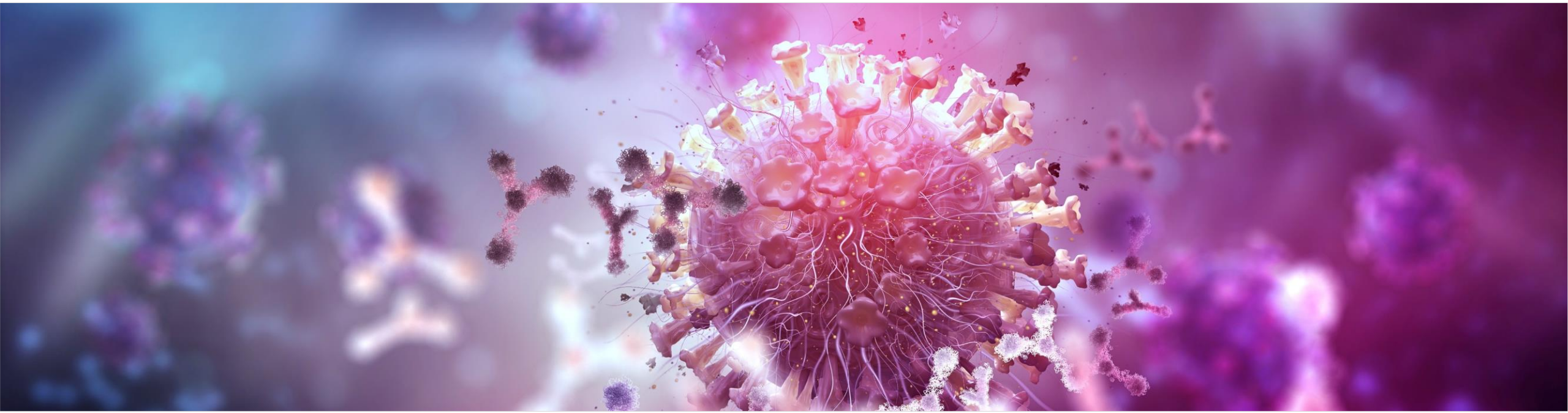

# ViruSure GmbH

## Impact of prior knowledge on viral clearance strategies

08th April 2024

Katy Lorineau, Head of GLP



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# Today’s special


1. What is it?
2. Where is it applicable?
3. How can it be used?

# What is Prior Knowledge?

The notion *Prior knowledge* can already be found in ICH guidelines (e.g. Q2 (analytical procedure validation), Q8 (pharmaceutical development), Q10 (Pharmaceutical Quality System), Q14 (analytical procedure development)…

ICH Q5A (R2) definition:

> *Prior knowledge refers to existing knowledge and includes internal knowledge (e.g., development and manufacturing experience), external knowledge (e.g., scientific and technical publications, including vendors' data, literature, and peer-reviewed publications), or the application of established scientific principles (e.g., chemistry, physics, and engineering principles).*

# Towards flexibility in viral clearance strategies

- Introduction of this new section offers possibility for new viral clearance strategies
- Annex 5 gives examples of process steps for which this concept can be applied
- Goals:
  - Encourage manufacturers to validation platform/in-house data
  - Reduce amount of product-specific virus clearance data
  - Save money
  - Save time
  - Save patients 

# Prior knowledge in ICH Q5A (R2)

Virus detection and identification: sections 3.2.1, 3.2.3

Function and regeneration of columns, section 6.2.6

Replacement of spiked runs without product in mock solution or prior knowledge can be applicable, section 6.2.7

Reduction of number of steps based on previous virus clearance data, section 6.6 and Annex 5

# Function and Regeneration of Columns

*"Over time and after repeated use, the ability of chromatography resins and membranes used in the purification process to clear virus may vary." (section 6.2.6)*

*"Viral clearance studies should be performed to support media/resin reuse."*



**Table 3**
Viral clearance database results for paired naïve and reused Protein A, AEX, and CEX chromatography resins against retrovirus, herpesvirus, reovirus, and parvovirus families.

