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Application Note 01

Cost analysis of 3M™ Polisher ST value in mAb manufacturing process replacing AEX polishing column

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Introduction

With increasing molecule diversity, biologic drug manufacturers are looking for platformable manufacturing solutions. With a diversified portfolio still dominated by monoclonal antibody (mAb) therapeutic proteins, an important focus is placed on process intensification which helps in lower capital expenditure investment and overall manufacturing cost. The number of molecules in the development pipeline and time to market are key factors for success.

These trends have led to a surge in demand for single-use products and solutions. Single-use technologies (SUTs) offer reduced capex, allow faster turnaround between batches, can improve changeover time between campaigns and flexibility to deal with molecular diversity in the pipeline, and can benefit the speed to market. The ability to use reliable platform technologies across different processes and molecules further decreases the time required for process development and production of clinical material.

In the past decade, cell line development and optimized cell culture technologies have shifted the bottleneck from upstream processes (USP) towards the downstream process (DSP) operations. Titers at commercial scale have moved from less than 2 g/L and low cell density ($<10\times10^6$ cell/mL) processes to titers of over 7 g/L. Now the yield of the downstream operations has become the limiting factor for the output capacity of the manufacturing plant. In order to fully utilize the increased therapeutic protein mass produced, the number of downstream unit operations needs to be reduced, and the footprint of individual steps must be decreased to minimize product losses. New single-use technologies such as 3M™ Polisher ST provide manufacturers with opportunities to intensify manufacturing operations.

One of the most important purification steps, especially for traditional mAb processes, is the primary capture chromatography operation. Significant efforts have been made to improve the productivity and efficacy of this affinity capture step. In 2015, 3M introduced chromatography to the clarification unit operation with the launch of 3M™ Emphaze™ AEX Hybrid Purifier, designed to work with the traditional platform processes either post centrifuge or as the second stage clarifier in a 2-stage depth filter train. Successful implementations of this technology have shown that reducing DNA-related impurities during clarification increases the performance of the capture step, and results in drastically reduced impurity levels in the elution pool.[1,2,3,4,5] These lower impurity concentrations can facilitate replacement of an anion exchange (AEX) flow through column by a single-use AEX capsule, resulting in further significant cost savings and operational flexibility.

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Single-use AEX Chromatography

Figure 1: *Traditional resin-based column (left) and holder with one BC16000 capsule* of 3M™ Polisher ST (right).*

Single-use chromatography technology offers the flexibility which a multiproduct manufacturing facility requires. Several studies have demonstrated that the capital investment required with a single-use facility is lower, and these facilities offer substantial time and labor savings by eliminating cleaning and cleaning validation procedures.[6,7]

3M™ Emphaze™ AEX Hybrid Purifier has been widely adopted in upstream production processes bringing chromatography in clarification. With 3M™ Polisher ST, 3M is now bringing a single-use chromatography solution to the downstream manufacturing space, allowing replacement of multi-use chromatography columns as shown in Figure 1.

In a multiproduct and multimodality facility, the downstream operations must keep pace with the product output from the upstream process. When using existing technologies, the size of the downstream unit operations increases proportionately with the batch size or product output.

Some of the recent technology innovations which have been tested and used in the industry include high capacity chromatography resins with higher loadings and lower residence time. Current single-use chromatographic membrane adsorbers have known limited usage in full scale downstream processes so far, primally due to capacity limitations and their cost effectiveness being restricted to small and intermediate scales. Sensitivity of the performance of AEX ligands to the process conditions, including pH, conductivity and buffer types, has hindered their application in the development of true platform processes.

To improve the overall process economy and allow adoption of single-use equipment at any scale, disposable chromatography solutions need to show high throughput capacities and improve the product yield. In this application note, we discuss strategies for replacing the downstream polishing AEX column with an advanced single-use AEX solution like 3M Polisher ST in traditional and modern mAb manufacturing processes.

* Production capsules will be available at a later date

3M™ Polisher ST in the Biopharmaceutical Process

3M™ Polisher ST is a synthetic, hybrid polishing solution containing two complementary AEX-functional media: a quaternary ammonium (Q) functional nonwoven and a guanidinium-functional membrane (Figure 2). The novel guanidinium functionality of the downstream AEX polishing membrane mimics the amino acid arginine, one of the three positively charged naturally occurring standard amino acids that make up proteins. The guanidinium group of arginine is observed to interact strongly with negatively charged groups of proteins, forming robust salt bridges that involve two hydrogen bonding interactions in addition to an electrostatic interaction.

