Evaluation of Viral Clearance for Monoclonal Antibody Chromatographic Process Steps

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Introduction

- Impurities such as viruses are typically present during the manufacturing of biological drugs. The viruses may be present in the unprocessed material from the upstream collected harvest, such as released from cell culture media (endogenous) or accidentally introduced during processing (adventitious).
- Viral clearance is required by the FDA and international regulatory bodies as specified in the ICH Q5A documents.
- In this study we evaluated two chromatography steps in the purification of a monoclonal antibody (mAb) for viral clearance. Evaluation was performed with four model viruses commonly used for the testing of mAb products. The purification scheme for this antibody is shown below, and tested steps are noted in red:

- Studies were performed as spike/chase experiments, where a known quantity of virus is added to unprocessed material, and remaining virus is quantitated following processing. A schematic for chromatography step testing is shown in the Methods – General section.

Materials and Methods

Chromatography resins were chosen according to those commonly used in mAb purification platforms:

- TOYOPEARL® AF-rProtein A HC-650F gives a high (> 70 g/L-resin) dynamic binding capacity.
- TOYOPEARL Sulfate-650F is a salt-tolerant cation exchange resin (CEX) with high dynamic binding capacity (> 120 g/L-resin) that has been shown to be an effective aggregate, host-cell protein, and protein A removal step.
- TOYOPEARL NH_2-750F is a novel salt-tolerant anion exchange resin (AEX) that is an effective host-cell protein and DNA removal step.

Table 1

Resin	Type	Bead diameter	Pore size	Ligand
TOYOPEARL AF-rProtein A HC-650F	Protein A affinity	45 µm	100 nm	Protein A modified C-domain hexamer
TOYOPEARL Sulfate-650F	Cation exchange	45 µm	100 nm	Sulfate ($R—SO_4^-$)
TOYOPEARL NH_2-750F	Anion exchange	45 µm	100 nm	Polyamine $(R—NH_2^+)_n$

Materials and Methods

Four model viruses were chosen that are commonly used for the testing of mAb products:

Table 2

Virus	Strain	Family	Genome	Envelope	Size (nm)	Physio-Chemical Resistance	Indicator Cell Line
Murine Leukemia Virus (MuLV)	TEK-1	Retorviridae	ssRNA-RT	Yes	80 - 100	Low	PG-4
Pseudorabies Virus (PRV)	Aujeszky	Herpesviridae	dsDNA	Yes	120 - 200	Medium	Vero 76
Reovirus type 3 (Reo)	Abney	Reoviridae	dsRNA	No	60 - 80	Medium	LLC-MK2
Minute Virus of Mice (MVM)	Prototype (p)	Parvoviridae	ssDNA	No	20 - 26	High	324k

