Vaccine Upstream Processing –an overview

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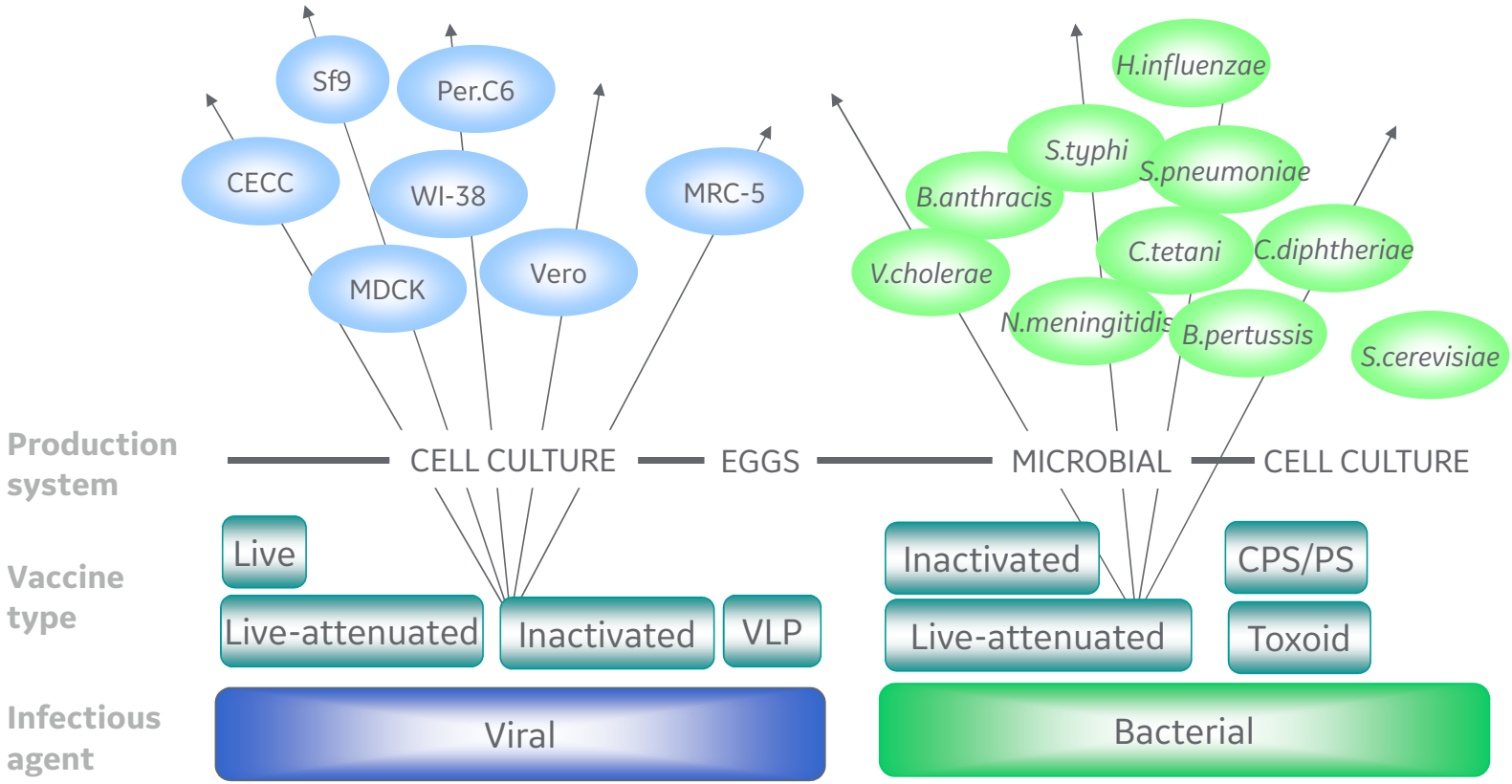
Outline of presentation

- Cell substrates for virus production
- Processing overview — batch, fed-batch, perfusion
- Cell culture using Microcarriers
- Scale up of Microcarrier cultures
- Conclusions

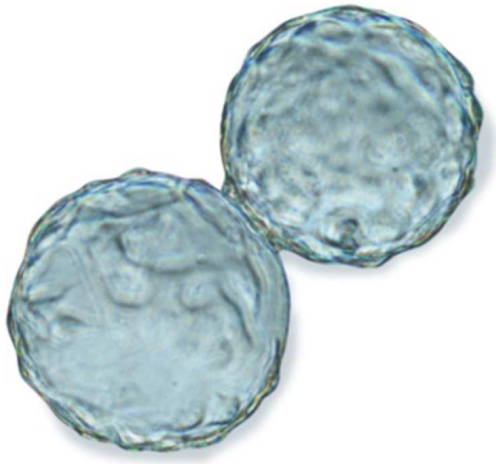
Cell substrates for virus production

Diversification of technology – low efficiency



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



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

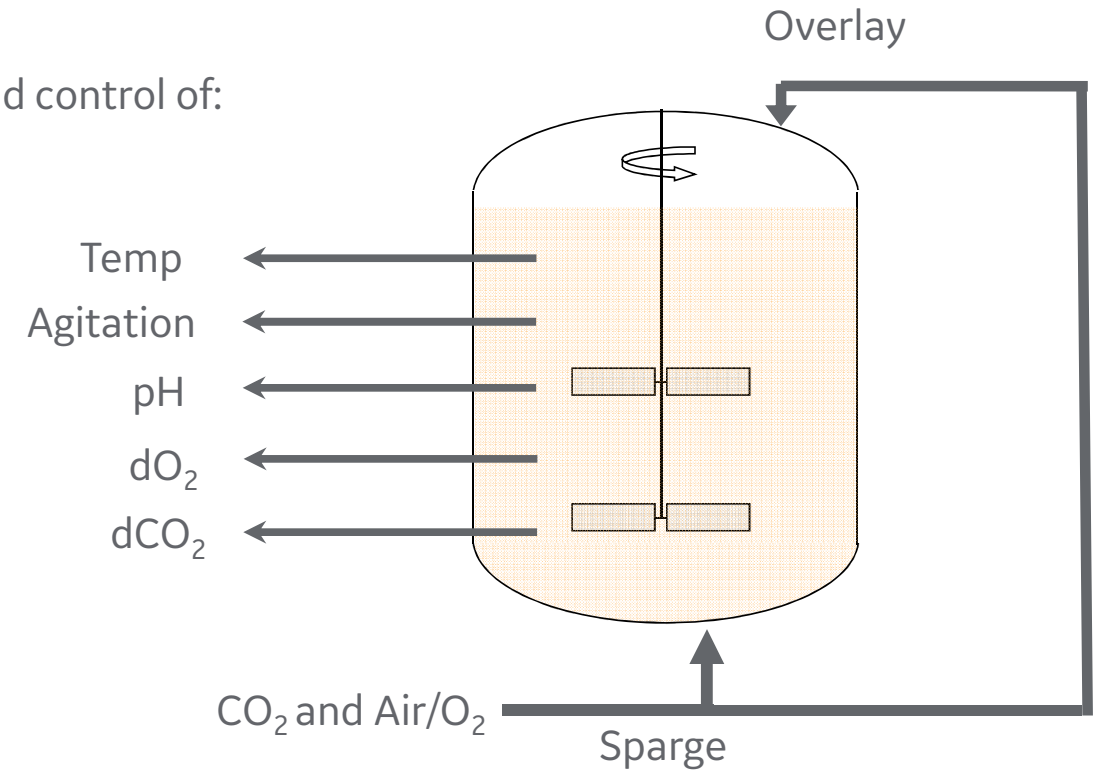
Processing overview—batch, fed-batch, perfusion

