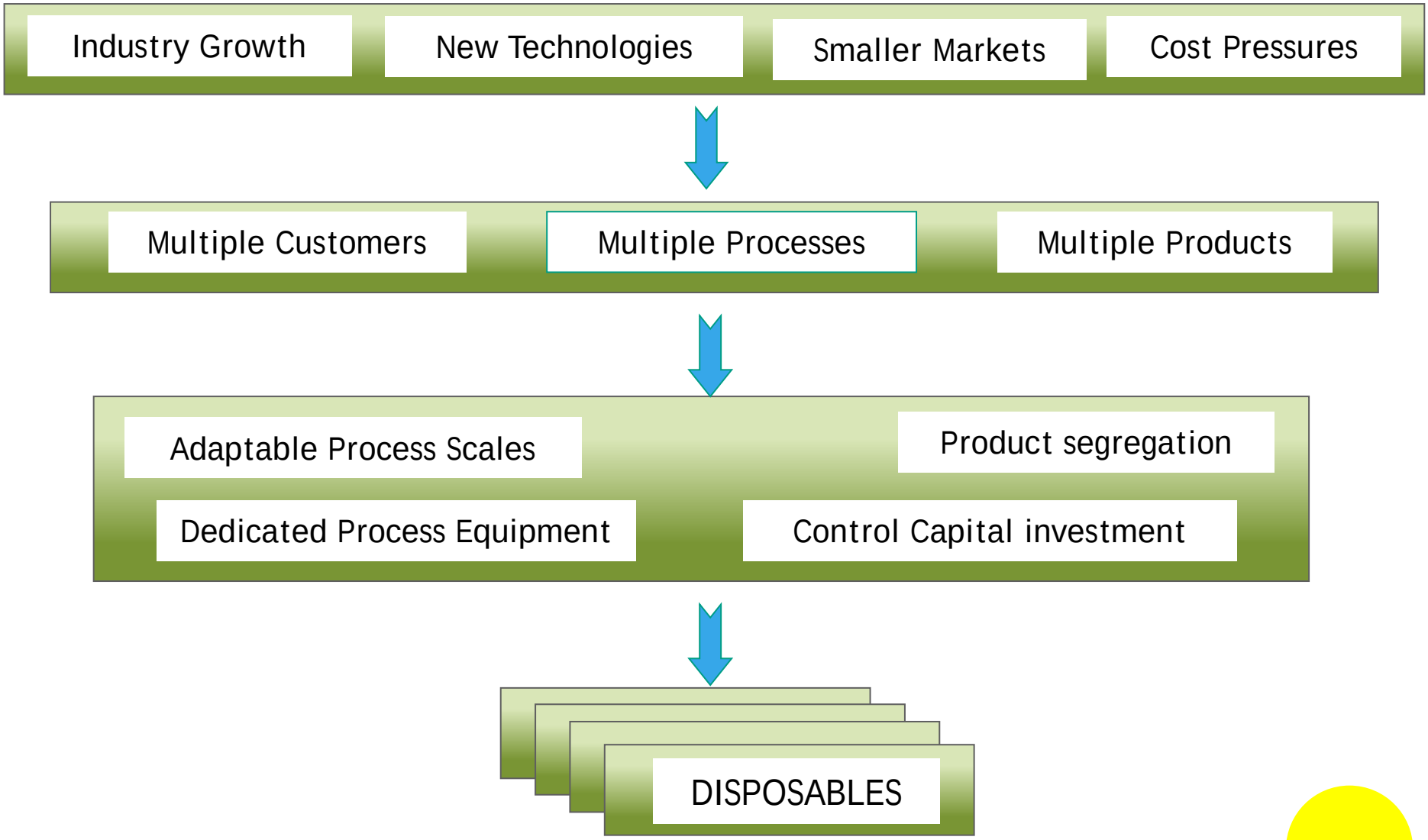
VIRUSES
BACTERIA
BIOLOGICS

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

BioPharma stake holders: challenges & strategies



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,

