How to successfully conduct a viral clearance study

- from design to execution -

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Outline

1. Introducing Charles River Laboratories and Cologne site
2. Biologics and the inherent need for viral clearance
3. Development of a Biologic
4. Viral clearance studies – Introduction
5. Viral clearance studies – Early phase
6. Viral clearance studies – Late stage design
7. Viral clearance for continuous processes
8. Summary

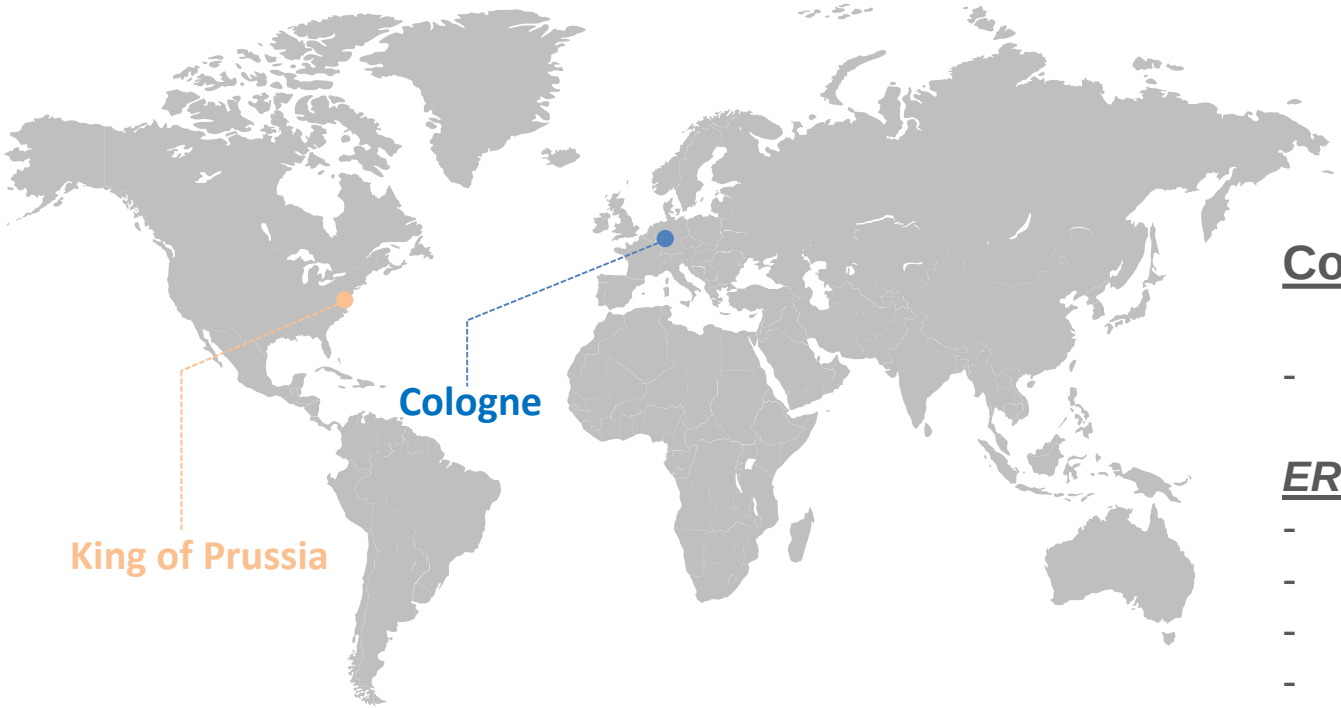
Charles River Laboratories – *„Every step of the way“*

Founded in 1947 by Dr. Henry Foster

A leading, full-service drug discovery and early-stage development company

Our scientists worked on ~70% of the drugs approved by the FDA in 2016

Charles River Laboratories – *„Every step of the way“*



Cologne / Erkrath site:

- VC part of Biologics group

ERK:

- *in vitro* testings
- cell line characterization
- release testing
- qPCR analysis

COL:

- viral clearance

Biologics and the inherent need for viral clearance

Biologics vs. synthetic drugs

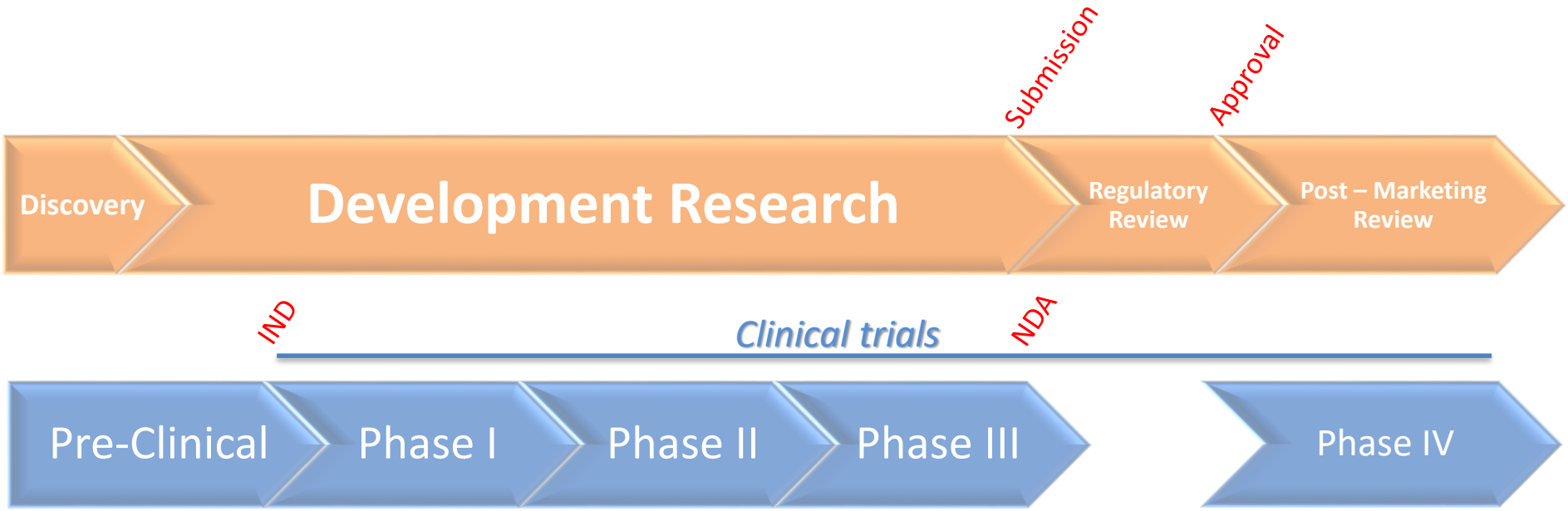
- strictly defined chemical process vs. highly variable “living” system
- prone to degradation / inactivation processes, modifications and contamination
- production using biological sources
    - microorganisms (bacteria; fungi / yeast)
    - animal and plant cells / cell cultures
    - tissues / organs
    - blood
    - urine

Biologics and the inherent need for viral clearance

Biologics vs. synthetic drugs

- active components
  - peptides / proteins (e.g. antibodies; vaccines; hormones; enzymes…)
  - oligonucleotides / nucleic acids (e.g. antisense-RNA…)
  - cells (e.g. cell therapy; wound patches…)
  - tissues (e.g. collagen wound patches…)
  - virus particles (e.g. vaccines; gene therapy vectors…)
- oral application vs. **injection / infusion**

Development of a Biologic



- *in vitro*
- *in vivo* (animal)
- toxicity

- first in human
- healthy volunteers
- side effects
- dose ranging
- formulation
- PK / PD
- safety

- first in patients
- proof of concept
- optimal dose
- efficacy
- safety

- confirming efficacy
- multi site trials
- safety

- monitoring public use
- drug-drug interaction
- safety

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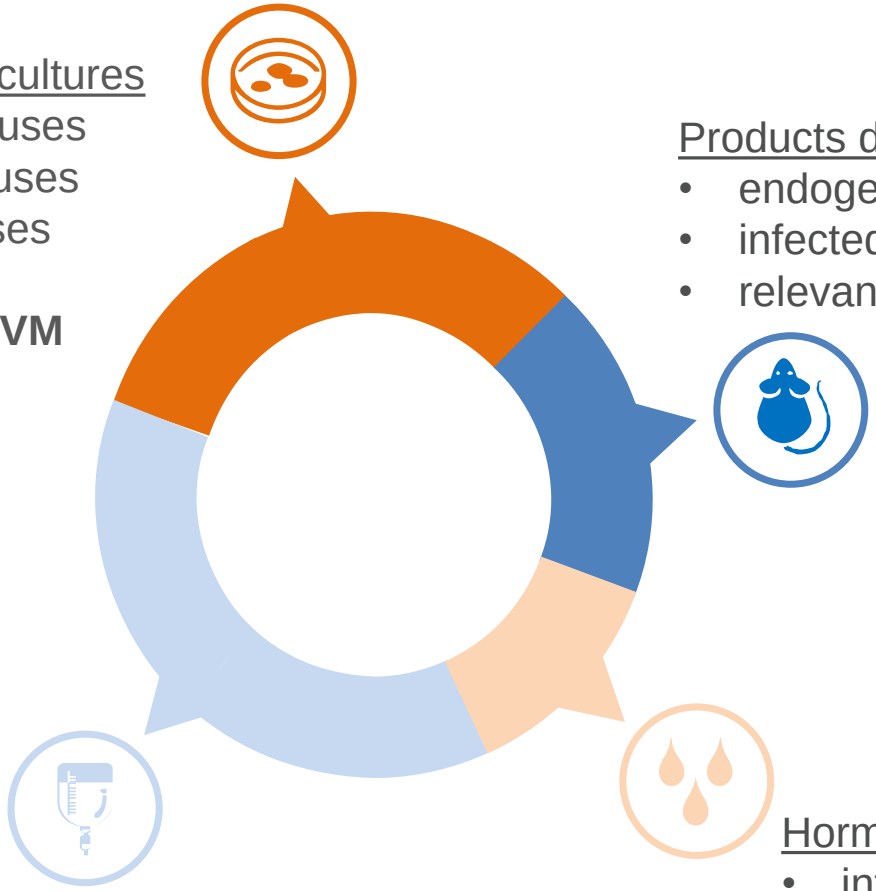
Viral clearance studies - Introduction

- Provide evidence that the production process will efficiently **inactivate/remove viruses known to contaminate** the starting material (**relevant viruses**)
- Provide indirect evidence that the production process might **inactivate/remove novel or unpredictable** virus contaminants (**model viruses**)

Viral clearance studies - Introduction

Antibody from cell cultures
- endogenous viruses
- adventitious viruses
- introduced viruses
- [Mycoplasma]
- relevant: e.g. **MVM**



Products derived from animal tissues
- endogenous viruses
- infected donor animals
- relevant: **PPV**