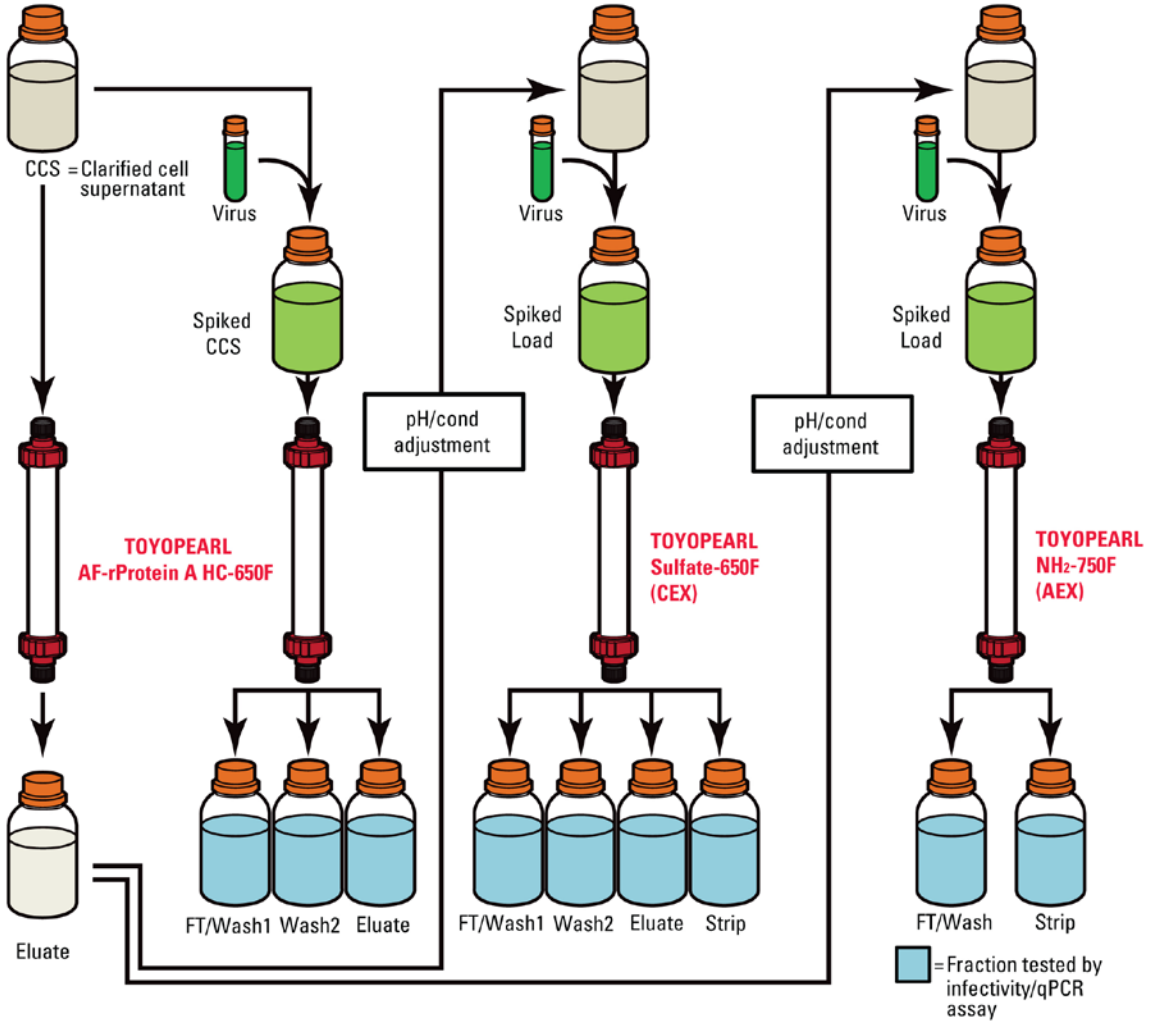
Materials

- Omnifit® Benchmark columns, 6.6 mm ID × 25 cm
- Tris base, ACS grade
- Acetic acid, glacial
- Sodium chloride, ACS grade
- Sodium hydroxide, ACS grade
- Deionized water
- HEPES free acid (ACS grade)
- Bis-Tris base (ACS grade)
- TBL-mAb-01, humanized IgG_1 clarified cell supernatant (CHO-expressed), pI 8.2
- ÄKTA® Explorer 100 with Unicorn® v 5.2 software