All culture systems have certain needs in common

- Absence of a competing organism
- Suitable temperature
- Suitable pH
- Suitable osmolality
- Well-mixed environment
- Gas transfer
- Not too much shear
- Nutrients to consume

A bioreactor provides a well-controlled environment to meet these needs

Online measurement and control of:



Bioreactors may also have the ability to measure and control glucose concentration, as well as to measure biomass (viable cell density)

What factors contribute to batch failure?

Nutrient depletion

- Cells have nothing left to “eat” (i.e., glucose, essential amino acids, vitamins)

Accumulation of toxic metabolites

- Could be inhibitory to cell growth or protein production

Shear sensitivity of the cells

- Cell death, or lysis that releases HCP and other “garbage” into media
- Often advisable to stop batch and purify a “cleaner” harvest stream before too much viability drop and cell lysis

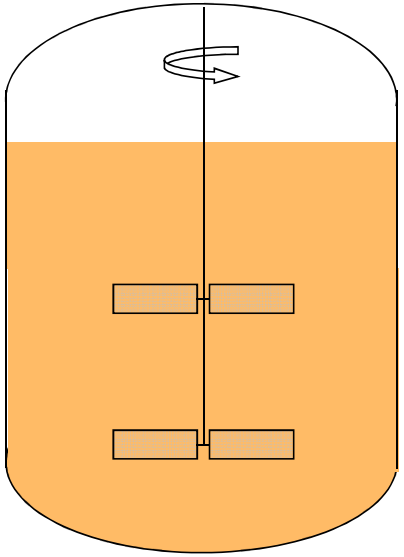
Contamination

Loss of stability of production clone

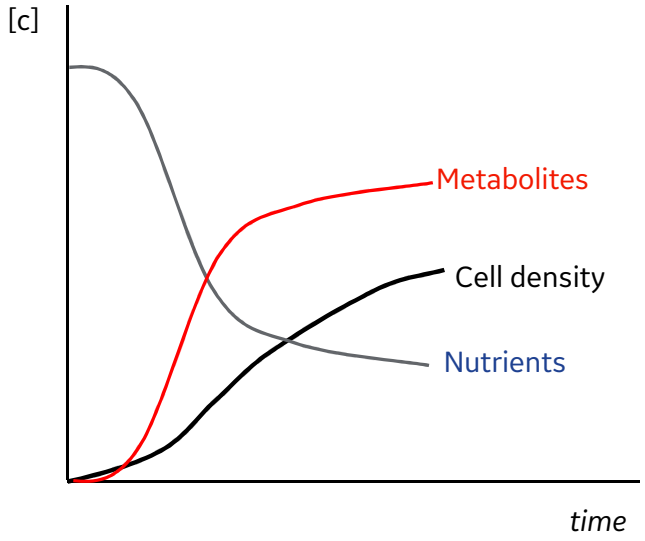
- Cells do not produce product anymore; sometimes seen with increasing generations

HCP = host cell protein

Batch process—all nutrients added into base medium along with inoculum at start of batch

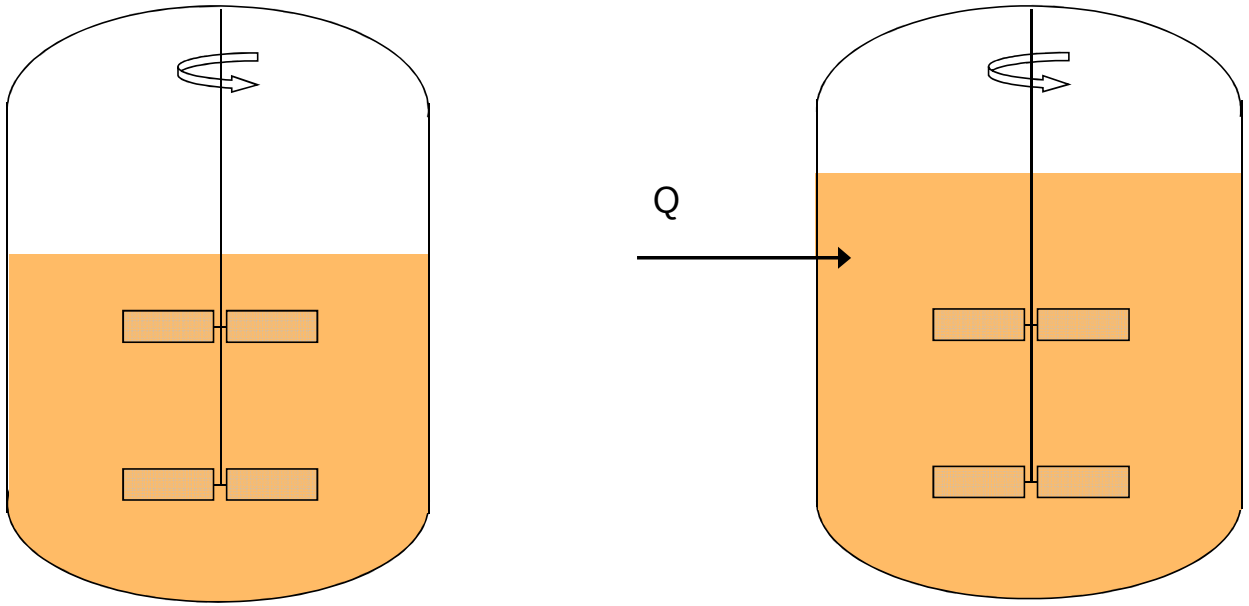


A high cell/product density is challenging to reach—osmolality constraints, because nutrients are added at start



