Vaccine Upstream Processing – an overview

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Imagination at work

New mood of optimism is sweeping through the vaccines business. . .

Healthcare needs and economics

Emerging technologies expand vaccine applications to new disease areas

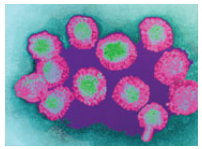
New set of innovative and high priced vaccines
Eg. rotavirus, HPV, and meningitis

The high profile promotion of vaccines in developing countries by the GAVI, Gates Foundation, DCVMN, PATH etc

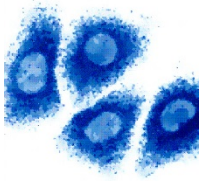
How Vaccines are manufactured

The Vaccines

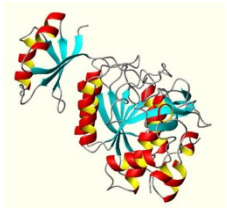
Bacteria based



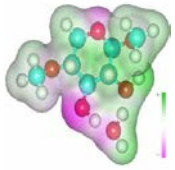
Virus based



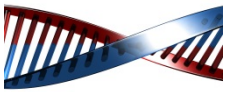
Protein based



Polysaccharide based



DNA based



The Manufacturing process

Cell culture / Fermentation

⬇

Purification

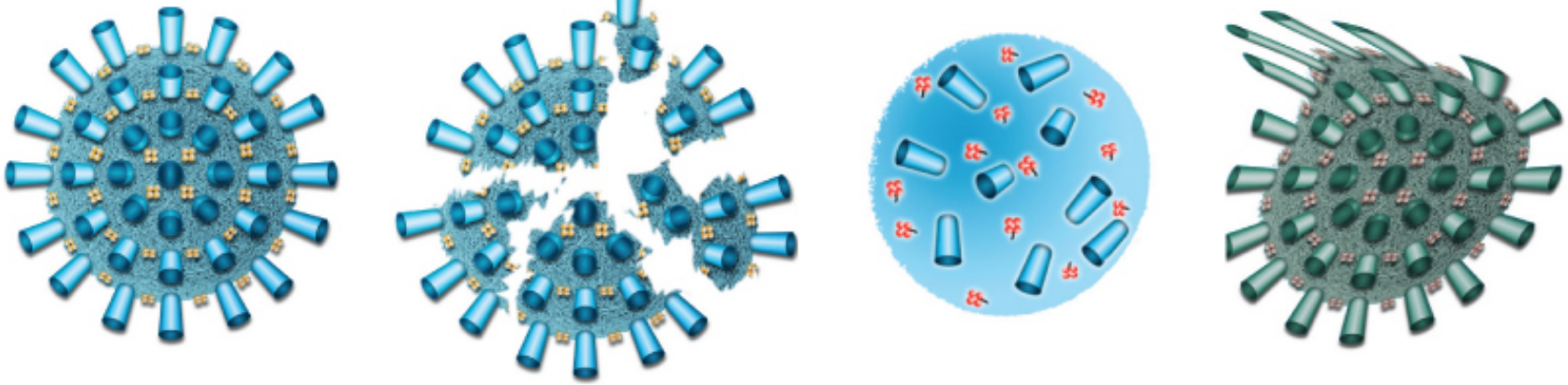
⬇

Fill and Finish

Analysis (QC/QA)

Number and order of the different steps depends on the specific vaccine production

Different types of marketed influenza vaccines.



