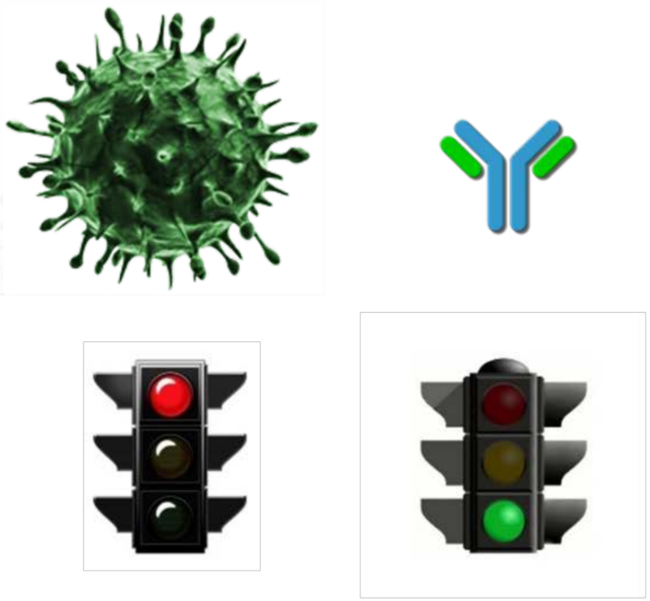
Theory 4:

- Mechanistic principles of (Parvo-) Virus retention
- Challenges of implementing virus filtration into continuous manufacturing
- Virus filters as bioprocess subject – current hot topics (ATMPS, facility segregation)

Dr. Sebastian Teitz, Product Manager & Scientific Coordinator, Asahi Kasei Bioprocess Europe,
s.teitz@akbio.eu, www.ak-bio.com

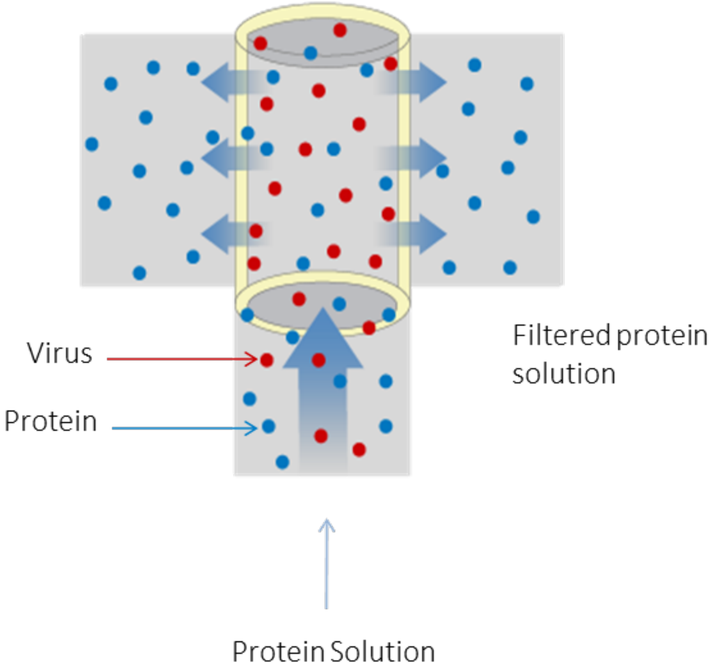
Mechanistic Principles of Parvovirus Retention

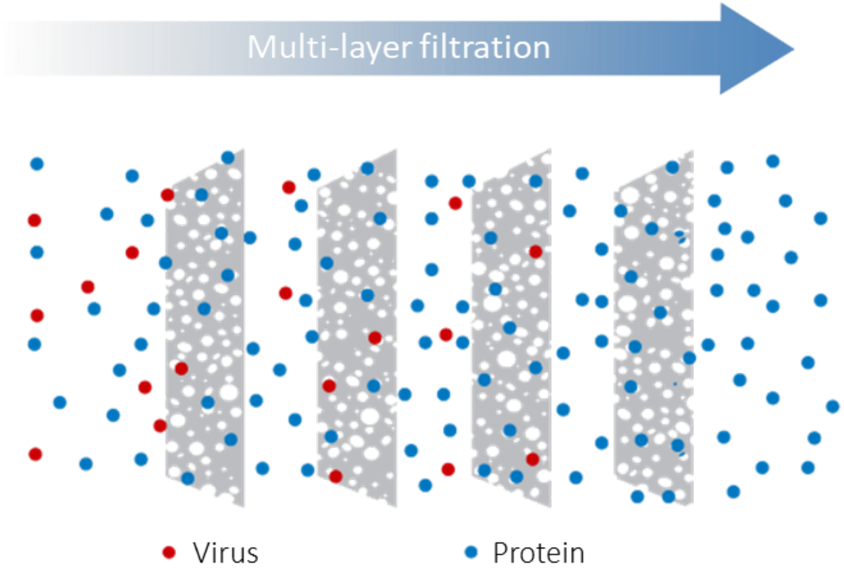
How does it work ?



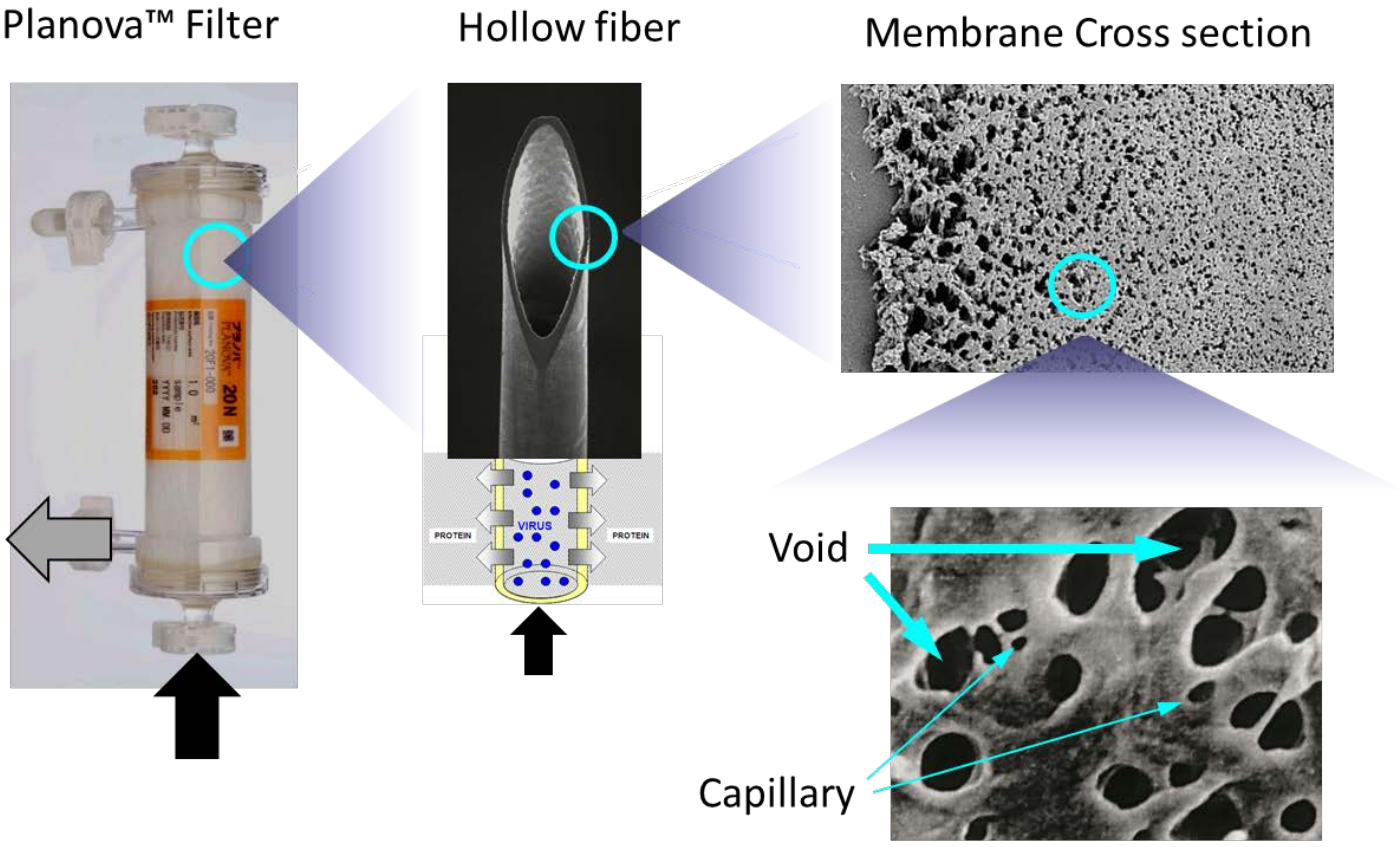
Mechanistic Principles of Parvovirus Retention

Size exclusion enabled by pore structures across the membrane that trap viruses and allow protein molecules to pass through


Virus
Protein
Filtered protein solution
Protein Solution


Multi-layer filtration
● Virus
● Protein

Mechanistic Principles of Parvovirus Retention


Planova™ Filter
Hollow fiber
Membrane Cross section
PROTEIN
VIRUS
PROTEIN
Void
Capillary

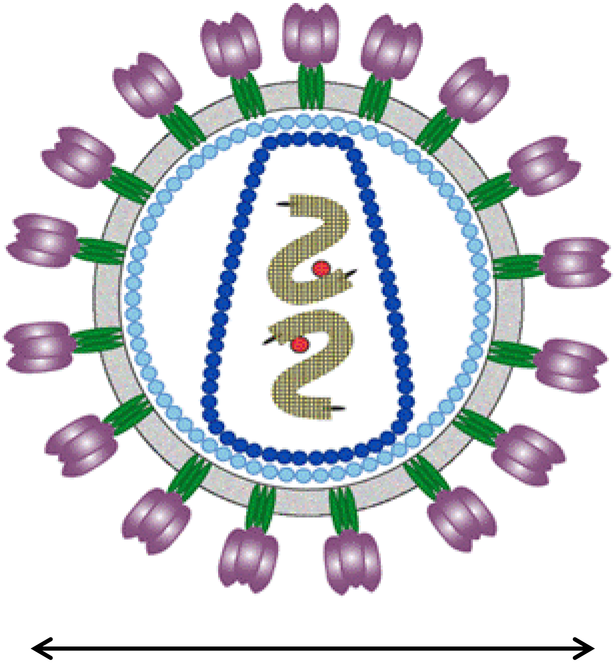
Hollow fibers have a three-dimensional network structure consisting of voids connected by capillaries.

Mechanistic Principles of Parvovirus Retention

Parvovirus

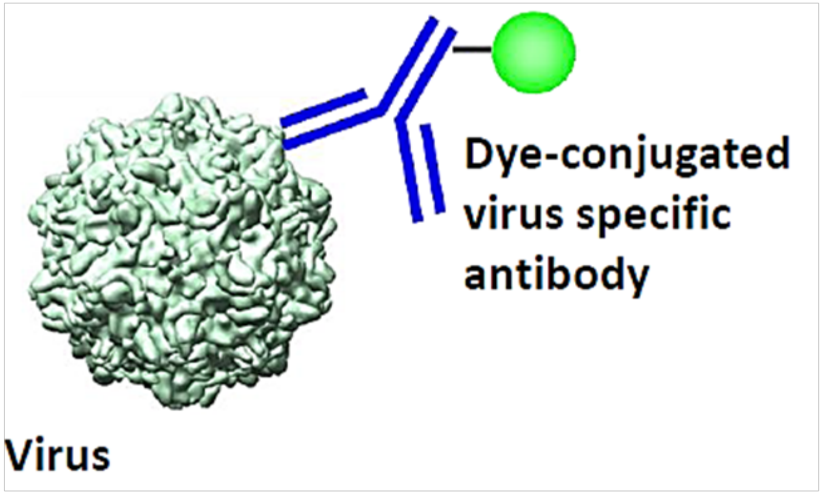
~18 – 26 nm

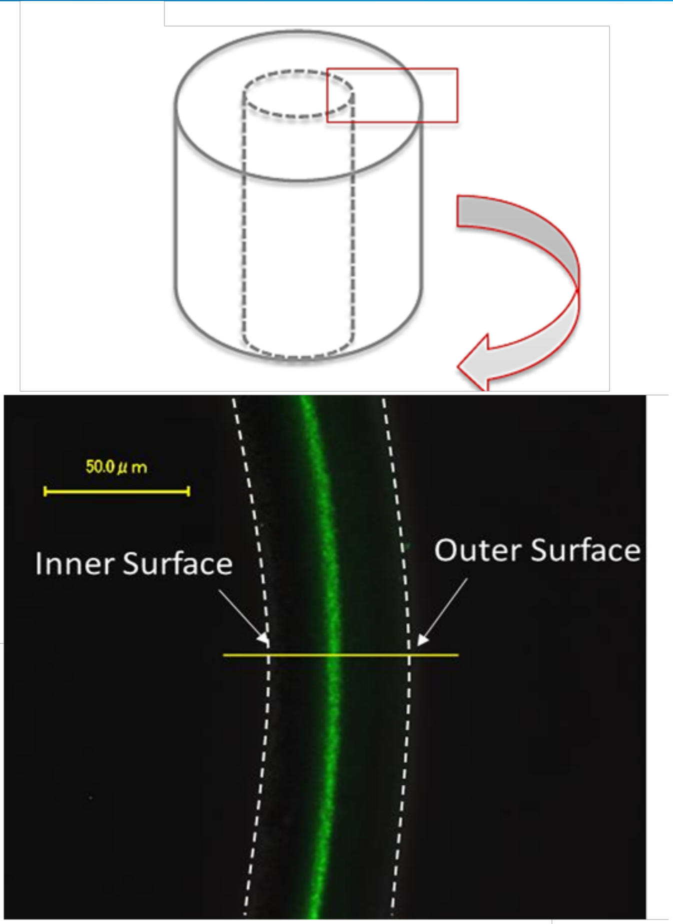
Retrovirus



~80 – 110 nm

Mechanistic Principles of Parvovirus Retention


Dye-conjugated virus specific antibody
Virus


50.0 μm
Inner Surface
Outer Surface

Cross sectional view

Porcine Parvovirus (PPV): 18 - 22 nm

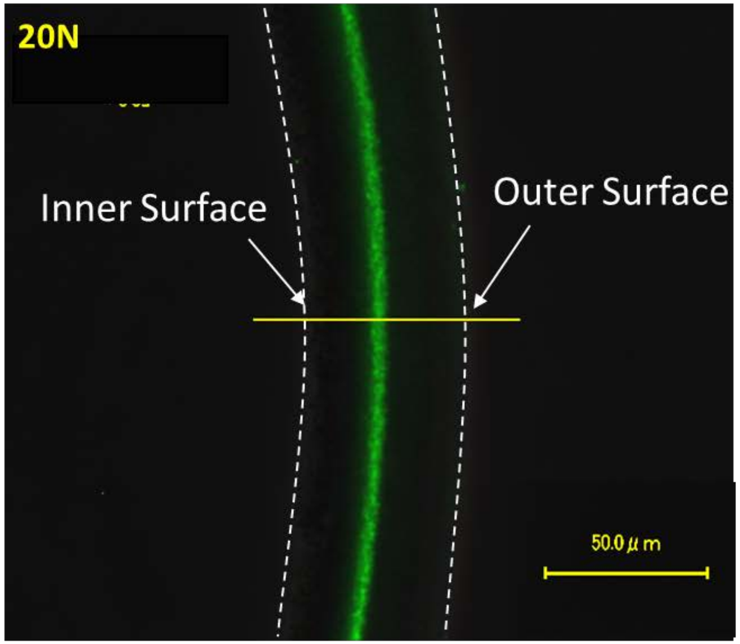
Load: 12.32 $\log_{10}$ ($TCID_{50}/m^2$)

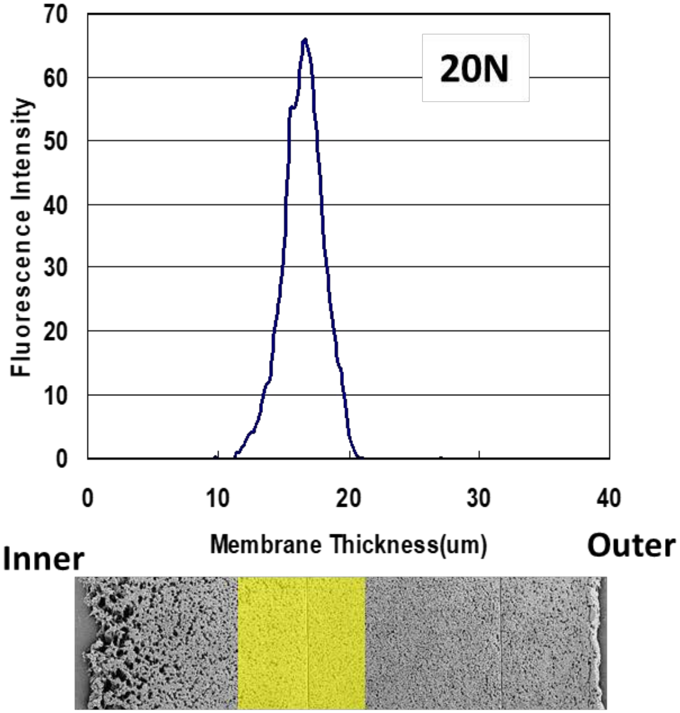
Immuno fluorescent Staining for PPV
FITC conjugated anti porcine parvovirus

5 g/L IgG, 100 mM NaCl, pH 4.5

Mechanistic Principles of Parvovirus Retention

Planova 20N


20N
[redacted]
Inner Surface
Outer Surface
50.0 µm



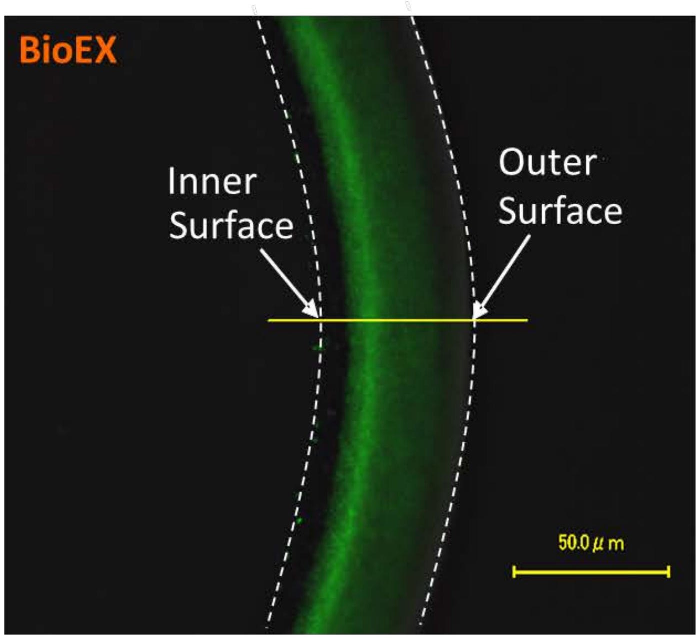
Virus load:12.32 log (TCID50/m2)

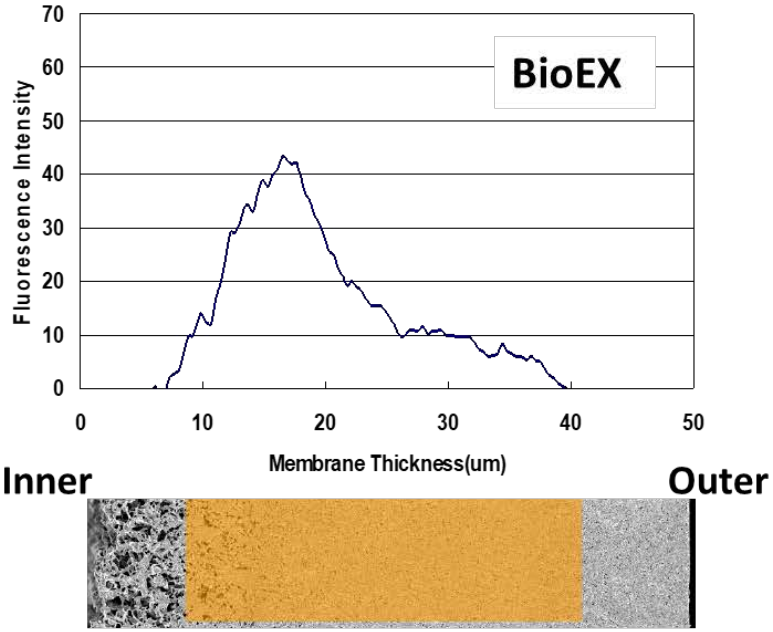
Immuno fluorescent Staining for PPV
FITC conjugated anti porcine parvovirus

