Development, Up- & Downscaling of a Virus Filtration Step

Franz Nothelfer, Nothelfer Consulting

Development, Up- & Downscaling of a Virus Filtration Step Overview Presentation

- Points to consider at start of development
- Upscaling of virus filtration step
- Downscaling of virus filtration step
- Conclusions

01-No

Development, Up- & Downscaling of a Virus Filtration Step
Points to Condsider at Start of Development (1)

Process

- Fed batch process or continuous process
- Position of virus filter in DSP
- Process conditions
  - product concentration – filterability of product
  - product purity – filterability of product
  - chemical/physical conditions of product solutions – product stability
- Virus segregation strategy

Upscaling Issues

- Possible maximum filter area in production facility
- Maximum product volume that can be processed in production facility (tank capacity)
- Filtration with pressurized tanks or with pumps
- Processing time – unlimited or time limitation (e.g. processing of several batches per week)

Product quality

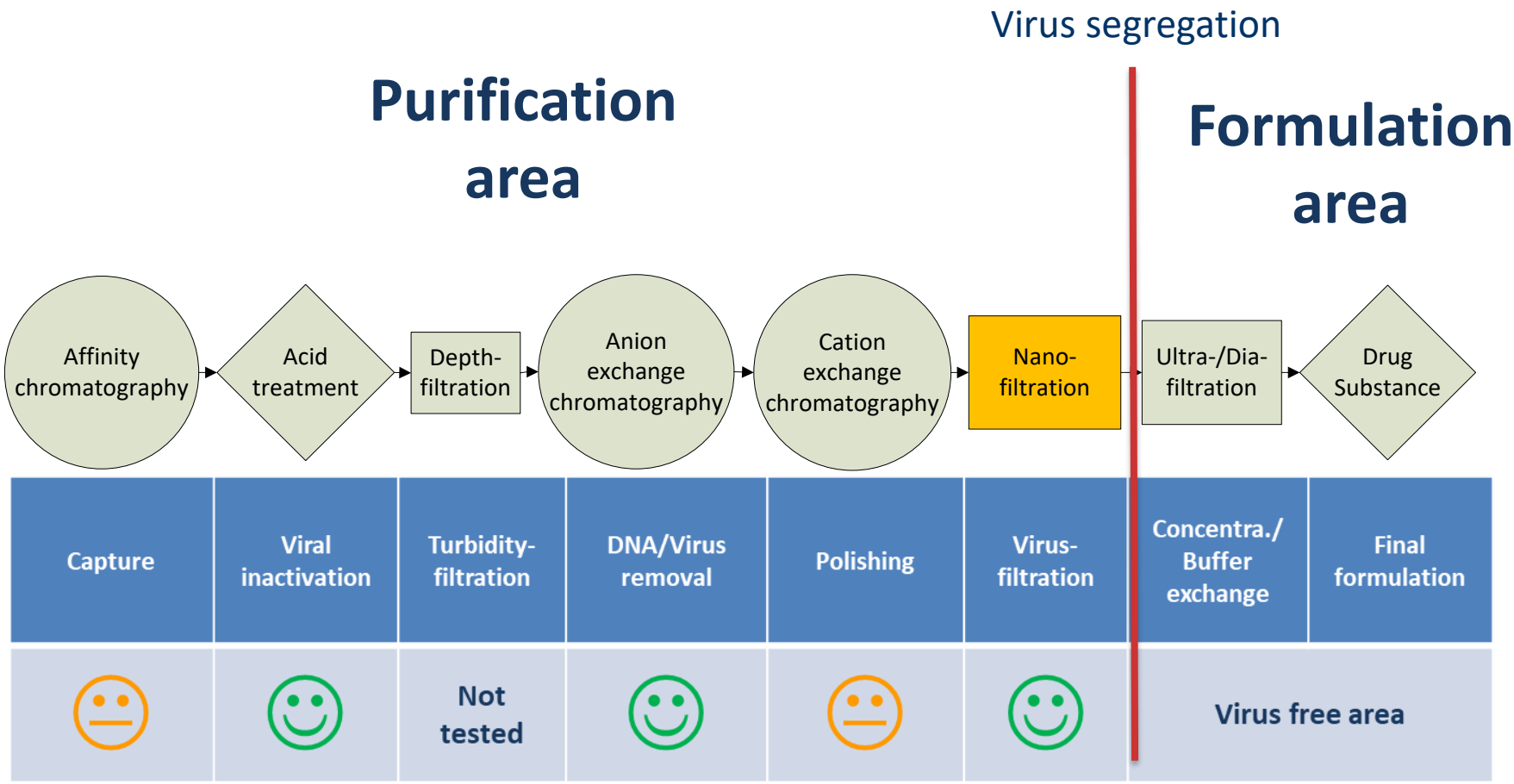
- Product stability at processing conditions – instability of product (e.g. oxidation – deaminidation – product degradation – increase of multimers)
- Product purity (e.g. host cell proteins – multimers – DNA etc.)