Methods - General

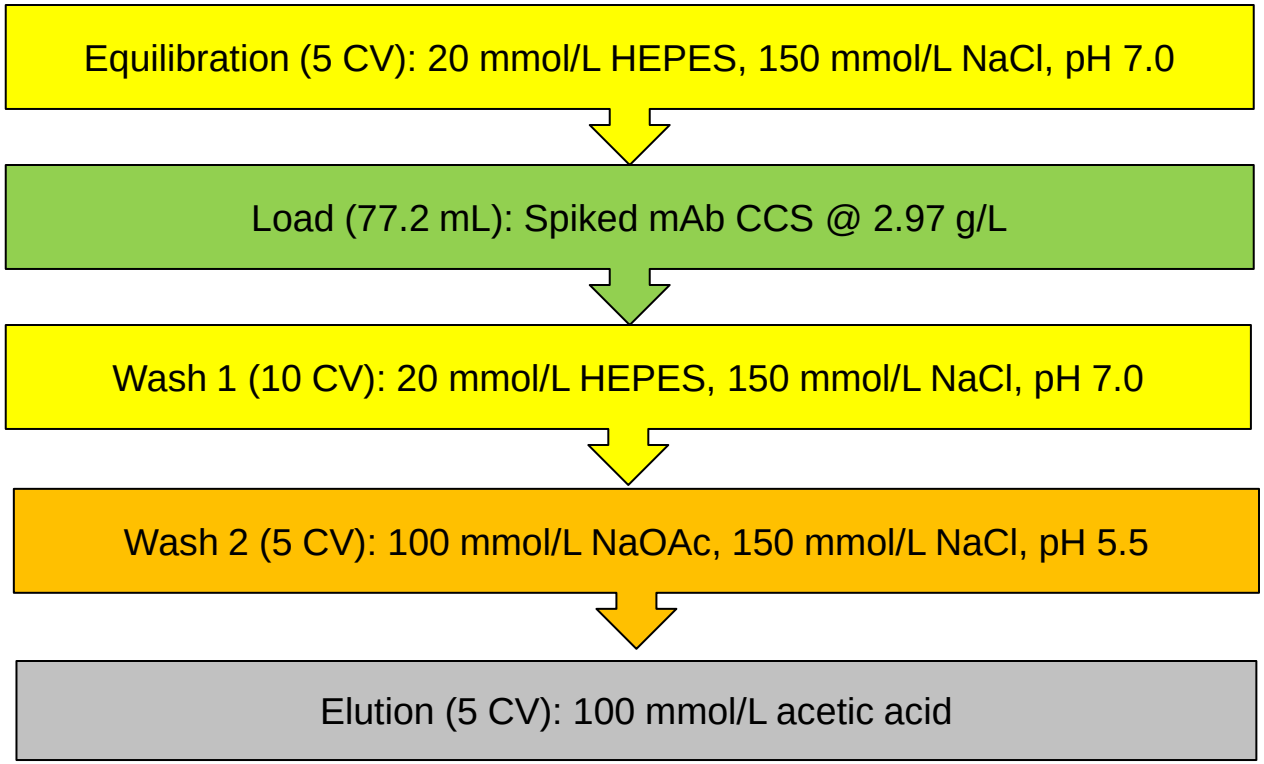
- Chromatography steps were evaluated by spiking feed stock material with 1- 5% (v/v) of virus stock followed by processing.
- Fractions from each run were collected and analyzed as described in the results section for each step.
- All runs were performed with 15 cm bed height columns, and run at 300 cm/hr (3 min residence time).
- Viral assays were performed by Charles River Laboratories according to established procedures.
  - For qPCR assays, the number of viral equivalent (VE) genomes was determined for each sample.
  - For infectivity assays, the 50% tissue culture infectious dose ($TCID_{50}$) was determined for each sample using the indicator cell line shown in Table 2.

