BIOTECH

Application Note

USD 2971[1]

Clarification of Influenza Virus Harvest with Seitz® Depth Filter Sheet V100P: A Compact and Economical Solution for Maximal Virus Yield and Robust Protection of Downstream Purification Steps

Contents

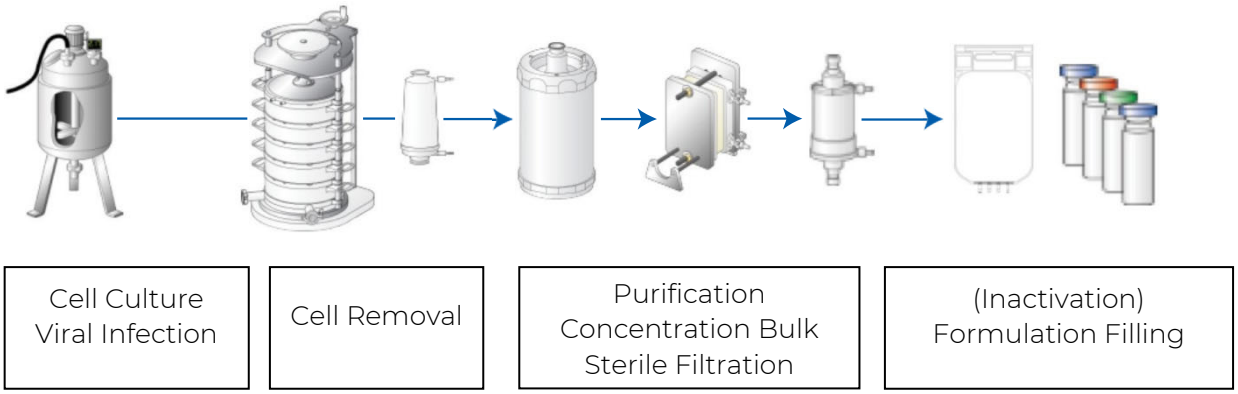
1 Summary

For most biological drugs, including viral vaccines, the removal of cells and cell debris after cell culture is a critical step with a large impact on the further purification process. An efficient clarification step separates the virus from the cells, cell debris and many impurities including insoluble precipitants, aggregates and other materials found in typical cell cultures. This step needs to combine high capacity for impurity removal, high product yield, ease of scale-up and maximally protect any further downstream operation, making the overall process as efficient and economical as possible. In principle, cellulose-based depth filters are the first choice clarification solution for cell culture feed streams, e.g. in monoclonal antibody production. With virus and nanoparticle products however, standard cellulose-based depth filters have been shown to perform sub-optimally for some applications, demonstrating particularly low product yields. Pall has developed a special depth filter sheet, grade V100P, based on our successful, established Seitz P-series depth filter sheet range. The V100P grade is demonstrating good removal of contaminating particles combined with low retention for viruses, increasing the virus yield post-filtration. The filter sheet reduces turbidity and protects the downstream purification steps, e.g. a bioburden-reducing membrane (0.45 µm). A throughput above 200 L/m2 for fluids with limited dirt capacity (~120 NTU) is expected. For higher turbidities, pre-filtration with commercially available depth filters, such as Pall's Seitz K900P, potentially in a double-layer configuration, is recommended. The positioning of the V100P depth filter in a typical virus production process is shown in Figure 1.

Depth filter V100P was tested on two live influenza harvests against current benchmark technologies and demonstrated high product yield with high impurity removal capacity, which would provide for an economic depth filter solution for post-bioreactor feed solutions up to 2000 L+ scale.

Figure 1

A typical virus manufacturing platform showing the position of the V100P depth filter, integrated in Stax™ capsules, as a cell removal step



2 Materials

2.1 Influenza Harvest Material

Material A: Influenza virus A/Puerto Rico/8/1934 (H1N1) was produced by infection of human embryonic kidney (HEK)-293 cells as described by Le Ru *et al* [1]. The material was centrifuged before filtration in the following experiments.