Development, Up- & Downscaling of a Virus Filtration Step Downstream Process for mAbs

Capture → Viral Inactivation → Depth filtration → AEX → CEX → Viral Filtration → UF/DF

Downstream Plattform Process for mAbs

Development, Up- & Downscaling of a Virus Filtration Step
Virus Segregation – mAb Process



☺ - effective to complete virus removal/inactivation

😐 - no to moderate virus removal

Development, Up- & Downscaling of a Virus Filtration Step Prerequisite for Retrovirus Grade Filters

- Virus filter should perform robust and predictable
- The virus filtration has to be performed within 4 hours*
- Good performance at 2 bar pressure to use compressed air in a vessel. For higher pressure a pressure regulated pump system is necessary*
- Easy handling in manufacturing (no additional filter skids or equipment)
- Easy filter testing before and after use
- COGS for virus filter.

* specific BI requirements

Development, Up- & Downscaling of a Virus Filtration Step Assessment Criteria for Parvovirus Grade Filter Selection

Parameter	VF1	VF2	VF3	VF4
Parvovirus clearance				
Scalability				
Process performance				
Flow characteristics				
Regulatory aspects (RSF)				
Price per m^2				
Robustness at validation study				
Handling / Filter testing				
Experience				

Score Card for Virus Filter Selection @ Boehringer Ingelheim

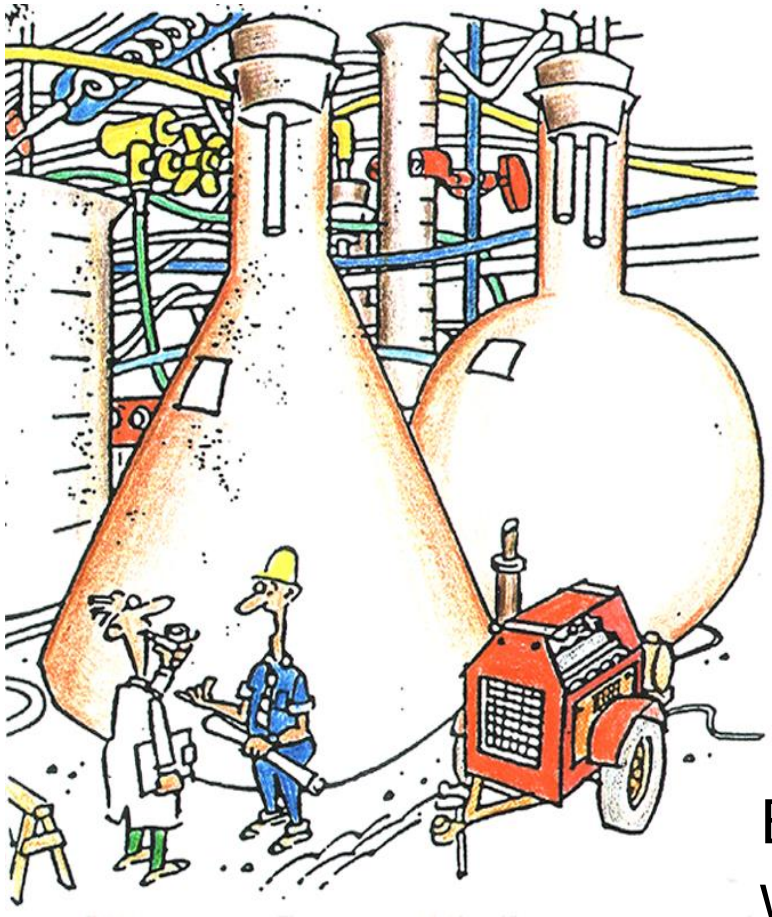
Development, Up- & Downscaling of a Virus Filtration Step
Conclusion Development of Viral Filtration Step