| | *Protein A* | | | *Anion Exchange* | | | *Cation Exchange* | | |
|---|---|---|---|---|---|---|---|---|---|
| Virus Family | Number of paired records | Naïve resin LRV mean ($log_{10}$) | Used resin LRV mean ($log_{10}$) | Number of paired records | Naïve resin LRV mean ($log_{10}$) | Used resin LRV mean ($log_{10}$) | Number of paired records | Naïve resin LRV mean ($log_{10}$) | Used resin LRV mean ($log_{10}$) |
| Retrovirus | 44 | 2.80 | 3.19 | 39 | 5.19 | 5.26 | 24 | 3.63 | 3.79 |
| Herpesvirus | 23 | 2.70 | 3.22 | 33 | 5.72 | 5.61 | 19 | 5.39 | 5.51 |
| Reovirus | 6 | 1.23 | 1.87 | 26 | 5.66 | 5.76 | 21 | 3.10 | 3.37 |
| Parvovirus | 14 | 2.08 | 2.55 | 35 | 4.64 | 4.75 | 17 | 1.62 | 1.68 |

# Chromatography media: Clearance Studies strategy

PDA Journal
of Pharmaceutical Science and Technology

**Retrospective Evaluation of Cycled Resin in Viral Clearance Studies—A Multiple Company Collaboration**

John Mattila, Sherrie Curtis, Yenny Webb-Vargas, et al.

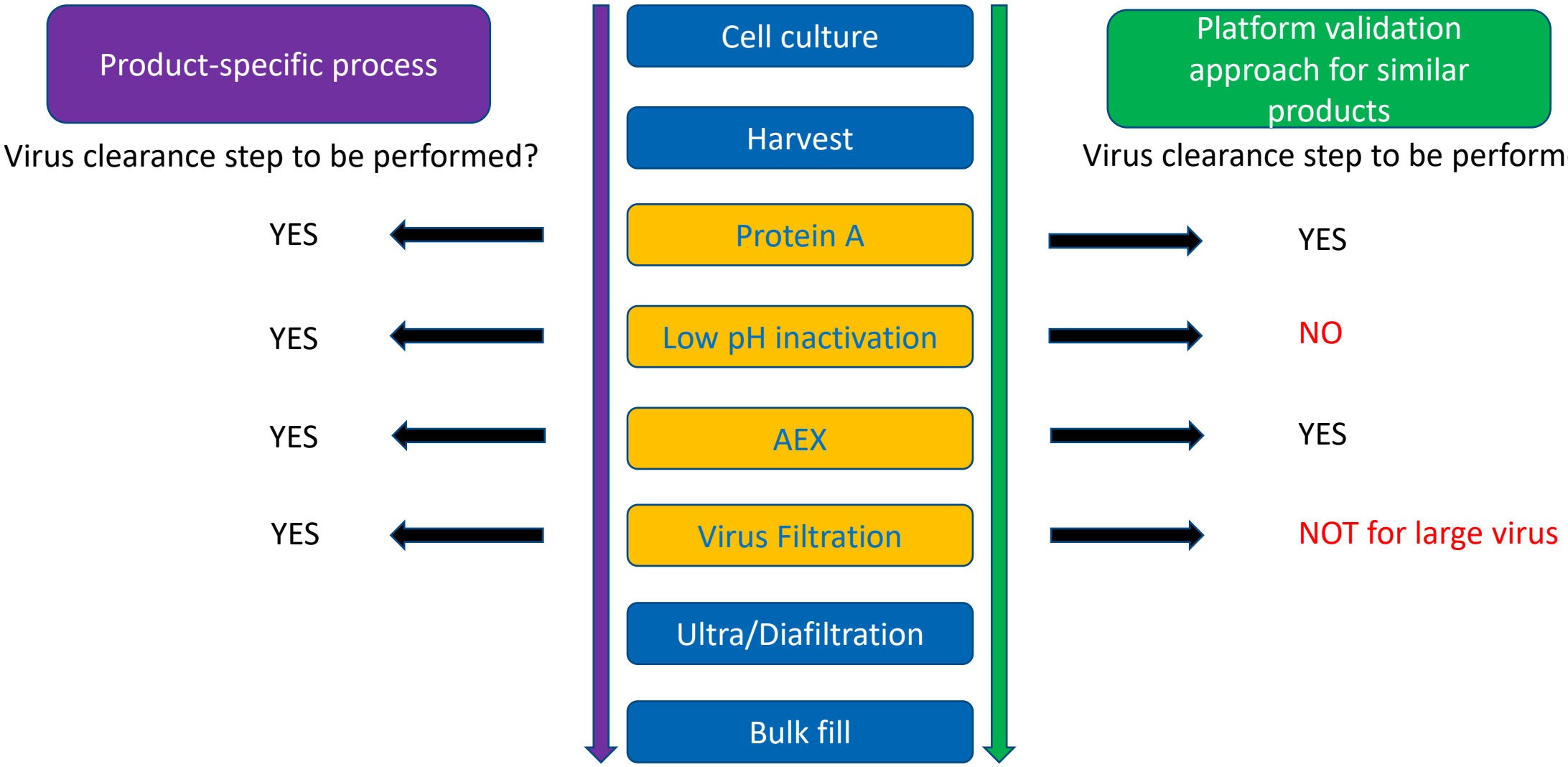
*PDA J Pharm Sci and Tech* **2019**, *73* 470-486
Access the most recent version at doi:10.5731/pdajpst.2018.009605