Process duration ~ 3-4 d seed cultures

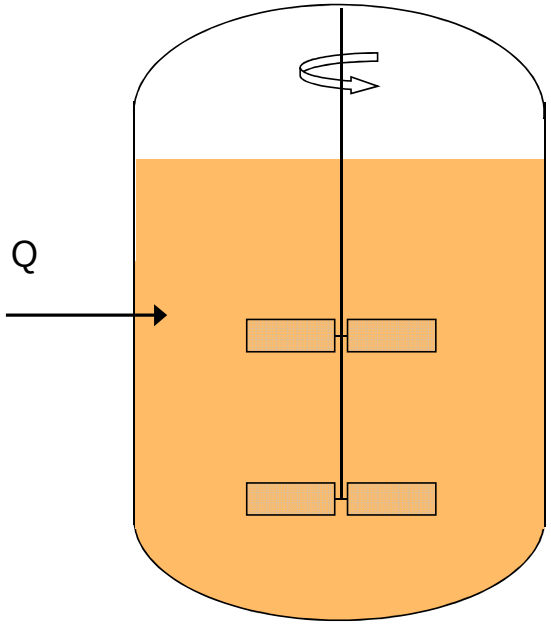
Fed-batch process—concentrated nutrients added over time as cells grow



Inoculation day

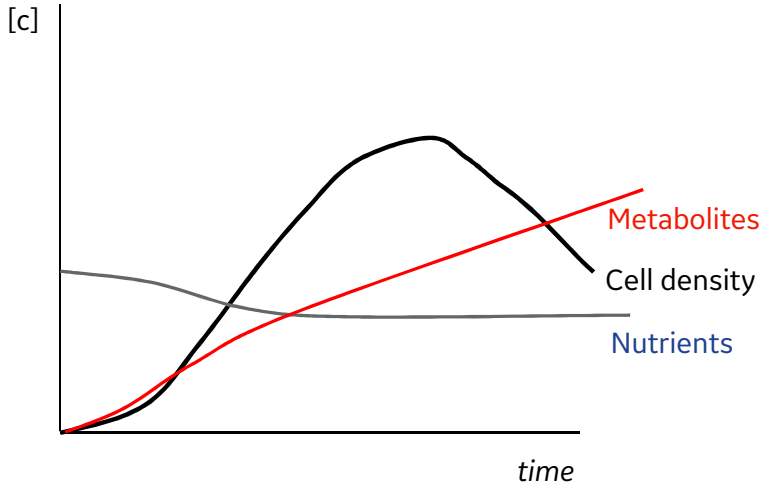
Harvest day

Fed-batch process—feeding over time allows stable nutrient concentration



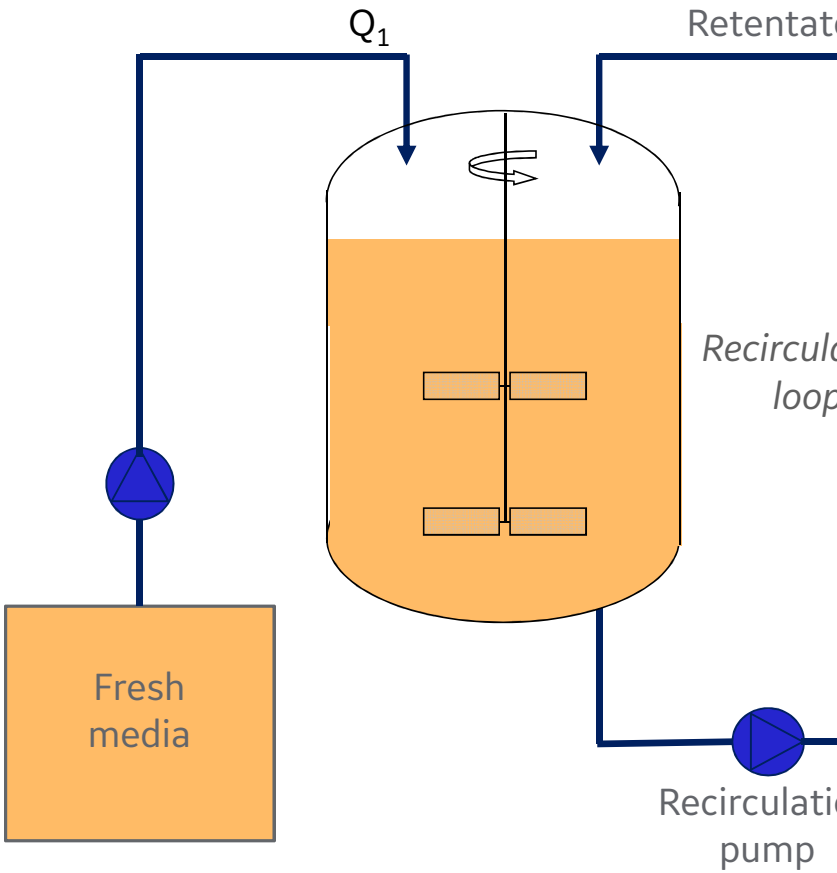
Growth of cells prolonged, resulting in higher titer

Metabolites could reach levels toxic to cell growth, yielding lower titers (assuming no change in cell-specific productivity)