- Virus filtration is preferable the last step prior formulation

Rationale:

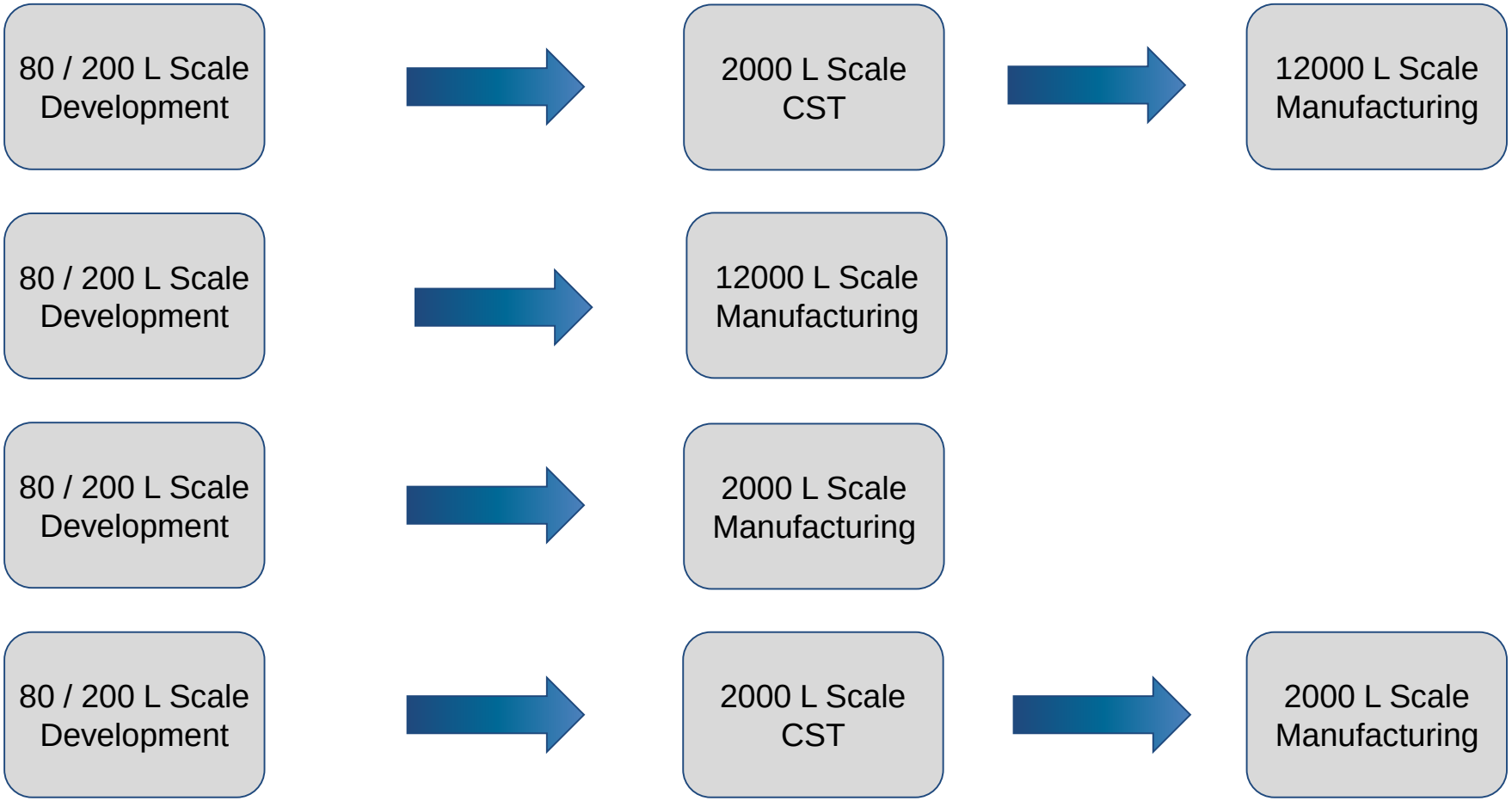
- highest purity of product
- low volume of product solution
- high stability of the product
- usually no time restrictions for testing the virus filter for integrity