- Collaborative study for Protein A (97 paired observations) and AEX (144 paired observations) steps
- Evaluation of virus type, resin type, number of reuse cycles and virus challenge
- Clearance capability not impacted by resin cycling

| | | |
|---|---|---|
| Protein A resin | → | Virus removal reported to be highly consistent for a given product<br>Product-specific studies not expected |
| AEX resin | → | Product-specific studies not necessarily necessary<br>In-house experience required and detailed justification |
| CEX resin | → | Product-specific studies not necessarily necessary<br>In-house experience required and detailed justification |

**It must be assured that virus potentially retained by resin/media would be inactivated or removed before reuse.**

# Product-specific process validation vs. platform validation approach



# What to consider to apply prior Knowledge?

1. Inactivation or removal mechanism — Published data

2. Process parameters must be well understood — Worst-case parameters

3. Process intermediates — Comparable composition

4. Product/virus interactions — Impact on viral clearance?

*"The representativeness of the prior knowledge for the specific process step should be well-understood."*

# Annex 5: examples to reduce product-specific validation effort

- **Platform validation** principles require robust viral clearance across products, established and well-characterised process conditions.
- **Platform validation** → New product viral clearance claims based on prior knowledge including in-house experience:
  - Discussion of **all relevant platform data** available
  - **Rationale** to support platform validation approach
  - **Comparison of new product** and its process with other in-house products, process conditions and process intermediates
  - Process steps such as inactivation by detergent treatment, low pH and removal by virus filtration, **dedicated steps for viral clearance**, are suitable candidates for this approach.

  Examples are only suggestions and should not be used as template or as sole basis for regulatory submission.

# Inactivation of X-MuLV by Triton X-100 - Data

TRANSFUSION

BLOOD COMPONENTS

**Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies**

Herbert O. Dichtelmüller, Lothar Biesert, Fabrizio Fabbrizzi, Rodrigo Gajardo, Albrecht Gröner, Ilka von Hoegen, Juan I. Jorquera, Christoph Kempf, Thomas R. Kreil, Dominique Pifat, Wendy Osheroff, Gerhard Poelsler ... See fewer authors

First published: 26 August 2009 | https://doi.org/10.1111/j.1537-2995.2009.02222.x | Citations: 82