Viral Clearance Testing Schematic



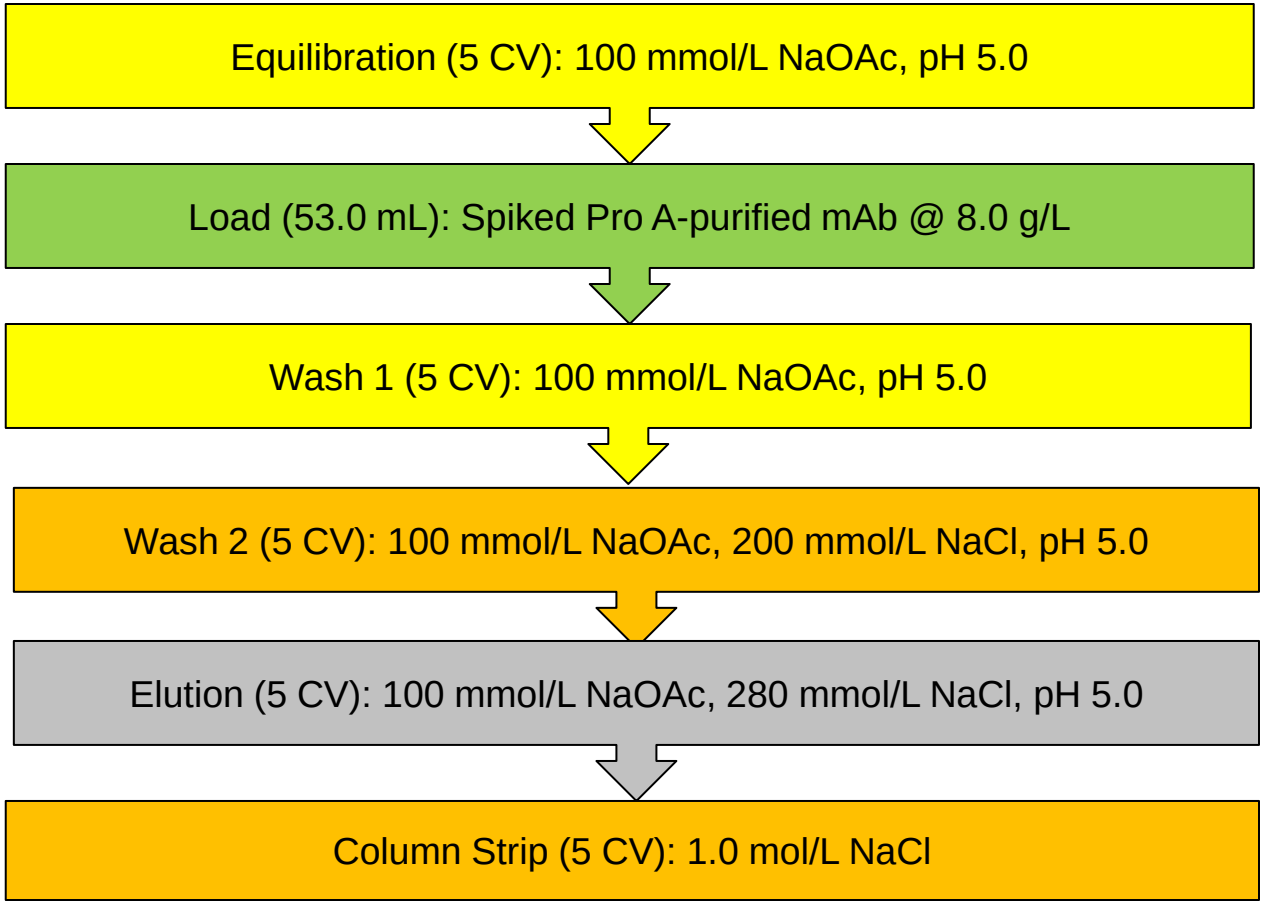
Purification of mAb with Protein A Resin

Clarified cell supernatant (CCS) containing 3.0 g/L mAb was spiked with 1% (v/v) virus stock and processed according to the following method:



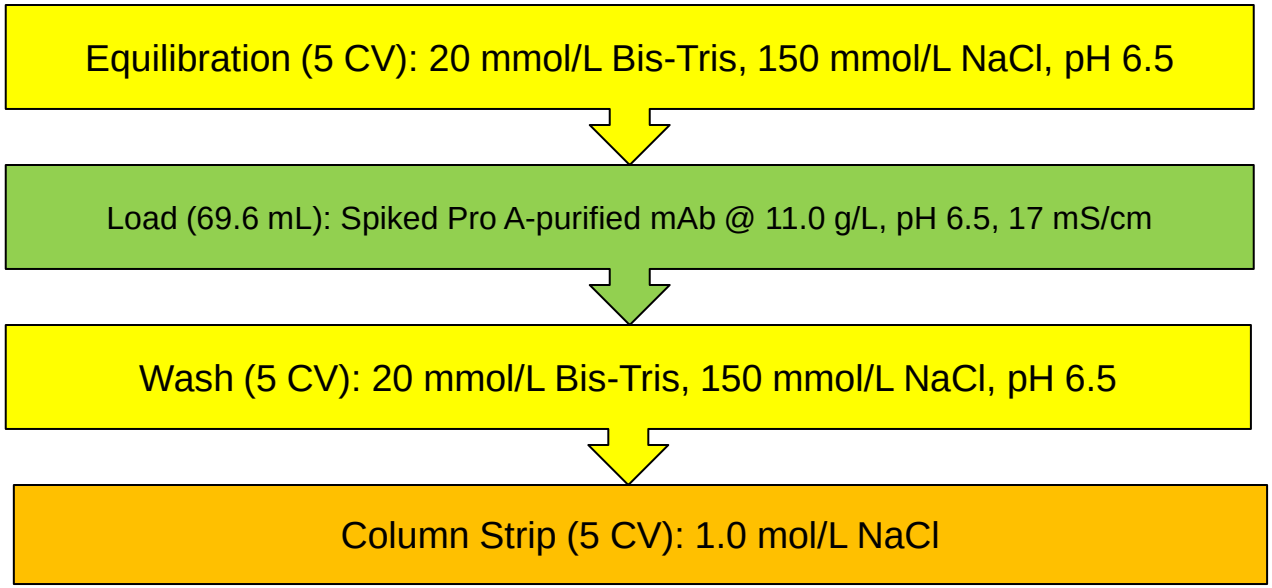
Viral Clearance with a CEX Resin in Bind-and-Elute Mode