Process duration ~ 2 wk

Perfusion—rate is in vessel volumes per day (VVD) Reactor volume remains stable

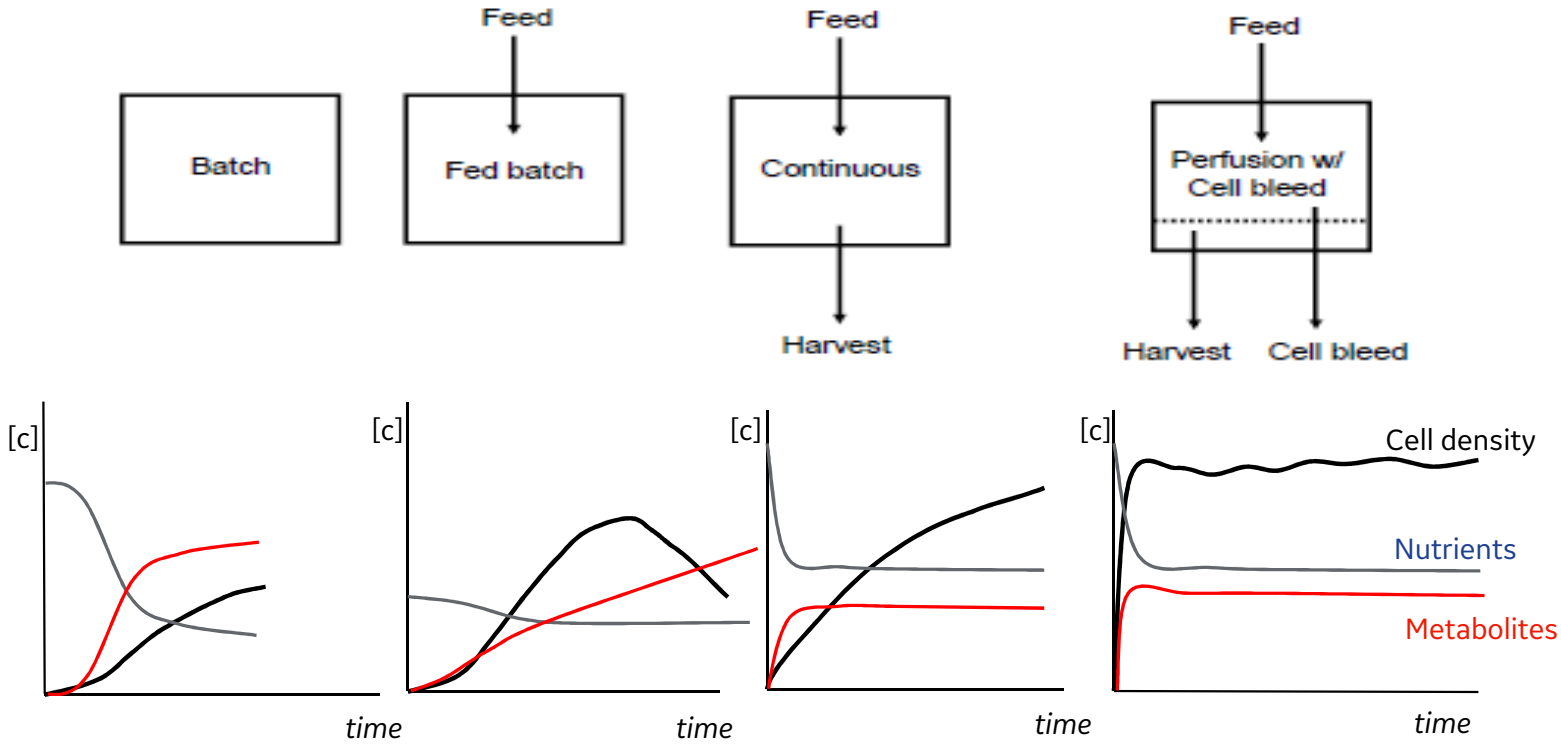


Permeate/Centrate

- Permeate is the spent media waste if purpose of perfusion is to increase cell density in bioreactor
- Permeate is the product if protein is secreted into media in production bioreactor

** Multiple options for perfusion devices

Summary of batch, fed-batch, and perfusion processing for product manufacture



Bioreactors – Fixed vs Disposabled

Control and scalability

Stainless steel

WAVE

10L

XDR

2000L

Cell culture using Microcarriers

Scale up of adherent cell cultures

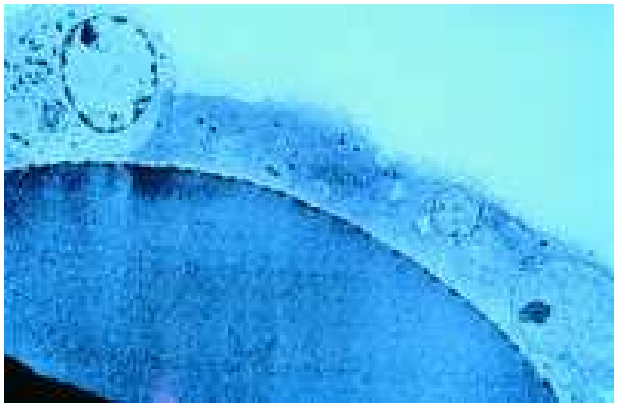
Increase volume

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)

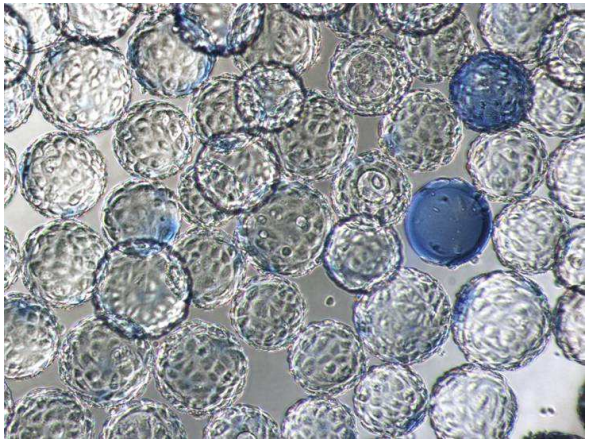
Why Microcarriers in vaccine production?



- 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

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



Introduction to Cytodex™ 1 and 3 Gamma

- Delivered gamma sterilized and ready to use. Supplied dry to save storage space and facilitate transportation.

Conventional process:

Simplified process:

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

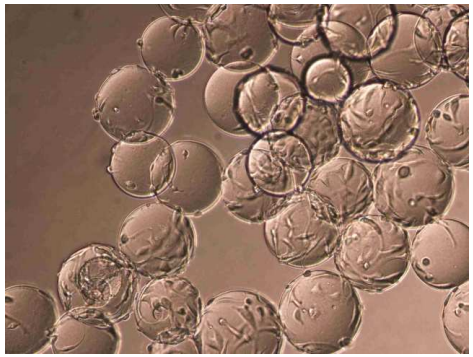

Media
Sera
Supplements
Buffers and process liquids
HyClone
AdvanceSTEM™
HyClone
HyPure™ WFI QUALITY
WATER
HyClone
DMEM/HIGH GLUCOSE
HyClone
HyCell CHO Medium
HyClone
HyCell - STEM-EF
HyClone
CDM4PerMAb™
HyClone
HyCell CHO
HyClone
CDM4NS0™
HyClone
HyPure™ Cell Culture Grade Water
HyClone
FetalClone® II
HyClone
HyQTase™
HyClone
HyClone
FETAL BOVINE SERUM
CHARACTERIZED
HyClone

