Clarification of mammalian cell culture feed streams using depth filters and flocculants; a case study

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Introduction

Merck has developed an all-synthetic depth filtration media (Millistak+® HC Pro D0SP and X0SP) intended to improve the process consistency and efficiency of midstream clarification steps by reducing soluble process impurities such as host cell proteins (HCP) and host cell DNA (HCDNA). These media have been tested in this study and were evaluated against benchmark Millistak+® HC D0HC and X0HC filters. Using a Chinese hamster ovary (CHO)-derived human monoclonal antibody (HuMAbs) culture at VCD: ~1.4 x 10^7 cells/mL, Viability: ~95% and HuMAb concentration: ~0.9 g/L (Figure 1), both Millistak+® HC Pro D0SP and Millistak+® HC D0HC filters showed no effect on HuMAb concentration; i.e. 100% HuMAb recovery, with a higher HCP clearance when Millistak+® D0HC filter was used (16% for D0HC vs 4% for D0SP, Figure 2). Both filters showed a minimal effect on HCDNA clearance (HCDNA log reduction: -0.3 for D0HC and -0.5 for D0SP, Figure 2). Table 2 shows filter sizing for these filters when used to filter a 500 L batch. The Millistak+® HC Pro X0SP filter increased HCP reduction compared to Millistak+® HC X0HC filter (~91% compared to ~63% respectively), at the cost of HuMAb recovery (Figure 3). HuMAb recovery post Millistak+® HC X0HC was ~94% while the recovery post Millistak+® HC Pro X0SP filter was ~70%. The data suggests that Millistak+® HC Pro X0SP filters are suitable for processes challenged by hard-to-remove HCP impurities during downstream processing. Both Millistak+® HC X0HC and Millistak+® HC Pro X0SP filters were highly efficient in removing HCDNA to ~5 logs (Figure 3B). Table 3 shows filter sizing for these filters when used to filter a 500 L batch.

Flocculation is a simple method to pre-clarify high-density CHO cell culture feed streams, allowing for an efficient clarification process that would otherwise not be feasible using traditional depth filters. Here two flocculants were utilized in a dose response study; 1) poly-diallyldimethylammonium chloride (pDADMAC), and 2) Clarisolve® mPAA, modified poly(allyl amine), a polycationic stimulus-responsive flocculation polymer. The dose of each flocculant that resulted in lowest turbidity post centrifugation was selected as the final treating dose (Figure 4A and 5A). Clarisolve® filters, which are designed for higher dirt holding capacity, were used as primary filters, followed by Millistak+® HC X0HC or Millistak+® HC Pro X0SP filters as secondary filters. Harvest material treated with 0.05% pDADMAC polymer and filtered with Clarisolve® 40MS filter showed similar HCP reduction (~16%) to Millistak+® HC D0HC filtrate with minimal (~1%) effect on HuMAb recovery (Figure 4B). The combination, however, showed an efficient HCDNA (~3 logs) clearance (Figure 4C). Harvest material treated with 0.2% Clarisolve® mPAA polymer and filtered with Clarisolve® 60HX filter, on the other hand, showed ~55% reduction of HCP, ~95% HuMAb recovery and ~3 logs HCDNA reduction (Figure 5A and B). The data are intriguing as they are comparable to the results when D0HC > X0HC filters were used (Figure 3A). This means lower numbers of filters (5 vs 13) can be used with similar efficiency, a benefit in large scale manufacturing. When combined with Millistak+® HC X0HC or Millistak+® HC Pro X0SP filters, Millistak+® HC Pro X0SP filter was more efficient in removing HCP (for pDADMAC treated feed: 64% vs 28% reduction and for mPAA treated feed: 92% vs 71% reduction, Figure 6), however, HuMAb recovery showed a lower impact for Millistak+® HC X0HC filters (for pDADMAC treated feed: 84% vs 71% recovery and for mPAA treated feed: 79% vs 68% recovery Figure 6A). Both filters were highly efficient in removing HCDNA to ~4.5-5 log fold (Figure 6B). Tables 4-6 show filter sizing for different combinations of flocculants and secondary filters when used to filter a 500 L batch.

