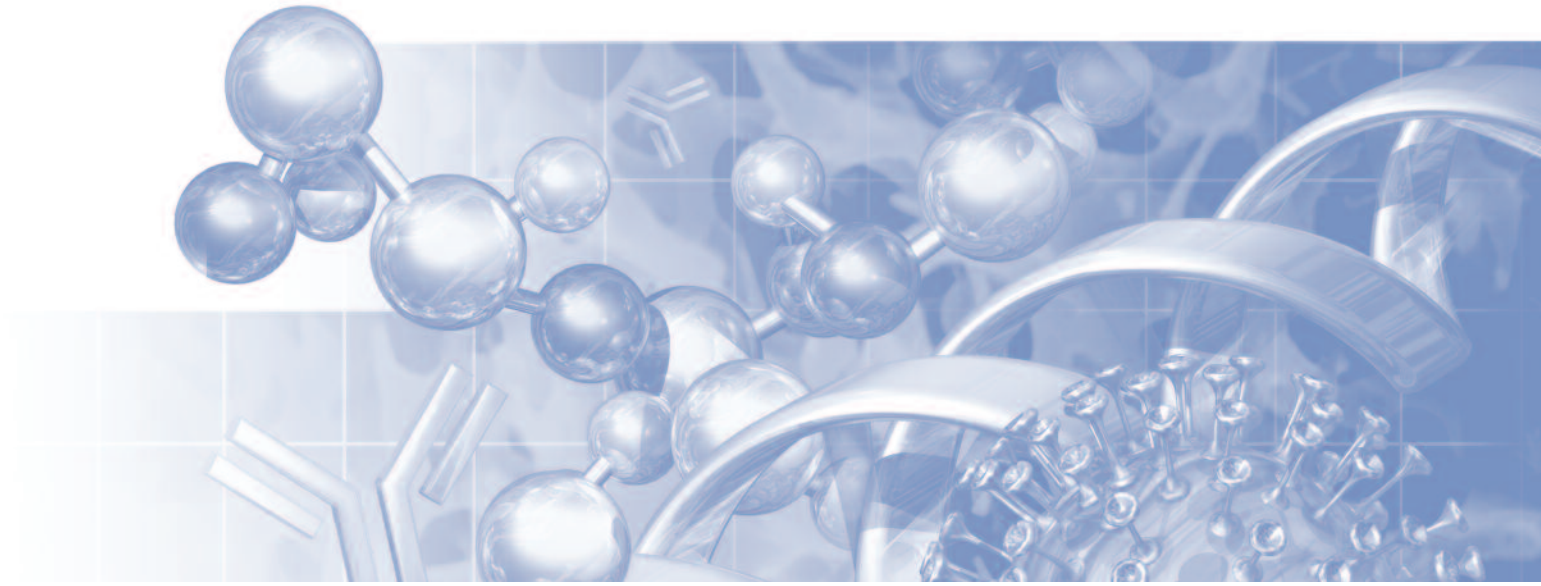
PALL Life Sciences

Application Note

USTR 2827

Implementation of Mustang® Q Membrane Chromatography as a Polishing Step (Residual DNA Removal) in Monoclonal IgG_1 Production from CHO Cell Culture

In collaboration with ProBioGen AG, Berlin, Germany



*Filtration. Separation. Solution.*SM

1. Summary

Mustang Q membrane chromatography was evaluated as a polishing step following protein A affinity and cation exchange chromatography to remove residual host cell DNA during a Monoclonal antibody (MAb) purification process at 250 L cell culture production-scale. Data indicated efficient DNA clearance by the Mustang Q membrane chromatography step during the process (<16 pg/mL), while keeping an excellent MAb recovery (>96%). Additionally, contribution of the Mustang Q membrane chromatography polishing step to HCP removal was shown. Based on a virus-spiking scale-down study using two model-viruses (Murine Leukemia Virus (MuLV) and Minute Virus of Mice (MVM)), effective MuLV virus removal was demonstrated using Mustang Q membrane chromatography, while MVM virus clearance needed further optimization. Overall viral clearance capacity was sufficient for manufacturing of a safe drug substance for clinical trials.