Upscaling of Virus Filtration Step Challenges

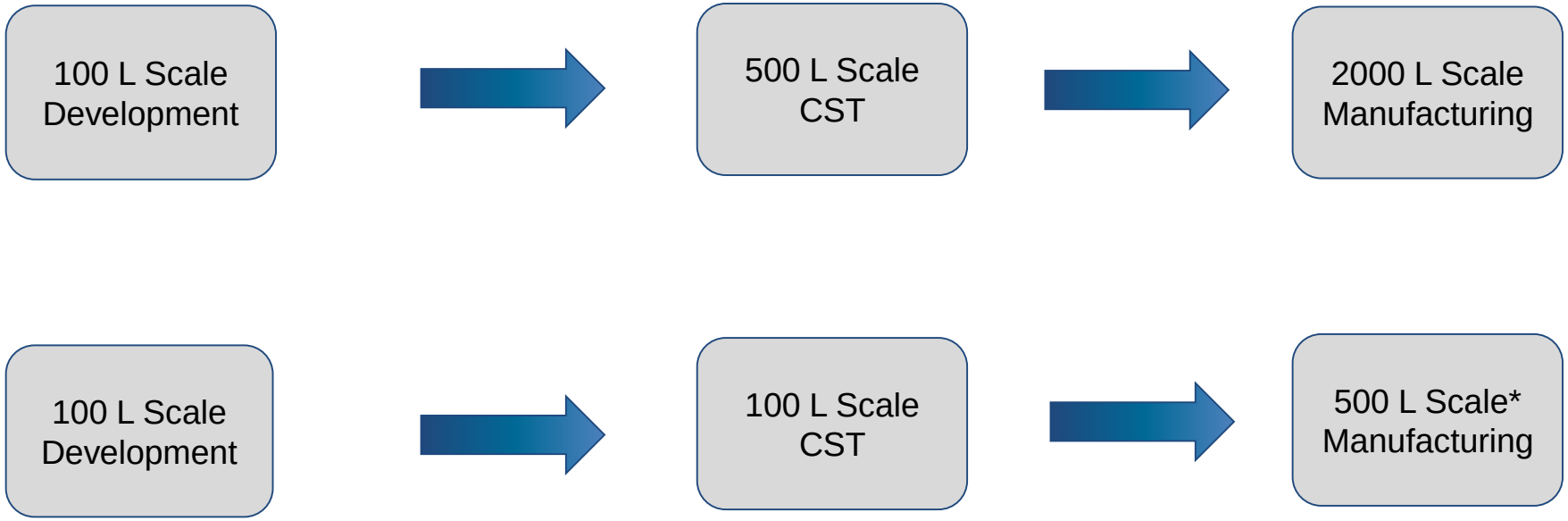


Boss, we‘re having trouble with sizing to production scale

Upscaling of Virus Filtration Step
Typical Scale up Steps in Stainless Steel Facility



Upscaling of Virus Filtration Step
Typical Scale up Steps in Single-Use Facility



* High potent proteins

Development, Up- & Downscaling of a Virus Filtration Step
Commercial Available Virus Filters

Virus Filter	Company
Planova 20 N	Asahi Kasei
Planova BioEx	Asahi Kasei
Viresolve Pro	Merck Millipore
Pegasus™ SV4	Pall
Virosart HF	SartoriusStedim

Commercial available Parvovirus grade filters

Development, Up- & Downscaling of a Virus Filtration Step
Commercial Available Virus Filters – Filter Areas

Virus Filter	Available Filter Areas
Planova 20 N	0.001 – 0.01 – 0.12 – 0.3 – 1 – 4 m²
Planova BioEx	0.0003 – 0.001 – 0.01 – 0.1 – 1 – 4 m²
Viresolve Pro	0.00031 – 0.017 – 0.07 – 0.22 – 0.51 – 1.53 m²
Pegasus™ SV4	0.00096 – 0.0058 –0.25 – 2.25 m²
Virosart HF	0.00017 – 0.0005 – 0.02 – 0.2 – 0.8 – 2.4 m²

Up Scale of Virus Filtration Process
Scale Up Principles / Prerequisites

Prerequisite

- no change in product quality during scale up
- no change of process conditions / time
- has to be fit in production plant

Scale up principles

- expand membrane area
- keep flow rate constant (L/m^2)
- increase diameter of piping to prevent high shear forces
- use same pump type as used in development or use pressurized vessels

First meeting with responsible people in production during development.

