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Monoclonal Antibody Aggregate Polish and Viral Clearance Using Hydrophobic Interaction Chromatography

SPECIAL REPORT

Monoclonal Antibody Aggregate Polish and Viral Clearance Using Hydrophobic-Interaction Chromatography

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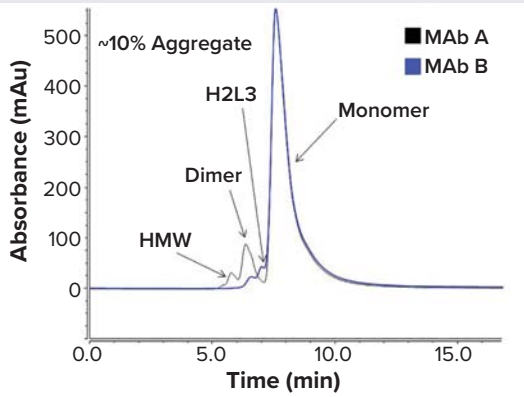
Therapeutic monoclonal antibodies (MAbs) have played an important part in the personalized medicine revolution, making significant clinical impact in fields such as oncology, immunology, neurology, and infectious diseases. To date, approximately 100 MAbs have been designated as drugs, and the rate of approvals is increasing rapidly. MAbs currently are a $100 billion industry, and their demand is likely to be high and continue to dominate the biologics market for years to come.

As a result of such increasing demand, the past three decades have seen significant improvements to the productivity of large-scale MAb biomanufacturing. Resin manufacturers invested greatly in developing better polishing chromatography solutions to clear residual impurities, provide additional virus clearance, and ultimately meet critical quality attributes (CQAs) required for antibody drug products. For MAb processes, at least one anion-exchange (AEX) polish step typically is required. Depending on a specific residual impurity profile and process challenges being addressed, an AEX step can be preceded or followed by a second polish step, typically using either cation-exchange (CEX) or hydrophobic-interaction chromatography (HIC).

The ultimate goal of downstream purification is to reliably and predictably produce a safe drug product suitable for therapeutic use in humans. To this end, biomanufacturing process- and product-related impurities such as host cell proteins (HCPs) from upstream cell culture, residual DNA, leached protein A from the affinity step, process leachables and extractables, adventitious and endogenous viruses, endotoxins, antibody aggregates, and other antibody variants all must be removed to acceptable levels in conformance with regulatory guidelines. Among all the impurities mentioned, aggregate removal can be especially challenging if levels are high. The FDA generally recommends that MAb aggregates be reduced to <1% for later phase clinical campaigns. For reference, a typical MAb process will start from 1–5% aggregates after protein A capture, but this percentage can be >10% for certain challenging MAbs. To address the need for aggregate clearance in MAb downstream processing, Thermo

Figure 1: MAb A and MAb B were run on the UltiMate™ 3000 HPLC system using MAbPac™ SEC-1 LC column, with run conditions of isocratic elution using 50 mM sodium phosphate pH 6.8, 250 mM NaCl. Aggregate and monomer peaks are highlighted. Both MAb A and MAb B contain high aggregate levels ~7–12%. The clinical processes developed for MAb A and MAb B aggregate polish are highlighted in the box below.