Cell culture media and serum

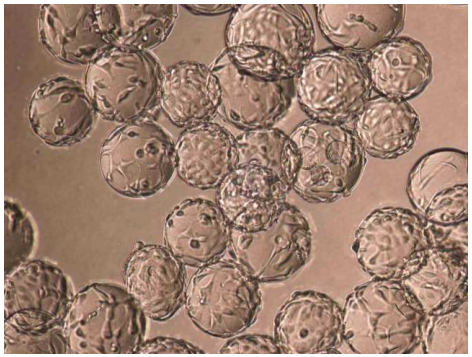
- Serum - Ensure quality, traceability and origin
- Classical media
- Animal origin free media
- Complex media containing hydrolysates
- Chemically defined media

The effect of cell culture media

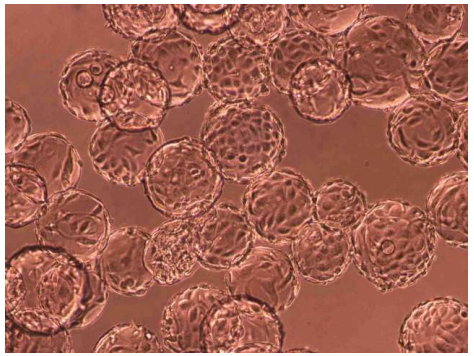
Medium 1



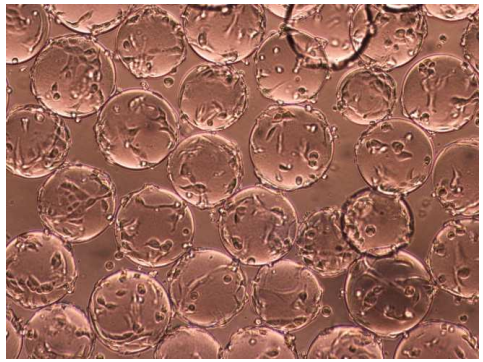
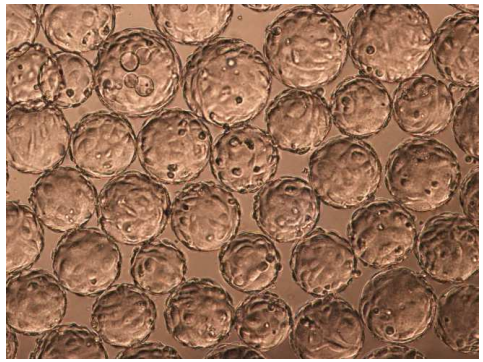
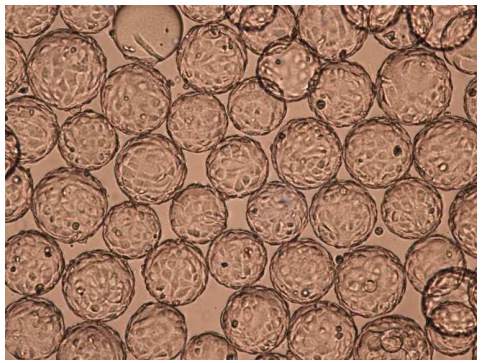
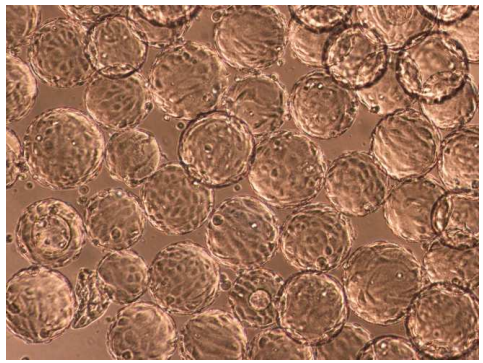
Medium 2



Medium 3

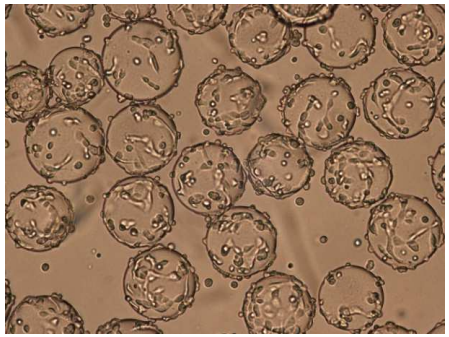


Serum-free expansion of Vero cells

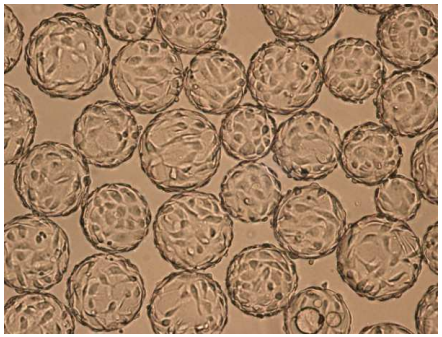
	No supplements	Supplemented with Soy peptone
Cytodex™ 1 (DEAE surface)		
Cytodex 3 (collagen surface		