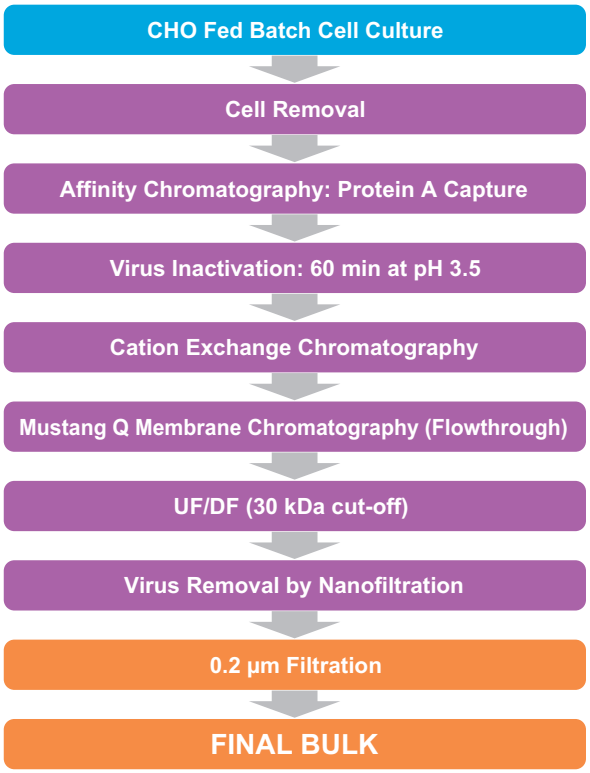
2. Introduction

A GMP-compliant manufacturing process (Figure 1) was established at 250 L culture-scale for the supply of a monoclonal gamma-globulin class 1 (IgG_1) material for toxicological studies and later-on for phase I clinical trials. Current platforms for MAb purification mostly consist of three packed bed chromatography steps including Protein A as a capture step and typically cation exchange or hydrophobic interaction as intermediate steps. Membrane chromatography is increasingly used as a polishing step to remove contaminants (residual DNA, Host Cell Proteins) and provides additional process-specific virus clearance. Mustang membrane chromatography capsules are disposable, easy to use and offer effective solutions for polishing.

The Mustang Q membrane chromatography step implemented here for host cell DNA removal was conducted in “negative mode”. Lab-scale development using Mustang Q Acrodisc® unit as well as intermediate-scale and production-scale on Mustang Q capsules of 10 mL and 60 mL bed volume respectively, are described here.

Figure 1

ProBioGen’s Monoclonal IgG_1 Production Process from CHO Fed-batch Cell Culture



3. Materials and Methods

3.1 Cell culture

Monoclonal IgG_1 was produced in serum-free CHO (Chinese Hamster Ovary) cell culture at 50 L and 250 L fed-batch scale for toxicological trials and GMP production runs for phase 1 clinical trials by ProBioGen AG, Berlin, Germany.

3.2 Lab-scale optimization of process conditions on Mustang Q unit:

Unit: 0.18 mL Mustang Q Acrodisc unit (Pall)

Running buffer: 20 mM Na Phosphate pH 7.0, 0.2 M NaCl

DNA elution buffer: 20 mM Na Phosphate pH 7.0, 1 M NaCl

Load for the DNA spiking experiment: 5.5 µg Calf Thymus DNA in running buffer

Load for the process test experiment: 68 mL of a cation exchange (CEX) chromatography column eluate at 3.2 mg IgG/mL and 22-25 mS/cm.

Flow rate: 10 bed volumes/min (1.8 mL/min).