Goal: Information regarding filter type, first results of development, rough estimation on filter area

Transfer of the filtration process after verification runs (end of development)

Transfer meeting with production
Hand-over of process description
- Filter type & filter area
- Product load (L/m² or g/m²)
- Pressure & flux profiles
- Tested process ranges
- Buffer volumes for equilibration and post-wash

Scale down model for viral filtration is needed for:

- Process Characterization Studies
- Virus Clearance Studies
- Re-filtration Studies

Scale Down of Virus Filtration Challenges Scale Down Model

Step	Manufacturing	Scale Down
Pre-testing	- Integrity test - Forward flow test - Pressure hold test	- Forward flow test - (Pressure hold test)
Pre-filtration	- Inline	- Offline
Filter setting	- Multiple filters in parallel	- One filter
Filtration	- Pump - Compressed air - Variablity in production lots: product concentrations, pH, conductivity	- Compressed air - Only one production lot is used
Wash	- Variation in volume	- Maximum wash volume
Post- testing	- Integrity test - Pressure hold test - Gold particle test	- Forward flow test - Pressure hold test - Gold particle test
Unknown	- Interruptions during filtration, Pressure deviations - Re-filtration - etc.	- Blocking (virus spike)

Scale Down of Virus Filtration
Comparison Production Scale / Downscale

Process Parameter	Process Scale: 12,000 L	Validation scale
Filter membrane area:	32,000 cm^2	10.0 cm^2
Load pool:	Product pool from previous step	
Load capacity:	$\leq$ 250 L m^{-2}	> 250 L m^{-2}
Inlet pressure:	$\leq$ 1.0 bar	0.8 bar
Buffer volume: equilibration wash	$\geq$ 50 L m^{-2} $\geq$ 50 L m^{-2}	50 - 55 L m^{-2} 45-55 L m^{-2}
Down scale factor:	3,200 (correlated to membrane area)	

Scale Down of Virus Filtration
Set Up of Down Scale

Standard run with worst case scenario:

Maximum load volume (plus 5-10%)

Minimum pressure (minus ~0.3 bar)

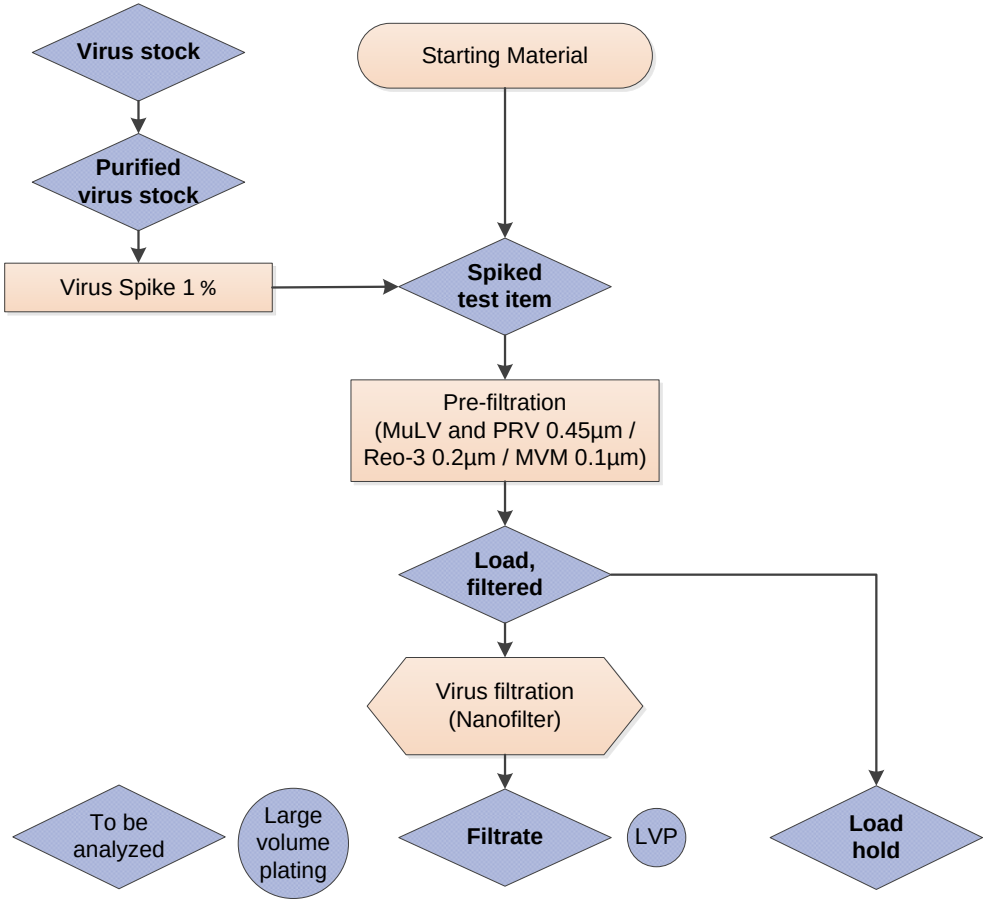
Maximum wash volume (plus 10- 20%)