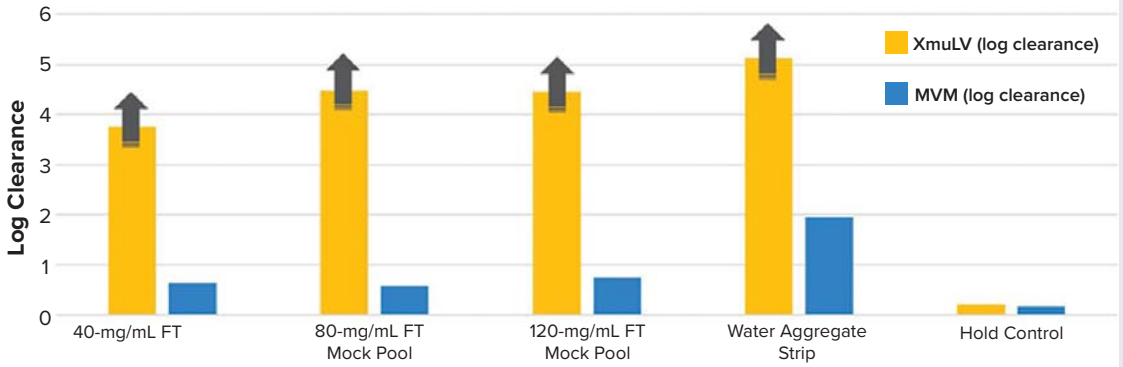


MAb Clinical Processes

MAb A Clinical Process: Mixed-mode in bind–elute mode, monomer recovery 90%, 25 g/L resin loading, 6-minute residence time

MAb B Clinical Process: Cation-exchange in bind–elute mode, monomer recovery 65–70%, 40–45 g/L resin loading, 6-minute residence time

Figure 2: POROS Benzyl Ultra FT viral clearance (MAb A); complete XmuLV and minimal MVM clearance



Fisher Scientific offers a wide range of polish purification tools based on POROS through-pore resin technology. POROS HQ and XQ are AEX resins and POROS HS and XS are CEX resins with different characteristics and selectivity that can be leveraged to remove low-to-moderate levels of MAb aggregates (**1, 2**). Herein, we discuss a family of POROS HIC resins with novel ethyl and benzyl chemistries for the successful polish of two challenging drug products: MAbs A and B, both with high levels of aggregates of >10% (Figure 1). In addition to aggregate clearance, viral clearance strategy on POROS HIC for MAb A and MAb B processes is discussed extensively, including a novel prediction technique that uses parvovirus surrogate mock virus particles (MVPs) from MockV Solutions as well as live viral clearance data using xenotropic murine leukemia virus (XmuLV) and minute virus of mice (MVM).

POROS HIC Case Study: Two Clinical MAbs Requiring Aggressive Strategy for Aggregate Clearance

MAb A and MAb B are two clinical-stage MAbs with difficult downstream processes. Significant aggregates remained after protein A capture and AEX flowthrough (FT) polish, requiring an additional polishing step. For this second polish step, MAb A uses a mixed-mode bind–elute (BE) process, with low resin load density, slow flow, and high yield, whereas MAb B uses a cation-exchange BE process, with high load density, slow flow, and low yield (Figure 1).

We asked the biologics company whether we could design and develop more productive processes for both MAb A and MAb B using POROS HIC resins and compared our approaches with existing, optimized clinical processes. For MAb A, we designed and verified two strategies: one using POROS Benzyl resin in BE mode and the other using POROS Benzyl Ultra resin in FT mode. Our processes were four times and 12 times more productive than the original mixed-mode process, respectively (Table 1). For full process development details, please refer to our previous publications (**3**). For MAb B, we optimized a POROS Benzyl Ultra BE process that improved yield significantly and shortened residence time compared with the existing clinical CEX process (Table 2).

Table 1: Overall comparison of the clinical mixed-mode process for MAb A compared with the two POROS HIC processes; process performance metrics have been improved, resulting in ~4× and ~12× higher productivity.

