Demonstrating the scalability of Cytiva P-grade depth filters for CHO cell culture clarification

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Abstract

Clarification using depth filtration is widely adopted in the manufacturing of mAb as the first step to remove the cells to prepare cell culture harvest for downstream chromatography and purification. Clarification is a critical step with strong impact on product recovery and subsequent downstream purifications steps. Turbidity and filter capacity/filter life have been the industry standards to evaluate depth filtration process and depth filters. We demonstrate the scalability of Stax™ mAx depth filters from lab-scale capsule to manufacturing-scale capsule. Consistent performance is achieved across the range of scales as measured by filtration throughput, turbidity reduction, impurity removal, mAb yields, and particle counts.

Design/materials and methods

Cell culture growth and harvest

- 500 L stirred-tank reactor (STR) with Chinese hamster ovary (CHO) cell culture producing anti-HER2 mAb in fed batch mode. Cell culture was harvested on day 13 with cell density of 24.93 × 10^6/mL, viability of 64.4%, and crude turbidity of 2835 nephelometric turbidity units (NTU).

Cytiva Stax mAx depth filter clarification platform

- Scalability of P-grade portfolio was tested in various formats in a standard CHO clarification application. Stax mAx clarification platform is a single-use, robust depth filtration solution for the economic clarification of monoclonal antibody cell cultures without the need for centrifugation or process additives. The platform applies two stages of filtration, each with two layers of pharmaceutical grade depth-filter media with sequentially finer filter grades.

Features	Benefits
Low hold-up volume	Greater product recovery and lower post use rinse volume requirements than traditional modules and housings.
Seamless linear scalability	Greater flexibility and assurance of process success from < 1 L to 20 000 L. Scalable through our entire line of equipment. Provides assurance of meeting process design and requirements.
Minimized risk	Stax capsules are based upon the design of Supradisc™ II modules, which provide benefits in process stability due to their high mechanical robustness.
No housings	Easier to use and manipulate while eliminating operator safety issues.
Completely disposable	Eliminates need for cleaning and cleaning validation.
Encapsulated design	Reduces operator exposure to potential biohazards.
Intuitive operation	Reduces operator training and increases time to acceptance.
Small footprint	Enables use near other equipment and reduces cost to install.

General materials

- Depth filter formats used in the study were 60 mm depth filter sheets in Velapad stainless steel housings (VP60), Supracap™ 50 (SC50; 22 cm^2), Supracap 100 5" (SC100; 250 cm^2), SC100 10" (500 cm^2), Stax medium capsule (0.5 m^2) and Stax large capsule (1.0 m^2).
- Peristaltic pump used for lab scale depth filter formats and Quattroflow 150 (QF150) diaphragm pump was used for manufacturing-scale depth filter format.

Clarification conditions

- Depth filters were flushed with 50 L/m^2/layer of 1× PBS at 300 LMH feed flux rate.
- CHO cell culture clarification was performed at 75 L/m^2/h (LMH) feed flux.
- Two stage Stax mAx platform clarification (PDP8>PDE2) was performed with stage 1 and stage 2 separated to assess the scalability over each stage.
- Product recovery chase of 25 L/m^2 was performed.

Analytics

- mAb titer was evaluated using the Octet platform based on bio-layer interferometry (BLI) technology. HCP was evaluated using HCP ELISA. DNA was evaluated using PicoGreen assay kit.
- Particle size distribution was measured using Beckman Multisizer 4e Coulter counter.

SC50 depth filter capsules

SC100 depth filter capsules

Stax disposable depth filter systems

Scalability

Depth filter loading

- Both stage 1 and stage 2 showed clarification, as shown by the continuous rise in inlet pressure (Fig 1).
- Stage 1 loading capacity was ~ 74 L/m^2 with loading for stage 1 depth filter defined by turbidity breakthrough (Fig 2).
- Stage 2 loading capacity for SC50 to Stax format was within a CV of 15%.
- Post stage 2, filtrate was clarified using Supor™ EKV sterile membrane filters with similar loading capacity (data not shown).

