Efficient Virus Clearance Across the Merck Downstream Purification Portfolio

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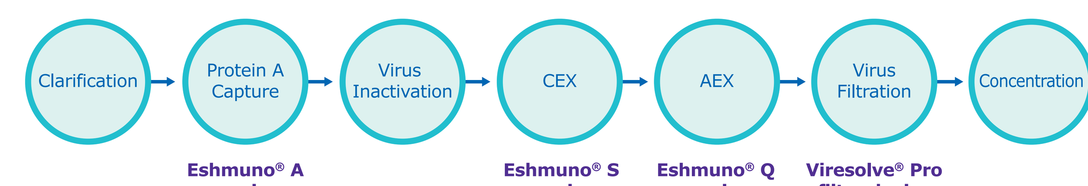
Introduction

The downstream purification process of any biologic has several objectives: purity, yield, and safety for humans or animals. A critical component of safety assurance is reducing virus to levels that meet stringent regulatory requirements. Virus reduction can be achieved through multiple complementary approaches and most processes rely on a combination of technologies that are designed primarily to achieve purification targets, but may also offer opportunities for virus reduction.

The purpose of this project was to establish capabilities for producing a monoclonal antibody feed enabling the development of new filtration and chromatography products. The purified antibody should have industry-representative titer, yield and purity, achieved using the Eshmuno® chromatography resin platform in combination with Viresolve® Pro filtration devices as seen in **Figure 1**.

Figure 1. Monoclonal Antibody Process Operations and Purification Media



Each unit operation was optimized to maximize step purity and yield. Following optimization, virus clearance was assessed using two viruses: Minute Virus of Mice (MVM), and Xenotropic Murine Leukemia Virus (X-MuLV), **Table 1**.

Table 1. Virus Properties

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

Methods

All aspects of clone development, selection, cell culture media selection, scalable production in 3-200L Mobius® bioreactors, and clarification over Clarisolve® 20MS depth filters were optimized as part of the benchmark process development.

Table 2 summarizes the properties of the feeds used in each step. Media were selected with consideration to the efficiency of purification with a view to minimizing dilution or modification for downstream purification.

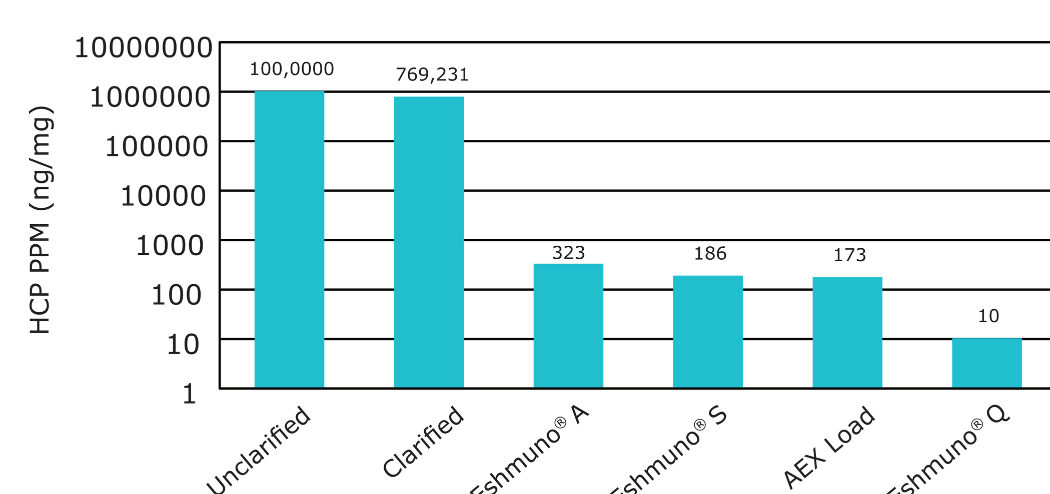
Table 2. Feed Specifications

Step *Media evaluated*	Feed	Protein Conc (mg/mL)	pH	Conductivity @ 25°C (mS/cm)
Protein A Eshmuno® A resin	Clarified Supernatant pool	1.3	7.2	12-15
CEX Eshmuno® S resin	Post Protein A	23.2	5.5	3-4
AEX Eshmuno® Q resin	Post CEX	11	8	6
Nanofiltration Viresolve® Pro Solution	Post AEX	10	5	6

Purification Summary

The purification scheme met mAb yield and purity targets.