Methods

A HyPerforma SUB 50 bioreactor was inoculated with CHO-expressing HuMAb at seeding density of ~0.4 x 10^6 cells/mL and viability of 99% (Figure 1A). The culture was harvested at day 9 post-inoculation with a VCD: ~1.4 x 10^7 cells/mL and viability of ~95% (Figure 1B and Table 1).

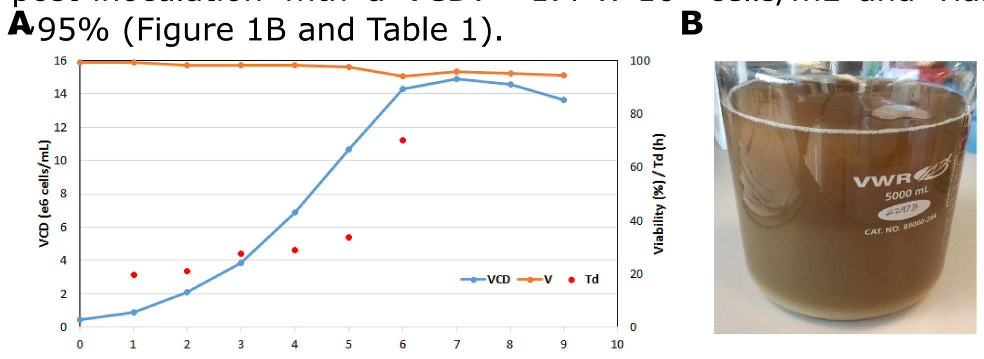

Figure 1: A) Viable cell density (VCD), viability (V), and doubling time (Td). **B**) HuMAb culture had a turbidity ~2817 NTU at day 9 post inoculation; i.e. harvest day.

Table 1: Harvest material turbidity, HuMAb, HCP and HCDNA concentration on harvest day.

The harvest was either left untreated or treated with different doses of pDADMAC or Clarisolve® mPAA polymers, then filtered using different primary and secondary filters (See Process Flow Diagram (PFD), then analyzed for HuMAb concentration, HCP and HCDNA (See analytical testing plan in PFD).

Test Sample	Feed pH	Harvest Turbidity (NTU)	HuMAb (mg/mL)	HCP (mg/mL)	HCDNA (µg/mL)
Direct harvest	6.8	2817	0.93	0.3	4.5