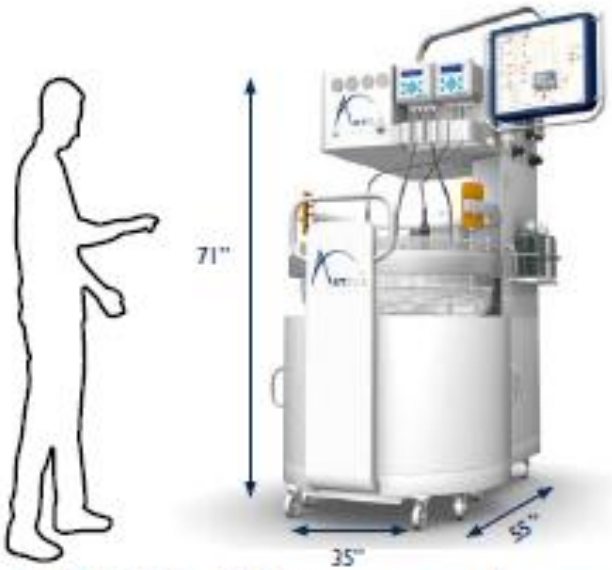
fixed-bed bioreactor (iCELLis)

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
  5-10m^2 scale: (117x 850cm^2 RBs)

Case Study I

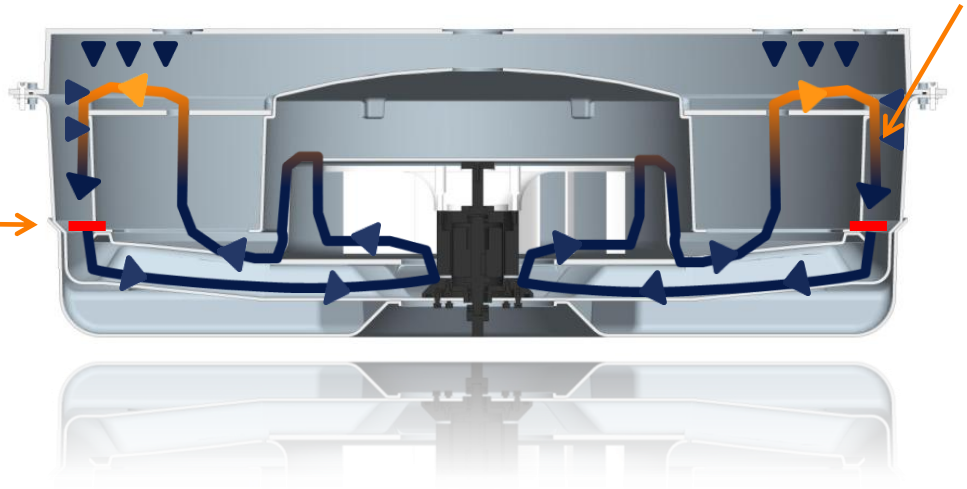
Principle: Fixed Bed & Waterfall oxygenation