Hormones from body fluids
- infected donor
- relevant: e.g. **HCMV**

Extracts from blood/plasma
- infected donor
- relevant: e.g. **HIV, HBV, HCV**

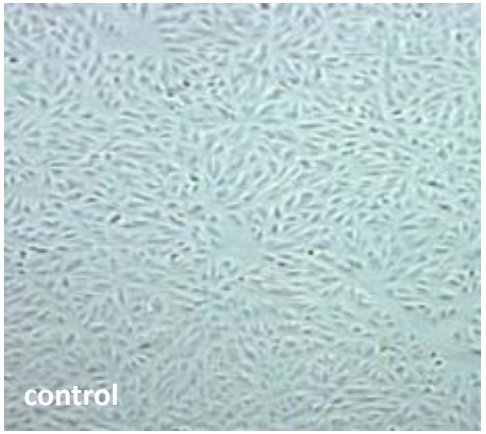
Viral clearance studies - Introduction

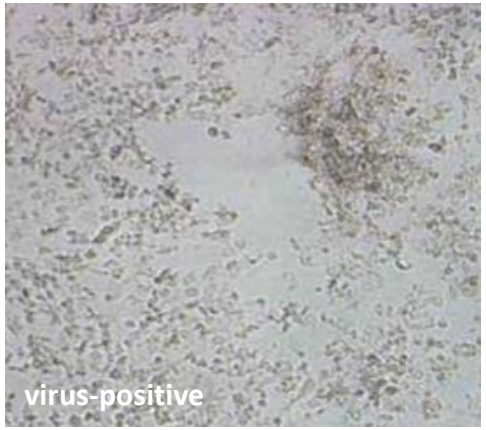
Typical model viruses used for spiking (Biotechnology)

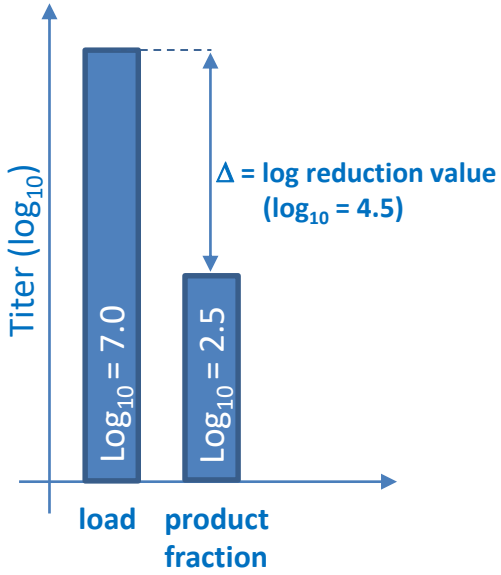
Envelope	Genome	Virus	Size [nm]	Stability
yes	ssRNA	Murine leukemia virus	80 – 100	low
	dsDNA	Pseudorabies virus	120 – 200	low – medium
no	dsRNA	Reovirus type 3	60 – 80	medium
	ssDNA	Minute virus of mice	20 – 26	very high

Viral clearance studies - Introduction

- Spiking of the start material for the respective step.
  - spike = high titer virus stock solution
- Determination of total virus load in start material (input).
  - tissue culture infectious dose 50 ($TCID_{50}$)
- Execution of the downscaled process step.
- Determination of total virus load in product-containing fraction (output).
- Assessment of the process step.







Viral clearance studies – Early phase

- development of the manufacturing steps and definition of correlating parameters
- risk assessment / determination of potential viral load (e.g. TEM)
- identification and/or introduction of potential steps contributing to viral clearance

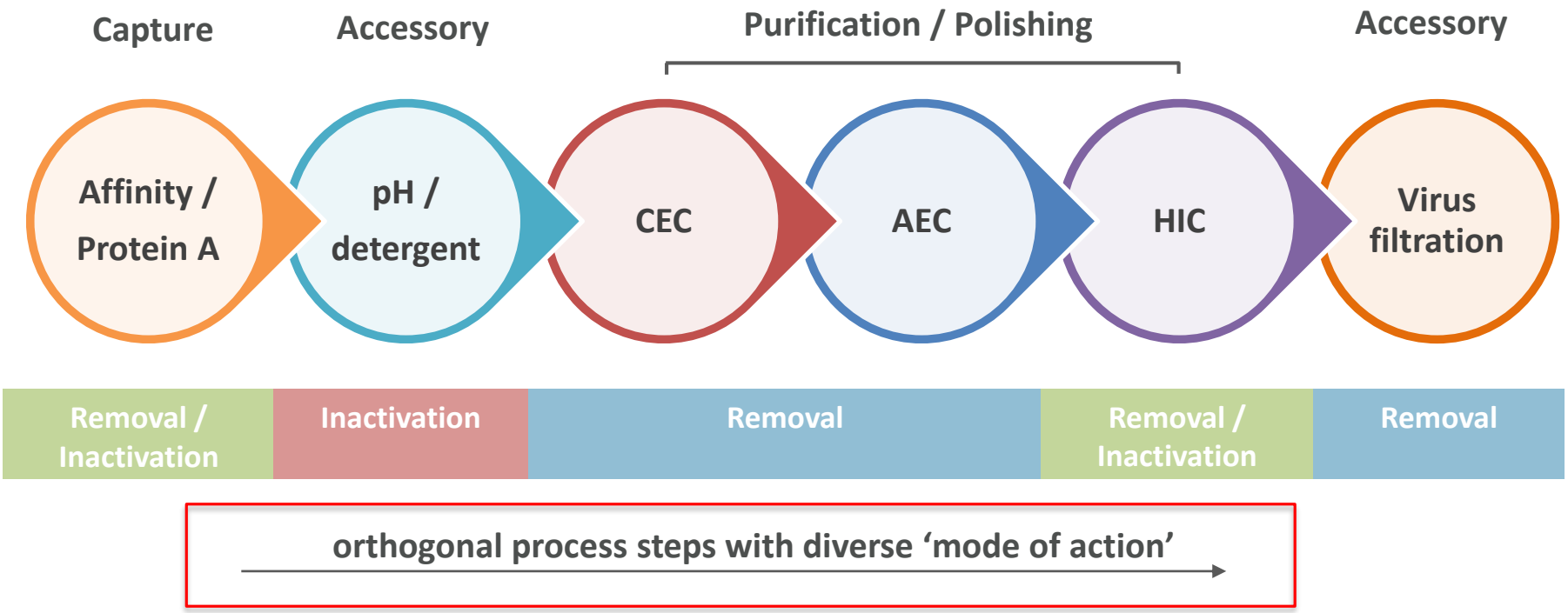
Capturing / Purification steps

fortuitous techniques, i.e. part of the manufacturing process (e.g. chromatography)

Accessory / Hold steps

deliberate techniques, solely introduced to remove or inactivate virus particles (e.g. pH treatment; virus filtration)

Viral clearance studies – Early phase

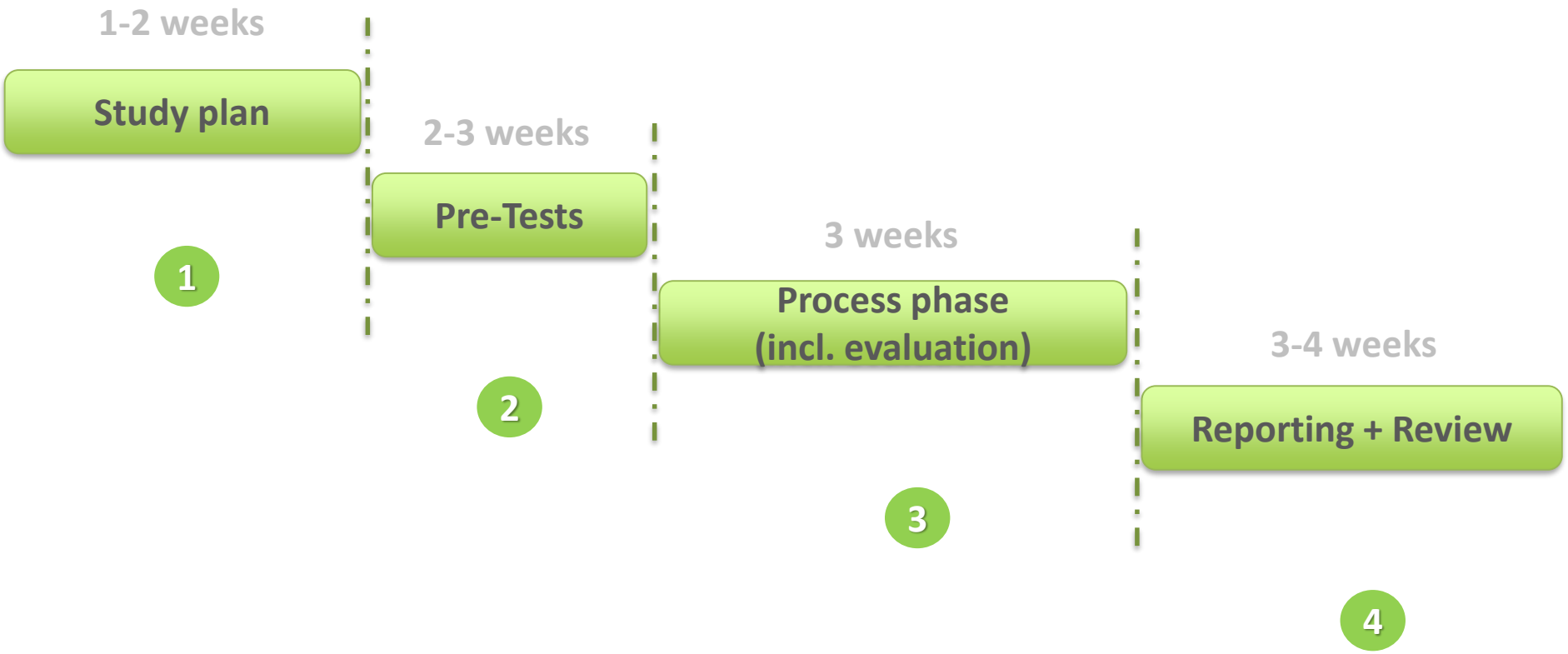


Viral clearance studies – Early phase

Biotech:

- investigation on 2 to 3 process steps
- mostly: inactivation, chromatography, virus filtration
- reduced virus panel (MuLV and MVM)
- reduced analysis (only product-containing fraction)
- fresh resin
- duplicate runs

Viral clearance studies – Early phase



Viral clearance studies – Early phase

1 Study initiation / Drafting the study plan

- **implementation of the down scale parameters**
- sampling of pre-test items (intermediates from DS process or manufacturing; no buffer!)
- identification of critical process parameters
- every process has tolerance ranges for parameters like protein concentration/load, pH, pressure, conductivity, temperature etc.
- **your task**: identify the most critical ones and determine the "worst case condition" (↓ ↑) which should be applied during the VCS

Viral clearance studies – Early phase

1 Study initiation / Drafting the study plan

Affinity / Protein A chromatography:

Mechanism of VC:
- removal (and inactivation)

Effective against:
- enveloped / non-enveloped viruses

Critical parameters:
- flow rate ↓ / residence time ↑
- load ↓ / resin capacity ↑
- wash volume ↓
- conductivity (load/wash ↓; elution ↑)

pH inactivation / detergent treatment:

Mechanism of VC:
- physico-chemical inactivation

Effective against:
- enveloped viruses

Critical parameters:
- pH ↑ ↓ (towards neutral)
- detergent concentration ↓
- incubation period ↓
- incubation temperature ↓

Viral clearance studies – Early phase

1 Study initiation / Drafting the study plan

Anion exchange chromatography:
(non-binding; HCP/DNA-removal)

Mechanism of VC:
- removal

Effective against:
- enveloped / non-enveloped viruses

Critical parameters:
- pH ↓ and conductivity ↑
- flow rate ↑ / residence time ↓
- load ↑ / resin capacity ↓

Cation exchange chromatography:
(binding; polishing)

Mechanism of VC:
- removal

Effective against:
- enveloped / non-enveloped viruses

Critical parameters:
- pH ↓ and conductivity (load/wash ↓; elution ↑)
- flow rate ↓ / residence time ↑
- load ↓ / resin capacity ↑

Viral clearance studies – Early phase

1 Study initiation / Drafting the study plan

Virus filtration:

Mechanism of VC:

- removal

Effective against:

- enveloped / non-enveloped viruses

Critical parameters:

- protein load
- filtration volume
- pressure / pressure releases & interruptions
- post-wash volume

Points to consider

- freeze and thaw cycle
  - unexpected aggregation
  - flow decay
  - blocking
  - insufficient volume processed
  - fresh (unfrozen) material
  - include additional pre-filtration steps
  - analytical data on protein content prior and post pre-filtration (?)

