DNA impurity removal at the clarification stage is the key to mAb purification process efficiency improvement

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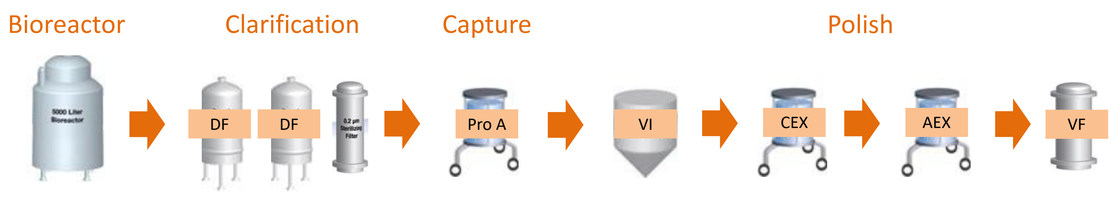
Introduction

Development of robust platform manufacturing processes for therapeutic proteins is required to rapidly advance a pipeline of bio-pharmaceutical candidates towards commercial scale.

In mAb purification, the protein A capture step is the key step that determines the robustness and the efficiency of the entire downstream process. Variability in the post protein A purity is a challenge for many biopharmaceutical manufacturers, as it results in the need for elaborate and highly engineered downstream purification processes. The specific downstream purification strategies may differ not only from company to company, but also within one company's product pipeline. Overall, most mAb manufacturing processes have a sequence of process steps similar to the scheme below:



The main advantage of the protein A capture column, and the reason for its widespread use, is its ability to remove 99.9%-99.99% of HCP from the process stream. However, in modern mAb processes with high cell densities and high titers, the purity after the protein A column is typically in the 95-98% range.

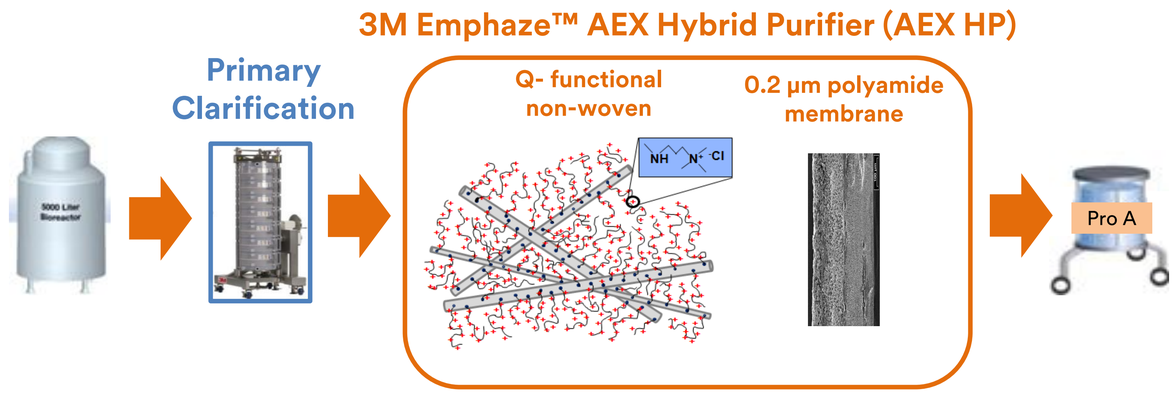
We present a chromatographic clarification strategy that significantly increases performance and robustness of Protein A capture chromatography. The advantage of this approach is demonstrated on mAb purification processes starting from the Bioceros CHO^{BC} ® cell line.

Chromatographic Clarification using 3M Emphaze™ AEX Hybrid Purifier

Conventional clarification strategies typically rely on depth filtration, sometimes preceded by a centrifugation step. The primary objective of these steps is to retain cells and other insoluble impurities, such as cell debris. The performance of these steps is often evaluated by the turbidity of the clarified cell culture fluid and the ability to protect a 0.2 µm sterilizing grade membrane. The limitation of these technologies is their ability to clear soluble impurities that may interfere with downstream chromatography.

