



# What Does Robustness Mean in Nanofiltration?

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# Definition of Robustness



OXFORD

- ✓ For an object: **sturdy** in construction
- ✓ For a system: able to withstand or overcome “**adverse conditions**”



WIKIPEDIA  
The Free Encyclopedia

- ✓ Property of being **strong** and healthy in **constitution**
- ✓ For a system: ability of **tolerating perturbations** that might affect the system's functional body

- 1) Robustness & Virus Removal**
- 2) Robustness & Filtration Flux/Capacity**
- 3) Robustness & Nanofilter Quality**

# Planova Nanofilters



**Planova™ 20N**




**Planova™ BioEX**

- ✓ Parvovirus removal nanofilters
- ✓ Launched respectively in 2001 and 2009
- ✓ Size exclusion mechanism and “multi-layer” filtration

## 1) Robustness & Virus Removal

- ✓ **Goal:** tolerating perturbations to keep LRV > 4 log
- ✓ **Main “perturbations”:** concentration, pH, conductivity, clogging/fouling, process pause

## Impact of Protein Concentration:

 **Parvovirus LRV  
(Antibody Concentration)-20N**

IgG Conc. (mg/ml)	PPV LRV	Filtration Time (hr)	Filtration Volume (L/m <sup>2</sup> )	Throughput (kg/m <sup>2</sup> )
1	≥ 5.67	6.4	350	0.35
5	≥ 5.37	6	280	1.40
10	≥ 6.00	6	240	2.40
20	≥ 5.50	6	148	2.96
30	≥ 5.58	6	98	2.94
50	≥ 5.67	5	24	1.20

100 mM NaCl, pH 4.5,  
Pressure; 78.4 kPa,  
Serum-free PPV 0.5 vol%

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- ✓ Increase of concentration → Decrease of flux
- ✓ Increase of mass throughput up to a certain point
- ✓ No impact on PPV LRV (> 4 log)

*Tomoko Hongo-Hirasaki, Asahi Kasei Medical, 2011 PDA Conference*

**Virus safety assured even if change of conc. during process development (phase I/III) → process optimizations possible !**

## Impact of pH:



**Parvovirus LRV (pH) - BioEX**

pH	PPV LRV	Filtration Time (hr)	Filtration Vol. (L/m <sup>2</sup> )
4.5	≥ 5.28	6.4	300
5.6	≥ 5.56	9.3	215
6.7	≥ 5.14	9.0	150
7.6	≥ 5.40	9.6	155

30 mg/ml IgG, 100 mM NaCl  
Serum-free PPV 0.5 vol%,  
Pressure; 294 kPa

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- ✓ Impact on flux
- ✓ No impact on PPV LRV (> 4 log)

*Tomoko Hongo-Hirasaki, Asahi Kasei Medical, 2011 PDA Conference*

**Virus safety assured even if change of pH during process development (phase I/III) → process optimizations possible !**




## Impact of Ionic Strength:

 Parvovirus LRV (Ionic Strength) - 20N

NaCl Conc. (mM)	PPV LRV	Filtration Time (hr)	Filtration Volume (L/m <sup>2</sup> )
1	≥ 5.84	6.0	152
10	≥ 6.34	6.0	200
100	≥ 6.00	6.0	240
200	≥ 5.67	6.0	220
500	≥ 6.00	6.0	190

10 mg/ml IgG, pH 4.5  
Serum-free PPV 0.5 vol%,  
Pressure; 78.4 kPa

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 Parvovirus LRV (Ionic Strength) - BioEX

NaCl Conc. (mM)	PPV LRV	Filtration Time (hr)	Filtration Vol. (L/m <sup>2</sup> )
50	≥ 5.48	6.0	300
100	≥ 5.28	6.0	300
250	≥ 5.28	9.5	280
500	≥ 5.92	9.5	160

30 mg/ml IgG, pH 4.5  
Serum-free PPV 0.5 vol%,  
Pressure; 294 kPa

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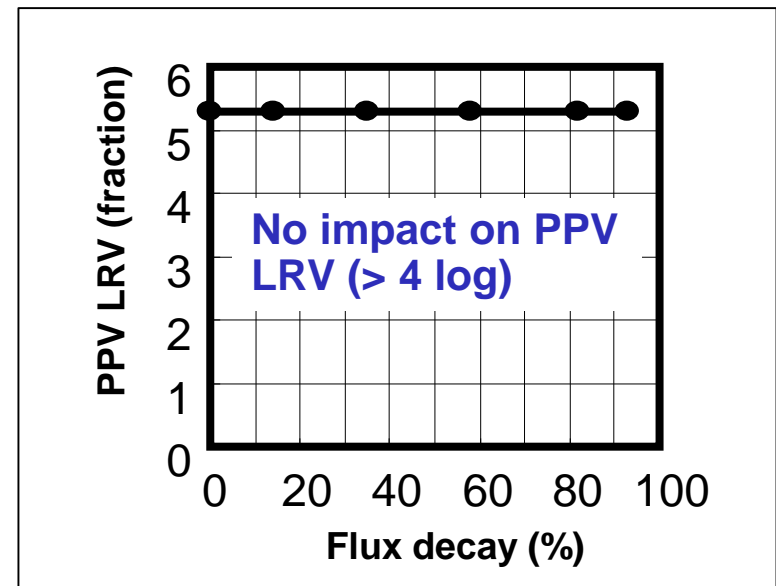
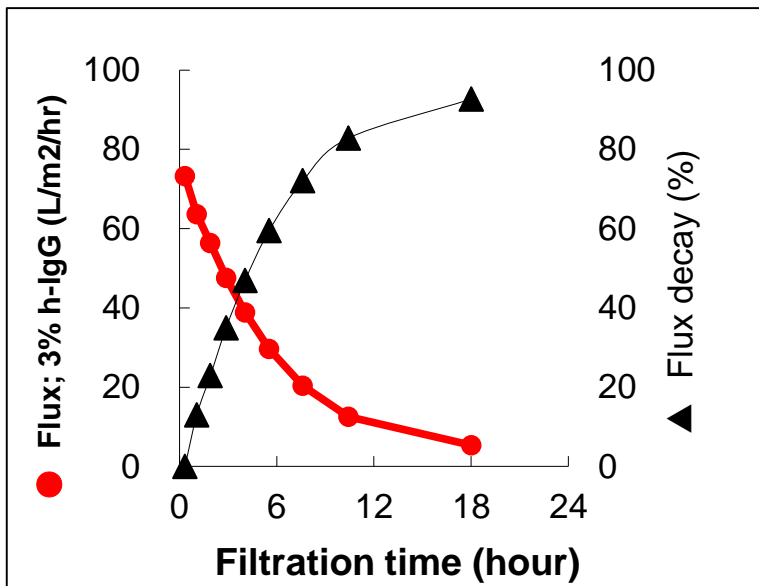
Tomoko Hongo-Hirasaki, Asahi Kasei Medical, 2011 PDA Conference

- ✓ Impact on flux
- ✓ No impact on PPV LRV (> 4 log)

Virus safety assured even if change of cond. during process development (phase I/III) → process optimizations possible !