Protein A-purifed mAb was spiked with 1% (Reo, MVM) or 5% (MuLV, Reo) (v/v) virus stock and processed according to the following method:



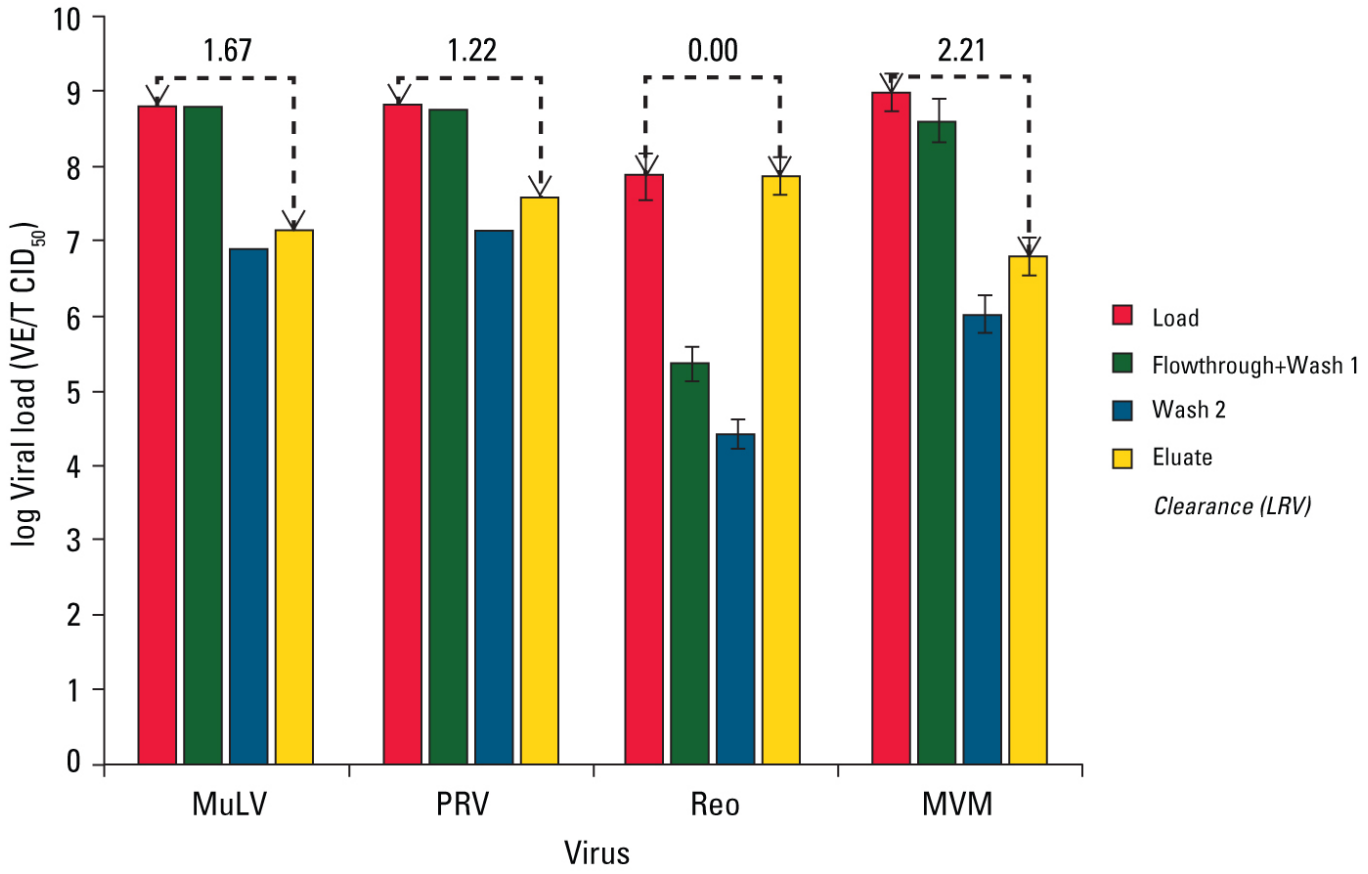
Viral Clearance with a AEX Resin in Flowthrough Mode