3.3 Implementation of manufacturing process runs on Mustang Q unit at 50 L and 250 L cell culture-scale (Table 1, Figure 2)

Unit: 10 mL Mustang Q CLM05 capsule or 60 mL Mustang Q CL3 unit (Pall)

Initial lab-scale conditions were slightly modified with regard to the conductivity of the running buffer and the process solution:
Running buffer: 20 mM Na Phosphate pH 7.0
Load: CEX chromatography column eluate diluted 2-fold in running buffer (resulting in final conductivity of ~11-12.5 mS/cm)
DNA elution buffer: 20 mM Na Phosphate pH 7.0, 1 M NaCl

Table 1

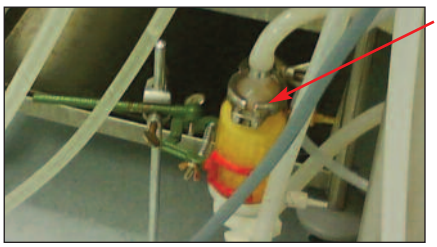
Process Conditions for Mustang Q membrane chromatography implemented at 50 L (Tox 2) and 250 L culture-scale (Tox 1 and GMP)

Process conditions	Production run Tox 1	Tox 2	GMP
Culture-scale	250 L	50 L	250 L
Process volume	225 L	44 L	238 L
Mustang Q membrane volume	60 mL capsule (CL3)	10 mL capsule (CLM05)	60 mL capsule (CL3)
DNA binding capacity	1.2-1.5 g	200-250 mg	1.2-1.5 g
Expected DNA load (process)	Low mg-range	µg-range	Low mg-range
Flow rate	36 L/h 10 MV*/min	30 mL/min** 3 MV*/min**	36 L/h 10 MV*/min

** MV: membrane volume according to supplier specification*
*** Low flow rate due to pressure limitations at the chromatography system*

Figure 2

Manufacturing run on a 60 mL bed volume Mustang Q capsule



Courtesy of Dr Martin Suhr, ProBioGen AG.

All chromatography runs were performed using ÄKTA◆ systems (GE Healthcare).

3.4 Scale-down virus-spiking study on Mustang Q Acrodisc unit

Two model viruses, Murine Leukemia Virus (MuLV) and Minute Virus of Mice (MVM) (Table 2), were selected for a spiking study to demonstrate the viral clearance capacity of the final DSP process in a scale-down model.

Table 2

Model viruses used for the virus spiking study

Virus	Genome	Enveloped	Family	Size [nm]	Chemical resistance
MuLV	RNA	yes	Retro	80-110	Low
MVM	DNA	no	Parvo	~20	Very high

Membrane chromatography unit: 0.18 mL Mustang Q Acrodisc unit

Running/process buffer: 20 mM Na Phosphate pH 7.0

Load: Process solution spiked with MuLV or MVM virus solution (5% volume spike)

3.5 Analytics

IgG_1 purity was assessed using a Size Exclusion Chromatography column (Superdex◆ 200 5/150 GL, GE Healthcare)

IgG_1 concentration was determined by UV_{280nm} measurement.

Residual DNA was measured by use of the Picogreen◆ Assay (Invitrogen) or by quantitative PCR (qPCR) performed by an external service provider.

Quantification of Host Cell Proteins (HCPs) was done by ELISA using a kit from Cygnus Technologies (HCP Host Cell Proteins, #F015).

Virus spiking study was performed by an external service provider under GLP using a process scale-down model. The virus titer was determined by endpoint titration and/or large volume plating.

4. Results and Discussion

The final goal of the Mustang Q membrane chromatography step evaluated in the current process was to achieve low levels of residual DNA in the final MAb product according to the < 150 pg/dose rule.

