Virus Removal by Simple Depth Filtration in a Two-Step Downstream Process for mAb Production

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Findings

- mAb purification processes relying on 3 chromatographic steps ensure excellent product quality and patient safety.
- Antibody sequences with 2 chromatographic steps enable economic improvement maintaining product quality.
- Virus removal by anionic adsorption on depth filters resulted in 2.0 – 5.0 log_{10} reduction factors for MuLV and 2.5 – 7.0 log_{10} reduction factors for MVM.
- Virus removal by depth filtration opens access to a two column step purification platform.

DSP Platform for mAb Products

Generic development platforms for the robust purification of monoclonal antibodies and antibody-related molecules are often driven by facility needs and different strategies of the companies. Most of them include an affinity step using Protein A and two bind/elute or flow through polishing steps. As many platforms were improved over time, one polishing step might be sufficient for adequate process robustness and appropriate product quality of the drug substance.

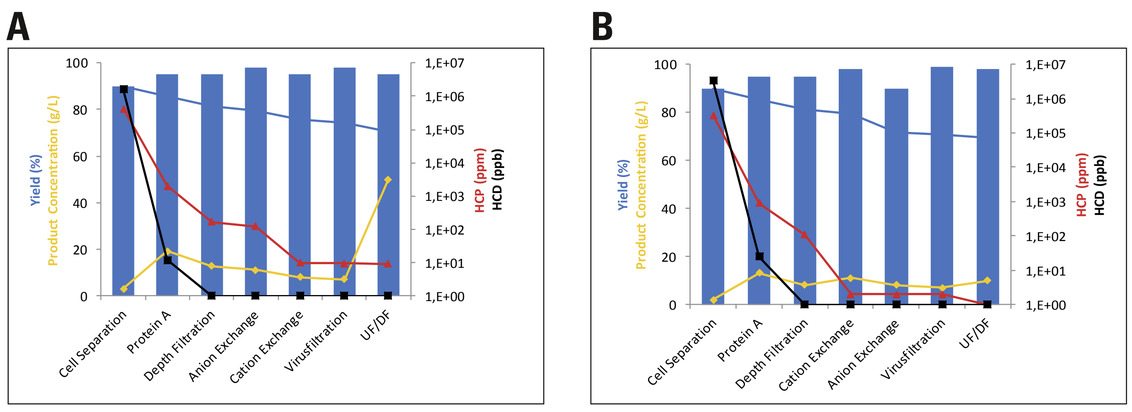

Figure 1: Performance of downstream processes with 3 column steps (A: mAb 1, B: mAb 2). Anion exchange step was needed for virus safety. Step yields given in bars, total yields in lines. Assays: HCP (F550, Cygnus); HCD (qPCR, Rentschler).

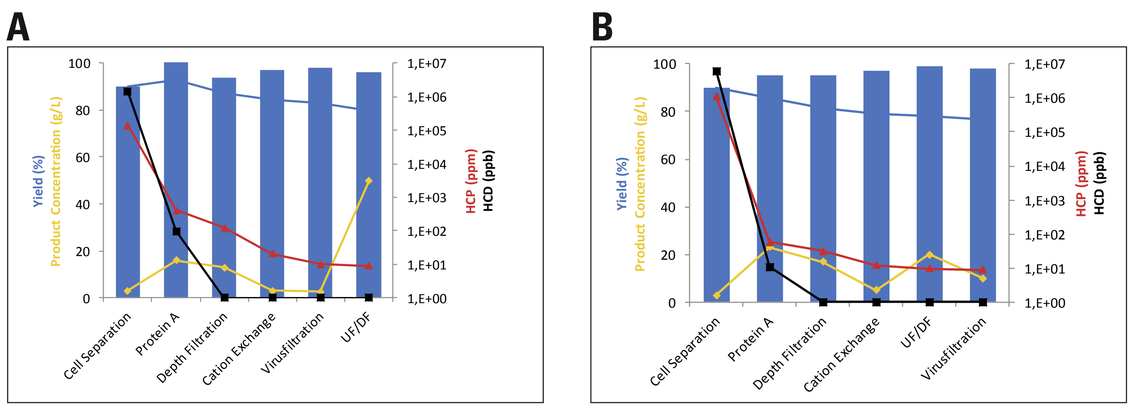

Figure 2: Production overview for downstream processes with 2 column steps (A: mAb-fusion-protein 3, B: mAb 4). Step yields given in bars, total yields in lines. Assays: HCP (F550, Cygnus); HCD (qPCR, Rentschler).