308 studies on plasma-derived products

RF between >2.9 and >6.5 log for the Triton X-100–TNBP combination

RF between >4.0 and >6.6 log for the same combination with polysorbate 80 added

| ViruSure Internal Data on X-MuLV inactivation by Triton X-100 or Triton X-100 + TnBP | |
|---|---|
| Number of studies | 10 |
| Triton concentration | 0.3% to 1% |
| Temperature | 14-25°C |
| Inactivation time | 60-120 minutes |
| Reduction factors | ≥ 4.3 log to ≥ 5.7 log |

ViruSure Data: 2010 to 2022

# X-MuLV inactivation by Triton X-100 Treatment as Example 1

| Parameters | Interaction virus/product | Mechanism | Product intermediates |
|---|---|---|---|
| Concentration | Aggregates could form from cell debris or virus aggregates and get entrapped | Lipid bilayers solubilisation | Non-ionic nature of Triton, no impact from pH, ionic strength |
| Detergent or SD | Harvested cell culture fluid is clarified via filtration step | Solubilisation of lipid envelope of virus leading to virus inactivation | Robust steps on multiple products |
| Impurities | | | |
| Cell culture media components | | | |

# S/D or Detergent treatment parameters and consensus

**Parameters with High potential Effects:**

Agent, concentration, incubation time, temperature, pre-treatment by ≤ 0.2 µm

**Parameters with Low potential Effects:**

lipid content, type of product, total protein content, pH, ionic strength, buffer salt in HCCF, interaction virus & product

Triton X-100 with clarified HCCF at a concentration of ≥ 0.5% for ≥ 60 minutes at ≥ 15°C effectively inactivates X-MuLV

Or

1% Triton X-100 and 0.3% TnBP for ≥ 30 minutes

Or

1% Polysorbate and 0.3% TnBP for ≥ 6 hours at ≥ 23°C effectively

# X-MuLV inactivation by Low pH – Published data

AsahiKASEI BIOPROCESS

**Bracketed Generic Inactivation of Rodent Retroviruses by Low pH Treatment for Monoclonal Antibodies and Recombinant Proteins**

Kurt Brorson,[1] Sherrie Krejci,[2] Kitty Lee,[2] Elizabeth Hamilton,[1] Kathryn Stein,[1]* Yuan Xu[2]

[1]Division of Monoclonal Antibodies, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, Maryland 20892
[2]Genentech, Inc., 1 DNA Way, South San Francisco, California 94080; e-mail: xu.yuan@gene.com

Received 17 July 2002; accepted 3 October 2002

- Low pH is a common step after Prot A for mAb purification
- Bracketed generic clearance: study defined the range of selected parameters which deliver consistently RF ≥ 4.6 log
- Performed on 1 product but can apply to others as long as key parameters are held constant
- The bracketing concept raised the possibility to define acceptable range which could be applied to all mAbs and Recombinant Proteins.

DOI: 10.1002/bit.28379 • Corpus ID: 257583099

**Characterization of ionic strength for X-MuLV inactivation by low pH treatment for monoclonal antibody purification**

Jena Daya, Valerie Cusick, J. Mattila • Published in Biotechnology and... 15 March 2023 • Medicine, Chemistry

TLDR Overall, robust and effective inactivation of X-MuLV at pH 3.65–3.80 can be achieved by manipulating either the pH or the NaCl concentration of theload material, and pH has a large effect when the load material has no additional NaCl. Expand

- pH is ≤3.8.
- Incubation time is ≥30 min.
- The incubation temperature is ≥14°C.
- The buffer system is acetate or citrate.
- Total protein concentration is ≤40 mg/mL.
- NaCl concentration is ≤500 m*M*.
- The p*I* of the mAb or RP is between approximately 3 and 9.