Viral clearance studies – Early phase

1 Study initiation / Drafting the study plan

Virus filtration:

Mechanism of VC:

- removal

Effective against:

- enveloped / non-enveloped viruses

Critical parameters:

- protein load
- filtration volume
- pressure / pressure releases & interruptions
- post-wash volume

Points to consider

- protein - spike interactions
  - virus and/or buffer
  - unexpected aggregation
  - flow decay / blocking
  - U/C virus
  - virus-specific pre-filtration (membrane type!)
  - mock spiking and filterability studies
  - reduce spike ratio (increase sensitivity!)
  - U/C virus in process buffer (recovery assay!)
  - [lower spike ratio with concentrated virus]

Viral clearance studies – Early phase

1 Study initiation / Drafting the study plan

Virus filtration:

Mechanism of VC:

- removal

Effective against:

- enveloped / non-enveloped viruses

Critical parameters:

- protein load
- filtration volume
- pressure / pressure releases & interruptions
- post-wash volume

Points to consider

- Pre-filter: coupled / de-coupled mode
  - evaluation of virus filter
  - pre-filter usually not quality controlled
  - reduction of virus load by pre-filter
    vs.
  - blocking of de-coupled virus filter
  - pre-filtration – spiking – virus filtration
  - VC in coupled mode and determination of reduction by pre-filter
  - coupled mode with in-line spiking

Viral clearance studies – Early phase

1 Study initiation / Drafting the study plan

Virus filtration:

Mechanism of VC:

- removal

Effective against:

- enveloped / non-enveloped viruses

Critical parameters:

- protein load
- filtration volume
- pressure / pressure releases & interruptions
- post-wash volume

Points to consider

- constant flow or constant pressure
  - constant flow → pressure likely to increase during filtration
  - constant pressure → flow decay over time
  - process-specific interruptions
  - failure-related interruptions

Viral clearance studies – Early phase

2 Pre-Tests

- cytotoxicity assay and interference assay
- virus inactivating agents (detergents, enzymes, extreme pH) / inactivating properties
- recovery assays (start material ; buffer)

Optionally:

- adaptation of assay sensitivity after determination of effects on viral replication
- change of propagation cell line if toxic/interfering properties affect determination of the load titer and/or cannot be compensated by assay sensitivity
- change of test system if product interacts / cross-reacts with virus or indicator cells

Viral clearance studies – Early phase

3 Process

- awareness of responsibilities
- (control runs)
- spare test items, additional columns, filters, buffers etc.
- sample analysis using cell cultures → sterile working conditions (!)
- unambiguous labeling and prompt transfer of process samples after collection
- determination of fraction size
- communicate changes (e.g. additional dilutions / pH- & conductivity adjustments)
- schedule: include some back up time

Viral clearance studies – Early phase

4 Reporting

- process conducted by the sponsor vs. test facility
- non-GLP study vs. GLP study
    - feasibility study / additional data
    - application / approval process
- guidelines
    - biotech products (e.g. ICH, WHO)
    - medical devices (e.g. ISO)

Viral clearance studies – Late stage design

Clinical trials

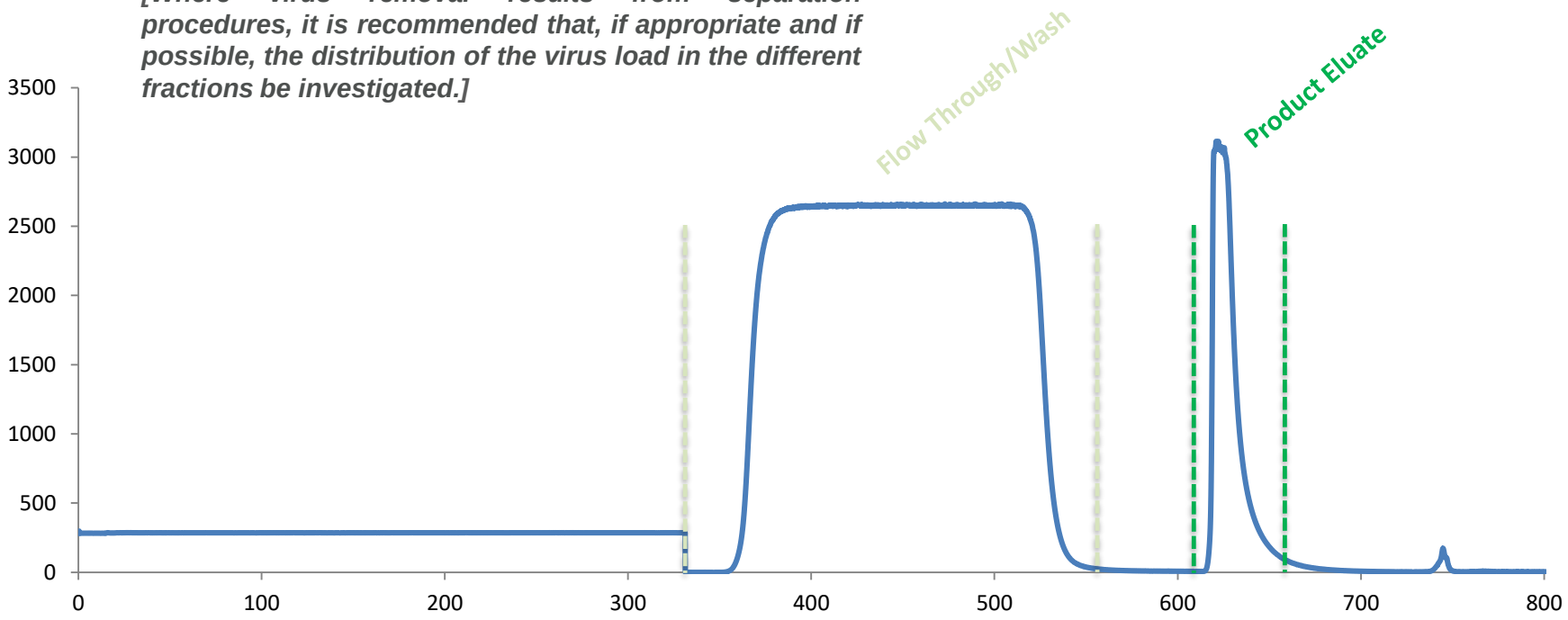
Biotech:

- investigation on at least 3 process steps
- mostly: inactivation, chromatography, virus filtration
- extended virus panel (MuLV, PRV, Reo-3 and MVM)
- extended analysis for virus balancing e.g. flow through, wash steps, pre- and post-eluate fractions, strip / CIP / regeneration fractions
- used resin runs
- carry over runs (viral carry over from batch to batch)

Viral clearance studies – Late stage design

ICH Q5A [1)]

[Where virus removal results from separation procedures, it is recommended that, if appropriate and if possible, the distribution of the virus load in the different fractions be investigated.]



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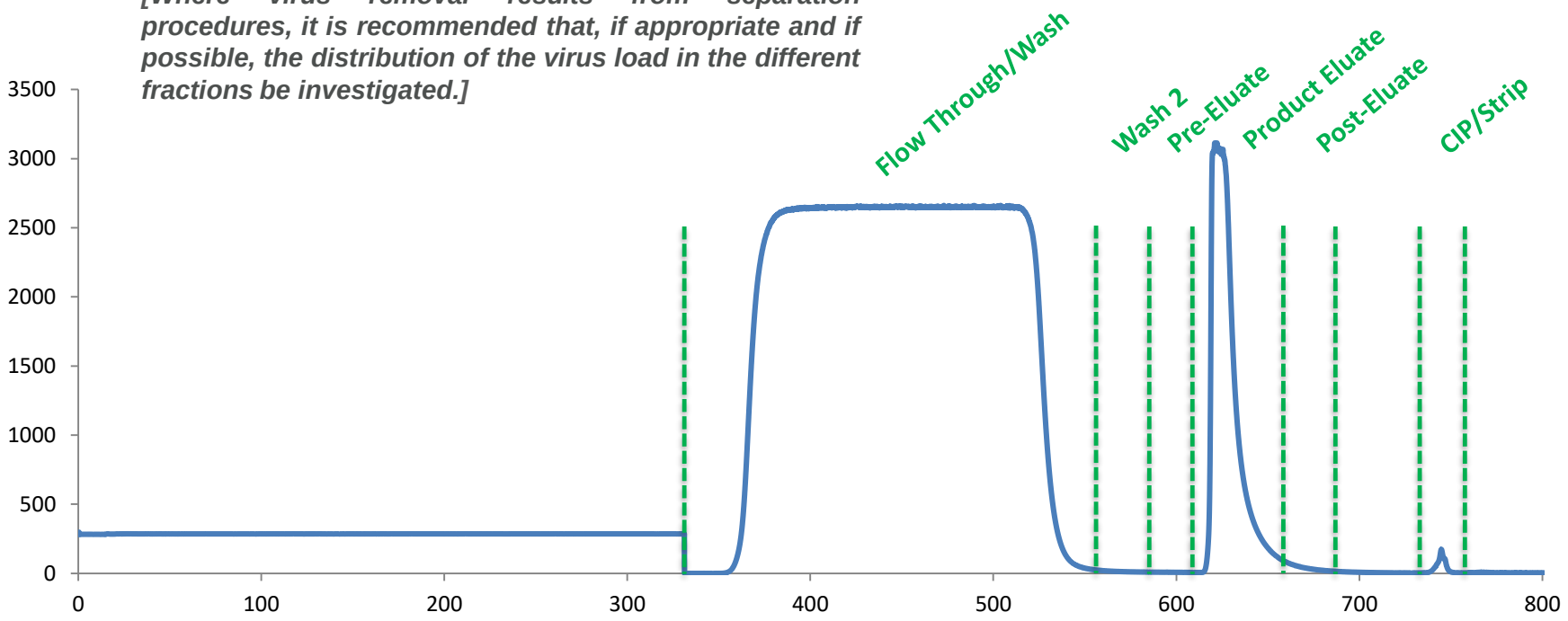