The effect of medium supplementation

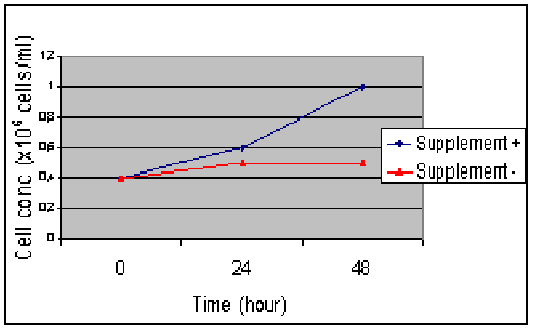
-Supplement



+Supplement



Comparison

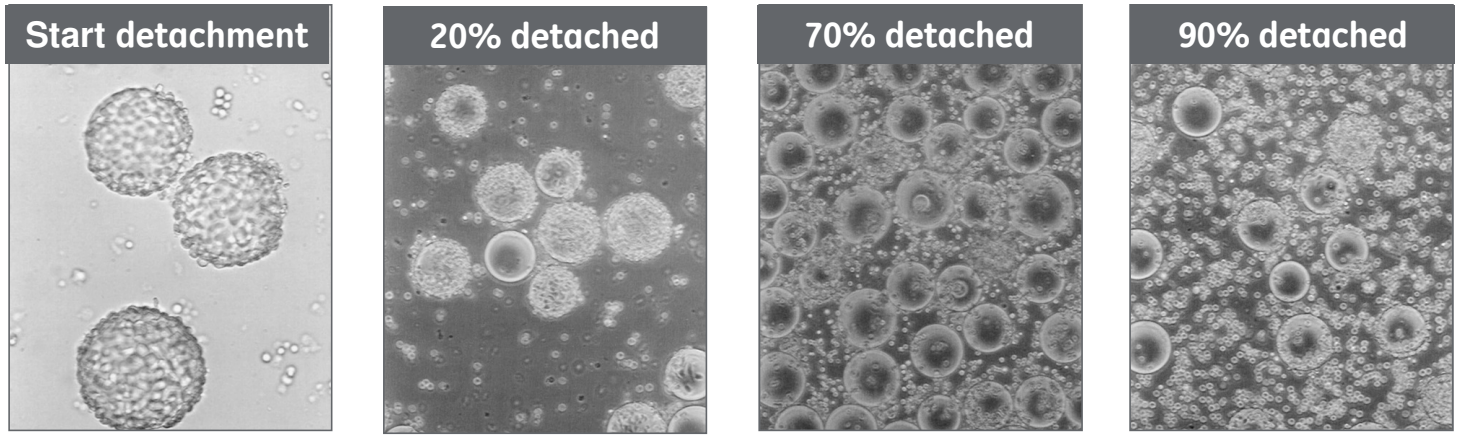


Scale up of microcarrier cultures

Subcultivation – Scale up

Procedure

- 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

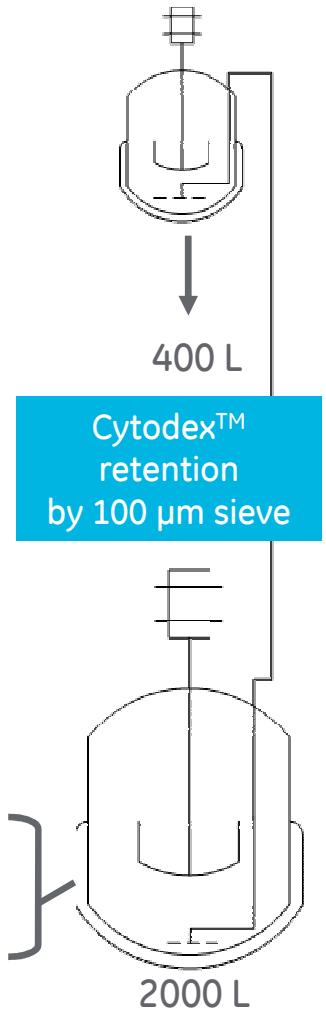


Scale-Up

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

At 90% detachment inhibit trypsine

Minimise shear stress transfer by pressure overlay

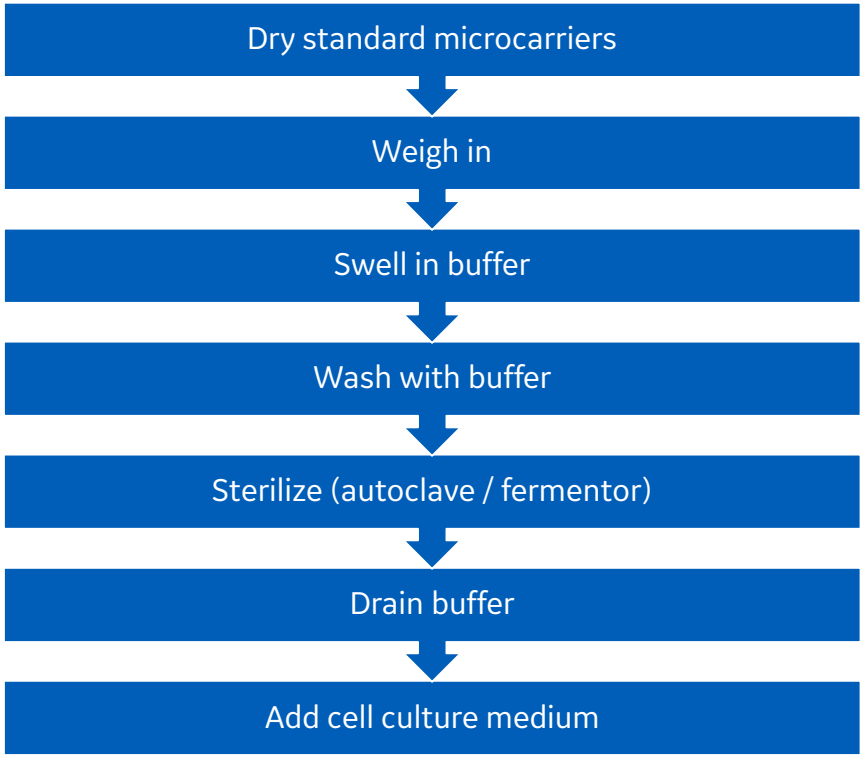


Bead to bead transfer

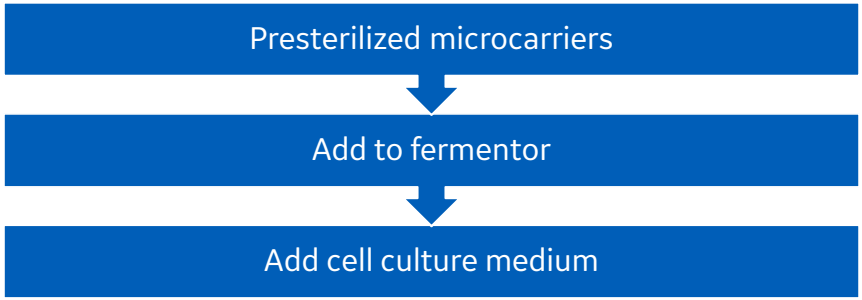
Transfer to 2000 L tank

Change in workflow with presterilized microcarriers

Conventional method



Simplified process