Whole virus **Split virus** **Subunit** **Live attenuated**

The evolution of vaccine processes

1st generation processes:

Focus on upstream, optional inactivation

2nd generation processes:

Separations based on centrifugation, filtration

Currently developed processes:

Quality based approach: Quality by Design

Focus on entire process incl. purification and virus safety

Outline of presentation

Cell substrates for virus production

Cell culture using Microcarriers

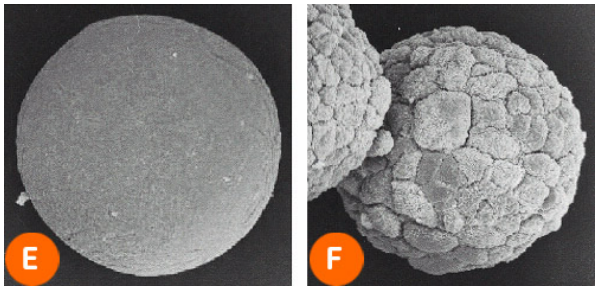
Scale up of Microcarrier cultures

Conclusions

Cell substrates for virus production

Selecting a cell line for virus production

- Cell substrate evolution from primary to diploid to continuous cell lines…
- Modern options: **Vero, MDCK, EBx™, AGE, PER.C6™ …**
- Requirements
  - Suitable for GMP production
  - Good safety track record
  - Good virus propagation
  - Broadly and highly permissive
  - Scalable to high volume production



from: Pereira et al. Biotech Bioeng; 2004; 85; 5

Vero cells

- Accepted by regulatory authorities for viral vaccine production
- Used for production of live attenuated viral vaccines
- Long track record for production of polio and rabies vaccine
- The cell line was derived in 1962 from kidney epithelial cells of the African Green Monkey
- Available from ATCC at passage level 121
- Most vaccine manufacture is performed with cells at passage levels in the 130's or 140's
- Non-tumorigenic at vaccine production passage levels
- Anchorage dependent, can be expanded on Cytodex™ microcarries

MDCK and Vero cells

	MDCK	Vero
+	• Higher productivity • Technically easier • Less risk for propagation of adventitious viruses	• Platform cell line (can be used for several virus vaccines) • Good safety record • Used for several marketed vaccines
−	• Potential tumorigenicity/ oncogenicity • New cell substrate • Restricted to influenza	• Lower productivity • Technically challenging • Potential propagation of adventitious viruses

Cell culture using Microcarriers

Scale up of adherent cell cultures

Increase volume

Increase number of units

Genetic Engineering News, 2007

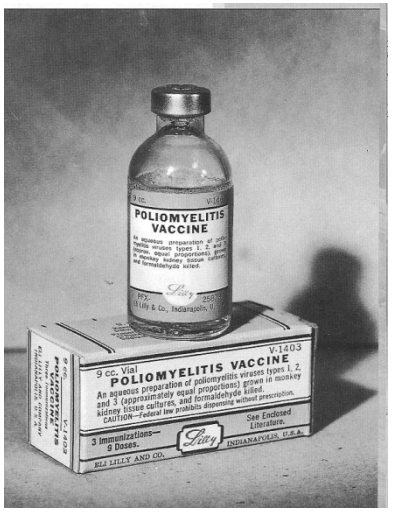
One 2500 L bioreactor with a carrier concentration of 3 g/L (Cytodex™ 1) provides the same surface area as 40 000 roller bottles (850 cm^2/bottle)

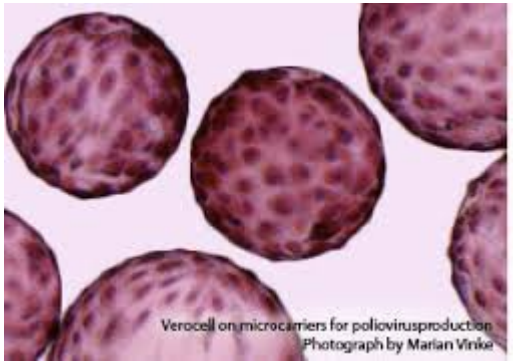
Why Microcarriers in vaccine production?