## Impact of Clogging/Fouling on Planova™ BioEX:

**Theory:** smaller pores clog first → virus passage increases with clogging

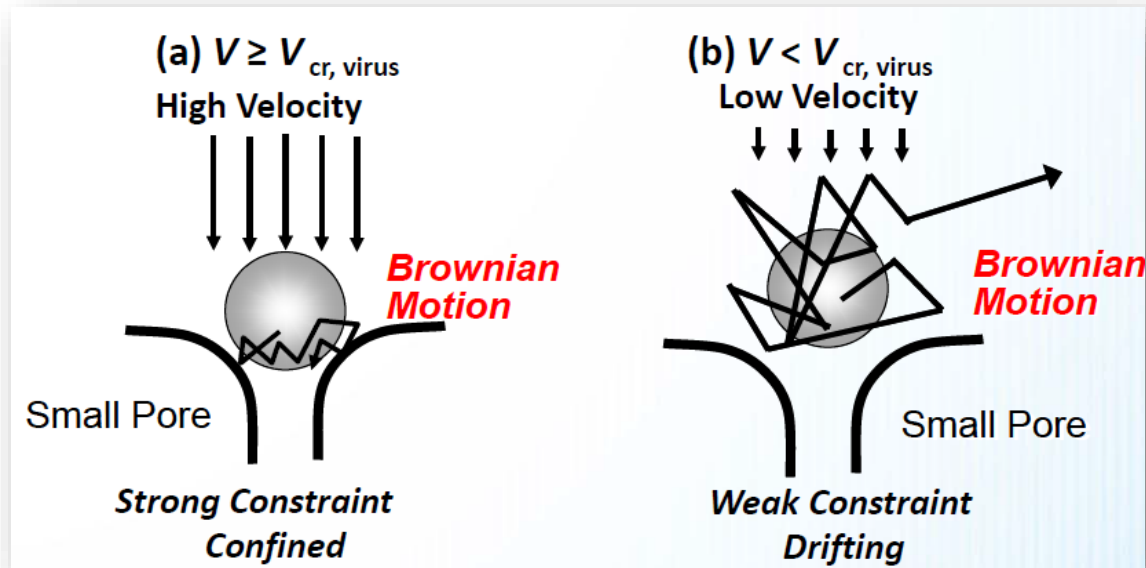


*Koichiro Yanagida, Asahi Kasei Medical, 2009 Planova Workshop – adapted*

**Virus safety assured even if unexpected production problem leading to big flux decay (nevertheless deviation must be addressed...)**

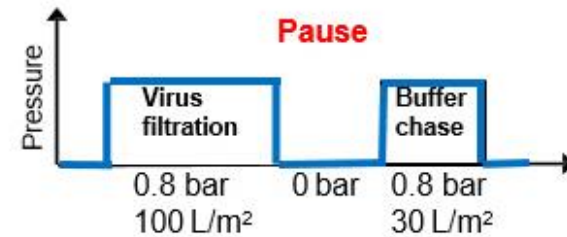
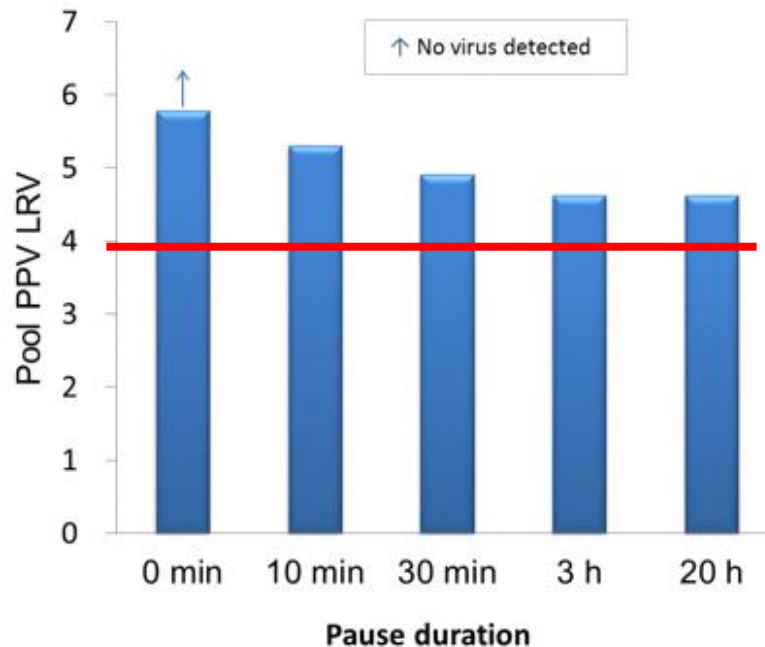
## Impact of Process Pause:

- ✓ Without pressure/flow, the ability for the virus to move increases !



- ✓ The pause can be accidental (e.g. power shutdown) or part of the process (e.g. preparation time prior to the buffer post-washing)