MAb A Process	Mixed-Mode BE (Clinical)	POROS Benzyl BE Resin	POROS Benzyl Ultra FT
Load monomer purity (%)	90	89	89
Load density (g/L resin)	25	32	120
Monomer purity pool (%)	99	99	>99
Monomer recovery (%)	90	>99	98
HCP (ppm)	NA	120–12 ppm	100–35 ppm
Residence time (min)	6	2	1.2
Pool volume (50–50 mAu)	5 CV	4 CV	NA
Productivity (g/L/h)	7	27	89

MAb A Impurity Clearance and Viral Clearance Study: In a separate study, we performed high-throughput screening for MAb A and demonstrated

Figure 3: MAb A POROS Benzyl bind–elute process viral clearance study; complete XmuLV and modest MVM clearance

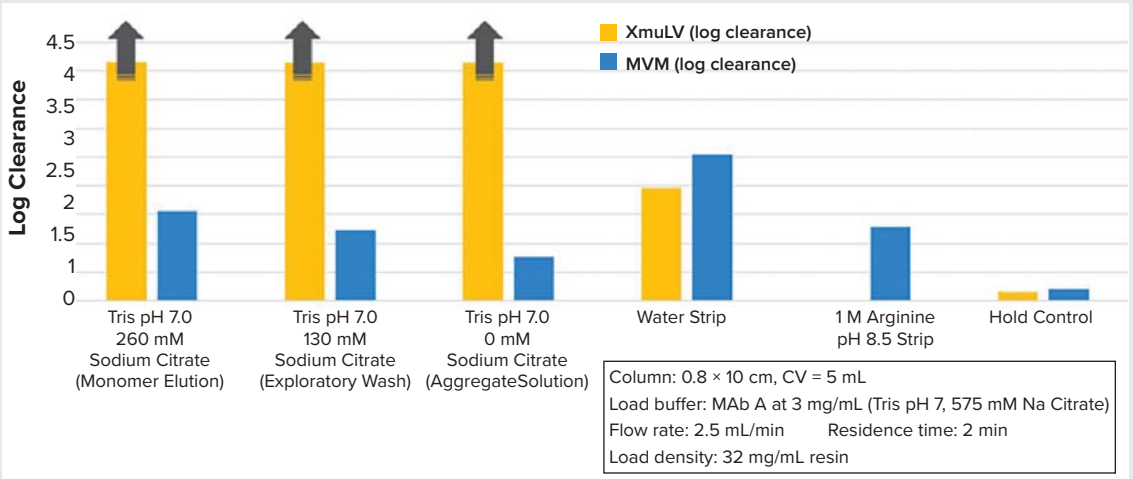


Table 2: Comparing the clinical cation-exchange bind–elute process for MAb B with the POROS Benzyl bind–elute process; POROS Benzyl resin achieved complete clearance of a difficult H2L3 aggregate species while improving yield by 20% and decreasing residence time threefold.

MAb B Process	Cation-Exchange BE (Clinical)	POROS Benzyl Ultra BE Resin	POROS Ethyl BE Resin
Load monomer purity (%)	90	90	NA
Load density (g/L resin)	40	40	18
Monomer purity pool (%)	>98	>98	NA
H2L3 (%)	<1	<1	NA
Monomer recovery (%)	>65	>85	NA
Residence time (min)	6	2	NA
Pool volume (50–50 mAu)	4 CV	4 CV	NA

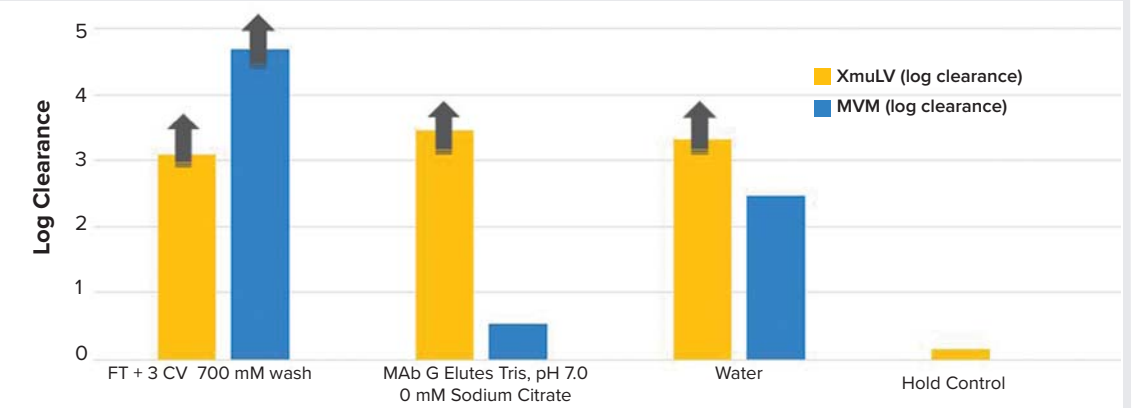
enhanced selectivity of POROS Benzyl and POROS Benzyl Ultra resins for clearing MAb A aggregates using sodium citrate (**3**). Compared with results from the clinical mixed-mode process, POROS Benzyl resin in BE mode demonstrated higher load capacity and lower residence time (from six to two minutes) with 10% higher monomer recovery, while maintaining aggregate and HCP clearance performance (Table 1). Overall productivity of the process increased about fourfold while achieving product CQAs.

