## Conclusion

With POROS® HQ resin, robust viral clearance can be attained under high-salt conditions. Viral clearance under higher-conductivity conditions allows for increased flexibility when designing a purification scheme. Because increased flow rates and decreased bed heights have no impact on viral clearance performance, researchers can opt for increased productivity or smaller column volumes, both of which provide cost-of-goods benefits to AEX FT operations. Improved salt tolerance reduces the need to dilute the feed stream or include a diafiltration step prior to loading on the POROS® HQ column, reducing process volumes and yielding a more efficient and cost-effective process.


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### References


"A Guide to Planning Your Viral or TSE Clearance Study" (2007) A publication of the BioReliance Corporation (PDF available at www.bioreliance.com/library/?ID=90)

"Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" (1993) A memo from the Director of the Center for Biologics Evaluation and Research, US Department of Health & Human Services (PDF available at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/UCM062745.pdf)

"Design and Performance of Viral Clearance Studies"(2006) A publication from Charles River Laboratories (PDF available at http://info.criver.com/flex_content_area/documents/bp_pc_r_viral_clearance_studies.pdf)

Parra SC, Gebski C (2011) Benefits of a revised approach to anion exchange flow-through polish chromatography. *Biopharm Int* 24:s16–s20.

Willkommen H (2008) Enhanced Speed to Clinic by a Reduced Virus Safety Package. Presented at BioProduction 2008, Antibody Production and Downstream Processing, in Dusseldorf, Germany.

Zhou JX, Tressel T (2005) Membrane Chromatography as a Robust Purification System for Large-Scale Antibody Production, Bioprocess International, September 2005, pp. 32–37.

Zhou JX, Tressel T (2006) Basic concepts in Q membrane chromatography for large-scale antibody production. *Biotechnol Prog* 22:341–349.


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# POROS® HQ 50 Chromatography Resin

## Viral clearance capability and recommendations

### Introduction

To assure product safety, regulatory agencies require a viral clearance assessment of the purification process for all mammalian biopharmaceutical products. Chromatography steps are commonly used in the biotech industry during downstream purification, and it is beneficial to understand the viral clearance capabilities of each of these steps to ensure the process is optimized for the best virus removal possible. Anion exchange (AEX) chromatography products are commonly used in the biotech industry as a polish step during downstream purification in product flow-through (FT) mode to bind impurities. This publication demonstrates the viral clearance capabilities of the various steps of a model AEX chromatography unit operation in the FT mode and also discusses recommendations and considerations for designing viral clearance processes. In addition, salt tolerance of viral binding/eluting is presented to show viral clearance capability under higher-conductivity conditions.

### Virus selection, scale-down model, and experimental design

A viral clearance study should mimic the intrinsic and extrinsic viral risks associated with the product and include a selection of model viruses that vary in size, shape, genome type, and physicochemical resistance. Viruses for this study were selected based on a manufacturing process for a monoclonal antibody or recombinant protein product expressed in mammalian cell culture. Four common viruses that represent a range of viral characteristics were used: murine minute virus (MVM), xenotropic murine leukemia virus (xMuLV), pseudorabies virus (PRV), and respiratory enteric orphan III (REO-3).

The POROS® HQ FT step was optimized to achieve typical step recovery at conditions normally used in industrial biotherapeutic operations: neutral pH and low salt. AEX FT steps are typically run between pH 7 and 9 for most monoclonal antibody products. These studies were conducted at pH 7.0, which is at the lower end of the range to be more stringent. At a higher operating pH, virus particles will be more highly charged and bind more tightly to an AEX resin than at the lower pH.

Polyclonal human IgG (Sigma Cat. No. G4386, 155–160 kDa, pI ~6.9) was used as the column load material for the model process. The step recovery of the polyclonal human IgG was >90% in the FT/wash 1 pool. After the post-load wash, the column was washed with higher-conductivity solutions in a stepwise manner. The salt concentrations of the wash solutions evaluated and the corresponding conductivity values are summarized in Table 1. The column format was 0.46 cm (D) x 20 cm (L), 3.3 mL. The study was conducted at 300 cm/hr at room temperature. The column was loaded with 100 mg IgG per milliliter of resin in FT mode, with a 5% virus spike (load volume: 74 mL, 3.9 mL virus). The column was washed with 11 column volumes (CVs) of wash 1, followed by 6 CVs each of washes 2 through 4, followed by a strip with 2 M NaCl. For each wash step, the entire pool was collected and evaluated for viral content. The viral log reduction or clearance was then calculated.