## Impact of Process Pause on Planova™ 20N:



IgG concentration: 10 mg/mL

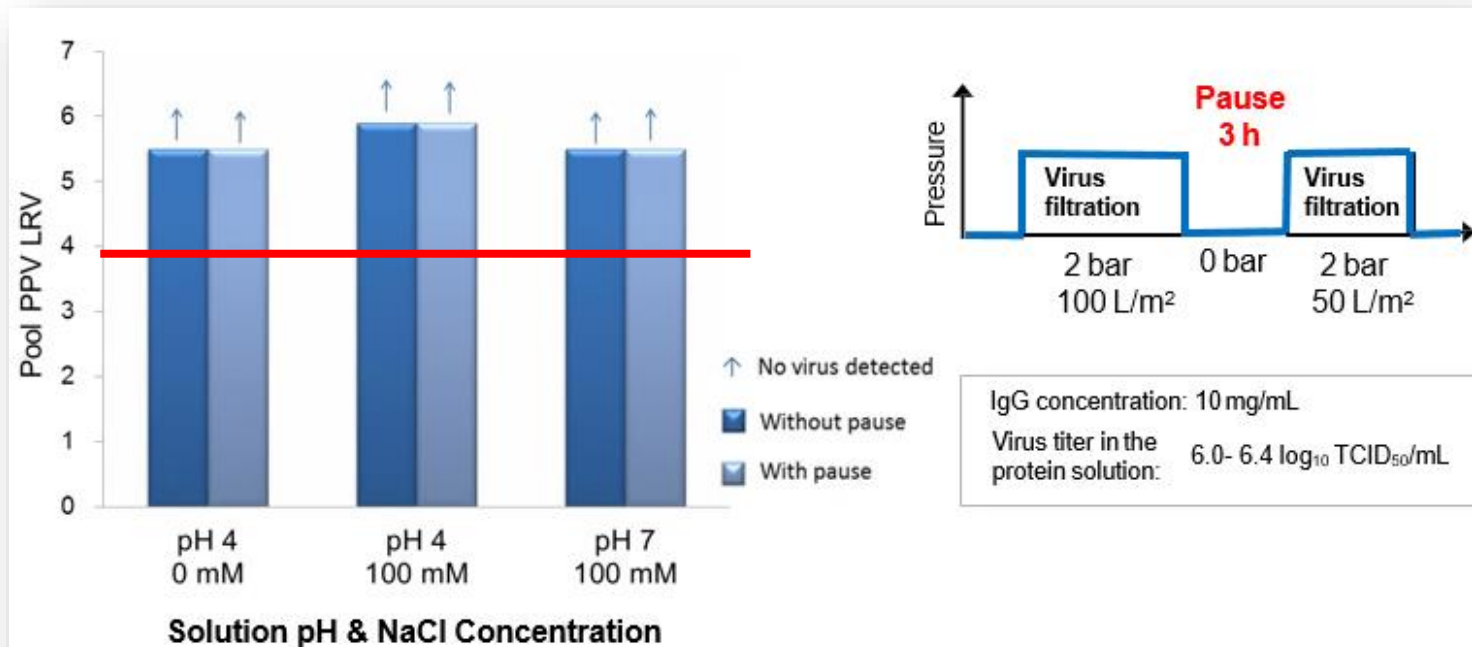
Solution condition: pH 4, 100 mM NaCl

Virus titer in the protein solution: 6.4 - 6.7 log<sub>10</sub> TCID<sub>50</sub>/mL

### Observations

- Pool LRV > 4 is maintained even at 20 h of pause
- Minimizing pause duration minimizes the impact

## Impact of Process Pause on Planova™ BioEX:



### Observation

- Complete PPV clearance is observed under all conditions

## Impact of Process Pause:

- ✓ Dependent on many factors: Solution conditions, Protein Molecules, Pause duration, **Virus challenge conditions (no overloading !!)**
- ✓ May affect any filter type and any process
- ✓ Evaluation on a case-by-case basis
- ✓ Most processes can achieve a parvovirus **LRV > 4 log even with a process pause**
- ✓ Important to understand the impacts (validation or supporting data)
- ✓ **Process pauses are completely manageable !**

## 2) Robustness & Filtration Flux/Capacity

- ✓ **Goal: tolerating perturbations to keep**
  - Small Flux decay or High  $V_{max}$
  - Consistent Flux ( $L/m^2/h$ ) or Filtration Capacity ( $L/m^2$ )
  
- ✓ **Main “perturbations”:** protein, concentration, virus spiking

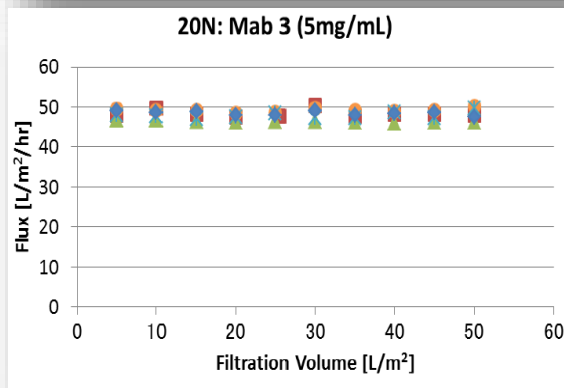
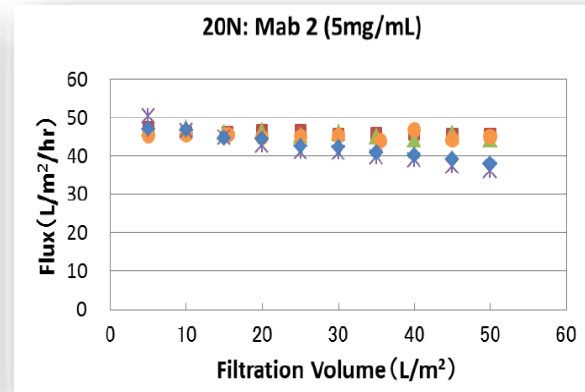
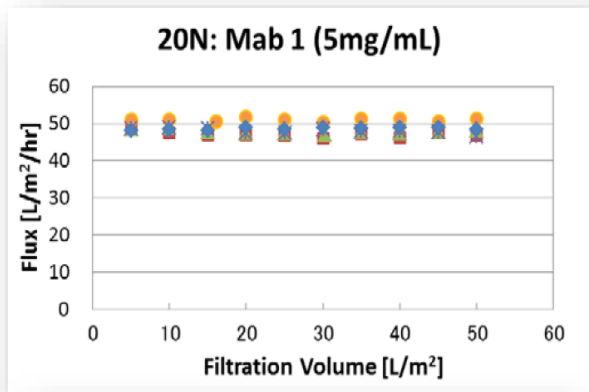