Host cell DNA-related impurities such as chromatin have been shown to interfere with the protein A capture step and negatively impact its performance (1, 2). The presence of chromatin can cause co-elution of host cell protein impurities with the protein of interest.

By introducing a chromatographic clarification step using the 3M Emphaze AEX Hybrid Purifier before the capture step, the challenge of the soluble impurities can be addressed.



3M Emphaze AEX Hybrid Purifier captures soluble and insoluble contaminants during clarification using the Q-functional non-woven material. The non-woven is constructed of a sparse polypropylene fiber scaffold with grafted positively charged Q-functional polymer.

In a typical mAb process, the 3M Emphaze AEX Hybrid Purifier will result in a 30% reduction of HCP and > 4 LRV reduction of DNA. Due to the removal of DNA-related impurities early on, the performance of the protein A capture step is increased, which typically results in over 10x lower HCP concentration in the elution pool, compared to traditional clarification (3).

References

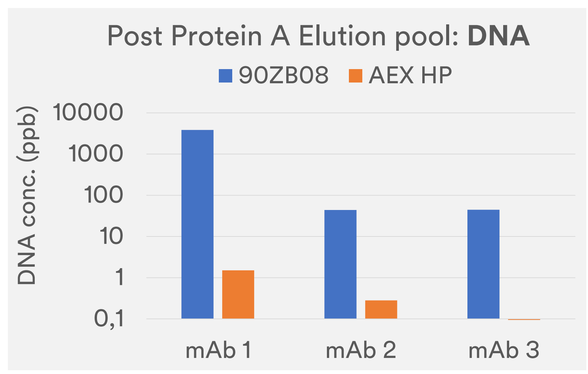
(1) Emerging Challenges to Protein A, P. Gagnon, BioProcess International, 2013

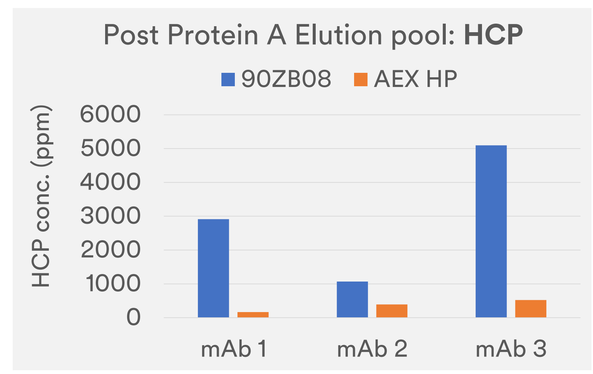
(2) Nonspecific interactions of chromatin with immunoglobulin G and protein A, and their impact on purification performance, P. Gagnon *et. Al, Journal of Chromatography A, 2014*

(3) Anion-Exchange Chromatographic Clarification: Bringing Simplification, Robustness, and Savings to MAbPurification, A. Castro-Forero., et. al., BioProcessInternational, June, 2015

Increase in Protein A HCP Clearance

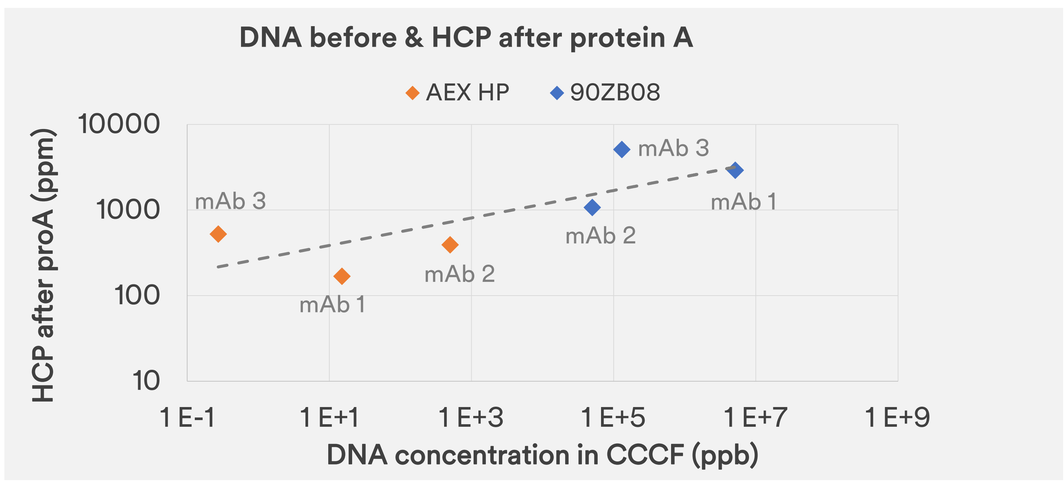
Clarification using the chromatographic solution results in significant reduction in process related impurities in the Protein A elution pools across 3 different mAbs. Furthermore, highly consistent post Protein A impurity concentrations enable platformability and rapid process development across the entire candidate pipeline.