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# ViruSure internal data: X-MuLV inactivation by Low pH

| ViruSure Internal Data on X-MuLV inactivation by Low pH | |
|---|---|
| Number of studies | 33 (out of 52) |
| pH range | 3.2 – 3.85 |
| Temperature | 10-20°C |
| Inactivation time | 30-150 minutes |
| Reduction factors | ≥ 4.3 log to ≥ 6.5 log |

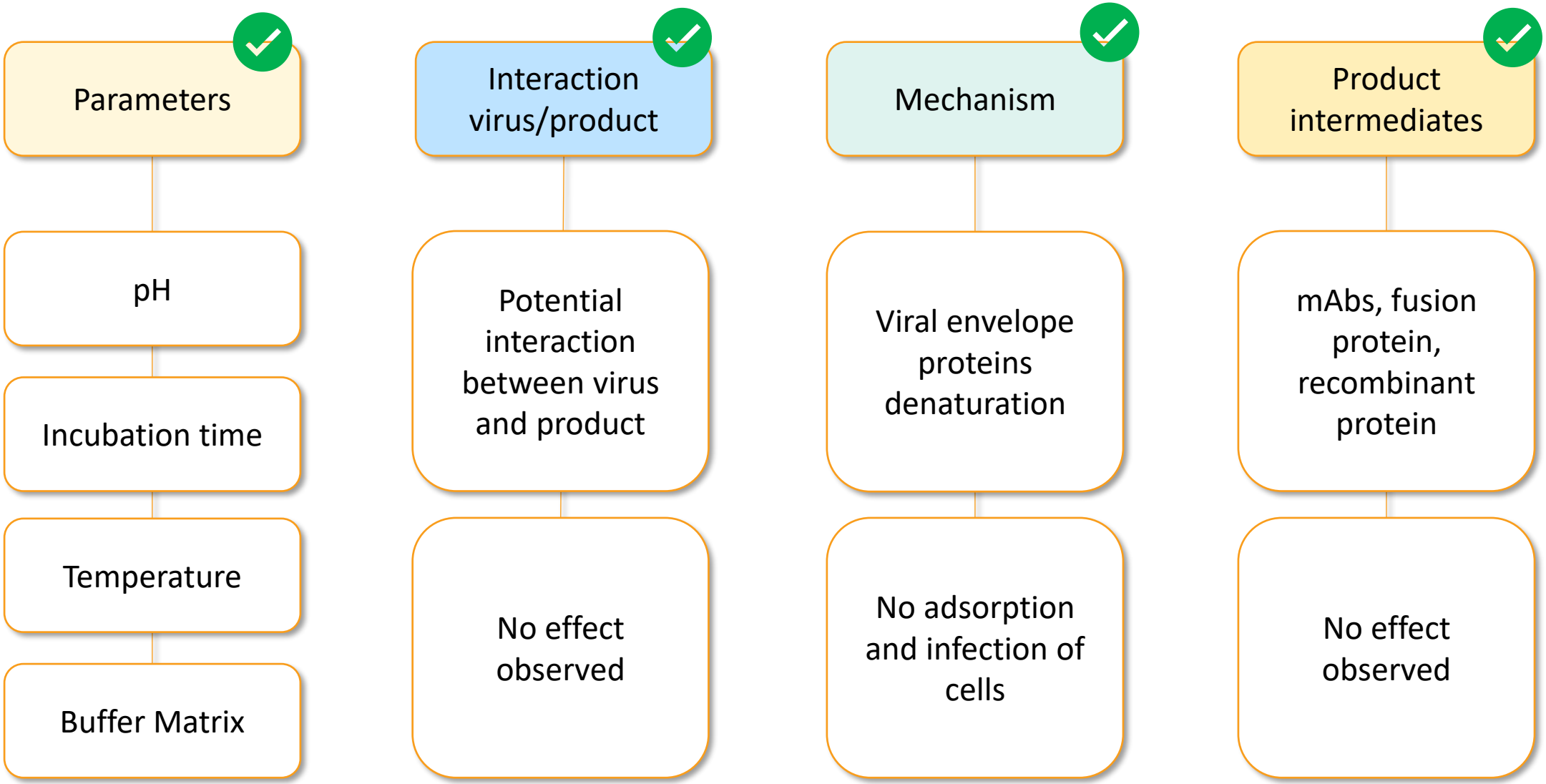
ViruSure database: 2008 to 2023

Note:

19 studies with residual infectivity:

- pH too high (n= 13)
- Where < pH 3.6 (n= 6), very few positive wells detected in large volume plating testing, however RF remains high.