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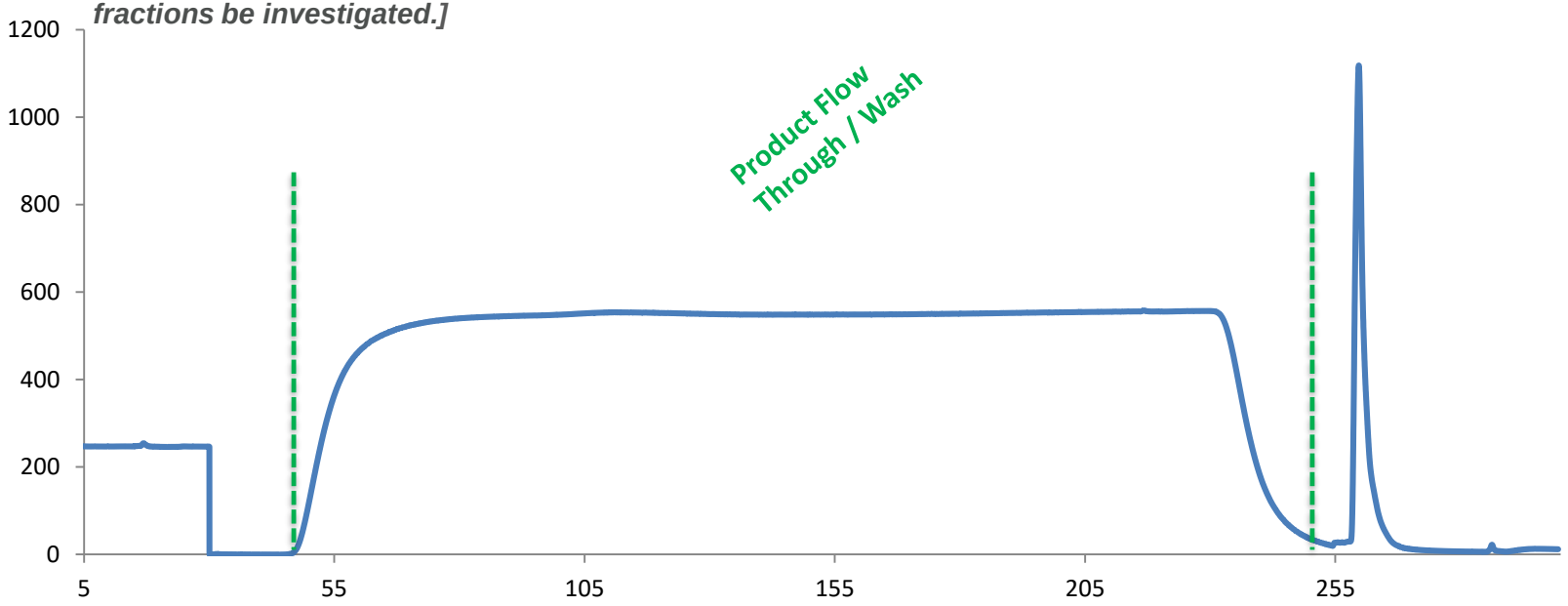

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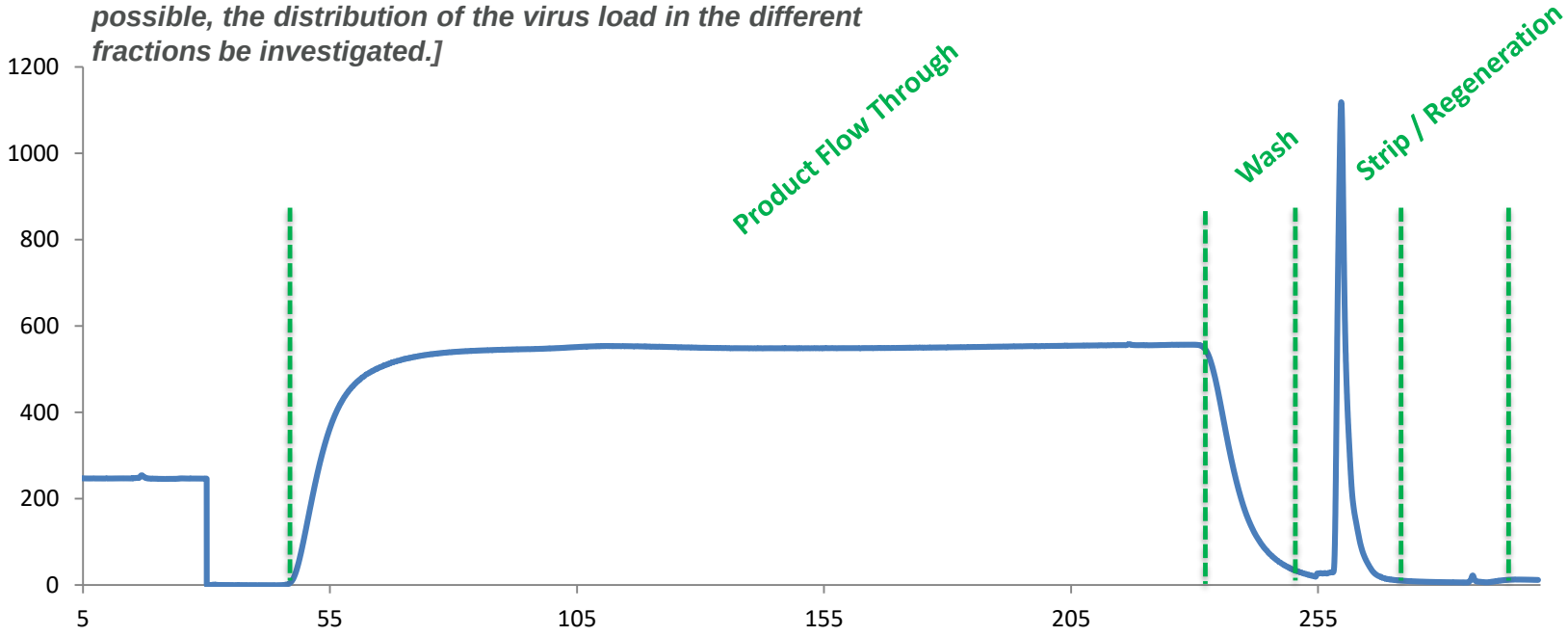
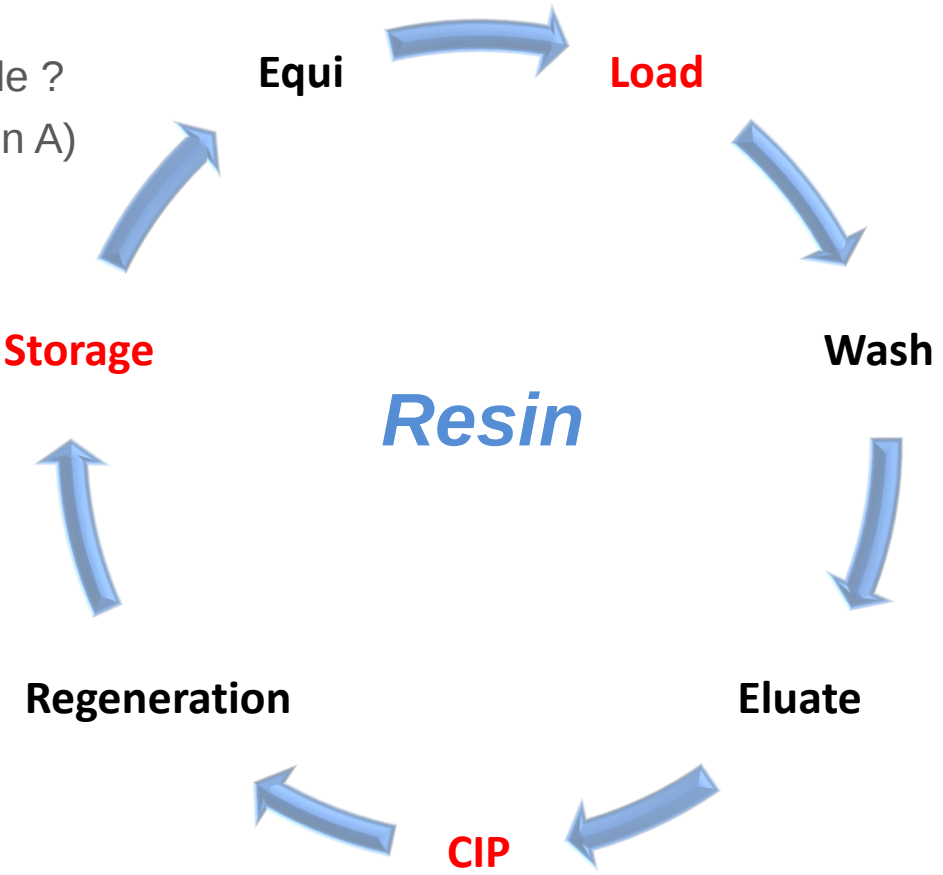


Viral clearance studies – Late stage design

Used resin:

- alteration of resin properties during life cycle ?
- load material ←→ resin ligands (e.g. protein A)
- CIP: extreme pH / harsh conditions



Points to consider

- used resin testing for Phase III mandatory

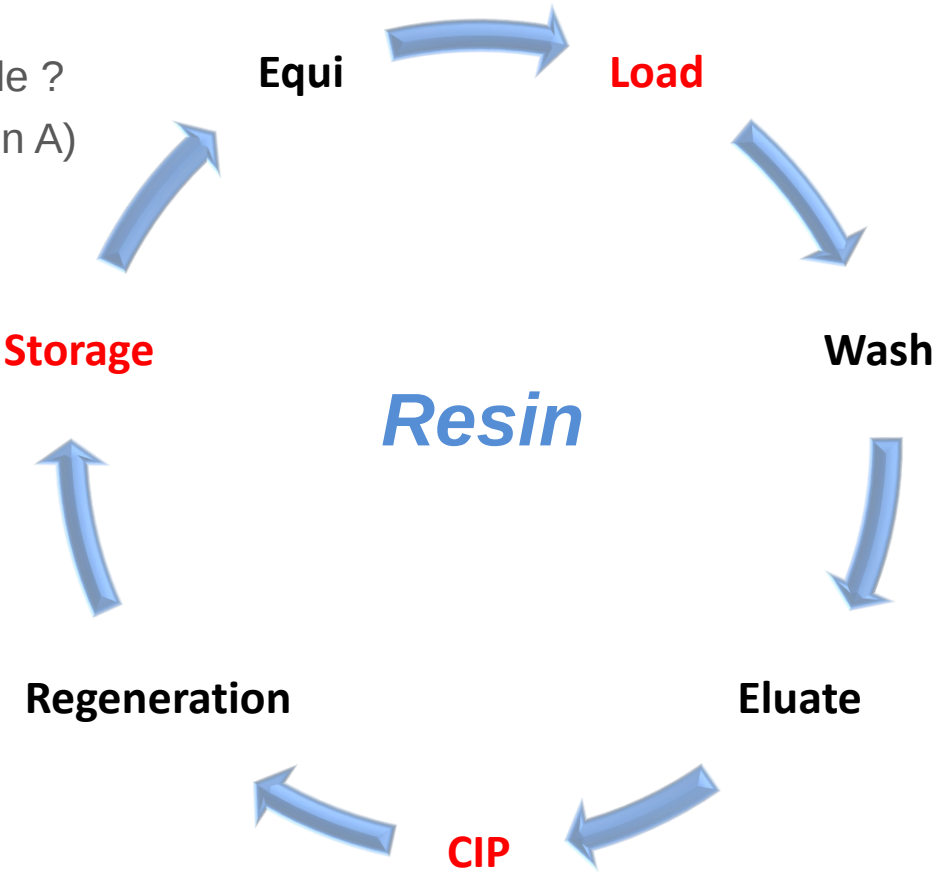
ICH Q5A [1)]

[Over time and after repeated use, the ability of chromatography columns and other devices used in the purification scheme to clear virus may vary. Some estimate of the stability of the viral clearance after several uses may provide support for repeated use of such columns…..]

