Virus Clearance on Eshmuno® A Affinity Chromatography Resin Using Clarified CHO Feed

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Summary

Eshmuno® A is a rigid, high capacity, acid and alkaline resistant Protein A affinity chromatography resin designed for the purification of Fc-containing proteins, including monoclonal antibodies. This study compared retrovirus and parvovirus clearance on Eshmuno® A resin with that of an established Protein A affinity resin, ProSep® Ultra Plus.

Using Eshmuno® A resin, approximately 3.1 logs of parvovirus removal and ≥4.9 logs of retrovirus reduction was measured by infectivity assays. Quantitative Polymerase Chain Reaction (qPCR) analysis revealed that 3.7 logs of retrovirus were removed by the Eshmuno® A resin and the remaining reduction was due to virus inactivation by the low pH protein elution conditions.

Overall, these data confirm that the Eshmuno® A affinity chromatography resin can make a notable contribution to process virus clearance targets using a minimally purified monoclonal antibody stream.

Introduction

Protein A chromatography is frequently used early in the purification of biopharmaceutical molecules to concentrate Fc-containing proteins from clarified cell harvest. Protein A chromatography is one of the several key steps in virus removal in a typical mAb purification process. The goal of this study was to benchmark virus removal capabilities of Eshmuno® A resin with those of an established commercially available resin, ProSep® Ultra Plus.

Eshmuno® A Protein A affinity chromatography resin is a rigid, high capacity, acid and alkaline resistant affinity chromatography resin for purification of Fc-containing proteins, including but not limited to, monoclonal antibodies.

ProSep® Ultra Plus resin based affinity chromatography media is designed for large-scale, cost-effective purification of high titer therapeutic antibodies due to its high dynamic binding capacity at high flow rate.

Virus clearance across these two resins was evaluated using two viruses typically used in clearance studies with monoclonal antibody feeds: Minute Virus of Mice (MVM), a non-enveloped parvovirus and Xenotropic Murine Leukemia virus (X-MuLV), a model retrovirus. These viruses were spiked into a model feed and reduction across the protein A resins were assessed using both infectivity and qPCR assays.

Experimental details

Feed

The cell culture harvest material was generated by clarifying serum free Chinese Hamster Ovary (CHO) cell harvest with Millistak+® Pod depth filters followed by processing over Opticap® XL150 Express filters. Purified monoclonal antibody was then spiked into the clarified CHO harvest to a final concentration of 1.0 mg/mL to simulate a CHO monoclonal antibody cell culture feed with conductivity of 10.6 mS/cm and pH 7.5.

Viral Spiking

The CHO monoclonal antibody cell culture feed was spiked with either X-MuLV to a final concentration of $1x10^6$ $TCID_{50}$/mL (0.5% (v/v)) or MVM to $2x10^6$ $TCID_{50}$/mL (0.1% (v/v)). Spiked feed material was then filtered through 0.45 µm (X-MuLV) or 0.22 µm (MVM) filters to assess monodispersity before challenging the protein A resins. Duplicate columns of each protein A resins were tested with each virus. During elution of the bound protein, eluent fractions were collected and protein concentration was determined. Those samples with OD greater than 0.2 Au at 280 nm were assayed for infectivity. Because the elution conditions were expected to inactivate the more labile X-MuLV, selected eluent fractions from the X-MuLV tests were evaluated by qPCR in order to differentiate virus removal and inactivation.

Table 1. Properties of Viruses Used for Clearance Studies

Virus Family	Virus	Size (nm)	Enveloped	Physico-chemical resistance
Parvoviridae	MVM	18-26	No	High
Retroviridae	X-MuLV	80-110	Yes	Low

