POROS MabCapture A 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. In general, viral clearance using protein A chromatography steps is very process-dependent and difficult to predict, with reported clearance levels ranging from no clearance to over 4-logarithmic units of clearance.

At many industry conferences, viral clearance contract laboratories and industrial biopharmaceutical companies discussed trends in viral clearance accumulated from many studies. The experts noted that viral clearance from a protein A chromatography step can be difficult to predict. However, of increasing use and effectiveness is the incorporation of an intermediate wash step. Post-load washing with high-salt washes; varying pH and other additives, was shown to increase the amount of impurities removed during the process operation. Viral studies indicated that the intermediate or secondary wash also provided additional viral clearance, making the step more predictable. This publication demonstrates the viral clearance capabilities of a model protein A affinity chromatography unit operation, and also discusses recommendations and considerations for designing viral clearance processes.

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. Viral clearance of parvovirus and retrovirus is commonly tested prior to phase 1 for biological products derived from mammalian cell lines, including monoclonal antibody products. The two most commonly used viruses that present with a range of viral characteristics were chosen for this study: minute virus of mice (MVM) and xenotropic murine leukemia virus (xMuLV). MVM is a non-enveloped, single-stranded DNA parvovirus that ranges in size from 18 to 26 nm. XMuLV is a highly charged, enveloped, single-stranded RNA retrovirus that ranges in size from 80 to 120 nm.

The protein A chromatography step was developed to mimic conditions normally used in industrial biotherapeutic operations. 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 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 control run was performed with a 1% virus spike, whereas the intermediate wash runs were performed with a 5% viral spike to further challenge the evaluation. Based on published data, we evaluated the effect of two post-load, intermediate wash solutions on Thermo Scientific™ POROS™ MabCapture™ A resin's viral clearance capability.

- Intermediate wash 1: 25 mM Tris, 10% propylene glycol (PG), 1 M urea, pH 9.0
- Intermediate wash 2: 25 mM Tris, 25 mM NaCl, 5 mM EDTA, 0.5 M tetramethylammonium chloride (TMAE), pH 5.0

Pool collection was designed to be more stringent than normal elution criteria, to again challenge the clearance. Although the peak elutes in approximately 1.5 column volumes (CVs), the entire 8 CVs of elution was pooled and tested. For each wash step, the entire pool was collected and evaluated for viral content. The viral log reduction or clearance was then calculated.

Viral clearance on POROS MabCapture A chromatography resin

Table 1 summarizes viral clearance data obtained on POROS MabCapture A resin with and without an intermediate wash. Less than 1-logarithmic unit viral clearance of MVM and xMuLV was observed under the control condition that did not include an intermediate wash. This is not necessarily unexpected for a protein A step, nor indicative of all processes. The results show that the use of the intermediate wash 1 solution increased MVM clearance by 44% and xMuLV clearance by 222%, compared to the control run that did not include an intermediate wash. The use of intermediate wash 2 solution increased MVM clearance by 18% and xMuLV clearance by 51%, compared to the control run. Although not all protein A steps achieve good clearance (as demonstrated by this mock process that employed an aggressive elution collection (<1-logarithmic unit)), these results cannot be extrapolated to every protein A process—different or less aggressive pooling conditions can offer improvements. Depending on the process conditions, it is possible to attain good viral clearance using protein A, especially with the inclusion of intermediate wash steps prior to elution.

Table 1. Viral clearance of POROS MabCapture A resin with and without intermediate washes.

	MabCapture A chromatography step	log (MVM clearance)	log (MuLV clearance)
No wash, 1% spike	FT/wash	0.2	1.7
	Elution	**0.9**	**0.7**
Intermediate wash 1, 5% spike	FT/wash	0	1.0
	Wash 1: 25 mM Tris, 10% PG, 1 M urea, pH 9.0	0.7	0.9
	Elution	**1.3**	**1.6**
	% increase from no-wash process	44%	222%
Intermediate wash 2, 5% spike	FT/wash	0.1	0.9
	Wash 2: 25 mM Tris, 25 mM NaCl, 5 mM EDTA, 0.5 M TMAE, pH 5.0	0.9	1.1
	Elution	**1.1**	**0.7**
	% increase from no-wash process	18%	51%

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 sodium phosphate, 150 mM NaCl, pH 7.5, 36 mg IgG per milliliter of resin, 1% or 5% virus spike; intermediate washes: varied conditions; elution: 75 mM acetic acid. The entire elution pool was collected and evaluated for viral content.

Conclusion

With POROS MabCapture A resin, robust viral clearance can be attained. Viral clearance from protein A affinity chromatography steps can be difficult to predict, but the addition of intermediate washes can improve clearance and make performance more predictable. Understanding how process conditions can impact virus partitioning or removal in a protein A affinity chromatography operation will provide increased flexibility when designing a purification scheme and help maximize viral clearance.

Speak to a technical specialist about how these resins can improve your current process at **bp@thermofisher.com**.

References

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Ordering information

Product	Quantity	Cat. No.
POROS MabCapture A resin	10 mL	4374732
	50 mL	4374730
	250 mL	4374729
	1 L	4374735
	5 L	4374728
	10 L	4374731

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