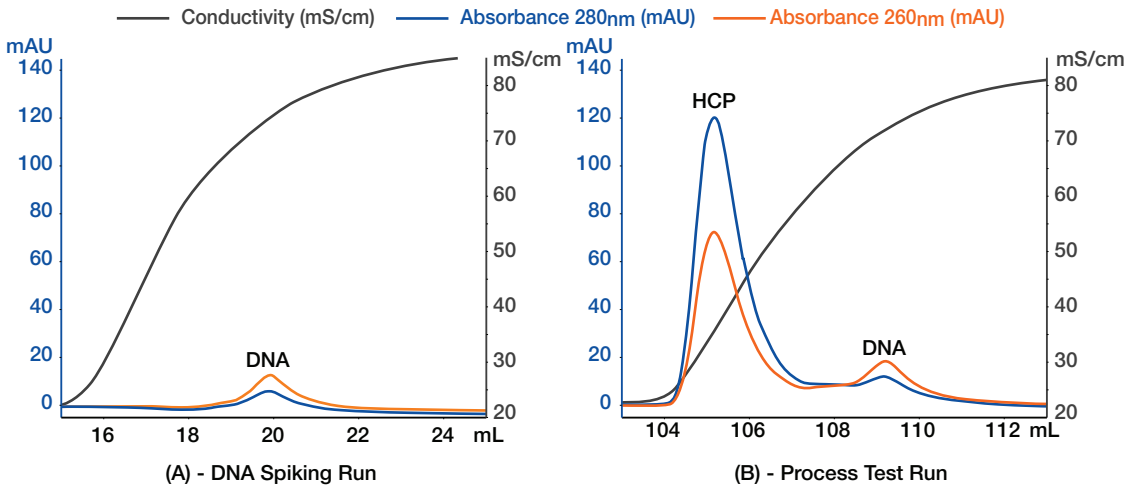
4.1 Lab-scale optimization of Mustang Q membrane chromatography process conditions for residual host cell DNA removal

A preliminary lab-scale DNA spiking experiment using calf thymus DNA was carried out to investigate load and elution conductivity values for DNA elimination with a Mustang Q Acrodisc unit (typical DNA binding capacity of 3.6 mg). A DNA load of 5.5 µg DNA was chosen as a process representative impurity load of this process step based on Picogreen data. The chromatography profile confirmed adsorption of DNA in 20 mM Na Phosphate pH 7.0, 0.2 M NaCl followed by a desorption in the same buffer containing 1 M NaCl (75 mS/cm) (Figure 3A, zoom of the ÄKTA profile showing DNA elution).

The same conditions were applied in a process test experiment where a cation exchange (CEX) chromatography column eluate was directly loaded onto the Mustang Q membrane chromatography unit (Figure 3B, zoom of the elution step). It confirmed the retention of residual DNA and its elution at 72 mS/cm.

Figure 3

DNA-spiking and process test experiments on 0.18 mL Mustang Q Acrodisc unit



Running buffer: 20 mM Na Phosphate pH 7.0, 0.2 M NaCl
DNA elution buffer: 20 mM Na Phosphate pH 7.0, 1 M NaCl
Loading conditions for:
- DNA spiking run (A): 5.5 µg Calf Thymus DNA in running buffer
- Process test run (B): cation exchange chromatography column eluate

Conclusion of 4.1

Optimization of the process conditions on a Mustang Q Acrodisc unit evidenced the elimination of residual DNA in negative mode (retention of DNA onto the Mustang Q membrane and elution at 75 mS/cm). Potential elimination of HCP peak in negative mode was also seen eluting earlier in the salt gradient.

4.2 Implementation of Mustang Q membrane chromatography process conditions to manufacturing-scale

Process conditions in previous section were based on a load conductivity of ~22-25 mS/cm of the CEX eluate. Effective virus clearance using anion exchanger in negative mode is dependent on the conductivity of the feedstock. Therefore the process conditions were slightly modified to reduce the load conductivity to ~11 mS/cm by implementation of a 1:2 dilution of the CEX eluate. Furthermore the running buffer was changed to 20 mM Na Phosphate, pH 7.0.

The modified conditions were applied to run the manufacturing processes on 10 mL and 60 mL Mustang Q capsules at 50 L and 250 L culture-scales, respectively. The 50 L culture-scale was produced for toxicology studies (Tox - 50 L) and the 250 L scale was produced for toxicology (Tox - 250 L) and GMP (GMP - 250 L) batches.