# Robustness & Flux/Capacity

## Impact of Protein:

Mabs with different pI tested with different pH & Conductivities

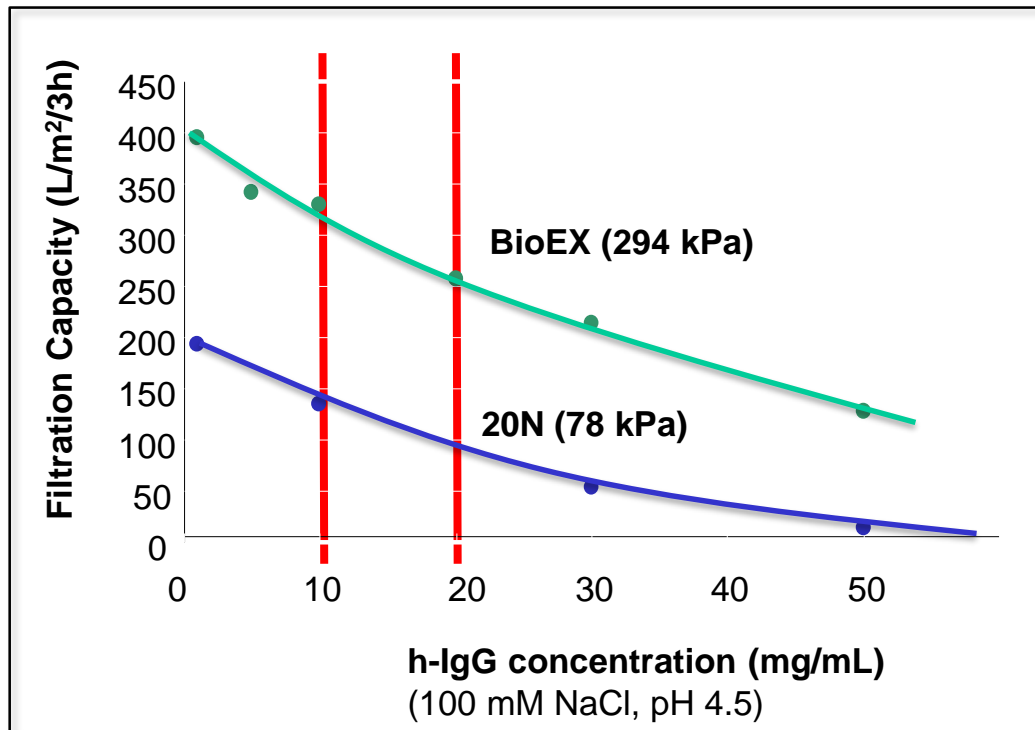
■ #1 (pH4, 20mS/cm) ▲ #2 (pH8, 20mS/cm) ● #3 (pH4, 4mS/cm) ✖ #4 (pH8, 3mS/cm) ◆ #5 (pH5.5, 7mS/cm)



- ✓ **Similar levels of flux** for the 3 mAbs
- ✓ **Consistent** flux with no or small decay
- ✓ Ideal for **platform** or multi-product plant
- ✓ Same trend for Planova™ BioEX

Josh Goldstein, Janssen,  
2014 Planova Workshop conference

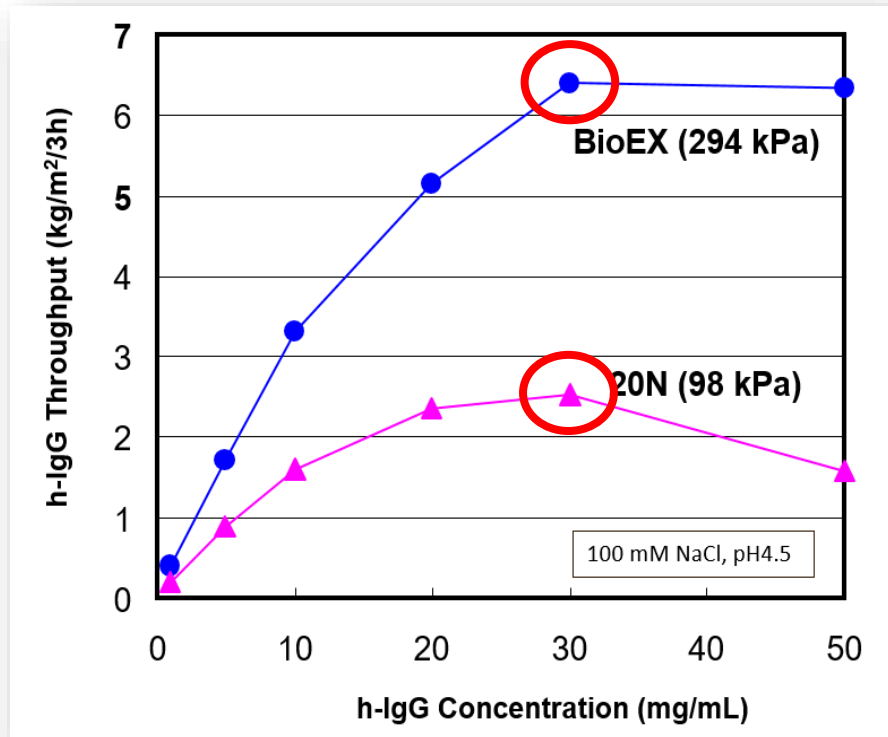
## Impact of Concentration:



- ✓ Impact of concentration on flux/capacity
- ✓ But only ~ 30% flux decrease while concentration x 2 for 10-20 g/L

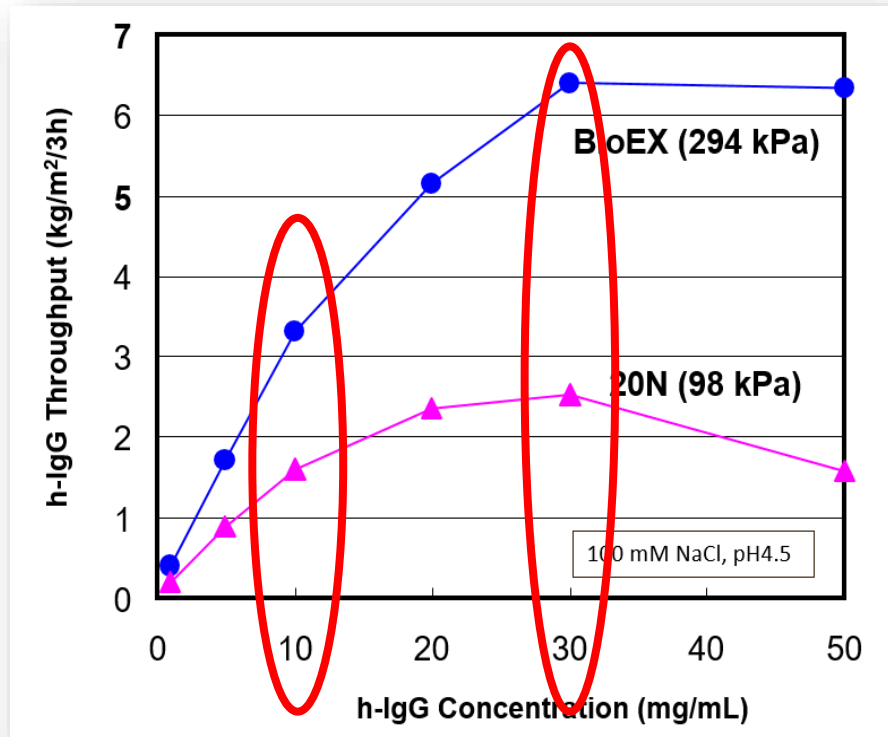
Planova filters can be considered robust against concentration increase up to a certain point

## Impact of Concentration:



- ✓ Optimal concentration to get the maximum mass throughput (kg or g /m²)
- ✓ Case by case !