- Necessary for adherent cell lines
- Proven scalable technology (1000's of L)
- Large volume to surface ratio (less waste problem)
- Cost effective surface supply/m2
- Separates cells from secreted products
- Microporous carriers allow polarization & differentiation
- Increased productivity of functional product

The history of Polio vaccine processes

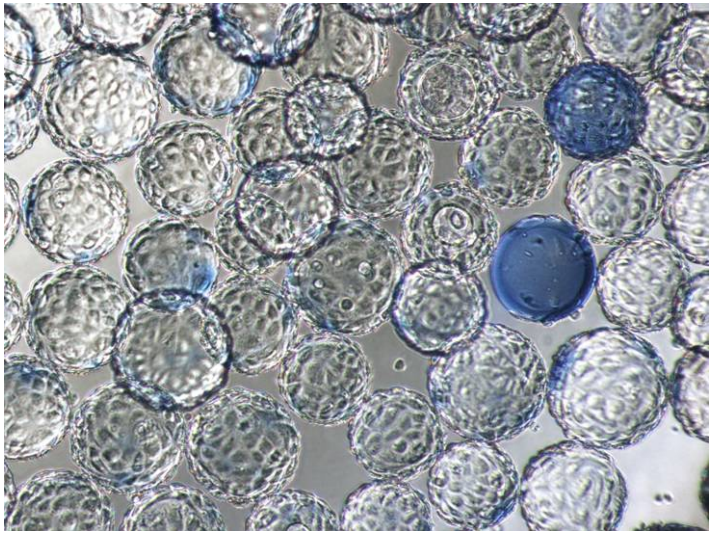
- 1955: Inactivated Polio vaccine (IPV) launched (Salk Type)
- 1960: Attenuated Polio vaccine launched (Sabin type)
- 1960s: Collaboration between Prof. Van Wezel (RIVM/NVI Netherlands) and GE (former Pharmacia) around microcarrier cultures of primary monkey cells.
- 1970s: New IPV purification method using chromatography resins
- 1980s: Switch to Vero cell production
- 2010s: Updating the IPV processes using modern technology





Cytodex™ specifications

	Cytodex 1	Cytodex 3
Matrix	Sephadex™	Sephadex
Particle diameter (µm)	200	175
Effective surface area (m^2/g dry)	0.44	0.27
Relative density	1.03	1.04
Swelling volume (mL/g dry weight)	18	14
Surface modification	DEAE	Gelatine



Vero cells on Cytodex 1, stained with trypan blue

Viruses produced in microcarrier cultures

- Adenovirus
- Bovine rhinotrachteritis
- Endogenous C type
- Equine rhinopneumonitis
- Foot and mouth
- Group B arboviruses
- HAV
- Herpes
- Influenza
- Japaneese encephalitis
- Marek's
- Papova virus
- Polio
- Polyoma
- Pseudorabies
- Rabies
- RSV
- Rous sarcoma
- Rubella
- Sendai
- SV40
- Sindbis
- Small pox
- Vaccinia
- Vesicular stomatitis