The final conditions were not further optimized at this development stage. Therefore the 60 mL Mustang Q capsule was selected for the 250 L culture-scale by taking into account a safety factor (potential biological variation of the feedstock composition).

4.2.1 DNA reduction during IgG_1 purification process including the Mustang Q membrane chromatography polishing step

Prior to the Tox- and GMP-production runs, two development runs at 10 L culture-scale (10 L – Dev) were carried out. Residual DNA was measured by use of the Picogreen assay. Data for both runs showed that the DNA content was already in the low ng range after the CEX chromatography step (Table 3). Therefore this assay could not be applied for analysis after Mustang Q membrane chromatography due to the limit of detection.

Table 3

DNA levels after the first and second chromatography steps of the process, measured using Picogreen assay, during 2 development runs at 10 L culture-scale

	Development run			
	10 L – Dev 1		10 L – Dev 2	
Process step	DNA ng/mL	IgG_1 mg/mL	DNA ng/mL	IgG_1 mg/mL
Cell culture supernatant	5951	n/a	7538	n/a
After first chromatography (Protein A)	19.0	6.6	22.2	7.2
After second chromatography step (Cation exchanger)	4.2	3.4	2.2	3.4

n/a = Not applicable

Samples derived from the Mustang Q membrane chromatography step (flowthrough) had to be analyzed by use of a qualified qPCR method (assay sensitivity below 1 pg).

Analysis of a final product sample withdrawn after pooling the product of both development runs (10 L scale) resulted in 12 pg residual DNA/mL thus confirming efficient DNA removal by Mustang Q membrane chromatography.

This finding was confirmed later on as the data obtained for the production runs showed that residual DNA was efficiently removed to a concentration close to the pg/mL range (Table 4).

Table 4

DNA levels after the Mustang Q membrane chromatography polishing step, measured using qPCR assay, at 50 and 250 L culture-scale

	Production run		
Process step	Tox - 250 L	Tox - 50 L	GMP - 250 L
Final bulk	< 40 pg/mL*	< 17 pg/mL*	< 16 pg/mL*

** result below lower limit of quantification of the assay (LLOQ)*

Conclusion of 4.2.1

The Mustang Q membrane chromatography polishing step was efficient to remove host cell residual DNA in the process. This step was therefore implemented in the GMP purification process at 250 L scale for the production of IgG_1 for early clinical trials.

4.2.2 IgG_1 recovery after polishing step on Mustang Q capsules at manufacturing-scale

Determination of the product concentration by UV280 nm measurement showed high IgG_1 recovery (>96%) for the Mustang Q membrane chromatography step, independently of the process-scale (Table 5).

Table 5

Process conditions and IgG_1 recovery for Mustang Q membrane chromatography implemented at 10 L, 50 L and 250 L culture-scales

Run	Load volume (mL)	Mustang Q capsule (mL)	Conductivity (mS/cm)	IgG_1 concentration (g/L)	Protein load (g product/mL bed volume)	IgG_1 recovery (%)
250 L - Tox	37600	60	12.9	1.7	1.07	96.1%
50 L - Tox	5609	10	11.8	2.4	1.34	97.9%
250 L - GMP	40150	60	12.5	1.7	1.14	104.2%

High consistency was achieved for the three production runs (Tox, GMP) regarding load conductivity and protein load (g product/mL bed volume). The variation of the product recovery between 96 and 104% may be explained by accuracy of the volume determination which was done by weight measurement of the process/ product solutions.

Conclusion of 4.2.2

High IgG_1 recovery (>96%) was achieved for the Mustang Q membrane chromatography polishing step performed in negative mode.

4.2.3 HCP removal during IgG_1 production process including the Mustang Q membrane chromatography polishing step

Although the membrane chromatography step was not specifically designed for HCP removal in this process, the HCP data showed that the Mustang Q membrane chromatography polishing step contributed to HCP depletion to finally meet the established rule of < 100 ppm at the end of the process (Table 6). Additionally, HCP removal was quite consistent over the different cell culture batches (50 L and 250 L).