Material B: Influenza virus A/Puerto Rico/8/1934 (H1N1) was produced by infection of Madin-Darby canine kidney (MDCK) cells grown in roller bottles on Glasgow's minimal essential medium(GMEM) supplemented with 10% fetal calf serum. The material was used without further processing as bulk for the following experiments.

2.2 Filters

Table 1

Overview and description of filters used

Filter Medium	Description	Retention Rating*	Permeability @ 100 kPa (L/m²/min)
Seitz V100P depth filter sheet	Cellulose based depth filter media containing perlite	2-4 µm	149
Seitz K100P depth filter sheet	Cellulose based depth filter media containing perlite and diatomaceous earth	1-3 µm	149
HDC®II filter	Polypropylene 'tapered pore' high dirt capacity filter	4.5-1.2-0.6 µm (series)	70
Fluorodyne® II Grade DBL filter	PVDF bioburden reducing membrane filter	0.45 µm	255
Competitor sheet 1	Cellulose based depth filter media containing perlite and diatomaceous earth	0.2-0.6 µm**	74**
Competitor sheet 2	Cellulose based depth filter media containing perlite and diatomaceous earth	0.6-9.0 µm**	650-1200

* For depth filter media, it is not possible to talk about an exact retention rate. The given values are estimations only indicating the range.

** Estimated values from competitor literature.

Table 2

Surface area of different filter formats and recommended operation flow rates

Device	Surface (cm²)	Flow Rate LMH (L/m²/h)	Flow Rate (mL/min)
Supracap™ 50 depth filter capsules	22	100	3.6
Membrane disc holder 47 cm	11	100	1.8
VELApad™ 60 filter housing	22	100	3.6
Supracap 100 depth filter capsules	1000	100	167

2.3 Equipment and Set Up

The filtration trials were carried out on a filtration system as is described in Figure 2. A peristaltic pump with compatible tubing, adapted to the required flow rate, pressure sensors and a weigh balance or graduated cylinder were used.

2.4 Buffers and Solutions

Pre-filtration and post-filtration, the filters were flushed with PBS buffer, pH 7.4, 15 mS/cm.

3 Methods

3.1 Filter Installation

The filters were installed according to the set up shown in Figure 2 and the instructions for use issued with the products. Filters were pre-rinsed with a minimum volume of 50 L/m² buffer.

The capsule was vented during filling by allowing the air to escape through the vent (Figure 3). The vent was tightened as soon as all excess air had escaped the assembly, and the liquid had reached the level of the vent. After rinsing, the filtration device was drained by opening the vent and pouring the excess liquid out.

3.2 Filtration and Sampling

The filtration device was filled and vented as described above. During filtration, the flow rate was set to 100 LMH. A sample of the initial feedstock was taken for virus titer determination and turbidity measurement. During filtration, pressure and turbidity were monitored as a function

of time, and the filtration stopped when either a pressure of 1.5 bar was exceeded, when a significant increase in turbidity was noticed, when the feedstock ran out or a predefined volumetric throughput was reached (200 L/m²).

3.3 Post-Rinsing

Preliminary trials had indicated that the virus yield could be significantly increased with a post-filtration buffer rinse step (Figure 4). Therefore, after filtration the filter capsules were emptied (see section 3.1: Filter Installation) and rinsed with ~23 L/m² buffer (recommended PBS-buffer or process-specific buffer). This rinse was pooled with the filtrate and the virus titer was determined on the pool.