Cell culture media and serum

Serum - Ensure quality, traceability and origin

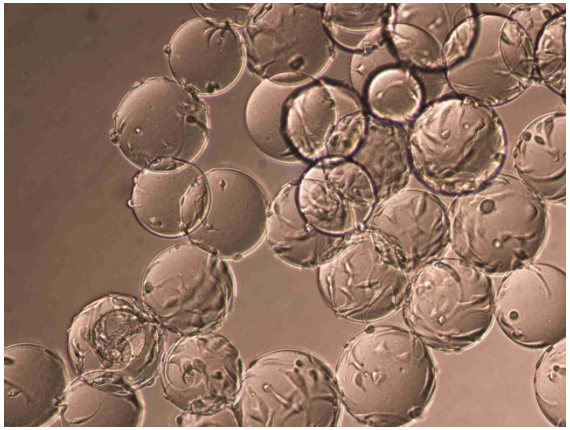
Classical media

Animal origin free media

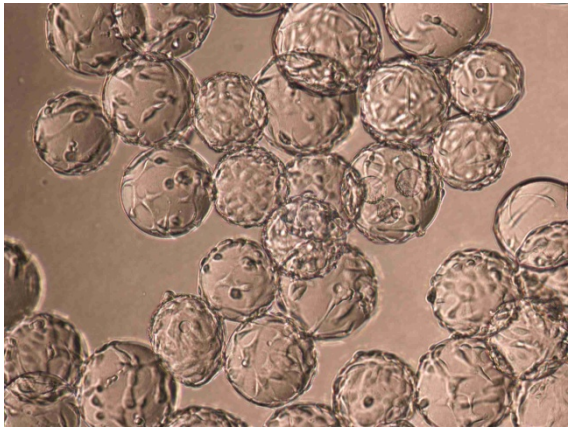
Complex media containing hydrolysates

Chemically defined media

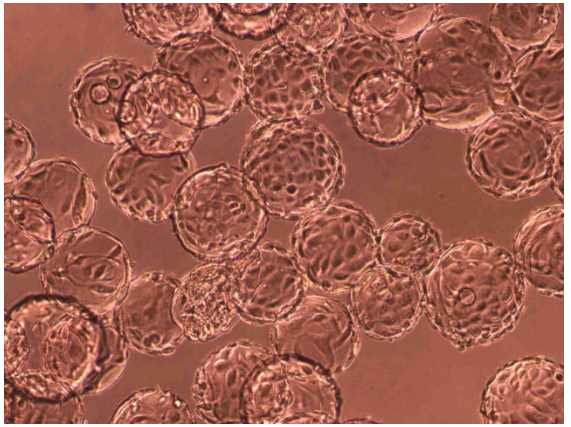
The effect of cell culture media



Medium 1

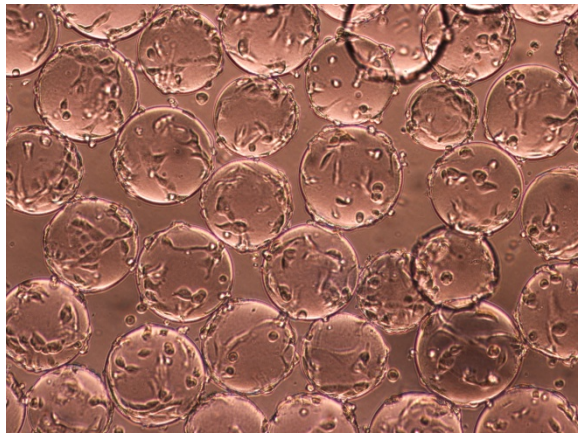
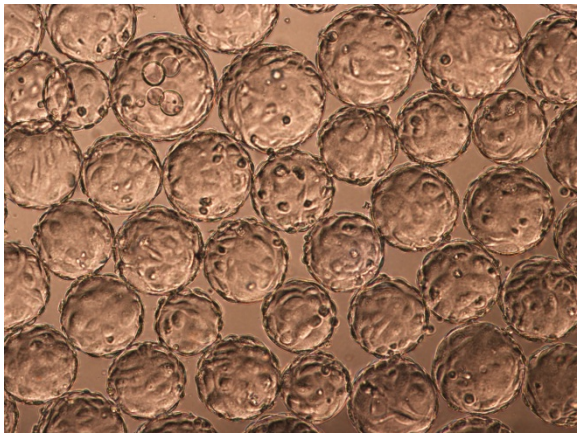
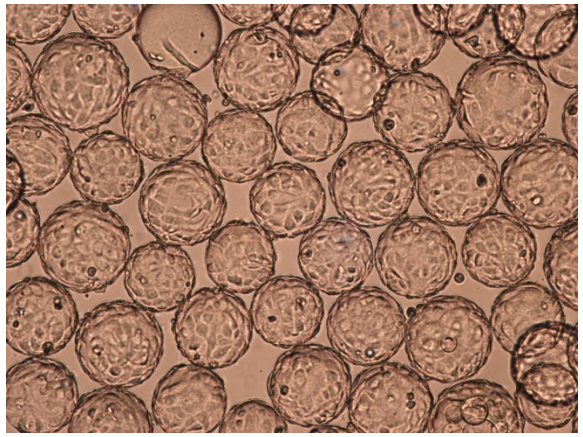
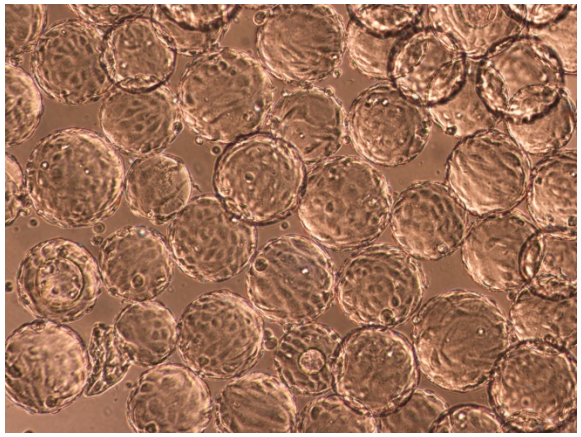