Table 6

Host Cell Protein (HCP) removal during the IgG_1 production process at 50 and 250 L culture-scales, including after the Mustang Q chromatography step

	HCP level (ppm) of production run		
Process step	250 L – Tox	50 L – Tox	250 L – GMP
Clarified Cell Culture supernatant	181,592	110,117	113,665
After Protein A chromatography	1074	ND*	295
After CEX chromatography	70	86	39
After Mustang Q membrane chromatography	45	41	25
After nanofiltration	25	33	27

**ND: not determined*

Conclusion of 4.2.3

The Mustang Q membrane chromatography polishing step contributed to HCP depletion.

4.2.4 Viral clearance during IgG_1 production process including the Mustang Q membrane chromatography polishing step

Potential contribution of the Mustang Q membrane chromatography polishing step to virus depletion was studied using two virus-spiking tests on Mustang Q Acrodisc units (Table 7).

The viral clearance capacity of the membrane chromatography step was demonstrated for MuLV (log10 reduction factor > 4). Lower LRF values were obtained for MVM under the process conditions tested (LRF ~ 1). As the MVM clearance may be affected by composition of the feedstock, load conductivity could be further optimized.

Table 7

Results of viral clearance during the virus-spiking scale-down study

Membrane adsorber	Log_{10} reduction factor (LRF) (MVM)	Log_{10} reduction factor (LFR) (MuLV)
Test 1	1.30 ± 0.41	4.60 ± 0.29
Test 2	1.12 ± 0.33	> 5.16 ± 0.27

However, the overall viral clearance capacity of the production process was quite high with a LRF ≥ 9 for MVM and LRF ≥ 13 for MuLV (Table 8). A moderate reduction was shown for MVM during affinity chromatography (LRF > 3), effective reduction for MuLV during low pH treatment and during the Mustang Q membrane chromatography polishing step (LRF > 4) and effective reduction, for both viruses applying the 20 nm nanofiltration (LRF > 4).

Table 8

Assessment of the virus clearance capacity of the manufacturing process. Data expressed as Log_{10} values.

Process steps	Log_{10} reduction factor (LRF) for MuLV	Log_{10} reduction factor (LRF) for MVM
Cell removal	Not tested	Not tested
Affinity chromatography	Not tested	3.16 ± 0.36
Low pH treatment	4.23 ± 0.27	Not tested
CEX chromatography	Not tested	Not tested
Mustang Q membrane chromatography	4.60 ± 0.29	< 2
TFF	Not tested	Not tested
Nanofiltration	≥ 4.49 ± 0.28	≥ 6.17 ± 0.26
0.22 µm filtration	Not tested	Not tested
Final assessment		
Virus clearance capacity	LRF ≥ 13.3	LRF ≥ 9.3

Conclusion of 4.2.4

Overall viral clearance capacity of the entire process was sufficient for manufacturing of a safe drug substance for clinical trials phase 1.

5. General Conclusions

- A Mustang Q membrane chromatography polishing step was successfully implemented in a flowthrough mode for removal of residual DNA in an IgG_1 purification process at production-scale, following two conventional column chromatography steps (protein A and cation exchange).
- It was efficient to remove host cell residual DNA below the LLOQ of the qPCR assay applied for analysis.
- Additionally, it showed a high IgG_1 recovery (>96%), independent of the scale applied.
- Contribution of the Mustang Q membrane chromatography polishing step to HCP removal was shown.
- Effective MuLV virus removal using Mustang Q membrane chromatography was demonstrated based on a virus-spiking scale-down study while MVM virus clearance needed further optimization.
- Overall viral clearance capacity was sufficient for manufacturing of a safe drug substance for clinical trials.
- Mustang membrane chromatography capsules are disposable and offer speed and ease-of-use for polishing applications

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