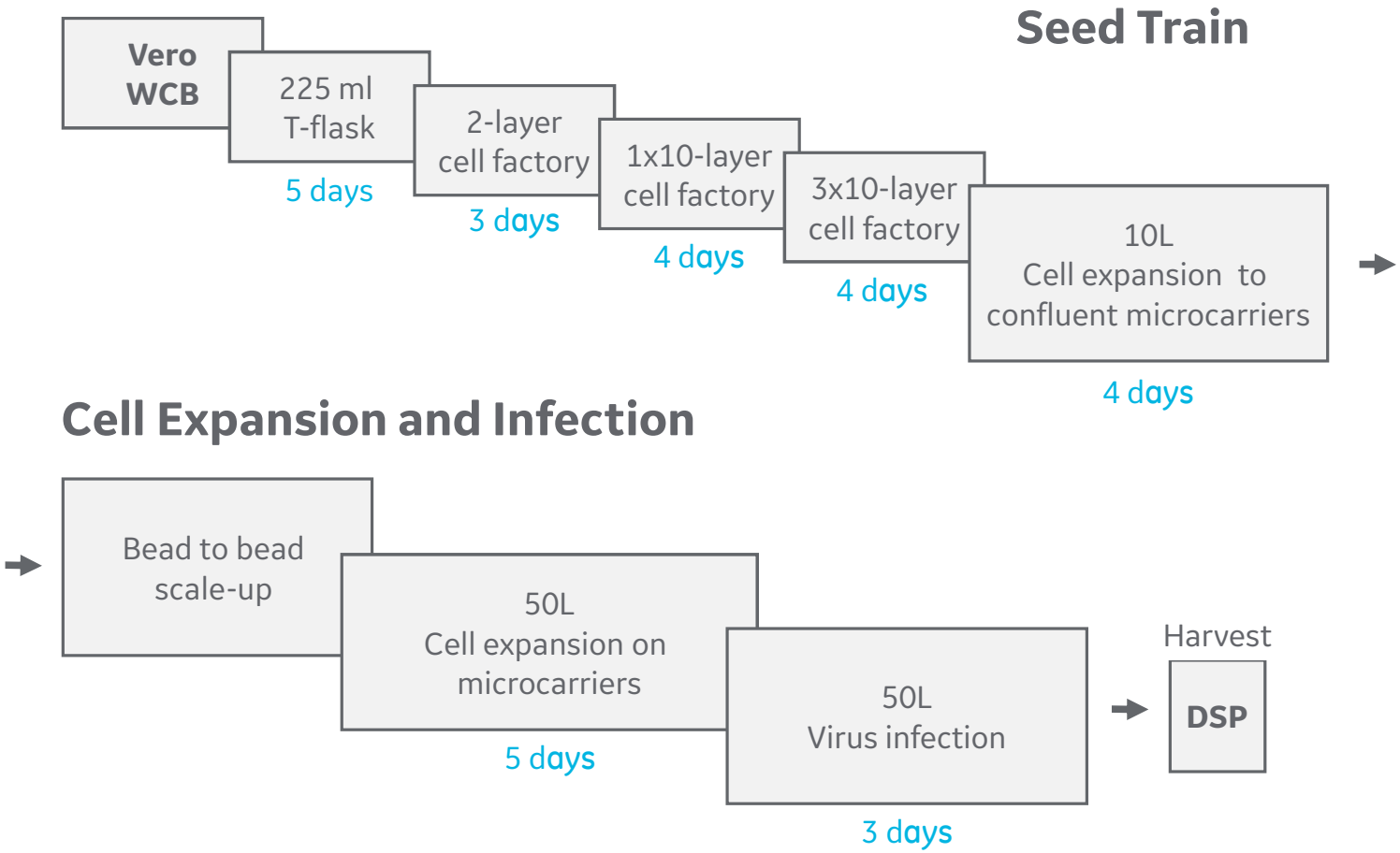
Propagation of virus

Cell Culture

Cell line:	Vero (ATCC-CCL81)
Cell culture media:	DMEM/Hams F-12
Medium supplements:	5% FCS, 0.2% Soy peptone, 0.2% Pluronic F-68
Microcarrier:	Cytodex™ 1 at 3 g/L
Cell detatchment:	Trypsin
Virus:	Influenza A/Solomon Island/3/2006(H1N1)
MOI:	0.004

<u>Equipment:</u>
Cell factory, 2 layers, 10 layers
WAVE Bioreactor™ system 20/50
WAVE Bioreactor system 200

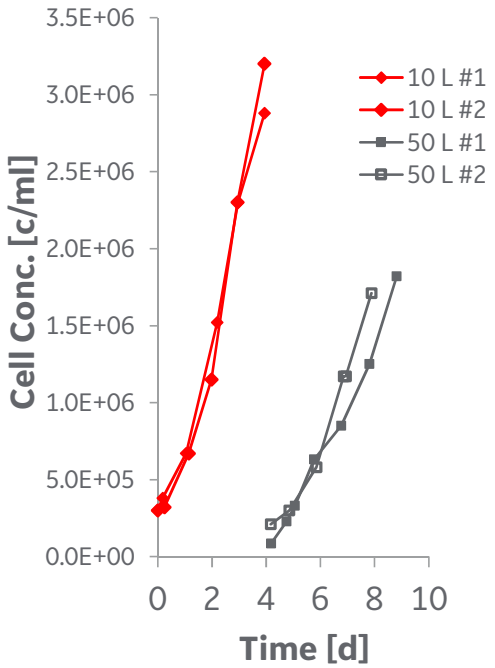
Overview



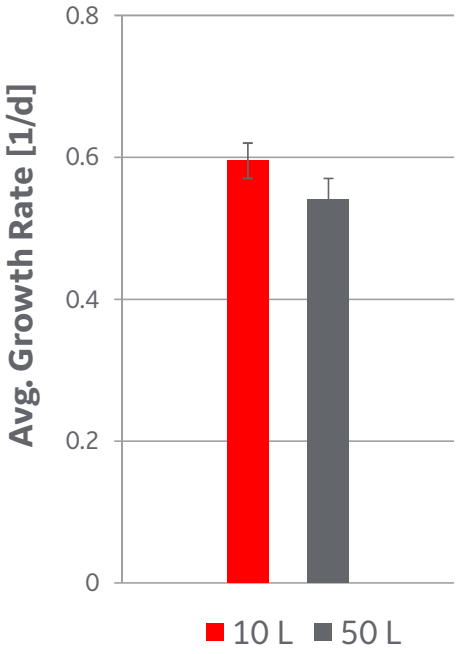
Cell Growth

- Cell growth in seed and production reactor
- Upstream process in disposable bioreactors
- Procedure for bead to bead scale-up
- Comparable cell growth rate at 10 L and 50 L scale