## Impact of Concentration:



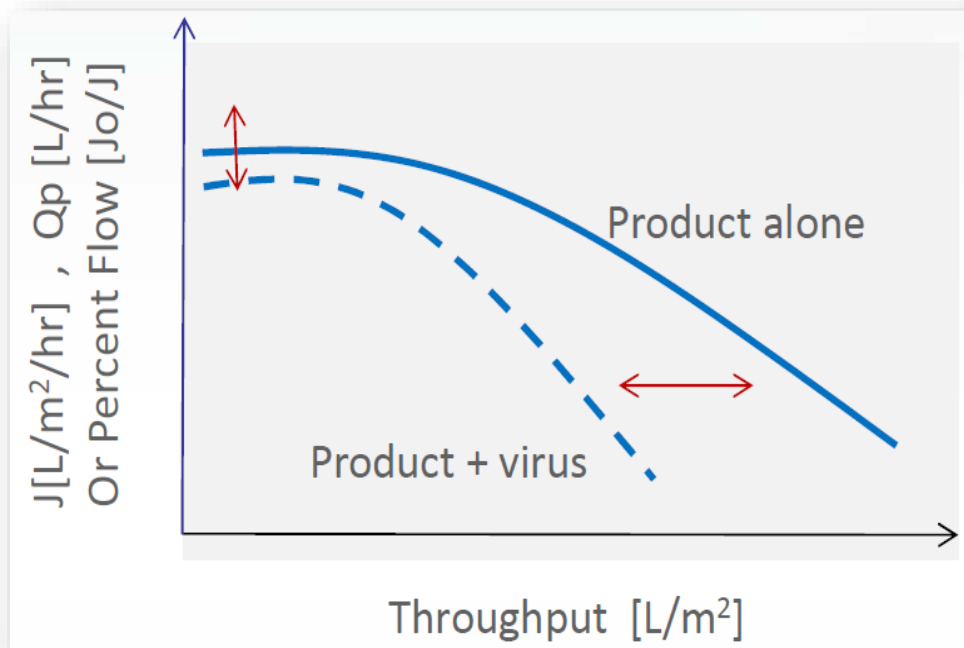
### 10 kg IgG nanofiltration:

- ✓ **10 mg/mL:**
  - 20N: 1.5 kg/m²  
→ 6.7 m²
  - BioEX: 3.5 kg/m²  
→ 2.9 m²
- ✓ **30 mg/mL:**
  - 20N: 2.5 kg/m²  
→ 4 m²
  - BioEX: 6.5 kg/m²  
→ 1.5 m²

Increase of protein mass throughput (g/m²):

→ smaller filtration surface → more cost effective

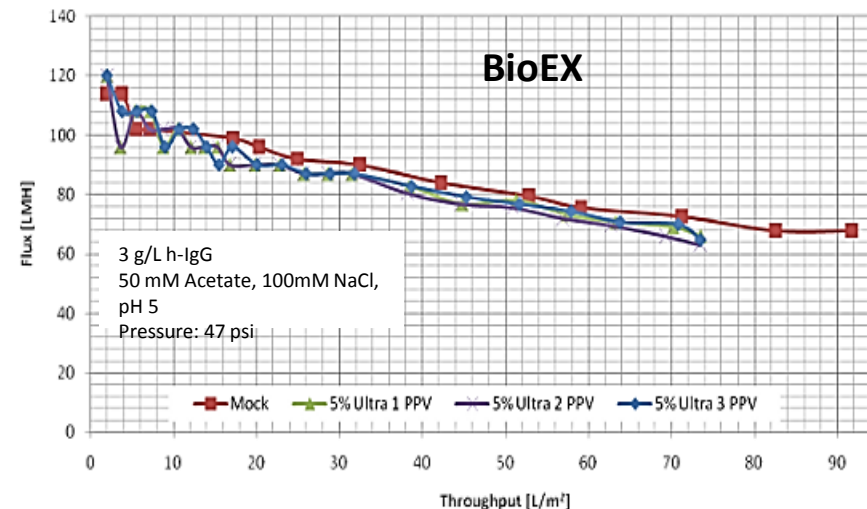
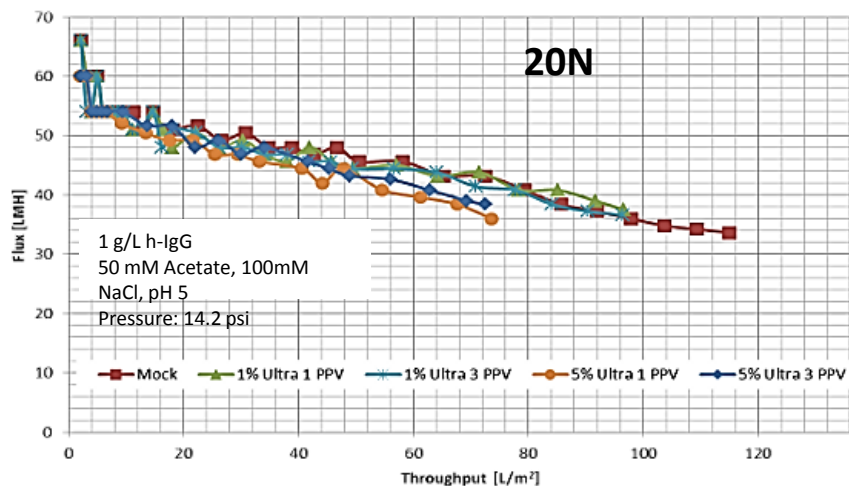
## Impact of Virus Spiking:



- ✓ Trend of most of the nanofilters on the market
- ✓ **Oversizing** of filtration surface area
- ✓ **What about Planova filters?**

## Impact of Virus Spiking:

Preparation of Ultra 1, 2, 3 and experiments conducted at Wuxi AppTec Inc.



- ✓ No impact of virus spiking material on flux
- ✓ Consistent flux with small decay
- ✓ No oversizing of the nanofilter !

## **3) Robustness & Nanofilter Quality**

The last 2 important questions:

- ✓ Is the **Robustness** kept during **Process Scale-up** ?
- ✓ Is the **Robustness** kept from one **Nanofilter Lot** to another nanofilter lot ?