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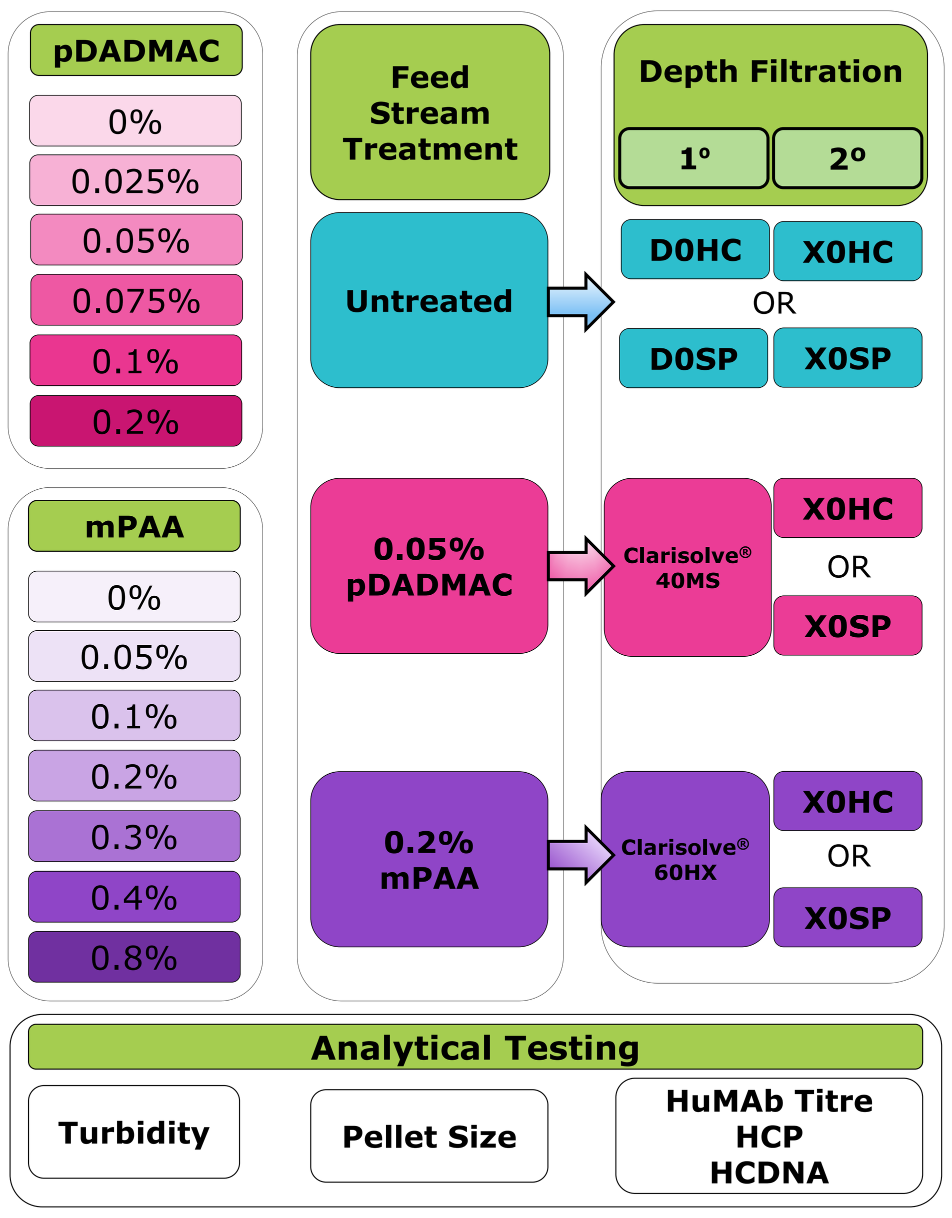
Methods Cont'd



Results

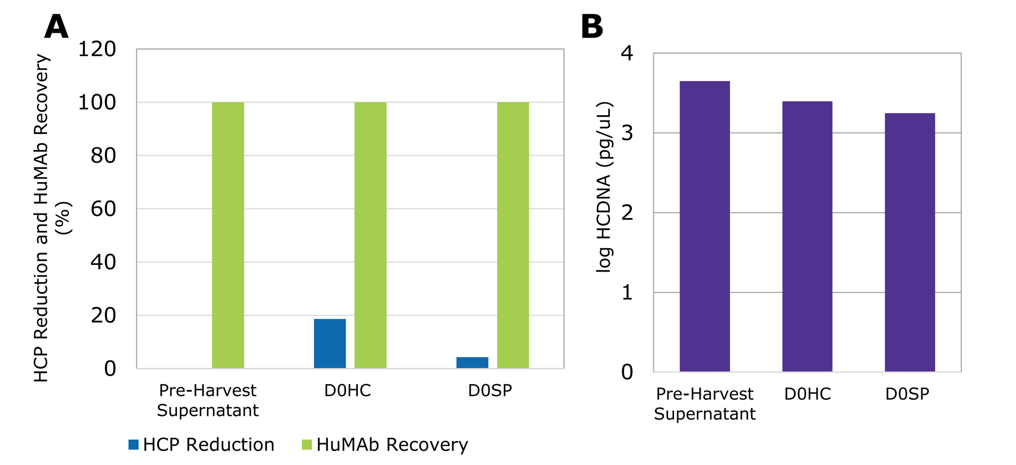


Figure 2: Effect of D0HC or D0SP Primary Filters on HCP Reduction and HuMAb Recovery (**A**) and HCDNA Reduction (**B**).