Viral clearance studies – Late stage design

Used resin:

- alteration of resin properties during life cycle ?
- load material ←→ resin ligands (e.g. protein A)
- CIP: extreme pH / harsh conditions



Points to consider

- used resin testing for Phase III mandatory
- usually single run, BUT 4 viruses
- end of life cycle resin (~ **100** cycles?)
- important factor when defining the small scale modell

Viral clearance studies – Late stage design

Carry over:

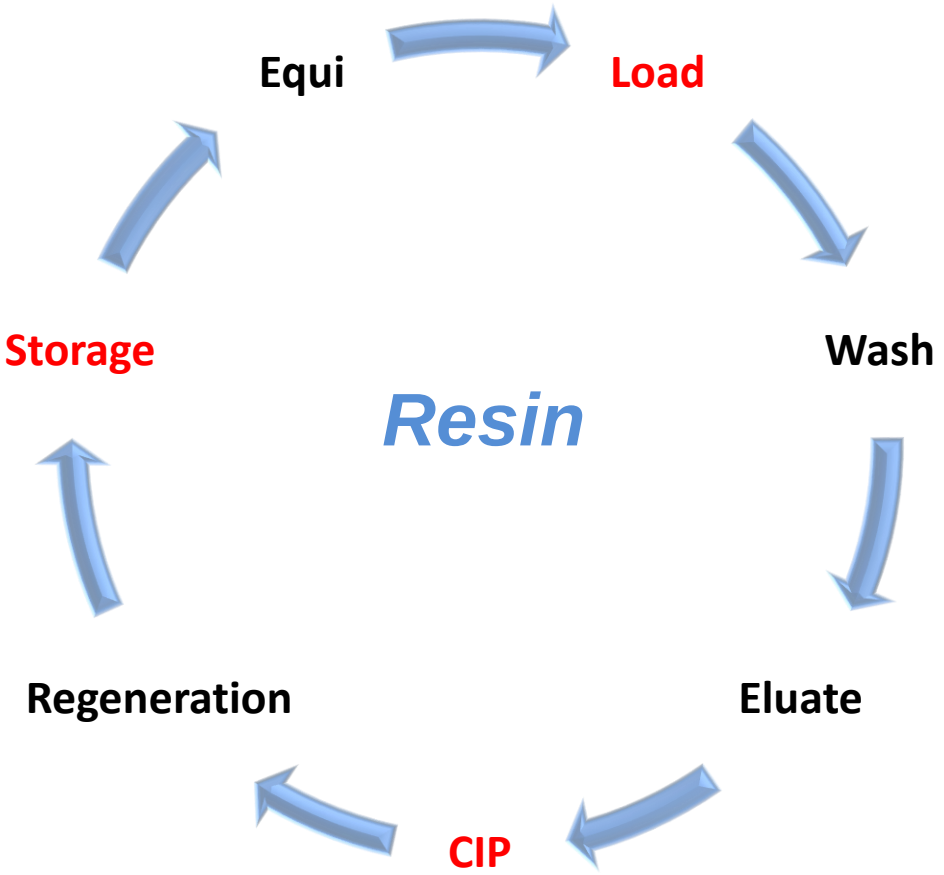
- resin re-use vs. disposable
- batch to batch cross-contaminations
- virus accumulation on the resin
- examination of the system

Points to consider

- carry over runs for Phase III mandatory

ICH Q5A [1)]

[…Assurance should be provided that any virus potentially retained by the production system be adequately destroyed or removed prior to reuse of the system. For example, such evidence may be provided by demonstrating that the cleaning and regeneration procedures do inactivate or remove virus.]



Viral clearance studies – Late stage design

Carry over:

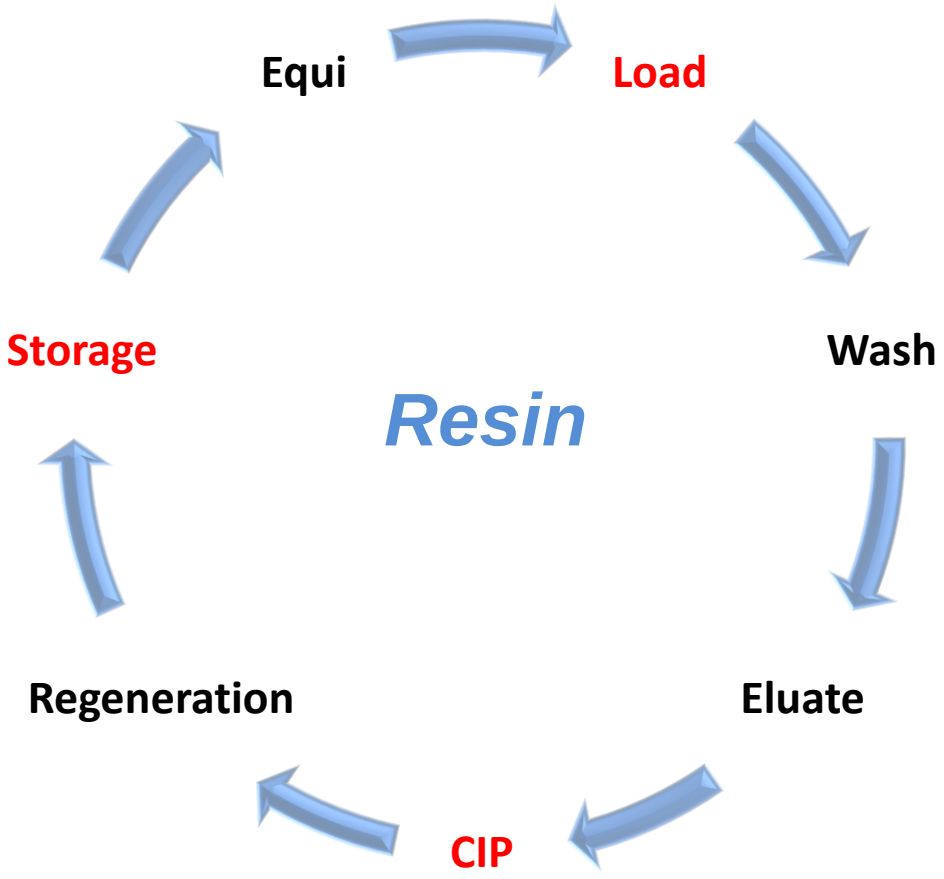
- resin re-use vs. disposable
- batch to batch cross-contaminations
- virus accumulation on the resin
- examination of the system

Points to consider

- carry over runs for Phase III mandatory

WHO Technical Report Series [2)]

[Since sanitization is an essential part of the production process, it must be validated to the same extent as virus inactivation or elimination steps…Finally, an attempt should be made to demonstrate that no infectious virus remains on the resin, usually by subjecting it to the next purification cycle. These validation experiments need to be done with fresh resin as well as with resin that has been used for the specified maximum number of cycles.]



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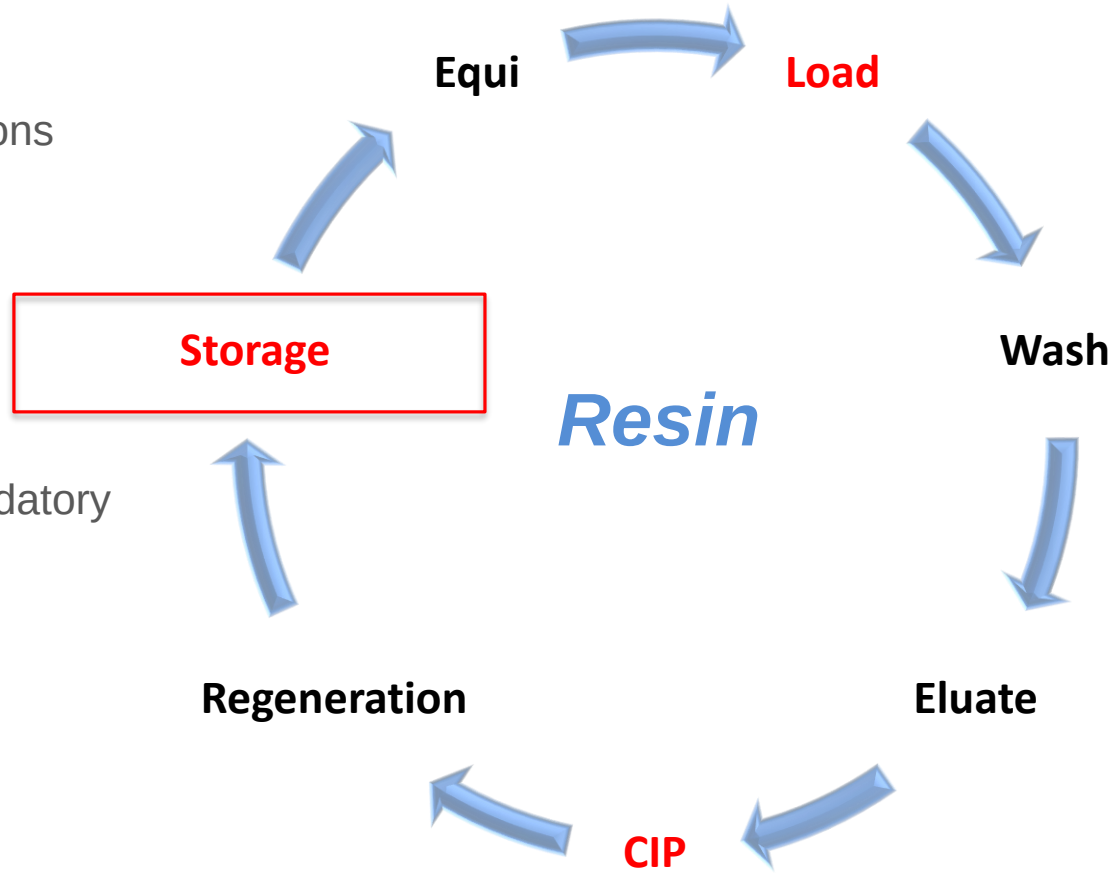
Viral clearance studies – Late stage design