# Robustness & Nanofilter Quality

## METHOD DETAILS

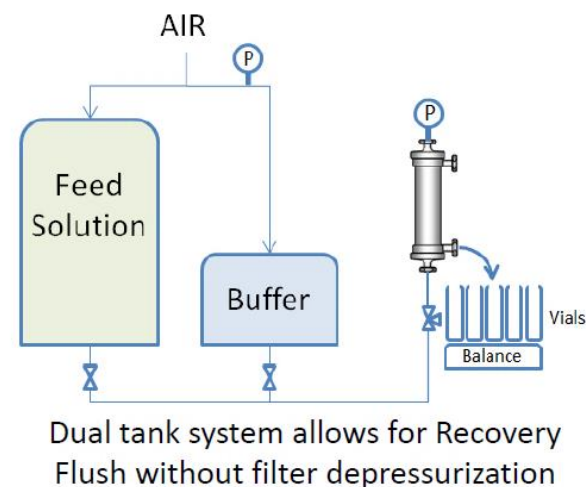
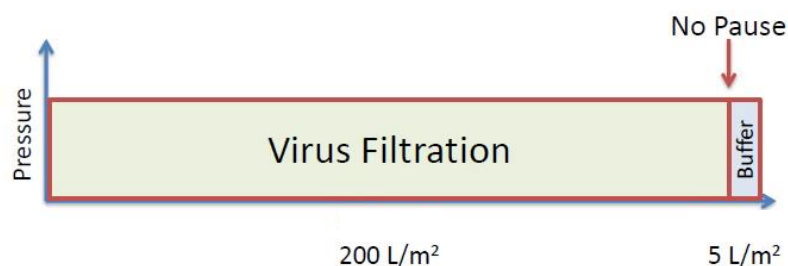
Filter Types: Planova™ 20N and BioEX

Feed Solution: 1 g/L Bovine Serum Albumin in 20mM Phosphate, 50mM NaCl, pH 7

Feed Volume: 200 L/m<sup>2</sup> with 5 L/m<sup>2</sup> Recovery Flush

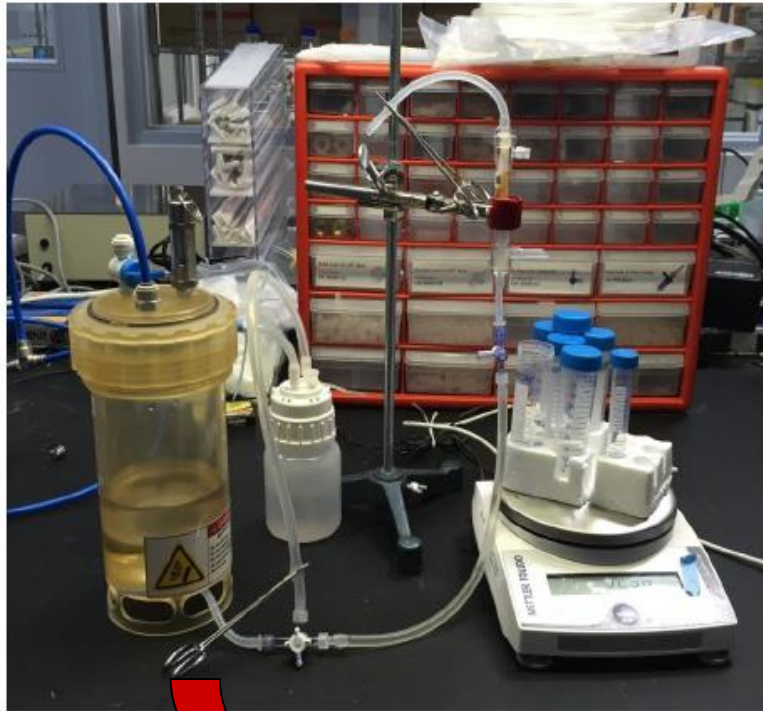
Virus Spike: ~6 log<sub>10</sub> PFU/mL PP7

Pressure: 0.98 bar (14.2 PSI)  
3.40 bar (49.7 PSI)



# Robustness & Nanofilter Quality

AsahiKASEI  
BIOPROCESS

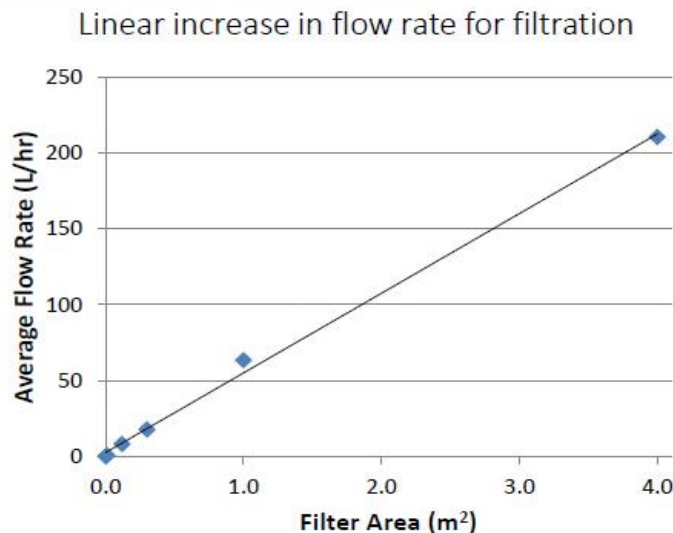


# Robustness & Nanofilter Quality

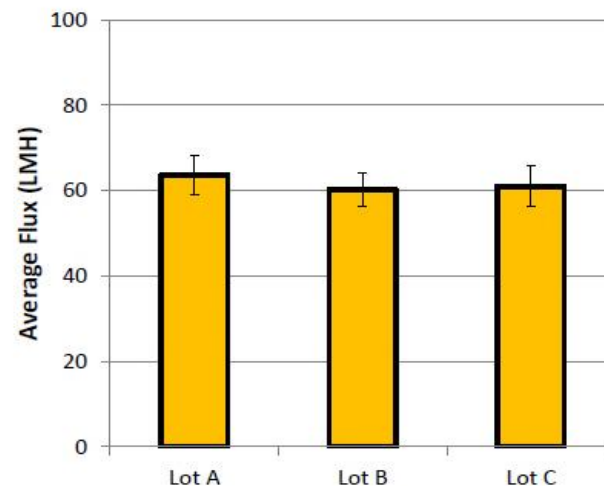
AsahiKASEI  
BIOPROCESS

## 20N FLUX PERFORMANCE

AsahiKASEI  
BIOPROCESS



No significant variation between different filter lots



Process developed at small scale is indicative of large scale filter performance

[www.ak-bio.com](http://www.ak-bio.com)