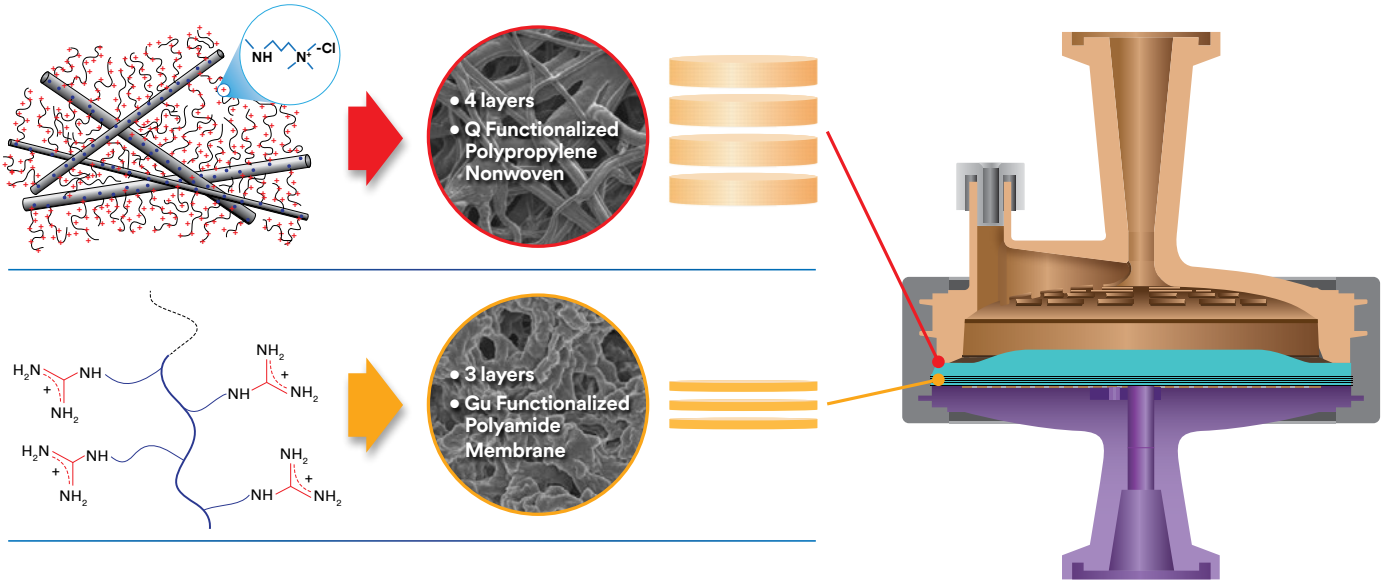


Figure 2: *Multi-layer capsule construction of 3M™ Polisher ST.*

The Q-functional nonwoven provides reduction of turbidity (when present), DNA, and endotoxin, and adds to the product's total charge capacity. The guanidinium functional ligand reduces host cell protein impurities and provides robust viral clearance in a wide range of operating conditions, including high conductivity, low pH and polyvalent buffers. The expanded operating window of the guanidinium ligand allows more freedom in designing DSP polishing trains and may ease the transition to true platform processes. The combination of the different functional layers results in a very high charge capacity, allowing typical mAb loadings of 10 kg/m^2, which is about 100x higher than the typical loadings of resin-based columns. This allows deployment of 3M Polisher ST in the downstream process at all scales, including full commercial manufacturing.

Modeling the Process

In this application note we explore the potential impact of replacement of an AEX flow-through column by 3M™ Polisher ST on the cost of monoclonal antibody therapeutic manufacturing. This work describes hypothetical scenarios. Depending on product and process conditions, the effects covered in this application note may not be realized or may not be realized to the degree shown in the scenarios.

We modeled a typical single-use large scale manufacturing facility using the commercial Biosolve Process™ software package from Biopharm Services limited. The facility and process have the following attributes:

- Reactor setup: 1800 L working volume X 6 single-use bioreactors
- mAb titer = 5 g/L
- 1 reactor is harvested at a time.
- Facility output of 100 batches per year
- Downstream operations utilize single-use systems as much as possible

Recovery and loading of the different process steps:

- Depth filter recovery 90%
- AEX column recovery 95%
- 3M Polisher ST recovery 99%
- Column loading 100 g/L
- 3M Polisher ST loading 10 kg/m^2

The recovery of 3M Polisher ST assumes that 50% of membrane's protein binding capacity (based on dynamic binding capacity for BSA) is used up by unwanted binding of the target mAb:

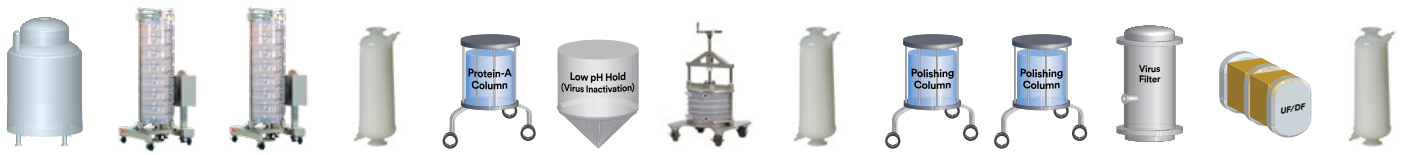
- 12 mg/cm^2 BSA capacity
- 1 g/cm^2 mAb load
- 6 mg/cm^2, half capacity used to bind mAb
- 99% recovery

Biosolve Process is an expansive model utilizing an enormous dataset from the industry in terms of operational strategy and cost. It is not possible to account for every scenario and detail. This investigation covers the key potential advantages of deploying of the 3M Polisher ST in the biopharmaceutical manufacturing process. The 3M Polisher ST is modeled as a single-use solution at about three times the cost of the standard AEX resin. The flow rate was aligned to the recommended flux of 1 mL/min/cm^2 for the product. A production sized BC16000 capsule* was used for all modeling.

The basic performance metrics of the different process scenarios are summarized in Figures 3, 4 and 5. More detailed information on the process sequence and cost breakdown are included in the Appendix. The DSP yield of the base process is 52%. The summary tables (1-4) include the overall yield, Cost of Goods Sold (COGS) in US dollars per gram of mAb and the process mass intensity (PMI) index. This last key performance indicator is the total mass of materials, including buffers and water, used in the process, divided by the mass output of the product of interest.[8,9] The PMI is seen as an indicator for the environmental impact of the process. Lower PMI values are associated with a lower footprint, less waste and generally also lower costs.

* Production capsules will be available at a later date

Base process: total yield: 52 %



Parameter	DF 1	DF 2	Membrane	Capture	VIN	DF	Membrane	AEX column	CEX column	Virus filter	UF/DF	Membrane
Size	14 pc	7 pc	2 pc	226 L	NA	2 pc	1 pc	62 L	62 L	2 pc	6 m²	1 pc
Load	100 L/m²	200 L/m²	1000 L/m²	40 g/L	NA	400 L/m²	1000 L/m²	100 g/L	50 g/L	250 L/m²	NA	1000 L/m²
Yield %	90	90	98	90	98	90	98	95	95	98	98	98

Figure 3: *Base process with traditional depth filters and resin-based AEX column.*

Overall Yield	PMI	COGS $/g
52%	**9.576**	**137,73**

Table 1: *Yield, PMI and COGS for base process.*

Scenario 1: *Replacing a multi-use AEX column by a high capacity single-use AEX solution (3M™ Polisher ST) - Figure 4*

3M™ Polisher ST is an advanced single-use solution containing two complementary AEX-functional media: A Q-functional nonwoven and a guanidinium-functional membrane. Due to its high capacity and unique guanidinium functionality, the 3M Polisher ST offers higher mAb loading than a traditional flow-through Q resin. The high capacity and convective flow of the membrane enables downsizing of the AEX polishing unit operation, while achieving an equivalent effluent quality in terms of turbidity, DNA and HCP levels. In addition, mAb recovery of 99% may be achieved with 3M Polisher ST due to the small size of the system.