Several pressure releases at load and wash

Duplicate runs per virus

Verification runs before VC study

Sampling:



Parameters for the verification of the scale down model

Comparison of:

- product yield
- product quality
- filtration rate
- product pool volume (L/m²)

Scale Down of Virus Filtration
Verification of the Scale Down Model - Results

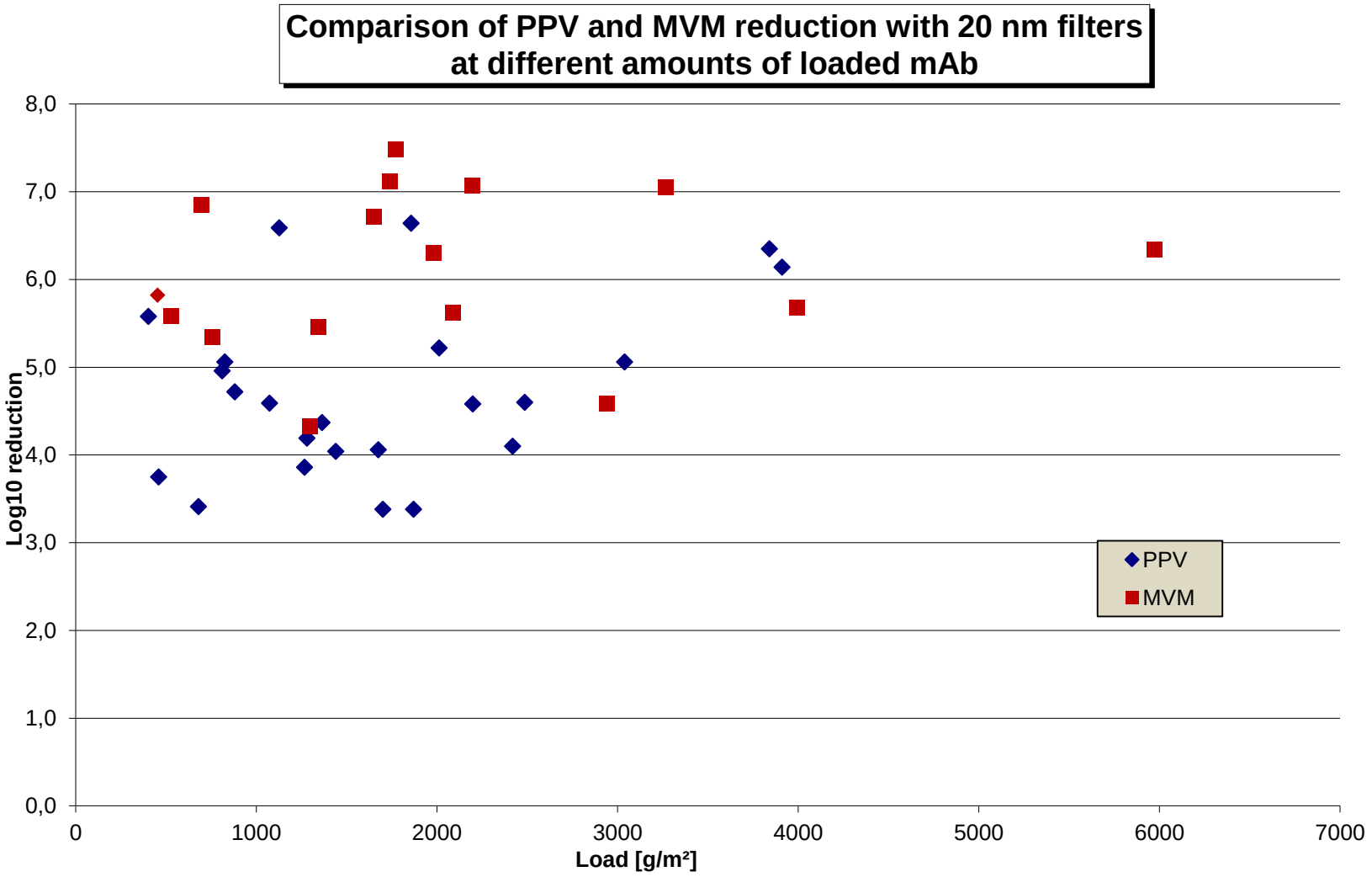
Step Yield

Process step		Step Yield	Protein conc.
		%	mg mL^{-1}
Virus Filtration	Process scale (12,000 L #1)	101	12.50
	Process scale (12,000 L #2)	99.2	15.00
	Process scale (12,000 L #3)	99.5	13.20
	Scale down (Validation study)	97.9	15.58
	Acceptance Criteria	> 95	---

Product Quality

Process step		Monomer	leach Prot.A	HCP
		%	ng mg^{-1}	U mg^{-1}
Virus Filtration	Process scale (12,000 L #1)	99.0	n.d.	n.d.