Carry over:

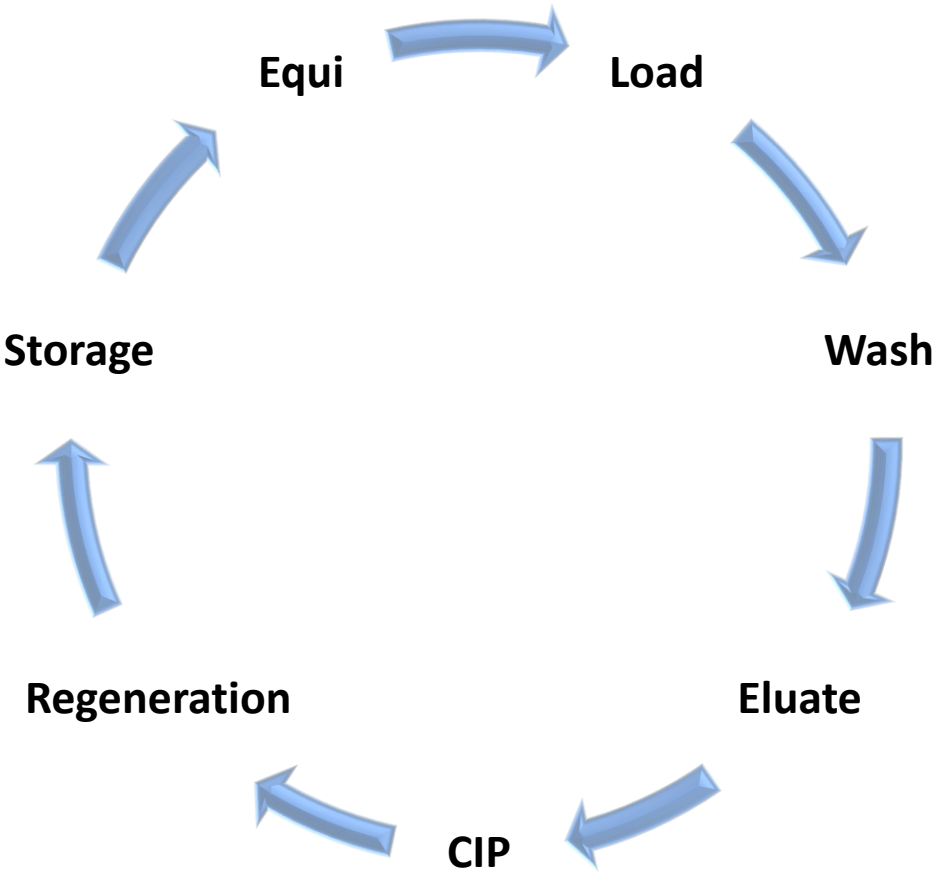
- resin re-use vs. disposable
- batch to batch cross-contaminations
- virus accumulation on the resin
- examination of the system

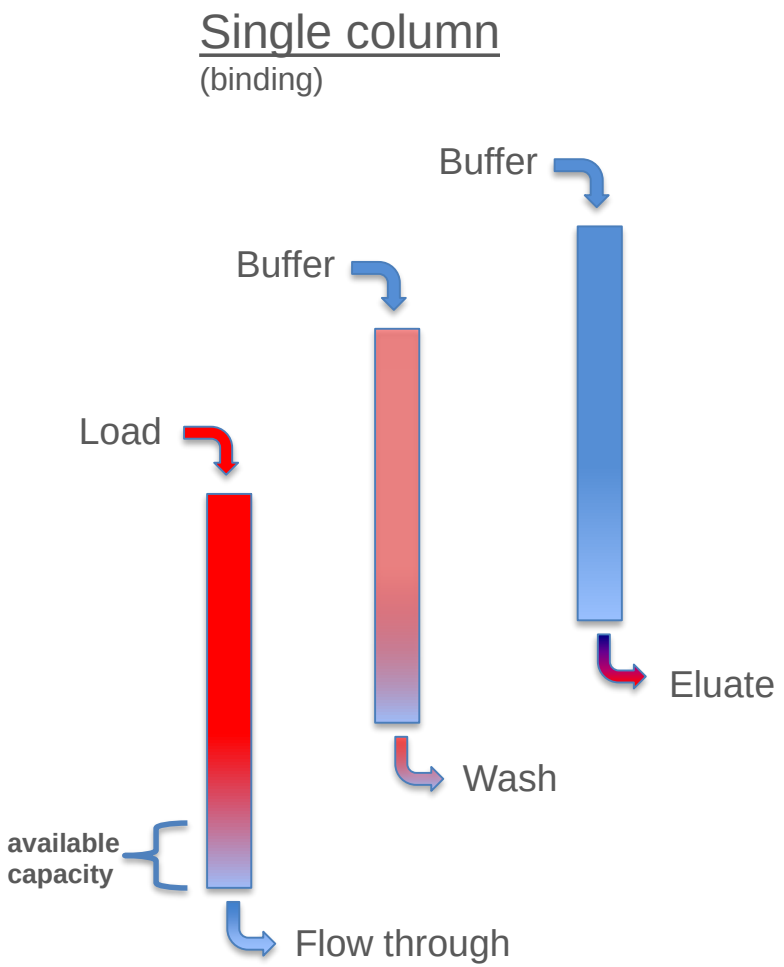
Points to consider

- carry over runs for Phase III mandatory
- additional load material required
- fresh and used resin
- storage vs. direct re-use



Viral clearance for continuous processes





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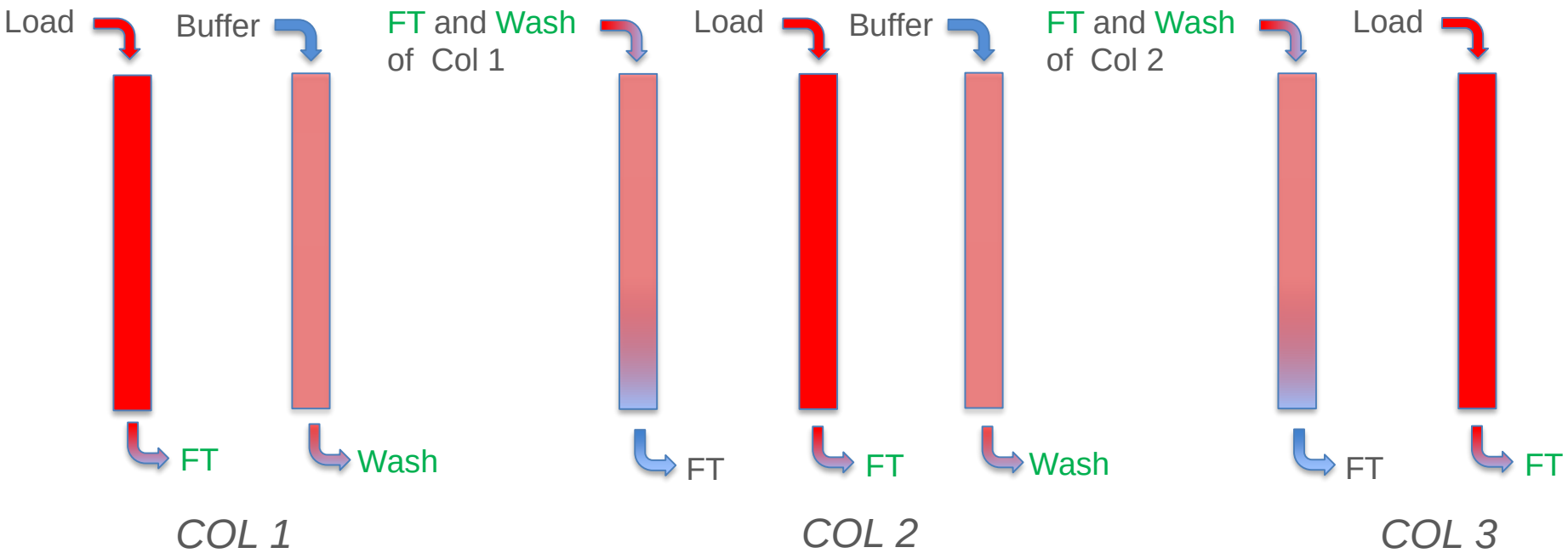
Viral clearance for continuous processes

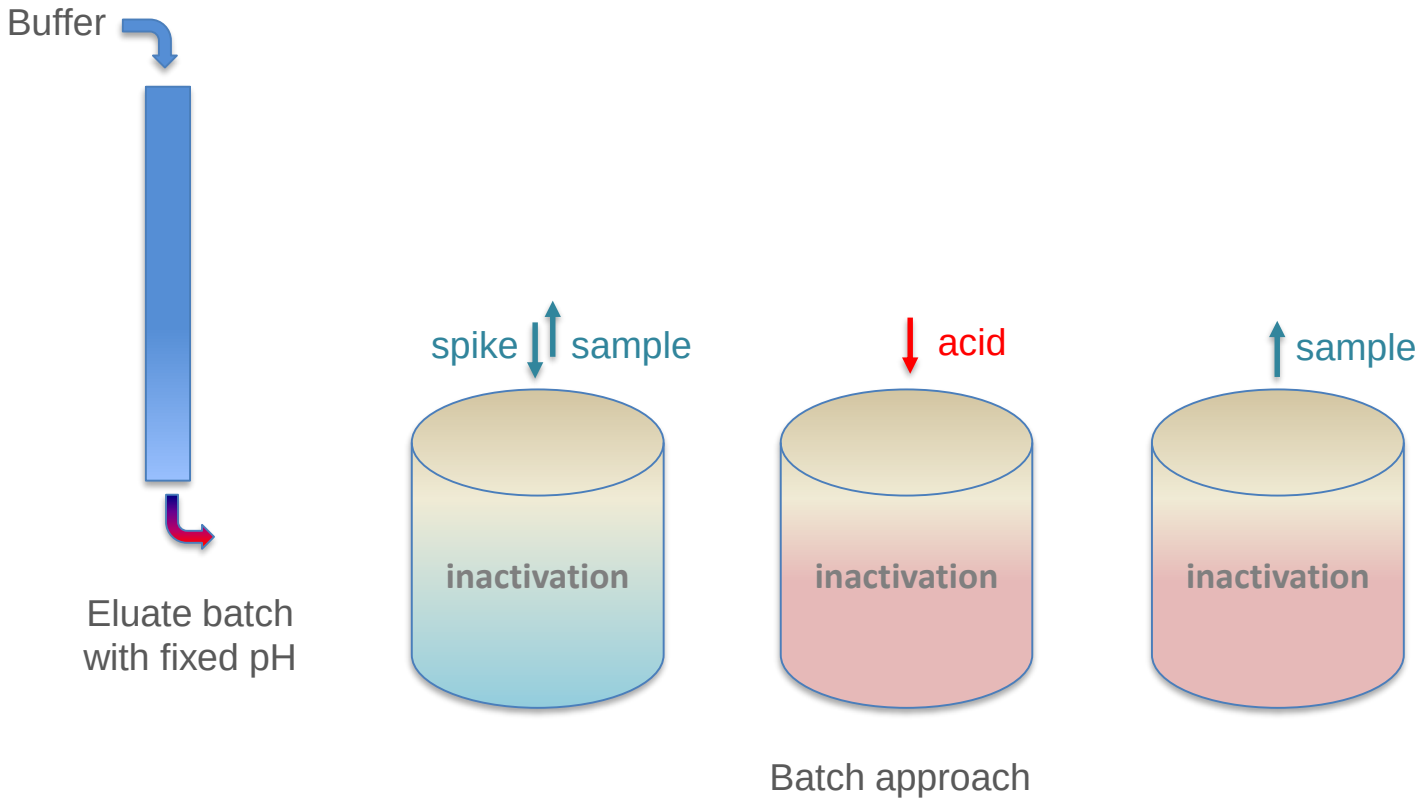
Multiple columns
(binding)