Table 2: Filter sizing for Millistak+® HC D0HC or Millistak+® HC Pro D0SP filters to filter direct harvest.

Test Fluid	Test Filter	Test Filter Area (cm²)	Test Endpoint Pressure (psi)	Test Load (L/m²)	Test Average Flux (L/m²/hr)	Turbidity In (Feed) (NTU)	Turbidity Out (Filtrate) (NTU)	Batch* Filter Area Minimum (m²)	Batch* Suggested Filter Configuration	Suggested Configuration Filter Area (m²)
Direct Harvest	D0HC	23	23	109	126	2817	669	3.10	5 x 1.1m²	5.5
Direct Harvest	D0SP	23	8	180	127	2817	13.2	1.83	4 x 0.77m²	3.08

* 500 L

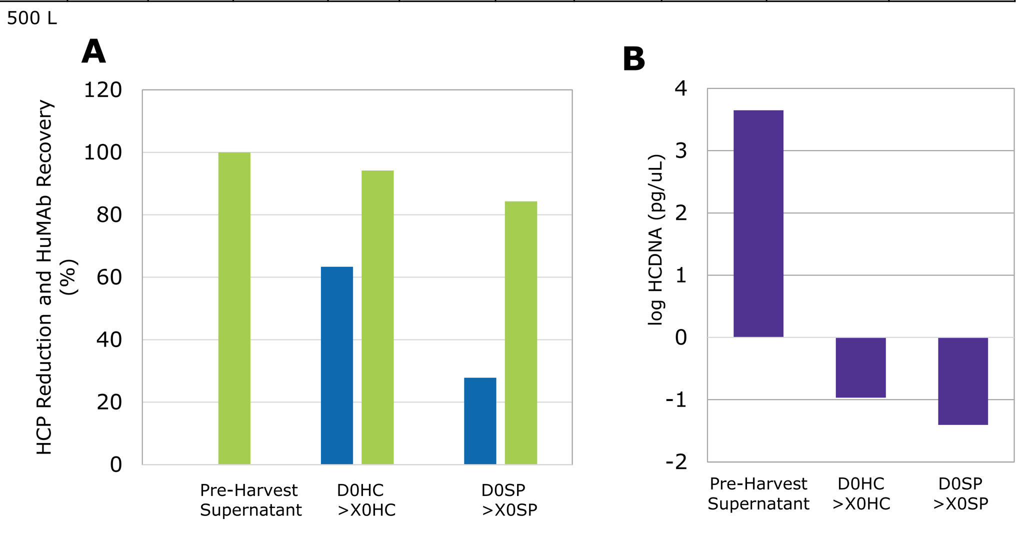


Figure 3: Effect of Secondary Filters on HCP Reduction and HuMAb Recovery (**A**) and HCDNA Reduction (**B**).

Table 3: Filter sizing for Merck's Millistak+® X0HC or Millistak+® X0SP filters to filter D0HC or D0SP filtrate respectively.

Test Fluid	Pre-filter >Test Filter	Test Filter Area (cm²)	Test Endpoint Pressure (psi)	Test Load (L/m²)	Test Average Flux (L/m²/hr)	Turbidity In (Feed) (NTU)	Turbidity Out (Filtrate) (NTU)	Batch* Filter Area Minimum (m²)	Batch* Suggested Filter Configuration	Suggested Configuration Filter Area (m²)
Direct harvest	D0HC >X0HC	5	23.5	45	223	426.0	1.32	5.96	8 x 1.1m²	8.8
Direct harvest	D0SP >X0SP	5	12.5	400	236	19.0	2.52	0.73	2 x 1.1m²	2.2