# Low pH Treatment on X-MuLV as Example 2



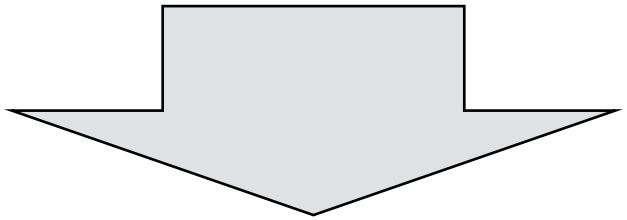
# Low pH treatment parameters and consensus

**Parameters with High potential Effects:**

pH, incubation time, temperature, buffer matrix

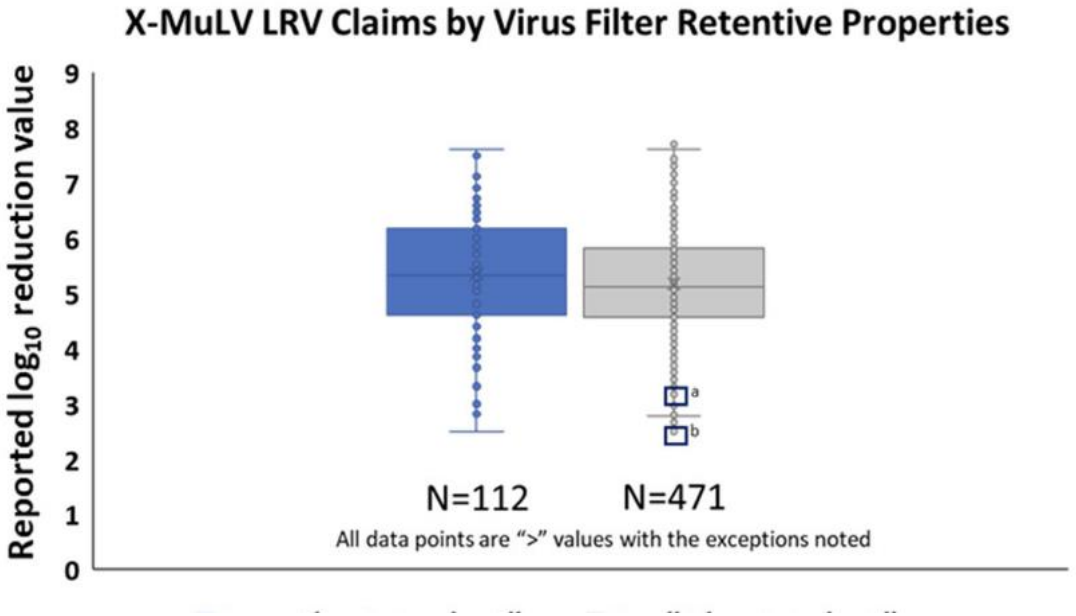
**Parameters with Low potential Effects:**

Product concentration, formation of protein aggregates, type of product, NaCl concentration (if less than 500 mmol/L)



Low pH at ≤ pH 3.6, ≥ 15°C for ≥ 30 min at ≤ 500 mmol/L NaCl
Acetate buffer mostly used