iCELLis 1000

Waterfall Media

Average media fill = 50-60L

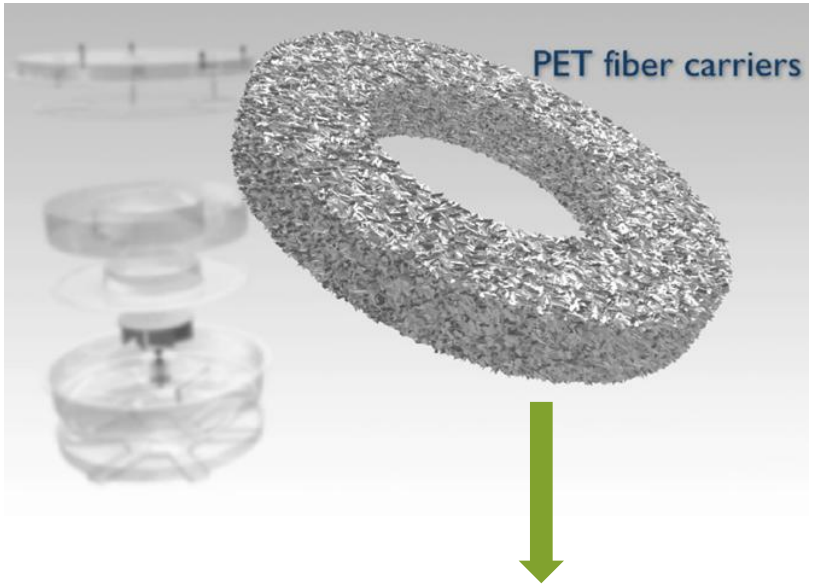
Oxygenation

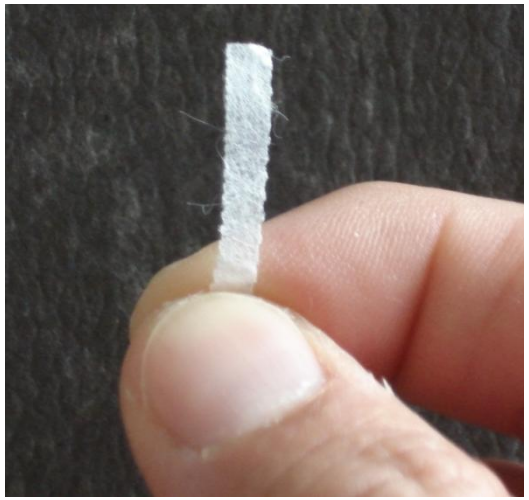


Head-space gasing:
$K_L a$: 7-20/hr

Media level

PET Carriers

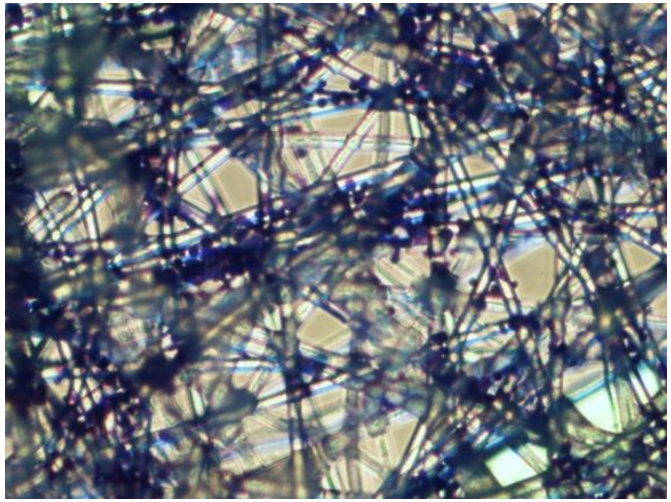




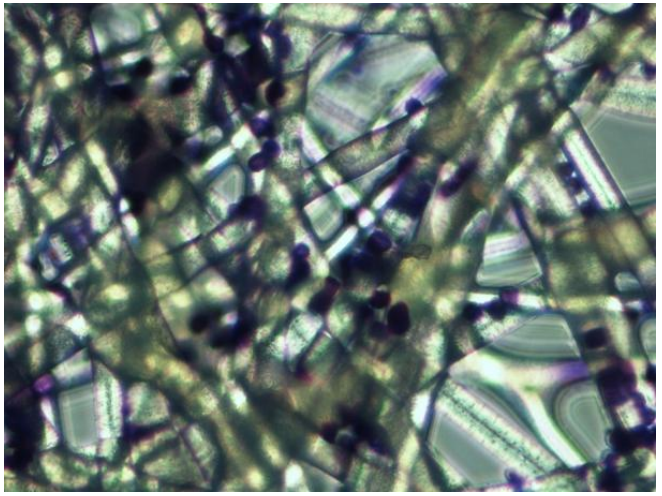
- In-house carrier production
- Microfibers of PolyEthyleneTerephtalate (PET)
- Medical-grade, non-woven
- Compressed into doughnut-shaped basket
- Carriers for adherent & suspension (entrapment) cell culture

1 carrier = 11.2cm^2 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/cm^2	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

Case Study I

Virus Production in iCELLis bioreactor

- Paramyxovirus model (enveloped) virus
- Comparison T-flask versus iCELLis bioreactor
- Analysis with:

  - Infectious particle titer ($TCID_{50}$ and EID_{50})
  - Genomic particle titer

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

Virus production T-flask versus iCELLis bioreactor

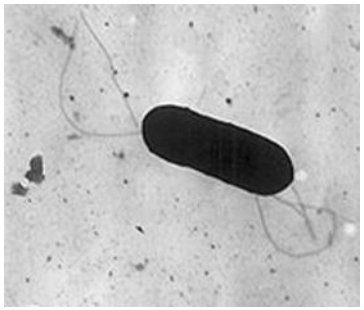
Normalization: Growth surface			
	Assay Type		
Vessel	$TCID_{50}$	EID_{50}	Virus Counter
T-flask	1	1	1
Nano200	11.2	5.5	11.2