5 g/L IgG, 100 mM NaCl, pH 4.5

Mechanistic Principles of Parvovirus Retention

Planova BioEX


BioEX
Inner Surface
Outer Surface
50.0 μm

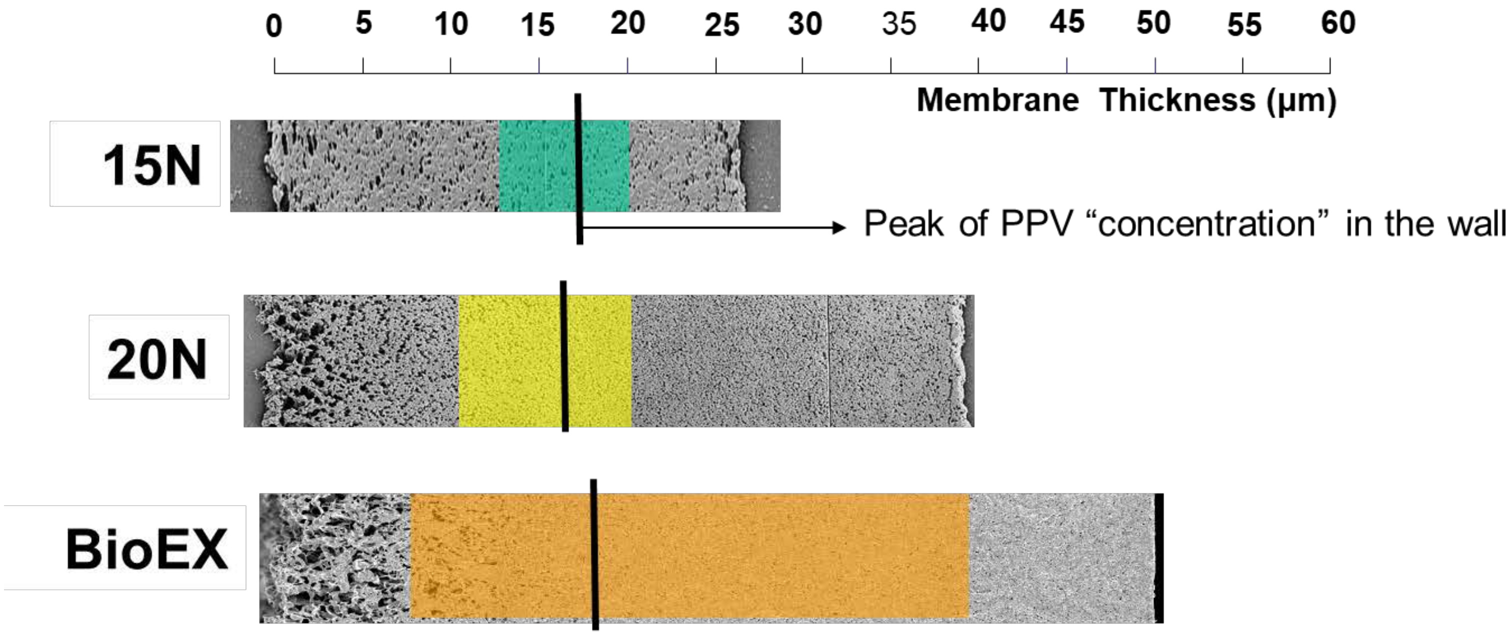


Virus load:12.32 log (TCID50/m2)

Immuno fluorescent Staining for PPV
FITC conjugated anti porcine parvovirus

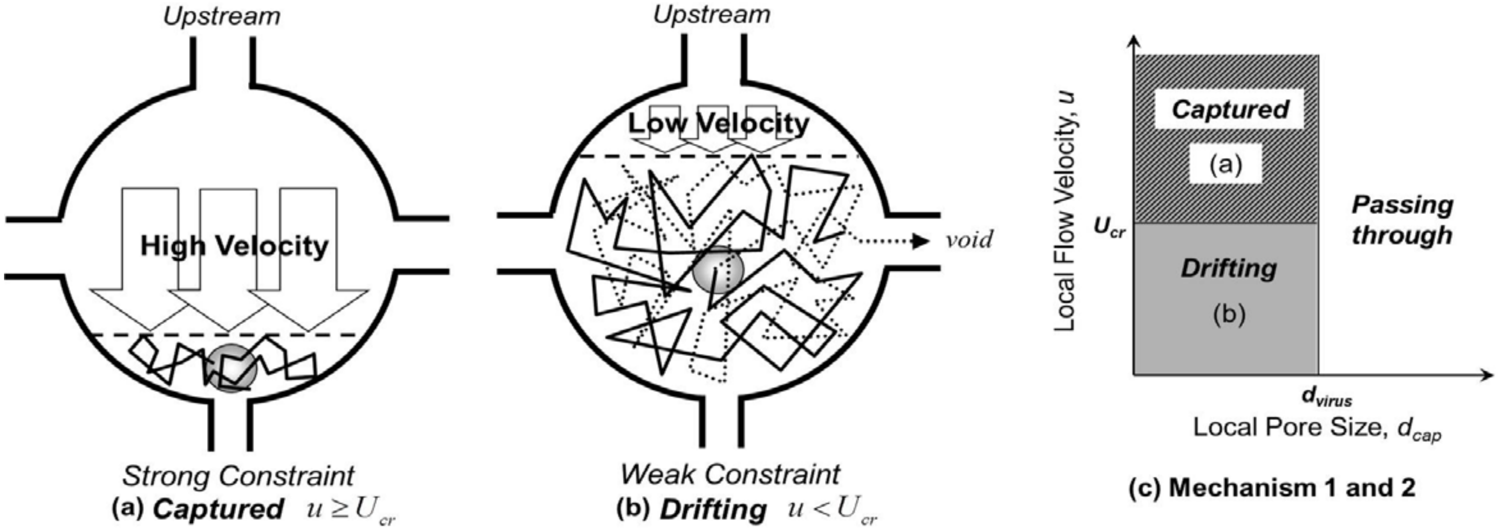
5 g/L IgG, 100 mM NaCl, pH 4.5

Mechanistic Principles of Parvovirus Retention


0
5
10
15
20
25
30
35
40
45
50
55
60
Membrane Thickness (µm)
15N
Peak of PPV “concentration” in the wall
20N
BioEX

All nanofilters on the market have a similar size exclusion mechanism. Different filter brand, pore structure & thickness: different permeability, protein loading capacity, parvovirus removal.

Mechanistic Principles of Parvovirus Retention


Upstream
High Velocity
Upstream
Low Velocity
void
Local Flow Velocity, u
U_{cr}
Captured
(a)
Drifting
(b)
Passing through
d_{virus}
Local Pore Size, d_{cap}

Strong Constraint
(a) **Captured** $u \geq U_{cr}$

Weak Constraint
(b) **Drifting** $u < U_{cr}$

(c) **Mechanism 1 and 2**

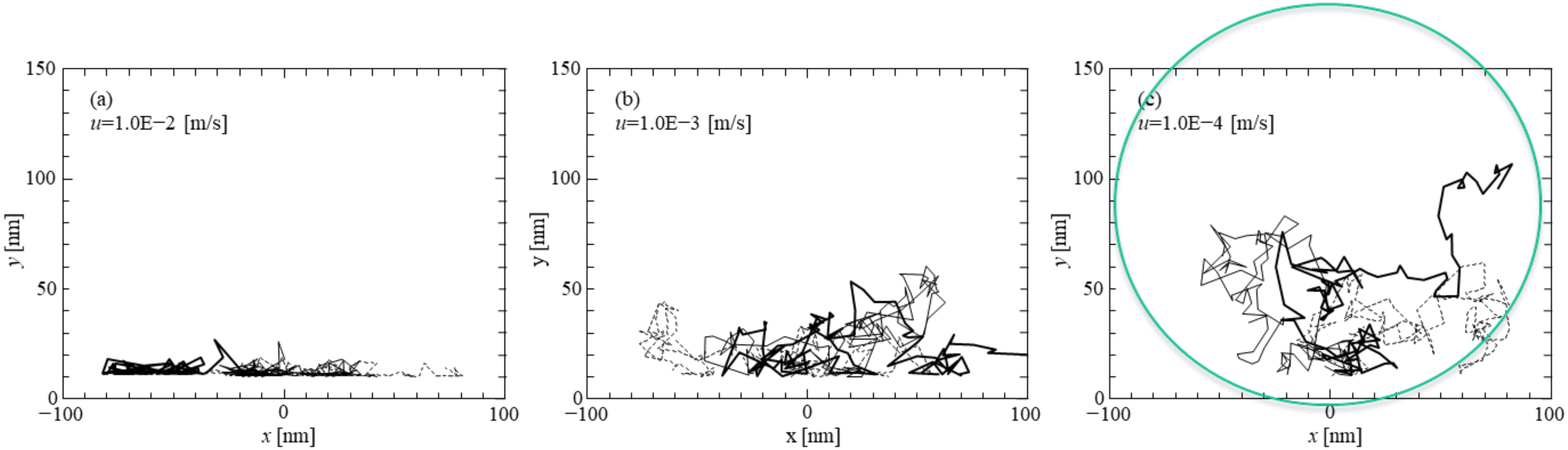
u; Flow velocity is controlled by filtration pressure
U_{cr}; Critical velocity to overcome the Brownian motion

Mechanism 1: Size exclusion
Mechanism 2: Hydrodynamic force

A Yamamoto, T Hongo-Hirasaki, Y Uchi, H Hayashida and F Nagoya.
Effect of hydrodynamic forces on virus removal capability of Planova™ filters,
AIChE Journal, 2014, 60(6): 2286–2297

Mechanistic Principles of Parvovirus Retention

Virus behavior simulation under different level of flow velocity

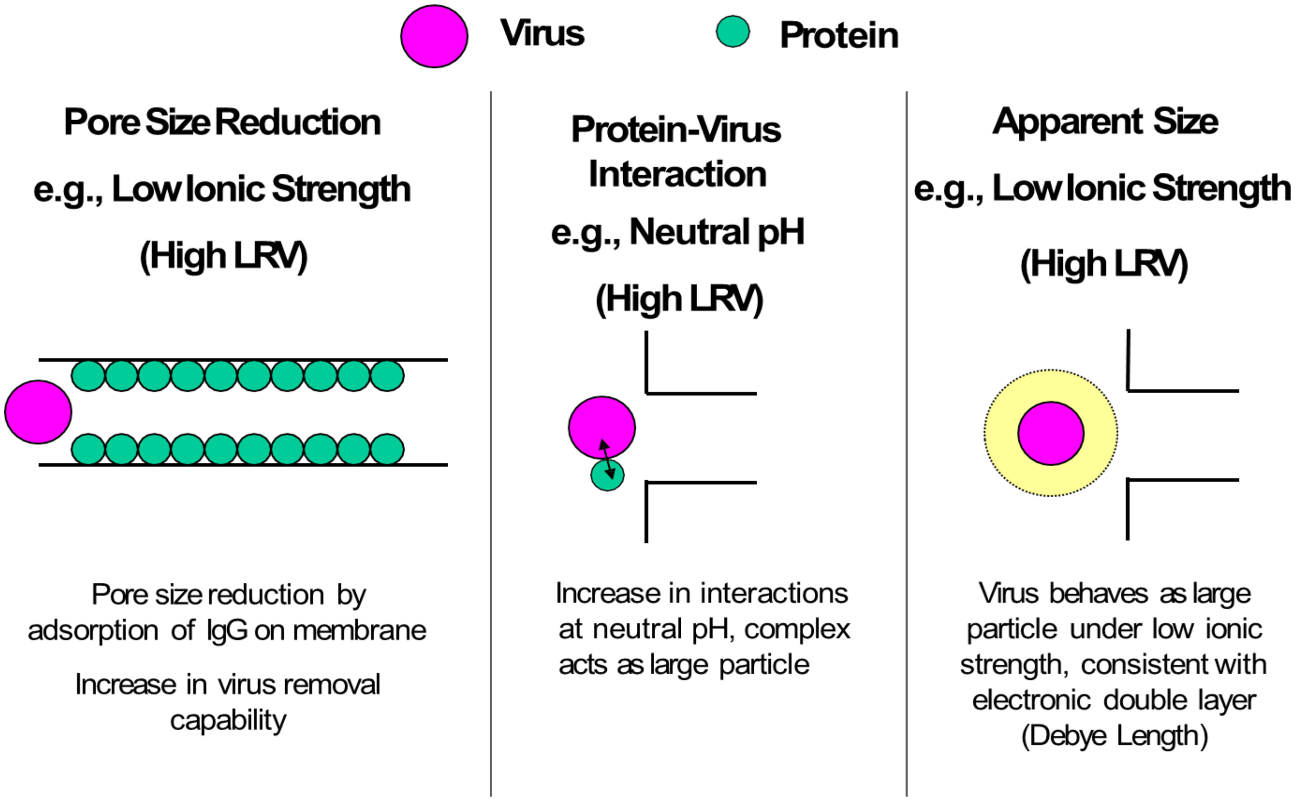


The degree of virus mobility was controlled by flow velocity.
The movable distance of virus increased with pressure decrease.

A Yamamoto, T Hongo-Hirasaki, Y Uchi, H Hayashida and F Nagoya.
Effect of hydrodynamic forces on virus removal capability of Planova™ filters,
AIChE Journal, 2014, 60(6): 2286–2297

Mechanistic Principles of Parvovirus Retention

Mechanism 3: physicochemical property & effect of solution condition (hypothesis)



Pore Size Reduction

e.g., Low Ionic Strength

(High LRV)

Pore size reduction by adsorption of IgG on membrane

Increase in virus removal capability

Protein-Virus Interaction

e.g., Neutral pH

(High LRV)

Increase in interactions at neutral pH, complex acts as large particle

Apparent Size

e.g., Low Ionic Strength

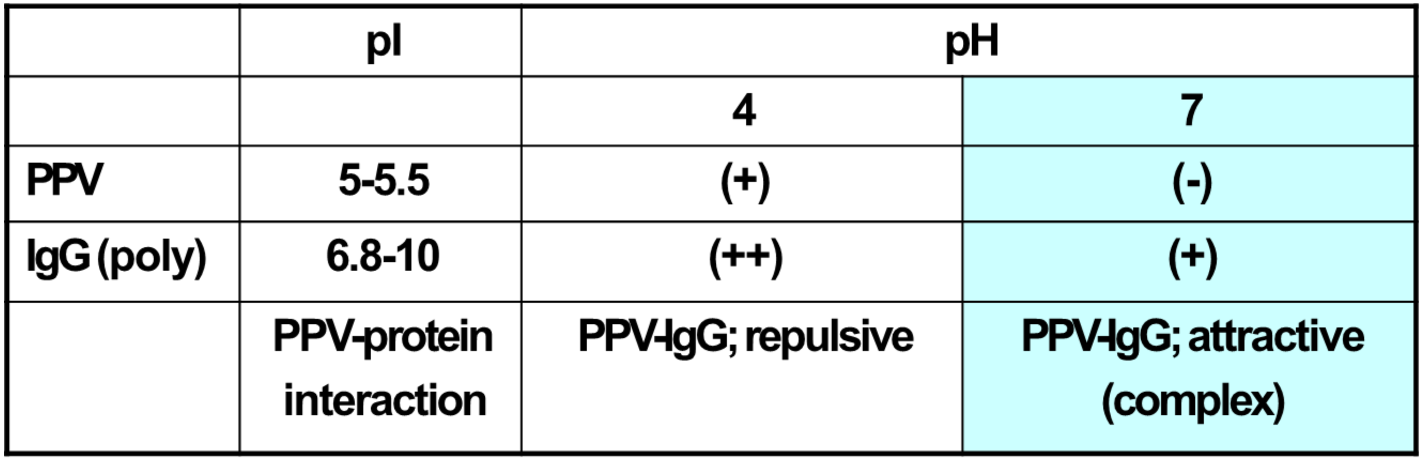
(High LRV)

Virus behaves as large particle under low ionic strength, consistent with electronic double layer (Debye Length)

Tomoko Hongo, Asahi, PDA 2013

Mechanistic Principles of Parvovirus Retention

Mechanism 3: physicochemical property & effect of solution condition (hypothesis)

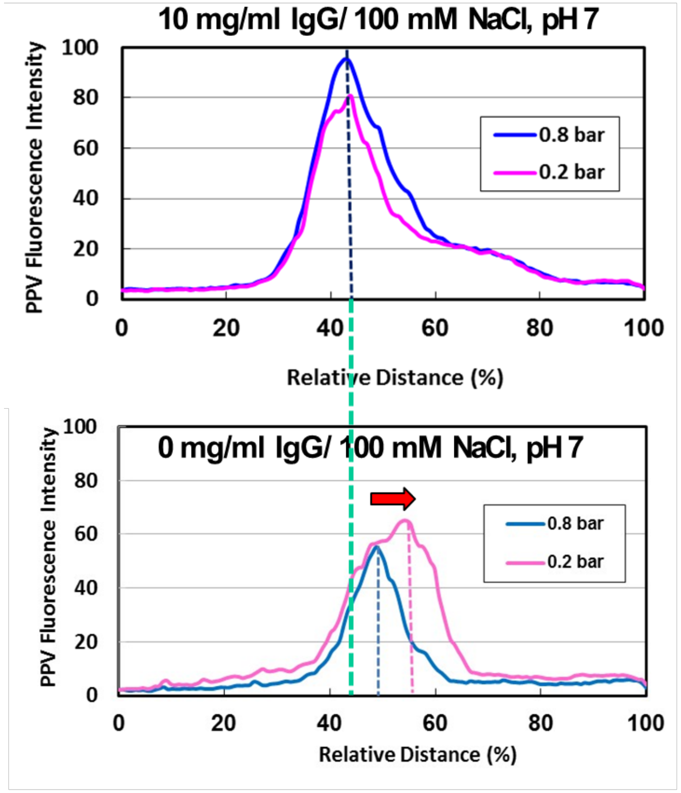


	pI	pH	
		4	7
PPV	5-5.5	(+)	(-)
IgG (poly)	6.8-10	(++)	(+)
	PPV-protein interaction	PPV-IgG; repulsive	PPV-IgG; attractive (complex)

Tomoko Hongo, Asahi, PDA 2013

Mechanistic Principles of Parvovirus Retention

Mechanism 3: physicochemical property & effect of solution condition (hypothesis)



0 or 10 mg/ml IgG/ 100 mM NaCl, pH 7,
0.5 vol% serum-free PPV spiking, 230～250 L/m^2

	PPV LRV (pool)		Relative Distance of Peak Position (%)	
Pressure (bar)	IgG(+)	IgG(-)	IgG(+)	IgG(-)
0.2	≥ 5.6	3.9	42	55
0.8	≥ 5.6	≥ 5.3	42	49

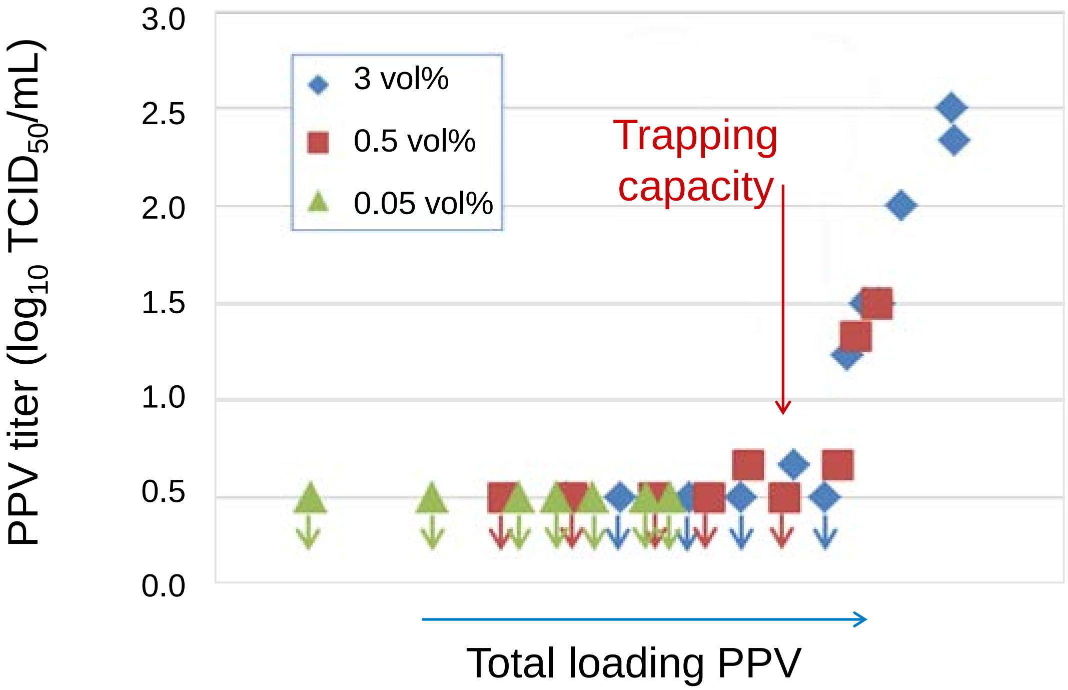
IgG (-)	PPV LRV		
Pressure (bar)	pH 4	pH 5	pH 6
0.2	3.7	3.7	3.8
0.8	≥ 4.5	≥ 4.7	≥5.0

- Peak position without IgG shifted to outer side under low pressure.
- PPV behavior with/ without IgG was different at pH 7.
- At pH7, PPV with IgG may behave larger size.

Tomoko Hongo, Asahi, PDA 2013

Mechanistic Principles of Parvovirus Retention

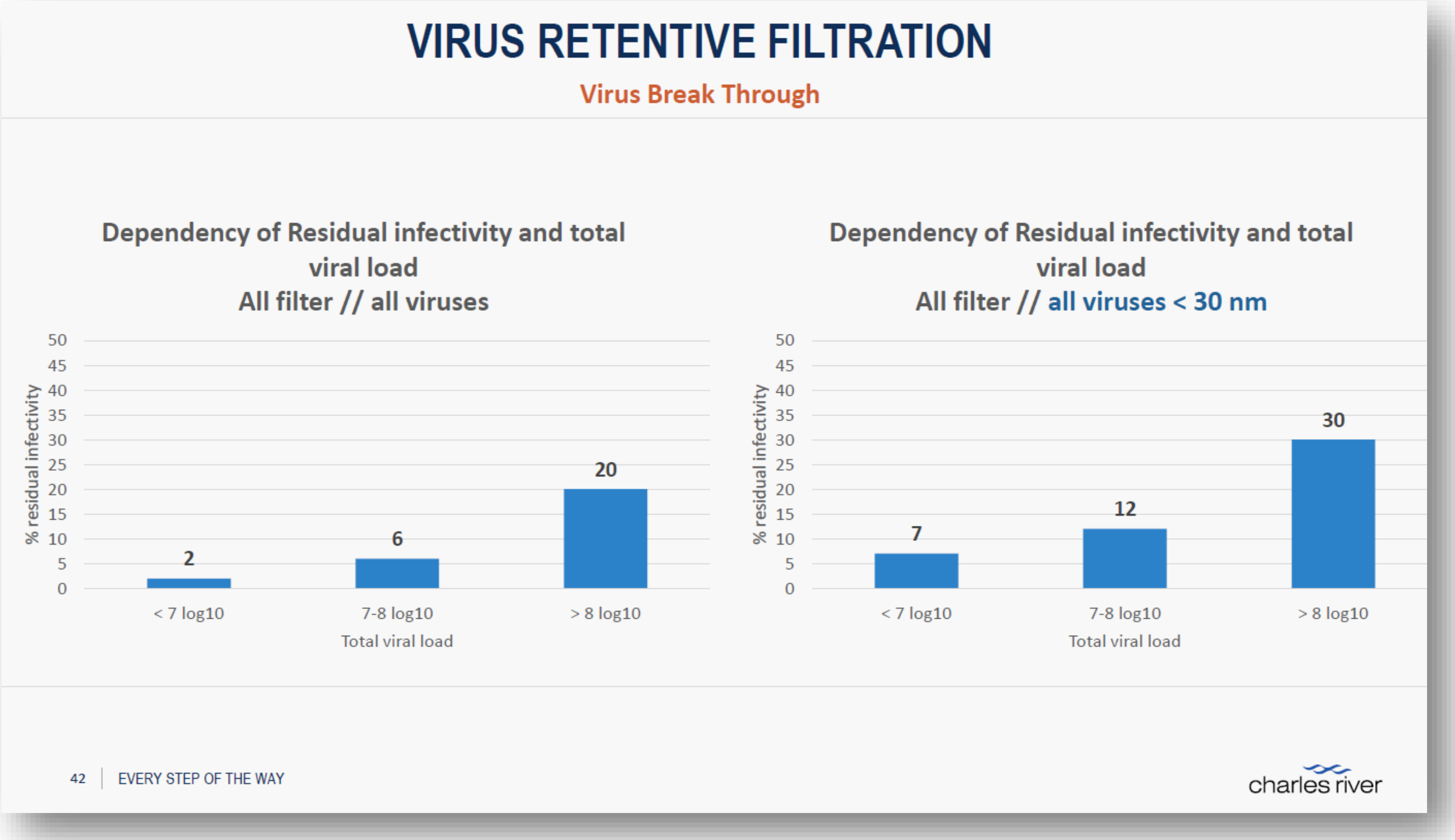
Virus filters have a finite virus capture capacity



10 mg/mL human IgG
Serum-free PPV spiking

Tomoko Hongo-Hirasaki, Asahi, 2014 Planova Workshop
(adapted)

Mechanistic Principles of Parvovirus Retention



VIRUS RETENTIVE FILTRATION

Virus Break Through

42 | EVERY STEP OF THE WAY

charles river

Horst Ruppach, Charles River, 2017 Planova Workshop (adapted)

Questions ?