Protein A-purifed mAb was spiked with 1% (Reo, MVM) or 5% (MuLV, Reo) (v/v) virus stock and processed according to the following method:



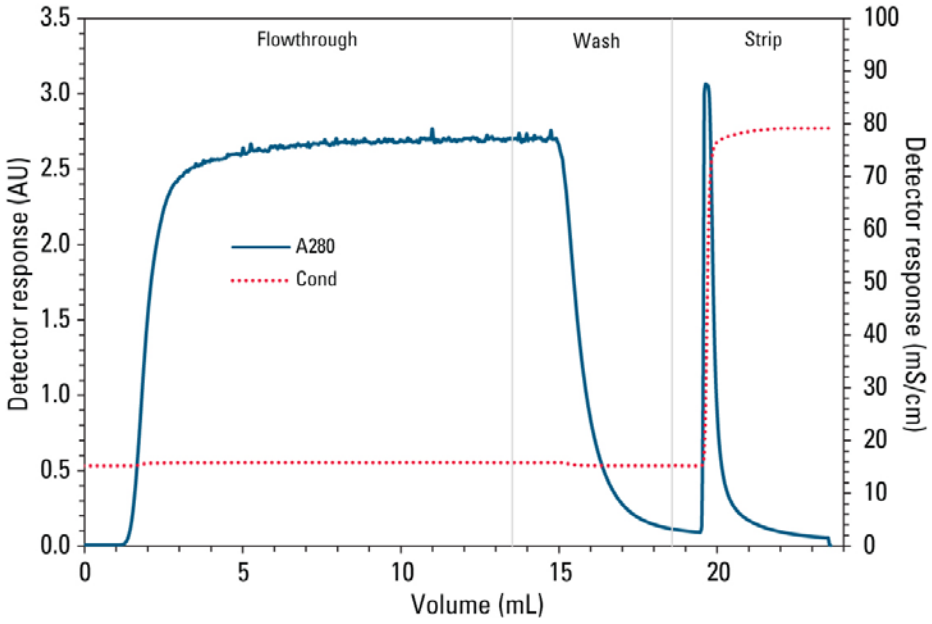
Analysis of Viral Level from Eluate of Protein A Resin

Protein A chromatography gave poor (< 2 logs) to no (< 0.5 logs) viral clearance for all viruses. This level of viral clearance is typical of protein A resins.