Figure 2

Experimental set up

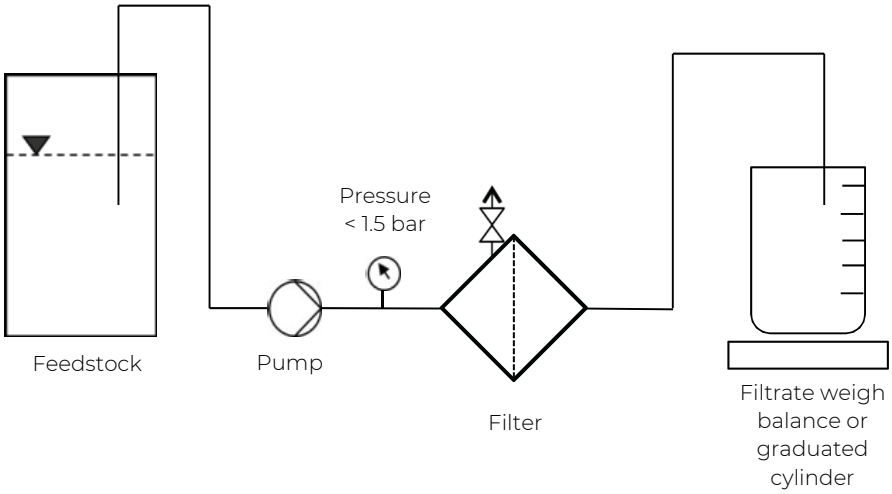


Figure 3

Detail of the Supracap 50 (left) and 100 (right) depth filter capsules with vent, Inlet and outlet detailed

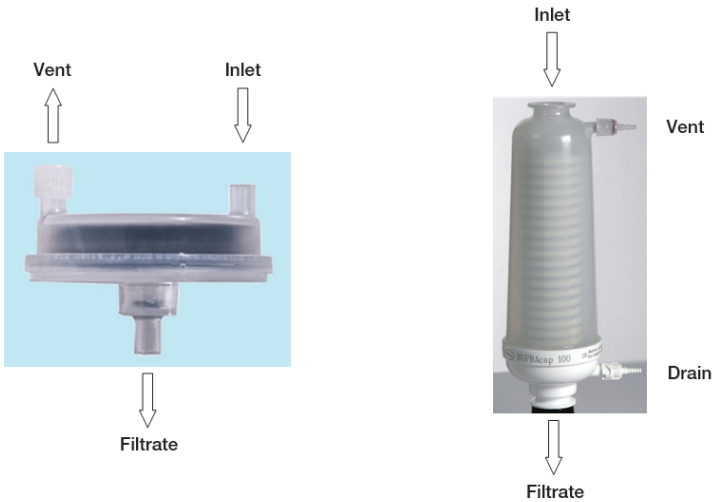
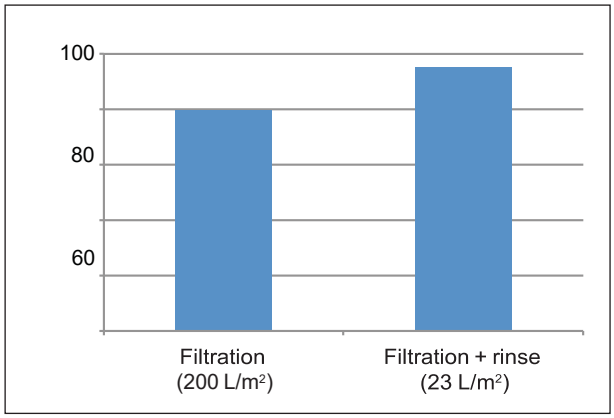


Figure 4

Impact of post-filtration rinse on the overall product recovery for material A on depth filter sheet V100P



3.4 Assays

Influenza virus was quantified using a classical hemagglutination assay on chicken red blood cells. The assay was performed according to the protocol described by Genzal *et al* [2] and Its standard deviation was generally 0.18 log HA units/mL. Turbidity was measured using a Hach♦ 2100P turbidimeter.

4 Results

4.1 Performance of V100P Compared to Current Available Products

Current viral vaccine manufacturing platforms often incorporate a train of polypropylene-based membrane filters with decreasing porosity, to remove the cells. A reference train combining HDC II series membranes (4.5 µm + 1.2 µm + 0.6 µm) was used as a benchmark throughout the experiments.