	Process scale (12,000 L #2)	99.1	n.d.	n.d.
	Process scale (12,000 L #3)	99.1	n.d.	n.d.
	Scale down (Validation study)	99.0	n.d.	n.d.
	Acceptance Criteria	> 96		

Development, Up- & Downscaling of a Virus Filtration Step
Results of Virus Filtration Studies



Scale Down of Virus Filtration
Re-filtration Study

Why have re-filtration studies to be performed for a virus filter?

Virus filter does not pass the integritiy test in production.
Consequence if no re-filtration study was performed →
the virus filtered product pool has to be discarded.

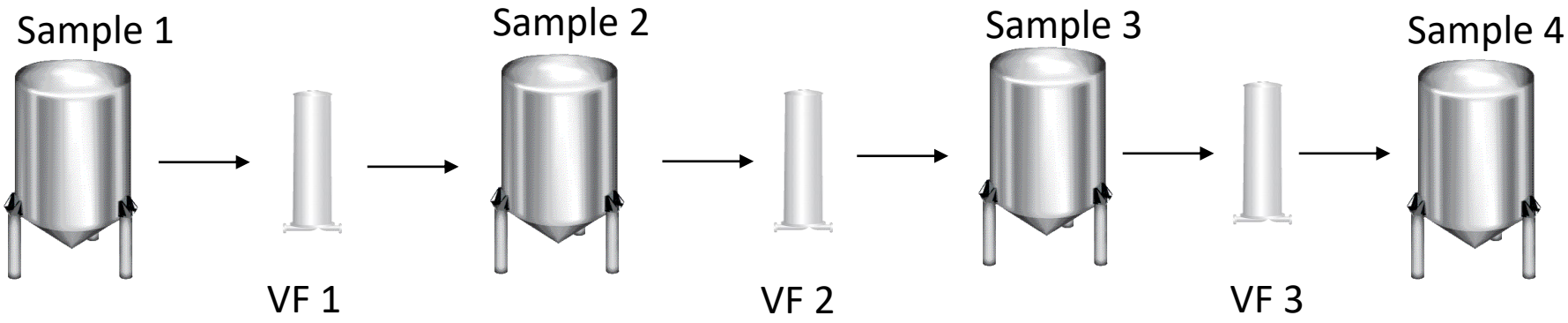
Performance of the re-filtration in small scale at least 2x better is 3x.

During the re-filtration steps the protein concentration, the concentration of the buffer ingredients and the product quality ideally is not affected.