Chromatographic Profile of a AEX Flowthrough Mode using TOYOPEARL NH_2-750F

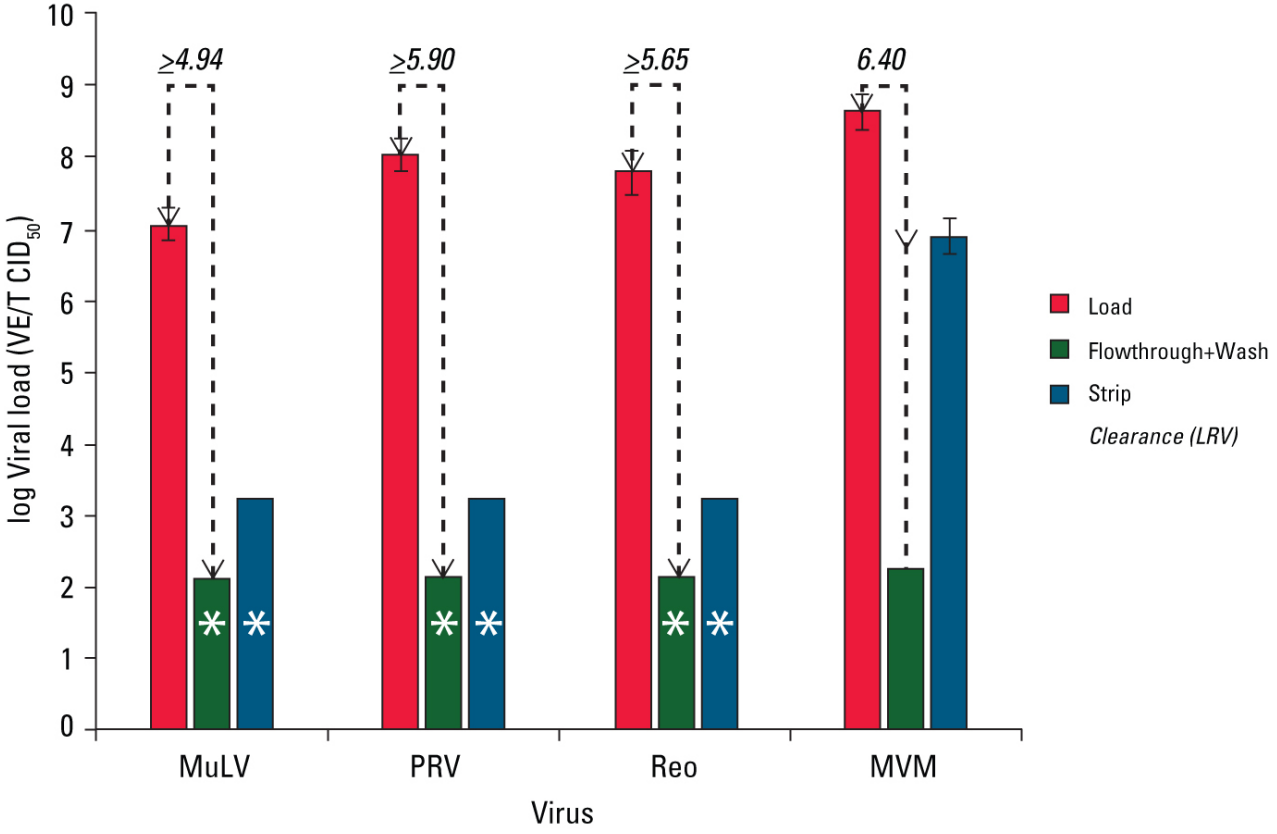
Chromatography steps were run with each virus separately and process fractions were evaluated for viral titer. A representative chromatogram for the AEX step is shown.



Resin:	TOYOPEARL NH_2-750F
Column:	6.6 mm × 15 cm (5.1 mL)
Mobile phase A:	20 mmol/L Bis-Tris, 150 mmol/L NaCl, pH 6.5
Mobile phase B:	1.0 mmol/L NaCl
Flow velocity:	300 cm/hr (3 min residence time)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Injection vol.:	69.6 mL
Sample:	Spiked, protein A-purified mAb, 11.0 g/L, pH 6.5, 17 mS/cm
Sample load:	765 mg (150 mg/mL-resin load ratio)
Instrument:	ÄKTA Explorer 100