We then optimized an even higher productivity process using POROS Benzyl Ultra resin in FT mode with similar impurity clearance ability (Table 1). A <2-minute residence time combined with a resin load density >100 g/L resulted in a 12-fold increase in productivity. Moreover, using a HIC FT process has the added benefit of a robust AEX-HIC straight-through process design with no additional conductivity or pH adjustments and no intermediate hold up required between AEX and HIC (data not shown).

In addition to the impurity clearance study, we tested the ability of the optimized MAb A POROS HIC BE and FT processes to clear retrovirus XmuLV and parvovirus MVM. In the POROS Benzyl Ultra FT process, we observed complete clearance of XmuLV (>4 log) and minimal clearance of MVM (<1 log) up to 120 g/L_r load at 2-minute residence time (Figure 2). XmuLV is more hydrophobic than MVM and thus bound tightly to the resin in FT mode. MVM is less hydrophobic and did not bind. It is interesting that a water strip was insufficient to remove bound XmuLV from POROS Benzyl Ultra resin.

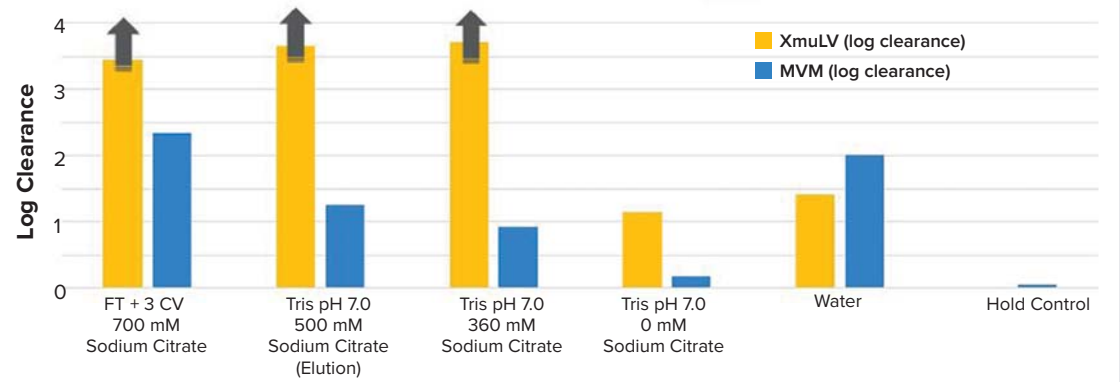
In the POROS Benzyl BE process, MAb A load was bound at high salt levels, and we performed specific monomer elution with 260 mM sodium citrate. We observed complete clearance of XmuLV (>4 log) and partial clearance of MVM (1.5 log) in the elution fraction. We then performed sequential washes of decreasing salt concentrations to 130 mM sodium citrate, then buffer alone where bound aggregates eluted, followed by a water strip and finally a 1M arginine strip. It is interesting to note that water and arginine but not buffer alone were sufficient to remove the highly hydrophobic XmuLV from POROS Benzyl resin. The differential stripping behavior for XmuLV is consistent with the relatively less hydrophobic character of POROS Benzyl resin compared with POROS Benzyl Ultra resin.

