

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

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Abstract

Biopharmaceutical products such as monoclonal antibodies (mAbs) which are derived from mammalian systems require evidence of viral clearance in the manufacturing process. Viral spiking studies are utilized to determine the efficacy of viral clearance in downstream purification methods (1).

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 has been shown to be physicochemically similar to live MVM and can be used as an alternative to MVM for viral clearance studies (2).

Cygnus Technologies' Type C Retrovirus Like Particles (RVLP) are endogenously produced during CHO cell expression. Endogenous viral clearance is generally demonstrated with a model mammalian 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 Ltd partnered with Cygnus Technologies to demonstrate viral clearance using HCPure™ mixed-mode and Q PuraBead® Anion Exchange Chromatography resins with MVM-MVP and RVLP spiking studies.

1 Study outline

A CHO derived IgG was partially purified by Protein A capture, viral inactivation with low pH hold and intermediate purification with CEX chromatography (SP PuraBead® HF) in bind and elute mode. The SP eluate was buffer exchanged by TFF into 50 mM Tris pH 8 and supplied to Cygnus Technologies with HCPure™ and Q PuraBead® HF columns packed in to Astrea Bioseparations SNAP® Laboratory Glass Columns at 10 cm bed height.

Cygnus Technologies spiked MVM-MVP or RVLP into the feedstock before triplicate purification runs with each adsorbent in flow through mode. Viral clearance was assessed by qPCR methods provided by Cygnus Technologies.

Carry over experiments with no viral load were performed to confirm the strip and CIP were sufficient between replicate runs on the same column.



2 Primary capture with Protein A

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

Pressure increases from multiple cycles was recovered using the extended wash regime recommended by the supplier which utilizes an acidic wash (100 mM arginine pH 2.3) and a reducing agent wash (50 mM Tris, 100 mM NaCl, 1% SDS, 10 mM DTT pH 10.4).

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.

Step	Buffer
Equilibration	PBS
Load	CHO harvest
Post-load wash	PBS
High salt wash	PBS + 1.8 M NaCl
EQ 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
CIP	0.1 M NaOH

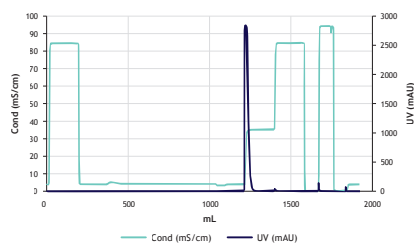
3 Intermediate purification with SP PuraBead® HF

The neutralized Protein A elution was diluted to 4 mS/cm before loading 40 mg/mL adsorbent on to an 18 mL SP column packed in a 15 mm diameter SNAP® column.

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

The elution fraction contained only 25% of the host cell protein impurities (HCP) but 100% of the IgG.

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



	IgG concentration (mg)	Aggregate (%)	HCP concentration (ppm)	HC DNA concentration (ppb)
CHO IgG feedstock	2695	0.5	68	1056

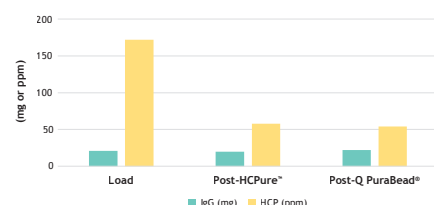
4 IgG polish with HCPure™ or Q PuraBead®

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 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.

Both the HCPure™ and Q PuraBead® HF adsorbents showed good IgG recovery (95 - 105%) and HCP clearance from 172 ppm in the load to 50 ppm.

HCP reduction from IgG with HCPure™ or Q PuraBead®



5 Viral clearance testing

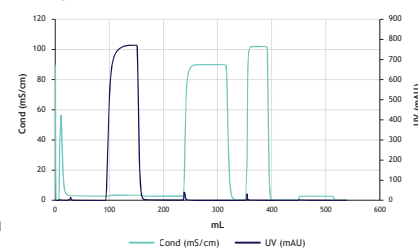
Triplicate runs on the 10 cm bed height HCPure™ and Q PuraBead® HF columns were performed with CHO IgG spiked with RVLP or MVM-MVP in 50 mM Tris pH 8.

The columns were equilibrated with 50 mM Tris pH 8 before loading the spiked feedstock at 20 mg IgG/mL adsorbent and collecting the UV peak in the flow through.

The columns were regenerated with 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.

Carry over experiments confirmed the success of the strip and CIP.

The virus concentration in the load, flow through and strip were measured by qPCR and the log clearance calculated.

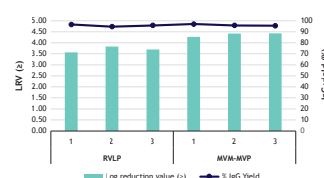


6 Viral clearance results

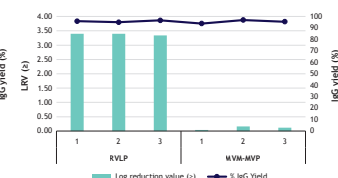
Excellent recovery of IgG was seen for all runs (94 - 97%). Viral clearance to below the limit of detection was seen for both model viruses with the Q PuraBead® adsorbent and for the RVLP for the HCPure™ adsorbent, corresponding to ≥3.3 log for RVLP and ≥4.2 log for MVM-MVP. No clearance of MVM-MVP was seen for HCPure™ under these conditions.

Therefore, for the CHO IgG used in this example, a suitable purification method could be a Protein A capture step followed by HCPure™ for host cell impurity removal followed by Q PuraBead® HF for viral clearance.

Viral clearance from CHO IgG on Q PuraBead®



Viral clearance from CHO IgG on HCPure™



Conclusions

A spiking study was performed by Cygnus Technologies on HCPure™ and Q PuraBead® HF for viral clearance with two model virus proteins MVM-MVP and RVLP. The Q PuraBead® 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.

The viral spiking agents and qPCR methods used by Cygnus Technologies are available off the shelf.

In addition, Astrea Bioseparations' adsorbents are available pre-packed into the Astrea Bioseparations SNAP® Laboratory Glass Columns range making them a great tool for viral clearance studies within your own laboratory.

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