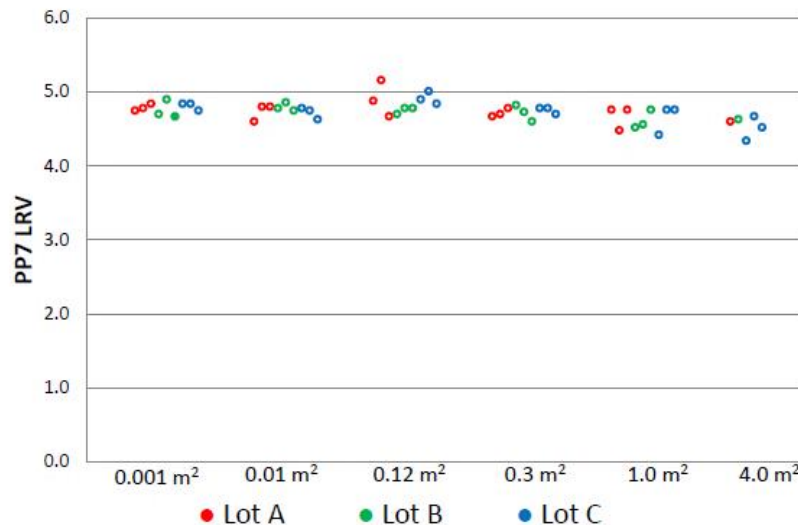
© 2015 Asahi Kasei Bioprocess America, Inc.  
Slide 15

*Brian Buesing, Asahi Kasei Bioprocess, 2015 Planova Workshop.*

**Consistent flux observed at all scales and no lot to lot variability**

# Robustness & Nanofilter Quality

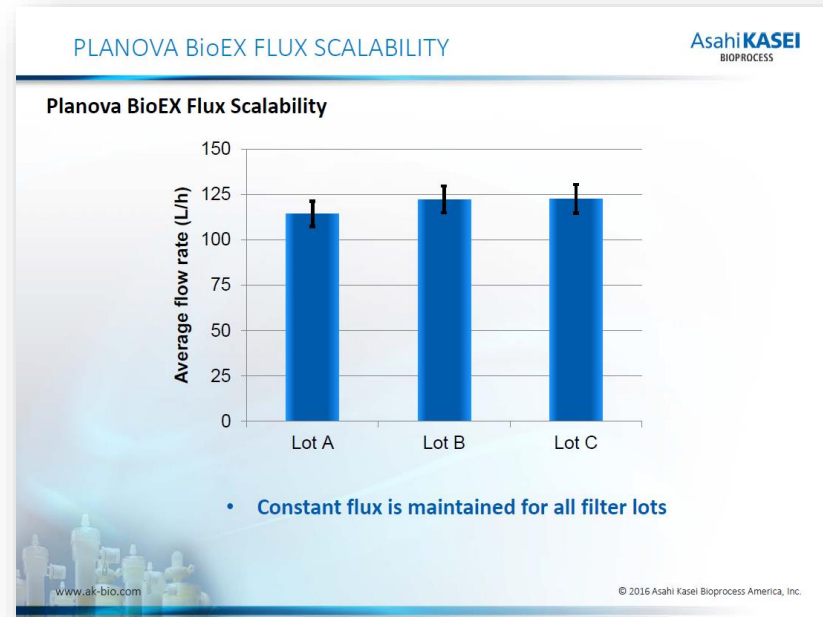
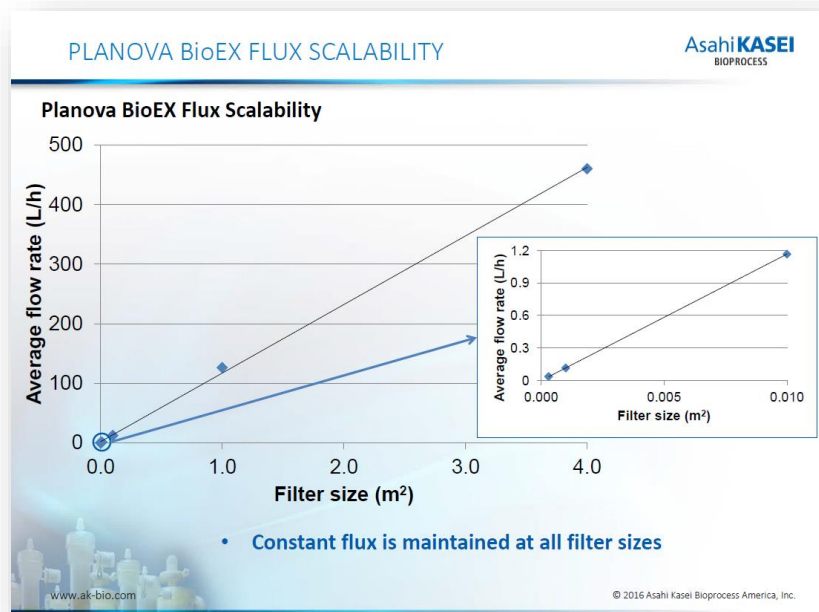
## PP7 REMOVAL USING 20N FILTERS



- Complete clearance in all but one pool sample
- LRV uniformly greater than 4.3
- Variation in LRV is an artifact of load titer variation