Medium 2

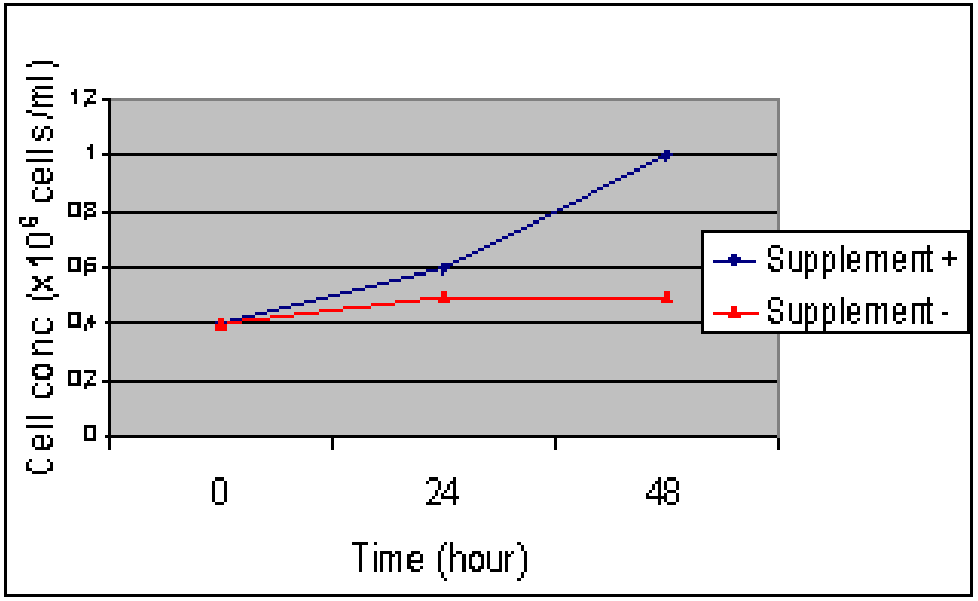


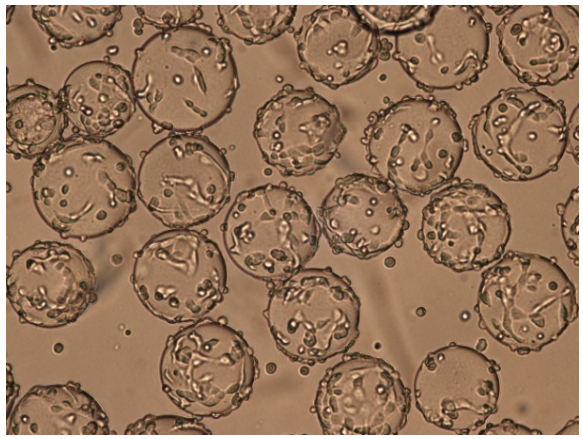
Medium 3

Serum-free expansion of Vero cells

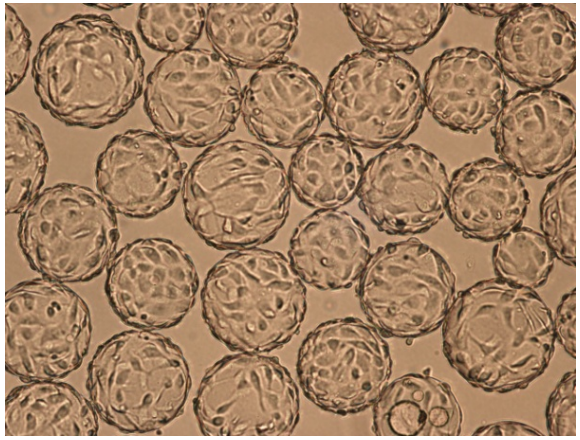
	No supplements	Supplemented with Soy peptone
Cytodex™ 1 (DEAE surface)		
Cytodex 3 (collagen surface)		