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



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Case Study II

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

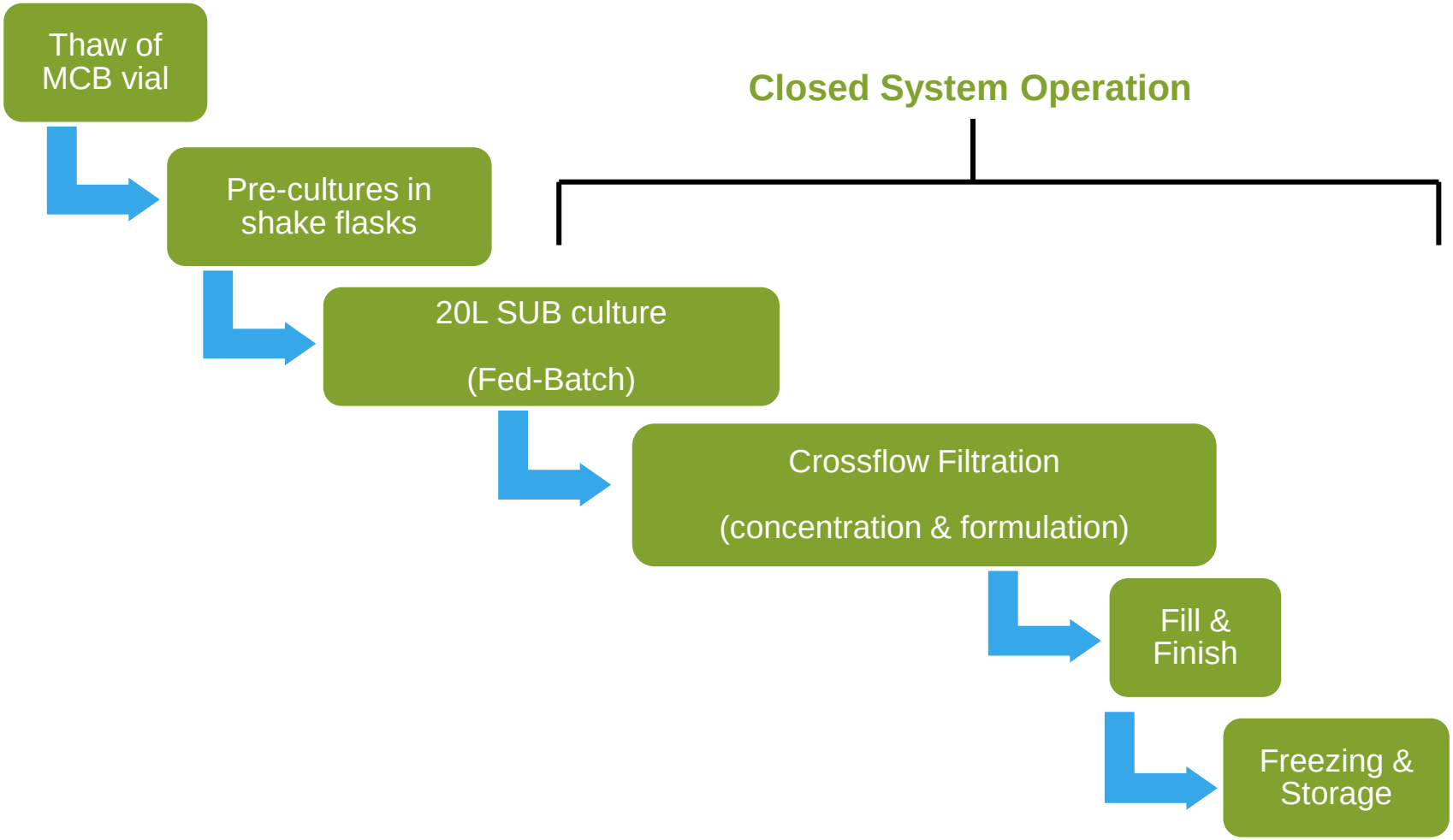
ADVAXIS

Sartorius SUB Technology

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