**Table 1. POROS® HQ flow-through process buffers.** The salt concentrations evaluated and the corresponding conductivity values are shown.

| Process step | Buffer composition | Buffer conductivity (mS/cm) |
|---|---|---|
| Equilibration/wash 1 | 20 mM Bis-Tris propane, 25 mM NaCl, pH 7.0 | 5 |
| Load | 5 mg/mL IgG in 20 mM Bis-Tris propane, 25 mM NaCl, pH 7.0 | 5 |
| Wash 2 | 20 mM Bis-Tris propane, 50 mM NaCl, pH 7.0 | 8 |
| Wash 3 | 20 mM Bis-Tris propane, 100 mM NaCl, pH 7.0 | 13 |
| Wash 4 | 20 mM Bis-Tris propane, 200 mM NaCl, pH 7.0 | 22 |

## Results and discussion

POROS® HQ demonstrated significant viral clearance in FT mode for all four model viruses under typical FT/wash operations, as summarized in Table 2. No virus was detected in the FT/wash 1 pools for three of the model viruses tested, xMuLV, PRV, and REO-3. The conductivity ranges with significant viral clearance are higher than currently reported for other anion exchangers:

- Viral clearance of >5.2 LRV was reported for MVM up to 100 mM NaCl (~13 mS/cm, pH 7.0).
- Clearance of 5.0 LRV was reported for xMuLV up to 200 mM NaCl (~22 mS/cm, pH 7.0). No virus was detected in FT and wash pools up to 100 mM NaCl.
- Clearance of >5.5 LRV was reported for PRV, and >4.1 LRV for REO-3, up to 200 mM NaCl (~22 mS/cm, pH 7.0). No virus was detected in FT and wash pools up to 200 mM NaCl for both viruses.

One of the main attributes of POROS® chromatography resins is that their performance is independent of flow rate due to the bead morphology. In Table 3, POROS® HQ resin's viral clearance was tested with increasing flow rates from 300 cm/hr to 600 cm/hr at a higher-conductivity load (13 mS/cm). The data show that the operating flow rate has no impact on viral clearance up to 600 cm/hr, and POROS® HQ provides excellent salt tolerance for viral clearance for xMuLV at both flow rates, giving researchers even more flexibility for process design. Obtaining robust viral clearance at higher conductivity means that AEX FT loads can be diluted less, resulting in a smaller process volume.

Although a 20 cm bed-height column run at 300 cm/hr or slower is an industry standard for process chromatography, an alternative and beneficial approach to AEX FT is the use of a short, 5 cm length packed-bed column capable of being operated at a significantly higher flow rate. This format enables volumetric throughput capability similar to that of a membrane adsorber and increases flexibility when designing a purification scheme. High-flow, packed-bed chromatography can provide a processing time similar to those of membrane adsorber applications, with the added benefits of reusability, scalability, and the ability to implement initial process design from early-phase manufacturing to commercial manufacturing, reducing overall process costs and time to market.

Viral clearance using this new, optimized chromatography approach is summarized in Table 4 and Figure 1. POROS® HQ resin demonstrated good viral clearance capability for xMuLV at up to 150 mM NaCl (18 mS/cm) at pH 7.0. The MVM model virus showed good clearance up to 50 mM NaCl (8 mS/cm) in this new AEX FT format, suggesting that a shorter column run at a faster operational flow rate can achieve good viral and impurity clearance. Interestingly, the conductivity of the load appears to have an effect on both viruses, and clearance is specific to the virus type but independent of the operating flow rate and bed height, as typically seen on POROS® resins. MVM is a poorly charged virus, so low concentrations of the counter ion are required to neutralize the charge and decrease the binding. However, it is a small virus and can easily access the pores, so binding performance is flow rate–independent. xMuLV, on the other hand, is significantly larger and highly charged. With the higher salt concentration and conductivity (18 mS/cm), the hydrodynamic radius of the virus is most likely changing, allowing for more optimal perfusion into the bead.