To evaluate its performance, various lots of the depth filter V100P were compared to the HDC II membrane benchmark (Figure 5) and to other cellulose-based depth filter products in the market of similar permeabilities or retention rates (Figure 6). Overall, depth filter sheet V100P resulted in a virus product yield ranging from as good as, to slightly below the HDC II membrane benchmark. However, it performed much better than the alternative cellulose-based depth filters available. Furthermore, it had a larger volumetric throughput than the benchmark, leading to a compact sizing of the manufacturing scale product and a better turbidity reduction, which will translate to better protection of the downstream process steps.

Figure 5

Comparison of performance of different lots of the depth filter sheet V100P v benchmark filter train (HDC II membrane). (a) turbidity, (b) capacity, (c) yield

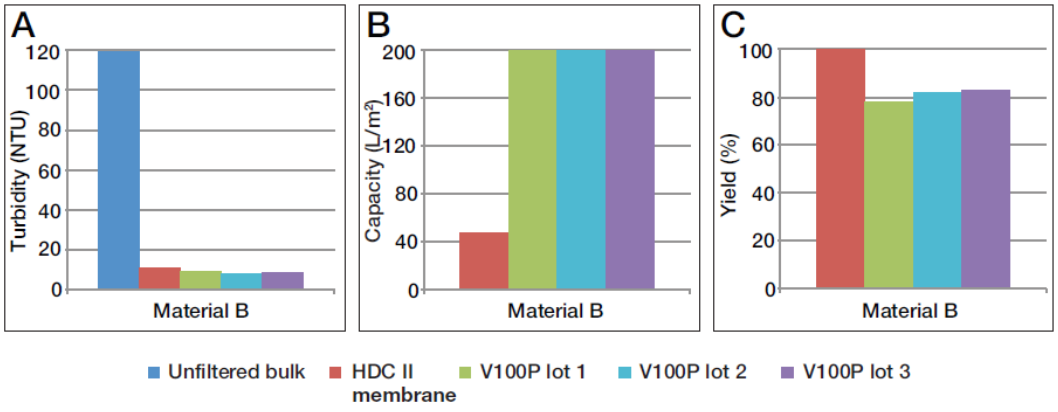
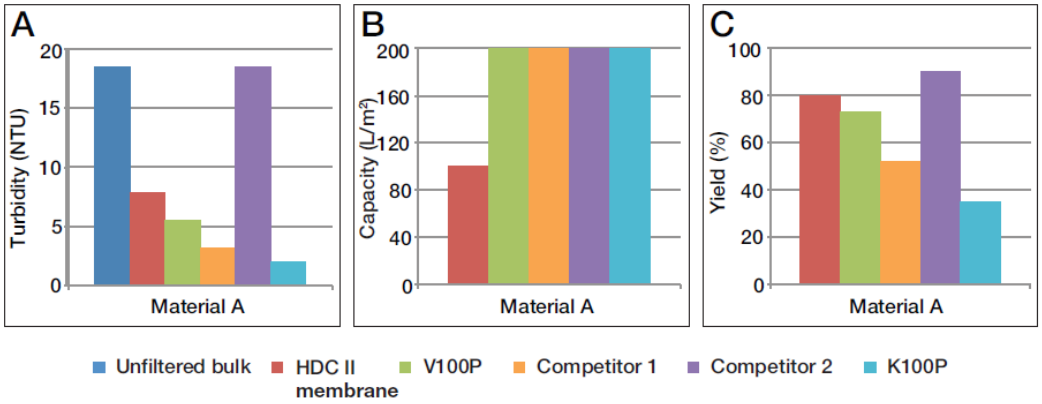


Figure 6

Comparison of performance of the depth filter sheet V100P v benchmark of filter train (HDC II membrane) and other commercial depth filters available. (a) turbidity, (b) capacity, (c) yield



4.2 Scalabilty of Performance

Pall's range of Supracap 50, Supracap 100 and Stax encapsulated depth filter modules provide great flexibility and assurance of scalability for process volumes from less <1 L to > 1000 L for typical cell removal applications. The scale-up performance of the range (Figure 7) has been shown to be scalable to within ± 15% of capacity (Collins *et al* [3]).