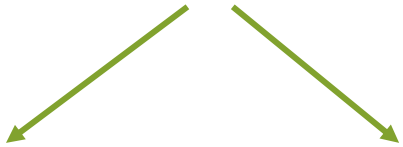
Case Study II

Process flow



Listeria: SUB Production

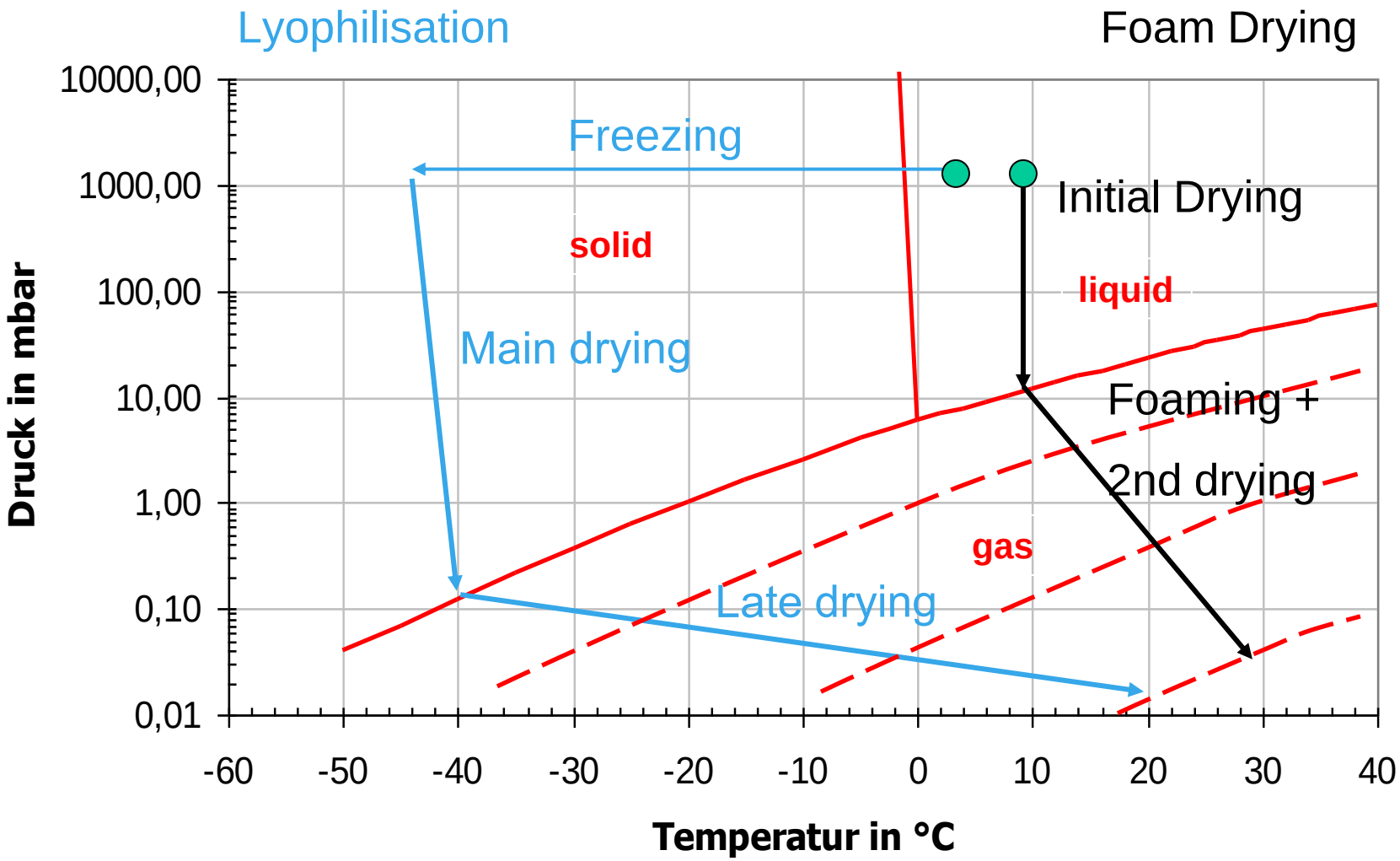
Sample	OD_{600}	VCC [cfu/mL]	Weight [kg]
Fermentation broth	5.2	$7.6x10^9$	20.92
Drug substance	37.0	$9.4x10^{10}$	1.54
Concentration factor	7.1	12.4	13.6