Cell concentration

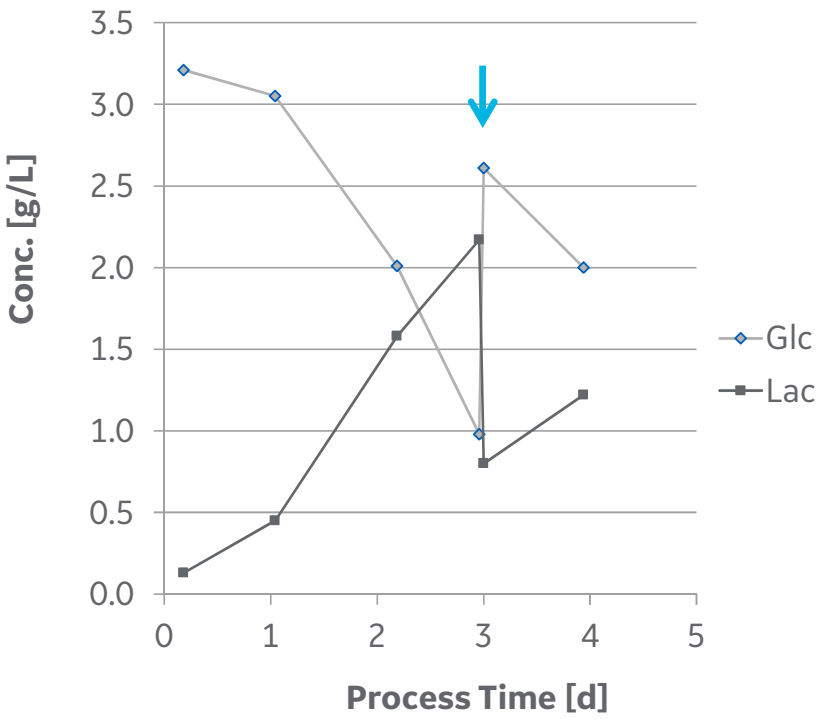


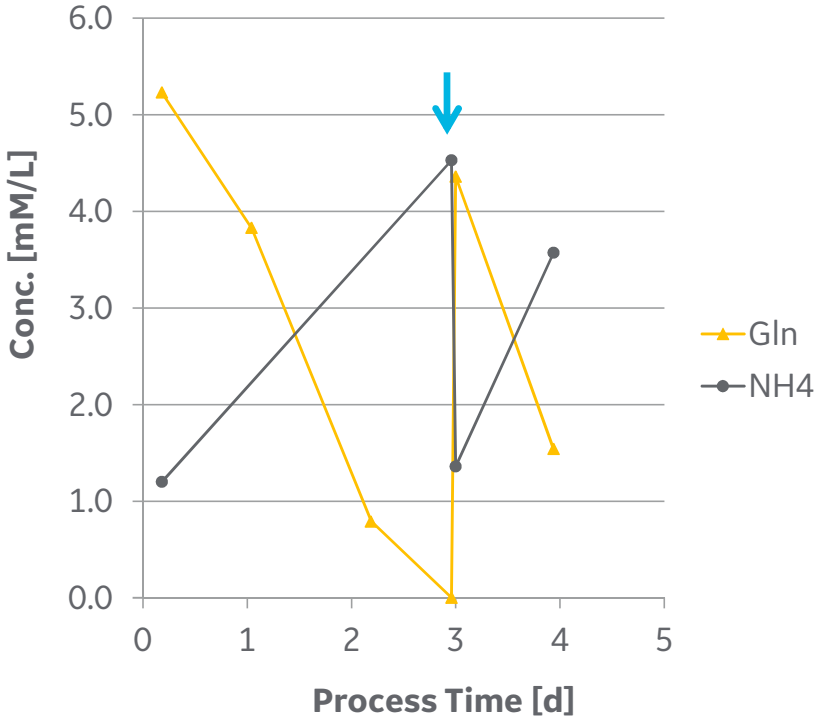
Growth rate



Cell Metabolism

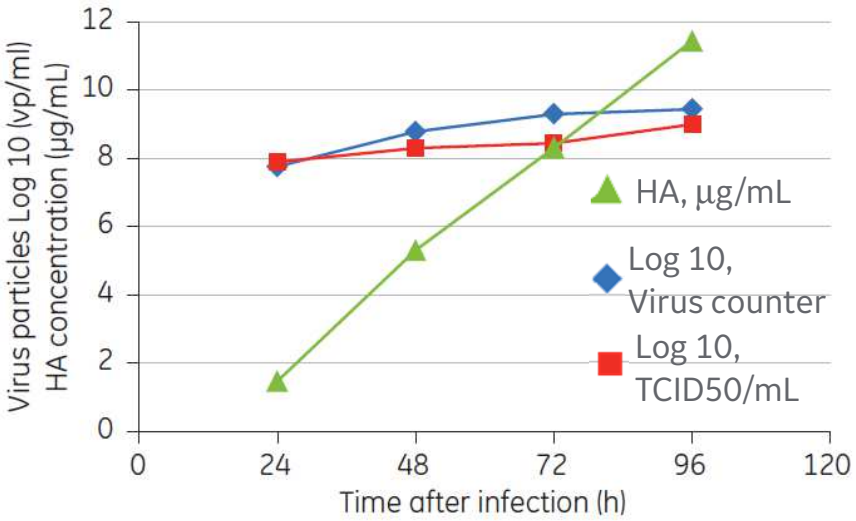
Depletion of glucose and glutamine prevented by medium exchange



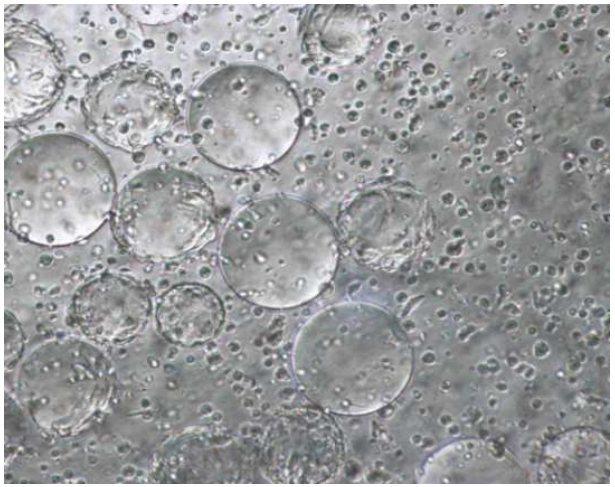


Virus growth kinetics

HA concentration and virus titer during culture



Cytopathic effect at harvest



- HA concentration at harvest was close to 12 µg/mL
- ViroCyt™ virus counter data correlates well with $TCID_{50}$

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