* 500 L

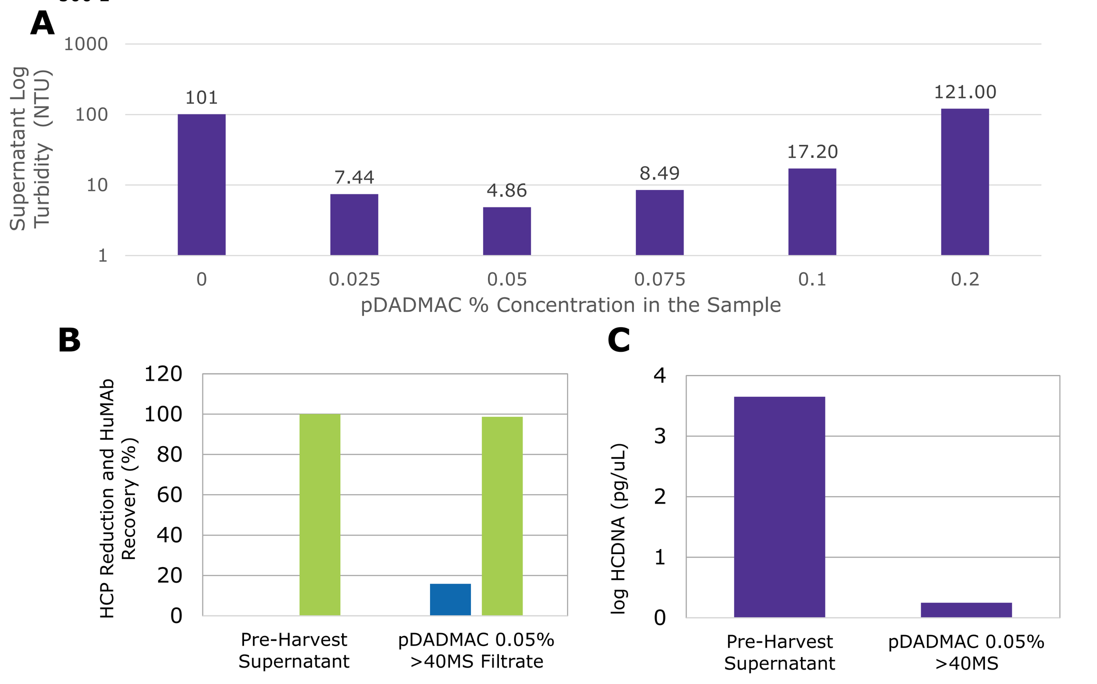


Figure 4: pDADMAC Dose Response Study (**A**), Effect of Primary Filters ± 0.05% pDADMAC polymer on HCP Reduction and HuMAb Recovery (**B**) and HCDNA (**C**).

Results Cont'd

Table 4: Filter sizing for Clarisolve® 40MS filters to filter 0.05% pDADMAC Treated Harvest Material.

Test Fluid	Pre-filter >Test Filter	Test Filter Area (cm²)	Test Endpoint Pressure (psi)	Test Load (L/m²)	Test Average Flux (L/m²/hr)	Turbidity In (Feed) (NTU)	Turbidity Out (Filtrate) (NTU)	Batch* Filter Area Minimum (m²)	Batch* Suggested Filter Configuration	Suggested Configuration Filter Area (m²)
0.05% pDADMAC treated Harvest	Clarisolve® 40MS	23	16	221	156	2355	5.6	2.14	5 x 1.1m²	2.75

