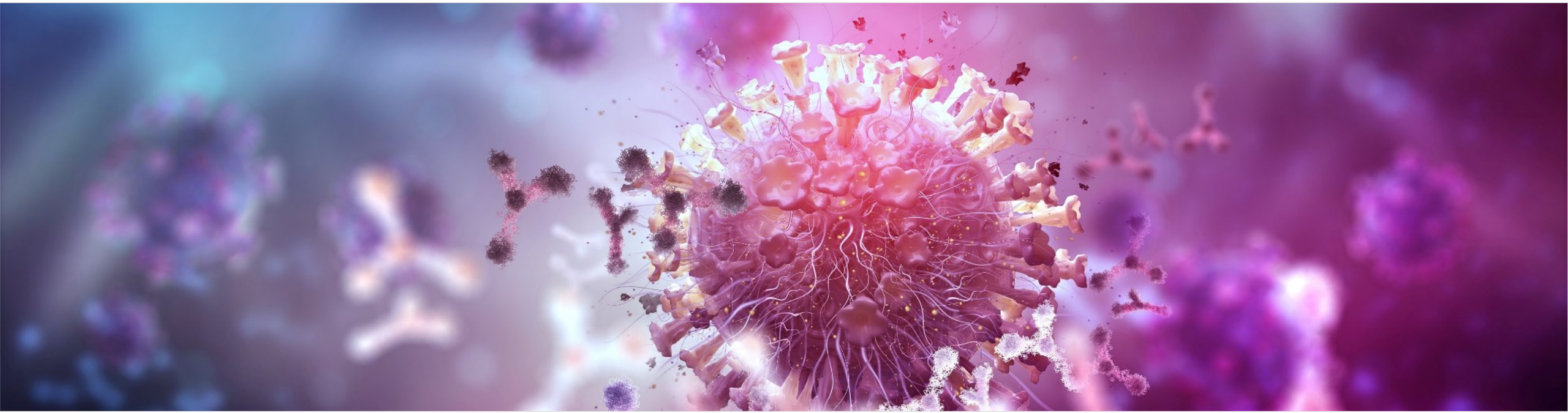


Virus Clearance Studies at ViruSure:
Basic Principles and New Approaches

ViruSure Workshop, Vienna, September 2022

Katy Lorineau, Head of GLP group

1

The Basic Principles of Virus Validation Studies

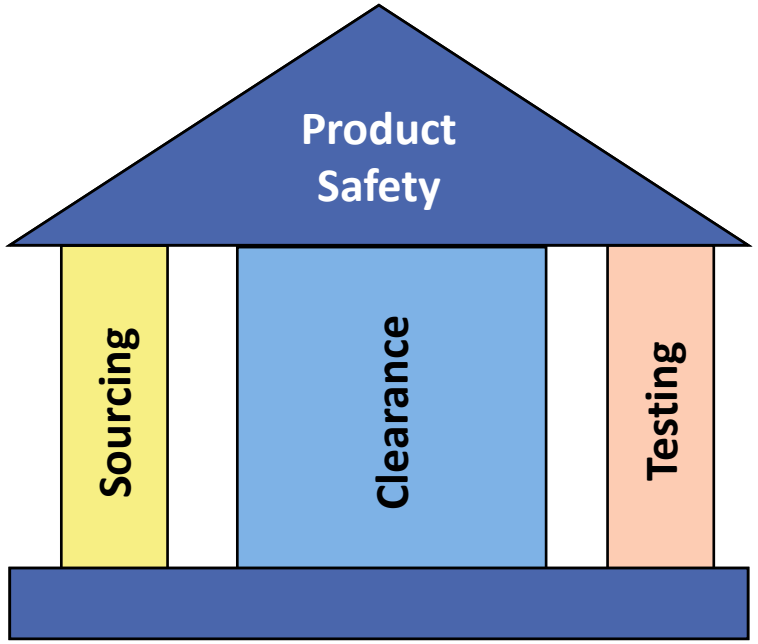
Sourcing, testing & virus clearance contribute to the patient safety

Sourcing
Based on plasma experience maximum

2 logs

Testing
Based on limits of sensitivity, a virus load in the order of 2-3 logs could still be present (Waytes et. al. *Dev Biol Stand* 2000;102:37-51)

2-3 logs



Clearance: Virus inactivation or removal

e.g. > 5 logs inactivation per step analysed

In comparison to sourcing & testing, the level of risk reduction through virus clearance is significantly greater!