This model shows the effect of deploying the 3M Polisher ST in the process, by increasing the load to 10 kg/m² and having an increased product recovery from 95% to 99%. Due to its ability to operate in moderate levels of turbidity, the depth and membrane filtration steps after virus inactivation and neutralization (VIN) can be eliminated. In this hypothetical scenario, the overall DSP yield increases to 62%, driving the cost of manufacturing down by 21%. In case the AEX column is replaced by the single-use AEX step, without elimination of the depth filter and the membrane, the cost is reduced by 10%. This means that about half of the cost savings is due to the elimination of the filtration steps, and half is due to the transition of the AEX step itself to a SUT.

Scenario 1: total yield: 62 %

Parameter	DF 1	DF 2	Membrane	Capture	VIN	3M Polisher ST	CEX column	Virus filter	UF/DF	Membrane
Size	14 pc	7 pc	2 pc	226 L	NA	**1 x BC16000**	157 L	3 pc	8 m²	1 pc
Load	100 L/m²	200 L/m²	1000 L/m²	40 g/L	NA	**10 kg/m²**	50 g/L	250 L/m²	NA	1000 L/m²
Yield %	90	90	98	90	98	**99%**	95	98	98	98

Figure 4: *Model process with traditional depth filters and single-use AEX step.*

Overall Yield	PMI	COGS $/g
62%	**8.246**	**109,41**
(+10% compared to base)	(-14% compared to base)	(-21% compared to base)

Table 2: *Yield, PMI and COGS for scenario 1.*

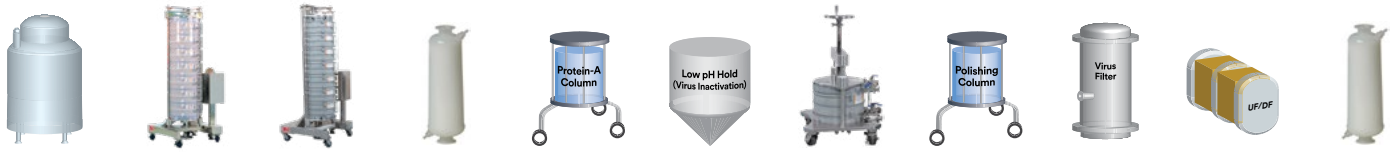
Scenario 2: *Replacing a multi-use AEX column by a high capacity single-use AEX solution (3M™ Polisher ST) with chromatography deployed in clarification (3M™ Emphaze™ AEX Hybrid Purifier) – Figure 5*

In this second hypothetical scenario (Figure 5), we compare the benefits of incorporating the newer 3M™ Emphaze™ AEX Hybrid Purifier chromatographic clarification technology with the traditional platform process using cellulose-based depth filters only.[10]

Due to the superior removal of cell debris and chromatin, and the built-in 0.2 µm membrane, 3M Emphaze AEX Hybrid Purifier offers better protection of the sterilizing grade membrane behind, which allows downsizing of this step. In this example, one 20" membrane capsule is used instead of two. By applying chromatographic clarification, lower turbidity and impurity levels (> 10x lower HCP, > 1000x lower DNA) are obtained after the capture step.[2,3,4,5,10] If a depth filter is present after the VIN step to protect a resin-based column, it can be eliminated.[10,12]

In the scenario where 3M Emphaze AEX Hybrid Purifier is used, the lower impurity levels in the VIN may allow even higher loadings of the single-use AEX step with 3M™ Polisher ST, potentially significantly exceeding the recommended 10 kg/m². In the model used here, further increased loadings would not result in additional downscaling, since the minimum number of a single large BC16000 capsule had already been reached in scenario 1.

Scenario 2: total yield: 67%



Parameter	DF 1	3M Emphaze AEX Hybrid Purifier	Membrane	Capture	VIN	3M Polisher ST	CEX column	Virus filter	UF/DF	Membrane
Size	14 pc	**4 pc**	**1 pc**	226 L	NA	**1 x BC16000**	157 L	3 pc	8 m²	1 pc
Load	100 L/m²	**400 L/m²**	**3000 L/m²**	40 g/L	NA	**10 kg/m²**	50 g/L	250 L/m²	NA	1000 L/m²
Yield %	90	**97**	**98**	90	98	**99%**	95	98	98	98

Figure 5: *Model process including chromatographic clarification and single-use AEX step.*

The tables below include the manufacturing cost when 3M Emphaze AEX Hybrid Purifier is applied by itself (table 3), and then in combination with 3M Polisher ST (table 4) for a fully optimized process.

Overall Yield	PMI	COGS $/g
63%	**8.765**	**127,36**
(+11% compared to base)	(-8% compared to base)	(-8% compared to base)

Table 3: *Clarification including 3M™ Emphaze™ AEX Hybrid Purifier, DSP with AEX column (without 3M™ Polisher ST).*

Overall Yield	PMI	COGS $/g
67 %	**7.602**	**106,14**
(+15% compared to base)	(-21% compared to base)	(-23% compared to base)

Table 4: *Clarification including 3M™ Emphaze™ AEX Hybrid Purifier, DSP with 3M™ Polisher ST.*

Cost breakdown

As the data tables of the scenarios indicate, cost savings can be obtained by implementing the presented single-use technologies. An important question is where these savings come from. The consumable costs rise by implementing more advanced SUT steps, as expected. This increasing cost is compensated for by a decrease in labor cost. The capital cost, materials cost and other cost show no significant differences between the scenarios (Figure 6 and 7).

The implementation of single-use technologies is expected to reduce the capital cost, since capsule holders are significantly less expensive than chromatography columns. Indeed, when looking at the capital cost of the AEX step alone, scenario 1 with 3M™ Polisher ST shows a capital cost saving of 16% compared to the base process. By including the elimination of the filtration steps after VIN, the capital cost saving increases to 37%. With the parameters used in our model, the capital cost of the subsequent CEX step rose by a similar percentage, mainly due to the need for a larger column required to bind the increased product mass. In general, significant capital cost savings can be expected when implementing 3M Polisher ST, unless the CEX column is operated at maximum capacity in the base process, like in this model. Optimized yields directly translate to increased plant output capacities but need to be considered when sizing downstream equipment.