* 500 L

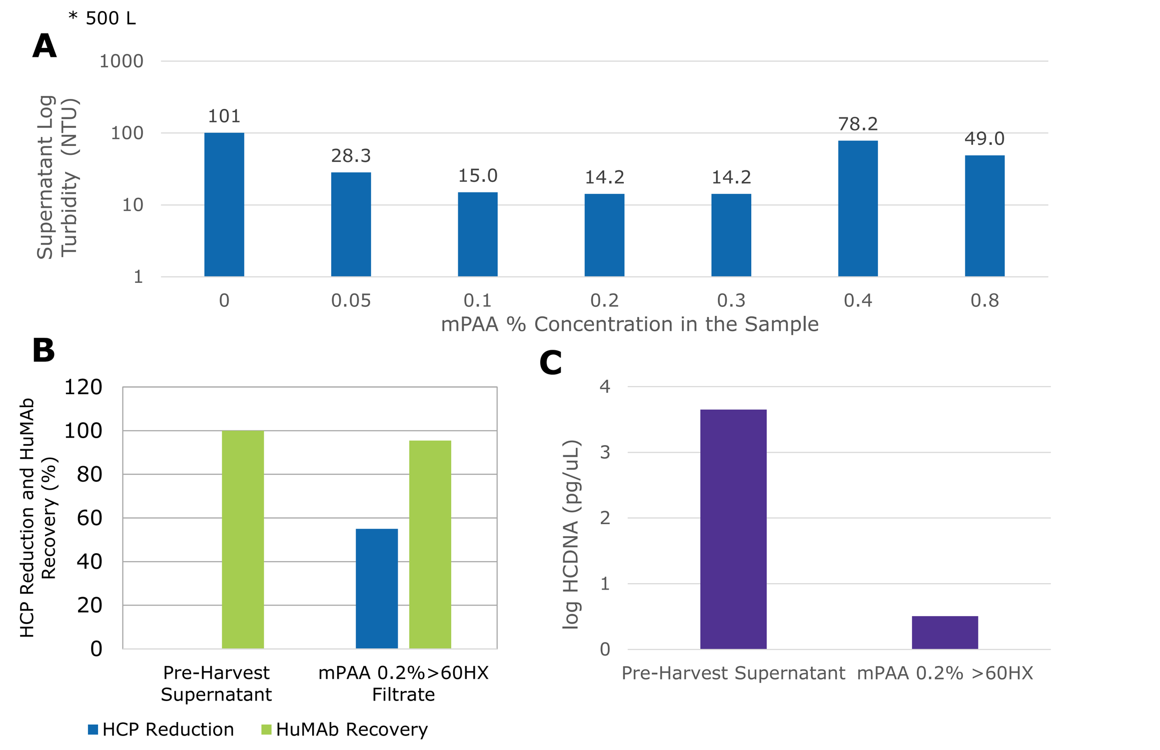
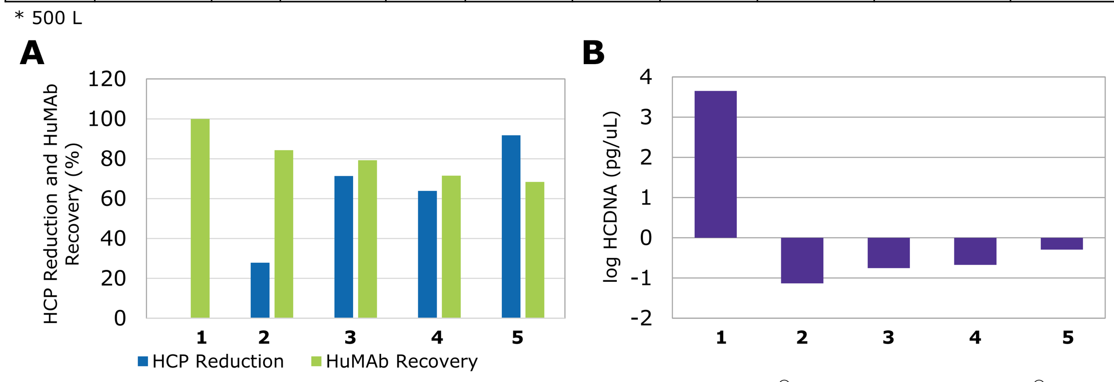


Figure 5: Clarisolve® mPAA polymer Dose Response Study (**A**), Effect of Primary Filters ± 0.2% Clarisolve® mPAA polymer on HCP Reduction and HuMAb Recovery (**B**) and HCDNA (**C**).

Table 5: Filter sizing for Clarisolve® 40MS filters to filter 0.2% mPAA Polymer Treated Harvest Material.