Figure 4: MAb B POROS Benzyl Ultra bind–elute process viral clearance study; complete XmuLV and minimal MVM clearance



Column: 0.8 × 10 cm, CV = 5 mL Load buffer: MAb B (Tris pH 7, 700 mM sodium citrate) Flow rate: 2.5 mL/min Residence time: 2 min

Figure 5: MAb B POROS Ethyl bind–elute process viral clearance study; complete XmuLV and modest MVM clearance



Column: 0.8 × 10 cm, CV = 5 mL Load buffer: MAb B (Tris pH 7, 700 mM sodium citrate)
Flow rate: 2.5 mL/min Residence time: 2 min

MAb B Impurity Clearance and Viral Clearance Study: MAb B also was difficult to polish from an aggregation standpoint, with >5% aggregation consistently post AEX-FT and a highly unique cysteine-mediated H2L3 aggregate species (one light chain more than the monomer) that did not behave similarly to dimer or higher molecular-weight aggregate species. To clear H2L3 and dimer species completely, we developed MAb B's clinical process as a CEX-BE step that tolerated a 30–40% yield loss. Using POROS Benzyl Ultra resin in BE mode, we optimized a process for MAb B that decreased residence time from six to two minutes, while yielding a 20% increase in monomer recovery (Table 2).

In preliminary studies, we also observed complete clearance of MAb B H2L3 and other aggregate species in FT mode on POROS Benzyl Ultra resin, though further salt type and concentration optimization was required (results not shown). Finally, we tested stability of MAb B in high-salt buffers and did not observe de novo aggregate formation over a seven-day time course at 4 °C and 25 °C (results not shown).

We tested the ability of the POROS Benzyl Ultra BE process for MAb B to clear retrovirus XmuLV and parvovirus MVM (Figure 4). MAb B was loaded with 700 mM citrate, and specific monomer elution was performed using buffer with no salt added. Note that both XMuLV and MVM bound POROS Benzyl Ultra resin under those load conditions, but buffer alone removed only MVM — not XMuLV. For the MAb B monomer elution fraction, we again observed complete XMuLV clearance and minimal MVM

MVM Mock Virus Particle (MVP) Kit

Commercial kits for analyzing the removal of impurities such as host cell proteins and residual host DNA are used routinely to ensure a quality-by-design (QbD) approach to biologics manufacturing. The information amassed from these types of kits feeds decision-making processes throughout the evolution of a downstream purification process — from early stage process development to late-stage commercial manufacturing. However, today's analytical toolbox lacks an easy-to-implement kit that can provide information about process capability for viral clearance. That is mainly because viral clearance studies require propagation of live mammalian viruses. The cost, time, logistics, and environmental challenges demand specialized laboratories, personnel, and expertise for such studies, leading many drug developers to outsource them or postpone that work until late in process validation or just before filing a biologics license application (BLA).