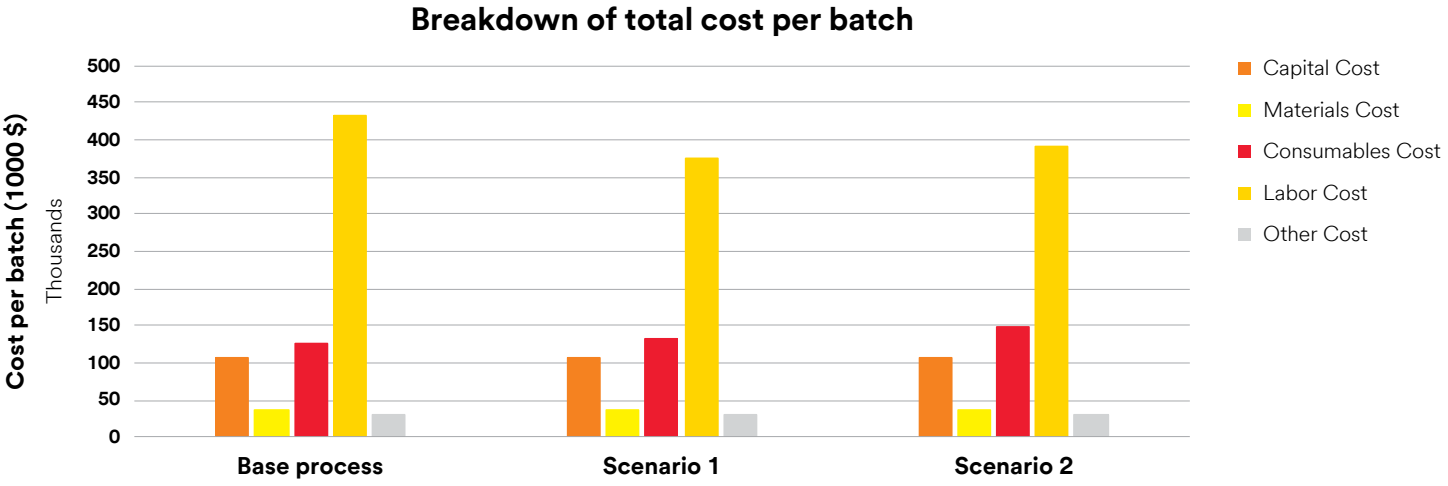


Figure 6: *Total cost per batch results for different scenarios.*

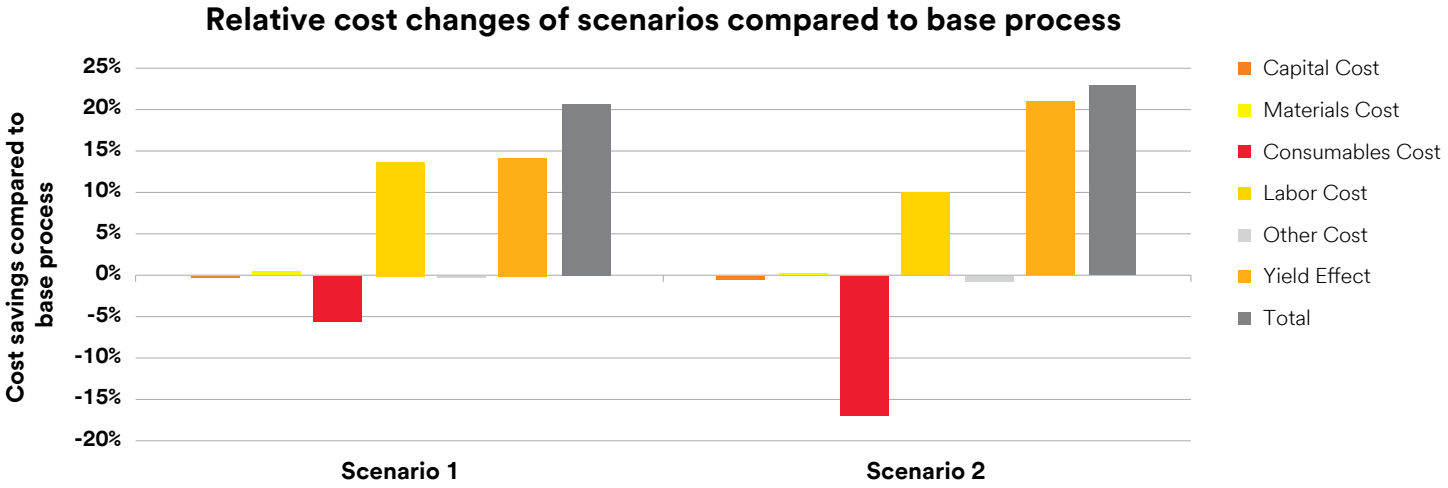


Figure 7: *Relative cost changes for modelled scenarios, compared to base process. Cost savings are shown as positive values, while increased costs are shown as negative values.*

The total yield of the DSP process is one of the most important factors for the cost of goods per gram of mAb. Implementing single-use devices with a small footprint and eliminating steps result in strongly reduced product loss. The increased capacity in kg of mAb produced per year, as shown in Figure 8, is the primary reason why process simplification and intensification are crucial for biopharmaceutical manufacturers. The cost savings also correlate well with a decreasing PMI value.

The results presented in this section can help prioritize cost saving efforts when making the transition to single-use purification technologies.