**Table 2. Viral clearance on POROS® HQ with increasing conductivity.** Column format: 0.46 cm (D) x 20 cm (L), 3.3 mL; flow rate: 300 cm/hr; room temperature; load: 5 mg/mL IgG in 20 mM Bis-Tris propane, 25 mM NaCl, pH 7.0, 100 mg IgG per milliliter of resin, 5% virus spike. Wash 1: 11 CVs; washes 2 through 4: 6 CVs. The entire pool was collected and evaluated for each step.

| Virus type/Description | Process step | Viral clearance ($log_{10}$) |
|---|---|---|
| **MVM:** Non-enveloped, ssDNA, 18–26 nm Total virus loaded: 6.68 x $10^7$ PFU | FT/wash 1 | 5.91 |
| | Wash 2 | 6.78 |
| | Wash 3 | 5.22 |
| | Wash 4 | 0.96 |
| **xMuLV:** Enveloped, ssRNA, 80–120 nm Total virus loaded: 6.91 x $10^7$ FFU | FT/wash 1 | >4.95 |
| | Wash 2 | >5.71 |
| | Wash 3 | >5.71 |
| | Wash 4 | 5.04 |
| **PRV:** Enveloped, dsDNA, 120–200 nm Total virus loaded: 3.81 x $10^7$ PFU | FT/wash 1 | >4.69 |
| | Wash 2 | >5.45 |
| | Wash 3 | >5.45 |
| | Wash 4 | >5.45 |
| **REO-3:** Non-enveloped, dsRNA, 60–80 nm Total virus loaded: 1.68 x $10^8$ PFU | FT/wash 1 | >3.33 |
| | Wash 2 | >4.09 |
| | Wash 3 | >4.09 |
| | Wash 4 | >4.09 |

**Table 3. Viral clearance on POROS® HQ with increasing flow rate.** Column format: 0.46 cm (D) x 20 cm (L), 3.3 mL; flow rate: 300 cm/hr and 600 cm/hr; room temperature; load: 5 mg/mL IgG in 20 mM Bis-Tris propane, 100 mM NaCl, pH 7.0 (13 mS/cm), 100 mg IgG per milliliter of resin, 5% virus spike. RT = residence time.

| HQ step | MVM clearance ($log_{10}$) | | xMuLV clearance ($log_{10}$) | |
|---|---|---|---|---|
| | 300 cm/hr 4 min RT | 600 cm/hr 2 min RT | 300 cm/hr 4 min RT | 600 cm/hr 2 min RT |
| FT/wash | 2.30 | 2.15 | >5.13 | >5.13 |

**Table 4. Impact of conductivity, flow rate, and bed height on viral clearance using POROS® HQ resin.** The IgG load salt concentrations evaluated, and the corresponding conductivity values, are summarized. Column format: 0.46 cm (D) x 5 cm (L), 0.83 mL or 0.46 cm (D) x 20 cm (L), 3.3 mL; flow rate: 1,000 cm/hr for the 25 mM, 50 mM, and 150 mM NaCl runs, and 300 cm/hr for the 100 mM NaCl run; room temperature; load: 5 mg/mL IgG in 20 mM Bis-Tris propane, pH 7.0 (salt variable), 100 mg IgG per milliliter of resin, 5% virus spike. The entire FT/wash pool was collected and evaluated for each condition.

| Load NaCl concentration (mM) | Load conductivity (mS/cm) | Viral clearance ($log_{10}$) | |
|---|---|---|---|
| | | xMuLV | MVM |
| 25 | 5 | 4.04 | 4.98 |
| 50 | 8 | 4.95 | 4.34 |
| 100* | 13 | >5.13 | 2.30 |
| 150 | 18 | >5.26 | 0.83 |

*The 100 mM NaCl run was executed using a 20 cm bed-height column at 300 cm/hr.

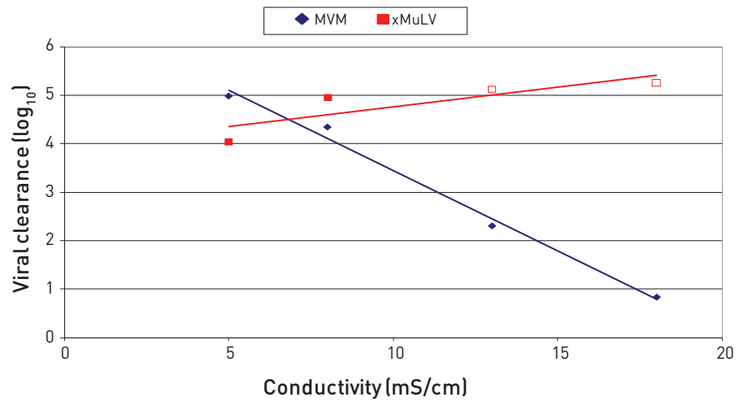


**Figure 1. Effect of load conductivity on viral clearance capability of POROS® HQ resin.** Solid data points denote actual results. Open data points denote results in which no virus was found in the sample and the clearance was calculated using the Poisson distribution calculation.