Application Note: Viral clearance from mAbs with HCPure™ and Q PuraBead® adsorbents

Biopharmaceutical products, such as monoclonal antibodies (mAbs) derived from mammalian systems, require evidence of viral clearance in the manufacturing process for approval. Viral spiking studies are utilized to determine the efficacy of viral clearance in downstream purification methods.

Minute Virus of Mice (MVM) is a small non-enveloped parvovirus which has been found as an adventitious contaminant of CHO derived drug products and which is commonly used for viral clearance studies. Cygnus Technologies has developed a BSL-1 compatible surrogate of MVM which and can be used in viral clearance studies to predict the removal of MVM.

Type C Retrovirus Like Particles (RVLP) are endogenously produced during CHO cell expression. Viral clearance is generally demonstrated using a model mammalian virus, Xenotropic Murine Leukemia Virus (XMuLV); however, the establishment of RVLP quantification methods have made it possible to track the removal of actual endogenous RVLP throughout the downstream process.

Astrea Bioseparations partnered with Cygnus to perform MVP and RVLP Spiking Studies on HCPure™ mixed-mode and Q PuraBead® Anion Exchange Chromatography resins, analyzing the samples to demonstrate MVP/RVLP concentration and Log Reduction Values (LRV's) for each study.

Study outline

A CHO derived IgG1 was partially purified by Protein A capture, viral inactivation with low pH holds and intermediate purification with CEX (SP PuraBead®) in bind and elute mode. The SP PuraBead® eluate was buffer exchanged by TFF into 50 mM Tris pH 8 and supplied to Cygnus with HCPure™ and Q PuraBead® columns packed in to Astrea Bioseparations SNAP® columns.

Cygnus technologies spiked MVM-MVP or RVLP into the feedstock before triplicate purification runs with each adsorbent. Viral clearance was assessed by q-PCR based methods available from Cygnus (M221, M231).

Primary capture with Protein A

Multiple batches of CHO IgG harvest were prepared in shake flasks (total ~ 28 L) and processed on a 9 mL GORE® Protein Capture Device with Protein A over 20 cycles. A typical run for the Protein A purification is shown.

Step	Buffer
Equilibration	PBS
Load	CHO Harvest
Post-load wash	PBS
High salt wash	PBS + 1.8 M NaCl
Equilibration wash	PBS
Arginine wash	PBS + 0.1 M Arginine pH 7.5
Elution	100 mM sodium citrate pH 3.4
Strip	0.1 M citric acid
Clean-In-Place	RO, 0.1 M NaOH, RO, PBS

The Protein A elution was held at pH 3.4 for 1 hour for viral inactivation before neutralization with 1 M Tris to pH 6.

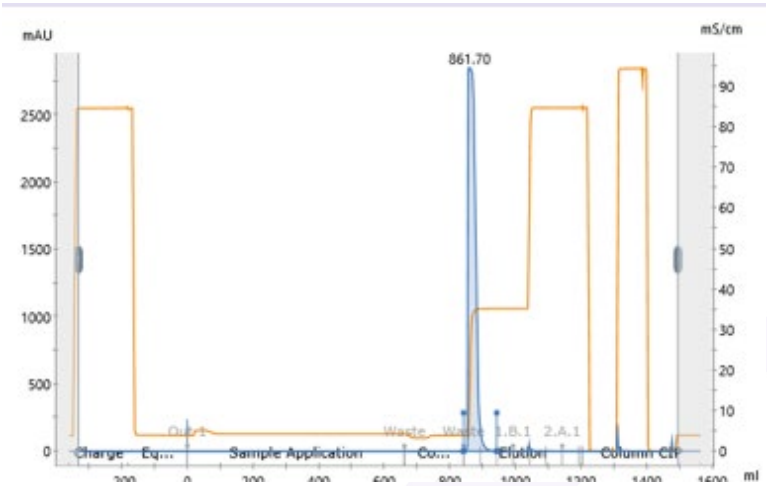
Intermediate purification with SP PuraBead® HF

The CHO IgG was further purified by cation exchange with SP PuraBead® HF. The neutralized Protein A elution was diluted to 4 mS/cm before loading 40 mg/mL adsorbent on to an 18 mL SP PuraBead® HF column packed into a 15 mm diameter SNAP® column.

The SP PuraBead® adsorbent was equilibrated and washed post-loading with 25 mM sodium citrate pH 6 and eluted with 25 mM sodium citrate, 350 mM NaCl pH 6, followed by a strip with 1 M NaCl.

The elution fraction contained 25% of the HCPs, but 100% of the IgG.

The final composition of the feedstock for the viral clearance study post-TFF is shown below.



IgG concentration (mg/mL)	% Aggregate	HCP levels (ppm)	HC DNA levels (ppb)
2.84	0.5	68	1056

IgG polish with HCPure™ or Q PuraBead® HF

Q PuraBead® is a strong anion exchange adsorbent which binds negatively charged analytes at low conductivity. In this work, the adsorbent is being used in flow-through mode at a pH below the PI of the IgG so that the target protein (IgG) passes through the column, while any negatively charged impurities are bound for removal.