Challenges of implementing virus filtration into continuous manufacturing

AsahiKASEI

Challenges of Implementing Virus Filtration into Continuous Manufacturing

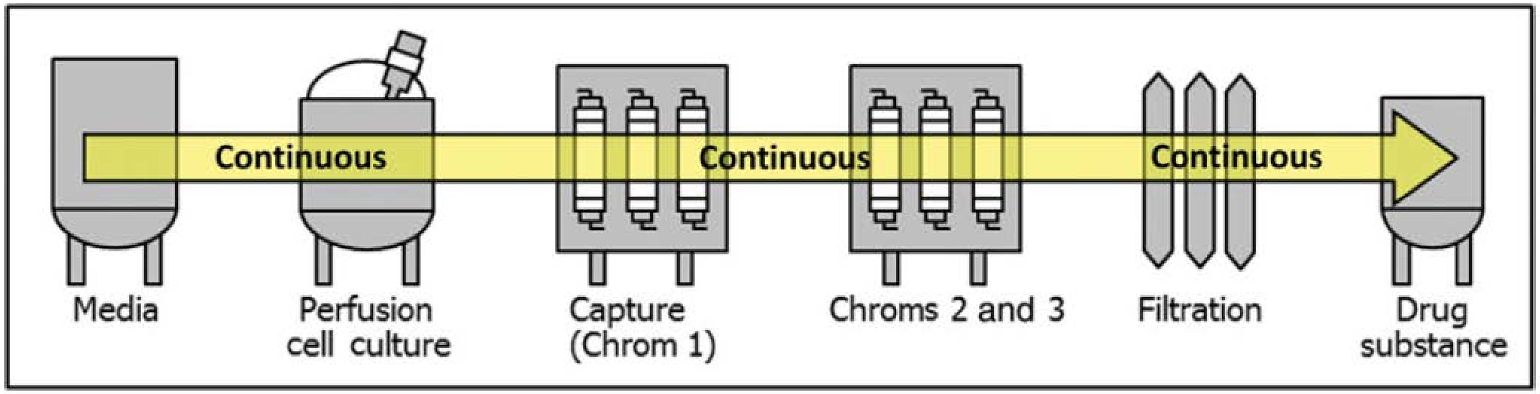
Daniel Strauss, PhD
Principal Scientist
Asahi Kasei Bioprocess America

Agenda

1) Virus filtration integration
2) Fluctuating solution conditions
3) Viral clearance validation studies

What are Continuous Processes

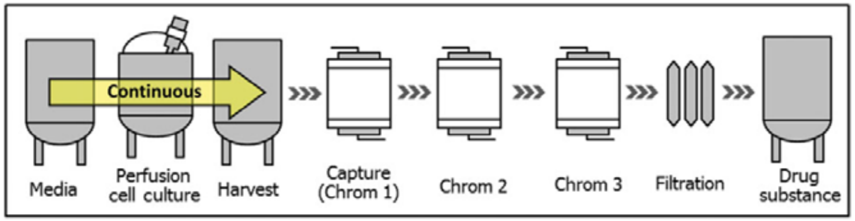
Fully Integrated Continuous Process


Continuous
Continuous
Continuous
Media
Perfusion cell culture
Capture (Chrom 1)
Chroms 2 and 3
Filtration
Drug substance

(Konstantinov and Cooney, 2015)

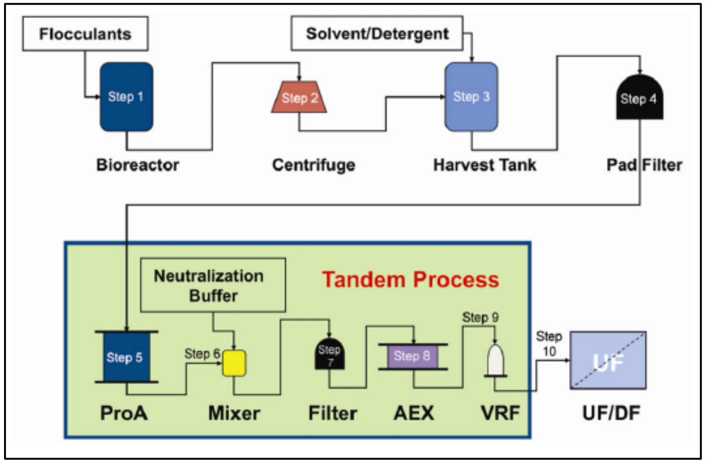
Hybrid Processes

Upstream


Continuous
Media
Perfusion cell culture
Harvest
Capture (Chrom 1)
Chrom 2
Chrom 3
Filtration
Drug substance

(Konstantinov and Cooney, 2015)

Downstream


Flocculants
Solvent/Detergent
Step 1
Step 2
Step 3
Step 4
Bioreactor
Centrifuge
Harvest Tank
Pad Filter
Neutralization Buffer
Tandem Process
Step 5
Step 6
Step 7
Step 8
Step 9
Step 10
UF
ProA
Mixer
Filter
AEX
VRF
UF/DF

(Shamashkin, *et al.*, 2015)

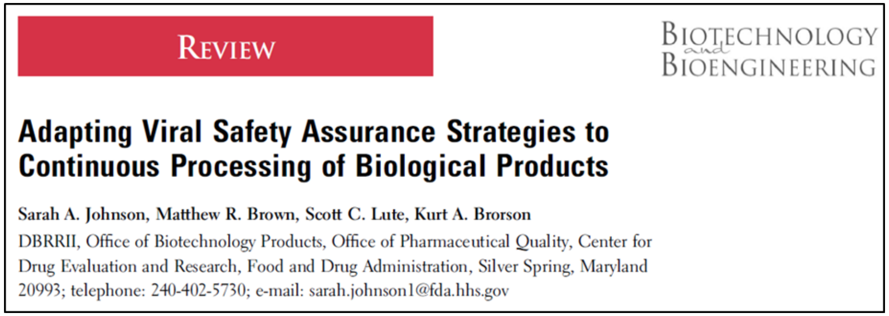
Viral Filtration Processes

Amenable to Continuous Processing

- Flow-through process
- Constant flow-rate operation
- Viral clearance is robust
- Can be run for long times

There is very little data published for continuous virus filtration processes!

Reviews:



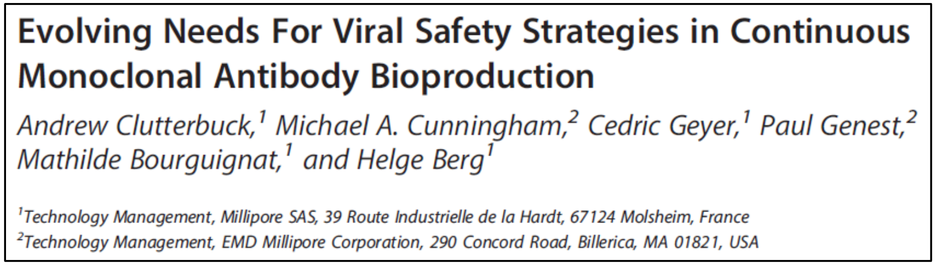
REVIEW

BIOTECHNOLOGY and BIOENGINEERING

Adapting Viral Safety Assurance Strategies to Continuous Processing of Biological Products

Sarah A. Johnson, Matthew R. Brown, Scott C. Lute, Kurt A. Brorson

DBRRII, Office of Biotechnology Products, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland 20993; telephone: 240-402-5730; e-mail: sarah.johnson1@fda.hhs.gov



Evolving Needs For Viral Safety Strategies in Continuous Monoclonal Antibody Bioproduction

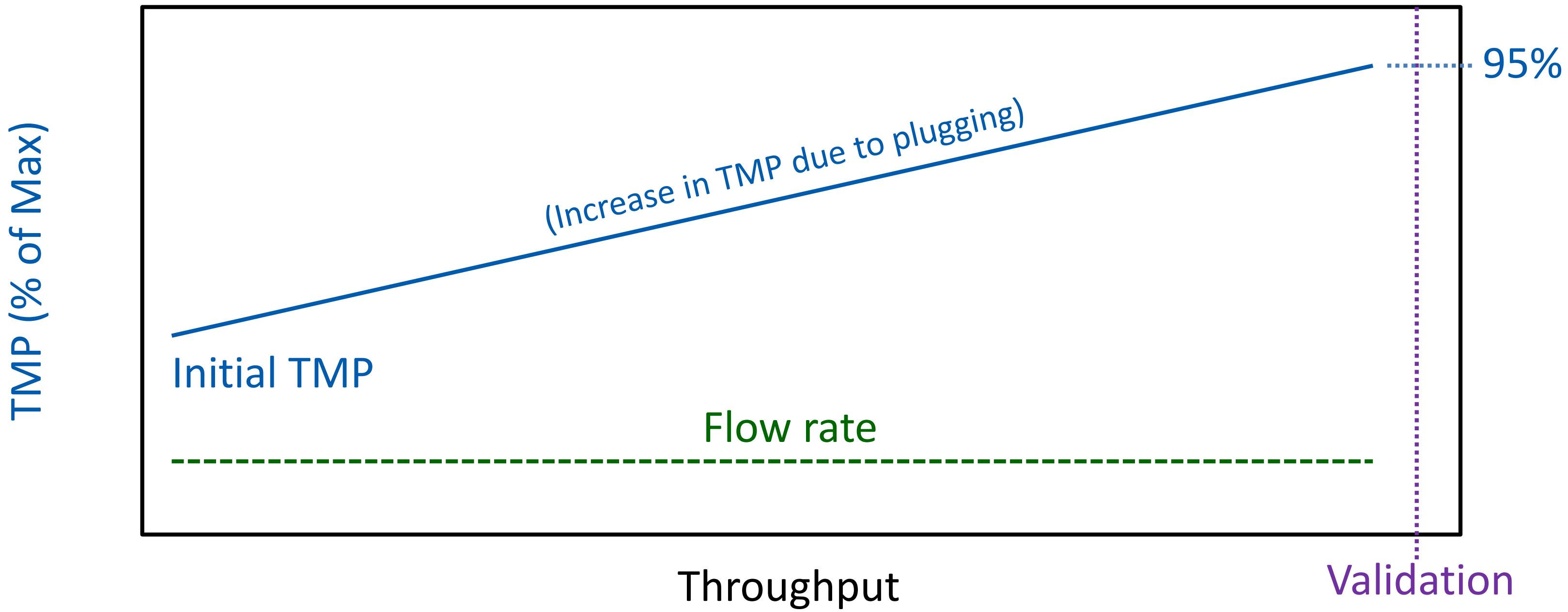
Andrew Clutterbuck,[1] Michael A. Cunningham,[2] Cedric Geyer,[1] Paul Genest,[2] Mathilde Bourguignat,[1] and Helge Berg[1]

[1]*Technology Management, Millipore SAS, 39 Route Industrielle de la Hardt, 67124 Molsheim, France*
[2]*Technology Management, EMD Millipore Corporation, 290 Concord Road, Billerica, MA 01821, USA*

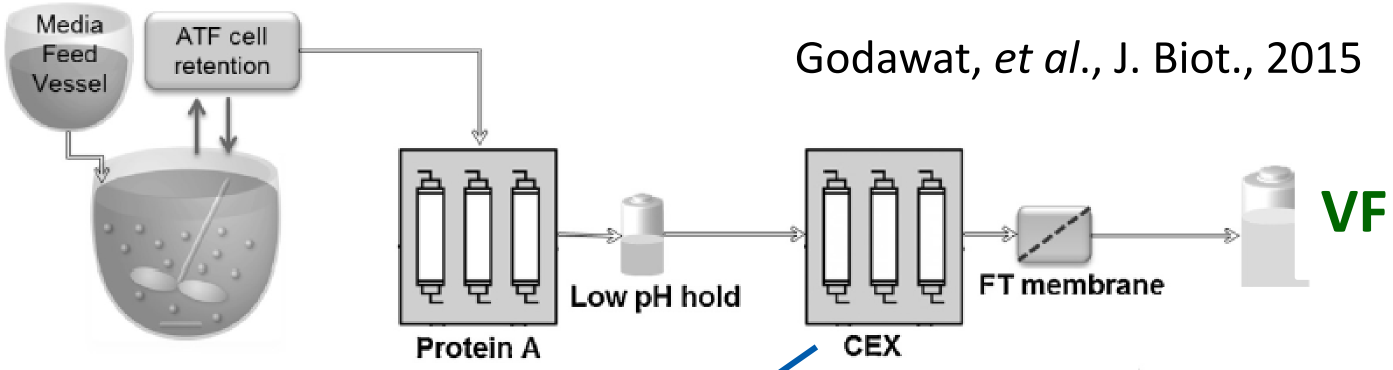
Viral Filtration Processes

Example Continuous Virus Filtration Process

- Constant flow – set by process
- Initial transmembrane pressure based on vendor recommendation
- Switch to new filter:
  - Validated throughput, OR
  - Maximum TMP

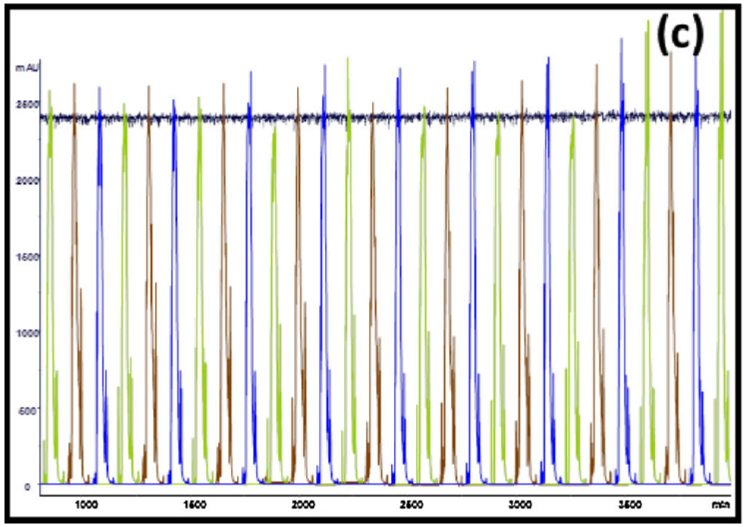


Feedstock Variation


Media Feed Vessel
ATF cell retention
Protein A
Low pH hold
CEX
FT membrane
VF

Godawat, *et al.*, J. Biot., 2015

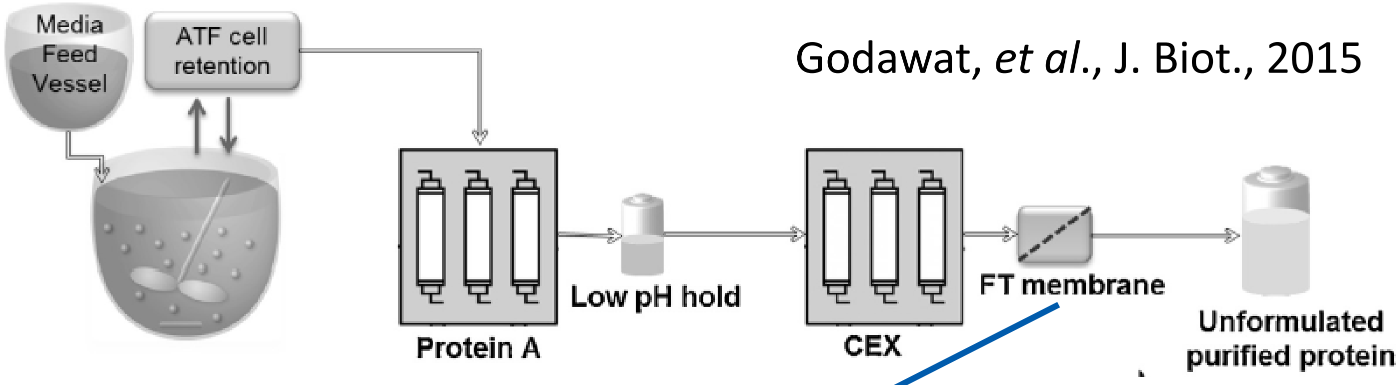
Output from a Continuous CEX Unit Operation



Solution Variations

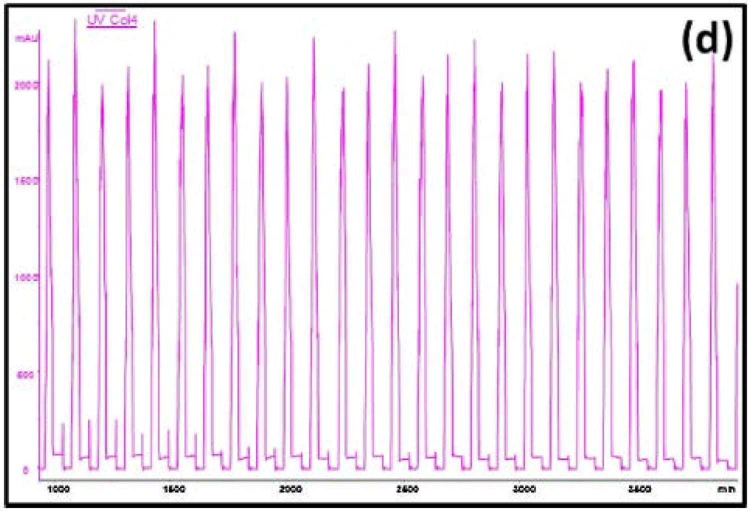
- Product Concentration
- Salt Concentration
- pH
- Impurities

Feedstock Variation


Media Feed Vessel
ATF cell retention
Protein A
Low pH hold
CEX
FT membrane
Unformulated purified protein

Godawat, *et al.*, J. Biot., 2015

Output from a Flow Through Q Membrane



Solution Variations

- Product Concentration
- Salt Concentration
- pH
- Impurities

Viral Clearance Robustness

Protein Concentration

IgG Conc. (g/L)	PPV LRV	
	Planova 20N	Planova BioEX
1	≥ 5.67	≥ 5.42
5	≥ 5.37	≥ 5.78
10	≥ 6.00	≥ 5.35
30	≥ 5.58	≥ 5.28
50	≥ 5.67	≥ 5.10

Salt Concentration

NaCl Conc. (mM)	PPV LRV	
	Planova 20N	Planova BioEX
1	≥ 5.84	N/A
50	N/A	≥ 5.48
100	≥ 6.00	≥ 5.28
200-250	≥ 5.67	≥ 5.28
500	≥ 6.00	≥ 5.92

(Hongo-Hirasaki, PDA Virus and TSE Safety Forum, 2011)

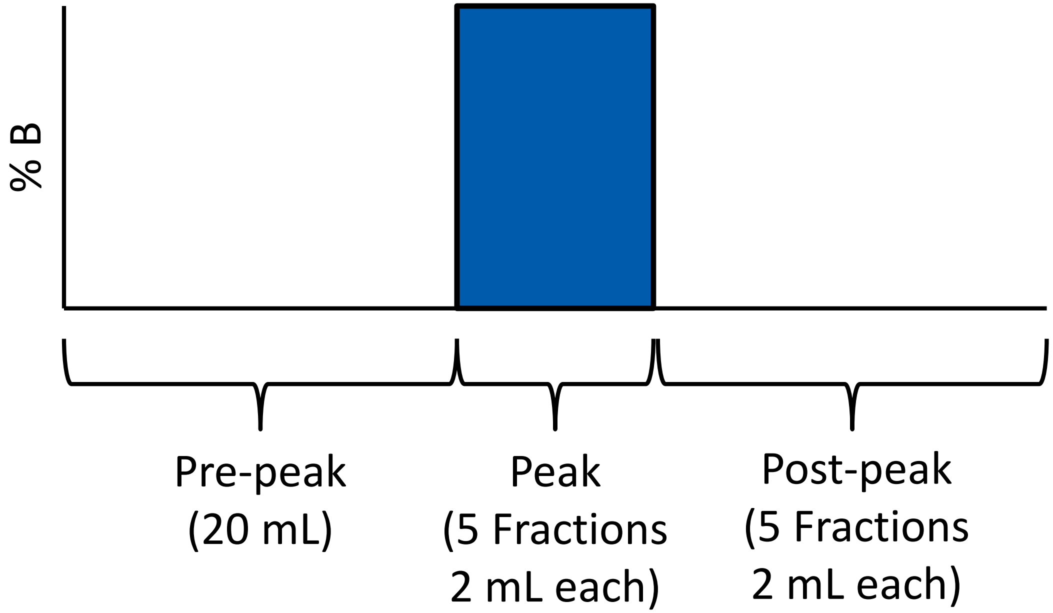
- Virus filters provide excellent viral clearance over wide ranges of conditions
- But what about the effects of fluctuating conditions during the filtration?