Total production capacity versus yield

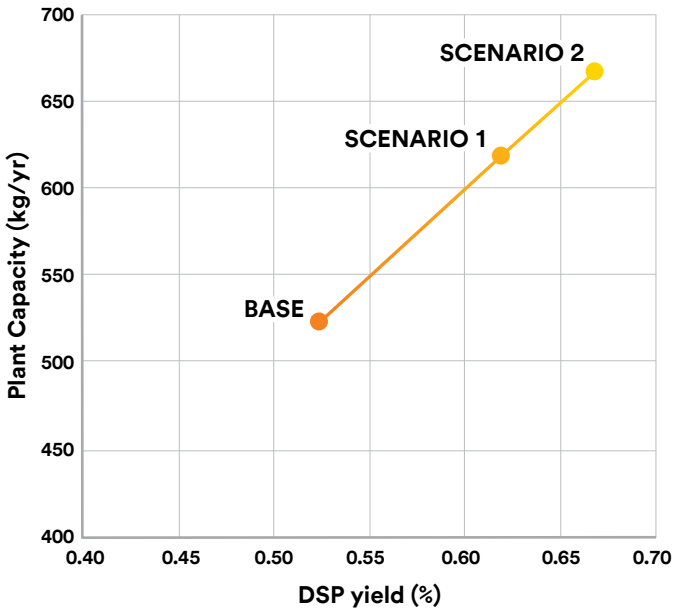


Figure 8: *DSP yield has a direct impact on the overall plant production capacity.*

Sensitivity analysis of process parameters

Some of the input parameters were varied to study their effect on the total COGS. In the base scenario, the loading of the AEX column was modified to a lower value of 50 g/L or a higher value of 200 g/L, compared to the original setting of 100 g/L. The maximum number of cycles, or resin lifetime, was changed to 50 and 200, next to the base setting of 100 cycles. These changes did not affect the cost much, with total COGS of 137.6 $/g for 200 cycles at 100 g/L loading and 144.1 $/g for 100 cycles at 50 g/L loading.

Changing the loading of 3M™ Polisher ST from 10 kg/m² to 5 kg/m² or 20 kg/m² did not affect the cost, because only one large BC16000 capsule was needed to process the batch volume in all three variations. The only factor which may significantly affect the total COGS is the recovery of the single-use AEX step, as shown in Figure 9. Higher product losses translate to higher costs per gram of mAb produced. Even at a worst-case condition of 95% yield, the cost savings are still outstanding (-17% for scenario 1 compared to the base scenario).

Effect of AEX yield on total cost

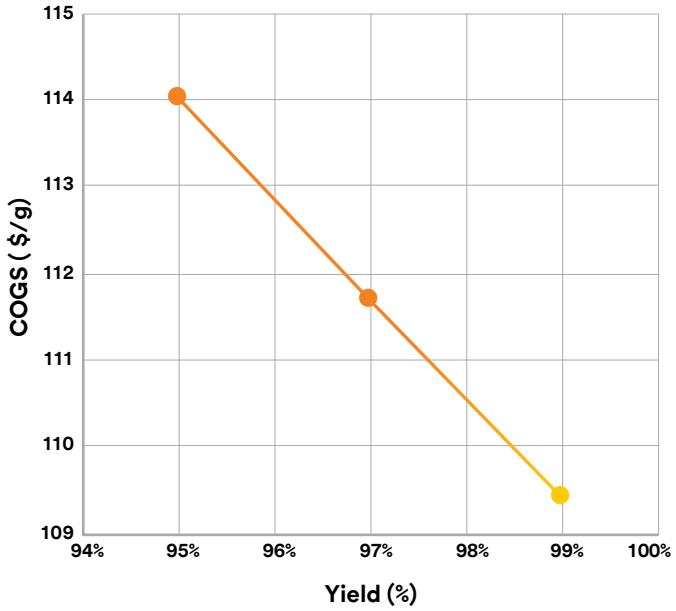


Figure 9: *Yield of the single-use AEX step has an important impact on the total cost. The data shown here were calculated with the scenario 1 model.*

Conclusion

With its high recommended loading of 10 kg/m^2, 3M™ Polisher ST can replace the multi-use AEX chromatography column. 3M Polisher ST's performance in AEX polishing unit operations can provide process simplifications that decrease the size and number of process steps and, thus, improve the productivity of the process.

Single-use chromatographic clarifiers and purifiers like 3M™ Emphaze™ AEX Hybrid Purifier are effective in reducing process- and product-related impurities prior to downstream column chromatography. Implementing 3M Polisher ST in the DSP polishing steps can further reduce HCP, residual CHO DNA and provide viral clearance.

Implementing 3M Emphaze AEX Hybrid Purifier and 3M Polisher ST can eliminate the need for a resin based AEX step and can enable process compression. The process becomes more productive and the cost of the mAb manufacturing decreases, as shown for the hypothetical process modeling scenarios discussed in this note (Figure 10).

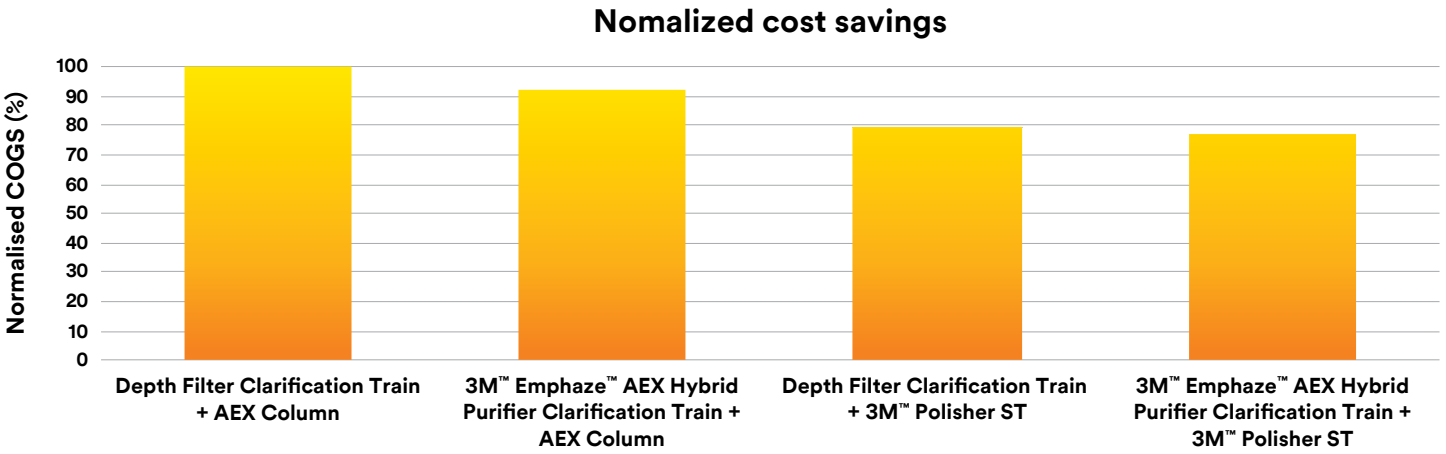


Figure 10: *Introduction of single-use solutions enables significant cost savings, as shown by process modelling.*