What define virus clearance studies?

GUIDELINES

- CPMP/BWP/268/95 (Note for Guidance on Virus Validation Studies
- ICH Q5A (CPMP/ICH/295/95)
- EMEA/CHMP/BWP/398498/2005

GLP GUIDELINES

- Quality system
- Data integrity
- GDP
- Audits
- Deviation procedure

STUDY DIRECTOR

- Study responsible
- Study design and study plan
- Experienced team
- Direct communication to Sponsor
- Data analysis
- Final report

SPONSOR

- Validated Downscaled process
- Process Parameters
- Tech Transfer
- Provision of GMP batch samples for pre-study and runs

ASSAYS:

- Validated TCID50 assays
- Virus stocks
- Large panel of viruses
- Operators Training

BSL2 (BSL 3 Facility in 2023)

- Qualified Equipment
- Validated CS
- Segregation concept

Virus inactivation steps

Blood products

- Solvent detergent
- Lyophilisation/Dry Heat
- Pasteurisation
- Methylene Blue/Light
- ß-Propriolactone/UV
- Octanoic Acid

Recombinant products

- **High/low pH**
- **Solvent detergent**
- **Lyophilisation/Dry Heat**
- **Heat**
- **Pasteurisation**
- **ß-Propriolactone/UV**
- **Octanoic Acid**

Others

- Gamma Irradiation
- Formaldehyde
- Glutaraldehyde
- Guanidine Hydrochloride
- Oxidative Treatment H_2O_2
- Organic solvents
- UV
- Urea

Virus removal steps

Chromatography

- Anion Exchange Column
- Cation Exchange Column
- Affinity column
- Hydrophobic Interaction
- Mixed Mode