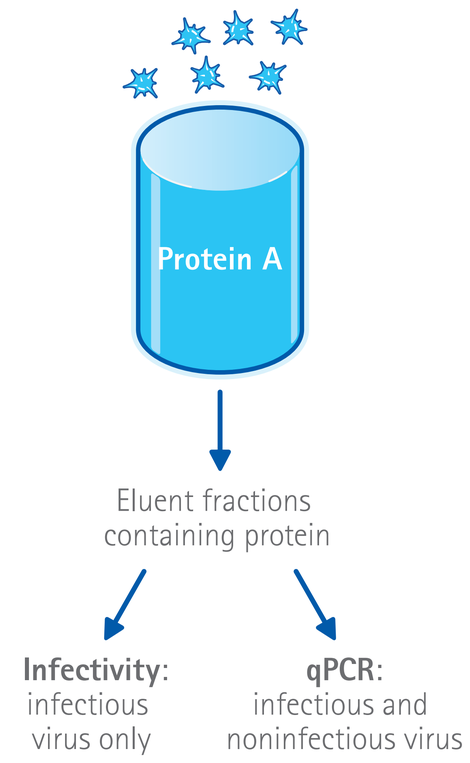
Experimental Set-up

Equipment: Watson Marlow peristaltic pump
Column: 1.0 cm id. x 5 cm, 3.93 mL

Buffers

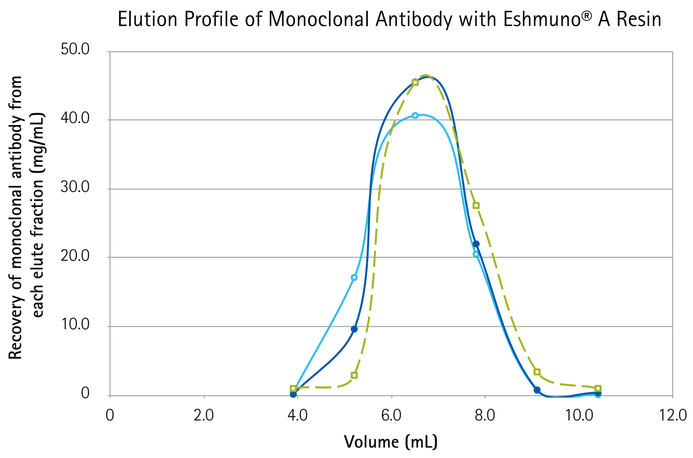
Equilibration buffer: 1XPBS: (3 CV, flow rate: 1.31 mL/min)
Intermediate wash buffer: 0.1 M Citric acid, pH 5.5: (4 CV, flow rate: 1.31 mL/min)
Elution: 0.1 M Citric acid, 20 mM NaCl, pH 3.5: (5CV, flow rate: 0.65 mL/min)
Sanitization buffer for Eshmuno® A columns: 0.1M NaOH
Sanitization buffer for ProSep® Ultra Plus columns: 0.15 M Phosphoric acid.
All the buffers were filtered through 0.22 µm Millipore Express® filters.

Figure 1. Schematic of X-MuLV Sample Collection and Titer Assays



Results

Figure 2. Eshmuno® A Resin Elution Profile



Most of the monoclonal antibody eluted around 7.0 mL for each of the MVM and X-MuLV spiked runs. Virus clearance results and calculated Log reduction Values (LRV) are shown in **Tables 2** and **3**.

Table 2. MVM Viral Clearance Results for Eshmuno® A and ProSep® Ultra Plus resin using CHO monoclonal antibody cell culture feed.

Eshmuno® A and ProSep® Ultra Plus resin showed similar levels of parvovirus removal with monoclonal antibody clarified CHO feed. Approximately 3.1 logs of virus removal was observed with each resin.

Media	Test	Virus Load Hold (log $TCID_{50}$/mL)	Virus Load Eluted (log $TCID_{50}$/mL)	LRV
Eshmuno® A resin	1	8.4	5.4	2.9
	2	8.4	5.0	3.4
ProSep® Ultra Plus resin	1	8.4	5.1	3.2
	2	8.4	5.2	3.2

Table 3. X-MuLV Viral clearance Results for Eshmuno® A and ProSep® Ultra Plus resin using CHO monoclonal antibody cell culture feed.

No infectious X-MuLV was detected in the protein A elution fractions following processing over either Eshmuno® A or ProSep® Ultra Plus resins indicating the retrovirus had either been removed by the resin or inactivated by the elution conditions. LRVs of greater than 4.8 logs were determined from infectivity assays.

Media	Titer Assay	Test	Virus Load Hold (log $TCID_{50}$/mL)	Virus Load Eluted (log $TCID_{50}$/mL)	LRV
Eshmuno® A resin	Infectivity (inactivation and removal)	1	7.7	≤2.9	≥4.9
		2	7.7	≤2.9	≥4.9
	qPCR (removal only)	1	9.9	6.2	3.7
		2	9.9	6.1	3.8
ProSep® Ultra Plus resin	Infectivity (inactivation and removal)	1	7.7	≤3.0	≥4.8
		2	7.7	≤3.0	≥4.8
	qPCR (removal only)	1	10.3	6.9	3.4
		2	10.3	6.9	3.4

By measuring the quantities of retrovirus genomes in the various fractions, qPCR analysis resolved that most of the retrovirus (3.4-3.8 logs) was removed by the resin. Subtracting the level removed from the total reduction indicated that the low pH protein elution conditions contributed at least 1.5 logs of X-MuLV reduction.

Virus removal capabilities of Eshmuno® A resins are in line with other commercially available protein A resins and offer an attractive option for contributing to process virus clearance targets.

Conclusion

- **Both Eshmuno® A and ProSep® Ultra Plus protein A chromatography resins showed approximately 3.1 logs of parvovirus removal in a clarified monoclonal antibody model feed. These results demonstrate that both Eshmuno® A and ProSep® Ultra Plus protein A chromatography resins are moderately effective in reducing parvovirus load.**
- **No infectious X-MuLV was detected in the protein containing elution fractions with measured LRVs of ≥4.9 logs. qPCR analysis revealed that 3.3-3.8 logs of X-MuLV were removed by each resin and at least 1.5 logs of retrovirus reduction was due to virus inactivation caused by the low pH protein elution conditions.**
- **These results indicate that Eshmuno® A protein A affinity chromatography resin can make an important contribution to help reach process virus reduction targets for both endogenous retrovirus as well as adventitious virus such as MVM.**

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