Test Fluid	Pre-filter >Test Filter	Test Filter Area (cm²)	Test Endpoint Pressure (psi)	Test Load (L/m²)	Test Average Flux (L/m²/hr)	Turbidity In (Feed) (NTU)	Turbidity Out (Filtrate) (NTU)	Batch* Filter Area Minimum (m²)	Batch* Suggested Filter Configuration	Suggested Configuration Filter Area (m²)
0.2% mPAA treated Harvest	Clarisolve® 60HX	23	25	204	168	6390	3.3	2.19	5 x 1.1m²	2.75

* 500 L



1. Pre-Harvest Supernatant, **2.** pDADMAC polymer 0.05% > Clarisolve® 40MS filter >Millistak+® X0HC filter, **3.** pDADMAC polymer 0.05% > Clarisolve® 40MS filter > Millistak+® X0SP filter **4.** mPAA polymer 0.2% > Clarisolve® 60HX filter > Millistak+® X0HC filter, **5.** mPAA polymer 0.2% > Clarisolve® 60HX filter> Millistak+® X0SP filter

Figure 6: Effect of Secondary Filters on HCP Reduction and HuMAb Recovery (**A**) and HCDNA (**B**) when 0.05% pDADMAC Treated Harvest> 40MS or 0.2% mPAA Treated Harvest > 60HX used as feed.

Table 6: Filter sizing for Millistak+® X0HC or X0SP filters to filter 0.05% pDADMAC Treated Harvest > Clarisolve® 40MS filtrate.

Test Fluid	Pre-filter >Test Filter	Test Filter Area (cm²)	Test Endpoint Pressure (psi)	Test Load (L/m²)	Test Average Flux (L/m²/hr)	Turbidity In (Feed) (NTU)	Turbidity Out (Filtrate) (NTU)	Batch* Filter Area Minimum (m²)	Batch* Suggested Filter Configuration	Suggested Configuration Filter Area (m²)
0.05% pDADMAC treated harvest	Clarisolve® 40MS >Millistak+® X0HC	5	7.8	400	238	15.4	1.31	0.76	1 x 1.1m²	1.1
0.05% pDADMAC treated harvest	Clarisolve® 40MS >Millistak+® X0SP	5	6.8	400	240	15.4	1.24	0.43	1 x 1.1m²	1.1
0.2% mPAA treated Harvest	Clarisolve® 60HX >Millistak+® X0HC	5	7.2	400	238	3.5	0.84	0.72	1 x 1.1m²	1.1
0.2% mPAA treated Harvest	Clarisolve® 60HX >Millistak+® X0SP	5	5.7	400	240	3.5	0.87	0.88	1 x 1.1m²	1.1

* 500 L

Summary

Table 7 : Sizing and effect of Primary/Secondary Filters ± pDADMAC or Clarisolve® mPAA polymers on HCP Reduction, HuMAb Recovery and HCDNA.

Filter Train	HCP Reduction %	HuMAb Recovery %	HCDNA Log Fold Reduction	Batch* Suggested Filter Configuration	Suggested Configuration Filter Area (m2)
D0HC	19	100	-0.34	5 x 1.1m²	5.5
D0HC >X0HC	63	94	-4.71	8 x 1.1m²	8.8
D0SP	4	100	-0.49	4 x 0.77m²	3.08
D0SP >X0SP	91	70	-5.15	2 x 1.1m²	2.2
pDADMAC 0.05% >40MS	16	99	-3.49	5 x 1.1m²	2.75
pDADMAC 0.05% >40MS >X0HC	28	84	-4.87	1 x 1.1m²	1.1
pDADMAC 0.05% >40MS >X0SP	64	72	-4.49	1 x 1.1m²	1.1
mPAA 0.2% >60HX	55	95	-3.23	5 x 1.1m²	2.75
mPAA 0.2% >60HX >X0HC	71	79	-4.41	1 x 1.1m²	1.1
mPAA 0.2% >60HX >X0SP	92	68	-4.03	1 x 1.1m²	1.1

* 500 L

This case study shows promising results and provides alternative paths for CHO cell culture clarification, based on the nature of HCP and of the protein of interest.