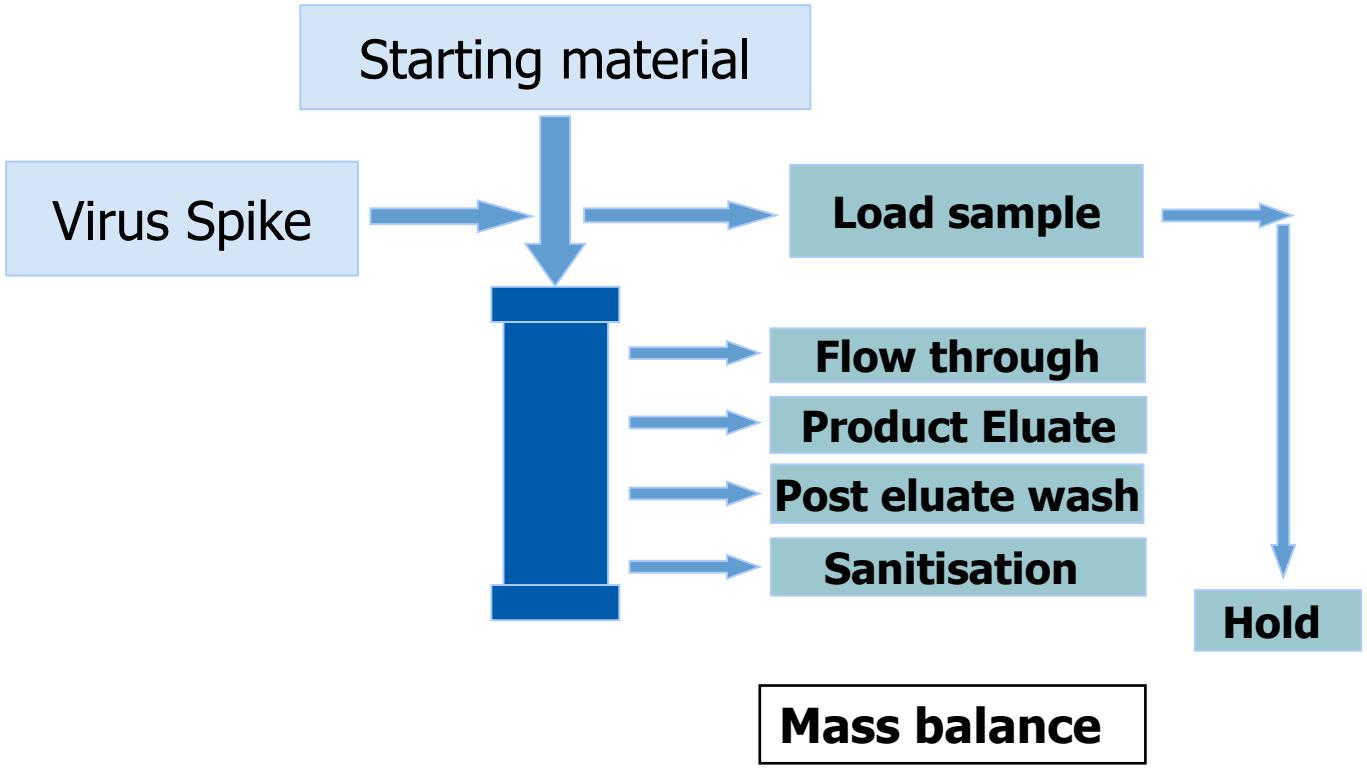
Precipitation

- **Ammonium sulphate**
- **Ethanol**
- **Glycine**
- **Acrinol**

Filtration

- Virus Removal filters
- Membrane Absorber Filter
- Depth filtration

Chromatography process flow chart



- Infectivity assays on collected samples to determine log reduction values (LRV)
- Duplicate runs
- Not all viruses behave the same!
- Side fractions tested for mass balance analysis
- RF determination using Total virus load SSM vs Eluate

2

Potential changes in the next ICH Q5A revision

ICH Q5A: viral safety evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

- Timelines: Nov. 2023 Regulatory consultation/discussion and adoption, presented by Johannes Blumel (PDA virus safety, June 2022)
- Revised topics:
  - New classes of biotechnology products
  - Introduction of NAT
  - New clearance validation approaches
  - Changes in clearance studies
    - Nanofiltration & parvoviruses
    - Study of resin lifetime

Small non-enveloped model viruses are worst case for retrovirus removal

- Parvovirus (20-25 nm) is the better worst-case model for **size exclusion**-based filtration compared to Retrovirus (100 nm)
- Reduction Factors from Parvovirus is more reflective of true filter performance.
- PDA Technical report (section 6.4.2)

Current Research in Biotechnology 4 (2022) 190–202

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An updated analysis of viral clearance unit operations for biotechnology manufacturing ☆

Opeyemi O. Ajayi [a], Sarah A. Johnson [a], Talia Faison [a], Nicole Azer [a,c], Jackie L. Cullinan [a], Jessica Dement-Brown [b], Scott C. Lute [a,*]

WILEY TRANSFUSION

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Transfusion. 2020 Nov; 60(11): 2661–2674. PMCID: PMC7754444

Published online 2020 Aug 19. doi: 10.1111/trf.16022 PMID: 32815181

Nanofiltration as a robust method contributing to viral safety of plasma-derived therapeutics: 20 years' experience of the plasma protein manufacturers