The effect of medium supplementation





- supplement



+ supplement

Scale up of Microcarrier cultures

Bioreactors – Fixed vs Disposabled

Control and scalability

Stainless steel

WAVE

10L

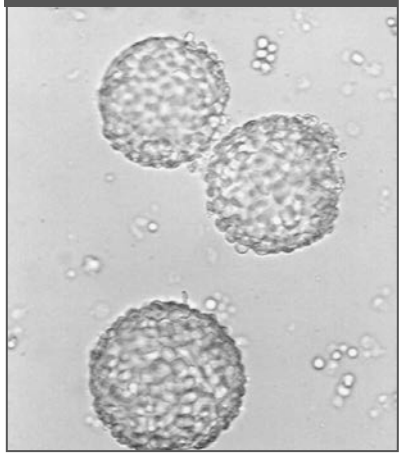
2000L

XDR

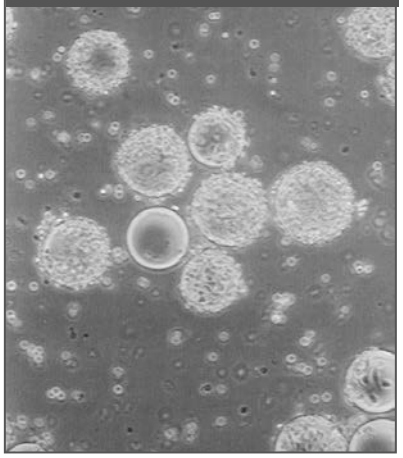
Subcultivation – Scale up

- Wash culture
- Add Trypsin. Optimal concentration and time of incubation need to be tested
- Inhibit trypsin when 90% of cells are detached
- Easy Cytodex™ retention by using 100µm stainless steel sieve