Liquid Dried product

Case Study II

Introduction: Lyophilisation versus Foam Drying



Freeze-Drying versus Foam-Drying

Cake

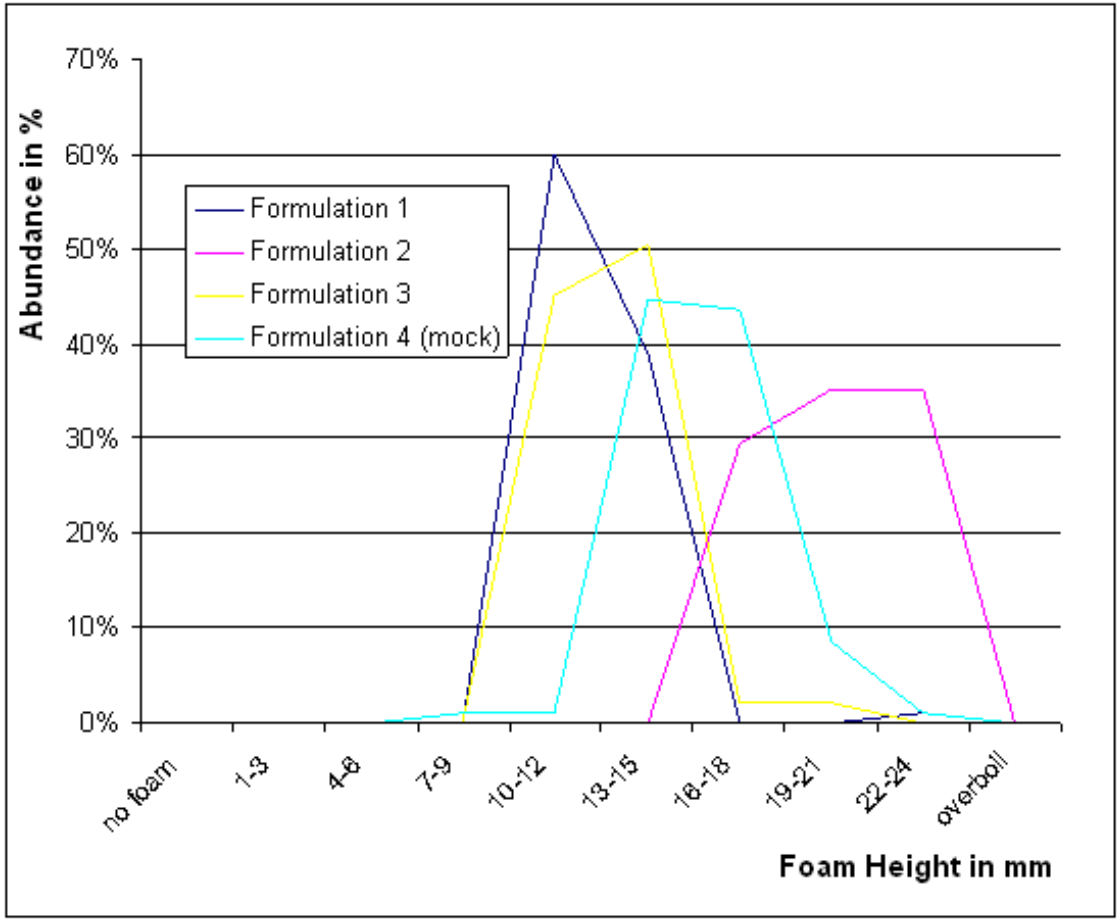
foam

- Control of formulation (foam matrix), temperature and vacuum programs are crucial parameters

- QC:
  - appearance
  - residual moisture
  - foam hight
  - live titer / stability

Case Study II

Formulation versus foam height distribution (a)