Fluctuating Solution Conditions

Collaboration between Asahi Kasei and FDA:

Do fluctuating solution conditions impact virus removal by VF?

Simulated peaks running virus filters using an AKTA:


% B
Pre-peak
(20 mL)
Peak
(5 Fractions
2 mL each)
Post-peak
(5 Fractions
2 mL each)

Buffer A: baseline condition

Buffer B: Same as A except one variable

Fluctuating Solution Conditions

Buffer A (Baseline condition):

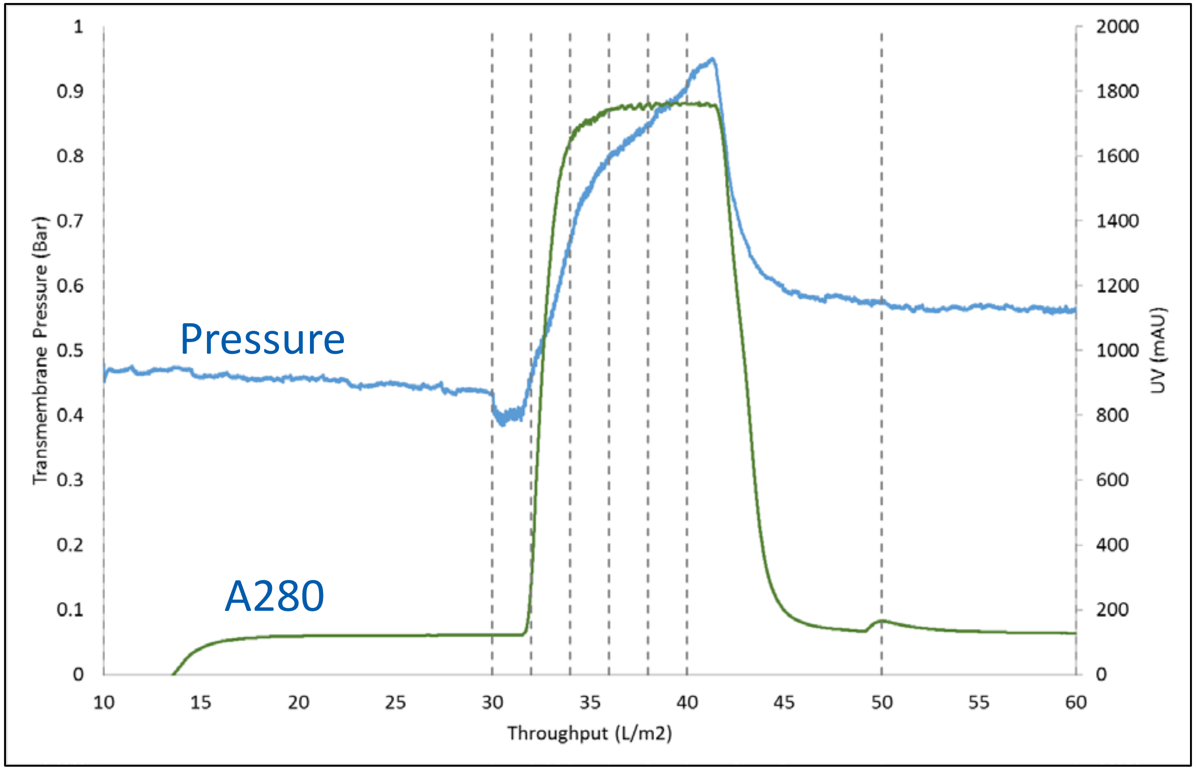
- 1 g/L Human Gamma Globulin (HGG, SeraCare)
- 20 mM Acetate, 50 mM NaCl, pH 6.0
- Spiking with PP7 bacteriophage at ~ 7 log PFU/mL

Buffer B:

- 10 g/L Human Gamma Globulin
- 20 mM Acetate, 50 mM NaCl, pH 6.0
- Spiking with PP7 bacteriophage at ~ 7 log PFU/mL

Protein Peak

Planova 20N with High Protein Peak



- 0.001 m^2 filters
- Planova 20N at 0.5 mL/min = 30 LMH
- Planova BioEX at 1.0 mL/min = 60 LMH

Effect of Protein Peak

Planova 20N

Sample	PP7 Titer (log PFU/mL)	$LRV_{Instantaneous}$
Average Load	6.0	N/A
Pre-peak	≤ 0.0	≥ 6.0
Peak F1	≤ 1.0	≥ 5.0
Peak F2	≤ 1.0	≥ 5.0
Peak F3	≤ 1.0	≥ 5.0
Peak F4	≤ 1.0	≥ 5.0
Peak F5	≤ 1.0	≥ 5.0
Post-Peak F1	≤ 0.0	≥ 6.0
Post-Peak F2	≤ 0.0	≥ 6.0

Protein peaks have little or no impact on virus removal

Effect of Protein Peak

Planova BioEX

Sample	PP7 Titer (log PFU/mL)	$LRV_{Instantaneous}$
Average Load	6.7	N/A
Pre-peak	≤ 0.0	≥ 6.7
Peak F1	≤ 1.0	≥ 5.7
Peak F2	≤ 1.0	≥ 5.7
Peak F3	≤ 1.0	≥ 5.7
Peak F4	≤ 1.0	≥ 5.7
Peak F5	≤ 1.0	≥ 5.7
Post-Peak F1	≤ 0.0	≥ 6.7
Post-Peak F2	≤ 0.0	≥ 6.7

Protein peaks have little or no impact on virus removal

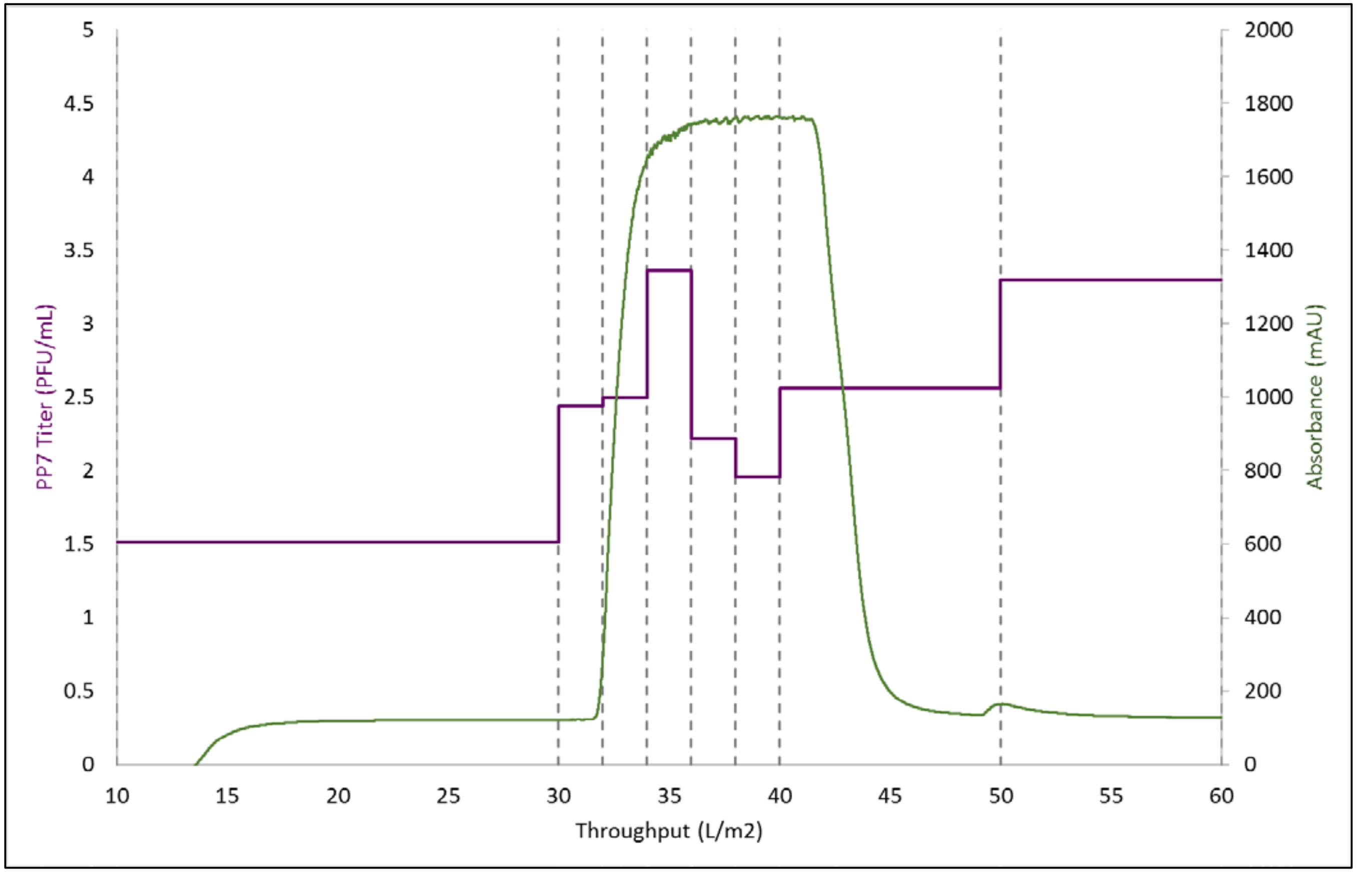
Effect of Protein Peak

Planova 20N – Run 2 (Higher than recommended load titer)

Sample	PP7 Titer (log PFU/mL)	$LRV_{Instantaneous}$
Average Load	8.1	N/A
Pre-peak	1.5	6.6
Peak F1	2.4	5.7
Peak F2	2.5	5.6
Peak F3	3.4	4.8
Peak F4	2.2	5.9
Peak F5	2.0	6.2
Post-Peak F1	2.6	5.6
Post-Peak F2	3.3	4.8

Effect of Protein Peak

Planova 20N – Run 2 (Higher than recommended load titer)



Protein peaks may impact virus removal under challenging conditions

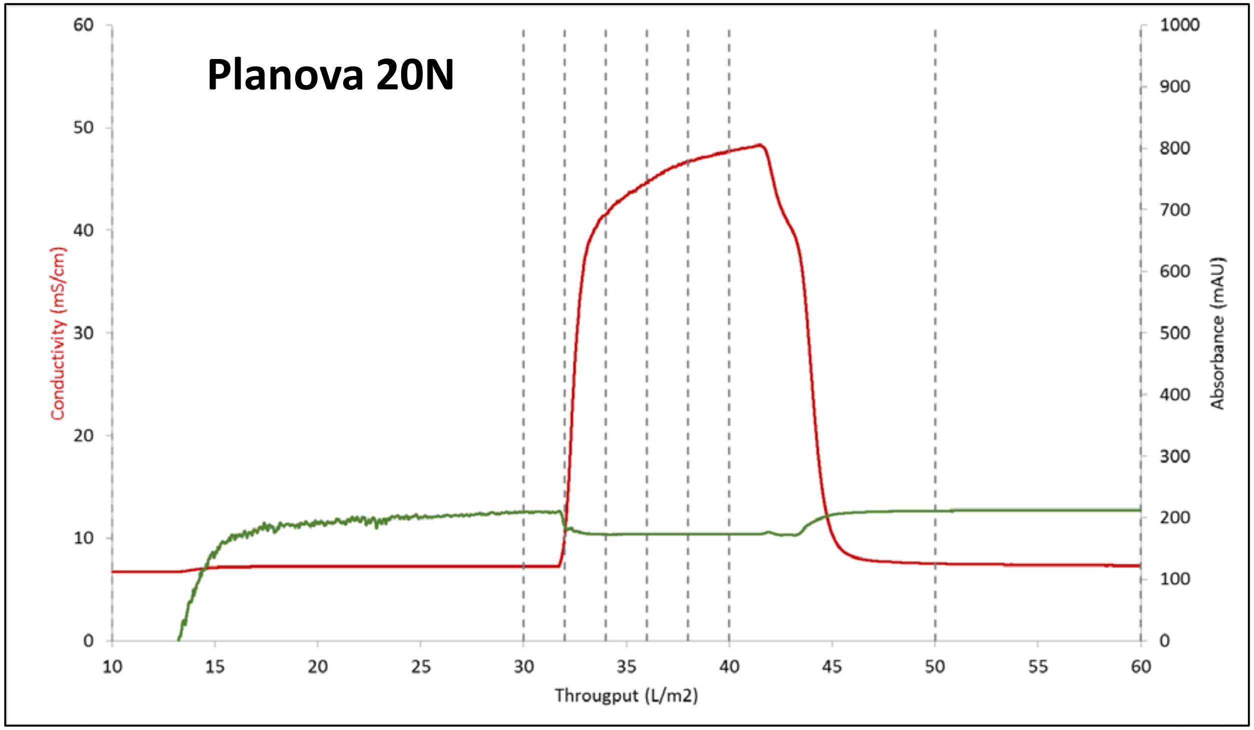
Effect of Conductivity Peak

Buffer A:

- 1 g/L HGG in 20 mM Acetate, pH 6.0, ~7 log pfu/mL PP7
- 10 mM NaCl

Buffer B:

- Same with 500 mM NaCl

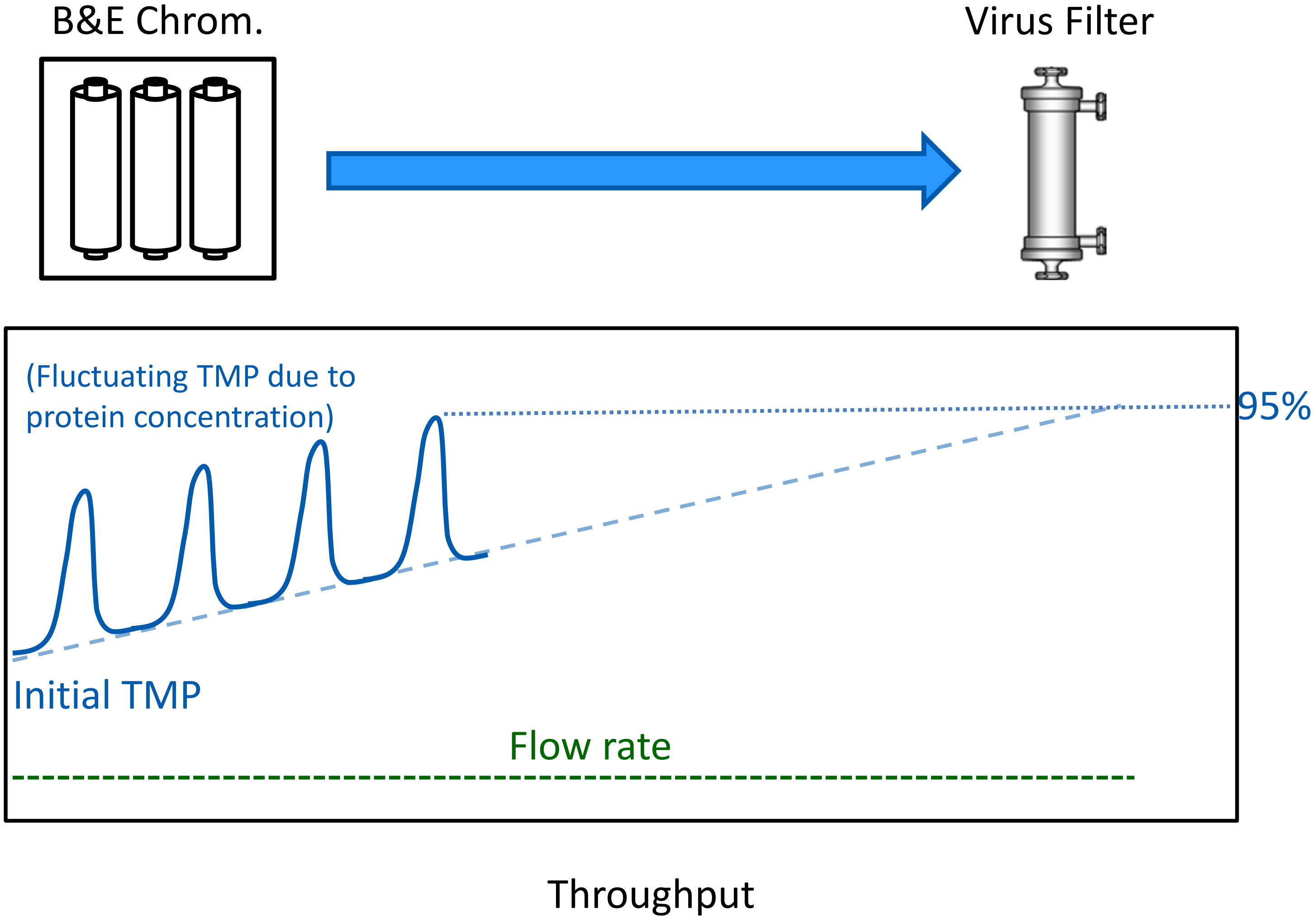


Effect of Conductivity Peak

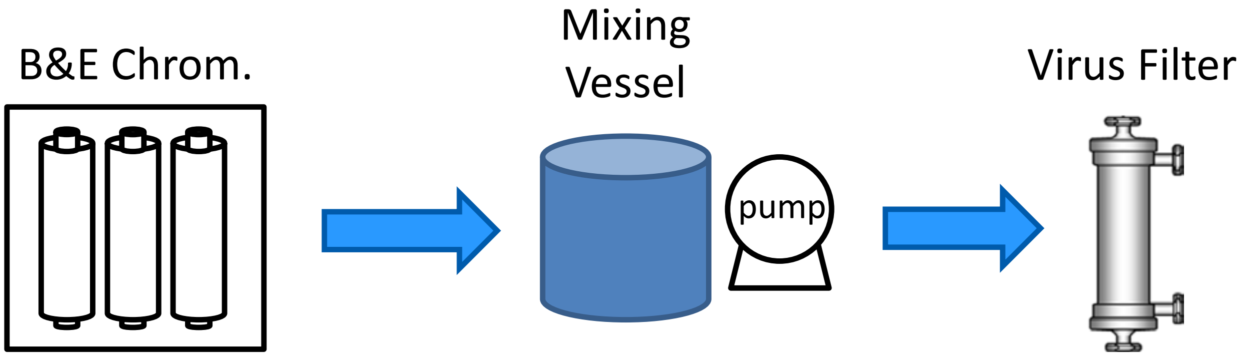
Sample	Planova 20N		Planova BioEX	
	PP7 Titer (log PFU/mL)	$LRV_{Inst.}$	PP7 Titer (log PFU/mL)	$LRV_{Inst.}$
Average Load	7.8	N/A	7.6	N/A
Pre-peak	≤ 0.0	≥ 7.8	≤ 0.0	≥ 7.6
Peak F1	≤ 1.0	≥ 6.8	≤ 1.0	≥ 6.6
Peak F2	≤ 1.0	≥ 6.8	≤ 1.0	≥ 6.6
Peak F3	≤ 1.0	≥ 6.8	≤ 1.0	≥ 6.6
Peak F4	≤ 1.0	≥ 6.8	≤ 1.0	≥ 6.6
Peak F5	≤ 1.0	≥ 6.8	≤ 1.0	≥ 6.6
Post-Peak F1	≤ 0.0	≥ 7.8	≤ 0.0	≥ 7.6
Post-Peak F2	≤ 0.0	≥ 7.8	≤ 0.0	≥ 7.6

Conductivity peaks have little or no impact on virus removal

Impact of Feedstock Variation



Mixing Tank


B&E Chrom.
Mixing Vessel
pump
Virus Filter

Mixing Vessel

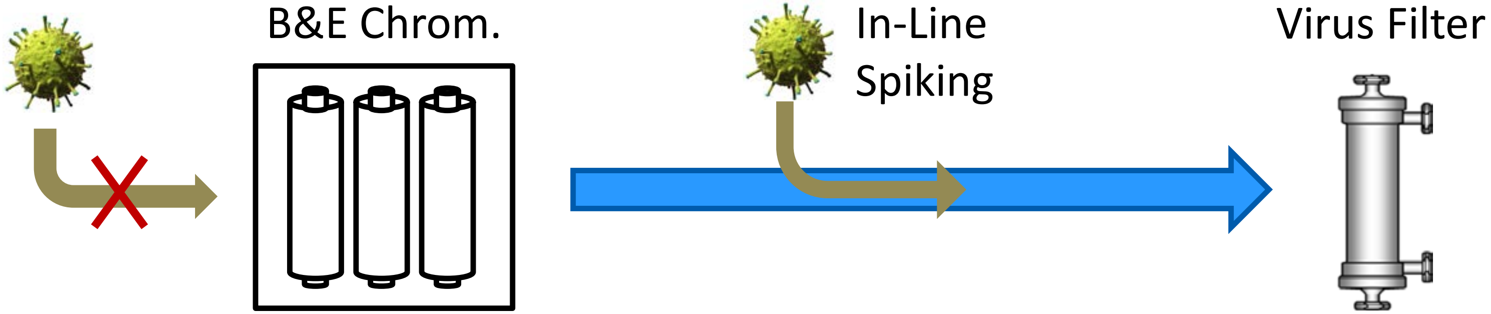
- Sufficient volume to mix multiple elution peaks
- Advantages:
    - Better utilization of filter area – no pressure spikes
    - Avoid fluctuating backpressure on upstream steps
    - Potential impact on validation strategy