HCP Levels in Process Intermediates



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Protein A Affinity Chromatography

Eshmuno® A resin is a rigid, high capacity, acid and alkaline resistant Protein A affinity chromatography media for the purification of Fc-containing proteins such as monoclonal antibodies. Following preliminary optimization of load, wash and elution conditions, Eshmuno® A resin delivered robust performance under a variety of conditions, consistently removed high levels of HCP, and could be loaded to 40 g/L with protein recoveries of at least 95%.

Duplicate columns were challenged with clarified harvest pool co-spiked with MVM at 1E+06 $TCID_{50}$/mL (0.1% (v/v)) and XMuLV at 5E+05 $TCID_{50}$/mL (~1% (v/v)). Columns were washed in 100 mM citric acid pH 5.6, then protein was eluted in 100 mM acetic acid pH 3.0. Samples were assessed for titer by infectivity assays (MVM and XMuLV) or qPCR (XMuLV only). Protein A viral clearance results are summarized below and show moderate removal (~2.5 log) of both MVM and XMuLV, and approximately 0.5-1 log inactivation of XMuLV, attributed to the low pH elution conditions.

Eshmuno® A Column	Sample	MVM Infectivity Virus Load (log $TCID_{50}$)	MVM Infectivity LRV	XMuLV Infectivity Virus Load (log $TCID_{50}$)	XMuLV Infectivity LRV	XMuLV qPCR Virus Load (log GC)	XMuLV qPCR LRV
Eshmuno® A resin: C1	Hold	8.31	2.5	6.94	3.1	8.51	2.5
	Flow-through	8.54		6.92		8.41	
	Wash	5.7		4.02		4.3	
	Eluted	5.78		3.85		***6.05***	
Eshmuno® A resin: C2	Hold	8.31	2.5	6.94	3.5	8.51	2.5
	Flow-through	8.29		6.72		8.31	
	Wash	6.19		4.63		4.34	
	Eluted	5.85		3.47		***6.05***	

Low pH Virus Inactivation

This step is dedicated to the reduction of enveloped virus and a short hold at low pH fits well in the process following elution at low pH from Protein A column. Duplicate aliquots of post Protein A elution pool were adjusted to **pH 3.6 with 100 mM Acetic Acid** then challenged with XMuLV at 1E+06 $TCID_{50}$/mL (~1% (v/v)), and incubated at room temperature for 60 mins. Rapid virus inactivation was observed and after 5 minutes, no infectious virus was detected, resulting in LRVs of at least 4 log.