# External knowledge on virus filtration

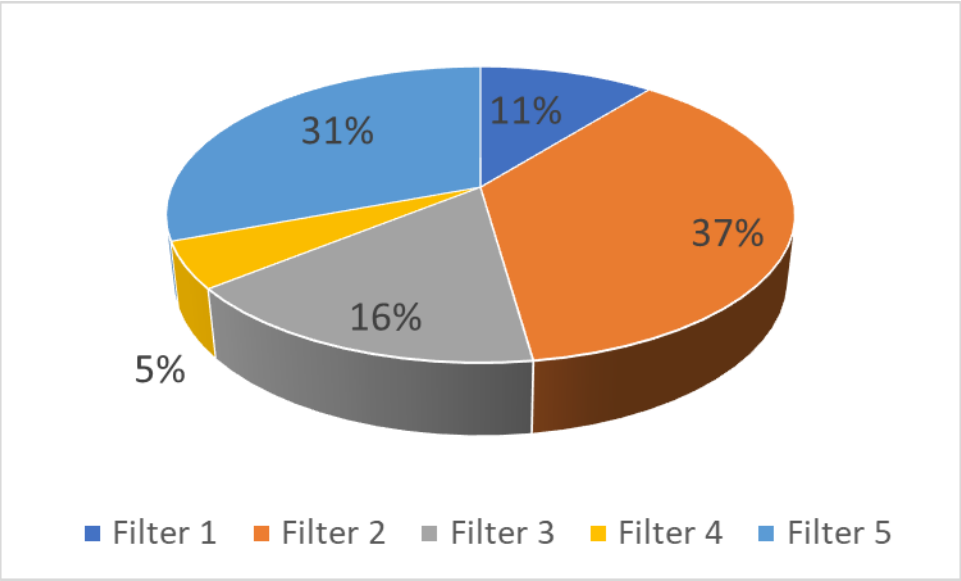



Biotechnol Bioeng. 2022;119:743–761; Sarah A. Johnson

- FDA meta-analysis performed on the viral clearance data from monoclonal antibody (mAb) regulatory submissions from 1990 to 2015
- In all 112 large virus filter studies: complete removal
- In 469/471 studies with small virus filters: complete removal
- Detected breakthrough: no clear explanation if filter-related or study-related