- loading beyond resin capacity
- excess protein load and chase is administered onto second column
- challenge for VCS: definition/sampling of load and calculation of initial load titer

Viral clearance for continuous processes

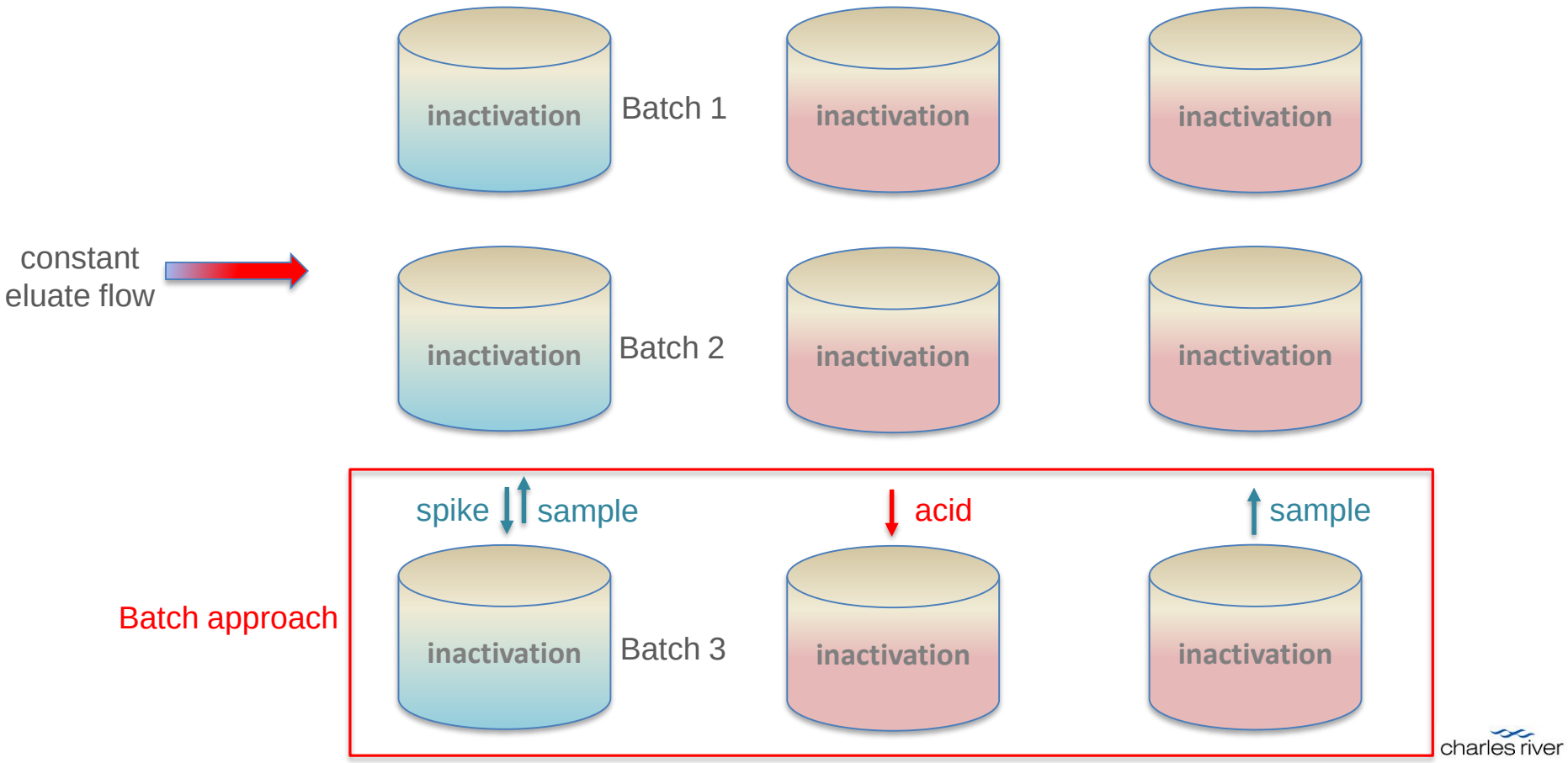
Single column → Hold step
(binding)



Viral clearance for continuous processes

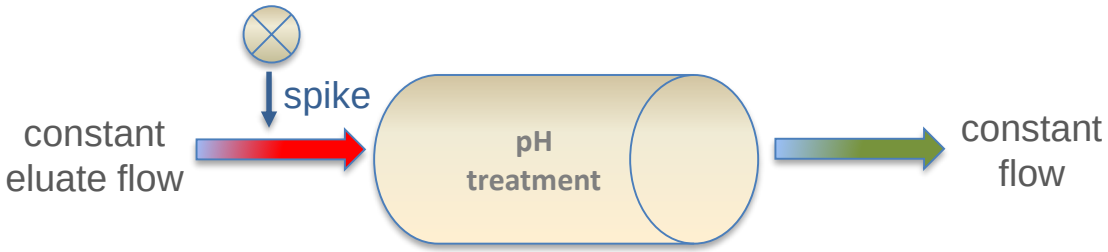
Multiple columns → "batch" Hold step

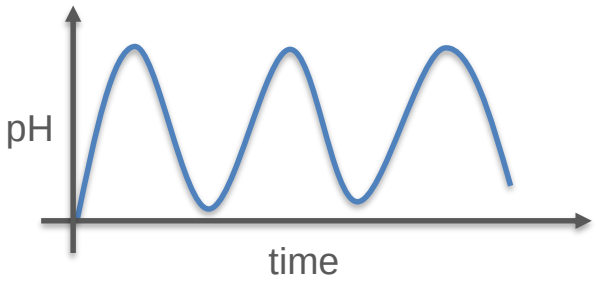
(binding)



Viral clearance for continuous processes

Multiple columns → "continuous" Hold step
(binding)



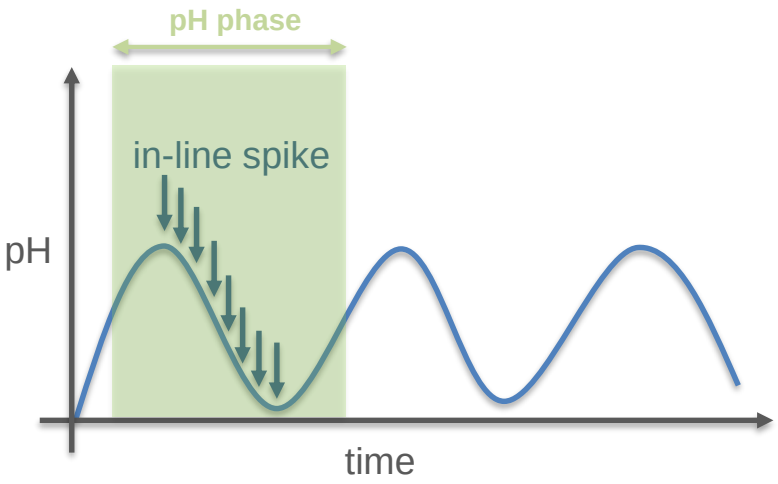


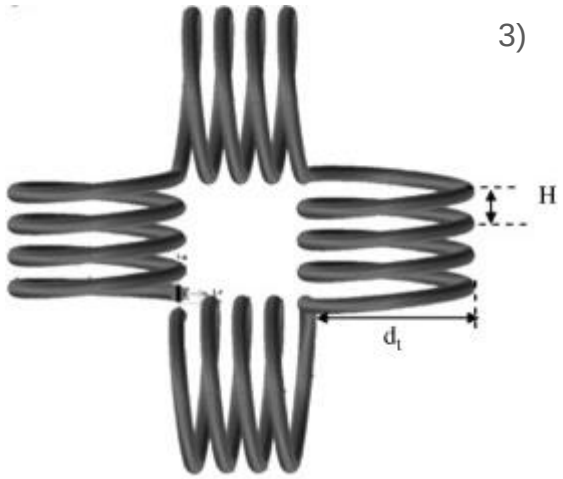
Challenge for VCS:

- no batch volume to spike → inline spiking
- pH fluctuations → compensation necessary

Viral clearance for continuous processes

Multiple columns → "continuous" Hold step
(binding)