Scale Down of Virus Filtration
Re-filtration Study

Parameters for re-filtration study have to be the same as in production scale
- filtration time
- flow rate (L/m²)
- inlet pressure
- product concentration

Analytical testing
- product quality e.g. multimers, oxidation, deamidation, degradation
- product concentration after each filtration



Scale Down of Virus Filtration
Process Characterization - Definition

Process Characterization

Identifying and quantifying all significant sources of variation, especially characterization of variation inherent to the materials and technology as applied to the specific product design

Process Characterization Studies

should identify and control sources that can effect the process

Potential sources of process variation

- Equipment
- Process
- Materials
- Measurement
- People
- Environment

Scale Down of Virus Filtration
Process Characterization - Definition Parameters

Term	Definition
Critical process parameter (CPP)	A critical process parameter is a parameter whose variability has an impact on a critical quality attribute and is hard to be controlled within the PAR and therefore should be monitored or controlled to ensure the process yields product of the desired quality.
Critical quality attribute (CQA)	A critical quality attribute is a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the process produces the desired finished product quality.
General process parameter (GPP)	A general process parameter is a parameter whose variability has no impact on a critical quality attribute or on process performance.
Key process parameter (KPP)	A key process parameter is a parameter whose variability has an impact on process performance (e.g. titer, yield, duration) and therefore should be monitored and controlled to ensure the process remains in a state of control. It does not affect a critical quality attribute.
Normal operating range (NOR)	Normal operating range is a defined (acceptable) range, within the proven acceptable range, specified in the manufacturing instructions as the range within which a process parameter is controlled, while producing unit operation material or final product meeting release criteria and critical quality attributes.
Performance indicator (PI)	Performance indicators are measurable output values used to quantify/assess process performance (e.g. product yield) and quality attributes to reflect the performance of a process and respective product quality.
Process parameter (PP)	A process parameter is an input value to control the process which is specified in process description and batch record.
Proven acceptable range (PAR)	A proven acceptable range is a characterized range of a process parameter for which operation within this range, while keeping other parameters constant, will result in producing a material meeting relevant quality criteria. In general operations, the PAR will be wider than the NOR (see NOR, above).
Well controlled critical process parameter (WC-CPP)	A well controlled critical process parameter is a parameter whose variability has an impact on a critical quality attribute but is readily and strictly controlled within the operating range with final product meeting release criteria and critical quality attributes.

Scale Down of Virus Filtration
Analytical Methods to Determine Product Quality

	CQA	Relevance for purification process
Glycosylation	Galactosylation (G0/G1/G2)	No
	High mannose content	No
	Sialic acids (NANA)	Yes
	Sialic acids (NGNA)	Yes
	Aglycosylated forms	No
	Afucosylated forms	No
Chemical modifications	Deamidated Isoforms	Yes
	Aspartate isomerization	Yes
	Oxidated isoforms, Met-oxidation	Yes
	Glycation	No
Folding/Conformation	Free thiol	Yes
	Mispaired disulfide bonds	Yes
	Secondary and higher order structure	Yes
Product related impurities	Soluble aggregates	Yes
	Fragments	Yes
Pharmaceutical attributes	Visible and sub-visible particles	Yes

+ process related impurities and additional pharmaceutical attributes

- Low/high pressure is considered worst case for virus retentive filtration. Have pressure release, pressure fluctuation, low/high pressure been validated as worst case conditions?
- Is validated pressure range, pressure release, process stop and interruption time representative for large scale manufacture?
- Taking into account the pressure ranges observed in the virus clearance studies, pressure limits in large scale manufacturing should be specified.
- High volume loads and high recovery flush volumes may impact removal of viruses. Limits should be specified based on results of virus validation study.

Development, Up- & Downscaling of a Virus Filtration Step Conclusion

- Make sure during development, that the developed virus filtration step can be scaled up and fits into the manufacturing facility e.g process volumes, size of tanks, process time (if several batches per week have to be managed)
- Make sure the equipment used in development is also available in manufacturing scale
- Scale down models are essential for

  - Process Characterization Studies
  - Virus Clearance Studies
  - Re-filtration Studies

Many thanks to the former colleagues at Boehringer Ingelheim especially to Helmut Winter, Carina Maigler, Franz Hepp, Michael Hinderhofer, Hans-Georg Bischof and Roland Pfänder.