Nathan J. Roth, [1] Herbert O. Dichtelmüller, [2,†] Fabrizio Fabbrizzi, [3,†] Eckhard Flechsig, [2] Albrecht Gröner, [4] Mary Gustafson, [5] Juan I. Jorquera, [6] Thomas R. Kreil, [7] Dominika Misztela, [8] Elisa Moretti, [3] Mila Moscardini, [3] Gerhard Poelsler, [2] John More, [9] Peter Roberts, [9] Andreas Wieser, [7] and Rodrigo Gajardo [6]

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REVIEW

BIOTECHNOLOGY BIOENGINEERING WILEY

Virus filtration: A review of current and future practices in bioprocessing

Sarah A. Johnson[1] | Shuang Chen[2] | Glen Bolton[3] | Qi Chen[4] | Scott Lute[1] | John Fisher[4] | Kurt Brorson[5]

Robust and complete clearance of X-MuLV by filtration step

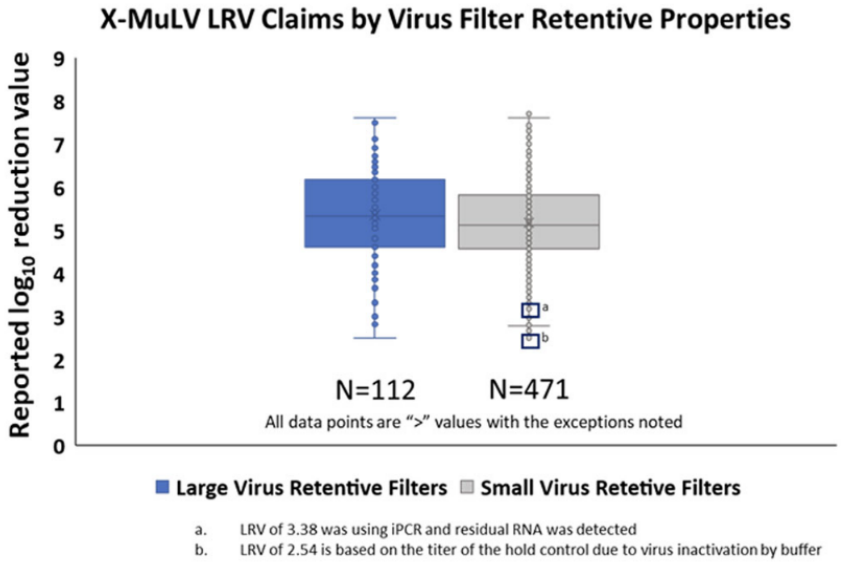


FIGURE 1 Xenotropic murine leukemia virus (X-MuLV) log reduction value (LRV) claims by virus filter retentive properties in FDA submissions (1990–2015) demonstrated robust and complete clearance for larger viruses in all 112 large virus retentive filter records and 469 out of 471 records related to small virus retentive filters. Two instances of reported noncomplete X-MuLV clearance values with small virus retentive filters were determined to be study related

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
- 2 detected breakthrough: no clear explanation if filter-related or study-related

1st and 2nd generation of virus filters are effective for removal of large viruses

- Cumulative data from BLA submissions from 1995 through January 2021:

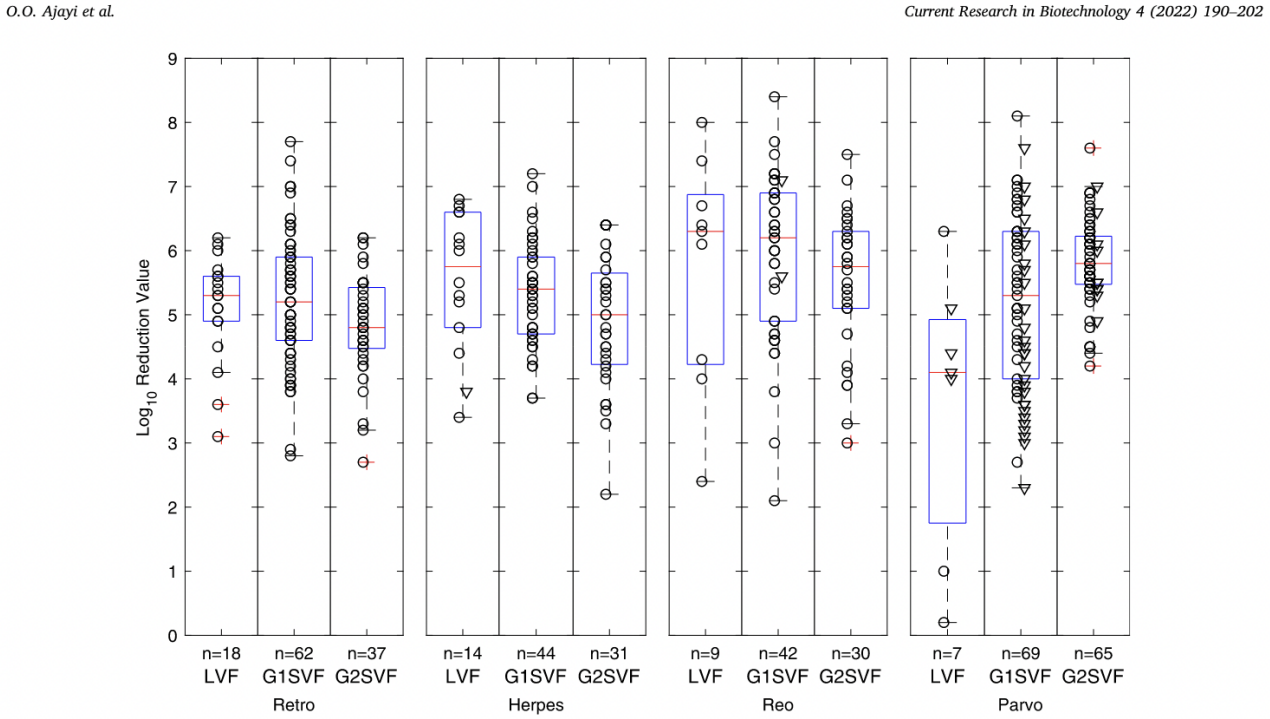


Fig. 4. Log_{10} Reduction Value (LRV) distribution for database virus retentive filtration records of retrovirus, herpesvirus, reovirus and parvovirus that were assessed via an infectivity assay. Each marker represents individual records. LVF = large virus filters, G1SVF = 1st generation small virus filters, G2SVF = 2nd generation small virus filters. Box plot shows the lower and upper range (bottom and top bars), distribution median (red line) and distribution outliers (+). n = total number of records assessed. **LEGEND**: ○– records indicating viral content in post-process pool below assay LOD, ▽ – records indicating infectious virus detected in post-process pool. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

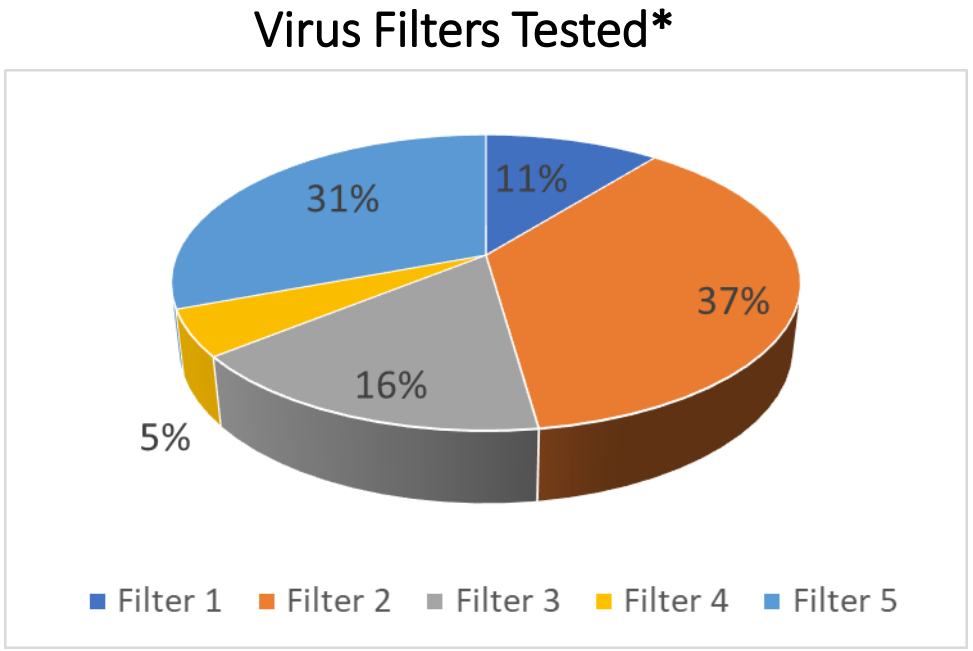
Filters	Type	Number of records
Large virus filters	Ultipor® DV50, Viresolve® NFR	61
G1 small virus filters	Planova® 15 N and 20 N, Viresolve® NFP, Virosart® CPV, Ultipor® DV20	266
G2 small virus filters	Planova® BioEx, Viresolve® Pro	178