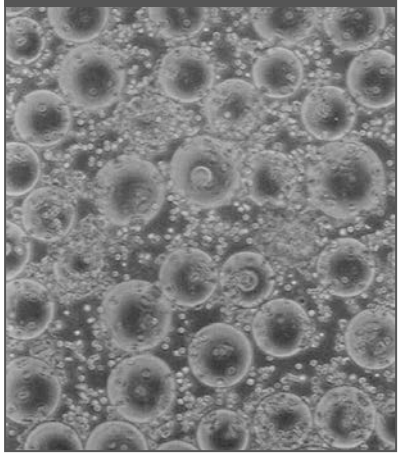
Start detachment



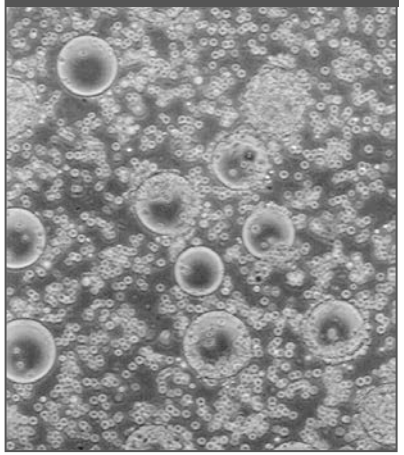
20% detached



70% detached



90% detached

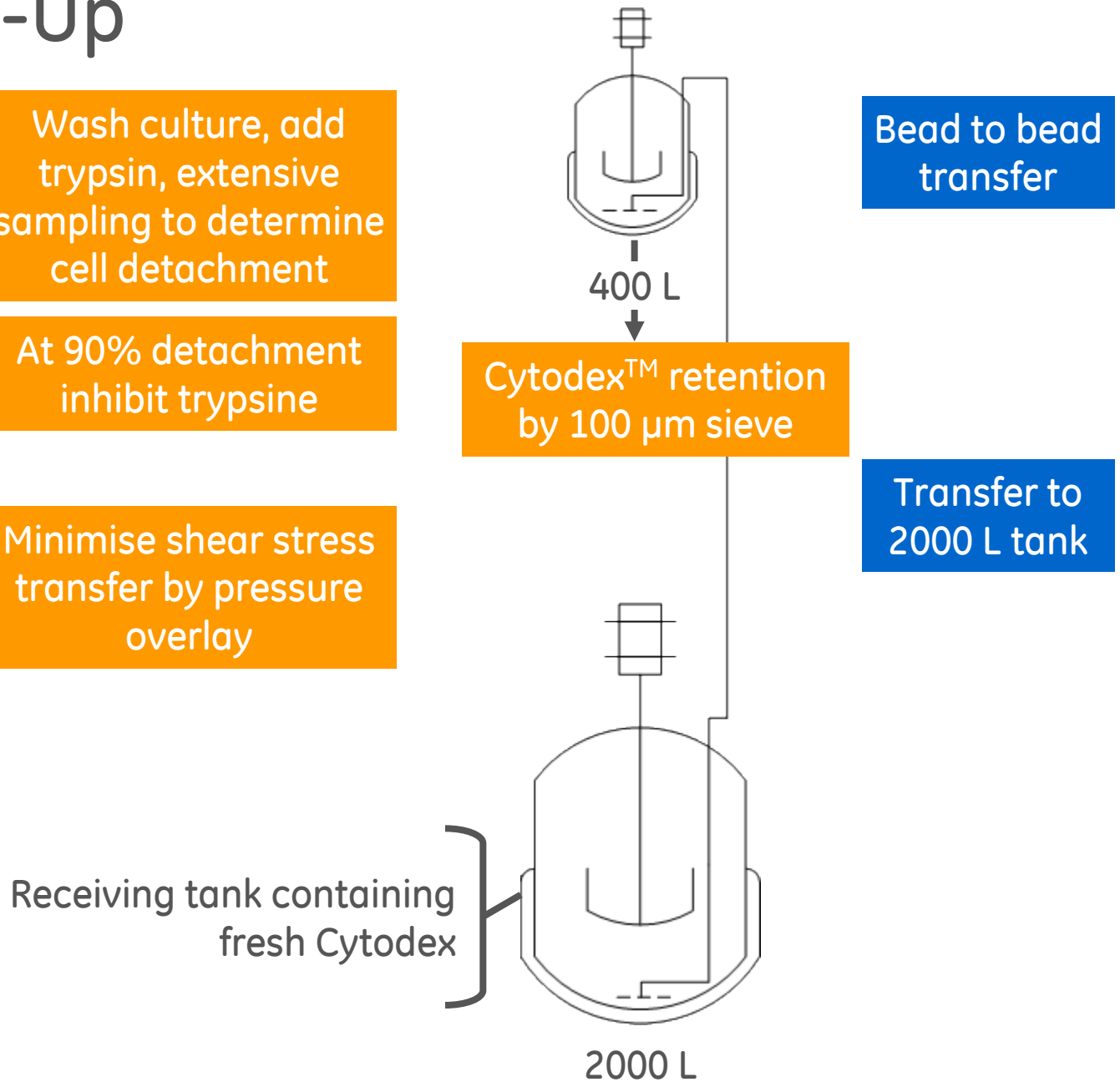


Scale-Up

Wash culture, add trypsin, extensive sampling to determine cell detachment

At 90% detachment inhibit trypsine

Minimise shear stress transfer by pressure overlay



Bead to bead transfer