Antibody downstream processes based on 3 chromatographic purification steps (Protein A, AEX, CEX, Figure 1) including a charged depth filter ensure excellent quality of the drug substance.

Purification sequences with 2 chromatographic steps (Protein A and CEX, Figure 2) including a charged depth filter enable more economic processes with increased product yield maintaining equivalent results in product quality.

Virus Removal by Adsorptive Depth Filtration

Depth Filtration is a widely used unit operation in state-of-the-art platform purification processes for clearance of precipitates and process-related impurities like HCD and HCP. It is also attractive for being applied as additional virus removal step based on its adsorption capacity caused by its positively charged surface properties.

The virus removal potential of depth filters (3M, Meriden, USA) is shown emphasizing its anionic adsorption capability. Log_{10} reduction values (LRV) were determined at two process relevant conditions, pH 5.5 and pH 7.0 ("low salt"). For minimizing electrostatic attraction 1 N NaCl ("high salt") was added at pH 7.0 in MuLV spiked runs to investigate the virus retention by size or other interactions. NaCl addition was omitted for MVM spiked runs due to its small size compared to the filter pores.

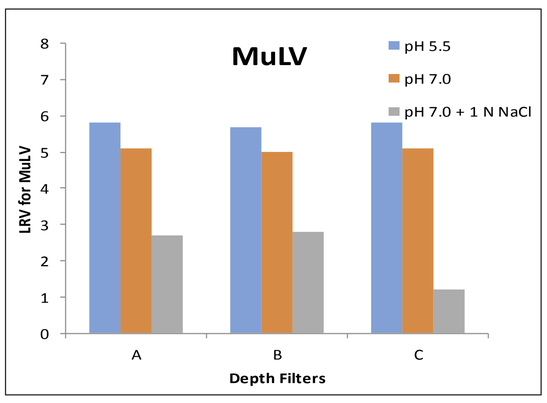

Figure 3: LRV of depth filters for MuLV.

Depth Filters	
A	Zeta Plus™ EXT 60ZA05A
B	Zeta Plus™ EXT 90ZA05A
C	Emphaze™ AEX FNW

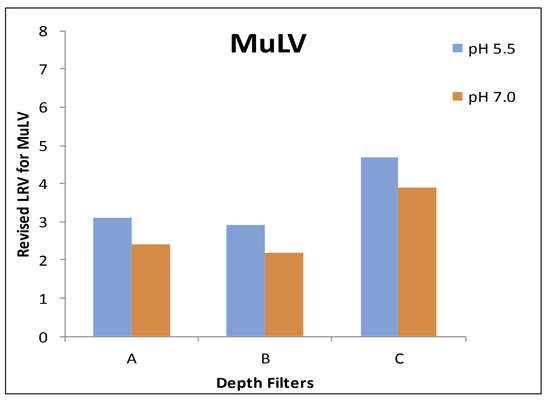

Figure 4: Revised LRV of depth filters for MuLV (Revised LRV = LRV (low salt - high salt).

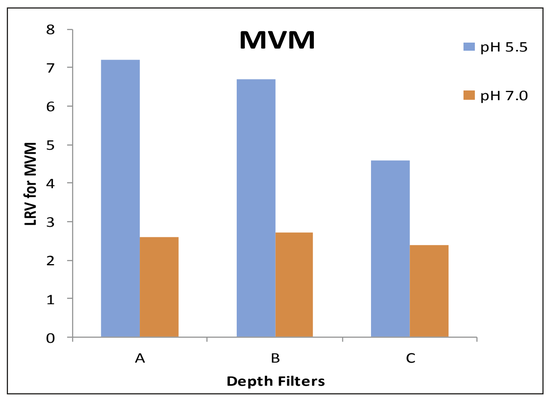

Figure 5: LRV of depth filters for MVM.

The contribution of anionic adsorption for additional virus removal by depth filters was shown, resulting in 2.0 – 5.0 log_{10} reduction factors for MuLV and 2.5 – 7.0 log_{10} reduction factors for MVM. Here, we demonstrated the orthogonal virus removal mechanisms of depth filtration compared to classical virus filtration. The additional virus removal step in the mAb purification sequence increases the overall virus safety of the process and promotes the consideration of a two column step purification platform.

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