The performance of depth filter V100P using the Supracap 100 module (0.1 m²) was compared to that of the scale-down tool Supracap 50 capsule (22 cm²). The latter is typically used for initial screening studies and is also the tool used in the experiments described above. Comparable performance was observed for filtrate turbidity and overall filter capacity (Figure 8). The overall yield was noticeably higher for the higher scale device, which could be attributed to either HA assay variation or optimized flow distribution through the larger device.

Figure 7

The current range of scalable depth filter tools from Pall available from bench to manufacturing.

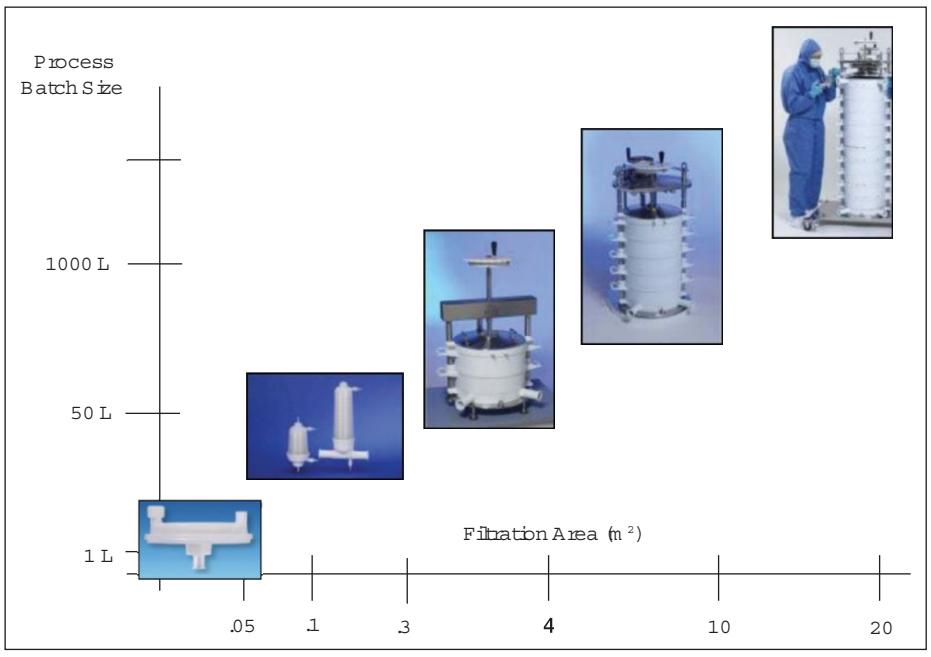
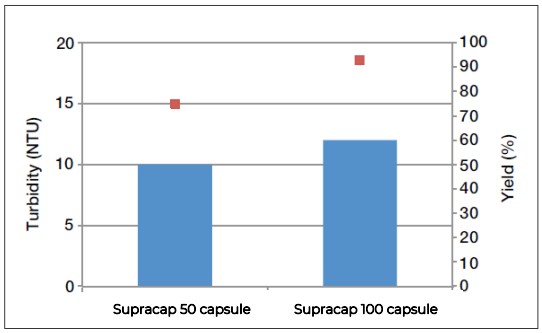


Figure 8

Comparison between filtrate turbidity (bars) and yield (dots) for the scale-down tool, Supracap 50 capsule, and the manufacturing tool, Supracap 100 capsule.