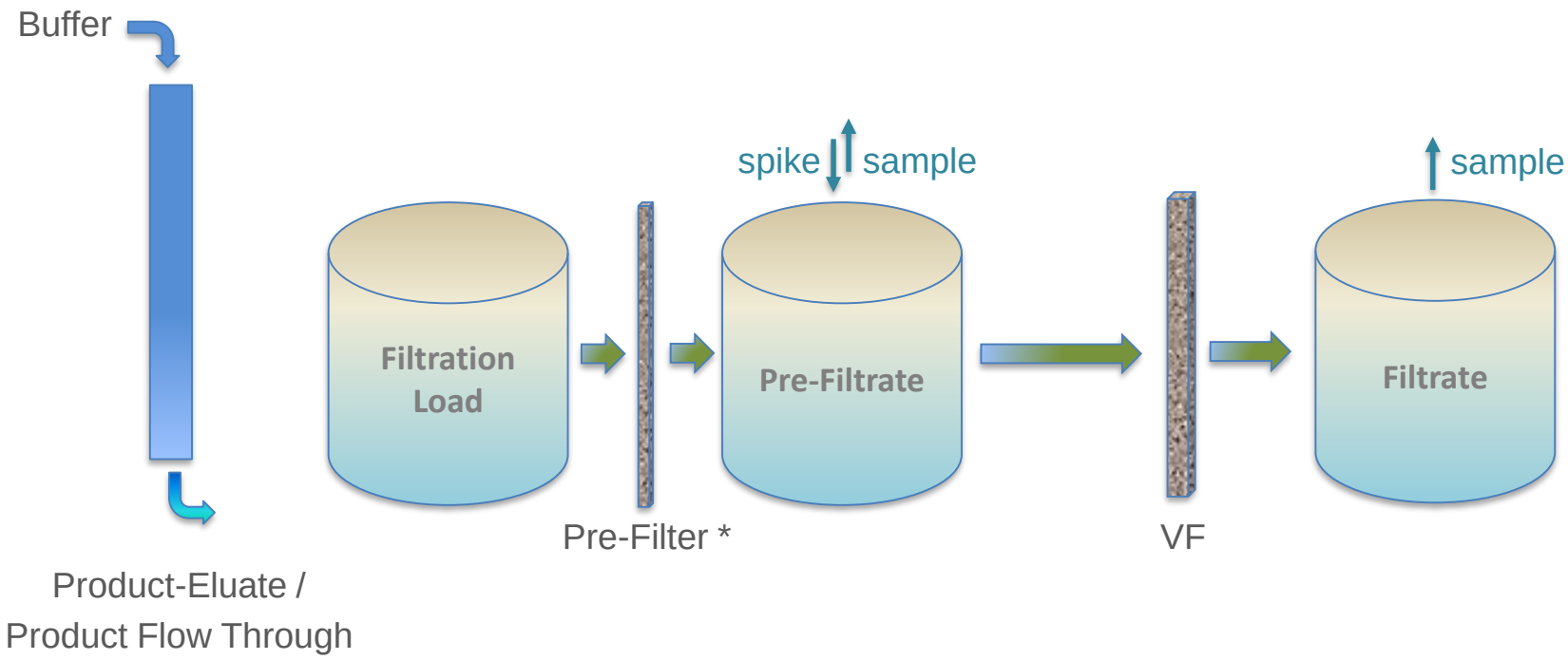


- homogenisation ("pH dampening")
- different inactivation kinetics
- no "single point" sampling
- sampling of representative fraction ("phase")

- constant flow and continuous spiking
- control of residence time ("dwell time")
- tube → laminar flow → non-uniform velocity
- coiled flow inverter (residence time; mixing)

Viral clearance for continuous processes

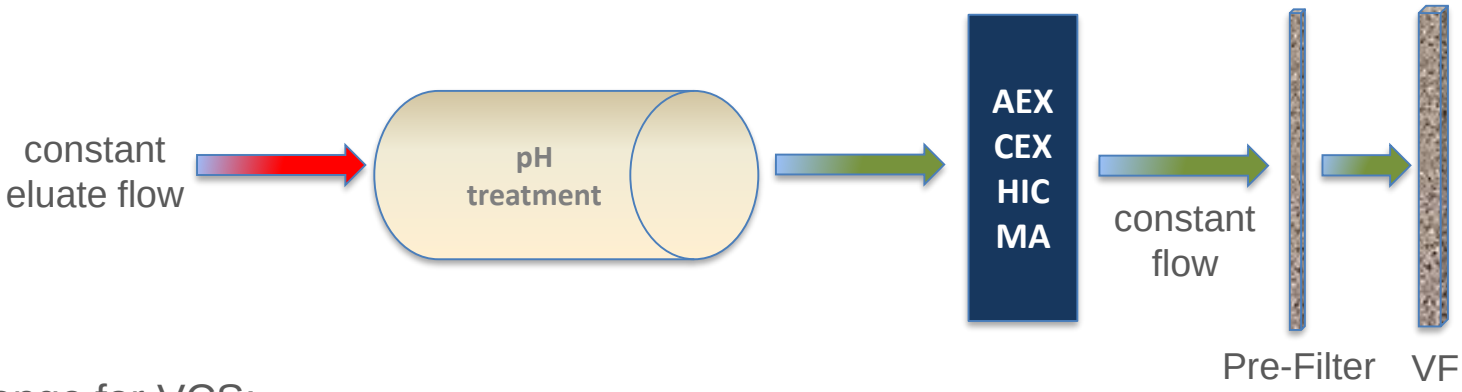
Single column → Hold step → *[XXX]* → "batch" virus filtration
(binding)



*** process-specific pre-filtration**

Viral clearance for continuous processes

Multiple columns → "continuous hold step" → *[XXX]* → "continuous" virus filtration
(binding)



Challenge for VCS:

- batch volume vs. continuous load material "production"
- in-line pre-filtration
- constant flow, BUT very low flow rates and minimal pressure (!)
- extensive process times (days!)
- process (flow) interruptions common

Viral clearance for continuous processes

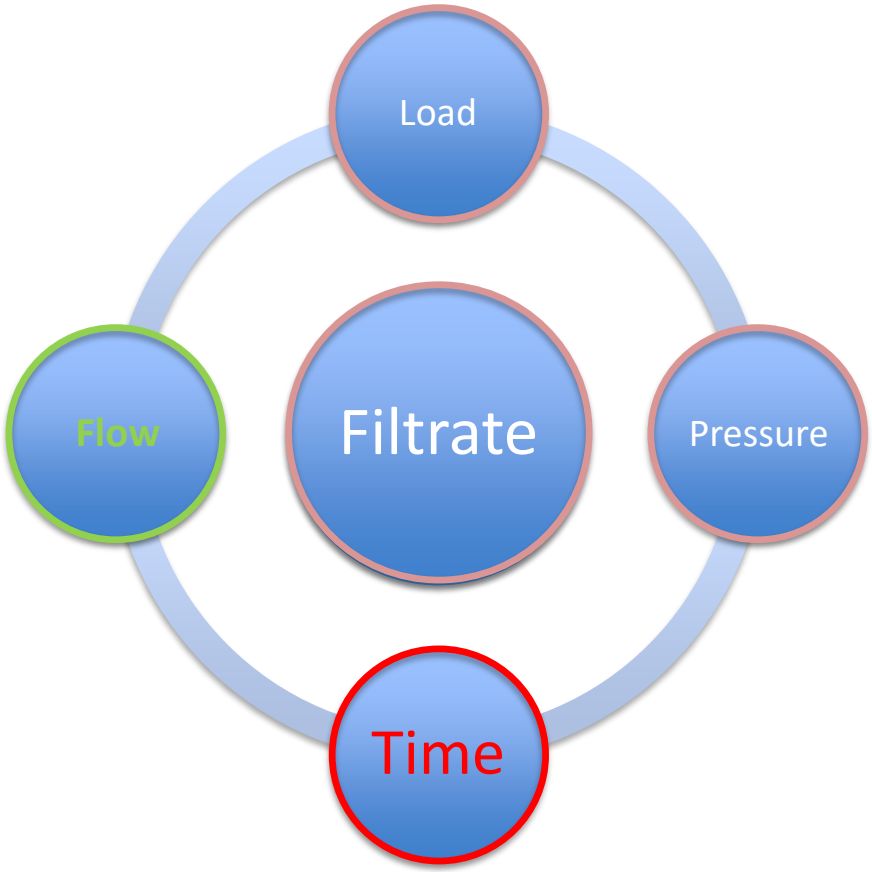
→*batch volume*

(simulate constant flow but not constant production)

- batch spiking ✓
- load / hold sampling ✓
- flow / flow rate ✓
- in-line pre-filtration not* possible **X**

  (*depending on pre-filter and virus size)
- representative load material **?**
- pressure **?**
- filtrability **?**

- possible solution:
  - pre-filtration prior to spiking
  - in-line pre-filtration and in-line spiking



Viral clearance for continuous processes

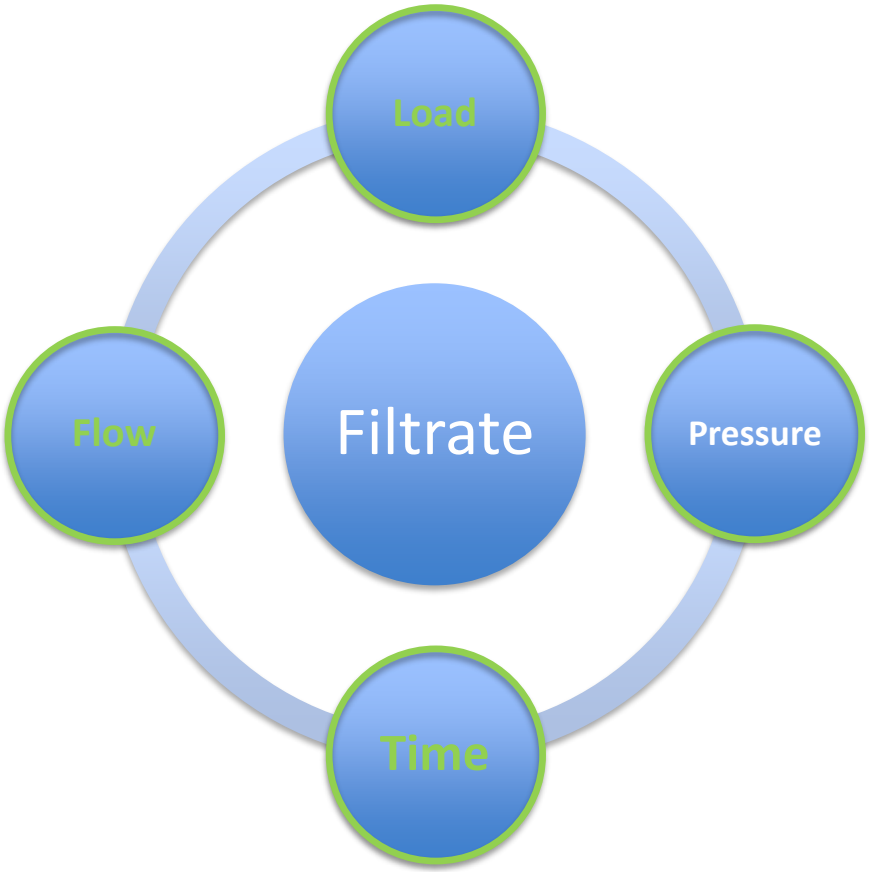
→*continuous load*

- representative load material ✓
- flow / flow rate ✓
- batch spiking **X**
- load / hold sampling **X**
- in-line pre-filtration ✓
- pressure* ✓

  (* depending on virus / test item interaction)
- filtrability* ✓

  (* depending on virus / test item interaction)

- possible solution: in-line spiking



Summary

- for some processes “standardization” is available and general statements possible (e.g. virus filtration with MuLV or low pH treatment under specific conditions)
- but: most VC approaches are as individual as your process (“case by case”)
- there is no one-fits-all and no simple “right & wrong” in the study design
- important: always know what you are doing / testing and especially **why** (!)
- different and “unconventional” approaches maybe accepted by authorities by providing a scientific rational why tests where executed in a specific manner
- non-GLP and R/D data and feasibility studies can be used to justify your VC approach

Thank you very much for your attention

References

1) ICH Harmonized Tripartite Guideline Q5A (R1) 1999: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin

2) WHO Technical Report, Series No. 924, 2004, Annex 4; Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products chapter 4.2.1, page 184

3) Figure extracted and modified from “Coiled flow inverter as an inline mixer” *(Mridha & Nigam; Chemical engineering science; Vol. 63; 2008)*