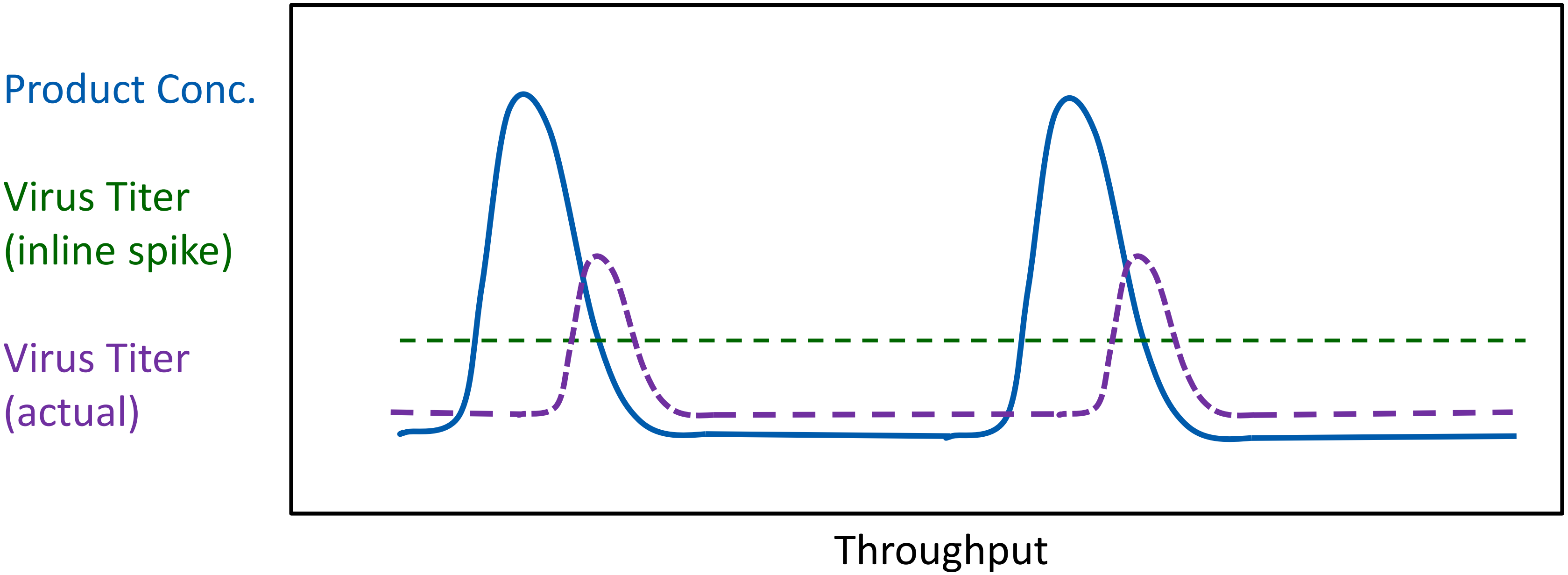
Viral Clearance Validation Studies

Viral Clearance Validation Challenges:

- In-line spiking
- Complex equipment setup
- Long filtration runs (multiple days)
    - Laborious
    - Virus and product stability issues
- Startup and stop of process may differ from steady-state
- Product profile through full process may change

In-Line Spiking


B&E Chrom.
In-Line
Spiking
Virus Filter



In-Line spiking captures protein fluctuations, but not virus fluctuations

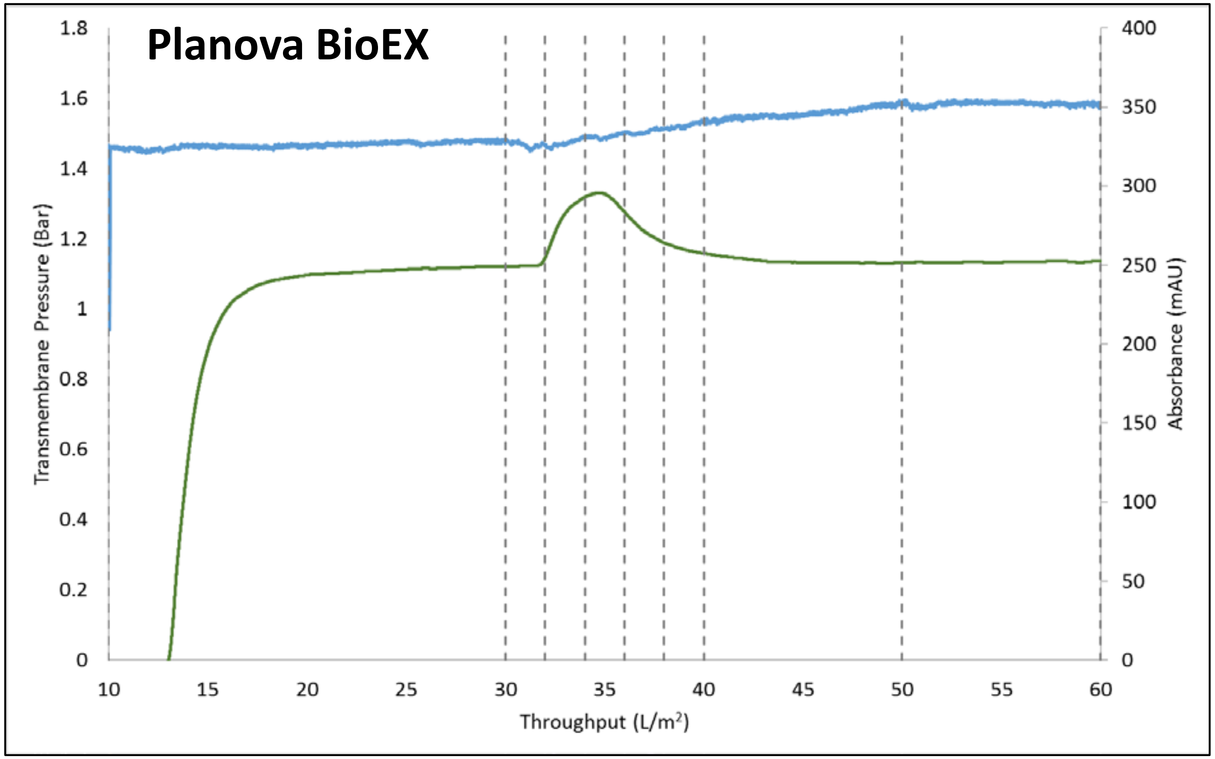
Effect of Virus Peak

Planova 20N

- Load A: 6.7 log PFU/mL
- Load B: 8.8 log PFU/mL

Planova BioEX

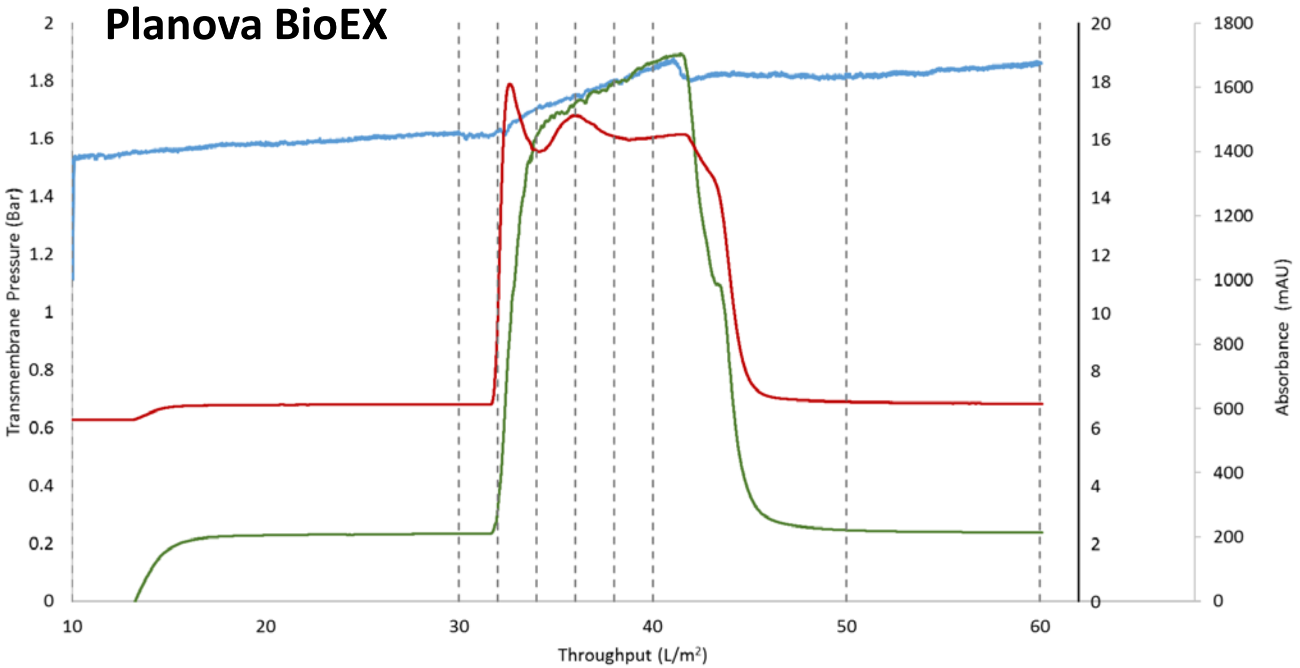
- Load A: none
- Load B: 7.8 log PFU/mL



All pools and fractions had complete clearance!

Effect of Protein, Salt, and Virus Peak

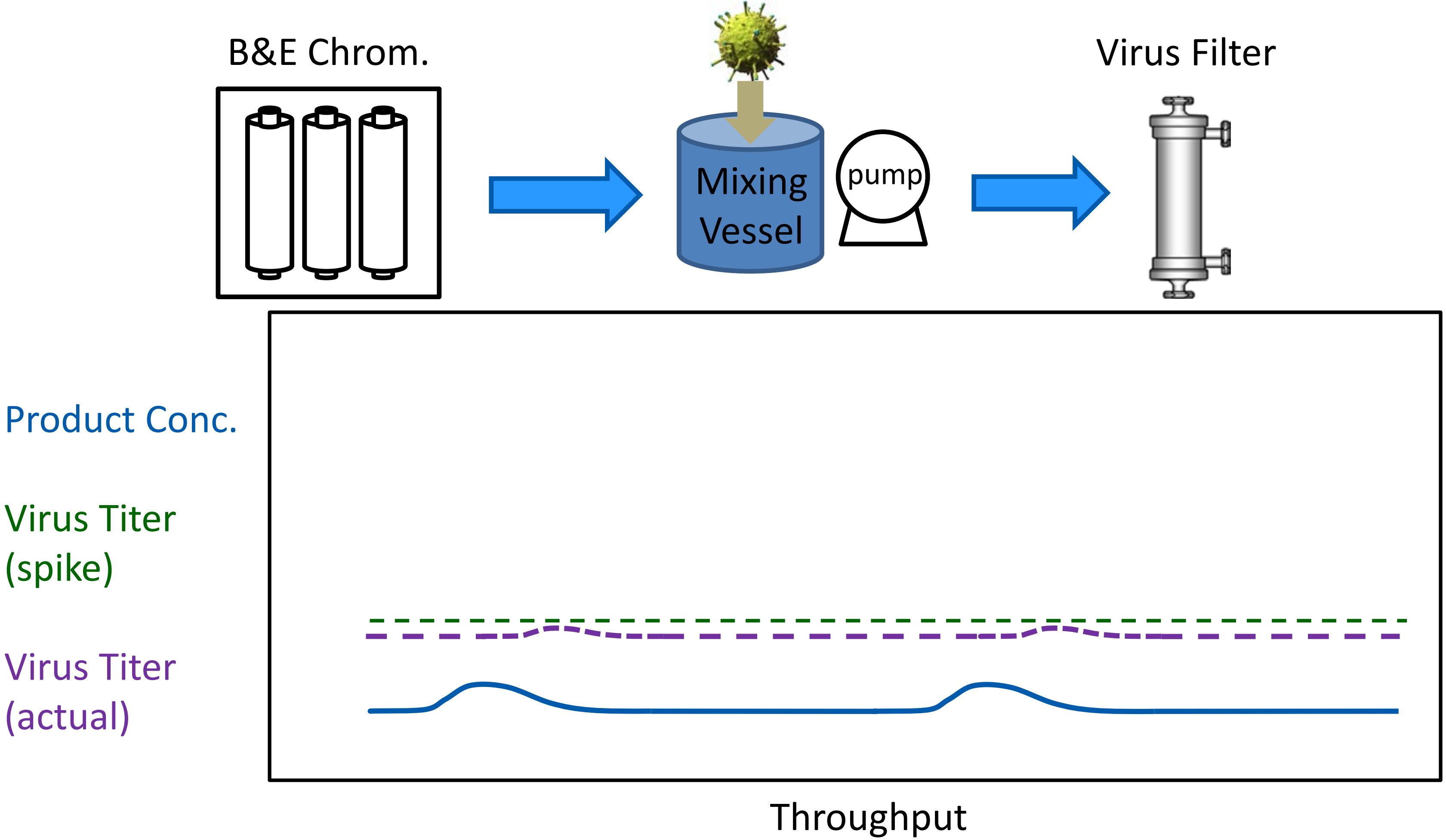
Load A	1 g/L HGG	50 mM NaCl	7.0 log PFU/mL
Load B	10 g/L HGG	500 mM NaCl	7.9 log PFU/mL


Planova BioEX

(Only performed on Planova BioEX so far)

All pools and fractions had complete clearance!

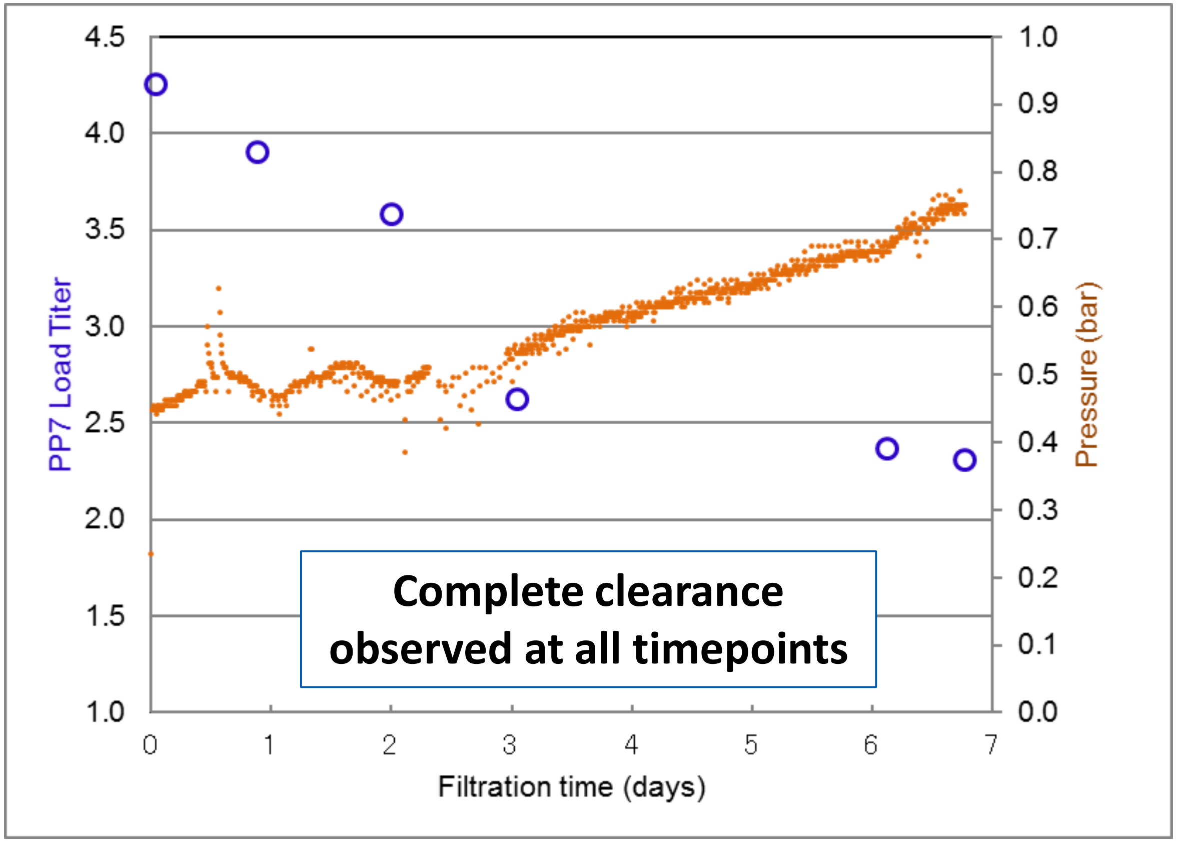
Viral Clearance Validation


B&E Chrom.
Mixing Vessel
pump
Virus Filter
Product Conc.
Virus Titer (spike)
Virus Titer (actual)
Throughput

With sufficient mixing, batch spiking may be representative

Long Term Validation Studies

Planova 20N



Complete clearance observed at all timepoints

Conditions:
0.001m^2 Planova 20N
0.15 g/L HGG
50 mM Acetate, pH 6.0
20 mM NaCl
0.5 mL/min = 30 LMH

- **Load titers decreased over the course of the run, limiting potential LRVs**

Summary

- **Continuous processes are coming!!!**

- Virus filtration implementation into a fully integrated continuous process has significant challenges associated
  - Integration strategy
  - Viral clearance validation
  - Integrity testing
  - Automation

- Virus filtration itself is highly robust…

  But we still need to get better at demonstrating its capabilities

Virus Filters as Bioprocess Subject - Current Hot Topics

PLANOVA BioEX
Catalog No. EX1-0000
Effective surface area 1.0 m²
Production number E111111****
Expiration date Jun. 01, 2099
Spinning series number ***
Assembly number ****-**

AsahiKASEI
BIOPROCESS

RAW MATERIAL SAFETY CONCEPTS – CASE STUDIES FOR MEDIA TREATMENT

Sebastian B. Teitz, PhD
Product Manager & Scientific Coordinator
Asahi Kasei Bioprocess Europe

CAACB Meeting on Raw Material Treatment,
April 2018, Cambridge, USA

www.ak-bio.com

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Contents

Introduction

- Raw Material Safety

Case Studies

- High Volume Media filtration
- Porcine Cirovirus
- Mycoplasma Treatment by Nanofiltration
- Nanofiltration of Microbial Fermentation Media Components

Conclusion

- Considerations

Introduction

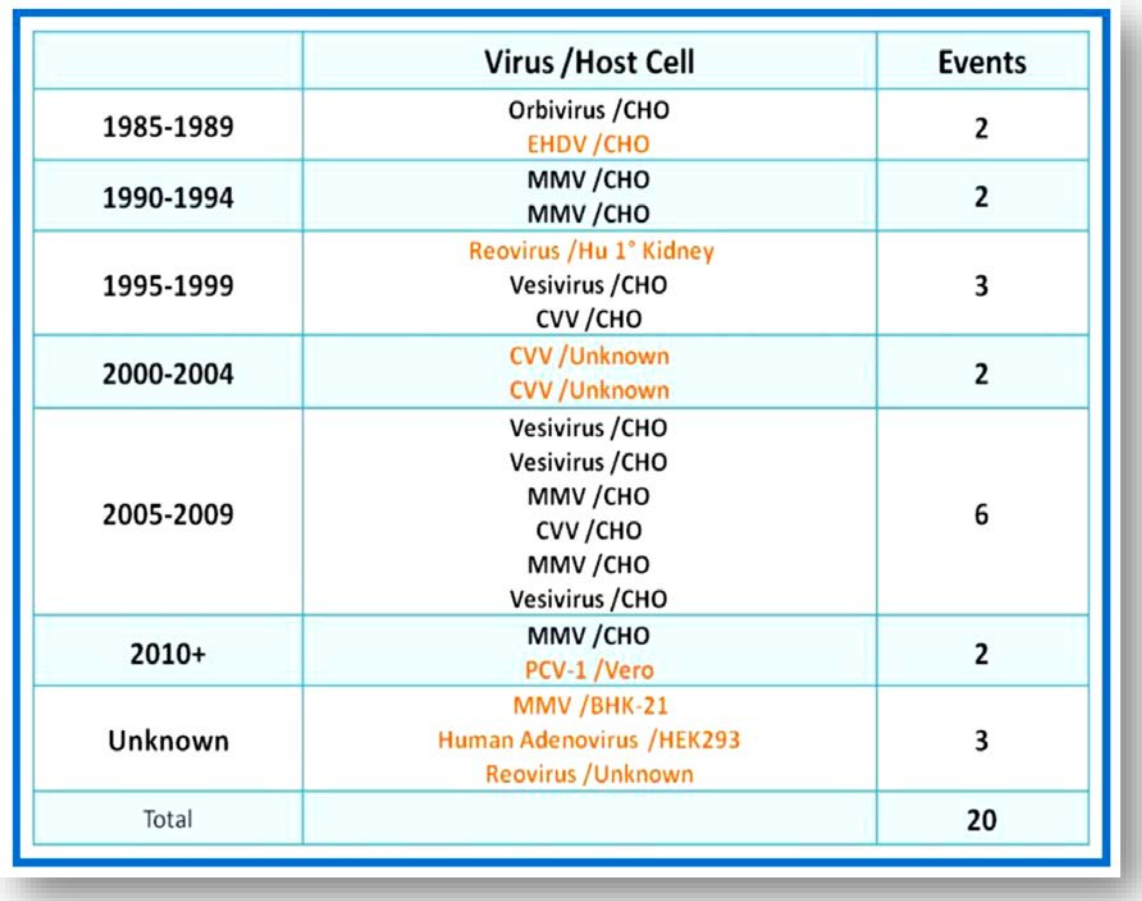
the Why? - 1) Plasma derived Products

Contamination by blood-borne pathogens
→ 10.000s of patients affected

1989	1992		1993	1994	1995/1996/1997
HIV	**B19**	**HAV**	**HCV**	**HBV**	**HAV**
PPSB	F-VIII	F-VIII	IvIg	PPSB	F-VIII

the Why? - 2) Recombinant Proteins

→ Impactful events!
- Shortage in drug supply to patient.
- Competitors product fast-tracked.



	Virus /Host Cell	Events
1985-1989	Orbivirus /CHO EHDV /CHO	2
1990-1994	MMV /CHO MMV /CHO	2
1995-1999	Reovirus /Hu 1° Kidney Vesivirus /CHO CVV /CHO	3
2000-2004	CVV /Unknown CVV /Unknown	2
2005-2009	Vesivirus /CHO Vesivirus /CHO MMV /CHO CVV /CHO MMV /CHO Vesivirus /CHO	6
2010+	MMV /CHO PCV-1 /Vero	2
Unknown	MMV /BHK-21 Human Adenovirus /HEK293 Reovirus /Unknown	3
Total		20

Mike Wiebe, CAACB, IBC Viral Safety, Huntington, Feb 25 2013

the Why? - 2) Recombinant Proteins

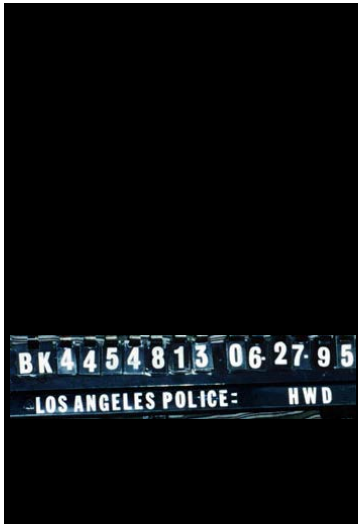
2) Recombinant proteins

Associated / suspected contamination, e.g. by rodents at some point in the supply chain.