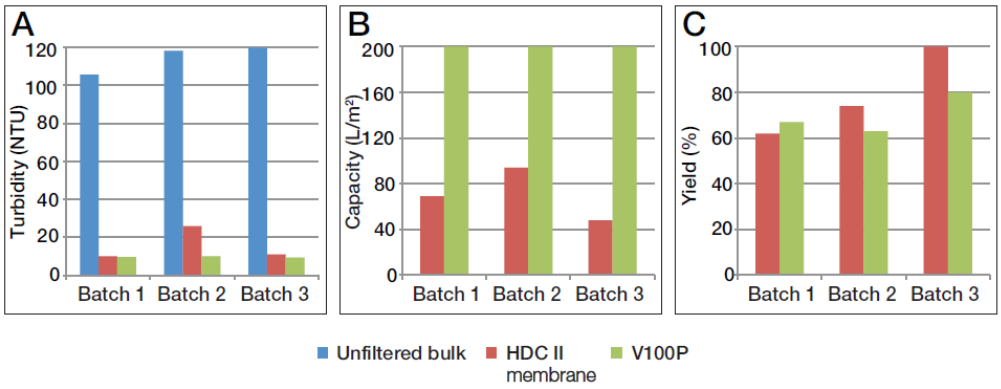
4.3 Batch to Batch Variation: Process Robustness

Batch failure rates in the manufacture of biologicals including vaccines are relatively high compared to other industries and are often a consequence of non-robust manufacturing processes. This can lead to shortage of important biological products as reported by the Food and Drug Administration (FDA) [4].

The performance of depth filter V100P was compared to the reference filter train for three different manufacturing batches of material B. The results in Figure 9 indicate that V100P has a more reliable and reproducible performance and can neutralize some of the upstream variations for the downstream process.

Figure 9

Impact of upstream variations on the cell removal efficiency of depth filter sheet V100P v benchmark filter train (HDC II membrane). (a) turbidity, (b) capacity, (c) yield.

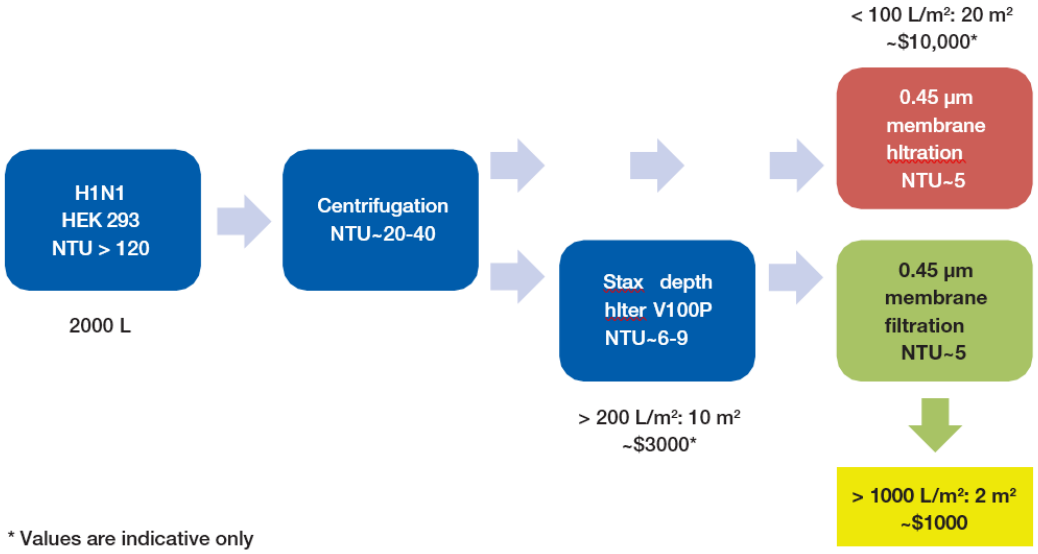


4.4 Protection of Downstream Processing: Post-Clarification Filtration on a Bioburden Reducing Membrane

The impact of the cell removal step on V100P on the downstream process and the related economics was demonstrated using the Fluorodyne II grade DBL bioburden-reducing membrane (0.45 µm). The membrane capacity during filtration without and with preceding cell removal on V100P was measured, and found to be at least 10-fold higher, resulting in a 10-fold reduction of the sizing of this filter (Figure 10). Typically, the cost of bioburden reducing and sterilizing-grade membranes or chromatography products is higher than that of cellulose-based depth filter products. The overall benefit of the implementation of such a step is therefore a cost reduction, in addition to achieving a more robust overall process.