One record reported the detection of Herpesvirus (no root cause identified)

Two records reports the detection of Reovirus (no root cause identified)

Data from: Updated analysis of viral clearance unit operations for biotechnology manufacturing; O.O. Ajayi et al. Current Research in Biotechnology 4 (2022) 190–202

Nanofiltration runs at ViruSure show consistently complete removal of large viruses



* 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; RF is > 4.8 log

Filter 3: slight breakthrough with X-MuLV (1 pos well/(104)); RF is > 5.5 log

Filter 5: slight breakthrough with X-MuLV (1 pos well/(104) & 2(968) pos wells); RF is > 5 log

If Parvovirus RF can be used for larger viruses…

- **Advantages:**
  - Less runs, Less Time, Less cost!
  - Higher Reduction Factors due to higher Parvovirus Titre
  - Less problems with overlying inactivation (e.g. detergents, low/high pH) – no virus envelope affected by test matrix
- **Disadvantages:**
  - In case of virus breakthrough with Parvovirus, no robust filtration data for Retrovirus due to a low RF – critical for risk evaluation, for total VRF.

Q5A(R1) recommendation on columns re-use

6.2.6 Function and regeneration of columns

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.
Assurance should be provided that any virus potentially retained by the production system would 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.

Actual recommendations for testing of column re-use

- Column Resin at maximal cycling time should be compared with New Column Resin.
- Old column resin from large scale manufacturing with real maximal life span or “artificially aged” in a downscale version by performing automated recycling runs (e.g. using the ÄKTA)
- Old and new resin compared in single runs, as long as the removal capacity is in the same range.
- Test of old resin can be done at a later stage, when material at maximal lifetime from large scale production is available
- Carry-over studies for regeneration of resin

FDA database on Resin aging studies

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

RESIN TYPE	Max lifetime	Cycle number vs Records number	
Protein A	301	> 100 (58%)	> 200 (15%)
AEX	300	> 100 (47%)	> 200 (0.1 %)
CEX	300	> 100 (50%)	> 200 (0.1 %)

Studies show that Virus removal is comparable between “new” and “old” Protein A resins

- **Old and new column resin performance for virus removal is comparable.**
  - Extensive FDA Database confirms no difference with between removal with old and new Protein A Resin: “Discontinuation of need for aged Protein A resin studies.“
- **No virus is left on the resin, which might accumulate and elutes in the product fraction of following runs.**
  - Check via carry-over Studies
  - Perform Sanitization Kinetic Studies (with and without resin)

Resin aging studies potentially no longer recommended in ICH Q5A(R2)

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- Walter Tabotta (HTS)

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- Quality team
- Equipment team
- R&D team
- PCR team
- Animal Facility
- GMP team