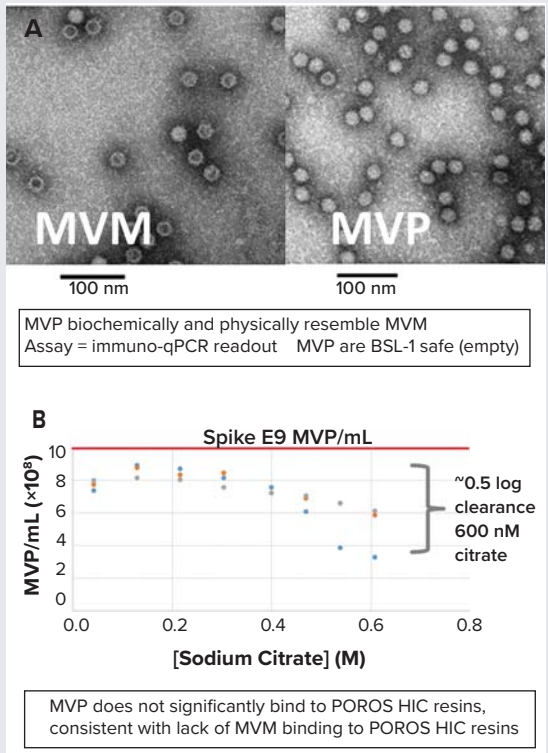
Viral surrogates such as bacteriophages have been used for over a decade in virus filtration applications because they do a satisfactory job at mimicking the size and physical properties of mammalian viruses (**4**). However, they fail to mimic the overall physiochemical and surface properties of the live mammalian viruses used in viral clearance spiking studies. Moreover, off-the-shelf quantification assays and consistent sources of bacteriophage spiking material are not readily available in standardized kit formats.

MockV Solutions commercialized the MVM mock virus particle (MVP) kit, which uses an immuno-qPCR assay to quantify a noninfectious MVM-surrogate viral particle. These MVPs have similar morphology and physiochemical characteristics to MVM (**4**). The ability of MVM MVPs to predict MVM clearance has been studied for virus filters (**5**) and ion-exchange chromatography (**6**). POROS AEX resins have shown superior salt tolerance in FT mode retention of MVP for a viral vaccine polish application (**7**).

clearance. Water strip did not remove XmuLV from POROS Benzyl Ultra resin, a finding consistent with results obtained for the MAb A FT process on the same resin.

To test virus-resin hydrophobic interaction, we performed MAb B BE experiments using POROS Ethyl resin without further co-optimizing for H2L3 or aggregate clearance. At 700 mM sodium citrate load phase, we saw both strong XmuLV and moderate MVM binding to POROS Ethyl resin. Using sequential salt drops of 500 mM and 360 mM, then buffer alone, both XmuLV and MVM were stripped from the resin using the minimal buffer wash with no salt added. The relative ease of elution for even the highly hydrophobic XmuLV virus is consistent with

Figure 6: MVP mimics MVM, with transmission electron microscopy (A). High-throughput screening of MVP binding to POROS Ethyl, Benzyl, and Benzyl Ultra resins under increasing sodium citrate concentrations (B) for a load spike of 1×10^9 MVP/mL MVP did not bind to any of the HIC resins under all conditions tested.



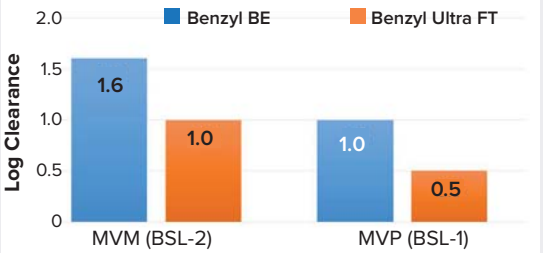
POROS Ethyl resin being the least hydrophobic member of the POROS HIC resin family.

Viral Clearance Prediction and Strategy Using MVPs by MockV Solutions

In the HIC virus clearance studies discussed above, we observed minimal to modest clearance of MVM in all POROS HIC processes conducted in BE and FT modes. To better understand MVM hydrophobicity, binding, and clearance for the three POROS HIC resins, we used MockV's MVM MVP in high-throughput screening (HTS) format. Binding was tested under increasing concentrations of sodium citrate (Figure 6).

Clearance of the MVP on all three HIC resins was <0.5 log, even under the highest salt concentrations (600 mM sodium citrate for MAb A). Overall, our observations point to the low hydrophobicity of MVP and (by extension) MVM — a finding supported by Johnson et al. (**8**). Our finding also is consistent with our live viral clearance observations above in which minimal binding of MVM was detected for POROS Benzyl Ultra resin in FT mode polish of MAb A.