Analysis of Viral Level from AEX Flowthrough Mode using TOYOPEARL NH2-750F

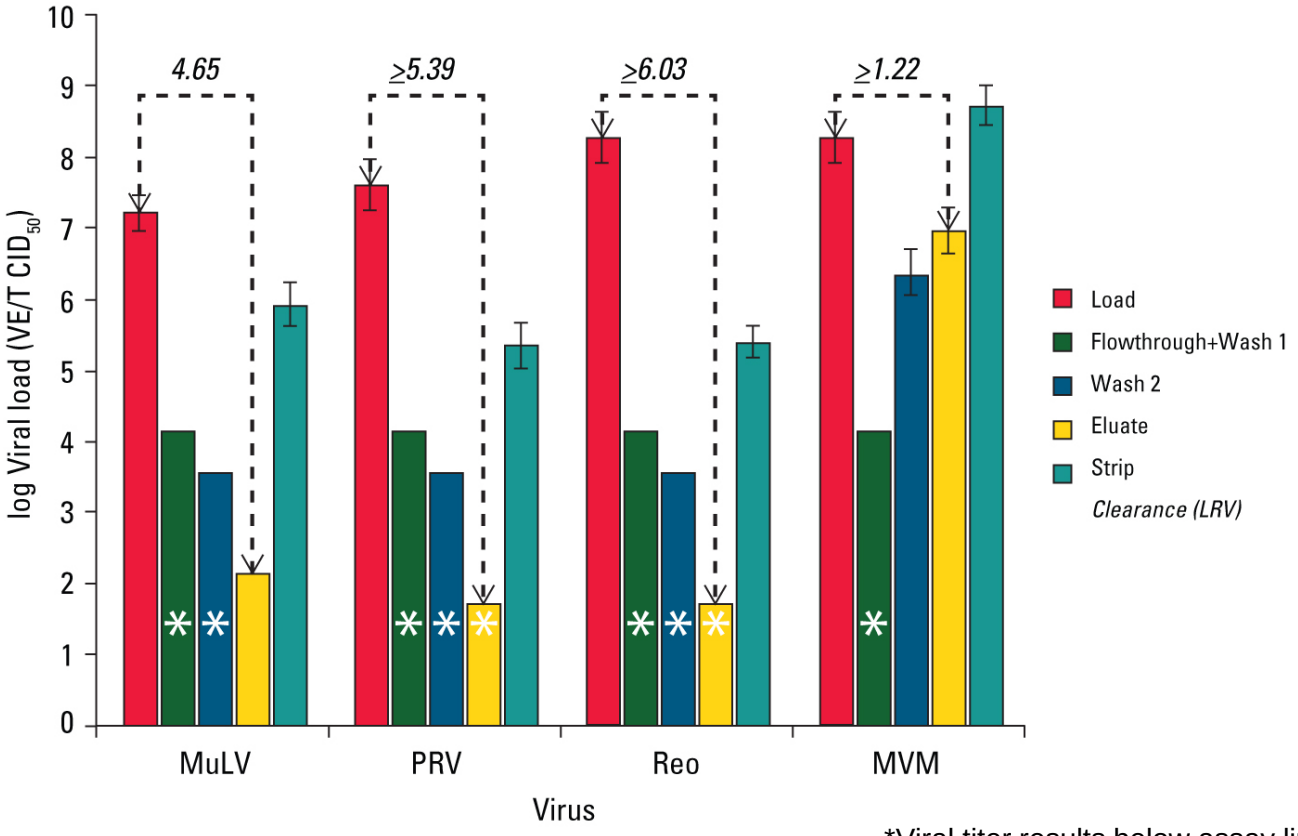
TOYOPEARL NH2-750F resin effectively removed all viruses with a clearance of > 4 logs.



*Viral titer results below assay limit of detection

Analysis of Viral Level from CEX Bind-and-Elute Mode using TOYOPEARL Sulfate-650F

The use of TOYOPEARL Sulfate-650F significantly reduced viral levels (> 4 logs) for all viruses except MVM, which had poor viral clearance (< 2 logs).



*Viral titer results below assay limit of detection

Conclusions

- TOYOPEARL AF-rProtein A HC-650F did not provide effective viral clearance. Typically, Protein A steps do not contribute robust virus removal to a purification process.
- TOYOPEARL NH_2-750F effectively removed all tested viruses (> 4 LRV). This removal is in the presence of a physiological salt concentration (150 mmol/L NaCl) and a relatively low pH for an AEX step (pH 6.5).
- TOYOPEARL Sulfate-650F significantly reduced viral levels (> 4 logs) for all viruses except MVM.

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