Figure 10

Impact of a cell removal filtration on V100P on the downstream process



5 Discussion

The clinical relevance of nanoparticle drugs and vaccines, based on virus or virus-like-particles, has never been so high. Not only are they still a successful approach to developing vaccines, but in addition they hold great promise in a myriad of therapeutic targets including cancer, Alzheimer's disease and AIDS, with the first gene therapy product approved by the FDA in 2017. Virus production processes have seen a big evolution on the upstream side, using reliable and controlled cell lines, growing on better defined culture media and using larger batch sizes. The prevalence of cell culture-based influenza vaccines is poised to further intensify virus production. However, due to their size and other properties, several of those nanoparticles present a challenge to the downstream purification process, not at least to the primary purification post bioreactor. An efficient clarification step separates the nanoparticles from cells, cell debris and many impurities, including insoluble precipitants, aggregates and other materials found in typical cell cultures. This step needs to combine high capacity for contaminant removal, high product yield, ease of scale-up and to maximally protect any further downstream operation, making the overall process as efficient, reproducible and economical as possible.

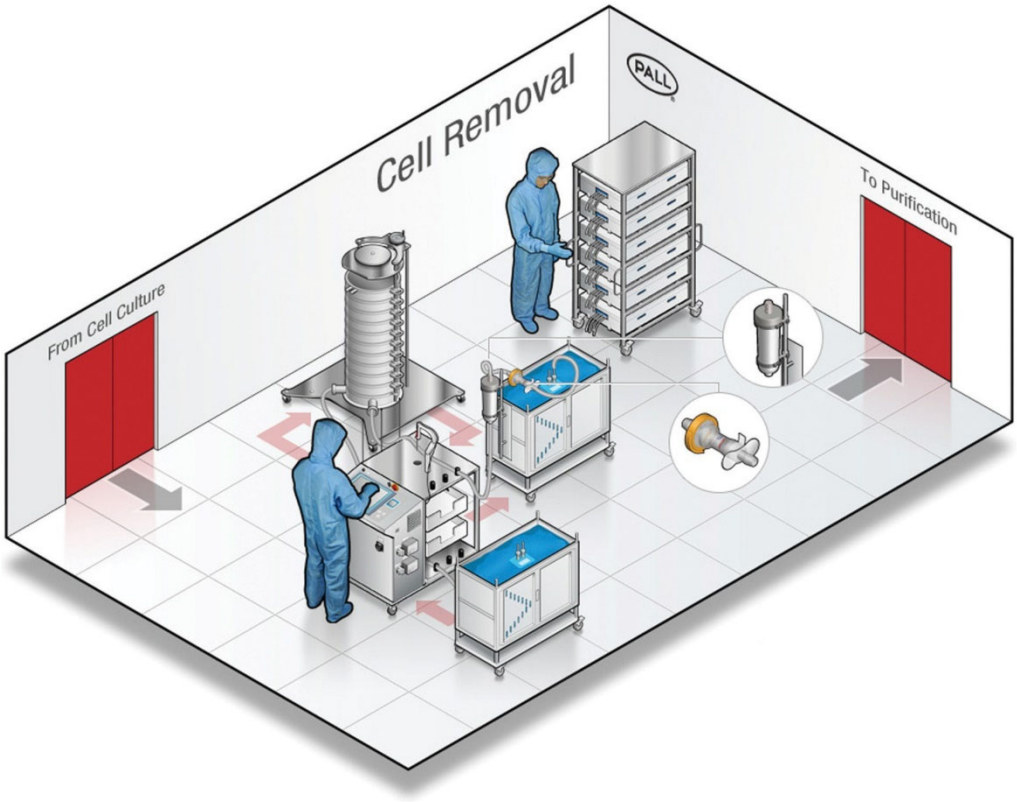
Cellulose-based depth filters have proven to meet all these objectives for typical biotech feed streams and are a preferred solution used for many protein-based drugs. Compared to other clarification techniques including tangential flow filtration or centrifugation, manufacturing operators appreciate the simplicity and compactness of the solution and the absence of time-consuming installation and cleaning steps linked to the single-use operation. A typical manufacturing-scale solution is shown in Figure 11.