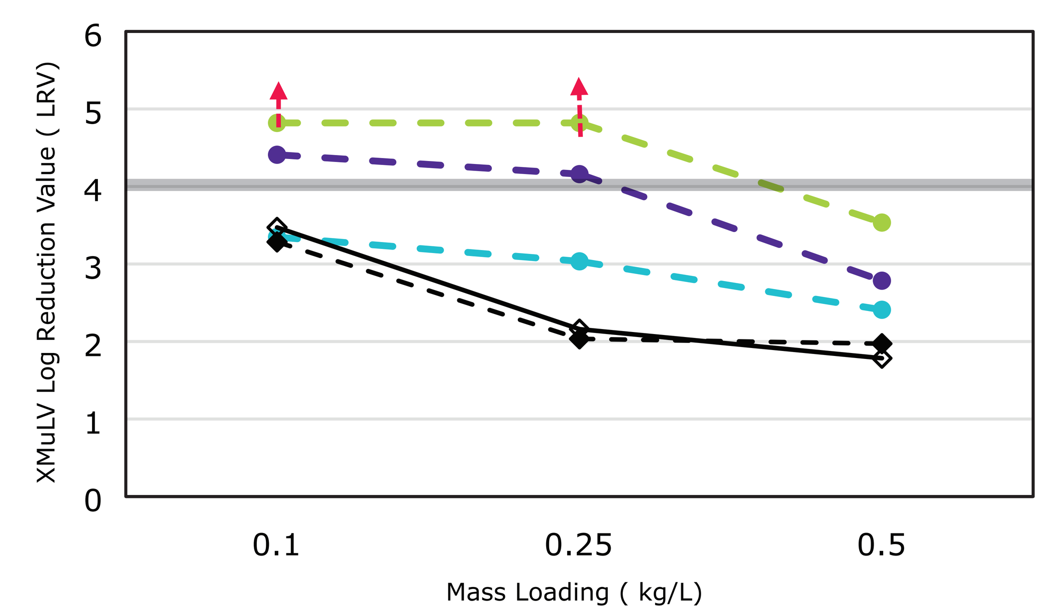
Time of incubation at pH 3.6 (min)	Replicate # 1 XMuLV LRV	Replicate # 2 XMuLV LRV
0	3.2	3.5
5	≥4.2	≥4.2
10	≥4.7	≥4.7
30	≥4.9	≥4.9
60	≥4.9	≥4.9

Cation (CEX) and Anion (AEX) Exchange Chromatography

Eshmuno® S resin is a strong cation exchange media with tentacle structure enabling fast, efficient purification. Excellent purification across **Eshmuno® S** resin was achieved with binding at pH 5.5 and elution with Tris pH 8.5, 10 mS/cm. These conditions avoided dilution of the feed before AEX polishing.

Eshmuno® S resin did not contribute to virus safety targets as less than 1 log virus was removed **(data not shown)**. However, purification over CEX media was critical to achieving acceptable viral clearance over **Eshmuno® Q** AEX resin as shown in the graph.

Clearance of MVM and XMuLV across **Eshmuno® Q** resin was evaluated at pH 7.5, 8.0 and 8.5. MVM removal was insensitive to loading pH, with LRVs of at least 5 log out to the 0.25 kg/L target loading **(data not shown)**. XMuLV removal was highly dependent on pH with effective reduction at the target 0.25 kg/L loading only achieved at pH 8.0 and pH 8.5, the optimal conditions for HCP removal.



Virus Filtration

In most biologic processes, virus filtration is the dedicated step for reduction of both enveloped and non-enveloped virus by size exclusion. Post AEX pool was pH adjusted then processed over a pre-filter **Viresolve® Pro Shield**, spiked with MVM at 2E+06 $TCID_{50}$/mL (~0.2% (v/v), then processed over **Viresolve® Pro** filter device at constant pressure of 30 psi.

At the 5.0 kg/m² target loading, processing was stopped, and product recovered. No virus was detected downstream of the filtration devices, even following product recovery, resulting in reduction of greater than 5.9 log.

Device	Sample description	LRV
Viresolve® Pro # 1	Pool at 5.0 Kg/m²	≥5.93
	Pool+ wash	≥5.93
Viresolve® Pro # 2	Pool at 5.0 Kg/m²	≥5.93
	Pool+ wash	≥5.93

Virus Clearance Summary

The purification scheme achieved virus clearance targets with a margin for safety. Importantly, the project confirmed our downstream purification technologies can be run in a connected process to deliver viral clearance performance required by customers.

Unit Operation	Target Loading	MVM LRV	XMuLV LRV
Protein A (Eshmuno® A resin)	40 mg/mL	2.5	2.5
Low pH hold at pH 3.6	NA	n/a	≥4.9
CEX (Eshmuno® S resin)	80 mg/mL	0	0.9
AEX (Eshmuno ® Q resin)	250 mg/mL	≥5.3	≥4.9
Virus Filtration (Viresolve® Pro Solution)	5 kg/m²	≥5.9	≥5.9*
Cumulative mean LRV		**13.7#**	**18.2#**

*Not tested, LRV from MVM used as surrogate for XMuLV #conservative estimate when no virus detected downstream

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