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Appendix

A. Base process

Table 5: *Base process - process sequence*

No	Process Stage	Unit Op Name	Conc (g/L)	Yield (%)	Duration (hr)	Adjusted Duration (hr)	Mass In (g)	Mass Out (g)	Vol In (L)	Vol Out (L)	Particles In	Particles Out	Target Out	Capacity Out (kg/year)
		Feed	0.0											
1	Upstream	N-2 Seed	0.0	0.0	52.5	8.8	0	0	2	20	0	0	0	0
2	Upstream	N-1 Seed	0.0	0.0	52.5	8.8	0	0	20	200	0	0	0	0
3	Upstream	Production	5.0	1.0	278.5	46.4	0	10000	200	2000	0	0	10	1000
4	Recovery	Primary depth filter	4.3	0.9	3.5	3.5	10000	9000	2000	2112	0	0	9	900
5	Recovery	Secondary depth filter	3.7	0.9	3.2	3.2	9000	8100	2112	2168	0	0	8	810
6	Purification	Filtration (0.2um)	3.6	1.0	2.1	2.1	8100	7938	2168	2184	0	0	8	794
7	Purification	Protein A	7.9	0.9	7.4	7.4	7938	7144	2184	905	0	0	7	714
8	Purification	Virus Inactivation	7.6	1.0	5.5	5.5	7144	7001	905	923	0	0	7	700
9	Recovery	Depth filtration	6.7	0.9	3.4	3.4	7001	6301	923	939	0	0	6	630
10	Purification	Filtration (0.2um)	6.5	1.0	2.4	2.4	6301	6175	939	947	0	0	6	618
11	Purification	AIEX Flow Through	6.3	1.0	7.4	7.4	6175	5928	947	947	0	0	6	593
12	Purification	IEX Bind & Elute	11.3	1.0	11.1	27.1	5928	5632	947	499	0	0	6	563
13	Purification	Viral Filtration	10.8	1.0	6.1	6.1	5632	5519	499	509	0	0	6	552
14	Purification	UF/DF	50.0	1.0	11	11	5519	5409	509	108	0	0	5.4	541
15	Purification	Filtration (0.2um)	45.6	1.0	3	3	5409	5301	108	116	0	0	5.3	530

Table 6: *Base process - cost of goods breakdown (USD per batch)*

	N-2 Seed	N-1 Seed	Production	Primary depth filter	Secondary depth filter	Filtration (0.2um)	Protein A	Virus Inactivation	Depth filtration	Filtration (0.2um)	AIEX Flow Through	IEX Bind & Elute	Viral Filtration	UF/DF	Filtration (0.2um)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Equipment (Total)	473554	1293761	4865370	226190	189561	113531	1506010	101546	154885	102739	760226	870806	107312	554851	78341
Capital	4432	12108	45533	2117	1774	1062	14094	950	1449	961	7115	8149	1004	5193	733
Materials	979	2423	16162	1133	1118	1108	5885	1017	823	821	1243	1333	836	993	821
Consumables	1406	4573	16664	6606	3768	3015	24558	1558	2978	3309	8417	15998	22998	6814	2889
Labour	12291	14124	65699	15855	14734	13654	50867	22155	11608	11380	55071	83092	13498	36928	11529
Other	1112	3043	11503	861	738	527	3950	414	375	244	1852	2179	269	1386	186
	3%	5%	21%	4%	3%	3%	14%	4%	2%	2%	10%	15%	5%	7%	2%
Perfusion Factor	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Capital Charge	4432	12108	45533	2117	1774	1062	14094	950	1449	961	7115	8149	1004	5193	733
Materials	979	2423	16162	1133	1118	1108	5885	1017	823	821	1243	1333	836	993	821
Media	160	1604	15343	0	0	0	0	0	0	0	0	0	0	0	0
Buffer	0	0	0	30	15	4	5066	1	4	2	424	514	3	148	2
Direct RM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bought WFI & PW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CIP	0	0	0	285	285	285	0	197	0	0	0	0	15	26	0
QC tests	819	819	819	819	819	819	819	819	819	819	819	819	819	819	819
Consumables	1406	4573	16664	6606	3768	3015	24558	1558	2978	3309	8417	15998	22998	6814	2889
Resins/MA	0	0	0	0	0	0	17542	0	0	0	1632	7215	0	0	0
Bags	1406	4573	16664	1253	1092	793	5726	1558	2213	2198	6785	8783	2198	4156	1778
Filters	0	0	0	5353	2676	2222	1290	0	765	1111	0	0	20800	2658	1111
Packages	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Labour	12291	14124	65699	15855	14734	13654	50867	22155	11608	11380	55071	83092	13498	36928	11529
Process	5159	5928	27576	6655	6184	5731	21351	9299	4872	4777	23115	34877	5665	15500	4839
Quality	5172	5943	27644	6671	6199	5745	21403	9322	4884	4788	23172	34962	5679	15538	4851
Indirect	1960	2253	10479	2529	2350	2178	8113	3534	1852	1815	8784	13253	2153	5890	1839
Other	1112	3043	11503	861	738	527	3950	414	375	244	1852	2179	269	1386	186
Insurance/other	228	623	2342	109	91	55	725	49	75	49	366	419	52	267	38
Waste mgmt	1	1	6	6	4	2	6	2	2	2	5	8	3	4	1
Maintenance	222	605	2277	106	89	53	705	48	72	48	356	407	50	260	37
Utilities	661	1814	6879	640	554	417	2515	316	226	145	1125	1344	164	855	111
TOTAL (USD)	20220	36271	155560	26572	22133	19366	99354	26095	17234	16716	73697	110751	38605	51313	16158
Total (USD/Gram normalized for the output)	3.81	6.84	29.35	5.01	4.18	3.65	18.74	4.92	3.25	3.15	13.90	20.89	7.28	9.68	3.05