A single mouse feces can contain >10.000 i.u. of parvovirus.
(Besselsen et al. Comp Med (2008) 58: 140)

the Why? - 3) Viral Vaccines contamination events by Mycoplasma*

- Initially detected by elevated total DNA content in final doses
- Investigation confirmed M.arginini (+ some M.fermentans & M.hyorhinis) as contaminants
- Suspected sources: bovine (BSA, M.arginini), porcine (Trypsin, M.hyorhinis), human (commensal, M.ferementans)


[redacted]
BK 4454813 06 27 95
LOS ANGELES POLICE: HWD

[redacted]

[redacted]
BK 4454813 06 27 95
LOS ANGELES POLICE: HWD

*Eric Sarcey, Sanofi Pasteur, CAACB Workshop on Contamination with Difficult to Detect Bacteria, Boston, April 2016

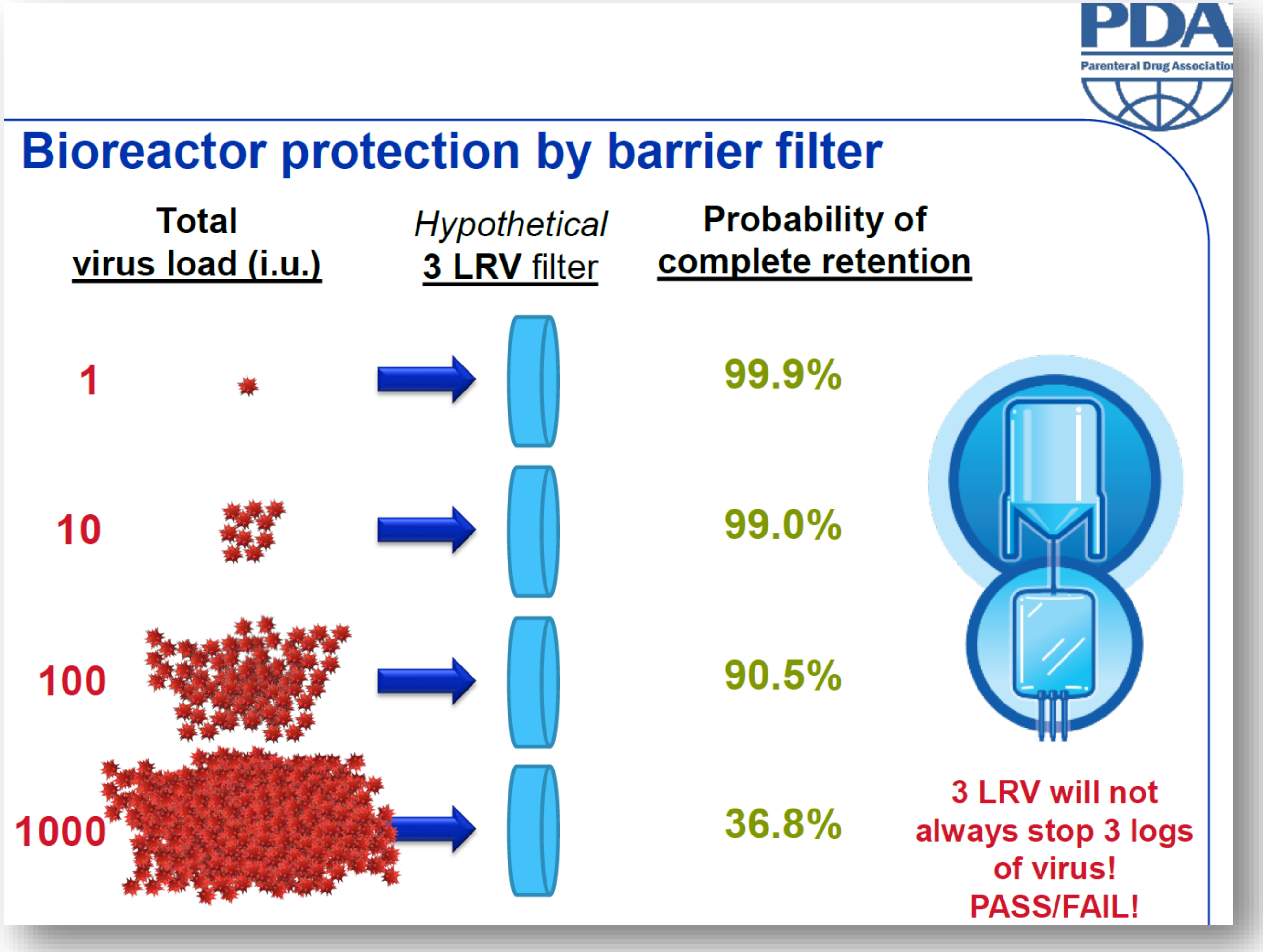
the Why? - 4) Viral Vaccines contamination event with Porcine Circovirus*

- PCV1 identified as contaminant of the paediatric Rotarix vaccine through MPS
- 100.000s of children were exposed to a live virus.
- All Vero-cell banks back to MCB (1983) found positive for PCV1
- Suspected entry point: porcine Trypsin, used during MCB generation.

GUILTY!

*Delwart et al., Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus, J Virol. 2010 Jun;84(12):6033-40

Raw Material Safety - Considerations



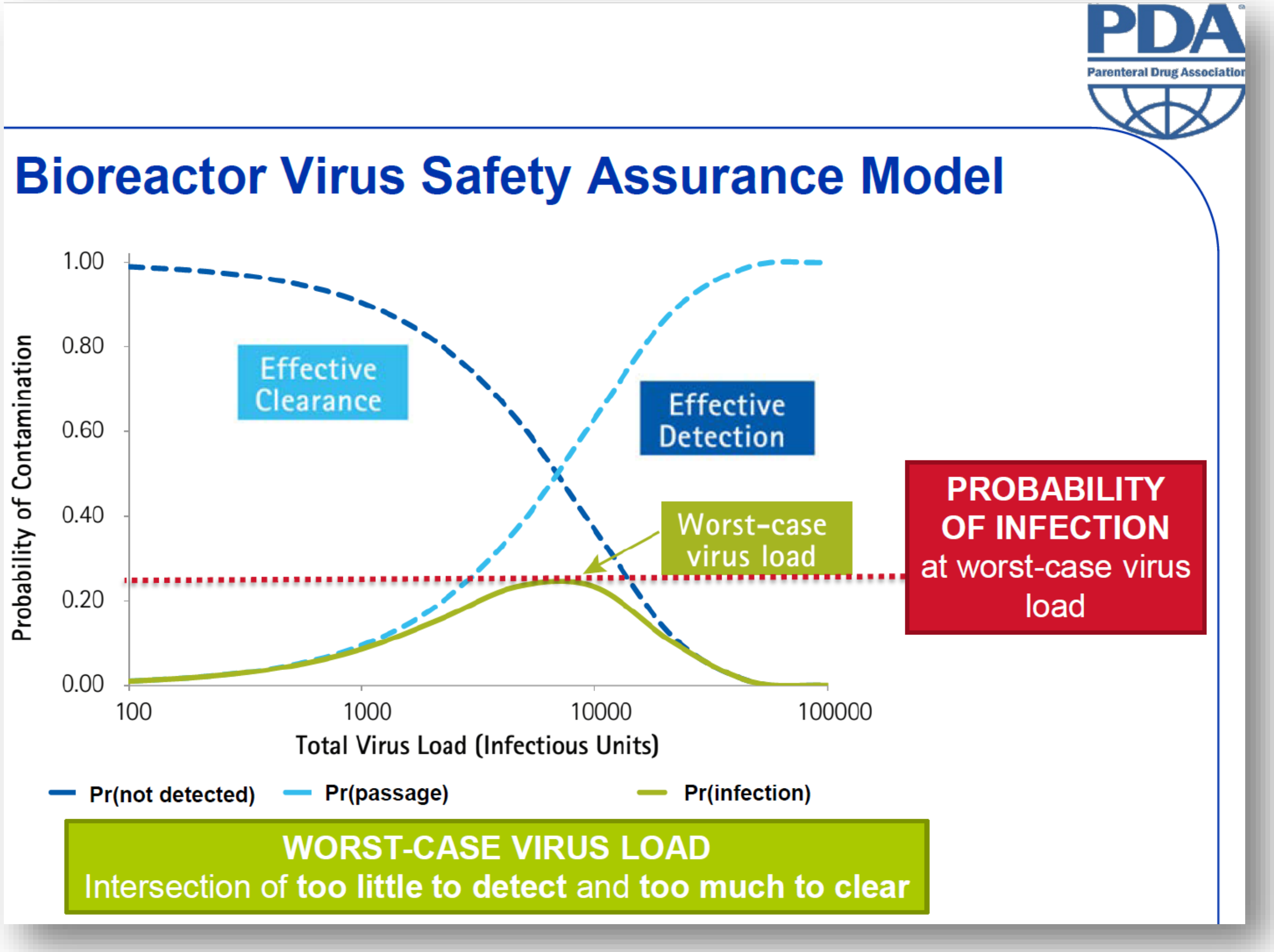
Bioreactor protection by barrier filter

Total virus load (i.u.)	*Hypothetical* 3 LRV filter	Probability of complete retention
1		99.9%
10		99.0%
100		90.5%
1000		36.8%

3 LRV will not always stop 3 logs of virus!
PASS/FAIL!

Barrier & Beyond | Damon Asher | 2014 PDA Europe Mycoplasma | Sep 2014

Raw Material Safety - Considerations



Bioreactor Virus Safety Assurance Model

PROBABILITY OF INFECTION at worst-case virus load

WORST-CASE VIRUS LOAD
Intersection of **too little to detect** and **too much to clear**

Barrier & Beyond | Damon Asher | 2014 PDA Europe Mycoplasma | Sep 2014

Raw Material Safety – Asahi‘s Stance

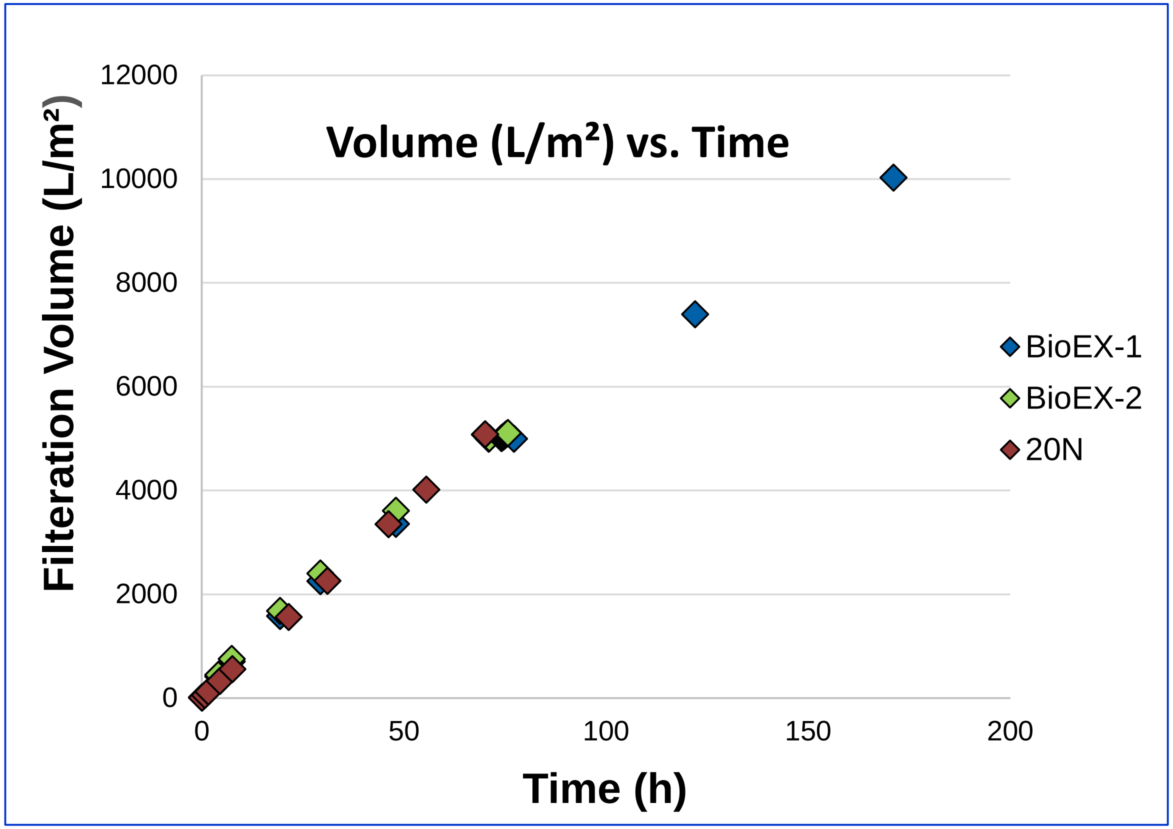
- Specific nanofilter for USP ? → NO. Same virus as in DSP to be removed!
- “Low cost” nanofilter? → NO. High quality nanofilter required
- Much higher flux ? → NO → 1) Nano-pore limitation
  2) or with sacrificing LRV
- High volumetric loads? → YES! Unlike in DSP, higher L/m^2 loads feasible

↓ ↓ ↓

Longer filtration times required

Case Studies

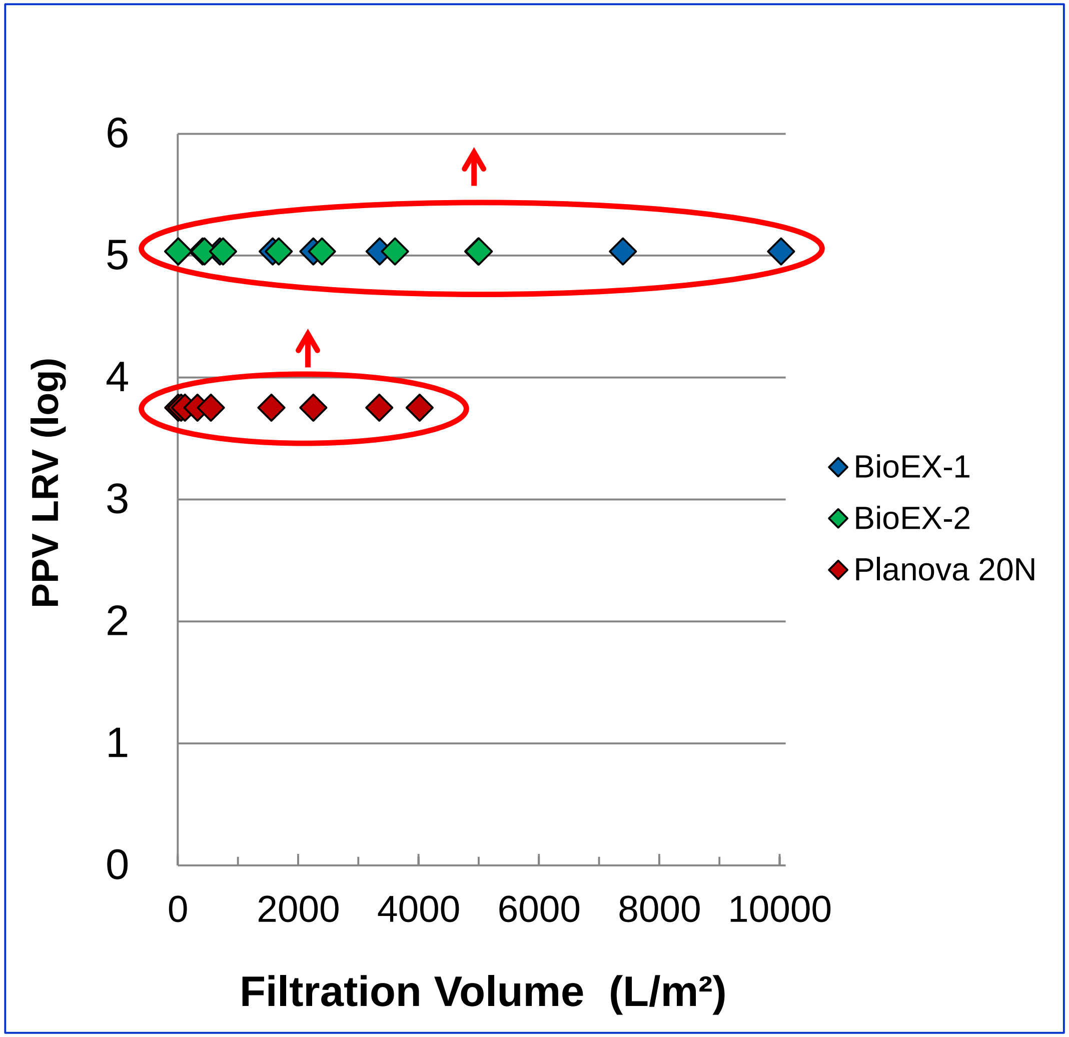
1) CD-CHO Medium Filtration



- ✓ No impact of the virus spike on Filtration Volume
- ✓ **Consistent** performance
- ✓ **20N:**
  2 000 L/m² in 1 day
  5 000 L/m² in 3 days
- ✓ **BioEX:**
  same as 20N
  + 10 000 L/m² in 7 days

Konstantin Agolli, Asahi Kasei, BioInnovation 2016, Berlin, February 10th, 2016

1) CD-CHO Medium Filtration

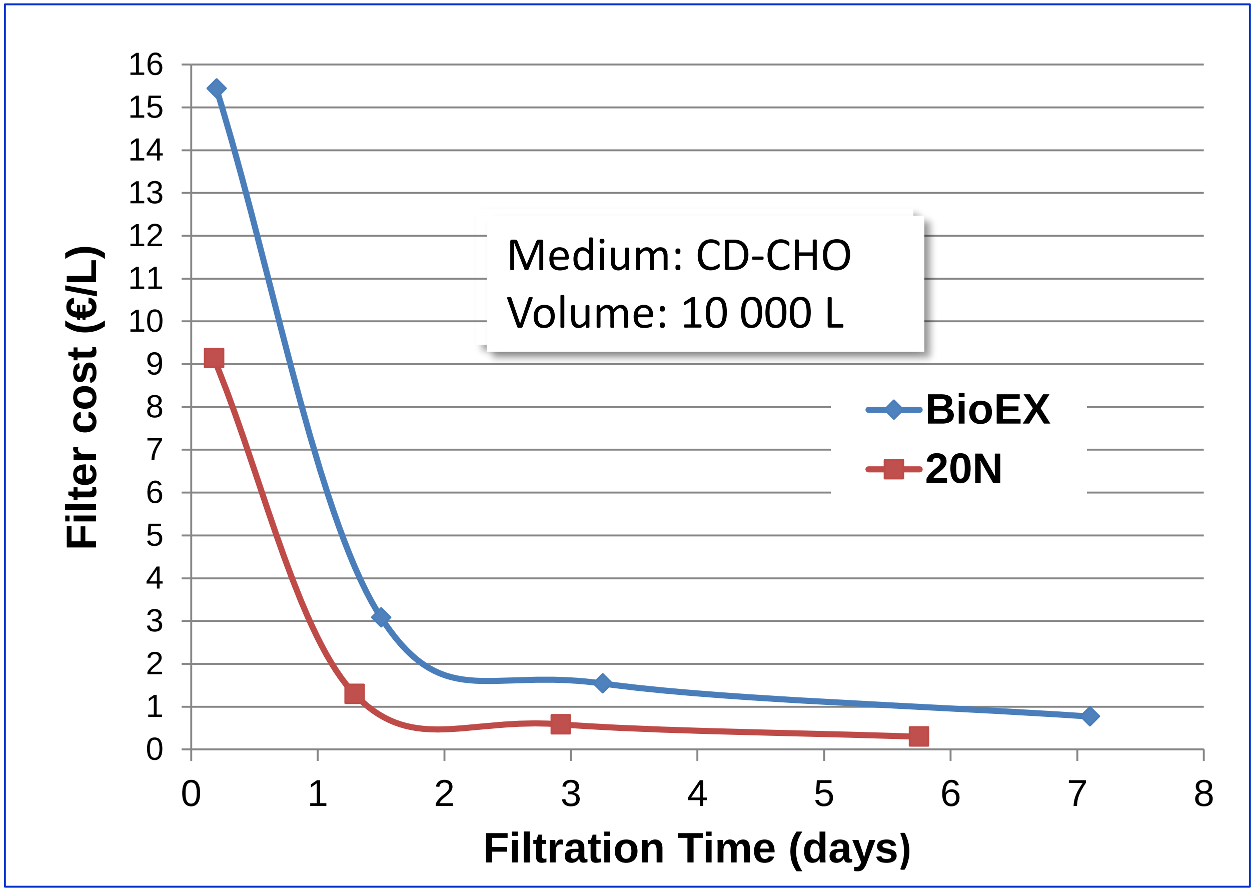


- ✓ **No virus detected (↑)**
- ✓ Difference in PPV LRV is due to differences in assay sensitivity

Konstantin Agolli, Asahi Kasei, BioInnovation 2016, Berlin, February 10th, 2016

1) CD-CHO Medium Filtration

Filter cost (€/L medium) vs. Filtration time (days)



- ✓ Price of CD-CHO: < 30 €/L
- ✓ **BioEX**: After 1.5 days, NF cost < 10 % medium cost
- ✓ **20N:** After 1 day, NF cost < 10 % medium cost
- ✓ **The longer the filtration time, the more cost effective !**

Konstantin Agolli, Asahi Kasei, BioInnovation 2016, Berlin, February 10th, 2016

2) Porcine Circovirus Nanofiltration

Planova 12.5 nm & 10 nm qPCR - removal data in DMEM

Planova 12.5 nm Filtration

Sample Code	PCV Loads- Run 1 (log_{10})			PCV Loads- Run 2 (log_{10})		
	-DNase	+DNase	RF*	-DNase	+DNase	RF*
SSM	7.17	6.85	-	7.17	6.86	-
PreF	7.00	6.83	-	7.00	6.83	-
NF2	6.64	4.59	**2.24**	6.69	4.54	**2.29**