- Robust Viral Clearance across all filter sizes and lots

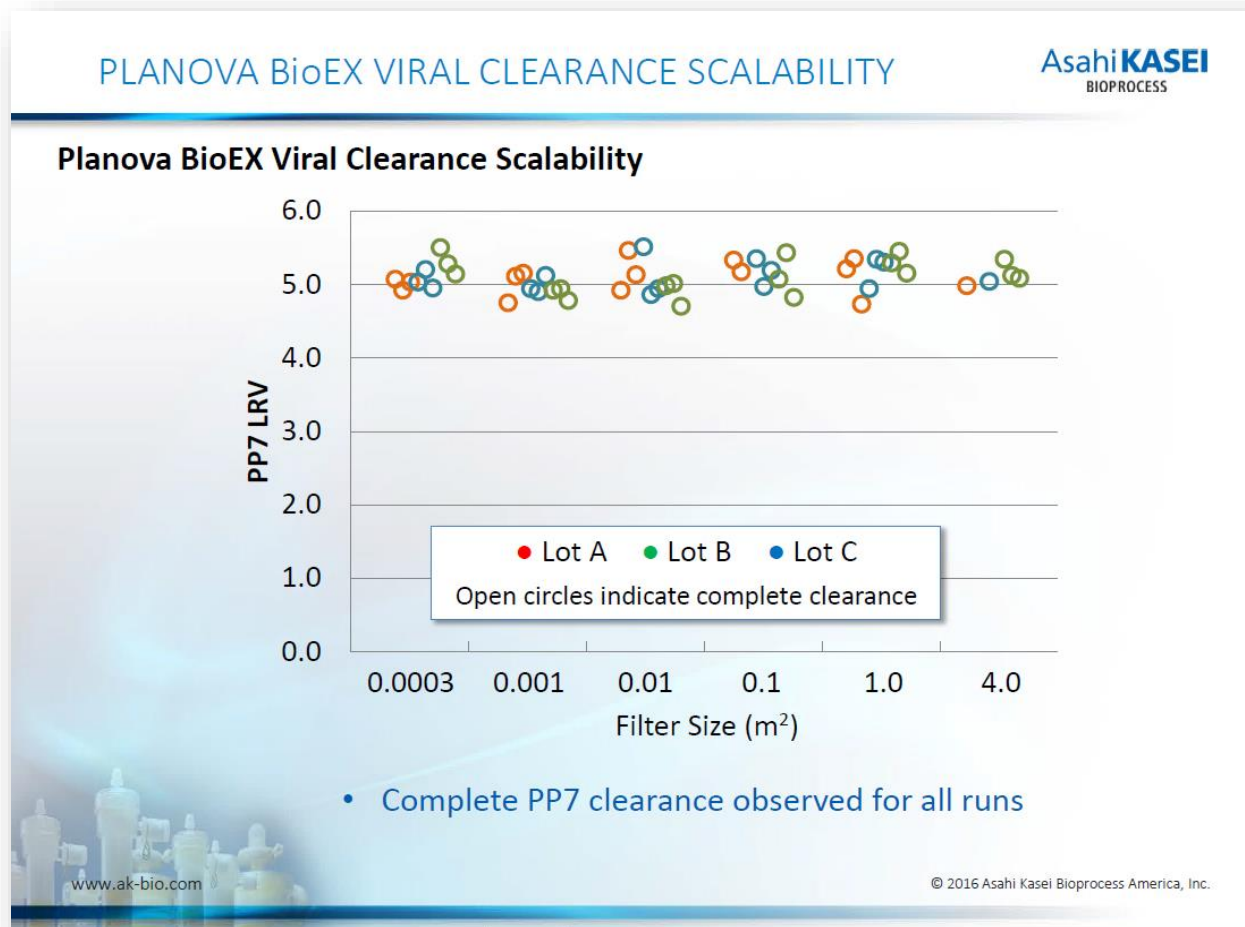
# Robustness & Nanofilter Quality



*Daniel Strauss, Asahi Kasei Bioprocess, 2016 Planova Workshop.*

**Consistent flux observed at all scales and no lot to lot variability**

# Robustness & Nanofilter Quality



*Daniel Strauss, Asahi Kasei Bioprocess, 2016 Planova Workshop.*

**Robust Viral Clearance across all filter sizes and lots**



## Conclusion:

*“ Virus removal nanofiltration not absolute  
filtration... “only” Robust 😊 ”*

## 1) Process Development:

- ✓ Possibility to change conditions (buffer, concentration, pH, cond.) to optimize nanofiltration or other purification steps
- ✓ Or faster development w/o optimizations because “it simply works”

## 2) Virus Clearance Validation:

- ✓ High LRV achievable
- ✓ No oversizing of the nanofilter

## 3) Manufacturing & Commercial Production:

- ✓ Process pause manageable
- ✓ Virus safety ensured despite accidents or purification problems
- ✓ Multi-product platform approach
- ✓ Slight production deviations absorbable
- ✓ Reliable performances



# Acknowledgements

**Thank you to my colleagues in Japan, US and Europe !**



どうもありがとう

**Domo Arigato!**

*(thank you very much)*

**Questions ??**

## Appendices

# Planova Filters



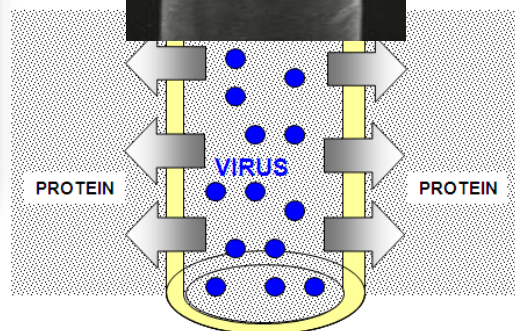
Filter	Planova™ N	Planova™ BioEX
Hollow fibers	cellulose	<b>PVDF</b>
Pore size (nm)	15, 20, 35, (75)	Parvovirus removal
Filtration surface (m <sup>2</sup> )	0.001 → 4.0	0.0003 → 4.0
<b>Max TMP (kPa)</b>	<b>98</b>	<b>343</b>
Water flux (LMH)	20N: 66 @ 98 kPa	170 @ 343 kPa
<b>SIP/Autoclaving</b>	<b>NO</b>	<b>YES</b>

# Planova Filtration Mechanism

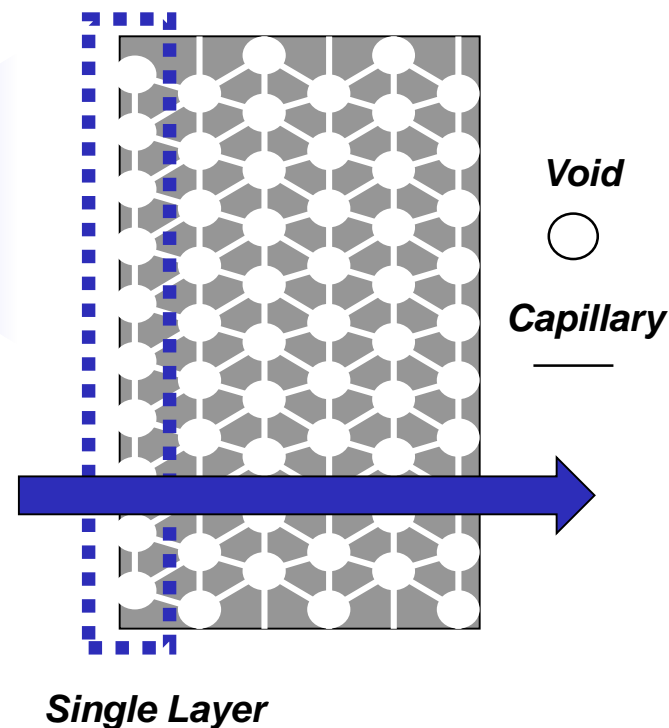
Planova™ Filter



Hollow fiber



Membrane structure:  
3D network



Size Exclusion & “Multi-layer” Filtration

## Planova™ Virus removal filters



## BioOptimal™ MF-SL TFF microfilter





## Systems & Equipment

Filtration, Chromatography, IBD™



## BioCradle

Microcarriers for Cell Culture



## Cellufine™

Cellulose Beads  
Chromatography media  
(made by JMC Corporation)