B. Scenario 1: Depth Filter Clarification Train + 3M™ Polisher ST

Table 7: *Scenario 1 - Process sequence*

No	Process Stage	Unit Op Name	Conc (g/L)	Yield (%)	Duration (hr)	Adjusted Duration (hr)	Mass In (g)	Mass Out (g)	Vol In (L)	Vol Out (L)	Particles In	Particles Out	Target Out	Capacity Out (kg/year)
		Feed	0.0											
1	Upstream	N-2 Seed	0.0	0.0	52.5	8.8	0	0	2	20	0	0	0	0
2	Upstream	N-1 Seed	0.0	0.0	52.5	8.8	0	0	20	200	0	0	0	0
3	Upstream	Production	5.0	1.0	278.5	46.4	0	10000	200	2000	0	0	10	1000
4	Recovery	Primary depth filter	4.3	0.9	3.5	3.5	10000	9000	2000	2112	0	0	9	900
5	Recovery	Secondary depth filter	3.7	0.9	3.2	3.2	9000	8100	2112	2168	0	0	8	810
6	Purification	Filtration (0.2um)	3.6	1.0	2.1	2.1	8100	7938	2168	2184	0	0	8	794
7	Purification	Protein A	7.9	0.9	7.4	7.4	7938	7144	2184	905	0	0	7	714
8	Purification	Virus Inactivation	7.6	1.0	5.5	5.5	7144	7001	905	923	0	0	7	700
9	**Purification**	**3M™ Polisher ST**	**7.1**	**1.0**	**0.9**	**0.9**	**7001**	**6931**	**923**	**970**	**0**	**0**	**7**	**693**
10	Purification	IEX Bind & Elute	10.5	1.0	6.9	6.9	6931	6585	970	628	0	0	7	658
11	Purification	Viral Filtration	10.0	1.0	5.7	5.7	6585	6453	628	643	0	0	6	645
12	Purification	UF/DF	50.0	1.0	11.3	11.3	6453	6324	643	126	0	0	6	632
13	Purification	Filtration (0.2um)	46.1	1.0	2.4	2.4	6324	6198	126	134	0	0	6	620

Table 8: *Scenario 1 - Cost of Goods Breakdown (USD per batch)*

	N-2 Seed	N-1 Seed	Production	Primary depth filter	Secondary depth filter	Filtration (0.2um)	Protein A	Virus Inactivation	3M™ Polisher ST	IEX Bind & Elute	Viral Filtration	UF/DF	Filtration (0.2um)
	1	2	3	4	5	6	7	8	**9**	10	11	12	13
Equipment (Total)	473432	1293428	4864120	246762	189512	117125	1518623	108765	**636902**	1220495	116143	539182	81943
Capital	4437	12121	45583	2312	1776	1098	14231	1019	**5969**	11437	1088	5053	768
Materials	1105	2549	16288	1259	1244	1234	6011	1143	**1024**	1657	985	1147	947
Consumables	1406	4573	16664	6606	3768	3015	24558	1558	**7673**	19073	33413	7257	2889
Labour	12301	14134	65747	15867	14745	13664	50905	22171	**12574**	89520	13338	37037	11401
Other	1113	3046	11515	910	739	535	3986	431	**1506**	3079	311	1358	195
	3%	5%	23%	4%	3%	3%	15%	4%	**4%**	18%	7%	8%	2%
Perfusion Factor	1	1	1	1	1	1	1	1	**1**	1	1	1	1
Capital Charge	4437	12121	45583	2312	1776	1098	14231	1019	**5969**	11437	1088	5053	768
Materials	1105	2549	16288	1259	1244	1234	6011	1143	**1024**	1657	985	1147	947
Media	160	1604	15343	0	0	0	0	0	**0**	0	0	0	0
Buffer	0	0	0	30	15	4	5066	1	**79**	712	4	176	2
Direct RM	0	0	0	0	0	0	0	0	**0**	0	0	0	0
Bought WFI & PW	0	0	0	0	0	0	0	0	**0**	0	0	0	0
CIP	0	0	0	285	285	285	0	197	**0**	0	37	26	0
QC tests	945	945	945	945	945	945	945	945	**945**	945	945	945	945
Consumables	1406	4573	16664	6606	3768	3015	24558	1558	**7673**	19073	33413	7257	2889
Resins/MA	0	0	0	0	0	0	17542	0	**5000**	9089	0	0	0
Bags	1406	4573	16664	1253	1092	793	5726	1558	**2673**	9984	2213	4156	1778
Filters	0	0	0	5353	2676	2222	1290	0	**0**	0	31200	3101	1111
Packages	0	0	0	0	0	0	0	0	**0**	0	0	0	0
Other	0	0	0	0	0	0	0	0	**0**	0	0	0	0
Labour	12301	14134	65747	15867	14745	13664	50905	22171	**12574**	89520	13338	37037	11401
Process	5168	5938	27621	6666	6194	5740	21385	9314	**5282**	37608	5603	15560	4790
Quality	5169	5940	27631	6668	6196	5742	21393	9318	**5284**	37621	5605	15565	4791
Indirect	1964	2256	10496	2533	2354	2181	8126	3539	**2007**	14291	2129	5913	1820
Other	1113	3046	11515	910	739	535	3986	431	**1506**	3079	311	1358	195
Insurance/other	228	623	2344	119	91	56	732	52	**307**	588	56	260	39
Waste mgmt	1	1	6	7	4	2	7	2	**4**	11	4	4	1
Maintenance	222	606	2279	116	89	55	712	51	**298**	572	54	253	38
Utilities	662	1816	6886	669	555	422	2535	326	**896**	1909	197	842	116
TOTAL (USD)	20362	36424	155797	26955	22272	19546	99690	26323	**28745**	124766	49136	51852	16200
Total (USD/Gram normalized for the output)	3.29	5.88	25.14	4.35	3.59	3.15	16.09	4.25	**4.64**	20.13	7.93	8.37	2.61