Turbidities

- Filtrate pool turbidities for repeat 1 and 2 of each depth filter format are shown (Fig 2).
- All the filtrate pool turbidities are similar for SC50 to Stax depth filter formats.

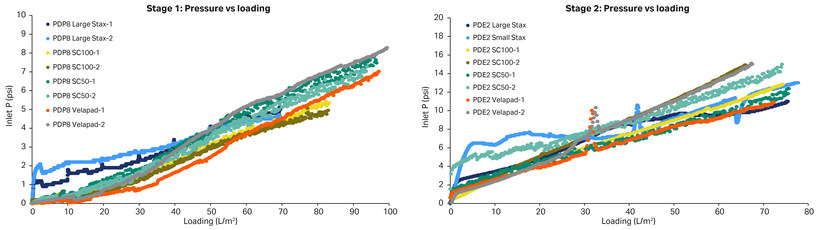
mAb yields

- mAb yields are measured post-product recovery chase.
- Stage 1 and stage 2 depth filter mAb yields were similar for all scales and formats of depth filters (Fig 3).

Impurity removal

- Stage 2 DNA removal was similar for SC50 to stax depth filter format, ~ 94% DNA removal was observed for stage 2 filters (Fig 4).
- Stage 2 HCP removal was 45% to 57% for SC50 and SC100 depth filter, but stage 2 stax depth filter had HCP removal of 30%.
- Stage 1 depth filter did not impact DNA or HCP removal (data not shown).

Fig 1. Stage 1 and stage 2 depth filter inlet pressures during CHO cell culture clarification.



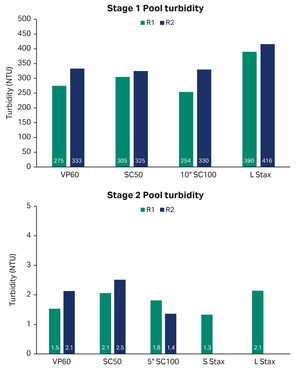


Fig 2. Stage 1 and stage 2 depth filter filtrate pool turbidities during CHO cell culture clarification.

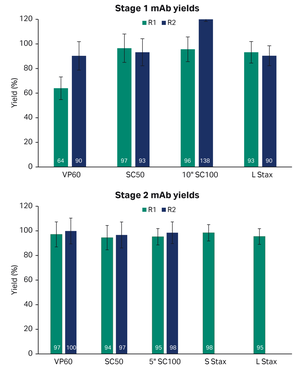


Fig 3. Stage 1 and stage 2 depth filter mAb yields during CHO cell culture clarification.

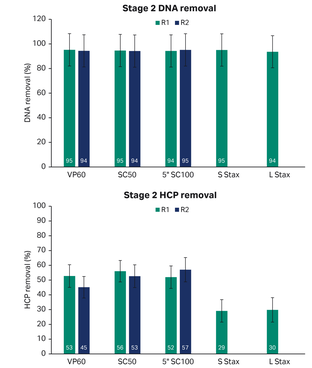


Fig 4. Stage 2 depth filter DNA and HCP removal capacity during CHO cell culture clarification.

Conclusions

- Loading capacity of stage 1 and 2 depth filters were similar for SC50 to Stax filter capsules. Stage 2 loading capacity within CV of 15%.
- Pool turbidities were similar across all the scales and formats of stage 1 and stage 2 depth filters.
- mAb yields were similar across all the scales and formats of stage 1 and stage 2 depth filters.
- DNA removal was similar across all the scales and formats of stage 2 depth filters.
- HCP removal was similar for SC50 (22 cm^2) and SC100 (250 cm^2) capsules of stage 2 depth filter; HCP removal was lower for stax capsules compared to small depth filter capsules.

Reference: https://cdn.cytivalifesciences.com/api/public/content/UMRuoYtuAku8U5tWu6hkfg-pdf

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