Planova 10 nm Filtration

Sample Code	PCV Loads- Run 1 (log10)			PCV Loads- Run 2 (log10)		
	-DNase	+DNase	RF*	-DNase	+DNase	RF*
SSM	7.13	6.71	-	7.13	6.71	-
PreF	6.86	6.76	-	6.86	6.76	-
NF2 with conc.	NA	≤2.70	**≥4.06**	NA	≤2.70	**≥4.06**

* RF calculated relative to PreF

2) Porcine Circovirus Nanofiltration

Planova 15N qPCR - removal data in DMEM

Single 15N Filtration

Sample ID	PCV Loads- Run 1 (log10)			PCV Loads- Run 2 (log10)		
	-DNase	+DNase	RF	-DNase	+DNase	RF
SSM	7.07	6.66	-	7.07	6.66	-
PreF	6.86	6.71	0.00	6.86	6.71	0.00
NF2 (1x 15N)	6.57	5.15	**1.56**	6.34	5.24	**1.47**

Serial 2x 15N Filtration

Sample ID	PCV Loads- Run 1 ($\log_{10}$)			PCV Loads- Run 2 ($\log_{10}$)		
	-DNase	+DNase	RF	-DNase	+DNase	RF
SSM	6.99	6.85	-	6.99	6.85	-
PreF	6.86	7.04	0.00	6.86	7.04	0.00
NF2 (2x 15N)	6.50	4.25	**2.79**	6.65	4.25	**2.79**

Natascha Hodosi, ViruSure, 17th Planova Workshop,
Washington D.C., June 13th, 2014

2) Porcine Circovirus Nanofiltration

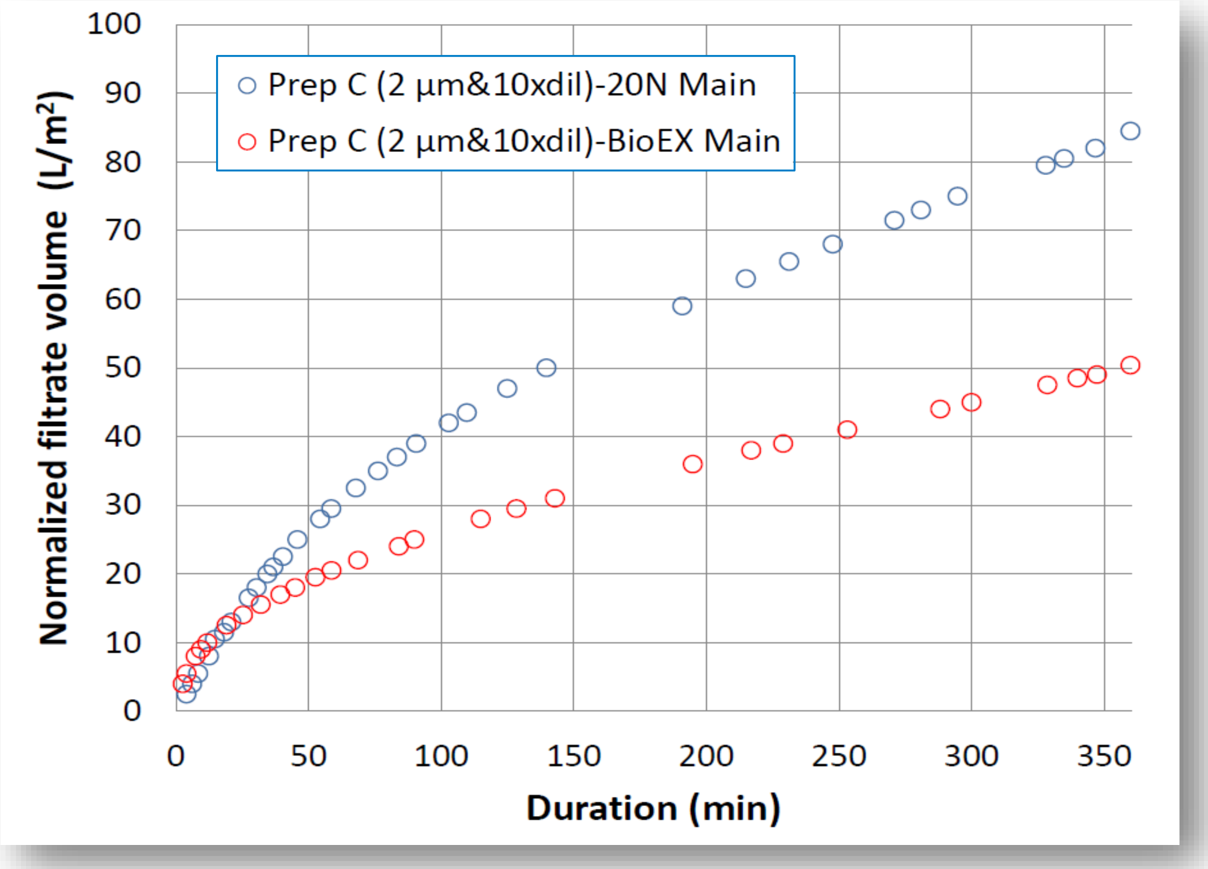
Planova qPCR - removal data in a mAb preparation

Sample ID	Planova 20N		Planova 15N		BioEX	
	+DNase	RF*	+DNase	RF*	+DNase	RF*
SSM	6,62	-	5.33	-	6.42	-
Hold control	6.20	-	5.05	-	6.39	-
PreF	6.59	-	5.58	-	6.21	-
NF1	≤4.18	**≥2.41**	≤3.58	**≥2.00**	≤ 4.18	**≥2.44**
NF2	≤3.98	**≥2.61**	≤3.38	**≥2.20**	≤ 3.98	**≥2.64**
NF3	≤3.78	**≥2.81**	≤3.18	**≥2.40**	≤ 3.78	**≥2.84**

Andy Bailey, ViruSure, 14th Planova Workshop, Cologne, Nov 09th, 2011

3) Mycoplasma Nanofiltration

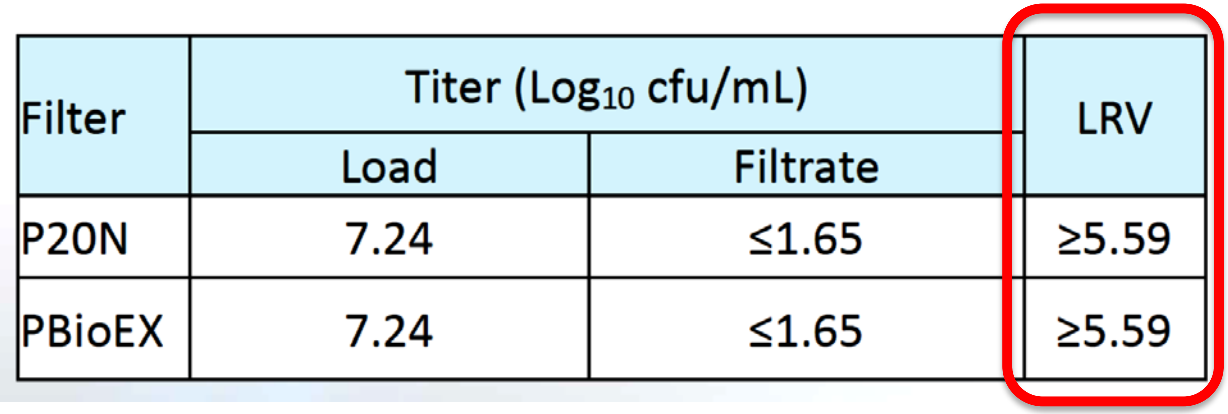
Filtration Profile of diluted *Acholeplasma laidlawii* preparation (incl. beef heart broth, yeast extract, horse serum…)



Masayasu Takahara, Asahi Kasei, 19th Planova Workshop, Philadelphia, Sept 22nd, 2016

3) Mycoplasma Nanofiltration

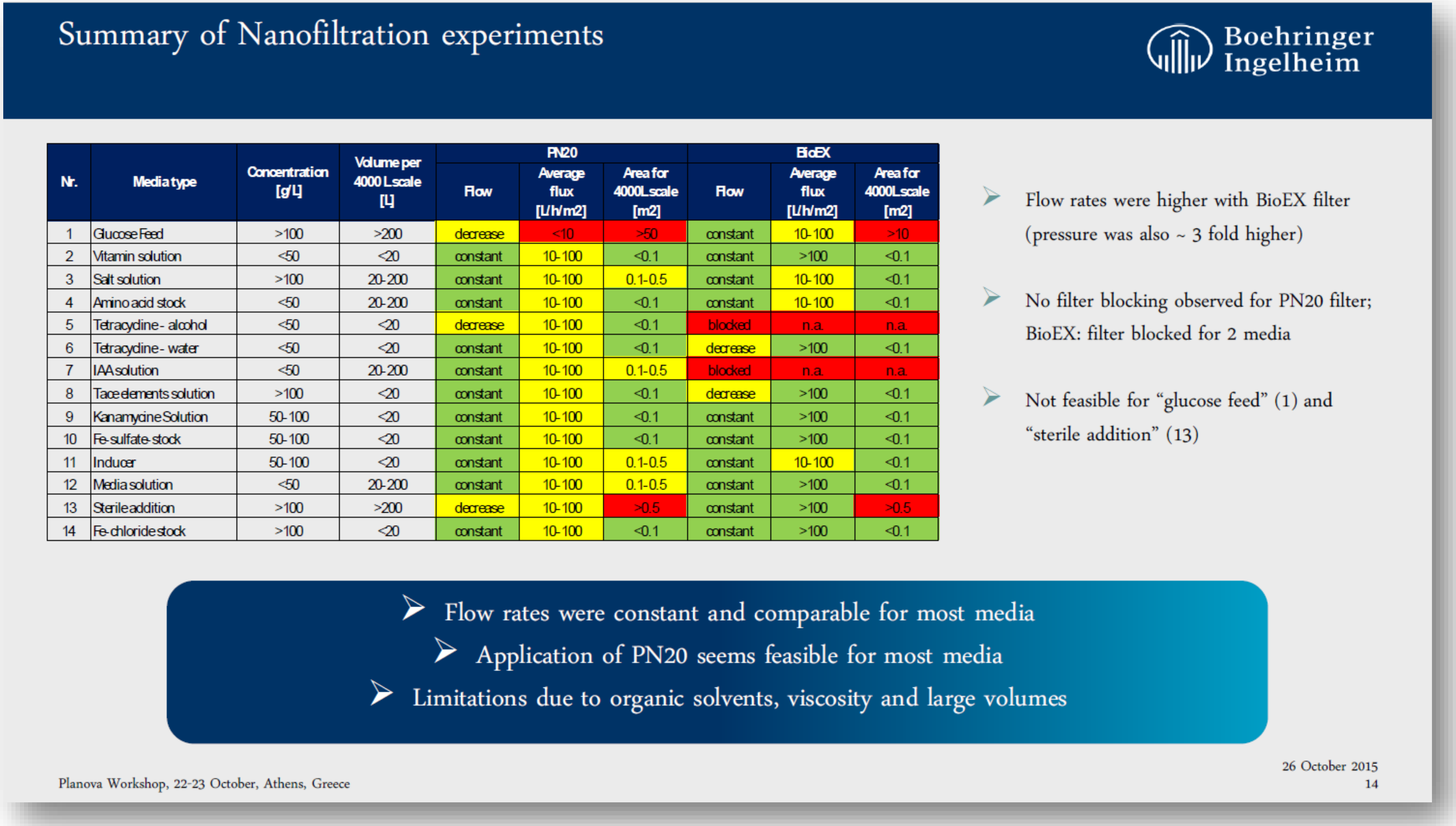
Removal of *Acholeplasma laidlawii* preparation by Planova filters



Filter	Titer ($\log_{10}$ cfu/mL)		LRV
	Load	Filtrate	
P20N	7.24	≤1.65	≥5.59
PBioEX	7.24	≤1.65	≥5.59

Masayasu Takahara, Asahi Kasei, 19th Planova Workshop,
Philadelphia, Sept 22nd, 2016

4) Nanofiltration of Microbial Fermentation Media Components



Summary of Nanofiltration experiments

Boehringer Ingelheim

Nr.	Media type	Concentration [g/L]	Volume per 4000 L scale [L]	PN20			BioEX		
				Flow	Average flux [L/h/m2]	Area for 4000L scale [m2]	Flow	Average flux [L/h/m2]	Area for 4000L scale [m2]
1	Glucose Feed	>100	>200	decrease	<10	>50	constant	10-100	>10
2	Vitamin solution	<50	<20	constant	10-100	<0.1	constant	>100	<0.1
3	Salt solution	>100	20-200	constant	10-100	0.1-0.5	constant	10-100	<0.1
4	Amino acid stock	<50	20-200	constant	10-100	<0.1	constant	10-100	<0.1
5	Tetracycline - alcohol	<50	<20	decrease	10-100	<0.1	blocked	n.a.	n.a.
6	Tetracycline - water	<50	<20	constant	10-100	<0.1	decrease	>100	<0.1
7	IAA solution	<50	20-200	constant	10-100	0.1-0.5	blocked	n.a.	n.a.
8	Tace elements solution	>100	<20	constant	10-100	<0.1	decrease	>100	<0.1
9	Kanamycine Solution	50-100	<20	constant	10-100	<0.1	constant	>100	<0.1
10	Fe-sulfate-stock	50-100	<20	constant	10-100	<0.1	constant	>100	<0.1
11	Inducer	50-100	<20	constant	10-100	0.1-0.5	constant	10-100	<0.1
12	Media solution	<50	20-200	constant	10-100	0.1-0.5	constant	>100	<0.1
13	Sterile addition	>100	>200	decrease	10-100	>0.5	constant	>100	>0.5
14	Fe-chloride stock	>100	<20	constant	10-100	<0.1	constant	>100	<0.1

- Flow rates were higher with BioEX filter (pressure was also ~ 3 fold higher)
- No filter blocking observed for PN20 filter; BioEX: filter blocked for 2 media
- Not feasible for "glucose feed" (1) and "sterile addition" (13)

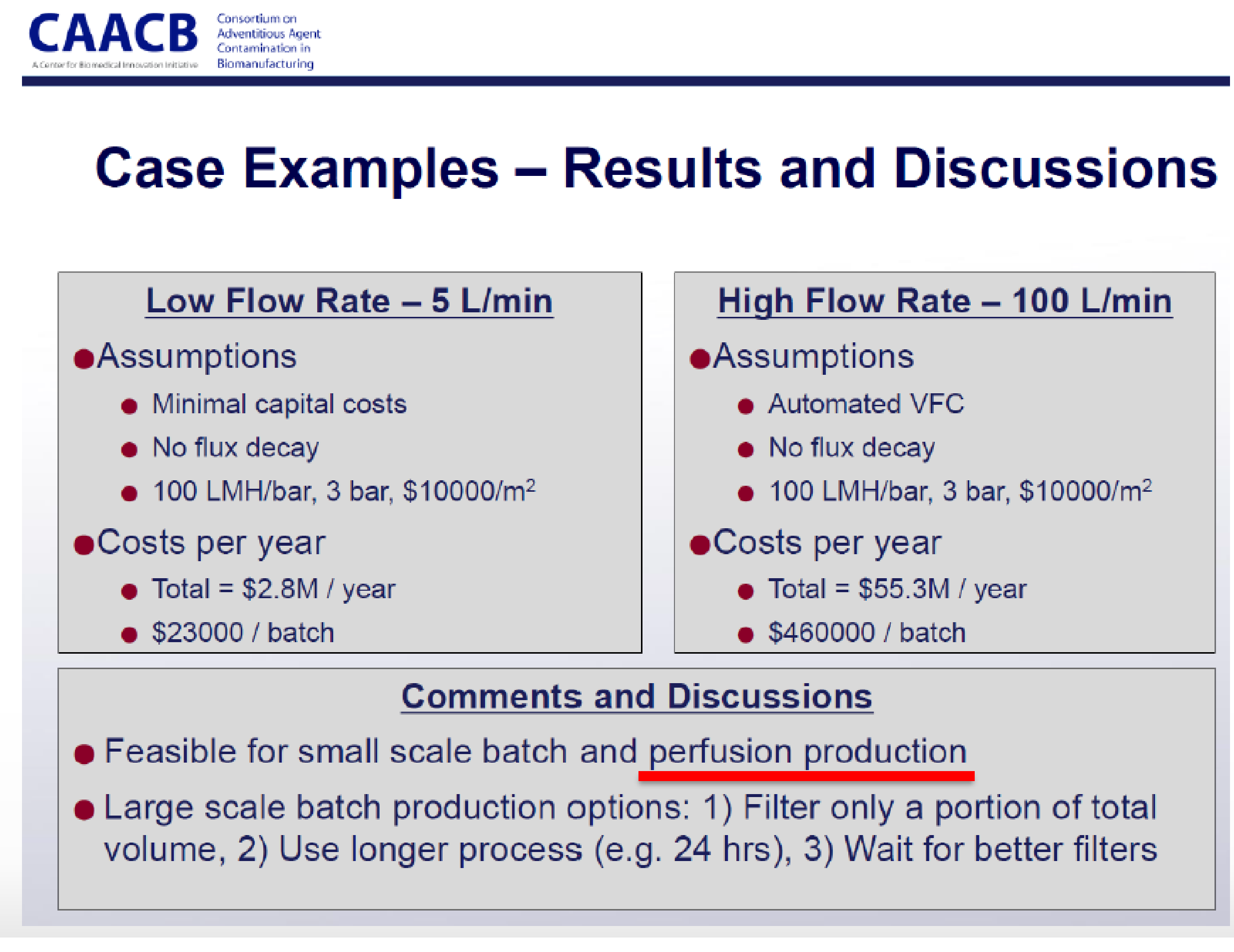
- Flow rates were constant and comparable for most media
- Application of PN20 seems feasible for most media
- Limitations due to organic solvents, viscosity and large volumes

Planova Workshop, 22-23 October, Athens, Greece

26 October 2015
14

Considerations

Considerations - Commercial Aspects Media Treatment



CAACB Consortium on Adventitious Agent Contamination in Biomanufacturing

A Center for Biomedical Innovation Initiative

Case Examples – Results and Discussions

Low Flow Rate – 5 L/min

- Assumptions
  - Minimal capital costs
  - No flux decay
  - 100 LMH/bar, 3 bar, $10000/m^2$
- Costs per year
  - Total = $2.8M / year
  - $23000 / batch

High Flow Rate – 100 L/min

- Assumptions
  - Automated VFC
  - No flux decay
  - 100 LMH/bar, 3 bar, $10000/m^2$
- Costs per year
  - Total = $55.3M / year
  - $460000 / batch

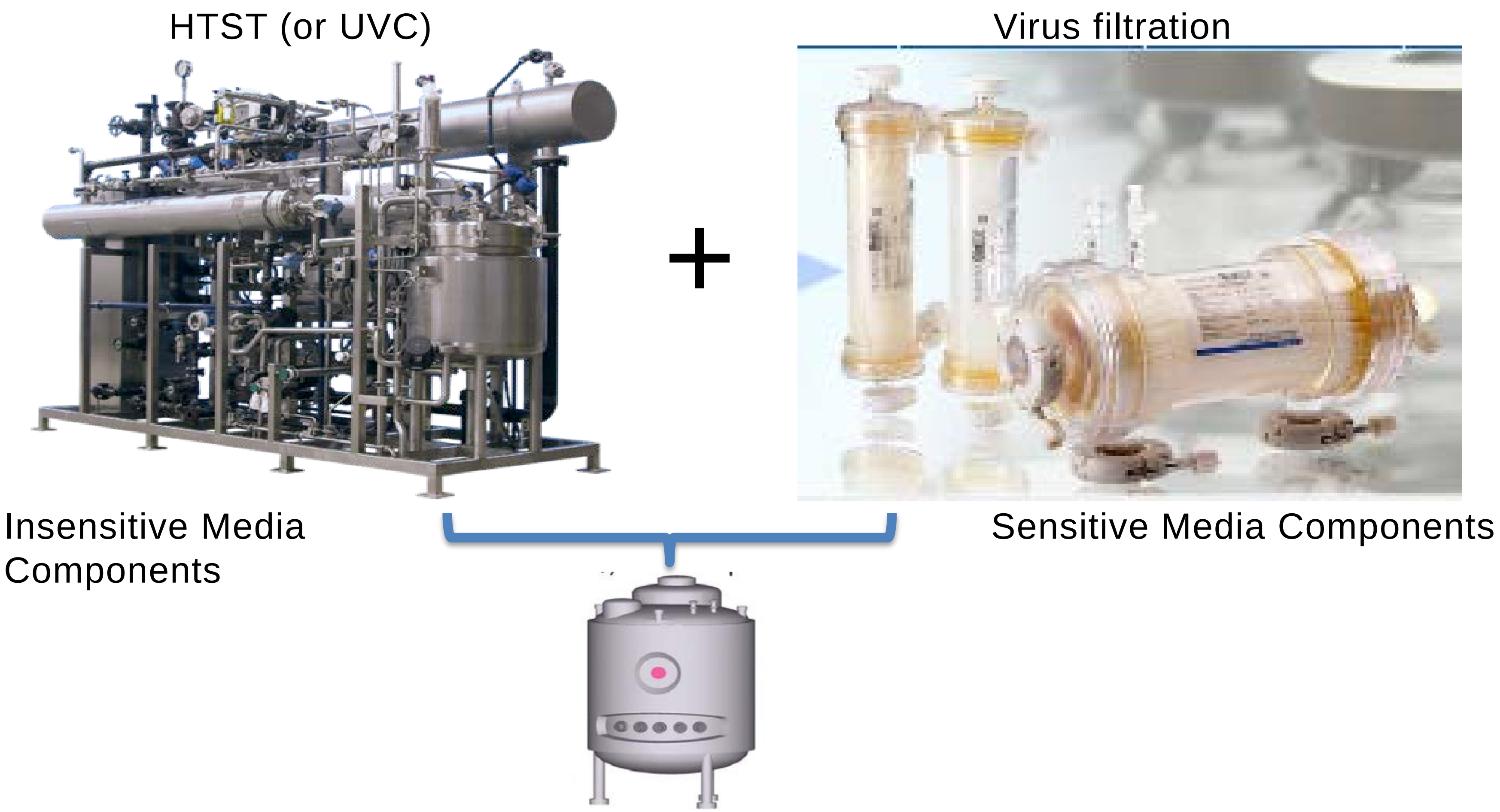
Comments and Discussions

- Feasible for small scale batch and perfusion production
- Large scale batch production options: 1) Filter only a portion of total volume, 2) Use longer process (e.g. 24 hrs), 3) Wait for better filters