Transfer to 2000 L tank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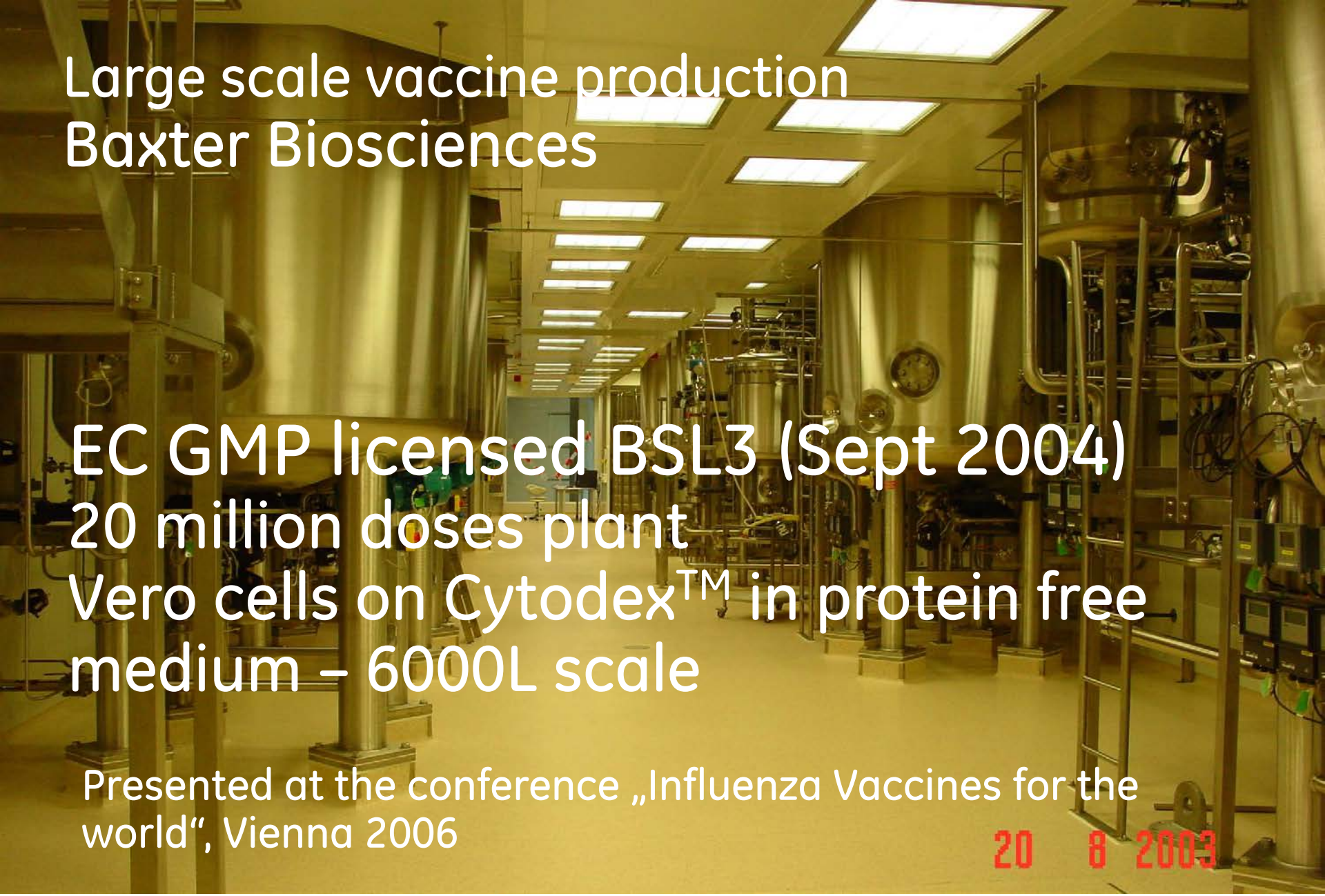

Large scale vaccine production
Baxter Biosciences

EC GMP licensed BSL3 (Sept 2004)
20 million doses plant
Vero cells on Cytodex™ in protein free medium – 6000L scale

Presented at the conference „Influenza Vaccines for the world“, Vienna 2006

Thank you!

www.gelifesciences.com

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