HCPure™ is a mixed-mode adsorbent with electrostatic and hydrophobic character, designed for HCP removal from a range of expression systems. This adsorbent is mainly used in flow-through mode at low to moderate conductivity (< 18 mS/cm) primarily utilizing hydrogen bonding to bind impurities whilst the target protein passes through the column. As the binding mode of HCPure™ is more complex than ion exchange, optimization of pH and conductivity conditions is required for each target protein.

An example purification run with the same buffer conditions to be used for the viral clearance study was performed on 1 mL pre-packed HCPure™ and Q PuraBead® HF columns with CHO IgG after Protein A purification and buffer exchange in to 50 mM Tris pH 8. No SP PuraBead® HF step was used for this feedstock.

The columns were equilibrated with 50 mM Tris pH 8 before loading 21 mg IgG in flow-through mode. The UV peak was collected and assessed for IgG recovery by A280 and HCP by ELISA (Cygnus CHO HCP F550-1).

Both the HCPure™ and Q PuraBead® HF adsorbents showed good IgG recovery and HCP clearance.

Run Description	IgG (mg)	HCP (ppm)
Load	21	172
HCPure™ UV peak	20	58
Q UV peak	22	54

HCPure™ viral clearance results

Triplicate runs on a 7.85 mL HCPure™ column were performed with CHO IgG spiked with RVLP or MVM_MVP in 50 mM Tris pH 8 in flow-through mode. The column was equilibrated with 50 mM Tris pH 8 before loading the spiked feedstock and collecting the UV peak in the flow-through. The column was regenerated with a low pH, high salt wash (50 mM sodium citrate, 1 M NaCl, pH 3) followed by cleaning with 0.5 M NaOH to ensure any bound materials were removed between runs.

The IgG recovery was determined by A280 and the viral clearance using the Cygnus q-PCR methods. The IgG recovery was excellent for all runs. The viral clearance showed very good RVLP removal, but no clearance of MVP was seen under these conditions.

Run Description	% IgG Yield	Log reduction value (>)
RVLP HCPure™ Run 1	96	3.4
RVLP HCPure™ Run 2	95	3.4
RVLP HCPure™ Run 3	97	3.34
MVP HCPure™ Run 1	94	0.04
MVP HCPure™ Run 2	97	0.16
MVP HCPure™ Run 3	96	0.12

Q PuraBead® HF viral clearance results

Triplicate runs on a 7.85 mL Q PuraBead® HF column were performed with CHO IgG spiked with RVLP or MVM-MVP in 50 mM Tris pH 8 in flow-through mode. The column was equilibrated with 50 mM Tris pH 8 before loading the spiked feedstock and collecting the UV peak in the flow-through. The column was regenerated with a high salt wash (50 mM sodium citrate, 1 M NaCl), followed by cleaning with 0.5 M NaOH to ensure any bound materials were removed between runs.

The IgG recovery was determined by A280 and the viral clearance using the Cygnus q-PCR methods. As seen with HCPure™, the IgG recovery levels were also excellent for all runs. The viral clearance also demonstrated very good viral reduction for both virus models under these conditions.

Run Description	% IgG Yield	Log reduction value (>)
RVLP Q Run 1	97	3.56
RVLP Q Run 2	95	3.83
RVLP Q Run 3	96	3.69
MVP Q Run 1	97	4.27
MVP Q Run 2	96	4.42
MVP Q Run 3	96	4.43

Conclusion

The results demonstrate that Astrea Bioseparations' HCPure™ and Q PuraBead® HF adsorbents can be very effective at viral clearance, whilst maintaining excellent recoveries of target protein.

The spiking study was performed using Cygnus technologies on HCPure™ and Q PuraBead® HF for viral clearance with two model virus proteins MVM-MVP and RVLP. The Q PuraBead® HF adsorbent showed ≥ 3.56 log reduction for RVLP and ≥ 4.27 log reduction for MVM-MVP. HCPure™ showed ≥ 3.34 log reduction for RVLP but no clearance of MVM-MVP under these conditions.

Further work may demonstrate improved MVM clearance using HCPure™ by optimizing the pH and conductivity.

In the case study outlined within this application, HCPure™ acts as an effective intermediate polishing step to reduce HCPs, followed by Q PuraBead® as an orthogonal polishing step, removing any residual impurities, as well as viral clearance.

+44 (0) 1223 433 800 | astreabioseparations.com

sales@astrea-bio.com | techsupport@astrea-bio.com | quality@astrea-bio.com

Global bases in North America, Canada and Cambridge UK HQ:
Horizon Park, Barton Road, Comberton, Cambridge, CB23 7AJ, UK

Astrea Bioseparations