C. Scenario 2: 3M™ Emphaze™ AEX Hybrid Purifier Clarification Train + 3M™ Polisher ST

Table 9: Scenario 2 - Process sequence

No	Process Stage	Unit Op Name	Conc (g/L)	Yield (%)	Duration (hr)	Adjusted Duration (hr)	Mass In (g)	Mass Out (g)	Vol In (L)	Vol Out (L)	Particles In	Particles Out	Target Out	Capacity Out (kg/year)
		Feed	0.0											
1	Upstream	N-2 Seed	0.0	0.0	52.5	8.8	0	0	2	20	0	0	0	0
2	Upstream	N-1 Seed	0.0	0.0	52.5	8.8	0	0	20	200	0	0	0	0
3	Upstream	Production	5.0	1.0	278.5	46.4	0	10000	200	2000	0	0	10	1000
4	Recovery	Primary depth filter	4.3	0.9	3.5	3.5	10000	9000	2000	2112	0	0	9	900
5	**Recovery**	**3M™ Emphaze™ AEX Hybrid Purifier**	**4.1**	**1.0**	**3.0**	**3.0**	**9000**	**8730**	**2112**	**2144**	**0**	**0**	**9**	**873**
6	Purification	Filtration (0.2um)	4.0	1.0	2.4	2.4	8730	8555	2144	2152	0	0	9	856
7	Purification	Protein A	8.5	0.9	7.4	7.4	8555	7700	2152	905	0	0	8	770
8	Purification	Virus Inactivation	8.2	1.0	5.5	5.5	7700	7546	905	923	0	0	8	755
9	**Purification**	**3M™ Polisher ST**	**7.7**	**1.0**	**0.9**	**0.9**	**7546**	**7470**	**923**	**970**	**0**	**0**	**7**	**747**
10	Purification	IEX Bind & Elute	11.3	1.0	6.9	6.9	7470	7097	970	628	0	0	7	710
11	Purification	Viral Filtration	10.8	1.0	5.7	5.7	7097	6955	628	643	0	0	7	695
12	Purification	UF/DF	50.0	1.0	11.3	11.3	6955	6816	643	136	0	0	7	682
13	Purification	Filtration (0.2um)	46.3	1.0	2.5	2.5	6816	6680	136	144	0	0	7	668

Table 10: Scenario 2 - Cost of Goods Breakdown (USD per batch)

	N-2 Seed	N-1 Seed	Production	Primary depth filter	3M™ Emphaze™ AEX Hybrid Purifier	Filtration (0.2um)	Protein A	Virus Inactivation	3M™ Polisher ST	IEX Bind & Elute	Viral Filtration	UF/DF	Filtration (0.2um)
	1	2	3	4	**5**	6	7	8	**9**	10	11	12	13
Equipment (Total)	472585	1291115	4855421	246321	**257063**	116875	1502931	108570	**635763**	1205335	115935	571027	81796
Capital	4426	12092	45474	2307	**2408**	1095	14076	1017	**5954**	11289	1086	5348	766
Materials	1105	2549	16288	1259	**1330**	1232	6011	1143	**1024**	1657	985	1157	947
Consumables	1406	4573	16664	6606	**19373**	1889	24558	1558	**7673**	19073	33413	7257	2889
Labour	12329	14167	65901	15904	**29472**	13830	51016	22223	**12603**	89729	13369	37162	11480
Other	1110	3039	11488	908	**890**	533	3945	430	**1502**	3041	310	1434	194
	3%	5%	22%	4%	**8%**	3%	14%	4%	**4%**	18%	7%	7%	2%
Perfusion Factor	1	1	1	1	**1**	1	1	1	**1**	1	1	1	1
Capital Charge	4426	12092	45474	2307	**2408**	1095	14076	1017	**5954**	11289	1086	5348	766
Materials	1105	2549	16288	1259	**1330**	1232	6011	1143	**1024**	1657	985	1157	947
Media	160	1604	15343	0	**0**	0	0	0	**0**	0	0	0	0
Buffer	0	0	0	30	**101**	2	5066	1	**79**	712	4	186	2
Direct RM	0	0	0	0	**0**	0	0	0	**0**	0	0	0	0
Bought WFI & PW	0	0	0	0	**0**	0	0	0	**0**	0	0	0	0
CIP	0	0	0	285	**285**	285	0	197	**0**	0	37	26	0
QC tests	945	945	945	945	**945**	945	945	945	**945**	945	945	945	945
Consumables	1406	4573	16664	6606	**19373**	1889	24558	1558	**7673**	19073	33413	7257	2889
Resins/MA	0	0	0	0	**0**	0	17542	0	**5000**	9089	0	0	0
Bags	1406	4573	16664	1253	**2573**	778	5726	1558	**2673**	9984	2213	4156	1778
Filters	0	0	0	5353	**16800**	1111	1290	0	**0**	0	31200	3101	1111
Packages	0	0	0	0	**0**	0	0	0	**0**	0	0	0	0
Other	0	0	0	0	**0**	0	0	0	**0**	0	0	0	0
Labour	12329	14167	65901	15904	**29472**	13830	51016	22223	**12603**	89729	13369	37162	11480
Process	5175	5946	27659	6675	**12369**	5804	21411	9327	**5289**	37659	5611	15597	4818
Quality	5188	5962	27733	6693	**12402**	5820	21468	9352	**5304**	37760	5626	15638	4831
Indirect	1966	2259	10510	2537	**4700**	2206	8136	3544	**2010**	14310	2132	5927	1831
Other	1110	3039	11488	908	**890**	533	3945	430	**1502**	3041	310	1434	194
Insurance/other	228	622	2339	119	**124**	56	724	52	**306**	581	56	275	39
Waste mgmt	1	1	6	6	**12**	2	6	2	**4**	10	4	3	1
Maintenance	221	605	2274	115	**120**	55	704	51	**298**	564	54	267	38
Utilities	661	1811	6870	668	**634**	420	2512	325	**894**	1887	196	888	115
TOTAL (USD)	20377	36420	155815	26985	**53473**	18578	99606	26372	**28757**	124789	49164	52358	16277
Total (USD/Gram normalized for the output)	3.05	5.45	23.33	4.04	**8.01**	2.78	14.91	3.95	**4.31**	18.68	7.36	7.84	2.44

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