With nanoparticles however, the standard cellulose-based depth filters have seemed to perform poorly, giving particularly low product yields. Our research hence focused on gaining a better understanding of the mechanisms responsible for retention and/or transmission of large viruses or VLPs, which are expected to include size-exclusion and adsorption. The outcome was the development of depth filter sheet V100P, by optimizing the depth filter component mixture, which typically includes cellulose, perlites and other compounds.

V100P was tested on two influenza feed materials against current benchmark technologies and demonstrated high product yield with high contaminant capacity, that would provide for a robust and economic depth filter solution for post bioreactor feed solutions up to typically 2000 L scale. Overall, across the data generated, it is clear there is a balance between increased turbidity reduction and reduced virus yield, probably linked to size exclusion of the virus particles. However, for depth filter sheets with equal permeability, it can be observed that the new sheet recipe offers a significant improvement on the yield. Further research to the exact mechanisms involved is continuing.

Figure 11

Typical manufacturing-scale cell removal operation including related equipment for product hold and transport, controlled process operation and inclusive data acquisition.



6 Ordering Information

Supracap 50 Capsule (< 1 L)	Effective Filter Area	Connections
SC050V100	22 cm^2	Luer lock

Supracap 100 Capsules (1 – 100+ L)	Description	Effective Filter Area	Connections
NP5LV1006	127 mm (5 in.) capsule	0.05 m^2	
NP6V1006	254 mm (10 in.) capsule	0.1 m^2	13 mm single barb hose barb inlet and outlet
NP5LV1001	508 mm (20 in.) capsule	0.05 m^2	
NP6V1001	762 mm (30 in.) capsule	0.1 m^2	
NP7V1001	127 mm (5 in.) capsule	0.2 m^2	
NP8V1001	254 mm (10 in.) capsule	0.3 m^2	1-1½ in. sanitary flange inlet and outlet

Stax Capsules (100-2000+ L)	Description	Effective Filter Area	Connections
SXSV100404SP	Small capsule	0.5 m^2	
SXMV100408SP	Medium capsule	1.0 m^2	
SXLV100416SP	Large capsule	2.0 m^2	For use with the Stax chassis (SXPSC)

7 References

(1) Le Ru, A., Jacob, D., Transfiguracion, J., Ansorge, S., Henry, O., Kamen, A.A. Scalable production of influenza virus in HEK-293 cells for efficient vaccine manufacturing. *Vaccine 28* (2010) 3661-3671

(2) Genzel, Y., Behrendt, I., König S., Sann, H.,Reichl, U. Metabolism of MDCK cells during cell growth and influenza virus production in large-scale microcarrier culture *Vaccine 22* (2004) 2202-2208

(3) Collins, M., Lari, H., Anderson, S., Leibnitz, R., Kumar, A. and Kuriyel, R. Investigating flow distribution and its effects on scale-up. Performance of a multicapsule depth filtration system. *BioProcess International* 7 (9) (2009) 46-52

(4) https://www.fda.gov/drugs/drug-safety-and-availability/drug-shortages, *accessed June 2021*

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PALL CORPORATION

Corporate Headquarters
Port Washington, NY, USA
+1-800-717-7255 toll free (USA)
+1-516-484-5400 phone

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone

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