# ViruSure data: internal knowledge

**Virus Filters Tested***

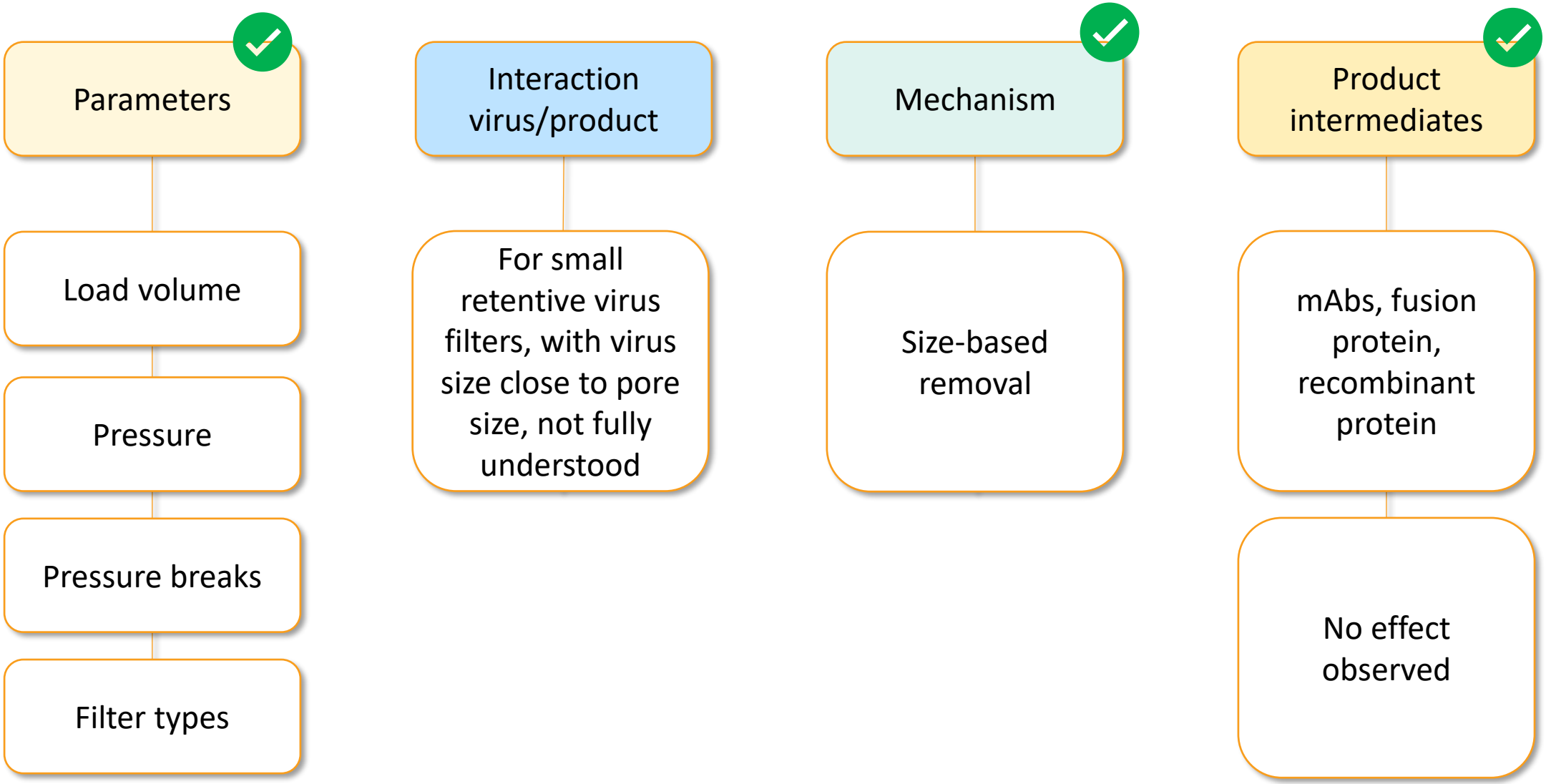



* Based on data generated at ViruSure for studies between 2005 and 2018 ( 494 experiments)

| Filter Type | Total runs | Total runs with Residual infectivity | |
|---|---|---|---|
| | | PPV/MMV | Others (> size) |
| Filter 1 | 53 | 18 | 0 |
| Filter 2 | 183 | 22 | 2 |
| Filter 3 | 81 | 1 | 1 |
| Filter 4 | 25 | 2 | 0 |
| Filter 5 | 152 | 6 | 2 |

**Filter 2:** slight breakthrough for BVDV (1 pos well/(104)); 1 filter failure
**Filter 3:** slight breakthrough with X-MuLV (1 pos well/(104))
**Filter 5:** slight breakthrough with X-MuLV (1 pos well/(104) & 2(968) pos wells)

# Virus Filtration: claim for large virus clearance as Example 3



# Virus Filtration parameters (using small virus retentive filters) and consensus

**Parameters with High potential Effects:**

Volumetric throughput of load material, post-wash, pressure/flow

**Parameters with Low potential Effects:**

Type of product, product concentration, pH, ionic strength, buffer matrix

**Option 1:** apply parvovirus RF to large viruses and envelopped viruses
BUT: underestimate virus removal in case of parvovirus passage (virus breakthrough)
**Option 2:** in-house data from both parvovirus and large viruses can build a plateform large viral clearance claim for commonly used small virus retentive filters

Thorough understanding of parameters with high effect should be in place.

If prior knowledge and in-house experience from other products used **to claim parvovirus removal**, at least one product-specific validation run (one confirmatory run) using parvovirus should be performed using worst-case conditions.

# Conclusions

- ICH Q5A (R2) emphasizes the use of prior knowledge
- Possibility to decrease the number of validation runs to be performed
- In-house data must be demonstrated
- Annex 5 gives examples on how some steps can be used from a platform validation approach
- Other steps can follow this process assuming good external (large industry experience) and internal/in-house knowledge (data from similar products)

# Thank you to the GLP team ☺

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감사합니다 Gracias Danke

Благодаря 谢谢 Tack धन्यवाद Dziękuję

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Děkuju Grazie Ευχαριστώ Merci Köszönöm

ありがとうございました Teşekkür ederim