Figure 7: MVM and MVP clearance for MAb A BE and FT processes on POROS HIC resins; MVM was spiked as a single virus, and clearance was measured by infectivity assay. MVP was spiked as a single agent and tracked by immuno-qPCR using the MockV analytical kit. MockV MVP clearance at mirrored MVM live virus clearance for all processes tested.



MVP clearance is comparable to MVM clearance in both MAb A FT and bind–elute polish processes.

Similarly, for the POROS Benzyl BE operation, negligible MVM binding was observed during MAb A loading at 600 mM citrate. Taking advantage of this result, the addition of a high-salt chase in the BE process could improve the MVM clearance achieved in the elution pool. We also performed parallel column studies using MVP and the optimized POROS HIC processes for MAb A (Figure 7). We showed that for both Benzyl Ultra FT and Benzyl BE processes, MVP clearance mirrored MVM clearance. Taken together, our results highlight the utility of MockV's MVM MVP kit as a process development and prediction tool for live MVM clearance.

Discussion

HIC is an excellent tool for the downstream purification of biotherapeutics. Thermo Fisher Scientfic has designed a family of POROS HIC resins to confer high selectivity for impurity removal. They are highly efficient for aggregate polish and offer orthogonal HCP and viral clearance capabilities. Here we showed highly productive POROS HIC polishing processes in both BE and FT modes that outcompeted legacy clinical processes designed for two highly challenging case studies (MAbs A and B).

We demonstrated complete XmuLV and partial MVM viral clearance for both MAb A and MAb B processes. Finally, we used MockV Solutions MVM MVP viral surrogate to better understand MVM virus and HIC interactions. Because these MVPs physiochemically resemble live MVM used for spiking studies, we also presented data highlighting the utility of MVP for predicting MVM clearance both in high-throughput screening and column studies using optimized POROS HIC polish processes. As predicted, the clearance of MockV MVPs closely paralleled MVM clearance in all POROS HIC studies. In conclusion, POROS HIC is a highly productive and platform-ready purification step that confer orthogonal selectivity for aggregate and impurity removal as well as viral clearance potential.

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References

1 Welsh J, et al. A Practical Strategy for Using Miniature Chromatography Columns in a Standardized High-Throughput Workflow for Purification Development of Monoclonal Antibodies. *Biotechnol Prog.* 30(3) 2014: 626–635; doi:10.1002/btpr.1905.

2 Kang K, et al. Development of an Acidic/Neutral Antibody Flow-Through Polishing Step Using Salt Tolerant Anion Exchange Chromatography. *Pharm. Bioprocess.* 3(8) 2015: 477–487.

3 Li JJ, et al. Improving Aggregate Removal to Enhance Clinical Manufacturing of MAbs. *BioPharm Int.* 28 September 2018.

4 Aranha-Creado H, Brandwein H. Application of Bacteriophages as Surrogates for Mammalian Viruses: A Case for Use in Filter Validation Based on Precedents and Current Practices in Medical and Environmental Virology. *PDA J. Pharm. Sci. Technol.* 53(2) 1999: 75–82.

5 Cetlin D, et al. Use of a Noninfectious Surrogate to Predict Minute Virus of Mice Removal During Nanofiltration. *Biotechnol. Progress* 34(5) 2018: 1213–1220.

6 Herbig K, et al. Modeling Virus Clearance: Use of a Noninfectious Surrogate of Mouse Minute Virus As a Tool for Evaluating an Anion-Exchange Chromatography Method. BioProcess Int. 17(5) 2019: 34–41.

7 Clearing the Way for Viral Clearance. *BioProcess Int.* Ask the Expert Webinar; https://bioprocessintl.com/sponsored-content/clearing-the-way-for-viral-clearance.

8 Johnson S, et al. A Step-Wise Framework to Design a Chromatography-Based Hydrophobicity Assay for Viral Particles. *J. Chromatog. B* 2017; doi:10.1016/j.jchromb.2017.08.002.

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