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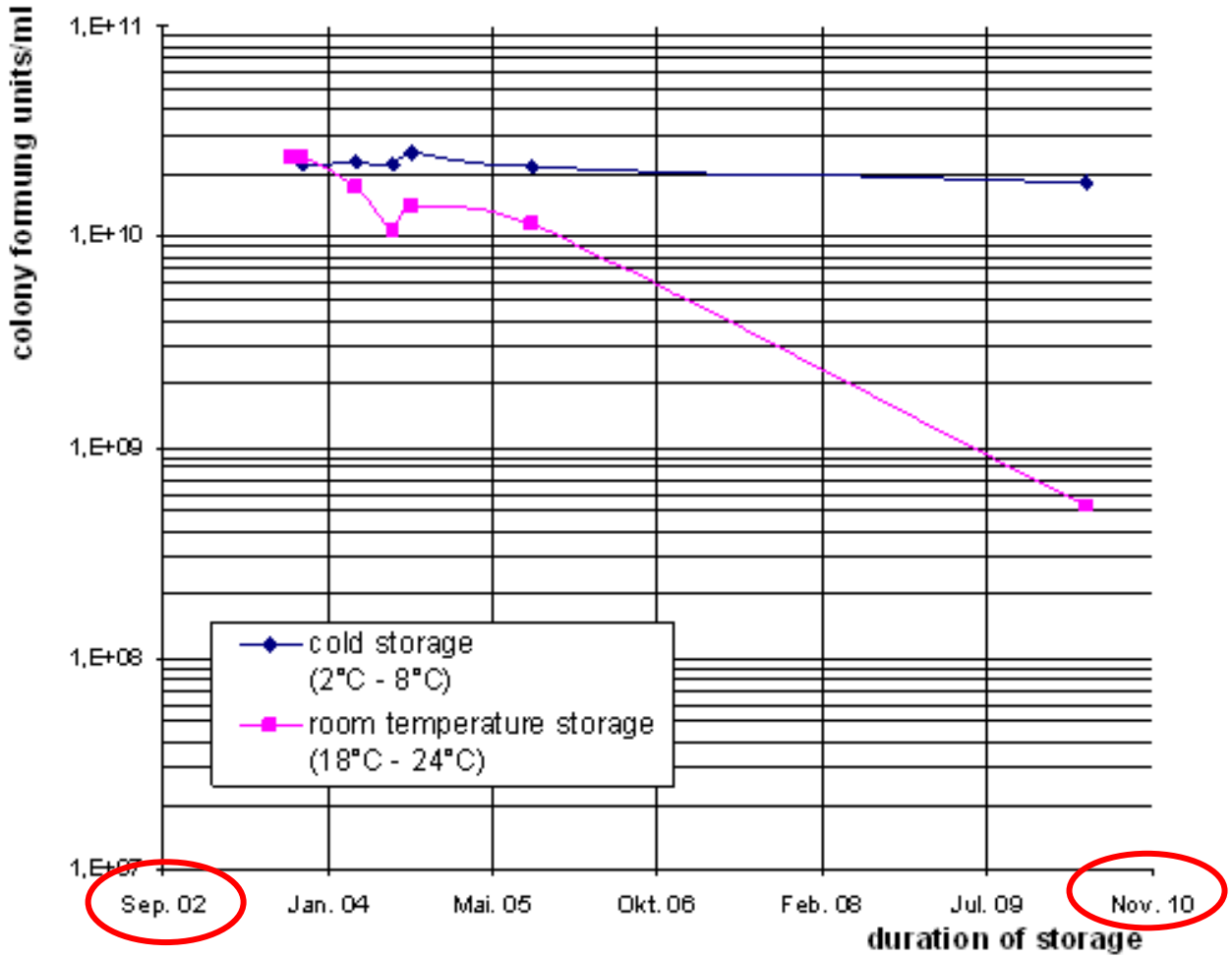
Case Study II

Recovery of foam-dried live *Listeria*

	VCC				
	before drying		in foam		
	in 1 ml	absolut per vial	per vial	Recovery in %	Residual Moisture in %
Formulation 1	**5.4E+10**	**2.3E+10**	**8.7E+09**	**38**	**5.0**
Formulation 2	4.6E+10	1.9E+10	8.6E+09	44	5.2
Formulation 3	5.4E+10	2.3E+10	7.4E+09	33	5.7
Formulation 4 (mock)	n.a	n.a	n.a	n.a	5.2

Stability data: *Salmonella sp.*



Case Study II

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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

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