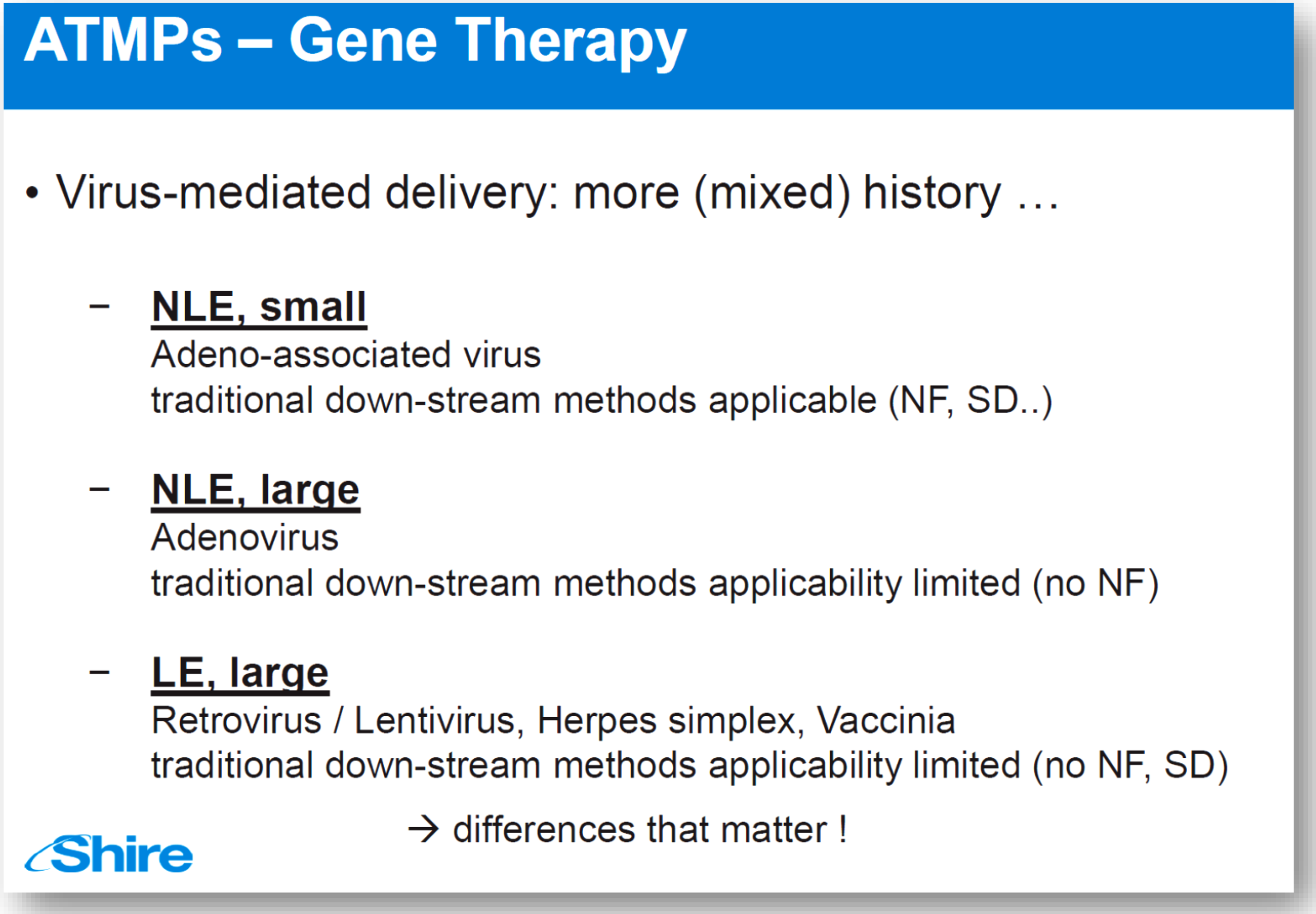
Economic Analysis Of Media Treatment Unit Operations To Mitigate Risk Of Virus Contamination In Biomanufacturing, CAACB Workshop, Cambridge, May 08, 2015

Considerations - Media Treatment - Way Forward?

Large scale recombinants:


HTST (or UVC)
Virus filtration
+
Insensitive Media Components
Sensitive Media Components

Considerations - ATMPs

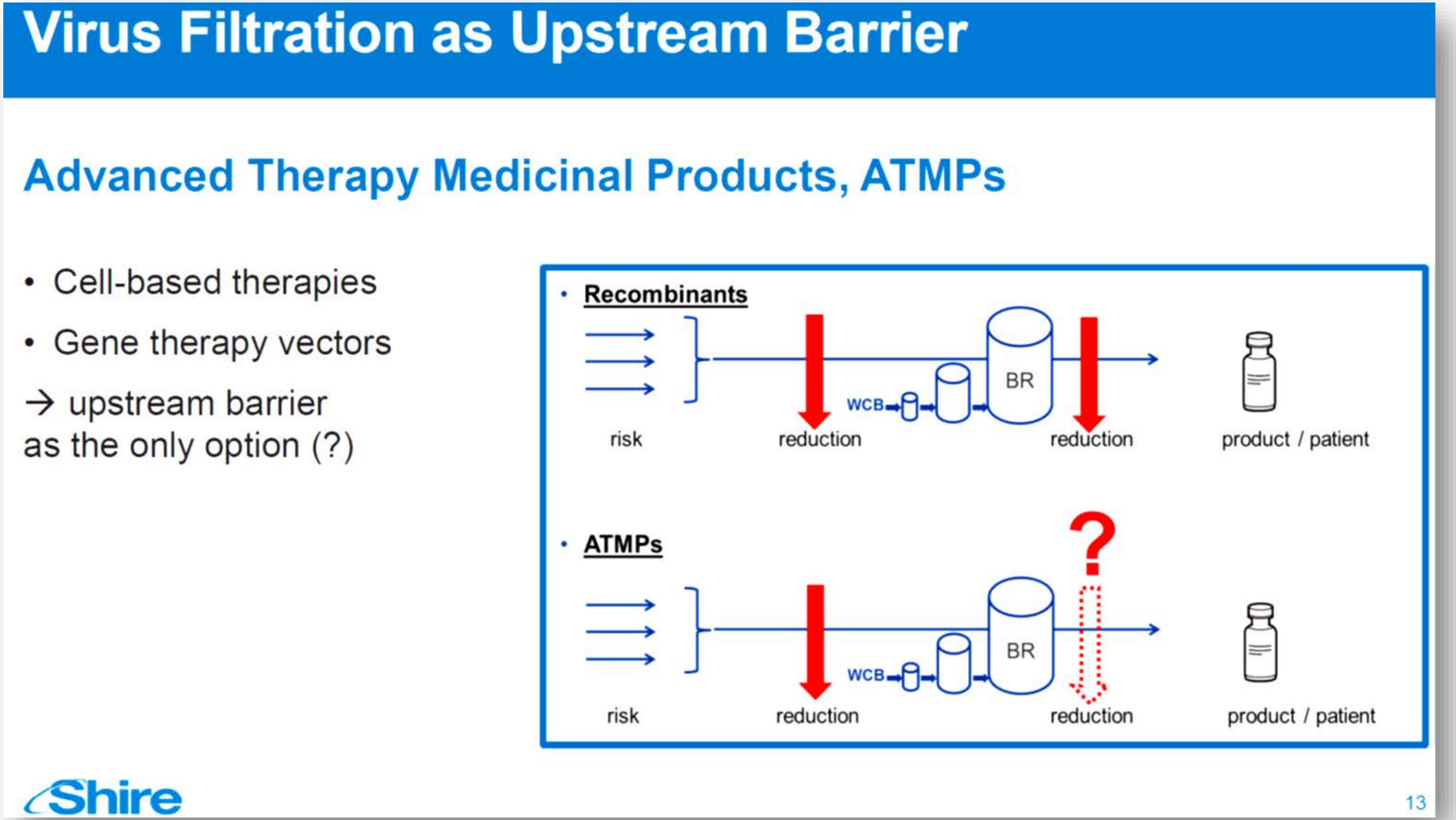


ATMPs – Gene Therapy

- Virus-mediated delivery: more (mixed) history …
    - **NLE, small**
      Adeno-associated virus
      traditional down-stream methods applicable (NF, SD..)
    - **NLE, large**
      Adenovirus
      traditional down-stream methods applicability limited (no NF)
    - **LE, large**
      Retrovirus / Lentivirus, Herpes simplex, Vaccinia
      traditional down-stream methods applicability limited (no NF, SD)

→ differences that matter !

Considerations - ATMPs



Virus Filtration as Upstream Barrier

Advanced Therapy Medicinal Products, ATMPs

- Cell-based therapies
- Gene therapy vectors

→ upstream barrier
as the only option (?)

Recombinants
risk
reduction
WCB
BR
reduction
product / patient
ATMPs
risk
reduction
WCB
BR
?
reduction
product / patient

Shire

13

Acknowledgements

ViruSure: Andy Bailey, Natascha Hodosi
Boehringer Ingelheim: Simon Haidinger
Shire: Andreas Wieser, Thomas Kreil
BioReliance: Kate Smith, Isobel Radigan, Doli Patel, Hidenori Coy Meiseki, Yuki Shimazoe, Steven McDade
Asahi Kasei: Masayasu Takahara, Bixente Martirene, Konstantin Agolli, Tomoko Hongo Hirasaki, Daniel Strauss

Virus Filters as Bioprocess Subject - Current Hot Topics

Virus Filters as Bioprocess Subject - Current Hot Topics

Facility Segregation

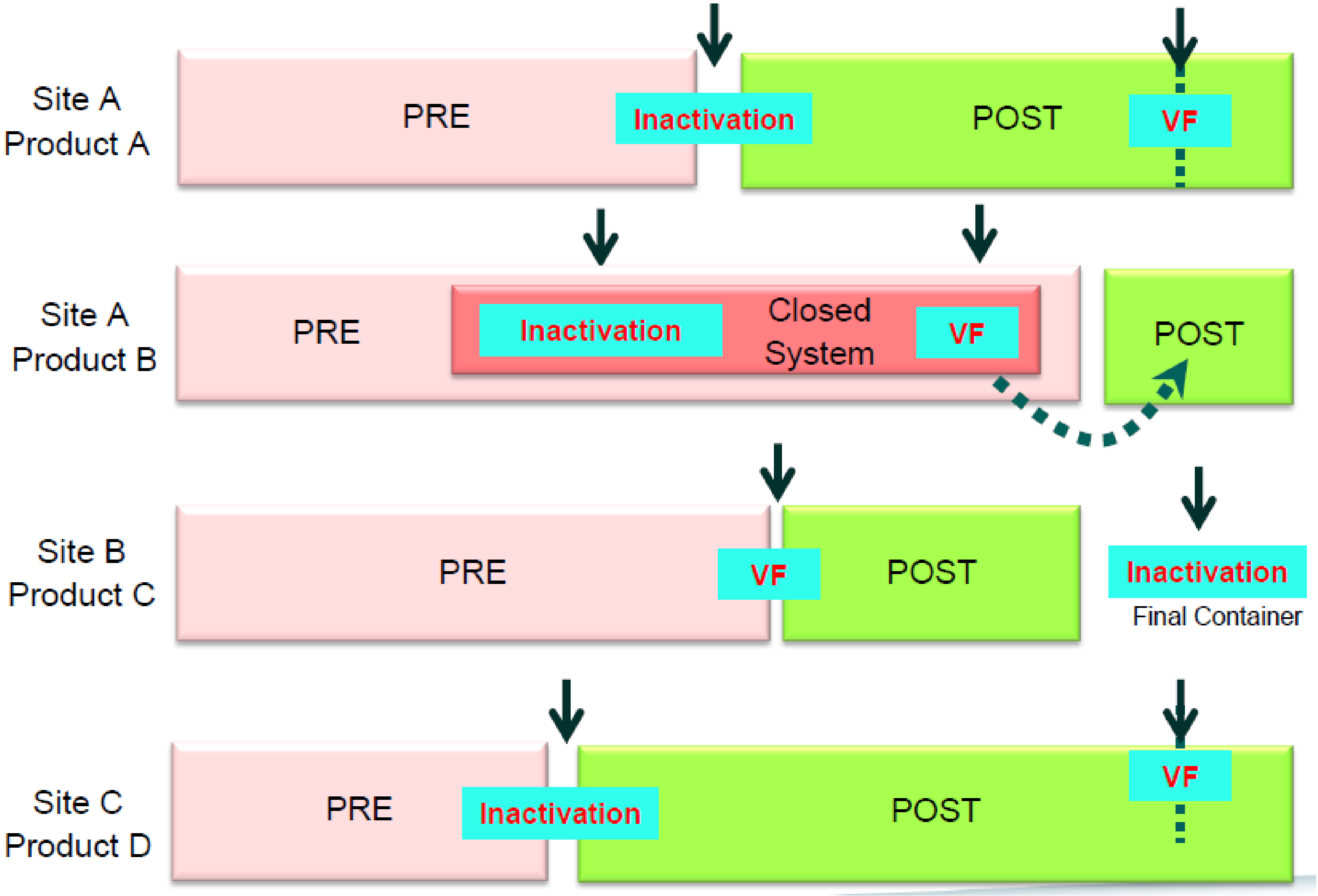
Why?

→ Regulatory pressure - companies are asked to segregate pre- & post-virus processes by agencies

Although…

No clear regulatory guidance! *"…the nature of the product as well as the equipment used will determine the level of segregation needed to avoid cross--contamination."* European GMP Guidance, Annex 2

Facility Segregation



Site A
Product A

PRE

Inactivation

POST

VF

Site A
Product B

PRE

Inactivation

Closed System

VF

POST

Site B
Product C

PRE

VF

POST

Inactivation

Final Container

Site C
Product D

PRE

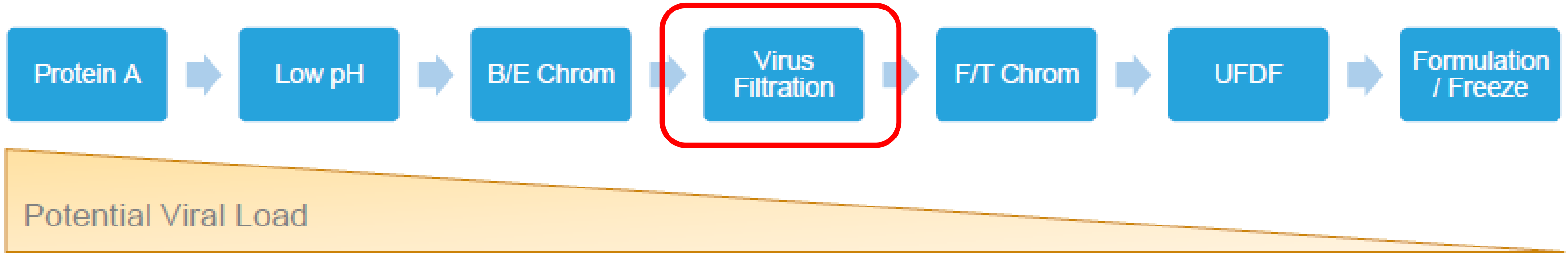
Inactivation

POST

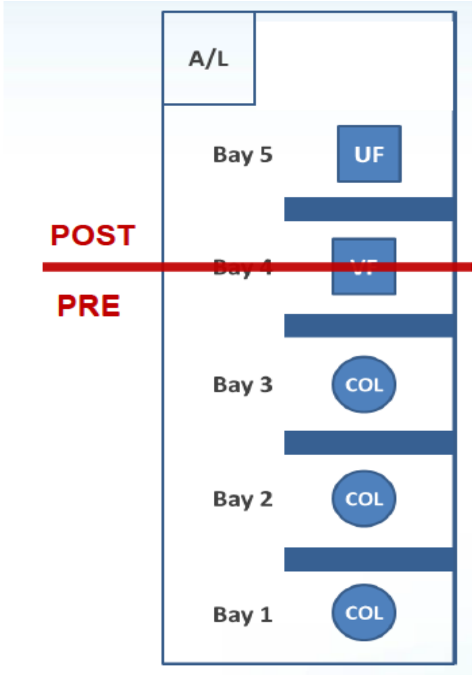
VF

Facility Segregation

VF is often THE point of segregation.


Protein A
Low pH
B/E Chrom
Virus Filtration
F/T Chrom
UFDF
Formulation / Freeze
Potential Viral Load

Ballroom facilities:
everything in one room
→ need for closed systems and aseptic assembly options


A/L
Bay 5
UF
POST
Bay 4
VF
PRE
Bay 3
COL
Bay 2
COL
Bay 1
COL

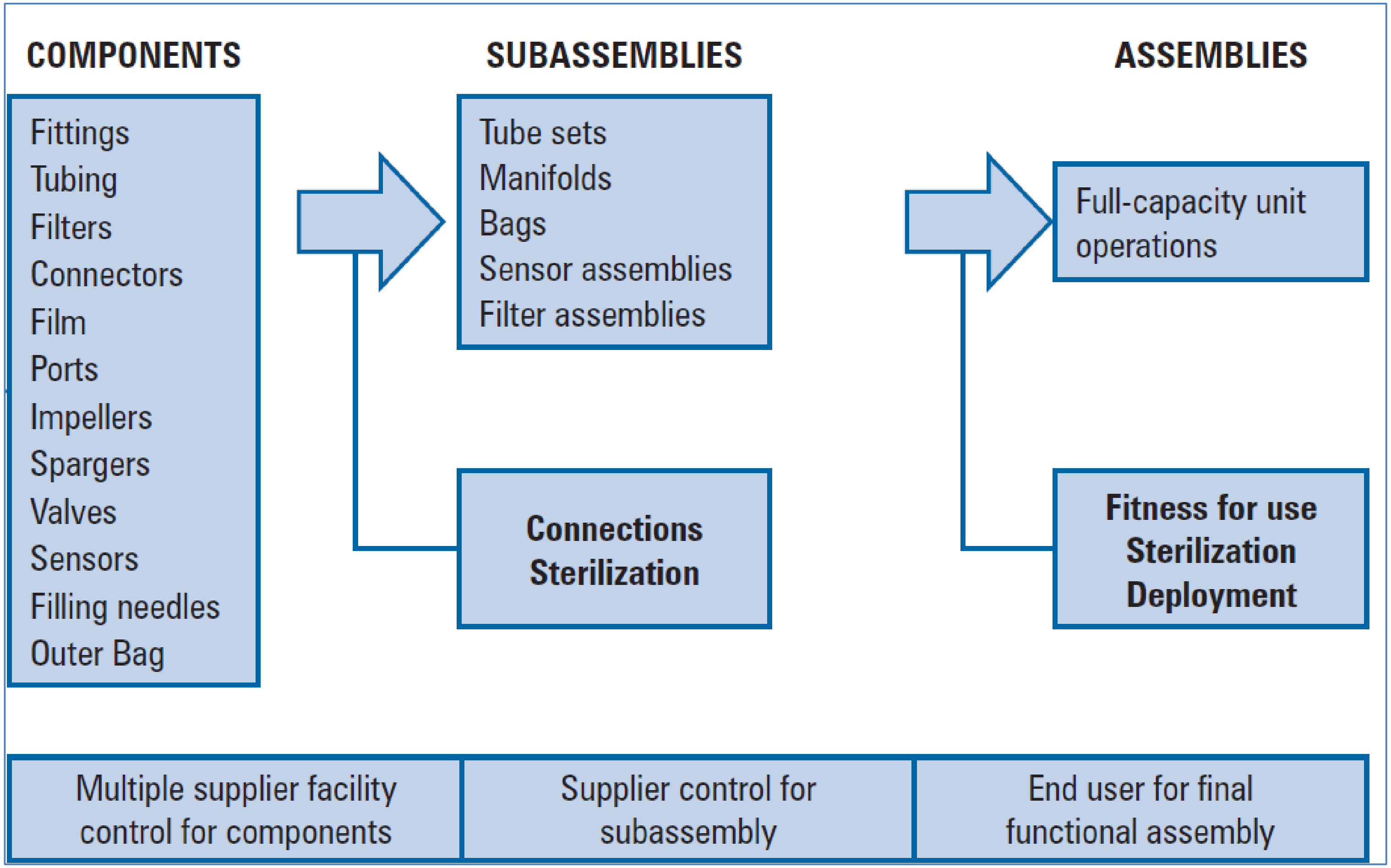
Facility Segregation → Single-Use

Drivers:

- Process economics
- Ease of use
- Change over time and safety for multi-product facilities (fewer multiple-use equipment)
- Flexibility

Facility Segregation → Single-Use

Single-use system anatomy



COMPONENTS

- Fittings
- Tubing
- Filters
- Connectors
- Film
- Ports
- Impellers
- Spargers
- Valves
- Sensors
- Filling needles
- Outer Bag

SUBASSEMBLIES

- Tube sets
- Manifolds
- Bags
- Sensor assemblies
- Filter assemblies

Connections
Sterilization

ASSEMBLIES

Full-capacity unit operations

Fitness for use
Sterilization
Deployment

Multiple supplier facility control for components	Supplier control for subassembly	End user for final functional assembly

(PDA Technical Report No. 66 - Application of Single-Use Systems in Pharmaceutical Manufacturing)

Virus Filters as Bioprocess Subject - Current Hot Topics

Industry Approaches to Facility Segregation for Viral Safety

Paul W. Barone
MIT Center for Biomedical Innovation

2017 CAACB Spring Workshop | April 11 - 12 | Boston, MA

MIT CBI Center for Biomedical Innovation CAACB

MIT Massachusetts Institute of Technology

Industry Approaches to Facility Segregation for Viral Safety

Paul W. Barone
MIT Center for Biomedical Innovation

2017 CAACB Spring Workshop | April 11 - 12 | Boston, MA

Questions ?