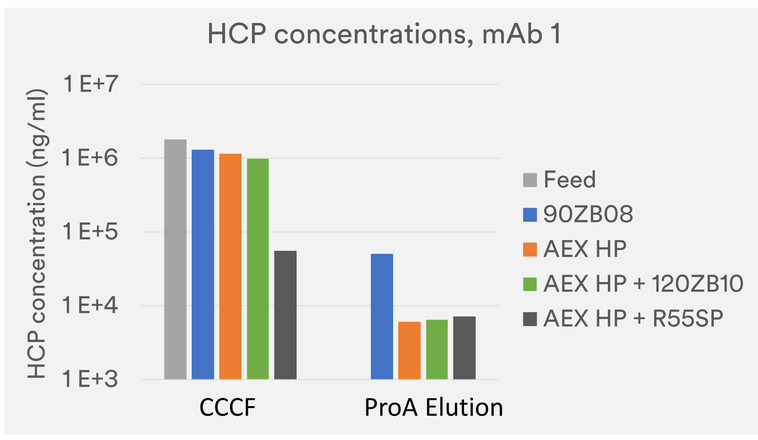
DNA Interference with Protein A Capture

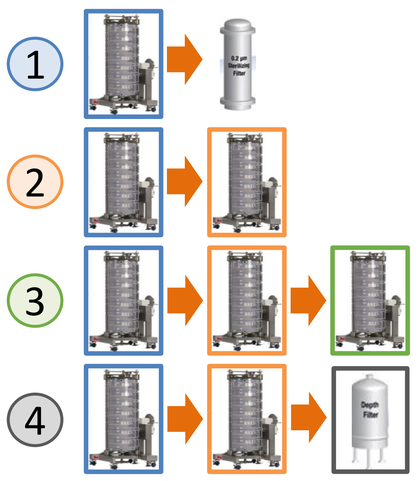
We studied the effects of DNA interference with Protein A chromatography on this capture step's ability to remove HCP.



On a log-log scale, the HCP impurity levels after the capture step are correlated with the amount of DNA in CCCF being loaded onto Protein A. Reduction of DNA by chromatographic clarification directly results in lower HCP levels in the downstream process.

No Impact from HCP Reduction at Harvest





Effect of HCP reduction at harvest on post Protein A HCP levels were evaluated by introduction of additional adsorptive depth filtration steps after Emphaze AEX HP.

- **Highly charged large surface area depth filter – 3M ZetaPlus™ 120ZB10**
- **Adsorptive activated carbon filter - 3M ZetaPlus™ R55SP**

None of these HCP clearance enhancements at harvest produced a meaningful reduction of HCP in the Protein A elution pool.

Conclusions

This work demonstrates that chromatographic clarification reduces key host cell impurities before and after the capture step. The strategy is robust, as demonstrated across 3 different mAb biosimilars expressed in the Bioceros CHO^{BC} ® cell line.

Removal of DNA-related impurities at harvest is the key: it improves the efficiency of the protein A column and results in lower HCP levels in the eluate. At the clarification step, DNA should be the main concern. Reduction of the HCP content in the CCCF may not result in any downstream impurity reduction.